PHYTOPLANKTON DYNAMICS IN GALVESTON BAY: ASSESSING RESPONSES TO FRESHWATER INFLOWS

A Thesis

by

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ABSTRACT

Increased freshwater use in estuarine watersheds is a concern for productivity downstream in ecologically and economically important estuaries worldwide. In Galveston Bay (TX), the seventh largest estuary in the United States, population growth in two large metropolitan areas (Houston and Dallas-Fort Worth), continues to alter the quantity and quality of freshwater inflows (FWI). We report here on the influence of FWI on pelagic and benthic phytoplankton in Galveston Bay in spring and summer over 3 years (2010 to 2012), intended to capture periods of high and low FWI, respectively. A year of severe drought that persisted throughout 2011 allowed us to also examine consequences of prolonged low flows. We followed the response of pelagic phytoplankton (biomass, community composition) to the addition of nutrients using assays, and the response of benthic phytoplankton (biomass, community composition), in addition to corresponding nutrient fluxes and sediment oxygen consumption via core incubation methods.

Log response ratios indicated bay-wide nitrate+ammonium (NA) and nitrate+phosphate (NP) co-limitation of pelagic phytoplankton, in addition to recurrent N or A limitation. Further, nutrient limitation of phytoplankton standing stock was more frequently observed during drought than non-drought years. Diatoms, cyanobacteria, and chlorophytes were dominant in 2010 and 2011, but dinoflagellates became particularly prominent in spring 2012 as FWI alleviated prolonged drought conditions. We also observed resilience of the benthic microalgal (BMA) community to drought, but not in

the benthic boundary layer (BBL) phytoplankton community. BMA communities primarily consisted of diatoms throughout, while BBL phytoplankton communities differed with each sampling event. Fluxes differed before and after the drought, and the results here imply that resilience of the water column system is at risk in future drought events, though further study is necessary. We observed that drought itself does not have a significant effect on pelagic or benthic phytoplankton community composition, though timing of the beginning of the drought in relation to annual phytoplankton growth cycles could play a role. Rather, the increase in availability of freshwater inflows following the drought appeared to be more influential on community structure, than the lack of inflows and the resources they bring.

DEDICATION

This thesis is in dedication to my daughter, Penelope. Thank you for giving me the clarity I sought for in my life, and the fire to finish. I hope in time to help you appreciate the wonders of this world, and the beauty of science. You are my heart, and I love you, more than you'll ever know.

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NOMENCLATURE

A	Ammonium
AICc	Akaike Information Criterion Corrected
BBL	Benthic Boundary Layer
BMA	Benthic Microalgae
CGB	Central Galveston Bay
CHEMTAX	Chemical Taxonomy
DIN	Dissolved Inorganic Nitrogen
DISTLM	Distance based Linear Modeling
DMSO	Dimethyl sulfoxide
DNRA	Dissimilatory nitrate reduction to ammonium
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
FIRe	Fluorescence Induction and Relaxation
F_m	Maximum Fluorescence Yield
FWI	Freshwater Inflows
F_v/F_m	Photosynthetic efficiency of chlorophyll fluorescence
G	Gulf
HPLC	High Performance Liquid Chromatography
HPO_4	Phosphate

IP	Ion Pairing
N	Nitrate
NA	Nitrate + Ammonium
NH_4	Ammonium
NO_3	Nitrate
NP	Nitrate + Phosphate
P	Phosphate
PCO	Principal Coordinates
PERMANOVA	Permutational analysis of variance
PSII	Photosystem II
Si	Silicate
SOC	Sediment Oxygen Consumption
SPARROW	Spatially Referenced Regressions on Watershed attributes
TB	Trinity Bay
TOC	Total Organic Carbon
TWDB	Texas Water Development Board

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1. INTRODUCTION AND LITERATURE REVIEW

Estuaries are ecosystems that much of the urbanized inland depends on for food, recreation, and waste disposal. These ecosystems make up more than three quarters of the United States coastline along the Atlantic Ocean and Gulf of Mexico, and drain the surrounding urbanized and industrialized watersheds (Pinckney et al., 2001). Coastal and estuarine watersheds support about 75% of the global human population, with approximately 60% residing within 100 km of the coastline (Paerl, 2006; Vitousek et al., 1997). Populations in these areas are dependent on estuaries as a source for food, recreation, and waste disposal (Bricker et al., 1999; Lester and Gonzalez, 2011). Increasing demand on freshwater sources within the estuarine watersheds to support human populations may lower the freshwater inflow (FWI) river discharge and consequently alter nutrient and sediment loading in estuaries (Dorado et al., 2015; Roelke et al., 2013). This may in turn have serious consequences for phytoplankton, which support higher trophic levels, and other ecosystem services (Feyrer et al., 2015; Flemer and Champ, 2006; Paerl et al., 2010).

Galveston Bay, a subtropical estuary on the Texas coast in the northwest Gulf of Mexico, is the seventh largest estuary in the United States (Pinckney, 2006; Yeager et al., 2007). The average depth of the 1,554-km^2-area bay is 2-3 meters, and is distinguished as a vertically mixed estuary with a residence time of 40-88 days (Bianchi et al., 1999; Lester and Gonzalez, 2002; Pinckney, 2006; Pinckney et al., 1996; Santschi, 1995). The watershed of Galveston Bay includes, in addition to major agricultural areas,

the Dallas-Fort Worth and Houston metropolitan areas. The Bay receives its majority of freshwater inflow (FWI) and nutrient input via the Trinity and San Jacinto Rivers, draining the Dallas-Fort Worth and Houston metropolitan areas respectively, in addition to smaller inputs from groundwater, streams and adjacent bayous. Though the percentage of contributions from each source varies, the majority of freshwater input originates from the Trinity River, at 55% (Guthrie et al., 2012). Galveston Bay also receives wastewater inputs from 60% of the main industrial activity in Texas (Galloway et al., 2003; Örnólfsdóttir et al., 2004a). Flows to the Bay follow a seasonal pattern, with peak annual discharge typically occurring in the spring months, and minimum annual discharge in the summer months (USGS monthly mean discharge from 2000 to 2012 at the Trinity River gage 08066500 in Romayor, TX).

Houston and Dallas-Fort Worth, both in the top ten most populous cities in the United States, are expected to increase in population by approximately 40 and 76 percent by 2070, respectively (TWDB, 2013b) . Overall, the state is expected to see an 82 percent increase in population, with an approximate 71 percent increase in municipal freshwater demand by 2060 (TWDB, 2012). It is therefore reasonable to expect that the San Jacinto River, which is nutrient fortified from sewage treatment plants and industrial facilities in Houston, will be responsible for a greater proportion of the riverine derived FWI to Galveston Bay (Quigg, 2009).

The Bay is home to commercial fisheries (such as shrimp, oysters, blue crab, spotted seatrout, and red drum, among others), in addition to being an important site of recreational tourism and fishing. Galveston Bay commercial and recreational fishing

supplies, respectively, one-third and more than half of the state's income for those categories (Lester and Gonzales 2011, and references therein).

The time period for the proposed study, 2010 to 2012, captured what has been called by the State Climatologist the most intense one-year drought (2011) in the state of Texas' recorded history (Nielsen-Gammon, 2011). During 2011, a number of records were broken, but those of particular importance to this study include: the driest March, the third driest July, and the warmest summer (June to August; Nielsen-Gammon 2011). By the end of March, more than 43% of state was reported to be in "extreme drought," and by the end of September, the entire state was in some form of drought, with 85.75% in "exceptional drought," the driest category assigned by the U.S. Drought Monitor (Nielsen-Gammon, 2011). Subsequent increases in freshwater use, both private and commercial due to population increase, in addition to predicted increase in probability and severity of droughts, is a concern for productivity this economically important estuary (Lester and Gonzalez, 2011; Stocker et al., 2014; TWDB, 2012). According to the Texas Water Development Board (TWDB), as of 2012, current freshwater demand could not be met in the event of a drought and would result in substantial economic losses to the state, approximately $11.9 billion annually (TWDB, 2012).

This study aimed to resolve the complexity of relationships between phytoplankton communities and freshwater inflows in Galveston Bay. The 2011 drought illustrates the worst-case scenario for Galveston Bay under increased population growth, and corresponding reduced freshwater inflows. By observing phytoplankton responses with and without freshwater inflows, we may be able to determine what factors

associated with inflows are most important to the health of the phytoplankton communities, which can then be used to direct resource management strategies. Our overall objective was to determine the response of phytoplankton populations in Galveston Bay to changes in freshwater inflow supply between 2010 and 2012. This was done:

1) By following the response of the phytoplankton (biomass, community composition) in surface waters to the addition of nutrients using assays (Section 2) and
2) By following the response of the benthic phytoplankton (biomass, community composition) as well as the corresponding nutrient fluxes and sediment oxygen consumption (Section 3).

2. PHYTOPLANKTON NUTRIENT LIMITATION: RESPONSES DURING NORMAL AND DROUGHT YEARS IN GALVESTON BAY (TEXAS, USA)

2.1. Summary

Increased freshwater use in estuarine watersheds is a concern for productivity downstream in ecologically and economically important estuaries worldwide. In Galveston Bay (TX), the seventh largest estuary in the United States, population growth in two large metropolitan areas (Houston and Dallas-Fort Worth), continues to alter the quantity and quality of freshwater inflows (FWI). We report here on the influence of nutrient limitation on phytoplankton biomass (or standing stock) and community structure in spring and summer over 3 years (2010 to 2012). The experimental design was intended to capture periods of high and low FWI, respectively. A year of severe drought that persisted throughout 2011 allowed us to also examine consequences of prolonged low flows. Six nutrient-addition treatments were tested: N (as nitrate), A (as ammonium), P (as phosphate), Si (as silicate), NA, and NP, along with a control (no additions) at six sites. Log response ratios indicated baywide NA and NP co-limitation, along with frequent N or A limitation, but not P or Si limitation of phytoplankton standing stock. Further, nutrient limitation of standing stock was more frequently observed during drought than non-drought years. High Performance Liquid Chromatography (HPLC), coupled with CHEMTAX, was utilized to characterize phytoplankton communities. Diatoms, cyanobacteria, and chlorophytes were dominant

in 2010 and 2011, but dinoflagellates became prominent in 2012, particularly in the spring after FWI alleviated prolonged drought conditions. While FWI may be a very important source of nutrients to phytoplankton in estuaries, these findings suggest other hydrographic features play a role.

2.2. Introduction

Coastal and estuarine watersheds support about 75% of the global human population, with approximately 60% residing within 100 km of the coastline (Paerl, 2006; Vitousek et al., 1997). Populations in these areas are dependent on estuaries as a source for food, recreation, and waste disposal (Bricker et al., 1999; Lester and Gonzalez, 2011). Increasing demand on freshwater sources within the estuarine watersheds to support human populations may lower the freshwater inflow (FWI) river discharge and consequently alter nutrient and sediment loading in estuaries (Dorado et al., 2015; Roelke et al., 2013). This may in turn have serious consequences for phytoplankton, which support higher trophic levels, and other ecosystem services (Feyrer et al., 2015; Flemer and Champ, 2006; Paerl et al., 2010).

Nutrient availability varies spatially and temporally within and between estuarine systems (Cloern et al., 2014). The resultant nutrient limitation of estuarine phytoplankton has been shown to be related to changes in FWI, nutrient ratios, and benthic fluxes (Doering et al., 1995; Fisher et al., 1999; Örnólfsdóttir et al., 2004a; Örnólfsdóttir et al., 2004b). Peak nitrogen concentrations in temperate estuaries typically

co-occur with high FWI in the spring, and are generally higher closer to the river mouth, decreasing with increasing salinity (Cloern, 1996; Fisher et al., 1999; Pinckney, 2006). Nutrients investigated as limiting for primary productivity in marine systems include nitrogen, phosphorus, and in some cases, silicate (Hecky and Kilham, 1988). Nutrient addition bioassays are commonly used in assessing the influence of nutrient forms on the community structure and growth rates of phytoplankton populations (Downing et al., 1999; Elser et al., 2007; Fisher et al., 1999; Richardson et al., 2001). It has been proposed that nitrogen is lost via denitrification without any process to counterbalance and resupply it (Fisher et al., 1999; Howarth and Marino, 2006), causing phytoplankton in temperate estuarine systems to be nitrogen limited. However, more recent studies have demonstrated several processes that need to be considered as compensatory to denitrification, including anammox bacteria, dissimilatory nitrate reduction to ammonium, regeneration, and recycling (Dong et al., 2011; Fisher et al., 1982; Gardner et al., 2006; Lin et al., 2011; Miller et al., 1995; Mortazavi et al., 2012; Ward, 2013). Furthermore, internal cycling of nitrogen can be important in fueling primary production when FWI is diminished by drought or seasonal low flow (Bruesewitz et al., 2013; Paerl, 2006).

Riverine sediment discharged to the estuary brings phosphorus adsorbed to the particles of sediment and turbidity due to lack of flocculation, potentially resulting in phosphorus and/or light limitation in areas closer to the river mouth (Fisher et al., 1999). Increasing salinity desorbs phosphorus, making it more bioavailable in temperate estuaries (Fisher et al., 1999; Howarth and Marino, 2006). While P limitation is observed

in the nearby northern Gulf of Mexico adjacent to the Louisiana shelf (Quigg et al., 2011), it is not observed in tropical systems such as the west coast of Florida due to the higher sorption of phosphorus to carbonate sediments (Bianchi, 2007). Sediment fluxes of phosphorus to the water column in Gulf of Mexico estuaries are lower than those estuaries found on the eastern United States and Europe due to reduced residence times and loading (Bianchi, 2007). Low flow or drought conditions likely reduce overall phosphorus concentrations in Gulf of Mexico estuaries, as it is largely supplied by rivers to estuarine systems (Bianchi, 2007).

Rivers also supply 80% of the dissolved silicon delivered to the global ocean, by way of estuaries (Tréguer et al., 1995). While silicate (Si) can be considered limiting for siliceous phytoplankton (Hecky and Kilham, 1988), it is generally not a primary limiting nutrient because the effects exhibited by phytoplankton to Si addition are typically an enhancement of the limitation by primary limiting nutrients, either nitrate (N) or phosphorus (P) (Fisher et al., 1999). Within estuaries, limitations of different nutrients can undergo a spatial and seasonal "switch" between nitrogen and phosphorus due to external supply from rivers and hydrodynamic mixing, as shown by mesocosm experiments (Doering et al., 1995).

Evidence of co-limitation in estuarine phytoplankton studies has recently become increasingly common (Davidson and Howarth, 2007; Downing, 1997; Elser et al., 2007), such that discussion of co-limitation has been investigated (Harpole et al., 2011). Co-limitation is discussed herein as a limitation response exhibited by the phytoplankton at the biomass level, and revealed most simply as a change in total biomass or standing

stock (measured as chlorophyll *a*) following the addition of a combination of two nutrients. Though phytoplankton standing stock may illustrate overall nutrient limitation, community level (i.e. independent taxonomic group) changes are not apparent. Within the community level, resource (e.g. nutrients, light) competition and resulting shifts in dominant taxonomic groups can be codependent on season, and nutrient concentrations and ratios (Klausmeier et al., 2008; Klausmeier et al., 2004; Litchman et al., 2007; Roelke et al., 1997; Tilman et al., 1986). Previous research has demonstrated drought and low riverine discharge induced shifts in phytoplankton community structure (Breckenridge et al., 2015; Cloern et al., 1983; Nichols, 1985; Putland et al., 2014). Examining community composition in addition to nutrient limitations of standing stock may reveal more information on how phytoplankton are impacted by droughts.

FWI regimes are likely to become more variable, as projected global changes in precipitation over the course of the 21st century in sub-tropical and mid-latitudes will demonstrate increased intensity, decreased mean precipitation, and increases in time between rainfall events, making droughts more prevalent in these areas worldwide (Meehl et al., 2007; Stocker et al., 2014). These detrimental conditions will be exacerbated by more frequent and enduring heat waves, or multiple consecutive days during which temperature exceeds the 90th percentile (daily minimum or maximum) relative to a reference period in the late 20th century (Hartmann et al., 2013; Meehl et al., 2007; Perkins and Alexander, 2013). Studies examining biological responses to changing freshwater availability upon which densely populated areas depend are becoming increasingly important. During the period of this study, the most intense one-

year drought (October 2010 – December 2011) in the state of Texas was observed (Nielsen-Gammon, 2011). By the end of March 2011, more than 43% of state was reported to be in "extreme drought," and by the end of September 2011, the entire state was in some level of drought, with 86% in "exceptional drought," the driest category assigned by the U.S. Drought Monitor (Nielsen-Gammon, 2011). As of 2012, current water demands would not be met in the event of a future drought, resulting in approximately $11.9 billion in annual economic losses to the state (TWDB, 2012).

This study investigated nutrient limitation of phytoplankton communities in Galveston Bay (Texas, U.S.A.) in relation to FWI. The watershed includes the Houston and Dallas-Fort Worth metropolitan areas, both amongst the top 10 most populous cities in the U.S., with respective populations expected to increase by approximately 40 and 76 percent by 2070 (TWDB, 2013b). The Bay is home to commercial and recreational fishing (such as shrimp, oysters, blue crab, spotted seatrout, and red drum, among others), and tourism. These provide one-third and more than half of the state's income for these ecosystem services, respectively (Lester and Gonzales, 2011, and references therein). Overall, the state is expected to see an 82% increase in population, with an approximate 73% increase in municipal freshwater demand by 2060 (TWDB, 2012). Our overall objective was to use nutrient addition bioassays to determine the responses of phytoplankton standing stock and community structure along gradients from riverine sources (Trinity and San Jacinto Rivers) to the ocean (Gulf of Mexico). Using multivariate statistical approaches, we determined the combination of environmental

factors most important in driving the response of phytoplankton communities, which in turn can be used to forecast system responses to changing FWI.

2.3. Materials and Methods

2.3.1. Study Site

Galveston Bay (Fig. 1), on the Texas coast in the northwest Gulf of Mexico, is the seventh largest estuary in the United States (Yeager et al., 2007). The average depth of the vertically mixed 1,554-km2 estuary is 2-3 m, with a residence time of less than 100 days (Bianchi et al., 1999; Pinckney, 2006; Pinckney et al., 1996; Santschi, 1995). Galveston Bay receives the majority of FWI input via the Trinity River (55%) and San Jacinto River and Buffalo Bayou (26%), in addition to smaller inputs from groundwater, streams and adjacent bayous (Guthrie et al., 2012). Galveston Bay also receives wastewater inputs from 60% of the main industrial activities in Texas (Galloway et al., 2003). USGS daily mean discharge from 2010 to 2012 at the Trinity River gage 08066500 in Romayor, TX (www.usgs.gov) is shown in Fig. 2.

