Nitrogen Source Tracking and Benthic Community Assessment in Relation to Oyster Aquaculture in the Delaware Inland Bays

By

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DEDICATION

This work is dedicated to family and friends who have supported me throughout my graduate studies. Thank you for your continuous encouragement to follow my dreams. I would especially like to thank my parents, Brian and Michelle, who instilled in me drive, courage, and confidence, which have been invaluable tools to help me get to where I am today. I would also like to thank my brother, Alex, who has always believed in me and helped push me to pursue my dreams. Thank you to my four fuzzy ferrets who have given me snuggles and made me laugh through the most stressful times during my graduate program. And finally thank you to my loving partner, Jon. Without you I don’t know where I would be today. You have given me endless encouragement, showed me just how strong I can be, and kept me grounded during one of the most challenging things I have ever accomplished.

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ABSTRACT

The Delaware Inland Bays consist of three shallow bays located in southern Delaware. These bays are surrounded by highly developed areas and have low flushing rates, leading to anthropogenic activities resulting in water quality degradation. This results in loss of biodiversity and abundance of organisms within the bays. The ongoing degradation of the bays since the late 1800's has led to a dramatic decline in local *Crassostrea virginica* populations. Oysters are keystone species, which provide habitats for organisms, help to improve water quality and act as bioindicator for the ecosystem health. The goals of this research are two fold: i) determine the sources of nitrogen pollution in the bays using oysters as a bioindicator and ii) identify if the introduction of oyster aquaculture improves local biodiversity and abundance of macrobenthos. To achieve these goals, field study was conducted in Rehoboth, Indian River, and Little Assawoman Bays. Aquaculture gear was placed at one location in each bay. Stable isotope ratios of nitrogen of oyster tissue, water sample and soil samples were analyzed to identify the sources of pollution and to assess the health of the bays. A benthic community assessment of Polychaetes was used to identify the impacts of oyster aquaculture. The results of the stable isotope analysis indicate Indian River Bay has the highest levels of anthropogenic nitrogen loading. The results of the benthic community assessment indicate that there was no significant

impact to Polychaete abundance or species richness from the oysters and aquaculture gear. Little Assawoman bay did have significantly higher abundance and species richness than the other bays. This research is expected to help better understand the role of oyster aquaculture restoring the viability in natural habitat of the Delaware Inland Bays.

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LIST OF ABBREVIATIONS

%DO	Percent Dissolved Oxygen
DIB	Delaware Inland Bays
IR	Indian River Bay
LAW	Little Assawoman Bay
RB	Rehoboth Bay
SIA	Stable Isotope Analysis
PCA	Principal Component Analysis

CHAPTER 1: INTRODUCTION

Crassostrea virginica, commonly known as the Eastern oyster, is a species of bivalve native to the east coast of the United States. The Eastern oyster is a reef-forming organism, creating habitat through the building of benthic structures (Jones et al., 1994). Acting as ecosystem engineers, Eastern oysters perform many services. Some of these services include providing habitat and protection for other organisms, providing foraging grounds for other organisms including both recreationally and commercially important species, and improving water quality through filtration (Zimmerman et al., 1989; Rossi-Snook et al., 2010; Harding et al., 1999; Grabowski et al., 2012). This commercially important species has been harvested as a source of protein since pre-colonial times. However, due to overharvesting, degradation of water quality and the introduction of pathogens, oyster populations in the United States have dramatically declined (Rothchild et al., 1994). In addition, invasive industrial harvesting techniques have degraded oyster beds to the point where they are no longer suitable environments for oysters to settle (Rothchild et al., 1994).

Oyster aquaculture in Delaware began in the 1800's and until the late 1970's oyster aquaculture existed in the Delaware Inland Bays (Ewart, 2013). However, due to disease and problems with the commercial fishermen, oyster aquaculture in the Delaware Inland Bays stopped in 1978 (Ewart, 2013). In 2017, DNREC reopened applications for bottom-leases to begin oyster aquaculture in the Delaware Inland Bays again. With the prospective for aquaculture in the bays, baseline data is needed to assess the potential impacts of the introduction of aquaculture.

One problem facing the reintroduction of oyster aquaculture in the Delaware Inland Bays is the development of land for human use. Development of land has led to many problems in the Delaware Inland Bays. Two examples of impacts are increased nutrient inputs and sedimentation (Marenghi et al., 2009). High nitrogen loading resulting from non-point source pollution severely impacts water quality in the bays (Walch et al., 2016). Additionally, siltation due to sediment erosion and the destruction of natural oyster beds through overfishing has led to benthic conditions which are not suitable for oyster settlement (Marenghi et al., 2009; Rothchild et al., 1994).

Restoring a degraded ecosystem such as the Delaware Inland Bays can be rather challenging. Continuous stress from anthropogenic activities decreases species diversity and abundance and reduces submerged aquatic vegetation (Marenghi et al., 2009). One practice used to restore degraded estuarine communities such as these is oyster restoration programs. Oysters are ecosystem engineers, providing habitat in the form of oyster reefs and improving water quality through filtration (Marenghi et al., 2009). Introducing oyster aquaculture into the Delaware Inland Bays is a prospective method for improving ecosystem health. The introduction of aquaculture will help improve water quality, provide habitat for other species, and possibly help in oyster restoration efforts (Ulanowicz & Tuttle, 1992; Rose et al., 2015).

Prospectively, oyster aquaculture in the Delaware Inland Bays will have both ecological and economic benefits to the area. This project focuses on assessing the potential ecological benefits of oyster aquaculture in the Delaware Inland Bays. The purpose of this research is to expand previous research in two ways: a) A new method of tracking nutrient inputs in the Delaware Inland Bays (DIBs) using stable isotope analysis and b) An assessment of benthic community dynamics through polychaete monitoring with the introduction of oyster aquaculture.

This research hopes to address the following three questions:

1. What are the sources of nitrogen loads in the Delaware Inland Bays?
2. Where are the sources of nitrogen loads located in the Delaware Inland Bays?
3. Can oyster aquaculture improve the degraded benthic community in the Delaware Inland Bays?

Research Objectives

The Delaware Inland Bays are three shallow inland bays, which are impacted by high nitrogen loading (Walch et al., 2016). The primary sources of nitrogen loading in the bays are anthropogenic non-point source pollution (U.S. EPA, 2011; Walch et al., 2016). Previous research has been conducted in the Chesapeake Bay to examine the nitrogen content through stable isotope analysis of oyster tissue. This research offers similar insight into the anthropogenic nitrogen pollution of the Delaware Inland Bays. In addition to high nitrogen loading, the Delaware Inland Bays also suffer from degraded benthic communities (Chaillou et al., 1996). The second part of this research aimed to assess the impacts of oyster aquaculture gear on the benthic community. The results of this research will aid in the successful achievement of the oyster aquaculture industry in the Delaware Inland Bays. The objectives of this research include:

1. Collecting and identifying polychaete under and around oyster aquaculture gear in order to observe the effects of the gear and oysters on the benthic communities.
2. Collecting oyster tissue samples for stable isotope analysis of nitrogen.
3. Collecting water and soil samples for nitrogen source tracking through stable isotope analysis.

4. Determining nitrogen pollution through nitrogen source tracking.
5. Mapping sources of nitrogen pollution using the results of the stable isotope analysis and ArcGIS software.
6. Monitor Delaware Inland Bays water chemistry through bi-weekly water quality and nutrient analysis.

Hypotheses:

H_o1: Oyster aquaculture sites in the Delaware Inland Bays do not show signs of nitrogen pollution through $\delta^{15}N$ enriched stable isotope analysis values.

H_a1: Oyster aquaculture sites in the Delaware Inland Bays do show signs of nitrogen pollution through $\delta^{15}N$ enriched stable isotope analysis values.

H_o2: Polychaete abundance and diversity will not be significantly different under oyster gear, 1 meter away from the oyster gear and 5 meters away from oyster gear.

H_a2: Polychaete abundance and diversity will be significantly different under oyster gear, 1 meter away from the oyster gear and 5 meters away from oyster gear.

This research will offer invaluable baseline data on the impact of oyster aquaculture in the Delaware Inland Bays. It will also provide efficient and effective ways of assessing nitrogen sources and benthic community dynamics, which can be easily replicated in future studies.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Bottom leases for oyster aquaculture in the Delaware Inland Bays became available in 2017 (DNREC, 2017), meaning oyster aquaculture will soon begin in the Delaware Inland Bays. Because oyster aquaculture is new to the bays, baseline data regarding aquaculture in the Bays needs to be collected. Oysters are ecosystem engineers (Jones et al., 1994), and the introduction of their presence in high volume may ecologically impact the Bays. This research has two purposes: to track nitrogen sources of pollution and to assess the impacts of oyster aquaculture on benthic communities. The questions this research attempts to answer are:

What are the sources of nitrogen loads in the Delaware Inland Bays?

Where are the sources of nitrogen loads in the Delaware Inland Bays?

Will oyster aquaculture improve the degraded benthic community in the Delaware Inland Bays?

While oyster aquaculture will provide economic benefits through industry and tourism (Ewart, 2013), this project will focus on assessing the potential ecological impacts of oyster aquaculture in the Delaware Inland Bays. The Delaware Inland Bays suffer from high nitrogen loading (Walch et al., 2016) and degraded benthic communities (Chaillou et al., 1996). Nutrient loading in the bays comes from non-point source pollution (U.S. EPA, 2011; Walch et al., 2016). Oysters can be used to determine sources and location of nitrogen pollution in the bays through stable isotope analysis (Fertig et al., 2010). This can be used as a tracking tool for nitrogen sources. Collecting baseline data for future studies to continue to track pollution will be important for the aquaculture industry. In addition, nutrient bioextraction, where oysters are grown and harvested from the bays to remove nitrogen can be used as a tool for controlling nitrogen loading (Rose et al., 2015).

Oysters are filter feeding organism, depositing nutrient rich feces and pseudofeces to the benthos (Grabowski et al., 2012). Changes in their presence can impact the benthic community structure. As benthic organisms are highly susceptible to changes in the environment, benthic community composition can be used as biological indicators of environmental health (Tagliapietra & Sigovini, 2010). Previously benthic community structure has been studied to assess the impact of oyster population declines (Grabowski et al., 2012). As oyster populations declined, there was a shift in benthic community (Grabowski et al., 2012). Community composition of benthic macrofauna under and around oyster aquaculture gear can be used as a benthic impact assessment to determine the environmental impacts of oyster aquaculture in the Delaware Inland Bays.

2.2 Oysters and Their Value

Eastern oysters, *Crassostrea virginica*, are a keystone species in coastal estuarine ecosystems. Acting as both autogenic and allogenic ecosystem engineers, these organisms both physically and chemically alter the environment that surrounds them. As defined by Jones et al. (1994), autogenic engineers "change the environment via their own physical structures," while allogeneic engineers "change the environment by transforming living or non-living materials from one physical state to another."

Oysters act as ecosystem engineers by creating oyster beds, which provide habitat for many ecologically important species (Zimmerman et al., 1989; Rossi-Snook et al., 2010). This includes several recreationally and commercially important species; for examples blue crabs, bluefish and striped bass (Harding et al., 1999). While oysters are often thought of solely for their value based on the services they provide for the finfish industry, as ecosystem engineers these organisms provide many ecological services (Grabowski et al., 2012). These nonmarket ecosystem services include improvement of water quality, reduction in turbidity, providing nursery habitat for both ecological and commercially important species, acting as a foraging ground and refuge from predators, and promoting nutrient cycling and sequestration (Ewart, 2013; Grabowski et al., 2012; Newell, 2004). Oysters are of such ecological significance that the decline of oyster reefs from estuary ecosystems has been shown to contribute to regime shift from coastal communities dominated by benthic flora and fauna to communities predominantly comprised of planktonic and microbial organisms (Grabowski et al., 2012).

Besides providing habitat for important fisheries species, oysters themselves are also a commercially important fisheries species. Commercial oyster fisheries begun in Delaware in the early 1800's, however oysters had been a food staple and product of commerce since pre-colonial times (Ewart, 2013). Delaware reached its peak in oyster fisheries production from 1947 through 1957, after the end of World War II (Ewart, 2013). In addition, during this time, 16.2 km^2 of benthic area in Rehoboth Bay and Indian River Bay was leased for oyster production (Ewart, 2013). Collapse of the industry began with the protozoan parasite Multi-Nucleated Sphere Unknown (*Haplosporidium nelson*), also known as MSX (Ewart & Ford, 1993). The parasite first appeared in Chesapeake and Delaware Bays, and then spread up and down the coast (Greer, 2017). The disease destroyed 95% of the oyster population, and the number of bottom leases declined due to the disease and the reduced availability of seed oyster (Ewart & Ford, 1993; Ewart, 2013). By 1978, seed oyster supply was unavailable and oyster production was nonexistent in the bays (Ewart, 2013). A second pathogen, *Perkinsus marinus*, a deadly parasite which causes “dermo,” has been effecting oyster populations in the bays since the 1990’s (Ewart, 2013). In 2017 DNREC announced oyster aquaculture applications for bottom-leases had become available. With this announcement, oyster aquaculture in the Delaware Inland Bays will begin again within the next few years (DNREC, 2017).

2.3 Delaware Inland Bays

The Delaware Inland Bays are shallow bays located in the southern part of the mid-Atlantic state of Delaware. This system consists of three interconnected inland bays including Little Assawoman Bay (LAW), Rehoboth Bay (RB), and Indian River Bay (IR) (Figure 1). Rehoboth Bay and Indian River Bay are tidally connected to the Atlantic Ocean by the Indian River Inlet (Walch et al., 2016). Little Assawoman Bay is the most inland bay. Its tidal connection is through the Ocean City Inlet from the Assawoman Bay 15 kilometers south in Maryland (Walch et al., 2016). Little Assawoman Bay is also connected to Indian River Bay through the Assawoman Canal.

With a typical depth of less than 2.1 m and poor flushing rates, these bays are highly susceptible to nutrient loading from the surrounding watershed (Walch et al., 2016; Chaillou et al., 1996). In a report published by Walch et al. (2016) on the state of the Delaware Inland Bays, nitrogen loadings in the bays far exceed the healthy limits. Indian River Bay averaged inputs greater than 6 times this limit. The largest source of this nutrient loading comes from non-point source pollution including fertilizer and animal waste from surrounding farmland, and human wastewater (U.S. EPA, 2011; Walch et al., 2016).

While nitrogen is an essential nutrient in aquatic ecosystems, excess nitrogen could lead to problems in the Delaware Inland Bays such as eutrophication and deterioration of water quality (Chaillou et al., 1996). To assess the health of the Delaware Inland Bays, stable nitrogen isotope analysis was proposed to track sources of nitrogen. Several characteristics of the Eastern oyster, including their filtering capabilities, their ability to tolerate the physical stress of be transported between sites, and the characteristic of being sessile, make this species a perfect bio-indicator for stable nitrogen isotope analysis (Fertig et al., 2010). In addition, the use of oysters as bio-indicators allows the researcher to minimize temporal and special variability, which would be seen in direct measurements (Fertig et al., 2009).

In addition to being used as a bio-indicator for nitrogen loading, oyster aquaculture may also provide a partial solution to the problem of reducing nitrogen pollution. According to the research by Rose et al. (2015), nitrogen removal by shellfish farms was a more favorable solution per acre than Best Management Practices (BMPs) for agricultural and storm water runoff. Therefore, oyster aquaculture should be considered as a possible solution to reduce nutrient loading and preventing eutrophication in the Delaware Inland Bays.

According to a report published by Chaillou et al. (1996), who examined the benthic community measured by EMAP's benthic index, more than 28% of the area of Maryland and Delaware's coastal bays had degraded benthic communities. As benthic communities are biological indicators of environmental conditions, they reveal the levels of stress an environment is facing (Tagliapietra & Sigovini, 2010). Benthic organisms have often been used as indicators of environmental health due to their diversity and wide range of physiological tolerances and responses to stressors (Chaillou et al., 1996). These organisms, subjected to contaminants and low levels of oxygen in the sediment, are relatively immobile, which prevents them from avoiding exposure (Chaillou et al., 1996). In order to assess the impact on the benthic community from the introduction of oyster aquaculture, a benthic polychaete assessment was proposed. Therefore, a change in composition of the degraded benthic community in the Delaware Inland Bays would indicate an impact of aquaculture on the ecosystem.

2.4 Stable Isotope Analysis

Atoms of the same element with the same number of protons but differ in the number of neutrons are called isotopes (Foord, 2014). These atoms have the same atomic number but different atomic masses (Foord, 2014). The word isotope derives from "iso," which means "same", and "topos," which means "place", because isotopes are forms of an element which occupy the same place in the periodic table (Fry, 2006).

Besides differences in atomic mass, another physical difference is stability. Protons have electrostatic repulsion, which is overcome by a strong nuclear force (Foord, 2014). However, too many neutrons can result in an unstable nucleus (Fry, 2006). A nucleus with the number of neutrons equal to or slightly more than the number of protons is important for stability of isotopes (Fry, 2006).

While isotopes have different physical properties, their chemical properties are nearly identical (Foord, 2014). However, isotopes with more neutrons can suffer from the kinetic isotope effect (Foord, 2014). The kinetic isotope effect is the tendency for heavier isotopes of an element to undergo reactions more slowly than the lighter isotopes of an element (Foord, 2014). The mass of the isotope can also change the vibrational mode, which is the movement of the bonds within a molecule (Foord, 2014). This could in turn effect how photons are absorbed (Foord, 2014).

Two of the most important isotopes used in analysis are carbon and nitrogen. In nature 98.89% of carbon is in the form of the lighter isotope, ^{12}C, and only 1.11% of carbon is in the form of ^{13}C (Fry, 2006). Nitrogen's heavy isotope, ^{15}N, only makes up 0.36% of nitrogen and the rest is made up of the ^{14}N isotope (Fry, 2006). There are many methods for isotope analysis and samples in solid, liquid, and gas forms can be analyzed for isotopes using isotope mass spectrometry with an elemental analyzer (Brenna et al., 1997; Tuinov, 2007). Solid unprocessed samples are analyzed for carbon and nitrogen using flash combustion elemental analyzers (Brenna et al., 1997).

Isotope values are often denoted in δ notation (Fry, 2006). For example, nitrogen stable isotopes are often denoted in $\delta^{15}N$ values. The units for this measurement are ‰ or permil, which is defined as parts per thousand (Fry, 2006). These values are calculated using the equation below (Fry, 2006):

$$\delta\, {}^{H}X = [(R_{\mathrm{SAMPLE}}/ R_{\mathrm{STANDARD}} - 1)]*1000$$

${}^{H}X$= Heavy isotope mass (ex: ^{13}C or ^{15}N)

R= Ratio of heavy to light isotope of element

Stable isotope analysis is an important tool used by many different fields of science (Rundel et al., 1990). When this tool first began being used, mainly geochemists and analytical chemists utilized this technique (Lajtha & Michener, 1995). However, this tool has become especially important in the fields of biology, ecology, and environmental science. Stable isotope analysis has been used to examine many areas within these fields including animal movement and migration, resource partitioning, host-parasite interactions, trophic interactions, plant water use and nutrient status, ecophysiological processes, anthropogenic pollution, and ecosystem fluxes of carbon, nitrogen and water (Boecklen, et al., 2011; Fry, 2006).

Stable isotope analysis is an excellent tool for tracking and identifying non-point source pollution. Non-point source pollution is especially difficult to monitor because it is random, erratic, complex, unseen, and dispersed (Ma et al. 2015). However, stable isotope analysis can account for spatial and temporal variations within nitrogen loads (Corbett et al., 2015; Fertig et al., 2009; Fry, 2006; Jona-Lasinio et al. 2015; Mayer et al., 2002).

As there are several contributors of non-point source pollutions in Delaware Inland Bays, stable isotope analysis is the perfect tool to assess pollution. Less than 30% of anthropogenic nitrogen inputs in the Mid-Atlantic and New England are exported to the ocean (Mayer et al., 2002), which can contribute to high nitrogen loading in inland bodies of water. When assessing a watershed, tracking nitrogen pollution can be difficult using typical methods due to tides and rapid cycling (Fry, 2006). However, isotope values persist because they are integrated into the tissues of the organisms in the area (Fry, 2006). Therefore, isotopic signatures in organisms present in an ecosystem can be used to evaluate nitrogen pollution within the watershed. One important use for stable isotope analysis is to map pollution plumes (Fry, 2006; Costanzo et al., 2005). In polluted ecosystems, high $\delta^{15}N$ values indicate nitrogen pollution from within the watershed (Fry, 2006). According to Fry (2006), isotope studies in aquatic ecosystems “provide a good geographic context for ecological study in a fluid medium where boundaries are hard to visualize.” This is especially important in this study, as the Delaware Inland Bays consist of three distinct but interconnected bodies of water.

This study used nitrogen signatures of oyster tissue samples, particulate organic matter from filtered water samples, and soil samples from areas with different land uses within the watershed to map pollution plumes. By mapping these values, this research provided baseline data for future research in the Delaware Inland Bays. For example, this research showed the spatial variations of particulate organic matter which can be used in future studies on food web dynamics and nutrient transport (Kendall et al., 2001). In addition, this study can be replicated in the future after oyster aquaculture has been established in the Delaware Inland Bays in order to fully understand the impact on nutrient loading and the benthic community.

2.5 Benthic Community Assessment

Benthic community assessments are often used to evaluate the health of an ecosystem. A healthy benthic community in the mid-Atlantic is characterized by high biodiversity of benthic flora and macrofauna (Grabowski et al., 2012). Benthic communities are made up of a several different types of organisms including many invertebrate species (Tagliapietra & Sigovini, 2010). Benthic organisms play important roles in ecosystems because they are a fundamental part of the food web. They act both as a food source for larger organisms and as decomposers, helping bacteria break down organic matter (Tagliapietra & Sigovini, 2010).

