WILD OYSTERS, *CRASSOSTREA VIRGINICA*, IN THE HUDSON RIVER ESTUARY: GROWTH, HEALTH AND POPULATION STRUCTURE

by

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Abstract

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It has been estimated that the Hudson River Estuary (HRE) once had 350 square miles of oyster beds. Overharvesting and pollution during the Industrial Age ultimately led to the near eradication of the species from the estuary. Oysters are known for their filtering effects in minimizing eutrophication and oyster reefs provide habitat to many species of fish, invertebrates and algae. Today, there are no known functional oyster reefs in the HRE, but individual oysters can be found attached to rock and other hard substrate along shorelines. Their distribution, abundance, growth, and health were unknown.

This study identifies locations of where wild oysters, *Crassostrea virginica*, can be found living in the HRE. They were found to exist in geographically separate areas of the estuary identified as Hudson River (HDS), East River, Queens (ERQ), East River, Bronx (ERB), Hackensack River (HKS) and western Long Island Sound (LIS). Two known oyster diseases, MSX and Dermo, were found to be present in the HRE oysters, with the highest prevalence at the HKS location where 100% of oysters sampled tested

positive for MSX. Annual shell growth did not differ among the HRE populations and oysters were found to have the highest condition index at the LIS location. It was discovered that HRE oysters have significantly lighter shells than that of oysters sampled from Delaware Bay. An analysis of metals resulted in highest overall metal concentrations at HKS and significantly different chromium and nickel concentrations at the LIS location between two sampling years. A genetic analysis using mitochondrial DNA and microsatellite markers indicated that HRE oysters show some genetic differentiation from one another, in addition to Delaware Bay and Rhode Island oysters, and that the populations do not exhibit low genetic diversity. Though there is a long history of pollution in the HRE, existing wild oysters in the East River and western Long Island Sound appear to be tolerating their environmental conditions and provide assurance that oyster restoration efforts in these areas of the estuary could be successful.

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OVERVIEW

Oysters, *Crassostrea virginica*, have been a primary fishery in estuaries on the East Coast of the United States from Native American times to the present. It has been estimated that the estuary of the lower Hudson River once had 350 square miles of oyster beds (Kurlansky 2006). Overharvesting and pollution during the Industrial Age ultimately led to the near eradication of the species from the estuary (Waldman 1999). Today the Hudson River Estuary (HRE) lacks the benefits a healthy oyster population can provide by filtering the water and removing phytoplankton to help minimize eutrophication. In addition, oyster reefs provide hard substrate and vertical relief on otherwise flat soft-sediment bottoms that offers habitat to many species of fishes, invertebrates, and algae (Lenihan & Peterson, 1998). For these reasons, the presence of oyster reefs has been linked to the ecological health of an estuary and oyster restoration projects have been developed in areas where they have been extirpated. Currently, there have been anecdotal reports of random isolated findings of oysters in the HRE (e.g., Waldman 1999). However, rigorous assessments of the distribution, abundance, growth and health of the existing wild populations have not yet occurred.

This is the first study to document the status of wild oysters, *Crassostrea virginica*, living in the HRE since their decline in the early 1900's. The information provided by this study should help guide local organizations as to where to locate future restoration projects. These groups may also choose to protect the wild oysters found during this study or develop enhancement projects based from these populations.

Biology of the Eastern Oyster, Crassostrea virginica

The Eastern Oyster lives in estuaries and behind barrier islands along the east coast of North America, from the Gulf of St. Lawrence, Canada to Key Biscayne, Florida, extending southward into the Yucatan Peninsula of Mexico and the West Indies to Venezuela (Stanley & Sellers 1986). There are no other species of *Crassostrea* sympatric with the Eastern oyster in the Mid-Atlantic region (Stanley & Sellers 1986). The Eastern Oyster is a dioecious alternate hermaphrodite (Thompson et al. 1996); it exhibits sex reversal. Oysters are usually male when they first mature and become female as they grow. However, there is also evidence that the process is reversible in subsequent years (Thompson et al. 1996). The adaptive significance of sex reversals in oysters and the factors influencing them are not clear, however there are studies that indicate that food limitation and other environmental stresses result in a more male dominated population (Thompson et al. 1996).