2.3.2. Sample Collection and Preparation

Surface water samples were collected during March (spring) and July (summer) in 2010, 2011, and 2012 from 6 stations around the Bay aboard R/V Phyto I (Fig. 1):

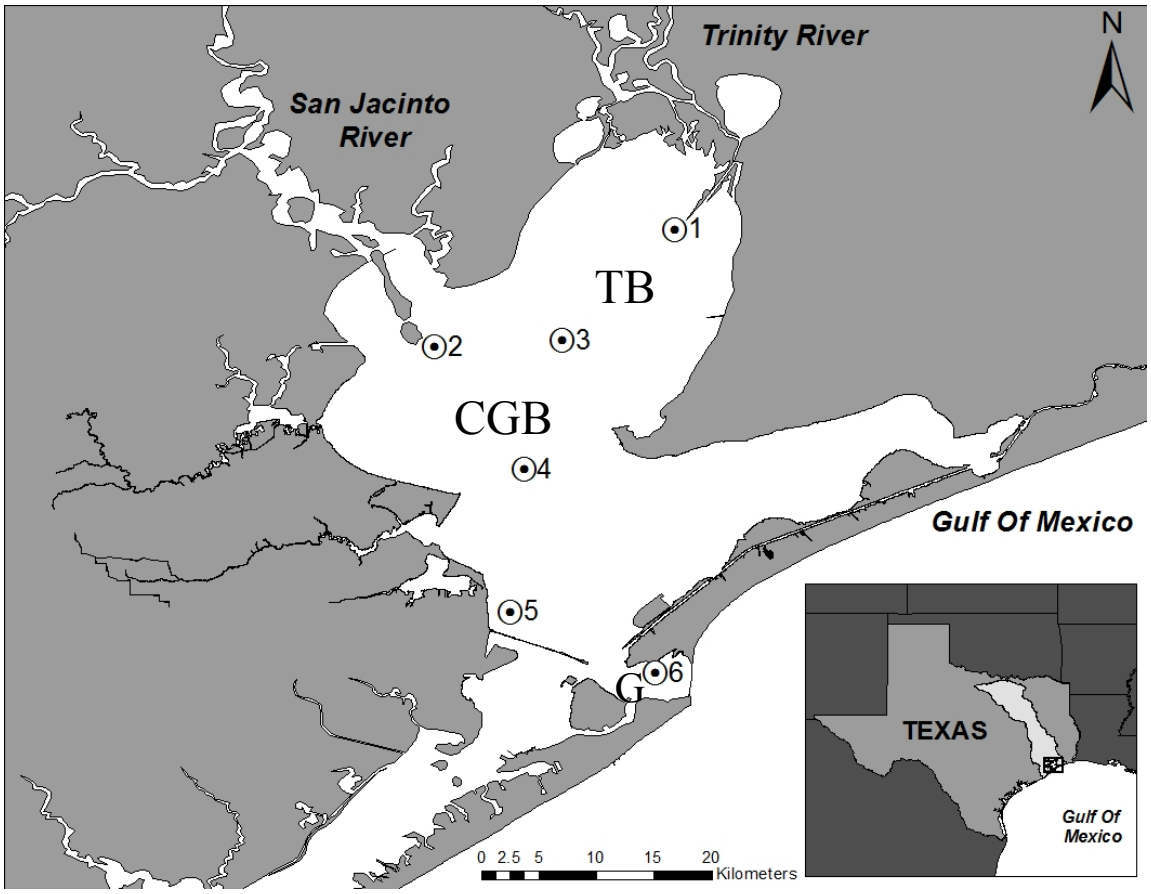

Figure 1. **Bioassay sampling map of Galveston Bay, Texas with significant sub-bay clusters designated as Central Galveston Bay (CGB), Trinity Bay (TB), and Gulf station (G).**

Stations 1 and 3 (Trinity Bay, or TB), Stations 2, 4, and 5 (Central Galveston Bay, or CGB), and Station 6 (Gulf, or G). The samples were processed for chlorophyll (chl) a, phytoplankton community pigments using High Performance Liquid Chromatography (HPLC), dissolved nutrients, total organic carbon (TOC), and dissolved organic carbon (DOC). Parameters measured in the field included salinity (presented herein using the unit-less practical salinity scale) and temperature (˚C) using a calibrated Hach Hydrolab, and Secchi depth (m).

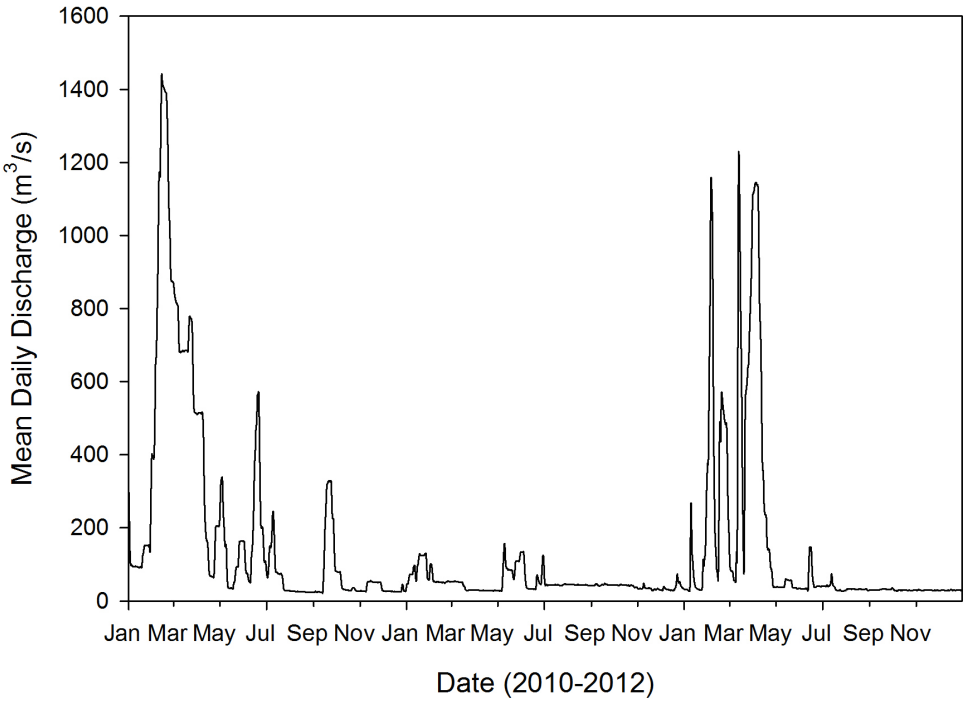

Figure 2. Mean daily freshwater discharge (m^3/s) from the Trinity River, using the USGS gage at Romayor, Texas from 2010 to 2012.

Water samples (3.8L) from each station were collected in 4L acid washed cubitainers; triplicates of the 7 treatments per station were prepared immediately upon return to campus. Nutrients added (4 mL; nitrate (N), ammonium (A), phosphate (P), silicate (Si), NA, NP, and a control (no nutrients added)), vitamins (4 mL), and trace metals (4 mL) were added to the cubitainers using the concentrations prescribed for f/2 media (www.ncma.org). The cubitainers were incubated in a shaded (50% sunscreen) corrals for 7 days in the Texas A&M University Galveston small boat basin, floating in surface waters, at in situ temperatures, diel cycles, and turbulence.

2.3.3. Sample Processing

Water collected from each station prior to start of the bioassays was processed according to the following procedures. Samples were vacuum filtered (maximum pressure 130 kPa) using 47mm Whatman GF/F filters, which were frozen until fluorometric analysis (chl-*a*, stored at -20˚C) and HPLC analysis (pigments, stored at -80°C). Dissolved nutrients measured in the filtrate were sent to the Geochemical and Environmental Research Group at Texas A&M University (College Station, Texas) for analysis using standard protocols (see Dorado et al. (2015)). To measure DOC, pre-combusted, pre-weighed 47mm Whatman GF/F filters were used. Filtrate for DOC analysis was stored in amber glass vials at -20˚C to prevent photochemical degradation of the DOC in the filtrate (Miller and Moran, 1997). TOC was measured on an unfiltered sample. At the end of the bioassays, we again collected samples and processed them for chl-*a* and pigment analysis.

Chl-*a* (corrected for pheophytin-*a*) was fluorometrically (Turner Designs 10-AU) measured for phytoplankton standing stock according to the methods in Arar and Collins (1997) with one modification. The pigments were extracted from the filters using a solution of 60/40 ratio of 90% acetone/DMSO (Jeffrey et al., 1997). TOC and DOC concentrations were measured using a Shimadzu TOC-5000/ASI-5000 instrument operating under standard procedures set by APHA (1998).

HPLC analyses were conducted according to the procedures in Pinckney et al. (1996) with these specific details. The pigment filters were lyophilized for 24 hours and

then extracted in a -20˚C cryo-cooler container in a dark freezer for 24 hrs using 100% acetone, along with carotenal (internal standard, unknown concentration). Blank samples were prepared simultaneously. Pigment extracts were filtered (with a 0.2 µm PTFE filter attachment) and centrifuged for 5 minutes at 2795 g. An ammonium acetate ion pairing (IP) solution (1 M) was added in a 4:1 extract:IP ratio. Batches were run using 80% methanol + 20% 0.5M ammonium acetate as Solvent A, and 80% methanol + 20% acetone as Solvent B.

In conjunction with the pigment data acquired with HPLC, the program CHEMTAX V1.95 (CHEMical TAXonomy; (Mackey et al., 1996)) was employed to estimate the relative contributions of different phytoplankton groups to the total community. Galveston Bay samples were analyzed in multiple datasets per station, targeting diatoms, dinoflagellates (including gyroxanthin-containing), cyanobacteria, chlorophytes, cryptophytes, and haptophytes-3 and -4, using the Schlüter matrix for estuarine phytoplankton (Schlüter et al., 2000). Each of these was defined as a separate phytoplankton group for the purpose of statistical analysis (except haptophytes-3 and -4 which were grouped as haptophytes). This approach has been used previously in Galveston Bay (Örnólfsdóttir et al., 2004a; Örnólfsdóttir et al., 2004b; Roelke et al., 2013). Additionally, we employed the validation method described by Latasa (2007), performing three successive reruns using the newly generated output ratios as input, and the final community composition did not change after further reruns.

2.3.4. Statistical Analyses

PRIMER V6.1.15 and PERMANOVA V1.0.5 were utilized to perform statistical analyses (Anderson et al., 2008; Clarke and Warwick, 2001). We investigated null hypotheses by using permutational analysis of variance (PERMANOVA) such that these were rejected when $p < 0.05$. PERMANOVA analyses used the following design parameters as recommended by Anderson et al. (2008): Type III (partial) Sums of Squares, 9999 permutations, residuals permuted under reduced model. Unrestricted permutation of data was selected when testing significance of a single factor in both main and pairwise testing. These parameters increase the statistical power of the resulting p-value, ensure independence of all hypotheses tested, and are appropriate for unbalanced designs (Anderson et al., 2008).

To preserve inherent variability in the biological data (community composition and biomass), data was not transformed or normalized, and were analyzed using Bray-Curtis resemblance matrices to reduce bias associated with inclusion of zero values as recommended by Clarke and Warwick (2001). Abiotic variables, including temperature, salinity, Secchi depth, dissolved nutrients, and total and dissolved organic carbon, were evaluated for collinearity using draftsman plots, and were excluded from the analysis if correlations were greater than 0.90. Data from the abiotic variables were square-root transformed to decrease outlier influence before normalization and then analyzed using Euclidean distance resemblance matrices.

2.3.4.1. Correlating in situ Phytoplankton to Environmental Conditions Prior to Treatment

A combined subset of predictor environmental variables correlated to the variability of the initial (in situ) phytoplankton standing stock and community structure variability was determined using distance-based linear modeling (DISTLM). The "BEST" selection model was chosen to test all possible combinations of predictor variables. As the number of possible predictor variables exceeded that of the biological variables, the Akaike Information Criterion corrected (AICc) selection criterion was determined to be most suitable. Models with the lowest AICc values were considered to optimally evaluate environmental predictors of variability in the in situ phytoplankton standing stock and communities. The model produced also calculated the correlation and proportion of variability explained for individual predictor variables to the in situ standing stock and community composition variability using marginal tests. These correlations were considered significant when $p < 0.05$.

Principal coordinates analysis (PCO) ordination plots were used to visualize multivariate spatial and temporal variation of in situ phytoplankton community composition in two dimensions. Interpretations of variability represented by a PCO are determined to be adequate by the amount of variability explained by the two axes shown in the ordination, with ≥70% being a reasonable depiction (Anderson et al., 2008). Relationships between significantly correlated predictor variables identified by DISTLM

and the relative abundance of in situ phytoplankton groups were determined using Spearman correlation vectors on the PCO plots.

2.3.4.2. Assessing Phytoplankton Standing Stock Response to Nutrient Limitation

To determine if the phytoplankton standing stock responded to nutrient addition(s), we first calculated the log response ratio following the methods of Harpole et al. (2011). We compared the phytoplankton response (as chl-*a* (μg/L)) in each treatment to that of the corresponding control after a 7 day incubation, and then calculated the natural log of the ratio. Treatments with limiting nutrients were considered those that had ratios that exceed the threshold of 1.385 (i.e. 38.5% greater than the control) as set by Harpole et al. (2011). This method is similar to that employed by Downing et al. (1999) and Fisher et al. (1999), though in the log response ratio, change relative to time (growth rate) is not considered as we were interested in the change relative to the control only. This method is also more stringent than a Student's t-test, allowing for the examination of the most important nutrients limiting to phytoplankton biomass.

Treatments were considered to be "co-limiting" if a limitation response was elicited from a treatment involving the addition of two nutrients together (Fisher et al., 1999). Evidence of co-limitation was further evaluated for additivity per Harpole et al. (2011). Treatments deemed to be co-limiting were considered to be "super-additive" if the combination of the two nutrients increased standing stocks in a treatment above that

of either nutrient alone. Furthermore, co-limiting treatments were considered to be "sub-additive" if the combination did not elevate standing stocks above that of both nutrients alone.

2.3.4.3. Assessing Phytoplankton Community Response to Nutrient Limitation

Shifts in phytoplankton community structure between bioassay nutrient enriched treatments and controls were determined using PERMANOVA pairwise tests with fixed factors including station (6 levels: Stations 1 through 6), time point (6 levels: spring 2010, 2011, 2012; summer 2010, 2011, 2012) and treatment (7 levels: Control, A, N, NA, NP, P, and Si). Assessment of community composition of the treatments were considered to be significantly different from the community of the control if $p < 0.05$. PCO ordination plots were also used to project the community variability after exposure to bioassay treatments in a two-dimensional space in the form of centroids. Centroids were used to visualize the overall average variability in the community composition among stations and treatments between drought and non-drought conditions.

We examined the response of phytoplankton group-specific limitation (i.e. diatoms, dinoflagellates, chlorophytes, etc.) between drought and non-drought years. This was determined by quantifying for each phytoplankton group the number of stations and treatments during drought and non-drought years in which a treatment elicited an increase in relative abundance over the control. The treatment with the

greatest frequency and increase of group abundance compared to the control was determined to be the primary limiting treatment for said phytoplankton group.

2.4. Results

2.4.1. Variability in Freshwater Inflows

Flows to Galveston Bay from the Trinity River, the major contributor of freshwater to the estuary, follow a seasonal pattern, with peaks in the spring and lows in the summer, except in 2011 (Fig. 2). Mean monthly discharge showed that March 2011 (42 m^3/s) was 14 times lower than the mean monthly discharge in March 2010 (704 m^3/s) and 2012 (479 m^3/s), but similar to the typical low flow periods in the summer (Fig. 2). During the drought period of 2011, average monthly flow was approximately 51 m^3/s, compared to 236 and 149 m^3/s in 2010 and 2012, respectively. Freshets (defined herein as FWI >250 m^3/s) were frequently observed in non-drought years, with 5-7 significant pulses recorded annually.

2.4.2. Variability in Abiotic Parameters

Peak water temperature occurred in the summer of 2011 (30.5 – 31.4˚C (±1˚C)) and the minima (16.5 – 17.8˚C) was recorded in spring 2010. Salinity was highest during the drought (2011) across all stations ranging from 22 – 31 (Table 1). In non-drought

years, salinity increased with distance from the river mouths (Table 1). Water clarity, as indicated by Secchi depth (m), was generally highest during the drought (0.6 -1.2 m), with minimum clarity in spring 2012 (0.2 – 0.5 m, Table 1). Also during spring 2012, maximum DOC concentrations (6.6 – 11.3 mg/L) were measured. DOC concentrations were generally lower during the drought (3.2 – 5.5 mg/L), with the exception of Station 2 in summer 2012 (Fig. 1, Table 1). TOC varied between 3.9 – 9.3 mg/L at most stations and times, except during 2012 when higher values up to 24.7 were observed in the upper bay.

Nutrient concentrations in Galveston Bay at the time of the bioassays exhibited spatiotemporal trends (Table 1). Maximum concentrations for these nutrients were higher in the upper bay, at either Station 1 (Si and NO3-), Station 2 (P), or Station 3 (NH4+), whereas minimum concentrations for P, Si, NO3-, and NH4+ all occurred in the lower bay (Stations 4, 5, and 6; Fig. 1). P and Si generally demonstrated higher concentrations during summer (0.71 – 7.8 μmol/L, and 22.6 – 122 μmol/L, respectively) than spring, in all years (Table 1). NO3- concentrations were commonly low (<1 μmol/L) across stations throughout the study (Table 1); however, maximum concentrations (0.07 – 33.4 μmol/L) occurred more often in summer and non-drought years. Maximum NH4+ concentrations (1.9 – 6.7 μmol/L) were exhibited in both spring and summer in all years across stations, whereas minimum concentrations (0.22 – 0.34 μmol/L) were typical in spring of non-drought years (Table 1).

Table 1. Environmental parameters measured at each station per sampling event prior to start of the bioassays. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_3^-, NO_2^-, and NH_4^+. Abiotic data is significantly variable across stations ($p < 0.01$), and sampling events ($p < 0.01$).

