RESPONSE OF BENTHIC MICROALGAL COMMUNITY COMPOSITION AT EAST BEACH, GALVESTON BAY, TEXAS TO CHANGES IN SALINITY AND NUTRIENTS

A Dissertation

by

ALYCE REBEKAH LEE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2009

Major Subject: Oceanography

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ABSTRACT

Response of Benthic Microalgal Community Composition at East Beach, Galveston Bay, Texas to Changes in Salinity and Nutrients. (May 2009)

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Benthic microalgal community composition on an ephemerally submerged sandflat at East Beach, Galveston Island, Texas was studied to determine the spatial and temporal variability of total biomass and community composition and its responses to experimental manipulations of two environmental factors (salinity and nutrients). Four field studies were conducted between August 2004 and February 2005. The community consisted of two major algal groups, diatoms, and cyanobacteria with two less abundant groups, green algae, and phototrophic bacteria. Spatial variability showed that patch sizes of 12 - 25 m were detected over larger scales with smaller scale (cm) patches of approximately 28 - 201 cm^{-2} contained within the larger patches. The second study examined the spatio-temporal variability of BMA over a 21-month period in a 1,000 m^2 area. Sampling location and date explained a significant amount of the variability in the abundances of algal groups, which were positively correlated with the water content of the sediments and negatively correlated with temperature (sediment and water). All of the algal groups showed a seasonal pattern with higher abundances measured in the

winter months and lower abundances found during the summer. BMA biomass (100 mg Chl *a* m^{-2} or greater) maxima occurred at temperatures less than 22° C and sediment water content greater than 15% (g water g $sediment^{-1}$).

BMA response to different salinities and nutrient (N+P) amended sediments was assessed in four bioassays conducted over a 6-month period (Aug. 2004, Oct. 2004, Dec. 2004, and Feb. 2005). In the salinity study, the treatments that were either 100% or partially diluted with deionized water had the lowest BMA biomass over all. Chlorophyll *a* and fucoxanthin were significantly affected by salinity with higher abundances found in salinities that averaged 15 with a preference for salinities greater than 22. Chlorophyll *b* was affected by salinity with higher abundances measured in the treatments with lowest salinity (DL and DI); and was affected by the time of year. This would suggest that this algal group prefers an environment with salinity <2 but can easily adapt to environments with higher salinities. BMA abundances were not significantly affected by the nutrient amended sediment, but were significantly affected by stations with higher water content, and during the cooler months (Dec. 2004 and Feb. 2005).

DEDICATION

I dedicate this dissertation to my father who began his education at Texas A&M University in 1936. His support and faith in my ability to accomplish this difficult task saw me through my darkest moments.

ACKNOWLEDGEMENTS

I want to thank my co-chairs Dr. James L. Pinckney and Dr. Daniel Thornton for their patience with the one of the most difficult challenges I have faced thus far. I especially appreciate Dr. Tammi Richardson for her invaluable knowledge, advice, and assistance throughout this process. I also want to express my gratitude to my other committee member, Dr. Stephen Davis, for his assistance during my research. In addition, I want to thank Dr. Gilbert Rowe and Kimberly Roberts for their assistance while sampling in Galveston. Storage and workspace were provided by Dr. Rowe and Kimberly provided assistance with sampling, laboratory equipment and materials and her couch.

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CHAPTER I

INTRODUCTION

Benthic microalgae (BMA) play a critical role in estuarine and coastal environments, providing a source of labile carbon for higher trophic levels (Admiraal 1984) and are one of the major primary producers (Haines and Montague 1979; Wainright et al. 2000). Their annual primary production may constitute one third to one half of the total primary production for an estuary with values ranging from 21 – 341 g C m^{-2} yr^{-1} in salt marshes (Sullivan and Moncreiff 1988). In addition to the importance BMA in benthic food webs, they play a vital role in pelagic food webs, when physical processes, such as tidal flows along with wave action, resuspend sediments and BMA. Suspended algae are a significant food source for secondary producers in pelagic environments where as much as 30-90% of the chlorophyll *a* may be attributed to benthic species (Guarini et al. 1998). In Ria de Arosa, Spain, Varela (1985) found that the total annual benthic production was 79 g C m^{-2} $year^{-1}$, whereas the water column production was only 6 g C m^{-2} $year^{-1}$ in the same area, suggesting that suspended BMA may be the primary food source for secondary consumers.

BMA display both temporal and spatial variability in abundance and primary productivity in intertidal sandflats, with communities forming clumps or patches of abundance. The variability in size and density of BMA patches may be caused by a

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This dissertation follows the style of *Estuaries and Coasts: Journal of the Estuarine Research Federation*.

variety of biotic and abiotic factors including, but not limited to, biogeochemical cycling within the sediment (Hopner and Wonneberger 1985; Hillebrand and Kahlert 2001), grazing pressure, salinity (Underwood et al. 1998; Chan and Hamilton 2001), grain size (Sandulli and Pinckney 1999; Watermann et al. 1999; Mitbavkar and Anil 2002), light (Kromkamp et al. 1995; MacIntyre and Cullen 1996), vertical migration (Kingston 1999) and seasonal change (Plante et al. 1986). The relatively short generation times of these organisms makes them very responsive to changes in ecosystem conditions (Cahoon 1999).

Spatial variability is necessary in ecological studies because of the important contribution to the stability of the ecosystem over large (hundreds of meters) and small (meters and centimeters) (Thrush 1991). Conducting field studies to examine the spatial patterns are necessary in order to determine the mechanics of what processes are determining these patterns (Thrush 1991). As investigators increase their knowledge of experimental design to capture the ecological complexity and variability of an ecosystem, this will aid in addressing the large-scale concerns of eutrophication, climate change, and how this will ultimately affect loss of bio-diversity within the system (Hewitt et al. 2007).

Bioturbation

Biological processes such as bioturbation can affect the nutrient concentration in porewater by increasing the vertical and horizontal mixing of sediments (Cullen 1973; Cadee 2001). BMA are predominantly found within the top 5 mm of the sediment surface; however, depth distributions may change due to various environmental factors.

Sediments examined on the continental slope off Cape Hatteras, NC, found viable diatoms up to 14 cm deep in the sediment, indicating high rates of both deposition and bioturbation (Cahoon et al. 1994). They attributed the bioturbation to head-down deposit feeders which are abundant on this slope and frequently found at depths of 14 cm or more (Cahoon et al. 1994).

Bioturbation can affect BMA spatial distributions because of nutrient exchange at the sediment-water interface which alters the denitrification/nitrification cycle (Knox 1986). An incubation study showed that the amphipod *Corophium volutator* added to the microcosm increased the absolute denitrification rate but also the denitrification relative to the consumption of oxygen (Pelegri et al. 1994). Another study confirmed this and showed that the addition of amphipods (*Monoporeia affinis*) negated the effects that algae have on suppressing the rate of denitrification in the sediment (Tuominen 1999). Decreasing denitrification rates allows for more nitrogen to be taken up by BMA, which out-compete bacteria with faster uptake and growth rates (Risgaard-Petersen et al. 2004). Therefore, bioturbation may affect BMA biomass and thereby affect the spatial variability in biomass and community composition.

Grain Size

Sediment grain size and type has been correlated with the spatial patterns of benthic organisms (Thrush 1991). Thrush stated that sediment characterization needs to be determined to examine its impact on the density of the organisms. A study conducted in the sandy intertidal sandflats in Barnstable, MA found the patch sizes for microalgal biomass to be 30 – 191 cm^2 which were attributed to sediment properties, with silty fine

sands having larger patches than muddy sediments (Sandulli and Pinckney 1999). Cahoon (1999) summarized studies of BMA biomass from several sites with differing grain size characteristics and found a negative correlation between BMA biomass and the proportion of fine sediments (< 125 mm dia.). He suggested that the fine sediments would prohibit BMA productivity within estuarine environments because the deposition and resuspension of the sediments would shade the BMA and reduce the light available for production. A study conducted in the Tagus Estuary found the diatoms were the dominant algal group found in the sandier sediments while the mudflats had higher taxonomic diversity (Brotas and Plante-Cuny 1998). Together, these studies attributed the spatial variability in BMA biomass to grain size differences.

Vertical Migration

Most benthic microalgal species (especially pennate diatoms) have the unique ability to migrate within the sediments, allowing them to regulate their vertical position. Some proposed advantages for this motility include the avoidance of wave action, access to more or less light, or access to higher nutrient concentrations (Kingston 1999; 2002). BMA primary productivity exhibits variability within sediments because of the rhythmic migratory patterns, and show a general absence of photoinhibition (Barranguet et al. 1998). These organisms may be migrate deeper into the sediment to prevent photoinhibition by high irradiances at the sediment surface (Sundback et al. 1996). Diatoms migrate upwards to optimal light conditions when the depositional sediment load is increased due to dredging or other anthropogenic influences (Wulff et al. 1997).

Light

BMA productivity and biomass respond to long-term changes in the seasonal solar irradiance. Annual primary production is greatly dependent on light availability with seasonal differences due to changes in the ambient irradiance at the sediment surface (Hargrave et al. 1983; Guarini et al. 2002). Competition for light may shift the community composition from one dominant algal group to another. Sandy sediments have a photic zone 2 – 3 times deeper than muddy sediments because of less absorption (Billerbeck et al. 2007) leading to greater productivity per unit biomass. While diatoms can grow at varying light intensities, cyanobacteria may be dominant at high irradiances and UV exposure (van der Grinten et al. 2005) They suggested that this was due to interference competition between the two groups. However, one reason for cyanobacteria being dominant at higher irradiances could be due to the mycosporine-like amino acids. One study showed that there was small sunscreen effect to UV radiation attributed to the mycosporine-like amino acids in *Gloeocapsa* sp (Garcia-Pichel et al. 1993). The pigment scytonemin, found in cyanobacteria, is also known for its photoprotective properties against high UV radiation (Sinha and Hader 2008).

Community Composition

BMA communities in intertidal sandflats are primarily composed of diatoms, cyanobacteria, green flagellates (euglenophytes and chlorophytes) and dinoflagellates (Pomeroy 1959; Sullivan and Moncreiff 1988; Barranguet et al. 1997; Cahoon 1999). In studies examining BMA community composition and distribution, diatoms tend to dominate community composition at a variety of locations, including mudflats, salt

marsh muds, and sandy sites, with euglenophytes and cyanobacteria being less abundant (Underwood and Paterson 1993; Brotas and Plante-Cuny 1998; Cartaxana et al. 2006). The species that comprise certain microalgal classes can be identified by their biomarker photosynthetic pigments or pigment combinations though the use of high performance liquid chromatography (HPLC) (Jeffrey et al. 1997; Jeffrey et al. 1999). Reverse-phase HPLC is a reliable method for characterizing the concentration of photopigments and their degradation products (Mantoura and Llewellyn 1983), providing an alternative to the time-consuming process of identifying algal groups using microscopy (Brotas and Plante-Cuny 2003). Chlorophyll *a* is a proxy for algal biomass (as it is found in all algal groups). Other photosynthetic pigments used as biomarkers in sediments include chlorophyll *c*, fucoxanthin, diadinoxanthin, diatoxanthin and ß-carotene for the diatoms, chlorophyll *b* and the lack of lutein for flagellated green alga, specifically euglenophytes, and zeaxanthin and myxoxanthophyll for filamentous cyanobacteria (Riaux-Gobin et al. 1987; Brotas et al. 1995; Brotas and Plante-Cuny 1998; Cartaxana et al. 2006). A preliminary study conducted for this dissertation using HPLC-based assessment of community composition showed that the predominant algal groups inhabiting the sandflats at East Beach, Galveston Bay, Texas were diatoms, cyanobacteria, green flagellates and photosynthetic bacteria (characterized by the presence of bacteriochlorophyll *a).*

Nutrients

BMA spatial and temporal heterogeneity or "patchiness" has also been attributed to nutrient sources within the sediment and the overlying water column (Hopner and

Wonneberger 1985; Underwood et al. 1998; Mitbavkar and Anil 2002). The abundances of several diatom and cyanobacteria taxa have been significantly correlated with certain nutrient species in the porewater and overlying water column (Underwood et al. 1998; Mitbavkar and Anil 2002). The source and concentration of different nutrients, specifically nitrogen (N) species necessary for BMA growth, significantly affect their spatial heterogeneity. Intertidal sediments are usually not N limited because of the relatively high inorganic and organic N concentrations found at depth within the sediments. Inorganic nutrients (ammonium, nitrate, nitrite, phosphate and silicate) have distribution patterns that show spatial heterogeneity over small scales (meters) (Marinelli et al. 1998). This could partially explain the spatial variability of BMA in estuarine sediments. For example, in a southern California estuary, nutrients within the sediment and the water column were temporally variable with N concentrations correlated with the time of year, and maximum concentrations found in the spring and declining through fall (Boyle et al. 2004). They found the highest nutrient concentrations (nitrate in water and N in sediments) during the rainy season when river input into the estuary was greatest. Underwood et al. (1998) found that BMA biomass did not respond to nutrient enrichment along a gradient, but differences were found in the abundances of individual species, indicating that BMA community composition may shift in response to changes in nutrient concentrations. Hopner and Wonneberger (1985) showed that the distribution of nutrients diffusing out of the sediment affects BMA patchiness. They stated that the ratio of the nutrient demand is similar to the elementary composition of diatoms (ca. 10:1). The N:P ratios of the porewater were 8.3:1 when converted to efflux ratios based

on diffusion coefficients, which is in the range of the demand ratio of the N:P When examining the N:P efflux ratios with the oxygen activity from the diatoms, they found that the oxygen activity was highest at the N:P ratio of 10 which is similar to the ratio of the N:P demand. These studies illustrate that nutrients within the sediment and the overlying water column can affect the spatial and temporal variability of the BMA biomass.

Salinity

Increasing salinity in estuaries is a growing concern in Texas due to a reduction in freshwater inflow attributed to the rising demands from agriculture and municipalities within the watersheds. As salinity increases due to decreased freshwater inflow, the BMA community composition may respond with a reduction in biomass or community composition, which in turn could affect higher trophic levels. Evaporation can also increase salinity resulting in an overall increase in salinity as well as from the decreased freshwater inflow. One of the Texas estuaries, the Nueces Bay, exhibits extreme variation in salinity due to decreased inflow and increased evaporation, anywhere from 300 in 2001 down to 11 in 2003 (Fejes et al. 2005). However, average salinities in the upper delta of the Nueces are over 120. Another study in the same bay found extreme conditions with salinities as high as 300 (Alexander-Mahala et al. 2000). Although these studies examined how evaporation affected salinity, they did not specifically address the effects on BMA structure or function.

BMA may adapt to salinity fluctuations by shifting the community composition, biomass, or both. Individual groups respond differently to salinity fluctuations, which

may alter the competitive hierarchy within the community, thereby resulting in a shift in community structure. Underwood et al. (1998), investigating the effect of salinity gradients, reported that the relative abundance of several diatom species shifted along the salinity gradient. In their study, the diatom genera shifted along the oligo- and meso- and polyhaline sites, with *Navicula gregaria* and *Navicula phyllepta* abundant at the low- to mid-salinity range while *Pleurosigma angulatum* and *Plagiotropis vitrea* were abundant at the higher salinities. Their results are consistent with two other studies that revealed a correlation between salinity and abundance for diatom distributions (Admiraal 1984; Clavero et al. 2000). Another study by Underwood and Provot (2000) isolated diatoms from the Colne estuary and monocultures grown in artificial media revealed certain diatoms had maximum growth rates at salinities of 20 to 35 with a significant decrease in the growth rate at higher salinities depending on the species (Admiraal 1984; Underwood 2002). A study by (2002) examined diatoms along a salinity gradient and found that most of the assemblages measured using cluster analysis were found at salinities greater than 25, but two of the assemblages were found in the lower salinities.

Cyanobacteria are sometimes, but not always, the only algal group observed in extremely hypersaline lagoons, and have been the only algal group observed in some Bahamian salt ponds (Paerl et al. 2000). Hypersalinity is when the salinity is greater than the ocean (Dahl 1956; Paerl et al. 2003). Diatoms have also been observed in cultured strains that corresponded well to field samples and grew well in brackish as well as hypersaline conditions (Clavero et al. 2000). However, cyanobacteria, unlike

diatoms, are able to fix nitrogen and through their evolution have adapted to various stressful environments, such as high temperature, high irradiance, ice ages, and fresh and saline waters (Apte and Alahari 1994). Other tropical and temperate hypersaline lagoons have phototrophic communities comprised of diatoms (fucoxanthin) and purple phototropic bacteria (bacteriochlorophyll *a*), but in lower abundances compared to cyanobacteria (zeaxanthin) (Pinckney and Paerl 1997; de Lomas et al. 2005). In one study site where the salinity increased from ca. 50 in May 2000 to over 100 in June of the same year, meiofauna and microfauna were almost absent in the area that contained the compacted microbial mat (de Lomas et al. 2005). A study in hypersaline environments in Baja California, Mexico, found that diatoms were unable to adapt to salinities >175, with cyanobacteria present in greater abundances at higher salinities (Clavero et al. 2000).

Organisms have the ability to adapt to higher salinities by physically and biochemically regulating their cell structure through osmotic regulation (Kirst 1990). Organisms are affected by changes in salinity one of three ways, impacting cellular water potential, uptake or loss of ions, and 'change of the cellular ionic ratios due to selective ion permeability of the membrane" (Kirst 1990). One reason cyanobacteria can readily adapt to higher salinities is they contain Mycosporine-like amino acids (MAAs) which is a low molecular weight water soluble compound known to function as a photoprotective compound but may function as a way to osmoregulate the cells(Oren 1997). In addition to adjusting the chlorophyll *a* or carotenoid content, cyanobacteria also synthesize solutes such as sucrose as osmolytes to acclimate to salt stress (Singh et

al. 2002). Osmolytes (sucrose and trehalose) have also been measured to increase in cyanobacteria with increased salinity (Portwich and Garcia-Pichel 1999). While increasing salinity allows cyanobacteria a competitive advantage over diatoms, cyanobacteria are not the preferred food source for most of the primary consumers and have been shown to have negative impact on microalgal herbivores (Armitage and Fong 2004). Thus, even though BMA abundance might be high in hypersaline environments, the energy transferred to higher trophic levels might be low because of poor palatability and food quality.

Statement of Purpose

This dissertation examined the spatio-temporal distribution of the BMA community in an estuarine sandflat at East Beach, Galveston, Texas, and determine what factors may influence the variability. Previous studies have indicated that both nutrient concentrations and salinity can affect shifts in BMA community composition. However, very few studies have assessed the influence of salinity and nutrients on BMA community structure and function in a high intertidal marine environment. This dissertation examined the response of a BMA community to manipulative experiments using different salinities and nutrient enrichment to determine if either of these factors regulates the spatiotemporal distributions of BMA biomass and community composition.
Objective 1: Determine the spatiotemporal variability of BMA biomass of the different groups that comprise the total biomass at East Beach over a 21-month period between March 2003 and February 2005.

Objective 2: Determine how salinity affects BMA biomass and community composition using manipulative experiments over a range of salinities.

Objective 3: Determine the effect of nutrient (N and P) enrichment on BMA biomass and community composition using manipulative experiments.

Study Site

The study site is a sandflat at East Beach (29º 20.025'N and 094º 44.226'W), on the northeastern end of Galveston Island, Texas (Fig. 1). This site was chosen because the ease of access and because of Galveston Bay's location to metropolitan areas. Examining the benthic microalgae at this site would assist in providing information on the ecological health of the area. This high intertidal flat is influenced minimally by normal tides but experiences periods of water saturation due to episodic rain events and wind/storm induced tidal surges. The sediment consists of fine (76%), very fine (21%), and coarse sands (3%) (pers. obs). Galveston Bay has an average depth of 2 – 3 m and the mean tidal range is 0.3 m ((NOAA) ; Lester and Gonzalez 2003). The oyster reefs within the bay area are shallower, but volume of the bay overall has increased over the last 50 years due to subsidence, dredging and sea-level rise (Lester and Gonzalez 2003). The Galveston Bay watershed extends north to Dallas-Fort Worth and receives approximately 60% of the state's wastewater effluent ((TRW) 1995). The bay receives 54% of river run-off inputs from the Trinity River into Trinity Bay (which is adjacent and connected to Galveston Bay) and an additional 28% of river input from the San Jacinto River ((TRW) 1995). The circulation pattern of Galveston Bay indicates that the majority of water that inundates the study site comes from the San Jacinto tributary with

some mixing of water from the Trinity River (Lester and Gonzalez 2003). The San Jacinto-Brazos basin provides 10% of the freshwater inflow with most of it entering the West Bay. Thereby, the freshwater flow and the amounts of nutrients affecting East Beach during periods of flooding would likely come from the San Jacinto tributary, San Jacinto-Brazos basin, West Bay, Dickinson Bayou and the smaller tributaries on the western side of Galveston Bay. The study site is situated near the mouth of the bay where circulation patterns would suggest that during the incoming tide, the Gulf of Mexico greatly influences the salinity and freshwater flow near the site. The outgoing tidal circulation pattern mixes water from Trinity Bay, East Bay and the San Jacinto tributary (Lester and Gonzalez 2003). The study site (ca. 1000 m^2) was examined routinely over a 33-month period and a portion of the site (ca. 300 m^2) was submerged approximately 50% of the time that the site was observed. In addition, in that same area of the study contained two types of vegetation, *Salicornia sp.* and *Spartina sp.* The *Spartina sp.* was observed in the portion of the site (ca. 300 m^2) that was submerged more frequently than the rest of the area, while the *Salicornia sp.* was found sparsely throughout the rest of the site.

Figure 1: The top two photos show an aerial view of Galveston Bay, Texas and the sampling location in reference to Galveston Bay. The bottom two photos show the mat formation typical of East Beach and a panoramic view of the site with north in the center of the photo.

CHAPTER II

SPATIAL VARIABILITY OF BENTHIC MICROALGAL COMMUNITY COMPOSITION AND BIOMASS AT EAST BEACH, GALVESTON, TEXAS

Introduction

Benthic microalgae (BMA) are photosynthetic organisms and one of the major contributors to the carbon cycle in estuarine and coastal ecosystems (Admiraal 1984; Sullivan and Moncreiff 1988; Moncreiff et al. 1992; Cahoon 1999). BMA exhibit patchy distributions {Sandulli, 1999 #540;Admiraal, 1984 #15;Guarini, 1998 #221} and the size and density of these patches vary due to biotic and abiotic factors such as grazing, nutrient concentration, competition, and sediment grain size (Whittaker and Levin 1977; Fleeger et al. 1984; Cahoon 1999). Sandulli and Pinckney (1999) defined spatial pattern as "the areal variation of species densities in their environment". The community can form small and large-scale spatial patterns within islands of individuals or algal groups that may vary in relative abundances from an area compared to a neighboring area. These mosaics are not closed systems with heterogeneity caused by biotic and abiotic factors.

The purpose of this study was to examine which algal groups comprise the BMA community at East Beach, Galveston, Texas, and quantifies the spatial variability (patch sizes) of both total biomass and algal groups over both small (cm^2) and large-scales (m^2). The working hypothesis was that the BMA composition would exhibit both large and small spatial variability (i.e., patches within patches) as measured by the changes in the relative abundances of diatoms and cyanobacteria.