Oyster reproduction occurs by external fertilization of eggs and sperm released into the water column in response to temperature cues during the warmer periods of the year. It has been found that as oysters grow larger during their 12-15 year lifespan, they divert more energy to egg production. Therefore, a larger oyster will expel more eggs into the water column than a smaller oyster. Each female produces 23 million to 86 million eggs per spawning (Stanley & Sellers 1986). There is a 2-3 week period where free-swimming larvae are passively transported by currents. In the last few days they move towards the headwaters of the estuary by remaining on the bottom during ebbtide and moving into the water column during floodtide (Babb 2005) being stimulated to rise

by the increasing salinity of early floodtide (Carriker 1951). The younger stages show a more uniform vertical disposition than the older stages, which remain near the bottom. The younger stages also ebb and flow with the tide, tending to ascend into the surface layers in response to the swifter current velocities of mid flood and mid ebb and remain in areas of sharp haloclines, if present (Carriker 1951).

Predators of the Eastern oyster include starfish, *Asterias forbesi*, the oyster drills, *Urosalpinx cinerea, Eupleura caudate, Thais haemastoma*, the boring sponge, *Cliona celata*, blue crab, *Callinectes sapidus* and polyclad flatworm, *Stylochus ellipticus*. A commensalist is the oyster pea crab, *Pinnotheres ostreum*.

The most common oyster drill, *U. cinerea,* is found on the entire east coast of the U.S. This gastropod bores a hole into its prey's shell by a combination of mechanical and chemical action from an accessory boring organ. Then the oyster flesh is extracted through the hole. The oyster drill is limited to salinities of greater than 12 to 17 ppt. Feeding ceases in most species of drills below about 10˚C and above 30˚C. The maximum feeding rate is at 25˚C (White & Wilson 1996).

The blue crab will crush small spat (with a shell height less than 15 mm) with their claws whereas the shells of larger oysters are usually chipped around the edge with the chelae. Oyster flesh is extracted with mouth parts and chelae tips. Blue crabs cease feeding when temperatures are below 13˚C (White & Wilson 2006).

Starfish cannot invade oyster beds where the salinity falls below 16 to 18 ppt. They open oysters by force and secrete an anesthetic substance from their stomachs to numb the oysters and cause them to gape. The starfish then extends its stomach through the gape and begins to digest the oyster (White & Wilson 2006).

Environmental factors such as food supply, temperature, substratum, pH, salinity, light, depth, turbidity, and population density (crowding) are known to influence growth in bivalves. Oysters can tolerate a range in levels of salinity and pH, but growth is stunted at salinity levels of 7.5 ppt and is almost nonexistent at 5 ppt and below (Chanley 1958). Ten ppt is the minimum salinity needed to grow with 12-27 ppt being the range for normal growth in juveniles and adults (Chanley 1958) Oysters will spawn in waters with a pH range of 6-10. Sperm released outside of that range lose viability. For normal embryonic development, the pH level should be 6.75-8.75 and the optimum pH for growth of oyster larvae is 8.25-8.5 (Calabrese & Davis 1970). *C. virginica* growth rates vary with temperature throughout a wide range (6-32°C) with the highest rates generally observed around 25°C (Galtsoff 1964). Therefore oysters in the warm waters of lower latitudes grow more rapidly. High concentrations of seston or suspended material can reduce the pumping rate and clog the gills of an oyster. If seston accumulates, it may smother and kill the oysters. Silt is especially harmful to oyster eggs. It is usually difficult to isolate any individual growth factor because all variables are not independent of each other.

The Oyster, an Ecosystem Engineer

Oysters feed by capturing suspended material, primarily phytoplankton, with cilia on their gills. Phytoplankton can be abundant in estuaries due to eutrophication which is primarily the result of nutrient loading from anthropogenic sources, especially inputs of nitrogen and phosphorous compounds from agricultural fertilizers, urban sewage, animal waste and atmospheric fallout (Kirby & Miller 2005). Estuaries in which oysters live

tend to be naturally nutrient rich because land-derived nutrients are entering the water by run-off. Nutrient loads contribute to excessive phytoplankton growth, resulting in algae blooms, which are both a source and sink of dissolved oxygen (DO) as the algae grow, settle, respire, die and decompose (Miller & St. John 2006).

If phytoplankton is not sufficiently consumed by suspension feeders such as *C. virginica*, it will accumulate in the water column or on the seafloor where it may undergo decomposition, which further decreases dissolved oxygen (Kirby & Miller 2005). Hypoxia (< 3 mg O_2/L) can occur when DO has declined to levels detrimental to aquatic organisms in the ecosystem. Microbial decomposition is intensified during summer, when temperatures are higher and the availability of labile carbon has been enhanced by recent deposition of algae from plankton blooms (Lenihan & Peterson 1998).

