The Thesis Committee for Aubrey Rain Lashaway

Certifies that this is the approved version of the following thesis:

Water Quality and Eukaryotic Plankton Dynamics in the Mission-Aransas Estuary, Texas from 2011-2012

APPROVED BY

SUPERVISING COMMITTEE:

_____

Deana L. Erdner, Supervisor

_____

James W. McClelland

_____

Tracy A. Villareal

Water Quality and Eukaryotic Plankton Dynamics in the Mission-Aransas Estuary, Texas from 2011-2012

by

Aubrey Rain Lashaway, B.S.; M.S.

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Marine Science

The University of Texas at Austin

August 2013

Dedication

I dedicate this work to my loving parents (Richard and Angela Lashaway) and sister (Vada Lashaway). Thank you for believing in me and for your constant encouragement. I won the family lottery! I was born with the winning ticket; and a major reason I was able to live out my childhood dreams is because of you. Love forever and always, Aubrey.

Acknowledgements

Eternal thanks go out to the following individuals for their help and support. I would like to begin by thanking my Lord and Savior Jesus Christ who gave me strength in the midst of doubt, stress, and overwhelmingly long nights. My parents who have shown endless support and who have been full of hopeful words and to the rest of my loving family. Next, I would like to thank the Kotsis family (Manny, Brenda, Ted, Elaine, Mike, Jill, Zachary, and Eleni), my second family, for encouragement, love, yummy Greek food and wonderful family festivals. I would like to acknowledge my committee members, Drs. Deana Erdner, Jim McClelland, and Tracy Villareal for indispensable guidance and support. Thanks to Dr. Jennifer Moon, Sara Pelleteri, Dana Sjostrom, Lynn Ulch, Linda Fuiman, Jill Smith, Colleen McCue, Colbi Gemmell, Courtney Holloway, Dr. Ken Dunton, and Julie Findley for giving me the opportunity to teach and for mentoring and nourishing my love for science education. Also, thank you to Krystal and Lomenick Cartwright for allowing me to become a part of their daily lives in Port Aransas, Texas. It has been a wonderful experience to tutor Lomenick and you have made me feel just like one of the family (P.S. Krystal, I will miss your wonderful dinners!). I would like to recognize my лучшая подруга Caitlin Bumford and to thank her for moving back to Texas on Groundhog's Day 2011! Caitlin has been an integral part of my life since meeting her at The University of Michigan in 2003. We have endured, persevered, and shared countless numbers of important life milestones together, and now we share two Alma Maters! The city of Austin, Texas also yielded a number of caring and helpful friends (Laura Burrus, Julie Wilke, Jennifer Jackson, Jessica Parker,

Tricia Johnson, Shawn Izadi, Dr. Damon Talley) as well as, many wonderful memories. I want to thank my dear adult Bible fellowship friends (Mr. Joe Fahl, Mrs. Ginny Fahl, Melody Perez, Carla Estrada, Jebp Hafey, Jonathan Storey, Lisa Brumit, Mike Brumit, Adam Garcia, Jenna Watson, Laura Pearson, Julia Koch, Katrina Larson, Cullen Moody, Israel Jaime, Lisa Goben) at Yorktown Baptist Church in Corpus Christi for helping South Texas feel like 'home' and for introducing me to the Corpus Christi Municipal Band. Thank you to Rae Mooney and Lindsay Pollard for guidance in every aspect of this project, for helping me learn all of the sampling protocols, and for piloting the monthly estuarine sampling trips on the C-Hawk and to the river sites; Cammie Hyatt for teaching me everything about zooplankton; Dr. Jena Campbell for helping me troubleshoot the flowCAM; Dr. Edward Buskey for allowing me constant use of his laboratory and equipment; Drs. Lindsay Scheef and Jianhong Xue for indispensable knowledge and help with coding multivariate statistical analyses in R and MATLAB; Catalina Cuellar for guidance with the t-RFLP protocol; Dr. Chang Jae Choi for guidance in the Erdner laboratory; and to Dr. Jim McClelland, Dr. Christopher Shank, Dr. Tracy Villareal, Cecil Harkey, Katie Swanson, Qiyuan Liu, Kimberly Bittler, Frank Ernst and Tal Herman-Aizen for kind assistance in the lab, field, and with data analysis; Betty Lopez (for lovely birthday surprises!) and the rest of the custodial staff for keeping our work environment neat and clean; Jamey Pelfrey, Heather Herrick, Johnny Reyes, Patty Webb, Liz Dehart, Veril Barr, Jim Aschmutat, and John Shedd for helping with registration, housing, maintenance, library book reservations and for computer and classroom technology assistance. Funding for this project was provided by the MA-

NERR Graduate Research Assistantship and a University of Texas Research Grant. Lastly, but not least, my UTMSI cohort (Qiyuan Liu, Shuting Liu, Claire Griffin, Ellen Knapke, Kellie Hoppe, John Mohan, Catalina Cuellar, Eva Salas) for all of the group study sessions!

Non tam pares quam superiores: Each successive year must not be equal to, but better than, before. Hail to the Victors! Go Blue.

The Lord Is My Shepherd: A Psalm of David
Psalm 23: 1-6 ESV

"[1]The Lord is my shepherd; I shall not want. [2]He makes me lie down in green pastures. He leads me beside still waters. [3]He restores my soul. He leads me in paths of righteousness for his name's sake. [4]Even though I walk through the valley of the shadow of death, I will fear no evil, for you are with me; your rod and your staff, they comfort me. [5]You prepare a table before me in the presence of my enemies; you anoint my head with oil; my cup overflows. [6]Surely goodness and mercy shall follow me all the days of my life, and I shall dwell in the house of the Lord forever."

Abstract

Water Quality and Eukaryotic Plankton Dynamics in the Mission-Aransas Estuary, Texas from 2011-2012

Aubrey Rain Lashaway, MSMarineSci

The University of Texas at Austin, 2013

Supervisor: Deana L. Erdner

As the base of the food chain, plankton affect the cycling of nutrients and organic matter within ecosystems and support production at higher trophic levels. The overall goal of this project was to examine how natural water quality fluctuations, such as changes in nutrients, temperature, and salinity, influence estuarine plankton community structure. To achieve this, I examined water quality as well as the diversity and biomass of eukaryotic plankton communities in a subtropical estuary located within the Mission-Aransas National Estuarine Research Reserve. The sampling sites included in this study consisted of three bay (Copano Bay West, Copano Bay East, Aransas Bay) and two river (Mission River Estuary, Aransas River Estuary) estuary sites. Water samples were collected monthly at the five sites from September 2011 to August 2012 and analyzed for a suite of abiotic and biotic variables. Eukaryotic plankton diversity and community

structure were evaluated by using the terminal restriction fragment length polymorphism (t-RFLP) method.

Although a narrow salinity gradient was present at the sampling sites, seasonal changes in water quality conditions were observed. In the river estuaries, water quality parameters defined three significant temporal periods at the Mission River Estuary site, whereas only one month differed at the Aransas River Estuary site, indicating little seasonal variation. The Copano Bay sites exhibited a seasonal pattern consisting of four periods, marked by a distinct fall (October, November, December) grouping, while Aransas Bay showed a seasonal pattern consisting of three periods, with no fall group. Even though the water quality conditions define different monthly groupings in the bay and river estuary sites, the same parameters – DOC, TDN, and pH – are the strongest drivers of the patterns at all of the sites.

Seasonal and spatial distinctions in the Mission-Aransas Estuary eukaryotic plankton community composition were determined using t-RFLP. Frequent shifts in composition were apparent across samples collected at approximately bi-weekly to monthly intervals. There were significant differences (ANOSIM, $p < 0.05$) in community composition between the Aransas and Mission River Estuary and Aransas Bay sites. Although the overall ANOSIM tests show significance between eukaryotic plankton communities monthly and between the bay water quality periods, none of the pairwise comparisons were significantly different. However, the ANOSIM R-statistic for the monthly pairwise comparisons displays a general increasing trend over time from

sampling, further highlighting the dynamic nature of the microbial eukaryotic assemblage within sites.

Table of Contents

List of Tables

List of Figures

List of Figures (continued)

List of Figures (continued)

Introduction

Estuaries are amongst the most biologically productive natural ecosystems in the world (Schelske and Odum 1962, Baban 1997, Wilson 2002, Leandro *et al.* 2007). Estuaries are partially enclosed bodies of water along coastlines where fresh water and salt water meet and mix. They act as a transition zone between oceans and continents. (Pritchard 1967). Within an estuary there are usually three overlapping zones: an open connection with the ocean where marine water dominates, a central area where saltwater and freshwater mix, and a tidal river zone where, typically, freshwater predominates. These systems support a great variety of marine resources, house an abundance of both freshwater and marine animals (e.g. fish, crustaceans, and molluscs), and are of great economic importance to local and global human populations. Although offshore areas such as the Grand Banks support the largest single fisheries in the world, estuaries are more important to total world fishery yields (Houde and Rutherford 1993). In the United States alone, nearly two-thirds of the three million tons of fish and shellfish harvested annually come from estuaries (Lellis-Dibble *et al.* 2008). Due to the many goods and services estuarine systems provide, many of the world's largest cities (e.g. New York and London) were founded near estuaries, and about 60% of the world's population now lives along estuarine and coastal environments (Canuel *et al.* 2012). Consequently, the health of many estuaries is threatened by human alteration of their hydrology, sprawling urbanization and pollution from industries that have taken a heavy toll on estuarine ecology (Barbier *et al.* 2011).

In most coastal ecosystems, phytoplankton dominate ecosystem primary production (Cloern 2001) and are of fundamental importance in supporting the surrounding planktonic consumers in estuarine food webs. Plankton communities occupy an essential role in aquatic ecosystems and have been a central focus for aquatic microbial scientists for decades, yet their community composition, rich diversity, and mechanisms that determine their patterns are not well known in estuarine ecosystems (Riley 1976, Sanders 1987, Dustan and Pinckney 1989, Dauer *et al.* 2000, Cowlishaw 2004, Morris *et al.* 2002, Fuhrman *et al*. 2006, Eiler *et al.* 2009). Estuarine plankton dynamics and distribution can vary substantially over hours, days, seasons, years, and decades (Marques *et al.* 2007b, Molinero *et al*. 2008) and are challenging to study due to the ecological complexities of the system (i.e. geomorphology, tidal influences, salinity ranges) (Dauer *et al*. 2000). Traditional methods (i.e. light microscopy) used for plankton composition studies are difficult to employ efficiently because they require significant time, resources, and manpower (Culverhouse *et al.* 2006). Recently, the development and use of molecular approaches in marine plankton studies has increased our understanding of the diversity of planktonic prokaryotes and eukaryotes, allowing for relatively fast and inexpensive data collection (e.g. Vigil *et al.* 2009). In addition to revealing plankton species diversity, molecular techniques can contribute to our understanding of the microbial food web and the processes that predict plankton size structure under given environmental conditions (Diez *et al.* 2001). Spatiotemporal variability in coastal plankton communities has important consequences for the structure

and function of ecosystems, including nutrient cycling, the fate of primary production, and food web dynamics (Brett and Goldman 1996).

Compared to the ecology of large organisms where community diversity research has been ongoing for nearly a century (Keddy and Weiher 2001, Eiler *et al*. 2011), microbial ecology has only just begun to explore theoretical frameworks to predict changes in the microbial world (Horner-Devine *et al.* 2004, Prosser *et al.* 2007). Now, microbial ecologists are exploring whether microbial communities are distinct in different habitats, and if environmental metrics associated with microbial communities show explainable patterns (Horner-Devine *et al.* 2007, Prosser *et al.* 2007). In other words, spatiotemporal patchiness of plankton due to biotic mechanisms (such as grazing and competition for space or food) and abiotic mechanisms (such as salinity, wind, or temperature) is driving the development of system models for explaining plankton community diversity (Pinckney and Dustan 1990, Pinckney *et al*. 1998, Diehl *et al.* 2002, Blanchette *et al.* 2007).

Historically the majority of estuarine and lagoonal plankton studies have occurred in temperate ecosystems including areas such as Chesapeake Bay, San Francisco Bay, and areas of northern Europe (Boynton *et al.* 1982, Harding 1994, Jonge *et al.* 1994, Cloern 1996, Conley 2000, Kemp *et al*. 2005). The Chesapeake Bay, for instance, is a plankton based ecosystem in which the zooplankton act as trophic intermediates between the very productive phytoplankton and bacteria, and higher trophic levels, including many economically important fish and shellfish species (Odebrecht *et al.* 2005).

Currently, there is a growing interest in the plankton dynamics occurring in warm-water ecosystems, and more studies have occurred as noticeable anthropogenic alterations have arisen in these areas (Turner and Rabalais 2003, Brodie and Mitchell 2005). The Mission-Aransas estuary is considered a subtropical estuary. Subtropical Gulf of Mexico estuaries, as compared to temperate estuaries, lack strong seasonal changes in temperature (generally including 3-5 months of average temperatures at or exceeding 28^0C), have high solar radiation, and shallow depths (Montagna and Kalke 1992, Buskey 1993). Thus, subtropical systems have reduced seasonal patterns of primary production (Koch *et al*. 2012), and the ability to detect changes in the plankton, directly linking them to pertinent environmental drivers, is especially difficult (Montagna and Kalke 1992). Along the Texas coast there are seven major estuarine systems, all of which are isolated by barrier islands from the Gulf of Mexico (Longley 1994). The barrier islands establish essential estuarine and lagoonal habitat for many commercially important fisheries (e.g. finfish and shellfish) that ultimately depend on plankton communities for survival (Steele and Bert 1994). These systems follow a decreasing freshwater inflow gradient from the border of Louisiana to Mexico (Longley 1994). The Mission-Aransas Estuary is considered a neutral estuary, lying in the central portion of the Texas Coastal Bend. This estuary is located at the boundary where precipitation surpasses evaporation to the north and evaporation surpasses precipitation to the south (Pulich and Blair 1997), and has an average inflow rate of approximately 10^8 m^3 y^{-1} (Montagna *et al.* 2011). However, the plankton dynamics, community structure and corresponding environmental drivers of sub-tropical Gulf of Mexico estuaries are little-

studied, especially along the Texas Gulf Coast (Buskey 1993, Livingston 2001 and 2003).

As Texas coastal populations continue to grow, it becomes increasingly difficult to locate estuarine ecosystems with minor structural and functional impacts. However, the National Estuarine Research Reserve (NERR) system, a network of twenty-eight reserves representing different biogeographic regions of the United States, establishes areas for long-term research, water-quality monitoring, education and coastal stewardship. The Mission Aransas National Estuarine Research Reserve (MANERR) is an 185,708 acre region of south Texas composed of terrestrial, wetland and marine environments (Morehead *et al*. 2007). Due to low population density surrounding the Reserve , it is located on one of the most pristine areas of Texas coastline, and the two main rivers that flow into the Mission-Aransas Estuary (Mission and Aransas Rivers) are dam free (Johns 2004). This makes it an ideal site for studying the baseline or 'natural' function of estuaries.

The Mission-Aransas estuary is a physically, chemically, and biologically diverse brackish water habitat located within the MANERR. One of the central goals of the MANERR has been to understand the influence of plankton communities on the structure and function of the Mission-Aransas Estuary (Morehead *et al*. 2007), as they are the key trophic link between nutrient inputs and higher trophic levels (Hays *et al*. 2005). In addition, they are valuable indicators of environmental conditions (Beaugrand 2004, Bonnet and Frid 2004), since they respond directly and sensitively to many physical, chemical and biological changes that occur in estuarine ecosystems (Leandro *et al.* 2007).

The overall goal of this project is to examine how natural water quality fluctuations, such as changes in nutrients, temperature, and salinity, influence the Mission-Aransas Estuary plankton community structure. The hypotheses of this study are three-fold:

(**i**) there is water quality structure at the Mission-Aransas Estuary sampling sites seasonally and spatially,

(**ii**) there is seasonal and spatial distinction in Mission-Aransas Estuary eukaryotic plankton communities, and

(**iii**) eukaryotic plankton communities are correlated with water quality conditions.

This study will augment previous and ongoing plankton process-oriented studies within the Mission-Aransas Estuary and will help to develop a clearer understanding of abiotic and biotic controls on plankton communities in sub-tropical estuaries.

Materials and Methods

Study Sites

Mission-Aransas National Estuarine Research Reserve

The Mission-Aransas National Estuarine Research Reserve (MANERR) is relatively young, inaugurated in May of 2006, and is one of the most pristine estuarine systems in the United States (Evans *et al*. 2012). The Mission-Aransas estuary (**Figure 1**), located within the MANERR, is a relatively shallow (0.6-3m) subtropical estuary that is typical of the Western Gulf of Mexico. It is fed by the Mission and Aransas Rivers and connected to the Gulf of Mexico by an inlet at Port Aransas. In addition, Aransas Bay is hydraulically connected to San Antonio Bay, which receives freshwater inflow predominantly from the San Antonio and Guadalupe Rivers (Bishop 2012). Water residence times due to low elevations and low freshwater inputs can be as long as 3 years during average weather conditions (Armstrong 1982) for the lower river reaches and the bays. However, during large storm events water in the estuary is exchanged very quickly (Mooney and McClelland 2012). There is generally a large salinity gradient within the system, and the restricted inlet at Port Aransas means that large freshwater inputs tend to be retained within the system for long periods of time. Generally, evaporation exceeds precipitation in this area. Armstrong (1982) estimated the average precipitation as 88.6 cm $year^{-1}$ and the average evaporation as 151.3 cm $year^{-1}$. Further, the MANERR provides significant infrastructure in terms of continuous monitoring for a variety of physical and chemical variables, as well as a regular biological monitoring program. All of these characteristics make it an excellent study site.

Mission and Aransas River Estuaries

The lower reaches of the Mission and Aransas Rivers are tidally influenced and served as the two riverine sampling sites of this study (**Figure 1**). The Mission River runs southeast to its mouth on Mission Bay, an inlet of Copano Bay. The Aransas River flows generally south and has a highly winding course, also entering Copano Bay. Estuarine conditions in Copano Bay and the lower reaches of the rivers vary widely in salinity and hydrologic condition, depending on the frequency and magnitude of regional rain events, with freshwater residence times that vary dramatically between low and high flow. Stream flow in the Mission and Aransas Rivers is generally low with episodic rainfall driving a few large export events each year. Johnson (2009) concluded that the freshwater residence times in the tidal reaches of the Mission and Aransas Rivers can be several months. The Mission and Aransas watersheds differ in their size, land use, and land cover characteristics. The Aransas watershed drains 2,146 km^2 with the majority of land use land cover as cultivated crops. In contrast, the Mission watershed drains 2,675 km^2 with the majority of land use land cover as shrub land.

Sample Collection

Water Quality

Samples for all analyses were collected at five sites (**Figure 1**) in the Mission-Aransas Estuary: Aransas River Estuary (ARE; 28.0750N, -97.2204W), Mission River Estuary (MRE; 28.1850N, -97.2127W), Copano Bay West (CW; 28.0502N, -97.1203W), Copano Bay East (CE; 28.0756N, -97.0204W), and Aransas Bay (AB; 27.5847N, -

97.0143W); the latter three sites are part of the System-Wide Monitoring Program (SWMP) in the Mission-Aransas Estuary.

A monthly sampling program of the SWMP sites was conducted on board the small boat *C-Hawk* from September 2011-August 2012; additional sampling was conducted in between the regularly scheduled trips, whenever a large rainfall event occurred. Riverine sites were accessed via bridge locations at each river. Water temperature, salinity, pH, dissolved oxygen concentration, and turbidity were measured with a Sonde 6600V_2 (YSI).

Nutrients

Inorganic and organic nutrients were measured at each site using a standard operating procedure for all NERR systems. At each station, two water samples were collected using a Van Dorn Sampler. Two 10mL sub-samples from each Van Dorn sample were collected and filtered on site using a hand syringe and ~0.45µm mixed cellulose ester membrane filter. Samples were stored in a 15mL capped tube on ice while in the field and frozen on return to the laboratory, for no more than 30 days, until analysis. Inorganic nutrients including nitrate + nitrite, silicate, ammonium, and soluble reactive phosphorus were measured using a SEAL QuAAtro AutoAnalyzer.

Organic nutrient samples were divided into dissolved and particulate fractions. In general, particulate organic matter was defined as organic matter that cannot pass through a filter with a pore size of 0.7µm; whereas dissolved organic matter was defined as organic matter that can pass through a filter with a pore size of 0.7µm. Water was

collected using a Van Dorn sampler and placed into two 1L polycarbonate bottle on ice while in the field. In the laboratory, each water sample was filtered through a pre-combusted glass fiber filter (GF/F). The filtered water was frozen for determination of dissolved organic matter, and, the filters were dried for the determination of particulate organic matter. Dissolved organic carbon and nitrogen were measured using a Shimadzu DOC/TN Analyzer. Particulate organic carbon and nitrogen samples were sent to The University of New Hampshire for analysis.

Eukaryotic Plankton Community Composition

Water collected with the Van Dorn sampler was filtered through a ~0.45µm pore size 25mm diameter membrane filter, and the filter was then placed into 360µL of ATL Buffer (Qiagen, Inc.) in a 2mL microcentrifuge tube and stored at -80ºC until DNA extraction. A total of 81 samples were analyzed. DNA extractions, PCR amplifications, and terminal restriction fragment length polymorphism (t-RFLP) analyses followed the procedure described in Vigil *et al.* (2009) with slight modifications. At the time of DNA extraction, 0.5mm diameter zirconia/silica beads were added to each tube, and the mixture was vortexed at maximum speed for approximately 1 minute to disrupt cells. The bottom of the tube was punctured with a heated 20G needle, and the lysate was separated from filter debris and beads by centrifugation at 2000 x g for 2 minutes at 25ºC into a clean 2mL microtube. Subsequent DNA extractions steps followed the manufacturer's procedure using the DNeasy Blood and Tissue kit (Qiagen).

Polymerase chain reaction (PCR) amplification of DNA fragments was performed in an Eppendorf Mastercycler using the fluorescently labeled forward primer Euk-A-FAM (5'-56FAM-AAC CTG GTT GAT CCT GCC AGT-3') and the fluorescently labeled reverse primer Euk-570R-HEX (5'-5HEX-GCT ATT GGA GCT GGA ATT AC-3') (Vigil *et al*. 2009). Each 25µl reaction contained 2.5µL of 10×PCR buffer solution (Takara), 2.0µL dNTP mixture (Takara), 1.25µL of each primer, and 0.25µL of Taq polymerase (Takara), to which 2µL of extracted DNA was added. All samples were amplified using the following protocol: initial denaturation at 95°C for 3 min, 35 cycles of amplification (95°C for 60sec, 60°C for 60sec, 72°C for 60sec), and a final extension at 72°C for 10min. Triplicate reactions were run in parallel for each sample, and successful amplification was verified by agarose gel electrophoresis of 5µL of each reaction. Successful reactions were combined, purified, and concentrated using the MinElute Reaction clean-up kit (Qiagen). One-half of the purified DNA (10µl) was digested using the restriction enzyme Mnl I (New England Biolabs). All restriction enzyme reactions were incubated at 37°C for approximately 4 hours. DNA sample concentrations (ng/µL) were quantified using a GE NanoVue spectrophotometer. For t-RFLP analysis, samples containing 60ng of digested DNA in a 4µL volume were sent to The University of Texas at Austin ICMB Core Facilities DNA Sequencing Laboratory for fragment analysis.

Raw t-RFLP data were analyzed using GeneMarker v. 1.70 (SoftGenetics, State College, PA). Peak identification and sizing were performed by GeneMarker, using a minimum peak height threshold of 5 RFU and t-RF fragment size of 50 bp. The peak

height, peak area, normalized peak area (individual peak_area/total_peak_area), and bp length of each t-RF were extracted for all samples. For each sample, individual t-RF peak height was normalized to the total peak area of the sample, to allow for comparisons across samples. T-RFs with a relative abundance of less than 5% were considered rare and omitted from the data analysis to eliminate potential errors and focus on the dominant members of the communities (Nazaries *et al*. 2013). Reproducibility of the t-RFLP patterns was evaluated by comparing the patterns derived from the September 2011 Mission River Estuary site, for which DNA extractions, PCR amplifications, and t-RFLP analyses were conducted in duplicate (Hartmann and Widmer 2008).

Statistical Analyses

In order to determine if distinct eukaryotic phytoplankton assemblages occurred at each sampling site, eukaryotic phytoplankton community composition (t-RFLP data) was visualized using a heatmap and compared using the Analysis of Similarity (ANOSIM) test. The heatmap which included all of the sampling sites and dates helps to distinguish patterns across sampling sites and allows for visual comparisons of all of the samples (Yunker *et al.* 2005, Vigil *et al.* 2009).

Terminal restriction fragment length polymorphism (t-RFLP) analysis is a strong comparative molecular technique that is frequently used to describe microbial community structure (Hartmann and Widmer 2008). The profile of a series of terminal restriction fragments (t-RFs) provides an estimate of the number of phylotypes (i.e. defined as DNA fragments of unique length) in a microbial community, and the fluorescence intensity of

each peak reflects the relative abundance of each phylotype (Vigil *et al*. 2009). However, within the same t-RF, there may be >1 phylogenetically similar species; therefore, t-RFs are commonly referred to as operational taxonomic units (OTUs). Intensity (area) of each peak was normalized as a percentage of the total peak area. The one-way ANOSIM test provides a way to test statistically whether there is a significant difference between two or more groups of samples. If the assigned groups are meaningful, samples within groups should be more similar in composition than samples from different groups (Clark 1993). The one-way ANOSIM method uses the Bray-Curtis measure of similarity (Chao *et al.* 2006). The ANOSIM test functions directly on a dissimilarity matrix and uses only the rank order of dissimilarity values. The distance rank is based on the rank order of dissimilarity values and allows for further insight into the within- and between-eukaryotic plankton patterns. If two groups of sampling units are really different in their species composition, then the dissimilarities between the groups will be greater than those within the groups (Chao *et al.* 2006). A p-value of less than 0.05 suggests that there is more similarity within the sampling sites than one would see by chance. The ANOSIM R-statistic describes where the most similar samples are found, either within or between comparative groups: R = 1 when the highest similarity is found within comparative groups; R = 0 when there is no relationship and comparative groups are randomly mixed; and R = -1 when the highest similarity is found between the comparative groups. Bonferroni correction was employed for the pairwise ANOSIM analyses, to adjust for multiple tests.

