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Gary Alon Blakeney

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Characterization of sediment microbial communities and analysis of biogeochemical responses to eutrophication in Southeastern estuaries

By

Gary Alon Blakeney

A Master’s Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Biological Sciences
in the Department of Biological Sciences

Mississippi State, Mississippi

December 2014
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2014
Characterization of sediment microbial communities and analysis of biogeochemical responses to eutrophication in Southeastern estuaries

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Estuaries are valuable economic resources to humans. However, changes in these ecosystems, such as alteration in nutrient availability, can impose eutrophic pressures. Traditionally, estuaries have been monitored through chemical analyses that measure factors such as sediment oxygen demand (SOD) and reduced forms of Fe(II), Mn(II), and H₂S. These methods are time consuming and have been proven to be unreliable. This study was conducted to determine if using T-RFLP analysis could be used as a proxy for some of the current methods used to monitor eutrophication. Using SPSS to perform a principle component analysis, it was determined that connections can be made between the genetic fingerprints of microbial communities in estuaries and the biogeochemical markers that are currently used to monitor eutrophication. This method can potentially replace the current methods by allowing scientists to measure more sites rapidly and with reproducibility.
DEDICATION

I would like to dedicate this study to my wife Josie, my family, and most of all God, without whom I would never have finished.
ACKNOWLEDGEMENTS

I would like to acknowledge those who helped me through this process. Dr. Janet Donaldson for being patient even when I was frustrating and for allowing me to work in her lab. Dr. Karen McNeal for allowing me to work on this project and all the experience I gained from the time working in her lab and on this project. I would like to acknowledge Dr. John Brooks for help with the TRFLP analysis, and allowing me to use his lab space in order to complete this project. This project was financially supported by the United States Environmental Protection Agency Grant No. 83604801 and the National Science Foundation Grant No. DGE-0947419 at Mississippi State University. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Environmental Protection Agency or the National Science Foundation.
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CHAPTER I  
INTRODUCTION AND BACKGROUND

1.1 Introduction

Estuaries, which are defined as the brackish, enclosed waters that connect one or more rivers to the sea, are an important source of life on this planet (Pritchard 1963). These large bodies of brackish water act as a natural filter for water as it transitions from fresh to salt water, which in turn creates a nutrient sink and a very diverse ecosystem (Wild-Allen et al. 2013). If changes in the nutrient status of the estuary occur, the viability of the estuary is negatively affected. This could lead to a decrease in the amount of resources that are provided by the ecosystem.

The diverse nutrients acquired within estuaries make them natural reservoirs for many biological species. Due to the general protective nature of their location, many animals use these areas as natural nesting or spawning locations. For example, Tampa Bay, which is located in Florida, USA, serves as a refuge for many species of filter feeders, such as oysters and clams, as well as many species of migratory birds and even manatees. There are a number of species of fish that rely on this location in order to spawn such as Red Drum, Spotted Seatrout, Snook, Southern flounder, Florida pompano, striped mullet as well as many others (Kunneke and Palik 1984). Fish that are dependent on estuaries as nursery areas generally migrate into temperate estuarine systems during the postflexion larval stage (Miskiewicz 1986; Strydom et al. 2003) or during the juvenile
stage, depending on the species. Consequently, estuaries play a pivotal role in the early life-history strategies and survival of early developmental stages of fish that rely on these habitats as nursery areas (Wasserman and Strydom 2011). In addition to wildlife, they harbor many plant species that could not survive in other ecosystems such as the open ocean. It is necessary to carefully monitor the overall health and integrity of these delicate environments. The aim of this study was to compare and contrast various monitoring methods to determine if a novel approach of monitoring the biological communities of an estuary can provide a more efficient method of determining the state of the estuary.

1.2 Background

It is estimated that approximately 60% of the global population now live along coasts and estuaries; this increase in population has also led to an increase in pollution in these sites (Wollanski 2007). Pollution and nutrient input will increase the trophic state of a given body, which will increase primary production, or eutrophication (Paerl et al. 2003). This increase in pollution can have detrimental effects on the cycles of carbon, nitrogen, and phosphorus (Smith et al. 1999). This could result in a loss of biodiversity, which can cause a decrease in the productivity or amount of biodiversity of the estuary (Meyer-Reil and Koster 2000, Smith et al. 1999).

Eutrophication is the process by which increasing nutrients causes a change in the nutritional status of a given body of water. Most frequently, the increase in the availability of mineral macronutrients (particularly nitrogen and phosphorus), as well as carbon, can cause an alteration in the cycling of these nutrients. If not properly regulated, this can lead to reduced water quality, loss of biodiversity, and a decrease in the overall
health of the estuarine ecosystem (Meyer-Reil and Koster 2000; Sims et al. 2013; Paerl et al. 2003; Harris 1999). It could be argued that eutrophication is the largest water quality problem, which is directly attributed to the influx of nutrients such as N and P (Smith et al. 1999). For water quality management, the U.S. Environmental Protection Agency (USEPA) has extensively employed a series of guidelines known as Total Maximum Daily Load (TMDL) to define the maximum amount of a pollutant (from point and nonpoint sources) that can enter a water body, which will allow the body to return to compliance as per section 303(d) of the Clean Water Act of 1972 (Sims et al. 2013; Yagow et al. 2006; Muñoz–Carpena et al. 2006). Water quality is a synthesis term for assessing the physical, chemical, biological and aesthetic characteristics of a water body against reference values to determine if the water is safe for specific uses (Wild-Allen et al. 2013).

There are a significant number of ecological models available that can give researchers insight into how eutrophication is affecting an estuary. These models help to provide a basis for first order nutrient budgets of a system by providing a visual representation of the chemical processes that are occurring within a system over time. (Fisher et al. 1988; Azevedo et al. 2008; Robson et al. 2008). Figure 1.1 and 1.2 (Wild-Allen et al. 2013) are both perfect examples of that visualization. These maps indicate how important environmental nutrients, chemicals, and biological species can impact an estuary at any given time of the year. For instance, these figures provide information related to how the amounts of nitrogen present in a system, as well as the amount of chlorophyll and sedimentary oxygen, alter over the course of the year. A researcher can easily make correlations as to how these nutrients impact not only each other, but how
they affect the overall health of an estuary. One of the most important compounds depicted are the nitrogen species. These can have a huge impact on not only the environment but also the microorganisms present in the system.

Microorganisms are responsible for a large portion of the cycling of nutrients, especially nitrogen. They can directly catalyze nitrification and denitrification, which allows the fixation of nitrogen for use by other organisms, or release of N\textsubscript{2} back into the atmosphere, respectively (Francis et al. 2007). The process of the N cycle is dynamic and there are multiple pathways with which microbes are involved. The process of nitrification occurs as follows: NH\textsubscript{3}→NO\textsubscript{2}→NO\textsubscript{3}, while the process of denitrification occurs as follows: NO\textsubscript{3}→NO\textsubscript{2}→NO→N\textsubscript{2}O→N\textsubscript{2} (Francis et al. 2007).

Certain microbial species are also involved with the cycling of other metals such as iron, manganese and sulfur. The oxides of these metals are used as electron acceptors when the organisms degrade organic matter (Thamdrup et al. 1994). These processes are generally anaerobic and as such would likely be identified within the sediment core. Thamdrup et al. (1994) gives the following equations to detail the reaction:
\[ 2\text{FeOOH}+\text{H}_2\text{S}\rightarrow 2\text{Fe}^{2+}+\text{S}^0+4\text{OH}^- \]
which describes the reduction of Fe oxides to Fe by H\textsubscript{2}S, and
\[ \text{MnO}_2+\text{H}_2\text{S}\rightarrow \text{Mn}^{2+}+\text{S}^0+2\text{OH}^- \] as well as
\[ \text{MnO}_2+2\text{Fe}^{2+}+2\text{H}_2\text{O}\rightarrow \text{Mn}^{2+}+2\text{FeOOH}+2\text{H}^+ \] to describe the reduction of Mn oxides to reduced Mn species and the production of Fe oxides. Sulfates are generally reduced anaerobically to produce H\textsubscript{2}S and follow a linear trend in relation to depth (Thamdrup et al. 1994). Since Fe\textsuperscript{2+}, Mn\textsuperscript{2+}, and H\textsubscript{2}S species are byproducts of microbial metabolism, quantification is directly correlated to microbial communities present in the sediment.
<table>
<thead>
<tr>
<th>Season</th>
<th>DIN mg/m³</th>
<th>Chlorophyll mg/m³</th>
<th>Sediment Oxygen %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>[Image]</td>
<td>[Image]</td>
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<tr>
<td>Winter</td>
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<tr>
<td>Summer</td>
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Figure 1.1 Modeled seasonal mean near surface dissolved inorganic nitrogen (left), chlorophyll (middle), and surface sediment oxygen % saturation (right) (Wild-Allen et al. 2013).
Figure 1.2  Monthly mean modeled salinity, nitrate, ammonia, chlorophyll, and oxygen along the axis of the estuary for December (summer-low flow) and October (spring-peak flow) (Wild-Allen et al. 2013).
1.3 Current Methods Used to Monitor Estuaries

With the importance of these ecosystems established, it is important to understand how these areas are monitored. There are numerous ways in which one can monitor an estuary, ranging from bioindication to chemical analysis. Currently, the US EPA and environmental researchers measure the content of oxygen, certain nutrients, metals, and carbon in the system. These include ammonium, nitrate, total phosphorous, total nitrogen, iron, and sulfur to name a few. Measurements of the sediment oxygen demand provide a rate for the amount of oxygen that is required of a system by both biological and chemical processes in the sediment and is given as a rate of oxygen exchange between benthic sediments and the surrounding water. Though each method provides vital information, no single resource is completely self-contained or exclusive. Active management is a high priority that is often hampered by limited understanding of complex multidisciplinary estuarine interactions (Davis and Koop 2006). An example of this is the difference between bioindication and chemical techniques to determine the health of an estuary. Bioindication relies on the fact that organisms must be in an environment for a period of time, adjusting to alterations in the environment, while chemical analyses provide more of a snapshot of the processes occurring within the system (Dziock et al. 2006). The limitations of these methods are described in this section.