Materials and Methods

Experimental Design. This study, conducted in June 2002, examined the small (cm^2) and large-scale (m^2) spatial variability of BMA community composition. Sediment cores (butyrate core tubes 47.8 cm^2) were collected using two different sampling techniques along two perpendicularly transects oriented 50 m long by 22 m wide at East Beach (Fig.2). The study site is a sandflat at East Beach (29º 20.025'N and 094º 44.226'W), on the northeastern end of Galveston Island, Texas (Fig. 1). This site was chosen because the ease of access and because of Galveston Bay's location to metropolitan areas. Examining the benthic microalgae at this site would assist in providing information on the ecological health of the area. This sand flat is not tidally influenced but experiences periods of water saturation due to episodic rain events and wind/storm induced tidal surges. The sediment consists of fine (76%), very fine (21%), and coarse sands (3%) (pers. obs). Grain size was not determined at each sample location and the site was not vegetated at the time this study was conducted. Samples were collected mid-day; however, a study done in Baffin Bay, TX indicated that the pigment chlorophyll *a* was independent of the daily photosynthetic changes and did not change during the course of the day (Blanchard and Montagna 1992). Therefore, the time of day that the samples were collected for pigment determination would not significantly affect the pigment concentration.

Samples for measurements of large-scale spatial patterns were collected at ca. 4 m intervals on Transect 1 and every 6.5 m on Transect 2 for a total of 13 samples and transported back to the laboratory for analysis. Each of the core tubes was sub-sampled

in triplicate using a smaller coring tube (0.95 cm^2). The upper 3 mm of sediment was extruded from each tube and placed in 2.0 ml microfuge tubes, frozen, and stored in the dark at -80 °C for photopigment analyses.

The spatial distribution at the smaller scale (cm^2) was determined using samples collected in a localized area at the mid-point of each transect. Four tissue culture plates (12-5.0 cm^2 wells per plate) were arranged in a 2 x 2-grid pattern covering an area ca. 241.6 cm^2. The four culture plates were pushed into the sediment simultaneously, filling all 48 wells with sediment in a defined grid pattern. The sediment in each well was sub-sampled using a small butyrate core tube (0.95 cm^2). The upper 3 mm of sediment was extruded from each tube and treated as described above.

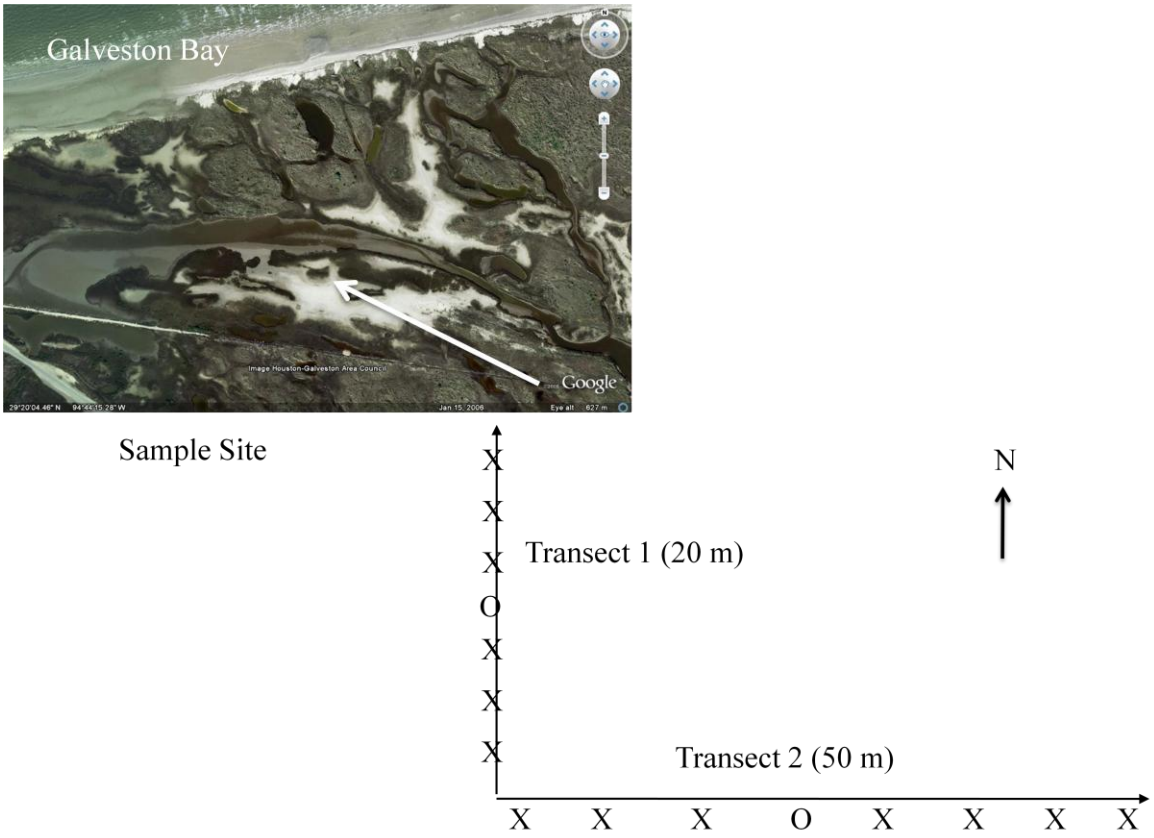

Figure 2: Diagram detailing the length, orientation, and sample locations for each transect (1 and 2) at East Beach, Galveston, Texas. The "X" indicates where along each transect the sample cores were collected to determine the larger (m^2) scale variability. The "O" indicates where the well culture plates were placed at each transect to collect the samples for the smaller (cm^2) scale variability. The picture shows the approximate location where the transects were established on this sandflat.

Photopigment Analyses. HPLC was used to determine chemotaxonomic photosynthetic pigments for BMA. The sediment samples were placed in 100% acetone (2 ml), sonicated (10s), and extracted at -20° C for 18-24 h. Filtered extracts (300 µl) were injected into a Shimadzu HPLC equipped with a monomeric (Rainin Microsorb-MV, 0.46 x 10 cm, 3 µm) and a polymeric (Vydac 201TP54, 0.46 x 25 cm, 5 µm) reverse-phase C18 column in series. A nonlinear binary gradient was used for pigment separations (Pinckney 1996). Absorption spectra and chromatograms (440 nm) were acquired using a Shimadzu SPD-M10av photodiode array detector. Diagnostic photopigment peaks were identified by comparing retention times and absorption spectra with pure crystalline standards, including chlorophylls *a*, *b*, *β*-carotene (Sigma Chemical Co.), fucoxanthin, and zeaxanthin (Hoffman-LaRoche and Company). Other pigments were identified by comparison with extracts from phytoplankton cultures and quantified using the appropriate extinction coefficients (Jeffrey et al. 1997). Fucoxanthin and zeaxanthin were considered indicators for diatoms and cyanobacteria, respectively, and chlorophyll *a* is indicative of the total biomass (Millie et al. 1993; Pinckney et al. 1995b).

Spatial Auto-correlation. Geary's C and Moran's I spatial autocorrelation indices were used to determine the patch sizes for small scale variability (Sokal and Oden 1978). Spatial auto-correlation determines if one variable is significantly different or dependent from the neighboring location. For this study, the spatial auto-correlation values were calculated for each transect (48 contiguous wells) using the software SAAP 4.3 (Exeter Software, Setauket, NY). Geary's C index measures the similarity between

neighboring locations and ranges from 0 to 2, with values closer to 0 indicating a strong positive correlation and values closer to 2 indicating a strong negative correlation. A value of 1 indicates the absence of spatial autocorrelation. Moran's I index ranges from -1 to +1 (strong negative to strong positive autocorrelation, respectively) and a value of 0 indicates the absence of spatial autocorrelation (Sokal and Oden 1978). Correlograms, which plot the indices (I and C) vs. distances between the wells, were constructed to illustrate spatial auto-correlation between neighboring sample locations. The point at which the value crosses the expected value, E(I) or E(C), indicates the patch size (El-Shaarawi and Piegorsch 2002). The statistical significance of each test assesses whether the coefficients are dependent on neighboring values (Oden 1984). The correlogram is considered significant if the significance level of at least one coefficient is lower than the p-value (El-Shaarawi and Piegorsch 2002).

Statistical Analyses. Replicate sediment samples were not collected; therefore, a non-parametric Friedman's test was performed to examine the large-scale variability. The concentrations of photopigments were averaged for the 3 sub-samples collected from each large core to provide a "best estimate" value for factor levels. The non-parametric Friedman's test was performed using three diagnostic photopigments (chlorophyll *a*, fucoxanthin, and zeaxanthin) as dependent values and the sample locations (1 - 5, 10 – 17) as the independent values. The other statistical analysis was used to determine the minimum sample size required to estimate the population mean for chlorophyll *a* with an accuracy of ± 20% using the equation (Eckblad 1991):

$$\text{Sample Size} = \left[\frac{(\text{Coefficient of variation} \times \text{t value})}{\text{Accuracy}} \right]^2$$

Equation 1: Calculation used for determining the number of samples needed to represent the population. The accuracy refers to how accurate the investigator wants to be within the population mean. The t-value (p=0.05) is from the student's T-distribution and sample variance is the spread of values from the population mean.

Results

Concentrations of three diagnostic pigments were measured in 13 sediment cores collected from each transect and statistically analyzed to determine significant differences in pigment concentrations between cores. Results from the non-parametric Friedman's test indicated that the pigment concentrations (chlorophyll *a*, fucoxanthin, and zeaxanthin) were significantly different ($p < 0.001$) with respect to sample location (distance). Chlorophyll *a* had the highest abundance over the 13 samples, followed by fucoxanthin and then zeaxanthin (Table 1).

Table 1: The mean and standard deviation values for the diagnostic pigments measured in the 13 samples collected for the large-scale study.

Pigment Abundance	Mean (mg m^{-2})	SD
Chlorophyll *a*	109.1	61.6
Fucoxanthin (F)	16.9	8.4
Zeaxanthin (Z)	5.8	4.6
F/Z Ratio	4.3	2.6

Plots of pigment abundances vs. distance along the transects suggested patch sizes for chl *a* were ca. 12 m and 25 m for Transects 1 and 2, respectively (Fig. 3). Patch sizes were similar for both diatoms (fucoxanthin) and cyanobacteria (zeaxanthin) (Fig. 3). The ratio of fucoxanthin to zeaxanthin (F/Z ratio) was used to indicate the abundance of diatoms relative to cyanobacteria and provide a relative measure of community composition. The F/Z ratio (Fig. 3) was nearly constant along Transect 1 and suggested a homogenous community. However, in Transect 2, the relative abundance of the diatoms peaked at distance of 30 m, indicating a change in the BMA community composition. Two main differences between the two transects were that the patch sizes were larger and the distance interval between the patches was greater in Transect 2 than in Transect 1.

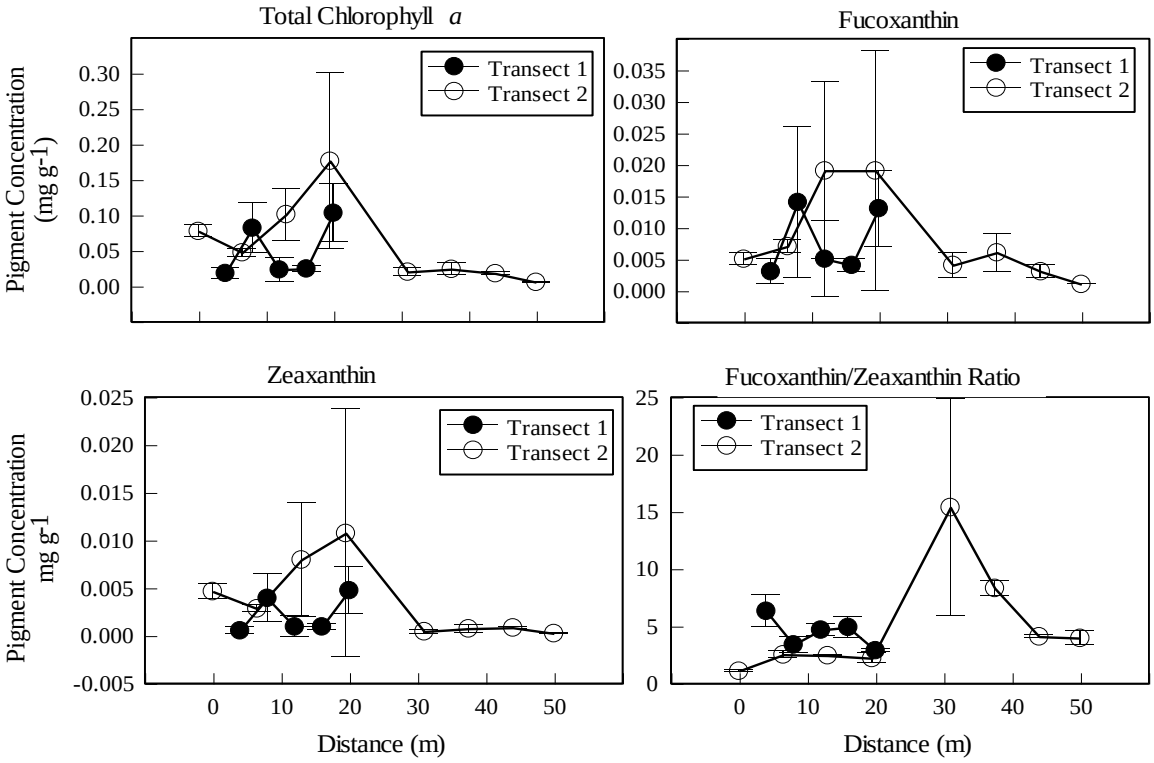

Figure 3: The large-scale (m^2) distribution patterns for chlorophyll *a*, fucoxanthin, zeaxanthin, and the fucoxanthin to zeaxanthin ratio in Transects 1 and 2.

Spatial autocorrelation analysis was done to examine whether the BMA exhibited small-scale (cm^2) variability in addition to the large-scale it was revealed that BMA also exhibited the smaller scale variability. Correlograms for each algal group were constructed using the distance interval between samples (based on the center of the wells) (Fig. 4). Patch sizes were determined by the distance interval at which the autocorrelation value crossed the expected value (E(I) = 1.0 for Moran's I and E(C) = 0.0 for Geary's C). The correlograms indicated that the patch size radii (areas) for chl *a* ranged from 3 – 8 cm (28 – 201 cm^2), fucoxanthin ranged between 4 – 8 cm (50 – 201 cm^2), and zeaxanthin ranged between 4 – 6 cm (50 – 113 cm^2) (Table 2). Overall, the patch sizes for Transect 2 were larger than Transect 1 and each correlogram was not significant (Bonferroni approximation, $p>0.05$). Therefore, as the results from the Bonferonni indicate a p-value > 0.05 for each index, each pigment displays spatial patchiness and the correlograms show the size of these patches.

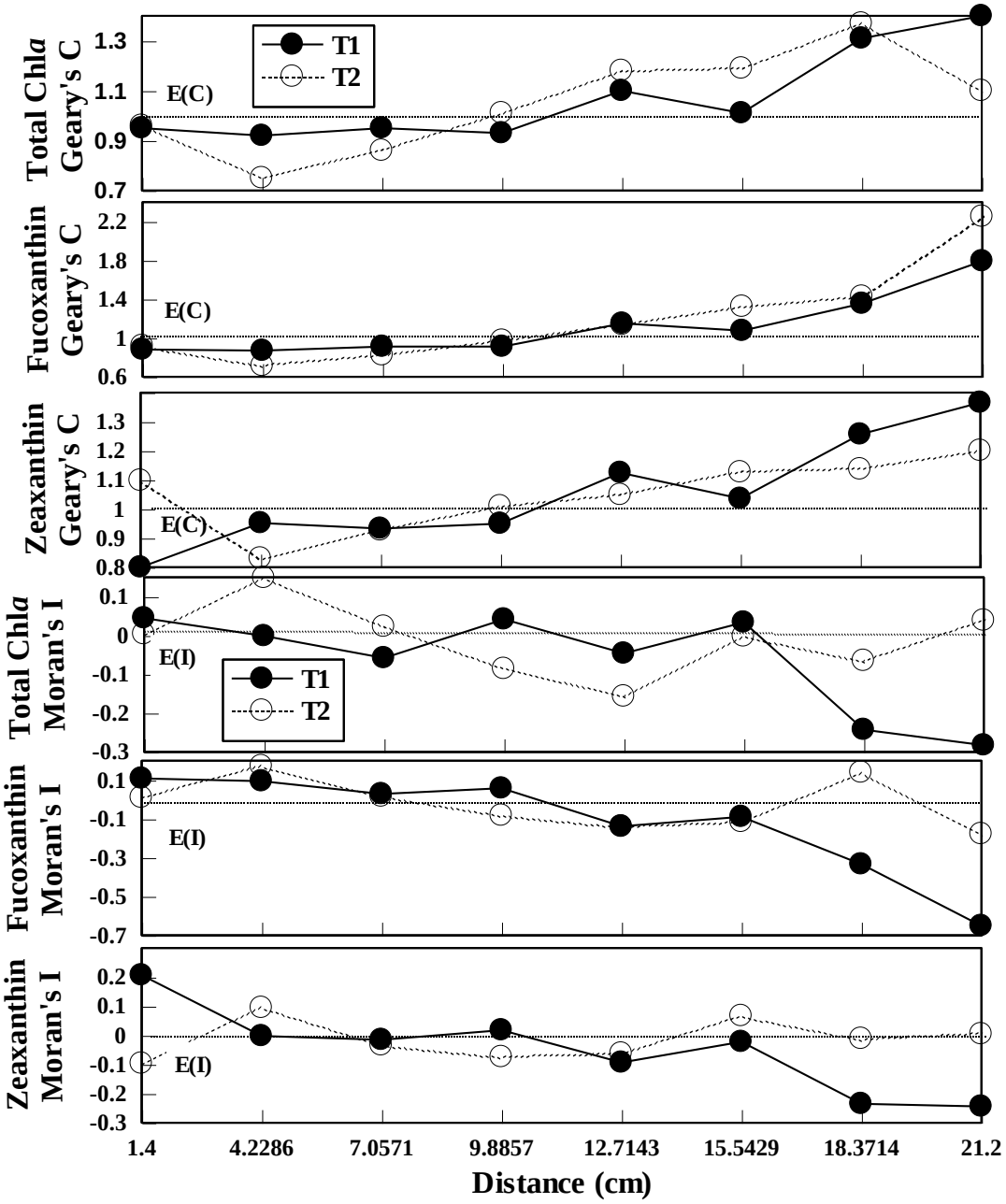

Figure 4: Correlograms constructed for chlorophyll *a*, fucoxanthin, and zeaxanthin that show patch sizes (radii) ranging from 4-8 cm^2 resulting from the Geary's C and Moran's I indices.

Table 2: Results from the Geary's C and Moran's I indices for both Transects 1 and 2. Patch sizes (radii) range from 3 - 8 cm (28 – 201 cm^2). The overall correlogram significance (Bonferroni adjustment) is listed for each index.

	Geary's C Index			Moran's I Index		
	Chl *a*	Fuco	Zeax	Chl *a*	Fuco	Zeax
Transect 1	5 – 7	4 – 6	4 – 5	3 – 5	4 – 5	4 - 6
Bonferroni approx.	0.535	0.081	0.340	0.231	0.031	0.230
Transect 2	5 – 6	4 – 6	4 – 6	5 – 8	5 – 8	4 - 5
Bonferroni approx.	0.029	0.026	0.403	0.107	0.041	0.500

The mean of each pigment was determined and a summary of the patch sizes show that each of the pigments had higher abundances in Transect 1 than in Transect 2 (Table 3). The relative abundances of chlorophyll *a* (chl *a*), fucoxanthin (fuco), zeaxanthin (zeax) and the fucoxanthin to zeaxanthin ratio (F/Z ratio) were plotted using a contour-mapping program (Surfer v. 8.0, Golden Software) to illustrate distribution patterns for the two transects (Fig. 5). The distribution of abundances for each pigment appears to overlap with each map showing the same distribution pattern. The F/Z ratio showed a relatively homogeneous distribution in each transect, except for one patch in Transect 2, ca. 4 cm in diameter, and two smaller patches in Transect 1 (Fig. 5). These areas (that appear darker in color) suggest a shift in the relative abundance of diatoms compared to cyanobacteria.

Table 3: Small-scale spatial variability measured in the contour plots for both Transects 1 and 2. The mean (± 1 SD) relative abundance (mg m^{-2}) measured for each pigment based on the data collected from each well of the cell culture plates (48 wells from each transect). The diameter of the patch sizes are in cm.

	Transect 1		Transect 2	
Pigment	Mean±SD	Patch Size (cm)	Mean±SD	Patch Size (cm)
Chlorophyll *a*	168±96	2 - 7	108±80	2 - 7
Fucoxanthin (F)	9.4±5.4	3 - 6	9.6±7.9	5 - 6
Zeaxanthin (Z)	5.8±3.6	4 - 6	4.8±3.7	2
F/Z ratio	1.8±1.7	2 - 4	2.4±3.1	4

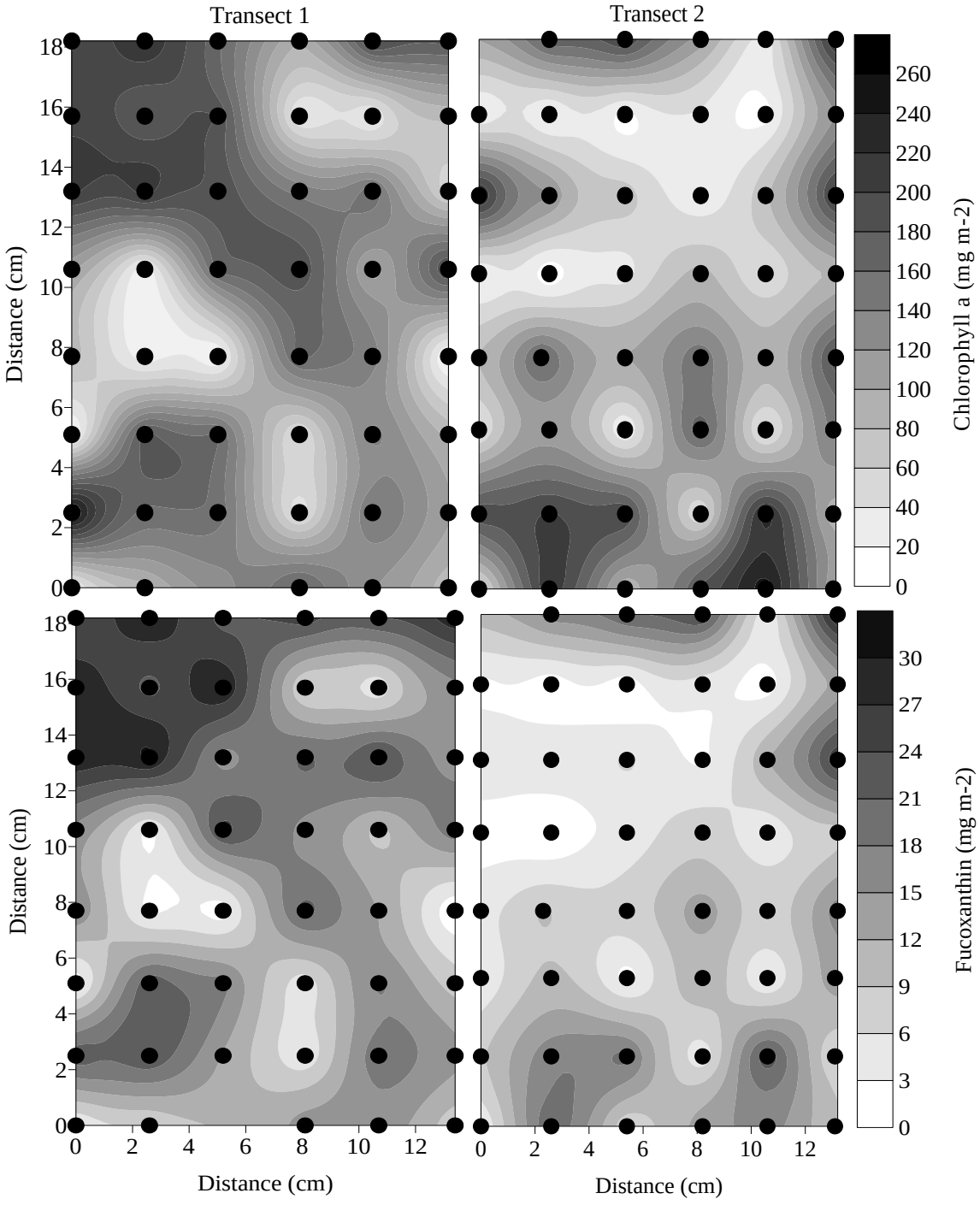

Figure 5: Contour plots indicating the distribution of chlorophyll *a*, fucoxanthin, zeaxanthin, and fucoxanthin to zeaxanthin ratio relative abundances in the 48 wells from each transect, covering an area of 241.6 cm^2.