The oyster has become a recommended species in helping to control against the effects of eutrophication due to their historical abundance in estuaries and scientific evidence of their strong filtering ability. However, recent studies have suggested that the filtering power of oysters has been overestimated (Pomeroy 2006). Newell (1988) estimated that in pre-colonial times the oyster population of Chesapeake Bay would have cleared a volume of water equal to that of the bay in 2 to 4 days and with the reduced oyster populations, it would now take 325 days to do the same. However, further analysis has shown that this estimate assumed summer filtration rates on spring blooms when temperatures are cooler and also assumed that the oysters had access to all the phytoplankton in the bay (Pomeroy 2006). Using the March filtration rate of Newell et al. 2005 of 0.45 l g^{-1} h^{-1} in replacement of 5 l g^{-1} h^{-1} in his original estimate, gives an approximate time of 34 days for the large oyster population of pre-colonial times to filter

the upper 9 m of Chesapeake Bay assuming the oysters have access to all the bay water (Pomeroy 2006). In addition, there are other limiting factors to oyster filtration rates that were not accounted for such as the interference of sediment or low water flow where oysters are filtering the same water repeatedly.

Nevertheless, oysters and oyster reefs contribute both biologically and physically to the estuarine ecosystem. Oyster reefs develop as multiple generations settle one upon another and the resulting vertical structure can protect wetlands from wave action. The roughness of the reef increases flow turbulence, improving resuspension and further facilitating the removal of particulate matter by the oysters (Nelson et al. 2004). Oyster reefs also provide refuge, habitat and nesting sites to many species of fish. These benefits, along with their filtering capacity, though it may be more limited than originally suggested, warrants the restoration of oysters to estuaries. In areas like the Chesapeake Bay and Delaware Bay, the maintenance and restoration of oyster reefs is also critical to a commercial fishery. For the HRE, acknowledgment of the biological importance of the restoration of oysters is coupled with their intrinsic value and local environmental stewardship to the overall return of health to the estuary.

Study Site: The Hudson River Estuary

The Hudson River starts at Lake Tear of the Clouds in the Adirondack Mountains and ends at New York City. The 248-km stretch below Troy, New York, is commonly referred to as the Hudson River Estuary (Hellweger et al. 2004). It is also known simply as the Hudson Estuary, the Hudson-Raritan Estuary or near Manhattan as New York Harbor. The system includes a network of tidal straights (Arthur Kill, Kill Van Kull and

the Harlem and East Rivers), open and enclosed bays (Raritan, Jamaica and New York Bays) and tidal mud flats and beaches (O'Shea & Brosnan 2000). The Hudson River constitutes approximately 87% of the total freshwater flow into the estuary (Mueller et al. 1982). The Raritan, Passaic and Hackensack Rivers in New Jersey drain most of the remaining watershed (O'Shea & Brosnan 2000).

The Hudson River once supported rich commercial fisheries, but most of these were shut down in 1976 because of contamination by polychlorinated biphenyls (PCBs). The River still supports a great biodiversity of more than 210 fish species, but only American shad (a species that spends most of its life outside the system) and blue crabs are fished commercially (Levinton & Waldman 2006).

Water Quality of the Hudson River Estuary

Pollutants are discharged to the Hudson River from municipal and industrial wastewater treatment plants, combined sewer outflows, urban storm water, tributaries and nonpoint sources including dry and wet atmospheric deposition and land runoff (Brosnan et al. 2006). The bacterial decomposition of high organic carbon loads from untreated sewage can deplete dissolved oxygen (DO) which is necessary for respiration by oysters and all aerobic forms of aquatic life (Brosnan et al. 2006).

Nutrient loading per area of estuary to the saline portion of the Hudson is probably the highest of any major estuary in North America (Howarth et al. 2006). The HRE suffered from low DO concentrations over much of the Twentieth Century. Summer average DO levels in the Hudson River were typically between 2 and 4 mg/L and were as low as 1 mg/L in surface and bottom waters off of Manhattan from circa