There are multiple ways in which benthic organisms can be used to assess ecosystem health. Firstly, the presence or absence of benthic organisms, which are sensitive to environmental factors, can be used as indicator species (Tagliapietra & Sigovini, 2010). Secondly, some species of benthic organisms can be directly assessed for environmental signals which indicate the health of an ecosystem, such as exposure to chemical contaminates through analysis of the organism and their tissues (Tagliapietra & Sigovini, 2010).

Previous studies have utilized polychaete surveys as a form of benthic community assessment, especially around sites altered for aquaculture purposes. For example, Schafer et al. (1995) utilized both foraminifera and polychaete surveys in order to assess benthic impacts at Canadian aquaculture sites. In a study published by Lewis and Nelson (2008), a benthic community assessment was used to assess the impact of hypoxic conditions caused the deposition of organic matter by mussel rafts.

Several factors make a benthic community assessment through polychaete surveys an ideal technique for this research. One factor is that it can be easily replicated. This technique requires inexpensive equipment. A trained technician using a dissecting microscope and dichotomy key can identify specimens. Secondly, this method can be completed over a long period of time. Specimens can be collected during the field season and preserved for later identification. Additionally, aquaculture sites experience a high rate of deposition of organic matter (Schafer et al., 1995), therefore it is crucial to study the organisms which will be directly impacted by the implementation of aquaculture. This is especially important as polychaetes are indicators of environmental health.

2.6 Tourism and the Economy in Delaware Inland Bays

Tourism makes up a large part of Delaware's economy. Every summer, millions of people travel to this small state to vacation at Delaware's beaches. In 2015 alone, Delaware attracted 8.5 million visitors (Delaware Tourism Office, 2015). This tourism generates hundreds of millions of dollars in state and local taxes and fees each year (Delaware Tourism Office, 2015). Without tourism, Delaware households would pay nearly $1,500.00 more in taxes annually (Delaware Tourism Office, 2015). The implementation of oyster aquaculture may have economic benefits for the tourism industry as well. A study conducted by Kecinski et al. (2016) shows that experienced oyster consumers preferred aquaculture oysters opposed to wild caught oysters. This could lead to a lucrative, branded market with unique, local Delaware Inland Bay brand oysters. Oyster aquaculture will also provide a locally sourced seafood supply and preserve the working waterfront and coastal community heritage, which may be attractive for tourists (Ewart, 2013). In addition, oyster reefs will offer an ecotourism opportunity and may improve recreational fisheries by improving water quality and acting as a nursery for recreational species (Ewart, 2013).

The following chapter provides the methods used in this study to assess nitrogen loads and the benthic community structure. Protocols for water quality and nutrient analysis; stable isotope analysis of oyster tissue, water samples, and soil samples; and benthic community assessment through Polychaete collections are described.

CHAPTER 3: METHODS AND MATERIALS

3.1 Study Location

Field testing was conducted in the Delaware Inland Bays in the southern Delaware (Figure 1). Oyster aquaculture gear was deployed in each Inland Bay: Rehoboth Bay, Indian River Bay, and Little Assawoman Bay. The gear consisted of one set of metal aquaculture cages (Ketchum Triple Stack cage, 0.61m x 0.91m x 0.76m, mesh size 12.7mm x 12.7mm mesh) (Figure 2a) and one set of double-stacked aquaculture trays with lids (Aqua Trays 0.91m x 0.91m, mesh size 12mm x 12mm) (Figure 2b) The trays were suspended approximately 10 cm from the bottom and were anchored using PVC pipes placed in each corner. The cages were anchored using one anchor at either end of the gear. Approximately 50 market-sized oysters were deployed in June 2016 and 250 oysters of various sizes were deployed in May 2017.

A control set of gear was added in 2017, with no oysters deployed. One set of double-stacked aquaculture trays with lids and one set of metal aquaculture cages were deployed at the Indian River site, on the opposite side of the research area markers.

3.2 Water Quality and Nutrient Analysis

Environmental parameters such as water quality and weather data were collected in order to assess the environmental factors, which could influence the outcomes of the research.

Water quality was monitored weekly using a handheld YSI (YSI Inc., Yellow Springs, OH 45387) to collect temperature, salinity, pH, and dissolved oxygen (Figure 3a). Chlorophyll-a concentrations was measured using a Flourometer (Handheld Aquaflour, Turner Designs, State)

(Figure 3b). Turbidity was measured using a turbidity meter (WQ770 Turbidimeter, Global water, College Station, Texas) (Figure 3c).

Nutrient analysis in 2016 occurred weekly. Samples were collected in the field, kept on ice and brought back to DSU Aquatic Sciences Laboratory for analysis using a HACH R3900 Laboratory VIS Spectrophotometer (Hach, Loveland, CO 80539) (Figure 4a). Nitrate (HACH Method 8171), ammonia (HACH Method 8155), orthophosphate (HACH Method 8048), alkalinity (Total Alkalinity Alkaphot Palintest Method) and total nitrogen (HACH Method 10071) were analyzed weekly. In 2016, hardness (Hardness Hardicol Palintest Method) was measured three times throughout the project, once in the beginning, halfway through and once at the end of the project. Nitrite (HACH method 8507) and total phosphorus (HACH Method 8190) were measured alternately bi-weekly in 2016. In 2017, hardness (Hardness Hardicol Palintest method) was measured three times throughout the project, once in the beginning, halfway through and once at the end of the project.

Nutrient analysis in 2017 also occurred weekly. Samples were collected in the field and analyzed in the field using YSI Photometer 9500 (YSI Inc., Yellow Springs, OH 45387) (Figure 4b). Nitrate (Nitrate Nitratest Powder Palintest method), ammonia (Ammonia Palintest method), nitrite (Nitrite-N Nitricol Palintest method), phosphate (Phosphate LR Palintest method), and alkalinity (Total Alkalinity Alkaphot Palintest method) were analyzed weekly.

Time of collection, tide and weather during collection was recorded using https://www.weatherforyou.com (WeatherForYou.com LLC, 2018) and the Weather Channel App for iPhone (TWC Product and Technology, LLC, 2018). In addition, water quality and nutrient analysis was taken directly before and after the occurrence of any significant weather events, such as hurricanes.

3.3 Stable Isotope Analyses

Collection of oysters for stable nitrogen isotope analysis occurred three times throughout each field season. Stable isotope analysis methods are based on a proven protocol from Fertig, et al. (2010). Five to seven oysters from each bay were collected in June, August and October. Upon collection, the oysters were transported on ice to the Aquatic Sciences Laboratory at DSU where they were frozen at -20 ^{O}C until prepared for stable isotope analysis.

To be prepared for analysis, oysters were thawed and dissected to remove the gills, mantel, and adductor muscle. Tissue samples were rinsed and dried at 80^{O}C for a minimum of 48 hours until completely dry. Once dry, the samples were weighed, then ground and homogenized. Sub-samples of tissue (1.0 ± 0.2 mg) were packed into tin capsules for analysis. Samples were analyzed at Dr. Deb Jaisi's Stable Isotopes Lab at the University of Delaware, Newark, Delaware.

Water and soil samples collection occurred once at the end of the season. A total of 18 water sample (6 per bay) and 10 soil samples (3 per bay + 1) were collected in 2016. In 2017, 15 water samples (5 per bay) and 9 soil samples (3 per bay) were collected.

During the 2016 field season, one liter of water sample was taken in each bay on the north, south, east and west shores, at the oyster gear site and at the inlet of each bay. During the 2017 field season, one liter of water samples per site were taken from 6 sites around the bays. A Niskin bottle was used at a depth of 1m to collect water samples.

During the 2016 field season, three soil samples were taken around the field sites at each bay, one in a commercial area, one in a residential area, and one in the salt marsh area surrounding the site. An agricultural sample was also taken from a site around Indian River.

During the 2017 field season, soil samples were taken from commercial, residential, salt marsh and agricultural areas around the three bays. Soil samples were collected using a shovel to obtain the top 10 cm of soil. Samples of the top 10 cm of soil were taken at each collection site until a 50 ml plastic centrifuge tube was full.

Water samples were transported on ice to the Aquatic Sciences Laboratory at DSU and frozen at -20^{O}C until preparation for stable isotope analysis. Samples were processed using a protocol for particulate organic matter from Levin and Currin (2012). To prepare for analysis, water samples were filtered at low pressure (5 in. Hg) vacuum through an ashed 47 m glass fiber filter (GF/F). Samples were treated with 1N HCl and rinsed with Millipore water. Samples were dried at 80^{O}C until completely dry. Once completely dry, sub-samples were packed into tin capsules for analysis. Samples were analyzed at Dr. Deb Jaisi's Stable Isotopes Lab at the University of Delaware, Newark, Delaware.

Soil samples were collected in the field, transported on ice to the Aquatic Sciences Laboratory at DSU and frozen at -20 ^{O}C until preparation for stable isotope analysis. Samples were processed using a protocol for sediment from Levin and Currin (2012). Samples were dried at 80^{O}C until completely dry, then ground and homogenized into a fine powder (450 micron). The sample was then acidified with 1N HCl and dried again. Once completely dry, sub-samples were packed into tin capsules for analysis. Samples were analyzed in Dr. Deb Jaisi's Laboratory at the University of Delaware, Newark, Delaware.

The samples were analyzed for δ^{15}N values using the protocol by Ladin et al. (2015). Analysis was conducted at Dr. Deb Jaisi's Laboratory at University of Delaware, Newark, Delaware using a Costech Elemental Analyzer (Valencia, California, USA) connected via a

Thermo Scientific ConFlow IV to a Thermo Delta V (Bremen, Germany) isotope ratio measuring mass spectrometer. Isotope ratios of parts per thousand were calculated using the equation below:

$$\delta\, ^{15}N = [(R_{SAMPLE/}\, R_{STANDARD} - 1)]*1000$$

$$R = {}^{15}N/{}^{14}N$$

The $\delta^{15}N$ values of the samples were calibrated against the USGS standards, USGS 40 and 41. Values for $\delta^{15}N$ in USGS 40 is 4.52% and in USGS 41 is 47.57%. The internal standard acetanilide was used in all runs. Samples and correction standards were run in duplicate or more. Nitrogen isotope values were reported as $\delta\, ^{15}N$ relative to atmospheric N_2 (0.0%).

3.4 Benthic Community Assessment

Polychaete collections occurred monthly from June through October. Core samples were taken using a PVC pipe and cap. The sample volume was 47.88 cm. A total of twenty-four core samples were collected from each bay during each sampling date. Eight core samples were taken under the gear (4 under the cages and 4 under the trays), eight core samples 1 meter away from the gear and eight core samples 5 meters away from the gear.

Each core sample was sieved though a 1 mm sieve and all Polychaetes were collected and placed in a 15% ethanol solution. After 15 minutes, the specimens were rinsed using tap water then fixed in a 10% Formalin Rose Bengal solution. After several days in the Formalin Rose Bengal solution the specimens were rinsed in tap water, then in 70% ethanol and preserved in a 70% ethanol solution. After preservation, the Polychaetes were identified using a Polychaete Identification guide from the Virginia Institute of Marine Science (VIMS, 2011). Species richness and abundance (N) were calculated for each site during each sampling season.

Species Richness= Total number of species present

Abundance= Total number of organisms present

3.5 Statistical Data Analysis

A Principal Component Analysis (PCA) was performed in R 3.3.2 (R Development Core Team, Vienna Austria) on the water quality data to determine important factors. Important factors were graphed with standard deviation for both field seasons. A multivariate general linear model was performed in SPSS 25 (IBM Corp., Armonk, New York) for the macro-benthic species richness and total abundance analyses. A Tukey post-hoc test was performed to determine differences between the bays and distances from gear. A p-value ≤ 0.05 was used to determine significance. Using ArcMap 10.5.1 (Environmental Systems Research Institute, Redlands, California), isotopic data was mapped to create an isotope map indicating the locations and values of samples indicating nitrogen pollution.

3.6 Potential Limitations

There are some limitations, which may affect the outcome of this research. Firstly, the study was conducted at the locations that were not directly in the proposed areas of aquaculture in each bay. Bottom leases did not become available at the proposed aquaculture sites until after the second year of the study. Secondly, the scale of the research is significantly smaller than that of an industrial aquaculture set up, permits were issued for one piece of each gear per site. Thirdly, the sizes of oysters varied between years, this was due to the availability of oysters being limited. Fourthly, because this is a field study and there are uncontrollable environmental factors which could have impacted the outcomes of this research. However, water quality and weather were monitored to account for these limitations.

CHAPTER 4: RESULTS

The following chapter provides important findings from this study. The first section provides results on the water quality and nutrient monitoring. These results were analyzed using principle component analyses, calculating the maximum and minimums of parameters, and graphing important parameters. The second section reports polychaete survey results. This data was analyzed using a general linear model and graphed. The third section provides the mapped results of the stable isotope analysis for oyster tissue, water samples, and soil samples are provided.

4.1 Water Quality and Nutrient Analysis

4.1.a 2016 Results

Water quality and nutrient analysis parameters were checked for normal distribution. Parameters were plotted to check for correlations. A PCA was preformed to reduce data, reduce dimensionality and create new components. Results of the PCA were examined for the proportion of variance of each component.

The first component accounted for 53.4% of variation and the second component accounted for 26.6% of the variation. The third component accounted for 16.4% and every other component accounted for less than 3%. The plot of the components (Figure 5) illustrates that the first three components could be considered for use. For the purposes of this study, components which accounted for more than 20% of variation were used. From Figure 5 and the results of the component analysis it was determined the first two components provide adequate explanation of the results.

The first component is influenced to a greater degree by DO% in the negative direction and slightly less by turbidity and alkalinity in the positive direction (Figure 6). This means turbidity and alkalinity relate to component one positively, indicating high values, and DO% relates to component one negatively, indicating low values (Figure 6). Component two is influenced to the greatest degree by DO%, turbidity and alkalinity in the negative direction and slightly by temperature in the positive direction (Figure 6). This means DO%, turbidity and alkalinity relate to component two negatively, indicating low values, and temperature relates to component two positively, indicating high values (Figure 6). The results of the PCA indicates the factors which influence the differences between the sites are DO%, turbidity, alkalinity and temperature.

As seen in Figure 7, there are slight groupings of the three sites. Indian River's water quality is mainly influenced by component 1 in the negative direction, indicating DO% is important water quality parameter in this bay (Figure 7). Little Assawoman's water quality is influenced by component 1 in the positive direction and to a lesser degree influence by component 2 in the negative direction, this indicates that dissolved oxygen, alkalinity and turbidity are important water quality parameters (Figure 7). Rehoboth's water quality is centered around zero for both components (Figure 7). It is apparent from the results of the PCA that the water quality parameters described above, influence the Indian River, Little Assawoman, and Rehoboth bays differently in the 2016 field season.

Water quality and nutrient parameters measured, varied greatly between the three bay sites and throughout the field season (Table 1). DO% was highest during the month of October and was lowest in September for Indian River and Little Assawoman Bays and June for Rehoboth Bay (Figure 8). Little Assawoman consistently had the lowest levels of nitrate throughout the 2016 field season (Figure 9). Orthophosphate was not stable throughout the field season and varied greatly among all sites (Figure 10). Turbidity increased throughout the field season in Little Assawoman (Figure 11). Turbidity stayed relatively stable in Rehoboth, below 10 NTU (Figure 11). Indian River had unstable turbidity levels, as shown in Figure 11, September had relatively high turbidity. Overall, the results of the water quality and nutrient analysis for the 2016 field season indicates that Little Assawoman bay had the most stable water quality during this season than the other two bay sites.

USGS data collected during the 2016 field season at the USGS Massey's Landing site in Millsboro, Delaware shows readings were similar to the data collected in this study. USGS water temperature ranged from 12.0°C to 28.7°C and DO mg/L ranged from 4.6 mg/L to 10.9 mg/L (USGS, 2018). Data collected at the Indian River field site show water temperatures ranged from 13.26°C to 30.68°C and DO mg/L ranged from 2.91 mg/L to 10.75 mg/L. Data collected at the Rehoboth field site show water temperatures ranged from 21.73°C to 31.42°C and DO mg/L ranged from 2.43 mg/L to 7.97 mg/L.

4.1.b 2017 Results

Water quality and nutrient analysis parameters were checked for normal distribution. Parameters were plotted to check for correlations. A PCA was preformed to reduce data, reduce dimensionality and create new components. Results of the PCA were examined for the proportion of variance of each component.

The first component accounted for 61.3% of variation and the second component accounted for 24.0% of the variation. The third component accounted for 12.2% and every other component accounted for less than 6%. The plot of the components (Figure 12) illustrates that the first three components could be considered for use. For the purpose of this study, components which accounted for more than 20% of variation were used. From Figure 8 and the results of the component analysis, it was determined the first two components provide adequate explanation of the results.

The first component is influenced by DO% in the positive direction, meaning it is related positively to component one indicating high values (Figure 13). The second component is influenced by turbidity and alkalinity in the negative direction, meaning turbidity and alkalinity relate negatively to component two indicating low values. The results of the PCA indicates the factors which influence the differences between the sites are DO%, turbidity and alkalinity.

As seen in Figure 14, there are slight groupings of water quality in Indian River and Little Assawoman Bays. Indian River's water quality data is clustered around zero for component one (Figure 14). Little Assawoman's water quality is influence by component two in the positive direction. Rehoboth's water quality data is not influenced by either component (Figure 14). It is apparent from the results of the PCA that the water quality parameters as described above, influenced the Indian River, Little Assawoman, and Rehoboth Bays differently during the 2017 field season.

Water quality and nutrient parameters measured varied greatly between the three bay sites and throughout the seasons (Table 2). DO% during the 2017 field season was lowest during the month of July for all three bays (Figure 15). Nitrate was lowest in June and increased in October for the three bays (Figure 16). Phosphate, similarly to the previous year, varied greatly among all sites throughout the field season and was not stable (Figure 17). Turbidity increased from June through September in the bays and decreased in October (Figure 18). Unlike the previous year, water quality was more unstable throughout the 2017 field season.

4.2 Benthic Community Assessment

4.2.a 2016 Results

The results of the Polychaete survey during the 2016 field season indicate the highest abundance of polychaetes were found at the Little Assawoman site and the lowest abundance of Polychaetes in Rehoboth Bay (Figure 19). During the 2016 field season, sampling at the Rehoboth bay site yielded a total of 3 polychaetes throughout the entire season. Conditions of substrate and water quality may have impacted the benthic conditions, which will be discussed in the next chapter. The most abundant species found at both Little Assawoman and Indian River Bays sites were the Capitellidae family (Figure 19). The top six families of Polychaetes identified include Glyceridae, Spionidae, Spirorbidae, Orbiniidae, Capitellidae, and Oweniidae (Figure 5).

The results of a multivariate general linear model indicate that there is a significant difference between the number of species identified (p-value < 0.001) and total abundance of organisms (p-values < 0.001) collected per bay during the 2016 field season (Table 3). There was no significant different between distances from oyster gear for number of species (p-value = 0.279), however there was near significance between distances for the total abundance of organisms (p-value = 0.072) (Table 3). The Post-Hoc Tukey tests indicate there is a significant difference between the number of species collected in all three bays (p-values ≤ 0.001) (Table 4). The Post-Hoc Tukey tests also indicate there is significant difference between the total abundance of organisms collected between Rehoboth Bay and Little Assawoman Bay (p-value < 0.001), and Indian River Bay and Little Assawoman Bay (p-value <0.001) (Table 4). There was no significant difference between the number of organisms collected between Rehoboth Bay and Indian River Bay (p-value = 0.126) (Table 4).

4.2.b 2017 Results

During the 2017 Polychaete survey, a fourth site (control) was added to the survey at Indian River Bay. The results of the Polychaete survey during the 2017 field season indicate the highest abundance of Polychaetes were found at the Little Assawoman site, similarly to the 2016 season (Figure 20). The lowest abundance of Polychaetes were found at the control site and the second lowest at the Indian River site (Figure 20). Unlike the 2016 field season, over 190 organisms were collected throughout the field 2017 season at the Rehoboth site. The most abundant species found at the Little Assawoman, Indian River and Rehoboth sites were the Glyceridae family (Figure 20). The second most abundant was the Capitellidae family (Figure 20). The top six families of Polychaetes identified include Glyceridae, Spionidae, Chaetopteridae, Orbiniidae, Capitellidae, and Oweniidae (Figure 20).

The results of a multivariate general linear model indicate there is a significant difference between both the number of species identified (p-value = 0.001) and total abundance of organisms collected per bay (p-value < 0.001) during the 2016 field season (Table 5). There was no significant difference between distances from oyster gear for both total abundance of organisms (p-value = 0.526) or number of species (p-value = 0.530) (Table 5). The Post-Hoc Tukey tests indicate there is significant difference between the number of species collected between Indian River Bay and Little Assawoman Bay (p-value = 0.039), and the control site and Little Assawoman Bay (p-value = 0.001) (Table 6). There was significant difference between the total abundance of organisms collected between Little Assawoman Bay and the control (p-value < 0.001), Little Assawoman Bay and Indian River Bay (p-value < 0.001), and Little Assawoman Bay and Rehoboth Bay (p-value < 0.001) (Table 6).

4.3 Stable Isotope Analysis

4.3.a 2016 Results

The 2016 nitrogen stable isotope analysis results for the oyster muscle tissue, water samples and soil samples were mapped using Arcmap 10.5.1 (Environmental Systems Research Institute, Redlands, California) from the samples collected in June and later in October. The average nitrogen stable isotope values for October samples of the oyster muscle tissue was 10.24 ±0.05‰ in Rehoboth Bay, 10.72 ± 0.05‰ in Indian River Bay, 9.99 ± 0.19‰ in Little Assawoman Bay (Figure 21a).