1.3.1 Detection of Chemicals

Many of the current technologies to evaluate the activity of estuaries rely on chemical information to describe the status of nutrient input into a wetland ecosystem. However, these methods are limited as they only provide partial evidence of water
quality. These chemical analyses include quantifying the amount of carbon, nutrients, and metals present, such as iron, nitrogen, phosphorous, sulfur etc., as well as measuring the amount of sediment oxygen demand (SOD) present in the system (Ranjard et al. 2000; Paerl et al. 2003; Shin et al. 2000). Shin et al. (2000) define SOD as “the areal uptake rate (mmol O₂/m²-day) due to the decomposition of organic matter in the bottom sediments and is dependent on microbial activity and a variety of chemical oxidation processes.” SOD is one of a multitude of measures used to determine the level of eutrophication. Changes in organic matter coincide with the changes in concentrations of nutrients available in the system. The demand for oxygen in the sediment increases as the microbial communities in the sediment adapt to recycle the nutrients found in the decaying algal matter (Paerl et al. 1998; Sims et al. 2013; Meyer-Reil and Koster 2000). This creates hypoxic or anoxic conditions in the system that can have detrimental impacts on the other types of fauna present including fish, and the impacts are manifested as large fish kills (Smith et al. 1999). Increased organic matter present should result in higher demands for oxygen in the sediments by the microorganisms responsible for removing the organic material. Examining the communities in the sediment as a whole could theoretically monitor this change.

Three of the most important elements to analyze within the sediments are Fe, Mn and S. Iron and sulfur are closely related to each other in the biogeochemical processes of sediments. Some trace metals can be absorbed or co-precipitated with the iron-sulfide minerals in anoxic sediments (Hong-Bin et al. 2008). This is critical especially in anaerobic sediments, as anaerobic oxidation of organic matter in marine sediment is coupled to oxygen reduction via a cascade of redox processes and transport of
intermittent electron donors and acceptors (Risgaard-Petersen et al. 2012). Dissolved hydrogen sulfide that is produced during bacterial dissimilatory sulfate reduction may quickly react with sedimentary reactive iron compounds to form iron monosulfide (Wijsman et al. 2001; Rickard 1997). These iron monosulfide molecules can then react with dissolved sulfide, elemental sulfur or polysulfides to form pyrite (FeS$_2$) (Berner 1970; Luther 1991; Rickard 1997; Wijsman et al. 2001). The detection of Fe$^{2+}$ and Mn$^{2+}$ can indicate if a site is anoxic.

1.3.2 Bioindication

Bioindication refers to the use of plants, animals, and microbial species to predict or indicate the quality and health of a given area (Sims et al. 2013, Dziock et al. 2006). There are multiple forms of bioindicators and each has a special niche that it characterizes. They include: classification, status, environmental, biodiversity, and target (validation) indicators (Dziock et al. 2006). These indicators can be used to determine the dependency of species survival as it relates to their environment (Dziock et al. 2006). Plants and large birds, such as egrets, are commonly used as indicator species (Dziock et al. 2006; Boncompagni et al. 2003). A study conducted by Boncompagni et al. (2003) analyzed the eggs and feathers of egrets to determine the presence of multiple pollutants including As, Cd, Cr, Pb, Hg, Mn, Se, and Zn. In addition to the feathers and eggs, they evaluated the prey and sediments in order to analyze the pollutants in the ecosystem in China and Pakistan. However, a major limitation to the use of these bioindicators is that the effect is due to exposure to the pollutant in the estuary over long periods of time, ranging from weeks to years. This, unfortunately, is not useful in determining the overall health of the estuary at any given point in time. Bocompagni et al (2003) showed that
there was a marked similarity to the concentrations of heavy metals found in the tested materials between the three sites analyzed in their study. This makes it difficult to tell where the contamination is occurring. It was possible, however, to determine the extent of pollution at the sites by measuring the relative concentrations of the heavy metals. They also found that the concentrations differed between species, which could pose a problem when trying to determine the status of an estuary.

Given that bioindication is a useful tool to examine the health of estuaries, there is an ever-expanding need to use microbes as a marker for evaluating estuarine health. The analysis of microbial populations in natural habitats, such as soil, is one of the cornerstones of current research on the function of natural ecosystems (Van Elsas and Boersma 2011). Microbes have an important role in the cycling of nutrients and decaying matter and are therefore useful indicators for the biological activities occurring within these complex communities. Additionally, microbes are excellent indicators for nutrient cycling and degradation as they are more sensitive to changes in the ecosystem than plants and animals (Paerl et al. 2003). As the status of the system changes with regard to nutrient input, structure of the microbial community will adjust in response to changes in the environment. Therefore, microbial indicator information is useful in the determination of estuarine health (Sims et al. 2013; Clement et al. 1998; Lukow et al. 1999). The benefits of using microbes as indicators is that they can be sampled from specific parts of the bay and reflect the changes in the nutrients much more rapidly than higher order species. This is because the growth cycles of these organisms are much shorter and they produce or consume chemicals that can be analyzed in a field laboratory relatively quickly. In particular, monitoring the microbial populations related to
manganese oxidation could prove useful in determining if a site is eutrophic. These include cyanobacteria, the sheathed (*Leptothrix*-like) and budding (*Hyphomicrobium*-like) bacteria, *Pseudomonas*, and *Metallogenium* (Nealson 2006).

![Microbially Mediated Nitrogen, Carbon, and Sulfur Cycles (Sims et al. 2012)](image)

Other key microbial populations that can be monitored are those related to nitrogen cycling. Nitrification is generally performed by two physiologically distinct groups of obligate aerobes: ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Sulfate reduction, an important biogeochemical process in the recycling of sulfur is performed by sulfate reducing bacteria (SRB); these bacteria require anaerobic conditions, carbon, and a continuous source of sulfate ions (Sims et al. 2013).

There is evidence to suggest that it may even be possible for single organisms to utilize many of these chemicals as their terminal electron acceptor (Lovley et al. 2004). The Proteobacteria phylum is a prime example of this. These organisms can be found in
multiple ecosystems and are involved in nutrient cycling, especially iron, manganese, and sulfur. This makes them prime candidates to examine as markers for environmental processes and should be considered in the monitoring effort.

Table 1.1 Ability of various microbial species to utilize multiple terminal electron acceptors (adapted from Lovley et al. 2004)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Source</th>
<th>Oxidation with Fe(III)</th>
<th>Fe forms reduced</th>
<th>Other Electron Acceptors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Geobacter argillaceus</em></td>
<td>Subsurface Clay Beddings</td>
<td>No Data</td>
<td>Fe(III)-Cit, Fe(III)-NTA, Fe(III)-P, PCIO, Smectite</td>
<td>AQDS, Fum, nitrate, Mn(IV), S0</td>
<td>Shelobolina et al., 2004</td>
</tr>
<tr>
<td><em>Geobacter bemidjiensis</em></td>
<td>Subsurface sediment</td>
<td>Complete</td>
<td>Fe(III)-Cit, Fe(III)-NTA, Fe(III)-P, PCIO</td>
<td>AQDS, Fum, Mal, Mn(IV)</td>
<td>Shelobolina et al., 2004</td>
</tr>
<tr>
<td><em>Geobacter bremensis</em></td>
<td>Freshwater ditch</td>
<td>ND</td>
<td>PCIO</td>
<td>Mn(IV), S0, Fum, Mal</td>
<td>Straub and Bucholz-Cleven, 2001</td>
</tr>
<tr>
<td><em>Geobacter chapellei</em></td>
<td>Deep subsurface</td>
<td>Complete</td>
<td>PCIO, Fe-NTA</td>
<td>Mn(IV), AQDS, Fum</td>
<td>Coates et al., 2000; Lovley et al., 1990</td>
</tr>
<tr>
<td><em>Geobacter grhiciae</em></td>
<td>Aquatic sediments</td>
<td>Complete</td>
<td>PCIO, Fe(III)-Cit</td>
<td>Aqds</td>
<td>Coates et al., 2000</td>
</tr>
<tr>
<td><em>Geobacter humireducens</em></td>
<td>Contaminated wetland</td>
<td>Complete</td>
<td>PCIO, Fe(III)-Cit</td>
<td>Mn(IV), AQDS, nitrate, Fum, S0</td>
<td>Coates et al., 1998b</td>
</tr>
<tr>
<td><em>Geobacter hydrogenophilus</em></td>
<td>Contaminated aquifer</td>
<td>Complete</td>
<td>PCIO, Fe(III)-Cit</td>
<td>AQDS, Fum</td>
<td>Coates et al., 2000</td>
</tr>
</tbody>
</table>

1.3.2.1 Algae as an Indicator

Direct links exist between algal biomass and nutrient loading and are the most widely used indicator of eutrophication in aquatic ecosystems (Sims et al. 2013; Paerl et al. 2003; Yagow et al. 2006). Other studies have also provided correlations to the overall
state of the estuary based on the amount of algae present (Stevenson et al. 2002; Sims et al. 2013; Harris 1999; Paerl et al. 2003; Paerl et al. 1998; Meyer-Reil and Koster 2000). These studies involved quantifying the amount of chlorophyll $a$, $b$, and $c$ present and linking the concentration detected to the status of eutrophication, as greater chlorophyll equals more biomass, greater concentrations of nutrients and greater potential for hypoxia or anoxia. However, these studies only provide information for the activity within the water column and not within the sediment (Paerl et al. 2003; Harris 1999; Sims et al. 2013). As the algae die and settle to the bottom sediments, microbes remove the resulting organic deposits. This will potentially create an anoxic environment that can be detected by measuring the concentrations of reduced iron, manganese, and sulfur.

Harmful algal blooms affect nearly every coastal nation (Hallegraeff 1993) and it is now understood that there are more toxic algal species than previously thought that impact the fisheries resources (Anderson et al. 2002). Some of the impacts include paralytic shellfish poisoning (PSP), ciguatera fish poisoning (CFP) and various other harmful algal bloom (HAB) phenomena such as fish kills, loss of submerged vegetation, shellfish mortalities, and widespread marine mammal mortalities (Anderson et al. 2008).

With the mounting evidence that algal blooms are impacting a large number of estuaries around the world, it is important that these data be included in any monitoring effort by researchers. This is the view of many researchers and there seems to be a consensus on some aspects of the relationship between eutrophication and HABs (Smayda 1989; Anderson et al. 2002; Glibert and Burkholder 2006; Glibert et al. 2008; Anderson et al. 2008). There are multiple ways to determine how algae are affecting estuarine health such as the algal growth potential (AGP) and the limiting nutrient algal
assay (LNAA), both of which offer reliable evidence about limited nutrients in the wetland system (McCormick and Stevenson 1998; Stevenson et al. 2002; Sims et al. 2013). There are a number of Biotic indices that include the Myxophycean, Chlorophycean, and Euglenophycean indexes which use the presence of algal species to indicate the pollution of organic waste in the water (Palmer 1969; Sims et al. 2013).