Station	Sampling Event	Temperature (°C)	Salinity	Secchi (m)	Chl-*a* (µg/L)	P_i (µmol/L)	$HSiO_3^-$ (µmol/L)	NO_3^- (µmol/L)	NH_4^+ (µmol/L)	NO_2^- (µmol/L)	DIN:P_i	DOC (mg/L)	TOC (mg/L)
St. 1	Spring 2010	16.5	0.2	0.4	10.5	0.39	96.29	33.41	2.23	0.33	92	6.79	6.59
	Summer 2010	29.7	8.7	0.7	8.7	2.85	99.32	0.01	0.34	0.02	0.13	6.64	6.41
	Spring 2011	17.7	15.1	0.8	36.3	0.83	11.10	0.34	0.31	0.15	0.97	5.53	6.48
	Summer 2011	30.5	22.1	0.5	14.3	3.02	75.95	0.05	0.22	0.07	0.11	6.81	8.15
	Spring 2012	17.8	2.2	0.2	16.8	1.59	67.21	26.94	0.71	2.24	18.8	10.51	10.96
	Summer 2012	27.6	8.7	0.5	26.8	3.41	121.80	0.13	0.36	0.05	0.16	8.45	11.44
St. 2	Spring 2010	18.2	4.8	0.5	33.5	0.33	35.61	0.01	1.55	0.06	4.9	7.50	9.09
	Summer 2010	28.9	10.3	0.6	4.7	3.31	100.45	0.01	0.73	0.06	0.24	6.06	7.18
	Spring 2011	17.8	21.9	0.6	189.1	1.61	13.08	15.60	6.71	1.56	14.8	5.10	4.79
	Summer 2011	31.4	24.3	0.6	21.1	1.66	22.57	0.07	0.62	0.08	0.46	5.76	6.67
	Spring 2012	19.2	8.2	0.4	14.2	0.81	66.93	0.29	2.52	0.06	3.5	11.29	15.26
	Summer 2012	28.5	9.2	0.6	18.1	7.80	93.55	32.40	0.52	20.08	6.8	4.69	19.99
St. 3	Spring 2010	17.1	3.6	0.7	30.5	0.24	50.35	0.19	0.44	0.20	3.5	6.63	7.66
	Summer 2010	29.8	11.0	0.6	16.4	3.04	94.26	0.05	0.83	0.03	0.30	5.92	7.12
	Spring 2011	17.6	19.9	0.5	55.3	0.60	6.46	0.40	1.14	0.14	2.8	5.48	6.79
	Summer 2011	30.8	24.4	0.6	20.4	1.44	25.77	0.06	0.41	0.10	0.39	5.81	7.16
	Spring 2012	18.5	3.1	0.3	13.9	1.40	68.88	18.64	1.07	0.82	14.7	9.55	13.13
	Summer 2012	27.7	14.2	0.5	28.9	3.77	103.01	0.26	1.85	0.09	0.58	7.23	9.68
St. 4	Spring 2010	17.2	6.2	0.6	30.4	0.47	45.54	5.09	1.62	0.26	14.8	7.15	7.53
	Summer 2010	31.0	14.9	0.6	3.8	3.23	69.74	0.02	0.64	0.09	0.23	4.97	6.10
	Spring 2011	18.1	23.3	1.0	37.7	1.32	10.55	4.69	5.66	0.65	8.3	4.72	4.68
	Summer 2011	30.4	25.8	0.5	18.9	1.89	29.86	0.61	1.05	3.83	2.9	5.25	6.13
	Spring 2012	17.8	11.7	0.4	15.6	0.81	55.93	0.53	2.86	0.06	4.3	9.21	8.93
	Summer 2012	30.3	15.5	0.6	47.5	4.46	77.46	1.85	0.48	5.71	1.8	6.21	24.73
St. 5	Spring 2010	17.6	10.6	0.9	14.6	0.49	2.28	0.06	0.25	0.11	0.86	6.37	7.47
	Summer 2010	29.8	16.5	0.8	5.2	3.02	69.98	0.04	0.43	0.06	0.18	5.01	5.71
	Spring 2011	18.6	23.2	1.2	104.9	0.55	2.61	0.05	0.26	0.11	0.76	5.99	9.26
	Summer 2011	31.4	27.0	0.5	13.0	0.75	42.67	0.06	2.41	0.04	3.3	4.08	5.04
	Spring 2012	18.0	15.0	0.5	11.8	0.38	46.77	0.00	0.50	0.02	1.4	8.50	8.92
	Summer 2012	30.6	18.0	0.7	30.1	3.17	37.65	0.27	0.23	0.15	0.21	5.67	9.87
St. 6	Spring 2010	16.8	15.3	0.6	16.3	0.23	22.43	0.09	1.30	0.02	6.1	5.35	6.48
	Summer 2010	29.9	19.1	0.8	11.7	4.01	77.92	0.07	5.53	0.13	1.4	4.77	5.12
	Spring 2011	18.0	27.2	1.0	32.7	0.66	4.07	0.05	1.07	0.22	2.0	3.70	3.86
	Summer 2011	29.9	30.9	1.0	15.1	0.71	30.33	0.96	0.60	1.69	4.6	3.16	4.47
	Spring 2012	18.0	21.0	0.5	8.4	0.20	23.83	1.04	0.31	0.03	6.9	6.64	8.24
	Summer 2012	30.6	25.8	1.2	7.8	0.87	29.89	0.27	0.79	0.03	1.2	4.47	4.89

2.4.3. Variability in Biological Parameters

Average phytoplankton standing stock (chl-*a*) in the bay ranged from 8.4 – 76 µg/L and 13.4 – 27 µg/L in spring and summer, respectively (Table 1). The seasonal minima and maxima occurred in 2011 and 2012 respectively, with the average spring maximum elevated by standing stock at Stations 2 and 5 (189 and 105 µg/L chl-*a*, respectively). DISTLM analysis of in situ standing stock identified salinity, N, DIN:Pi, Total N, Secchi, and DOC as the combination of environmental parameters that optimally predicted variability (69% explained) of phytoplankton standing stock (AICc = 223.1). Of these variables, salinity, Total N, Secchi, and DOC significantly explain variability ($p < 0.01$).

Overall, the phytoplankton community structure in Galveston Bay during our study was primarily composed of four phytoplankton groups: diatoms, dinoflagellates, chlorophytes, and cyanobacteria, with only minor contributions of haptophytes and cryptophytes (Table 2). The largest percentage component was often diatoms, with some exceptions (Table 2). Diatoms constituted the majority of the in situ community structure in spring before the drought, with the exception of chlorophytes at Station 3. Pre-drought summer in situ communities were predominantly a mixture of chlorophytes (Stations 1, 3 and 6), dinoflagellates (Station 2), diatoms (Station 4), and cyanobacteria (Station 5, Table 2). During the drought, diatoms again predominated in situ community composition with few exceptions: chlorophytes at Station 1 in spring, and cyanobacteria at Stations 3 and 5 in the summer (Table 2). Dinoflagellates overwhelmingly dominated

Table 2. Percent contributions of phytoplankton groups to *in situ* community structure as determined using relative abundances from CHEMTAX. Bolded numbers indicate largest contribution to community composition.

Station	Sampling Event	Cyanobacteria	Chlorophytes	Dinoflagellates	Haptophytes	Cryptophytes	Diatoms
St. 1	Spring 2010	2.42	25.95	3.36	0.93	17.99	**49.36**
	Summer 2010	29.24	**34.23**	13.04	0.16	7.76	15.57
	Spring 2011	7.47	**42.14**	3.45	3.85	2.64	40.45
	Summer 2011	12.79	21.30	0.64	1.91	12.32	**51.04**
	Spring 2012	2.22	**66.34**	15.64	0.00	11.44	4.36
	Summer 2012	20.82	**33.31**	8.63	3.49	14.70	19.04
St. 2	Spring 2010	0.35	32.28	4.79	7.00	1.94	**53.65**
	Summer 2010	22.06	27.67	**38.24**	0.00	8.15	3.88
	Spring 2011	5.07	5.65	4.83	3.98	0.00	**80.47**
	Summer 2011	19.85	9.60	16.86	0.46	7.71	**45.52**
	Spring 2012	0.36	4.58	**92.96**	0.11	1.98	0.00
	Summer 2012	12.83	24.46	4.79	0.84	2.30	**54.76**
St. 3	Spring 2010	0.99	**46.15**	1.75	0.31	10.13	40.66
	Summer 2010	15.78	**50.15**	6.45	0.00	11.70	15.92
	Spring 2011	4.46	19.86	9.05	2.83	0.00	**63.80**
	Summer 2011	**53.58**	13.19	6.97	1.14	4.87	20.25
	Spring 2012	3.25	**50.89**	29.74	0.65	14.24	1.23
	Summer 2012	17.11	32.33	1.20	0.64	0.00	**48.72**
St. 4	Spring 2010	0.80	33.05	11.17	0.97	2.12	**51.88**
	Summer 2010	21.01	27.45	6.89	1.08	9.53	**34.05**
	Spring 2011	3.86	8.17	6.35	3.13	0.00	**78.49**
	Summer 2011	13.88	22.01	5.33	3.59	15.35	**39.84**
	Spring 2012	0.39	4.77	**88.39**	0.00	6.45	0.00
	Summer 2012	8.12	23.90	6.04	0.23	0.97	**60.75**
St. 5	Spring 2010	2.50	28.76	21.13	1.37	2.18	**44.06**
	Summer 2010	**38.19**	29.22	4.66	0.89	6.12	20.92
	Spring 2011	2.62	22.11	12.68	1.32	0.00	**61.28**
	Summer 2011	**36.08**	16.68	7.88	0.00	6.23	33.14
	Spring 2012	0.26	4.09	**94.56**	0.00	1.08	0.00
	Summer 2012	16.57	26.26	3.83	0.22	0.00	**53.12**
St. 6	Spring 2010	0.90	16.10	5.96	1.07	3.67	**72.31**
	Summer 2010	23.16	**32.80**	5.50	1.45	7.58	29.51
	Spring 2011	0.84	14.09	3.37	0.73	0.00	**80.98**
	Summer 2011	4.88	14.31	8.89	1.40	4.08	**66.44**
	Spring 2012	0.14	10.75	**80.55**	0.00	8.56	0.00
	Summer 2012	**39.95**	4.59	5.51	4.67	13.33	31.95

the in situ community in spring following the drought (more than 80%) in 4 out of the 6 stations. The two stations excluded in this trend were in the Trinity River basin, where chlorophytes constituted the in situ majority, and dinoflagellates were at least 10% of the community (Table 2). Post-drought summer saw the return of diatoms to predominance

bay wide, with the exception of Station 1 (chlorophytes) and Station 6 (cyanobacteria). The DISTLM model identified the combination of temperature, salinity, and DOC (AICc = 255. 52) as the significant environmental variables ($p < 0.01$) that best predict variability in the phytoplankton communities, explaining approximately 46%.

Dinoflagellate and cryptophyte abundances in the spring of 2012 (Fig. 3A) were positively correlated with DOC and TOC (Fig. 3B). Increased cyanobacterial abundance in the summer (Fig. 3A), was positively correlated with salinity and temperature (Fig. 3B), whereas chlorophyte abundance in spring 2012 was inversely correlated with these parameters (Fig. 3A). During seasons when diatom and haptophyte abundances were increased, the concentration of NO2- (Fig. 3B) was also higher. Temperature and salinity both positively correlate with groups that are highly abundant in the summer (Table 1). DOC significantly explains the shift in phytoplankton community structure in spring 2012 (Stations 2, 4-6) from that of the other time points (Fig. 3B), which corresponds to maximum DOC concentrations temporally across stations (Table 1).

2.4.4. Nutrient Limitation of Phytoplankton Standing Stock

Enrichment with N (as nitrate) and A (as ammonium) in the bioassays caused the most significant increases in phytoplankton standing stock (Table 3). N limitation was observed in ~42% of the 36 bioassays, and at all stations at least once throughout the study; it was most common in summer. A limitation was present in about 27% of the bioassays, occurring at all stations but Station 2, and was most common in spring of the

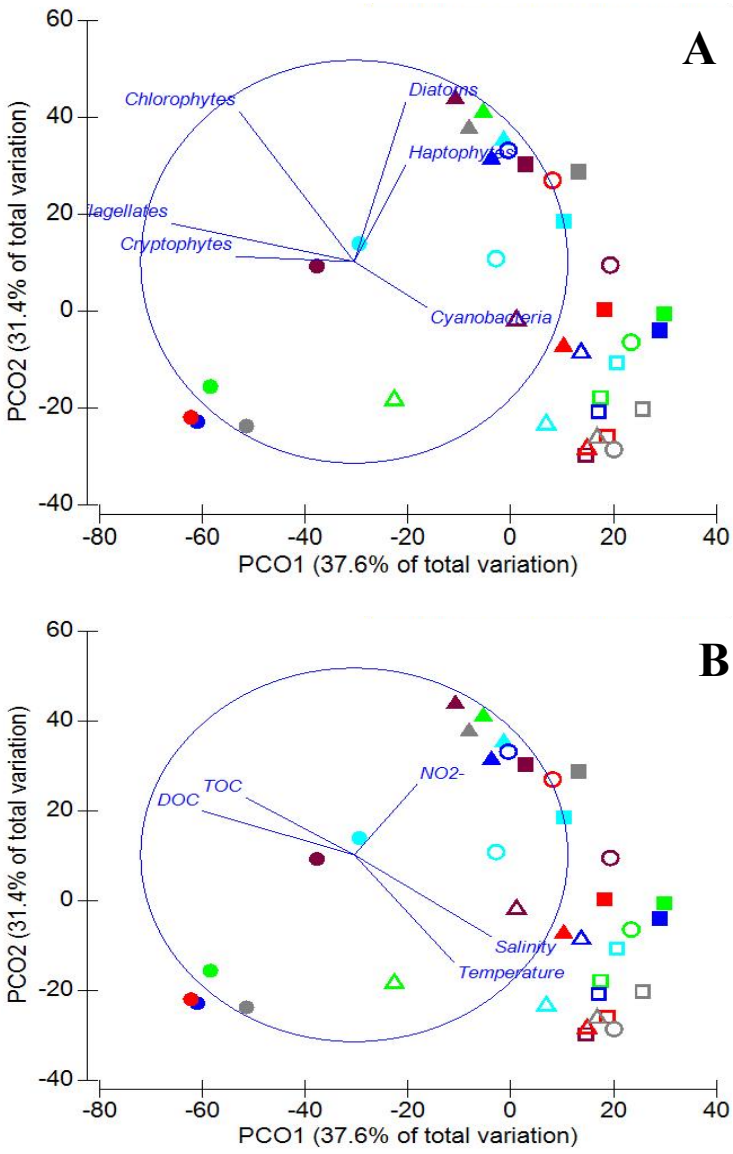

Figure 3. PCO ordination plots of phytoplankton community composition prior to the bioassays, with Spearman vectors overlaid. Direction and length of vector indicates positive and strength of correlation, respectively. Symbols represent Station – Season – Year as follows: St. 1 (light blue), St. 2 (green), St. 3 (purple), St. 4 (dark blue), St. 5 (red), St. 6 (gray); spring (filled), summer (open); 2010 (triangles), 2011 (squares), and 2012 (circles). (A) Spearman vectors illustrate the phytoplankton groups correlated to communities at stations and sampling events. (B) Spearman vectors show correlations of environmental variables, indicated by DISTLM to best explain the variability in the community composition, to communities at stations and sampling events.

drought. Co-limitation by N and P was the most pervasive, occurring in 80% of the bioassays (Table 3). NP co-limitation was most common in spring prior to and during the drought. N and A co-limitation occurred in 50% of the bioassays at 4 of the 6 stations (Stations 1, 4, 5, and 6), though it was absent entirely during the drought.

Treatments found to be limiting were most common in lower bay stations (4-6; Table 4). Station 4 frequently exhibited limitation in the N and A treatments, whereas at Station 5, A, NA, and NP treatments were most often limiting. Stations 5 and 6 illustrated NP co-limitation throughout the study. Temporal variation in the limiting treatments overall show N and NA limitations to be more frequent in the summer, whereas A limitation was most common in the drought in spring. NP co-limitation was absent from Stations 1, 2, and 3, except during spring 2012. Overall, nutrient limitation was observed more frequently during the drought than during non-drought (Table 4).

PERMANOVA tests show that only NP treatments did not have different effects on biomass between drought and non-drought conditions, whether it was limiting ($p = 0.09$) or non-limiting ($p = 0.11$). All other nutrient enriched treatments showed differences in biomass during the drought, regardless of whether they were limiting or not, with $p < 0.01$. Non-drought biomass of these other treatments (A, N, NA, P, and Si) were on average at least double that of their drought counterparts (not shown).

2.4.5. Nutrient Limitation Driven Shifts in Community Composition

In each row of Fig. 4, a representative station of three significantly different sub-

bay phytoplankton communities is presented. These were identified using

PERMANOVA pairwise testing of community composition at the end of each bioassay

Table 3. Log response ratios of each treatment by Station and Sampling Event. Bolded ratios denote the treatment exceeded the threshold of 1.385 as set by Harpole et al. (2011). Bolded and italic ratios indicate the ratio is at the threshold. Ratios with an asterisk (*) were further examined for co-limitation, with * signifying sub-additivity, and ** signifying super-additivity.

Station	Sampling Event	A	N	NA	NP	P	Si
St. 1	Spring 2010	0.399	0.208	0.103	0.915	0.222	0.493
	Summer 2010	1.174	**1.983**	**1.542***	**2.535****	0.038	0.142
	Spring 2011	**1.549**	1.312	1.284*	**3.187****	0.200	0.353
	Summer 2011	0.378	0.882	**1.960****	**2.135****	-0.161	0.280
	Spring 2012	0.639	0.411	0.817	1.117**	-0.026	0.017
	Summer 2012	0.804	1.204	1.316	**1.623****	0.004	0.010
St. 2	Spring 2010	0.963	0.915	0.907*	**1.843****	0.004	-0.299
	Summer 2010	0.875	0.783	1.025	**1.773****	0.151	0.294
	Spring 2011	0.578	-0.021	0.907	**1.433****	0.122	0.706
	Summer 2011	0.342	0.540	0.502	0.754	0.271	0.474
	Spring 2012	0.363	-0.472	0.236	-0.212	-0.101	0.018
	Summer 2012	1.143	**1.468**	**1.683**	**1.799****	0.051	0.006
St. 3	Spring 2010	0.483	0.418	0.571	**1.586****	-0.186	0.092
	Summer 2010	1.238	**1.770**	**1.967**	**2.709****	0.348	0.111
	Spring 2011	***1.385***	1.119	-0.415*	**2.611****	-0.031	0.311
	Summer 2011	1.136	**1.487**	**1.783**	**2.707****	0.096	0.136
	Spring 2012	0.921	0.767	0.852	0.940	0.214	0.094
	Summer 2012	**1.678**	**1.948**	**2.416**	**1.809**	0.026	0.361
St. 4	Spring 2010	1.049	1.169	1.083	**2.114****	0.057	0.095
	Summer 2010	**1.538**	**1.448**	**1.418***	1.059*	0.125	0.168
	Spring 2011	**1.550**	**1.390**	1.287*	**2.601****	0.425	0.421
	Summer 2011	0.805	**1.431**	**1.629**	**2.017****	-0.043	-0.168
	Spring 2012	**1.856**	**1.990**	**2.314***	**2.428****	0.246	0.138
	Summer 2012	1.278	0.929	**1.895****	0.571*	0.605	0.104
St. 5	Spring 2010	**1.773**	1.242	**1.530***	**2.430****	-0.042	-0.144
	Summer 2010	1.329	1.353	**1.568***	**2.502****	0.039	-0.145
	Spring 2011	**1.946**	**1.509**	**1.807***	**3.444****	0.414	0.080
	Summer 2011	**1.643**	**1.605**	**1.688***	**2.523****	0.254	0.014
	Spring 2012	0.755	1.007	1.167	**1.670****	-0.392	-0.489
	Summer 2012	0.775	**1.449**	**2.008****	**2.054****	0.067	-0.067
St. 6	Spring 2010	1.065	1.142	1.031*	**2.255****	0.067	0.026
	Summer 2010	1.099	**1.525**	1.223*	**2.470****	-0.318	-0.081
	Spring 2011	1.096	**1.464**	**1.936**	**2.511***	0.126	0.269
	Summer 2011	1.361	1.195	0.907*	**2.949****	-0.115	-0.313
	Spring 2012	1.229	1.039	**1.510**	**1.738****	-0.024	-0.114
	Summer 2012	**2.268**	**2.418**	**2.271***	**3.111****	0.845	1.110

Table 4. Treatments, by Station and Sampling Event that demonstrated significant shifts in community composition from the control at the end of bioassay incubation (S), nutrient limitation as indicated by the log response ratio (L), both (S&L), or did not have community composition data (ND).

Treatment	Station	Spring 2010	Summer 2010	Spring 2011	Summer 2011	Spring 2012	Summer 2012
A	St. 1			S&L		S	
	St. 2	S	S				S
	St. 3	ND	S	S&L		S	S&L
	St. 4	S	S&L	S&L		S&L	
	St. 5	S&L	S	S&L	L		
	St. 6	S					L
N	St. 1	ND	S&L	S		S	
	St. 2	S	S				S&L
	St. 3	ND	S&L	S	L		L
	St. 4		L	S&L	L	S&L	
	St. 5	S	S	S&L	L		L
	St. 6	S	S&L	L			L
NA	St. 1	S	S&L	S	L	S	
	St. 2	S	S				S&L
	St. 3	ND	S&L	S	L	S	L
	St. 4	S	S&L		L	S&L	L
	St. 5	S&L	S&L	S&L	L		L
	St. 6	S	S	L		L	L
NP	St. 1		S&L	S&L	L	S	L
	St. 2	L	S&L	L			S&L
	St. 3	L	L	S&L	L		L
	St. 4	L	S	S&L	L	S&L	
	St. 5	S&L	S&L	S&L	L	L	L
	St. 6	S&L	S&L	S&L	L	L	L
P	St. 1			S			
	St. 2						
	St. 3	ND					S
	St. 4						S
	St. 5	S					
	St. 6						
Si	St. 1	ND	S	S			
	St. 2						
	St. 3	ND					
	St. 4	S		S			
	St. 5		S				
	St. 6						

among stations. Sub-bay groups are defined by statistically similar ($p > 0.05$) stations, as follows: Stations 1 and 3 (TB), Stations 2, 4, and 5 (CGB), and Station 6 (Gulf). In CGB, Stations 4 and 5 are significantly different ($p < 0.05$), though both are statistically similar

to Station 2. In spring 2010 (Fig. 4A, D, and G), the overall communities at the end of the bioassays were predominantly composed of diatoms and chlorophytes in CGB and Gulf, (Fig. 4D and G, respectively) with the addition of cryptophytes in TB (Fig. 4A). Diatoms largely dominate communities in spring of the drought (Fig. 4B, E, and H), and similar in composition, with the exception of increased cyanobacteria abundance in TB (Fig. 4B) compared to CGB and Gulf (Fig. 4E and H, respectively). In post-drought spring 2012, however, chlorophytes dominated in TB (Fig. 4C), and dinoflagellates in CGB (Fig. 4F), while diatoms continued to dominate at the Gulf (Fig. 4I). The phytoplankton community of TB did not see a recovery of cryptophytes to the levels observed in spring 2010 (Fig. 4A).