1910-1970 (Brosnan et al. 2006). Regional waterways such as the East River and Arthur Kill began experiencing increased DO beginning in the 1940s (Brosnan et al. 2006). The abatement of sewerage discharges into the estuary began when the Metropolitan Sewerage Commission, established in 1906, began a water quality monitoring program and required the construction of 35 wastewater treatment plants (Metropolitan Sewerage Commission 1912; Suszkowski 1990; Brosnan et al. 2006). In addition, the New York State Environmental Bond Act in the mid 1960s provided $1.7 billion under the Pure Waters Program for the construction of municipal wastewater treatment plants (Brosnan et al. 2006). From the mid-1930s to the late 1980s the amount of untreated sewage discharge declined as treatment plants provided primary treatment, which typically removes 30 percent of the biochemical oxygen demand (BOD) and total suspended solids (TSS) load (Brosnan et al. 2006). In 1972, the Clean Water Act forbade the use of rivers, lakes and harbors as sources for sewage discharge without proper treatment. After this, wastewater treatment plants were upgraded to secondary levels of treatment, to remove 85 percent of BOD and TSS (Brosnan et al. 2006).

Dissolved oxygen did not increase in the Hudson River until the late 1970s, but by the late 1990s, summer average DO levels were typically between 5 and 7 mg/L (Brosnan et al. 2006). Levels of 5 mg/L and above are acceptable for shellfishing, bathing and other recreational use as defined by NYSDEC (Table 1-1). By the mid 1990s all discharges from sewage treatment plants in the New York City region received secondary treatment and DO levels in the most recent years have met the New York State standard as Class 1 for water quality goals, which sets a limit of 4 mg/L for DO, the standard for secondary contact recreation (Howarth et al. 2006). The estuary usually

meets this goal, but does not reliably meet the 5 mg/L standard for primary contact recreation (Howarth et al. 2006).

The City of New York has been collecting water quality data in the Harbor since 1909. The Harbor can be separated into four geographic regions based on water flow and nutrient runoff from freshwater rivers and streams: The Inner Harbor, the Upper East River – Western Long Island Sound, Jamaica Bay and Lower New York Bay – Raritan River (Figure 1-2). Temperature is consistent throughout the estuary with water temperatures as high as 25˚C in the summer months, but average 17˚C annually. Salinity decreases northward in the Hudson River due to the greater freshwater influence with distance from the Atlantic Ocean. There is large variation in the production of phytoplankton, measured as chlorophyll *a*, which is problematic in the Lower Harbor and Jamaica Bay. Chlorophyll *a* is a plant pigment that can be used as an estimate of phytoplankton abundance. Quantifying chlorophyll *a* concentrations in estuaries can be used as an indirect measure of relative food quantity available for filter feeders (Coen & Luckenbach 2000). Levels above 20 μg/L are considered eutrophic.

History of the New York Oyster Fishery

In colonial times, an oyster bed about 1 mile in diameter, known as the "Great Beds" was present just beyond where the Arthur Kill and Raritan River meet in the lower HRE (MacKenzie 1990). Oyster beds were also found along the shore of Brooklyn and Queens, in Jamaica Bay, in the East River, and on all shores of Manhattan. They extended along the Hudson as far up as Ossining, and along the Jersey shore down to

Keyport, in the Raritan, and Hackensack rivers, and on many reefs surrounding Staten Island, City Island, Liberty Island and Ellis Island (Kurlansky 2006).

Evidence of the historical local consumption of oysters is evident by the numerous mounds of shell known as shell middens that have been left behind. These piles have been found all over New York City, in areas that were once along the shoreline before landfilling of the Manhattan shore. Some middens have been dated to before European settlement, when as many as 15,000 Native Americans lived in what is today New York City and as many as 50,000 others lived in the Lenape region, between the Delaware and the Hudson (Kurlansky 2006). Other middens have dated to an even earlier period, like those at Croton Point Park that showed that humans fished there nearly six millennia ago (Anonymous 2001, Limburg et al. 2006). But many date to when the oyster industry was booming, like the deposit that was discovered during the excavation for the 2005-2006 reconstruction of Manhattan's South Ferry Terminal located near Battery Park. Here the shells date from approximately 1825-1840 in a location that was once directly on the Hudson River before landfilling of the Manhattan shore.

In the early 1600s, the Dutch settled and made New Amsterdam their capital at the tip of southern Manhattan. Settlers could feast on the plentiful oysters that extended throughout New York Harbor, East and Harlem Rivers, and up the Hudson as far as Stony Point (Limburg et al. 2006). The oysters then were large enough to be cut into two or three pieces and were so accessible by the seashore that the poorest people in Manhattan were living off of oysters and bread (Kurlansky 2006).

The demise of oysters began in the mid-1700s when most of the streams and brooks in Manhattan were turned into sewers. The edges of lower Manhattan were expanded by landfill and did not have proper drainage. In addition to pollution, the overharvesting of oysters began where they were being taken faster from their beds than they could reproduce. .