The relationship between water quality parameters was evaluated using Hierarchical Clustering and Principle Component Analysis (PCA). Hierarchical clustering is useful for visualizing dissimilarity among specific groups that occur from large data sets. I utilized hierarchical clustering with Ward's Method (which employs the minimum variance method, ANOVA, between samples) and Euclidean distance which is characteristic to this procedure (Dodson *et al*. 2009). A data matrix of Mission-Aransas Estuary water quality parameters was constructed to examine the similarities among river estuary (**Figure 2**) and bay estuary (**Figure 3**) sites, with each site representing a specific sample location and time in the Mission-Aransas Estuary. The water quality parameters in these analyses included temperature, salinity, pH, dissolved oxygen, turbidity, silicate, phosphate, dissolved organic carbon, and total dissolved nitrogen. The storm event was excluded from these analyses, as it did not change the orientation of the significant clusters when being included into the analysis (**Figure 4**). The significance of the clusters that are formed at each step of the hierarchical cluster analysis was shown by a scree plot. This plot has a point for where each cluster joins another, and the natural break in the scree plot determines the number of significant clusters (Digby and Kempton 1987). The same data matrix employed for the hierarchical cluster analyses was used to construct principal component analyses to examine the distribution of samples relative to water quality parameters explaining most of the variance in the matrix and to provide support for the hierarchical cluster analysis results. The points in the principal component plots are labeled with the matching colors associated with the corresponding cluster designations from the hierarchical clusters.

To evaluate if eukaryotic plankton community composition was correlated with water quality conditions, river and bay estuary eukaryotic plankton communities were grouped by environmental seasons, as described by the hierarchical cluster analyses, and evaluated using one-way ANOSIM and pairwise comparisons with Bonferroni Correction. Incorporation of community and water quality data into the aforementioned multivariate statistical analyses has proved successful for identifying the relationship(s) between key water quality and biological characteristics in subtropical estuarine studies (Wang *et al*. 2006, Aßmus *et al*. 2009).

Results and Discussion

Water Quality

During the study period, water quality generally showed similar trends, with salinity, temperature, turbidity, and pH largely overlapping among the different sampling sites (**Table 1, Figures 5, 6, 7, 8**). However, three nutrients including silicate, dissolved organic carbon and total dissolved nitrogen were consistently higher at the Mission River Estuary site. The Mission River Estuary salinity decreased from 21.0 to 7.9 ppt during April 2012 (**Figure 5**) due to the one storm event recorded during the 2011-2012 study period. This storm also impacted the Copano Bay West site, decreasing salinity from 34.3 to 13.3 ppt. However, compared to a recent study in the MANERR by Mooney and McClelland (2012), the storm event recorded in April 2012 was relatively small and had little impact on the salinity in the rest of the bay. After April 2012, salinity gradually increased, taking approximately five months to return to pre-storm conditions. The generally high salinity values amongst the sampling sites can be attributed to a severe drought that affected the region during my sampling period. Since the onset of this study, average precipitation in the Mission-Aransas Watershed has reached record lows, totaling only twelve inches of rain in 2011 (NOAA 2012), about one-third the average annual precipitation.

Observations of estuarine water quality fluctuations during an extreme dry year are not well documented. Salinity is a key water quality parameter for describing estuarine systems, and understanding the variation of salinity in estuaries under different conditions (e.g. drought, climate change, human alterations) is important for management

and understanding of the biological communities (McLaughlin *et al.* 2007). For instance, studies in the Sabine-Neches estuary (located in the northeastern part of Texas, along the Texas-Louisiana border) show that algal and fish community compositions are influenced by changing salinities, with lower species abundances during drought conditions (Bianchi 1998, Tolan 2013).

The Mission River Estuary showed the most variability amongst the sampling sites in terms of water quality. Phosphate concentrations (**Figure 9**) ranged from 0.2 to 4.0 µM and dissolved oxygen concentrations (**Figure 10**) ranged from 10.1 to 2.0 mg L^{-1} with highest phosphate concentrations measured during the storm event and lowest dissolved oxygen concentrations measured just after the storm event. Silicate concentrations (**Figure 11**) in the Mission River Estuary were on average 2.5 times greater than in Copano Bay West, Copano Bay East, and the Aransas River Estuary, and 5 fold higher than in Aransas Bay. Dissolved organic carbon concentrations (DOC) (**Figure 12**) ranged from 11.3 to 7.3 mgC L^{-1} in the Mission River Estuary and were on average 1.5 times greater than concentrations in the Aransas River Estuary and Copano Bay West, and 3 times greater than concentrations in Copano Bay East and Aransas Bay. Total dissolved nitrogen concentrations (**Figure 13**) ranged from 62.1 to 35.7 µM and were approximately 1.7 times greater than concentrations in the Aransas River Estuary and Copano Bay West, and 2 times greater than concentrations in Copano Bay East and Aransas Bay.

Temporally, river flow to the Mission-Aransas Estuary fluctuates episodically and dramatically, with high flows during wet years and low flows during dry years (Mooney

and McClelland 2012). However, this study predominantly spanned a dry year (drought), capturing only one small storm event in April 2012. Dissolved organic matter concentrations and patterns of variability were different in both rivers and the bays. On average, DOC and TDN (mostly comprised of DON as DIN concentrations are often undetectable until storm events) were higher in the Mission River Estuary when compared to all other sampling sites during the duration of this study. A recent study by Klein *et al*. (2008) indicates that MRE DOC concentrations vary depending on sampling location and reached a maximum in the estuarine river portion. Comparatively, Mooney and McClelland (2012) observed ranges in dissolved organic matter concentrations that were generally higher in the Mission River.

Short-term episodic storm events, like the April-2012 event in the Mission River, can cause rapid changes in water quality conditions. After the storm, there were short lived (~ 1 month) decreases in pH and dissolved oxygen, and a spike in phosphate. Pollutants from storm water runoff and point-source wastewater discharges contain organic materials and nutrients that contribute to consumption of dissolved oxygen. A study on the Peace River in Florida revealed that within one week of a passing storm, dissolved oxygen levels fell to below 1 mg L^{-1} (Stevens *et al.* 2006). However, the low dissolved oxygen event in the Peace River following the passage of the storm was short lived. Approximately one month later, the dissolved oxygen concentrations had returned to near-normal conditions, that was near or exceeding 4 mg L^{-1} (Stevens *et al*. 2006), comparable to what is observed in this study.

Fluctuations in nutrient concentrations, such as dissolved phosphate, nitrogen and silicate, due to storm events are also well described (Bowes *et al.* 2003, Fink *et al*. 2004, Bernal *et al.* 2005, Mooney and McClelland 2012). It has also been suggested that dissolved phosphorus, nitrogen and silicate can accumulate within watershed soils and along riparian margins throughout extended dry periods (Bhaduri *et al.* 2000, Bowes *et al*. 2003). Buildup of these nutrients subsequently washes off during the next storm event presenting a measureable increase in the dissolved nutrient concentrations within the affected estuarine system (**Figures 9, 11, 13**). In particular, pulses of dissolved organic matter can affect the functioning of the estuarine aquatic ecosystem through its influence on acidity (Eshleman and Hemond 1985, Evans *et al*. 2005), light absorbance, energy and nutrient supply. During extended, dry, warm periods, plant debris found in soil and water environments will undergo chemical reactions, decay and transformations into dissolved organic materials that are acidic in nature (Oliver *et al*. 1983). When these materials are flushed through aquatic ecosystems during a storm event, pH levels can be affected, even slightly, possibly explaining what was detected in the Mission River (**Figure 8)**.

Water Quality Structure

Although narrow environmental gradients were present at the sampling sites, seasonal changes in water quality parameters were observed. Hierarchical cluster analyses revealed three major seasonal groups amongst the river estuaries (**Figure 2**) and four amongst the bay estuary sites (**Figure 3**). The different periods identified by the water quality conditions are hereafter referred to as "environmental seasons," to

distinguish them from seasons as traditionally based on calendar months. In the river estuaries, there was a lack of temporal structure at the Aransas River Estuary site, whereas three distinct periods were defined within the Mission River Estuary (**Figure 2**). The three Mission River Estuary clusters were designated as winter/spring (December, February, March, April), summer (May, June, July), and late summer/fall (August, September, October, November). The month of January was unique, as its water quality parameters resembled the late summer/fall cluster.

At the bay estuary sites, Copano Bay East and West shared four distinct environmental seasons (**Figure 3**) while Aransas Bay was defined by three. At the Copano Bay sites, the environmental seasons included a distinct fall cluster that was absent at Aransas Bay. The four clusters of Copano Bay East and West were designated as fall (October, November, December), winter (January, February March), spring (April, May), and summer (June, July, August, September). However, the water quality parameters of Copano Bay East in March resembled the spring cluster, whereas, the environmental parameters in Copano Bay West resembled winter. The three clusters of Aransas Bay were designated as summer (July, June, August, September), fall/winter (October, November, December, January, February, March), and spring (April, May).

The three main parameters driving the river and bay estuarine environmental seasons were the same. A principal component analysis for the river estuary sites (**Figure 14**) shows that the first principal component (explaining 39.9% of the total variance) is most strongly influenced by pH, total dissolved nitrogen, dissolved organic carbon, and silicate (**Table 2**). Similar analysis of the bay estuary sites (**Figure 15**)

shows that that PC1 (42.5% of the total variance) is strongly driven by total dissolved nitrogen, dissolved organic carbon, pH, and phosphate (**Table 3**).

Generally, seasonal cycles of precipitation and river flows contribute to the spatial and seasonal variability in estuarine water quality structure. Currently, Texas is experiencing 'extreme' drought conditions, which can seriously reduce the amount of water flowing within river and between bay systems (Tallaksen *et al*. 1997). Droughts are historically common in Texas and have dramatic effects on downstream flows to the coast (Copeland 1966). Low river flows and high evaporation rates cause the shallow Texas coastal bays and estuaries to experience high salinities, low nutrient movement, and high water residence times. Nonetheless, a seasonal pattern in water quality was observed at four of this study's sampling sites: MRE, AB, CE and CW.

Estuarine hydrodynamics depend upon tides, riverine inputs and, for those estuaries located in South Texas, wind. Also, seasonal temperature and irradiance can change in predictable ways (Russel and Montagna 2007), leading to expected thermal seasonality. Freshwater exchange from the rivers to the bays was minor during this study; during drought years, the flow direction of the Mission River is either upstream or zero (no net flow), except during storm events (Tolan *et al.* 2011). Thus Mission-Aransas Estuary hydrodynamics were primarily driven by meteorological conditions and astronomical tides (Ward and Armstrong 1997). However, due to the Mission-Aransas Estuary's relatively shallow depths (0.6-3m), wind exerts a much greater influence on the estuarine circulation than do astronomical tides (Armstrong 1987, Ward and Armstrong 1997). In general, periods of upstream flow in the Mission and Aransas Rivers were

associated with relatively strong winds, (Tolan *et al*. 2011). Further, Ward and Armstrong (1997) describe that wind generated tides result in considerable exchange of water between the Gulf of Mexico and the Mission-Aransas Estuary.

The Texas coast experiences four wind seasons (Spring, Winter, Summer, and Fall; NREL 2012). In the spring (March, April, May), the coastal region of Texas exhibits the greatest thermal contrasts between the land and ocean and wind speeds exceed those from the winter (December, January, February). As spring progresses toward summer (June, July, August), wind speeds diminish and are at their lowest, until fall (September, October, November) wind speeds advance toward the cooler winter months (NREL 2012). Accordingly, the mean wind speed along the Texas coast is noticeably greater in November than in September, but still less than that of the mean wind speeds measured throughout the spring. Shideler (1984) determined that wind was the dominant process regulating daily/seasonal estuarine particle resuspension along the Texas coast. As particles are resuspended, nutrient enrichment to the ecosystem can occur (Fanning *et al*. 1982). Fanning *et al*. (1981) reported that storm related effects (such as wind) explained the changes in nutrients and sediment load to Southern Mobile Bay, Alabama.

When comparing the wind seasons to the environmental seasons (defined by the water quality parameters), the Copano Bay estuary sites exhibited a one month lag; whereas, Aransas Bay, only having three environmental seasons, showed no distinguishable difference between the fall and winter wind seasons. Similarly, the three Mission River environmental seasons showed no distinguishable difference between the

winter and spring wind seasons. Thus, the changes in wind seasons along the Texas coastline may provide partial explanation to the water quality seasonality observed amongst the Mission-Aransas Estuary sampling sites. However, the mismatch between environmental seasons and wind seasons in Aransas Bay and the river estuary sites indicates other driving forces in the seasonality, possibly due to the effects of the drought and the changes in climate. Copano Bay is predominantly a closed system, receiving considerable inputs from the Mission and Aransas Rivers during high flow events; however, Aransas Bay is connected to San Antonio Bay, which receives freshwater inflow predominantly from the San Antonio and Guadalupe Rivers, Corpus Christi Bay, which receives freshwater inflow from the Nueces River, and the Gulf of Mexico via the Ship Channel (Bishop 2012). The connectivity between these 4 systems may also influence the mixing and seasonality of Aransas Bay. The Mission and Aransas Rivers have considerably different watersheds, with the Mission River watershed draining 500 km^2 more area than the Aransas River watershed and having fewer waste water treatment plants, only 3 compared to 10 (Mooney and McClelland 2012). The Mission River estuary site also experienced a storm event during this study. These differences may be additional factors attributing to the varying seasonality of the system.

Eukaryotic Plankton Communities

One important constraint in the comparison of microbial communities is the degree of reproducibility between replicate samples. Under the working conditions of this study, t-RFLP patterns of replicate samples were highly reproducible. A comparison

of the fragment patterns (**Figure 16**) using the Bray-Curtis Similarity measure yielded a similarity of 0.87 between the two replicate Mission River Estuary samples. The Bray-Curtis Similarity index ranges from 0 to 1, with the value of 1 indicating identical OTUs in each sample. A study by Osborn *et al*. (2000) also showed high reproducibility when utilizing the t-RFLP method to investigate microbial community composition using environmental DNA samples isolated from soils. Their results showed the majority of t-RFs to be common to all profiles, with only one or two additional t-RFs observed in the replicated analyses (Osborn *et al.* 2000).

T-RFLP patterns across all sites were assembled into a single heatmap (**Figure 17**) to allow for visual comparisons among all samples. There were no OTUs that persisted at all of the sampling sites throughout the entire year. However, a number of OTUs were detected on the same sampling date at each of the sample locations (e.g. 95, 286, 378 bp). Patterns within the heatmap suggest frequent shifts in OTUs at all sampling sites over time. A comparison of Mission-Aransas Estuary samples collected at approximately bi-weekly to monthly intervals indicated rapid seasonal changes in the dominant OTUs present and considerable variations in eukaryotic community composition across sampling sites. Several OTUs (e.g. 81, 91, 282 bp) represented the most abundant taxa on one sampling date and would subsequently reach undetectable levels only a few weeks to months later. Several OTUs including the t-RF length of 85 bp were dominant multiple times in thirteen different samples throughout the estuary.

This study revealed a highly dynamic eukaryotic microbial assemblage within and among the estuarine sampling sites investigated in this study. Large and frequent

(biweekly to monthly) shifts in the dominant taxa were observed at all sampling sites. DNA fragment analyses, such as the t-RFLP method, have proven to be an appropriate approach for assessing eukaryotic community shifts and dominant species shifts in natural environments (Diez *et al.* 2001, Countway *et al*. 2005, Yu *et al.* 2005, Vigil *et al*. 2009). Large changes in the eukaryotic community composition on relatively short time scales have been previously documented. Vigil *et al*. (2009) noted rapid transitions between dominant taxa occurring on 1 to 2 week intervals, consistent with the findings of this study.

Overall, the composition of the eukaryotic plankton communities was significantly different between sites (one-way ANOSIM $p < 0.05$). Overall one-way ANOSIM tests of sampling sites ($p = 0.001$, $R = 0.088$) (**Figure 18**) indicated that differences between the sites were significantly greater than those within a site. However, post-hoc pairwise ANOSIM tests resulted in only two significant pairwise comparisons between sites (**Table 4**); Aransas Bay was significantly different from both river estuary sites (MRE and ARE).

Aransas Bay is oriented laterally and is surrounded by Redfish Bay to the southwest, Copano Bay to the west, Mesquite Bay to the northeast, and Saint Charles Bay to the north (East 2001). Although there are no major freshwater inputs flowing directly into Aransas Bay, the Aransas River and Mission River flow into Copano Bay, which flows into Aransas Bay. In addition, Aransas Bay is hydraulically connected to San Antonio Bay, which receives freshwater inflow predominantly from the San Antonio and Guadalupe Rivers (Bishop 2012). Water exchange via either or both Copano Bay or San

Antonio Bay could contribute to eukaryotic plankton communities in Aransas Bay. A study by Bishop (2012) indicated that the average Mission and Aransas River discharge was much lower as compared to the average San Antonio and Guadalupe River discharge (e.g. the Aransas River discharge averages 35 times below the average Guadalupe River discharge). Also, numerous studies have suggested that plankton dynamics and patterns are influenced by estuarine salinity gradients (Ahel *et al.* 1996, Sin *et al*. 2000, Bouvier and Giorgio 2002, Pommier *et al.* 2007). Bouvier and Giorgio (2002) observed a clear pattern of bacterioplankton across a salinity gradient in the Chesapeake Bay with certain species dominating in the lower saltwater regions and others in the upper freshwater regions, similar to the areas being studied in this system. Thus, water and nutrient contributions from San Antonio Bay to Aransas Bay as well as distance between the river estuary sampling sites and Aransas Bay may drive the differences between their eukaryotic plankton communities.

Eukaryotic plankton communities show subtle monthly structure, as the overall one-way ANOSIM test is significant ($p = 0.001$, $R = 0.1433$) (**Figure 19**). However, this structure is lost in the pairwise comparisons, as none were significantly different between sampling months in the post-hoc tests, partly due to the conservative nature of the Bonferroni Correction (**Table 5**). However, plots of the R-statistic for each pairwise monthly comparison show that the R-statistic is positively correlated with the time period between samples (**Figure 20**). A higher R-statistic indicates greater dissimilarity between the samples being compared. This suggests that when samples collected closer in time are compared, their eukaryotic plankton communities more closely resemble one

another, but subsequently become more distinct from one another with longer sampling intervals.

Seasonal plankton dynamics in subtropical Gulf of Mexico estuaries are little studied compared with temperate Atlantic Ocean estuaries. Typically, Texas estuarine systems are impacted by large rainfall events (e.g. tropical storms, hurricanes) amidst long, dry periods, and freshwater inflows tend to have little seasonality as high flow events can occur almost any time of the year (Solis and Powell 1999, Mooney and McClelland 2012). Plankton communities in these ecosystems have the ability to respond rapidly to perturbations (e.g. grazers, storm events) as subtropical waters have a warmer temperature regime with greater light availability throughout the year, providing better growth conditions (Bledsoe and Philips 2000, Philips *et al*. 2002, Murrell and Lores 2004), and contributing to the subtle temporal shifts in eukaryotic plankton communities. These characteristics combined tend to make subtropical estuarine systems less predictable than the seasonality of temperate estuaries, which experience stronger seasonality.

Mortality in estuarine food webs due to plankton grazers (e.g. larval fish, ctenophores, ciliates, copepods, oysters, viruses), changes in available resources, or avoidance capabilities generally keeps balance with production and growth, resulting in rapid turnover of subtropical eukaryotic plankton communities on the scale of hours to days (Marques *et al.* 2007b, Molinero *et al*. 2008, Strom 2008). Pelagic grazing pressures from meso- and microzooplankton generally maintain a balance with plankton production in shallow Texas estuaries (e.g. Laguna Madre, Nueces Estuary) (Buskey and

Stockwell 1993, Buskey and Hyatt 1995), but also have the potential to rapidly consume the current communities (Landry and Hassett 1982, Aberle *et al*. 2012). In shallow Texas estuaries, the mixing induced by wind and tidal water as well as the plankton community's ability to respond rapidly to predation and resource changes create more homogenous conditions and subtle spatiotemporal differences.

Correlation of Environmental Seasons and Eukaryotic Plankton Communities

Rapid reshaping of the eukaryotic plankton species composition due to short-term perturbations could help explain the results of this study and how even in the midst of drought conditions, a seasonality signal is present. To determine if the eukaryotic plankton community composition responded to changes in environmental conditions, I compared the similarity of communities grouped by the environmental seasons as defined by the water quality parameters. There were no significant differences among river estuary sampling sites grouped by environmental season (one-way ANOSIM test: $p = 0.3$, $R = 0.053$) (**Figure 21, Table 6**); but, community structuring along the environmental seasons was evident among the bay estuary sites (overall one-way ANOSIM test: $p = 0.008$, $R = 0.106$) (**Figure 22**). However, the structure was subtle, as the post-hoc pairwise tests indicated that none of the seasonal groups of communities were significantly different (**Table 7**).

It is widely accepted that estuarine ecosystems are more variable environmentally, as compared to freshwater and marine ecosystems (McLusky and Elliott 2004). Variability in plankton communities is often attributed to a combination of physical and

chemical environmental factors, which makes it seemingly difficult to diagnose which factor is shaping the community (Crump *et al.* 2004, Nemergut *et al*. 2011). Therefore, relative importance of these environmental gradients to eukaryotic plankton community structure has yet to be fully evaluated for many estuarine systems. Marshall and Alden (1990) showed that plankton assemblages in riverine stations more closely resembled other riverine assemblages than those within the corresponding Chesapeake Bay system. However, during salt intrusion events due to drought and/or incoming tidal currents, species of Chesapeake Bay plankton were observed inhabiting the riverine areas.

Relationships between species distribution and environmental parameters suggest that plankton groups adapt to these forms of environmental variations (Goncalves *et al*. 2010a). Analyses linking environmental gradients to species' presence can lead to useful tools for early detection of environmental change in aquatic ecosystems (Herrmann and Stottlemyer 1991, Painchaud *et al*. 1995, Crump *et al.* 2003, Crump *et al.* 2004). For instance, a phytoplankton study conducted on Swedish lakes recognized three types of lake conditions consistently: acid humic lakes, very acid impoverished lakes, and subarctic lakes (Fangstrom and Willen 1987). The principal component analyses used in their study allowed for a straightforward display of the locations of the lakes and the phytoplankton species along distinct environmental gradients (Fangstrom and Willen 1987). Similarly, a study completed on the Scottish Loch Lomond showed phytoplankton communities connected to environmental variables and revealed that seasonal factors in the associated variables predict changes in the phytoplankton communities (Habib *et al*. 1997). Overall, descriptions of plankton abundance and

structure have shown to be influenced by the environmental conditions present at different habitats.

Conclusions

Although environmental gradients have been clearly linked to the distribution and patterns of estuarine plankton patterns along the temperate Atlantic east coast, the importance of environmental gradients to estuarine plankton species along the Gulf of Mexico coast is not well studied. The microbial eukaryotic communities assessed in this study did not correlate clearly with the environmental seasons or water quality parameters. The one-way ANOSIM results indicated the presence of a subtle seasonality signal in the microbial community composition from September 2011 to August 2012. However, OTUs did change rapidly (e.g. heatmap results), likely responding to minor changes in the environmental conditions and/or biological interactions. Correlation between environmental seasons and microbial eukaryotic community composition was not present amongst all of the sampling sites which may be attributed to the high variability observed among samples as seen in the heatmap, the large number of samples, the lack of seasonality at some sites such as ARE, and the multiple trophic levels included in the t-RFLP analysis. Findings in this study suggest that the succession of a microbial eukaryote species from dominant to relatively undetectable over a short period of time is possible in an estuarine environment. The outcome of this study suggests that very subtle changes in water quality conditions may be sufficient to result in changes in the microbial eukaryotic community within the Mission-Aransas Estuary. However, this study was merely focusing on the bottom-up processes affecting the eukaryotic plankton communities. Top-down influences such as pelagic grazing pressures from meso- and microzooplankton (e.g. larval fish, ctenophores, ciliates, copepods), oysters and viruses

are also very important and may result in the rapid turnover of subtropical eukaryotic plankton communities.

Low river flows and high evaporation rates cause the shallow coastal bays and estuaries of southern Texas to experience high salinities, low nutrient movement, and high water residence times. Nonetheless, amidst a shallow salinity gradient, a seasonal pattern in water quality was observed at four of this study's sampling sites: MRE, AB, CE and CW. In the shallow estuaries located along the South Texas coast, the estuarine hydrodynamics largely depend upon wind as well as tides and riverine inputs. When freshwater exchange from rivers is low, the seasonal tides and wind dynamics can strongly influence the seasonality of estuarine water quality.

Water quality structure was still present in the midst of a shallow salinity gradient and driven by a series of three parameters including: TDN, DOC, and pH. These results suggest that salinity is, in fact, not the major driving factor in drought influenced water quality structure throughout the Mission-Aransas Estuary. However, salinity may be an important factor in the eukaryotic plankton community structure observed. Little eukaryotic plankton community structure was detected in this study except between Aransas Bay and both river estuary sites (MRE and ARE). Salinity differences between the river estuary sampling sites and Aransas Bay may be driving the significant differences between their eukaryotic plankton communities, as salinity differences were greatest between these sites. Thus, water and nutrient contributions from San Antonio Bay and the Gulf of Mexico to Aransas Bay may be creating a distinct gradient between these plankton communities.

Investigation of the plankton community composition occurring in the rivers and bays of the Mission-Aransas Estuary provided a description of the spatial and temporal variation in environmental conditions, and explored the relationship between the eukaryotic plankton community and water quality from September 2011 through August 2012. Because of its natural climate variability, Texas may provide a model of the changeable future conditions, which can be extreme in the forms of flood and drought, expected for other coastal areas. Furthermore, plankton can be used as indicators of climatic changes due to their rapid response to environmental changes. As higher trophic levels depend on phytoplankton and primary production, changes in species composition, size structure, and food quality could have "knock-on" effects on the estuarine shellfish and finfish populations and the biodiversity of the system Also, given current uncertainties about the necessary amounts of freshwater inflows and its effects on aquatic environmental physical and chemical variables on plankton community structure, it is crucial that we understand how different components of the ecosystems respond to these changes. While we still lack a clear understanding of the interplay of forcing factors (e.g. macro- and micronutrients, physical parameters, trophic interactions) resulting in the eukaryotic plankton community changes, this project's observations add to our understanding of the spatial and seasonal variability in subtropical eukaryotic plankton communities in a setting with little environmental fluctuation.