Significant shifts in bioassay community structures were absent in summer 2011 (Table 4). Among the treatments, significant shifts in overall community structure were observed in the greatest proportion in the A bioassays (89%, Table 4). In all treatments except P, significant shifts in community structure from the control were observed most frequently pre-drought (2010; Table 4). However, of the four treatments found to be limiting, A, N, and NP were most often limiting during the drought, whereas NA demonstrated more instances of limitation post-drought. Generally, significant shifts in community composition and/or nutrient limitation were most common in summer 2010 and spring 2011 across treatments (Table 4).

Cyanobacteria were the only group which did not display change in response to nutrient limitation between drought and non-drought conditions. In both conditions, N

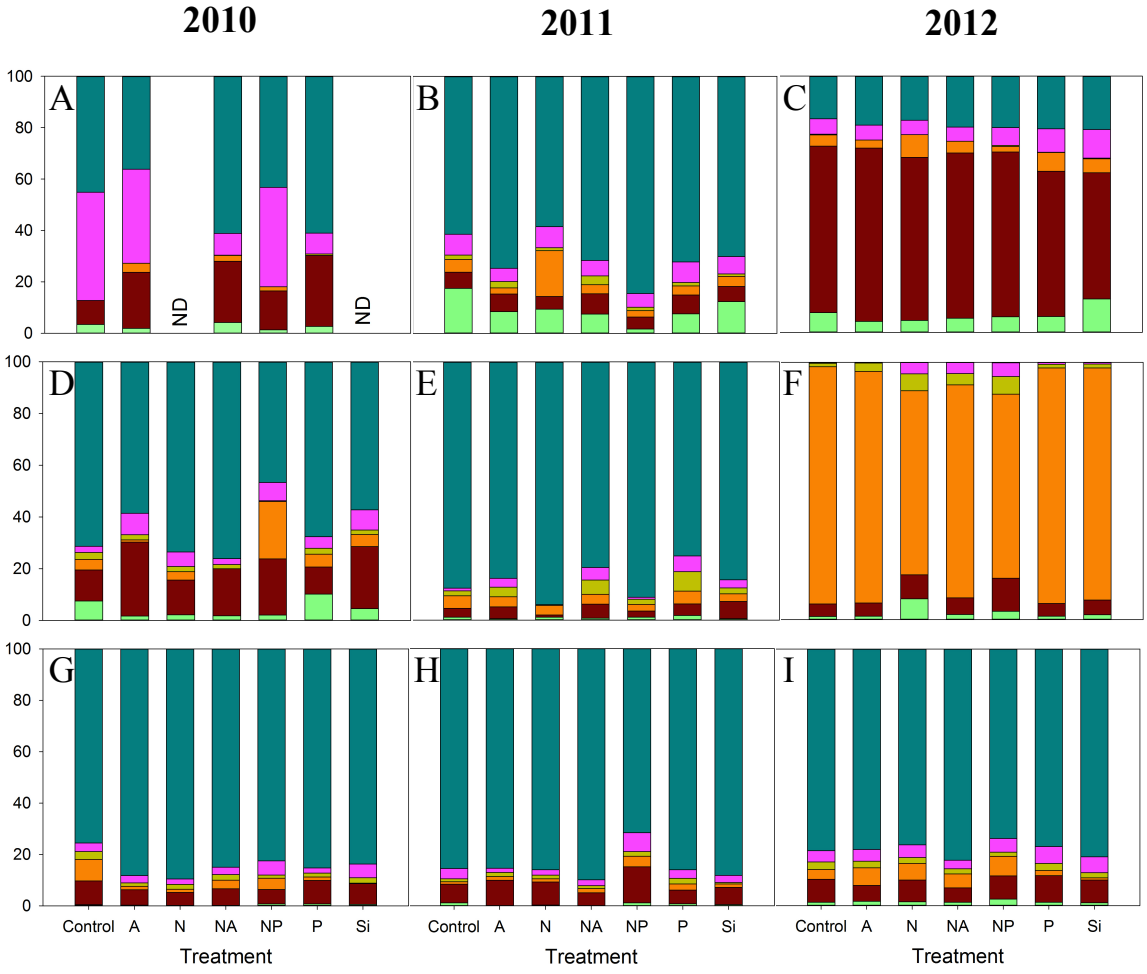

Figure 4. Phytoplankton group percent contribution at the end of the bioassays to total biomass based on relative community composition from CHEMTAX. Rows of panels are representative stations of each cluster: Trinity (A-C), Central Galveston Bay (D-F), and Gulf (G-I). Columns of panels are Spring of 2010 (A,D,I), 2011 (B,E,H), and 2012 (C,F,I). Phytoplankton groups presented: cyanobacteria (light green), chlorophytes (red), dinoflagellates (orange), haptophytes (yellow), cryptophytes (pink), and diatoms (blue).

treatments most frequently and greatly increased cyanobacterial abundance over the control (75% and 83% of the time, respectively; Fig. 4). Co-limiting treatments were

more prevalent in demonstrating recurrent and higher increased abundances of diatoms, dinoflagellates, and chlorophytes over the control during the drought (NP (11.8% ± 6.7 over control), NA (3.5% ± 4.9), and NA (5.1% ± 5.3), respectively). In comparison, these groups were most often elevated in abundance over the control by a single nutrient in non-drought (Si (5.5% ± 4.2 over control), A (4.7% ± 6.6), and N (6.2% ± 3.9), respectively; Fig. 4). Cryptophytes and haptophytes were most frequently and greatly abundant in A and Si treatments during the drought (67% and 75% of the time), respectively, and in NA and A (65% and 70%), respectively, during non-drought (Fig. 4).

2.4.6. Drought Effect on Nutrient Limitation and Community Composition

Examining water quality and community structure between drought and non-drought conditions, we found evidence of a drought effect on community structure at the end of the bioassays (Fig. 5). During the drought, the communities of TB and CGB did not change significantly with nutrient enrichment ($p = 0.07$), with the exception of the NP treatment ($p < 0.05$), though both sub-bays are significantly different from the Gulf (Station 6, TB $p < 0.01$, CGB $p < 0.05$). Outside of the drought, the sub-bays are all significantly different from each other ($p < 0.01$). A drought effect on phytoplankton communities in the bioassays performed is apparent temporally in Galveston Bay (Fig. 5A). Communities within all treatments were different between drought and non-drought ($p < 0.01$, Fig. 5B). It appears that the driving factor in community composition between

drought and non-drought is the shift in spring 2012 illustrated in Fig. 4 (panels C, F, and I).

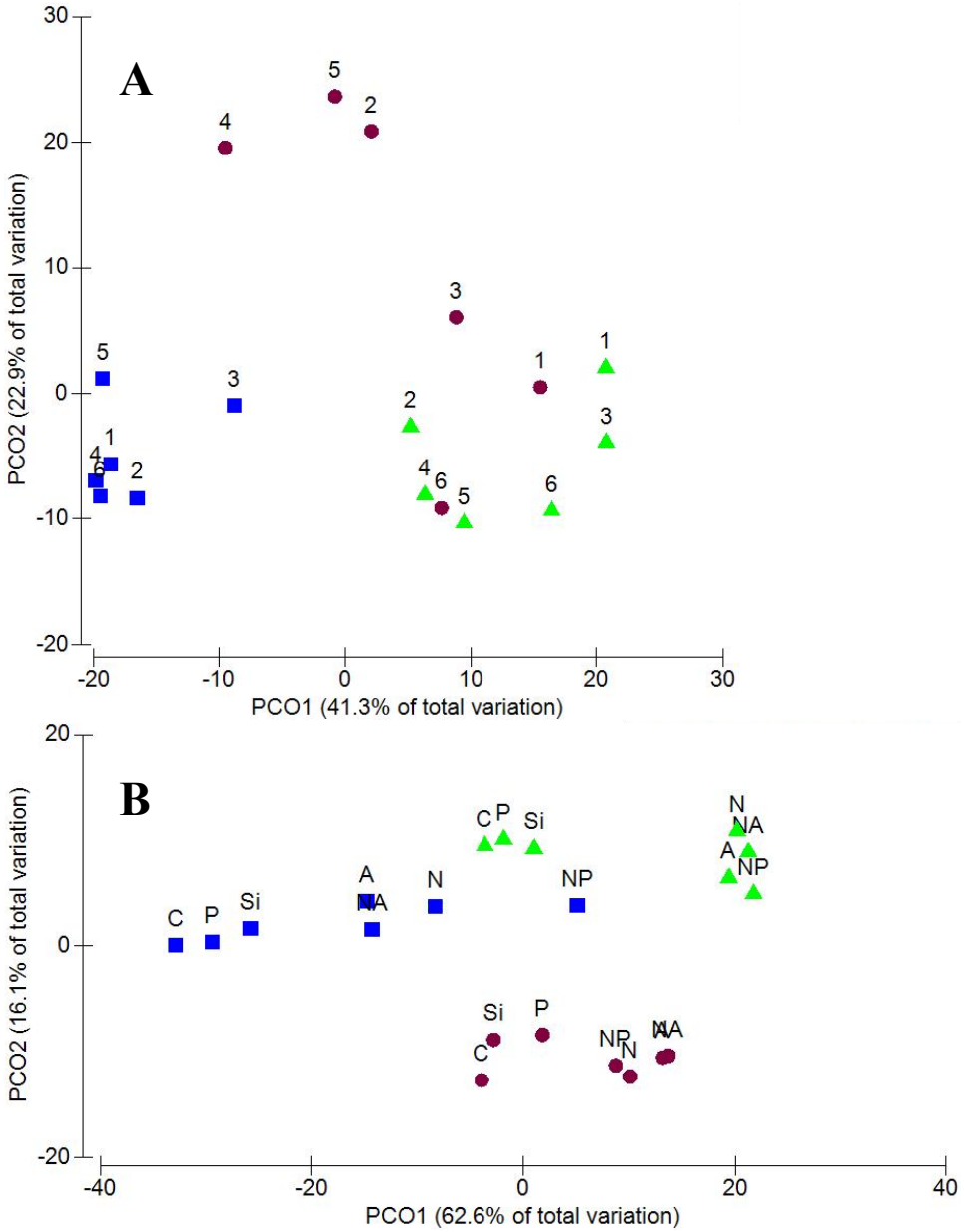

Figure 5. PCO ordination plots of centroids (Station, shown as numbers, and Treatment, shown as letters) based on community composition. (A) Spatially, the communities of the bioassays are significantly different between drought (blue

Figure 5. Continued. squares) and non-drought (green triangles, purple circles) ($p<0.01$). (B) Treatment centroids show a similar drought separation as observed spatially, and a significant drought effect exists ($p<0.05$).

2.5. Discussion

Coastal ecosystems are experiencing strong anthropogenic influences, particularly through runoff associated with urbanization, industry, and agriculture as most of the human population growth and development worldwide is taking place in coastal areas (e.g.,Paerl, 1997; Vitousek et al., 1997). Consequences of this include but are not limited to eutrophication, harmful algal blooms (HABs), hypoxia, and fish kills (Anderson et al., 2002; Bianchi et al., 2010; Paerl et al., 2010; Paerl and Otten, 2013). Continued development (TWDB, 2013a) will likely not only continue to alter water quality, but also quantity. Concurrently climate change is likely to result in increased severity and frequency of drought events (Meehl et al., 2007; Stocker et al., 2014), ultimately altering nutrient availability in estuarine systems. Phytoplankton standing stock is known to be typically primarily N-limited though P-limitation has been reported in some estuaries (Downing et al., 1999; Howarth and Marino, 2006; Piehler et al., 2004), despite nutrient loading. Less often investigated is how nutrient additions impact coastal phytoplankton communities (identified using HPLC, cell counts, etc.), yet studies that have, reveal shifts in the community composition (Doering et al., 1995; Fisher et al., 1999; Örnólfsdóttir et al., 2004a; Örnólfsdóttir et al., 2004b). An investigation of 50 years of fish kills along the Texas coast found that Galveston Bay was a hot spot for fish kills (Thronson and Quigg, 2008), indicative of an ecosystem under pressure. Hence, in

this study, we examined the response of the phytoplankton community to different nutrients across an estuarine gradient before, during, and after a period of severe drought.

2.5.1. Response to Nutrient Limitation

Overall, the outcome of the bioassays indicated that total phytoplankton standing stock in Galveston Bay is primarily nitrogen-limited, which is consistent with previous findings (Armstrong and Hinson Jr., 1973; Fruh, 1969; Örnólfsdóttir et al., 2004a; Pinckney, 2006) and findings in other temperate estuaries (Paerl et al., 2010; Paerl et al., 2014). We found that both nitrate and ammonium frequently limited phytoplankton, but also NP and NA co-limitation – the latter has not previously been reported in Galveston Bay. During the drought, nutrient limitation was overall more common than in non-drought years, though there was a delayed response to the lack of FWI into the bay in terms of the composition phytoplankton community.

In this study, NP bioassays were overwhelmingly super-additive (Table 3), that is, the combination of N and P increased chl-*a* concentrations above that of both N and P alone (Harpole et al., 2011). Serial co-limitation as defined by Harpole et al. (2011) describes a standing stock increase response to a second nutrient only after the addition of the nutrient that is primarily limiting to the community. In our study, the two were added together simultaneously, which according to the definitions of Harpole et al.

(2011) suggest synergistic co-limitation. However, these classifications are defined in the context of only N and P.

A recent review of nitrogen uptake in the form of NO_3^- and NH_4^+ discusses the preference of NH_4^+ as a phytoplankton cell's first source of nitrogen before it will uptake NO_3^- and the mechanisms regulating transport of either nutrient into the cell (Glibert et al., 2015). With the preference of NH_4^+ to NO_3^- in terms of being energetically favorable, it is reasonable to expect that NH_4^+ would have more instances of limitation, however this is not the case. In this study co-limitation is more prevalent than limitation of either nutrient alone, though N (as nitrate) limitation is almost as common as the co-limitation. Supply of NO_3^- and NH_4^+ together can induce concomitant growth compared to growth on either substrate alone (Britto and Kronzucker, 2002; Weissman, 1964). However, it has been shown in San Francisco Bay that NH_4^+ can inhibit the uptake of NO_3^- at concentrations greater than 4 μmol/L (Dugdale et al., 2007), which here only occurred during summer 2010 (St. 6) and spring 2011 (St. 2 and 4; Table 1). In contrast to NP treatments, NA bioassays were most often sub-additive (Table 3), or rather, the combination of N and A did not elevate concentrations of chl-*a* above that of both nutrients alone, suggesting that A inhibition of N uptake could potentially be occurring.

The higher incidence of co-limitation rather than single limitation in regards to these two nutrients could also be the result of serial nutrient use by the community: first of NH_4^+, which could be inhibiting the uptake of NO_3^- in the bioassays (due to its concentration being greater than 4 μmol/L), and then when NH_4^+ concentrations have been sufficiently drawn down, uptake of NO_3^- can begin, inducing higher growth rates

(Glibert et al., 2015). In the spring when NO_3^- is typically high, communities are mostly diatoms, which are NO_3^- opportunists (Glibert et al., 2015; Litchman et al., 2007), whereas NH_4^+ tends to favor cyanobacteria, dinoflagellates (which tend to form harmful algal blooms), and chlorophytes (Glibert et al., 2014). In conjunction with our findings and the ability of NH_4^+ to inhibit the uptake of NO_3^-, the possibility of a different type of serial co-limitation warrants further study in Galveston Bay.

2.5.2. Community Composition Changes

Overall, community composition prior to the bioassays in spring 2010 and 2011 did not appear to be different from each other, with diatoms making up the majority (Fig. 3A, Table 2). The absence of a trend change in the phytoplankton community during the drought could also be the result of the timing of its beginning (Wetz et al., 2011). In the Neuse River Estuary, North Carolina, Wetz et al. (2011) determined that the seasonal time during which droughts are most severe may determine the effect on the phytoplankton populations, and thus water quality. A lack of riverine derived nutrients related to drought that is most severe during a time when primary productivity is mostly supported by recycled nutrients will not demonstrate a long-term effect on primary productivity (Wetz et al., 2011). However, if a drought peaks in severity when phytoplankton productivity is dependent on riverine derived nutrients, such as in the fall and winter, annual blooms may fail to develop to the detriment of the ecosystem, as was observed in the Neuse River Estuary (Wetz et al., 2011). The 2011 Texas drought began

in October of 2010 and reached the height of its severity one year later in October 2011, well outside of the typical spring blooming season in Galveston Bay (Nielsen-Gammon, 2011). Both at the time of the start of the drought and the peak of its severity, the communities could have been subsisting on recycled nutrients. Thus, when the spring input of riverine nutrients and organic matter was inhibited by drought, there was no outside forcing to change the community structure (Malone et al., 1996).

However, in spring 2012 there was a distinct shift in community composition. Differences were observed between stations in the three sub-bays in Galveston Bay identified by PERMANOVA based on community structure ($p<0.01$): chlorophytes were dominant in Trinity Bay (TB), dinoflagellates became more important in Central Galveston Bay (CGB), and diatoms were prevalent at the Gulf station (Fig. 4). This spatial variability in dominant phytoplankton taxa has been previously documented in Galveston Bay (Dorado et al., 2015; Örnólfsdóttir et al., 2004a; Örnólfsdóttir et al., 2004b; Roelke et al., 2013), the Neuse River Estuary, NC (Paerl et al., 2014), and the Swan River Estuary in Australia (Chan and Hamilton, 2001). Diatoms frequently comprise the bulk of the phytoplankton community, particularly in the upper estuary, during high inflow and cooler temperatures (i.e., winter and spring), whereas cyanobacteria are favored in the lower estuary and in times of low inflow in the warmer months (Dorado et al., 2015; Paerl et al., 2010; Paerl et al., 2014; Roelke et al., 2013). Dinoflagellates are important in the mid- to lower-regions of the Bay, as they are sensitive to hydraulic displacement and have slower growth rates (Dorado et al., 2015; Paerl et al., 2014; Roelke et al., 2013). Chlorophytes, which are mostly important in TB,

were overwhelmingly dominant in spring 2012 compared to other sampling events. It has been suggested that chlorophyte biomass is regulated more so by flow and salinity than nutrients (Chan and Hamilton, 2001; Paerl et al., 2014). In Chesapeake Bay, community composition shifts to dinoflagellates (several of them harmful species) have been linked to an increase in the ratio of dissolved organic carbon to dissolved organic nitrogen (Glibert et al., 2001). In this study, the alteration of the community composition to dinoflagellates in spring 2012 was significantly correlated to an elevation of DOC in CGB (Fig. 3), which can be used as a source of ancillary carbon for the cell in mixotrophic phytoplankton such as dinoflagellates (Neilson and Lewin, 1974a).