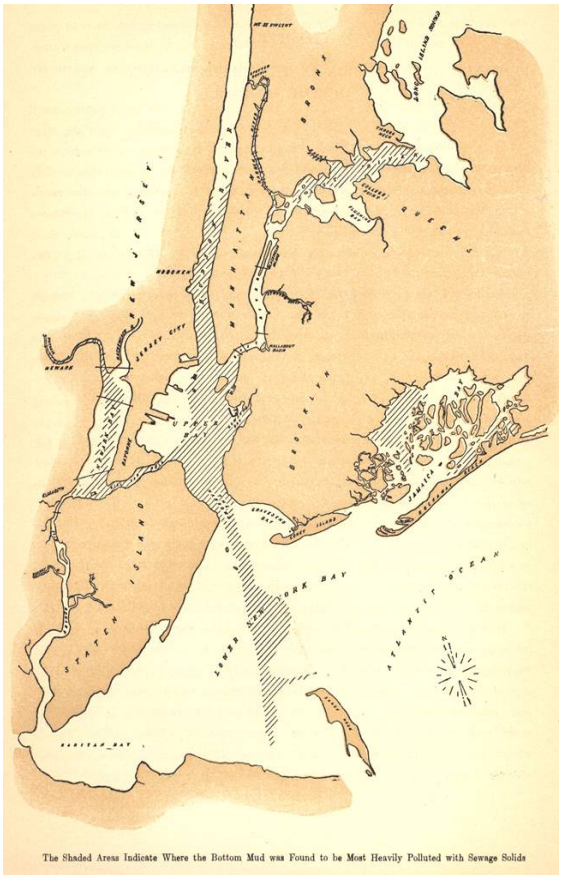
As early as 1810, the oyster beds of Staten Island were showing signs of exhaustion and by the 1820s; most New York beds had been overharvested. The practice of oyster cultivation was begun where oysters were collected at an early stage from one area and planted in another area where maximum growth could be achieved. But once oystermen changed from wild to cultivated oysters, the "big" New York oyster became a rarity. Still, by 1880, the oyster beds of the HRE were still producing 700 million oysters a year (Kurlansky 2006).

In the early 1900s, the link between oysters and diseases such as cholera and typhoid became apparent. The market-sized oysters of the Passaic River and Newark Bay were too polluted to be consumed. Maps published from the Metropolitan Sewerage Commission of New York c. 1910 indicate that oyster reefs existed only where sewage sludge did not (Figure 1-1). Gross sewerage pollution, including seas of floating garbage, was reported in the early 1900's within 15 miles of Manhattan (Metropolitan Sewerage Commission 1912; Brosnan et al. 2006). By 1910, 600 million gallons of untreated sewage were dumped into New York City water every day (Kurlansky 2006). In 1915 and again in 1921, the city closed down all the shellfish beds in Jamaica Bay (Kurlansky 2006). Outbreaks of typhoid linked to oysters from Raritan Bay in 1904 and Jamaica Bay in 1918 closed most of the oyster fishery by 1925 (Brosnan et al. 2006). In 1927, the last

of the Raritan Bay beds was closed and oystering in the HRE came to an end (Kurlansky 2006).

Figure 1-1. Maps from the Metropolitan Sewerage Commission of New York displaying the extent of oyster reefs and sewerage pollution in the Hudson River Estuary (c. 1910).

Oyster reefs (c. 1910)



Sewerage Pollution (c. 1910)

Table 1-1. New York State Department of Environmental Conservation (NYSDEC) Fecal Coliform and Dissolved Oxygen Standards for Saline Waters.

Class	Best Usage of Waters	Fecal Coliform	Dissolved Oxygen (never less than)
SA	Shellfishing and all other recreational use	No standard	5.0 mg/L
SB	Bathing and other recreational use	Monthly geometric mean = > 200 cells/100mL from 5 or more samples	5.0 mg/L
I	Fishing or boating	Monthly geometric mean = > 2,000 cells/100mL from 5 or more samples	4.0 mg/L
SD	Fish survival	No standard	3.0 mg/L

Figure 1-2. New York City Department of Environmental Protection (NYCDEP) Harbor Water Quality Survey Sampling Site Map dividing the estuary into four distinct regions; Inner Harbor, Upper East River – Western LIS, Lower Bay and Jamaica Bay. Note: Passaic River and Hackensack River labels are reversed.