Data from this project may aid the efforts of resource managers and policymakers to determine the potential resiliency and response of eukaryotic plankton communities within the Mission-Aransas Estuary, and it has provided an initial study of how

fluctuating water quality parameters contribute to estuarine eukaryotic plankton community dynamics.

Table 1: Water quality data for all of the samples collected during this study. pH is unit-less and dates are given as mm.yyyy.

Collection Date	T (°C)	S (ppt)	pH	DO (mg L^{-1})	Trb (NTU)	DOC (mgC L^{-1})	TDN (mgN L^{-1})	Si (µM)	P (µM)
AB									
09.2011	28.11	40.87	8.10	5.88	9.67	2.70	21.43	71.11	0.18
10.2011	21.94	38.41	7.93	6.68	26.70	4.10	31.43	124.38	0.85
11.2011	21.13	36.09	7.95	7.24	2.57	2.90	20.00	37.75	0.24
12.2011	15.90	35.59	7.99	8.54	2.47	3.50	25.71	46.63	0.54
01.2012	15.09	35.29	8.02	8.05	0.10	3.80	23.57	50.00	0.02
02.2012	18.75	35.36	8.05	7.43	10.40	3.20	22.14	0.88	0.15
03.2012	21.17	30.38	7.98	7.11	13.83	3.10	22.86	27.55	0.18
04.2012	23.67	29.38	8.08	8.19	19.20	3.00	20.71	49.19	0.02
04.2012 (Storm Event)	22.16	28.50	8.09	6.96	14.90	3.40	22.86	25.31	0.04
05.2012	25.55	29.36	8.11	6.41	14.93	3.20	18.57	37.15	0.06
06.2012	30.55	35.30	8.22	5.76	10.30	2.30	11.43	28.16	0.01
07.2012	29.35	34.69	8.21	6.00	3.93	3.30	20.71	65.10	0.06
08.2012	30.02	39.81	8.26	5.88	8.70	2.50	14.29	46.31	0.09
CE									
09.2011	28.01	37.71	7.97	6.05	8.17	4.10	29.29	130.05	1.03
10.2011	20.90	37.81	7.98	7.33	12.00	4.60	32.14	118.24	1.11
11.2011	21.33	38.95	7.99	6.98	11.37	4.60	30.71	112.11	1.22
12.2011	16.33	37.89	8.00	8.44	12.21	4.60	32.14	95.43	1.24
01.2012	15.87	38.08	7.89	7.76	14.93	4.70	31.43	27.25	0.29
02.2012	19.25	35.94	8.01	8.18	13.73	3.90	27.86	2.11	0.40
03.2012	20.92	30.01	7.95	7.35	38.97	3.20	25.00	46.81	0.41
04.2012	23.29	27.05	8.09	8.11	6.80	3.40	21.43	80.16	0.18
04.2012 (Storm Event)	22.57	26.15	8.03	7.00	29.27	3.40	20.71	32.24	0.25
05.2012	25.39	26.35	8.03	6.71	5.77	3.50	17.86	36.18	0.25
06.2012	30.74	30.27	8.08	6.04	9.93	3.70	20.71	69.03	0.13
07.2012	29.33	29.71	8.07	6.00	6.13	3.80	22.86	112.23	0.36
08.2012	29.96	34.68	8.11	5.79	17.97	3.80	22.14	99.68	0.51
CW									
09.2011	27.97	34.24	8.02	6.00	12.73	5.20	37.14	150.89	1.12
10.2011	20.49	35.17	8.05	7.54	16.47	5.80	37.14	176.83	0.97
11.2011	20.64	37.65	8.06	7.22	5.17	5.30	37.86	147.80	1.33
12.2011	16.38	39.15	7.93	8.07	4.10	5.10	35.71	93.56	1.37
01.2012	14.37	38.99	7.84	7.90	3.80	5.10	37.14	53.97	0.31
02.2012	19.67	38.65	7.97	7.49	26.23	5.00	33.57	49.12	0.28
03.2012	20.63	34.28	7.89	6.94	12.93	4.10	30.71	20.69	0.41

Table 1: (continued)

4.2012	23.04	13.29	8.16	8.67	2.93	2.9	22.86	85.3	1.11
4.2012 (Storm Event)	22.74	13.29	8.06	7.24	17.1	4	22.14	89.31	0.63
5.2012	25.08	24.19	8	6.46	24.77	4	23.57	28.04	0.39
6.2012	30.89	28.41	8.09	5.74	14.13	4.5	28.57	114.16	0.22
7.2012	29.52	29.17	8.06	6.05	8.93	4.8	28.57	301.5	0.15
8.2012	30.05	32.9	8.05	5.95	8.2	5	31.43	132.14	0.25
ARE									
9.2011	29.1	35.01	8.2	6.7	17.4	8.8	45.71	226.89	0.2
10.2011	24.83	33.92	7.96	7.18	7.3	7.1	40	190.58	1.02
11.2011	14.1	37.61	7.92	8.43	24.1	6.4	39.29	213.18	1.44
12.2011	19.15	38.2	7.91	7.64	48.45	6.4	39.29	102.89	1.14
1.2012	16.27	38.93	7.79	8.25	13.7	6.1	34.29	59.09	0.46
2.2012	17.82	36.51	7.78	7.17	22.6	5.7	32.14	57.21	0.46
3.2012	23.43	35.36	7.9	6.94	17.8	5.4	30.71	62.21	0.37
4.2012	24.24	22.87	7.9	7.03	62.2	4.1	29.29	90.5	0.74
5.2012	25.12	21.35	7.8	6.92	30.2	4.4	32.86	71.32	0.94
6.2012	29.73	26.56	8.06	6.36	7.45	4.7	34.29	57.56	0.74
7.2012	31.07	28.51	8.13	6.25	10.55	4.7	32.86	287.07	0.34
8.2012	29.08	33.97	8.11	6.56	11.3	5.6	39.29	182.4	0.43
MRE									
9.2011	28.54	32.9	8.1	4.98	10.6	10.5	56.43	249.87	0.2
10.2011	26.44	32.38	8.19	7.82	4.15	11	55.71	347.24	0.27
11.2011	13.48	33.72	7.94	8.43	29.17	11.3	55.71	334.71	0.22
12.2011	17.28	36.58	7.93	8.18	8.13	8.1	40.71	102.02	0.7
1.2012	15.53	33.44	7.85	8.37	9.7	10.4	57.14	332.3	0.83
2.2012	17.21	26.45	8.18	10.07	9.73	8.8	46.43	94.71	1.6
3.2012	22.38	31.75	7.99	7.62	20.8	7.3	35.71	245.38	0.15
4.2012	24.4	20.96	7.93	7.5	11.3	7.9	47.14	164.07	1.07
4.2012 (Storm Event)	24.67	7.91	7.66	3.43	52.7	8	60.71	440.02	4.01
5.2012	27.86	12.72	8.29	1.95	8.07	8.8	47.86	109.03	1.47
6.2012	30.35	18.41	8.22	4.93	13.7	8.3	47.14	282.32	1.04
7.2012	29.55	19.77	8.2	3.95	6.6	8.8	53.57	283.88	0.84
8.2012	29.8	29.16	8.1	3.92	10.3	9.5	62.14	296.8	0.17

Table 2: Loading matrix of river estuary environmental parameters for 2011-2012 on the first four principal components (PCs). Parameter units are the same as in Table 1.

Parameters	PC1	PC2	PC3	PC4
T	0.60034	-0.62061	-0.42723	-0.03022
S	-0.45622	0.68034	-0.36825	-0.21898
pH	0.82371	-0.26684	0.05631	-0.11657
DO	-0.63305	0.57349	0.16822	-0.15739
Trb	-0.59243	-0.12172	-0.01055	0.76758
DOC	0.69040	0.62599	0.25357	0.08753
TDN	0.78975	0.47812	0.23253	0.15399
Si	0.68637	0.49349	-0.13579	0.25511
P	-0.13610	-0.33603	0.89347	-0.08481

Table 3: Loading matrix of bay estuary environmental parameters for 2011-2012 on the first four principal components (PCs). Parameter units are the same as in Table 1.

Parameters	PC1	PC2	PC3	PC4
T	-0.72531	0.64773	0.04246	0.10306
S	0.39144	0.00088	-0.68949	0.59015
pH	-0.76661	0.31551	-0.22429	-0.00035
DO	0.68962	-0.62774	-0.04254	-0.19186
Trb	-0.01985	-0.11060	0.84806	0.49203
DOC	0.80152	0.45991	0.13932	-0.14935
TDN	0.91283	0.30756	0.06210	0.05096
Si	0.27312	0.87178	0.03328	-0.14638
P	0.72787	0.34973	-0.00349	0.13564

Table 4: Pairwise comparisons of Mission-Aransas Estuary eukaryotic plankton communities between sampling sites. Significant pairwise comparisons (one-way ANOSIM, $p<0.05$ after Bonferroni correction for 10 pairwise tests) are underlined.

Pairwise Comparisons	R-Statistic	p-value
AB vs ARE	0.23964	0.003
AB vs CE	0.03652	0.143
AB vs CW	0.04321	0.107
AB vs MRE	0.23634	0.003
ARE vs CE	0.15825	0.014
ARE vs CW	0.02555	0.164
ARE vs MRE	0.05287	0.278
CE vs CW	-0.02338	0.740
CE vs MRE	0.13449	0.016
CW vs MRE	0.07399	0.094

Table 5: Pairwise comparisons of monthly Mission-Aransas Estuary eukaryotic plankton communities. There were no significant pairwise comparisons (one-way ANOSIM, $p<0.05$ after Bonferroni correction for 66 pairwise tests).

Pairwise Comparisons	R-Statistic	p-value
September vs October	-0.16400	0.914
September vs November	0.12600	0.135
September vs December	0.16875	0.201
September vs January	0.22400	0.029
September vs February	0.23200	0.072
September vs March	0.27373	0.033
September vs April	0.12281	0.137
September vs May	0.30526	0.018
September vs June	0.51875	0.024
September vs July	0.17237	0.112
September vs August	-0.09342	0.756
October vs November	0.05625	0.364
October vs December	0.04200	0.343
October vs January	0.03200	0.329
October vs February	0.36800	0.026
October vs March	0.10138	0.184
October vs April	0.26316	0.148
October vs May	0.13750	0.045
October vs June	0.27632	0.047
October vs July	0.35625	0.031
October vs August	-0.11579	0.821
November vs December	-0.05000	0.679
November vs January	-0.14800	0.932
November vs February	-0.19000	0.924
November vs March	0.12442	0.169
November vs April	0.31316	0.021
November vs May	0.13750	0.128
November vs June	0.07188	0.314
November vs July	0.03421	0.389
November vs August	-0.10000	0.786

Table 5: (continued)

Pairwise Comparisons	R-Statistic	p-value
December vs January	0.08355	0.235
December vs February	-0.08125	0.638
December vs March	0.31614	0.026
December vs April	0.35880	0.021
December vs May	0.14706	0.162
December vs June	0.27083	0.06
December vs July	-0.00919	0.509
December vs August	0.02574	0.364
January vs February	0.07465	0.267
January vs March	-0.09400	0.792
January vs April	0.42061	0.003
January vs May	0.19803	0.065
January vs June	0.03438	0.275
January vs July	0.20000	0.081
January vs August	0.14539	0.138
February vs March	0.04977	0.353
February vs April	0.39430	0.372
February vs May	0.17500	0.006
February vs June	0.03618	0.114
February vs July	0.09605	0.208
February vs August	0.01184	0.436
March vs April	0.19622	0.033
March vs May	0.19971	0.038
March vs June	0.31669	0.184
March vs July	0.10053	0.002
March vs August	0.24271	0.012
April vs May	0.14051	0.066
April vs June	-0.03762	0.587
April vs July	0.33610	0.002
April vs August	0.25000	0.006
May vs June	-0.08088	0.645
May vs July	-0.01339	0.524
May vs August	0.08119	0.114
June vs July	-0.06342	0.613
June vs August	-0.09926	0.684
July vs August	0.07980	0.154

Table 6: Pairwise comparisons of Mission-Aransas Estuaryeukaryotic plankton communities between river estuary environmental seasons. There were no significant pairwise comparisons (one-way ANOSIM, $p<0.05$ after Bonferroni correction for 3 pairwise tests).

Pairwise Comparisons	R-Statistic	p-value
Winter/Early Spring vs Late Spring/Early Summer	0.08872	0.225
Winter/Early Spring vs Late Summer/Fall	-0.04813	0.587
Late Summer/Fall vs Late Spring/Early Summer	0.17284	0.210

Table 7: One-way ANOSIM pairwise comparisons of Mission-Aransas Estuary eukaryotic plankton communities between bay estuary environmental seasons. There were no significant pairwise comparisons (one-way ANOSIM, $p<0.05$ after Bonferroni correction for 6 pairwise tests).

Pairwise Comparison	R-Statistic	p-value
Winter vs Spring	0.04720	0.155
Winter vs Summer	0.16278	0.009
Winter vs Fall	-0.04921	0.635
Summer vs Fall	0.10784	0.187
Spring vs Fall	0.22956	0.028
Spring vs Summer	0.10003	0.027

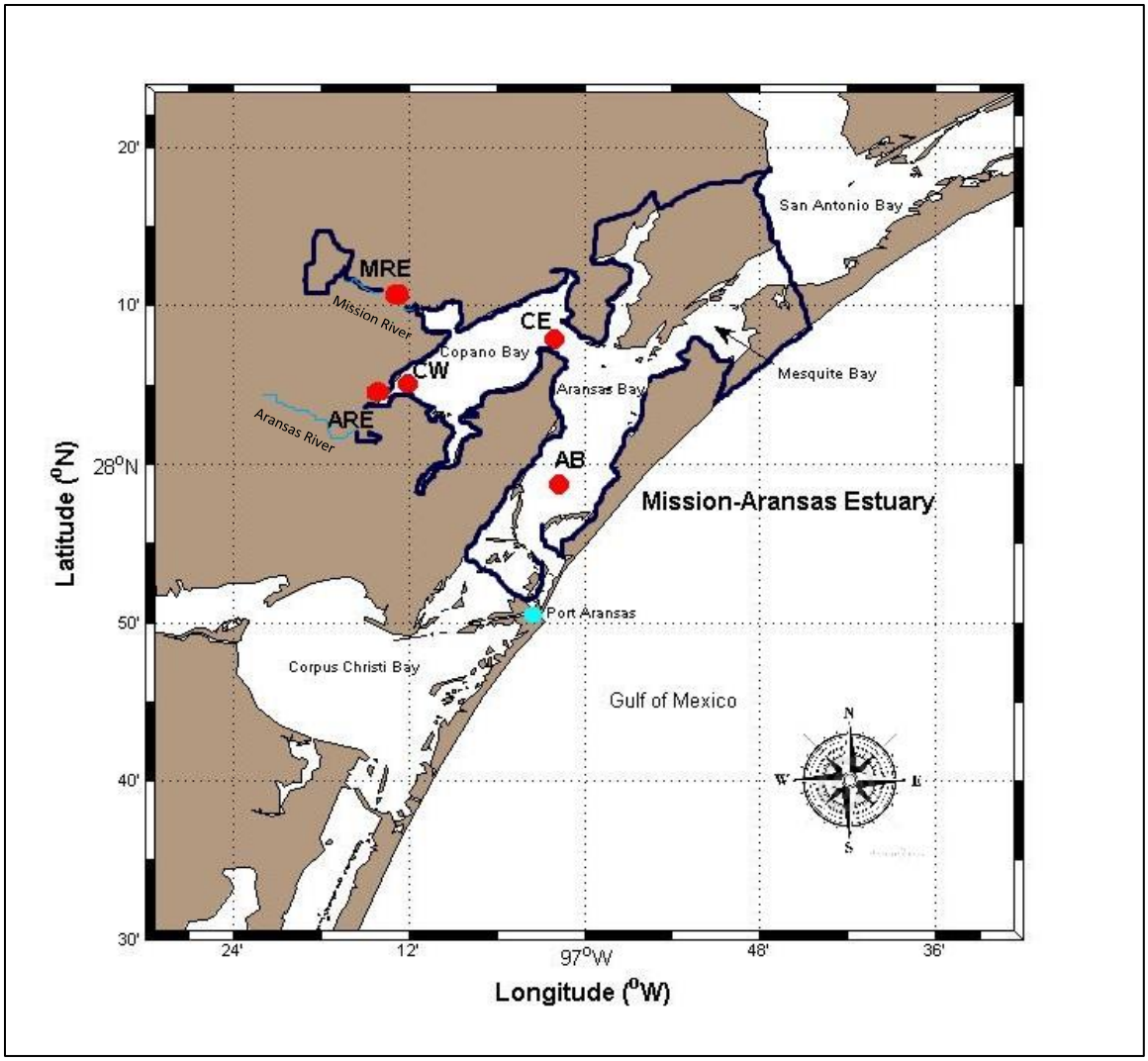

Figure 1: 2011-2012 Sampling sites in the Mission-Aransas Estuary, Texas. Red Dots = Sampling Sites; Dark Blue Border = MANERR Boundary; Teal Dot = Port Aransas, TX; Turquoise Lines = Rivers.

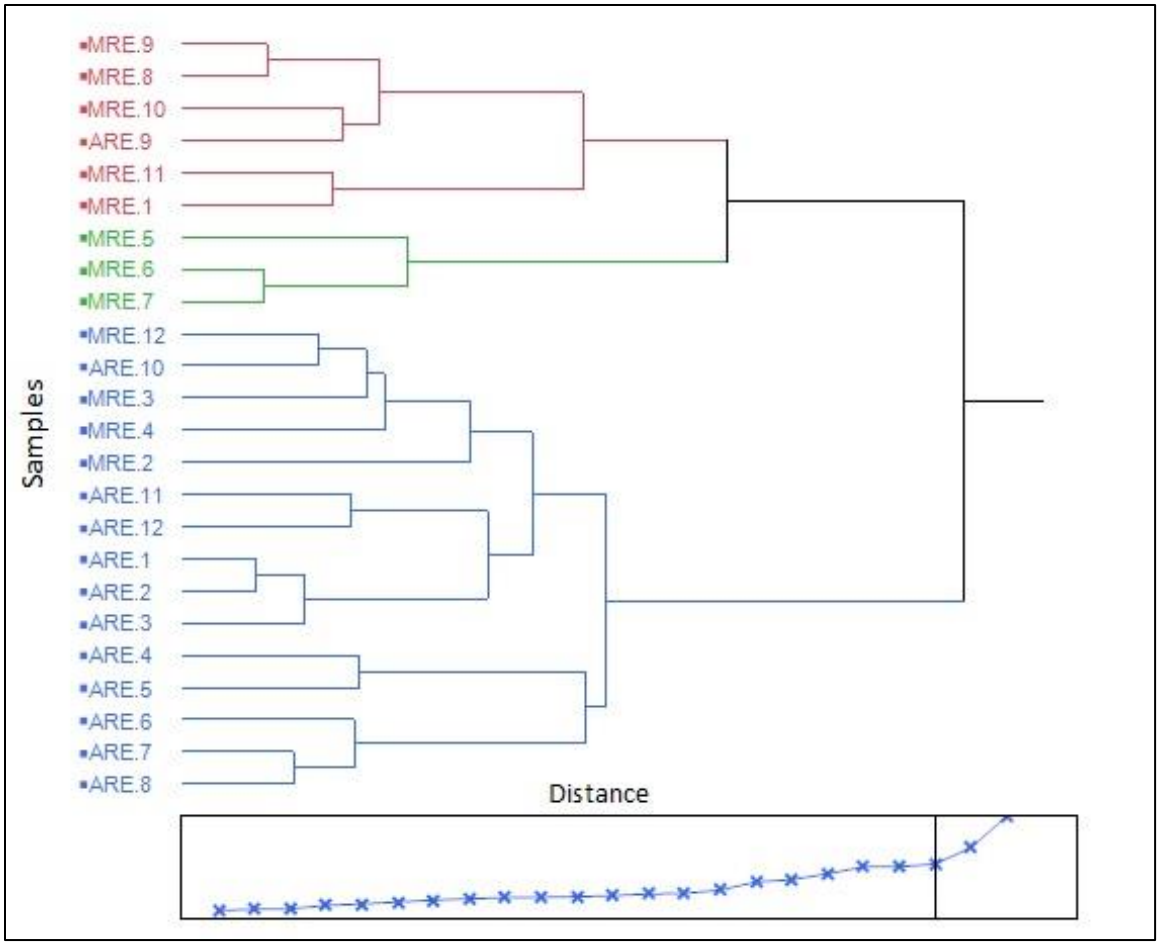

Figure 2: Hierarchical clustering of 2011-2012 water quality parameters at the river estuary sampling sites. Different colors represent significant seasons defined by the river estuary water quality parameters. The Mission River Estuary was defined by three seasons: Blue = winter/early spring (December, February, March, April), Green = late spring/early summer (May, June, July), and Red = late summer/fall (August, September, October, November). There were no distinct seasonal clusters for the Aransas River Estuary.

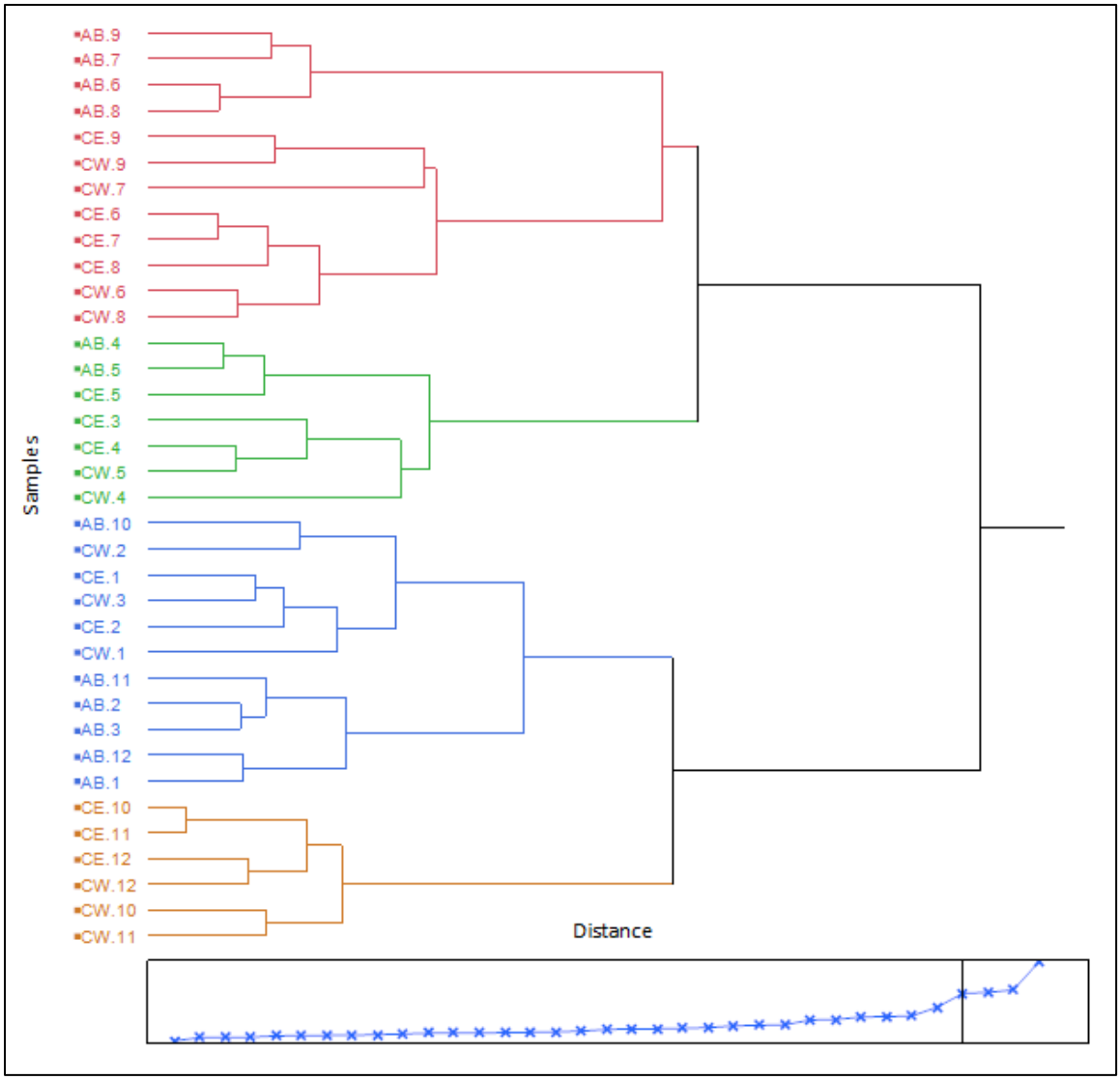

Figure 3: Hierarchical clustering of 2011-2012 water quality parameters at the bay estuary sampling sites. Different colors represent significant groupings defined by the bay estuary water quality parameters. The four clusters of Copano Bay East and West were designated as: Orange = fall (October, November, December), Blue = winter (January, February March), Green = spring (April, May), and Red = summer (June, July, August, September). The three clusters of Aransas Bay were designated as Red = summer (July, June, August, September), Blue = fall/winter (October, November, December, January, February, March), and Green = spring (April, May).