The average nitrogen isotope values for water samples were mapped. Nitrogen isotope values for water samples in Rehoboth Bay ranged from 5.59 ± 5.51‰ to 6.62 ± 1.19‰ (Figure 21b). Nitrogen isotope values for water samples in Indian River Bay ranged from 6.59 ± 2.77‰ to 8.35 ± 1.98‰ (Figure 21b). Nitrogen isotope values for water samples in Little Assawoman Bay ranged from 5.41 ± 0.20‰ to 6.92 ± 0.17‰ (Figure 21b). The map of water nitrogen isotope values shows a trend of the highest isotopic values in Indian River Bay and lowest values in Little Assawoman Bay (Figure 21b).

The average nitrogen isotope values for soil samples were mapped and labeled for the type of land use where the soil was collected from. Nitrogen isotope values for soil samples in Rehoboth Bay ranged from -0.63 ± .56‰ (residential) to 3.95 ± .62‰ (salt marsh) (Figure 21c). Nitrogen isotope values for soil samples in Indian River bay ranged from 3.70 ± .40‰ (commercial) 7.88 ± .44‰ (salt marsh) (Figure 21c). Nitrogen isotope values for soil samples in Little Assawoman bay ranged from 4.49 ± .10‰ (salt marsh) to 6.79 ± .81‰ (commercial) (Figure 21c). There were no trends in nitrogen isotope values as it pertains to locations where samples were obtained from the bay waters and land soils (Figure 21c).

4.3.b 2017 Results

The 2017 nitrogen stable isotope analysis results for the oyster muscle tissue, water samples and soil samples were mapped using Arcmap 10.5.1 (Environmental Systems Research Institute, Redlands, California). The average nitrogen s isotope values for October samples of the oyster muscle tissue was 11.85 ± 0.23‰ in Rehoboth Bay, 11.72 ± 0.09‰ in Indian River Bay, and 11.26 ± 0.19‰ in Little Assawoman Bay (Figure 22a).

The average nitrogen isotope values for water samples were mapped. Nitrogen isotope values for water samples in Rehoboth Bay ranged from -1.82 ± 15.07‰ to 5.79 ± 0.40‰ (Figure 22b). Nitrogen isotope values for water samples in Indian River Bay ranged from 4.29 ± 2.86‰ to 11.68 ± 7.22‰ (Figure 22b). Nitrogen isotope values for water samples in Little Assawoman Bay ranged from 5.10 ± 2.04‰ to 10.89 ± 1.24‰ (Figure 22b). Similarly, to the previous year's results, the highest nitrogen isotope value was found in Indian River Bay. However, the lowest nitrogen isotope value was found in Rehoboth Bay. There were some issues with the consistency of the data. Considering the high standard deviations of some samples, there is suspicion that the accuracy of the 2017 water sample data was compromised due to contamination of samples during analysis.

The average nitrogen isotope values for soil samples were mapped and labeled for the type of land use where the soil was collected from. Nitrogen isotope values for soil samples in Rehoboth Bay ranged from -13.26 ± 2.51‰ (residential) to -7.15 ± .86‰ (agricultural) (Figure 22c). Nitrogen isotope values for soil samples in Indian River bay ranged from -11.29 ± 3.62‰ (residential) 0.62 ± 1.49‰ (agricultural) (Figure 22c). Nitrogen isotope values for soil samples in Little Assawoman Bay ranged from -1.49 ± 1.36‰ (salt marsh) to 8.38± .14‰ (agricultural) (Figure 22c). There were no trends in nitrogen isotope values as it pertains to locations where samples were obtained from in bays and land use of soil (Figure 22c). Similarly, to the water samples during the 2017 field season there were some issues with the consistency of the soil sample data. Considering the high standard deviations of some samples, there is suspicion that the accuracy of the 2017 soil sample data was compromised due to contamination of samples during analysis. It is possible, there was water in the samples which effected the analysis.

Figure 23 shows the land use and land cover of the area surrounding the Delaware Inland Bays. As shown in Figure 23, the majority of the land use surrounding the bays is developed for single family homes and cropland. The light blue color indicates wetland area which can be seen along the shoreline of the bays (Figure 23). An important area to note around the Little Assawoman bay is the Assawoman Wildlife area located along the western shore between Miller Creek and Dirickson Creek (Figure 23).

4.4 Summary of Research Findings

The following hypotheses were proposed for this research:

Hypotheses:

H_o1: Oyster aquaculture sites in the Delaware Inland Bays do not show signs of nitrogen pollution through $\delta^{15}N$ enriched stable isotope analysis values.

H_a1: Oyster aquaculture sites in the Delaware Inland Bays do show signs of nitrogen pollution through $\delta^{15}N$ enriched stable isotope analysis values.

H_o2: Polychaete abundance and diversity will not be significantly different under oyster gear, 1 meter away from the oyster gear and 5 meters away from oyster gear.

H_a2: Polychaete abundance and diversity will be significantly different under oyster gear, 1 meter away from the oyster gear and 5 meters away from oyster gear.

Based on the finding of this research for nitrogen stable isotope analysis, the alternative hypothesis is accepted. The null hypothesis is rejected. The results of the stable isotope analysis show $\delta^{15}N$ enriched stable isotope analysis values.

Based on the finding of this research for the benthic Polychaete assessment, the null hypothesis is accepted. The alternative hypothesis is rejected. There was no significant difference between the distances from the gear for both Polychaete abundance and diversity.

The following discussion and conclusion chapter examines the significance of these results, some possible limitations within the study, and makes suggestions for future research.

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 Discussion

As oyster aquaculture is beginning in the Delaware Inland Bays, it is important to understand what impacts this industry will have on ecosystem dynamics. The goals of this research were to understand the impact of oyster aquaculture on the benthic community and determine the extent and sources of nitrogen pollution in the Delaware Inland Bays. The previous chapter discussed the findings from this research. This chapter discusses the significance and implications of these findings and suggestions for future research.

The results of the water quality monitoring indicate that these bays are highly susceptible to changes in water quality. According to U.S. EPA (2017) standards, the suggested value for inorganic nitrogen in tidal portions of the Delaware Inland Bays is 0.14 mg/L. One potential factor impacting the water quality in the Delaware Inland Bays' ecosystems is runoff from developed land used for agriculture, livestock, and housing developments. According to a study conducted in Rehoboth Bay by Volk et al. (2006), nitrogen loads from the surrounding watershed accounted for the highest percentage of nitrogen loading, followed by atmospheric deposition, and local wastewater treatment. The principle source of nitrogen loads in the Rehoboth Bay throughout the year is watershed runoff (Volk et al., 2006).

Another factor impacting the water quality is the tidal connection of the bays to the ocean. As seen in Figure 1, the shared tidal connection for the Rehoboth and Indian River Bays is the Indian River Inlet. Not shown in Figure 1, is the tidal connection for Little Assawoman Bay. Little Assawoman Bay is tidally connected through the Ocean City Inlet about 15 kilometers south. Open water areas are highly influenced by flushing due to tidal changes in the bays (Walch et al., 2016). Of the three study sites, Little Assawoman and Rehoboth Bays are located in protected coves, a significant distance from the inlets, which slows flushing rates. The Indian River site was located closest to its inlet, and yet this is the most polluted of the three bays. However, flushing rates in the Delaware Inland Bays continues to improve since the stabilization of the inlet in the 1930's (Walch et al., 2016). A water quality assessment of the bays in 2004 showed flushing volume improved by 11% -24% since the last assessment in 1988 (Walch et al., 2016). As flushing rates continue to improve, water quality will become more stable and nutrient loading will be reduced as more water is exchanged during tides.

While water quality monitoring did show the bays are susceptible to changes in water quality, there were some limitations on the water quality and nutrient analysis portion of this research. Firstly, the methods used for nutrient analysis give a relative value. The methods used were developed mainly for aquaculture purposes. However, because the focus of this research is aquaculture based and field-testing was required, these methods were chosen. Secondly, two different nutrient analysis techniques were used, making a direct comparison of data between the two years difficult. A second technique was used during the 2017 field season due to accessibility of equipment. During the 2017 field season, water samples needed to be analyzed in the field. As the Hach method requires laboratory equipment, the Palintest method was chosen as the YSI Photometer 9500 is a portable piece of field equipment. However, since both techniques give relative values, the results of both tests provided the overall water quality conditions in the three bays. Lastly, measurements for water quality and nutrient analysis were not taken at the same time during every collection which may have impacted parameters. For example, diel cycles of dissolved oxygen in aquatic ecosystems results in low oxygen conditions at night and early morning due to night time respiration (D'Avanzo & Kremer, 1994). Sonde data collected in

Rehoboth Bay during the 2016 field season, which sampled every 15 minutes, shows daily dissolved oxygen levels cycling (Appendix 1). The data exhibits the trend previously described, dissolved oxygen levels are lowest during the morning, after respiration has depleted oxygen during the nighttime. This confirms that there are large variations in dissolved oxygen levels throughout the day.

The results of the Polychaete survey show significant differences in both the number of species and number of organisms collected throughout the bays. One factor which may have contributed to the significant differences between Polychaete abundance and species richness were the variations in the bottom substrate. The bottom sediment in Little Assawoman and Indian River Bays were sandy and had few anoxic patches. Rehoboth Bay had areas of very thick clay sediment with anoxic conditions (personal observation). Overall, Little Assawoman's abundance of worms may be attributed to the fact that this bay is located further inland, has the most stable water quality, and the sediment conditions seem to be favorable.

Additionally, in the beginning of the 2016 field season there was an *Ulva lactuca* bloom, commonly known as sea lettuce, in Rehoboth Bay. The field site for this location at Camp Arrowhead was located close to shore in a protected cove. This led to the site being covered in decaying sea lettuce until late in the season when a storm washed the decaying vegetation out of the cove. As a result of the decaying vegetation, anoxic muddy conditions developed in the sediment. Previous studies in the Chesapeake Bay have shown that oxygen depletion due to eutrophication leads to a decrease in species richness and abundance of microbenthic organisms (Sturdivant et al., 2014). USGS data collected at Massey's Landing in Millsboro, Delaware confirm that the maximum and minimum dissolved oxygen levels at the Rehoboth Bay field site had a lower dissolved oxygen levels than other areas in Rehoboth Bay. Additionally, the maximum value for dissolved oxygen in the Indian River field site was similar to the USGS data for Massey's Landing. The comparisons of the data confirm that there were lower dissolved oxygen levels at the Rehoboth bay field site, most likely due to decay of vegetation causing anoxic conditions. Therefore, it is possible that the bloom of sea lettuce impacted the benthic community productivity during the 2016 field season.

During the 2017 field season, the conditions of the bottom sediment in the cove improved. There were noticeably fewer anoxic clay patches, which may have contributed to the differences seen in the Polychaete abundances and species compositions. While there were differences in benthic Polychaete communities in each bay, there were no significant differences in the benthic communities under and around each gear for both the 2016 and 2017 field seasons. Similar studies on the impact of aquaculture gear on benthic communities have found results which indicate oyster aquaculture gear has little to no effect on benthic community composition. A study by Crawford et al. (2003) found that there was no significant difference between benthic organisms in and around the shellfish farms studied. In a study conducted by Nugues et al. (1996), small changes in benthic communities were detected under gear when compared to uncultivated areas. A study conducted by Forrest and Creese (2006) found that anthropogenic farming operations contribute to disturbances in the benthic community, which are not caused by the presence of oysters in aquaculture gear.

One of the most abundant families of Polychaetes found in both 2016 and 2017 was the Capitellidae family. This family of Polychaete are common, opportunistic feeders often found in organically enriched sediments (Méndez et al., 2001; Silva et al., 2016). Capitellidae polychaetes are infaunal deposit feeders which promote decomposition of organic matter, often feeding in areas enriched with fish farm waste, sewage sludge, and paper mill effluents and oil (Méndez et al., 2001). The Capitellidae family can be used as indicator species, as they are a common species which inhabits polluted marine sediments (Méndez et al., 2001). The other most abundant group, Glyceridae, are another common family found worldwide (Böggemann et al., 2012). This cannibalistic species forms a semi-permanent borrow in sandy and muddy sediments (Böggemann et al., 2012). The two most abundant families found during each field season are common families, often found in a variety of environments.

Of the other families collected during this study, Oweniidae, Spionidae, and Orbiniidae were present both years. The Oweniidae family is a common tube dwelling Polychaete found throughout the world (Fiege et al., 2000). The Orbiniidae family is another commonly found family that burrows in marine sediments (Francoeur & Dorgan, 2014). One of the top species found during the 2017 field season, Chaetopteridae, is another common tube-dwelling organism, found throughout the world (Yueyun, & Xinzheng, 2017). These Polychaetes are found in a wide variety of habitats from subtidal zones to hydrothermal vents (Yueyun, & Xinzheng, 2017). Many of the Polychaetes collected in this study were common families which have a wide distribution globally.

A suggestion for future studies assessing the benthic Polychaete community is to mimic the actual scale of aquaculture in the bays. The impact may be different with a larger scale experiment. The scale of hundreds of pieces of gear compared to two pieces of gear may be significantly more substantial. In order to fully understand benthic community dynamics in the Delaware Inland Bays, benthic surveys should be continued throughout the bays at larger scale aquaculture sites. This is especially important since the Ulva bloom in Rehoboth Bay may have impacted the benthic community during the two-year duration of this study.

The stable isotope analysis values during the 2016 field season show a clear trend in both oyster tissue and water samples. The highest $\delta^{15}N$ values for the tissue and water samples were found in Indian River Bay. In polluted ecosystems, high $\delta^{15}N$ values indicate nitrogen pollution from within the watershed (Fry, 2006). Anthropogenic nitrogen pollution derived from human and animal waste has ^{15}N enriched isotope values ranging from 8‰ to 20‰ (Hou et al., 2013). Whereas agricultural fertilizers have ^{15}N depleted isotope values ranging from -3‰ to 3‰ (Hou et al., 2013). The highest $\delta^{15}N$ values indicates Indian River bay has the highest amount of human derived nitrogen. Previous research has shown that Indian River Bay has the highest levels of human derived nitrogen through other techniques (Walch et al., 2016). As the area surrounding Indian River Bay is highly developed, anthropogenic nitrogen runoff may be contributing to the nitrogen loads in the bay.

The stable isotope analysis values from the 2016 field season also show the lowest $\delta^{15}N$ values for both oyster tissue and water samples in Little Assawoman Bay. One possible reason is that during the sampling season Little Assawoman Bay had the most stable water quality. As Little Assawoman Bay is located further inland and has a distant tidal connection, it may not be as susceptible to changes in water quality due to tidal flushing. Another reason, is that the western shore of Little Assawoman Bay is the protected Assawoman Wildlife Area. This area may be acting as a buffer zone preventing pollution from entering the bay. Because this area is protected from development, there is less runoff from developed land entering the bay. According to Mayer et al. (2006), the width of buffer zones is significantly correlated with nitrogen removal and sequestration. This suggests the significant area on the western shore of Little Assawoman Bay could be contributing to the lower nitrogen isotope values in the oyster and water samples.

The stable isotope values for oyster tissue during the 2017 field season showed similar trends to the 2016 field season. Indian River and Rehoboth Bays, had the highest $\delta^{15}N$ values in the oyster tissue samples. Little Assawoman Bay had the lowest $\delta^{15}N$ values, similarly to the 2016 results. Due to an error during the analysis of the 2017 water and soil samples, isotope values showed high standard deviation. Therefore, this data may not be as reliable as the 2016 data. The error may be due to water content in the samples affecting the analysis. Although there were problems with the water and soil samples during the 2017 field season, the results of the 2016 stable isotope analysis clearly show this is a viable and effective way to monitor nitrogen pollution in the Delaware Inland Bays.

As oysters are filter feeders, they have great potential for nutrient bioextraction, which could be used as a tool to reduce the nitrogen loads in the Delaware Inland Bays. Both the 2016 and 2017 results for the oyster tissue stable isotope analysis show the oyster placed in the bays assimilated nitrogen throughout the field season. The results of this research indicate the oysters are up taking human derived nitrogen sources and removing them from the water column. Harvesting the oysters out of the Delaware Inland Bays will remove the nitrogen sequestered in their tissue and shell (Reitsma et al., 2017). Additionally, the oysters will aid in nitrogen removal by creating nitrogen rich biodeposits (Reitsma et al., 2017). As the nitrogen rich biodeposits enter the benthic community denitrification occurs through microbial activity (Reitsma et al., 2017). Additionally, long-term burial of deposits in benthic sediment sequesters the nitrogen and prevents it from reentering the water column (Reitsma et al., 2017).

5.2 Conclusions

The implementation of oyster aquaculture will have many lasting ecological impacts on the Delaware Inland Bays. By doing a preliminary assessment on the benthic community and nitrogen loading, this data can be used as a baseline for future studies to compare to after oyster aquaculture is in operation. Future studies could utilize this data to monitor, among other things, pollution reduction, benthic community dynamics, and sources of nitrogen pollution. The findings of this study show evidence of anthropogenic nitrogen pollution and high susceptibility to fluctuations in water quality and nutrients in the Delaware Inland Bays. The findings suggest that the implementation of oyster aquaculture will not have a significant impact on benthic community composition. In addition, the benthic polychaete study showed that benthic communities in the bays are impacted from effects of nutrient pollution, such as eutrophication.

Suggestions for future studies include more intensive sampling of soil and water samples for stable isotope analysis throughout the bays. Sampling soil and water throughout the field season and in more locations will give a more resolute picture of the locations and types of nitrogen inputs causing high nitrogen loading in the bays. Soil samples could be taken from sites with different land uses including agricultural sites, livestock farms, residential areas with septic systems, residential areas using fertilizers, commercial areas, transitional zones, and protected wildlife areas to assess the isotopic signatures of different land uses. Additionally, more intensive sampling of water for particulate organic matter may also be beneficial in tracking areas from which high nitrogen run-off discharges into the bays. Areas which have storm and waste water drainage and agricultural drainage through groundwater run-off may be of interest.

As this was only a two-year study, long term monitoring of both nitrogen loading and benthic communities in the Delaware Inland Bays can provide a better picture of natural fluctuation in these ecosystems. For example, the Ulva bloom at the Rehoboth site during the 2016 field season may have impacted the outcome of the benthic assessment. Therefore, a long-term study monitoring the bays will offer a more complete analysis of these factors and will not be impacted as greatly by natural anomalies which can influence short-term studies.

The implications of this research are wide reaching. Monitoring the impacts of oyster aquaculture on a degraded ecosystem like the Delaware Inland Bays gives researchers invaluable insight into the benefits of oyster aquaculture. Oyster aquaculture can reduce nitrogen loading through nutrient bioextraction, improve degraded ecosystems, and enhance biodiversity. Oyster aquaculture is a practical solution to many problems faced by polluted and degraded estuarine ecosystems. In addition to the ecological benefits of oyster aquaculture to ecosystems, economic benefits include the creation of a lucrative industry which can lead to tourism and job opportunities. Overall, this study strengthens the idea that oyster aquaculture will bring both ecologic and economic benefits to the Delaware Inland Bays.

REFERENCES

Boecklen WJ, Yarnes CT, Cook BA, James AC. 2011. On the Use of Stable Isotopes in Trophic Ecology. Annual Review of Ecology, Evolution and Systematics 42 (411-440).

Böggemann M, Bienhold C, Gaudron SM. 2012. A New Species of Glyceridae (Annelida: "Polychaeta") Recovered from Organic Substrate Experiments at Cold Seeps in the Eastern Mediterranean Sea. Marine Biodiversity 42: 47-54.

Brenna JT, Corso TN, Tobias HJ, Caimi, RJ. 1997. High-Precision Continuous-Flow Isotope Ratio Mass Spectrometry. Mass Spectrometry Reviews 16: 227-258.

Chaillou JC, DeMoss TE, Eskin R, Kutz FW, Magnien R, Mangiaracina L, Maxted J, Price K, Summers JK, Weisberg SB. 1996. Assessment of the ecological condition of the Delaware and Maryland coastal bays. U.S. Environmental Protection Agency, Region III, Annapolis, MD.

Corbett PA, King CK, Mondon JA. 2015. Tracking Spatial Distribuiton of Human-Derived Wastewater from Davis Station, East Antarctica, Using $\delta^{15}N$ and $\delta^{13}C$ Stable Isotopes. Marine Pollution Bulletin 90: 41-47.

Crawford MC, Macleod MKA, Mitchell IM. 2003. Effects of Shellfish Farming on the Benthic Environment. Aquaculture 224(1-4): 177-140.

Costanzo SD, Udy J, Longstaff B, Jones, A. 2005. Using Nitrogen Stable Isotope Ratios ($\delta^{15}N$) of Macroalgae to Determine the Effectiveness of Sewage Upgrades: Changes in the Extent of Sewage Plumes Over Four Years in Moreton Bay, Australia. Marine Pollution Bulletin 51(1-4): 212-217.

D'Avanzo C, & Kremer JN. 1994. Diel Oxygen Dynamics and Anoxic Events in an Eutrophic Estuary of Waquoit Bay, Massachusetts. Estuaries 17(1): 131-139.

Delaware Department of Natural Resources and Environmental Control 2017. DNREC's Division of Fish & Wildlife announces shellfish aquaculture leasing lottery for Inland Bays [Press Release]. Accessed on December 18th, 2017 through: https://news.delaware.gov/2017/03/31/dnrecs-division-of-fish-wildlife-announces-shellfish-aquaculture-leasing-lottery-for-inland-bays/

Delaware Tourism Office 2015. The Value of Tourism 2015: Bringing in More People, Bringing in More Revenue. Dover, DE.