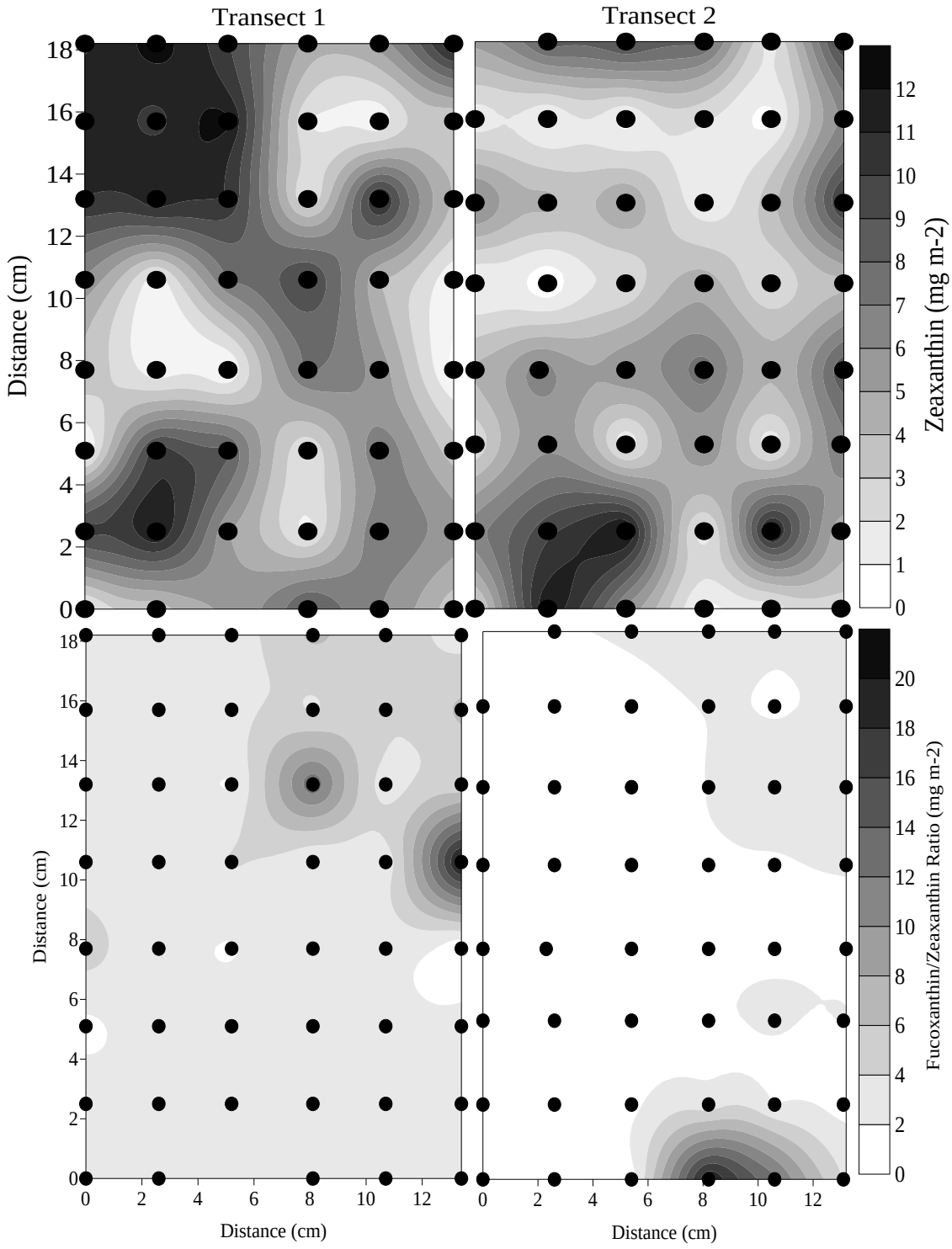

Figure 5: Continued.

The sample size determination revealed that ~ 50 samples should be collected within the sampling location for the sample mean to be within ± 20% of the population mean for transects 1 and 2 (Fig. 6). Estimating the sample mean within ± 20% for Transect 1, as indicated by a solid line, 50 samples are required, while Transect 2, indicated by a dotted line, requires a sample size of 75.

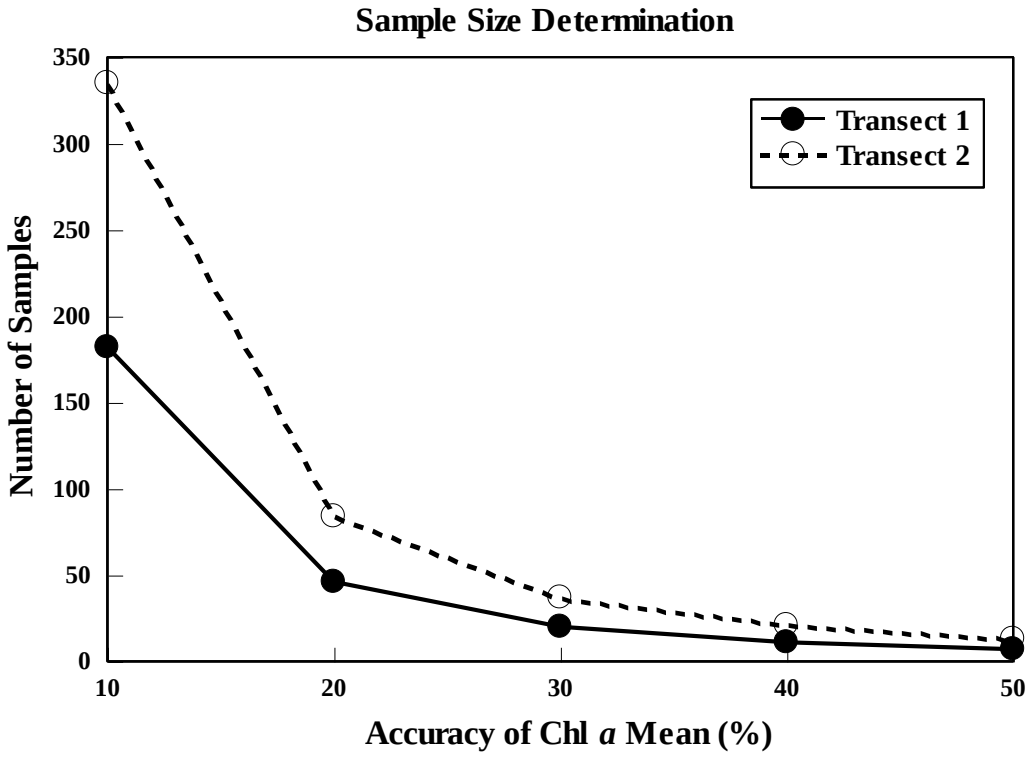

Figure 6: Sample size required to estimate the sample mean within certain percentage accuracy for both sampling transects 1 and 2 located at East Beach, on Galveston Island, Texas. In order for the sample mean to be within ±20 % of the true population mean, approximately 75 samples would have to be collected along transect 1 and roughly, 50 samples would be required from Transect 2.

Discussion

Benthic microalgae exhibit spatial variability in estuarine environments by forming aggregates (patches) (Decho and Castenholz 1986; Blanchard 1990; Guarini 1998; Guarini et al. 1998; Sandulli and Pinckney 1999). Studies have shown that several biotic and abiotic factors such as temperature, grain size (Cahoon et al. 1999), nutrient pulses (Hillebrand et al. 2000), and grazing pressure (Round and Eaton 1966) may control the spatial distribution of BMA biomass. The purpose of this study was to examine the large (m^2) and small (cm^2) scale spatial variability of the BMA community at East Beach. On a larger scale, without external influences, patches become more homogeneous (Levin and Paine 1974). A study by Jesus et al. (2005) examined spatial scales using PAM fluorescence and determined that the scale being examined, 2 cm, was too large to fully determine the spatial variability of BMA and argued that BMA should be studied at smaller scales. Blanchard (1990) also suggested that if the sampling scale is not correct, then this could lead to inaccurate conclusions because true heterogeneity is not observed. These studies would suggest that the patch sizes measured for the small-scale study are more accurate than the patches revealed from the large-scale study. Overall, the patches measured in this study for both the small and large scales were comparable to other studies in estuarine environments (Blanchard 1990; Sandulli and Pinckney 1999). The small-scale variability for BMA patch sizes measured at East Beach ranged from 28 - 201 cm^2, is similar to other studies and may be attributed to grain size. A study on sandy intertidal sandflats in Barnstable, MA found BMA patch sizes of 30 – 191 cm^2 and attributed the patches variations in sediment grain size. Silty

fine sands had larger patches than muddy sediments (Sandulli and Pinckney 1999). Cahoon (1999) looked at BMA biomass from several sites with differing grain size characteristics and found a negative correlation between BMA biomass and the proportion of fine sediments (< 125 mm dia.). He suggested that the fine sediments would prohibit BMA productivity within estuarine environments because of these sediments shading the BMA. Together, these studies attributed BMA spatial variability to grain size differences. However, the sediment at East Beach is predominantly fine sand to very fine sand with no measurable silt or clay material. Therefore, the spatial patchiness of BMA at East Beach is not likely attributable to variations in sediment grain properties.

Water flow and grazing are other factors that can affect BMA biomass and spatial patterns. Eckman (1979) examined the small-scale (mm^2 and cm^2) dispersion patterns on the intertidal sandflats in Skagit Bay, WA and argued that BMA patches could be an active response from the algae or a passive response to the type of transport. The sandflat at East Beach is aerially exposed and non-tidal, with periodic flooding during rain events and storm surges. BMA at this site generally do not undergo advection out of the area but could be resuspended and transported to adjacent areas of the sandflat. These occurrences, when severe, can erode BMA, grazers, and transport or export them to other areas. Decho and Fleeger (1988) showed a clear relationship between harpacticoid copepods and BMA during low tide on the microspatial scale (cm^2). They stated that the small aggregations of the two groups resulted from hydrodynamic processes occurring during low current flow and the irregular sediment

surface topography. In addition to the current flow, when the mudflat was exposed, small scale patchiness could be produced by high feeding rates from grazers seeking out areas of high BMA abundances (Decho and Fleeger 1988). While studies show that grazing pressure can negatively influence microalgal biomass, one study showed the opposite. Grazing pressure actually increased the relative abundance of microalgae to total algal biomass (microalgae and macroalgae), even though absolute biomass decreased with increased grazing (Roll et al. 2005). They stated that microalgae could tolerate grazing pressure in a low nutrient environment because of their fast growth rate compared to the macroalgae. Along the coast of France patch sizes for diatoms were found to be <4 - 113 cm^2 and diatoms partially influenced meiofaunal patch sizes because of feeding preference (Blanchard 1990).

Grazing pressure was not measured during this study and it cannot be ruled out as a significant factor affecting the spatial heterogeneity at this site. Many organisms feed on BMA (Cahoon 1999). Decho and Fleeger (1988) performed laboratory studies that showed harpacticoid copepods preferred sediments with "high concentrations of diatoms and/or their chemical exudates". A study in Barnstable Harbor, MA found that most copepods and benthic microalgae were spatially auto correlated. However, significant correlations between microalgal and copepod abundances were not found at the spatial scales studied (Sandulli and Pinckney 1999). Nematodes and harpacticoid copepods were significantly associated with certain microalgal densities in a study conducted on the West Atlantic coast of France (Blanchard 1990). Collectively, these

studies provide evidence that BMA are a major source of food for a variety of consumers and grazing is a plausible explanation for the spatial variability found at East Beach.

In summary, the results provided insight into the formulation of the experimental design needed to examine the spatial and temporal variability of BMA over a 1,000 m^2 on the sandflat at East Beach. Diatoms and cyanobacteria exhibited small (cm^2) and large (m^2) scale variability in a mosaic of patches within patches. On the large-scale, patches were ca. 12 - 25 m in distance along Transects 1 and 2, respectively, and the small-scale variability study indicated patches 3 - 8 cm in radius. For the small-scale study, patch sizes for the three pigments were smaller for Transect 1, with chlorophyll *a* measuring 25-154 cm^2, fucoxanthin and zeaxanthin at 50-130 cm^2. On Transect 2, chlorophyll *a* and fucoxanthin were 78-201 cm^2 and 50-201 cm^2, while zeaxanthin patches were the same as Transect 1. The two sample sets collected for the small-scale study showed that the diatom to cyanobacteria ratio was uniformly distributed with only one exception.

This study provides evidence that the BMA at East Beach exhibit spatial variability on two different scales. However, BMA also display temporal variability that lead to the next question of how the temporal variability is exhibited at East Beach, if at all. The results of this study provide a quantitative justification for establishing a 3 by 3-grid pattern of nine sampling stations covering a 1,000-m^2 area (20 m by 50 m) to capture the spatial variability in BMA biomass and community composition. The number of samples required to estimate the population mean ± 20% (ca. > 50 replicates) would be impractical from a logistical perspective. In an attempt to balance practicality

with accuracy, five replicates will be collected at each station monthly over two years. Even though the percent accuracy at each station will be <50% of the population mean, over the course of the study, enough samples will be collected to statistically determine if there is a significant trend in the data.

CHAPTER III

SPATIO-TEMPORAL PATTERNS OF BENTHIC MICROALGAL COMMUNITY STRUCTURE AT EAST BEACH, GALVESTON, TEXAS

Introduction

BMA display a random distribution pattern of aggregated clumps or patches in sandy estuarine sediments (Decho and Castenholz 1986; Blanchard 1990; Guarini et al. 1997; Guarini et al. 1998; Sandulli and Pinckney 1999). Environmental factors can alter BMA community composition, absolute abundances, and production rates that in turn affect this variability. These factors include, but are not limited to, nutrient concentrations within the sediment (Hopner and Wonneberger 1985; Hillebrand and Kahlert 2001), grazing pressure (Sandulli and Pinckney 1999; Hillebrand and Kahlert 2001) salinity (Underwood et al. 1998; Chan and Hamilton 2001) grain size (Watermann et al. 1999) light (Kromkamp et al. 1995; MacIntyre and Cullen 1996) and vertical migration (Kingston 1999). Determining the spatial distribution of BMA is necessary to understand the processes that affect the variability and provides a starting point from which to examine competitive interactions (Thrush 1991; Hewitt et al. 1993). The rates of change within a community and processes that cause these changes can also be addressed by understanding the spatial variability (Underwood et al. 2000). Understanding the processes that affect BMA spatial and temporal distribution patterns can provide better estimates of the abundance and community structure, which may be applicable to other sandy, intertidal ecosystems.

The purpose of this study was to examine the spatial and temporal distribution of BMA biomass and community composition over a 21-month period on an intertidal sandflat at East Beach, Galveston, Texas. The spatial distribution of the community was determined over a large area (1,000 m^2) along a gradient where sediment water content (porewater) was variable. Determining the spatial and temporal distribution patterns of BMA at this site may provide insights into which environmental factors regulate BMA community structure and function at East Beach, Galveston, Texas. The working hypothesis is that the BMA community composition exhibit spatiotemporal heterogeneity in conjunction with sediment moisture and time of year.

Materials and Methods

Experimental Design. This study was conducted over 21-months (March 2003 – February 2005). A 3 by 3-grid system with nine sampling locations was established with dimensions 50 m by 20 m (Figs. 7) covering an area ca. 1,000 m^2. To determine spatial and seasonal variability, five replicate sediment samples were collected at each of the nine stations at monthly intervals. Permanent markers (PVC pipe) were used to fix the sample locations.

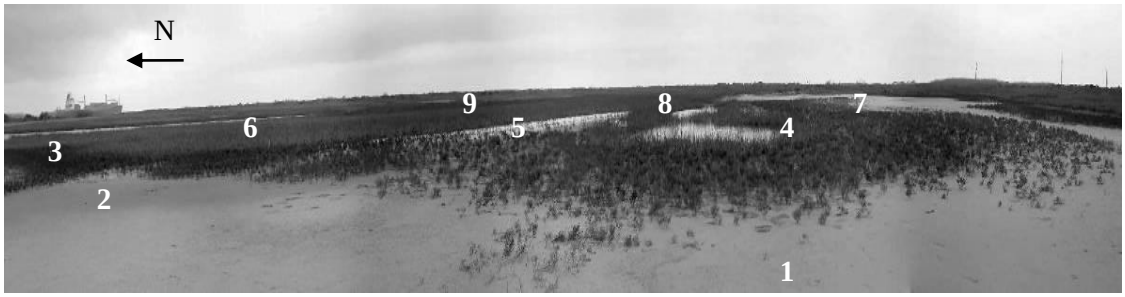

Figure 7: A panoramic view of the sampling site located at East Beach, Galveston, Texas. The site incorporates a 3 x 3 sampling grid over an area ca. 1,000 m^{-2}. The Gulf of Mexico is located approximately ESE with Galveston Bay adjacent to the north side of this site. Photograph by A. Lee 14-Dec-04.

Sample Collection and Core Sectioning. Five replicate sediment samples were collected ca. every month within a one-meter radius at each station, using a core-tube liner with a 1.1cm (ID), and 7.8 cm in length. A calibrated extruding device was used to section the upper 3 mm of sediment from each sample, then placed in 2-ml microfuge tube, and stored at -80° C. Three additional samples were collected at each station for sediment moisture content measurements (Tolhurst et al. 2003).

Sediment Water Content. The sediment moisture content was measured on three replicate sediment samples at each of the nine stations. The top 3 mm of each sample was extruded, placed in aluminum pans and dried for approximately 24 hours at 40º C to measure water content by weight loss. The percent difference between the wet and dry sample was calculated using the equation Wet weight – Dry weight/Wet weight * 100 (Tolhurst et al. 2003).

Photopigment Analyses. HPLC was used to determine chemosystematic photosynthetic pigments for benthic microalgae. The sediment samples were placed in 100% acetone (2 ml), sonicated (10 s), and extracted at -20 °C for 18-24 h. Filtered extracts (300 µl) were injected into a Shimadzu HPLC equipped with a monomeric (Rainin Microsorb-MV, 0.46 x 10 cm, 3 µm) and a polymeric (Vydac 201TP54, 0.46 x 25 cm, 5 µm) reverse-phase C18 column in series. A nonlinear binary gradient was used for pigment separations(Pinckney 1996). Absorption spectra and chromatograms (440 nm) were acquired using a Shimadzu SPD-M10av photodiode array detector. Pigment peaks were identified by comparison of retention times and absorption spectra with pure crystalline standards, including chlorophylls *a, b*, *β*-carotene (Sigma Chemical Co.), fucoxanthin, and zeaxanthin (Hoffman-LaRoche and Company). Other pigments were identified by comparison with extracts from phytoplankton cultures and quantified using the appropriate extinction coefficients (Jeffrey et al. 1997). Fucoxanthin and zeaxanthin were considered indicators for diatoms and cyanobacteria, respectively (Millie et al. 1993; Pinckney et al. 1995b). The ratio of fucoxanthin and zeaxanthin (F/Z ratio) was used assess changes in the abundance of diatoms relative to cyanobacteria and to measure relative changes in BMA community composition (Pinckney et al. 1995b).

Statistical Analyses. A two-factor multivariate analysis of variance (MANOVA) using the abundances of pigments (chlorophyll *a*, fucoxanthin, zeaxanthin, chlorophyll *b*, and bacteriochlorophyll *a*) as the dependent variables with date (21) and sampling station (9) as the main factors was used determine significant differences between dates and sampling stations. The data were not normally distributed (K-S test, $p<0.05$) and the variances were not homogeneous (Levene's test, $p<0.05$), therefore the data were transformed using ln (x+1). A multiple comparisons Dunnett's T3 *a posteriori* test was performed to compare the estimated means of the pigment abundances for station and date.

Results

MANOVA Results. The main factors were station (1 - 9) and date (March 2003 – February 2005). The main effects, station ($F = 1.085$, $p < 0.001$), date ($F = 2.895$, $p < 0.001$), and the interaction term ($F = 3.427$, $p < 0.001$), station and date, were all statistically significant (Pillai's Trace). The univariate tests (ANOVAs) indicated that each pigment was significantly different for station ($p<0.05$), date ($p<0.05$), and the interaction term (station and date, $p<0.05$) (Table 4).

Table 4: The univariate two-way test (ANOVA) results from a 21-month study observing spatial and temporal variability among the BMA community listed below with station, date, and the interaction term (station*date) as the main factors and the pigment abundances as variables.

	Station		Date		Station*Date	
	F value	p-value	F value	p-value	F value	p-value
Fucoxanthin (F)	297	<0.001	105	<0.001	13.3	<0.001
Zeaxanthin (Z)	92.3	<0.001	42.1	<0.001	9.66	<0.001
Chl_*b*	12.4	<0.001	45.1	<0.001	15.4	<0.001
Chl_*a*	156	<0.001	78.2	<0.001	9.90	<0.001
BChl_*a*	5.47	<0.001	31.6	<0.001	4.86	<0.001
F/Z Ratio	52.6	<0.001	28.8	<0.001	4.88	<0.001

Post-Hoc Comparison Results for Date. The *post-hoc* comparisons using the Dunnett's T3 test indicated that fucoxanthin, chlorophyll *a*, chlorophyll *b*, zeaxanthin, and bacteriochlorophyll *a* varied significantly with date ($p < 0.001$). Temporal variability was greater for chlorophyll *a*, fucoxanthin, and bacteriochlorophyll *a*, with higher pigment concentrations measured in the winter months than in the summer. Zeaxanthin and chlorophyll *b* did not exhibit the same temporal variability, they remained more uniform throughout the study. Chlorophyll *a*, fucoxanthin, and bacteriochlorophyll *a* displayed temporal variability with higher pigment concentrations (mg m^{-2}) measured in the winter months (October – May) compared to the summer (June – September) (Fig. 8). The community consisted of two dominant algal groups, diatoms, and cyanobacteria. The absolute abundance of diatoms was higher in the samples collected in the winter months and the relative abundance of cyanobacteria remained constant throughout the study. Therefore, it appears that diatoms are the dominant algal group at this site.