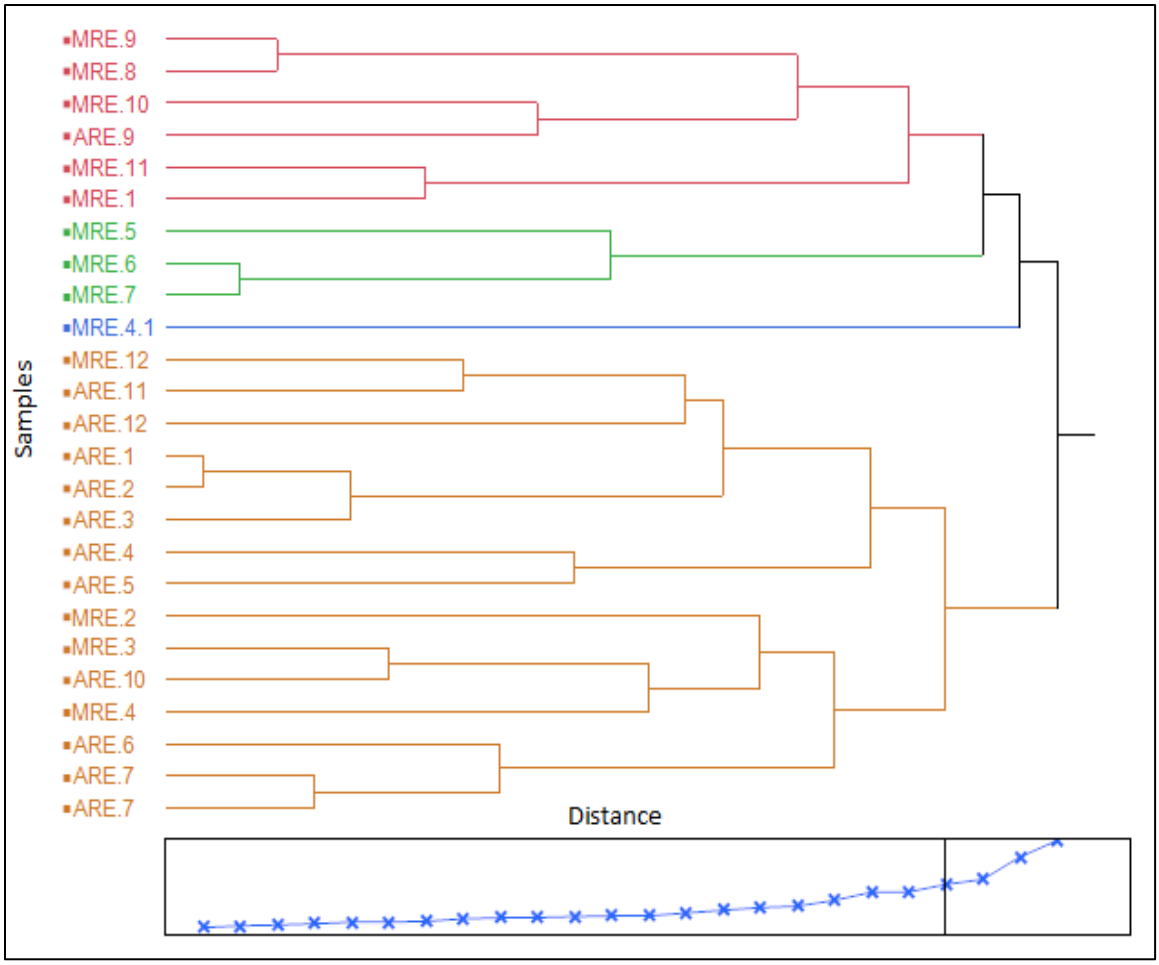

Figure 4: Hierarchical clustering of 2011-2012 water quality parameters at the river estuary sampling sites including the Mission River Estuary storm event. Different colors represent significant groupings defined by the river estuary water quality parameters. The Mission River Estuary was defined by three environmental seasons: Orange = winter/early spring (December, February, March, April), Green = late spring/early summer (May, June, July), Red = late summer/fall (August, September, October, November), and Blue = April storm event. There were no distinct seasonal clusters for the Aransas River Estuary.

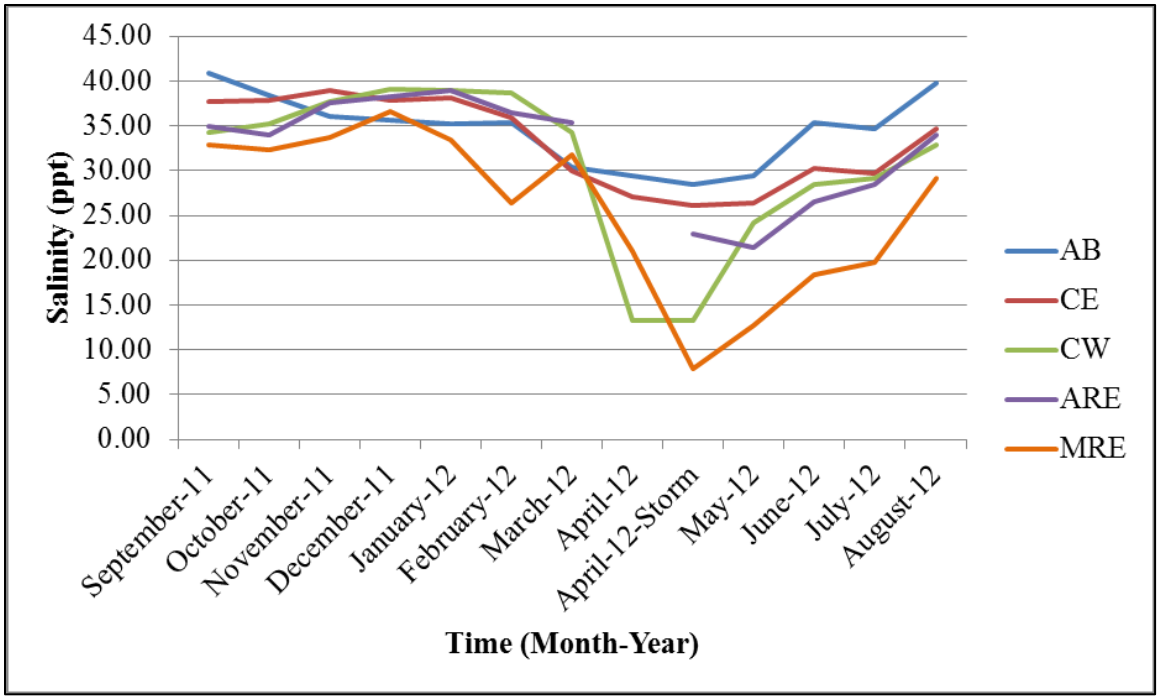

Figure 5: Salinity at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April-12-Storm event; therefore data is missing for that period.

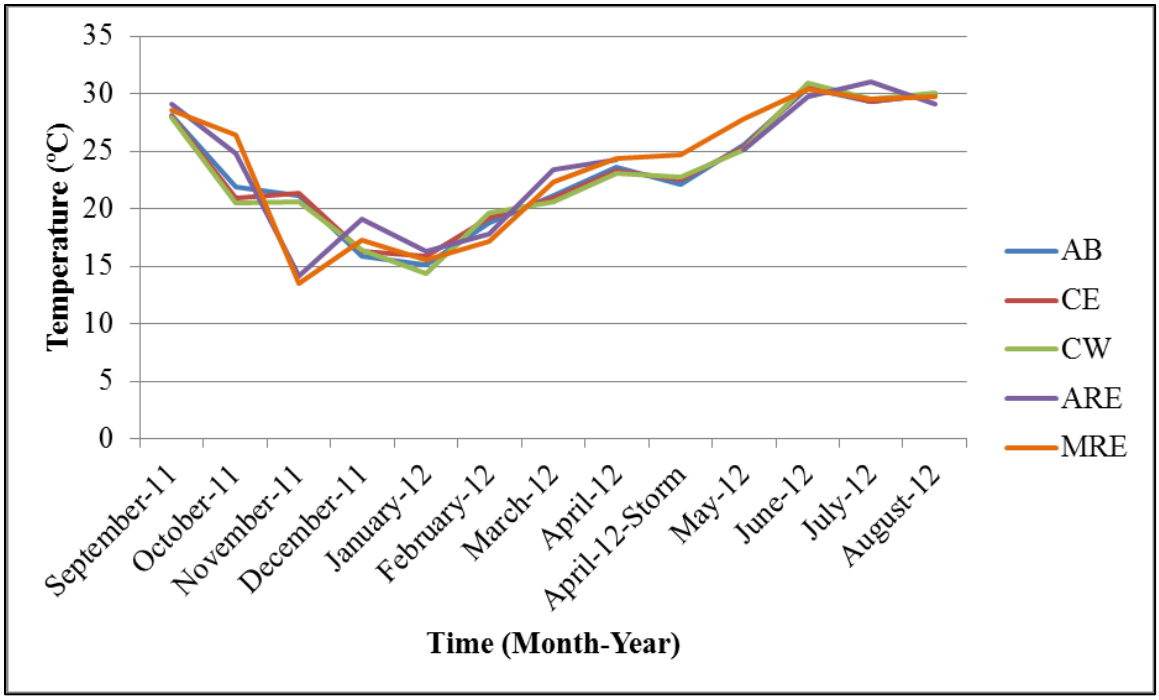

Figure 6: Temperature at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April-12-Storm event; therefore data is missing for that period.

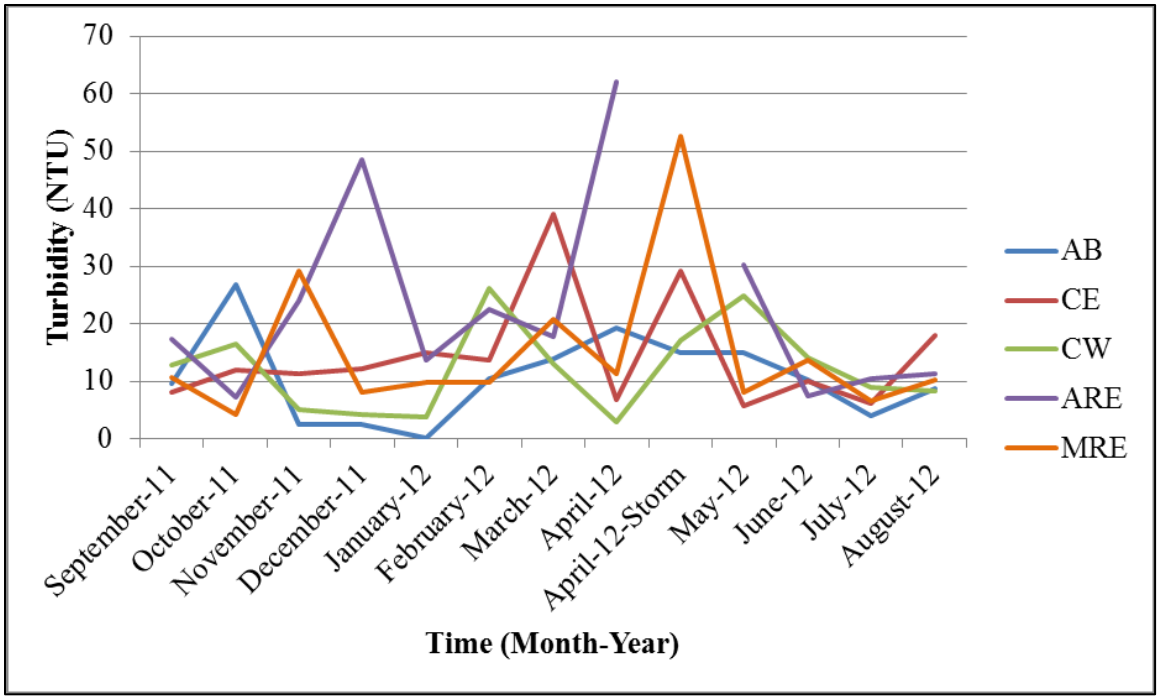

Figure 7: Turbidity at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April-12-Storm event; therefore data is missing for that period.

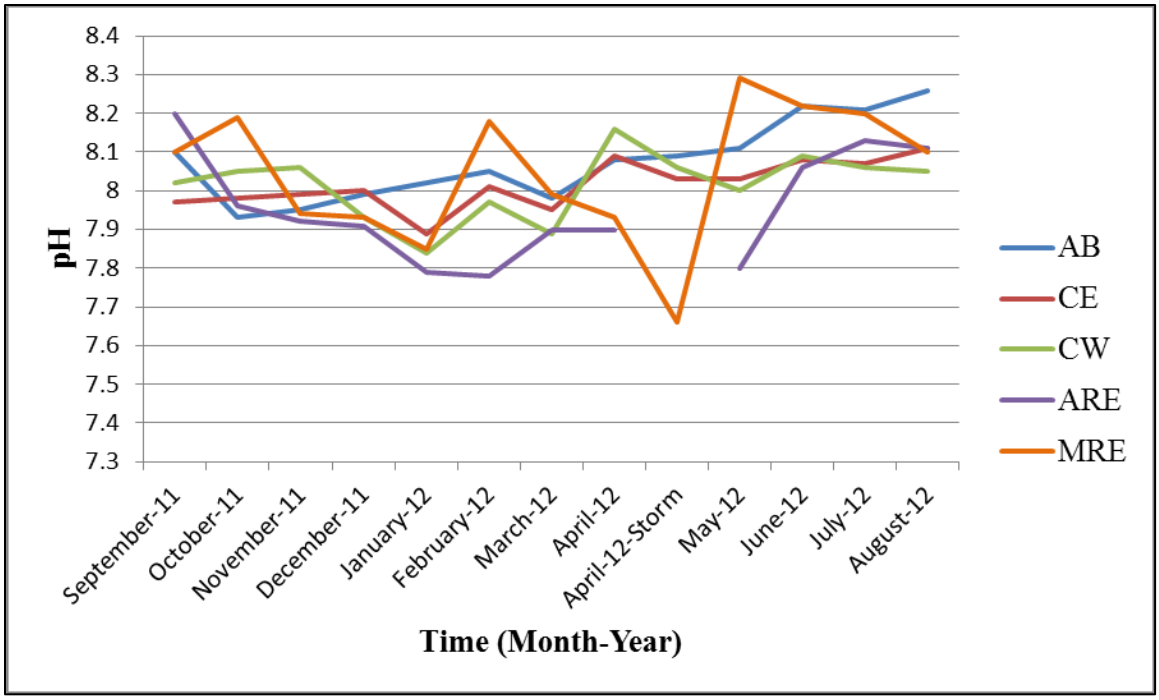

Figure 8: pH at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April-12-Storm event; therefore data is missing for that period.

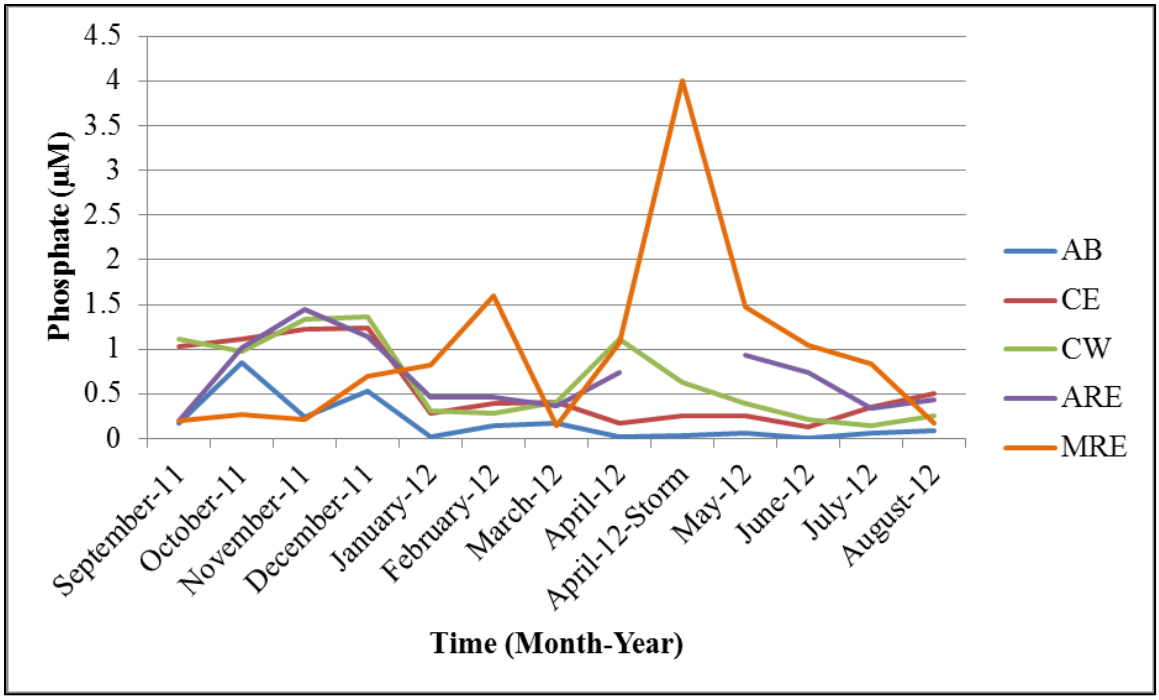

Figure 9: Phosphate concentrations at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April 12-Storm event; therefore data is missing for that period.

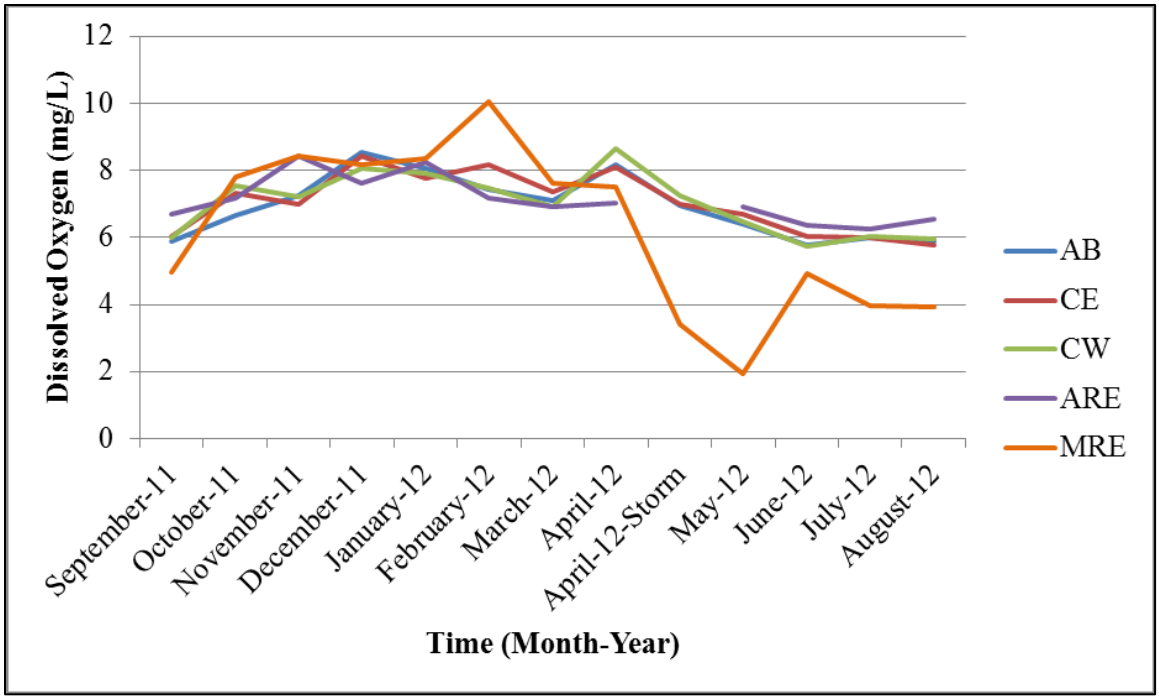

Figure 10: Dissolved oxygen concentrations at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April-12-Storm event; therefore data is missing for that period.

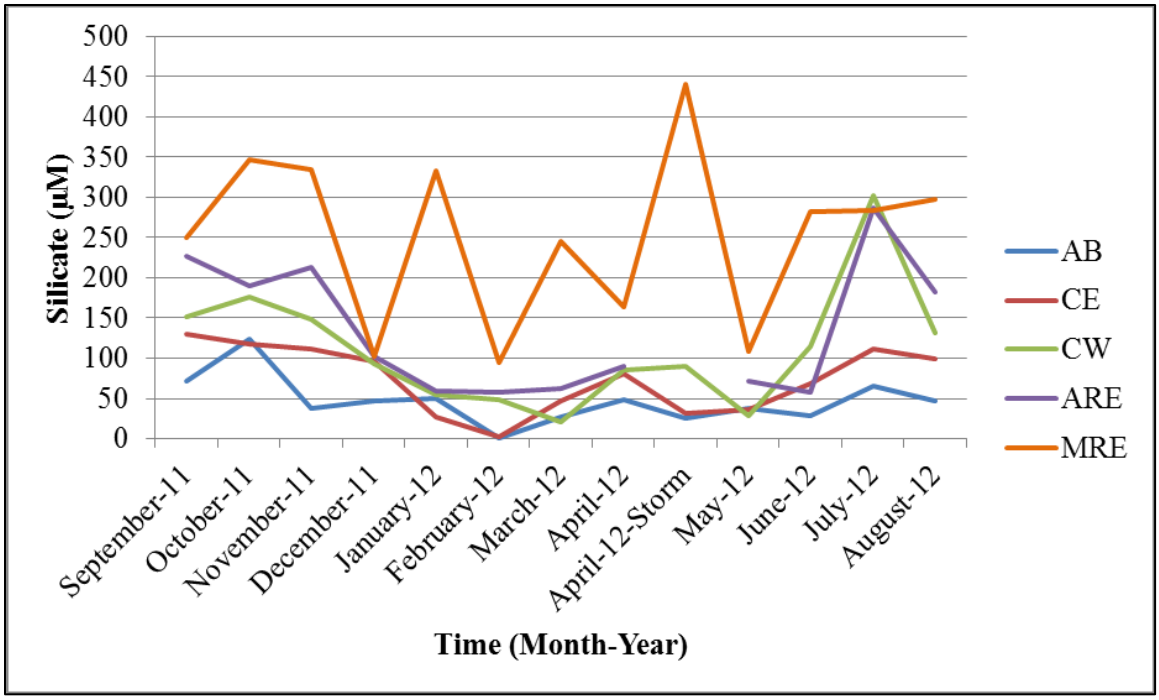

Figure 11: Silicate concentrations at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April 12-Storm event; therefore data is missing for that period.

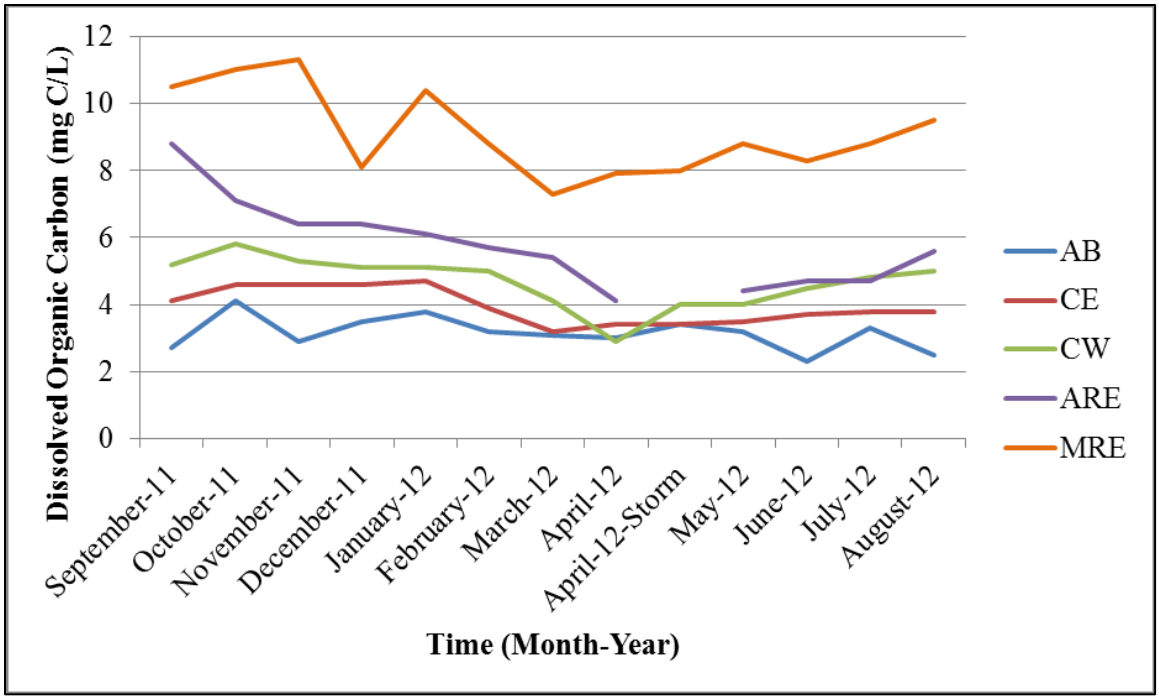

Figure 12: Dissolved organic carbon concentrations at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April-12-Storm event; therefore data is missing for that period.

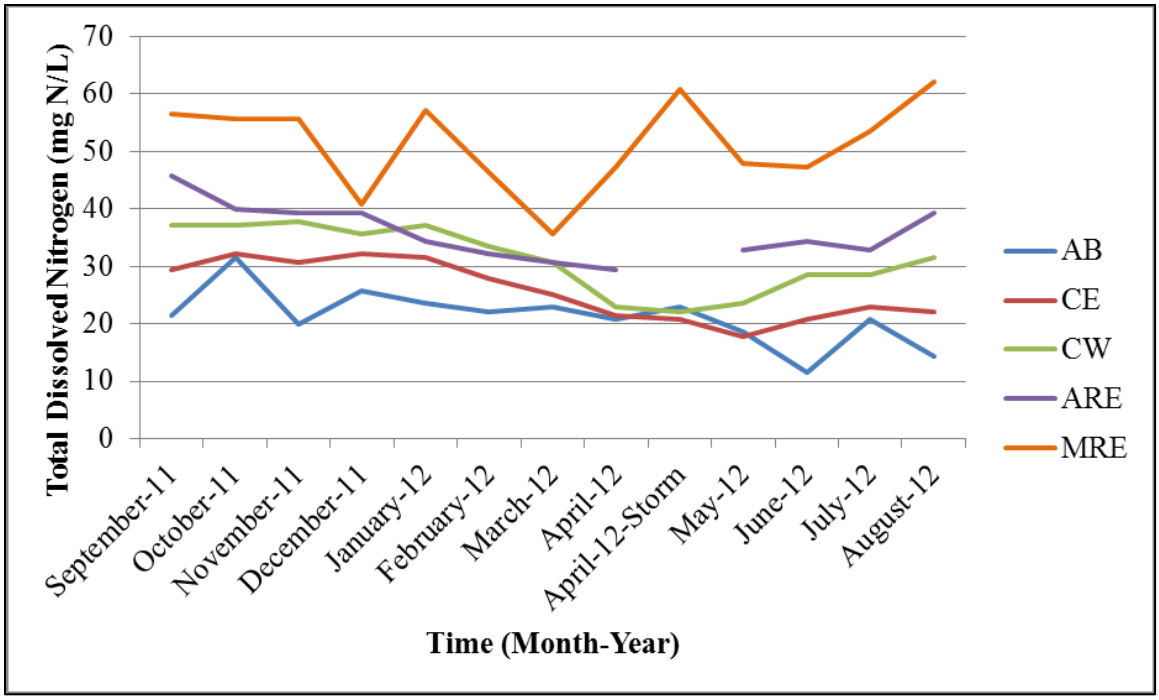

Figure 13: Total dissolved nitrogen concentrations at Mission-Aransas Estuary sampling sites during the 2011-2012 study period. The Aransas River Estuary did not experience the April-12-Storm event; therefore data is missing for that period.

