GENETICS AND MONITORING OF THE EASTERN OYSTER *Crassostrea virginica* WITHIN DELAWARE INLAND BAYS

by

SCOTT BORSUM

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This thesis is approved by the following members of the Final Oral Committee:

Dr. Gulnihal Ozbay, Committee Chairperson, Department of Agriculture and Natural Resources, Delaware State University
Dr. Dennis McIntosh, Committee Member, Department of Agriculture and Natural Resources, Delaware State University
Dr. Sigrid Smith, Committee Member, Department of Agriculture and Natural Resources, Delaware State University
Dr. Patrick Gaffney, External Committee Member, College of Earth, Ocean, and the Environment, University of Delaware
Mr. John Ewart, External Committee Member, Sea Grant Aquaculture Extension Agent, University of Delaware

DEDICATION

I would like to dedicate my work to both of my grandpas who past during my time as DSU, Chester Coe Swobe and Leslie Jack Borsum. I would not be able to pursue my dreams without their struggle, sacrifices, and support.

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Scott Borsum

Faculty Advisor: Dr. Gulnihal Ozbay

ABSTRACT

Oyster enhancement to recover ecological benefits has been occurring since the late 1990s within the Delaware Inland Bays (DIBs) using a disease resistant line. Locally recruited oysters have been observed along hardened shorelines but limited quantitative information exists on the status of past or present DIBs sub-population. The goals of this study were to 1) assess the current genetic diversity of local DIBs oysters, 2) identify possible larval sources via genetic profiles, and 3) establish baseline measurements of oyster density along the selected rip-rap locations. Genetic diversity was assessed using eleven microsatellites markers to determine allele frequencies between two groups of spat collected from within the DIBs. Genetic profiles from the DIB groups were compared with a hatchery bred oysters used in local restoration and local wild Delaware Bay oysters, to determine possible source populations. Genetic results show similar allele frequencies among the two DIB groups which are more similar to local wild oysters than hatchery strains. Surveys among intertidal rip-rap habitats documented the current density and size frequency of oysters to evaluate future demographic changes. Oyster densities were generally low at the sites monitored in my study but the highest densities were observed within mid Indian River Bay. Monitoring oyster genetics and density needs to continue and expand throughout the DIBs to better understand local population dynamics and enhancement effects, as restoration continues and commercial scale shellfish aquaculture develops in the region.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1. Background

Eastern oysters (*Crassostrea virginica*) historically numbered in the trillions along the vast coastal estuarine habitats of the eastern United States (McGreavy 2016). Signs of local overharvest began as early as the 1700s, and by the mid-19th century, the Northeast oyster fisheries were exploited (Kirby 2004). Disease outbreak and habitat degradation exacerbated this decline; today, only 1% of historic Northeast regional populations remain (Beck et al. 2011). Therefore, oysters are now ecologically extinct across much of their range altering the structure and function of estuarine ecosystems. Relatively numerous studies have quantified services from healthy oyster reefs and aquaculture, providing a strong foundation for estimating the economic and ecological value (Luckenbach 2005, Coen et al. 2007, Zu Ermgassen et al. 2013). Current oyster restoration projects aim to utilize functional benefits to support persistence of healthy local environments and communities.

Early efforts to mitigate the decline of oyster harvest were implemented in the early 1700s when permits, seasons, and size limits were established (MacKenzie 1996). Oyster shell removed during harvest was replaced sub-tidally to provide substrate for oyster larvae recruitment beginning in the 1800s and is still widely practiced. Modern oyster population management can be generally categorized into three strategies: protect existing populations, enhance habitat structure, and artificially enhance stock. Often, these three are used in combination to re-establish complex metapopulations connectivity which plays an important role in the persistence of this sedentary marine bivalve (Lipcius et al. 2008; Schulte et al. 2009; Munroe et al. 2012). Multiple publications have addressed important components to consider

from lessons learned in past oyster enhancement projects, as long-term persistence is difficult to achieved (Coen et al 2007; Kennedy et al. 2011; Bagget et al. 2015).

NOAA reviewed the status of the eastern oyster in 2007 and identified two key information needs. The first one called for more fisheries independent monitoring, due to the wide range of biases and limitations associated with harvest-based population metrics (Pikitch et al. 2004). Alternative monitoring strategies combine with existing may allow more robust interpretations of population dynamics. Secondly, the report encouraged more genetic analysis of local or regional population structure. Populations genetics examines variation in allele frequencies to better understand inbreeding effects and the genetic structure of sub-populations (Hartl 2000). The genetics field is advancing quickly making applications more accessible and many techniques exist. Microsatellites are often used to determine genetic relationships of subpopulations in ecological applications (Selkoe & Toone 2006). However, collaboration and advanced understanding of the environment, species, and select loci are required for accurate interpretation. These two key information needs guided the questions addressed in this study at the local scale within the three coastal lagoons of Delaware.

A variety of oyster enhancement projects to improve estuarine health have been occurring within the Delaware Inland Bays (DIBs) since the late 1990s but little assessment on their impacts exist. Ongoing recovery efforts use disease resistant hatchery lines to enhance the local population. Hatchery lines have a unique genetic signature due to artificial selection and small breeding population (Carlsson 2006). Previous research has found hatchery signatures among natural recruits, suggesting they are capable of impacting the local gene pool (Milbury et al 2004; Hare et al. 2006; Varney et al 2018). This study was designed to determine if any genetic

impacts of hatchery lines could be detected in a sample of natural recruits and form baseline genetic diversity and site-specific densities.

1.2. Statement of Project Objectives

The primary objective of this study was to assess the genetic diversity of spat recruits within the DIBs. Allele frequencies at eleven microsatellite markers were used to examine genetic relationships between two groups of DIBs spat. Two possible source populations in the wild Delaware Bay adults and the selectively bred hatchery adults used in local restoration were included to identify possible larval sources. It is expected to find genetic difference between the two DIBs grouping because they are influenced from different tidal inlets. All spat collected in the DIBs are expected to more closely resemble the allele frequencies of the NEH line, due to recruits originating from hatchery line gametes.

The second objective of this study was to quantify the number of oysters at select locations and establish baseline metrics for long-term monitoring. Transect surveys were conducted along sections of the artificially hardened intertidal shoreline to determine site-based oyster densities and size frequencies. Future studies can use the same methods to determine how sites are changing over time.

1.3. Limitations

The spat examined were collected during a 10-week period in the summer of 2016 which limits the scope of the genetic findings. This study does not aim to understand the drivers of oyster recruitment but simply to better understand the current genetic diversity and establish long-term monitoring protocols. Density measurements only occurred at select sites during 2017 and are not representative of the entire population. Expanded and continued monitoring of DIBs

oysters is encouraged to provide more information to understand the full impacts of restoration efforts and document status prior to commercial aquaculture.

CHAPTER 2

LITERATURE REVIEW

2.1. Species of interest

Eastern oysters (*Crassostrea virginica*) are filter-feeding, bivalves which engineer reefs that alter ecosystem dynamics and are historically important natural resource product in the United States. They occur in coastal and estuarine waters off the Western Atlantic, ranging from Newfoundland to the Caribbean (Carriker and Gaffney 1996). Oysters' capacity to adapt to a wide range of temperatures (4-36°C) and salinities (5-42ppt) has allowed for vast proliferation of the species, but they thrive in waters with temperatures of 20-30°C and lower salinities (10-28ppt) where pathogens are less virulent (NOAA 2007). Oysters, like many sessile marine organisms, are broadcast spawners with a bipartite life cycle, spending the early stages drifting in the water column. As with many marine invertebrates, oyster reproduction varies over time and location, but they are generally highly fecund with low survival. They are sequential hermaphrodites but sex determination is complex, with evidence for environmental, temporal, genetic, and spatial factors (Thompson et al. 1996).

Seasonal changes serve as triggers for the release of gametes in temperate locations (Barber et al. 1991; Davis and Chanley 1956). Non-feeding trochophore larvae form quickly after fertilization. Feeding veliger larvae develop within two days and use ciliary movements to position themselves in the water column. This movement is limited, so larval dispersal is driven by currents and tidal fluctuations (Dekshenieks et al. 1996). The length of the veliger stage is highly dependent on water parameters which can last from one week to two months, before developing into the pre-settlement pediveliger stage. Negative phototaxis develops, pushing pediveligers toward the benthos, and increasing exposure of the larval foot to suitable substrate.

Settlement occurs on many hard surfaces, although calcium carbonate and filter effluent from live oysters, may play some role in cueing metamorphosis (Zimmer-Faust et al. 1994). Evolutionarily, this allows recruitment on or near other successful oyster populations and facilitates the formation of beds. Settlement location is critical to survival, because an oyster never moves after settling on a substrate.

They feed using millions of rapid beating cilia to create currents, which pull suspended particles out of the water and across their specialized gills or ctenidia. They are capable of sorting eatable particles using a complex labyrinth of ciliary bands and mucus within the ctenidia and labial palps (Beninger et al. 2005). Particles that are benign or too large to consume are rejected and passed out of the oyster as pseudofeces, and sinks. Particle size, abundance, and consumption competition cause filtration rates to vary (Beninger and Veniot 1999).

Oyster commercial fishery on the east coast of the United States began as early as the 1600s, where oysters offered an easily accessible, reliable, and nutritious food source to growing coastal villages (Kirby 2004). Oysters were so abundant then, that it was difficult to navigate channels due to large reefs that rose out of the water. They soon became a staple to the new society's lifestyle driving a sizable economic engine. The market employed hundreds of people at every step of an oyster's journey from sea to table and provided a multimillion-dollar industry to the early economy (MacKenzie 1996). Even the discarded shells contributed to the economy, used to pave roads, provide calcium for poultry farms, as lime in fertilizers, and as plaster for houses. Oysters were firmly embedded in the East Coast culture by the 1700s. The demand was so high by the late 1750s, that signs of overfishing became apparent as harvest numbers began to dwindle. The effects of removing large breeding stocks and removing shell for spat settlement, limited recruitment success (MacKenzie 1996). Local governments realized how important this

industry was, which led to some of the earliest conservation efforts including requiring permits for harvest, protecting important spawning areas, enforcing seasons and size limitations, and replacing shell habitat back to the beds (Kurlansky 2006). The advent of dredging and fine tuning of steam boats during the Industrial Revolution had a devastating effect on oyster populations not only by producing higher yields, but from compromising the integrity of the oyster reef habitats (Rothschild 1994). Entire beds could be destroyed in a day and caused irreversible damage done to the subaquatic ecosystems. Railroads allowed transport of oysters to settlements expanding west, increasing demand. To help satisfy this demand, oysters were transplanted from areas of high abundance to areas had already been depleted o (MacKenzie 1996). Upwards of two million bushels a year were transplanted from the Chesapeake Bay cultivated and cultivated elsewhere.

Drastic declines of oyster reefs led to the realization that they are dynamic and important marine habitats providing numerous ecosystem service (Beck et. al. 2011). Reefs form when multigenerational aggregations thrive to build a three-dimensional structure. This in turn, provides shelter and food sources for many other living organisms, which yields a much more biodiverse ecosystem (Peterson et al. 2003). Consuming phytoplankton via filtration enacts top down population control and can prevent blooms, which can lead to eutrophic and toxic conditions (Fulford 2007). Clear water allows light to penetrate further into the water column and promotes important seagrass bed communities, which further increases diversity and clarity by slowing water, reducing suspend particles (Tallis 2009). Another benefit is the physical presents of dense groupings which can alter fluid dynamics (Woods et al 2005). Reef structure reduces wave action and stabilize sediment which slows down the erosion process of coastal

communities (Borsje 2011). A healthy and thriving reef can act as a storm barrier, taking the brunt of storm surge damage and minimizing inshore effects.

Oysters ecosystem services and ability to engineer ecosystems make it an ideal target for restoration, in theory, but success is limited (Coen et al. 2007). Methods can range from simply providing substrate for local recruitment to designing interconnected metapopulations with a network of source-sink oyster reefs (Lipcius et al.2008; Schulte 2009; Munroe et al. 2012). Selective breeding for disease tolerance to supplement declining numbers began period of high dermo and MSX mortality in the 1950s and 1960s. One such line developed at Rutgers University, the Northeast High Survival (NEH), has resistant to MSX and dermo pathogens (Guo et al. 2008). The line was produced from crosses of resistant lines from throughout the Northeast and mid-Atlantic regions. It has high survival in a wide range of habitats and has been used in numerous restoration efforts (Proestou 2016).

2.1. Restoration Theory

Anthropogenic impacts on the structure and function of ecosystems have been continuous for thousands of years, the effects of which we are only beginning to understand (Millennium Ecosystem Assessment 2005). Increased awareness of habitat loss and environmental pollution in the 1960s lead to sweeping regulations which enacted various environmental protections to encourage stewardship and restoration. Modern restoration theory aims to return ecosystem services, re-establish habitat connectivity, and maintain biodiversity at many levels of organization (Thrush et al. 2008). High biodiversity generates many weak ecological interactions with redundancies and overlaps (McNaughton 1977; McCann 2000). Theoretically, these functional overlaps allow systems to be more stable through resistance to natural and anthropogenic variation. This concept is known as the diversity-stability hypothesis and is

important to keep in mind when selecting species or habitats to restore (Sacande & Berrahmouni 2016).

Inclusion of a diverse group of stakeholders when planning restoration efforts is crucial, as each habitat and location present unique challenges. Realistic goals and variables to monitor should be clearly defined early on during project development to guide design (Kondolf and Micheli 1995; Kennedy et al. 2011). Prior to project implementation, baseline variables are measured to provide the foundation to interpret subsequent impacts of the project. Developing monitoring protocols are critical to understanding projects effectiveness but is an often over looked component due to time and cost (Iknayan et al. 2014). Monitoring protocols should include the use of repeatable metrics specific enough to determine impacts of restoration efforts relative to set goals (National Research Council 1992; Kennedy et al. 2011). Ideally, plans are periodically assessed and adjusted as needed to maximize the chances of achieving set goals (Perrow 2002). Setting an ecological historical reference point can be difficult to determine, but is fundamental to understanding the systems limiting factors and is an important consideration to set realistic goals (Kennedy et al. 2011; Balaguer et al. 2014).

Pre-historic (1100-1600) alterations to hydrology and sedimentation from small farming societies has been documented in Northeast flood plains (Stinchcomb et al 2011). Extensive habitat degradation in the eastern United States began in the 1700s, as large expanses of natural habitat was converted to monocrop farms, to produce cash crops which fueled the early American economy (Cochrane 1979). Ecosystems present before the Revolutionary and Civil Wars, the industrial revolution, and rapid urbanization have likely decreased in diversity and magnitude of ecosystem services they provide, yet expansion continues in these same areas to this day. The most densely populated stretch of the coastal zone in the eastern United States

occurs from Boston to Washington D.C. (Hinrichsen 1998). Watersheds encompassing these areas all drain into coastal plains and estuaries, common features throughout the eastern United States coast. Coastal ecosystems are often adversely impacted both indirectly through intense urbanization upstream, and directly from development due to proximity to the coast, where nearly 30% of the United States human population resides. (Wilson and Fischetti 2010).

The dynamic interaction of spatial and temporal patterns between tidal strength and river flow creates a uniquely complex habitat (Dalrymple and Choi 2007). Estuaries are one of the most productive and important ecosystems in the world having extremely high turnover rates, providing stopover habitats for birds, and providing nursery habitats for commercially important fisheries (Burger et al. 1997; David et al. 2016). They also protect against storm surges, while naturally mechanically and chemically filtering water and sequestering carbon (Nowicki and Oviatt 1990; Jones et al. 1994; Hopkinson et al. 2012). Loss of habitat has resulted in degraded water quality and decreased resilience to stressors, which can negatively impact local businesses and communities (Costanza 1997). Many restoration projects have aimed to return social and ecological function to coastal habitats along the Eastern United States (Elliot et al. 2016). Attempts to recover oyster stocks have occurred in every state bordering the Atlantic Ocean, whether it be for fisheries enhancement or more recent interests in returning ecosystem services (Coen and Luckenbach 2000). Historic oyster grounds in the Chesapeake Bay have received millions of dollars over the past 20 years during a state-federal partnership to restore oyster populations on (Wheeler 2018).