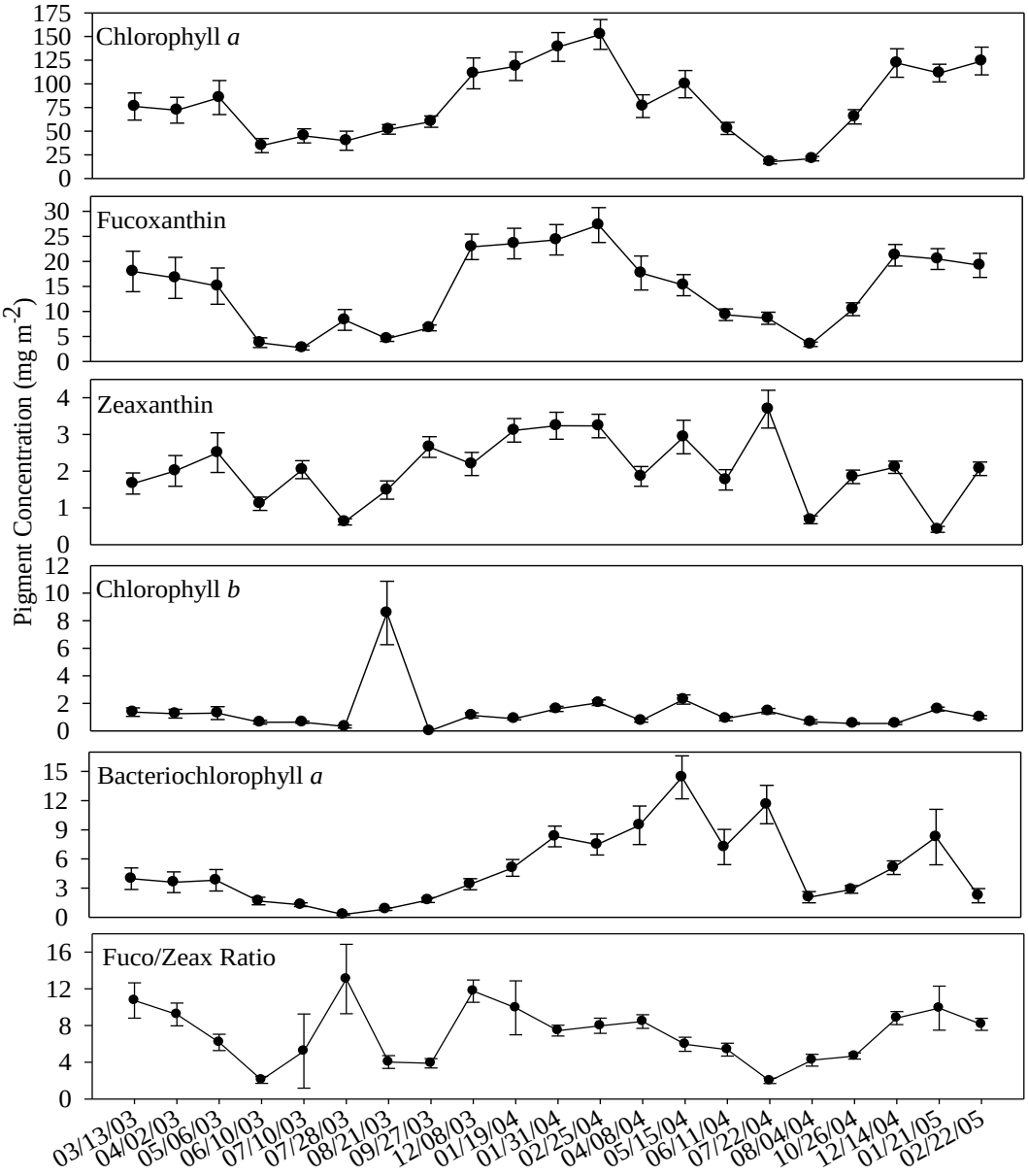

Figure 8: Pigment concentrations (mg m^{-2}) from chlorophyll *a*, fucoxanthin, zeaxanthin, chlorophyll *b*, bacteriochlorophyll *a*, and the fucoxanthin to zeaxanthin ratio measured during the 21-month study period. Values are the mean ± SE.

Post-Hoc Comparison Results for Station. BMA biomass was significantly different among the sample stations, with chlorophyll *a*, fucoxanthin, zeaxanthin, and bacteriochlorophyll *a* highest at Stations 9, 6, and 3 (Table 5). Chlorophyll *b* was highest at Stations 3 and 9 but Station 6 had one of the lowest concentrations for this pigment. In contrast, Stations 1 and 7 were significantly lower in concentration for all the diagnostic pigments compared to the other seven stations. The F/Z ratio revealed that the abundance of diatoms relative to cyanobacteria was higher at stations with higher sediment moisture (Stations 9, 6, and 3) (Table 5). The sediment moisture at East Beach ranged between 0% and 36.4% and temperature ranged between 8.1ºC and 39ºC. The sediment moisture (porewater content) at the sampling stations varied between stations, with two stations (1 and 7), having relatively little porewater compared to the other seven stations (Fig. 9). Stations 3, 6, and 9 had the highest sediment water content because they were sporadically under water due to rain events or tidal surges. A summary of the untransformed data revealed that all the diagnostic pigment abundances averaged higher in stations 3, 6, and 9, except for chlorophyll *b*, which measured highest in stations 1 and 7 (Fig. 10).

Table 5: Results of *a posteriori* mean comparisons (Dunnett's T3) for pigment concentrations at the different stations. The underline denotes that the means were not significantly different ($p<0.05$).

Pigment	Station
Chlorophyll *a*	9 6 3 4 5 8 2 7 1
Fucoxanthin (F)	9 3 6 4 8 5 2 7 1
Zeaxanthin (Z)	9 6 3 8 4 5 2 1 7
Chlorophyll *b*	9 3 2 4 5 8 6 1 7
Bacteriochlorophyll *a*	9 3 2 6 8 5 4 7 1
F/Z ratio	9 6 3 4 8 5 2 7 1

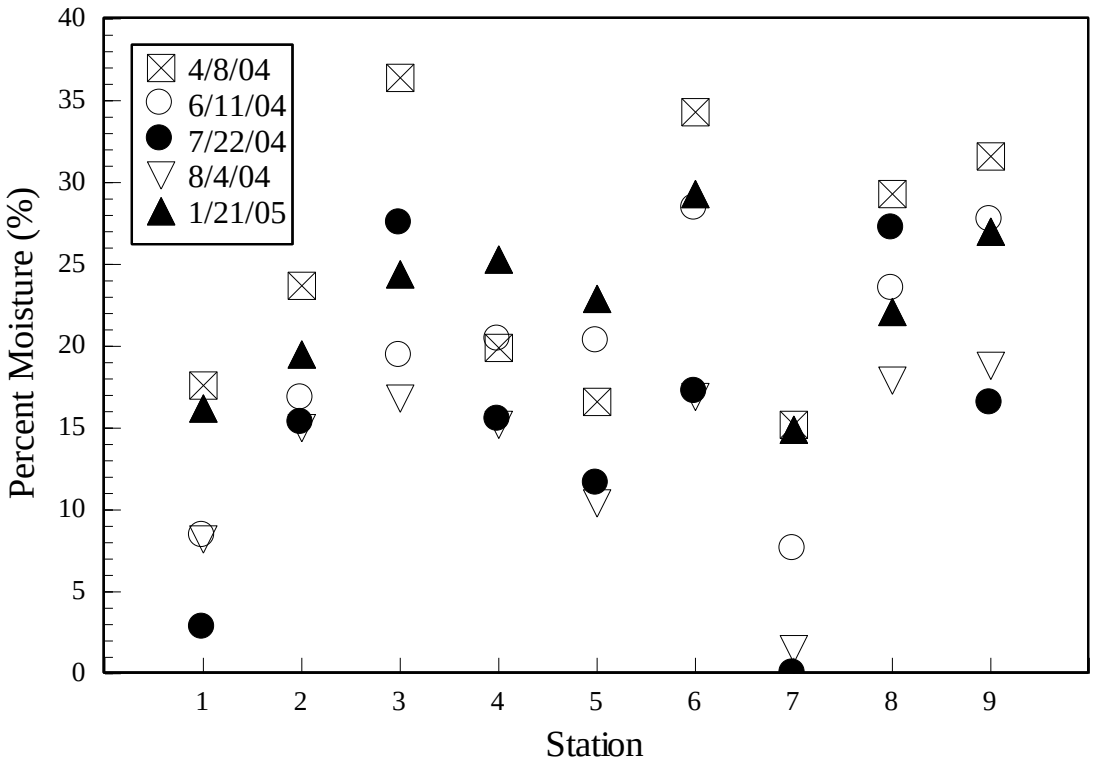

Figure 9: Average sediment water content for each sample location measured from March 2004 – January 2005. The sediments in Stations 3, 6, 8, and 9 had the highest water content, whereas, stations 1 and 7 had the lowest.

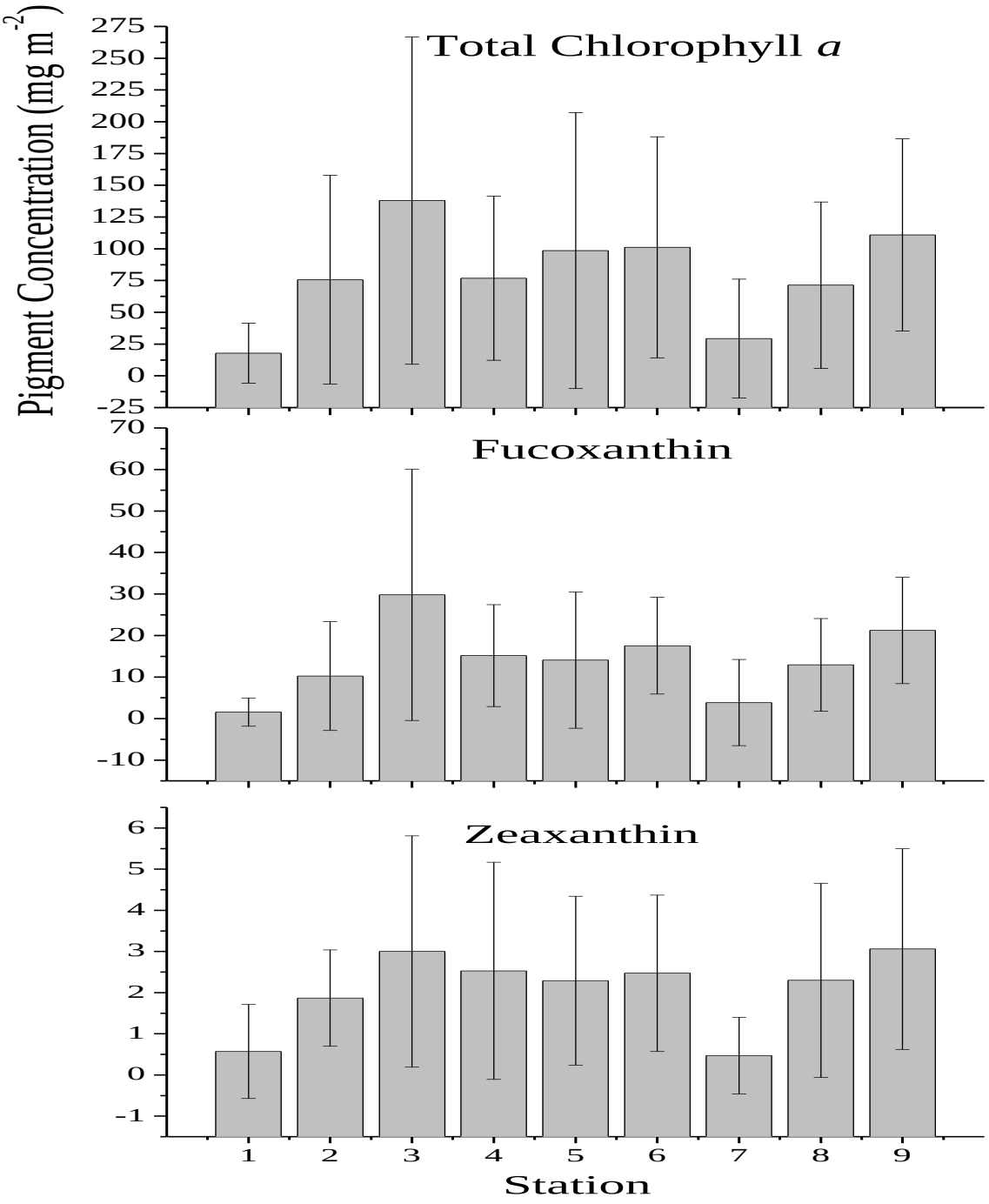

Figure 10: Average pigment concentrations (mg m^{-2}) for chlorophyll *a*, fucoxanthin, zeaxanthin, chlorophyll *b*, bacteriochlorophyll *a*, and the fucoxanthin to zeaxanthin ratio measured at each station over the 21-month study.

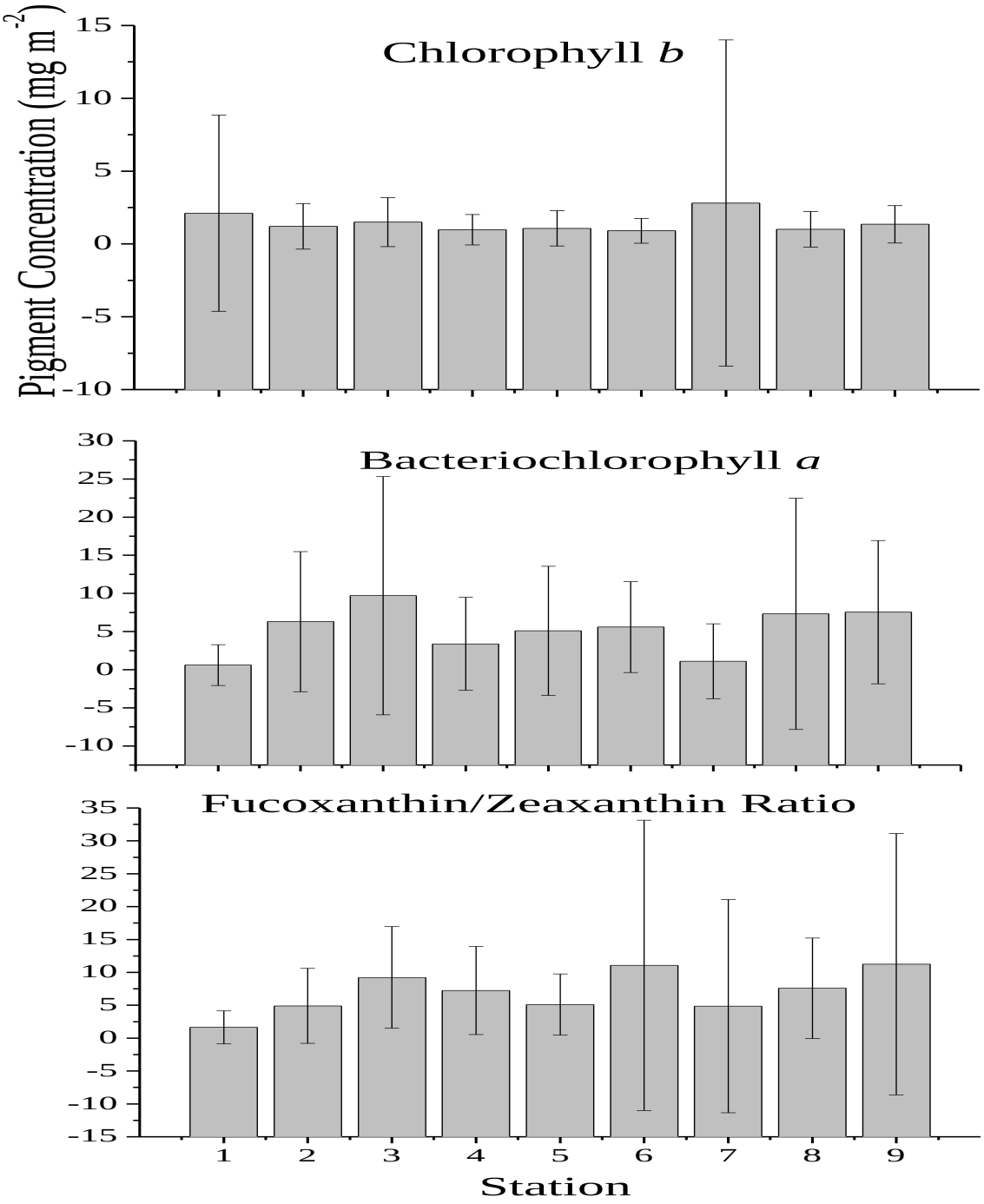

Figure 10: Continued.

Spearman's Rank Order Correlation Analysis. A Spearman's rank order correlation analysis was performed to examine whether sediment moisture, temperature (water and sediment) were correlated with pigment abundances. This analysis indicated a significant positive ($p<0.01$) association between all of the pigments (chlorophylls *a* and *b*, fucoxanthin, zeaxanthin, and bacteriochlorophyll *a* (Table 6) and sediment water content. In contrast, temperature (water and sediment) were negatively correlated for all the pigments, except zeaxanthin, and the F/Z ratio. In summary, these results show that BMA biomass was positively correlated with higher water content in the sediment and lower temperatures.

Table 6: Spearman's rank order correlation results indicating correlations between the diagnostic pigments and the major physical characteristics at the site.

	Sediment Moisture	Water Temp.	Sediment Temp.
Chlorophyll *a*	0.528**	-0.318*	-0.365**
Fucoxanthin (F)	0.712**	-0.465**	-0.503**
Zeaxanthin (Z)	0.387**	-0.115	-0.194**
Chlorophyll *b*	0.421**	-0.508**	-0.180*
Bacteriochlorophyll *a*	0.351**	-0.282	-0.201**
F/Z Ratio	0.413**	-0.337**	-0.451**

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Discussion

This 21-month study indicated that the BMA community displayed higher abundances in areas with higher sediment moisture. In contrast, a study by Urban-Malinga (2003) found higher chlorophyll *a* concentrations along the littoral zone compared to the waterline. Likewise, Perkins et al. (2003) did not find a significant relationship between chlorophyll *a* concentration (mg m^{-2}) and water content (%). The lowest percent water content was in Station 1 and the highest was in Station 3, which may indicate a difference in the sediments ability to retain moisture. One explanation for the moisture retention is grain size characterization of sediment. Well-sorted sandy sediment is characterized by grain sizes that are similar with higher porosity compared to poorly sorted sediment where the grain sizes are variable. Higher porosity allows more water to percolate down into the sediment compared to sediment with less pore spaces. In the sandy sediments of the Tagus estuary chlorophyll *a* concentrations in the upper 2 mm of the sediments ranged from 28.5 mg m^{-2} to 101 mg m^{-2} , fucoxanthin ranged from 9.9 mg m^{-2} to 41.6 mg m^{-2}, zeaxanthin ranged between 0.06 mg m^{-2} to 0.44 mg m^{-2}, and chlorophyll *b* ranged from 0.00 mg m^{-2} to 0.28 mg m^{-2} (Cartaxana et al. 2006). They stated that the sandy sediments in the Tagus estuary were lower in benthic microalgal biomass compared to the muddy sediments because of de-watering and that the wetter areas are conducive to microalgal growth because of nutrient supply, gas exchange and avoidance of desiccation. The sediments at East beach were fine grain to coarse grain sand with stations 1, 4, and 7 appearing sandier in texture compared to the other stations. This sediment type would provide a greater porosity and higher permeability allowing

for faster de-watering of the sediments that contained more coarse grain sand, which may explain the lower percent water content in Station 1. However, in another study in the Eden estuary, Scotland, chlorophyll *a* measurements showed a positive correlation with de-watered sediments (Perkins et al. 2003). As the sediment was de-watered after a 6 hr emersion period, the concentration of chlorophyll *a* at the subsurface enriched the top layers thereby increasing the bulk density of the sediments. They also determined that sandy sediments normally have lower benthic microalgal biomass than finer grain sizes (Perkins et al. 2003). These two factors, grain size and moisture content, may explain why the biomass at Station 3 was greater than Station 1.

Varying spatial dynamics of BMA can be due to sediment grain size and can be the main reason for microscale spatial heterogeneity (Wardle et al. 2001). The sediments at East Beach were predominantly very fine sand (4 φ) to fine sand (3 φ). The muddy sediments in the Tagus estuary showed a different response with higher chlorophyll *a* concentrations found in the summer compared to the winter months. The total chlorophyll *a* values at East Beach agree with the numbers reported by Cartaxana et al. (2006), with an overall average of total chlorophyll *a* at 74.55 mg m^{-2}, while the Tagus study had an overall average of 60 mg m^{-2} (Cartaxana et al. 2006). BMA were found to be higher in the sediments that had some sand versus muddy sediments in the Manukau Harbour because the sediment loading seems to affect the BMA biomass (Goldfinch and Carman 2000). In the Kandalakscha Bay, on the White Sea, BMA showed a random distribution pattern over a small scale <75 m. The distribution pattern became more homogeneous as the grain size increased (Thrush 1991; Watermann et al.

1999; Mitbavkar and Anil 2002). Their study agrees with a previous study by Herman et al. (2001) which also found that BMA biomass is lower in sandy sediments compared to muddy sediments because of less grazing (Cahoon and Safi 2002). The sediment loading was one reason why seasonal variation was not observed in an intertidal sandflat in Spain (Azovsky et al. 2000). A study conducted in the Sanggou and Jiaozhou Bays of the northern China found that the standing crop of BMA on tidal flats was higher in muddy sediments compared to sandy sediment because nutrients were higher and grazing pressure lower (Ning et al. 2003). The total annual production of BMA at this sandflat did not show any seasonal peaks. They concluded that this is due to sediment disturbance and high detrital supply (Ning et al. 2003).

The BMA showed a seasonal signal with higher pigment abundances measured in the winter. A decrease in ambient light leads to a drop in temperature. One study found that temperature had a greater affect on BMA gross production in the winter than in the summer when the temperatures were higher, and the same trend was found when production was normalized to chlorophyll *a* (Migne et al. 2004). Barranguet et al. (1998) found BMA adapted to the seasonal changes of light and temperature with lower production rates measured between October and February and the photoacclimation index (I_k) was highest in Aug., Sep. and Oct. (~300 - 400 µmol m^{-2} s^{-2}) compared to Dec. (~250 µmol m^{-2} s^{-2}) . At East Beach, the diatoms showed a definite decrease in biomass during the summer months, which could indicate a negative response to higher temperatures. Sediment temperatures at East Beach ranged from 8.1º C to 39º C. The lowest pigment abundances were measured in July and August of each year (2003 and

2004) and the highest values were found in cooler months usually January and February. The decline in pigment concentration during the summer months at East Beach does not agree with a study performed in the Severn Estuary (Underwood and Paterson 1993; Underwood 1994). This contrast is a result of the differences between sub-tropical (Galveston Bay, TX) and temperate (Severn Estuary, United Kingdome) climates. Guarini et al. (1998) found that the BMA biomass averaged 118 mg chl *a* m^{-2} in June with an average biomass measure at 85.7 mg chl *a* m^{-2} in January, stating this difference in biomass from January to June was due to seasonal variability. In contrast, the biomass at East Beach averaged 152.20 mg chl *a* m^{-2} in February 2004 and 17.68 mg chl *a* m^{-2} in July that same year.