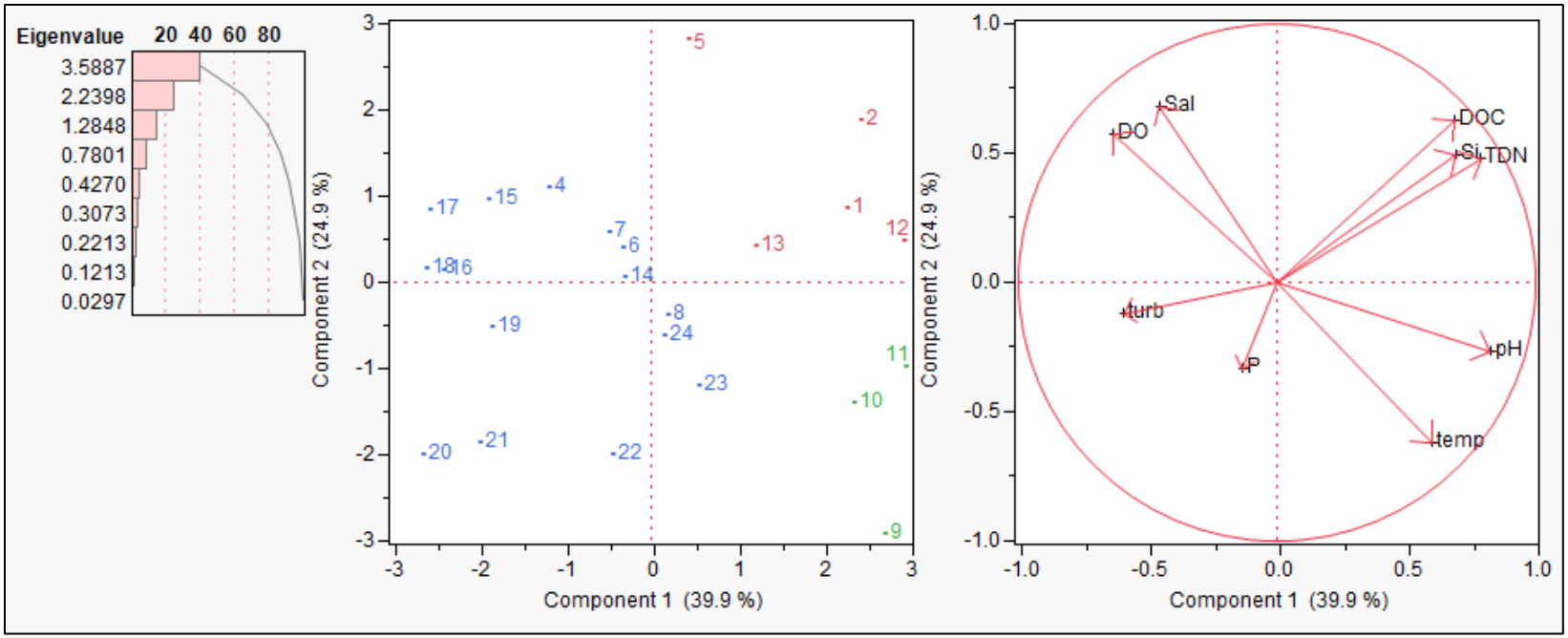

Figure 14: Principal component analysis (PCA) (Axis I and II) made on the loadings of environmental variables (right) and the scores of the river estuary sites (left) from September 2011 to August 2012.

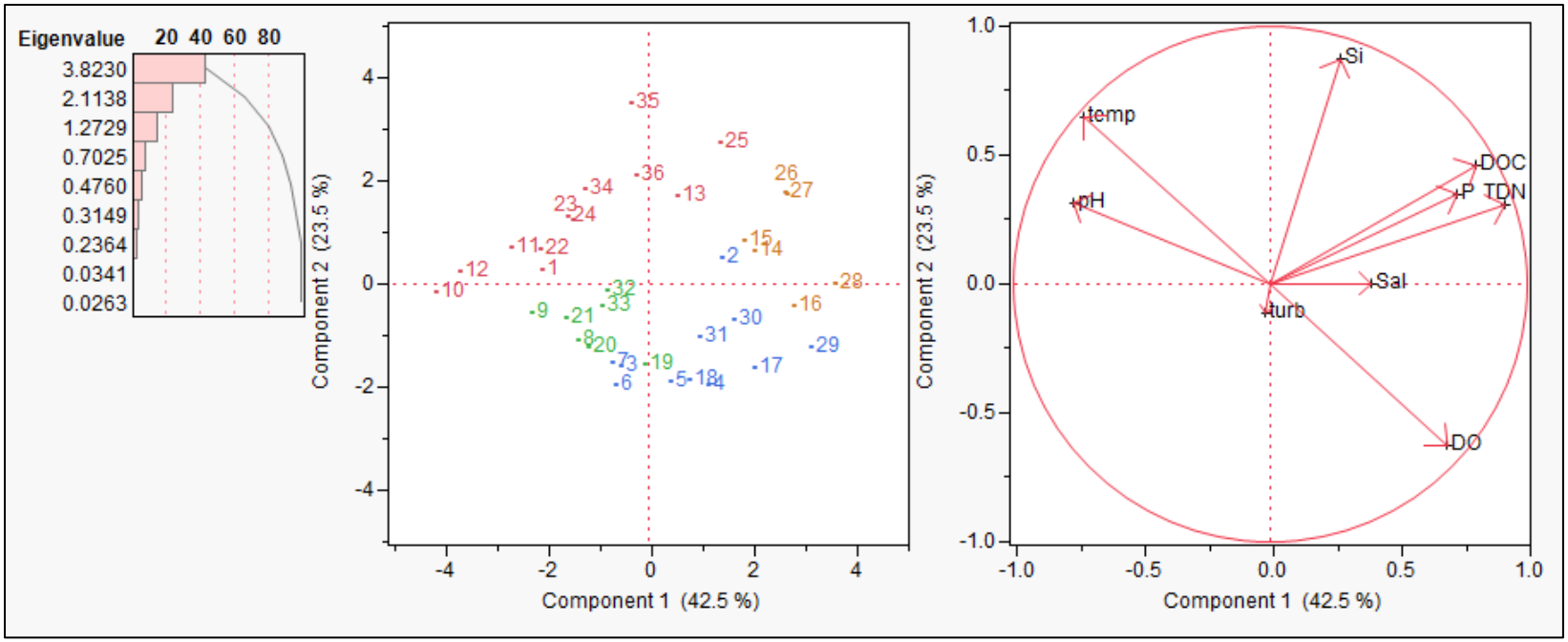

Figure 15: Principal component analysis (PCA) (Axis I and II) made on the loadings of environmental variables (right) and the scores of the bay estuary sites (left) from September 2011 to August 2012.

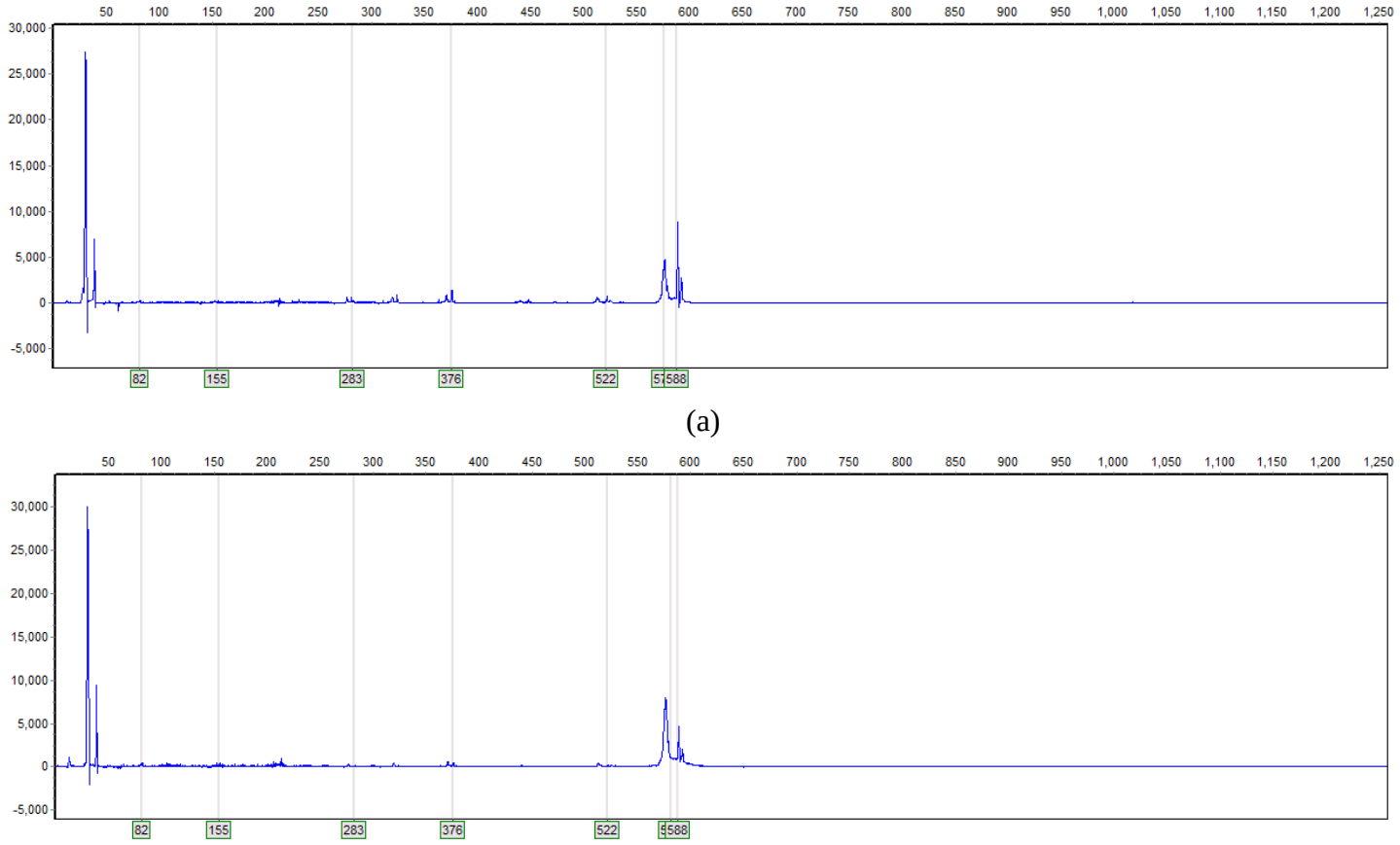

Figure 16: Raw chromatograms of t-RFLP results obtained from the first (a) and second (b) replicate of the September 2011 Mission River Estuary sampling site. Fragment intensity (relative fluorescence units, RFU) is shown on the vertical axis, fragment size (bp) along the horizontal axis, and an example of the signal from the size standard (bottom).

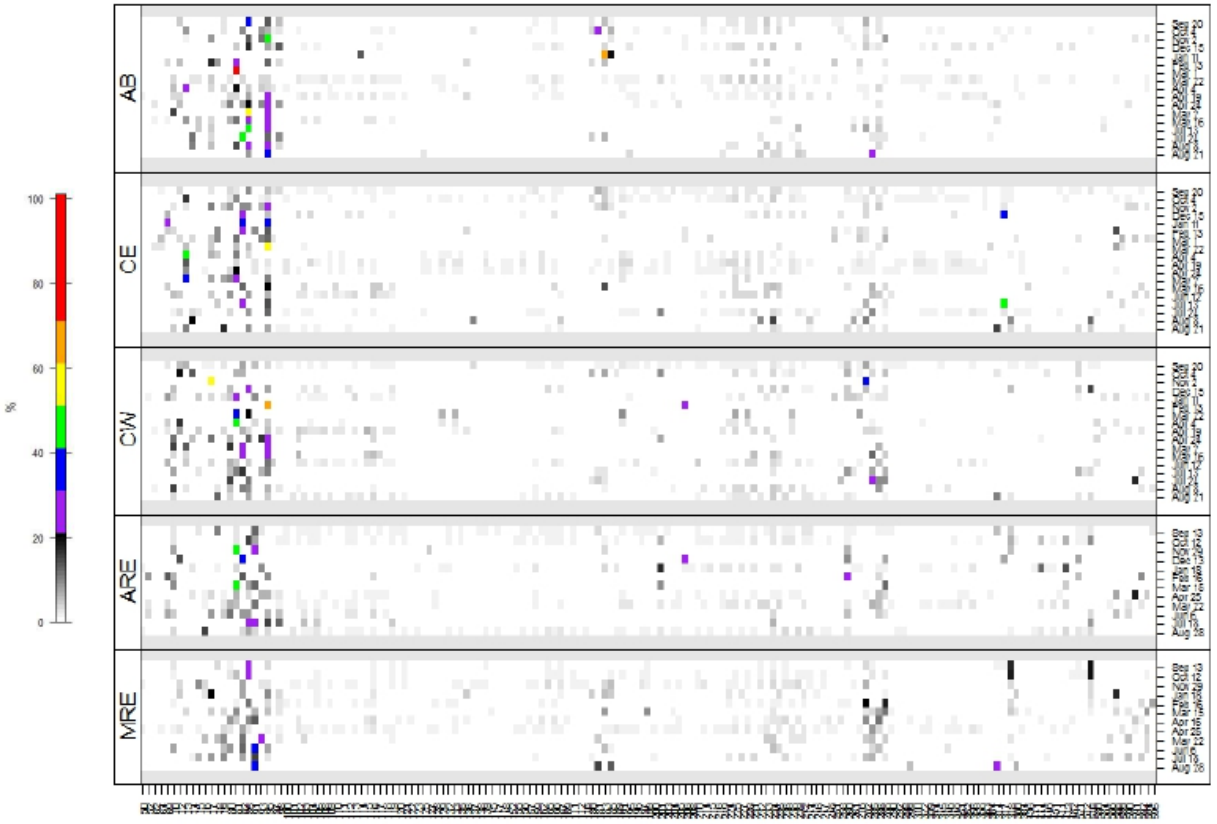

Figure 17: A heatmap showing all t-RFLP data from the 5 Mission-Aransas Estuary sampling sites from 2011-2012. The signal from each OTU is expressed as a percentage of the total signal from each sample. The color of the square corresponds to the signal intensity of each fragment: a white-black scale was used for signal strengths between 0-20% and data greater than 20% were binned into groups of 10% and color-coded. Rows show the data for each sample, labeled with the sample site on the left, sample date on the right. Columns show the data for a single fragment size across all samples. The gray lines separate data from different sampling locations.

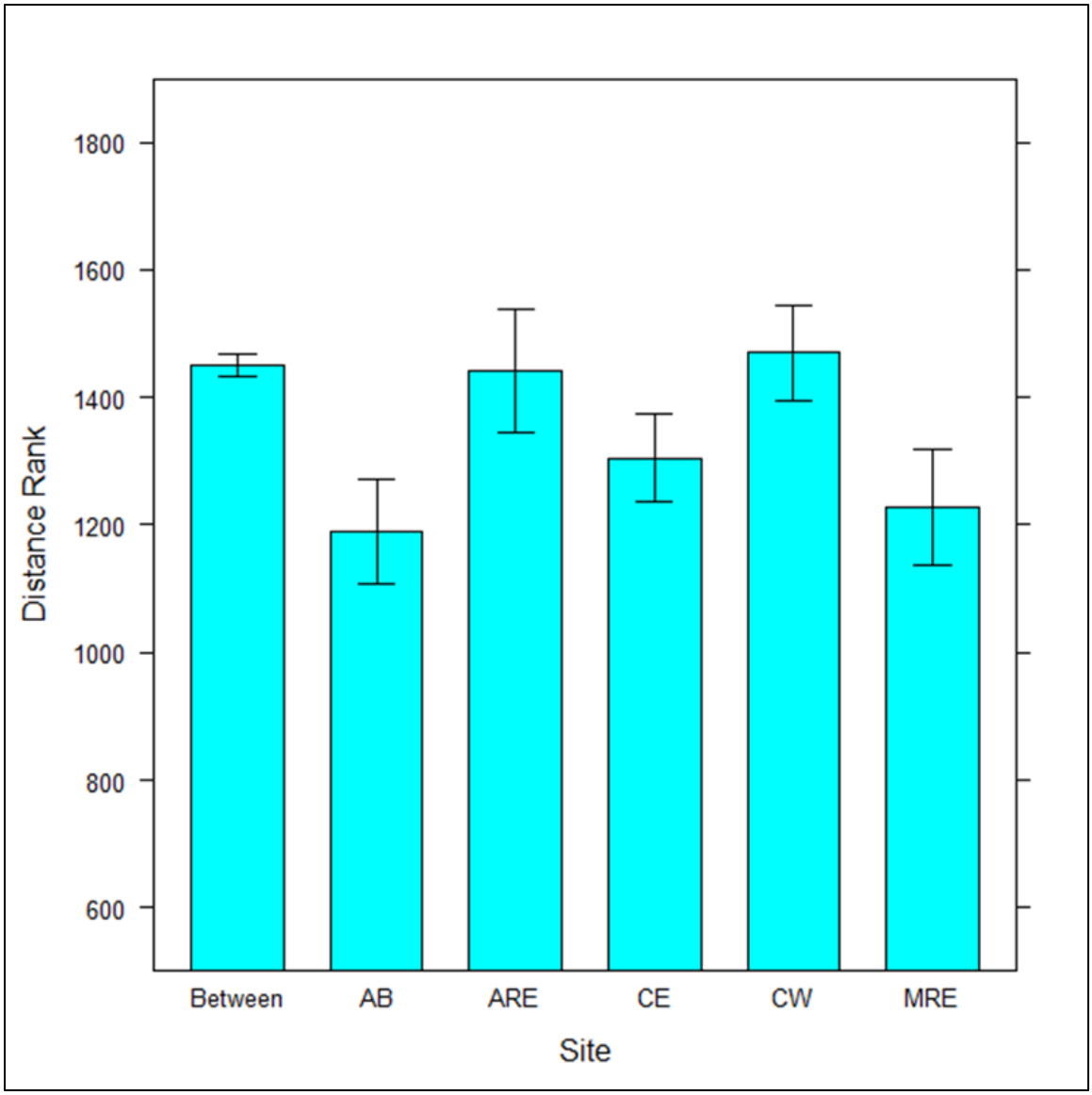

Figure 18: Between-site comparison of Mission-Aransas Estuary eukaryotic plankton community composition during the 2011-2012 study. This bar chart depicts the mean distance rank and standard error of the one-way ANOSIM ($p = 0.001$, $R = 0.088$, permutations = 999). The bars show the overall 'between' sites rank (left) and 'within' site ranks (right).

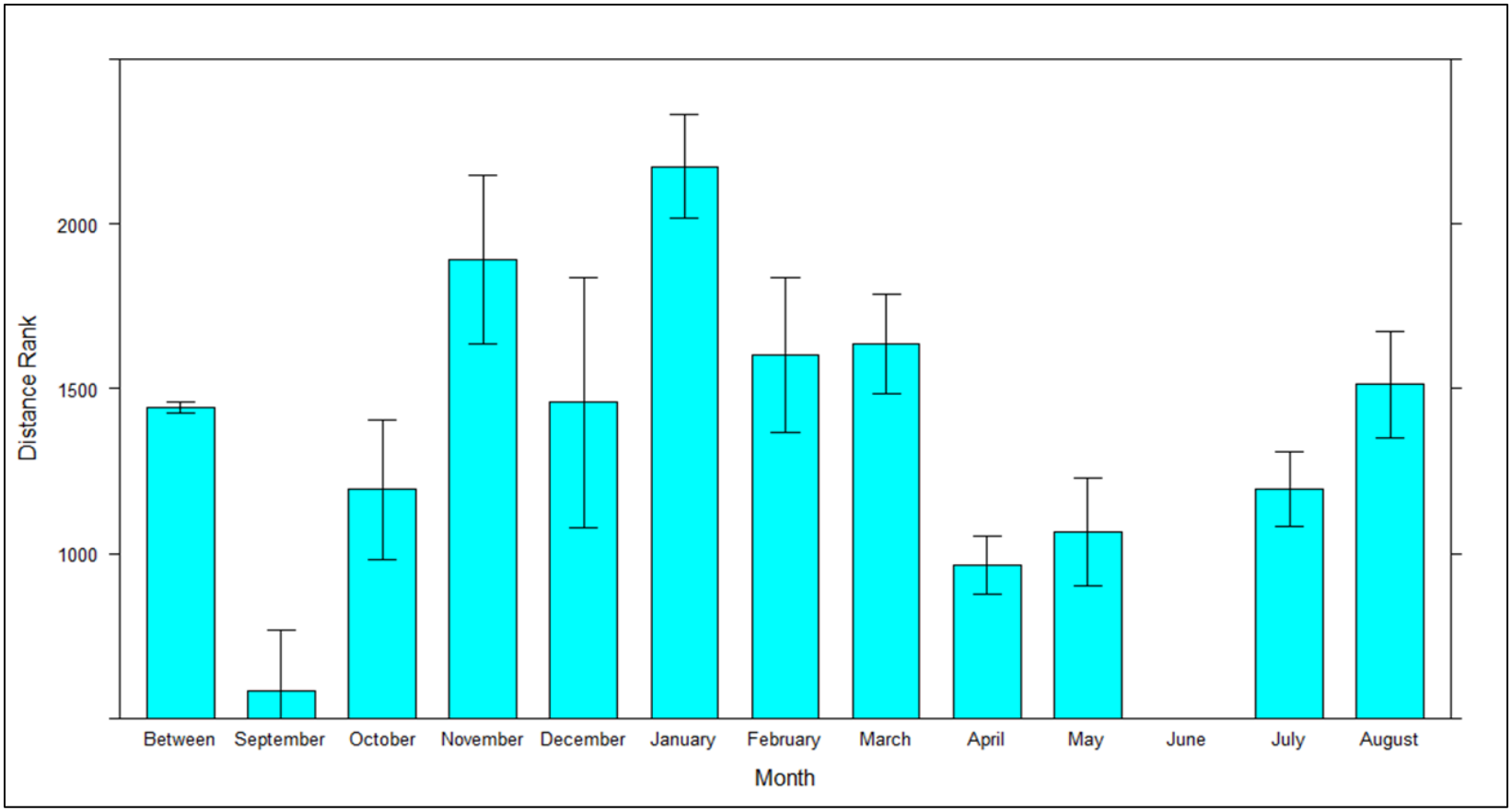

Figure 19: Between-month comparisons of Mission-Aransas Estuary eukaryotic planktoncommunity composition during the 2011-2012 study. This bar chart depicts the mean distance rank and standard error of the one-way ANOSIM (p-value = 0.001, R = 0.143, permutations = 999). The bars show the overall 'between' sites rank (left) and 'within' site ranks (right).

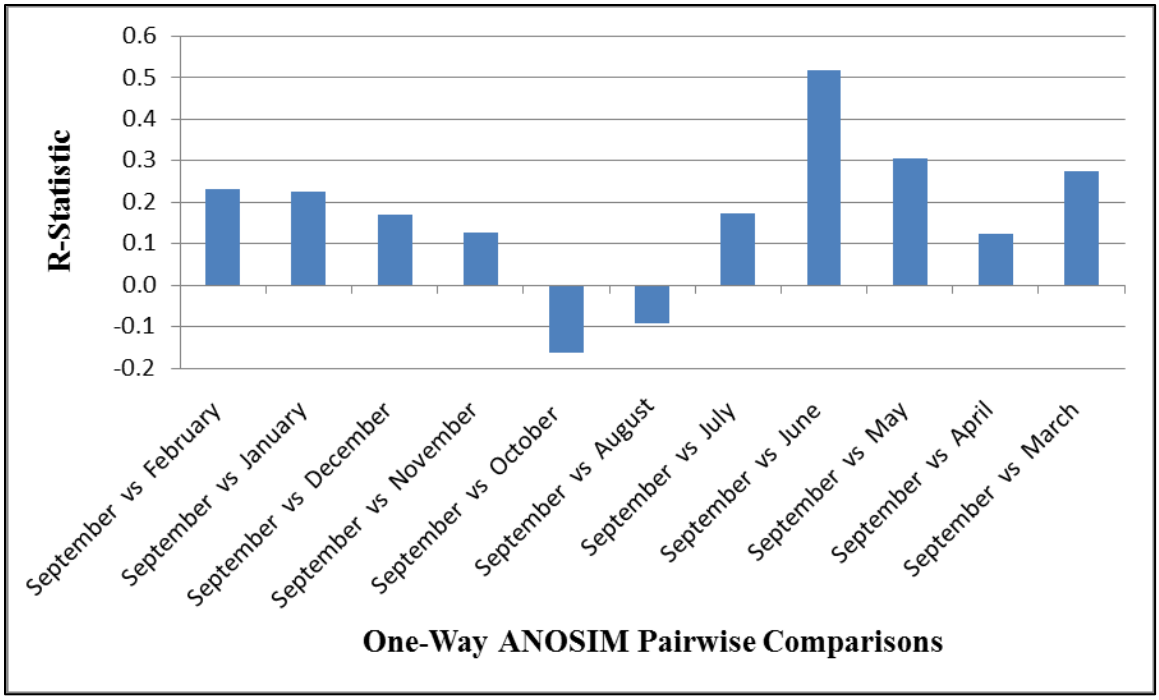

Figure 20: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for September 2011. The remaining R-statistic pairwise comparisons can be found in Appendix B. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

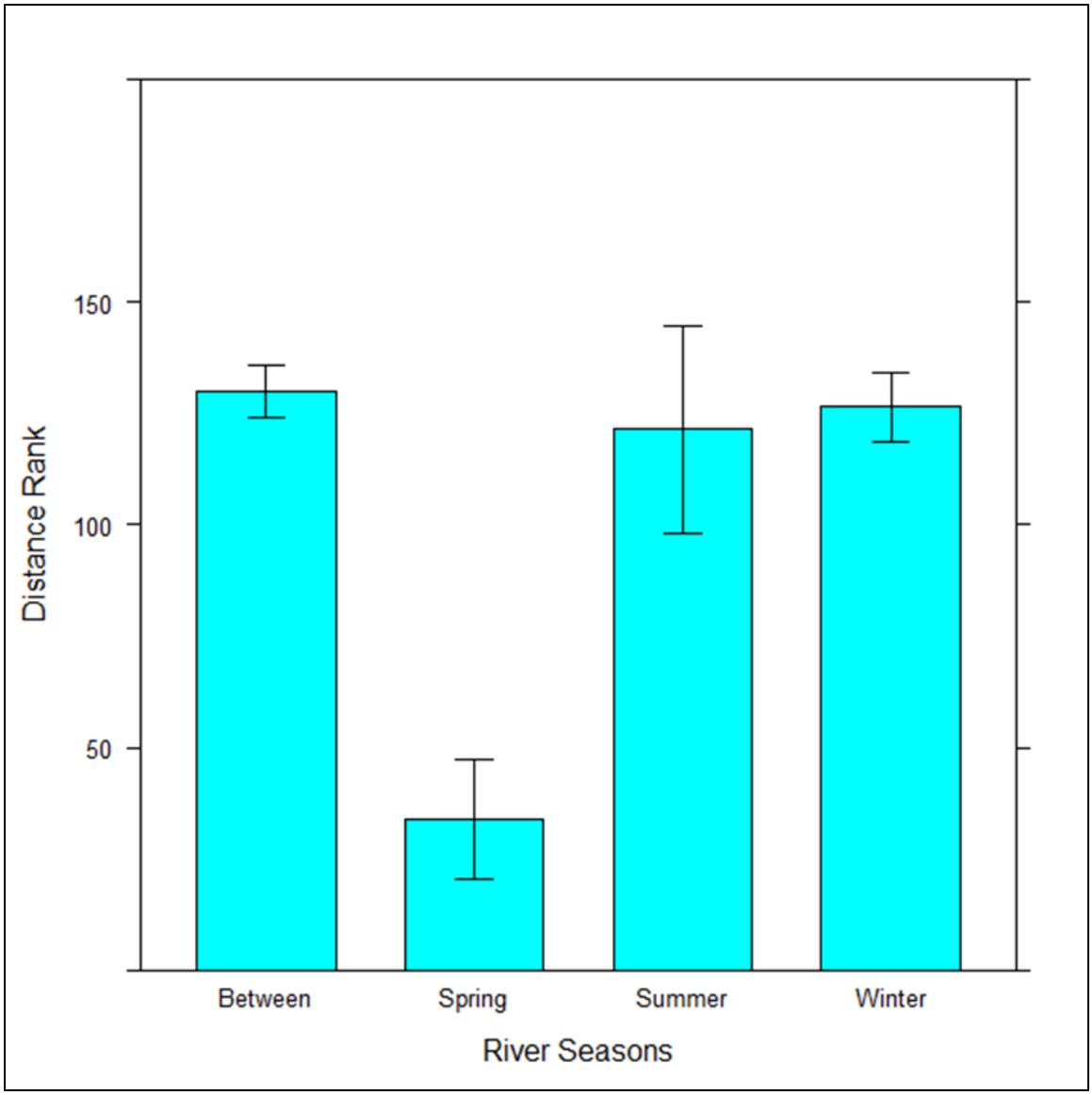

Figure 21: Between-environmental season comparisons of Mission-Aransas river estuary eukaryotic plankton communities (defined in **Figure 2**) during the 2011-2012 study. This bar chart depicts the mean distance rank and standard error of the one-way ANOSIM (p-value = 0.3, R = 0.053, permutations = 999). The bars show the overall 'between' sites rank (left) and 'within' site ranks (right).