The public oyster grounds of the Great Wicomico River were one of the first to be targeted for large scale restoration primarily due to naturally high recruitment. Habitat was enhanced with shell substrate using two different treatments of high and low relief reefs, then

seeded with adult transplants starting in 1996 (Southworth and Mann 1998). As opposed to wild transplants, disease resistant, selectively bred DEBY oysters from Virginia Institute of Marine Sciences (VIMS) line were used starting in 2002, (Carlsson et al 2008). A genetic analysis on recruits after the first year of the hatchery line supplementation, found minimal evidence of DEBY recruits using population assignment tests (Hare et al. 2006). The US Army Core of Engineers increased the size of the project in 2004, adding nine additional sites, but declared them no harvest sanctuaries (Schulte 2009). VIMS supplied an estimated 15.5 million "clutchless" individuals for seeding the newly formed reefs from 2004-2006 (Carlsson et al 2008). Long term surveys of population dynamics from 2000-2004 observed episodic high recruitment years followed by large mortality events (Southworth and Mann 2004). Also, habitat relief was better maintained up river where high accretion of natural shell mass occurred. Schulte et al. (2009) estimated 184.5 million oysters marking a 57-fold increases from the 1994 baseline, providing strong evidence for possibility of restoring metapopulations. He also noted that oysters were five time more likely to occur on high-relief reefs (Schulte et al. 2009). However, continued genetic analysis of the population from 2002-2006 found no evidence of enhancement due to DEBY (Carlsson et al. 2008). Carlsson et al. (2008) suggested absences of DEBY could be explained by high predation, scale of supplementation, or reduced fitness in the wild due to hatchery selection.

2.3. Population Genetics

Population genetics examines the variation of allele frequencies among and between individuals of sub-populations to make inferences about relatedness and the genetic structure. There are several processes which drive observed frequencies of alleles: natural selection, non-random-mating, mutation, genetic drift, and gene flow (Hartl 2000). The degree to which these

processes occur and their interactions give rise to unique genetic signatures over space and time. Human development drastically impacts how these processes occur through environmental degradation, transferring species, and artificial selection (Palumbi 2001). Advancements in sequencing technology and increased computing has increased the accessibility of genetic tools allowing the methods to be applied for useful questions in ecology. (Selkoe & Toonen 2006).

Many different forms of genetic markers have been identified for use in population genetics but microsatellites markers are commonly used in studies to analyze genetic variations in nature (Borsje et al 2011). Microsatellites are simple sequence basepair repeats (SSRs), which are genetically frequent, typically occur in non-coding region, and are highly polymorphic due to high mutation rates (Hartl 2006). Ecological questions such as relatedness and genetic structure of regions can be examined using variations of allele frequencies at each microsatellite marker.

A high mutation rates provides increased variations over relatively short evolutionary time making them ideal for studies of relatedness (Schlotterer 2000). A consequence of high mutation rates are null alleles. Null alleles occur when aa mutation alters the binding region used for primer amplification, causing that allele to not amplify during PCR (Eisen 1999). A single non-amplified allele creates a false positive as homozygotes and increases type I errors. Another important question to consider is, if the inheritance of alleles is randomly assorted. Although the SSR regions themselves do not code for proteins, they could in a similar region on the chromosome as an allele subject to selective pressures or another marker. The alleles are linked through proximity and therefore are less likely to be unchanged unless recombination occurs (Bachtrog et al. 1999). Selective pressures on a nearby gene causes retention of specific marker alleles. This linkage disequilibrium causes non-random assortment of alleles and failure of

mathematical assumption of independence. Careful selection of marker regions can control the impact of variation from null allele and linkage disequilibrium (Selkoe and Toone 2006).

The development of microsatellites markers for use in determining genetic variation in the eastern oyster is well documented and aided in decoding the eastern oyster genome (Carlsson et al. 2006; Hare et al. 2006; Rose et al 2006; Carlsson 2008; Gomez-Chiarri et al. 2015). Previous studies have resulted in development of microsatellites useful for genetic structure analysis and selective breeding (Brown et al. 2000; Reese et al. 2004; Wang and Guo 2007). Carlsson et al. (2006) found significant differences in genetic structure between hatchery strains and wild oysters using five highly polymorphic microsatellite markers. Hatchery strains show lower allele diversity due to inbreeding which could lead to reduced fitness in natural conditions (Carlsson et al. 2006).

2.4. Study Site: Delaware Inland Bays

The state of Delaware is a peninsula, flanked by two historically productive oyster grounds of the Mid-Atlantic region in Delaware Bay and the Chesapeake Bay (Beck 2011). The southern Delaware coast is exposed to the Atlantic Ocean and where three coastal lagoons called the Delaware Inland Bays (DIBs) are located (Figure 2.1). DIBs waters are no deeper than four meters with a total subaqueous area of approximately 83 km^2 (Price 1998). The DIBs are protected from the swell of the Atlantic by narrow barrier sand dunes but remain tidally influenced through a single maintained inlet, the Indian River Inlet (IRI).

The northern most bay, Rehoboth Bay (RB), connects to the Delaware Bay (DB) through a narrow canal to the north and joins the Indian River Bay (IRB) to the south. Both bays have low flushing rates of 80-100 days but water exchange is not consistent throughout (Price 1998). Tidal influences via the IRI exchange water regularly in eastern IRB and southern RB, while

backwaters are flushed through freshwater input driven by rainfall (115 cm yr) and groundwater discharge (0.6– 1.3 $m^3 s^{-1}$) (Andres 1992). The southernmost bay, Little Assawoman Bay (LAB), was historical tidally driven thought an inlet near Ocean City, Maryland. Presently, a narrow man-made channel, now connects LAB to the upper bays but has minimal hydrological influence (Scudlark et al. 2005) (Figure 2.1).

The 777 km^2 coastal plain watershed is comprised mainly of agricultural and rural lands but development along the DIBs is rapidly increasing (Price 1998). The population within the watershed doubled from 1990 to 2010 and is expected to increase by 46% from 2010 to 2040 (Walch et al. 2016). Anthropogenic activities have degraded the water quality through the input of excess nutrient from point and non-point sources, leading to eutrophic conditions. Vast improvements to total nitrogen and phosphorus loads were made by regulatory changes in the 1990s to meet total maximum daily loads, established by the Delaware Department of Natural Resources and Environmental Control (DNREC). The majority of point source pollution has been removed and management plans implemented to reduce impacts of agriculture and residential runoff (Walch et al. 2016). However, the cumulative impact to water quality has shifted the ecological community and tarnished habitat quality. Attempts to return the ecosystem to a more functional state are overseen by the Delaware Center for the Inland Bays (CIB). Established in 1994 as part of the National Estuary Program, it is a non-profit organization which implements restoration and encourages stewardship of the DIBs estuarine systems. Establishing health populations of filter feeding bivalves to leverage the ecosystem services they provide is one of their major objectives (Walch et al 2016).

The longest running bivalve restoration project is the oyster gardening program established in 2003. The goals of the project were to engage the local community, improve water

quality, and aid in establishing a self-sufficient breeding population (Walch et al. 2016). Sites are spread throughout the DIBs but concentrated mainly around the canals of Bethany Beach and Fenwick Island (Rossi-Snook 2010) (Figure 2.2). Oyster shell recycled from local restaurant is set with eyed-larvae from disease resistant hatchery lines. The spat on shell are then hung off local volunteer's docks where they are raised for two years before being used to stock restoration projects or living shorelines. A quarter acre oyster reef restoration plot at James Farm ecological preserve was the first to receive gardened oysters in 2002 (Ewart 2013). While growth and larval recruitment were observed, the project was ultimately discontinued in 2006, after complications from predation, freezing, and disease. Starting in 2008 oysters were transplanted to rip-rap structure as it provided increased water movement and protection from cownose rays (CIB 2014). Rip-rap is the stabilization of coastal shoreline using stacks of various size rocks to attenuate wave action. Survival in intertidal rip-rap habitat averaged around 50% but was highly variable between sites and years (Reckenbiel 2013). As of 2014 season, approximately 50,000 oysters were reported to have been transplanted to various DIBs intertidal rip-rap locations (CIB 2014). Oyster recruitment has been observed on DIBs rip-rap and warrants more study as a potential habitat for restoration of oyster reefs.

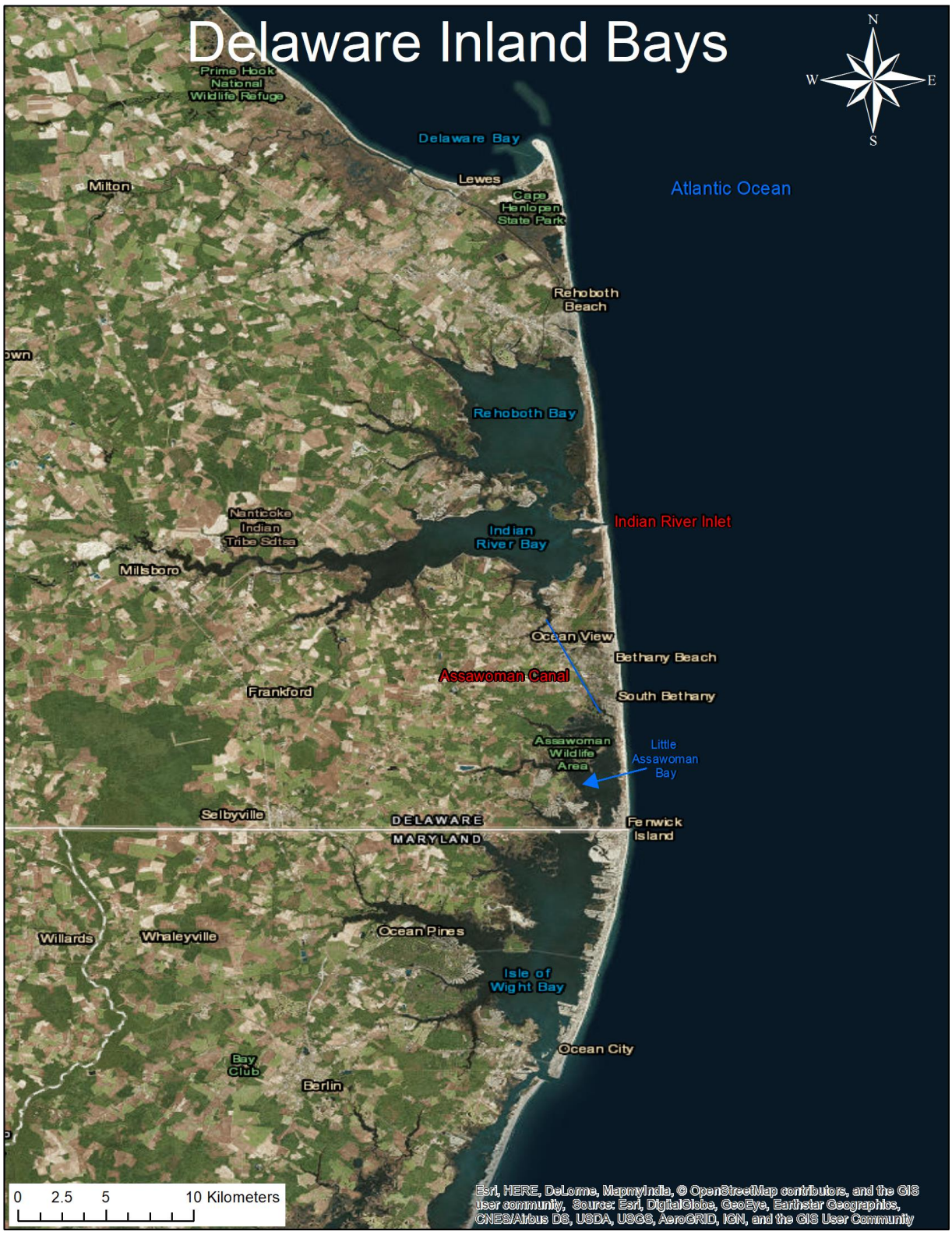

Figure 2.1. This figure shows the three Delaware Inland Bays locations along the southern coast of Delaware and a portion of Maryland's coastal bays. Image was created using ArcMap v10.6

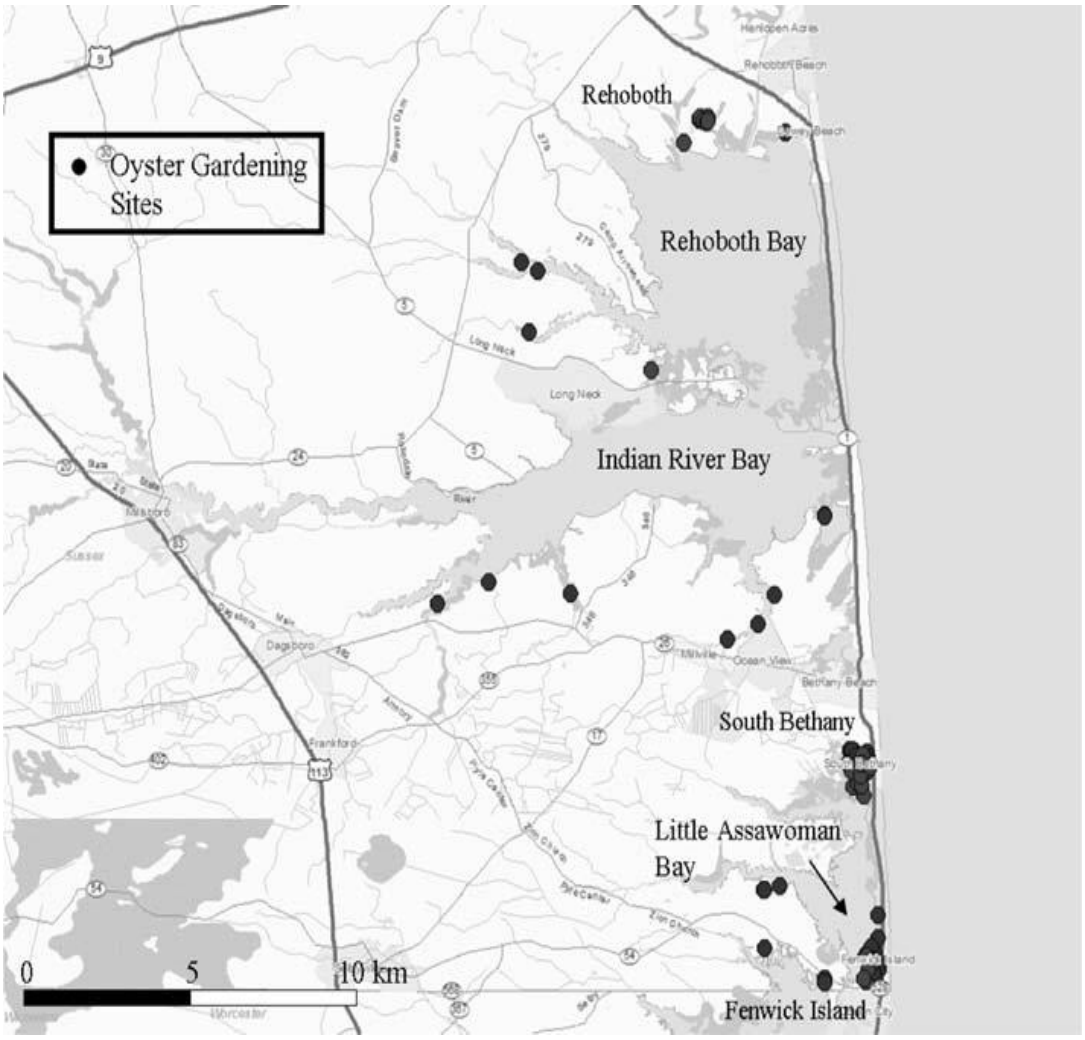

Figure 2.2. The locations marked show where oyster gardening occurred in 2007. During this year there were 102 sites with 78 occurring in Little Assawoman Bay (Rossi-Snook et al. 2010). The program peaked in 2013 with 120 sites and currently has 88 locations. The man-made canals in South Bethany and Fenwick Island account for the majority of gardening sites. Image provided in Rossi-Snook et al 2010.