Ewart JW, & Ford SE. 1993. History and Impact of MSX and Dermo Diseases on Oyster Stocks in the Northeast Region. NRAC Fact Sheet No. 200. Northeastern Regional Aquaculture Center. University of Massachusetts Dartmouth. Accessed March 9, 2018 through http://extension.umd.edu/sites/extension.umd.edu/files/_docs/programs/aquaculture/MSX%20and%20Dermo%20History.pdf.

Ewart, J. 2013. Shellfish Aquaculture in Delaware's Inland Bays: Status, Opportunities and Constraints. University of Delaware. Lewes, DE.

Fertig B, Carruthers TJB., Dennison WC, Jones AB, Pantus F, Longstaff B. 2009. Oyster and Macroalgae Bioindicators Detect Elevated $\delta^{15}N$ in Maryland's Coastal Bays. Estuaries and Coasts 32(4): 773-786.

Fertig, B, Carruthers TJ.B, Dennison WC, Fertig EJ, Altabet MA. 2010. Eastern oyster (*Crassostrea virginica*) $\delta^{15}N$ as a bioindicator of nitrogen sources: Observations and modeling. Marine Pollution Bulletin 60(8): 1288-1298.

Fiege D, Kröncke I, Barnich R. 2000. High Abundance of *Myriochele fragilis* Nilsen & Holthe, 1985 (Polychaeta: Oweniidae) in the Deep Sea of the Eastern Mediterranean. Hydrobiologia 426(1): 97-103.

Foord, E. 2014. Isotopes. Young Scientists Journal 15:46-48.

Forrest BM, Creese RG. 2006. Benthic Impacts of Intertidal Oyster Culture, with Consideration of Taxonomic Sufficiency. Environmental Monitoring and Assessment. 112(1-3): 159-176.

Francoeur AA, Dorgan KM. 2014. Burrowing Behavior in Mud and Sand of Morphologically Divergent Polychaete Species (Annelida: Orbiniidae). Biological Bulletin 226(2): 131-145.

Fry, B. 2006. Stable Isotope Ecology. New York, New York: Springer.

Grabowski JH, Conrad RF, Keeler AG, Opaluch JJ, Peterson CH, Piehler MF, Powers SP, Smyth AR. 2012. Economic Valuation of Ecosystem Services Provided by Oyster Reefs. BioScience 62 (10): 900-909.

Greer, J. 2017. Maryland Sea Grant: Chesapeake Quarterly. Accessed on April 10th, 2017 through http://ww2.mdsg.umd.edu/CQ/v08n2/main2/.

Harding, JM, Mann, R, & Clark, VP. 1999. Oyster Reefs in the Chesapeake Bay: A Brief Primer. Educational Series: No. 44. Virginia Institute of Marine Science, College of William and Mary. Accessed March 9, 2018 through https://publish.wm.edu/cgi/viewcontent.cgi?article=1984&context=reports

Hou, W., Gu, B., Zhang, H., Gu. J, & Han, B. 2013. The Relationship Between Carbon and Nitrogen Stable Isotopes of Zooplankton and Select Environmental Variables in Low-Latitude Reservoirs. Limnology 14: 97-104.

Jona-Lasinio G, Costantini ML, Calizza E, Pollice A, Bentivoglio F, Orlandi L, Careddu G, Rossi L. 2015. Stable Isotope-Based Statistical Tools as Ecological Indicator of Pollution Sources in Mediterranean Transitional Water Ecosystems. Ecological Indicators 55: 23-31.

Jones CG, Lawton JH, Shachak M. 1994. Organisms as Ecosystem Engineers. Oikos 69:373-386.

Kecinski M, Messer KD, Peo AJ. 2016. Consumer Preferences for the Provision of Water Quality Services by Oysters. Applied Economics & Statistics Research Report, University of Delaware, RR16-02.

Kendall C, Silva SR, Kelly VJ. 2001. Carbon and Nitrogen Isotopic Compositions of Particulate Organic Matter in Four Large River Systems Across the United States. Hydrological Processes 15: 1301-1346.

Ladin, ZS, D'Amico V, Jaisi DP, Shriver WG. 2015. Is Brood Parasitism Related to Host Nestling Diet and Nutrition? The Auk 132: 717-734.

Lajtha K, Michener RH. 1995. Stable Isotopes in Ecology and Environmental Science. Ecology 74(5): 1683-1684.

Levin LA, Currin C. 2012. Stable Isotope Protocols: Sampling and Sample Processing. Scripps Institution of Oceanography Technical Report. Accessed on October 6th, 2017 through https://escholarship.org/uc/item/3jw2v1hh.

Lewis J, Nelson M. 2008. Investigation of Benthic Conditions Under Mussel-Raft Farms. DMR Aquaculture Environmental Section. Maine Department of Marine Resources.

Ma G, Wang Y, Xiang B, Hu Y, Liu Y, He L, Wang T, Meng F. 2015. Nitrogen Pollution Characteristic and Source Analysis using the Stable Isotope Tracing Method in Ashi River, Northeast China. Environmental Earth Science 73: 4831-4839.

Marenghi F, Ozbay G, Rossi-Snook K, Chalabala EJ. 2009. Restoration Programs in Inland Bays Improves Ecosystems as Oyster Populations Recover. Global Aquaculture Alliance Global: Aquaculture Advocate p. 16-17.

Mayer B, Boyer EW, Goodale C, Jaworski NA, Van Breeman N, Howarth RW, Seitzinger S, Billen G, Lajtha K, Nadelhoffer K, Van Dam D, Hetling LJ, Nosal M, Paustian K. 2002. Sources of Nitrate in Rivers Draining Sixteen Watersheds in the northeastern U.S.: Isotopic Constraints. Biochemistry 57/58: 171-197.

Mayer PM, Reynolds SK, McCutchen MD, & Canfield TJ. 2006. Meta-Analysis of Nitrogen Removal in Riparian Buffers. Journal of Environmental Quality 36(4): 1172-1180.

Méndez, N, Linke-Gamenick I, Forbes VE, Baird DJ. 2001. Sediment Processing in *Capitella* spp. (Polychaeta: Capitellidae): Strain-Specific Differences and Effects of the Organic Toxicant Flouranthene. Marine Biology 138: 311-319.

Newell RIE. 2004. Ecosystem Influences of Natural and Cultivated Populations of Suspension-Feeding Bivalve Mollusks: A Review. Journal of Shellfish Research 23(1): 51-61.

Nugues MM, Kaiser MJ, Spencer BE, & Edwards DB. 1996. Benthic Community Changes Associated with Intertidal Oyster Cultivation. Aquaculture Research 27(12): 913-924.

Reitsma J, Murphy DC, Archer AF, & York RH. 2017. Nitrogen Extraction Potential of Wild and Cultured Bivalves Harvested from Nearshore Waters of Cape Code, USA. Marine Pollution Bulletin 116: 175-181.

Rose JM, Bricker SB, Ferreira JG. 2015. Comparative Analysis of Modeled Nitrogen Removal by Shellfish Farms. Marine Pollution Bulletin 91(1): 185-90.

Rossi-Snook K, Ozbay G, Marenghi F. 2010. Oyster (*Crassostrea virginica*) gardening program for restoration in Delaware's Inland Bays, USA. Aquaculture International 18(1): 61-67.

Rothschild BJ, Ault JS, Goulletquer P, Héral M. 1994. Decline of the Chesapeake Bay Oyster Population: A Century of Habitat Destruction and Overfishing. Marine Ecology Progress Series 111: 29-39.

Rundel PW, Ehleringer JR, Nagy KA. 1990. Review Stable Isotopes in Ecological Research. American Scientist, 78(3):277-278.

Schafer CT, Winters GV, Scott DB, Pocklington P, Cole FE, Honig C. 1995. Survey of Living Foraminifera and Polychaete Populations at Some Canadian Aquaculture Sites: Potential for Impact Mapping and Monitoring. Journal of Foraminiferal Research 25(3):236-259.

Silva CF, Shimabukuro M, Alfaro-Lucas JM, Fujiwara Y, Sumida PYG, Amaral ACZ. 2016. A New *Capitella* Polychaete Worm (Annelida: Capitellidae) Living Inside Whale Bones in the Abyssal South Atlantic. Deep Sea Research Part I: Oceanographic Research Papers 108: 23-31.

Sturdivant SK, Díaz RJ, Llansó R, Dauer DM. 2014. Relationship Between Hypoxia and Macrobenthic Production in Chesapeake Bay. Estuaries and Coasts 37(5): 1219-1232.

Tagliapietra D, Sigovini M. 2010. Benthic Fauna: Collection and Identification of Macrobenthic Invertebrates. Terre et Environment 88: 253-261.

Tuinov AV. 2007. Stable Isotopes of Carbon and Nitrogen in Soil Ecology Studies. Biology Bulletin 34(4): 395-407.

Ulanowicz RE, Tuttle JH. 1992. The Trophic Consequences of Oyster Stock Rehabilitation in Chesapeake Bay. Estuaries 15(3): 298-306.

U.S. Environmental Protection Agency. 2011. State of the Delaware Inland Bays. Accessed on January 17th, 2017 through https://www.epa.gov/sites/production/files/2015-09/documents/2011-state-of-the- bays.pdf.

U.S. Environmental Protection Agency. 2017. State Progress Toward Developing Numeric Nutrient Water Quality Criteria for Nitrogen and Phosphorus. Accessed on February 28th, 2018 through https://www.epa.gov/nutrient-policy-data/state-progress-toward-developing-numeric-nutrient-water-quality-criteria.

U.S. Geological Survey. 2018. Water-Year Summary for Site USGS 01484680. Accessed on April 22nd, 2018, through https://waterdata.usgs.gov/nwis/wys_rpt/?site_no=01484680&agency_cd=USGS.

Virginia Institute of Marine Science. 2011. Polychaete Key for Chesapeake Bay and Coastal Virginia. Gloucester Point, VA. College of William and Mary.

Volk JA, Savidge KB, Scudlark JR, Andres AS, Ullman WJ. 2006. Nitrogen Loads through Baseflow, Stormflow, and Underflow to Rehoboth Bay, Delaware. Journal of Environmental Quality 35(5): 1742-1755.

Walch M, Seldomridge E, McGowan A, Boswell S, Bason C. 2016. 2016 State of the Delaware Inland Bays. The Delaware Center for the Inland Bays, Rehoboth Beach Delaware. Rehoboth Beach, DE.

Yueyun W, Xinzheng L. 2017. A New Species of *Phyllochaetopterus* Grube, 1863 (Polychaeta: Chaetopteridae) from Hainan Island, South China Sea. Chinese Journal of Oceanology and Limnology 35(2): 360-366.

Zimmerman RJ, Minello TJ, Baumer TJ, Castiglione MC. 1989. Oyster Reef as Habitat for Estuarine Macrofauna. NOAA Technical Memorandum, NMFS-Sefc-249, p 16.

FIGURES AND TABLES

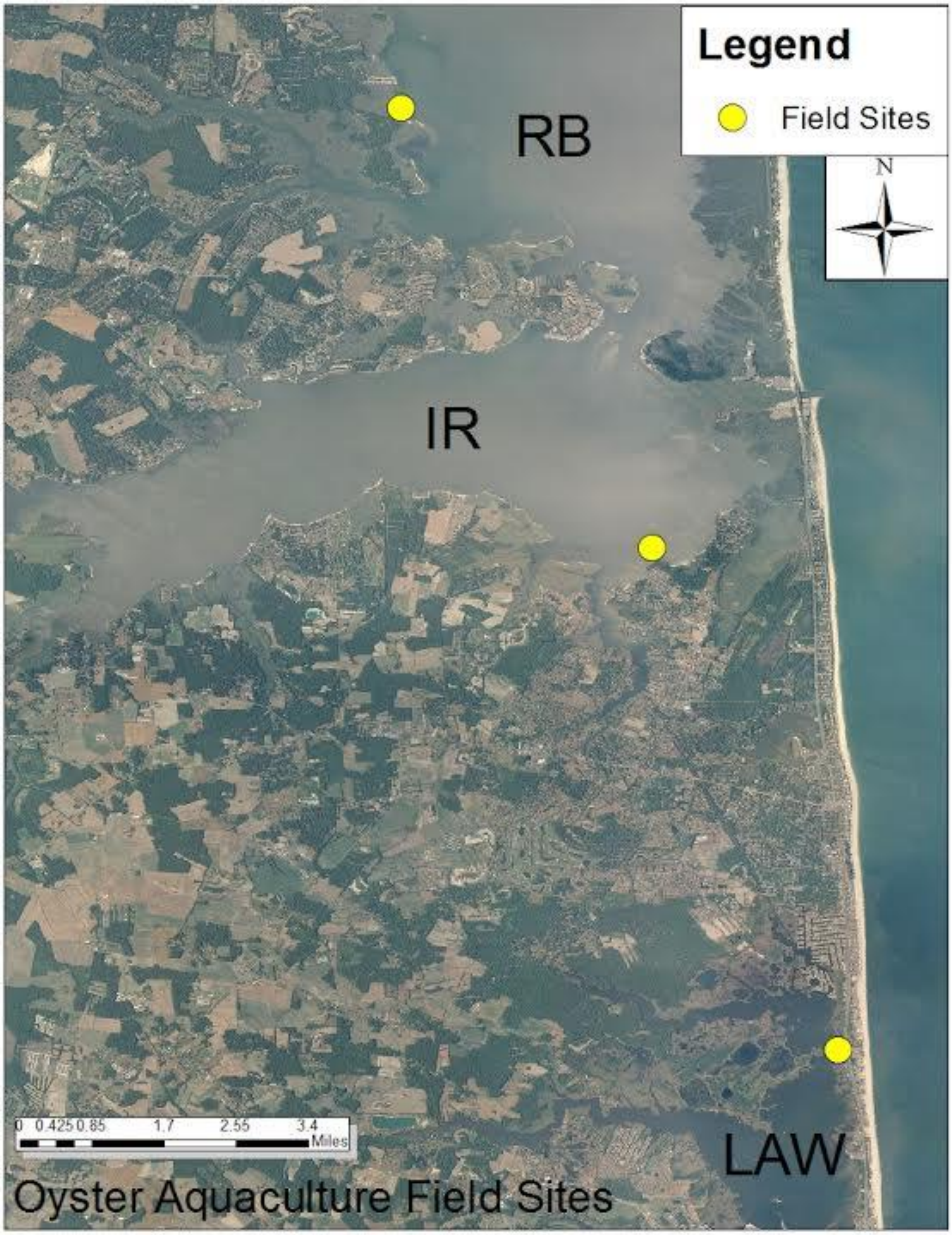


Figure 1. Field sites in this study for oyster aquaculture to be placed in the Delaware Inland Bays.

Figure 2. Oyster aquaculture gear used in this study a) metal aquaculture cages b) plastic aquaculture trays.

Figure 3. Equipment used for water quality monitoring a) Handheld YSI b) Handheld Aquaflour c) WQ770 Turbidimeter.

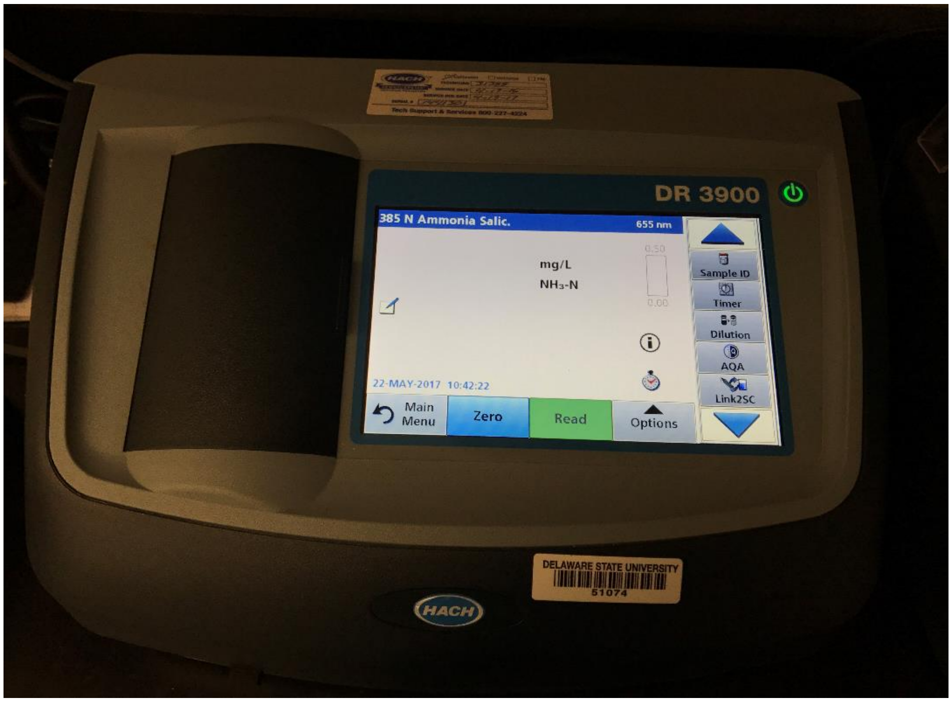


Figure 4a). HACH R3900 Laboratory VIS Spectrophotometer used for nutrient analysis.

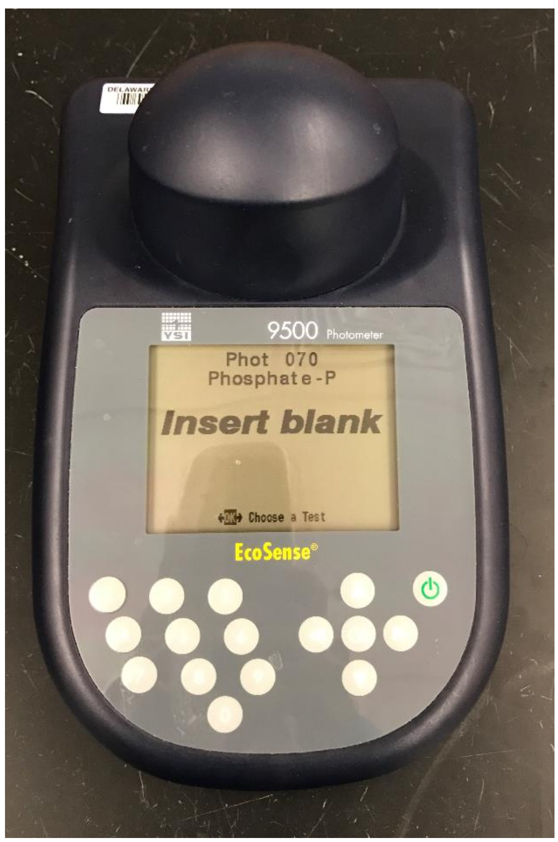


Figure 4b). YSI Photometer 9500 used for nutrient analysis.

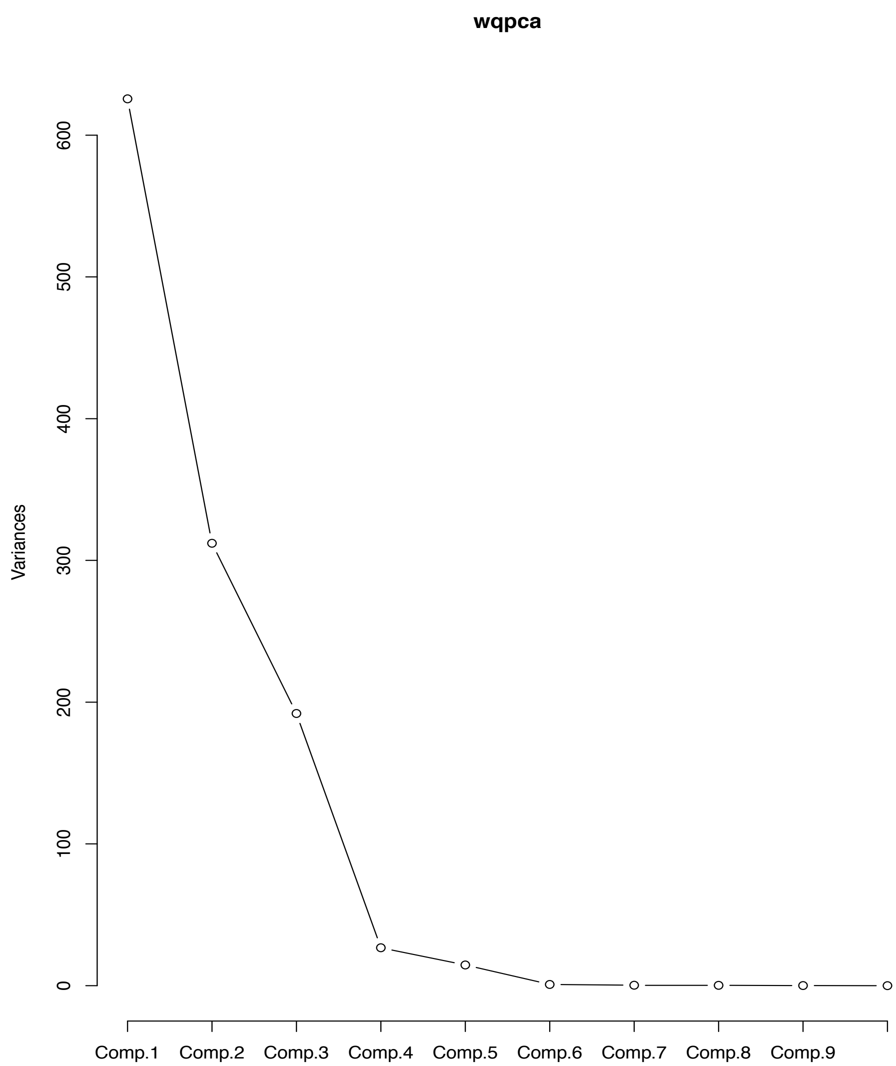


Figure 5. The amount of variances described by each component in a PCA for the water quality and nutrient analysis data for 2016.