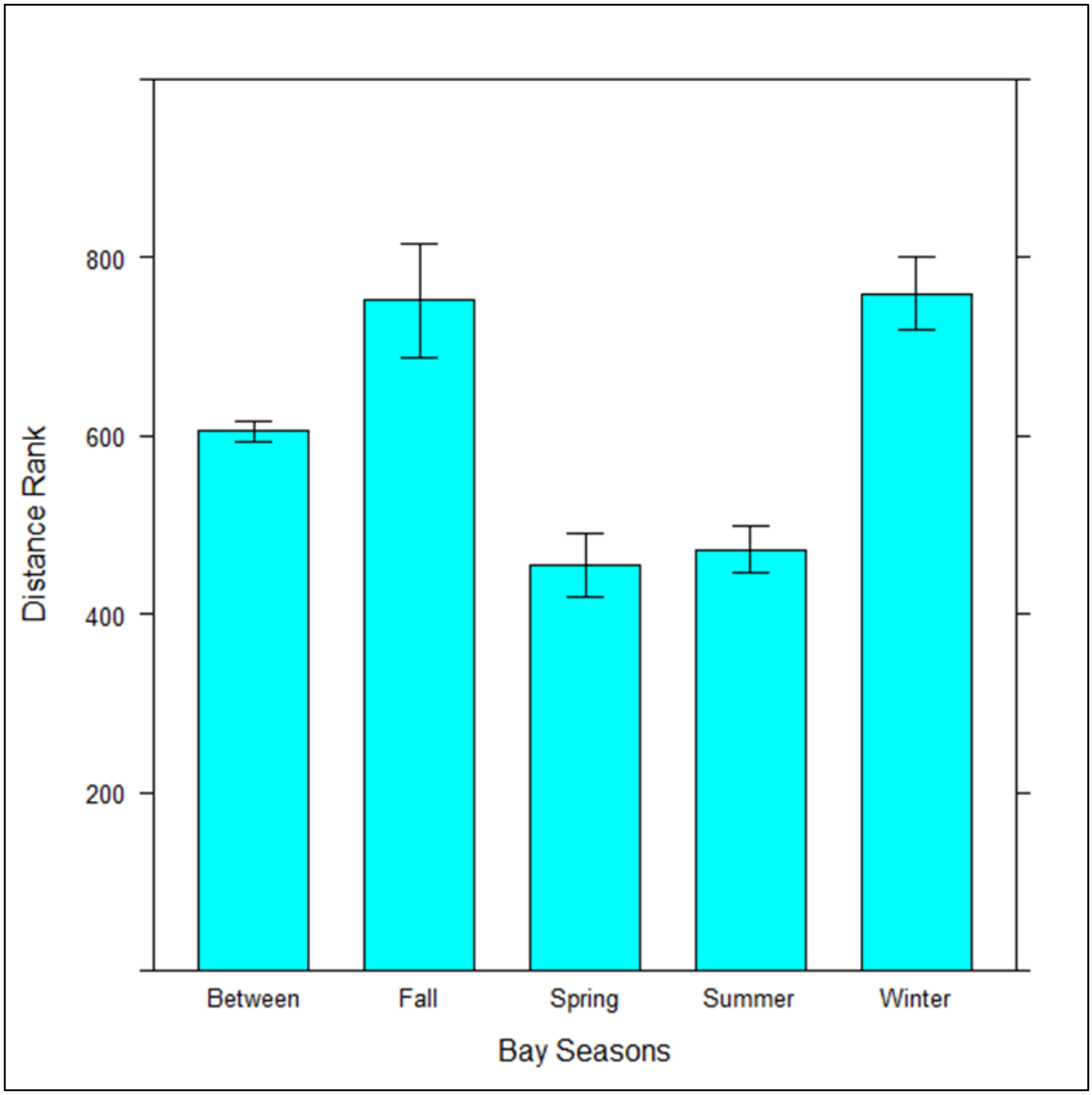

Figure 22: Between-environmental season comparisons of Mission-Aransas bay estuary eukaryotic plankton communities (defined in **Figure 3**) during the 2011-2012 study. This bar chart depicts the mean distance rank and standard error of the one-way ANOSIM (p-value = 0.008, R = 0.106, permutations = 999). The bars show the overall 'between' sites rank (left) and 'within' site ranks (right).

Appendices

Appendix A

Mission-Aransas Estuary Size Fractionated Chlorophyll-a

Although structurally and physically variable, coastal areas consisting of high fishery productivity are generally characterized by a combination of high primary productivity and short, efficient food chains (Chavez *et al*. 2011). For example, areas such as coastal upwelling zones are frequently comprised of large phytoplankton species that are often directly consumed by larval/juvenile fish (Ryther 1969), and in coral reef regions, where several fish species graze directly on the reef macroalgae (Russ 1991). This primary production by phytoplankton generates an energy flow through the food web (Day *et al*. 1989).

In estuarine systems, phytoplankton primary production is a major source of food energy supporting the tertiary production (Day *et al*. 1989) as plankton species diversity and composition are closely linked to these higher trophic levels (Mallin and Paerl 1994). With primary productivity as the cornerstone of the estuarine food chain, zooplankton act to transfer the energy captured by the phytoplankton to populations of shellfish and finfish that depend upon plankton for survival. Classic marine food chains demonstrate energy transfers directly from large primary producers, such as diatoms, to mesozooplankton, such as copepods, to consumers, such as fish (Pomeroy 1974). However, phytoplankton <20µm are not efficiently grazed by the mesozooplankton communities (Sherr *et al*. 1986). Instead, the phytoplankton of smaller size fractions is highly grazed upon by the microzooplankton community (e.g. ciliates, heterotrophic

dinoflagellates) (Calbet and Landry 2004). Thus, the efficiency of energy transfer through the planktonic food web is extremely dependent on the size structure of the phytoplankton community present in the ecosystem (Irwin *et al*. 2006).

Due to estuarine ecological and economic value, it is essential to understand the factors controlling or altering estuarine energy flows. One way of assessing the magnitude of planktonic trophic transfer is to determine spatiotemporal fluctuations in plankton communities (Kimmel *et al.* 2006, Litchman *et al.* 2010). Field observations of phytoplankton community size structure indicate that hydrography of the area and resource availability are important factors when accounting for plankton community biomass distributions (Tremblay and Legendre 1994, Li 2002). Phytoplankton in the smaller size-fraction are generally considered to have an advantage surviving under nutrient-limiting conditions due to high surface area to volume ratios, whereas those in the larger size fraction have adopted strategies, such as storage vacuoles, to thrive in areas with fluctuating nutrient and physical conditions (Litchman *et al.* 2009, Litchman *et al*. 2010).

Over the course of the season, alternating selective pressures such as nutrient limitation, grazers, light availability or fluctuating nutrient supply can select for different sizes, thus creating diversity in biomass distributions and energy flows through natural estuarine communities (Dziock *et al*. 2006, Sagert *et al*. 2008, Cabecinha *et al*. 2009, Hughes 2000, Sun *et al*. 2011). Specifically, benthic grazing may have a substantial influence on the chlorophyll distributions within the shallow Texas estuaries. Texas produces the second largest oyster harvest in the United States, with the southern most

commercial oyster fishery being located in the Mission-Aransas Estuary (Culbertson *et al*. 2004). As filter feeders, oysters remove plankton and other particles from bay waters, and when populations are abundant can regulate the availability of resources to other organisms (Newell 2004). In Texas coastal bays and estuaries, changes in estuarine plankton biomass via climate alterations may have knock-on effects by changing the feeding environment of larval fishes and the subsequent upper trophic levels comprised of important commercial fisheries (Doney *et al*. 2012). Therefore, understanding the effects of abiotic and biotic environmental forcing on plankton community dynamics and size structure is essential to the understanding of spatiotemporal fluctuations in food web structure and efficiency (Lindeman, 1942, Irwin *et al*. 2006).

For fractionated chlorophyll-*a* analyses, whole water was collected using a Van Dorn sampler, placed in a cooler to maintain ~ambient temperature, and transported back to the laboratory for processing. Samples for total chlorophyll a (chl) were collected on glass fiber filters (Whatman GF/F 0.7µm pore size) and two additional samples were collected on 5µm and 20µm pore size nylon filters, to allow for calculation of size fractionated chlorophyll a (<5µm, 5-20µm, and >20µm) concentrations. The filters were placed in glass scintillation vials, extracted with 10mL of 90% acetone for 48 hours at -20°C, and analyzed on a Turner Designs Trilogy Fluorometer. Each fraction is presumed to contain the following organisms: <5µm-small nanoplankton, flagellates, picocyanobacteria, small diatoms, small bacteria; 5-20µm-nanoflagellates, diatoms, small ciliates; >20µm-larger diatoms, dinoflagellates, larger ciliates, copepods, copepod nauplii, and larvae (Williams 1981 and Revilla *et al.* 2002).

A gradual seasonality of total chlorophyll-*a* concentrations demonstrated different seasonal peaks (**Figure A1**). The river estuary sites peaked in the late fall/winter and the bay estuary sites peaked in the spring. Total chlorophyll-*a* was highest at MRE (**Figure A2**), and the 5-20 µm size fraction was predominant. High January biomass in the >20 chlorophyll-*a* size fraction may be attributed to a bloom in *Rhizosolenia*.

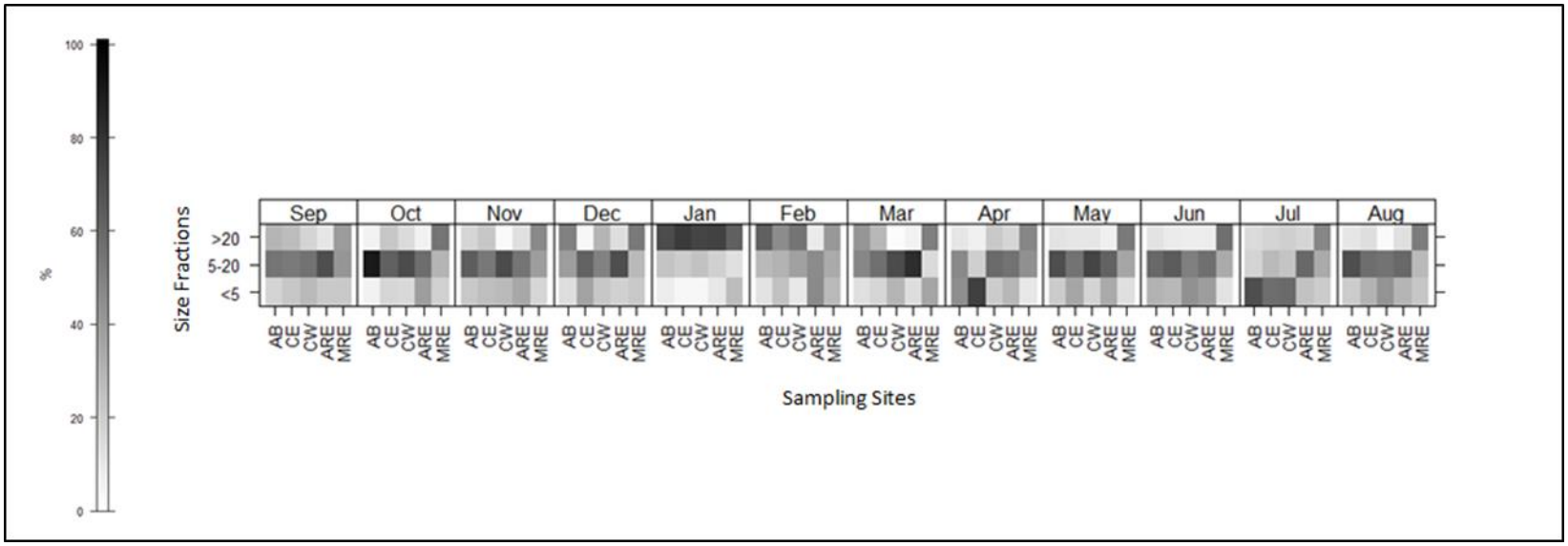

Figure A1: Heatmap summarizing all chlorophyll-*a* data from 5 sampling sites in the Mission-Aransas Estuary from 2011-2012. The signal from each size fraction is expressed as a percentage of the total signal from each sample. A white black scale was used from 0-100% with lighter colors being representative of smaller percentages.

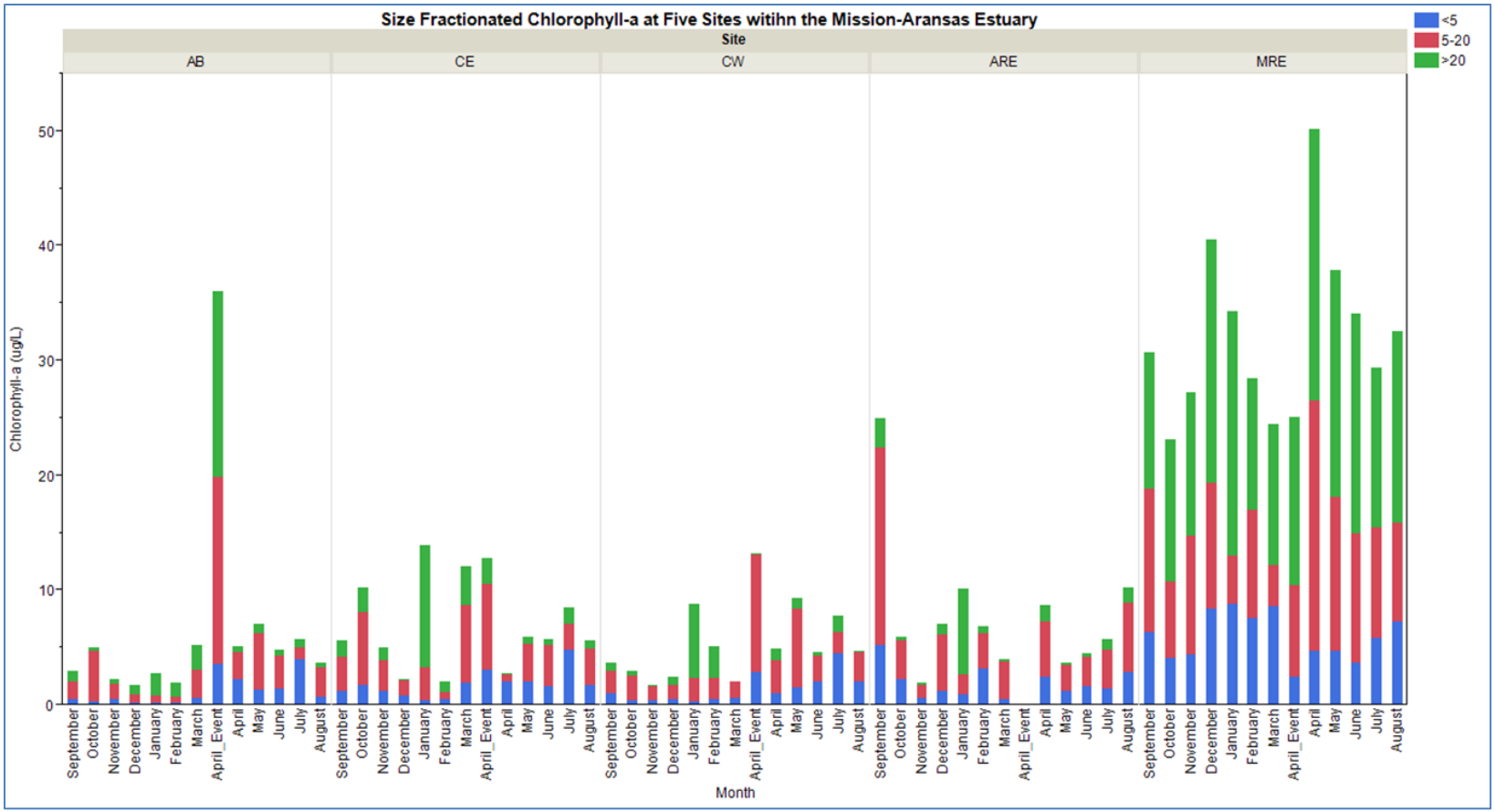

Figure A2: Monthly size fractionated (<5, 5-20, >20 µm) chlorophyll-*a* at Mission-Aransas Estuary sampling sites during the 2011-2012 study period.

Appendix B

ANOSIM Monthly Pairwise Comparison R-statistics

Although the overall one-way ANOSIM test shows significance (p-value = 0.001, R-statistic = 0.143, permutations = 999) between eukaryotic plankton communities monthly, the Bonferroni Correction (p-value = 0.0008) indicates that no pairwise comparisons were significantly different. However, the ANOSIM R-statistic for each monthly pairwise comparison displays a general seasonal trend in the sample communities overtime, illustrating a dynamic microbial eukaryotic assemblage within and among the estuarine sampling sites investigated in this study.

The ANOSIM R-statistic describes where the most similar samples are found, either within or between comparative groups. A higher R-statistic indicates greater dissimilarity between the samples being compared. This suggests that when samples collected closer in time are compared, their eukaryotic plankton communities more closely resemble one another, but subsequently become more distinct from one another with longer sampling intervals.

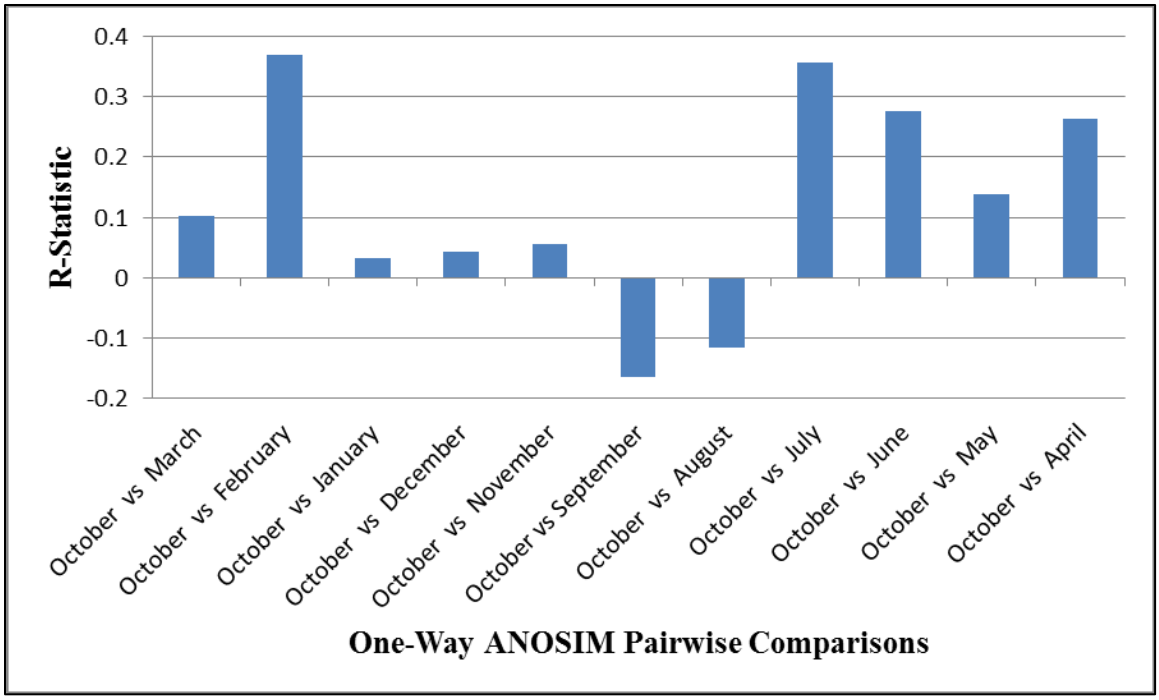

Figure B1: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for October 2011. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

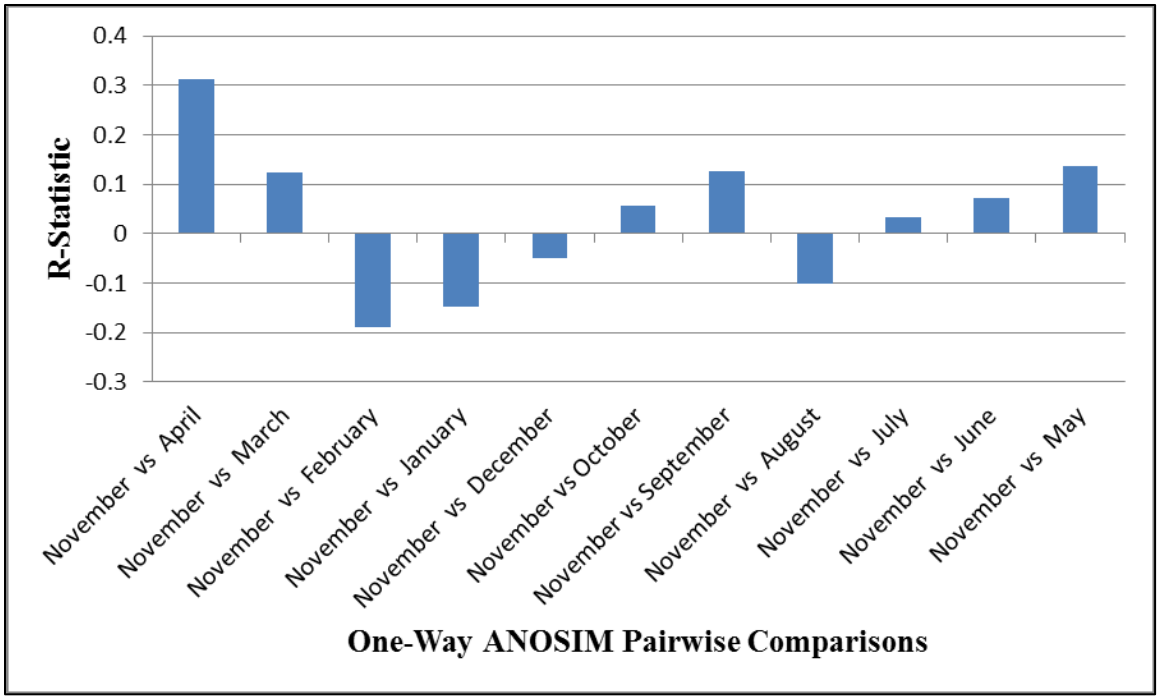

Figure B2: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for November 2011. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