2.5. Intertidal Surveys

The intertidal area encompasses habitat that is exposed to air during low tide but covered by water during high tides. They can be very productive habitats and many creatures have evolved to tolerate the daily fluxes and physical extremes (Newell 1976). Visible banding patterns are driven by species resilience to abiotic stressors with more diversity in the lower intertidal region. Studies seeking to test ecological theories often use this community due to the ease of manipulation and repeatability in a relatively small area (Connell and Slatyer 1977; Hewatt 1937; Lubchenco and Menge 1978; Sousa 1979). Paine (1969) first discribed the keystone species concept with his surveys of *Pisaster ochraceus* within the rocky intertidal of the Pacific Northwest.

Rocky intertidal habitats have been intensively surveyed using transects and quadrats to standardize sampling. Repeating measurements though time yields valuable insight to long-term ecological trends (Barry et al. 1995). The United States has artificially hardened 14% of tidal shorelines to protect property against storms, waves and coastal erosion (Gittman et al. 2015). Coastal armoring of intertidal zones has been occurring in the United states since the early 1900s creating artificial rocky intertidal habitat, yet very little long-term studies have been done to understand the ecological impact. A meta-analysis revealed similar biodiversity and species abundance in rip-rap and breakwaters but was highly variable across locations as was the environment (Gittman et al. 2016). However, a reduction in ecosystem services and increased abundance and diversity of invasive species were also observed. Alternatives to armoring included using local marsh grasses and natural breakwaters, called “living shorelines,” promote native habitat while providing the same benefits to coastal communities (Currin et al 2010).

Oyster shell is often used as the breakwater component of living shoreline to promote recruitment of wild oysters to the lower intertidal zone (Gittman et al. 2016). Oysters were historically abundant in intertidal habitat and harvested but are no longer targeted by commercial industries due to decreased number, intense labor, and low quality (Bahr and Lanier 1981). Intertidal reefs are common in southern states but limited in waters north of the outer banks (Capone et al. 2008). Taylor and Bushek (2008) reported the ephemeral nature of intertidal reefs in the Delaware Bay by documenting yearly shift of artificial intertidal habitat, constructed with oyster shell bags. They note a significant increase in biodiversity and suggest that shifting sands maybe the biggest threat to persistence of intertidal reefs.

CHAPTER 3

METHODS AND MATERIALS

3.1. Genetic Analysis

3.1.1. Sample Collection

Spat collectors were constructed using PVC, 20 X 20-cm ceramic tiles, and bricks using a design modified from the University of North Carolina Wilmington's spat monitoring project (UNCH weblink). Each collector had four tiles oriented vertically in the water column with the support of PVC leaders and was secured to the PVC frame using zip-ties (Figure 3.1). Zip-ties also held bricks to the bottom of the frame for stability and to prevent siltation by lifting the off the bottom. The ceramic tiles contained calcium carbonate and provide a hard substrate for the oyster larvae to settle on. Collectors (three per location) were placed in 12 locations throughout the DIBs during the summer of 2016 (Figure 3.2). Sites were selected in 2015 as part of an ongoing recruitment monitoring program (Ozbay 2017). Collectors were positioned near the shoreline in the low-intertidal zone to be exposed during low tides and submerged at high tides. Once installed spat collectors were left in place for 10 weeks. Tiles were replaced after 5 weeks to limit the impact of fouling on recruitment.

One location with spat collectors within each bay also had trial aquaculture cages which were part of a separate experiment measuring the growth and nitrogen uptake of NEH oysters in the different bays (Fuoco 2018) (Figure 3.2). Two 122 X 122 X 5-cm cages were initially stocked with 200 NEH oysters. However, these cages can be settled by oysters and were examined for additional spat to include in genetic analysis once removed from the water, during the final week of September in 2016.

Tiles with spat were identified and individuals counted after being removed from the DIBs locations. Tiles with spat were then labeled by origin and placed in flow through tanks at the University of Delaware, Lewes campus to grow for more tissue mass for DNA extraction. Spat were closely monitored to ensure survival and prevent mixing with spat settling from the intake pump located in the Broadkill River. After four months of growth, the DIBs spat were shucked and entire bodies preserved in glass vials of 95% ethanol. All DIBs spat were split into two groups based on the origin of saline water source which may also drive genetic divergence. The upper DIBs (UB) is comprised of RB and IRB where seawater enters through the Indian River inlet. The second grouping, lower DIBs (LB), is just LAB and receives seawater mainly sourced from an inlet near Ocean City, MD. Two likely larvae sources were sampled as references for genetic comparisons to the collected DIBs spat. Individuals gathered from the DIBs gardened NEH (46) oysters and DB (48) oysters harvested from the commercial seed beds were preserved for DNA extraction. The four groups defined (UB, LB, DB, NEH) were used throughout the genetic analysis to examine similarities and difference within and between groups.

Figure 3.1. A spat collector placed along the shoreline during a negative tide. The PVC frame supports the tiles in a vertical position to provide substrate for larvae settlement. It is attached to bricks to hold in place and to raise structure off the bottom to prevent siltation.

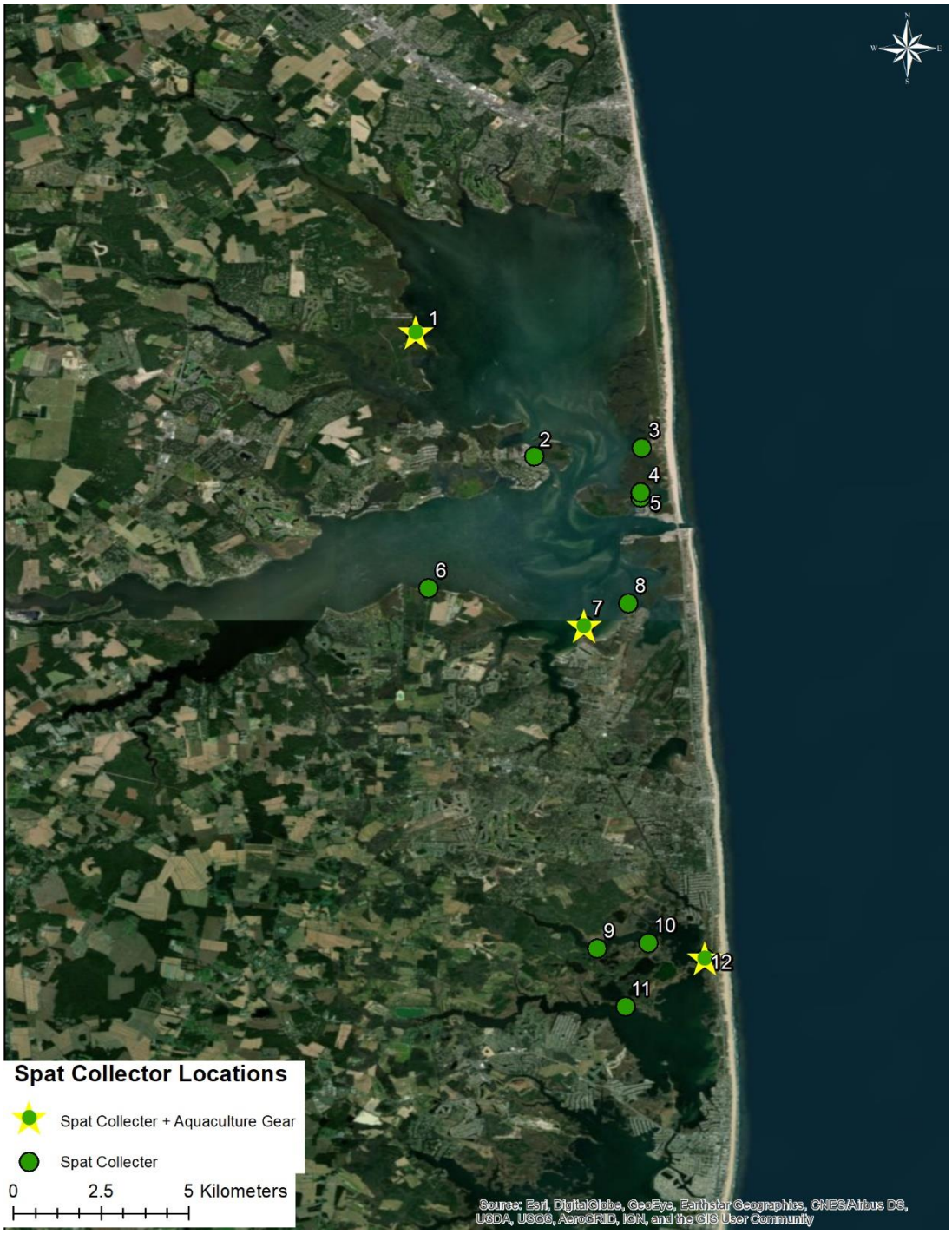

Figure 3.2. Locations where spat collectors were placed in the 2016 season are indicated with a green dot. Starred locations represent location where trial growth aquaculture cages. All aquaculture sites also had spat collectors. Locations identification key can be found in Table 3.1

Table 3.1. This table contains the names of the numbered site that received spat collectors or had aquaculture cages in Figure 3.2.

Identification Number	Location
1	Camp Arrowhead
2	Massey's Landing
3	Savages Ditch
4	Burtons Island - North
5	Burtons Island - South
6	Peninsula Golf & Country Club
7	Burtons Island-North
8	Burtons Island-South
9	Sassafras Landing
10	Strawberry Landin
11	Mulberry Landing
12	Narrows

3.1.2. DNA Extraction, Amplification, and Genotyping

Oyster abductor muscle was used in DNA extraction of each sample. Protocol from the High Pure PCR Template Kit (Roche Diagnostics, Mannheim, Germany) was followed with 150μL reduction in suggested elution buffer solution due consistently high yields of DNA concentration. DNA concentrations were measured using a nanodrop 2000 (Thermofisher scientific, Waltham, MA) and were diluted into aliquots of 100ng/ml for PCR reactions. Eleven polymorphic microsatellite markers were selected from a protocol using fluorescently labeled primers for multiplex reactions and suggested unpublished markers (Wang et al. 2010; X. Guo-personal communication). Amplification conditions reported by Wang et al. (2010) were used, but slightly modified to allow one extra minute of extension time and using consistent primer concentrations of .02μM per reactions.

Fluorescence from the labeled primers were recorded using capillary electrophoresis on an ABI 3130xl Prism Genetic Analyzer (Applied Biosystems, Foster City, CA).

Chromatographic outputs peaks were automatically identified for each marker, but all were manually scored to correct mistakes using GeneMapper v4.0 (Applied Biosystems, Foster City, CA). Heterozygote peaks were only scored when the fluorescence levels were at least 1/3 the height of the highest peak to limit the effects of stuttering and large allele dropout. Twenty individuals were randomly selected from the 195 samples to be processed twice to ensure repeated scoring of peaks on chromatograph outputs in Genemapper v4.0. Allele lengths were recorded in base pairs for each individual at each marker and used in the analyses.

3.1.3. Data Analysis

Preliminary descriptive genetic statistics were calculating in GenAlEx version 6.5 (Peakall and Smouse 2012). This free Microsoft Excel add-on uses macro functions to analyze genetics statistics and yields similar results to more complex programs (Dale et al. 2011). Allele frequencies for each marker and location were calculated and are used in all subsequent genetic analyses. Summary statistics provide an general overview of the genetic diversity and spread of alleles at each markers for each group. They include the number of alleles (N), number of effective alleles (Ne), Shannon's diversity index (I), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), and a fixation index (F). Unbiased He estimates were compared with Ho to determine if frequencies were equal or at Hardy Weinberg Equilibrium (HWE). The HWE is tested usig a chi-squared goodness-of-fit to examine deviations from the null where uHe is equal to Ho (Hedrick 2000). P-values were adjusted for multiple comparisons using sequential Bonferroni correction. Allele frequencies were used to calculate two indexes which evaluate the relationships between and within the four groups DB, NEH, UB, and LB.

Fst is an index which examine differences in groups using the correlation of alleles frequencies within a group relative to all the total samples for all groups (Wright 1951). Fst

values were estimated according to Weir and Cockerham (1984) and permutated 999 times to test significance. Population pairwise comparisons that significantly deviate from zero are considered structurally different. A different frequency-based index estimates relatedness of individuals within each group based on the probability of identify by descent. Using an equation defined in Lynch and Ritlands (1999), pairwise estimates of the coefficient of relatedness (CoR) was first calculated within each locus then summed across all loci for each sample. The mean within each group was calculated for comparisons. Bootstrap resampling with replacement was performed 999 times to account for error and to create a confidence interval mean. The CoR index ranges from 1-0, where individuals within a group are more similarly related with higher mean CoR values.

A multivariate method for determining population structure, has been suggested to work well with microsatellite analysis using the adgenet v2.0.0 package for R v3.4.3 (Jombart 2008, R core team 2013). Discriminant analysis of principal components (DAPC) uses a model which emphasizes variation between groups while minimizing influence of variation within groups to infer genetic clusters (Jombart et al. 2010). Sequential K-means clustering simulations of principal components produce a Bayesian Information Criteria (BIC) to determine the ideal number of clusters to maintain. Exploratory data analysis is used to examine effects of different numbers clusters, principal components (PCs), and discriminant functions. Steps to avoid overfitting of data and subsequent cross-validation methods, were used to produce a high preforming model. Outputs from the DAPC are used to assign membership probability of individuals to previously defined groups and to visualize population structure in an ordination plane.

3.2. Intertidal Riprap Surveys

3.2.1. Sampling Design

Hardened shorelines offer the most suitable habitat for oyster recruitment in the primarily muddy bottom of the DIBs. Previous research has qualitatively noted, sparse amounts of spat on intertidal rip-rap habitats of the DIBs (Reckenbeil 2013). A transect surveys was designed to establish location specific baselines of oyster densities and size frequency distribution within DIBs rip-rap habitats. This study was not designed to examine factors driving settlement, but to quantitatively capture the current state of oysters with rip-rap at various DIBs locations. Candidate sites were identified by searching Google Imagery (2017) on Google Maps for grey bands along the DIBs coastline, which are indictive of possible rip-rap hardened shoreline. Candidate sites were visited in-person to determine if they were publicly accessible, had a rocky rip-rap shoreline, and were large enough for repeat transects as well as, to qualitatively access oyster abundance (Figure 3.3). Candidate sites were omitted from final selection if they were not publicly accessible, had concrete slabs as opposed to rocks, not enough space for repeated transects, or if the rocks did not extend near the low tide water (Figure 3.4; Table 3.3). Final site selection did not factor in the qualitative abundance of oysters from the initial site visits. Unfortunately, not all candidate sites selected for surveys were able to be assessed due to logistics, labor, and weather constraints but all accessible candidate sites were qualitatively assessed for oyster abundance as high, medium, low, or none (Figure 3.4).

Sampling only occurred on days which met pre-defined constraints of tide levels and weather conditions to minimize influence of variation associated with environmental factors. Surveys were only conducted during predicted negative tides between -0.05ft and -0.5ft using monitoring station maintained by NOAA, at the Indian River Bridge, DE. Search time was also

standardized for one hour before and after the predicted peak low tide time. Replication was maximized within time restrictions but varied based on the site characteristics and numbers of oysters (Table 3.3). Surveys did not occur on days where the wind was over 15 knots or on overcast days. Training to develop a search image as well as calibration surveys were required in order to count and measure oysters on surveys to limit human sampling error.

Figure 3.3. The locations dotted blue were identified using Google Imagery (2017) for Google Maps as having rip-rap and appearing to have easy access. These select locations were further evaluated in person to determine if the size was suitable for repeat surveys to occur.