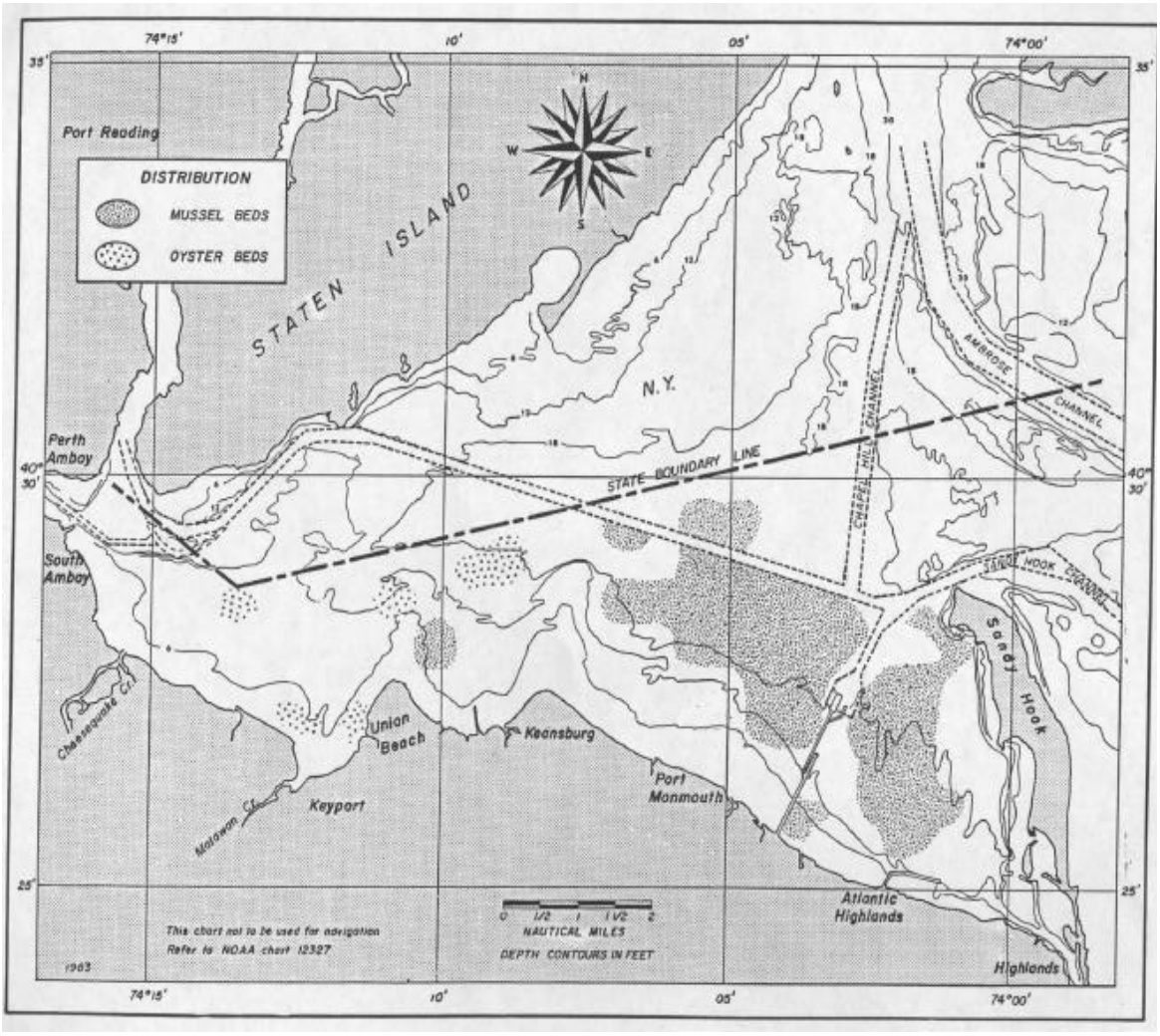
Chapter 2: Live Oyster Survey of the Hudson River Estuary

INTRODUCTION

The geographic extent and abundance of wild oysters either remaining in the Hudson River Estuary from historic populations or having been reestablished in recent times is unknown. The New Jersey Shellfish Inventory in 1983 of Raritan Bay (Figure 2-1) shows estimated areas of where oyster reefs were believed to be present based on scattered individual oysters found, but actual reefs were never confirmed. Waldman (1999) found live intertidal oysters in the East River at College Point, Queens, in the 1990s. The Meadowlands Environmental Research Institute, of the New Jersey Meadowlands Commission, recovered oysters in their trawl nets from the lower Hackensack River when conducting a fish inventory of the River between August 2001 and September 2003 (Bragin et al. 2005). Further up the estuary, in the Hudson River, near the Tappan Zee Bridge, live, young oysters were reported to be occasionally recovered during sampling of an archaeological study of fossil oyster beds (Carbotte et al. 2004). Other than these few documented reports, oysters were rumored to be found along shorelines of Raritan Bay after storm events and to be captured in the nets of fisherman near Piermont Pier, but the true extent of the range of oysters remained unclear.