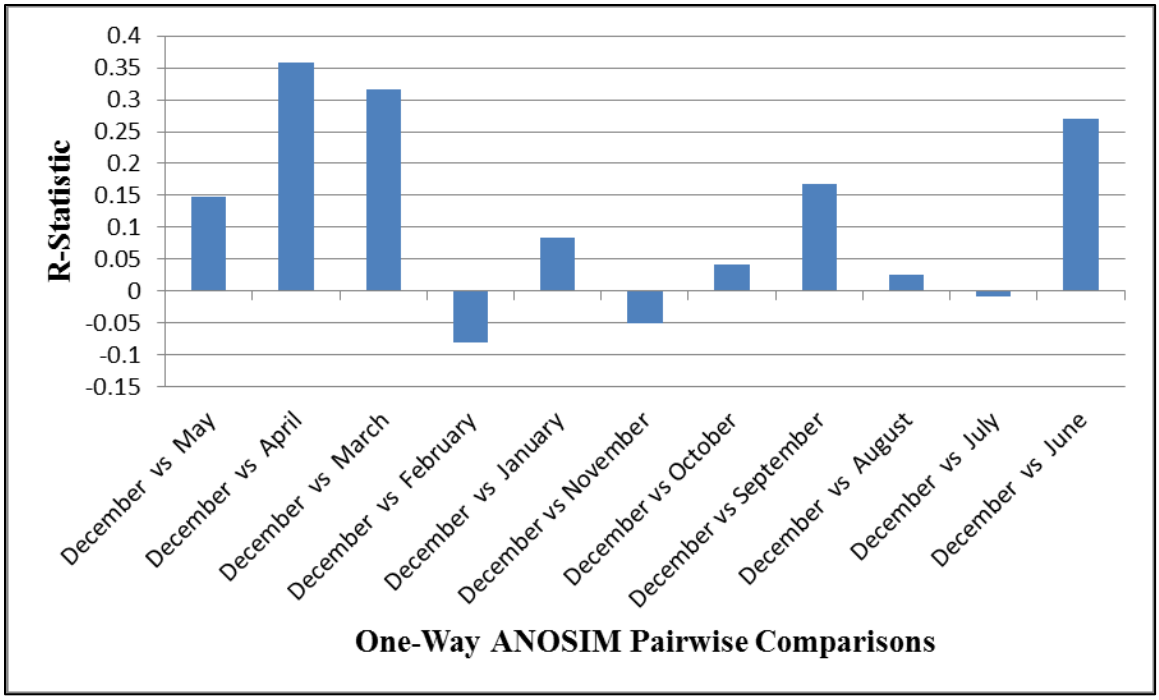

Figure B3: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for December 2011. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

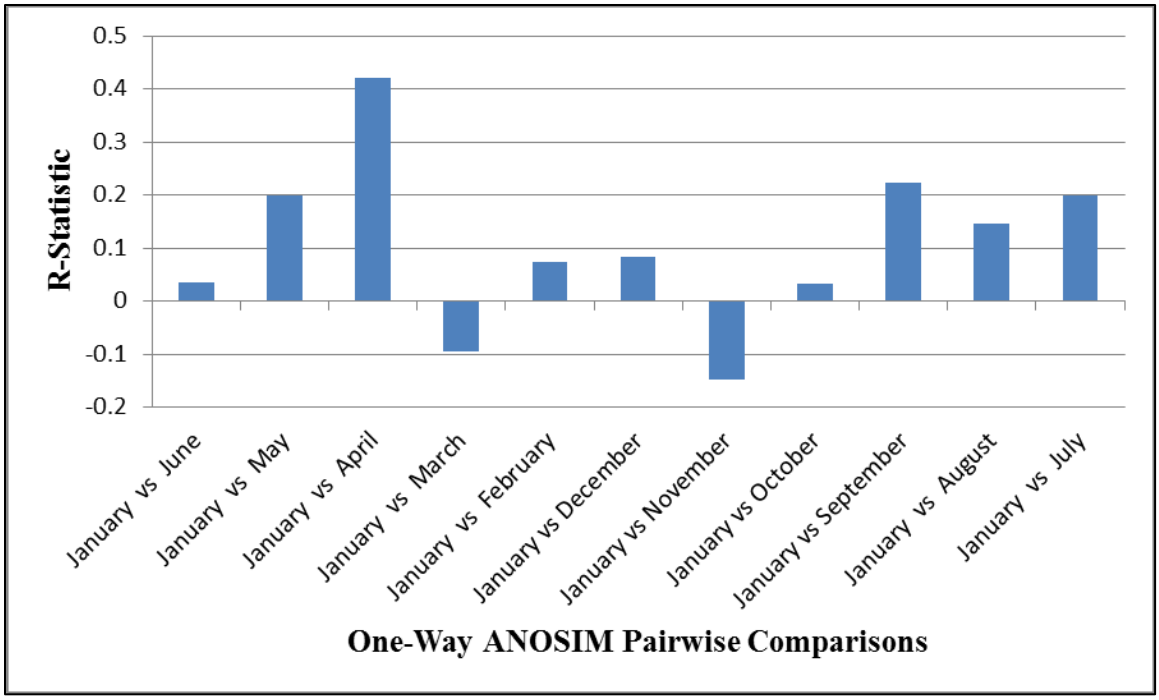

Figure B4: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for January 2012. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

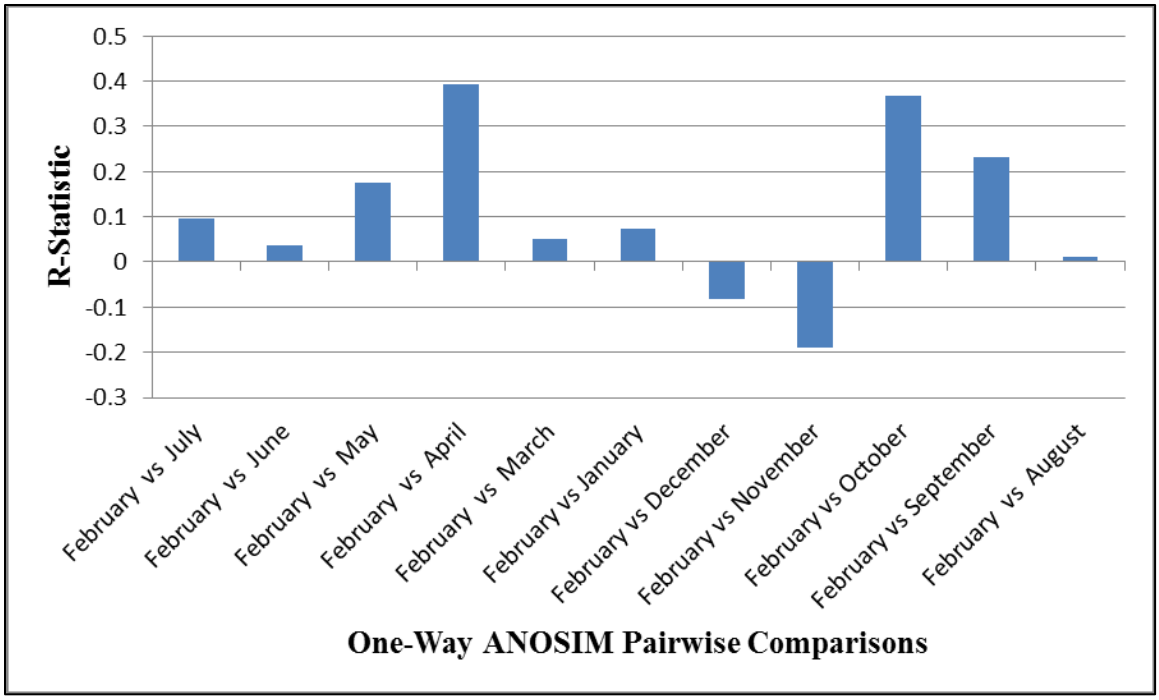

Figure B5: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for February 2012. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

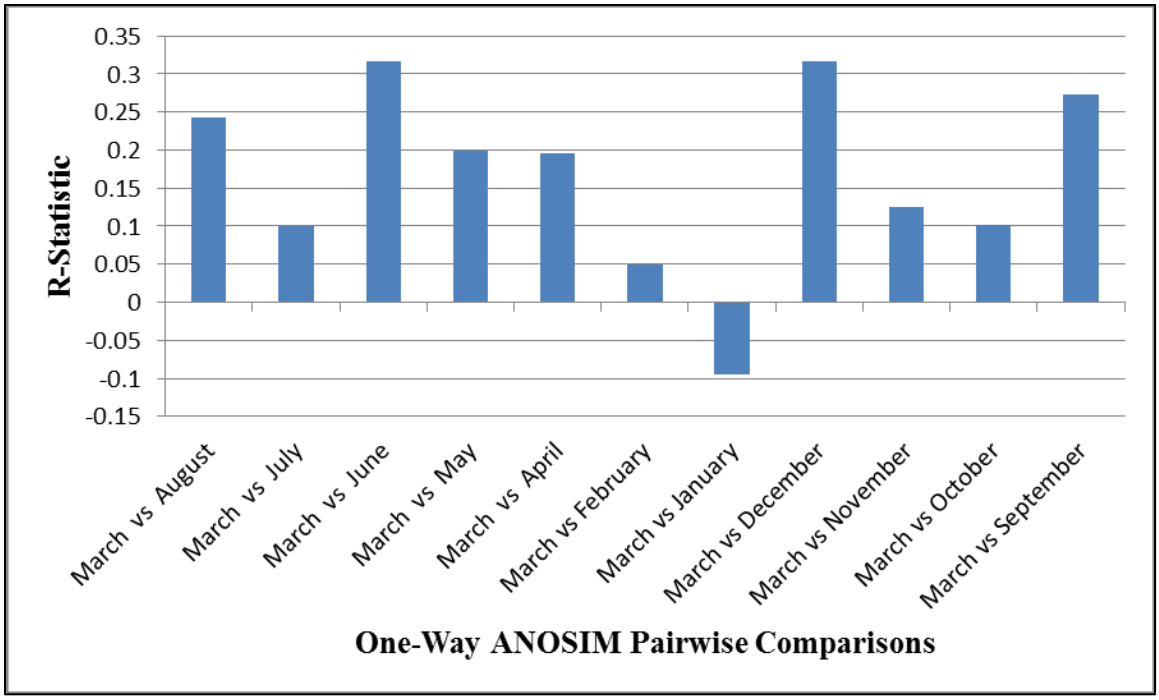

Figure B6: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for March 2012. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

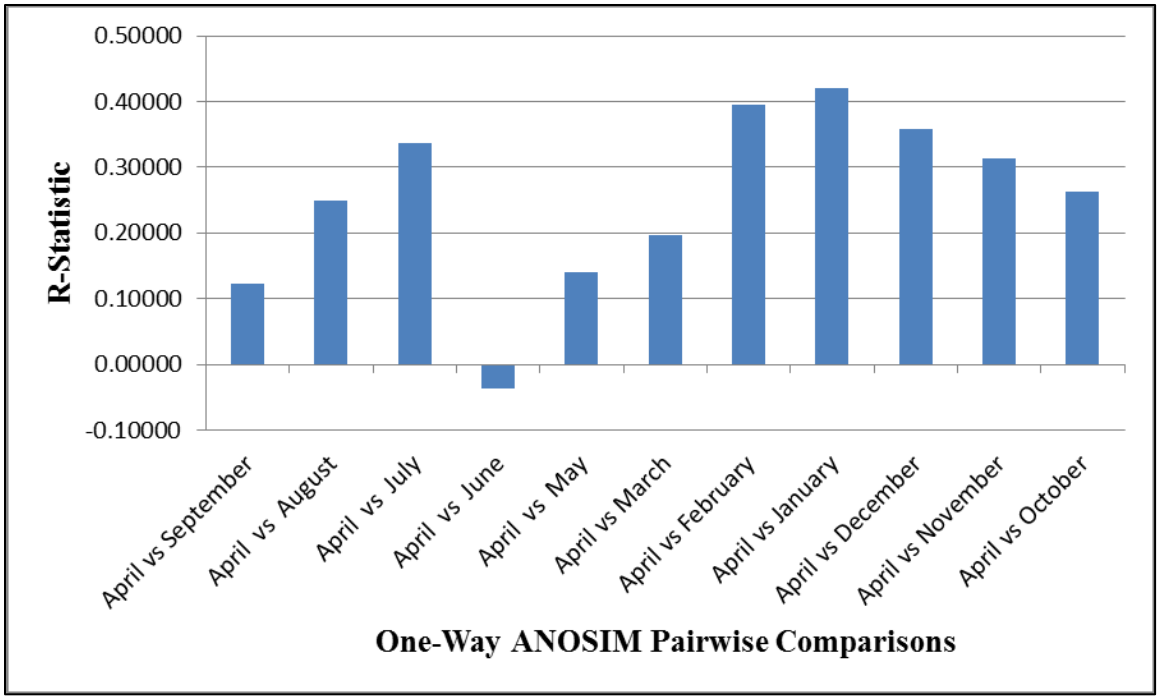

Figure B7: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for April 2012. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

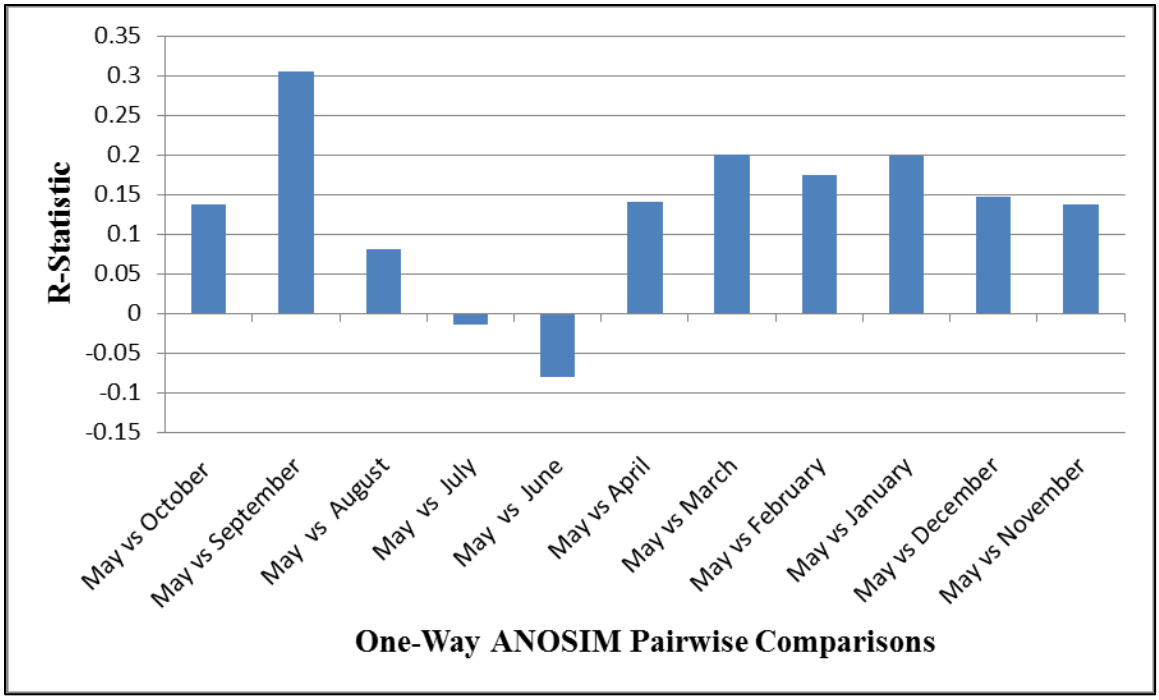

Figure B8: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for May 2012. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

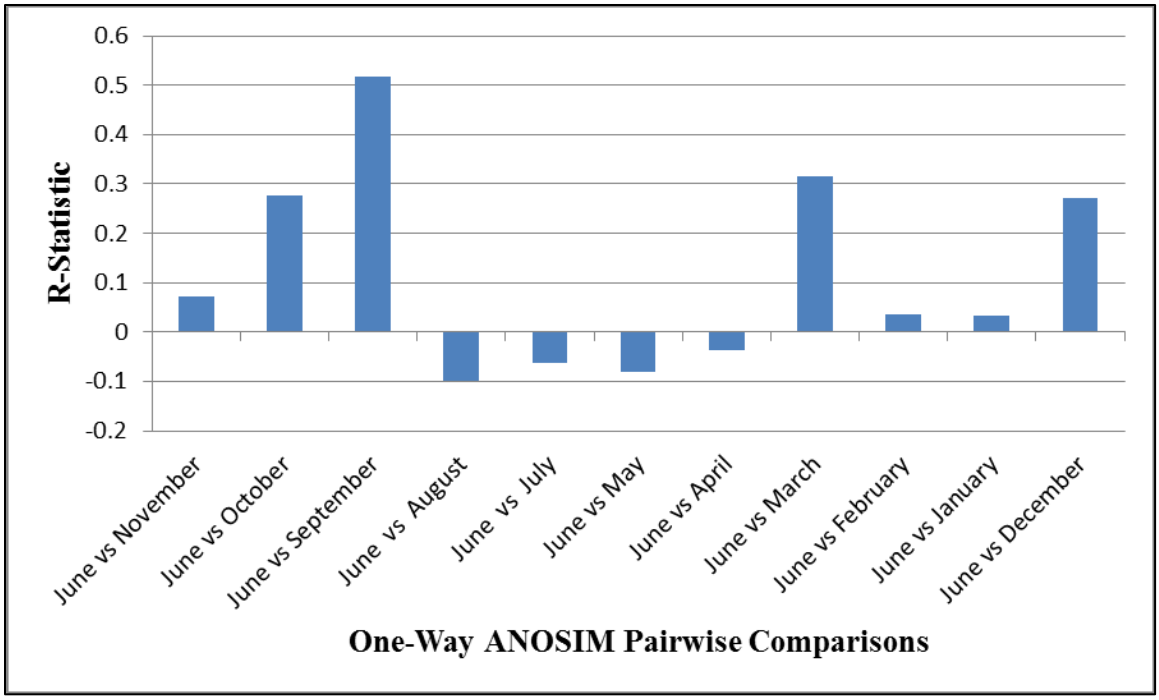

Figure B9: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for June 2012. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

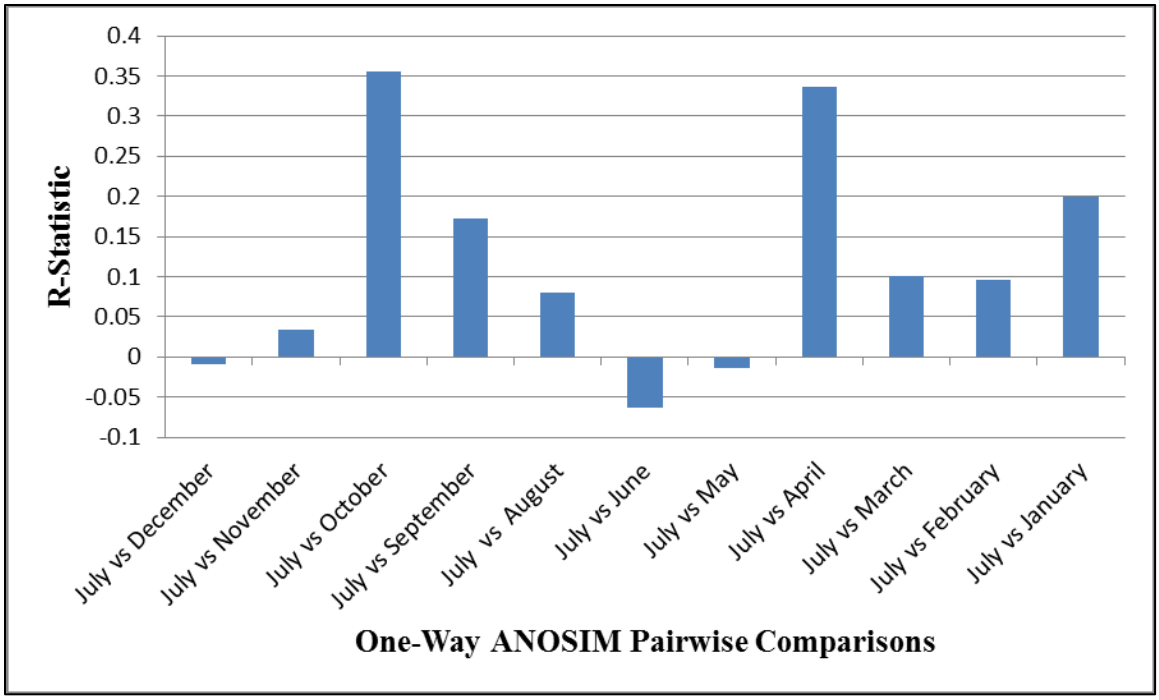

Figure B10: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for July 2012. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

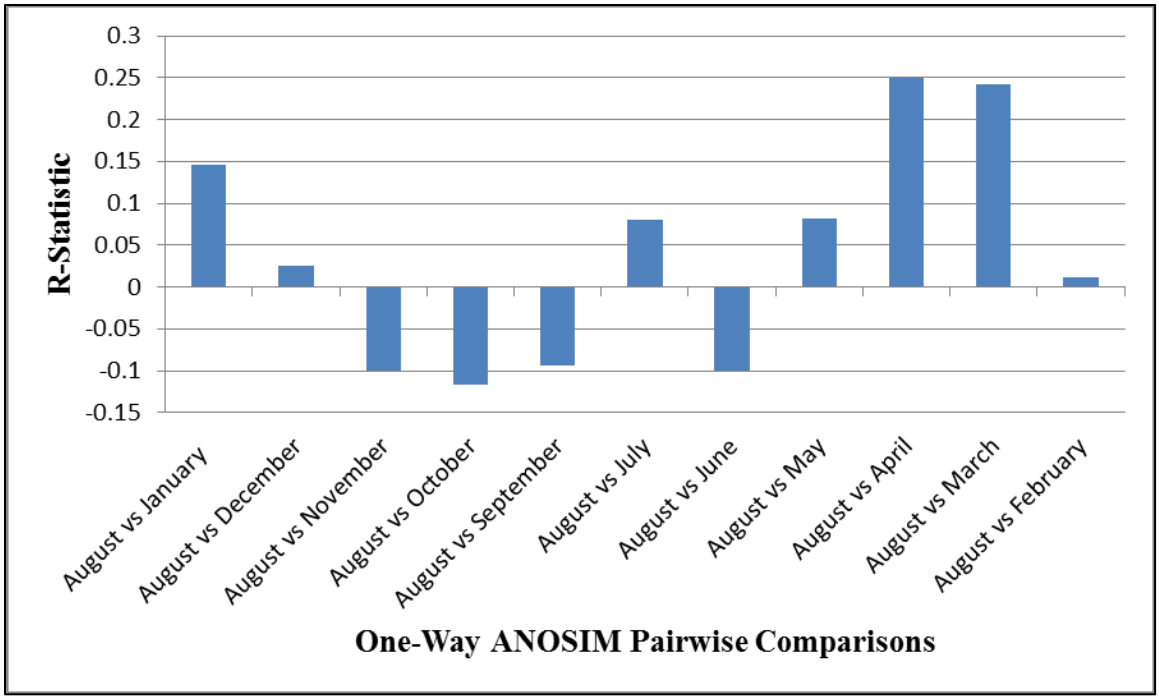

Figure B11: Mission-Aransas Estuary pairwise comparisons of the one-way ANOSIM R-statistic for August 2012. The x-axis is arranged with the smallest sampling interval at the center and the largest sampling intervals at the peripheries.

References

Aßmus, J., Melle, W. Tjøstheim, D., and Edwards, M. 2009. Seasonal cycles and long term trends of plankton in shelf and oceanic habitats of the Norwegian Sea in relation to environmental variables. *Deep Sea Research Part II.* 56: pp. 1895-1909.

Aberle, N., Bauer, B., Lewandowska, A., Gaedke, U., and Sommer, U. 2012. Warming induces shifts in microzooplankton phenology and reduces time-lags between phytoplankton and protozoan production. *Marine Biology*. 159: pp. 2441-2453.

Ahel, M., Barlow, R.G., and Mantoura, R.F.C. 1996. Effect of salinity gradients on the distribution of phytoplankton pigments in a stratified estuary. *Marine Ecology Progress Series*. 143: pp. 289-295.

Armstrong, N.E. 1982. Responses of Texas estuaries to freshwater inflows. In *Estuarine comparisons*, ed. Victor S. Kennedy, 103-120. New York: Academic.

Baban, S. M. J. 1997. Environmental monitoring of estuaries; estimating and mapping variousenvironmental indicators in Breydon Water Estuary, U.K., using Landsat TM imagery. *Estuarine, Coastal and Shelf Science.* 44: pp. 589-598.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., and Silliman, B.R. 2011. The value of estuarine and coastal ecosystem services. *Ecological Monographs*. 81: pp. 169-193.

Beaugrand G. 2004. Monitoring marine plankton ecosystems. Description of an ecosystem approach based on plankton indicators. *Marine Ecology Progress Series*. 269: pp. 69-81.

Beaugrand, G. 2005. Monitoring pelagic ecosystems using plankton indicators. *ICES Journal of Marine Science.* 62: pp. 333-338.

Bernall, S., Butturini, A., and Sabater, F. 2005. Seasonal variations of dissolved nitrogen and DOC:DON ratios in an intermittent Mediterranean stream. *Biogeochemistry*. 75: pp. 351-372.

Blanchette, C.A., Helmuth, B. and Gaines, S.D. 2007. Spatial patterns of growth in the mussel, *Mytilus californianus*, across a major oceanographic and biogeographic boundary at Point Conception, California, USA. *Journal of Experimental Marine Biology and Ecology*. 340: pp. 126-148.

Bianchi, T.S, Baskaran, M., and Ravichandran, M. Is the Sabine-Neches Estuary net heterotrophic or autrophic? A reply to the comment by Flinn *et al. Estuaries*. 21: pp. 839-841.

Bishop, K.A. 2012. Freshwater contributions and nitrogen sources in a South Texas estuarine ecosystem: a time-integrated story from stable isotope ratios in the eastern oyster (*Crassostrea virginica*). *M.S. Thesis*. The University of Texas at Austin.

Bledsoe, E.L. and Phlips, E.J. 2000. Correlation of phytoplankton standing crop in the Suwannee River and estuary to physical and chemical gradients. *Estuaries*. 23: pp. 458-473.

Bonnet D., and Frid, C. 2004. Seven copepod species considered as indicators of water mass influence and changes: results from Northumberland coastal station. *ICES Journal of Marine Science.* 61: pp. 485-491.

Bouvier, T.C. and Giorgio, P.A. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. *Limnology and Oceanography*. 47: pp. 453-470.

Bowes, M.J., House, W.A., and Hodgkinson, R.A. 2003. Phosphorus dynamics along a river continuum. *The Science of the Total Environment*. 313: pp. 199-212.

Boynton, W.R., Kemp, W.M. and Keefe, C.W. 1982. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. In: Kennedy, V.S. (ed.) *Estuarine Comparisons*. Academic Press, New York. pp. 69-90.

Brett, M.T. and Goldman, C.R. 1996. A meta-analysis of the freshwater trophic cascade. *Proceedings of the National Academy of Sciences.* 93: pp. 7723-7726.

Brodie, J.E. and Mitchell, A.W. 2005. Nutrients in Australian tropical rivers: changes with agricultural development and implications for receiving environments. *Marine and Freshwater Research*. 56: pp. 279-302.