The trophic relationship between the BMA and grazers could explain the seasonal difference in biomass. In a microcosm study in Louisiana, a decrease in benthic copepod grazing led to a reduction in copepod diversity and an increase in BMA biomass (Underwood 1994). Another study in Terrebonne Bay, Louisiana found that meiofauna was more effective at grazing during the summer compared to winter (Pinckney et al. 2003). They stated that grazing may not be the primary reason for the difference in biomass but may play a secondary role by altering the light environment and nutrient concentrations. The observed winter increase in biomass at East Beach could result from reduction in the abundance or grazing activity of benthic consumers during the winter months. Temperatures at East Beach are much higher than on the mudflats of higher latitude estuaries, especially during the winter months. Cooler winter temperatures may also explain the winter maximum in BMA biomass at East Beach.

The seasonal trend exhibited at East Beach agrees with a study conducted in the Northern Wadden Sea where a BMA spring bloom started in January and declined during the late summer (Guarini et al. 1998). In their study, BMA biomass ranged from 85.7 mg chl *a* m^{-2} in January to 118 mg chl *a* m^{-2} in June. Similarly, the BMA biomass East Beach averaged 138.95 mg chl *a* m^{-2} in January and 52.86 mg chl *a* m^{-2} in June, showing a similar seasonal trend. A study in the Mdloti Estuary in South Africa found that the correlation analysis suggested a decrease in temperature is significantly related to an increase in benthic chlorophyll *a* biomass (Mundree et al. 2003). A study in Baffin Bay, TX found that temperature had a negative effect on the photosynthetic efficiency and chlorophyll *a* abundance when increased by 6° C from May to July (Blanchard and Montagna 1992).

Nutrient concentrations in sediments also exhibit seasonal patterns and offer another plausible explanation for why BMA exhibit seasonal variability in biomass. BMA show rapid short-term responses to nutrient enrichment. In the Gulf of Trieste, seasonal patterns of BMA abundance have been attributed to the concentrations of inorganic nutrients concentration (Varela and Penas 1985). Posey et al. (2002) observed the direct and indirect effects of nutrient enrichments on the BMA in North Carolina. Nutrients fluxing out of the sediment explained much of the BMA patchiness in West German and Gulf of Trieste estuaries (Hopner and Wonneberger 1985). Another study in southern California showed a direct link between sediment nitrogen content and seasons, with a maximum nitrogen content found in the spring and a clear decrease during the summer (Boyle et al. 2004). The nutrient concentrations often exhibit the

same seasonal pattern at the sediment-water interface as the BMA (Welker et al. 2002). The porewater nutrient concentrations were not measured in the study conducted at East Beach and cannot be ruled out as one of the driving forces behind the observed spatiotemporal distribution patterns exhibited by the BMA.

In summary, the BMA community composition at East Beach exhibited a spatiotemporal shift over a 21-month period (March 2003-February 2005). Sediments with higher moisture content had higher biomass for all the major algal groups. A positive correlation between BMA biomass and water content indicated that sediment water content might at least partially explain differences in spatial distributions. Furthermore, a negative correlation between chlorophyll *a* and temperature (water and sediment) suggests seasonal changes in BMA biomass. Diatoms and cyanobacteria were the most abundant algal groups within the BMA community. The relative abundance of diatoms was higher at stations with higher moisture content and during the winter months while the relative abundance of cyanobacteria was relatively constant. These results suggest that the spatiotemporal variability of BMA biomass at East Beach is significantly correlated with sediment moisture and temperature. Should environmental conditions change, the spatiotemporal patterns of BMA biomass and community composition may also undergo corresponding changes. This in turn could affect the resource availability to the primary consumers within the area and overall energy transfer to the higher trophic levels.

CHAPTER IV

THE BENTHIC MICROALGAL COMMUNITY RESPONSE TO SALINITY MANIPULATIONS

Introduction

Human population growth and the subsequent demand for freshwater in the coastal zone and within the watersheds entering Texas bays and estuaries is increasing, promoting a forecast of increasing salinity in estuaries due to reduced freshwater input (Alexander and Dunton 2002; Montagna et al. 2002; Ji and Chang 2005). Increasing the salinity could result in a shift in benthic microalgal (BMA) biomass and community structure, possibly affecting energy transfer to higher trophic levels. Estuaries are dynamic systems where rapidly changing salinity can result from storm surge, precipitation, freshwater inflow, or evaporation (Montagna et al. 2002). For example in Nueces Bay, Texas, the mean annual river discharge into the estuary has decreased from 0.763 km^3 to 0.344 km^3, resulting in an average increase in salinity of ca. 2.5 (Alexander-Mahala et al. 2000). Average salinities in the upper delta of Nueces Bay can exceed 120 (Alexander-Mahala et al. 2000) during the summer months and have been measured as high as 300 (Fejes et al. 2005). These hypersaline conditions may influence BMA by inhibiting photosynthesis (Pinckney et al. 1995a; Garcia-Pichel et al. 1999; Liska et al. 2004). The shipping channel in Galveston Bay, Texas experienced a 33% increase in salinity (16 to 24) between 1973 and 2002 (Lester and Gonzalez 2003). This trend is expected to continue and it is reasonable to expect a 10% increase in salinity in the near future. Diatoms and cyanobacteria tolerate different salinities because of their

evolutionary adaptability to various environments (Clavero et al. 2000; Paerl et al. 2000). This is in part because of osmotic regulation of ions and organic solutes within the cell (Clavero et al. 2000). In the Colne estuary the salinity gradient ranges from 17.5 to 33.0, and is responsible for the observed differences in the relative abundances of several diatom species (Underwood et al. 1998).

Diatoms have a tolerance for salinity that ranges between 4 – 60 (Admiraal 1984). However, a number of diatom species are able to increase their numbers even at salinities of 75, but very few species can maintain this growth in salinities of 150 (Clavero et al. 2000). These studies would suggest that diatoms, in general, are able to adapt to a wide range of salinities except for extreme hypersaline environments. In contrast, cyanobacteria have a greater tolerance to hypersaline environments (Garcia-Pichel et al. 1998; Herbst and Blinn 1998; Paerl et al. 2000). Organisms are affected by changes in salinity one of three ways, impacting cellular water potential, uptake or loss of ions, and 'change of the cellular ionic ratios due to selective ion permeability of the membrane" (Kirst 1990). One reason cyanobacteria can readily adapt to higher salinities is they contain Mycosporine-like amino acids (MAAs) which is a low molecular weight water soluble compound are known to function as a photoprotective compound but may function as a way to osmoregulate the cells (Oren 1997). Other osmolytes that increase in cyanobacteria with increased salinity is intracellular concentrations of sucrose and trehalose (Portwich and Garcia-Pichel 1999). In addition to adjusting the chlorophyll *a* or carotenoid content, cyanobacteria also synthesize solutes such as sucrose as osmolytes to acclimate to salt stress (Singh et al. 2002). In Bahamian hypersaline lagoons,

cyanobacteria form microbial mats at salinities as high as 300 (Paerl et al. 2000). Cyanobacteria have a competitive advantage over diatoms in environments with higher salinities and temperatures (Watermann et al. 1999). Therefore, under hypersaline conditions, cyanobacteria appear to have a competitive advantage over diatoms. Rapid changes in salinity can affect the productivity of BMA in partially submerged intertidal sandflats. For example, in a Bahamian hypersaline lagoon, dominated by cyanobacteria, primary production was enhanced when "salinity-induced osmotic stress was relieved" (Pinckney et al. 1995a) . However, some cyanobacteria have a much broader salinity tolerance and are capable of photosynthesis at salinities as high as 300 (Pinckney et al. 1995a).

The purpose of this study was to examine the effects of different salinity conditions on BMA community structure and function at East Beach, Galveston, Texas. The BMA community at East Beach sandflat is predominantly comprised of two major algal groups, diatoms, and cyanobacteria. Green algae and phototrophic bacteria are also found at this location, but at much lower concentrations relative to the other two groups. The primary hypothesis for this research was that exposure to elevated or reduced salinities over a five-day period will alter BMA community composition such that the ratio of diatoms to cyanobacteria will decrease with increasing salinity. The five-day incubation was selected based on low growth rates ($0.06\ d^{-1} – 0.27\ d^{-1}$) measured in Savin Hill Cove (Gould and Gallagher 1990). Whether the growth rates are higher at East Beach, if the BMA are significantly affected by salinity, a measurable difference in biomass between the controls and treatments should be observable after five days.

Materials and Methods

Experimental Design. Four *in-situ* bioassays were conducted over a six-month period using sediment cores exposed to five different salinity concentrations (in triplicate) ranging between 0 and 47. The study site was an intertidal sandflat adjacent to a narrow channel connected to Galveston Bay (Fig. 11). Sediment samples were collected using 100 x 15 mm Petri dishes and placed in a shallow, translucent plastic containers (11.4 cm wide, 7.6 cm deep, ca. vol. = 776 cm^3) which were exposed to ambient environmental conditions (i.e., irradiance, temperature, rain, etc.) except inundation by tides (Fig. 12). Three additional sediment cores were collected at the sampling site prior to (Initial) and after (Final) each bioassay using 1.00 cm (ID) core tubes**.** The top 3 mm of each subsample was extruded, sectioned, frozen in 2.0 ml microfuge tubes, and stored at -80 °C.

The treatments were a mixture of control water (collected from the channel adjacent to the sampling site), deionized water, and Instant Ocean to create the following combinations: control, deionized water (DI), a 50% dilution of control water with DI, a 25% increase above the control water, and a 50% increase above the control water. Treated water was added to each container to a depth ca. 1.3 cm above the sediment. The salinity was increased using Instant Ocean added the control water and to increase the salinity by 25% and by 50%. This was the average depth of water covering the sediment from where the samples were collected. Salinity in the incubation containers was measured daily and the water in the incubation containers was replaced with newly-mixed water in an attempt to maintain constant conditions. The salinity of the control

water determined daily and the concentrations for the other treatments were based on this salinity. Salinity was measured using a refractometer.

Bioassays were conducted for six days on August 23 – 29, 2004; and five days on October 25 – 30, 2004, December 13 – 18, 2004 and February 21 – 26, 2005. The study was conducted over a 6-month period to determine if there was a seasonal response to the different salinities. The study period of 6 months vs. a yearly study was because Galveston Bay is a sub-tropical climate with two seasons, unlike the four distinct seasons experienced in the temperate climate of the northern part of the United States. Upon termination of each bioassay, three subsamples were taken from each Petri dish using a 1.00 cm (ID) butyrate core tube (0.80 cm^2). The top 3 mm of each subsample was extruded, sectioned, frozen in 2.0 ml microfuge tubes, and stored at -80 °C. Photopigment abundances were determined for all samples using HPLC.

Figure 11: Ariel photo view of site where samples were collected for salinity study.

Figure 12: Container setup for the salinity bioassays. The petri dish was used to collect the sediment and then placed in a shallow container filled with seawater in the correct salinity (control = ambient seawater, DI = deionized water, DL = ambient seawater diluted in half with deionized water, 25% = 25% increase above ambient seawater, and 50% = 50% increase above ambient seawater. The water in each container was exchanged daily with newly-mixed treatment water.

Photopigment Analyses. HPLC was used to determine the chemosystematic photosynthetic pigments for benthic microalgae. The sediment samples were placed in 100% acetone (2 ml), sonicated (10s), and extracted at -20 °C for 18-24 h. Filtered extracts (300 µl) were injected into a Shimadzu HPLC equipped with a monomeric (Rainin Microsorb-MV, 0.46 x 10 cm, 3 µm) and a polymeric (Vydac 201TP54, 0.46 x 25 cm, 5 µm) reverse-phase C18 column in series. A nonlinear binary gradient was used for pigment separations (Pinckney 1996). Absorption spectra and chromatograms (440 nm) were acquired using a Shimadzu SPD-M10av photodiode array detector. Pigment peaks were identified by comparison of retention times and absorption spectra with pure crystalline standards, including chlorophylls *a, b*, *β*-carotene (Sigma Chemical Co.), fucoxanthin, and zeaxanthin (Hoffman-LaRoche and Company). Other pigments were identified by comparison with extracts from phytoplankton cultures and quantified using the appropriate extinction coefficients (Jeffrey et al. 1997).

Statistical Analyses. A randomized complete block design multivariate analysis of variance (MANOVA) with three replicates of five diagnostic pigments (chlorophylls *a* and *b*, fucoxanthin, zeaxanthin, bacteriochlorophyll *a*) as the dependent variables. The blocking factor was bioassay date (Aug. 2004, Oct. 2004, Dec. 2004, and Feb. 2005) and the main factor was salinity treatment (control water, DI, 50% dilution of control water with DI water, 25% and 50% increase in salinity above control water). This analysis examined whether salinity treatment would have a significant effect on BMA community composition after removing the variability associated with the different dates on which the experiments were conducted. The data were normally distributed (K-S

test, $p>0.05$) and the variances were not homogeneous (Levene's test, $p<0.05$) for some pigments, therefore a multiple comparisons were performed using Dunnett's T3 tests to compare the estimated means of absolute abundances. Otherwise, a Bonferroni multiple comparisons was performed for the pigment abundances with homogeneous variances.

Incubation Artifacts. The containers for the incubations may affect the BMA community, thereby resulting in possible experimental artifacts. This artifact effect was tested by comparing the BMA pigment abundances measured in the control treatments at the end of the incubation period with the cores collected from an undisturbed area in close proximity to where the original samples were collected. A randomized-complete block design 2 factor MANOVA (treatment and date of experiment, as the blocking factor) was used to determine if BMA community composition was affected by the presence of core tubes. The salinity treatment (CNT and FINAL), and date of experiment effects were all significant (Pillai's Trace, $p<0.05$), clearly indicating core tube artifacts (Fig. 13). Univariate ANOVAs for the individual pigments indicated that only zeaxanthin differed in abundance between the treatments and all the pigments except bacteriochlorophyll *a* differed in abundance between the dates of the experiments. This would indicate that zeaxanthin was the pigment that exhibited an artifact from the container.

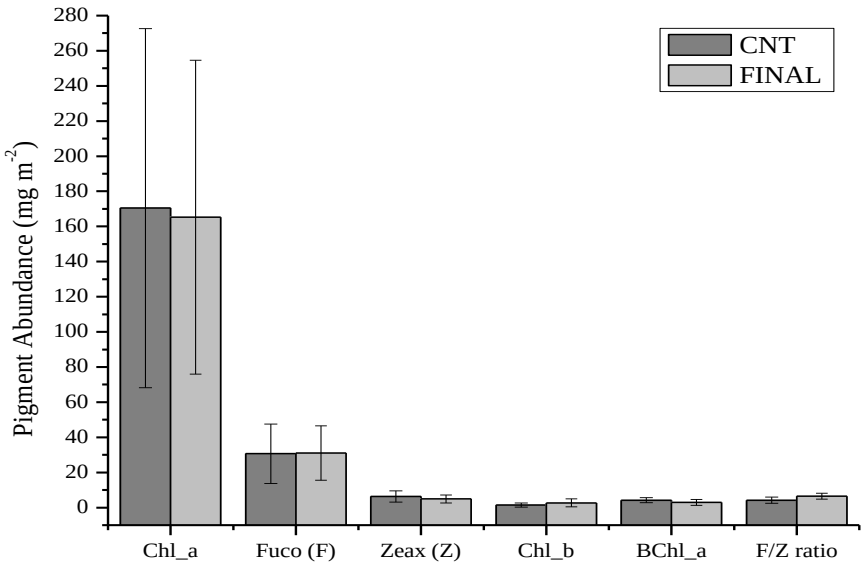

Figure 13: BMA pigment abundances between the control (CNT) and the final (FINAL) samples.

Results

Salinity Averages. During the course of the bioassay, salinity was measured daily in each container and averaged over the course of the bioassay. The average salinity reveals the variability between treatments and bioassay date (Table 7). As expected, salinity averaged highest in the Controls for August 2004 and measured lowest in February 2005. This would coincide with the amount of precipitation and/or evaporation for that time of year.

Table 7: The average salinity measured from each treatment during the bioassay. Salinity was measured daily prior to the exchange with new treatment water and averaged over the course of the bioassay. Average salinity values are listed in the parentheses.

Aug. 2004		Oct. 2004		Dec. 2004		Feb. 2005	
CNT	(30.2)	CNT	(26.7)	CNT	(24.4)	CNT	(14.2)
DL	(14.7)	DL	(15.1)	DL	(13.5)	DL	(7.0)
DI	(0.1)	DI	(0)	DI	(1.7)	DI	(0.2)
25%	(35.6)	25%	(29)	25%	(29.7)	25%	(17.7)
50%	(41.1)	50%	(34.5)	50%	(32.5)	50%	(22.1)

MANOVA and ANOVA Results. The BMA total biomass responded significantly to the main factor, salinity. A two-way randomized-complete block design univariate with three replicates of one diagnostic pigment, chlorophyll *a* as the dependent variable. The blocking factor was bioassay date (Aug. 2004, Oct. 2004, Dec. 2004, and Feb. 2005) and the main factor was salinity treatment (CNT, DI, DL, 25%> and 50%>). The results did indicate that the grand mean (± 1SE) of chlorophyll *a* (162.688 ± 3.283) was significantly affected by salinity. However, the blocking factor, bioassay date, was also significantly different ($p<0.05$) and is the reason that the group means comparisons cannot be performed (Table 8). The highest average chlorophyll *a* concentration was measured in the 50%> salinity treatment, followed by DL, then CNT, then 25%>, and finally DI.

The results from the two-way randomized-complete block design MANOVA analysis indicated that the BMA community did reach statistical significance (Pillai's Trace, $p<0.001$) for salinity and bioassay date as the blocking factor. As the date of the experiment was also significantly different ($p<0.001$), the group means could not be performed. The univariate tests for the four pigments (fucoxanthin, zeaxanthin, chlorophyll *b*, and bacteriochlorophyll *a*) indicated that all the pigments, except bacteriochlorophyll *a*, were significantly affected by the date of the experiment (Table 8). They revealed that the grand mean (± 1 SE) scores for fucoxanthin (29.996 ± 0.614) and chlorophyll *b* (1.426 ± 0.061) were significantly affected by salinity ($p<0.05$). However, in contrast zeaxanthin (5.868 ± 0.134) and bacteriochlorophyll *a* (4.345 ± 0.218) were not significantly affected by salinity. Since the group means comparisons

cannot be compared between treatments, the average abundances of each pigment per salinity treatment and bioassay date reveal that the abundances for chlorophylls *a* and *b*, fucoxanthin, and zeaxanthin are higher in the Feb. 05 bioassay compared to the Oct. 04 bioassay (Fig. 14). Bacteriochlorophyll *a* and the ratio of fucoxanthin to zeaxanthin abundances were relatively constant throughout the study.

Table 8: The results from the univariate randomized-complete block design two factor (ANOVAs) with salinity treatment as the main factor and date as the blocking factor with pigment abundances as the variable.

	Treatment		Date	
	F value	p-value	F value	p-value
Fucoxanthin (F)	9.084	<.000	237.549	<.000
Zeaxanthin (Z)	1.566	0.197	64.466	<.000
Chl_*b*	2.917	0.030	90.156	<.000
Chl_*a*	3.447	0.014	186.307	<.000
BChl_*a*	1.754	0.152	2.030	.121

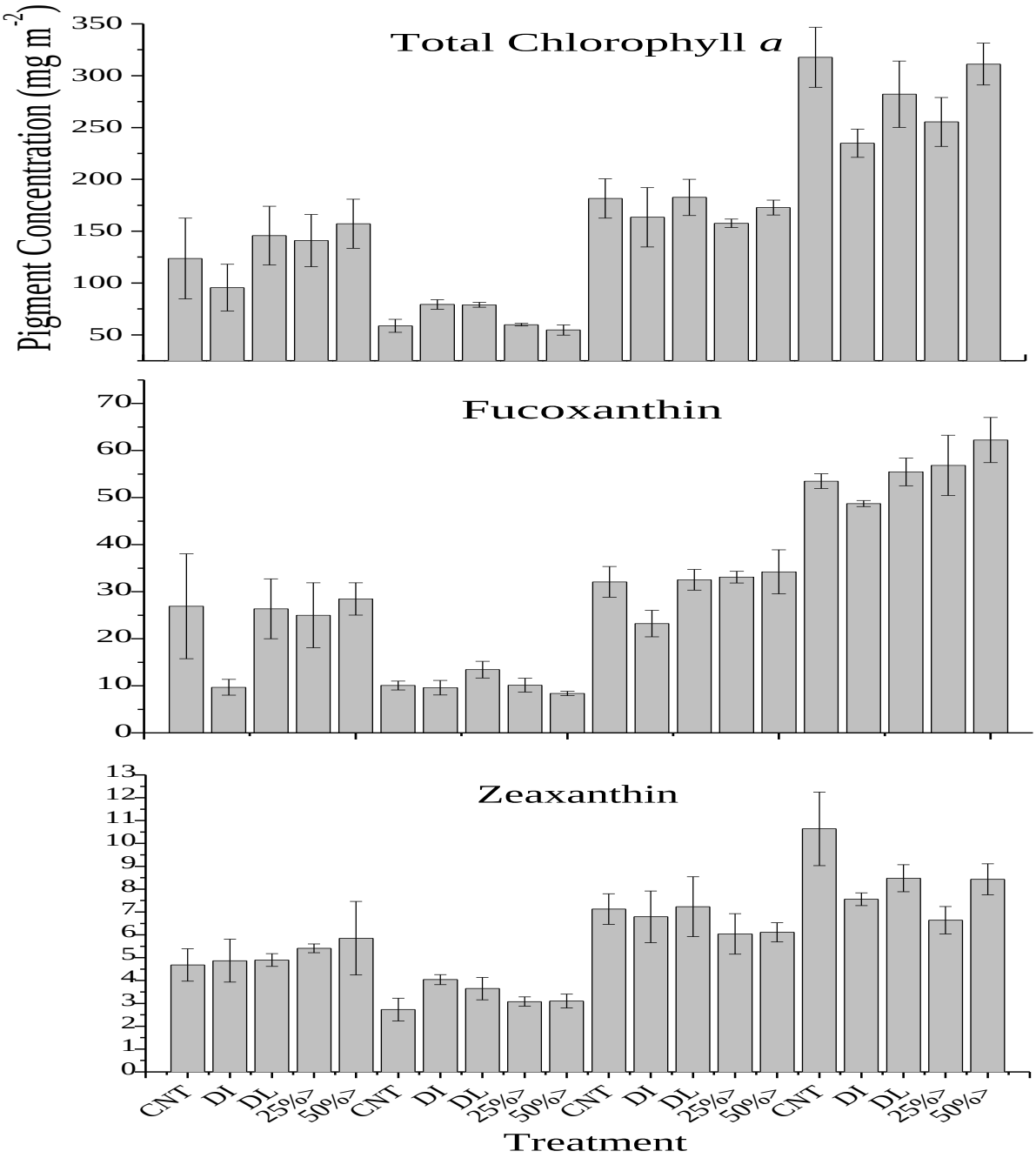

Figure 14: Average abundances of each treatment for each bioassay date. CNT – Control, DI – Deionized Water, DL – Control water diluted 50% by deionized water, 25%> - 25% increase above the control water, 50%> - 50% increase above control water.