1.3.2.2 Phospholipid Fatty Acid as an Indicator

Phospholipid fatty acid analysis (PLFA) involves sampling a whole community and examining the types of fatty acids present, which can then be analyzed to determine the microbial species present in a given system. PLFA can be useful, but currently there is a trend that uses genetic fingerprinting techniques to define the structure of the communities present in the soil sample (Piotrowska-Seget and Mrozik 2003). These studies determine the microbial communities present in the sediment, which can be a valuable tool to examine the relationship between microbial communities and the status of health in estuaries. Phospholipid Fatty Acids (PLFAs) and Community Level Physiological Profiles (CLPP) have been used to describe the functional diversity of the microbial communities in wetland restoration (Anderson et al. 2010; Sims et al. 2013).

However, there are limitations to using PLFAs to correlate the relationship between the communities and the overall status of the estuary. Multiple species may contain the same phospholipid fatty acids, which can result in problems with resolution of the profile. An example of this is the fatty acid 18:2ω6,9, which can also be found in certain mosses (Dembitsky and Rezanka 1995) and cyanobacteria (Lechevalier and Lechevalier 1988; Anderson et al. 2010). Some groups of organisms, including some methanogens, are even completely impossible to identify using simple PLFAs extractions.
(Kaur et al. 2005; Anderson et al. 2010). It is of the utmost importance to analyze samples using multiple techniques to gain a broader view of the impact these communities have on the surrounding environment. If PLFAs were to be used in monitoring programs, they should be complemented by molecular approaches (Anderson and Cairney 2004) to separate the different functional groups of organisms (Anderson et al. 2010).

1.3.2.3 Molecular Techniques to Identify Bioindicators

There are many types of molecular biological approaches that can be utilized in monitoring efforts such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), single-strand conformation polymorphism (SSCP), terminal restriction fragment length polymorphism (T-RFLP), amplified rDNA restriction analysis (ARDRA), amplified ribosomal intergenic spacer analysis (ARISA), and cloning (Anderson and Cairney 2004). Community fingerprinting techniques have commonly been applied to studies of bacterial ecology and have significantly increased our understanding of the role and diversity of bacteria in soil (Anderson and Cairney 2004). Several of these techniques are similar in the way that they assess microbial diversity in the natural sedimentary environment. DGGE and TGGE separate DNA fragments of the same size but of different sequence based on the melting behavior of DNA (Anderson and Cairney 2004). This is based on the different G+C content of the DNA sequences. DGGE require some form of a denaturant, usually in a linear gradient, to differentiate between different microbial species. TGGE is based on the same concept, however differs in the use of temperature rather than a chemical denaturant to break the antiparallel strands of the DNA. This can pose serious problems when using certain
types of primers. If the primers cannot withstand either the denaturant or the temperature, they could separate from the DNA and give false bands that could cause an over estimation of diversity (Muyzer 1999).

There are additional pitfalls with the use of molecular techniques, such as reproducibility, that have been outlined by others including Muyzer (1999) and Fromin et al. (2002). For instance, polymorphisms can result in skewed data. There are techniques that utilize these similarities to distinguish between species in a sample. A certain technique, known as Terminal-Restriction Fragment Length Polymorphisms (T-RFLP), can distinguish these sequence similarities through the utilization of restriction enzyme digestion (Marsh 1999). This technique has a much higher throughput, which allows researchers to examine multiple samples faster and more accurately than traditional gel based methods. Not only does it increase the amount of samples that can be processed, but can also vastly increase the accuracy of size determination between sequences (Anderson and Cairney 2004).

T-RFLP analysis is a culture independent- polymerase chain reaction (PCR) based method used to examine species variation in whole communities based on differences in the 16S rRNA gene sequence, which provides information about species richness/diversity (the number and quantity of species within a community), species evenness (the size of species populations), as well as spatial (distance) and temporal (time) differences between communities. This difference is based on the variation of restriction enzyme sites located within the 16S rRNA genes. This technique can be performed on sediment samples to give a genetic fingerprint of the communities in the samples and can give distinct fingerprints for many different samples and varying
community compositions (Kirk et al. 2004; Lukow et al. 2000). The quantitative measurement capability of this technique makes it a valuable tool to determine the relationship between microbial communities, the amount of nutrients present, and rate of SOD in the sediments to give us a better understanding of the impact of eutrophication on an estuarine system.

The technique utilizes universal fluorescently labeled forward and reverse primers that bind to conserved 16S rRNA sequences that are shared by multiple species of organisms. PCR is then used to amplify the entire 16S rRNA sequence, which is then followed by enzyme digestion. This digestion utilizes enzymes with small (approximately 4bp) recognition sites. As the enzymes cleave the resulting PCR product, each species should theoretically contain a distinct length of gene product between the fluorescently labeled primer and the cleavage site. This technique is highly reproducible as shown by the work of many others (Dunbar et al. 2001; Osborn et al. 2000; Liu et al. 1997).

1.4 Hypothesis and Objectives

The overall hypothesis of this research is that microbial communities can be used as a proxy for the current monitoring techniques of estuaries. The hypothesis was tested by analyzing nutrients in sediment and SOD data, which was compared to the microbial community present among various sites within three different estuaries. The estuaries tested were Choctawhatchee Bay, FL (CHB), Old Tampa Bay, FL (TB), and Bay St. Louis, MS (BSL). There should be a clear correlation between the amount of nutrients present and the microbial communities that are present. If there are high numbers of characteristic bacteria, such as nitrogen fixing species, iron oxidizing and reducing
species, manganese oxidizing and reducing species, and sulfur oxidizing and reducing species, then it may be possible to use communities as a proxy for SOD. The following research objectives were pursued to test this hypothesis.

Objective 1: Characterize the microbial community profile in two estuaries in Florida and one in Mississippi.

Objective 2: Correlate the biogeochemical data with the communities present in these estuaries.

This method can prove to be a more expedient, less expensive replacement for some of the current monitoring protocols, such as SOD. The sampling time may be reduced and allow for more sampling locations to be investigated during a comparable time frame. Therefore, the health of the estuary can be more closely monitored and regulated by environmental agencies, such as the EPA.
CHAPTER II
MATERIALS AND METHODS

2.1 Study Sites

Sediment cores were collected at 8 sites throughout Choctawhatchee Bay, FL and 11 sites were sampled throughout Old Tampa Bay, FL. Additionally 4 sites were sampled from Bay St. Louis, MS. These sites have large watersheds and are associated with densely populated regions. These sites were chosen as they are typically experiencing a large amount of nutrient enrichment due to the human population, agricultural land use, as well as dumping. The large watersheds have the potential for increased eutrophication, which makes these sites good candidates for this proposed research.
2.1.1 Choctawhatchee Bay, FL

Figure 2.1 Choctawhatchee Bay study sites sampled in July 2013. (Courtesy of EPA).
Figure 2.2  Choctawhatchee Bay watershed (Courtesy of Choctawhatchee Basin Alliance).
The Choctawhatchee Bay watershed (Fig. 2.2) covers nearly 13,856 km² (5,350 mi²) and stretches from northwest Florida to southern Alabama. Much of the watershed is composed of agricultural, silvicultural, and rural land, with a large portion connected to Eglin Air Force Base. Handley and DeMay (2007) state that “this impacts the ecosystem through additional storm water runoff, resource utilization, and similar pressures that are caused by development. Choctawhatchee Bay is more than 43 km (27 mi) long in an east-west orientation along the upper gulf coast of Florida in Okaloosa and Walton Counties. It varies from 2 to 10 km (1 to 6 mi) in width, with depths ranging from 3 to 13 m (10 to 43 ft). Tidal exchange in the bay is minimal (a range of about 0.15 m, or 0.5 ft) and flushing is limited to the narrow, shallow opening to the Gulf of Mexico at East Pass. The human population of the watershed is growing rapidly, with that of the Florida counties increasing 41% from 1980 to 1995.” This area was sampled at 8 sites randomly selected throughout the bay (Fig 2.1) during the summer of 2013 by MSU and the USEPA. Cores were collected by divers and were then subsequently sliced anaerobically to retrieve porewater and microbial community data. Sediment samples were stored at -20°C in the field and at -80°C upon return to MSU (Starkville, MS). Pore water samples were collected using centrifugation in anaerobic conditions. Concentration of trace elements such as manganese, iron, and sulfur were collected, as well as various other chemical data, such as chlorophyll, total suspended solids, total particulate organic carbon, dissolved organic carbon, aluminum, total Kjeldahl nitrogen, ammonia, total phosphorus, redox potential, pH, sediment oxygen demand, temperature, and dissolved oxygen.
2.1.2 Old Tampa Bay, FL

Figure 2.3 Location of study sites from Old Tampa Bay, FL (7-12 July 2012) (Map courtesy of the EPA).
Goodwin (1987) states “Tampa Bay is a shallow, Y-shaped embayment along the west-central coast of peninsular Florida, one of the most rapidly growing regions of the state. The bay occupies parts of Hillsborough, Manatee, and Pinellas Counties. It is bordered by the major cities of Tampa, St. Petersburg, Clearwater, and Bradenton. The population of the three-county area in 1982 was approximately 1.6 million, with a growth
rate of about 42,000 residents yearly since 1970. Tampa Bay has a total surface area of about 390 mi$^2$ and is the largest estuary in Florida. Its average depth is about 12 ft. The maximum depth, about 90 ft., is off the northern tip of Egmont Key at the mouth of Tampa Bay.” Dixon et al (1996) states, “the watershed to Tampa Bay includes approximately 5879 km$^2$ (Fig. 2.4). The water surface of the Bay itself is approximately 1031 km$^2$.” This site was sampled during the summer of 2012 (as indicated in Fig. 2.3). Cores were collected by divers, which were subsequently sliced under anaerobic conditions and stored at -20°C in the field, then transferred to -80°C upon return to MSU. Pore water samples were collected using a centrifugation method and biogeochemical data was analyzed colorimetrically for the concentration of trace elements such as Mn, Fe, and S were collected as well as various other chemical data, including chlorophyll, total suspended solids, total particulate organic carbon, dissolved organic carbon, aluminum, total Kjeldahl nitrogen, ammonia, total phosphorus, redox potential, pH, sediment oxygen demand, temperature, and dissolved oxygen.
2.1.3 St. Louis Bay, MS

Figure 2.5 St. Louis Bay, MS Study Sites (Courtesy Jonathon Geroux, MSU)
Figure 2.6  St. Louis Bay Watershed (ltmcp.org)
St. Louis Bay is an estuarine system that is located in the Gulf Coast region of MS that covers almost 9900 acres (Huddleston et al. 2003). This estuary is the recipient of water that drains from nearly half a million acres of Mississippi’s coastal watershed (Fig. 2.6). The Jourdan and the Wolf Rivers, as well as multiple bayous, feed into the bay (Huddleston et al. 2003). A large majority of the watershed is undeveloped and covered by forested land (Huddleston et al. 2003). This watershed also contains land that is used for agricultural purposes. There are also small population centers around the bay. The major population centers include Bay St. Louis, Pass Christian, Biloxi, and Gulfport, and Long Beach, MS (Huddleston et al. 2003). These urban areas house many industrial centers as well as military bases and the NASA Stennis Space Center. According to the US Census Bureau in 2013, Bay St. Louis had a population of 10,259 while Pass Christian and Long Beach contained populations of 4,613 and 15,300 respectively. Cores were collected, sliced under anaerobic conditions, and the solid phase was analyzed for reduced manganese, sulfur, and iron. Porewaters were analyzed for redox potential, pH, H$_2$S, and dissolved O$_2$ (Fig. 2.5).