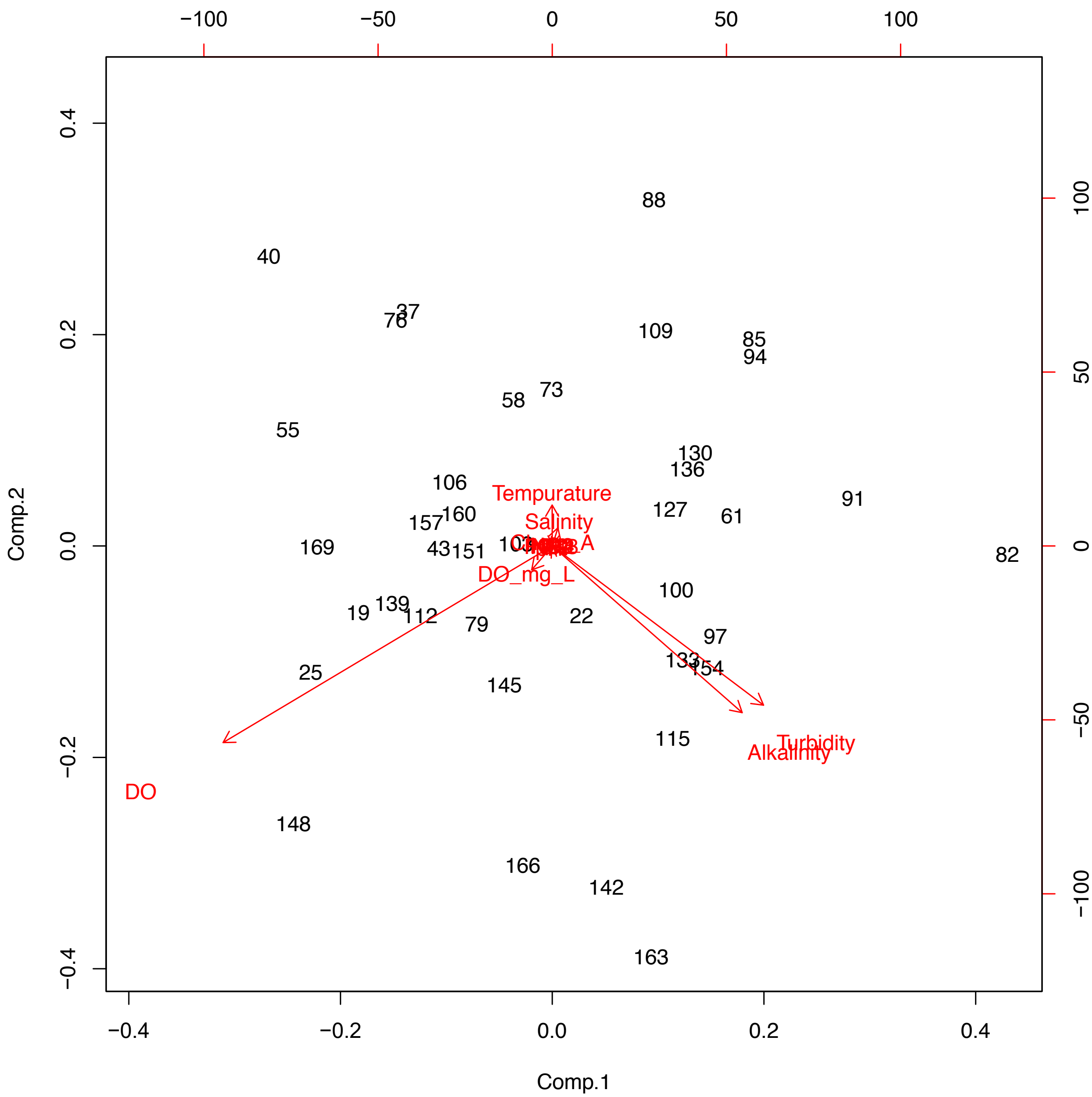


Figure 6. Biplot of components 1 and 2 which resulted from the PCA of the 2016 water quality data. The red lines with arrows indicate the influences of the variables in each component. The black numbers indicate a sample number.

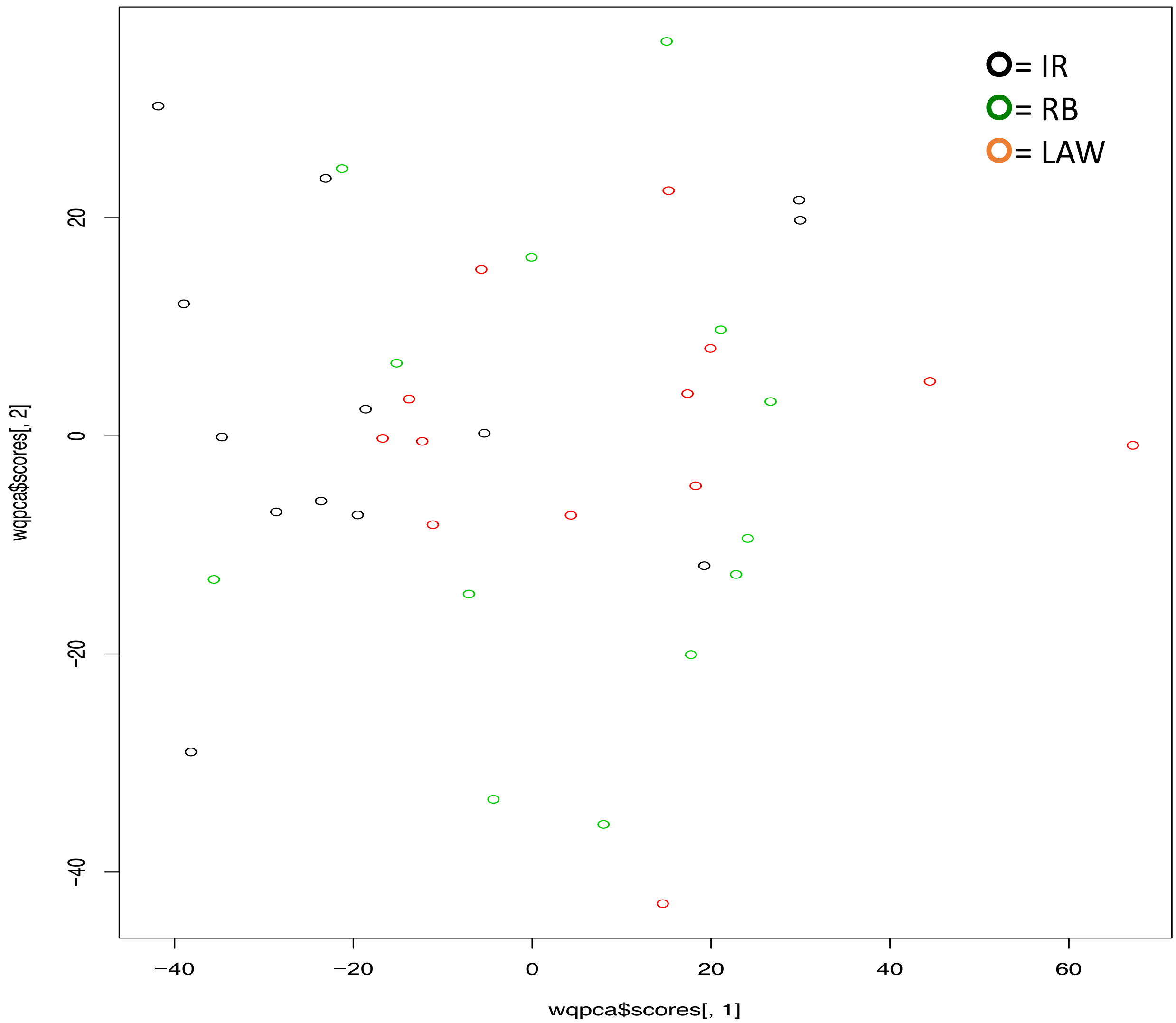


Figure 7. PCA plot for 2016 water quality PCA scores. The color of the circles represents the different sites. Black represents Indian River Bay, green represents Rehoboth Bay, and Red represents Little Assawoman Bay.

Table 1. Minimums and maximums of water quality parameters for Indian River (IR), Little Assawoman (LAW), and Rehoboth Bays (RB) during June through October 2016.

Parameter	Bay	Max	Min
Temperature (^{o}C)	IR	30.68	13.26
	LAW	31.23	12.49
	RB	31.42	21.73
pH	IR	9.08	7.48
	LAW	9.12	7.52
	RB	8.27	7.38
Salinity (ppt)	IR	34	15.87
	LAW	30	14.97
	RB	34	26.74
Ammonia (mg/L)	IR	0.46	0
	LAW	0.6	0
	RB	0.9	0
Nitrite (mg/L)	IR	0.014	0
	LAW	0.032	0.002
	RB	0.14	0.002
Alkalinity (mg/L)	IR	117	25
	LAW	125	52
	RB	122	53
Hardness (mg/L)	IR	263	254
	LAW	350	266
	RB	328	250
Total Nitrogen (mg/L)	IR	1.2	0
	LAW	2.8	0
	RB	2.7	0

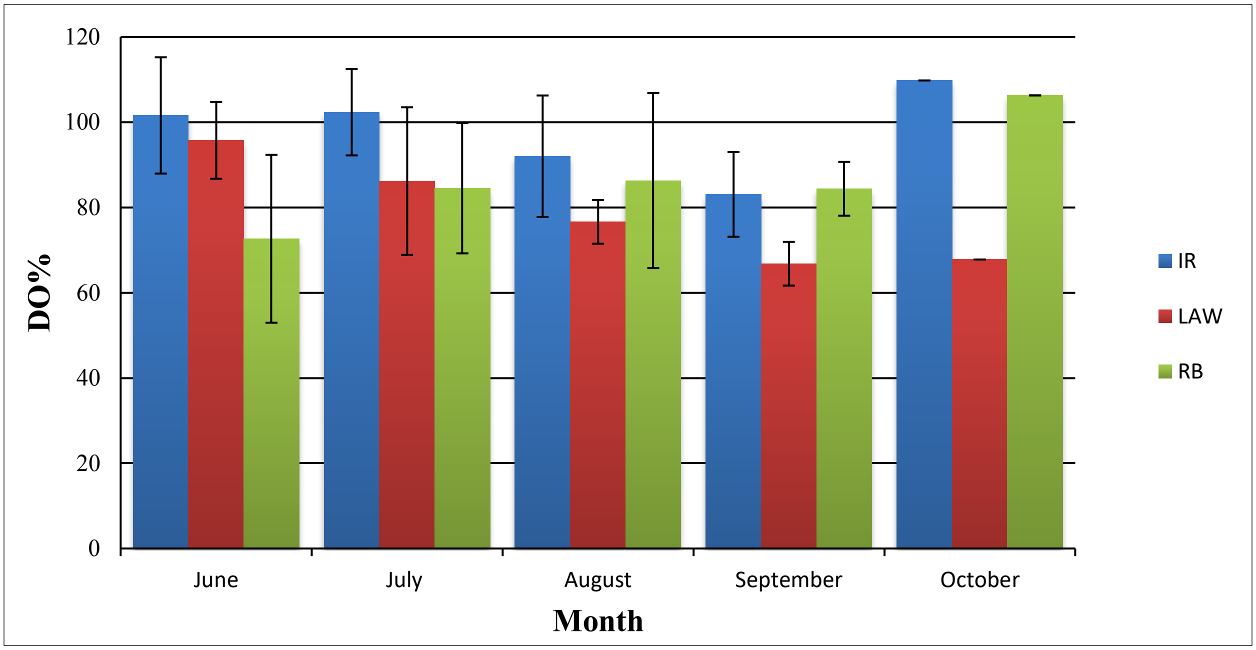


Figure 8. Monthly averages with standard deviation of DO% in Indian River, Little Assawoman, and Rehoboth Bays for June through October 2016.

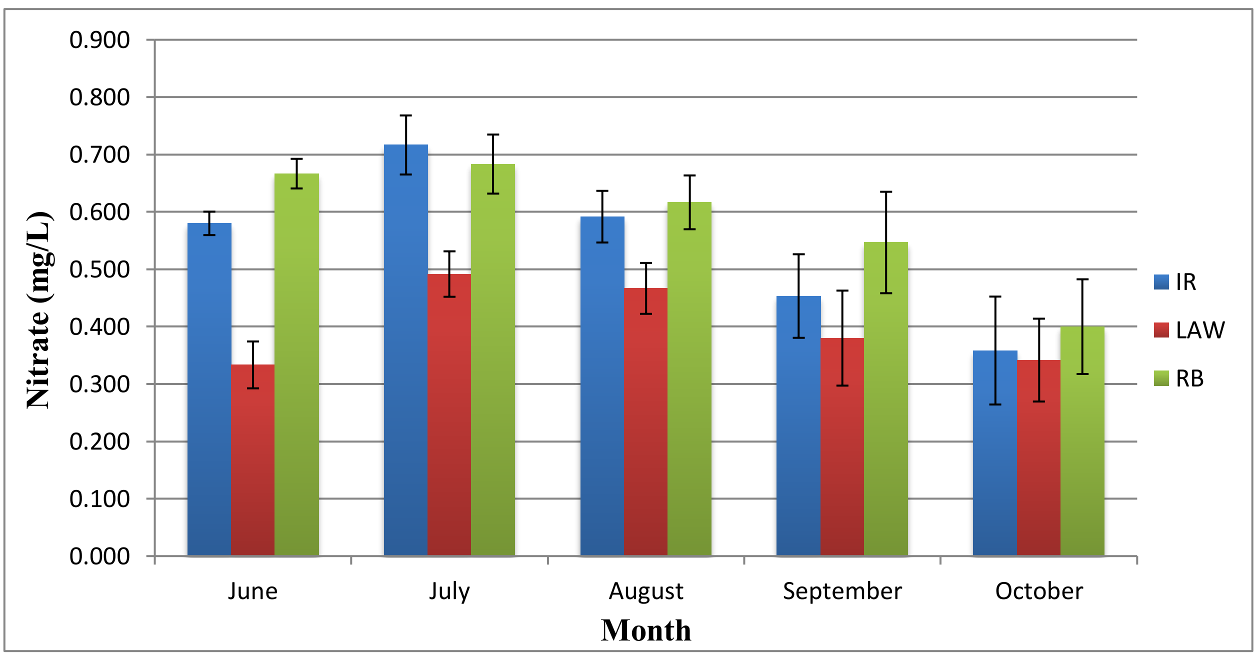


Figure 9. Monthly averages with standard deviation of Nitrate (mg/L) in Indian River, Little Assawoman, and Rehoboth Bays for June through October 2016.

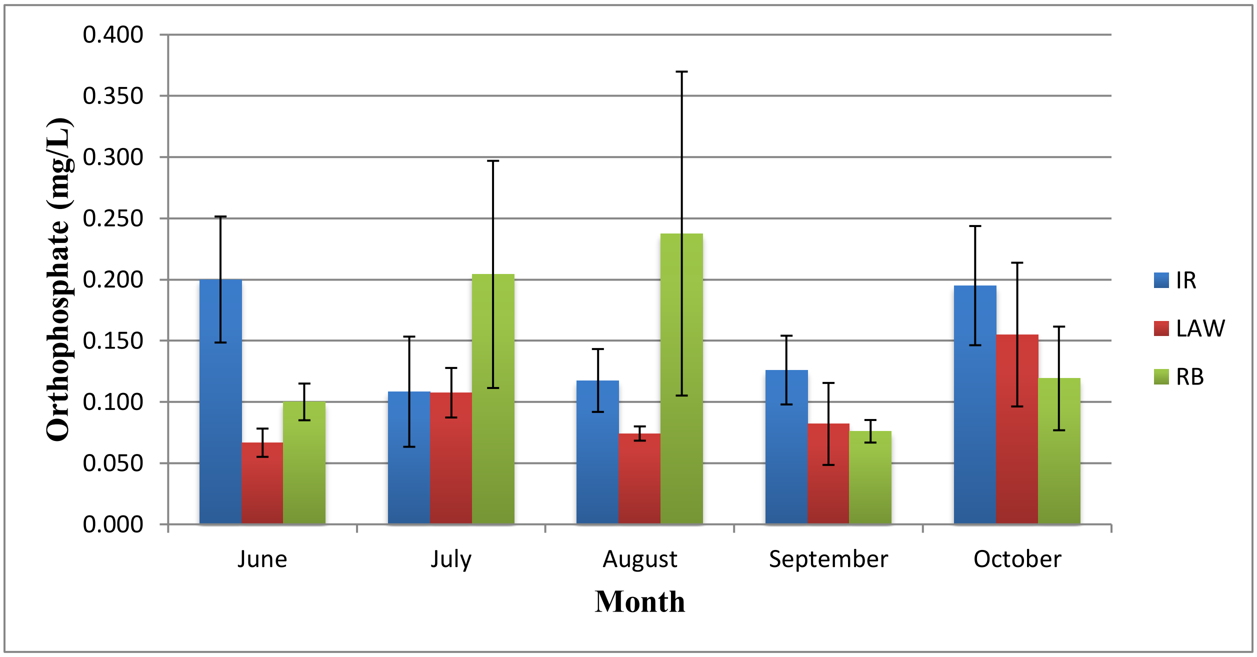


Figure 10. Monthly averages with standard deviation of Orthophosphate (mg/L) in Indian River, Little Assawoman, and Rehoboth Bays for June through October 2016.

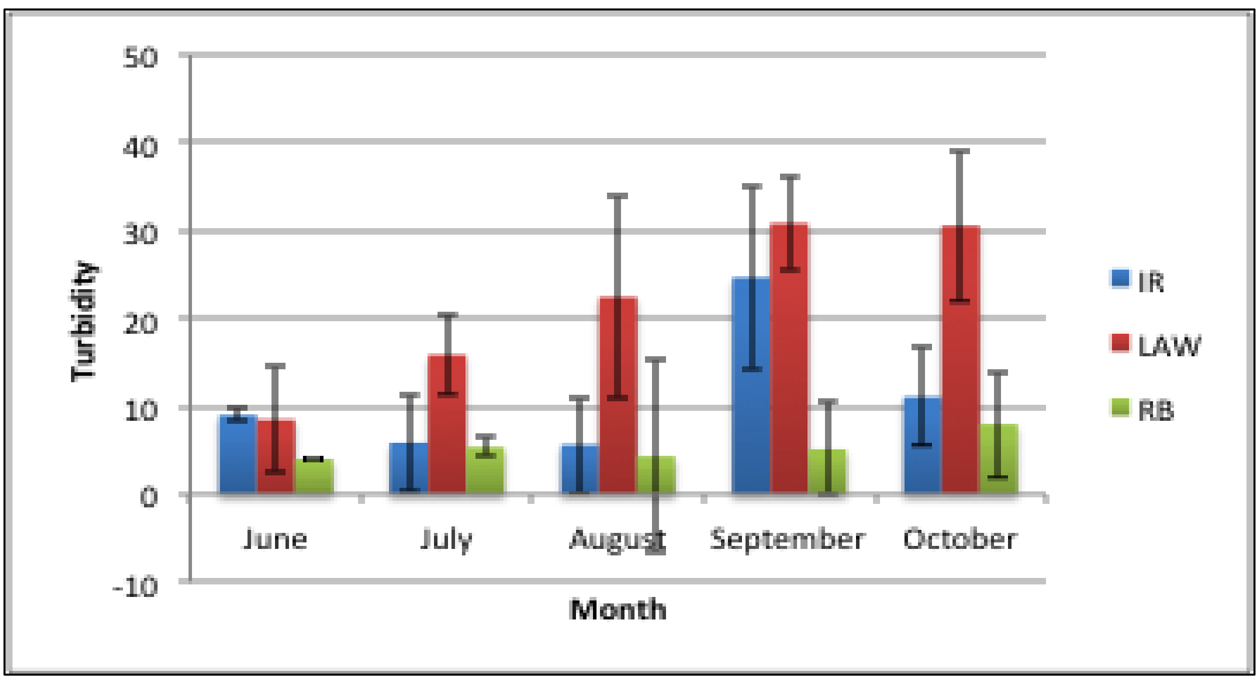


Figure 11. Monthly averages with standard deviation of Turbidity (NTU) in Indian River, Little Assawoman, and Rehoboth Bays for June through October 2016.