Buskey, E.J. 1993. Annual pattern of micro- and mesozooplankton abundance and biomass in a subtropical estuary. *Journal of Plankton Research*. 15: pp. 907-924.

Buskey, E.J. and Hyatt, C.J. 1995. Effects of the Texas (USA) 'brown tide' alga on planktonic grazers. *Marine Ecology Progress Series*. 126: pp. 285-292.

Buskey, E.J. and Stockwell, D.A. 1993. Effects of a persistent 'brown tide' on zooplankton populations in the Laguna Madre of South Texas. In: Smayda, T.J. and Shimizu, Y. (eds) Toxic phytoplankton blooms in the sea. Proc. 5th Int. Conf. Toxic Marine Phytoplankton. Elsevier, Amsterdam. pp. 659-666.

Caliman, A., Leal, J.J.F., Carneiro, L.S., Esteves, F.A., Bozelli, R.L., and Farjalla, V.F. 2007. Functional bioturbator diversity enhances benthic-pelagic processes and properties in experimental microcosms. *Journal North American Benthological Society.* 3: pp. 450-459.

Cabecinha, E., Cortes, R., Cabral, J., Ferreira, T., Lourenco, M., and Pardal, M. 2009. Multi scale approach using phytoplankton as a first step towards the definition of the ecological status of reservoirs. *Ecological Indicators.* 9: pp. 240-255.

Calbet, A. and Landry, M.R. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnology and Oceanography*. 49: pp. 51-57.

Campbell, L., Walpert, J.N., and Guinasso, N.L. 2008. A new buoy based in situ optical early warning system for harmful algal blooms in the Gulf of Mexico. *Nova Hedwigia.* 133: pp. 161-170.

Canuel, E.A., Cammer, S.S., McIntosh, H.A., and Pondell, C.P. 2012. Climate change impacts on the organic carbon cycle at the land-ocean interface. *Earth and Planetary Sciences*. 40: pp. 685-711.

Chapin, F.S. III, McGuire, A.D., and Randerson, J. 2000. Arctic and boreal ecosystems of western North America as components of the climate system. *Global Change Biology*. 6: pp. 1-13.

Chao, A., Chazdon, R. L., Colwell, R.K., and T. Shen. 2006. Abundance-based similarity indices and their estimations when there are unseen species in samples. *Biometrics*. 62: pp. 361-371.

Chavez, F.P., Messie, M. and Pennington, J.T. 2011. Marine primary production in relation to climate variability and change. *Annual Review of Marine Science*. 3: pp. 227-260.

Cloern, J.E. 1996. Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. *Review in Geophysics*. 34: pp. 27-168.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series.* 210: pp. 223-253.

Conley, D.J. 2000. Biogeochemical nutrient cycles and nutrient management strategies. *Hydrobiologia.* 410: pp. 87-96.

Copeland, B.J. 1966. Effects of decreased river flow on estuarine ecology. *Journal of the Water Pollution Control Federation*. 38: pp. 1831-1839.

Countway, P.D., Gast, R.J., Savai, P., and Caron, D.A. 2005. Protistan diversity estimates based on 18S rDNA from seawater incubations in the western North Atlantic. *Journal of Eukaryotic Microbiology.* 52: pp. 95-106.

Cowlishaw, R.J. 2004. Seasonal coupling between ciliate and phytoplankton standing stocks in the South Slough of Coos Bay, Oregon. *Estuaries.* 27: pp. 539-550.

Crump, B. C., C. S. Hopkinson, M. L. Sogin, and J. E. Hobbie. 2004. Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. *Applied and Environmental Microbiology.* 70: pp. 1494-1505.

Crump, B. C., G. W. Kling, M. Bahr, and J. E. Hobbie. 2003. Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. *Applied and Environmental Microbiology*. 69**:** pp. 2253-2268.

Culbertson, J., Robinson, L., Campbell, P., and Butler, L. 2004. Trends in Texas commercial fishery landings 1981-2001. Austin: Texas Parks and Wildlife Department.

Culverhouse, P., Williams, R. and Benfield, M. 2006. Automated image analysis of plankton: future perspectives. *Marine Ecology Progress Series*. 312: pp. 297-309.

Dauer, D.M., Ranasinghe, J.A. and Weisberg, S.B. 2000. Relationships between benthic community condition, water quality, sediment quality, nutrient loads, and land use patterns in Chesapeake Bay. *Estuaries.* 23: pp. 80-96.

Day, J. W., Hall, C.A.S., Kemp, W.M. and Yanez-Arancibia, A. 1989. *Estuarine Ecology*. John Wiley & Sons, New York, New York, USA.

Diehl, S. 2002. Phytoplankton, light and nutrients in a gradient of mixing depths: Theory. *Ecology*. 83: pp. 386-398.

Diez, B., Pedro´s-Alio, C. and Masanna, R. 2001. Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. *Applied and Environmental Microbiology*. 67: pp. 2932-2941.

Digby, P.G.N. & Kempton, R.A. 1987. Multivariate analysis of ecological communities. Chapman & Hall, London.

Dodson, S.I., Newman, A.L., Will-Wolf, S., Alexander, M.L., Woodford, M.P., and Van Egeren, S.V. 2009. The relationship between zooplankton community structure and lake characteristics in temperate lakes (Northern Wisconsin, USA). *Journal of Plankton Research*. 31: pp. 93-100.

Doney, S.C., Ruckelshaus, J., Duffy, J.E., Barry, J.P., Chan, F., English, C.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., and Talley, L.D. 2012. Climate Change Impacts on Marine Ecosystems. 2012. *Annual Review of Marine Science.* 4: pp. 11-37.

Dustan, P. and Pinckney Jr., J.L. 1989. Tidally induced estuarine phytoplankton patchiness. *Limnology and Oceanography*. 34: pp. 410-419.

Dziock, F., Henle, K., Foeckler, F., Follner, K., and Scholz, M. 2006. Biological indicator systems in floodplains—A review. *International Review of Hydrobiology*. 91: pp. 271-291.

East, J.W. 2001. Discharge between San Antonio Bay and Aransas Bay, Southern Gulf Coast, Texas, May-September 1999. USGS. pp. 1-6.

Eiler, A., Hayakawa, H., Church, M.J., Karl, D.M. and Rappe´ M.S. 2009. Dynamics of the SAR11 bacterioplankton lineage in relation to environmental conditions in the oligotrophic North Pacific subtropical gyre. *Environmental Microbiology*. 11: pp. 2291-2300.

Eshleman, K.N. and Hemond, H.F. 1985. The role of organic acids in the acid-base status of surface waters at Bickford Watershed, Massachusetts. *Water Resources Research*. 21: pp. 1503-1510.

Evans, A., Madden, K., Morehead, S., 2012. The ecology and sociology of the Mission-Aransas Estuary-An estuarine and watershed profile. pp. 1-183.

Evans, C.D., Monteith, D.T., and Cooper, D.M. 2005. Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. *Environmental Pollution*. 137: pp. 55-71.

Fangstrom, I. and Willen, E. 1987. Clustering and canonical correspondence analysis of phytoplankton and environmental variables in Swedish Lakes. *Plant Ecology.* 71: pp. 87- 95.

Fanning, K.A., Carder, K.L., and Betzer, P.R. 1982. Sediment resuspension by coastal waters: a potential mechanisms for nutrient re-cycling on the ocean's margins. *Deep Sea Research Part A. Oceanographic Research Papers.* 29: pp. 953-965.

Fink, D.F. and Mitsch, W.J. 2004. Seasonal and storm event nutrient removal by a created wetland in an agricultural watershed. *Ecological Engineering*. 23: pp. 313-325.

Fuhrman, J.A., Hewson, I., Schwalbach, M.S., Steele, J.A., Brown, M.V. and Naeem, S. 2006. Annually reoccurring bacterial communities are predictable from ocean conditions. *Proceedings of the National Academy of Sciences.* 103: pp. 13104-13109.

Gonçalves, A.M.M, De Troch, M., Marques, S.C., Pardal, M.A., and Azeiteiro. 2010a. Spatial and temporal distribution of harpacticoid copepods in Mondego Estuary. *Journal of the Marine Biological Association of the United Kingdom*. 90: pp. 1279-1290.

Habib, O.A., Tippett, R., and Murphy, K.J. 1997. Seasonal changes in phytoplankton community structure in relation physic-chemical factors in Loch Lomond, Scotland. *Hydrobiologia*. 350: pp. 63-79.

Harding, L. 1994. Long-term trends in the distribution of phytoplankton in Chesapeake Bay: roles of light, nutrients and streamflow. *Marine Ecology Progress Series*. 104: pp. 267-291.

Hays, G.C., Richardson, A.J., Robinson, C. 2005. Climate change and marine plankton. *Trends in Ecology and Evolution*. 20: pp. 337-344.

Herrmann, R. and Stottlemyer, R. 1991. Long-term monitoring for environmental change in U.S. National Parks and watershed approach. *Environmental Monitoring and Assessment.* 17: pp. 51-65.

Horner-Devine, M.C., Lage, M., Hughes, J.B., and Bohannan, B.J.M. 2004. A taxa–area relationship for bacteria. *Nature*. 432: pp. 750-753.

Horner-Devine, M.C., Silver, J.M. and Leibold, M.A. 2007. A comparison of taxon co-occurrence patterns for macro- and microorganisms. *Ecology.* 88: pp. 1345-1353.

Houde, E.D. and Rutherford, E.S. 1993. Recent Trends in Estuarine Fisheries: Predictions of Fish Production and Yield. *Coastal and Estuarine Research Federation*. 16: pp. 161-176.

Hughes L. 2000. Biological consequences of global warming: is the signal already apparent? *Trends in Ecology and Evolution*. 15: pp. 56-61.

Irwin, A.J., Finkel, Z.V. and Schofield, O.M.E. 2006. Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. *Journal of Plankton Research.* 28: pp. 459-471.

Johns, N. 2004. Bays in Peril, A forecast for freshwater flows to Texas estuaries. National Wildlife Federation.

Johnson, S.J. 2009. A general method for modeling coastal water pollutant loadings. *Ph.D. Dissertation*. The University of Texas at Austin.

Kalke, R.D. and Montagna, P.A. 1991. The effect of freshwater inflow on macrobenthos in the Lavaca River Delta and Upper Lavaca Bay, Texas. *Contributions to Marine Science.* 32: pp. 49-72.

Keddy, P.A. and Weiher, E. 2001. Ecological Assembly Rules: Perspectives, advances, retreats. Cambridge University Press, Cambridge, U.

Kemp, W.M., Boynton, W.R., Adolf, J.E. and Boesch, D.F. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology Progress Series*. 303: pp. 1-29.

Kimmel, D.G., Roman, M.R. and Zhang, X. 2006. Spatial and temporal variability in factors affecting mesozooplankton dynamics in Chesapeake Bay: evidence from biomass size spectra. *Limnology and Oceanography*. 51: pp. 131-141.

Klein, N., Bulloch, S., Mooney, R., Garlough, P., and McClelland, J. 2008. Nutrient and Organic Matter Dynamics in Tidally-influenced Freshwater Sections of the Mission and Aransas Rivers, South Texas. Research Education for Undergraduates Scientific Poster.

Koch, G. R., Childers, D.L., Staehr, P.A., Price, R.M., Davis, S.E., and Gaiser, E.E. 2012. Hydrological conditions control P loading and aquatic metabolism in an oligotrophic, subtropical estuary. *Estuaries and Coasts*. 35: pp. 292-307.

Landry, M.R. and Hassett, R.P. 1982. Estimating the grazing impact on marine mirco zooplankton. *Marine Biology*. 67: pp. 283-288.

Leandro, S. M., Morgado, F., Pereira, F., and Queiroga, H. 2007. Temporal changes of abundance, biomass and production of copepod community in a shallow temperate estuary (Ria de Aveiro, Portugal). *Estuarine, Coastal and Shelf Science*. 74: pp. 215-222.

Lellis-Dibble, K. A., McGlynn, K. E., and Bigford, T. E. 2008. Estuarine Fish and Shellfish Species in U.S. Commercial and Recreational Fisheries: Economic Value as an Incentive to Protect and Restore Estuarine Habitat. U.S. Dep. Commerce, NOAA Tech. Memo. NMFSF/SPO-90. pp. 1-94.

Li, W.K.W. 2002. Macroecological patterns of phytoplankton in the northwestern North Atlantic Ocean. *Nature*. 419: pp. 154–157.

Lindeman, R.L. 1942. The trophic-dynamic aspect of ecology. *Ecology.* 23: pp. 399-418.

Litchman, E., de Tezanos Pinto, P., Klausmeier, C.A., Thomas, M.K. and Yoshiyama, K. 2010. Linking traits to species diversity and community structure in phytoplankton. *Hydrobiologia.* 653: pp. 15-28.

Litchman, E., Klausmeier, C.A. and Yoshiyama, K. 2009. Contrasting size evolution in marine and freshwater diatoms. *Proceedings of the National Academy of Sciences of the United States of America.* 106: pp. 2665-2670.

Livingston, R.J. 2001. Eutrophication Processes in Coastal Systems CRC Press, London. pp. 1-327.

Livingston, R.J. 2003. Trophic Organization in Coastal Systems CRC Press, London. pp. 1-388.

Llope, M., Daskalov, G.M., Rouyer, T.A., Mihneva, V., Chan, K., Grishin, A.N., and Stenseth, N.C. 2011. Overfishing of top predators eroded the resilience of the Black Sea system regardless of the climate and anthropogenic conditions. *Global Change Biology*. 17: pp. 1251-1265.

Longley, W. L. 1994. Freshwater inflows to Texas bays and estuaries: ecological relationships and methods for determination of needs. Joint Estuarine Research Study Texas Water Development Board and Texas Parks and Wildlife Department.

Mallin, M.A. and Paerl, H.W. 1994. Planktonic trophic transfer in an estuary: seasonal, diel and community structure effects. *Ecology*. 75: pp. 2168-2184.

Marques, S.C., Pardal, M.A., Pereira, M.J., Gonçalves, F., Marques, J.C., and Azeiteiro, U.M., 2007b. Zooplankton distribution and dynamics in a temperate shallow estuary. *Hydrobiologia.* 587: pp. 213-223.

Marshall, H.G. and Alden, R.W. 1990. A comparison of phytoplankton assemblages and environmental relationships in three estuarine rivers of the lower Chesapeake Bay. *Estuaries*. 13: pp. 287-300.

McLaughlin, K., Ahn, J.H., Litton, R.M., And Grant, S.B. 2007. Use of salinity mixing models to estimate the contribution of creek water fecal indicator bacteria to an estuarine environment: Newport Bay California. *Water Research*. 41: pp. 3595-3604.

Molinero, J.C., Buercher, E., Dallot, S., and Nival, P. 2008. Climate control on the long term anomalous changes of zooplankton communities in the Northwestern Mediterranean. *Global Change Biology*. 14: pp. 11-26.

Montagna, P.A. and Kalke, R.D. 1992. The effect of freshwater inflow on meiofaunal and macrofaunal populations in the Guadalupe and Nueces Estuaries, Texas. *Estuaries*. 15: pp. 266-285.

Montagna, P.A., Ward, G., and Vaughan, B. 2011. The importance and problem of freshwater inflows to Texas estuaries. In: Griffin, R. (ed.), Water Policy in Texas: Responding to the Rise of Scarcity, The RFF Press, Washington, DC. pp. 107-127.

Mooney, R.F. and McClelland, J.W. 2012. Watershed export events and ecosystem responses in the Mission-Aransas National Estuarine Research Reserve, South Texas. *Estuaries and Coasts*. 35: pp. 1468-1485.

Morehead, S., Beyer, T.G., Dunton, K., 2007. Community Characterization of the Mission-Aransas National Estuarine Research Reserve and Surrounding Areas. UTMSI Report TR/07-001. University of Texas Marine Science Institute, Port Aransas, Texas, 42 leaves.

Morris, R.M., Rappe´, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A. and Carlson, C.A. 2002. SAR11 clade dominates ocean surface bacterioplankton communities. *Nature.* 420: pp. 806-810.

Murrell, M.C. and Lores, E.M. 2004. Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria. *Journal of Plankton Research.* 26: pp. 371-382.

Nazaries, L., Pam, Y., Bodrossy, L., Baggs, E.M., Millard, P., Murrell, C. and Singh, B.K. 2013. Microbial regulation of biogeochemical cycles: evidence from a study on methane flux and land-use change. *Applied and Environmental Microbiology*. 79: pp. 1-39.

Nemergut, D.R., Costello, E.K., and Hamady, M. 2011. Global patterns in the biogeography of bacterial taxa. *Environmental Microbiology*. 13: pp. 135-144.

Newell, R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review. *Journal of Shellfish Research*. 23: pp. 51-61.

National Renewable Energy Laboratory (NREL). 2013. Chapter 2: The national wind resource.

Odebrecht, C., Abreu, P., O. Moller Jr, O., Niencheski, L.F., Proenc, A., and Torgan, L.C. 2005. Drought effects on pelagic properties in the shallow and turbid Patos Lagoon, Brazil. *Estuaries.* 28: pp. 675-685.

Oliver, B.G., Thurman, E.M., and Malcom, R.L. 1983. The contribution of humic substances to the acidity of coloured natural waters. *Geochimica Et Cosmochimica Acta*. 47: pp. 2031-2035.

Osborn, M.A., Moore, E.R.B., and Timmis, K.N. 2000. An evaluation of terminal restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. *Environmental Microbiology*. 2: pp. 39-50.

Painchaud, J., Lefaivre, D., Therriault, J.C., and Legendre, L. 1995. Physical processes controlling bacterial distribution and variability in the upper St. Lawrence estuary. *Estuaries*. 18**:** pp. 433-444.

Phlips, E.J., Badylak, S., and Grosskopf, T. 2002. Factors affecting the abundance of phytoplankton in a restricted subtropical lagoon, the Indian River Lagoon, Florida, USA. *Estuarine Coastal and Shelf Science*. 55: pp. 385-402.

Pinckney, J.L. and Dustan, P. 1990. Ebb-tidal fronts in Charleston Harbor, South Carolina: Physical and biological characteristics. *Estuaries.* 13: pp. 1-7.

Pinckney, J.L., Paerl, H.W., Harrington, H.B. and Howe, K.E. 1998. Annual cycles of phytoplankton community-structure and bloom dynamics in the Neuse River estuary, North Carolina. *Marine Biology*. 131: pp. 371-381.

Pomeroy, L.R. 1974. The ocean's food web, a changing paradigm. *BioScience*. 24: pp. 499-504.

Pommier, T., Canback, B., Riemann, L., Bostrom, K.H., Simu, K., Lundberg, P., Tunlid, A., and Hagstrom A. 2007. Global patterns of diversity and community structure in marine bacterioplankton. *Molecular Ecology*. 16: pp. 867-880.

Pritchard, D.W. 1967. Salinity distribution and circulation in the Chespeake Bay estuarine system. *Journal of Marine Research*. 11: pp.106-123.

Prosser, J.I., Bohannan, B.J. and Curtis, T.P. 2007. The role of ecological theory in microbial ecology. *Nature Review Microbiology*. 5: pp. 384-392.

Pulich, Jr. W. and Blair, C. 1997. Current status and historical trends of seagrasses in the Corpus Christi Bay National Estuary Program study area. CCBNEP (20) Corpus Christi, Texas.

Revilla, M., Ansotegui, A., Iriarte, A., Madariaga, I., Orive, E., Sarobe, A. and Trigueros, J.M. 2002. Microplankton metabolism along a trophic gradient in a shallow temperate estuary. *Estuaries.* 25: pp. 6-18.

Riley, G.A. 1976. A model of plankton patchiness. *Limnology and Oceanography*. 21: pp. 873-880.

Russ, G.R. 1991. Coral reef fisheries: effects and yields. pp. 601-635 in P.F. Sale, editor. The ecology of fishes on coral reefs. Academic Press, San Diego, California, USA.

Russell, M.J. and Montagna, P.A. 2007. Spatial and temporal variability and drivers of net ecosystem metabolism in western Gulf of Mexico estuaries. *Estuaries and Coasts*. 30: pp. 137-153.

Ryther, J.H. 1969. Photosynthesis and fish production in the sea. *Science.* 166: pp. 72-76.

Sagert, S., Rieling, T., Eggert, A., and Schubert, H. 2008. Development of a phytoplankton indicator system for the ecological assessment of brackish coastal waters (German Baltic Seacoast). *Hydrobiologia*. 611: pp. 91-103.

Sanders, J.G., Cibik, S.J., D'Elia, C.F. and Boynton, W.R., 1987. Nutrient enrichment studies in a coastal plain estuary: changes in phytoplankton species composition. *Canadian Journal of Fisheries and Aquatic Sciences*. 44: pp. 83-90.

Schelske, C.L. and Odum, E.P. 1962. Mechanisms maintaining high productivity in Georgia estuaries. *Proceedings - Gulf and Caribbean Fisheries Institute*. 14: pp. 75-80.

Sherr, B.F., Sherr, E.B., Andrew, T.L , Fallon, R.D. and Newell, S.Y. 1986. Trophic Interaction between heterotrophic Protozoa and bacterioplankton in estuarine water analyzed with selective metabolic inhibitors. *Marine Ecology Progress Series.* 32: pp. 169-180.

Shideler, G.L. 1984. Suspended sediment responses in a wind-dominated estuary of the Texas Gulf Coast. *Journal of Sedimentary Petrology*. 54: pp.731-745.

Sin, Y., Wetzel, R.L., and Anderson, I.C. 2000. Seasonal variations of size-fractionated phytoplankton along the salinity gradient in the York River estuary, Virginia (USA). *Journal of Plankton Research*. 22: pp. 1945-1960.

Solis, R.S. and G.L. Powell. 1999. Hydrography, mixing characteristics, and residence times of Gulf of Mexico estuaries. *Biogeochemistry of Gulf of Mexico Estuaries*. John Wiley and Sons, Inc., New York. pp. 29–61.

Steele, P. and Bert, T.M. 1994. Population ecology of the blue crab, *Callinectes sapidus Rathbun*, in a subtropical estuary: population structure, aspects of reproduction, and habitat partitioning. *Florida Marine Research Publications*. 51: pp. 1-24.

Stevens, P.W., Blewett, D.A., and Casey, J.P. 2006. Short-term effects of low dissolved oxygen event on estuarine fish assemblages following the passage of Hurricane Charley. *Estuaries and Coasts*. 29: pp. 997-1003.

Strom, S.L. 2008. Microbial ecology of ocean biogeochemistry: a community perspective. *Science*. 320: pp. 1043-1045.

Sun C.C., Wang Y.S., Wu M.L., Dong J.D., Wang Y.T., Sun F.L., and Zhang Y.Y. 2011. Seasonal variation of water quality and phytoplankton response patterns in Daya Bay, China. *International Journal of Environmental Research and Public Health.* 8: pp. 2951-2966.

Tallaksen, L.M., Madsen, H., and Clausen, B. 1997. On the definition and modeling of streamflow drought duration and deficit volume. *Hydrological Sciences Journal*. 42: pp. 15-33.

Tolan, J.M. 2013. Estuarine community-level response to freshwater inflows. Water Resources Planning, Development and Management. Chapter 4. pp. 74-94.

Tolan, J.M., Nelson, J., German, D., and Kuhn, N. 2011. Multivariate methodology assessment of two tidal streams within the San Antonio-Nueces Coastal Basin: Final Report on the Mission and Aransas Rivers. Texas Parks and Wildlife Department.

Tremblay, J.E. and Legendre, L. 1994. A model for the size-fractionated biomass and production of marine phytoplankton. *Limnology and Oceanography*. 39: pp. 2004 -2014.

Turner R.E. and Rabalais N.N. 2003. Linking landscape and water quality in the Mississippi River basin for 200 years. *BioScience*. 53: pp. 563-572.

Vigil, P., Countway, P.D., Rose, J., Lonsdale, D.J., Gobler, C.J., and Caron, D.A. 2009. Rapid shifts in dominant taxa among microbial eukaryotes in estuarine ecosystems. *Aquatic Microbial Ecology.* 54: pp. 83-100.

Ward, D.H. and Armstrong, N.E. 1997. Ambient water, sediment, and tissue quality of the Corpus Christi Bay National Estuary Program area, present status and historical trends. CCBNEP-23. Texas Natural Resources Conservation Commission, Austin, Texas.

Wang, Y.S., Lou, Z.P., Sun, C.S., Wu, M.L., and Han, S.H. 2006. Multivariate statistical analysis of water quality and phytoplankton characteristics in Daya Bay, China, from 1999 to 2002. *Oceanologia.* 48: pp. 193-211.

Wilson, J.G. 2002. Productivity, fisheries and aquaculture in temperate estuaries. *Estuarine, Coastal and Shelf Science.* 55: pp. 953-967.

Williams, P.J.le B. 1981. Microbial contribution to overall marine plankton metabolism: Direct measurements of respiration. *Oceanologica Acta*. 4: pp. 359-364.

Yu, C., Ahuja, R., Sayler, G., and Chu, K. 2005. Quantitative molecular assay for fingerprinting microbial communities of wastewater and estrogen-degrading consortia. *Applied and Environmental Microbiology*. 71: pp. 1433-1444.

Yunker, M.B., Belicka, L.L. Harvey, H.R., and Macdonald, R.W. 2005. Tracing the inputs and fate of marine and terrigenous organic matter in Arctic Ocean sediments: A multivariate analysis of lipid biomarkers. *Deep Sea Research II.* 52: pp. 3478-3508.

Vita

Aubrey Rain Lashaway was born in Bryan, Ohio, but has lived in Pioneer, Ohio her entire life. After completing her work at North Central Local High School, Pioneer, Ohio, she entered The University of Michigan in Ann Arbor, Michigan. She received the degree of Bachelor of Science in Environmental Science with a concentration in Aquatic Ecology and a minor in the Russian Language in April 2007. Following graduation at The University of Michigan, Aubrey entered graduate school at The Pennsylvania State University. She received a Master of Science in Wildlife and Fisheries Science with a minor in Watershed Stewardship in May 2009. During the following year, Aubrey was employed as a technician for the National Park Service and the Boyer Hydrological Laboratory at The Pennsylvania State University. In August 2010, she entered the Graduate School at The University of Texas at Austin, and pursued her degree of Master of Science in Marine Science at The University of Texas Marine Science Institute in Port Aransas, Texas, graduating in August 2013.

Permanent Email Address: aubrain@gmail.com

This thesis was typed by the author.