Our findings suggest that estuarine phytoplankton community shifts in sub-tropical and mid-latitudes are likely dependent on when a drought begins (Wetz et al., 2011). These climatic fluctuations have been shown to overwhelm bloom formation and extent in terms of biomass, as well as shift community structure, in the Neuse River Estuary, which lies within the second largest estuarine complex in the United States (Paerl et al., 2010). In the case of Galveston Bay, community shifts were the result of increased FWI in spring 2012 following the 2011 drought, with dominant taxa changing from diatoms to chlorophytes in TB, and dinoflagellates in CGB. The shift to dinoflagellates in CGB following the drought presents an interesting potential issue, as the majority of fish kills from biotoxins in coastal Texas from 1951-2006 were the result of dinoflagellate HABs (Thronson and Quigg, 2008). Toxin producing HAB species are a threat to human health, especially in densely populated areas, by way of bioaccumulation in consumable fishery species, in addition to becoming aerosolized

upon cell lysis in wave action (Anderson et al., 2002). Worldwide, increasing anthropogenic inputs to coastal waters (particularly total N) have been linked to altering the stoichiometry of nutrients available to phytoplankton such that HABs are more likely to form (Anderson et al., 2002).

2.6. Conclusions

This study examined the complex relationships of nutrient limitation and phytoplankton community structure as affected by resource supply via FWI in Galveston Bay and during a drought period. Nutrient limitation was more common during the drought, and it was found that ammonium potentially plays a larger role than expected in the form of co-limitation with nitrate. The possibility of serial nutrient use as a result of nitrate inhibition by ammonium demonstrates the need for further investigation. The community shift observed in the spring after drought suggests that increased availability of FWI is more influential on spatial variability in community structure than a period of lack of inflows, as demonstrated by the shift from diatom dominance baywide during the drought, to chlorophytes and dinoflagellates in TB and CGB, respectively, in spring 2012. This is further supported by the importance of TOC and DOC availability, as well as salinity and NO2- as indicated by the DISTLM, all of which are brought into the system by FWI. With droughts projected to become more pervasive over the course of the next century in the sub-tropical and mid-latitudes (Meehl et al., 2007; Stocker et al., 2014), and populations in coastal areas continuing to grow, understanding the potential

risks to both human and ecosystem health as a result of phytoplankton community shifts in estuaries will only become more important.

3. COMPLEXITIES OF THE BENTHOS: SPATIOTEMPORAL VARIABILITY IN PHYTOPLANKTON AND NUTRIENT CYCLING RESPONSES TO FRESHWATER INFLOWS IN GALVESTON BAY (TEXAS, USA)

3.1. Summary

Downstream freshwater availability is changing in ecologically and economically important estuarine watersheds worldwide as they become increasingly more densely populated and precipitation becomes more variable. Assessments of biological responses to variable freshwater availability in these systems are becoming critically important. In Galveston Bay (Texas, USA), the seventh largest estuary in the United States, population growth in two large metropolitan areas (Houston and Dallas-Fort Worth), continues to alter the quantity and quality of freshwater inflows (FWI). We report here on the influence of FWI on biomass and community structure of benthic microalgae (BMA) and benthic boundary layer (BBL) phytoplankton, and nutrient fluxes in spring and summer over 3 years (2010 to 2012). The experimental design was intended to capture periods of high and low FWI, respectively. A year of severe drought that persisted throughout 2011 allowed us to also examine consequences of prolonged low flows. Nutrient fluxes investigated included: NO_3, NO_2, NH_4, Si, and P. High Performance Liquid Chromatography (HPLC), coupled with CHEMTAX V1.95, was utilized to distinguish phytoplankton communities. In this study we observed resilience of the BMA community to drought, but not in the BBL phytoplankton community. BMA

communities primarily consisted of diatoms throughout, while BBL phytoplankton communities differed with each sampling event. Fluxes differed before and after the drought, and while further study would be necessary to determine whether those processes returned to pre-drought conditions, the results here imply that resilience of the water column system is at risk in future drought events.

3.2. Introduction

Benthic microalgae (BMA, also called microphytobenthos) populations mediate nutrient fluxes into and out of the sediment, and as such, can be sources or sinks of recycled or new nutrients to the water column phytoplankton (Gardner et al., 2006; Twilley et al., 1999). The benthos has the potential to be an important driver in new production in upper trophic levels in estuarine systems. A collection of papers published in 1996 entitled "The Ecological Role of the Secret Garden" argued for reductionist studies of two-factor interactions of environmental parameters, owing to the intrinsic variability within singular parameters as a result of season and location, in addition to lack of independence of BMA from the water column (MacIntyre et al.). The studies also stated that BMA productivity has been correlated to a number of variables, and they did suggest measuring all possible environmental parameters and perform rigorous statistical analyses as an alternative route of investigation. Nonetheless, their argument strongly favored exploring two-factor interactions in order to highlight which interactions are most important in governing BMA communities.

Benthic phytoplankton communities (abundance, distribution, and primary productivity) can be structured by grain size (Cahoon et al., 1999; Forehead and Thompson, 2010; Shimeta et al., 2007; Watermann et al., 1999), nutrient availability (Forehead and Thompson, 2010; Pinckney et al., 1995), light (MacIntyre et al., 1996; Miller et al., 1996; Pinckney and Lee, 2008; Watermann et al., 1999), temperature, salinity, and mixing (Baillie and Welsh, 1980; Cahoon et al., 1999; de Jonge and van den Bergs, 1987; Delgado et al., 1991; Roman and Tenore, 1978). Furthermore, benthic nutrient fluxes can be affected by salinity (Gardner et al., 2006; Meiggs and Taillefert, 2011), sediment grain size and porosity (Cahoon et al., 1999; Grenz et al., 2000; Shimeta et al., 2007; Watermann et al., 1999), irradiance (Miller et al., 1996; Pinckney and Lee, 2008; Watermann et al., 1999), temperature (Fisher et al., 1982; Mortazavi et al., 2012; Zimmerman and Benner, 1994), and nutrients (Fisher et al., 1982; Pinckney et al., 1995; Watermann et al., 1999; Zimmerman and Benner, 1994). Overlap from these studies across the United States (e.g. Massachusetts, North Carolina, Texas, California, Georgia, and Alabama) suggests that multiple variables affect BMA and nutrient cycling simultaneously, that is, it is likely that the important variables are system specific, and that reductionist studies are not the solution to determining what drives the benthic communities and the nutrients they cycle.

A commonality among the majority of these parameters is that changes specific to them are affected by seasonal freshwater inflows (FWI) in some way. Sediments and nutrients are delivered to the estuaries via FWI, and they have been shown to affect phytoplankton (water column and benthic) biomass and community structure, and

nutrient fluxes on a seasonal basis, though no consistent pattern has been determined (Gardner et al., 2006; Meiggs and Taillefert, 2011; Pinckney et al., 1995). From these studies, it is apparent that the availability of FWI, their magnitude, mode, and duration, affects nutrient cycling and phytoplankton communities. With the impending increases in populations within the Galveston Bay watershed, the subsequent freshwater use, and the probability and severity of droughts, fundamental changes to coastal system food webs and interactions within them are likely (Meiggs and Taillefert, 2011).

High rates of primary productivity have been shown to be supported by efficient nutrient recycling through the coupling of benthic and water column processes (Fisher et al., 1982; Gardner et al., 2006; Grenz et al., 2000; Mortazavi et al., 2012), and the role that the BMA have in supporting secondary production important to human populations is likely to increase (Miller et al., 1996). Additionally, it has been suggested that in considerably low flow periods (24 to 101 m^3/s) in the Galveston Bay estuary, the sediments in Trinity Bay are likely to be the primary source of nutrients to support pelagic primary production (Mortazavi et al., 2012; Warnken et al., 2000). Thus, it is becoming more important to understand the role of the BMA and bottom-up controls with varying amounts of freshwater in situ, which provoke a number of interesting questions that we aim to answer in this study. This lack of consensus in the literature as to what factors drive variability in BMA and benthic nutrient fluxes suggests that the responses reported may be system specific.

However, in Galveston Bay it is not yet known what environmental parameters influence benthic phytoplankton distribution, abundance, community structure and

productivity, or the nutrient fluxes they cycle. The objective of this chapter is to understand the spatial and temporal variability of benthic phytoplankton communities and the nutrient cycles, their relationship to freshwater inflows, and the potential role of benthic nutrient fluxes in Galveston Bay productivity. The nutrient fluxes of interest in this study include nitrogen (in the forms of nitrate, nitrite, and ammonium), phosphate, and silicate. In this study, we examined sediment cores from cross-system sites representing the gradient of freshwater inflows into Galveston Bay to the Gulf over the course of 2010 to 2012 in March and July. This time series represents historically high and low freshwater inputs, and includes a year of severe drought (2011).

3.3. Materials and Methods

3.3.1. Study Site

Samples for water quality and water column data were collected in Galveston Bay as previously described (see section 2.3.1).

3.3.2. Core Collection and Incubation

Sediment cores were sampled from 4 stations (Fig. 6) biannually during the spring/high freshwater inflow (March) and summer/low freshwater inflow (July), and were used to assess benthic fluxes and cycling of nutrients and phytoplankton

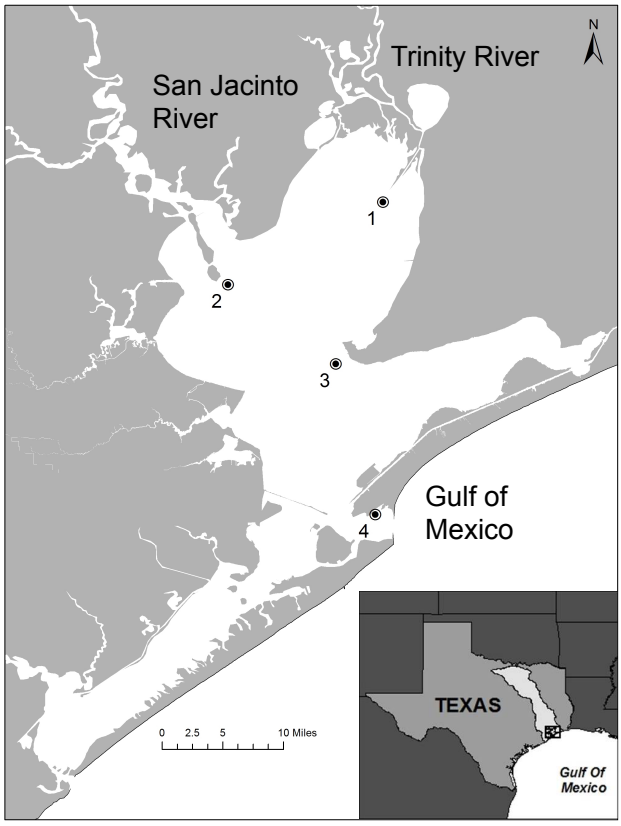

Figure 6. Sampling map of Galveston Bay, Texas. Stations of core collections indicated by numbers.

community composition following the methods of Liu et al. (2014). Five cores from each station were collected using a standard push-corer with a 30 cm tall polycarbonate core-sleeve (0.32 cm thick, 9.37 cm diameter), in addition to 2L of bottom water. The sediment material in the cores averaged 0.15 m in height (Table 5); they were capped and kept in a cooler filled with station water while transported back to the laboratory. Aliquots of water were taken from the benthic boundary layer (BBL; approximately one inch above the sediment) to filter for chlorophyll-*a*, accessory pigments, and dissolved

Table 5. Environmental parameters measured at each station per sampling event prior core collection. Dissolved nutrients were obtained from bottom water, approximately 2.5 cm above the sediment *within the cores prior to incubation*. Abiotic data is significantly variable across stations ($p < 0.01$), between seasons ($p < 0.01$), and among years ($p<0.01$). SD: standard deviation.

Sampling Event	Station	Depth (m)	Temperature (˚C)	Salinity	Secchi (m)	Core Height (m)		DO (mg/L)		NO_3^- (µMol/L)		HPO_4^- (µMol/L)		$HSiO_3^-$ (µMol/L)		NH_4^+ (µMol/L)		NO_2^- (µMol/L)	
						Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Spring 2010	1	2	15.64	0.19	0.32	0.149	0.018	8.85	0.73	26.26	0.51	0.67	0.21	88.25	2.40	3.49	1.26	0.50	0.09
	2	2	16.49	18.10	0.61	0.158	0.032	8.95	0.36	18.91	2.48	1.79	0.40	33.57	3.73	3.86	2.74	1.11	0.06
	3	2	20.5	13.72	0.15	0.162	0.037	8.64	0.42	2.72	0.47	1.06	0.38	58.19	9.76	17.63	16.86	0.29	0.07
	4	2.5	17.06	14.99	1.13	0.169	0.016	9.57	0.20	0.23	0.22	0.66	0.11	18.68	3.31	0.66	0.64	0.15	0.02
Summer 2010	1	2.8	30.85	9.13	0.71	0.197	0.014	6.59	0.13	0.72	0.33	3.93	0.45	100.18	18.33	22.23	6.78	0.19	0.03
	2	2.5	31.43	15.55	0.45	0.184	0.025	6.28	0.15	1.23	0.44	4.70	0.71	75.77	4.39	28.98	8.06	0.73	0.15
	3	2	30.95	14.84	0.51	0.153	0.037	7.02	0.18	0.09	0.06	3.42	0.28	102.78	4.85	1.44	0.68	0.08	0.02
	4	1.5	30.67	32.05	0.94	0.172	0.014	7.23	0.09	1.39	0.21	1.05	0.34	35.27	3.54	4.35	2.00	0.96	0.16
Spring 2011	1	2	20.34	17.23	0.79	0.150	0.000	8.35	0.28	0.31	0.18	0.92	0.09	38.35	3.27	3.89	3.42	0.03	0.01
	2	2	20.92	22.22	0.67	0.155	0.027	8.32	0.12	13.89	0.46	1.26	0.16	20.55	2.71	5.17	3.13	1.09	0.04
	3	1.5	20.34	18.99	0.45	0.150	0.000	8.31	0.27	0.07	0.05	0.76	0.29	25.57	5.63	4.59	4.13	0.06	0.01
	4	1	22.15	27.00	0.49	0.128	0.033	8.43	0.08	0.33	0.13	0.29	0.03	7.06	1.13	2.29	1.34	0.07	0.01
Summer 2011	1	2	29.84	21.81	0.61	0.136	0.016	7.20	0.06	5.07	2.91	3.49	0.81	55.70	11.07	1.98	1.43	4.46	2.58
	2	2	30.14	24.38	0.31	0.173	0.018	6.95	0.16	0.15	0.07	2.57	0.21	87.66	7.99	24.84	12.96	0.08	0.02
	3	1.5	29.68	27.47	0.42	0.175	0.012	7.48	0.13	0.06	0.04	2.91	0.68	59.64	7.92	2.61	3.41	0.04	0.02
	4	1.5	29.28	36.50	0.91	0.156	0.029	7.23	0.17	0.56	0.07	0.85	0.09	23.46	2.10	4.35	0.87	0.38	0.06
Spring 2012	1	1.5	24.08	0.55	0.21	0.124	0.027	8.01	0.08	24.70	0.92	1.88	0.09	88.38	3.77	3.14	1.10	1.29	0.21
	2	1.5	23.65	7.53	0.42	0.177	0.020	7.93	0.27	24.34	1.30	3.14	0.09	69.34	5.53	5.35	2.47	2.55	0.11
	3	1.5	21.07	6.69	0.37	0.148	0.032	7.07	0.28	2.97	0.48	1.33	0.82	62.07	3.58	5.99	5.33	1.17	0.17
	4	1.5	21.16	23.89	0.67	0.089	0.017	7.14	0.13	14.85	1.06	2.06	0.14	47.79	3.19	11.58	1.07	2.71	0.22
Summer 2012	1	1.5	28.49	8.3	0.5	0.126	0.024	6.57	0.12	0.06	0.08	3.00	0.04	124.23	2.65	0.57	0.36	0.28	0.03
	2	2	28.2	12.42	0.29	0.083	0.012	6.80	0.40	15.67	2.25	5.80	0.19	100.86	3.81	4.36	1.78	14.40	1.64
	3	1	29.7	12.92	0.59	0.143	0.013	7.10	0.07	0.57	0.23	3.95	0.26	110.31	3.46	1.20	1.57	0.24	0.10
	4	1.4	28.9	27.69	1.73	0.094	0.018	7.33	0.05	0.32	0.10	0.94	0.06	27.15	1.69	1.06	0.88	0.42	0.07

nutrients, and to assay for total phosphorus (TP) and total nitrogen (TN). Dissolved nutrients are reported herein as follows: NO_3^- as NO_3, HPO_4^- as P, $HSiO_3^-$ as Si, NH_4^+ as NH_4, and NO_2^- as NO_2.

The cores were then refilled with bottom water to eliminate headspace, minimizing disturbance of the surface of the core. Dissolved oxygen (DO) concentration was recorded for each core (Hach HQ40d Portable Meter with LBOD101 Luminescent/Optical Dissolved Oxygen (LDO) Probe). Cores were capped with stir bar lids covered with parafilm to ensure no gas exchange with the lab environment. They were incubated for 4 hours while maintaining in situ bottom temperature using a circulatory water bath system. Cores were incubated in the dark as was done in other studies (Cornwell et al., 2014; Enrich-Prast et al., 2016; Grenz et al., 2000). At the end of the incubation, the DO of each core was recorded, and water aliquots were again taken from the BBL. These samples were filtered for High Performance Liquid Chromatography (HPLC) pigments and dissolved nutrients (to determine fluxes). A Fluorescence Induction and Relaxation (FIRe) fluorometer was used to assess phytoplankton physiology. Sediment height was recorded and the top 1cm was collected for benthic HPLC assessment, and another top 1cm of sediment to assess for grain size analysis. The sediment for HPLC analysis was stored in a 15 mL conical centrifuge tube at -80˚C, while the grain size sediment was stored in a 50 mL conical centrifuge tube at room temperature until analysis.

3.3.3. Benthic Microalgae Pigments

The top 1 cm of sediment from a core was freeze-dried for 48 hours. The samples then received 5 mL of 100% acetone to extract the pigment and 1 mL of carotenal standard (internal standard, unknown concentration). The samples were homogenized and placed in a dark freezer for 12 hours. Following the extraction, the sample was homogenized again, and 1.5 mL of extract was pipetted and centrifuged for 5 minutes at 2795 g. Of this, 1 mL of clean extract was removed and placed into a 1.5 mL HPLC amber autosampler vial with 250 µL of ion-pairing 1M ammonium acetate solution. Benthic microalgal groups were identified using the following indicator pigments (µg/cm2): chlorophyll-*b* (chlorophytes), fucoxanthin (diatoms), peridinin (dinoflagellates), alloxanthin (cryptophytes), and zeaxanthin (cyanobacteria). Concentrations of BMA chlorophyll-a (µg/cm^2) were also determined via HPLC, and were used as a proxy for BMA biomass.