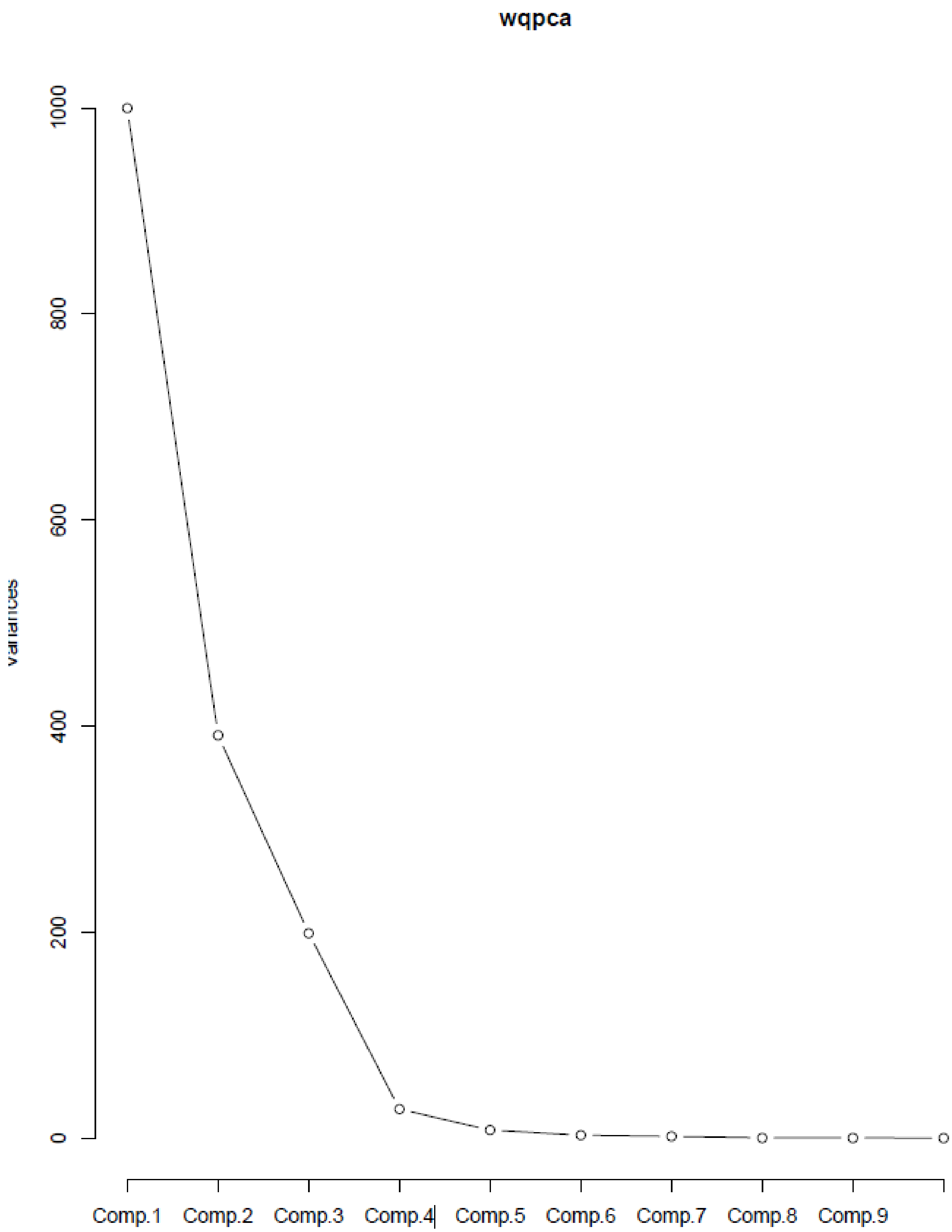


Figure 12. The amount of variances described by each component in a PCA for the water quality and nutrient analysis data for 2017.

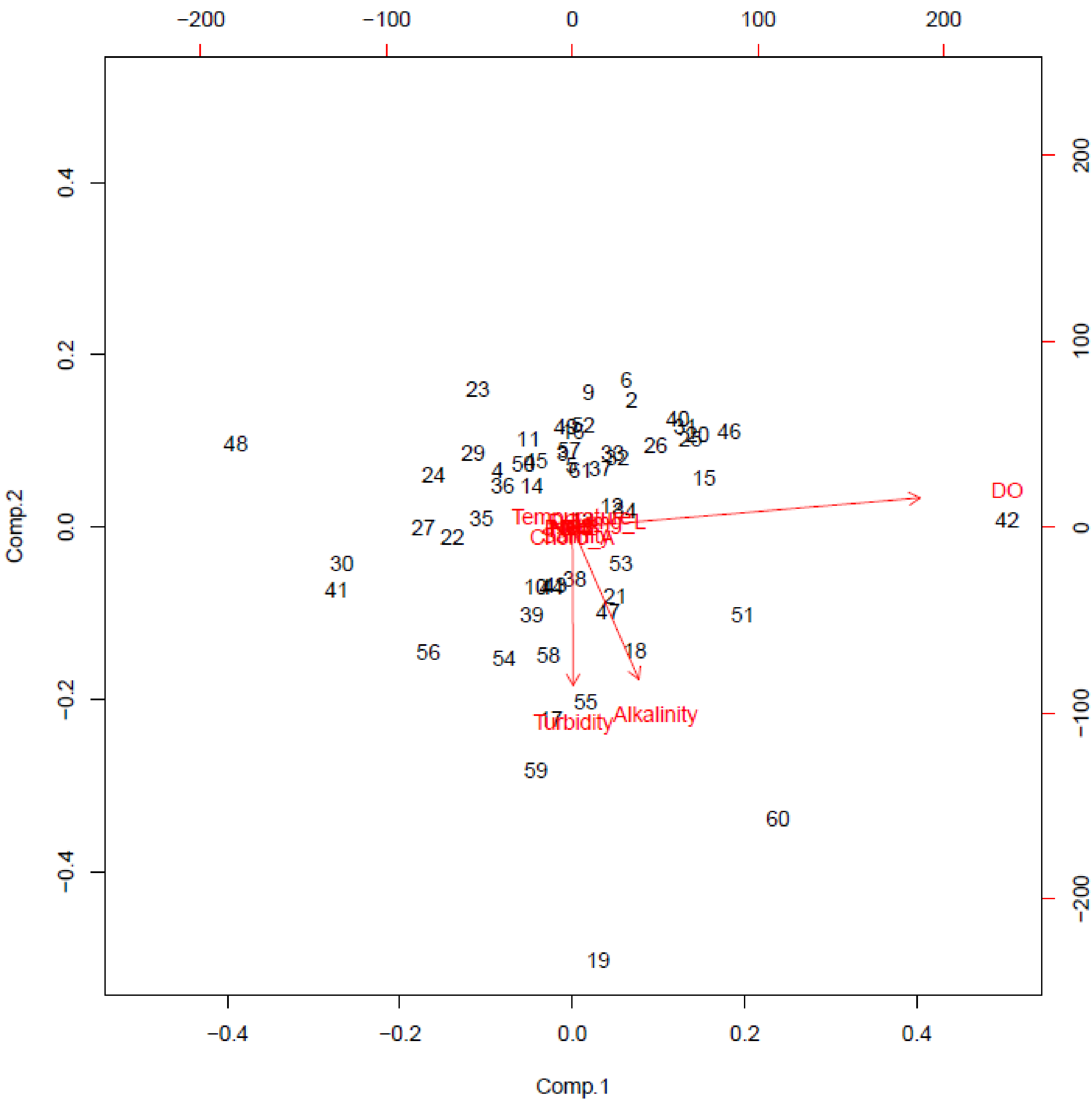


Figure 13. Biplot of components 1 and 2 which resulted from the PCA of the 2017 water quality data. The red lines with arrows indicate the influences of the variables in each component. The black numbers indicate a sample number.

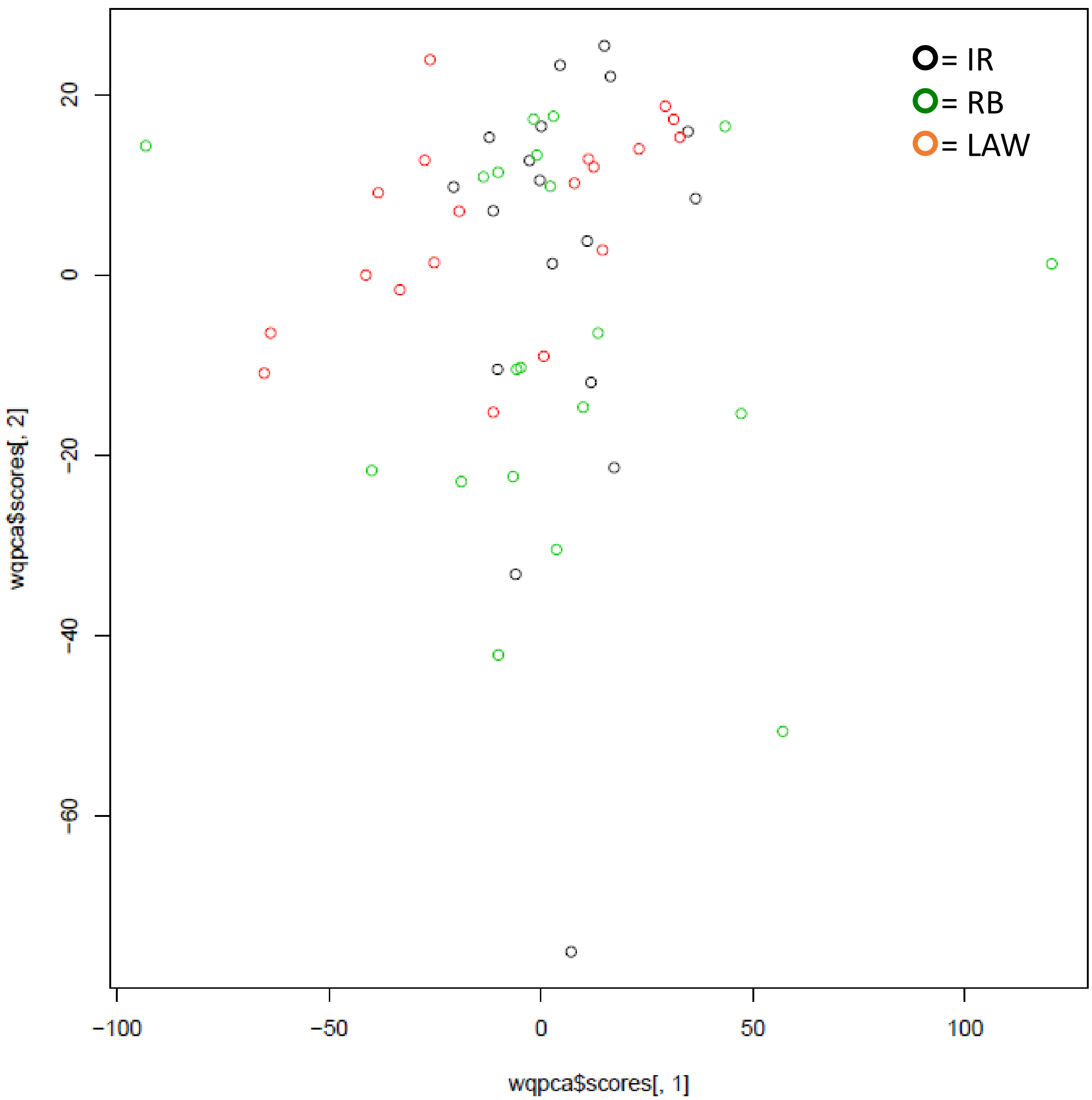


Figure 14. PCA plot for 2017 water quality PCA scores. The color of the circles represents the different sites. Black represents Indian River Bay, green represents Rehoboth Bay, and Red represents Little Assawoman Bay.

Table 2. Minimums and maximums of water quality parameters for Indian River (IR), Little Assawoman (LAW), and Rehoboth Bays (RB) during June through October 2017.

Parameter	Bay	Max	Min
Temperature (^{o}C)	IR	27.21	17.38
	LAW	28.82	19.50
	RB	31.42	21.73
pH	IR	9.02	7.03
	LAW	9.27	6.38
	RB	9.33	7.10
Salinity (ppt)	IR	33.28	23.75
	LAW	28.80	11.93
	RB	29.70	19.16
Ammonia (mg/L)	IR	1.00	0.05
	LAW	0.99	0.02
	RB	0.80	0.04
Nitrite (mg/L)	IR	0.023	0
	LAW	0.014	0
	RB	0.017	0
Alkalinity (mg/L)	IR	150	31
	LAW	99	42
	RB	138	18
Hardness (mg/L)	IR	352	211
	LAW	301	259
	RB	318	245

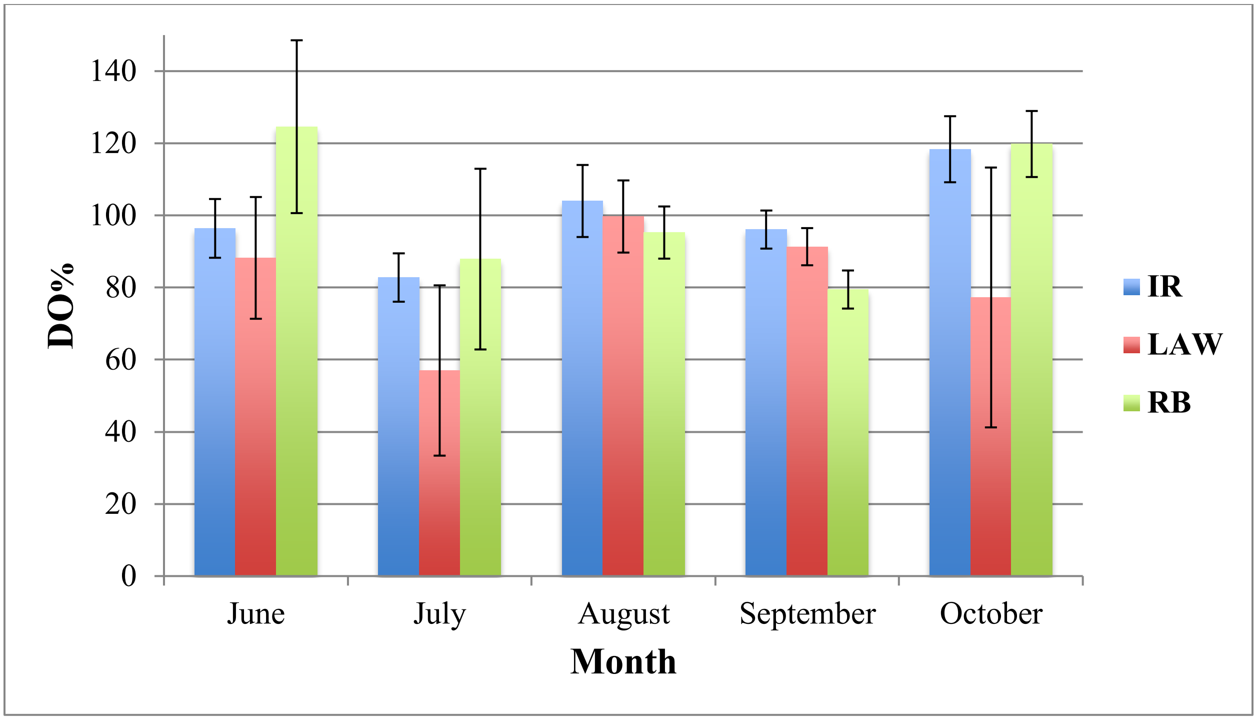


Figure 15. Monthly averages with standard deviation of DO% in Indian River, Little Assawoman, and Rehoboth Bays for June through October 2017.

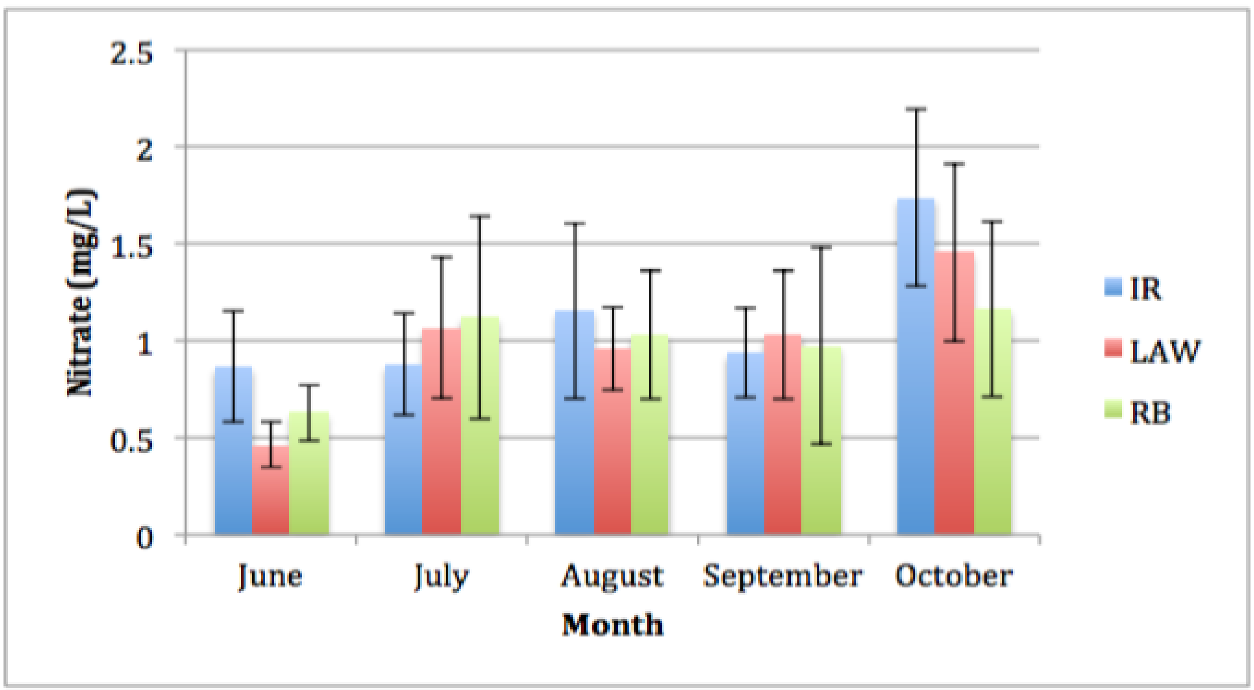


Figure 16. Monthly averages with standard deviation of Nitrate (mg/L) in Indian River, Little Assawoman, and Rehoboth Bays for June through October 2017.

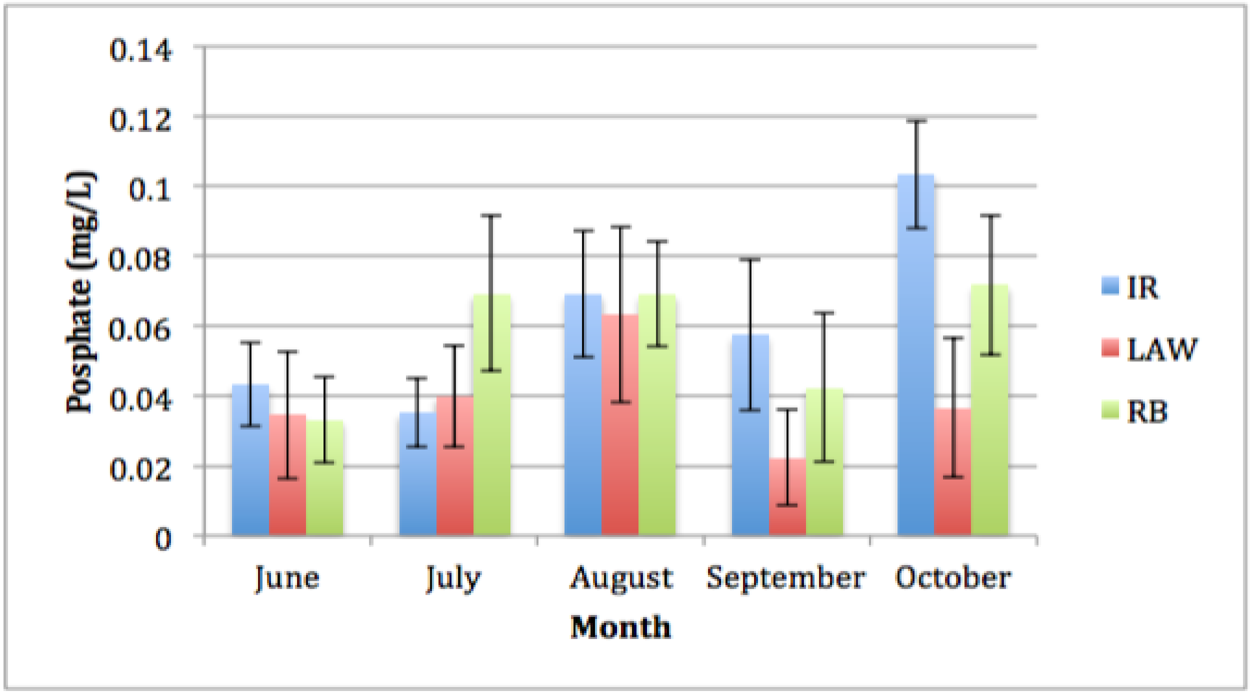


Figure 17. Monthly averages with standard deviation of Phosphate (mg/L) in Indian River, Little Assawoman, and Rehoboth bays for June through October 2017.

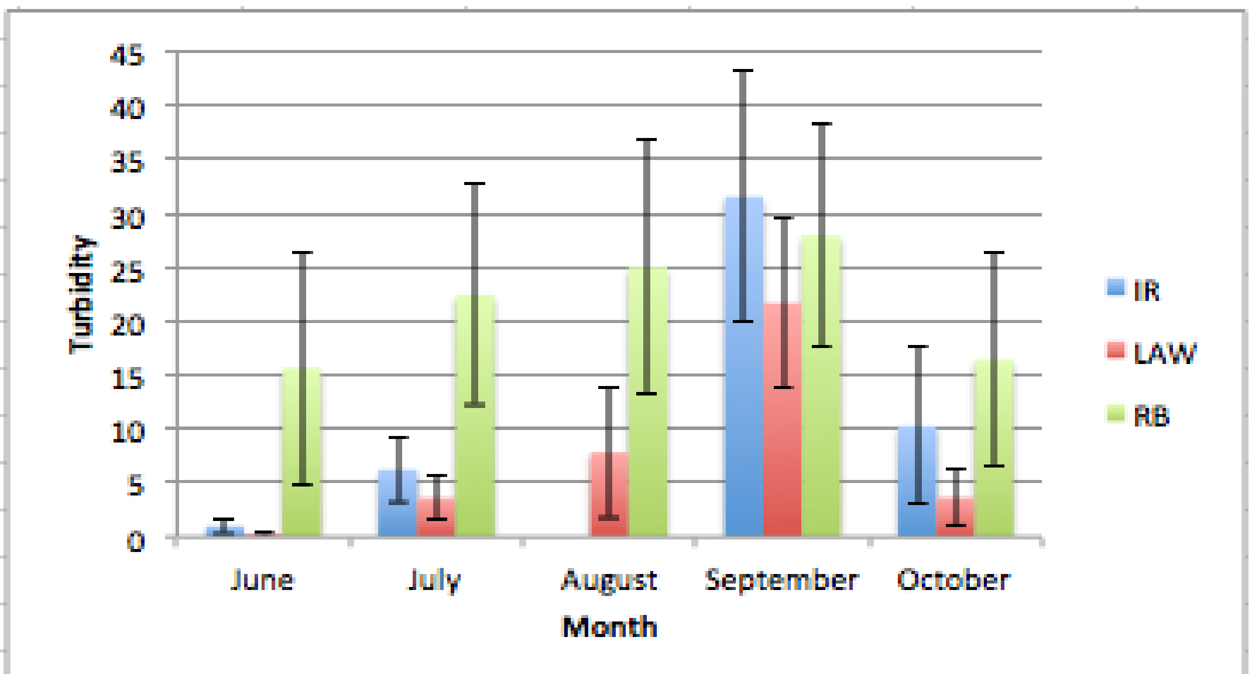


Figure 18. Monthly averages with standard deviation of Turbidity (NTU) in Indian River, Little Assawoman, and Rehoboth bays for June through October 2017.