2.2 Microbial Community Analysis

Ten cores from Choctawhatchee Bay, nine cores from Tampa Bay, and four cores from Bay St. Louis were collected and kept on ice until returning to the field lab to be processed. Cores were sliced in a glove box purged with nitrogen into 2cm sections down to 10 cm and stored at -20°C until return to MSU at which time they were transferred to -80°C. DNA was isolated from 1g sediment samples from the top and bottom sections of the cores using the Mo-Bio Power Soil DNA Isolation kit following
the manufacturer’s protocol (Mo Bio Laboratories, Inc., Carlsbad, CA). DNA quality was analyzed by using a NanoDrop ND-1000 spectrophotometer. PCR was then performed on the extracted DNA using the following program: 10 min at 95°C followed by 35 cycles of 95°C for 40 seconds, 60°C for 30 seconds, and 72°C for 33 min, followed by a final annealing step of 72°C for 33 min. The following Osborn (2000) primers specific to the 16S rRNA gene were utilized: 5’-(6-FAM)-CAGGCTAACACATGCAAGTC-3’, 5’-(HEX)-ACGGGGCGGTGTGATAAG-3’. The reaction volume was 45μL and contained the following for each sample from each core at both depths: 2μL BSA (1/100 NEB BSA, 0.1mg/ml), 5 μL 10x Gold Buffer, 6 μL MgCl₂ (25mM), 1μL dNTPs (10 mM final concentration), 0.6μL Taq Gold (5U/μL), 2 μL polyvinylpyrrolidone (PVPP), 26.4μL sterile PCR H₂O, and 1μL of each forward and reverse primer (1 μg/μL). 10μL of template DNA (5 μg/μL) was then added to the wells of a 96 well plate and the reaction was then allowed to proceed. PVPP is a chelating agent that is used to remove humic substances from the mixture incase any were left over from the DNA extraction. The presence of humic substances can inhibit the binding of primers (Braun et al. 2011). The PCR product was then quantified using a NanoDrop ND-1000 spectrophotometer and confirmed using gel electrophoresis. The PCR product was then column purified (QIAquick PCR Purification Kit, Qiagen, Valencia, CA) and digested with the restriction endonuclease HhaI at 37°C for 4 hours. This digestion was mixed with ROX-labeled GS500 size standard (Applied Biosystems) and loading buffer, denatured at 95°C for 5 min, then immediately transferred to ice. The samples were then separated on a 36cm 5% polyacrylamide gel containing 7M urea for 6hr at 3000V using an ABI377 genetic analyzer. The profiles were then analyzed with GENESCAN.
software and profiles were generated in triplicate and then PCA was performed to determine the correlation of the microbial community profiles.

2.3 Biogeochemical Data Analysis

Biogeochemical data was collected from pore water that was extracted from each 2cm section of a core down to 10cm; each section was examined independently. Samples were examined colorimetrically using a Milton Roy Spectronic 501 spectrophotometer to determine the concentration for reduced Fe and Mn following the methods of Stookey (1970), Burl and Kirby-Smith et al. (1979), Brewer and Spencer (1971), and Goto et al. (1962). Sections were centrifuged at 500x g to separate the sediment from the pore water. The porewater was then siphoned off the top of the sediment and stored in 4ml vials at 4°C until they were processed for biogeochemical analysis.

2.3.1 Fe²⁺ Extraction

For Fe²⁺ extraction, an ammonium acetate buffer was prepared using 400 g of ammonium acetate that was combined with 350 ml of 14.5M ammonium hydroxide and diluted to 1L with deionized water (DIW). Afterwards, an acid reducing solution was created using 0.514 g of ferrozine, 10 g of hydroxylamine hydrochloride and only sufficient water to dissolve the solid (approximately 2ml). Then 50 ml of 12M HCl was added, mixed, and allowed to cool prior to being diluted to 100 mL with DIW. In a 4 mL pre-labeled vial, 200 µL (0.2 mL) of sample pore water, 100 µL of acid reducing solution, and 1.8 mL of DIW was combined. The vial was capped and swirled gently to combine contents and heated in a 90°C oven for 30 minutes. Following incubation 200 µL of the ammonium acetate buffer was added to the samples, mixed, and cooled to room
temperature. Standards were prepared as follows: a 100 ppm standard was made by
dissolving 0.707 g Fe(NH$_4$)$_2$(SO$_4$)$_2$·6H$_2$O in 1L of deoxygenated 0.1N H$_2$SO$_4$. This was
followed by serial dilutions of 10, 25, 50, 100 and 500 fold to make the final
concentrations 10, 4, 2, 1, and 0.2 ppm respectively. Standards were then fixed with the
identical reagents used in sample preparation. The samples were examined using a
Milton Roy Spectronic 501 spectrophotometer set to at an absorbance of 562 nm. There
is a linear relationship between the Abs (562 nm) and Fe (II) up to an absorbance of ~1.0
(Stookey 1970). This yielded a quantitative measure of the amount of Fe (II) in pore
water samples. This analysis was conducted in triplicate for the sections.

2.3.2 Mn$^{2+}$ Extraction

For Mn$^{2+}$ extraction, a formaldoxime reagent was prepared by dissolving 2.0g
NH$_2$OH·HCl (hydroxylamine hydrochloride) in 1.0 mL of formaldehyde solution (37%)
and followed by dilution in 50 mL with DI water. This was mixed with 2 mL of
ammonium hydroxide (NH$_4$OH, 14.5M) to create a Formaldoxime-NH$_4$OH mixed
reagent (FMR). A 0.1M EDTA solution was prepared by dissolving 3.72g of Na$_2$-EDTA
in 100 mL DIW. A 10% Hydroxylamine hydrochloride solution was prepared by
dissolving 100g Hydroxylamine hydrochloride/ 1L DIW. These were compared to 0.2,
0.5, 1, 1.5, 2, 4, 5, and 10-ppm standards. For reactions, 500 μL of the pore water sample
and 1.5 mL of water were added to a 4 mL pre-labeled vial, and 171 μL FMR were added
to the 4 mL vial, mixed well and allowed to stand for at least 2 minutes. 100 μL EDTA
solution and 200 μL hydroxylamine solution was added and mixed well to combine.
This reaction proceeded for 10 minutes for the Mn$^{2+}$-formaldoximine color complex to
stabilize. Absorbance was measured at 450 nm using a Milton Roy Spectronic 501 spectrophotometer. The relationship between Abs (450 nm) and Mn is linear up to an absorbance of ~0.9 which provides quantitative data as to the amount of Mn present in a pore water sample (Burle and Kirby-Smith 1979).

2.3.3 Total Reduced Sulfur

Solid phase sulfur data was collected upon return to MSU using previously described acid volatile sulfur (AVS) and total reducible sulfur (TRS) methods (Cornwell and Morse 1987, and Canfield et al. 1986). A 5 g frozen sediment sample was weighed and quickly placed in a 125 mL wide-mouthed reaction flask along with a magnetic stir bar. The reaction flask was attached to the extraction condensers and N₂ gas was allowed to flow for 10 minutes (bubble rate ~ 2-3 bubbles/sec). To conduct the extraction, 40 mL chrome reductant, 20 mL of 12M HCl, and 10 mL of ethanol were added through the injection port. The flask was boiled for one hour, after which point the NaOH capture solution (70 mL 0.5 N NaOH in a graduated cylinder) was sampled for sulfide using the Cline’s method (Cline 1969).

2.3.4 Acid Volatile Sulfide

AVS follows the same procedure as TRS but differs in the fact that there was no chromium reductant used. Briefly, 5 g of weighed frozen sediment was placed in the reaction flask, condensers attached, and the nitrogen gas supply affixed. The system was allowed to return to anoxia, and then 20 mL of the HCl-SnCl₂ solution (5g SnCl₂ in 20 mL of 6N HCL) was added. The digestion proceeded for 1 hour at room temperature, and
then the extracted sulfide from the trapping solution was measured as per TRS by Cline’s method (Cline 1969).

2.3.5  **H$_2$S, O$_2$, pH, and Redox Potential Detection Using Microelectrodes**

After collecting sediment cores, the Unisense microelectrode profiling system (Brendstrup, Denmark) and the Sensor Trace Pro software program were used to collect H$_2$S, O$_2$, pH and redox potential (unisense.com). Probes were calibrated in the laboratory before being used in the field. A micromanipulator was used to lower the microelectrodes through the core in 1mm increments. Over a total depth of 10 cm, triplicate readings of oxygen, sulfide, pH and redox potential readings in sediment porewater were obtained (Revsbech et al. 1983; Revsbech and Ward 1984; Revsbech and Jorgensen 1986).

2.3.6  **Miscellaneous**

SOD as well as phosphate and other nutrient data was collected by the EPA and made available for use in this research. Grain size, porosity, aluminum, total suspended solids (TSS), total particulate organic carbon (TPOC), dissolved organic carbon (DOC), and chlorophyll data was collected by others at MSU and made available for this project as well. Temperature, salinity, and turbidity were measured using a SONDE (YSI Incorporated, Yellow Springs, OH).

2.4  **Mesocosm Experiment**

At Choctawhatchee Bay (CB), a mesocosm experiment was also performed. This experiment was used to determine the effect of aeration on microbial communities in the sediments. Three additional cores were selected from each of three sites in the bay (CB1, CB2, and CB3, see Fig. 2.1). The cores were placed upright in a fish tank that was
partially filled with water from the bay to ensure the cores stayed the same temperature, which was monitored approximately every 2 hours for 96 hours. One core from each site was completely oxygenated by bubbling continuously with oxygen for 96 hours. The second core was forced anoxic by bubbling the water with nitrogen continuously for 96 hours. The third and final core from each site had no manipulation for 96 hours.