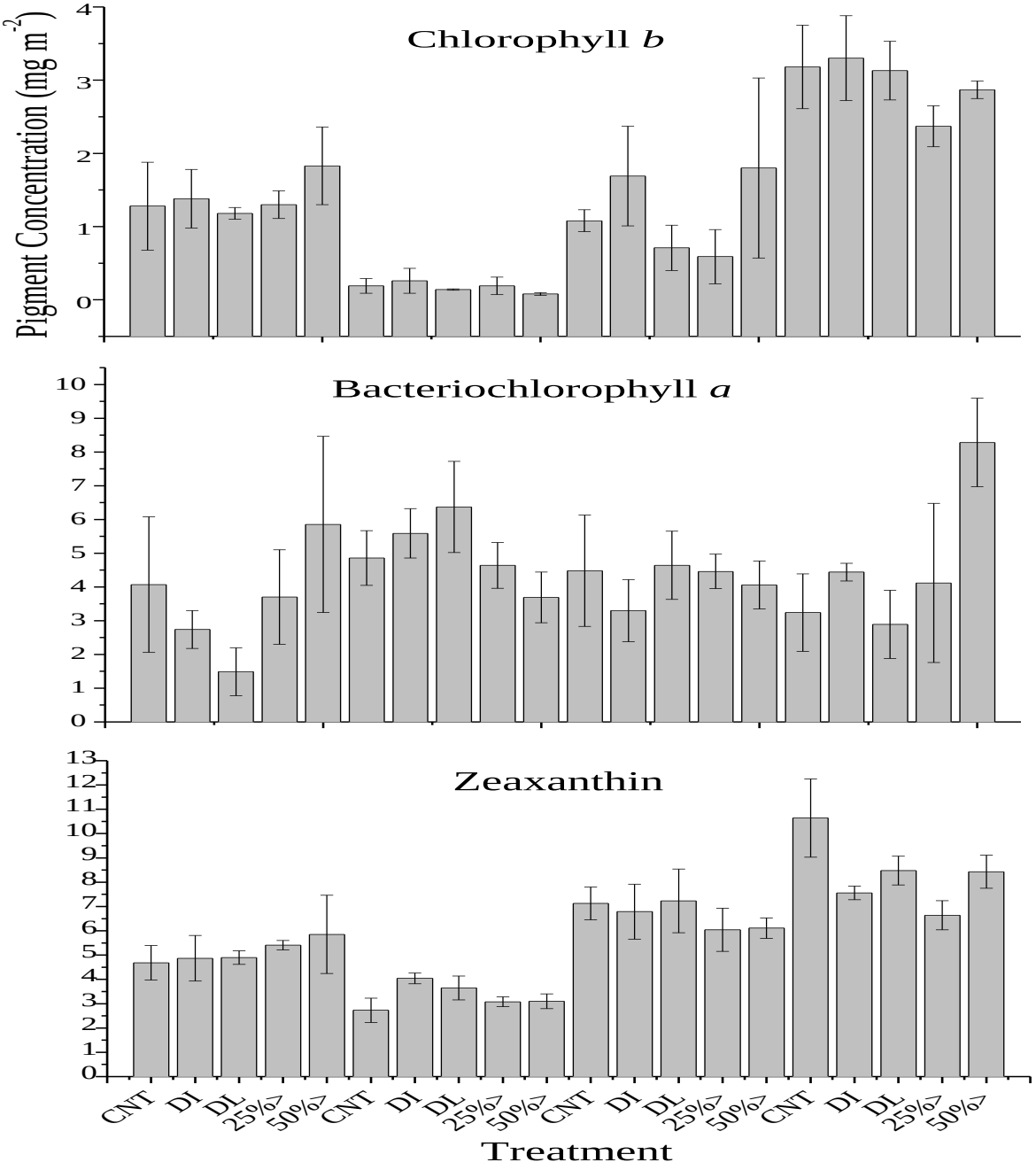

Figure 14: Continued.

A one-factor MANOVA was performed on each experimental date using the five pigment abundances as dependent variables and salinity as the independent variables. The results from the August 2004 bioassay (Pillai's Trace, $p < 0.05$), October 2004 (Pillai's Trace, $p < 0.05$), and the February 2005 (Pillai's Trace, $p < 0.05$) indicated that the pigment abundances are statistically significant. In contrast, the results from the December 2004 (Pillai's Trace, $p = 0.144$) indicated that the pigment abundances were not significantly different. The univariate tests for the five pigments (chlorophyll *a*, fucoxanthin, zeaxanthin, chlorophyll *b*, and bacteriochlorophyll *a*) indicated which photodiagnostic pigment was significantly affected by salinity for that experimental date (Table 9). Fucoxanthin was the only pigment significantly affected by salinity treatment in August 2004. All the pigment abundances except chlorophyll *b* were statistically significant in October 2004 and in February 2005.

Table 9: The results from the univariate one factor (ANOVAs) with salinity treatment as the main factor and pigment abundances as the variable.

	Aug. 2004		Oct. 2004		Feb. 2005	
	F Value	P Value	F Value	P Value	F Value	P Value
Fucoxanthin	3.916	0.036	6.109	0.009	4.793	0.020
Zeaxanthin	0.841	0.530	6.056	0.010	8.646	0.003
Chl_*b*	1.145	0.390	1.303	0.333	2.230	0.138
Chl_*a*	2.143	0.150	23.019	0.000	6.279	0.009
BChl_*a*	2.881	0.080	3.777	0.040	7.181	0.005

A *post-hoc* comparisons test was done on the estimated means for each bioassay date. A Bonferroni test was done on the estimated means that had homogenous variances while the Dunnett's T3 test was done on the estimated means that were not homogenous. The results are listed in Tables 10 - 12. In the August 2004 bioassay all the pigment abundances were highest in the 50%> treatment and for all the pigments except zeaxanthin, they were lowest in the DI or DL treatment. Zeaxanthin measured lowest in the CNT treatment. However, in the October 2004 bioassay, all the pigments measured highest in either the DI or the DL treatments and measured lowest in the 50%> treatment except for zeaxanthin which again measured lowest in the CNT treatment. In the February 2005 bioassay, fucoxanthin was highest in the 50%> treatment and significantly lower in the DI treatment. Zeaxanthin measured highest in the CNT and was significantly lower in the 25%> treatment, while chlorophyll *a* was significantly highest in the CNT compared to the DI treatment.

Table 10: Results of *a posteriori* multiple analyses of variance (Bonferroni, and Dunnett's T3) with salinity as the main factor and the diagnostic pigments as the independent values for the August 2004 bioassay. The underline denotes that the means were not significantly different ($p<0.05$). The means are ranked from highest to lowest.

	August 2004	N	Levene's Test
Fucoxanthin	50%> CNT DL 25%> DI	3	.172
Zeaxanthin	50%> 25%> DL DI CNT	3	.015
Chlorophyll *b*	50%> DI 25%> CNT DL	3	.048
Chlorophyll *a*	50%> DL 25%> CNT DI	3	.835
Bacteriochlorophyll *a*	50%> CNT 25%> DI DL	3	.239

Table 11: Results of *a posteriori* multiple analyses of variance (Bonferroni, and Dunnett's T3) with salinity as the main factor and the diagnostic pigments as the independent values for the October 2004 bioassay. The underline denotes that the means were not significantly different ($p<0.05$). The means are ranked from highest to lowest.

	October 2004	N	Levene's Test
Fucoxanthin	DL 25%> CNT DI 50%>	3	.180
Zeaxanthin	DI DL 50%> 25%> CNT	3	.299
Chlorophyll *b*	DI 25%> CNT DL 50%>	3	.006
Chlorophyll *a*	DI DL 25%> CNT 50%>	3	.080
Bacteriochlorophyll *a*	DL DI CNT 25%> 50%>	3	.363

Table 12: Results of *a posteriori* multiple analyses of variance (Bonferroni, and Dunnett's T3) with salinity as the main factor and the diagnostic pigments as the independent values for the February 2005 bioassay. The underline denotes that the means were not significantly different ($p<0.05$). The means are ranked from highest to lowest.

	February 2005	N	Levene's Test
Fucoxanthin	50%> 25%> DL CNT DI	3	.113
Zeaxanthin	CNT DL 50%> DI 25%>	3	.176
Chlorophyll *b*	DI CNT DL 50%> 25%>	3	.283
Chlorophyll *a*	CNT 50%> DL 25%> DI	3	.508
Bacteriochlorophyll *a*	50%> DI 25%> CNT DL	3	.081

The fucoxanthin to zeaxanthin ratio is an indicator of community composition. The average of this ratio did not vary greatly between treatments. The lowest ratio average was in the DI treatment at 3.58 and the highest was in 25%> treatment at 5.54. The control measured 4.19, the DL was 5.07 and the 50%> was 5.20. This would indicate that the ratio of fucoxanthin to zeaxanthin increased in the higher salinities. This ratio increased in the cooler months, with the ratio measuring 4.53 in Aug. 2004, 3.18 in Oct. 2004, 4.32 in Dec. 2004, and finally 6.83 in Feb. 2005.

Additional Cores Collected over a Range of Salinities. The results from the MANOVA analysis revealed that the main factor, salinity, did not reach statistical significance (Pillai's Trace, $p = 0.304$), thereby indicating that there were no measurable

changes within the BMA community with respect to salinity. Total biomass, salinity, and sediment type between several additional sediment samples from other locations around Galveston Bay and East Beach showed that the biomass was comparable, but there was no relationship between salinity, sediment, and BMA biomass. Three of the randomly sampled stations were muddy in appearance, the other three consisted of mostly sand, and the salinity varied depending on the site location (Table 13). The results from these external samples indicated that the BMA had relatively similar pigment abundances compared to East Beach, with the predominant algal pigments consisting of chlorophyll *a*, fucoxanthin, zeaxanthin, chlorophyll *b* and bacteriochlorophyll *a*. Samples WPT001 and WPT002 were collected in a small channel adjacent to the Bay, with salinities similar to East Beach. The pigment abundances were just as high for these two samples collected in August as the samples collected in Dec. 2004, with salinities much lower, around 5. WPT003 was collected along the sandy beach adjacent to the Gulf of Mexico had extremely low pigment abundances when compared to the other five samples. In summary, there did not appear to be a pattern between the sediment type, salinity, and BMA biomass and the BMA community at East Beach was similar to other nearby habitats.

Table 13: Table showing salinity and sediment type for the additional samples collected at six other sites around Galveston Bay.

Sample ID	Sample Date	Sediment Type	Salinity
WPT001	25 Aug 04	Muddy	26
WPT002	25 Aug 04	Sandy w/Shells	20
WPT003	25 Aug 04	Sandy	26
WPT004	25 Aug 04	Muddy	22
WPT005	15 Dec 04	Muddy	5
WPT006	15 Dec 04	Sandy w/Silt	5

Discussion

The purpose of this study was to examine the response of BMA at East Beach, Galveston, Texas to different salinities in four *in-situ* bioassays conducted over a 6-month period. The salinity fluctuated over the course of each bioassay due to rain events and evaporation. The two most abundant diagnostic pigments were fucoxanthin (diatoms) and zeaxanthin (cyanobacteria), while the other two pigments, chlorophyll *b* (green algae), and bacteriochlorophyll *a* (photosynthetic bacteria) were found in lesser abundances. The results showed that the BMA community was significantly affected by salinity and by the date of the experiment with the exception of zeaxanthin and bacteriochlorophyll *a*. Container artifacts influenced zeaxanthin with abundances higher in the sediment inside the core tubes compared to outside the core tubes. The results

also showed that bacteriochlorophyll *a* not significantly affected by salinity or by date of experiment.

These results suggest that diatoms proliferate in an environment with moderately higher salinities compared to cyanobacteria, which is in contrast to other studies showing where cyanobacteria dominate under hypersaline (> 100) conditions (Pinckney et al. 1995a). The F/Z ratios varied between treatments with higher ratios in the higher salinities and decreasing in CNT and DI samples. This indicates that the relative abundances of diatoms were higher in the higher salinities compared to the lower salinities. However, zeaxanthin abundances did not vary greatly between treatments and any change in the F/Z ratio was due to a change in the abundance of fucoxanthin. Herbst and Blinn (1998) found that at salinities between 50 and 75, diatom diversity dropped greatly with the number of taxa decreasing from 30 to 15 and filamentous cyanobacteria (*Oscillatoria spp*.) being observed between 50 – 100. Nübel et al. (2000) showed that cyanobacteria are the predominant algal group at salinity greater than 111. During this study, the average salinity measured from the CNT treatments was between 14 and 30 and the 50%> averaged between 22 and 41, which is not considered hypersaline. A study was done to examine the status and trends of water quality within Galveston Bay from 1969 – 2002 and salinity was one of the water quality parameters measured (Lester and Gonzalez 2003). The salinities used in the study at East Beach fall within the salinities reported in the Galveston Bay Status and Trends report, with the average salinities measured during drier periods of 28, with a high of 35, and average salinities of 11, with a low of 8 during the wetter periods (Lester and Gonzalez 2003). Furthermore,

the salinity in Galveston Channel (which is adjacent to East Beach) showed an increasing annual trend in salinity from ca. 17 in 1962 to 25 in 2002 (Lester and Gonzalez 2003). The salinity treatments used in this study are within the scope of work mentioned in the experimental design when compared to the annual trend, i.e. examining the effects that salinity would have on BMA biomass if the salinity were raised 25 and 50% above the controls. Therefore, diatoms at East Beach can easily acclimate to a salinity increase of 25% and even 50%, thereby outcompeting cyanobacteria within this salinity range. These results support other studies in that diatoms and cyanobacteria are able to tolerate a wide range in salinity (Admiraal 1977) and can adjust to fluctuations in salinity (Kirkwood and Henley 2006). While diatoms are productive over a wide range of salinities, one study resulted in the majority of diatoms failing to grow at salinities of 75 (Clavero et al. 2000). In hypersaline ponds, a large number of algal strains cease to grow at salinities of 75 and this agrees with the reported decrease in diversity at salinities of 5 – 75 (Herbst and Blinn 1998). Underwood et al. (1998) showed this same effect between biomass and salinity at concentrations >30. The highest salinity (41) in the study at East Beach measured in Aug. 04, which is 27% higher than the study conducted by Underwood et al. (1998), did not show a significant change in biomass between the salinity treatments (as illustrated in Table 10), indicating that the BMA biomass was not exposed to salinities high enough or long enough to effect an observable change. Therefore, it could be argued that even though the statistical analysis demonstrates a significant difference in fucoxanthin abundance between salinities in Aug. 04, the post-hoc comparisons analysis suggests otherwise.

The BMA community showed significant temporal variability for all the algal groups except the photosynthetic bacteria. BMA biomass was significantly higher in Feb. 2005 and lower in Oct. 2004. Diatoms dominated the community during each bioassay. Cyanobacteria abundances were significantly different for salinity treatments in the Oct. 04 and Feb. 05 bioassays with higher abundances measured in Feb. 05 and the lowest in the Oct. 04 bioassays. The results from this study revealed that the four pigments (chlorophylls *a* and *b*, fucoxanthin, and zeaxanthin) averaged higher abundances in February 2005 compared to October 2004. This suggests that all the algal groups responded positively to the cooler temperatures except bacteriochlorophyll *a* which was not affected by salinity or date of the experiment. This agrees with the data shown in a study conducted previously at this site (A.Lee, pers. obs.) where the pigments chlorophyll *a,* fucoxanthin, and zeaxanthin show a seasonal signal with higher abundances measured during the winter months compared to the summer months (Chap. 2, Fig. 8). In temperate intertidal mudflats, an increase in temperature during the day results in a decrease in BMA productivity and biomass (Blanchard and Guarini 1998). Kendrick et al. (1998) also reported that abundances of chlorophyll *a* in surface sediments were negatively correlated with temperature and salinity. This could explain the decrease in the pigment abundances in Oct. 04 compared to Feb. 05. The temperatures in Oct. for that week averaged 9º F higher than normal. Another study examined the effects of the mud surface temperature on biomass-specific photosynthetic capacity and found that the photosynthetic capacity was inhibited during the summer when temperatures were highest (Guarini et al. 1997). They concluded that the thermo-

inhibition was not the direct cause of the decrease in BMA but did decrease production. A study in the Mdloti Estuary in South Africa found that the correlation analysis suggested a decrease in temperature is significantly related to an increase in benthic chlorophyll *a* biomass (Mundree et al. 2003). A study in Baffin Bay, TX found that temperature had a negative effect on the photosynthetic efficiency and chlorophyll *a* abundance when increased by 6° C from May to July (Blanchard and Montagna 1992). However, a study conducted in the San Antonio Bay did not find a significant correlation between microalgal abundance and any of the physical parameters, temperature being ne of them (MacIntyre and Cullen 1996).

Another reason for the change in biomass during the Oct. 04 bioassay could be related to the amount of nutrients in the sediments. One study examined the sediment nutrients in a California estuary over a period of time and found that the nitrogen content in the sediment indicated a spring maximum with a decrease in the fall (Boyle et al. 2004). They stated that the macroalgae use primarily riverine nutrients in the spring and recycled nutrients from the sediment in the summer and fall. One study conducted in Trinity Bay within Galveston showed an decrease in ammonium concentrations during the summer months compared to the cooler winter months (Warnken et al. 2000). This too was related to riverine input. Sediment nutrients were not measured in this study; however, they could play a significant role in the amount available for nutrient uptake by the biomass. One could assume that nutrients were already in the sediment when the samples were collected and the only other source would have been from the daily exchange water. The control water was used to make up the other treatments with the

exception of the deionized treatment. If the nutrient concentration from the nearby channel fluctuated with decreased amounts in the summer and higher amounts due to river input in the winter, then this could explain the change in the temporal change in biomass. Nutrients would also be recycling during this study as the control water was not filtered to remove grazers nor were they removed from the sediment.

The potential impacts of differential grazing rates in the salinity treatments were not measured in this study. However, grazing pressure is another factor that can reduce BMA biomass (Admiraal and Peletier 1980) and may have contributed to the measured differences in BMA biomass in the salinity incubations (Brotas and Plante-Cuny 1998; Hillebrand 2002; Roll et al. 2005). Liess and Kahlert (2007) reported a negative correlation between periphyton and grazers. However, grazer impacts were reduced when nutrients were added because of enhanced algal growth (Liess and Kahlert 2007). Montagna et al. (1995) found that grazing rates increased as BMA biomass increased. A review by Liess and Hillebrand (2004) examined the interactions between grazers and benthic algae to determine the trophic interactions. They found that grazing significantly reduced species richness and diversity (Liess and Hillebrand 2004). One reason for the diatoms proliferating at higher salinities could possibly be attributed to a reduction in grazing pressure (Montagna et al. 2002). The study conducted in Upper Rincon Bayou, Texas, found that meiofauna abundances were lowest when salinities were highest and the highest abundances were found following a flood event (Montagna et al. 2002). While the results from these two studies differ, the higher BMA biomass at higher salinities at East Beach suggests that the grazers might not be negatively affected by the

salinity at this site. The salinity at East Beach was higher and the microalgal biomass was lower during the bioassays conducted in the warmer months, suggesting that the grazers might be the reason for the decrease in biomass. However, isolating the effect of salinity on benthic microalgae in estuarine systems can be very difficult due to other environmental factors such as seasonality and nutrients (Admiraal and Peletier 1980; Underwood and Provot 2000).

Zeaxanthin was the only pigment to show a response to the container artifact. This response was shown by an increase in abundance in the CNT compared to the FINAL samples. This would suggest that the abundances quantified for zeaxanthin were artificially elevated. The increase in abundance for zeaxanthin within the sediment inside the containers compared to the sediment outside the containers could be due to an increase in temperature. A study conducted by Watermann et al. (1999) examined the competition between diatoms and cyanobacteria with respect to grain size and temperature. They found that at temperatures at 25º C were dominated by the filamentous cyanobacteria. Temperature within the containers was not measured and the new water was exchanged early in the morning right after sunrise, before the ambient air temperature had reached the daily maximum. The overall abundances of zeaxanthin were low; therefore, the elevated abundances did not significantly affect the community composition.

In summary, an increase in salinity may shift benthic microalgae community structure, possibly altering the biomass and in turn affecting higher trophic levels. The community composition at this site was significantly affected by salinity treatment and

bioassay date. Zeaxanthin was the only pigment to illustrate a container artifact with an average abundance higher in the Controls (6.29 mg m^{-2} ± 3.19 STD) than in the Final samples (4.93 mg m^{-2} ± 2.29 STD). All of the pigments except zeaxanthin and bacteriochlorophyll *a* were significantly affected by salinity. Zeaxanthin abundances were artificially elevated due to the container effect, but the concentrations for this pigment overall were low, therefore, it cannot be stated conclusively whether zeaxanthin is significantly affected by salinity. The treatments that were either 100% or partially diluted with deionized water had the lowest BMA biomass over all. Chlorophyll *a* and fucoxanthin were significantly affected by salinity with higher abundances found in salinities that averaged 15 with a preference for salinities greater than 22. Chlorophyll *b* was affected by salinity with higher abundances measured in the treatments with lowest salinity (DL and DI); and was affected by the time of year. This would suggest that this algal group prefers an environment with salinity <2 but can easily adapt to environments with higher salinities. The seasonal response of BMA indicated that biomass peaked in the late fall and winter months. Bacteriochlorophyll *a* was the exception with uniform abundances measured in all treatments throughout the four bioassays but was not significantly affected by date. These data also suggested that BMA biomass might decrease following a rain event or with an increase in freshwater inflow, which significantly reduces the salinity. BMA at East Beach can easily adapt to a moderate increase in salinity should a greater demand for freshwater occur in the watershed. The primary hypothesis was that exposure to elevated or reduced salinities over a five-day period will alter BMA community composition such that the ratio of diatoms to

cyanobacteria will decrease with increasing salinity is null. The ratio increased. Finally, while there are subtle changes in biomass at the extreme salinity ranges (i.e. 0 compare to 22 or greater), salinity is not a factor in determining biomass or the spatio-temporal variability at this site.

These results suggest that BMA biomass would not decrease with a 10% increase salinity, should the demand for freshwater grow as expected in the Galveston Bay watershed. Further studies should be conducted to determine how much salinity would have to be increased in order to show a significant decrease in BMA biomass on this sandflat. The duration of exposure to different salinities could be very important and should be considered in future studies as well as the time of year over which these studies are conducted.

CHAPTER V

THE RESPONSE OF THE BENTHIC MICROALGAL COMMUNITY TO NUTRIENT ENRICHED SEDIMENT

Introduction

BMA spatial and temporal heterogeneity have been attributed to porewater nutrients (Hopner and Wonneberger 1985; Marinelli et al. 1998; Underwood et al. 1998). The source and concentration of different nutrients necessary for BMA growth, specifically nitrogen (N) species, may have a significant effect on their spatial variability. Ammonium (NH_4), nitrate (NO_3), nitrite (NO_2), phosphate (P), and silicate (Si) also show spatial heterogeneity in concentrations over small scales (meters) (Hopner and Wonneberger 1985; Marinelli et al. 1998; Underwood et al. 1998). For example, in a southern California estuary, nutrients within the sediment and the water column were temporally variable with N concentrations maximum in the spring and declining through fall (Boyle et al. 2004). Hopner and Wonneberger (1985) also demonstrated spatial variability with N and P diffusing out of the sediment and that it affects BMA patchiness. They stated that the ratio of the nutrient demand is similar to the elementary composition of diatoms (ca. 10:1). The N:P ratios of the porewater were 8.3:1 when converted to efflux ratios based on diffusion coefficients, which is in the range of the demand ratio of the N:P When examining the N:P efflux ratios with the oxygen activity from the diatoms, they found that the oxygen activity was highest at the N:P ratio of 10 which is similar to the ratio of the N:P demand. The autototrophs in intertidal sediments are usually not N limited because of the relatively high inorganic and organic nitrogen

concentrations found at depth in the sediments (Underwood et al. 1998). Underwood et al. (1998) found that BMA biomass did not respond to nutrient enrichment along a gradient but BMA community composition shifted in response to changes in nutrient concentrations. These studies illustrate that nutrients within the sediment and the overlying water column can affect BMA biomass, and could be one of the main driving forces behind the spatiotemporal variability of BMA.