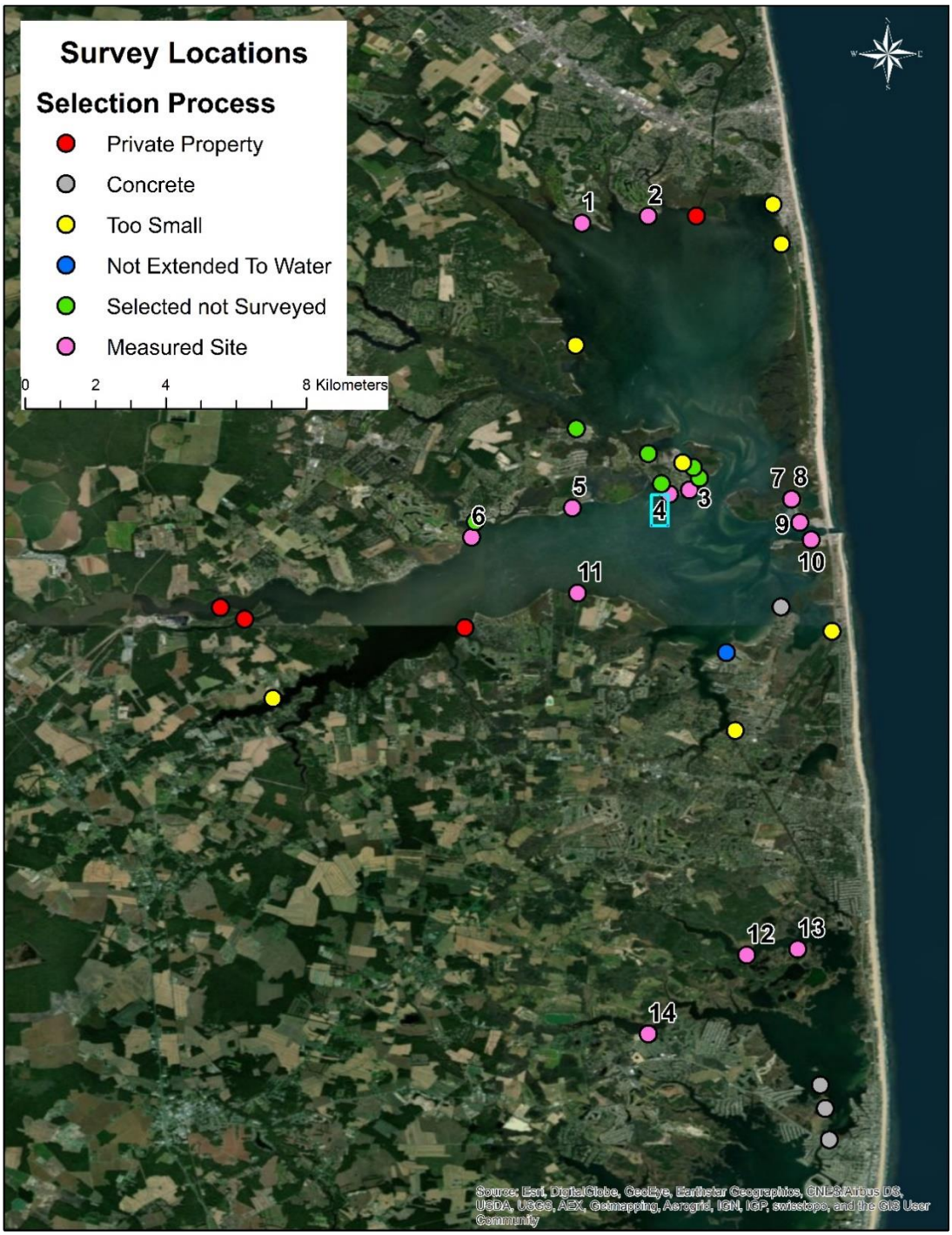

Figure 3.4. Locations from figure 3.3 are color coded based of their suitability for survey measurements to occur. Red, yellow, blue, and grey dots indicate locations that were not suitable due to access, rip-rap habitat was too small, or physical properties of the rip-rap. Green dots signify locations that were approved for surveys but were unable to be surveyed due to time and weather constraints. The numbered pink dots are locations where surveys occurred. The following Table 3.3 identifies the site names and number of transects conducted.

Table 3.3. The locations surveyed and the number of transects that were used at each site. Identification numbers correspond to labels for the pink dots on Figure 3.4.

Identification Number	Location	Number of Transects
1	Rehoboth Bay Mobile Homes	1
2	Rehoboth Bay Country Club	3
3	Pots Nests-Southeast	4
4	Pots Nest-South	3
5	White House Beach	3
6	Peninsula Golf & Country Club	1
7	Burtons Island-North	2
8	Burtons Island-South	3
9	Indian River Outlet - North	4
10	Indian River Outlet - South	3
11	Holts Landing	3
12	Sassafras Landing	3
13	Strawberry Landing	3
14	Swan Cove	1

Transects 16-20 meters long were laid parallel to the water along mean high tide, identified from physical features of rocks, which served as the upper boundary for the survey area. Entire delineated intertidal sections were systematically searched for oysters using a snake like pattern extending from the mean high tide transect to the low tide water level. The number and sizes of oysters were documented within each transect to use in density measurements and size frequency plots. Measurements of the physical characteristics were taken to estimate the area searched (Bagget et al. 2015). All the physical traits were taken at three evenly spaced intervals and averaged for each transect to be used in survey density measurements

A meter tape was drawn straight out from the mean high tide line to intersect perpendicularly with a vertical PVC pole at the low tide mark, forming a right triangle above a cross-section of rip-rap (Figure 3.5). The intersect value on the PVC pole represented height and the intersect on the meter tape represented width (Figure 3.5). A handheld bubble level was used

to ensure width measurement was parallel to the ground. The height and width were used to calculate the hypotenuse of the right triangle formed using the Pythagorean theorem and converted to an angle measurement using inverse sine. Roughness was calculated to account for the surface area of the interstitial space created from the rock stacks. Meter tape was laid from the upper limit to low tide in a straight line outlining the topography of rocks and the distance was recorded (cm) as “slack”. Without moving the meter tape, the slack pulled tight and new distance was recorded (cm) as “straight” (Figure 3.1). Dividing the slack by the straight creates a roughness index were 1 is perfectly flat surface and is a proxy for interstitial space.

The transect length and average width, were multiplied to calculate total area. The area was multiplied by average roughness as a proxy for total surface area where an oyster could settle (TSA). Density was calculated by dividing the total number of oysters counted by TSA for each transect. Transect densities were averaged within sites to represent mean site density mapped using ArcGIS v10.6 (ESRI, Redlands, CA).

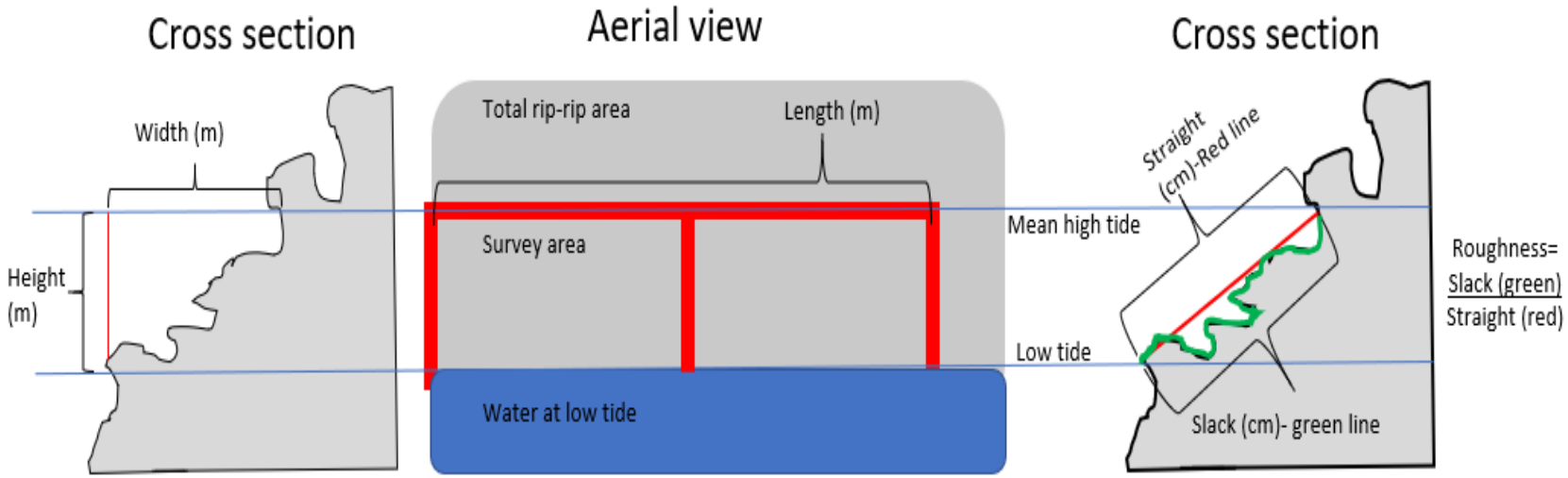

Figure 3.5. Diagram depicting how physical characteristic measurements height, width, and roughness were collected in the rip-rap. The aerial view is shows the area delineated by the transect at mean high tide (water parallel red line) and the three locations were cross section measurements are taken at the beginning, middle and end of each transect (perpendicular red lines).

CHAPTER 4

RESULTS

4.1 Microsatellite Analysis

Spat collection was low at each site with a max of 16 oysters settled (Table 4.1). The majority of spat sampled in LB were settled on the trial aquaculture cages at the narrows, but spat collectors in the same area only received a single spat. However, at other sites with aquaculture cages and tile collectors, some were on the associated tiles and none were found on the cages (Table 4.1).

Table 4.1. Total spat collected by site on all the tiles and overall for UB and LB groupings. The number in parentheses signifies spat that were collected from aquaculture cages placed nearby for a different research project. Each cage contained around 100 NEH oysters

Location	Spat Count	Location	Spat Count
Masseys	1	Sassafras Landing	0
Camp Arrowhead	13(0)	Strawberry Landing	2
Savages	0	Mulberry Landing	3
North Burtons	16	Narrows	1 (47)
South Burtons	5		
Holts	6		
Pasture Point	4 (0)		
VFW	3		
Upper Bays UB Total	48	Lower Bay LB Total	53

All samples amplified in at least 10 of the 11 microsatellite markers and amplification failures were rare (2.7%). The RUCV 61 locus had the most samples that failed across all populations (7%). Twenty randomly re-run samples only showed a 1.1% discrepancy in allele scoring. All mismatches were found in dinucleotide repeats and most likely were effects of stuttering. Allele numbers from initial scorings were used in the final spreadsheet for analysis.

Reduction in the number of alleles (Na) for NEH (10-14 alleles) is most noticeable in three highly polymorphic markers (RUCV 45,61, and 424), when compared to other populations (19-30 alleles; Table 4.3). Number of effective alleles (Ne) and Shannon's information index (I) also show similar declines in NEH at some markers, with only one (RUCV20) showing the opposite pattern. Significant deviations from the HWE after sequential Bonferroni corrections are seen in the direction of homozygote deficiency at RUCV 61 and 114 across most groups (Table 4.3).

Pairwise Fst values were high (0.045-0.050; Table 4.2) when NEH was compared to any other population and permutations found significant between population differences (Table 4.2; Hartl and Clark 1997). Smaller Fst values (0.007-0.009) exist between all other possible comparisons but were not significantly different from the null, which is zero (Table 4.2). The mean coefficient of relatedness (r) values showed very little intrapopulation relatedness (<0.01) in the Delaware Bay and two DIBs populations (Figure 4.1). NEH had a much higher level of within population relatedness (0.135) (Figure 4.1).

Table 4.2. The pairwise Fst results values are used to measure between population variation. Numbers below the diagonal blanks are the Fst values, while numbers above are p-values against the null of 0. P-values were calculated using 999 permutations. Fst values less than .05 have little genetic differences, while values over 0.25 have very high genetic differences. Values are significantly different from zero at 4 of the 6 comparisons and were highly significant (.001) with all comparisons to NEH. **DB** = Delaware Bay, **NEH** = Northeast High Survival line, **LB** = Lower bay, **UB** = Upper Bays

	DB	**NEH**	**LB**	**UB**	
DB		**0.001**	0.066	**0.044**	**DB**
NEH	0.046		**0.001**	**0.001**	**NEH**
LB	0.008	0.045		0.144	**LB**
UB	0.009	0.050	0.007		**UB**
	DB	**NEH**	**LB**	**UB**	

Table 4.3. Summary statistics for 11 microsatellite loci among 4 different populations of eastern oysters. Single loci F value is listed first. **N =** No. of Samples (If less than number under population names, then amplification failed, **Na** = No. of Different Alleles, **Ne** = No. of Effective Alleles, **I** = Shannon's Information Index, **Ho** = Observed Heterozygosity, **He** = Expected Heterozygosity, **HWE** = Hardy Weinberg Equilibrium, **F**= Fixation Index (He-Ho)/He= 1-(Ho/He). Bolded numbers represent significant p-values for the test of HWE after sequential Bonferroni correction for multiple test (p-value <.00025).

Group		RUC V60	RUC V197	RUC V61	RUC V73	RUC V21	RUC V75	RUC V114	RUC V374	RUC V45	RUC V424	RUC V20
	F	0.048	0.231	0.222	0.054	0.037	0.069	0.295	0.137	0.087	0.137	0.178
DB	**N**	48	46	46	48	48	48	45	48	48	46	48
	Na	7	3	20	4	5	4	6	6	23	28	10
	Ne	2.26	1.65	15.62	2.26	3.35	2.11	2.72	3.75	13.09	17.13	2.98
	I	1.13	0.68	2.85	1.02	1.29	0.85	1.26	1.44	2.79	3.06	1.43
	Ho	0.50	0.26	0.74	0.52	0.60	0.48	0.44	0.60	0.75	0.80	0.48
	He	0.56	0.39	0.94	0.56	0.70	0.53	0.63	0.73	0.92	0.94	0.66
	HWE	0.945	0.005	**0.000**	0.893	0.426	0.448	**0.000**	0.364	0.325	0.013	0.000
	F	0.10	0.34	0.21	0.06	0.14	0.09	0.30	0.18	0.19	0.15	0.28
NEH	**N**	46	44	44	45	46	46	45	45	46	46	46
	Na	3	4	14	3	5	4	4	5	12	10	8
	Ne	1.77	2.38	5.67	2.31	2.70	2.19	1.87	2.19	5.72	4.81	4.58
	I	0.73	1.00	2.10	0.96	1.19	0.89	0.92	1.07	1.99	1.76	1.70
	Ho	0.46	0.55	0.70	0.62	0.76	0.70	0.36	0.49	0.85	0.70	0.76
	He	0.44	0.58	0.82	0.57	0.63	0.54	0.47	0.54	0.83	0.79	0.78
	HWE	0.653	0.975	0.001	0.643	0.372	0.276	0.008	0.061	0.027	0.850	0.953
	F	-0.05	0.06	0.14	-0.10	-0.21	-0.28	0.24	0.10	-0.03	0.12	0.03
LB	**N**	53	53	46	53	53	53	53	52	53	53	53
	Na	6	9	21	5	6	7	6	7	22	31	6
	Ne	2.12	2.43	15.56	1.91	3.45	2.61	3.37	3.52	17.18	21.86	3.73
	I	1.06	1.31	2.88	0.94	1.36	1.15	1.43	1.45	2.95	3.23	1.45
	Ho	0.45	0.47	0.67	0.43	0.72	0.62	0.42	0.56	0.83	0.85	0.57
	He	0.53	0.59	0.94	0.48	0.71	0.62	0.70	0.72	0.94	0.95	0.73
	HWE	0.558	0.065	**0.000**	0.261	1.000	1.000	**0.000**	0.221	0.040	0.005	0.040
	F	0.14	0.20	0.28	0.09	-0.01	-0.01	0.41	0.22	0.12	0.11	0.23
UB												
	N	48	45	45	48	48	48	48	48	48	48	48
	Na	6	6	19	6	4	4	5	6	24	30	7
	Ne	2.07	1.98	13.06	1.80	3.14	2.19	3.46	3.61	17.07	21.33	2.56
	I	1.03	1.04	2.70	0.95	1.23	0.93	1.37	1.43	2.98	3.21	1.26
	Ho	0.52	0.33	0.69	0.38	0.73	0.58	0.54	0.69	0.88	0.79	0.50
	He	0.52	0.50	0.92	0.45	0.68	0.54	0.71	0.72	0.94	0.95	0.61
	HWE	1.000	0.114	**0.000**	0.003	0.197	0.679	0.003	0.080	0.432	0.209	0.027
	F	-0.01	0.33	0.25	0.16	-0.07	-0.07	0.24	0.05	0.07	0.17	0.18