Following the 96-hour incubation, the cores were sliced in an anaerobic chamber and stored at -20°C until arrival at MSU. T-RFLP analysis was then used as described in section 2.2 to determine the effect that these conditions have on the genetic fingerprints of each core.

2.5 Principle Component Analysis

Principle component analysis is a data reduction method that provides the ability to find useable patterns in large data sets. Data collected from the study was entered into SPSS (IBM Corp.) and the analysis was performed using Varimax rotation with Kaiser normalization. Only variables with an eigenvalue greater than one were selected as components. These were then plotted in a component plot to visualize the patterns within the data.
CHAPTER III
RESULTS

3.1 Tampa Bay (TB), FL

3.1.1 Chemical Data

3.1.1.1 Ammonia Data

The EPA collected ammonia data and the values for each extraction as well as the nutrient flux data are shown in Figs. 3.1-3.3. The data indicate that there tends to be more ammonia in the sediments of all sites relative to the amount of ammonia in the porewater at each site (Fig. 3.1). Site one contained the most ammonia of any site sampled. With respect to the sediment, most of the ammonia is found in the top layer of the core, which indicates the ammonia is not being buried, but rather is released into the surrounding waters. This is supported by the nutrient flux data that was supplied by the EPA. Figure 3.3 shows that the ammonia flux is positive, indicating a release of the nutrient from the sediment into the surrounding water column.
Figure 3.1  Tampa Bay Porewater Ammonia Concentrations.

Average concentrations (μg/L) from sediment collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

Figure 3.2  Tampa Bay Sediment Ammonia Concentrations.

Average concentrations (mg/kg) from porewater collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
Figure 3.3  Tampa Bay Ammonia Nutrient Flux Concentrations.

This data indicates how the ammonia is interacting with the sediment. A positive reading indicates a release of ammonia from the sediment into the water column. Values represent and average of 3 replicates.

3.1.1.2  Manganese Data

Manganese was extracted using Diffusion Gradients in Thin Films (DGTs) by the MSU research laboratory (Figs 3.4 and 3.5). Manganese can be used to determine if a site is anoxic as Mn(II) is usually produced under anaerobic conditions. The average concentration of manganese in Tampa Bay is around 15 (mg/kg). With the exception of site one, Tampa bay had a fairly consistent amount of Mn(II) in the porewater collected at both the top and bottom of the core (Fig. 3.4). This could indicate that the processes that are producing these manganese species are present throughout the bay.
Figure 3.4  Tampa Bay Porewater Manganese Concentrations.

Average concentrations (mg/L) from porewater collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

Figure 3.5  Tampa Bay Sediment Manganese Concentrations.

Average concentrations (mg/kg) from sediment collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
3.1.1.3 Phosphorus Data

Phosphorus is generally considered to be the limiting factor when determining if eutrophication is occurring. Healthy waters generally contain 0.02-0.03 mg/L or less of phosphate (USEPA 2006). Increases in the concentration of phosphates is generally due to the introduction of fertilizers through runoff. The EPA collected phosphorus data, which included porewater and sediment total phosphorus, ortho-p, and total phosphorus nutrient flux (Figs. 3.6-3.9). This element was found in small quantities throughout the bay with the first site containing the most (Fig 3.6). According to the established guideline of 0.03 mg/L or less, all sites were considered in normal range with the exception of site 1.

There are 3 main types of phosphates that are sampled for water quality. The first are orthophosphates, which are the inorganic forms of phosphate, such as $\text{PO}_4^{3-}$, $\text{HPO}_4^{2-}$, and $\text{H}_2\text{PO}_4^-$. The second are organically bound phosphates, which are found in human and animal wastes or in decaying organic matter. The third are condensed phosphates (or polyphosphates, $\text{P}_3\text{O}_{10}^{5+}$), which are used in industrial processes and were previously found in detergents, but have been discontinued. Total phosphate is a measure of the three phosphate species together (Fig. 3.7 and 3.8). The total phosphate (TP) measurement indicates that there are much larger concentrations of phosphorous containing compounds in the sediments than are found in the porewater in Tampa Bay (Fig. 3.7). The flux measurement (Fig. 3.8) indicates that there is a larger release of TP from the sediments than what is being deposited in the sediment. Sites 1, 2, and 4 are all releasing total phosphorous into the water column while sites 2 and 4 are releasing it at a higher rate than what is being deposited in sites 3 and 5. However, even though the
concentrations are very small (approx. 40mg/m²/day at sites 2 and 4), this release could still have an impact on the health of the estuary. This is supported by Figure 3.9, which indicates a larger efflux of phosphorus from the sediments at all sites sampled.

Figure 3.6  Tampa Bay Porewater Phosphate Concentrations.

Average concentrations (μg /L) from porewater collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
Figure 3.7  Tampa Bay Sediment Total Phosphorus Concentrations.

Average concentrations (mg/kg) from sediments taken from 11 sites in Tampa Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (top) and 8-10 cm (bottom).

Figure 3.8  Tampa Bay Total Phosphorus Nutrient Flux.

This data indicates how the total phosphorus is interacting with the sediment. A positive reading indicates a release of phosphorus from the sediment into the water column. A negative reading indicates that the phosphorus is being deposited into the sediment. Data represents an average of three replicates.
Figure 3.9  Tampa Bay Total Ortho-p Nutrient Flux.

This data indicates how the total ortho-p is interacting with the sediment. A positive reading indicates a release of ortho-p from the sediment into the water column. A negative reading indicates that the phosphorus is being deposited into the sediment. Data represents an average of three replicates.

3.1.1.4  Nitrogen Data

Nitrogen is the main contributor to eutrophication in estuaries. Normal concentrations should be less than 1 mg/L in unpolluted water (USEPA 2006). To determine whether the sites were at risk of becoming eutrophic, the EPA also collected Nitrogen data in the form of porewater nitrate, sediment total Kjeldahl nitrogen (TKN), and TKN nutrient flux (Figs. 3.10-3.12). Figure 3.10 indicated that the porewater nitrogen concentrations were varied throughout the estuary. Sites 4 and 5 had the highest concentrations in the bottom section, while sites 7 and 11 had the highest concentrations in the top section of the core. Figures 3.11 and 3.12 represent the TKN data collected; TKN represents the combination of organic and inorganic nitrogen, including ammonium, nitrate, and nitrite. These are important nitrogen species because in
conjunction with phosphorous, these are responsible for the overgrowth of algae in the estuary caused by eutrophication. Figure 3.11 indicates the amount of TKN found in the sediments of Tampa Bay. These concentrations were fairly consistent throughout the bay with the exception of sites 1 and 6. Site 6 had approximately double the amount of TKN than any other site, while site 1 contained nearly 3 times as much as site 6. Figure 3.12 indicates that these nitrogen species can either be absorbed or released by the sediment. Sites 1, 3, and 4 are all giving off nitrogen while sites 2 and 5 are absorbing nitrogen into the sediment.

![Figure 3.10](image.png)

Figure 3.10  Tampa Bay Porewater Nitrate/Nitrite concentrations.

Average concentrations (μg/L) from sediment collected from 11 sites in Tampa Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
Figure 3.11  Tampa Bay Sediment TKN Concentrations.

Average concentrations (mg/kg) from sediment collected from 11 sites in Tampa Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

Figure 3.12  Tampa Bay TKN Nutrient Flux.

This data indicates how the TKN is interacting with the sediment. A positive reading indicates a release of TKN from the sediment into the water column. A negative reading indicates that TKN is being deposited into the sediment. Data represents and average of three replicates.
3.1.1.5 Iron Data

The EPA collected porewater and sediment iron data (Fig. 3.13 and 3.14). The amount of iron that was found in the porewater could not nearly compare to the amount of iron in the sediment. This was to be expected, as iron is one of the most abundant elements in the earth’s crust. What was interesting about these measurements, however, was the fact that most of the iron that was found in the porewater was found near the top of the core, particularly with sites 7 and 11.

Figure 3.13 Tampa Bay Porewater Iron Concentration.

Average concentrations (mg/L) from sediment collected from 11 sites in Tampa Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
Figure 3.14   Tampa Bay Sediment Iron Concentration.

Average concentrations (mg/kg) from sediment collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

3.1.1.6  Sulfur Data

Collection of sulfur data is important for detecting anoxic conditions as hydrogen sulfide is only produced under anaerobic conditions. To determine if these sites were anoxic, sulfide data was collected using microelectrodes and acid volatile sulfide and total reduced sulfide data was collected using previous methods (Figs. 3.15-3.17) (Cornwell and Morse 1987, Canfield et al. 1986, and Cline 1969). The greatest concentration of sulfide was found at site one. Most of the sulfide was found in the lower sections of the sediment, where the conditions are more anoxic (Fig. 3.22) as well as more reducing (Fig. 3.18). Sulfide was detected at nearly identical concentrations at all the sites with the exception of site one.

Acid volatile sulfide (AVS) was determined to be solid phase sulfur. This can be produced by reacting dried sediment with 1N HCl, which will cause a release of sulfide.
This solid phase sulfur was found deeper within the sediment core. This was to be expected because once these materials become combined with iron or other metals, they become buried, and over time they are buried deeper. The data indicate that nearly all sites had greater amounts of AVS deeper in the core with the exception of sites 1, 2, 7, and 8 (Fig. 3.16). Sites 1 and 2 had similar concentrations while site 8 had no AVS detected in the lower sections of the core. Site 7 had nearly 4 times as much AVS in the top of the core than the bottom.

Total Reduced Sulfide (TRS) includes multiple forms of reduced sulfur including hydrogen sulfide (H$_2$S), carbon disulfide (CS$_2$), carbonyl sulfide (COS), dimethyl sulfide (C$_2$H$_6$S), methyl mercaptan (CH$_4$S), and dimethyl disulfide (C$_2$H$_6$S$_2$). Figure 3.17 indicates that the concentrations of TRS are less than AVS at every site sampled throughout the bay.

![Figure 3.15](TB_Porewater_Sulfide.png)

**Figure 3.15** Tampa Bay Porewater Sulfide Concentration.

Average concentrations (mg/L) from sediment collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
Figure 3.16  Tampa Bay Sediment Acid Volatile Sulfide Concentration.

Average concentrations (μg/gdw) from sediment collected from 11 sites in Tampa Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

Figure 3.17  Tampa Bay Sediment Total Reduced Sulfur Concentration.