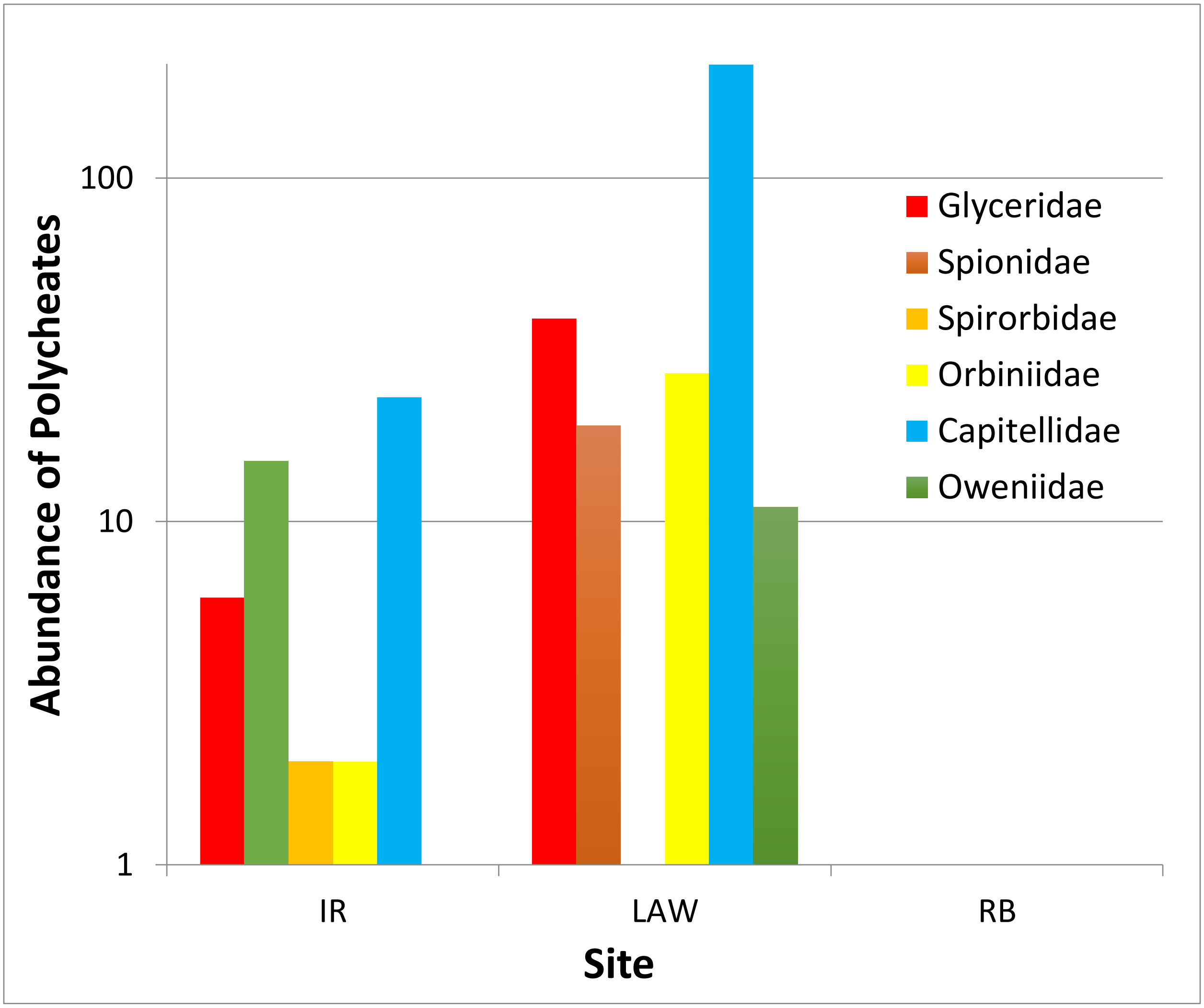


Figure 19. Total abundances of top six families of Polychaetes found during June through October 2016.

Table 3. Results of multivariate general linear model on distance from gear and sites (bays) for number of species and total abundance (N) of 2016 Polychaete data.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	# of species	172.786[a]	8	21.598	9.722	.000
	total N	4364.894[b]	8	545.612	17.737	.000
Intercept	# of species	250.811	1	250.811	112.893	.000
	total N	3332.058	1	3332.058	108.319	.000
Distance	# of species	5.919	2	2.960	1.332	.279
	total N	176.760	2	88.380	2.873	.072
Bay	# of species	155.664	2	77.832	35.033	.000
	total N	3738.788	2	1869.394	60.770	.000
Distance * Bay	# of species	9.277	4	2.319	1.044	.401
	total N	227.159	4	56.790	1.846	.146
Error	# of species	66.650	30	2.222		
	total N	922.850	30	30.762		
Total	# of species	501.000	39			
	total N	8817.000	39			
Corrected Total	# of species	239.436	38			
	total N	5287.744	38			

a. R Squared = .722 (Adjusted R Squared = .647)

b. R Squared = .825 (Adjusted R Squared = .779)

Table 4. Post-Hoc Tukey tests show differences between the sites (bays) for number of species and total abundance (N) of 2016 Polychaete data.

Multiple Comparisons

Dependent Variable		(I) Bay	(J) Bay	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
# of species	Tukey HSD	IR	LA	-2.5385*	.58463	.000	-3.9797	-1.0972
			RB	2.3846*	.58463	.001	.9433	3.8259
		LA	IR	2.5385*	.58463	.000	1.0972	3.9797
			RB	4.9231*	.58463	.000	3.4818	6.3644
		RB	IR	-2.3846*	.58463	.001	-3.8259	-.9433
			LA	-4.9231*	.58463	.000	-6.3644	-3.4818
total N	Tukey HSD	IR	LA	-18.8462*	2.17545	.000	-24.2092	-13.4831
			RB	4.3846	2.17545	.126	-.9784	9.7477
		LA	IR	18.8462*	2.17545	.000	13.4831	24.2092
			RB	23.2308*	2.17545	.000	17.8677	28.5938
		RB	IR	-4.3846	2.17545	.126	-9.7477	.9784
			LA	-23.2308*	2.17545	.000	-28.5938	-17.8677

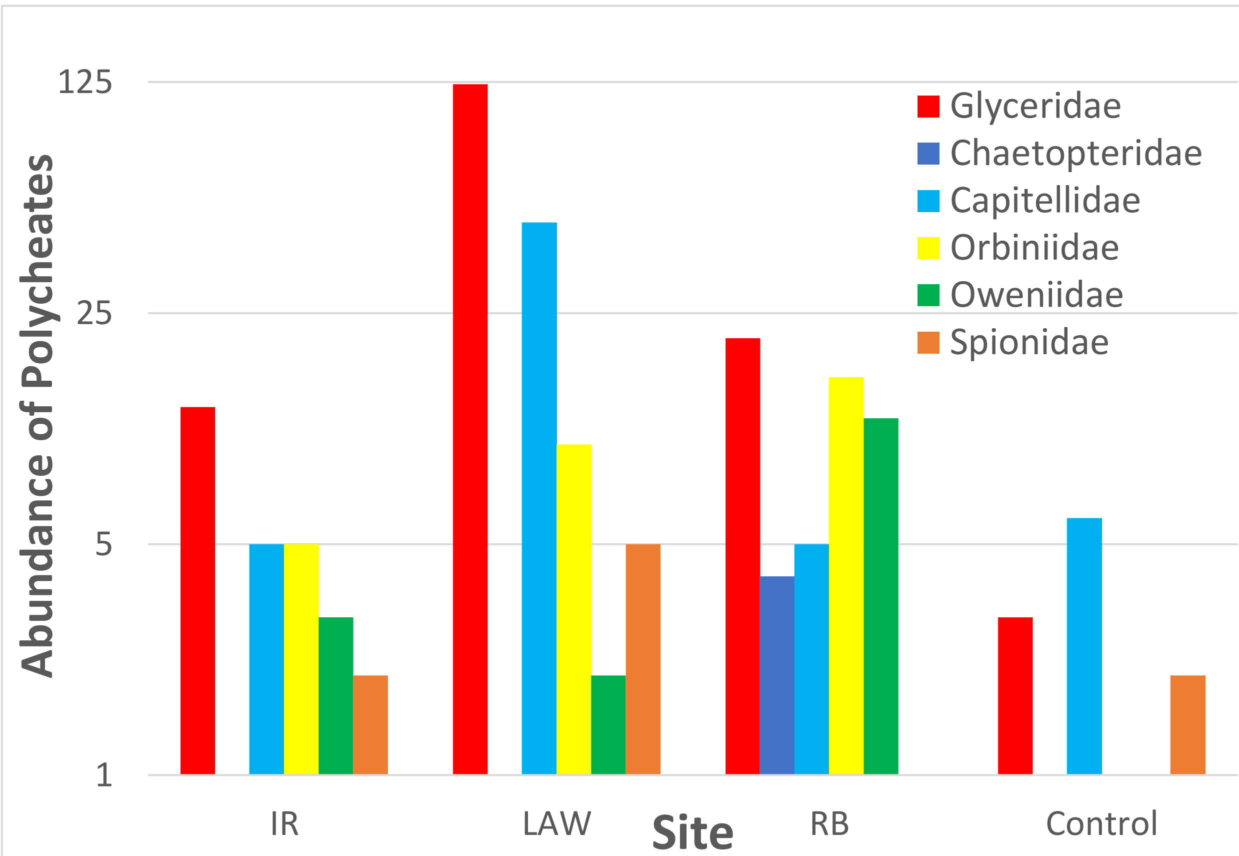


Figure 20. Total abundances of top six families of Polychaetes found during June through October 2017.

Table 5. Results of multivariate general linear model on distance from gear and sites (bays) for number of species and total abundance (N) of 2017 Polychaete data.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	# of species	29.750[a]	11	2.705	2.437	.021
	total N	1643.021[b]	11	149.366	9.792	.000
Intercept	# of species	167.321	1	167.321	150.752	.000
	total N	1736.000	1	1736.000	113.813	.000
Bay	# of species	20.993	3	6.998	6.305	.001
	total N	1512.005	3	504.002	33.042	.000
Distance	# of species	1.435	2	.718	.647	.530
	total N	19.918	2	9.959	.653	.526
Bay * Distance	# of species	7.737	6	1.289	1.162	.347
	total N	94.776	6	15.796	1.036	.418
Error	# of species	41.067	37	1.110		
	total N	564.367	37	15.253		
Total	# of species	255.000	49			
	total N	4194.000	49			
Corrected Total	# of species	70.816	48			
	total N	2207.388	48			

a. R Squared = .420 (Adjusted R Squared = .248)

b. R Squared = .744 (Adjusted R Squared = .668)

Table 6. Post-Hoc Tukey tests show differences between the sites (bays) for number of species and total abundance (N) of 2017 Polychaete data.

Tests of Between-Subjects Effects

Dependent Variable		(I) Bay	(J) Bay	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
# of species	Tukey HSD	Co	IR	-.6923	.44314	.412	-1.8842	.4996
			LA	-1.8462*	.44314	.001	-3.0381	-.6542
			RB	-1.0000	.44314	.127	-2.1919	.1919
		IR	Co	.6923	.44314	.412	-.4996	1.8842
			LA	-1.1538*	.41323	.039	-2.2653	-.0424
			RB	-.3077	.41323	.878	-1.4192	.8038
		LA	Co	1.8462*	.44314	.001	.6542	3.0381
			IR	1.1538*	.41323	.039	.0424	2.2653
			RB	.8462	.41323	.189	-.2653	1.9576
		RB	Co	1.0000	.44314	.127	-.1919	2.1919
			IR	.3077	.41323	.878	-.8038	1.4192
			LA	-.8462	.41323	.189	-1.9576	.2653
total N	Tukey HSD	Co	IR	-1.3692	1.64275	.838	-5.7878	3.0494
			LA	-14.0615*	1.64275	.000	-18.4801	-9.6429
			RB	-3.2923	1.64275	.205	-7.7109	1.1263
		IR	Co	1.3692	1.64275	.838	-3.0494	5.7878
			LA	-12.6923*	1.53187	.000	-16.8127	-8.5719
			RB	-1.9231	1.53187	.596	-6.0434	2.1973
		LA	Co	14.0615*	1.64275	.000	9.6429	18.4801
			IR	12.6923*	1.53187	.000	8.5719	16.8127
			RB	10.7692*	1.53187	.000	6.6489	14.8896
		RB	Co	3.2923	1.64275	.205	-1.1263	7.7109
			IR	1.9231	1.53187	.596	-2.1973	6.0434
			LA	-10.7692*	1.53187	.000	-14.8896	-6.6489

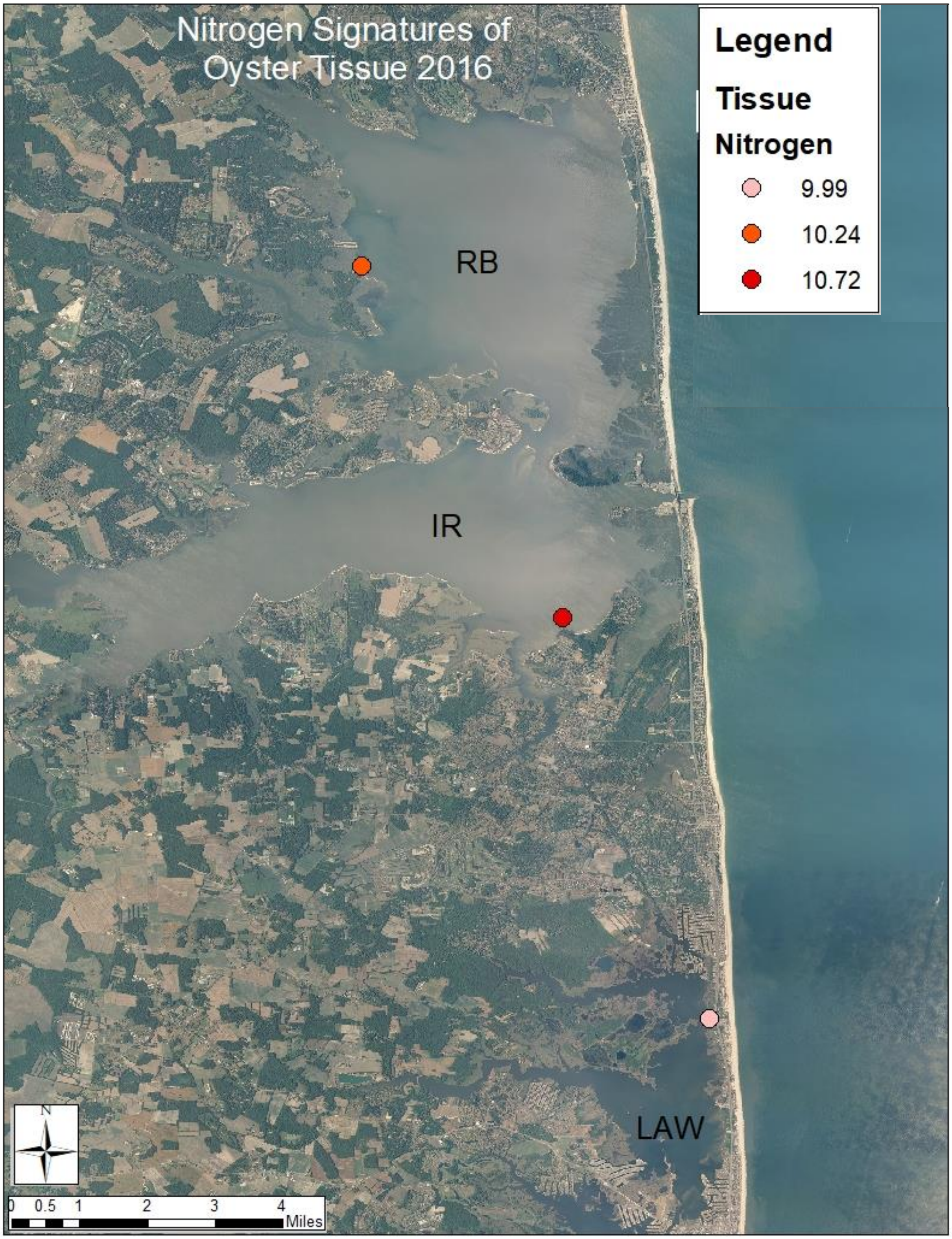


Figure 21a. Map of nitrogen signatures for oyster samples in 2016.

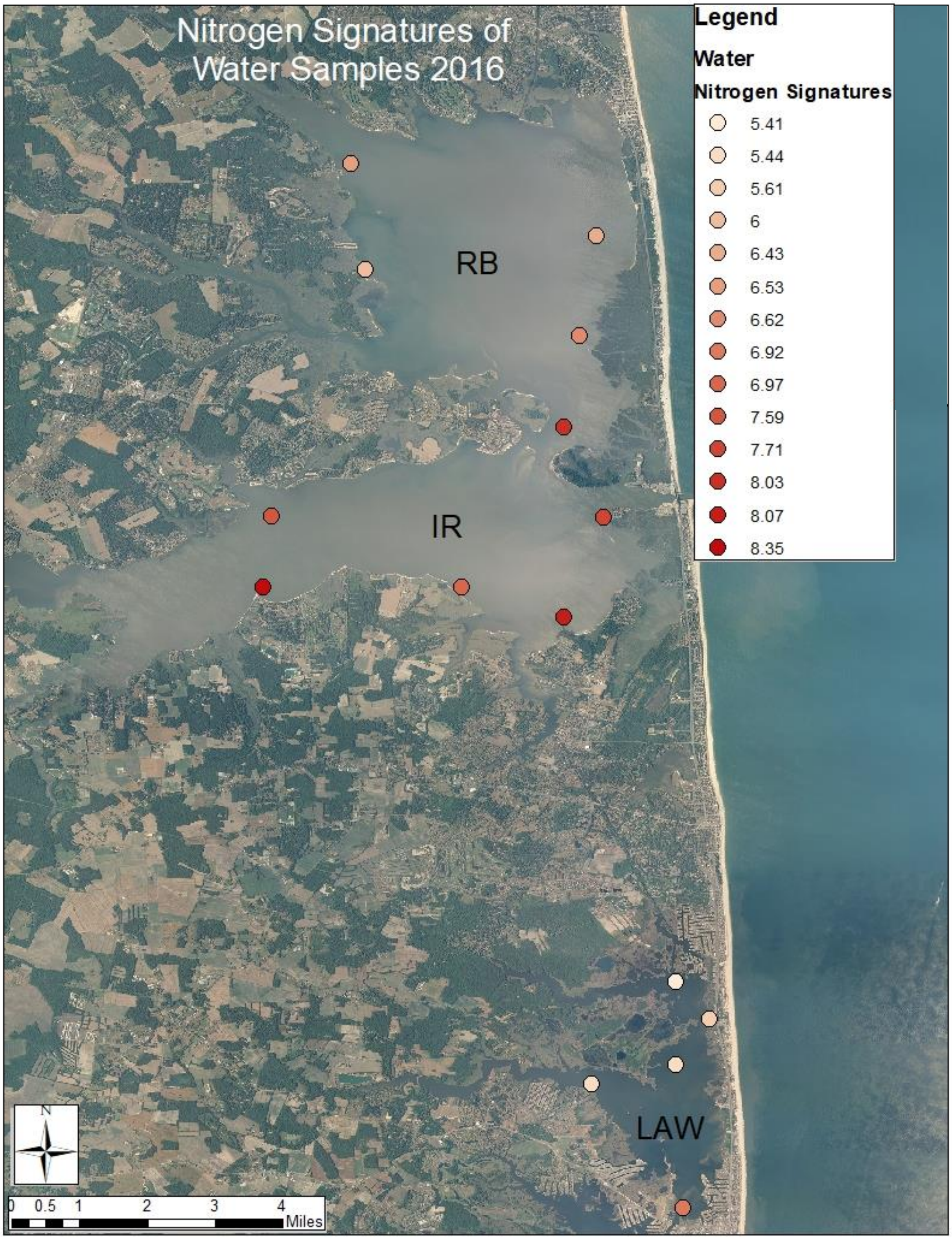


Figure 21b. Map of nitrogen signatures for water samples in 2016.