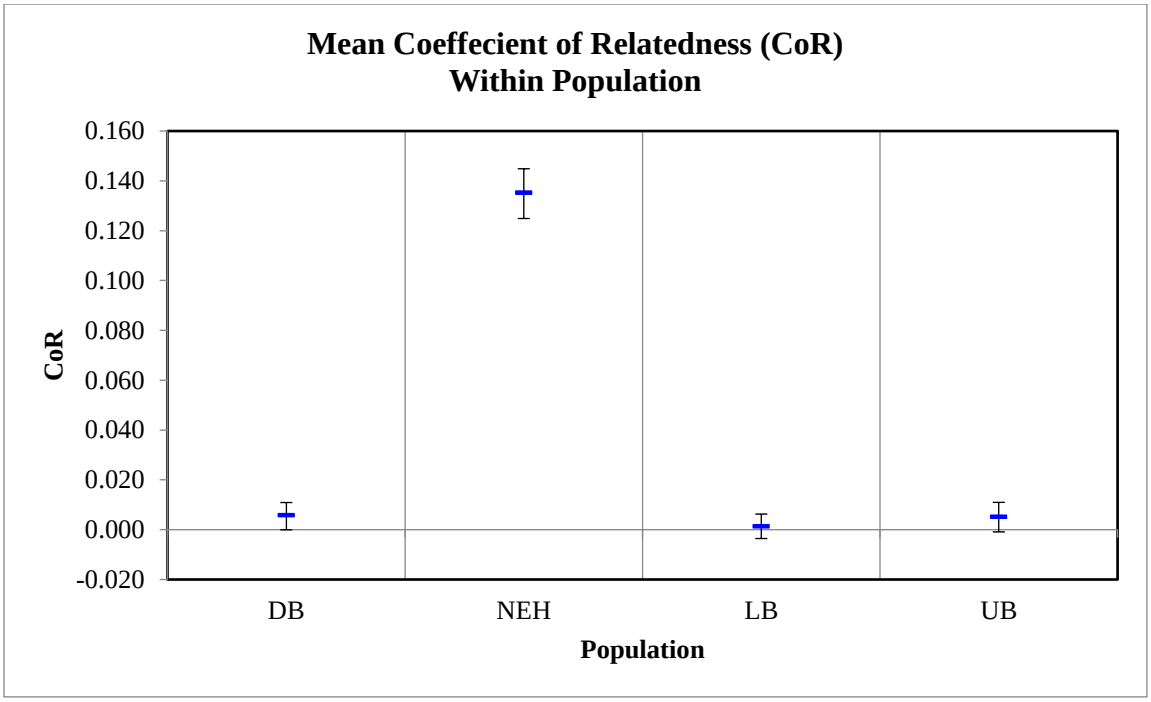

Figure 4.1. The mean coefficient of relatedness (CoR) was estimated according to Lynch & Ritlands (1999) within each population using pairwise comparisons to examine the within population variation. The relatedness coefficient was summed by locus for each sample and averaged across all individuals with a group yielding a CoR for comparison. Bootstrap resampling with replacement was done 999 times to produce a confidence interval around the mean. The CoR index is a degree of relatedness with 1 being identical and 0 being no relationship. NEH had the highest value meaning that individuals within NEH have more in common than the other three groupings. **DB** = Delaware Bay, **NEH** =Northeast High Survival line, **LB** = Lower Bay, **UB** = Upper Bays

Among the data set without population priors, four clusters were identified to be retained and used in the DAPC model. The α-score value used to correct for overfitting suggested 37 principal component (PCs) for optimal output. Cross validation of the overfitting corrected model showed highest predictive success when 60 PCs were used. The final model was adjusted to retain 60 PCs and to use three discriminant functions in population membership assignments and to visualize groups structure on an ordination plane.

Membership probabilities using the DAPC model shows a high percent of successful assignment with an overall assignment probability of 83.5%. Less accurate assignments were seen for DB and the two DIBs populations (DB=79.2%, UB=81.3%, LB= 79.2%), but NEH was correctly assigned 95.7% (Figure 4.2). The model had 79 of the 195 individuals that were admixed, meaning they have less than .9 probability of being correctly assigned to a single group. Most admixing was found outside the NEH strain (96.3%) and there was only two admix samples which NEH had more than 0.2 probability in groups other than its own. Ordination reveals the structure of pre-defined groups using the DAPC model's output, which maximizes between group variation (Figure 4.3). There is near perfect overlap between the two DIBs groups, with the DB slightly overlapping both (Figure 4.3). NEH is a unique cluster with two individuals from DB showing similar genetic structure (Figure 4.3).

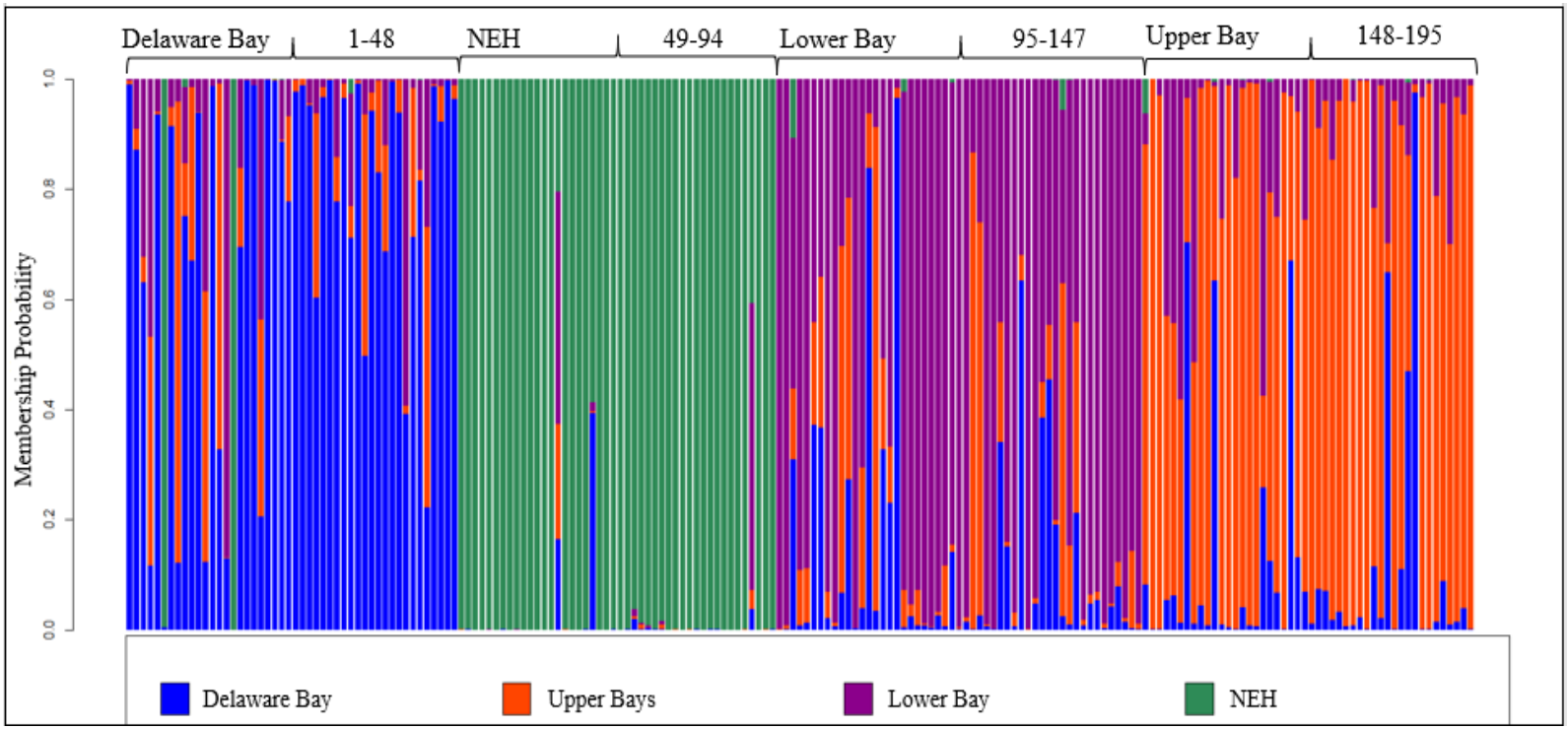

Figure 4.2. Individual outcomes of assignments to groups based on the DAPC clustering model. Each vertical bar represents an individual, and the amount of color symbolizes the probability of assignment to a population. Successful assignment was high (83.5%), and admixing was more common in all populations besides NEH.

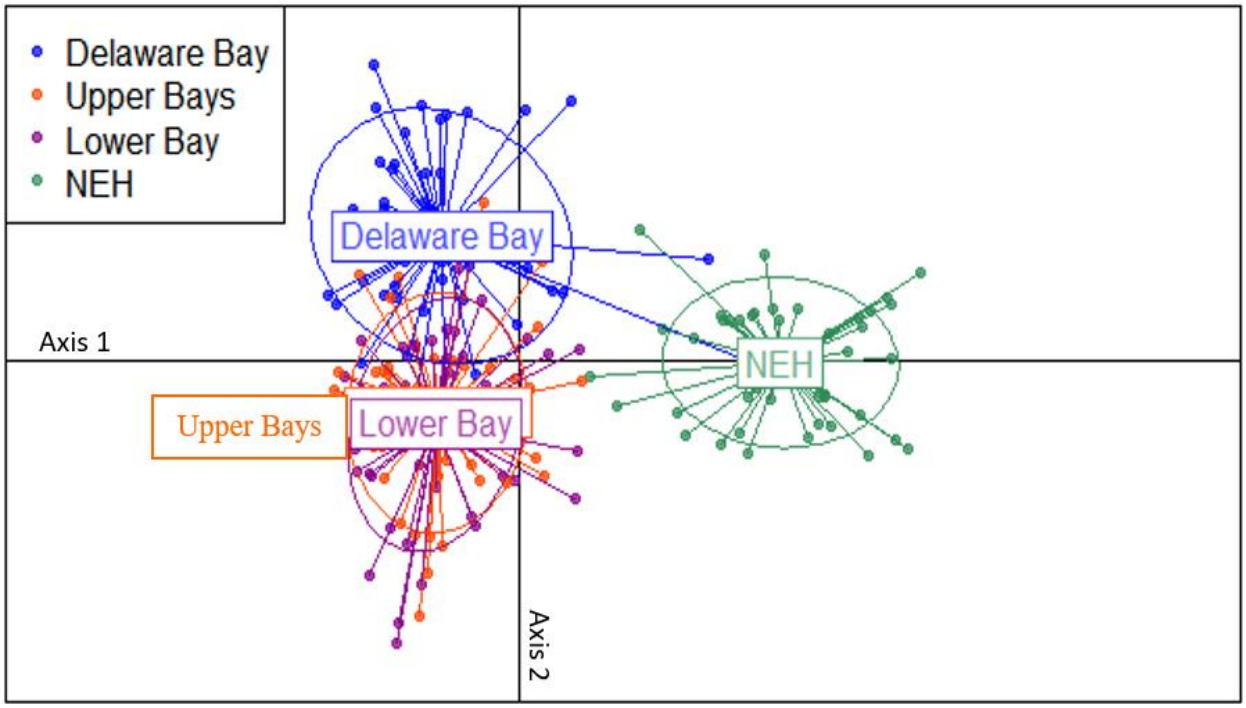

Figure 4.3. An ordination plot of the DAPC model provides a visual assessment of variation between genetic structure for each group. Clear separation of NEH is seen but Upper Bays and Lower Bays are overlapping. The optimal number of principal component and discriminate functions to retain after avoiding over-fitting and cross validations was 60 and three respectively.

4.2. Intertidal Rip-Rap Surveys

A total of 37 candidate sites were identified to be qualitatively assessed for relative density, after searching the entire DIBs coastline for signs of oyster rip-rap. Not all of the sites selected (4/37) were able to be evaluated, because they were inaccessible by land or were on private property (Figure 4.4). The concrete rubble near the southern inlet to LAB was visited using rented kayaks during low tide to determine the relative density (Figure 3.4). Six sites which fit the final selection criteria did not receive transects due to limited time and uncontrollable environmental factors. Fourteen locations received transect surveys; three only had a single transect due to time restriction or weather conditions (Table 4.4). Of the 19 locations which were exclusively qualitatively-defined, only five locations were identified with medium relative densities, which was the highest observed approximation. There were four medium density points within IRB and one within the concrete rubble near the southern entrance to LAB (Figure 3.4, Figure 4.4). Swan cove was the only location where no oysters were observed during the initial assessment.

Most of the surveyed sites occurred within IRB (9), due to the prevalence of large sections of coastal hardening using rip-rap. The survey locations within RB and LAB occurred in the tributaries, distant from the main source of saline water. Highest average densities observed by the transect surveys were in the middle of IRB (4.51-1.11 ind/m^2) and the north side of Burtons Island (1.72 ind/m^2) (Table 4.4, Figure 4.4). The Indian River Inlet site had among the lowest densities, similar to what was observed in RB and LAB locations (Table 4.4, Figure 4.4). The highest average count was Holts Landing (242.25 oysters), which was double the next closest Pots Nets-South (114 oysters). Relatively large intra-location variation exists in all parameters as evidenced by large confidence intervals (Table 4.4).

When it was possible to accurately determine length, oysters' sizes were measured to examine the relative contributions each age class makes relative to the total. Measurements were combined by bay and plotted in a genderless population pyramid to visualize the number of counts for each size class (Figure 4.5). Similar patterns of high abundance in small age classes with a steady decline in abundance as size increased were seen across locations. Not enough oysters were observed at the survey sites in LAB (5) to determine the size structure. All of the LAB oysters were slightly larger, measuring between five to ten centimeters long.

Table 4.4. The summary table shows the location means and 95% confidence intervals (CI) of parameters used in the density calculations. For each transect, density was calculated by dividing the count by the area which was first multiplied the transects mean roughness to account for total surface area. Roughness was measured three times per transect to produce a mean before then being averaged again by location. Locations that have only a single transect do not have confidence intervals. RB = Rehoboth Bay, IR = Indian River Bay, LAB = Little Assawoman Bay.