Average concentrations (μg/gdw) from sediment collected from 11 sites in Tampa Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
3.1.1.7  Redox Data

Redox potential is the measure of how high the potential is for substrates to be reduced in the porewater. This measurement can vary daily and even hourly. The more reducing an environment is, the higher the potential that some substrate other than oxygen will be used as a final electron receptor. These data were collected in 5-millimeter sections through the entirety of the core from the surface to the bottom using microelectrodes (Fig. 3.18). Throughout the bay the redox potential increased (became more negative) in relation to depth. Site 11 had nearly identical redox potential at both the surface and bottom of the core.

Figure 3.18  Tampa Bay Redox Potential.

The data represented are the average mV readings from 11 sites in Choctawhatchee bay. The more negative the reading, the higher the redox potential. These are the average of 3 mV readings collected at depths of 0-2 cm (red) and 8-10 cm (blue).
3.1.1.8 Temperature Data

Temperature data was collected using a SONDE to determine the change in temperature throughout the water column (Fig. 3.19). The temperature of the bay was fairly consistent throughout the entire bay, with variances of at most less than one degree. This suggests that temperature throughout the bay should be consistent enough to not interfere with the processes occurring in the sediment.

![Figure 3.19 Tampa Bay Water Temperature](image)

3.1.1.9 Salinity

Salinity is used in monitoring efforts because there is an inverse relationship between the amount of oxygen in an environment and salinity. Salinity was measured at each site throughout the bay using the SONDE (Fig. 3.20). Salinity was found to be very consistent throughout the bay as well, with variance at the bottom of the water column around 5 ppt. The top of the water column had the greatest variance between site 1 and 2.
of around 10 ppt. This could be explained by the input of fresh water from the river that feeds site 1. These measurements are within the normal salinity measurements (25 ppt) for this bay.

![Salinity graph]

Figure 3.20  Tampa Bay Salinity.

Data represents the amount of salinity in the top of the water column (blue) and the bottom of the water column (red).

3.1.1.10  pH Data

Using microelectrodes, pH data was collected from each site (Fig. 3.21). There was a trend in the water pH to transition from neutral at sites close to the freshwater source to more alkaline at sites closer to the ocean. This is not a significant increase (p=0.1) based on the average pH of the bay, approximately 8.
Figure 3.21  Tampa Bay Porewater pH.

Data represents the values of the pH of the water in the top of the water column (blue) and the bottom of the water column (red).

3.1.1.11  Oxygen Data

Dissolved oxygen is the measure of oxygen that is found in the water and according to the EPA anything less than 2ppm (125 μmol/L) is considered hypoxic. Dissolved oxygen (DO) was collected using microelectrodes and the EPA collected sediment oxygen demand data (Figs. 3.22 and 3.23). Dissolved oxygen is usually found on average around 5mg/L in the overlying water but in the porewater, the greatest concentrations were found in site 11 and were around 0.48 ppm (30 μmol/L) (Fig 3.22). The porewater with zero oxygen detected would be considered anoxic while the others would be considered hypoxic. From these measurements it is apparent that if a site contained any DO, it was detected only in the very upper portion of the sediment. Sediment oxygen demand data indicated that all the sites had a strong demand for oxygen.
Figure 3.22  Tampa Bay Porewater Dissolved Oxygen Concentration.

Average concentrations (μmol/L) from sediment collected from 11 sites in Tampa Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

Figure 3.23  Tampa Bay Sediment Oxygen Demand.

This data indicates how the processes in the sediment are impacting the oxygen in the surrounding water column. A positive reading indicates a release of oxygen from the sediment into the water column. A negative reading indicates that the oxygen is being deposited into the sediment.
3.1.1.12 Chlorophyll Data

Chlorophyll A is used as a measure of how much algal growth there is in an estuary. Anything less than 30μg/L is considered a normal algal level according from the values of Walmsley and Butty (1980) and Walmsley (1984). Figure 3.24 indicates that no site in Tampa could be considered eutrophic based on this measurement.

Figure 3.24 Tampa Bay Overlying Water Chlorophyll A Data.

Average concentrations (μg/L) from the water column collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from each site at the top of the water column (blue) and the overlying water (red).

3.1.1.13 Carbon Data

Dissolved organic carbon (DOC) data is of interest because this measurement is the concentration of organic carbon that is left in a water sample after the sample has been filtered. This indicates the availability of carbon for microorganisms. Figure 3.25 indicates that at the bottom of the water column, which includes the sediment water
interface, the amount of DOC is fairly consistent at every site throughout the bay with variances at approximately 1 mg/L.

Figure 3.25  Tampa Bay Overlying Water Dissolved Organic Carbon Concentration. Average concentrations (mg/L) from the water column collected from 11 sites in Tampa Bay, FL. Data represents averages of 3 samples taken from the top of the water column (blue) and the overlying water (red).

3.1.2  Tampa Bay Microbial Community Analysis

Microbial community analysis was performed on the sites throughout the bay. This was done by extracting the DNA and performing T-RFLP. The data was analyzed by T-RFLP Analysis Expedited (T-REX) software (Culman et al. 2009). Four unique T-RFLPs were found to be of interest (TRFLP_1, TRFLP_2, TRFLP_3, and TRFLP_4). These are potentially 4 unique species that were found throughout the study sites. The T-RFLP’s were selected based on the height of the peaks given by the genetic analyzer. These T-RFLPs were also found in all bays; therefore, the analysis focused on their correlation to the nutrient data collected (Fig. 3.26). The results from the chemical
analysis as well as the T-RFLP’s were analyzed using Principle Component Analysis (PCA). This analysis was used to determine how variance is distributed throughout the data and to determine which biochemical data are most closely related. This is important because it can give insight into the processes that are occurring in the estuary. This analysis separated the data into components that have the most variance. These components were the axis with the most variance between samples. The axis was then rotated to show variance among other axes. The samples represented in a loading plot indicate the relationship to each variable; the closer the data points are plotted, the more closely related they are. From the PCA correlations a strong correlation between TRFLP_1 and iron, manganese, sulfide, and sediment oxygen demand was detected. There also appeared to be a correlation between TRFLP_2-4 with DO, redox potential, and the pH of the bay.
Figure 3.26  TB Component Plot showing the correlation between the TRFLPs and the Chemical Data collected throughout the bay.

The proximity of the circles indicates their relation to each other based on their Pearson correlation. The stronger their relationship, the closer the circles. Temperature, manganese, iron, H2S, TRFLP_1 and SOD are all strongly correlated.
Table 3.1  Loadings assigned to the different variables found in Tampa Bay.

<table>
<thead>
<tr>
<th>Tampa Bay Rotated Component Matrix(^a)</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>TRFLP_1</td>
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</tr>
<tr>
<td>TRFLP_2</td>
<td>0.161</td>
</tr>
<tr>
<td>TRFLP_3</td>
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</tr>
<tr>
<td>TRFLP_4</td>
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</tr>
<tr>
<td>Iron</td>
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</tr>
<tr>
<td>H2S</td>
<td>0.781</td>
</tr>
<tr>
<td>Redox</td>
<td>0.007</td>
</tr>
<tr>
<td>Manganese</td>
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</tr>
<tr>
<td>pH</td>
<td>-0.779</td>
</tr>
<tr>
<td>SOD</td>
<td>0.209</td>
</tr>
<tr>
<td>Temp</td>
<td>0.530</td>
</tr>
<tr>
<td>DO</td>
<td>-0.019</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.\(^a\)

\(^a\) Rotation converged in 10 iterations.

The closer the number in the column is to 1.0, the more likely that variable is the component. The loadings represent the likelihood of that variable being the source of the most variability between the other variables.

Table 3.1 shows how the loadings were assigned to each of the variables found in Tampa Bay. These loadings are how SPSS determines how to assign components. From the table it is clear that component 1 was assigned to iron. This means that most of the variance in the data can be contributed to the iron data. Component 2 was assigned to Redox potential while 3, 4, and 5 were assigned to TRFLP_2, SOD, and TRFLP_1 respectively.
3.2 Choctawhatchee Bay (CB), FL

3.2.1 Chemical Data

3.2.1.1 Ammonia Data

As with Tampa Bay data, it is apparent that most of the ammonia is found in the upper sections of the core (Fig. 3.26). The amount of ammonia is much more varied by site than it was in Tampa Bay (Fig. 3.1). However the range of ammonia detected was similar in both bays. Choctawhatchee Bay had a greater difference between the amount of ammonia in the top and the bottom of the core. The exception was site 3, which had negligible concentrations of ammonia in the top of the core. Sites 4 and 6 had the lowest concentrations of ammonia, which was consistent with the other nutrient data collected (Figs. 3.27-3.30).

![CB Sediment Ammonia](image)

Figure 3.27 Choctawhatchee Bay Sediment Ammonia Data.

Average concentrations (mg/kg) from sediment collected from 8 sites in Choctawhatchee Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
3.2.1.2 Manganese Data

Manganese data was collected using DGTs (Fig. 3.27). Though differences were evident throughout the bay, sections within individual sites were fairly consistent. The concentrations increased as the sites moved from closer to the mouth of the river to the center of the bay (Fig. 2.1). Site 8 is centrally located in the bay and had the greatest concentration of manganese present (Fig. 2.1).

![CB Sediment Manganese](image)

**Figure 3.28 Choctawhatchee Bay Sediment Manganese Concentration.**

Average concentrations (mg/kg) from sediment collected from 8 sites in Choctawhatchee Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

3.2.1.3 Phosphorus Data

The EPA collected Phosphorus (Fig. 3.28) and the amounts of total phosphorus (TP) that were found in Choctawhatchee Bay were similar to the concentrations of phosphorous found in Tampa bay (Figs. 3.6 and 3.7). Phosphorous was detected in small
amounts at sites 4 and 6, which was consistent with the minimal amount of Mn data found at these two sites as well (Fig. 3.27).

Figure 3.29  Choctawhatchee Bay Sediment Total Phosphorus Concentration.

Average concentrations (mg/kg) from sediment collected from 8 sites in Choctawhatchee Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

3.2.1.4  Nitrogen Data

The EPA collected Total Kjeldahl Nitrogen data (Fig. 3.29). As stated previously, this is the main contributor to eutrophication in an estuarine system. Again with the exception of sites 4 and 6, nitrogen concentrations were fairly consistent throughout the bay. This bay differed from Tampa Bay in the amounts that were present at each site (Fig. 3.11). Choctawhatchee Bay contained on average as much TKN as site 1 in Tampa Bay. There also seemed to be a larger difference between the top and the bottom of the cores than there were in Tampa (Fig. 3.11). In Tampa Bay the average difference
between the top and bottom of the core was on average 217 mg/kg while in Choctawhatchee Bay the average difference was approximately 750 mg/kg.