The shell remains of historic oyster reefs still exist buried under sediment. Geophysical mapping of the Hudson River bottom in a five-mile stretch north of the Tappan Zee Bridge imaged oyster beds covering 30% of the estuary floor (Carbotte et al. 2004). Eroded oyster shell can be found washed up along many shorelines with an occasional shell having both valves intact. Shells that are still attached represent a more recent death and indicate that there may be living oysters nearby.

Figure 2-1. New Jersey Shellfish Inventory, 1983. Distribution of the blue mussel, *Mytilus edulis* and the Eastern oyster, *Crassostrea virginica*



METHODS

An intertidal survey of the HRE for the presence of oysters was conducted in 2006 and 2007 (Figure 2-2 and Table 2-1). Shorelines were walked between one hour before the low tide until one hour after the low tide on and around the full and new moon of the month when the lowest water levels were expected. Locations were first chosen based on previous reports of oysters. Then selected areas with public access to the water were investigated.

Figure 2-2. Map of survey locations in the Hudson River Estuary and the presence or absence of oysters

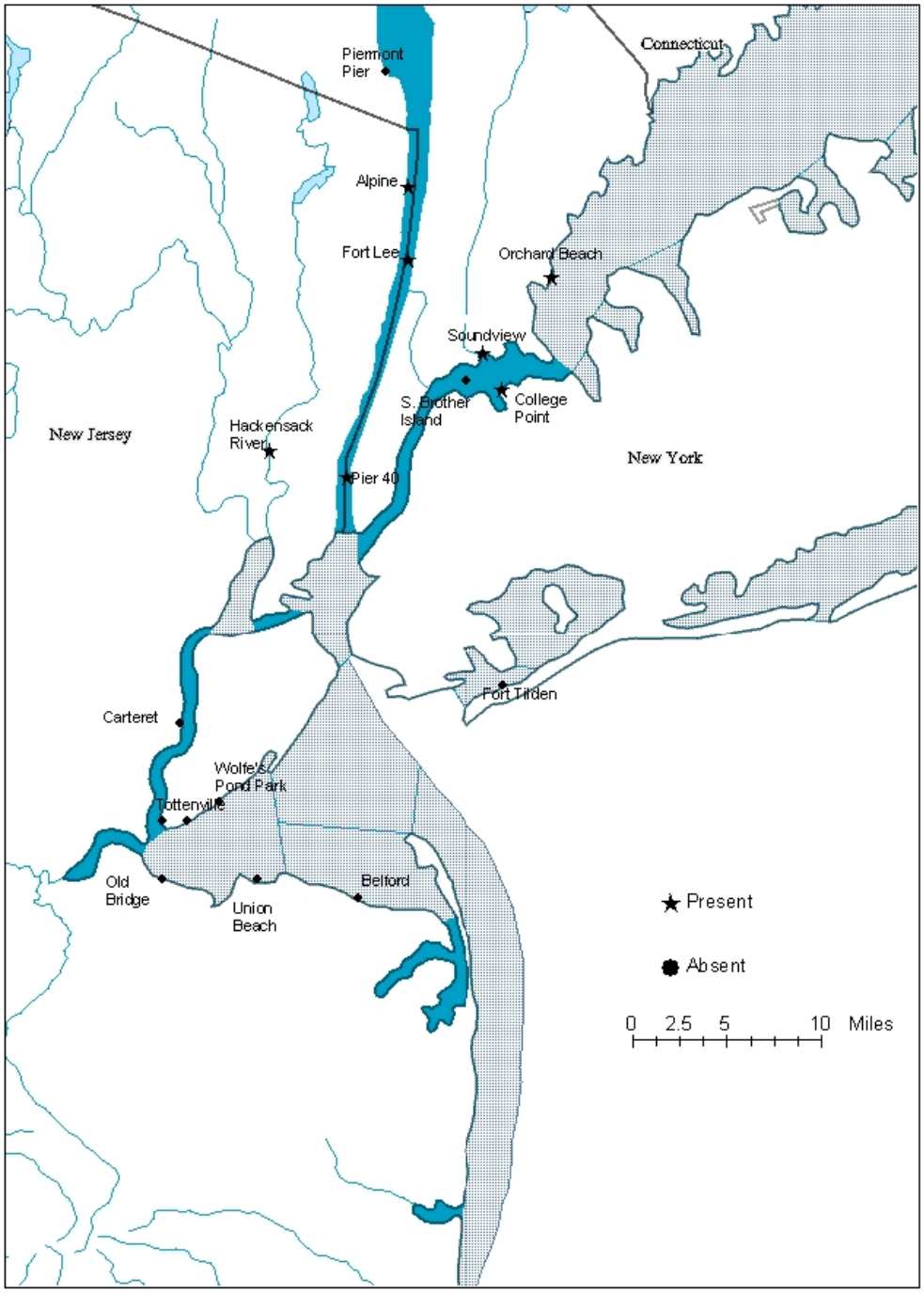


Table 2-1. Hudson River Estuary oyster survey location descriptions.

Location	Coordinates	Date	Area searched	Oysters
Piermont Pier, NY	41°02'28" N, 73°54'16"W	Aug. 2007	Entire length of pier (2400 feet)	Absent
Alpine, NJ (HDS)	40°56'54" N, 73°55'06"W	Sept. 2007	Beach area North of Kearney House and continuing North 350 feet	Present
Fort Lee, NJ	40°52'31" N, 73°56'47"W	Sept. 2007	Area adjacent to parking area (450 Feet)	Present
Secaucus, NJ (HKS)	40°45'47" N, 74°05'13"W	Sept. 2007	Laurel Hill County Park from boat launch south to end of exposed beach area (1200 feet)	Present
Pier 40 Manhattan, NY	40°43'50" N, 74°00'47"W	May 2007	Footings underneath pier searched by kayak	Present
S. Brother Island, NY	40°47'46" N, 73°53'47"W	July 2007	Entire perimeter	Absent
Soundview Park, Bronx (ERB)	40°48'35" N, 73°51'46"W	June 2006	Beach area from where Bronx River meets the East River and east 1600 feet	Present