There are various sources for nutrients that BMA require, from anthropogenic sources (industrial and agricultural) (Vitousek et al. 1997), atmospheric deposition (Paerl et al. 1990; Duce et al. 1991), as well as riverine and groundwater input (Jickells 1998). The dominant algal group can be dependent on the nutrient that is in excess. Diatoms become more dominant compared to cyanobacteria in high Si:N ratio, and when Si supply is low, then cyanobacteria become dominant even in low N:P ratio because of their ability to fix N (Sommer 1996). They found in tissue-culture experiments that when the N:P ratio is balanced (15:1) cyanobacteria and green algae are dominant when the Si:N or the Si:P ratios declined. When the system was N limited (5:1), diatoms had the advantage when the Si:N ratios were high and cyanobacteria had the advantage when the Si:N were low (Sommer 1996). Berman (Berman 2001) concluded that when the nutrient becomes limiting it may determine the dominance of one algal group over another, it is the availability of the nutrient and not the ratios between them that determines it.

Frequency of the nutrient supplies whether continuous or pulsed needs to be considered when conducting experimental studies. A mesocosm experiment conducted

in the Baltic Sea found that pulsed nutrients compared to a continuous source of nutrients were less harmful because it favored more chlorophytes and zooplankton than cyanobacteria (Lagus et al. 2007). One study in a semi-enclosed marine system found that a steady nutrient supply stimulated a diversity taxonomic community while a high pulse of nutrients had a negative effect on the diversity and can increase the potential for a harmful algal bloom (Spatharisa et al. 2007). However, another study found that by pulsing the nutrients into the system prohibited the growth of slow growing algae in phytoplankton communities (Roelke et al. 1999). They stated that this information could be used to prohibit slow-growing noxious blooms. Eutrophication, and increase in the rate of supply of organic matter to an ecosystem (Nixon 1995), can lead to the dominance of nutritionally replete algal species that can have detrimental consequences to the food web structure (Riegman 1995).

The purpose of this study was to determine if BMA in an intertidal sandflat at East Beach, Galveston, Texas, are nutrient limited and quantify the responses of major community components to nutrients added to the sediment. The working hypothesis tested was that the addition of nutrients (nitrogen and phosphorus) over a five-day period significantly alters the BMA total biomass and community composition.

Materials and Methods

Experimental Design. Four *in-situ* bioassays were conducted in triplicate at three stations (1, 2 and 3) over a six month period on East Beach, Galveston, Texas using commercially produced slow release fertilizer sticks (Jobe's Plant Food Spikes). This study used slow release fertilizer sticks to ensure that the BMA were continuously supplied with nutrients below the water-sediment surface, as these nutrients would diffuse into the porewater. Three stations were chosen because of the variability in sediment porewater content that averaged 10.6%, 18.1%, and 24.9% (by weight) in stations 1, 2, and 3, respectively (Fig. 15). The nutrient concentration of each fertilizer stick averaged 15.0% total nitrogen (N, both as NH_4+ and NO_3), 5.7% available phosphate (P, as water soluble P_2O_5), 6.8% soluble potash (K, as K_2O), and trace elements (as reported by the manufacturer). Each slow release fertilizer stick contained an average of 13,900 µmol N, 520 µmol P, and 940 µmol K. N in each stick was in the form of water-soluble nitrate and urea, water-soluble and water-insoluble nitrogen. Therefore, each stick averaged 1,720 µmoles of nitrate, 1,170 µmoles of urea, 3,590 µmoles of nitrogen from water soluble sources and 6,260 µmoles from water insoluble sources. These averages were calculated and provided from the manufacturer (Easy Gardener Products, Inc.) (pers. comm., Scott Ross). With respect to the P and K there was approximately 520 µmoles of P_2O_5 and 940 µmoles of K_2O in each stick (pers. comm., Scott Ross). The sticks release the nutrients over a 60-day period once inserted into the sediment/soil matrix. This study allowed the fertilizer sticks to amend the sediment for 5 days, after which the bioassays were terminated. Assuming a constant

dissolution rate for each stick during each bioassay, the amount of nutrients added to the sediment would be ca. 30 µmoles of nitrate, 20 µmoles of urea, 60 µmoles of nitrogen from water-soluble sources, 100 µmoles from water insoluble sources, 9 µmoles of P_2O_5, and 15 µmoles of K_2O per day. It would also be expected that there would be a concentration gradient with higher concentrations closest to the fertilizer stick and concentrations decreasing further away from the fertilizer stick.

Sediment for the incubations was separated from surrounding sediments using six clear core liners (7.8 cm in length and dia.) which were inserted ca. 5 cm into the sediment at each station (Fig. 16). Three of the six core liners were designated as control samples (CNT) and the other three as nitrogen and phosphorous (N+P) enriched samples. The nutrient enriched samples contained one fertilizer spike (ca. 5 cm in length and 6 mm in diameter) centrally located and inserted into the sediment flush with the sediment surface. Each control sample contained one wood dowel rod (ca. 5 cm in length and 7 mm in dia.) inserted into the sediment to simulate the experimental conditions in the spiked treatments. The bioassays were conducted on Aug. 23 – 28 (Aug. 2004), 2004, Oct. 25 – 29, 2004 (Oct. 2004), Dec. 13 – 17, 2004 (Dec. 2004) and Feb. 21 – 25, 2005 (Feb. 2005). Upon termination of the bioassay, the core-tube liners were collected with sediment intact and transported to the laboratory to be subsampled for photopigment analysis. Five subsamples were taken from each sediment core tube liner using a 1.00 cm (ID) butyrate core tube (0.80 cm^2). The top 3 mm of each subsample was extruded, sectioned, frozen in 2.0 ml microfuge tubes, and stored at -80 °C. Photopigment abundances were determined for all samples using HPLC. The core-

tube liners remained in the sediment throughout the incubation period, possibly affecting horizontal advection of porewater and nutrients. Therefore, three additional sediment cores (Final) were collected at each station after each bioassay and compared to the Controls (CNT) to test for any artifacts associated with the core-tube liners.

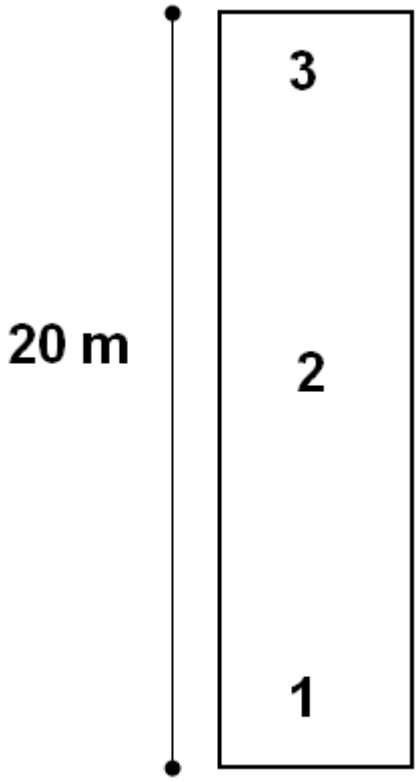

Figure 15: Top-down view of sample locations at East Beach, Galveston, Texas. The nutrient enrichment study was conducted at stations 1, 2, and 3. The site was 20 m in length.

Figure 16: Photograph showing the experimental set-up at one of the three stations used in the nutrient enrichment study. The three cores on the left were amended with slow release fertilizer sticks and the three cores located to the right were the control samples.

Photopigment Analyses. HPLC was used to determine chemosystematic photosynthetic pigments for BMA. The sediment samples were placed in 100% acetone (2 ml), sonicated (10s), and extracted at -20 °C for 18-24 h. Filtered extracts (300 µl) were injected into a Shimadzu HPLC equipped with a monomeric (Rainin Microsorb-MV, 0.46 x 10 cm, 3 µm) and a polymeric (Vydac 201TP54, 0.46 x 25 cm, 5 µm)

reverse-phase C18 column in series. A nonlinear binary gradient was used for pigment separations (Pinckney 1996). Absorption spectra and chromatograms (440 nm) were acquired using a Shimadzu SPD-M10av photodiode array detector. Pigment peaks were identified by comparison of retention times and absorption spectra with pure crystalline standards, including chlorophylls *a, b*, *β*-carotene (Sigma Chemical Co.), fucoxanthin, and zeaxanthin (Hoffman-LaRoche and Company). Other pigments were identified by comparison with extracts from phytoplankton cultures and quantified using the appropriate extinction coefficients (Jeffrey et al. 1997). Fucoxanthin is often used as the biomarker pigment representing diatoms and zeaxanthin is the primary pigment for cyanobacteria. These were the two major diagnostic pigments measured in the samples. Two other pigments measured in smaller concentrations were chlorophyll *b* and bacteriochlorophyll *a*, representing green algae, and photosynthetic bacteria, respectively.

Statistical Analyses. A three-way, randomized-complete block design multivariate analysis of variance (MANOVA) with replication was used to determine statistical significance on the BMA biomass using the abundances of five diagnostic pigments (chlorophyll *a*, fucoxanthin, zeaxanthin, chlorophyll *b*, and bacteriochlorophyll *a*) as the variables. The blocking factor was station (1, 2, and 3), and the two main factors were nutrient enrichment (control and N+P) and bioassay date (Aug. 2004, Oct. 2004, Dec. 2004, and Feb. 2005). This analysis examined whether nutrient enrichment and/or station location influenced BMA biomass and community structure. The data were not normally distributed (K-S test, $p<0.05$) therefore, the data were transformed

using the equation (ln (X + 1)). The variances were not homogeneous (Levene's test, $p<0.05$); therefore a Dunnett's T3 *post-hoc* test was used to compare the means.

Results

Incubation Artifacts. The placement of core tubes in the sediment for the incubations may also affect the BMA community, thereby resulting in possible experimental artifacts. This artifact effect was tested by comparing the BMA pigment abundances within the control treatments at the end of the incubation with cores collected from an undisturbed area in close proximity to the core tubes (the CNT and FINAL bars in Fig. 17). A randomized-complete block design 3 factor MANOVA (nutrient treatment, date of experiment (as the blocking factor), and sample location) was used to determine if BMA community composition was affected by the presence of core tubes. The experimental date, sample location, and interactive term (nutrient treatment and sample location) effects were all significant (Pillai's Trace, $p<0.05$). However, the nutrient treatment was not significant (Pillai's Trace $p=0.184$). The ANOVAs for individual pigments indicated that all five pigments differed in abundance between the three sampling locations and each sample date ($p<0.001$). Chlorophylls *a* and *b* were the only pigments significantly affected by the interaction term (nutrient treatment and sample location). Although the nutrient treatment was not significant but the interaction term was, there could be a significant change in community composition between stations because of the incubation artifact. Therefore, a multivariate analysis was done for each station (1, 2, and 3).

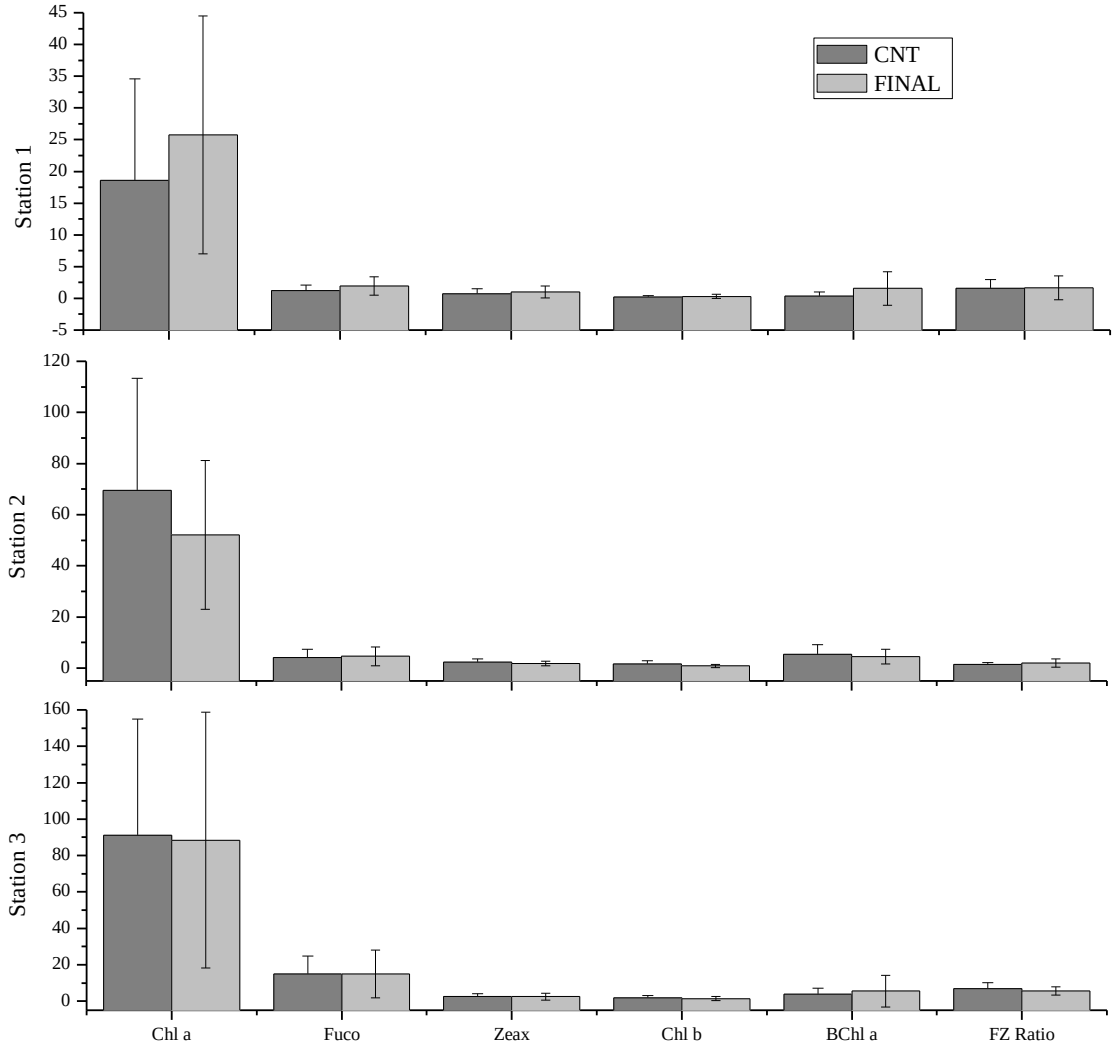

Figure 17: BMA pigment abundances between the control (CNT) and the final (FINAL) samples from each station.

The MANOVA was performed using the five pigments as the dependent variable and treatment and date as the independent variables for each station. In Stations 1 and 3, nutrient treatment was not significant (Pillai's Trace $p>0.05$), however sample date was significant for all three stations (Pillai's Trace $P<0.001$) (Table 14). The MANOVA was performed using the five pigments as the dependent variable and treatment and date as the independent variables for each station. In Stations 1 and 3, nutrient treatment was not significant (Pillai's Trace $p>0.05$), but sample date was significant (Pillai's Trace $p<0.001$) (Table 14). The univariate analysis indicated that all the pigments were significantly affected by sample date ($p<0.001$) at Stations 1 and 3, except bacteriochlorophyll *a* ($p=0.506$, 0.289, respectively). However, the two-factor MANOVA performed on all pigments for Station 2 indicated that the pigments were significantly different for sediment samples inside and outside of the tubes and for each sample date (Pillai's Trace, $p<0.05$). The ANOVA's indicated that zeaxanthin, chlorophylls *a* and *b* were significantly different for sediment samples inside and outside of the tubes ($p<0.05$). The pigment abundances measured higher in the controls compared to the final samples for all three pigments. In contrast, fucoxanthin and bacteriochlorophyll *a* were not significantly different for sediment samples inside and outside the core tubes ($p=0.424$ and $p=0.125$, respectively). All five pigments were significantly different with respect to sample date ($p<0.001$).

Table 14: Two-factor MANOVA results from each station using Treatment (CNT and N+P) and Experimental Date (Aug. 2004, Oct. 2004, Dec. 2004 and Feb. 2005) as the independent variables and pigment abundances as the dependent variables. Computed alpha 0.05.

	Station 1		Station 2		Station 3	
	F Value	p Value	F Value	p Value	F Value	p Value
Treatment	1.845	0.164	3.851	0.021	2.879	0.051
Date	6.357	<0.001	21.210	<0.001	5.899	<0.001

MANOVA and ANOVA Results. The BMA community did not show a significant response to nutrient enrichment. The results from the MANOVA analysis revealed that the station location and bioassay date were significant (Pillai's Trace, $p<0.001$) although, the nutrient treatments and the interaction term (station and nutrient) were not significant ($p=0.047$ and $p=0.453$, respectively, computed alpha = 0.01). These results indicate that the BMA community composition differed between the three sampling locations and the experimental dates but the nutrient addition treatments were not significantly different from the controls. The univariate tests indicated that the abundances of all five pigments were significantly different between the sampling locations and experimental date ($p<0.001$) (Table 15). Since the group means comparisons cannot be compared between nutrient treatments, station location, and experimental date, the average abundances of each pigment per salinity treatment and

bioassay date indicated higher abundances in the Dec. 04 and Feb. 05 bioassays and in Station 3 compared to Stations 1 and 2 (Fig. 18).

Table 15: The results from the univariate randomized-complete block design two factor (ANOVAs) with station (blocking factor) and date with pigment abundances as the variable. Computed alpha = 0.01.

	Date		Station	
	F value	p-value	F value	p-value
Fucoxanthin	94.875	.000	250.553	.000
Zeaxanthin	90.902	.000	74.732	.000
Chl_*b*	66.058	.000	97.150	.000
Chl_*a*	172.692	.000	225.712	.000
BChl_*a*	30.883	.000	72.579	.000

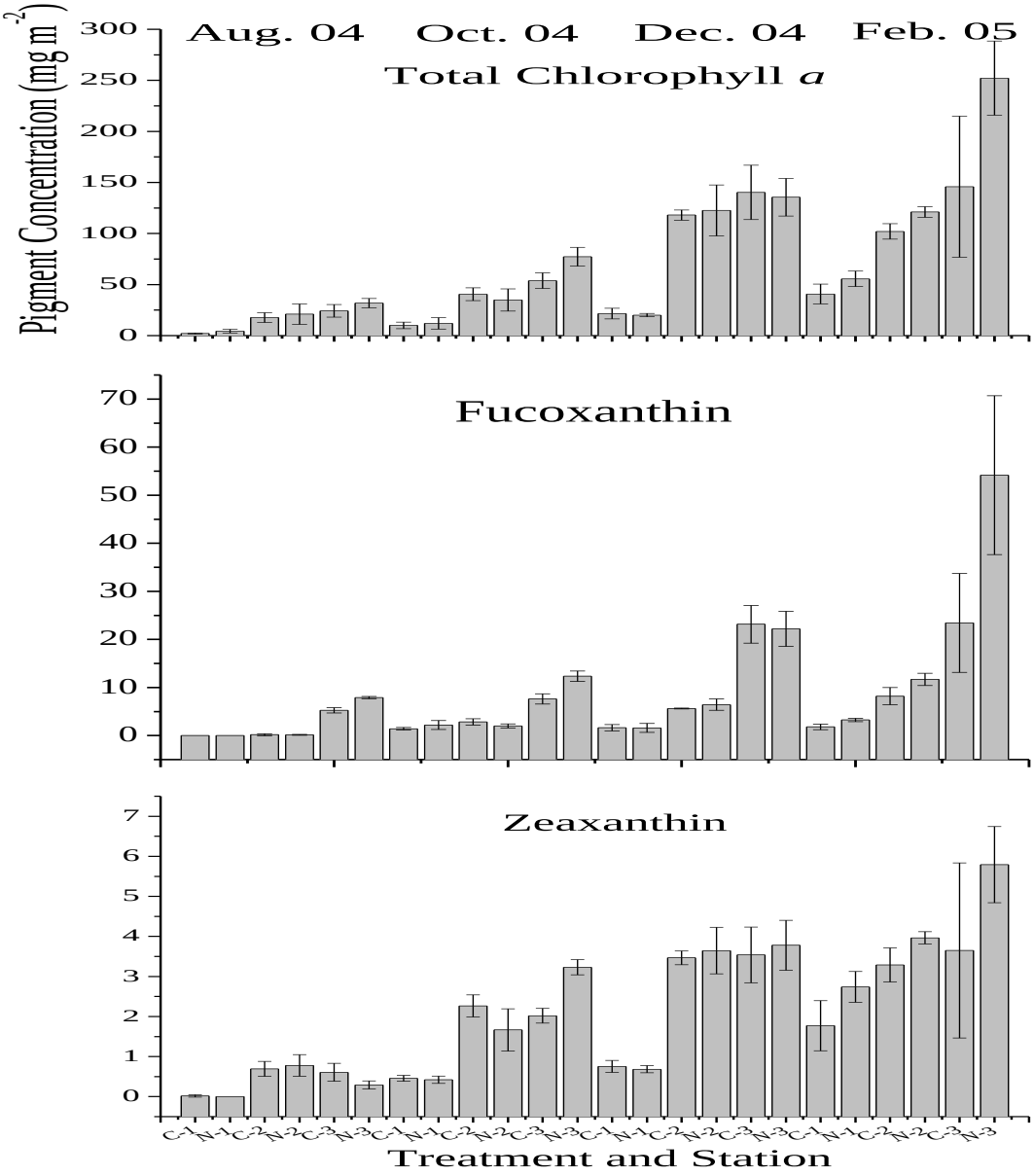

Figure 18: Graphs showing the average pigment concentration for total chlorophyll *a* from each station, treatment, and bioassay date. The abbreviations are as follows: S1 = Station 1, S2 = Station 2, S3 = Station 3, CNT = control, N+P = Nutrient Enriched, Initial = Samples collected prior to bioassay set-up and Final = samples collected after the bioassay was terminated.