Figure 3.30 Choctawhatchee Bay Sediment TKN Concentration.

Average concentrations (mg/kg) from sediment collected from 8 sites in Choctawhatchee Bay. Data represent averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

3.2.1.5 Iron Data

Sediment Iron data were collected by the EPA (Fig. 3.30). The concentrations of the iron in the sediment were fairly homogeneous throughout the bay with the exception of sites 4 and 6. The concentrations identified also exceedingly surpassed the amounts of iron found in the sediment of Tampa Bay (Fig. 3.14). There was also consistency in the amounts found in the top and the bottom of the core. The average difference between the concentrations in the top and bottom of the core was approximately 101 mg/kg of iron. This was not a significant difference in the average iron in the sediment (p=0.9)
Figure 3.31  Choctawhatchee Bay Sediment Iron Concentration.

Average concentrations (mg/kg) from sediment collected from 8 sites in Choctawhatchee Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

3.2.1.6  Sulfur Data

Sulfide data was collected using microelectrodes (Fig. 3.31). Sulfide concentrations were very consistent throughout Choctawhatchee Bay (3.35ppm or 88μmol/L) with the exception of site 4. This site had the greatest concentration of sulfide, which was found in the lower sections of the core. It was also interesting that the amounts of sulfide that were found in the top of the core were nearly identical to the amount of sulfide produced in the bottom of the core throughout the sites sampled. Another interesting note was the fact that even though site 4 contained among the lowest concentrations of other nutrients in the bay (Fig. 3.26-3.30), it had the highest concentration of sulfide. This bay also contained approximately the same amount of sulfide as the average concentrations sampled in Tampa Bay (3.37 ppm) (Fig. 3.15).
There was a large range of AVS that was found in the sediments of Choctawhatchee Bay from around 9 to 38 μg/gdw (Fig. 3.32). There was also far less overall in the bay than what was found in Tampa Bay (Fig. 3.16). Site 6 contained the most stable sulfur, which is interesting because other nutrients were found in some of the lowest concentrations here (Figs. 3.26-3.30).

![Figure 3.32 Choctawhatchee Bay Porewater Sulfide Concentration.](image)

Average concentrations (μmol/L) from porewater collected from 8 sites in Choctawhatchee Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
Average concentrations (μg/gdw) from sediment collected from 8 sites in Choctawhatchee Bay, FL. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

3.2.1.7 Redox Data

Redox potential was collected using microelectrodes (Fig. 3.33). This site was different from Tampa Bay (Fig. 3.18) as the sites contained reducing environments above the sediments as well as within. The sites clearly became more reducing as depth increased, which could be caused by an increased organic matter load, and reduce the amount of oxygen present (Fig. 3.36).
The data represented are the average mV readings from 8 sites in Choctawhatchee bay. The more negative the reading, the higher the redox potential. These are the average of 3 mV readings collected at depths of 0-2 cm (blue) and 8-10 cm (red).

3.2.1.8 pH Data

pH data was collected using a SONDE (Fig. 3.34). pH was more varied in Choctawhatchee Bay than it was in Tampa Bay (Fig. 3.21), but was still well within reasonable variances. The average pH for this bay is approximately 7.7 according to the University of Florida website (lakewatch.ifas.ufl.edu).
The data represent the pH values found in the top of the water column (blue) and the bottom of the water column (red).

3.2.1.9 Dissolved Oxygen Data

Dissolved oxygen data was collected using microelectrodes (Fig. 3.35). Site 3 contained more oxygen at the deeper depths than was present at the shallower depths of the core. This could have been due to human error or it could simply be an anomaly. Overall there was a complete absence of dissolved oxygen in the porewater throughout the sediments of the bay with the exception of sites 3 and 7.
Average concentrations (μmol/L) from porewater collected from 8 sites in Choctawhatchee Bay. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

### 3.2.1.10 Chlorophyll Data

Chlorophyll data was collected at MSU (Fig. 3.36). The concentrations were within a similar range as those collected at Tampa Bay (Fig. 3.24). The concentrations were below the 30μg/L limit set forth by Walmsley and Butty (1980) and Walmsley (1984) and are therefore not considered eutrophic.
Figure 3.37  Choctawhatchee Bay Overlying Water Chlorophyll A Concentration.

Shown are average concentrations (μg/L) from the water column taken from 8 sites in Choctawhatchee Bay. Data represents averages of 3 samples taken from each site at the top of the water column (blue) and the overlying water (bottom of the water column) (red).

3.2.1.11  Carbon Data

Carbon data was collected by MSU (Fig. 3.37). These readings were, on average, reduced in comparison to Tampa Bay. Site 4 had one of the highest organic carbon loads of any site in the bay. However, this site had some of the lowest readings for the other nutrient data that was collected (Figs. 3.26-3.30, 3.32, 3.34 and 3.35).
Figure 3.38 Choctawhatchee Bay Overlying Water DOC data.

Shown are average concentrations (mg/L) from the water column taken from 8 sites in Choctawhatchee Bay. Data represents averages of 3 samples taken from each site at the top of the water column (blue) and the overlying water (bottom of the water column) (red).

3.2.2 Choctawhatchee Bay Microbial Community Analysis

Community correlation analysis was performed on all sites sampled throughout the bay. The T-RFLP fragments were compared to the chemical data that was collected to see if there were any correlations. In Choctawhatchee Bay there were different correlations that those seen in Tampa Bay (Fig. 3.26 in comparison to 3.39). In Choctawhatchee Bay there were strong correlations between TRFLP_4 and the amount of sulfide present. There were also correlations between TRFLP_3 and iron and manganese. TRFLP_1 and TRFLP_2 were correlated equally between iron, manganese, redox, and sulfide (Fig. 3.39). These TRFLP fragments are equidistance from the chemical species. This type of correlation suggests that these TRFLPs are involved with the production of iron, sulfur and manganese. This is slightly different than what was found in Tampa Bay.
In Tampa (fig. 3.26), TRFLP_1 was more closely related to sulfur than it was in Choctawhatchee Bay (Fig. 3.39).

Figure 3.39  CB Component plot showing the correlation between the TRFLPs and the chemical data collected throughout the bay.

The proximity of the circles indicates their relation to each other based on their Pearson correlation. The stronger their relationship, the closer the circles. TRFLP_3, pH, manganese and iron are all closely correlated.
Table 3.2  Choctawhatchee Bay Component loadings

<table>
<thead>
<tr>
<th>Choctawhatchee Bay Component Score Coefficient Matrix</th>
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<td>Component</td>
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<td>TRFLP_1</td>
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<tr>
<td>Redox</td>
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<tr>
<td>Manganese</td>
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</tbody>
</table>

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

The closer the number in the column is to 1.0, the more likely that variable is the component.

Figure 3.40 shows how the components were assigned in this bay as well. The majority of the variance in this data came from the T-RFLP’s.

3.3 Influence of Oxygenation on Microbial Communities in Mesocosm

To determine the effect that oxygenation had on the microbial communities present in Choctawhatchee Bay, a mesocosm experiment was conducted on samples collected from sites 1, 2, and 3 (Fig. 3.40). The TRFLPs were analyzed with TREX and then a PCA was performed on the samples. The fragments were assigned loadings based on their oxygenation state. E3 (0-2cm of all anoxic cores) and E4 (8-10 cm of all anoxic cores) were anoxic samples, E5 (0-2cm) and E6 (8-10cm) were control samples, and E7
(0-2cm) and E8 (8-10cm) were the oxygenated samples. E6 and E7 are so strongly correlated it is hard to distinguish the two. This PCA separated the samples along the first component axis (IPCA1) based on oxygenation. Figure 3.40 shows E3 and E4, the anoxic samples were not as strongly correlated to each other as was expected. E5 and E6, the control samples, were also not correlated as strongly with each other. This indicates that some of the oxygenated samples (E7) correlated with the control samples (E6), which were loosely correlated with the anoxic samples (E4).

Figure 3.40  Mesocosm experiment TREX PCA in which the samples were separated along component 1 based on oxygenation.

E3 and E4 are the anoxic cores, E5 and E6 control, and E7 and E8 oxygenated cores.
3.4 **Bay St. Louis Chemical Data**

Iron, manganese, and sulfide data were collected in Bay St. Louis (Fig. 3.41-3.43). Iron and manganese were collected using the DGTs while sulfur was collected using microelectrodes. Figure 3.41 indicates that the concentrations of iron in the porewater were found to be greater in Bay St. Louis than in Tampa Bay. There was also more iron found in the bottom of sites 3 and 4 than were found in the top.

Manganese concentrations were found to be greater in the porewater of Bay St. Louis than in Tampa Bay as well (Fig. 3.42). The average porewater manganese found in Tampa bay was less than .5 ppm but in Bay St. Louis the average amount of manganese present was nearly 5 ppm, a 10-fold increase.

Sulfide concentrations were reduced in Bay St. Louis in comparison to both Tampa Bay and Choctawhatchee Bay (Fig. 3.43). The average sulfide level in Bay St. Louis was around .08 ppm while in Tampa the average concentration was 3.37 ppm (Fig. 3.15) and in Choctawhatchee Bay 3.35 ppm (88 μmol/L).
Figure 3.41  Bay St. Louis Iron Concentrations.

Average concentrations (mg/kg) from sediment collected from 4 sites in Bay St. Louis. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

Figure 3.42  Bay St. Louis Manganese Concentrations.

Average concentrations (mg/kg) from sediment collected from 4 sites in Bay St. Louis. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).
Figure 3.43   Bay St. Louis Sulfide Concentrations.

Average concentrations (ppm) from sediment collected from 4 sites in Bay St. Louis. Data represents averages of 3 samples taken from each site at a depth of 0-2 cm (blue) and 8-10 cm (red).