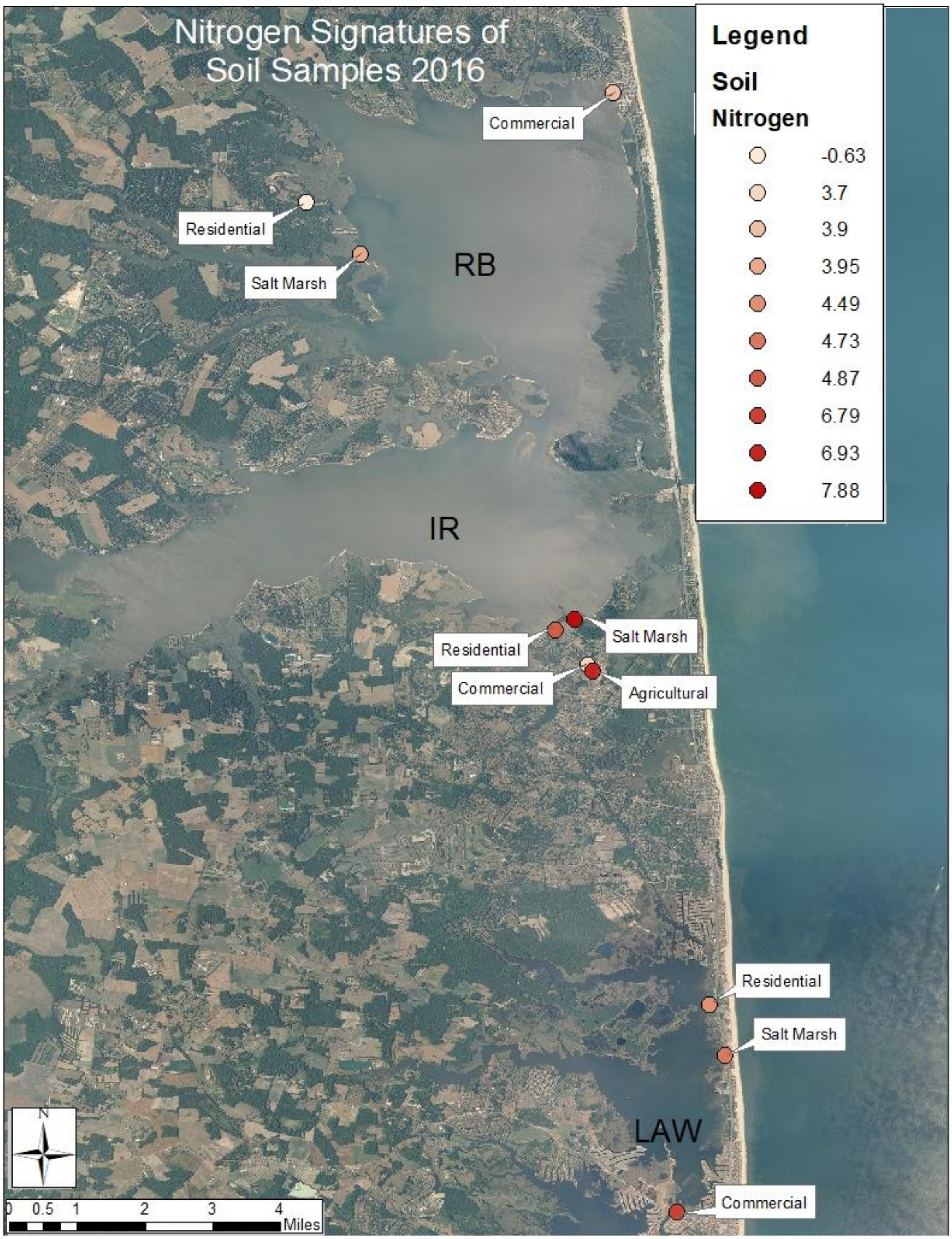


Figure 21c. Map of nitrogen signatures for soil samples in 2016.

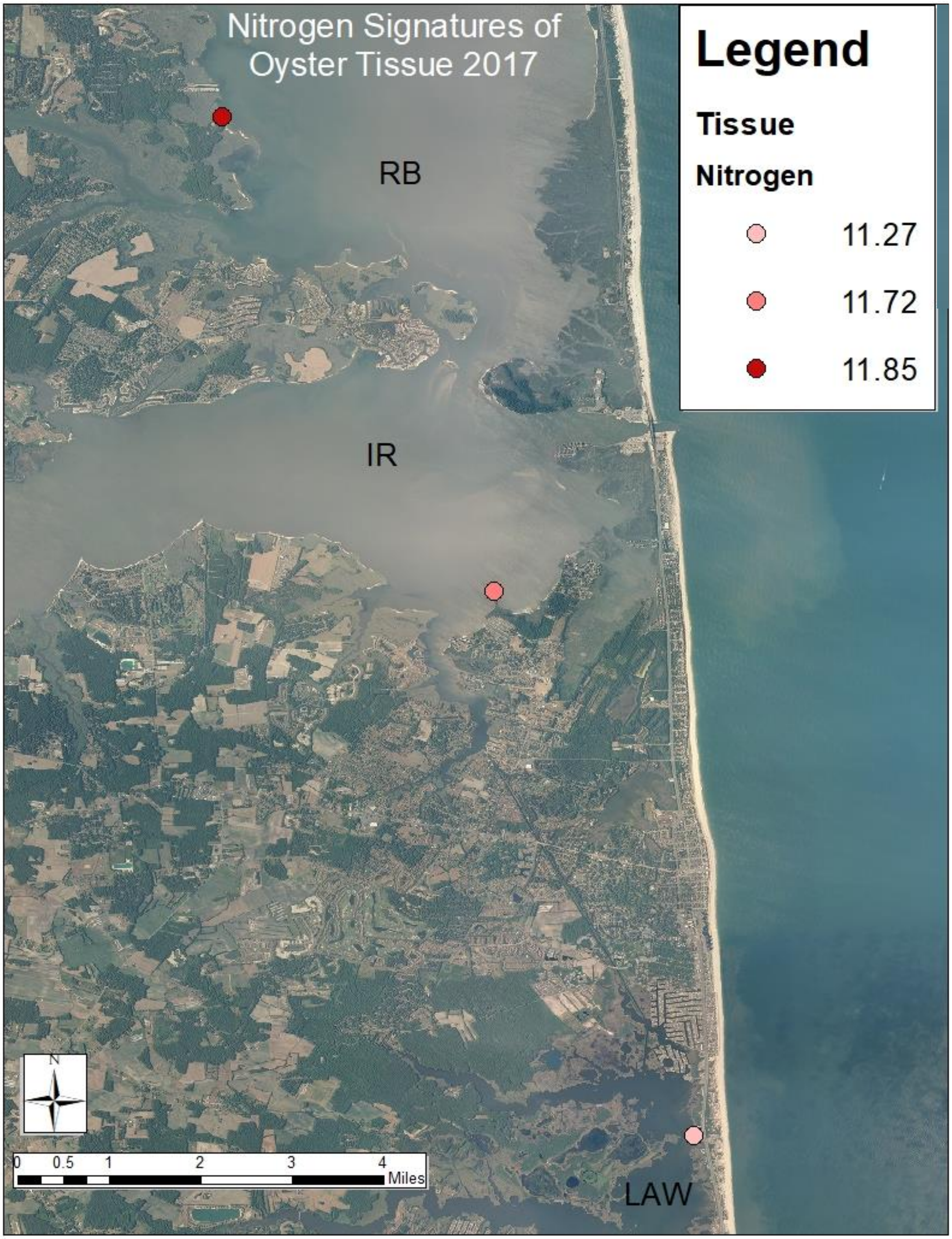


Figure 22a. Map of nitrogen signatures for oyster samples in 2017.

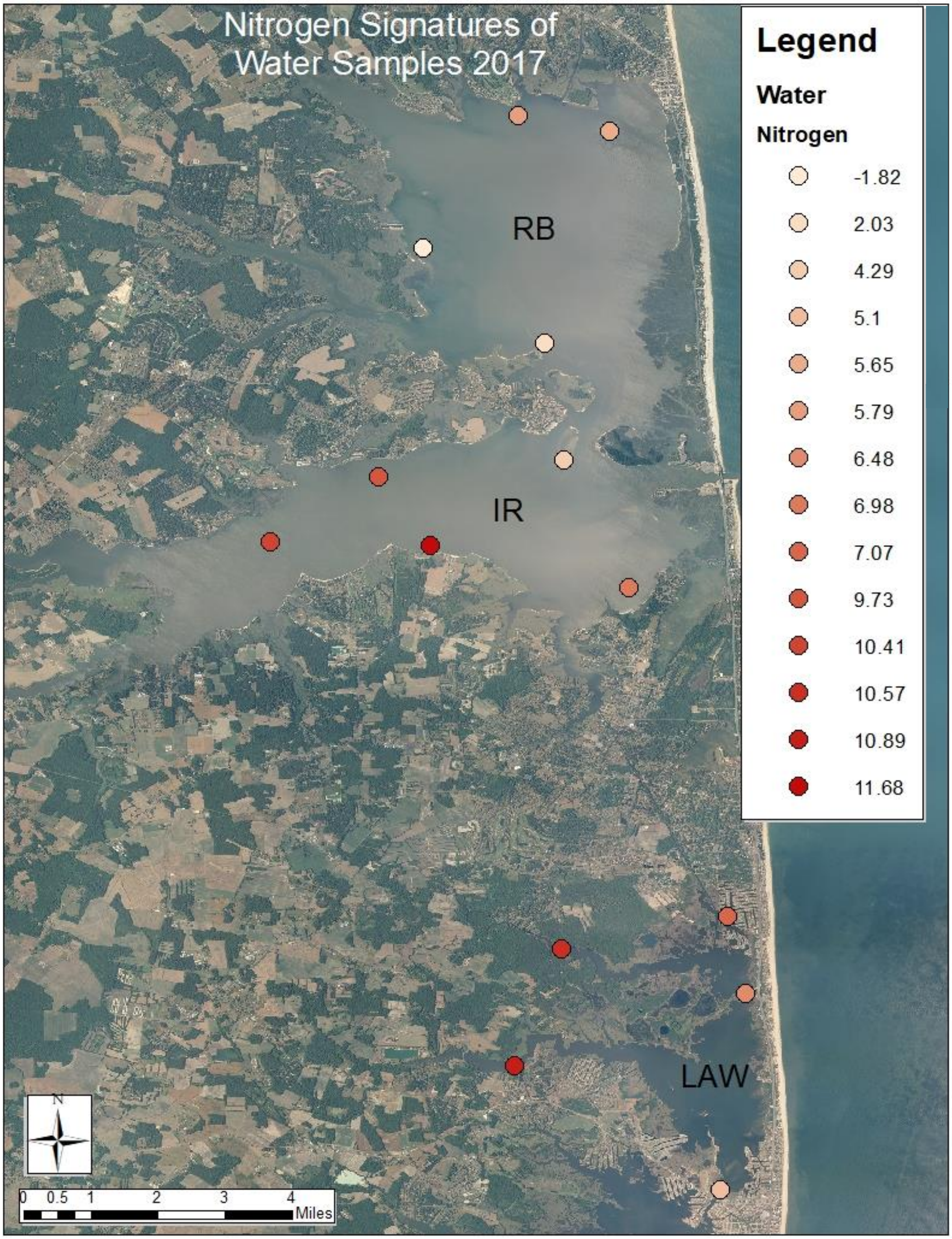


Figure 22b. Map of nitrogen signatures for water samples in 2017.

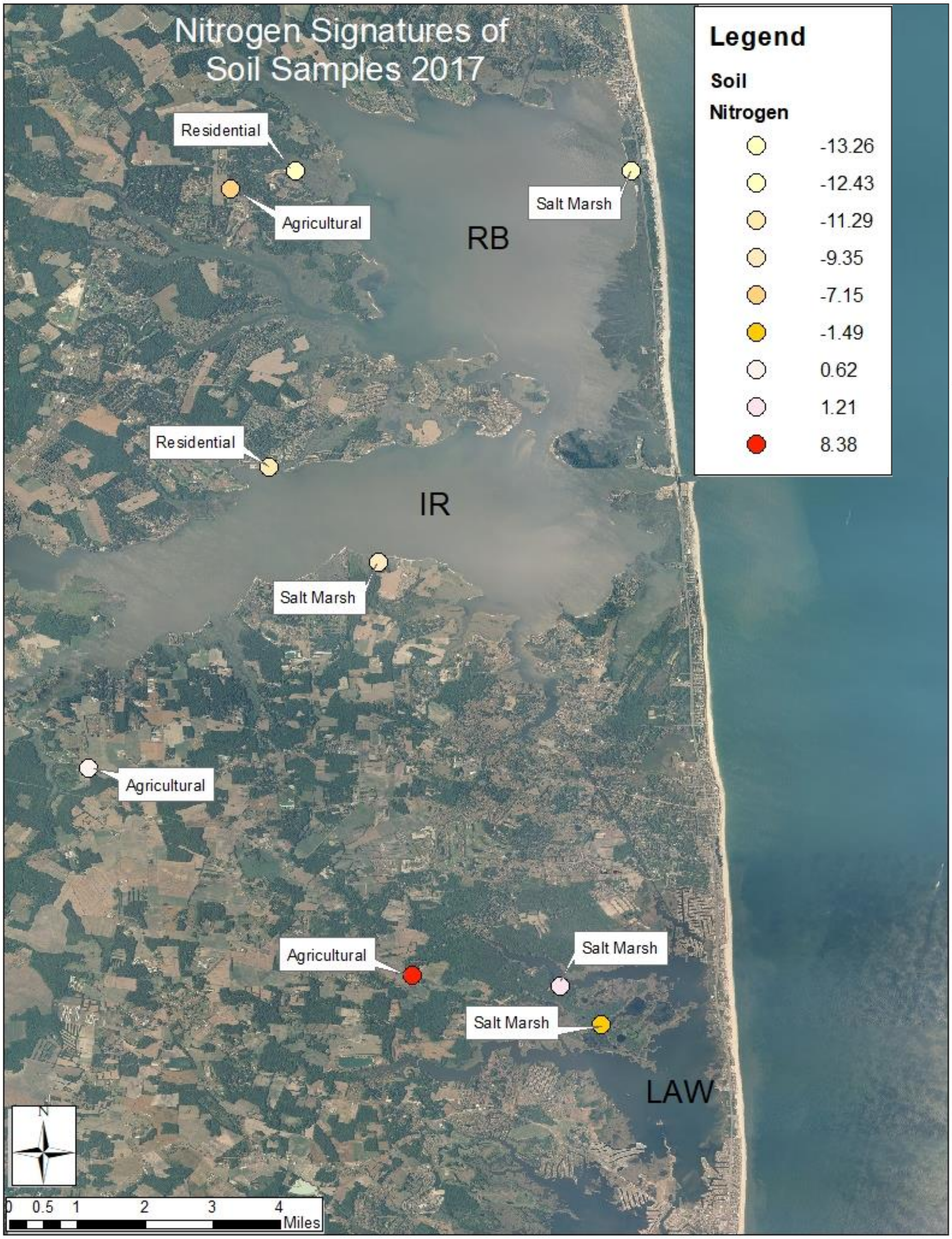


Figure 22c. Map of nitrogen signatures for soil samples in 2017.

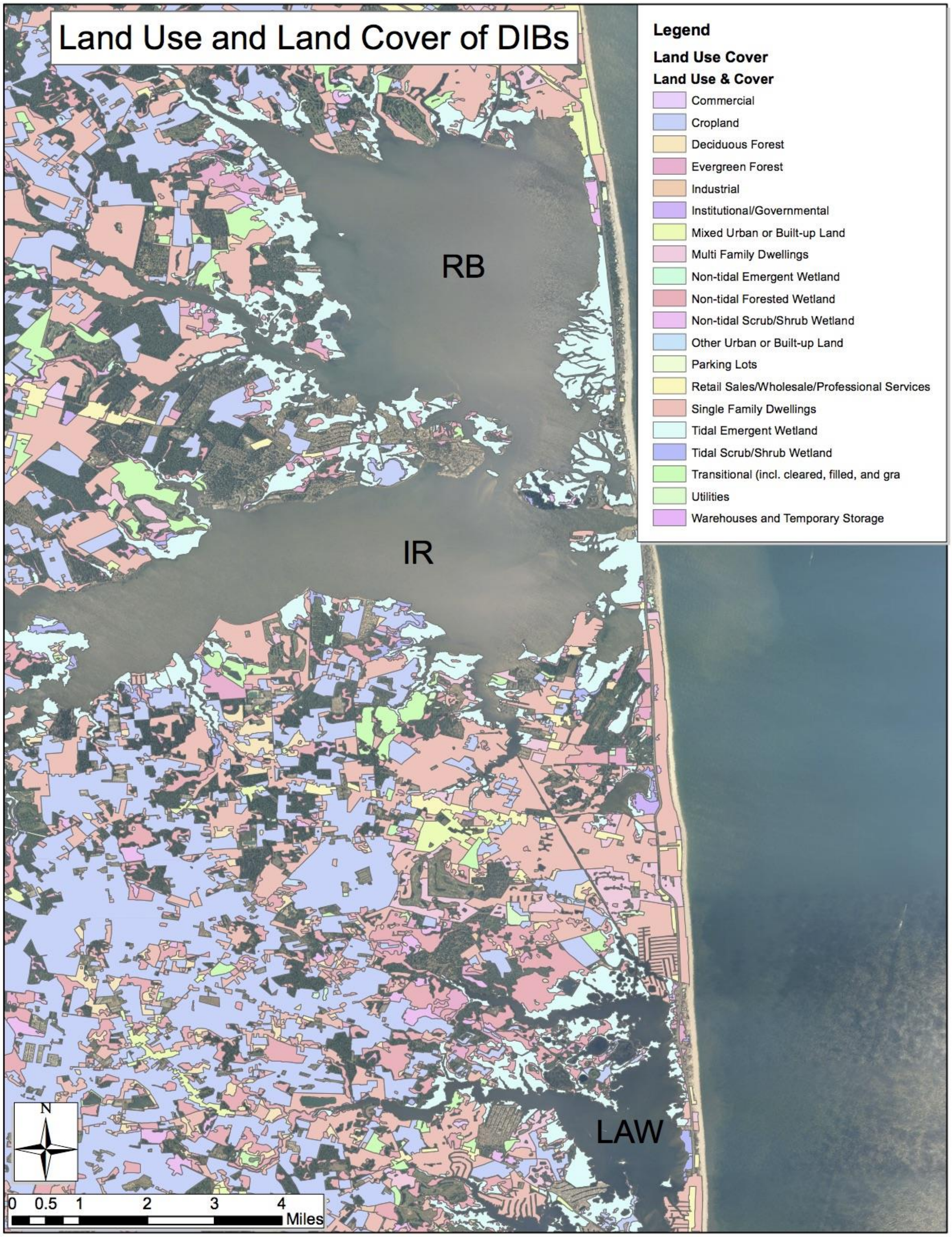
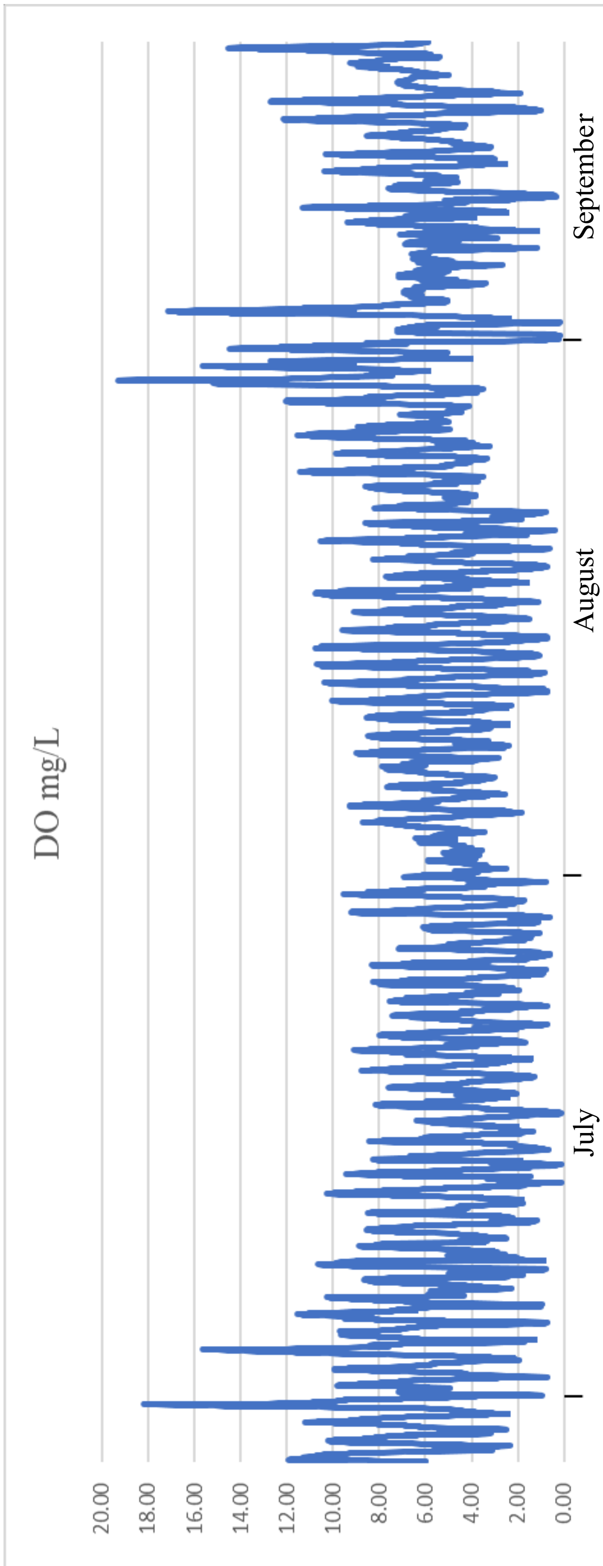


Figure 23. Map of land use and land cover for area surrounding the Delaware Inland Bays.

APPENDIX



Appendix 1. Sonde data for Rehoboth Bay during the 2016 field season showing Diel cycling of Dissolved oxygen mg/L.

DO mg/L