Bay	Location	Transects	Count (ind)		Roughness Index		Area (m^2)		Density (ind/m^2)	
			Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
RB	Rehoboth Bay Mobile Homes	1	43	na	1.17	na	79.6	na	0.54	na
	Rehoboth Bay Country Club	2	24.00	±9.70	1.64	±0.44	80.95	±6.31	0.29	±0.10
IR	Pots Nets-South	3	114.00	±0.92	1.84	±0.55	77.17	±24.36	1.60	±0.53
	Pots Nets-Southeast	3	34.67	±15.90	1.63	±0.12	74.37	±5.03	0.46	±0.18
	White House Beach	4	80.25	±41.22	1.74	±0.13	69.15	±12.09	1.11	±0.45
	Peninsula Golf & Country Club	1	420	na	1.58	na	93.2	na	4.51	na
	Burtons Island-North	2	91.33	±57.53	1.21	±0.07	57.63	±8.19	1.72	±1.22
	Burtons Island-South	3	49.00	±32.13	1.16	±0.04	56.33	±8.71	0.92	±0.66
	Indian River Inlet-North	3	9.67	±3.50	1.85	±0.08	131.77	±20.19	0.08	±0.04
	Indian River Inlet-South	3	13.67	±3.50	1.44	±0.12	63.20	±9.36	0.22	±0.07
	Holts Landing	4	242.25	±88.25	1.56	±0.20	70.73	±17.54	3.61	±1.29
LAB	Sassafrass Landing	3	0	±0.00	1.32	±0.16	27.33	±10.29	0	±0.00
	Strawberry Landing	3	2.00	±1.85	1.54	±0.22	23.30	±5.09	0.08	±0.07
	Swan Cove	1	0	na	1.64	na	42.19	na	0.00	na

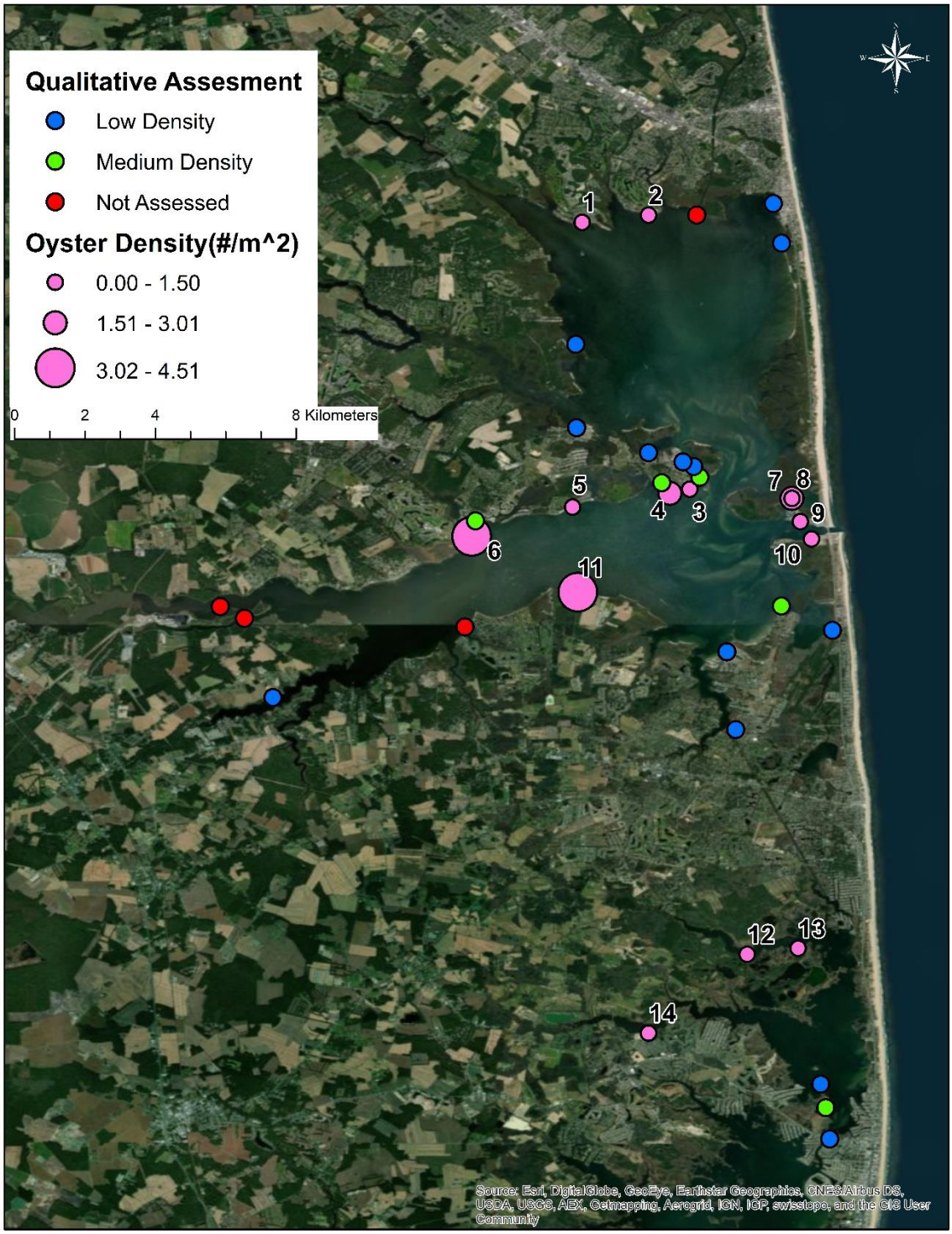

Figure 4.4. The results from all the candidate sites visited are shown in the map below. The locations which received transect surveys are numbered and represented by variously sized pink dots, which correspond with the observed average densities. The relative density at each candidate location qualitatively assessed prior to surveys are depicted with blue (low density) and green (medium density) dots. Some of the candidate locations were not accessible from land or on private property and could not be assessed (red).

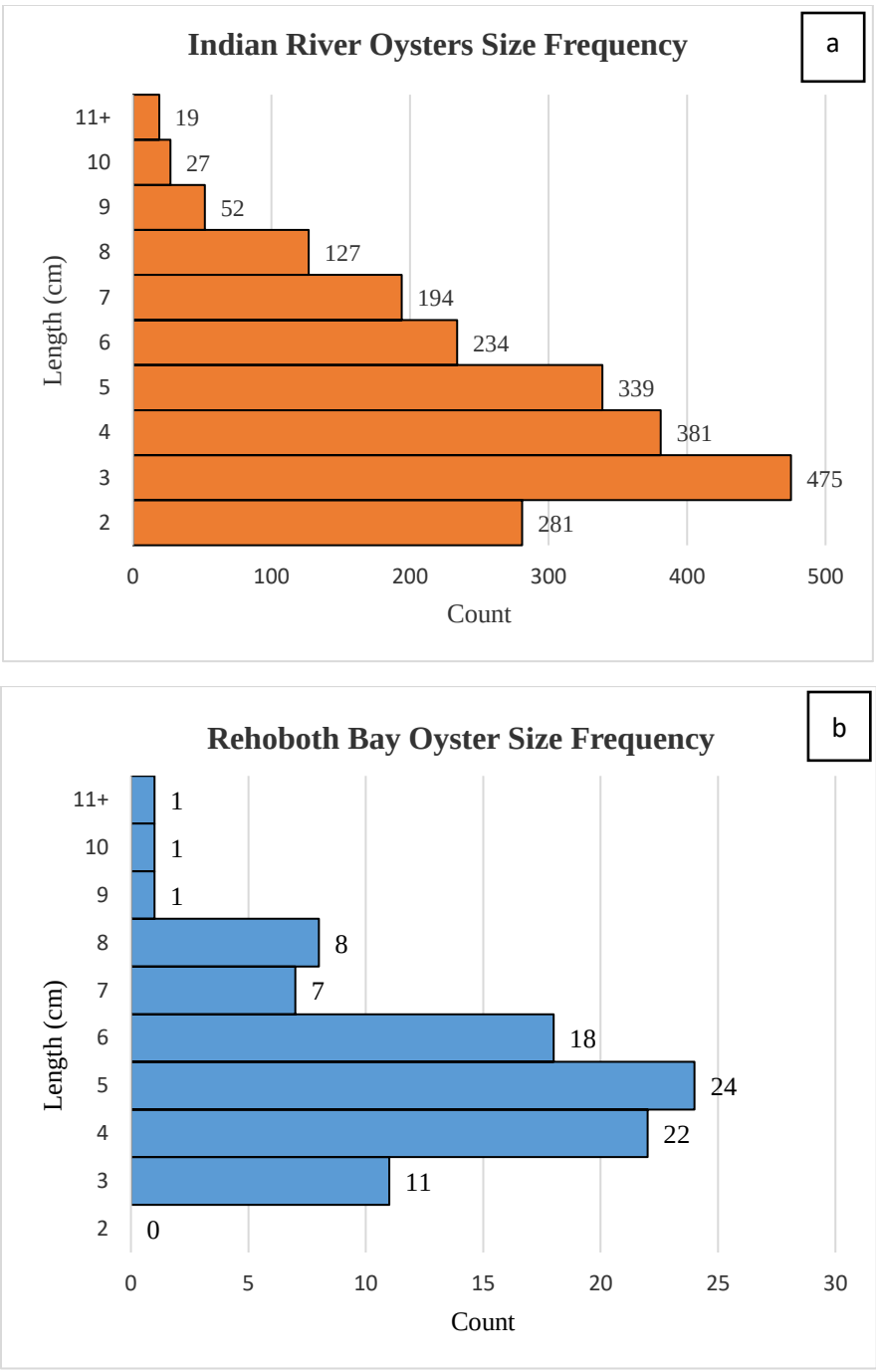

Figure 4.5. The size frequency of oysters counted during the survey are grouped by bay and displayed in a population pyramid format. (a) Indian River Bay (IRB) had 2129 oysters, (b) Rehoboth Bay (RB) had 93, and Little Assawoman Bay (LAB) only had 5, so that pyramid was not displayed. Similar patterns are seen in both graphs, as the majority of the population is in the smaller size class and steadily decreases with increasing size.

CHAPTER 5

DISCUSSION, CONCLUSIONS, AND FUTURE RECOMMENDATION

5.1. Discussion

5.1.1. Genetic Analysis

Genetic analysis provided a baseline for the genetic diversity of oyster, which naturally set within the DIBs. The genetic profile between the UBs and LB was found to be similar in all of the indices used, suggesting they are from a similar origin. Conversely, the DIBs groups differed significantly from the NEH while more closely resembling the wild DB across all analysis. This study was unable to detect any contribution of NEH to the wild population during the summer of 2016. A variety of factors including experimental design, environmental variation, and oyster life history are suggested to provide possible explanations of the observed results.

The monitoring program which developed the spat collector design, reports site averages around 500 spat per tile, but large annual variation exists (University North Carolina Wilmington Spat Monitoring Project 2013). Relatively low recruitment to the DIBs spat collectors (n=0-3 spat per tile) occurred during the summer of 2016. However, nearly all spat representing LAB (n=47) were found on aquaculture cages as opposed to tiles at the Narrows. It is possible that the live NEH oysters in aquaculture cages nearby influenced settlement behavior by passively sending cues to attract nearby larvae (Keough and Downes 1982). This is not the only factor influencing settlement, because no spat was found on the two other cages, but spat was captured on the tiles at those same sites.

Making comparisons between the bays would not have been possible if the aquaculture cage was not present in LAB. Subsequently, a large recruitment event could have easily been

missed if the trays were not in place. This highlights tiles limited effectiveness to quantify recruits in systems with low larval supply, because the inherent recruitment variation creates noise which masks any signal. (Ortega and Sutherland 1992, Gaines and Bertness 1992). A larger surface area or increased tile sampling effort would provide better resolution to monitor spat when recruitment is limited, however it would also increase material and labor costs. Regardless, the patterns in observed recruitment highlights the large spatial variation at small scales and the limited recruitment potential within the DIBs. Low levels of recruitment present a concern for long-term viability because of a naturally high juvenile mortality rate that leads to restricted population growth (Gosselin and Qian 1997). This justifies restoration approaches such as oyster gardening and stocking, which attempt to bolster the breeding population.

To ensure they all recruited during the same season, all DIBs spat used in genetic analysis consisted of spat collected only from tiles and the exterior of the aquaculture trays. However, relying solely on capturing natural set for samples puts the experiment at the mercy of varying natural conditions. Higher per site recruitment was expected, but enough samples were collected to provide meaningful inferences about the genetic variation among and between the groups defined by the research questions (Selkoe and Toonen 2006). Fortunately, many genetic markers have been identified for the eastern oyster in pursuit of breeding disease resistance, for genetic monitoring of diversity, and to improve culture for farming (Reece et al 2004, Quilang 2007, Zhang and Guo 2010). Methods were derived from Wang et al. (2010), because the multiplex design allows for high throughput sampling and was designed for family distinction in NEH lines.

High levels of amplification success are indicative of high-quality DNA extraction, and resampling found low levels of discrepancy in allele scoring; this ensures genotyping error is

limited. Therefore, all samples and markers were included in the analysis of groups genetic structure. General reductions of NEH allele characteristic measurements (N, Ne, and I) are expected from genetic drift caused by an artificially small breeding population (Lacy 1987). The selection imposed by breeding reduces variation, thereby increasing the remaining allele's frequencies (Doebly et al. 2006). This creates a unique genetic profile, relative to more admix wild populations.

The genetic similarity of the two DIBs groups and the unique genetic signature of NEH was a recurring pattern across all statistical indices and multivariate analyses preformed. The two DIBs groups Fst values were high and did not differ significantly, suggesting they are similarly structured. Low Fst values for any group compared against NEH showed significant differences between the genetic structure of the hatchery and wild strains. The DB was nearly significantly different from both DIBs groups, suggesting some similarities between their genetic profiles. The mean coefficient of relatedness estimates shows a similar pattern to Fst values. A higher degree of individual relatedness is seen within NEH than in any other group. All the other groups show values just above no relatedness. These results make sense given that NEH came from an artificial population with a controlled breeding, where alleles are not mixed randomly and are sheltered from natural selection processes. The pattern continues into the multivariate DAPC analysis, where the model was nearly 15% more accurate at correctly assigning individuals to NEH and represented little of the admixed percentage in any other groups. The DB and DIBs have more genetic structure overlap, because they have a wider range of alleles.

Wild animals typically have a larger potential breeding population and are exposed to environmental and spatial variations, which introduce numerous selective and random genetic drivers to increase diversity (Selkoe et al 2010). More genetically diverse populations have more

alleles, increasing the amount of possible genetic combinations of haploid cells (Hartl 2000). The only two individuals incorrectly assigned to NEH by the optimal DAPC came from DB. It is most like due to a combination of alleles which are scarce in DB but became common in NEH. The NEH line has an origin from the DB, so a rare allele in wild conditions was made more prevalent after many generations of selective breeding. This concept makes determining the contribution of NEH to natural set populations in the DIBs difficult, because the alleles are not unique to the region (Gaffney 2005).

This analysis was unable to detect any contribution of NEH descendants in the DIBs based on the population assignments from the DAPC model. However, it is highly possible that the sample sizes were not large enough to detect change (Gaffney 2005). Previous studies have detected small signals of successful settlement of hatchery strains using much higher sample sizes but still at very low frequencies (Milbury et al. 2004, Hare et al. 2006, Varney 2018). The DIBs spats were analyzed from the 2016 recruiting class, but the cultivation of the NEH line in oyster gardening has been occurring for fifteen years. Without previous understanding of the genetic structure of variation in recruitment, success could have resulted in spread of alleles in a unique way and over time led to a slightly different genetic structure of the population of DIBs compared to DB. Multiple introduction events are shown to promote population expansion and increased genetic variability (Roman and Darling 2007). It is also possible that purebred or hybrid NEH oysters were surviving in 2016 but were not captured at select sampling locations.

Adult and spat NEH oysters are known to survive well in a variety of locations and stressors, but larvae survival is unknown (Proestou et al. 2016). NEH oyster larvae are exposed to different selective pressures in the wild, which could reduce fitness and success of survival. In the presence of heavy disease loads, hybrids containing resistance have increased survival and

could proliferate the spread of disease-resistant alleles. The presence of disease-resistant alleles, even in low frequencies, could be the reason for anecdotal increases in the DIBs oyster populations (Ellis et al. 2000). However, in absence of disease, a resistant allele may have reduced fitness, shifting selective pressures.

Assessing the population structure in marine organisms is complicated by the bipartite life history, which is difficult to fully assess (Paulay and Meyer 2006). Oysters pose a particular problem because they have been moved around from areas of high density to low for centuries, with little documentation of the historic magnitude (McCay 1998).

5.1.2. Intertidal Rip-rap Surveys

If resource managers had an abundant supply of time and money to invest in accurately estimating population densities, it would still be met with great difficulty (Witmer 2005). All surveys are subject to some level of viability bias that needs to be recognized and accounted for when possible (Samuel et al. 1992). A simple technique to limit the influence of biases is in the design of survey methods to answer a specific research question or resource management situation. The purpose of this survey was simply to document current densities of oysters within the most suitable habitat in the DIBs and establish locations where measurement can be repeated through time to understand the dynamics.

Oyster distribution is known to be complex and influenced by a variety of physical, chemical, and biological components (Puckett et al. 2018). These transect surveys were not designed to explore the complex interaction of abiotic and biotic factors effecting the distribution of oyster. The research goal was to develop site-specific density measurements as baseline comparisons for future monitoring, allowing for better documentation of the temporal dynamics

of the oyster in this system. This survey was designed to limit the impact of zero inflating phenomena that occur when species are fairly cryptic or rare (Denes et al. 2015). This experimental design attempted to account for rarity by using large area sampling units to quantify oyster densities within rip-rap habitats.

It is important to keep in mind that the site selection process creates a bias by homogenizing habitats surveyed to locations which have rip-rap structures, are large, and are easily assessable. Rip-rap structure is inherently linked to human's, as an effort to protect the corresponding land from erosion. Typically, developed areas receive more armoring, and the corresponding land use may influence suitability for oyster settlement and survival. Most sites visited were in the IRB at residential communities, harbors, and natural areas where rip-rap is common. The other two bays have coastal armoring, but seawalls were more common and were not addressed in the scope of this study.