3.3.4. Benthic Boundary Layer Phytoplankton

Chlorophyll and Pigments – Chlorophyll-a concentration was determined as a proxy for BBL phytoplankton biomass using a Turner Designs 10-AU Flurometer. We followed the methods of Arar and Collins (1997), with the exception of extracting pigments in a 60/40 solution of 90% acetone/DMSO (Jeffrey et al., 1997). BBL aliquots following the incubation were used to assess phytoplankton community composition via

HPLC and the program CHEMTAX V1.95 (CHEMical TAXonomy; Mackey et al., (1996)) as a means to determine relative contributions of phytoplankton groups to the whole. Samples were analyzed in multiple datasets per sampling event to identify diatoms, dinoflagellates (incorporating gyroxanthin), cyanobacteria, chlorophytes, cryptophytes, and haptophytes-3 and -4, using the Schülter matrix as appropriate for estuarine phytoplankton communities (Schlüter et al., 2000). For statistical analysis, phytoplankton groups were defined as previously done (Örnólfsdóttir et al., 2004a; Örnólfsdóttir et al., 2004b; Roelke et al., 2013).

FIRe – The Fluorescence Induction and Relaxation (FIRe) fluorometer was used to measure a suite of parameters that assess physiological health of phytoplankton populations by evaluating the photosynthetic efficiency of sample phytoplankton (Kolber et al., 1998; Maxwell and Johnson, 2000; Sylvan et al., 2007). These parameters are useful in that their values have been previously used to suggest nutrient limitation and photoinhibition, and vary with community structure (Kolber et al., 1998; Suggett et al., 2009). These parameters included: maximum fluorescence yield (F_m), photosynthetic efficiency of chlorophyll fluorescence (F_v/F_m), the efficiency of electron transfer across photosystem II (PSII) (σ_{PSII}; $Å^2$/quanta), connectivity between reaction centers (p; unitless), and electron travel time across PSII (τ; μs) (Gorbunov and Falkowski, 2004; Kolber et al., 1998; Zhao and Quigg, 2014). F_v/F_m can be as high as 0.65 in healthy phytoplankton populations (Kolber et al., 1998). σ_{PSII} is lower in nutrient replete cells (Kolber et al., 1998), and demonstrated a decline when nutrient limitation is relieved (Sylvan et al., 2007). Additionally, nutrient limitation recovery is associated with

increased p and decreased τ (Kolber et al., 1998; Sylvan et al., 2007). Samples were dark adapted for 30 minutes to relax PSII prior to assessment (Kolber et al., 1998) and processed using a gain range of 200-2400 with 60 iterations and a 1000 ms delay between iterations. Filtered seawater was used as a blank to correct for background fluorescence.

3.3.5. Grain Size Analysis

The use of laser diffraction to obtain size classes of sediment grains is more efficient and accurate than historical sieve or pipette methods (Konert and Vandenberghe, 1997). Grain size analysis was performed using the Malvern Mastersizer 2000 according to Williams et al. (2013) to determine the sediment composition, including %gravel (>2000 μm), %sand (63 – 2000 μm), %silt (4 – 63 μm), and %clay (<4 μm).

3.3.6. Nutrient Fluxes and Sediment Oxygen Consumption

Fluxes of nutrients and oxygen consumption were calculated using the following equation:

$$\frac{\left(concentration_{post} - concentration_{pre}\right) * (sleeve\ height - sediment\ height)}{0.001/_{incubation\ time}}$$

Concentrations of nutrients were given in μmol/L, whereas DO was given in mg/L. Core sleeve and sediment height were both measured in meters, and the incubation time in hours. Nutrient fluxes herein are reported in $\mu mol/m^2/hr$, while sediment oxygen consumption is reported as $mg/m^2/hr$. Cores that demonstrated positive oxygen consumption were excluded from nutrient and oxygen statistical and trend analysis on the assumption that the cores were not adequately sealed. This exclusion included St. 3 (2 cores – spring 2011, and 4 cores – summer 2012) and St. 4 (5 cores – spring 2012).

3.3.7. Statistical Analyses

PRIMER V6.1.15 and PERMANOVA V1.0.5 were utilized to perform statistical analyses (Anderson et al., 2008; Clarke and Warwick, 2001). We investigated null hypotheses by using permutational analysis of variance (PERMANOVA) such that these were rejected when $p < 0.05$. When unable to obtain a reasonable number of unique permutations, Monte Carlo testing was used to gain a p-value for PERMANOVA testing involving sampling events at each station (Anderson et al., 2008).

To preserve inherent variability in the biological data (BMA and BBL community composition and biomass, FIRe data), data was not transformed or normalized, and were analyzed using Bray-Curtis resemblance matrices to reduce bias associated with inclusion of zero values as recommended by Clarke and Warwick (2001). Abiotic variables, including temperature, salinity, Secchi depth, dissolved

nutrients, dissolved oxygen, and sediment composition (%gravel, %sand, %silt, and %clay) were evaluated for collinearity using draftsman plots, and were excluded from the analysis if correlations were greater than 0.90. Of these variables, %sand was excluded from the analysis due to collinearity. Data from the abiotic variables were normalized and then analyzed using Euclidean distance resemblance matrices.

A combined subset of predictor environmental variables correlated to the variability of sediment-water interface fluxes and the BMA and BBL community structure was determined using distance-based linear modeling (DISTLM). The "BEST" selection model was chosen to test all possible combinations of predictor variables. As the ratio of number of samples to predictor variables was less than 40, the Akaike Information Criterion corrected (AICc) selection criterion was determined to be most appropriate (Anderson et al., 2008). Models with the lowest AICc values were considered to optimally evaluate environmental predictors of variability in fluxes and the BMA and BBL communities. The model produced also calculated the correlation and proportion of variability explained for individual predictor variables to the BMA and BBL community composition variability using marginal tests. These correlations were considered significant when $p < 0.05$. DISTLM analysis for BMA excluded the following parameters based on being influenced by external forces (i.e. phytoplankton, or sample collector bias): fluxes, water depth, and core height. DISTLM analysis for BBL community composition excluded FIRe parameters for the same reason.

Principal coordinates analysis (PCO) ordination plots were again used to visualize multivariate spatial and temporal variation of in situ BMA and BBL

phytoplankton community composition in two dimensions. Relationships between nutrient fluxes and sediment oxygen consumption with the abundance of BMA and BBL phytoplankton communities were determined using Spearman correlation vectors on the PCO plots.

3.4. Results

3.4.1. Environmental parameters

During our study, a priori selected stations were determined to be significantly different environmentally, in regards to water quality and physical parameters ($p<0.01$, Table 5). Bay-wide environmental conditions at these stations were statistically different between seasons ($p<0.01$) and among years ($p<0.01$). Average water depth during core collection was 1.78 m (Table 5). Salinity ranged from 0.19 – 36.5, increasing with distance from the river mouths (Table 5). Over the course of the study, secchi depth was on average 0.59 m, and was lowest in the spring (0.15 m) and highest in the summer (1.73 m, Table 5). Spatially, secchi depth decreased from the river mouth to the middle of the bay, but was highest at the Gulf exchange (Station 4, Table 5).

Mean bottom water dissolved oxygen over the course of the study ranged from 6.28 – 9.57 mg/L, with a higher seasonal average in spring compared to summer (Table 5). Of the bottom water nutrients assayed, an overall concentration maximum was observed in spring only for NO_3 (26.3 µMol/L, Table 5). Other nutrients investigated, P,

Si, NH_4, and NO_2, all demonstrated maximum concentrations in the summer (Table 5). Spatially, maximum mean nutrient concentrations were higher at Stations 1 and 2 (Table 5). There were no obvious patterns between years for the environmental parameters (Table 5).

Sand or silt predominated (>50%) core sediment composition in 17 of the 24 sampling events (Table 6). Of the sediment types analyzed, sand had the highest maximum mean (93.3%) in terms of proportion of core composition, while gravel was the lowest (1.64%, Table 6). However, proportion of sand generally increased with distance from the river mouths, whereas stations nearer to the river mouths had higher contributions of silt and clay (Table 6).

3.4.2. Benthic Microalgae

3.4.2.1. Biomass (chl-*a*)

BMA biomass (chlorophyll-*a* concentrations; µg/cm^2) differed among stations ($p<0.01$), though Stations 1 and 2 and Stations 3 and 4 are statistically similar ($p=0.10$; $p=0.27$). Biomass was higher on average at the lower Bay stations (St. 3 and 4; 1.24±0.7 µg/cm2 and 1.60±1.06 µg/cm^2, respectively). Seasonal and annual differences overall were not significant ($p=0.15$; $p=0.13$). Among stations, a season effect was apparent only at Station 4 ($p<0.01$). Generally, BMA biomass among stations was on average higher in the spring in the upper Bay (St. 1:

Table 6. Mean sediment grain size composition of each core as determined using the Malvern Mastersizer 2000. Size fractions are defined as: %gravel (>2000 µm), %sand (63 – 2000 µm), %silt (4 – 63 µm), and %clay (<4 µm). SD: standard deviation.

Sampling Event	Station	%Gravel		%Sand		%Silt		%Clay	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Spring 2010	1	0.092	0.206	11.66	6.59	59.99	3.03	28.26	6.46
	2	1.431	1.332	27.81	4.19	53.17	2.76	17.59	2.12
	3	0.000	0.000	21.31	4.74	50.82	4.31	27.87	2.37
	4	0.004	0.010	85.24	4.21	9.84	2.90	4.92	1.37
Summer 2010	1	0.050	0.112	28.98	7.41	43.82	4.56	27.15	5.37
	2	0.633	0.948	39.57	17.38	43.05	11.85	16.75	6.42
	3	1.635	3.105	71.11	9.41	18.20	4.17	9.05	3.01
	4	0.058	0.062	73.10	4.65	20.65	3.44	6.20	1.30
Spring 2011	1	0.000	0.000	6.85	3.90	65.06	4.73	28.09	4.12
	2	0.275	0.298	36.32	5.22	49.27	3.68	14.14	1.81
	3	0.000	0.000	9.02	2.04	50.93	2.15	40.06	1.36
	4	0.032	0.071	46.18	9.46	35.17	6.79	18.62	4.21
Summer 2011	1	0.089	0.186	7.44	2.44	48.24	1.96	44.23	4.13
	2	0.980	1.074	63.83	5.62	23.55	4.33	11.64	2.12
	3	0.057	0.127	91.95	3.18	4.10	2.15	3.90	0.99
	4	0.033	0.047	79.43	3.58	14.64	2.92	5.91	0.71
Spring 2012	1	0.347	0.556	51.48	3.29	37.61	1.79	10.57	2.82
	2	0.100	0.207	38.09	10.52	34.67	4.11	27.14	6.75
	3	0.000	0.000	88.10	4.21	6.31	2.00	5.60	2.22
	4	0.000	0.000	93.29	3.15	4.15	1.47	2.56	1.88
Summer 2012	1	0.925	1.385	54.16	3.32	36.05	2.96	8.86	0.52
	2	0.000	0.000	3.18	2.67	49.22	2.12	47.61	1.58
	3	0.006	0.015	89.05	1.09	6.22	0.67	4.73	0.66
	4	0.003	0.007	92.03	2.11	4.27	1.40	3.69	0.80

0.91±0.80 μg/cm^2; St. 2: 0.51±0.26 μg/cm^2), and higher in summer in the lower Bay (St. 3: 1.40±0.95 μg/cm^2; St. 4: 2.11±1.23 μg/cm^2, Fig. 7A). Annually, average BMA biomass was highest in 2012 among stations (1.15 – 1.87 μg/cm^2), with the exception of St. 2, which observed highest biomass in 2011 (0.63±0.23 μg/cm^2, Fig. 7A). Mean BMA biomass differed from all other sampling events in summer 2011 ($p<0.01$) and summer 2012 (except spring 2012, $p<0.05$, Fig. 7A).

3.4.3. Benthic Microalgae

3.4.3.1. Biomass (chl-a)

BMA biomass (chlorophyll-*a* concentrations; μg/cm2) differed among stations ($p<0.01$), though Stations 1 and 2 and Stations 3 and 4 are statistically similar ($p=0.10$; $p=0.27$). Biomass was higher on average at the lower Bay stations (St. 3 and 4; 1.24±0.7 μg/cm2 and 1.60±1.06 μg/cm^2, respectively). Seasonal and annual differences overall were not significant ($p=0.15$; $p=0.13$). Among stations, a season effect was apparent only at Station 4 ($p<0.01$). Generally, BMA biomass among stations was on average higher in the spring in the upper Bay (St. 1: 0.91±0.80 μg/cm^2; St. 2: 0.51±0.26 μg/cm^2), and higher in summer in the lower Bay (St. 3: 1.40±0.95 μg/cm2; St. 4: 2.11±1.23 μg/cm^2, Fig. 7A). Annually, average BMA biomass was highest in 2012 among stations (1.15 – 1.87 μg/cm^2), with the exception of St. 2, which observed highest biomass in 2011 (0.63±0.23 μg/cm^2, Fig. 7A). Mean BMA biomass differed from all other sampling

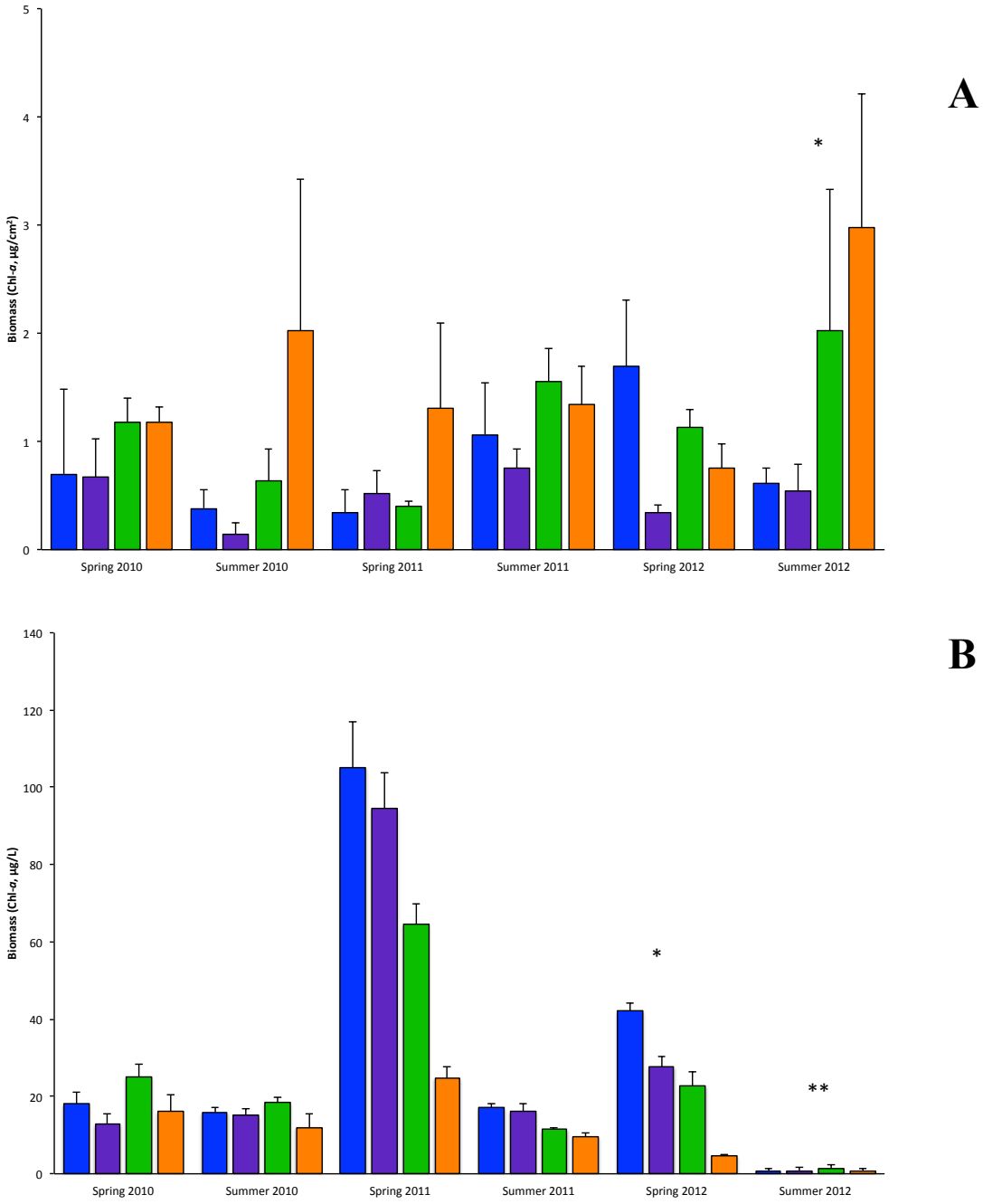

Figure 7. Mean phytoplankton biomass of benthic microalgae (A) and benthic boundary layer phytoplankton (B). Asterisks (*) denotes significant difference to all other sampling events * for p<0.05, while ** for p<0.01. BMA * excludes spring 2012. Colors represent stations as follows: St. 1 (blue), St. 2 (purple), St. 3 (green), St. 4 (orange).

events in summer 2011 ($p<0.01$) and summer 2012 (except spring 2012, $p<0.05$, Fig. 7A).

3.4.3.2. Benthic Community Structure

BMA communities at Stations 1 and 2 were statistically similar ($p=0.08$), whereas communities at the other station pairings were significantly different ($p<0.01$). Stations 1 and 2 BMA communities were comprised of a mixture of diatoms, cyanobacteria, and cryptophytes (Fig. 8A, B). Station 3 BMA communities were more diatom dominated (consistently >50%), with lesser contributions of cyanobacteria and cryptophytes, whereas Station 4 was almost exclusively comprised of diatoms (>80% throughout the study, Fig. 8C, D). Differences in community structure between seasons were marginally non-significant ($p=0.05$), as were annual differences in structure between 2010 and 2011 ($p=0.06$). However, community composition differed significantly between 2010 and 2012, and 2011 and 2012 ($p<0.01$). Closer examination of the community structure in spring across years revealed a significant difference in spring 2011 compared to both spring 2010 and 2012 ($p<0.01$), with spring 2010 and spring 2012 being statistically similar ($p=0.67$). This shift in community composition coincided with an increase in the proportion of diatoms at St. 1, 2, and 3, and an increase in contribution of cyanobacteria and cryptophytes at St. 4 (Fig. 8A-D). No such shifts were apparent across summers; all were different from one another ($p<0.01$). DISTLM

analysis found BMA community structure variability (~41% explained) driven overall by temperature, salinity, P, Si, NO_2, %Silt, and %Clay (AICc = 753.74). Of these parameters, salinity, P, Si, NO_2, %Silt, and %Clay were significant predictors of variability ($p<0.01$, except NO_2: $p<0.05$).