3.5 Bay St. Louis Microbial Community Analysis

    A microbial community analysis was performed on samples collected in Bay St. Louis (Fig. 3.41). This plot indicates a strong correlation between TRFLP_1 and the concentrations of sulfide and iron that were found in the bay. This is similar to the correlation that was seen in Tampa Bay and less so in Choctawhatchee Bay. It also shows a strong correlation between TRFLP_3 and 4 and the amount of manganese collected.
Figure 3.44  BSL Component plot showing the correlation between the TRFLPs collected and the chemical data collected throughout Bay St. Louis sites.
Table 3.3 Bay St. Louis Component Matrix

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>TRFLP_1</td>
<td>-0.091</td>
<td>0.924</td>
</tr>
<tr>
<td>Iron</td>
<td>0.325</td>
<td>0.893</td>
</tr>
<tr>
<td>H2S</td>
<td>-0.360</td>
<td>0.668</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.722</td>
<td>-0.358</td>
</tr>
<tr>
<td>TRFLP_2</td>
<td>-0.668</td>
<td>-0.018</td>
</tr>
<tr>
<td>TRFLP_3</td>
<td>0.953</td>
<td>-0.117</td>
</tr>
<tr>
<td>TRFLP_4</td>
<td>0.872</td>
<td>0.157</td>
</tr>
</tbody>
</table>

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a Rotation converged in 3 iterations.

Table 3.2 shows how the components were assigned at Bay St. Louis. Component one, which accounts for most of the variance was assigned to TRFLP_3 while the second component was assigned to TRFLP_1.

3.6 PCA of Nutrient Data in All Bays

A principle component analysis was performed on the nutrient data to determine the correlations of nutrient availability between the bays (Fig. 3.42). Data that was not collected in all the bays, such as nutrient flux, was not used in this analysis. Using this approach, there was a strong correlation between the iron and manganese concentrations. This is due to the fact that they are reduced under the same conditions, which means they should be correlated together.
Figure 3.45  PCA showing the relationship between the chemical data collected from all three bays.

The proximity of the circles indicates their relation to each other based on their Pearson correlation. The stronger their relationship, the closer the circles.
CHAPTER IV
DISCUSSION AND CONCLUSION

4.1 Discussion

Relationships were detected between manganese, iron, and sulfur in all three estuaries analyzed (Figs. 3.26, 3.39, and 3.42). These relationships are respective to the relative amounts of the chemicals that were found in the bays. The proximity of the points in the PCA show how closely related they are. In two of these three component plots, manganese and iron were very closely related, or produced under similar conditions. When Mn (II) is present at any concentration sites are typically anaerobic, as Mn(II) is generally only produced under anaerobic conditions. Anaerobic sites are predominated by anaerobic microorganisms, which use Fe (III) and Mn (IV) as the final electron acceptors and release Fe (II) and Mn (II) as byproducts. This oxidation of organic matter coupled to the reduction of Fe (III) and Mn (IV) can affect the quality of surface and groundwater (Lovley and Phillips1988). There has been evidence to prove that multiple species of organisms can reduce these compounds as well as single species that can utilize both (Lovley and Phillips 1988). When compared to the Ribosome Database Projects (RDP) sequence search (http://rdp.cme.msu.edu/index.jsp), sequence matches show that this species could potentially be from the Geobacteracea family of the phylum Proteobacteria. This is interesting because Lovley et al. (2004) stated that multiple species can reduce both iron, manganese, and sulfur. Since there is such a
strong correlation with the TRFLPs and the chemical species present, it may be possible to use this method to determine the health of an estuary.

In the BSL plot (Fig 3.41) there is a close relationship between iron and sulfur. Iron and sulfur are both reduced under the same conditions, which could be indicative of this relationship. Iron is often associated with sulfur in sediments as they can combine to form FeS complexes. Aqueous iron sulfide–complexes produced by the reaction of H$_2$S or HS$^-$ with Fe (II) are prevalent in the literature (reviewed in Li et al. 2012). In Figure 3.39, a greater separation between the H$_2$S and iron was detected. The distance between these data points could reflect an abundance of one compound (such as sulfide) over iron.

There is geochemical evidence suggesting that Mn(IV), Fe(III), and sulfate are the primary terminal electron acceptors for organic matter decomposition (Lovley and Phillips 1988). This provides proof that the microbial species in these sediments have to be better understood in order to determine the effectiveness of utilizing them in monitoring efforts. This is again supported by the RDP sequence analysis. When compared to the chemical data that was collected, there seems to be a pattern with respect to the TRFLPs collected at the sites as well. The data indicate that some of these TRFLPs are closely correlated to the amounts of iron, manganese, and sulfur collected at the study sites. This relationship can clearly be seen in Bay St. Louis (Fig. 3.41). TRFLP_1 is closely related to both iron and sulfur. This supports the idea that a single microorganism can utilize both sulfur and iron as the terminal electron acceptor in the degradation of organic matter in sedimentary systems (Lovley et al. 2004, Lovley et al. 1988). TRFLP_3 and 4 are closely related to the amounts of manganese that are present (Fig. 3.41). This also provides supporting evidence for the idea that multiple species are involved in the
reduction of these chemicals. This trend extends beyond Bay St. Louis, however, and is seen in the other plots as well, i.e. Figure 3.39.

In the Choctawhatchee Bay plot (Fig. 3.39), strong correlations between the TRFLPs and the amounts of iron, sulfur, and manganese. TRFLP_3 is strongly correlated to the amounts of iron and manganese while TRFLP_4 is more closely related to the amounts of sulfide present. This suggests that these organisms are involved with the reduction of these metabolites. This is again more supporting evidence that these organisms are so closely related to these metabolites that they could potentially be used to monitor for water quality. In Tampa Bay (Fig. 3.53) TRFLP_1 is related to concentrations of iron, manganese and sulfur present. This suggests that different organisms in different bays utilized these pathways to degrade organic matter. This also explains the difference between bays that is seen with the TREX generated PCA of TFLPs related to their respective bays (Fig. 3.51). These correlations also provide evidence that even when separated by location, these organisms may be monitored to evaluate water quality.

This is not conclusive however, because the correlations are not as consistent as theorized. In Tampa Bay (Fig. 3.26) TRFLP_3 and 4 are not as closely correlated to the amounts of iron, manganese, and sulfur as they are in other bays. This discrepancy could suggest that there is an abundance issue in the bay with respect to the organisms in the communities. It could also mean that those organisms do not prefer to use iron, manganese, or sulfur as their terminal electron acceptor other metabolites that the organisms prefer in other bays. There is evidence in the literature to suggest that some organisms utilize the Fe (III) reduction pathway as a minor pathway (Lovley and Phillips
If these organisms do not prefer to use iron, manganese or sulfur as the terminal electron acceptor, they may not always necessarily reflect the status of these nutrients in the estuary. This could prove problematic in monitoring efforts because if these chemicals are not their preference, they may not be reliable indicators of water quality. Additionally, the lack of consistency between the bays indicates that further analysis is needed to determine this correlation.

This study provides proof that analyzing the microorganisms present in an estuary may provide the needed information for determining the overall health of an estuary. T-RFLP analysis can provide a more reliable, and less expensive option to monitor estuaries. Additionally, the data presented suggest that only the top sections of a core sample are needed for analysis (with the exception of sulfide). The results of the mesocosm experiment showed that there is not a significant difference between the oxygenated cores and the anaerobic and control cores, which further supports the findings that only the top sections of the sediments are needed for a comprehensive analysis. Fossing et al. (2004) demonstrated that the degradation of organic matter requires oxygen but as more material is being degraded the organic load depletes the oxygen to such a degree that the upper few millimeters of the sediment become anaerobic. Even when the organic load is low, such as in winter, oxygen will penetrate at best 5mm into the sediment. During summer it is even shallower, approximately 1-2mm. The fact that there is little oxygen throughout the sediment could explain why there was not much difference between the T-RFLPs in each core in the experiment. This provides additional evidence that the presence (or absence) of certain organisms can be used to monitor the status of estuaries.
4.2 Conclusions

Estuaries are extremely valuable resources ecologically and economically. They provide natural spawning grounds for many species of wildlife and have proven to be valuable assets to the people that inhabit the watershed. With well over half of the earth’s human population inhabiting watersheds, it is important to carefully and accurately monitor estuaries to maintain the health of the aquatic environment. The slow moving shallow nature of these systems makes them natural sinks for nutrients. These nutrients settle when the water slows and allows for an explosion of algal growth that must be degraded, which can cause a depletion of the oxygen as the microbes break down this matter. Depletion of oxygen can be detrimental to the wildlife in the estuary and manifests as a massive loss of life.

This study focused on the task of identifying a more reliable means for monitoring the overall health of a given estuary as the current trends in water quality testing have proven to be slow, expensive, and somewhat unreliable. Current methods rely on monitoring for byproducts of microbial metabolism that are responsible for the degradation of this influx of organic matter, such as Fe(II), Mn(II), and sulfide.

This study analyzed the possibility of monitoring the microbial species present in an estuary as a means to determine the eutrophic status of the site. Chemical and microbial community data were collected from various sites at three different bays and correlations were analyzed. There were strong correlations in all three bays to the types of chemicals that are usually collected to monitor for water quality. The main three geochemical variables that were most strongly related to the microbial communities were iron, manganese, and sulfur. In every bay, certain T-RFLPs were correlated to the
amounts of these chemicals present. These correlations indicate that it may be possible to use microbial community data to provide insight into the status of an estuary.

The abundance of these T-RFLPs is the key to the successful application of this method to monitoring efforts. Considering that an estuary is an incredibly dynamic environment, it is difficult to determine exactly what is responsible for the processes that are occurring in the sediment. Abiotic processes are occurring simultaneously with biotic processes, sometimes to obtain the same end result. This increases the difficulty of determining why the processes are occurring. Using the abundance of certain T-RFLPs it may be possible to use this technique to monitor estuaries. Instead of trying to determine if a particular species is directly responsible it may be possible to determine if these species can be used as a biomarker for the chemical processes that are occurring. This could provide a valuable means for successful monitoring the health of the estuary.

4.3 Future Studies

Future studies are needed in order to evaluate the effectiveness of TRFLP as a means to determine the health of the estuary. Clonal libraries of the TRFLPs that were collected need to be created and analyzed to determine the species of the microorganisms present. There also needs to be experimentation that directly relates these organisms with SOD which is the main method used by the EPA to determine if eutrophication is occurring. These methods would provide further evidence that this method can be effective to use in current monitoring efforts. In order for this study to be effective, it is important to establish the usefulness of microbial communities as biomarkers for the current monitoring methods. After the clonal libraries are established, it is important to determine if key bacterial species are present when certain chemicals are high. Due to
the dynamic nature of the ecosystem that is under scrutiny, it is difficult to determine if
the processes are occurring abiotically or biotically. The species may not be directly
involved with producing the reduced forms of Mn, Fe, and S, however may be producing
precursors that are contributing to the abiotic reduction of these chemicals. This dynamic
nature indicates that future studies must take into account the fact that these abiotic
processes are involved.
REFERENCES