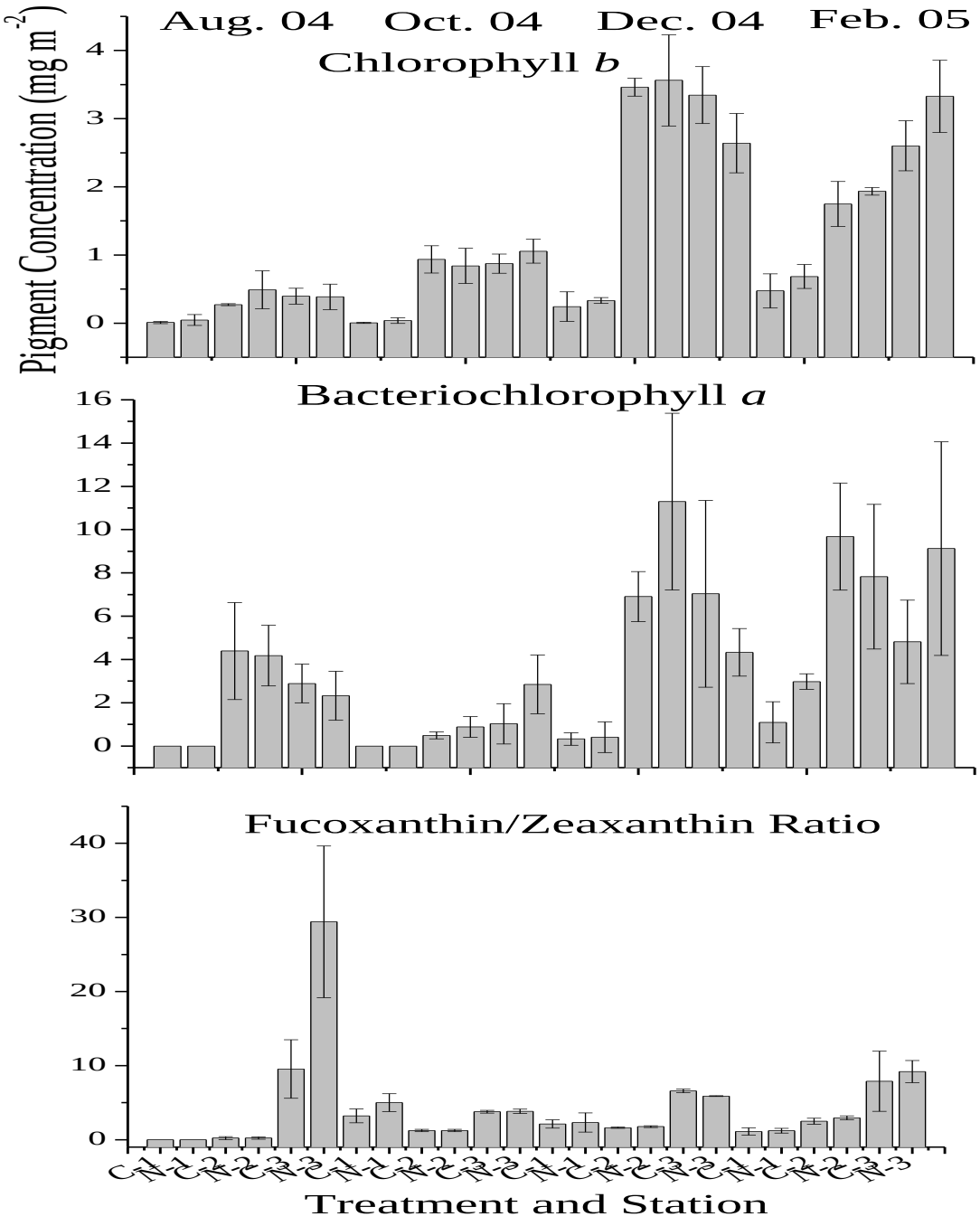

Figure 18: Continued.

A one-factor MANOVA was performed on each station using the five pigment abundances as dependent variables and nutrient treatment and date of the experiment as the independent variables. The results from Stations 1, 2 and 3 MANOVAs indicated that the pigment abundances were not statistically significant between nutrient treatment and the control (Pillai's Trace, $p=0.306$, 0.404, and 0.103, respectively). However, the pigment abundances were statistically significant between the experimental dates (Pillai's Trace, $p<0.05$) for all three stations (Table 15). The ANOVAs for each station revealed that all five pigment abundances were statistically different ($p<0.05$) with respect to date. Even though the nutrient treatment grand means for these three MANOVAs indicated that the nutrient treatment was higher than the control in every case, they were not significantly higher.

A *post-hoc* comparisons test was done on the estimated means for each station. A Bonferroni test was done on the estimated means that had homogenous variances while the Dunnett's T3 test was done on the estimated means that were not homogenous. In Station 1, all the pigments measured highest in Feb. 05 and lowest in Aug. 04 except for chlorophyll *b*, which measured lowest in Oct. 04 (Table 16).

Table 16 : Results of *a posteriori* multiple analyses of variance (Bonferroni, and Dunnett's T3) with date as the main factor and the diagnostic pigments as the independent values for Station 1. The underline denotes that the means were not significantly different ($p<0.05$). The means are ranked from highest to lowest.

	Station 1 Date	N	Levene's Test
Fucoxanthin	Feb. 05 Oct. 04 Dec. 04 Aug. 04	6	.080
Zeaxanthin	Feb. 05 Dec. 04 Oct. 04 Aug. 04	6	.586
Chlorophyll *b*	Feb. 05 Dec. 04 Aug. 04 Oct. 04	6	.052
Chlorophyll *a*	Feb. 05 Dec. 04 Oct. 04 Aug. 04	6	.346
Bacteriochlorophyll *a*	Feb. 05 Dec. 04 Oct. 04 Aug. 04	6	.285

In Station 2, fucoxanthin and zeaxanthin measured highest in Dec. 04 and lowest in Aug. 04. While chlorophylls *a* and *b* measured highest in Feb. 05 and lowest in Aug. 04, bacteriochlorophyll *a* also measured highest in Feb. 05 but was lowest in Oct. 04. However, chlorophyll *b*, chlorophyll *a*, and zeaxanthin indicated an incubation artifact in Station 2. Therefore, the pigment abundances for these three pigments could be artificially elevated because of this artifact, suggesting that these values are over estimated (Table 17).

Table 17: Results of *a posteriori* multiple analyses of variance (Bonferroni, and Dunnett's T3) with date as the main factor and the diagnostic pigments as the independent values for Station 2. The underline denotes that the means were not significantly different ($p<0.05$). The means are ranked from highest to lowest.

	Station 2 Date	N	Levene's Test
Fucoxanthin	Feb. 05 Dec. 04 Oct. 04 Aug. 04	6	.131
Zeaxanthin	Feb. 05 Dec. 04 Oct. 04 Aug. 04	6	.363
Chlorophyll *b*	Dec. 04 Feb. 05 Oct. 04 Aug. 04	6	.040
Chlorophyll *a*	Dec. 04 Feb. 05 Oct. 04 Aug. 04	6	.333
Bacteriochlorophyll *a*	Dec. 04 Feb. 05 Aug. 04 Oct. 04	6	.101

Finally, in Station 3, fucoxanthin, zeaxanthin, and chlorophyll *a* measured highest in Feb. 05 and lowest in Aug. 04. Chlorophyll *b* measured highest in Dec. 04 and lowest in Aug. 04 and bacteriochlorophyll *a* measured highest in Feb. 05 and lowest in Oct. 04 (Table 18).

Table 18: Results of *a posteriori* multiple analyses of variance (Bonferroni, and Dunnett's T3) with date as the main factor and the diagnostic pigments as the independent values for Station 3. The underline denotes that the means were not significantly different ($p<0.05$). The means are ranked from highest to lowest.

	Station 3 Date	N	Levene's Test
Fucoxanthin	Feb. 05 Dec. 04 Oct. 04 Aug. 04	6	.018
Zeaxanthin	Feb. 05 Dec. 04 Oct. 04 Aug. 04	6	.327
Chlorophyll *b*	Dec. 04 Feb. 05 Oct. 04 Aug. 04	6	.203
Chlorophyll *a*	Feb. 05 Dec. 04 Oct. 04 Aug. 04	6	.368
Bacteriochlorophyll *a*	Feb. 05 Dec. 04 Aug. 04 Oct. 04	6	.130

The fucoxanthin to zeaxanthin ratio is an indicator of community composition. The average of this ratio did vary greatly between treatments which was lower in the controls compared to the treatments (CNT = 3.32, N+P = 5.25). This would indicate that the ratio of fucoxanthin to zeaxanthin increased in the nutrient treated samples. This ratio also increased in the warmer months, with the ratio measuring 6.57 in Aug. 2004, 3.05 in Oct. 2004, 3.38 in Dec. 2004, and finally 4.14 in Feb. 2005. Again, zeaxanthin abundances were low overall, and any variability in the community was due to the fucoxanthin abundances.

Discussion

The purpose of this study was to examine the BMA community response at East Beach, Galveston, Texas to nutrient enriched sediment. The response of BMA to nutrient enriched sediment may indicate whether the BMA are nutrient limited or replete and if the spatial variability in BMA biomass and community composition found at this site is regulated by nutrients found in the sediments. Sediments are a major source of nutrients in shallow water systems (Marinelli et al. 1998) and are rarely nutrient depleted relative to the water column. Warnken et al. (2000) found that the sediments in Galveston Bay appear to be the primary source for nutrients especially during low periods of freshwater inflow. BMA have a lower nutrient requirement than phytoplankton because they use less energy for nutrient uptake compared to the phytoplankton as a result of the large nutrient pool found in the sediments (Reuter et al. 1986). The sediments at East Beach are aerially exposed, which suggests that the BMA community relies mostly on these sediments for their nutrients. If sediments are nutrient deficient, whether in nitrogen or phosphorus, this could decrease BMA biomass.

The BMA biomass was significantly affected by bioassay date and station, but was not significantly affected by the slow release fertilizer sticks. Other studies examining the effects of nutrient enrichment on BMA have shown that sediments amended with N+P have a greater effect than sediments amended with only N. This was observed in a eutrophic estuary where the BMA biomass showed a greater response to N+P amended sediments compared to N only amended sediments (Lever and Valiela 2005). This would imply that the BMA were P limited or that the N was in such excess

concentration that it alleviated the N limitation until P was limited. The addition of both nutrients would enhance growth and neither nutrient would be limiting. If the sediments at East Beach were nutrient limited in N, P, or N+P, then enriching the sediment with the slow release fertilizer would have increased the biomass in the N+P samples, as shown in this study.

Ambient nutrient concentrations of the porewater were not analyzed; however, a study conducted in central Galveston Bay, near Texas City, found that porewater concentrations were 10 times higher or more than in the water column and capable of supporting benthic primary production (An and Joye 2001). Another study measured ambient nutrient concentrations in the water column at several stations across Galveston Bay and found dissolved inorganic nitrogen (DIN) which included nitrate, nitrite and nitrogen at 43.6 µmol L^{-1} in March, 21.5 µmol L^{-1} in May and 1.9 µmol L^{-1} in July (Ornolfsdottir et al. 2004b). Phosphate levels did not vary as much, with 3.0 µmol L^{-1} in March, 2.0 µmol L^{-1} in May and 3.0 µmol L^{-1} in July, while concentrations of Silicate were 42.9 µmol L^{-1} in March, 38.1 µmol L^{-1} in May and 45.4 µmol L^{-1} in July (Ornolfsdottir et al. 2004b). An additional study by Ornolfsdottir (2004a) found similar ambient concentrations with DIN ranging between 0.32 – 2.91 µM and silica found at < 50 µM between 1999 and 2001. If these are the concentrations found in the water column, it can be assumed that based on An and Joye's study, the concentration of nutrients in the sediments at East Beach are higher by approximately a factor of 10 and therefore, much higher than the average concentration released from the fertilizer sticks, which were within the range found in the water column. A constant dissolution rate was

assumed over the 5 days for each bioassay with an average of 59.8 µmoles of nitrogen from water-soluble sources, 8.7 µmoles of phosphate and 15.6 µmoles of potassium entering the sediments. The ca. volume of sediment amended with the nutrients was 239 cm^3, based on the diameter of the core tube liner and the depth that the slow-release fertilizer stick penetrated the sediment. Since, there was not a significant change in BMA biomass between the CNT and the N+P samples, this could be because the nutrients did not diffuse completely throughout the core. This would provide evidence that the duration for the bioassay should be extended so that the nutrients are allowed to diffuse throughout the core.

Amending the sediments can also result in a shift within the BMA community. In a hypersaline pond in Lake Salada de Chiprana, an increase in nitrogen resulted in an increase in diatom biomass relative to cyanobacteria, while phosphorus addition increased the relative abundance of cyanobacteria with respect to diatoms (Camacho and de Wit 2003). In the East Beach study, the amended sediments did not have a significant effect on the community composition; however, the fucoxanthin to zeaxanthin ratio was higher in the nutrient amended sediments compared to the controls. This indicated an increase in diatoms over cyanobacteria. This positive effect on diatoms could indicate that they were nitrogen limited, while the cyanobacteria were not significantly affected by either nutrient. Another sediment enrichment study found that in two ponds, the Sage Lot and Green, with similar salinity ranges and grain size, chlorophyll *a* concentrations increased after the sediments were enhanced with both nitrogen and phosphorus (Lever and Valiela 2005). Whereas chlorophyll *a* concentrations showed little response to the

nitrogen only enrichment in the highly eutrophic Childs River (Lever and Valiela 2005). These findings show that even in a eutrophic environment, sediments can still be deficient in nutrients, thereby affecting BMA biomass and the community composition.

Since the BMA did show a significant different between stations and bioassay date, spatial variability was observed in the biomass with significantly different pigment concentrations measured between Stations 1, 2, and 3. The chlorophyll *a* concentration was lowest at Station 1 (2.08 mg m^{-2}) and measured highest (480 mg m^{-2}) in Station 3. However, the amount of chlorophyll *a* measured in these three stations over the course of this study compares to the amount of chlorophyll *a* measured in these same stations from another study conducted previously (Chapter 2) where the sediments were not nutrient amended. In the previous study, Station 1 had the least amount of porewater with a sediment water content averaging 10.62% and Station 3 averaged the highest at 24.89%. For the purposes of this study, it was assumed that the dissolution rate of the fertilizer stick was the same at all three stations throughout the bioassay period because this rate was not measured. It can be argued that the sediment water content would have significantly affected the dissolution rate of the fertilizer stick. A higher rate of dissolution would be expected in Station 3 compared to Station 1 due to the higher porewater content, which would have provided a greater amount of nutrients available for uptake by the BMA community. This may explain the significant difference in biomass between the three stations with Station 3 having the highest absolute abundances.

The difference in sediment water content between the three stations also indicates

a difference in the sediments ability to retain moisture, possibly due to a more well sorted sandy sediment for Station 1 compared to Stations 2 and 3, where poorly sorted sandy sediment would allow more moisture retention. The well-sorted sandy sediment would allow for the nutrient pulses in the porewater to percolate deeper into the sediment removing them from the photic zone where the BMA are predominant. The Tagus estuary had chlorophyll *a* concentrations in the upper 2 mm of the sediments from 28.5 mg m^{-2} to 101 mg m^{-2} (Cartaxana et al. 2006). In the sandy sediments of the Tagus estuary fucoxanthin ranged from 9.9 mg m^{-2} to 41.6 mg m^{-2}, zeaxanthin ranged between 0.06 mg m^{-2} to 0.44 mg m^{-2}, and chlorophyll *b* ranged from 0.00 mg m^{-2} to 0.28 mg m^{-2} (Cartaxana et al. 2006). They argued that the sandy sediments in the Tagus estuary were lower in benthic microalgal biomass compared to the muddy sediments because of de-watering. The sediments at East beach were fine grain to coarse grain sand. This sediment type would provide a greater porosity and higher permeability allowing for faster de-watering of the sediments that contained more coarse grain sand, which may explain the lower percent water content in Station 1. The other diagnostic pigment concentrations (fucoxanthin, zeaxanthin, chlorophyll *b*, and bacteriochlorophyll *a*) measured at East Beach were higher overall to the concentrations found in the Tagus estuary, Portugal. Fucoxanthin concentrations ranged from 0.00 mg m^{-2} to 64.59 mg m^{-2}, zeaxanthin ranged from 0.00 mg m^{-2} to 6.80 mg m^{-2}, chlorophyll *b* ranged from 0.00 mg m^{-2} to 4.04 mg m^{-2}, and bacteriochlorophyll *a* ranged from 0.00 mg m^{-2} to 32.59 mg m^{-2}. However, in contrast another study in the Eden estuary, Scotland, chlorophyll *a* measurements showed a positive correlation with de-watered sediments (Perkins et al.

2003). As the sediment was de-watered after a 6 hr emersion period, the concentration of chlorophyll *a* at the subsurface enriched the top layers thereby increasing the bulk density of the sediments. They also determined that sandy sediments normally have lower benthic microalgal biomass than finer grain sizes (Perkins et al. 2003). These two factors, grain size and moisture content, may explain why the biomass at Station 3 was greater than Station 1.

A temporal pattern as well as a spatial pattern was observed with the lowest pigment concentrations measured in Aug. 04 while the highest concentrations were measured in Feb. 05. Because there was a significant difference between the experimental dates, each station was analyzed to determine how the spatial variability affected the temporal variability during this study. At all three stations, all the pigments measured higher in Feb. 05 and/or Dec. 04. and lower in Aug. 04 and/or Oct. 04. Interestingly, the fucoxanthin to zeaxanthin ratio measured highest in Aug. 04 at 6.57, followed by Feb. 05 at 4.14, Dec. 04 was 3.38, and finally Oct. 04 was 3.05. These findings show a definite seasonal pattern with BMA total biomass greater in the winter months. Not every estuary shows this same pattern. The Colne estuary did not show any distinct seasonal pattern with BMA (Thornton et al. 2002). The chlorophyll *a* averaged from 18.8 mg m^{-2} and 119.9 mg m^{-2} for a study that was done on four sites on the estuary (Thornton et al. 2002). This is lower than the average total chlorophyll *a* found at East Beach, with averages ranging from 2.12 mg m^{-2} up to 253.53 mg m^{-2}. One reason would be the difference in sediment characterization between the two estuaries and the depth from which the biomass was measured. For example, the top 3-mm of the

sediments was measured at East Beach and the top 5-mm was in the Colne estuary. If a significant amount of biomass was found in depths greater than 3-mm in the sediments at East Beach, then the biomass measured was underestimated. The sediments at the Colne estuary are predominantly clays and silts while the sediments at East Beach very fine to coarse sand. Finally, nutrients (ortho-phosphate, nitrate, nitrite, ammonia, and silicate) along the East and West Bay are much lower than the nutrients measured in the rest of bay, ortho-phosphate and nitrate concentrations were inversely correlated to salinity at the station in Trinity Bay, and nitrate is not correlated to salinity at East and West Bays (Santschi 1995). He discovered that nutrients in Galveston Bay show a seasonal signal with concentrations dropping during the summer months because of increased denitrification, which could lead to a decrease in nutrients available for uptake by benthic primary producers within the top 3-mm of the sediment.

Another reason for the disparity of biomass between sampling dates, could result from grazing pressure. Grazing pressure could be greater during the summer than during the winter, which would result in higher biomass found in the winter months than during the summer months. Densities of meiofauna, dominated by nematodes followed by copepods, and microalgae were measured in several salt marsh sites in Galveston Bay (Wardle et al. 2001). Wardle examined the mean densities between interculm *Spartina alterniflora* and interplant areas. He found that the mean densities of the meiofauna, while not statistically different, were higher in the interculm areas and no statistical difference in chlorophyll *a* values measured between the two habitats. Hillebrand et al. (2000) study found that grazers were not that effective in an area of low nutrient and low

biomass, but stimulated grazing under the nutrient enrichment experiment. This same study also found that grazing pressure also reduced the variability of the BMA and the community structure. However, grazing pressure was not measured during this study. The incubation artifact observed in Station 2 for pigments zeaxanthin, chlorophylls a and b showed that these sediments were significantly different inside the core tubes compared to the sediments outside. In each case, the affected pigments were significantly higher in the CNT indicating that these values could be artificially elevated. Several causes could be attributed to the elevation. One reason for this artifact could be due to the exclusion of surface deposit feeders. Surface deposit feeders meet their metabolic requirements on intertidal mudflats because of the benthic microalgae (Lopez and Levinton 1987). The sediments at Station 2 had definite mat formation compared to the other two stations, even though the biomass was quantified at higher concentrations at Station 3. Another cause could be the nutrients within the sediments at Station 2 were sequestered within the core tubes. The core tube liner prevented the advection of nutrients outside the core tube. This did not occur at Station 1 because these sediments were sandier in texture allowing the nutrients to penetrate deeper into the sediment. The sediments in Station 2 contained more clay and silt and therefore could retain more nutrients because of the moisture retention.

In summary, an increase in nutrients could shift BMA community structure, possibly altering the biomass and in turn affecting the energy transfer the higher trophic levels. The five diagnostic photopigments were significantly affected by station and bioassay date, but was not significantly affected by nutrient enrichment, indicating that the sediments are not nutrient limited. These pigments also displayed spatiotemporal variability with higher concentrations measured in Station 3 compared to Station 1 and in the sediment cores analyzed in Feb. 2005 and Dec. 2004 compared to Aug. and Oct. 2004. The pigment concentrations were significantly different with respect to station and all the pigments except bacteriochlorophyll *a* measured highest in Station 3, and measured lowest in Station 1 corresponding to the percent water content, which averaged higher in Station 3 than in Station 1. The interaction between nutrient treatment and station location showed an incubation artifact, with results concluding that zeaxanthin, chlorophylls *a* and *b* were artificially elevated in the sediments at Station 2. The results from this study show that BMA biomass are not nutrient limited, therefore, should more nutrients enter Galveston Bay BMA biomass would not increase or show a shift within the community.

CHAPTER VI

CONCLUSION

These data suggest that should climate and environmental changes take place within the next decade with increases in temperature, salinity, and nutrients likely, the BMA community at East Beach, Galveston, Texas would adapt quite readily to these changes. An increase in salinity by 10%, which is foreseeable, would raise the average annual salinity of Galveston Bay from 25 to 27.5 and would not result in a reduction in BMA biomass or a significant shift in the community composition. Diatoms and cyanobacteria would still be the two most abundant groups within the sediments. Even though other areas with hypersaline conditions have observed a community with cyanobacteria as the most abundant algal group, it is unlikely that Galveston Bay would see an increase in salinity of that magnitude within the next decade. Local temperatures are not likely to increase to such an extent as to have a major impact on the BMA community composition. However, climatic conditions could change that could affect the amount of rainfall in the area, which would influence the amount of sediment water content and nutrients at East Beach. A decrease in rainfall could mean a more arid environment especially at East Beach, which showed a decrease in biomass in the more arid stations. The shift may be due to sediment water content and not necessarily an increase in salinity. Anthropogenic influences could also affect the area's hydrology thus altering the sediment water content and nutrients. This environmental change would not shift the BMA community dominated by diatoms and cyanobacteria to one

dominated by cyanobacteria alone. Thus, the higher trophic levels should not see a reduction in the more palatable food source.

Galveston Bay is a shallow dynamic estuary where the freshwater inflow varies with the seasons with higher inflow measured in the cooler winter months and spring. This results from an increase in precipitation, which increases runoff and in turn increases the nutrient concentration into the Bay and reduces the salinity. Therefore, during the cooler winter months and spring when precipitation is greatest, the salinity will decrease in the sediment porewater and overlying water column as the nutrient concentration will increase. These changes in the cooler months may show an increase in the absolute abundance of green alga. The community composition would not change from a diatom-dominated community to a cyanobacteria-dominated community as all four studies indicated that cyanobacteria remain constant and the diatoms showed greater variability with higher abundances in the winter compared to the summer. However, with the decrease in salinity during this time, green alga and may increase in absolute abundance, since it prefers cooler temperatures and lower salinities. The phototrophic bacteria would increase in absolute abundance with cooler temperatures, but this group does not appear to be significantly influenced by salinity. Total biomass as characterized by chlorophyll *a* would increase in absolute abundance during the winter months but may decrease somewhat because of the preference for higher salinities. The increase in nutrients with an increase in freshwater inflow would stimulate the absolute abundances of all the algal groups. During the summer months, which would increase the salinity, decrease the nutrients in the porewater, and decrease the sediment water

content, the community composition would not shift with an increase in cyanobacteria but would decrease in abundance. Spatial and temporal variability is demonstrated by the BMA at East Beach at scales of cm^2 and m^2 and demonstrated patches within patches. However, while salinity does significantly affect BMA biomass at the extreme salinities (0 or >41), nutrients nor salinity significantly impact BMA biomass or community structure at East Beach and are not the major driving forces behind the variability.

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