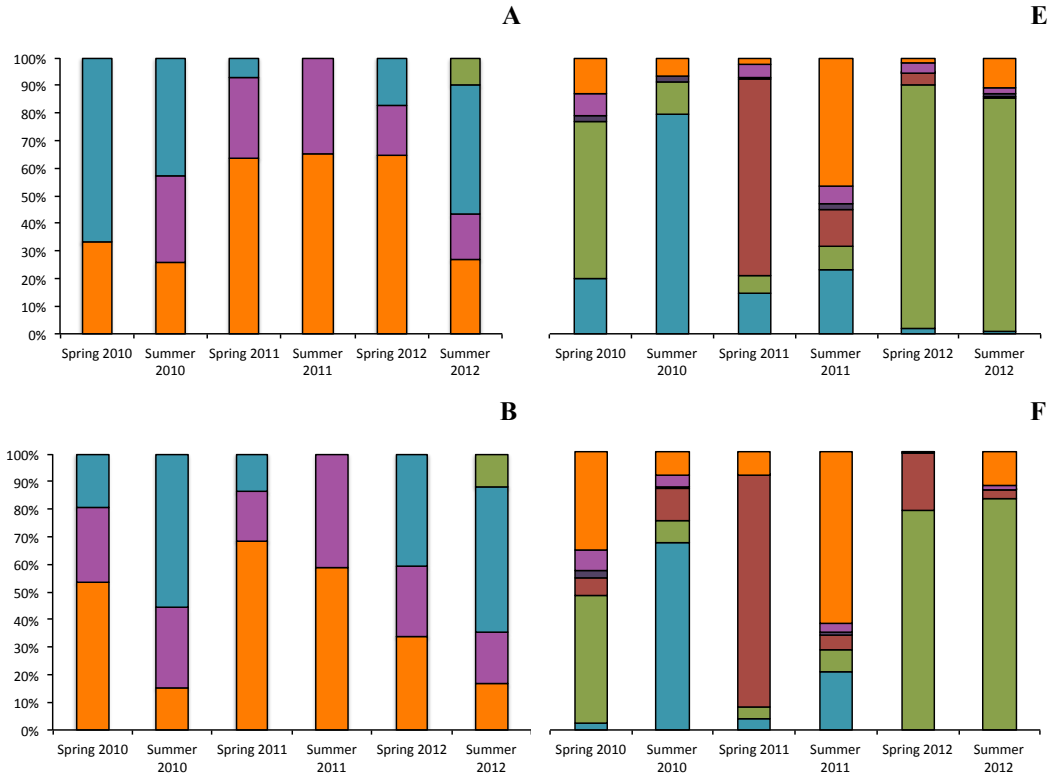

Figure 8. Phytoplankton group percent contribution to total biomass based on biomarker pigments for benthic microalgae, and relative community composition from CHEMTAX for benthic boundary layer phytoplankton. Columns of panels are representative of BMA (A-D), and BBL phytoplankton (E-H). Rows of panels are representative of stations: St. 1 (A, E), St. 2 (B, F), St. 3 (C, G), and St. 4 (D, H). Phytoplankton groups presented: cyanobacteria (blue), chlorophytes (green), dinoflagellates (red), haptophytes (purple), cryptophytes (pink), and diatoms (orange).

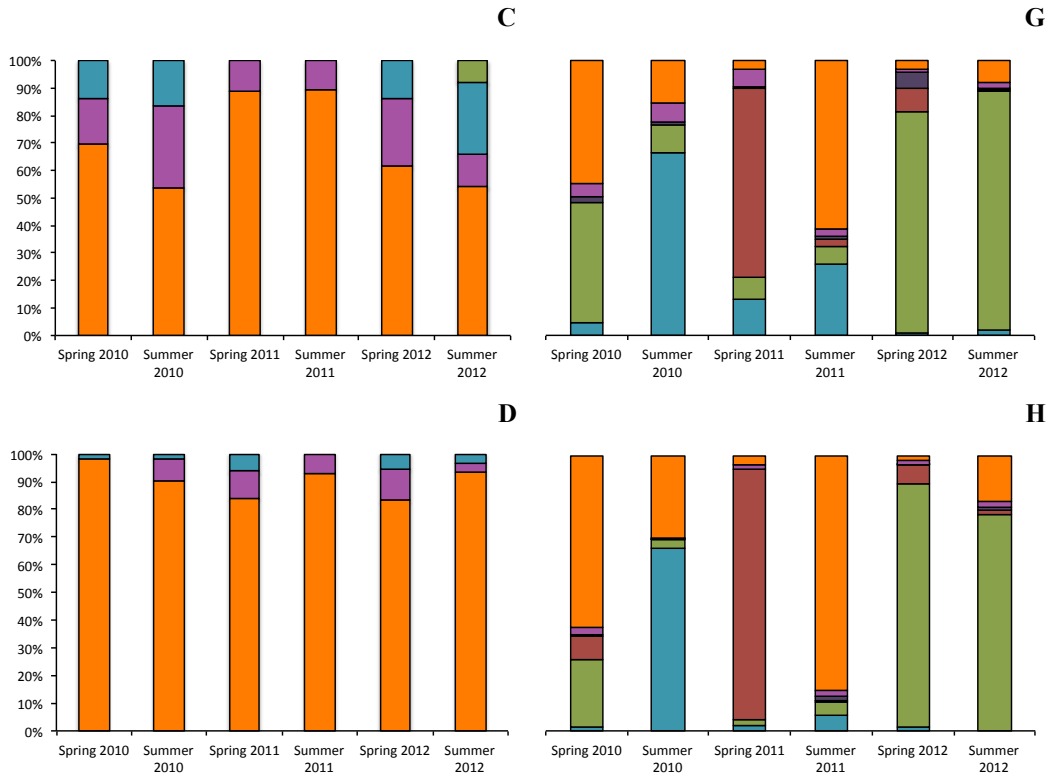

Figure 8. Continued.

3.4.3.3. Benthic Boundary Layer (BBL) Phytoplankton

Benthic Biomass (chl-a)

BBL biomass (chlorophyll-*a* concentrations; µg/L), that is, the biomass found immediately overlying the sediments, also differed among stations overall ($p<0.05$), with St. 4 being significantly different from St. 1, 2, and 3 ($p<0.05$) and St. 1, 2, and 3 statistically similar to each other ($p>0.70$). Overall, seasonal and annual differences were significant ($p<0.01$). BBL biomass was significantly different between seasons and

across years for all stations ($p<0.01$), with the exception of biomass at St. 4 between 2010 and 2011 ($p=0.13$). Average BBL biomass decreased with distance from the river mouths, with the exception of 2010, where biomass was greatest at St. 3 in both spring and summer (Fig. 7B). Additionally, mean BBL biomass was greater in the spring (38.1±31.9 µg/L) compared to summer (10.0 ±7.0 µg/L, Fig. 7B). Spring average BBL biomass was greatest in 2011 (72.1±32.9 µg/L), and different from all other sampling events ($p<0.01$, Fig. 7B). Minimum mean biomass was observed in summer 2012 for all stations (0.71 – 1.23 µg/L), and was significantly different from all other sampling events ($p<0.01$, Fig. 7B). Annually, average BBL biomass was highest in 2011 among stations (17.1 – 61.1 µg/L, Fig. 7B).

BBL Community Structure

Spatially, BBL communities overall were not significantly different among stations ($p=0.10$), however station communities were different among sampling events ($p<0.05$), with the exception of similarity between spring and summer 2012 at St. 1 ($p=0.07$) and St. 3 ($p=0.11$). Community structures demonstrated more differences across sampling events than dominance of particular groups at each station (Fig. 3E-H). Significant differences were observed seasonally, annually, and among sampling events overall ($p<0.01$). Chlorophytes predominated BBL community structure in 2010 at St. 1 and 2 (though almost equal with diatoms contribution at St. 3), and in 2012 across stations (Fig. 8E-H). Cyanobacteria proportions comprised more than half of the

community only in summer 2010, across stations (Fig. 8E-H). Similarly, dinoflagellates dominated BBL community composition (>50%) only in spring 2011 (Fig. 8E-H). Lastly, diatoms made up the majority of the community structure (>45%) at St. 3 and 4 in spring 2010, and at all stations in summer 2011 (Fig. 8E-H). DISTLM analysis identified temperature, salinity, secchi depth, DO, NO_3, NO_2, NH_4, and Si as the drivers of variability in BBL community structure (~43% explained, AICc = 845.25), with all but secchi being significant predictors of variability ($p<0.01$).

FIRe

FIRe parameters allow the assessment and comparison of BBL phytoplankton physiological health during sampling events. Overall, phytoplankton health demonstrated no spatial differences ($p=0.11$), but showed seasonal and annual variability ($p<0.01$ in both instances). Spring and summer in 2011, and 2011 and 2012 were statistically similar ($p=0.28$, $p=0.61$, respectively). Mean maximum fluorescence yield (F_m) was highest most often throughout the study at St. 3 (769 – 1371.76) and lowest most often at St. 4 (274.4 – 1158, Table 3). Over the course of the study, F_m ranged from 274.40±66.91 (St. 4, summer 2010) to 1371.76±95.92 (St. 3, summer 2012). On average, F_m was greater in spring (1094.1±247.9) than in summer (935±391.1), and among years was highest in 2012 (1215.6±126.8, Table 3). Average F_v/F_m (0.39 – 0.58) showed little difference between spring (0.49±0.06) and summer (0.48±0.05), and when examined annually, was highest in 2012 (0.53±0.04, Table 7). Overall spatial trends were not

Table 7. Mean phytoplankton physiology parameters from Fluorescence Induction and Relaxation (FIRe) analysis: maximum fluorescence yield (F_m), photosynthetic efficiency of chlorophyll fluorescence (F_v/F_m), the efficiency of electron transfer across PSII (σ_{PSII}; $Å^2$/quanta), connectivity between reaction centers (p; unitless), and electron travel time across PSII (τ; µs) SD: standard deviation.

Sampling Event	Station	F_m		F_v/F_m		σ_{PSII}		ρ		τ	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Spring 2010	1	1344.68	160.58	0.45	0.02	131.40	7.50	0.28	0.07	491.60	60.15
	2	1213.42	202.53	0.40	0.03	129.20	7.01	0.27	0.05	578.40	61.27
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	4	622.86	107.50	0.39	0.02	156.80	16.44	0.17	0.03	520.80	49.91
Summer 2010	1	423.74	28.82	0.45	0.04	206.40	5.59	0.16	0.08	444.00	101.99
	2	388.32	44.98	0.43	0.03	210.00	1.00	0.15	0.04	491.20	170.82
	3	659.28	52.38	0.47	0.02	212.60	3.85	0.11	0.03	416.00	89.60
	4	274.40	66.91	0.45	0.04	217.00	12.29	0.25	0.07	342.40	48.74
Spring 2011	1	911.50	96.84	0.48	0.02	251.40	5.94	0.10	0.02	366.20	33.54
	2	1248.68	153.00	0.50	0.02	264.00	5.39	0.21	0.02	397.60	48.77
	3	768.58	35.27	0.52	0.01	256.60	3.85	0.12	0.03	347.60	38.04
	4	1157.90	132.56	0.50	0.01	161.00	3.94	0.15	0.03	348.80	24.79
Summer 2011	1	1256.72	164.14	0.44	0.04	242.00	17.90	0.12	0.04	378.20	33.85
	2	1221.70	171.98	0.44	0.02	241.80	7.53	0.09	0.01	387.80	42.17
	3	986.08	63.86	0.45	0.04	264.40	11.28	0.13	0.05	329.40	30.12
	4	1052.58	216.67	0.46	0.05	249.20	11.78	0.17	0.06	366.60	25.26
Spring 2012	1	1194.98	171.01	0.56	0.01	212.40	2.19	0.30	0.01	371.20	13.70
	2	1211.56	47.55	0.53	0.00	185.20	4.66	0.21	0.02	357.40	25.07
	3	1217.76	120.87	0.58	0.02	236.80	2.59	0.23	0.03	354.80	20.50
	4	1142.60	136.60	0.47	0.02	243.40	15.98	0.20	0.02	348.80	53.17
Summer 2012	1	1196.62	41.07	0.55	0.01	251.00	1.87	0.10	0.02	392.80	47.49
	2	1284.98	139.73	0.56	0.00	235.60	4.67	0.14	0.00	380.60	14.05
	3	1371.76	95.92	0.48	0.02	267.40	6.11	0.09	0.01	398.00	59.55
	4	1104.30	43.89	0.55	0.01	265.80	2.17	0.15	0.03	329.60	46.63

apparent for F_v/F_m, cross section absorption of PSII (σ_{PSII}), or connectivity among reaction centers (p). Mean annual σ_{PSII} lowest in 2010 compared to other years (181±38.3 $Å^2$/quanta), and demonstrated smaller range in summer (220 – 275 $Å^2$/quanta) than in spring (120 – 273 $Å^2$/quanta, Table 7). p was on average greater in spring (0.21±0.07) than in summer (0.14±0.06), and ranged from 0.09 (St. 3, summer 2012) to 0.30 (St.1, spring 2012, Table 7). Among years, p was highest in 2010 (0.20±0.08, Table 7). Maximum transport time of electrons across PSII (τ) decreased overall over time, with the lowest in 2012 (451 μs).

3.4.4. Fluxes of Nutrients – Nitrogen, Phosphorus, Si – and Oxygen

Nutrient fluxes overall demonstrated spatial, seasonal, and annual differences ($p<0.01$), with spatiotemporal differences more nuanced among nutrient fluxes as flux magnitude and direction varied widely across stations without clear temporal trends (Fig. 9A,B; Table 8). However, some general trends are apparent. The greatest maximum influx of all nutrients was observed in 2010 (-177 – -0.49 μmol/m^2/hr), with the exception of NO_3, which occurred in summer 2012 at St. 2 (-39.9 μmol/m^2/hr; Table 8). Furthermore, the greatest maximum efflux for all nutrients was observed at St. 2 (21.8 – 441 μmol/m^2/hr), with the exception of NO_3, which occurred at St. 1 in summer 2011 (45.8 μmol/m^2/hr; Table 8). At St. 2, most of these flux values were observed in summer (2010 and 2012), with the exception of NO_2 (spring 2012; Table 8). In terms of flux variability, St. 2 also demonstrated the widest flux ranges for all nutrients but NO_3 (St. 1,

Table 8. Mean and standard deviation (SD) of nutrient fluxes (*μ*mol/m^2*/hr).* ND signifies No Data due to positive oxygen consumption fluxes.

Sampling Event	Station	NO_3^- Flux		NH_4^+ Flux		NO_2^- Flux		HPO_4^- Flux		$HSiO_3^-$ Flux	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Spring 2010	1	-21.16	7.11	14.95	46.60	-0.39	4.33	-4.11	7.17	-11.83	113.32
	2	-17.77	29.07	68.67	80.26	1.81	2.51	0.70	7.44	-14.99	43.46
	3	11.37	17.18	-62.57	113.96	2.53	1.35	-0.70	4.06	-176.92	105.75
	4	1.90	2.76	3.17	13.09	-0.49	0.44	-1.53	1.42	-43.17	35.16
Summer 2010	1	3.75	2.02	-93.99	63.32	0.68	0.66	-13.10	8.55	290.18	258.86
	2	-8.98	5.80	201.27	86.96	3.38	6.66	21.82	11.15	440.95	545.56
	3	7.93	1.48	26.28	21.92	1.78	0.77	4.36	11.60	13.84	101.51
	4	13.47	14.62	79.61	62.91	9.09	11.15	4.52	13.19	71.03	56.78
Spring 2011	1	6.32	1.93	9.94	30.95	1.04	0.47	1.18	1.87	10.74	34.96
	2	-19.08	32.10	-12.13	58.06	0.27	2.32	-0.37	1.72	25.56	28.23
	3	0.93	1.12	39.70	39.29	-0.16	0.44	-0.46	1.85	-8.72	52.07
	4	3.04	1.85	-1.73	28.73	-0.18	0.12	1.82	1.30	38.51	76.11
Summer 2011	1	45.77	185.42	4.02	82.20	0.35	144.76	7.84	54.56	25.47	676.08
	2	3.25	1.94	-15.38	100.07	0.18	0.30	-6.02	2.75	-20.94	49.04
	3	0.14	0.84	-24.94	48.22	0.07	0.21	-4.86	6.13	-63.18	99.37
	4	6.39	2.77	81.87	34.96	6.33	1.65	5.34	2.37	88.88	30.65
Spring 2012	1	39.75	39.89	28.60	36.41	2.54	3.46	4.15	7.83	117.46	239.29
	2	16.79	16.62	-8.30	69.80	142.37	319.11	10.02	1.79	-57.03	347.40
	3	-2.14	15.94	87.07	47.81	2.61	7.39	0.75	7.09	99.91	156.97
	4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Summer 2012	1	20.71	10.12	22.89	25.79	3.03	0.96	1.53	2.10	33.30	143.04
	2	-39.90	30.56	242.00	175.11	28.64	26.31	-0.10	7.01	232.49	214.29
	3	23.95	ND	-12.04	ND	0.26	ND	-5.68	ND	59.36	ND
	4	0.16	3.68	-12.44	41.55	2.59	3.19	-0.73	3.60	0.16	123.77

-21.2 – 45.8 μmol/m^2/hr; Table 8). DISTLM revealed DO, NO_3, Si, NH_4, NO_2, %Silt, and %Clay as the suite of environmental parameters that best predict variability in the fluxes (AICc: 220.4; 18.8% explained). Of these variables, DO, NO_3, Si, and NO_2 were identified as significant predictors ($p<0.01$, except DO: $p<0.05$)

Generally, maximum efflux of DIN ($NO_3 + NO_2 + NH_4$) increased with distance from the river mouths, though the greatest efflux occurred at St. 2 in summer 2012 (231; Fig. 9A). Conversely, maximum consumption of DIN generally decreased with distance from the river mouths, with maximum and minimum influxes in summer 2012: at St. 1 (-89.6 μmol/m2/hr) and at St. 4 (-9.69 μmol/m2/hr), respectively (Fig. 9A). St. 1, and 2 were sinks for DIN in 2010 and 2011, respectively, however, no clear trends are apparent across stations for when a station was a source or a sink for DIN (Fig. 9A).

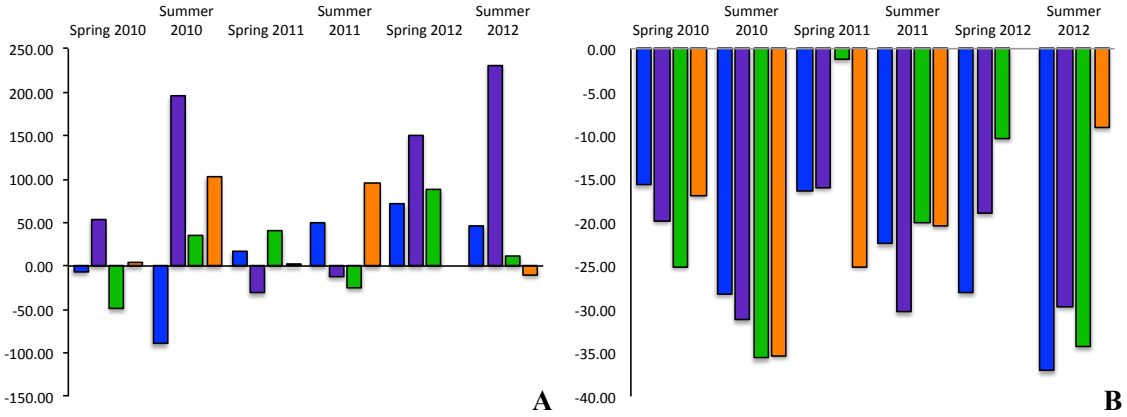

Figure 9. Mean dissolved inorganic nitrogen ($NO_3 + NO_2 + NH_4$) fluxes (µmol/m^2/hr) (A) and sediment oxygen consumption fluxes (mg/m^2/hr) (B). Error bars are standard deviation. Color represent stations as follows: St. 1 (blue), St. 2 (purple), St. 3 (green), St. 4 (orange).

Peak sediment oxygen consumption (SOC) occurred at all stations in summer, and overall, ranged from -37 (St. 1, summer 2012) to -1.30 (St. 3, spring 2011, Fig. 9B). Overall, SOC was greater in summer (-27.3±9.5 mg/m^2/hr) than in spring (-17.9±10.6 mg/m^2/hr; Fig. 9B). This trend held true across stations, with a general spatial trend of decrease from the river mouths for both seasons (Fig. 9B). With the exception of St. 1 (-32.5±11.1 mg/m^2/hr, 2012), annual SOC was greatest in 2010, ranging from -30.3±11.5 mg/m^2/hr to -25.5±7.4 mg/m^2/hr (Fig. 9B). Maximum consumption was observed in summer 2010 for St. 2, 3, and 4 (Fig. 9B).