A total of 33 locations received some level of oyster density designations throughout the entire survey process. The qualitative assessments contain less detailed information than the transect surveys but require significantly less effort, allowing for increased number of locations to visit. When data from both methods are combined, patterns of natural settlement were observed. The qualitative and quantitative studies found the highest levels of density to be within IRB. This is not surprising as IRB is the most flushed and has polyhaline conditions ideal of high oyster survival (La Peyre et al. 2009). A groundwater spring near Holts Landing State Park is influencing the salinity and maybe creating optimal conditions as overall, most oysters and highest average density was seen there (Russoniello et al 2013). Holts Landing is known to have received multiple plantings of oysters from the gardening program (Reckenbeil 2013). Both of these processes could be affecting the values seen in the center of IRB.

All of the locations visited in RB had oysters occurring at sparse levels throughout the rip rap. The two locations suitable for transects were of the northern portion, and values reflected similarly. The Long Neck peninsula that separates the two UBs had no sites that were transect surveyed but four that fit criteria. There are many trap-like embankments that warrants further investigation, because oyster was seen throughout the region. LAB was similar to RB with all the transect sites occurring in the northern portion of the bay. However, they were the only sites that fit the survey criteria in LAB. A piece of land that could not be surveyed due to hazardous concrete rubble scattered along the shoreline had medium and low densities of oysters. This area was qualitatively assessed by kayak, because it represents the largest possible habitat for oysters in LAB and is likely where most of the wild oysters within the bay occur. The oyster gardening program has the majority of its sites in LAB, and the Fenwick Island community is located directly across from the concrete rubble. This site was not sampled for genetic analysis, but it is possible that gardening oysters are settling there and thus warrants further investigation.

Oyster densities were much lower than what is reported in nearby intertidal waters such as the Chesapeake and Delaware Bays (Southworth et al. 2010). Oysters at these low levels are no capable of providing ecosystem services at a level that is impactful. The size frequencies mirrored what has been reported at many sites throughout the east coast (Theuerkauf and Eggleston 2015). Oysters that make it to settlement have high mortality with only a few surviving to large sizes. The number of large oysters is a proxy for females in the system as oyster change sex when they age.

5.2. Conclusions

It is difficult to determine the true effect of NEH oyster's contribution to natural set DIBs populations based on a single recruitment event during a specific time of year. Multiple years of

study are needed to fully understand local population dynamics and gene flow given the known natural variation in oyster recruitment. Oysters within the two DIBs locations were found to be genetically similar and deviated slightly from the DB oysters. It is likely that DIBs oysters have an origin from the DB but no signal of NEH was detected in this study. The DIBs have relatively low oyster densities but locations within the center of IRB and South LAB are where they are most abundant. Baseline distribution and genetic diversity of natural set oysters was completed to set up future comparisons and studies of oyster demographics in the DIBs. There is an opportunity to better understand how to return a functional oyster population in larvae limited systems, as oyster restoration continues to expands and oyster aquaculture begins.

5.3. Future Recommendations

Continued genetic and demographic monitoring of wild set oysters is needed to determine if the DIBs have consistently low recruitment or if periodic high influxes occur, as oysters commonly have episodic recruitment events (Ortega and Sutherland 1992). The sites which received transect surveys should be resampled at least every two years to track the demographic changes that occur as shellfish aquaculture industry enters the bay. This sampling method was designed to handle low levels of oyster counts, but if oysters become more abundant this method would be impractical, and method changes should be calibrated to ensure meaningful comparisons.

This study identified areas where oysters are more prevalent than in other parts of the bay. These locations in the central part of IRB, Long Neck peninsula, and the concrete rubble near the mouth of LAB are a larval sink and should be the focal points of restoration and natural set oyster research in the DIBs. Enhancing areas where survival and settlement appear to be already occurring naturally could allow for spillover effects as more and more larvae are

produced. The western portion of IRB was not very well represented in this survey design and warrants further study.

The genetic diversity of natural oysters should continue to be monitored as there is a unique opportunity to understand the impacts of aquaculture. Ideally, detailed documentation on information regarding the number of oysters imported and their origin will aid with interpretation of future analysis. Genetic sampling should not rely on natural set due to limited availability and samples should be collected with permission from areas where high density already occurs. Sampling at different times of year and across multiple years is needed to better understand the impacts of NEH oyster's contribution.

References

Andres, A. S. (1992). Estimate of Nitrate Flux to Rehoboth and Indian River Bays, Delaware, Through Direct Discharge of Ground Water. *State of Delaware Geological Survey, Open File No.35*. Retrieved from https://www.dgs.udel.edu/sites/default/files/publications/OFR35.pdf

Bachtrog, D., Weiss, S., Zangerl, B. & Schlotterer, C. (1999). Distribution of dinucleotide microsatellites in the Drosophila melanogaster genome. *Molecular Biology and Evolution*, 16, 602–610.

Bagget, L.P., Powers, S. P., Brumbaugh, R. D., Coen, L. D., Deangelis, B. M., Greene, J. K., Zu Ermgassen, P. S. E. (2015). Guidelines for evaluating performance of oyster habitat restoration. *Restoration Ecology*, *23*(6), 737–745. https://doi.org/10.1111/rec.12262

Balaguer, L., Escudero, A., Martín-Duque, J. F., Mola, I., & Aronson, J. (2014). The Historical Reference in Restoration Ecology: Re-defining a Cornerstone Concept. *Biological Conservation*, *176*, 12–20. https://doi.org/10.1016/j.biocon.2014.05.007

Bahr, L. M., & Lanier, W. P. (1981). The Ecology of Intertidal Oyster Reeds of the South Atlantic Coast: A Community Profile. *Fish & Wildlife Services, Office of Biological Services 81*, 118.

Barber, Bruce, J., Ford, S. E., & Wargo, R. N. (1991). Genetic variation in the timing of gonadal maturation and spawning of the eastern oyster, *Crassostrea virginia* (Gmelin). *Biological Bulletin*, *181*, 216–221. https://doi.org/10.2307/1542092

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., … Guo, X. (2011). Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. *BioScience*, *61*(2), 107–116. https://doi.org/10.1525/bio.2011.61.2.5

Beninger, P. G., & Veniot, A. (1999). The oyster proves the rule: Mechanisms of pseudofeces transport and rejection on the mantle of Crassostrea virginica and C. gigas. *Marine Ecology Progress Series*, *190*, 179–188. https://doi.org/10.3354/meps190179

Beninger, P. G., Cannuel, R., & Jaunet, S. (2005). Particle processing on the gill plicae of the oyster Crassostrea gigas: Fine-scale mucocyte distribution and functional correlates. *Marine Ecology Progress Series*, *295*(3), 191–199. https://doi.org/10.3354/meps295191

Brown, B.L., Franklin, D.E., Gaffney, P.M., Hong, M., Dendanto, D., Kornfield, I., (2000) Characterization of microsatellite loci in the eastern oyster, Crassostrea virginica. *Molecular Ecology* 9, 2217–2219.

Borsje, B. W., van Wesenbeeck, B. K., Dekker, F., Paalvast, P., Bouma, T. J., van Katwijk, M. M., & de Vries, M. B. (2011). How ecological engineering can serve in coastal protection. *Ecological Engineering*, *37*(2), 113–122. https://doi.org/10.1016/j.ecoleng.2010.11.027

Burger, J., Niles, L., & Clark, K. E. (1997). Importance of beach, mudflat and marsh habitats to migrant shorebirds on Delaware Bay. *Biological Conservation*, *79*(2–3), 283–292. https://doi.org/10.1016/S0006-3207(96)00077-8

Carriker, M.R. and P.M. Gaffney. (1996). A catalogue of selected species of living oysters (Ostreacea) of the world. *Chapt 1*- In V.S. Kennedy, R.I.E. Newell, and A.F. Eble (eds). *The Eastern Oyster Crassostrea virginica*. MD Sea Grant Publication. pp 1-18.

Capone, M., Grizzle, R., Mathieson, A. C., & Odell, J. (2008). Intertidal Oysters in Northern New England. *Northeastern Naturalist*, *15*(2), 209–214. https://doi.org/10.1656/1092-6194(2008)15[209:IOINNE]2.0.CO;2

Carlsson, J., Morrison, C. L., & Reece, K. S. (2006). Wild and aquaculture populations of the eastern oyster compared using microsatellites. *Journal of Heredity*, *97*(6), 595–598. https://doi.org/10.1093/jhered/esl034

Carlsson, J., Carnegie, R. B., Cordes, J. F., Hare, M. P., Leggett, A. T., & Reece, K. S. (2008). Evaluating Recruitment Contribution of a Selectively Bred Aquaculture Line of the Oyster, Crassostrea virginica used in Restoration Efforts. *Journal of Shellfish Research*, *27*(5), 1117–1124. https://doi.org/10.2983/0730-8000-27.5.1117

(CIB) Center for the Inland Bays. (2014). "DE Center for the Inland Bays Oyster Gardening Program" https://www.inlandbays.org/wp-content/documents/Oyster_Gardening_Program.pdf

Connell J.H., & Slatyer R.O. (1977). Mechanisms of Succession in Natural Communities and Their Role in Community Stability and Organization. *American Society of Naturalists*, *111*, 1119–1144. Retrieved from https://www.jstor.org/stable/pdf/2460259.pdf?refreqid=excelsior%3-Ae503842a8bca198056516d0beb71d94c

Cochrane, W.W. (1979). *The Development of American Agriculture: A Historical Analysis*. Minnianplois, Minnisota: U of Minnesota Press.

Coen, L. D., Brumbaugh, R. D., Bushek, D., Grizzle, R., Luckenbach, M. W., Posey, M. H., Tolley, S. G. (2007). Ecosystem services related to oyster restoration. *Marine Ecology Progress Series*, *341*, 303–307. https://doi.org/10.3354/meps341299

Coen, L. D., & Luckenbach, M. W. (2000). Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? *15*, 323–343.

Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., van den Belt, M. (1997). The value of the world's ecosystem service and natural capital. *Nature, 387*, 253-260.

Currin, C.A., Chappell, W.S, and Deaton, A. (2010). Developing alternative shoreline armoring strategies: The living shoreline approach in North Carolina, in Shipman, H., Dethier, M.N., Gelfenbaum, G., Fresh, K.L., and Dinicola, R.S., eds., 2010, Puget Sound Shorelines and the Impacts of Armoring—Proceedings of a State of the Science Workshop, May 2009: U.S. Geological Survey Scientific Investigations Report 2010-5254, p. 91-102.

Dale, J., Price, E. P., Hornstra, H., Busch, J. D., Mayo, M., Godoy, D., … Pearson, T. (2011). Epidemiological Tracking and Population Assignment of the Non-Clonal Bacterium, Burkholderia pseudomallei. *PLoS Negl Trop Dis*, *5*(12). https://doi.org/10.1371/journal.pntd.0001381

Dalrymple, R. W., & Choi, K. (2007). Morphologic and facies trends through the fluvial-marine transition in tide-dominated depositional systems: A schematic framework for environmental and sequence-stratigraphic interpretation. *Earth-Science Reviews*, *81*(3–4), 135–174. https://doi.org/10.1016/j.earscirev.2006.10.002

David, A. T., Goertler, P. A. L., Munsch, S. H., Jones, B. R., Simenstad, C. A., Toft, J. D., … Morgan, E. E. (2016). Influences of Natural and Anthropogenic Factors and Tidal Restoration on Terrestrial Arthropod Assemblages in West Coast North American Estuarine Wetlands. *Estuaries and Coasts*, *39*(5), 1491–1504. https://doi.org/10.1007/s12237-016-0091-3

Davis, N.W. and P.E. Chanley. (1956). Spawning and egg production of oysters and clams. Proc. Natl. Shellfish. Assoc. 46: 40-58

Denes, F. V. D., Silveira, L.F., & Beissinger, S. R. (2015). Estimating abundance of unmarked animal populations: accounting for imperfect detection and other sources of zero inflation. *Methods in Ecology and Evolution*, *6*, 543–556. https://doi.org/10.1111/2041-210X.12333

Doebley, J. F., Gaut, B. S., & Smith, B. D. (2006). Molecular Genetics of Crop Domestication. *Cell*, *127*, 1309-. https://doi.org/10.1016.j.cell.2006.12.006

Eisen, J.A. (1999). Mechanistic basis for microsatellite instability. In: Microsatellites: Evolution and Applications. *Oxford University Press*, Oxford, UK, pp. 34–48.

Elliott, M., Mander, L., Mazik, K., Simenstad, C., Valesini, F., Whitfield, A., & Wolanski, E. (2016). Ecoengineering with Ecohydrology: Successes and failures in estuarine restoration. *Estuarine, Coastal and Shelf Science*, *176*(August), 12–35. https://doi.org/10.1016/j.ecss.2016.04.003

Fulford R. S., Breitburg D. L., Newell, R. I., Kemp, W. M., & Luckenbach, M. (2007). Effects of Oysters Population Restoration Strategies on Phytoplankton Biomass in Chesapeake Bay: A Flexible Modeling Approach. *Marine Ecology Progress Series*, *336*, 43–61.

Fuoco M. (2018). Nitrogen Source Tracking and Benthic Community Assessment in Relation to Oyster Aquaculture in the Delaware Inland Bays. Delaware State University, Dover, DE 19901 USA. Natural Resources Program, Master Thesis, 74pp.

Gaffney, P. (2005). The role of genetics in shellfish restoration. *Aquatic Living Resources*, *19*, 277–282. https://doi.org/10.1051/alr

Gittman, R., Fodrie, F. ., Popowich, A. M., Keller, D. A., Bruno, J. F., Currin, C. A., … Piehler, M. F. (2015). Engineering away our natural defenses: an analysis of shoreline hardening in the US. *Frontiers in Ecology & Environment*, *13*(6), 301–307.

Gittman, R. K., Peterson, C. H., Currin, C. A., Joel Fodrie, F., Piehler, M. F., & Bruno, J. F. (2016). Living shorelines can enhance the nursery role of threatened estuarine habitats. *Ecological Applications*. https://doi.org/10.1890/14-0716.1/suppinfo

Gittman, R., Scyphers, S. B., Smith, C. S., Neylan, I. P., & Grabowski, J. H. (2016). Ecological Consequences of Shoreline Hardening: A Meta-Analysis. *BioScience*, *66*(763). Retrieved from https://doi.org/10.1093/biosci/biw091

Gomez-Chiarri M., Warren W.C., Guo X., Proestou D. (2015) Developing tools for the study of molluscan immunity: the sequencing of the genome of the eastern oyster, Crassostrea virginica. *Fish & Shellfish Immunology*. 46(1):2–4

Gosselin, L. A., & Qian, P.-Y. (1997). *Juvenile mortality in benthic marine invertebrates*. *MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser* (Vol. 146). Retrieved from https://www.int-res.com/articles/meps/146/m146p265.pdf

Guo, X., Wang, Y., DeBrosse, G., Bushek, D., & Ford, S. (2008). Building a Superior Oyster for Aquaculture. *The Jersey Shoreline*, 7–9.

Hare, M. P., Allen, S.K., Bloomer, P., Camara, M.D., Carnegie, R.**.,** Murfree, J., Luckenbach, M., Meritt, D., Morrison, C., Paynter, K., Reece, K.S. & Rose, C.G. (2006). A genetic test for recruitment enhancement in Chesapeake Bay oysters, *Crassostrea virginica*, after population supplementation with a disease tolerant strain. *Conservation Genetics*. 7:717–734.

Hartl D.L. (2000). *A Primer of Population Genetics 3rd Ed*, Sunderland, Massachusetts: Sinauer Assoc, Inc.

Hewatt, W. G. (1937). Studies on Select Marine Intertidal Communities of Monterey Bay, California. *The American Midland Naturalist*, *18*(2), 161–206. https://www.jstor.org/stable/pdf/2420496.pdf?refreqid=excelsior%3Abb91ef0f4b46a69ad14f69bf1ffeb822

Hinrichsen, D. (1998). *Coastal Waters of the World: Trends, Threats, and Strategies.* Washington D.C. Island Press.