General trends among stations between season were not apparent. St. 1 experienced significant differences between high and low flow only in 2010 ($p<0.01$), and St. 3 only in 2011 ($p<0.01$). At St. 2 these differences were observed all three years ($p<0.05$ for all pairs), and St. 4, though only analyzed for 2010 and 2011, had significant differences between seasons in both years ($p<0.01$ and $p<0.05$, respectively). Significant

differences among spring sampling events tend to be centered on 2010 or 2012. St. 1 demonstrated differences between 2010 and 2012 ($p<0.05$). Furthermore, at St. 2, fluxes in 2010 and 2011 were significantly different from 2012 ($p<0.05$), and at St. 3, 2010 was significantly different from 2011 and 2012 ($p<0.05$). Summers 2010 and 2012 were significantly different at St. 1 ($p<0.01$), whereas at St. 2, fluxes in summer 2011 were different from summer in 2010 and 2012 ($p<0.01$, $p<0.05$, respectively). In the lower bay (St. 3 and 4), fluxes in summer 2010 were different from 2011 at St. 3 ($p<0.05$), and summer 2012 was different from both 2010 and 2011 at St. 4 ($p<0.01$). This trend was also apparent for annual differences at these two stations ($p<0.01$ for both).

3.5. Discussion

The "Secret Garden" papers called for reductionist studies, looking at single environmental factors at a time. Parameters can covary or have more of an effect in combination with other parameters (and specific patterns cannot always be applied to the whole of a study area), and consequently, reductionist studies may not be the best way to design experiments in all cases. Parameters of the environment change in unexpected ways, and further studies looking at factor interactions should be undertaken.

The primary difference between phytoplankton of BMA and BBL phytoplankton is that the former inhabits the sediments, and the latter inhabit the water column immediately adjacent to the sediments. Depending on resuspension and deposition processes, the two are intricately linked, for one can influence the other's biomass and

community composition by physical forcing. And yet, they are drastically different in terms of quantities of previous research, where BMA are far more studied than BBL phytoplankton (e.g. (Forehead and Thompson, 2010; Hillebrand et al., 2000; MacIntyre et al., 1996; Pinckney and Lee, 2008; Shimeta et al., 2007) as they are important potential drivers of pelagic secondary production due to their role in benthic-pelagic coupling of nutrient cycling (Gardner et al., 2006; Twilley et al., 1999). The BBL in itself is well studied in other aspects, such as fluxes of nutrients, sedimentation process, and benthic-pelagic coupling through macrofaunal suspension feeders (e.g. (Fréchette et al., 1993; Gardner et al., 2006; McKee et al., 2004). However, few studies have directly examined the BBL for phytoplankton. Wetz et al. (2004), one such study, concluded that the BBL phytoplankton act as seeds for the spring bloom off the Oregon coast (USA). As possible precursors for a vital component of aquatic food webs, more research is needed to understand their spatiotemporal variability.

3.5.1. BMA

Spatially, BMA appear to be divided between the upper bay (St. 1 and 2) and the lower bay (St. 3 and 4), both in terms of biomass and community composition, slightly different from the trends previously reported in which the central bay was different from the river and estuary mouths (Pinckney and Lee, 2008), indicative of a dynamic system. BMA biomass generally increased with distance from the rivers, as did the contribution of diatoms to the overall community. Annually, biomass was greatest in 2012, and the

community composition was significantly different from that of 2010 or 2011, owing to an increase in contribution of cyanobacteria, cryptophytes, and chlorophytes in 2012 (Fig. 8A-D). However, overall BMA community variability does not reveal any separation of sampling events, either spatially or temporally (Fig. 10A). The shift in BMA biomass and community structure following the 2011 Texas drought (Nielsen-Gammon, 2011) suggests that relief from drought is more influential to BMA community composition and biomass than lack of freshwater and riverine derived nutrients.

3.5.2. BBL Phytoplankton

Biomass of BBL phytoplankton exhibited both spatial and temporal differences, whereas community structure and physiological health differed temporally. Among stations, BBL phytoplankton exhibited significant differences in regards to biomass (St. 4 being different from St. 1-3, $p<0.05$), being lower at the Gulf exchange than at the river mouths. Peak BBL biomass occurred in spring 2011, with the community primarily comprised of dinoflagellates. Minimum mean spring concentrations for all nutrients but NH_4 occurred in 2011, the drought year, with the added minor exceptions of NO_3 at St. 4 and P at St. 1 (Table 5). Dinoflagellates, as known mixotrophs, flourish and outcompete other phytoplankton groups in low nutrient environments, as they themselves are not good competitors for inorganic nutrients (Litchman and Klausmeier, 2008; Neilson and Lewin, 1974b). As a result, BBL community in spring 2011 separates from all other

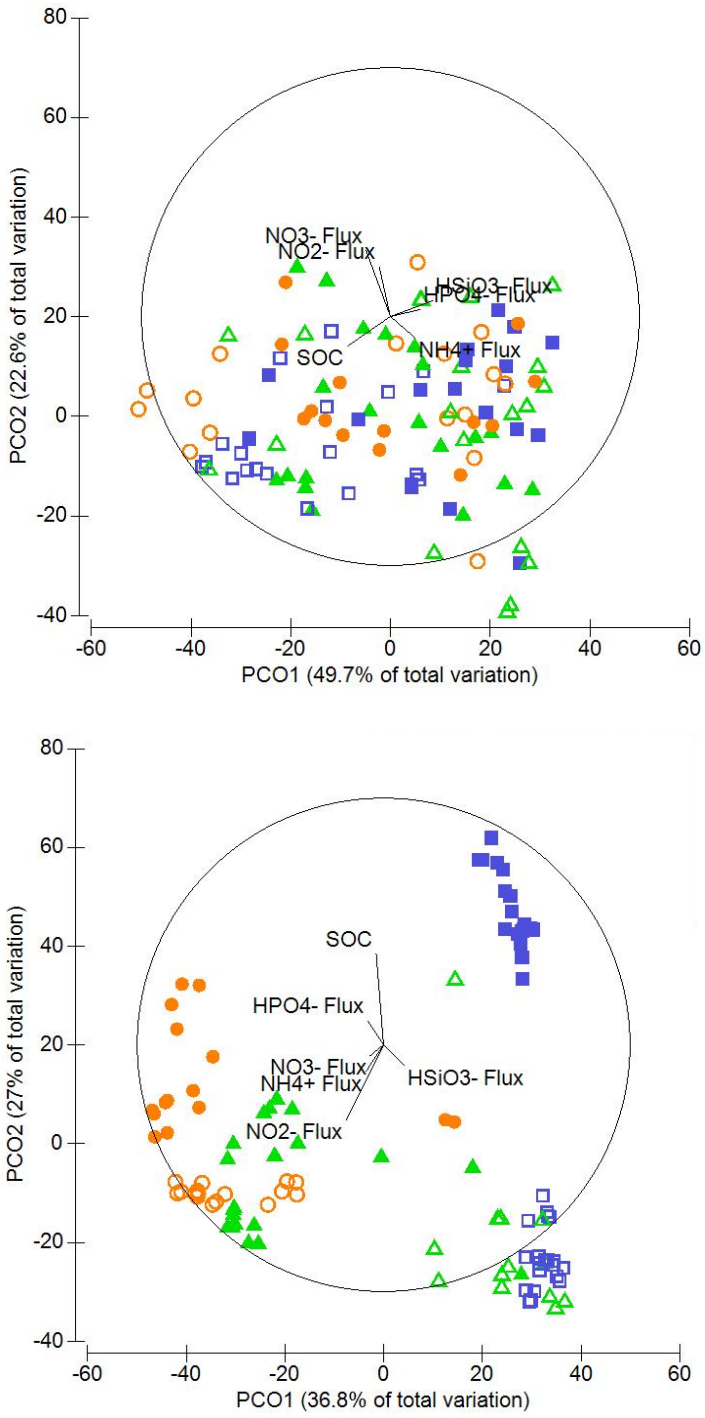

Figure 10. PCO ordination plots of samples based on community composition with Spearman vectors of fluxes overlaid. (A) BMA community (B) BBL phytoplankton community. Sampling events are represented as follows: 2010 (green triangles), 2011 (blue squares), 2012 (orange circles); spring (filled), summer (open).

sampling events (Fig. 10B) unlike the BBA community (Fig. 10A), suggesting BMA community resilience to changing environments such as droughts not found in BBL phytoplankton.

F_v/F_m ranged 0.39 to 0.58 over the course of the study (Table 7), with higher values indicating higher photosynthetic efficiency. Taxonomic indicator values of F_v/F_m previously described (Suggett et al., 2009) corroborate the community majority BBL phytoplankton, with cyanobacteria dominant summer 2010 having F_v/F_m of 0.43 – 0.47, and majority chlorophyte 2012 having the highest F_v/F_m, 0.47-0.56. The lowest mean F_v/F_m does not closely approach the theoretical threshold for physiological stress, 0.3 (Kolber et al., 1994; Sylvan et al., 2007), and p values generally declined over time, with the exception of spring 2012 when values were highest at St. 1 and 3. This suggests that over the course of the study, BBL phytoplankton were healthy. An increase in F_v/F_m during the spring 2012 sampling event could be the result of increased nutrient availability from increased freshwater inflows, comparable to relief from nutrient limitation seen in the Gulf of Mexico (Sylvan et al., 2007; Zhao and Quigg, 2014).

Though nutrients tended to be higher in concentration in the summer than in the spring, this could also be due to phytoplankton utilization during the pelagic spring bloom. However generally increasing σ_{PSII} values and decreasing τ indicate that the communities became more nutrient limited and/or photoinhibited over time (Kolber et al., 1998; Moore et al., 2008; Suggett et al., 2009; Sylvan et al., 2007). This disconnect among the results of FIRe parameters and what they indicate could be explained by a condition of "balanced growth" as seen in nutrient addition experiments in the Gulf of

Mexico (Zhao and Quigg, 2014) and the North Atlantic (Moore et al., 2008) where the addition of nutrients (or in this case, drought relief) did not demonstrate a dramatic increase in health metrics. Indeed, in this study, photosynthetic health was not significantly different between the year of the drought, 2011, and the year of drought relief, 2012.

3.5.3. Comparing BMA and BBL Phytoplankton

Spring 2011 demonstrated significant community shifts in both the BMA and BBL, but in different ways: BMA communities increased diatoms at St. 1-3, and increased cyanobacteria and cryptophytes at St. 4; BBL sharply increased in biomass, and was dinoflagellate dominated baywide. Predominance of diatoms in the sediment, combined with efflux of Si (except St. 3, -8.72) and minimum mean spring water column concentration of Si, suggests that BMA diatoms have a more consistent supply of the nutrient than that of the pelagic community, at least in the bay proper. At St. 4, where diatoms were still the major contributor to the BMA community, cyanobacteria and cryptophytes increased, which could be due to the environmental conditions of the station at the time: the lowest concentrations of P, Si, and NO2, lowest %Silt, and highest temperature and salinity of the sampling event were observed. All of these parameters were indicated by DISTLM as significant predictors of variability, with the exception of temperature.

3.5.4. Benthic Fluxes and Their Relationship to Phytoplankton Community Structure

In regards to the influence of freshwater inflows, it was expected that fluxes during the drought year, 2011, would be significantly different compared to 2010 and 2012, however this did not occur. Rather, 2010 and 2012 were significantly different from each other, and 2011 was statistically similar to both. Statistically, a difference is apparent in fluxes between high and low flow periods, with summer DIN, P, and Si fluxes averaging greater production than those in spring (Fig. 9A, Table 8). DIN fluxes were predominantly NH_4, with NO_3 being more important at St. 1 overall, and NO2 at St. 2, particularly in 2012 (Table 8). Ranges of nutrient fluxes and SOC observed in this study are comparable to those found in the Northern Gulf of Mexico (McCarthy et al., 2015; Roberts and Doty, 2015), San Francisco Bay (Cornwell et al., 2014; Grenz et al., 2000), and Cabiúnas Lake, a shallow oligotrophic lagoon in Brazil (Enrich-Prast et al., 2016). Similar to San Francisco Bay, DIN production and SOC increased with distance from the river, with a concomitant decrease in uptake of DIN (Cornwell et al., 2014). The sampling events in which the sediments produce DIN far exceed those where DIN is consumed, indicating that in Galveston Bay the sediments are an important source for DIN (Fig 9A). DIN production persevered through the 2011 drought in the spring, with the exception of St. 2, thus supporting pelagic phytoplankton during what would be the spring bloom (Fig 9A). Periods of decreased river flow (summer, and the 2011 drought), however, did not have the expected effect of dramatic increased nutrient efflux as previously described (Warnken et al., 2000; Zimmerman and Benner, 1994). This could

be explained by resuspension due to wind induced mixing in a shallow estuary, hindering sedimentation of BBL phytodetritus and thus nutrient remineralization (Grenz et al., 2000). Furthermore, our results do contrast with previous observations in Galveston Bay (Warnken et al., 2000; Zimmerman and Benner, 1994), as ranges of SOC (-1.3 - -37 mg/m2/hr) far exceeded those previously reported (-0.04 - -0.71 $mg/m^2/hr$).

While denitrification and dissimilatory reduction of nitrate to ammonium (DNRA) were not directly measured, hypotheses can be made in regards to these processes by comparing the rates of NO_3 flux to NO_2 and NH_4 fluxes, respectively. Potential denitrification was observed largely at St. 2, where rates of NO_2 production were paired with simultaneous NO_3 consumption, with the exception of spring 2012 where NO_2 production far exceeded that of NO_3 (Table 8). Other instances included St. 3 (spring 2012) and St. 4 (summer 2012). Potential DNRA, when rates of NH_4 production coincided with NO_3 consumption, was observed at St. 1-3, though only in either 2010 or 2012 (Table 8). An argument could be made for DNRA occurring at St. 4 due to NH_4 production surpassing that of NO_3, as the process is known to positively correlate with salinity (Gardner et al., 2006).

Fluxes identified by DISTLM predict variability of BMA included NO_3, P, and SOC (AICc: 734.92; 6.5% explained), whereas for BBL variability, the model identified only SOC as a significant predictor of variability ($p<0.01$; AICc: 801.4; 3.6% explained). The intent of this test was to investigate the cycling of nutrients between BMA and BBL phytoplankton, which has not been previously studied. However, there is a lack of nutrient flux overlap, or even trade-off of what nutrient fluxes are important

between BMA and BBL phytoplankton. This could be the result of the absence of clear spatiotemporal relationships between the fluxes and the BMA (Fig. 10A), which implies that the resilience of the sediment community and the variability of the BBL phytoplankton (Fig. 10B) are mismatched, thus obscuring the cycling of nutrients between the communities. Additionally, the fact that the stations themselves were environmentally different throughout the study may preclude cross system trends. Further study is required to determine this relationship, and could include examining the role of groundwater nutrients (Bowen et al., 2007; Valiela et al., 1990), pore water nutrients (Boynton and Kemp, 1985; Warnken et al., 2000), and isotope-labeled nitrogen species for estimations of nutrient transformation processes (Dong et al., 2011; Gardner et al., 2006; McCarthy et al., 2015).

3.6. Conclusions

Variability in freshwater inflows is becoming more apparent and drastic, as estimated precipitation fluctuations over the course of the 21st century in sub-tropical and mid-latitudes will exhibit decreased mean precipitation overall with longer time between rainfall events, making droughts more ubiquitous in these areas worldwide (Meehl et al., 2007; Stocker et al., 2014). Assessments of biological responses to changing freshwater availability in systems upon which increasingly more densely populated areas depend are becoming critically important. Additionally, examinations of the effect of anthropogenic alterations of nutrient loading (quantity and stoichiometry) to

these systems in relation to trophic transfers and thus ecosystem dynamics will be necessary to evaluate food web sustainability (Glibert, 2012). Failure to address these concerns risks impacting the resilience of ecosystems to both human and climate impacts (Bond et al., 2008). In this study we observed resilience of the BMA community to drought, but not in the phytoplankton community in the water column. Fluxes differed before and after the drought, and while further study would be necessary to determine whether those processes returned to pre-drought conditions, the results here imply that resilience of the water column system is at risk. Additional future work could include the effects of grazing on BMA biomass and community structure (Hillebrand and Kahlert, 2002; Hillebrand et al., 2000), and BMA and BBL species identification to evaluate the role of any specific species in food web dynamics. Finally, DISTLM results demonstrate the necessity of assessing the environment as a whole, rather than examining the effect of single parameters.

4. CONCLUSIONS

The objective of this study was to resolve the complexity of relationships between benthic and water column phytoplankton communities and freshwater inflows in Galveston Bay. The statewide drought in 2011 afforded a unique opportunity to observe responses of the phytoplankton community to drought, and as well as drought recovery. These responses allow the scientific community a contemporary in situ look at the future challenges our ecosystems will face as they cope with a warming earth, more variable precipitation and increased likelihood of drought, and thus more variable freshwater availability necessary for ecosystem function and sustainability (Longley, 1994; Meehl et al., 2007; Stocker et al., 2014). Temperature and salinity were identified as predictors of variability by DISTLM across the board, indicating that climate change and freshwater inflows are set up to be major drivers of ecosystem transformation.

We observed that drought in itself does not have a significant effect on pelagic or benthic phytoplankton community composition, though that could be a result of the timing of the beginning of the drought in relation to annual phytoplankton growth cycles (Wetz et al., 2011). Rather, in these communities, the increase in availability of freshwater inflows following the drought appeared to be more influential on community structure, rather than the lack of inflows and the resources they bring. In the pelagic community, post-drought spring brought a community shift of predominantly diatoms to chlorophytes and dinoflagellates in the estuary proper. Additionally, pelagic phytoplankton nutrient limitations included NP co-limitation as well as NA, with the

latter a new condition observed in the bay. We also propose that in NA co-limitation NH_4 could be inhibiting NO_3 uptake, as described by Glibert et al. (2015). Sediments during the study were on the whole a source for DIN, mostly in the form of NH_4, indicating that further study into the relationship of sediment sources of nutrients to the bay and pelagic phytoplankton should be considered. Benthic communities saw an increase of diatoms in drought spring, but highest biomass overall post drought. In contrast, benthic boundary layer phytoplankton peaked in biomass in drought spring, with a mostly dinoflagellate community. However, communities lacked a drought specific response, and were different each sampling event of the study. Overall, this suggests that following a drought there should be consideration of the delivery of nutrients and allochthonous carbon in terms of quantity, type, and proportion if detrimental post-drought effects are to be mitigated with proactive ecosystem and food web management (Anderson et al., 2002; Glibert et al., 2001).

4.1. Future Directions

As documented previously, nutrient delivery and availability to estuaries potentially drives phytoplankton community composition and abundance in the water column and the benthos (Chapters II and III, and references therein). Sources of nitrogen to coastal ecosystems are varied, and loading and cycling of nitrogen in estuaries has long been a subject of interest to researchers (Castro et al., 2003; Galloway et al., 2003; Howarth and Marino, 2006; Pinckney et al., 2001). Nonetheless, nitrogen is generally

considered to be a limiting nutrient to primary production in temperate estuaries, and some subtropical estuaries like Galveston Bay (Howarth and Marino, 2006; Lester and Gonzalez, 2011). While important, a nitrogen budget that balances inputs to and exports from Galveston Bay does not yet exist, but could be modeled after a budget similar nearby system, the Nueces Estuary (Brock, 2001). This knowledge is critical for making nutrient reduction efforts more economical to state and local budgets (Rebich et al., 2011). Models such as SPARROW (SPAtially Referenced Regressions On Watershed attributes) are predictive tools that may aid coastal management of resources in respect to nutrient loading to estuaries by identifying dominant sources of nutrients and their location (Preston et al., 2009; Preston and Brakebill, 1999).

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