Hopkinson, C. S., Cai, W.-J., & Hu, X. (2012). Carbon sequestration in wetland dominated coastal systems—A global sink of rapidly diminishing magnitude. *Current Opinion in Environmental Sustainability, 4*, 1–9. https://doi.org/10.1016/j.cosust.2012.03.005

Iknayan, K., Tingley, M., Furnas, B., & Beissinger, S. (2014). Detecting diversity: emerging methods to estimate species diversity. *Trends in Ecology & Evolution*, *29*(2), 97–106. https://doi.org/10.1016/j.tree.2013.10.012

Jones, C. G., Lawton, J. H., & Shachak, M. (1994). Organisms as Ecosystem Engineers, *OIKOS*, *69*(3), 373–386.

Kennedy, V. S., Breitburg, D. L., Christman, M. C., Luckenbach, M. W., Paynter, K., Kramer, J., … Mann, R. (2011). Lessons Learned from Efforts to Restore Oyster Populations in Maryland and Virginia, 1990 to 2007. *Journal of Shellfish Research*, *30*(3), 719–731. https://doi.org/10.2983/035.030.0312

Keough, M. J., & Downes, B. J. (1982). Recruitment of Marine Invertebrates: The Role of Active Larval Choices and Early Mortality. *Oecologia (Berl)*, *54*, 348–352. https://courses.pbsci.ucsc.edu/eeb/bioe108/wp-content/uploads/2016/01/Recruitment-of-Marine-Invertebrates1.pdf

Kirby, M. X. (2004). Fishing down the coast: historical expansion and collapse of oyster fisheries along continental margins. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(35), 13096–9. https://doi.org/10.1073/pnas.0405150101

Kondolf, G. M., & Micheli, E. R. (1995). Evaluating stream restoration projects. *Environmental Management*, *19*(1), 1–15. https://doi.org/10.1007/BF02471999

Kurlansky, M. (2006). *The Big Oyster: History on the Half Shell*. New York, NY: Ballantine Books.

La Peyre, M. K., Gossman, B., & La Peyre, J. F. (2009). Defining Optimal Freshwater Flow for Oyster Production: Effects of Freshet Rate and Magnitude of Change and Duration on Eastern Oysters and Perkinsus marinus Infection. *Esturaies and Coasts*, *32*, 522–534. https://doi.org/10.1007/s12237-009-9149-9

Lacy, R. C. (1987). *Loss of Genetic Diversity from Managed Populations: Interacting Effects of Drift, Mutation, Immigration, Selection, and Population Subdivision*. *Conservation Biology* (Vol. 1). Retrieved from https://www.jstor.org/stable/pdf/2385830.pdf

Lipcius, R. N., Eggleston, D. B., Schreiber, S. J., Seitz, R. D., Shen, J., Sisson, M., … Wang, H. V. (2008). Importance of Metapopulation Connectivity to Restocking and Restoration of Marine Species. *Reviews in Fisheries Science*, *16*(13), 101–110. https://doi.org/10.1080/10641260701812574

Lubchenco, J., & Menge, B. A. (1978). Community Development and Persistence in a Low Rocky Intertidal Zone. *Ecological Monographs*, *59*, 67–94. http://www.jstor.org/stable/2937

Luckenbach, M. W., Coen, L. D., Ross, P. G., & Stephen, J. A. (2005). Oyster reef habitat restoration: Relationships Between Oyster Abundance and Community Development based on Two Studies in Virgina and South Carolina. *Journal of Coastal Research*, *40*, 64–78.

Lynch, M., & Ritland, K. (1999). Estimation of Pairwise Relatedness with Molecular Markers. *Genetics*, *152*, 1753–1766.

National Research Council. (1992). *Restoration of Aquatic Ecosystems: Science, Technology, and Public Policy.* Washington, DC: National Academy Press

Newell, R.C. (1976) *Adaptation to Intertidal Life*. In: Newell, R.C., Ed., *Adaptation to Environment: Essays on the Physiology of Marine Animals*, Butterworth, Sydney, 1-82.

NOAA. (2007). Status review of the eastern oyster (Crassostrea virginica). *NMFS*, 105.

Nowicki, B. L., and C. A. Oviatt. 1990. Are estuaries traps for anthropogenic nutrients? Evidence from estuarine mesocosms. Marine Ecology Progress Series 66: 131-146.

Marenghi, F. P. 2009. Floating oyster (*Crassostrea virginica*) aquaculture as habitat for fishes and macroinvertebrates in Delaware's Inland Bays. Master's Thesis. Delaware State University, Dover, Delaware. 203 pp.

McGreavy, Bridie. (2016). "Resilience as Discourse". *Publications*. 53. https://digitalcommons.library.umaine.edu/mitchellcenter_pubs/53

MacKenzie Jr., C. L. (1996). History of oystering in the United States and Canada featuring the eight greatest oyster estuaries. *Marine Fisheries Review*, *58*(4), 1–78.

McCan, K. S. (2000). The Diversity-Stability Debate. *Nature*, *405*(228–232).

McCay, B. J. (1998) *Oyster Wars and the Public Trust*, Univ. Arizona Press, Tucson.

McNaughton, S. J. (1977). Diversity and Stability of Ecological Communities: A Comment on the Role of Empiricism in Ecology. *American Society of Naturalist*, *111*(979), 515–525.

Milbury, C. A., Meritt, D. W., Newel, R. I. E., & Gaffney, P. M. (2004). Mitochondrial DNA markers allow monitoring of oyster stock enhancement in the Chesapeake Bay. *Marine Biology*, *145*(2), 351–359. https://doi.org/10.1007/s00227-004-1312-z

Millennium Ecosystem Assessment. (2005). *Ecosystems and human well- being: current state and trends*. Washington D.C.: Island Press.

Munroe, D., Klinck, J. M., Hofmann, E. E., & Powell, E. N. (2012). The role of larval dispersal in metapopulation gene flow: Local population dynamics matter. *Journal of Marine Research, 70*(2–3), 441–467. https://doi.org/10.1357/002240128028518 69

Ortega, S., & Sutherland, J. P. (1992). Recruitment and Growth of the Eastern Oyster, Crassostrea virginica, in North Carolina. *Estuaries, 15*(2), 158–170. Retrieved from https://link.springer.com/content/pdf/10.2307/1352689.pdf

Ozbay, G. (2017). "Evaluation of Oyster Spat Settlement in the Delaware Inland Bays Design and Implementation Spat on Tile" *Department of Agriculture and Natural Sciences,* Report number: DSU-CARS-AQUA#2.

Palumbi, S.R. (2001). Humans as the world's greatest evolutionary force. *Science. 293*, 1786-1790

Peakall, R., Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update, *Bioinformatics, 28*(19), 2537–2539, https://doi.org/10.1093/bioinformatics/bts460

Peterson, C. H., Grabowski, J. H., & Powers, S. P. (2003). Estimated enhancement of fish production resulting from restoring oyster reef habitat: Quantitative valuation. *Marine Ecology Progress Series, 264*, 249–264. https://doi.org/10.3354/meps264249

Perrow, M.R. (2002) *Handbook of Ecological Restoration: Volume 1 Principles of Restoration,* New York City, New York: Cambridge University Press.

Pikitch, E. K., Santora, C., Babcock, E. A., Bakun, A., Bonfil, R., Conover, D. O., … Sainsbury, K. J. (2004). Ecosystem-based fishery management. *Science, 305*(5682), 346–347. https://doi.org/10.1126/science.1098222

Price, K. (1998). A Framework for a Delaware Inland Bays Environmental Classification. *Environmental Monitoring and Assessment, 51*(1–2), 285–298. Retrieved from https://link.springer.com/content/pdf/10.1023/A:1005951706152.pdf

Proestou, D. A., Vinyard, B. T., Corbett, R. J., Piesz, J., Allen, S. K., Small, J. M., … Gómez-Chiarri, M. (2016). Performance of selectively-bred lines of eastern oyster, Crassostrea virginica, across eastern US estuaries. *Aquaculture, 464*, 17–27. https://doi.org/10.1016/j.aquaculture.2016.06.012

Quilang, J., Wang, S., Li, P., Abernathy, J., Peatman, E., … Liu, Z. (2007). Generation and analysis of ESTs from the eastern oyster, Crassostrea virginica Gmelin and identification of microsatellite and SNP markers. *BMC Genomics, 8*(157), 1–11. https://doi.org/10.1186/1471-2164-8-157

Reckenbeil, B.A. (2013). Assessment of oyster restoration along human altered shorelines in the Delaware Inland Bays: An examination of riprap stocked with the eastern oyster

(Crassostrea virginica). Master's Thesis. Delaware State University, Dover, Delaware, 174 pp

Reece, K. S., Ribeiro, W. L., Gaffney, P. M., Carnegie, R. B., & Allen, S. K. (2004). Microsatellite marker development and analysis in the eastern oyster (Crassostrea virginica): Confirmation of null alleles and non-mendelian segregation ratios. *Journal of Heredity*, *95*(4), 346–352. https://doi.org/10.1093/jhered/esh058

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. https://doi.org/10.1007/s10499-009-9271-5

Rothschild, B. J., Ault, J. S., Goulletquer, P. & Heral, M. Decline of the Chesapeake Bay oyster population: A century of habitat destruction and overfishing. *Mar. Ecol. Prog. Ser. 111,* 29–40 (1994).

Russoniello, C. J., Fernandez, C., Bratton, J. F., Banaszak, J. F., Krantz, D. E., Andres, A. S., … Michael, H. A. (2013). Geologic effects on groundwater salinity and discharge into an estuary. *Journal of Hydrology*, *498*, 1–12. https://doi.org/10.1016/j.jhydrol.2013.05.049

Sacande, M. and Berrahmouni, N. (2016), Community participation and ecological criteria for selecting species and restoring natural capital with native species in the Sahel. *Restoration Ecology*, *24*: 479-488.

Samuel, M. D., Kirk Steinhorst, R., & Garton, E. O. (1992). *Estimation of Wildlife Population Ratios Incorporating Survey Design and Visibility Bias*. *The Journal of Wildlife Management* (Vol. 56).

Schlotterer, C. (2000). Evolutionary dynamics of microsatellite DNA. Chromosoma, 109, 365–371.

Schulte, D. M., Burke, R. P., & Lipcius, R. N. (2009). Unprecedented restoration of a native oyster metapopulation. *Science*, *325*(5944), 1124–8. https://doi.org/10.1126/science.1176516

Scudlark, J. R., Jennings, J. A., Roadman, M. J., Savidge, K. B., & Ullman, W. J. (2005). Atmospheric nitrogen inputs to the Delaware Inland Bays: the role of ammonia. *Environmental Pollution*, *135*, 433–443. https://doi.org/10.1016/j.envpol.2004.11.017

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters*, *9*, 615–629. https://doi.org/10.1111/j.1461-0248.2006.00889.x

Selkoe, K. A., Watson, J. R., White, C., Horin, T. Ben, Iacchei, M., Mitarai, S., Siegel, D., Gaines, T., Toone, R. J. (2010). Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Molecular Ecology*, *19*(17), 3708–3726. https://doi.org/10.1111/j.1365-294X.2010.04658.x

Sousa, W. P. (1979). Experimental Investigations of Disturbance and Ecological Succession in a Rocky Intertidal. *Source: Ecological Monographs*, *49*(3), 227–254.

Stinchcomb, G. E., & Driese, S. G. (2011). Pre-colonial (A.D. 1100-1600) sedimentation related to prehistoric maize agriculture and climate change in Eastern North America. *Geological Society of America*, *39*(4), 363–366. https://doi.org/10.1130/G31596.1

Southworth, M., & Mann, R. (1998). Oyster Reef Broodstock Enhancement in the Great Wicomico River, Virginia. *Journal of Shellfish Research, 17,* 1101-1114.

Southworth, M., & Mann, R. (2004). Decadal Scale Changes in Seasonal Patterns of Oyster Recruitment in the Virginia Sub Estuaries of the Chesapeake Bay. *Journal of Shellfish Research*, *23*(2), 391–402.

Southworth, M., Harding, J. M., Wesson, J. A., & Mann, R. (2010). Oyster (Crassostrea virginica, Gmelin 1791) Population Dynamics on Public Reefs in the Great Wicomico River, Virginia, USA. *Journal of Shellfish Research*, *29*, 271–290. https://doi.org/10.2983/035.029.0202

Tallis, H. M., Ruesink, J. L., Dumbauld, B., Hacker, S., & Wisehart, L. M. (2009). Oysters and Aquaculture Practices Affect Eelgrass Density and Productivity in a Pacific Northwest Estuary. *Journal of Shellfish Research*, *28*(2), 251–261. https://doi.org/10.2983/035.028.0207

Thompson, R.J., R.I.E. Newell, V.S. Kennedy and R. Mann. (1996). Reproductive processes and early development. In: V.S. Kennedy, R.I.E. Newell and A.F. Eble, The Eastern Oyster Crassostrea virginica. Maryland Sea Grant College, University of Maryland, College Park, Maryland. pp. 335-370.

Thrush, S. F., Halliday, J., Hewitt, J. E., & Lohrer, A. M. (2008). The Effects of Habitat Loss, Fragmentation, and Community Homogenization on Resilience in Estuaries. *Source: Ecological Applications*, *18*(1), 12–21. http://www.jstor.org/stable/40062107

University of North Carolina Wilmington (UNCW). *Oyster Spat Monitoring Project Data Summary.* UNCW online weblink. Updated 3/21/2013. http://www.ncoystermonitoring.org/datasummary.php

University of North Carolina Wilmington (UNCW). *Oyster Spat Monitoring Protocol.* UNCW online weblink. http://www.ncoystermonitoring.org/MonitoringProtocol.pdf

Varney, R. L., Watts, J. C., & Wilbur, A. E. (2018). Genetic impacts of a commercial aquaculture lease on adjacent oyster populations. *Aquaculture*, *491*(March), 310–320. https://doi.org/10.1016/j.aquaculture.2018.03.060

Walch, M., Seldomridge, E., McGowan, A., Boswell, S., & Bason, C. "2016 State of the Delaware Inland Bays", *The Delaware Center for the Inland Bays*. (2016).

https://www.inlandbays.org/wp-content/uploads/Final-CIB-State-of-the-Bays-2016-low-res.pdf

Wang, Y., & Guo, X. (2007). Development and characterization of EST-SSR markers in the eastern oyster Crassostrea virginica. *Marine Biotechnology*, *9*(4), 500–511. https://doi.org/10.1007/s10126-007-9011-7

Wang, Y., Wang, X., Wang, A., & Guo, X. (2010). A 16-microsatellite multiplex assay for parentage assignment in the eastern oyster (Crassostrea virginica Gmelin). *Aquaculture*, *308*, 28–33. https://doi.org/10.1016/j.aquaculture.2010.05.037

Wheeler, T.B. "New federal budget does not contain funds to build oyster reefs in Maryland or Virginia" *Bay Journal* 6 April 2018. Web

Wilson, G. W. & Fischetti, T. R. (2010). Coastline Population Trends in the United States: 1960 to 2008. *U. S. Cencus Bureau.* 28.

Witmer, G. W. (2005). Wildlife population monitoring: some practical considerations. *Wildlife Research*, *32*, 259–263. https://doi.org/10.1071/WR04003

Woods H, Hargis WJ Jr, Hershner CH, Mason P. (2005). Disappearance of the natural emergent 3-dimensional oyster reef system of the James River, Virginia, 1871–1948. *Journal of Shellfish Research 24*, 139–142.

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics*, *15*: 323-35

Zhang, L., & Guo, X. (2010). Development and validation of single nucleotide polymorphism markers in the eastern oyster Crassostrea virginica Gmelin by mining ESTs and resequencing. *Aquaculture*, *302*(1–2), 124–129. https://doi.org/10.1016/j.aquaculture.2010.02.012

Zimmer-Faust, R. K., & Tamburri, M. (1994). Chemical identity and ecological implications of a waterborne, larval settlement cue. *Limnology and Oceanography*, *39*(5), 1075–1087.

Zu Ermgassen, P. S. E., Spalding, M. D., Grizzle, R. E., & Brumbaugh, R. D. (2013). Quantifying the Loss of a Marine Ecosystem Service: Filtration by the Eastern Oyster in US Estuaries. *Estuaries and Coasts*, *36*(1), 36–43. https://doi.org/10.1007/s12237-012-9559-y