College Point, Queens (ERQ)	40°47'36" N, 73°50'58"W	July 2007	From access at Poppenhusen Ave and College Place west 500 feet	Present
Orchard Beach Lagoon (LIS)	40°52'03" N, 73°48'03"W	Sept. 2006	From kayak launch to the end of the lagoon (1000 feet)	Present
Carteret Waterfront Park, NJ	40°34'06" N, 74°12'50"W	July 2007	Rocks exposed at boat launch (50 feet)	Absent
Tottenville, Staten Island, NY	40°30'10" N, 74°15'17"W	Aug. 2007	Conference House Park from gazebo south to where Arthur Kill meets Raritan Bay (2600 feet)	Absent
Wolfe's Pond Park, Staten Island, NY	40°31'05" N, 74°11'11"W	Aug. 2007	Beach access from Chester Ave east 800 feet	Absent

Table 2-1 (continued).

Old Bridge Park, NJ	40°27'46" N, 74°15'18"W	June 2007	Area between two jetties (500 feet)	Absent
Union Beach, NJ	40°26'30" N, 74°04'42"W	June 2007	Beach area beginning at Walnut street continuing east 1600 feet	Absent
Belford Ferry Terminal, NJ	40°26'01" N, 74°04'42"W	Aug. 2007	Beach area from the terminal east 1100 feet	Absent
Fort Tilden, Jamaica Bay, NY	40°34'06" N, 73°52'59"W	Feb. 2008	Former Coast Guard Station Marina and area at base of Marine Parkway Bridge and Beach Channel Drive (200 feet)	Absent

RESULTS

The survey identified live oysters in the following locations:

East River, Bronx (ERB) at Soundview Park:

Soundview Park is located where the Bronx River opens into the East River in the Upper East River – Western Long Island Sound portion of the estuary. The NYC Parks Department first reported oysters here, which was confirmed in the summer of 2006. The abundance of oysters is low with 100-300 individuals to be found in a 100-meter intertidal stretch at any given low tide. Many are attached to tires that have become exposed though erosion of the shoreline of what was previously a landfill. Others can be found attached to rock, each other, or other bivalves.

The salinity here usually ranges 21-24 ppt (recorded monitoring data from the NYC Parks Department). The NYCDEP stations (E14, E6) in this portion of the East River (Figure 1-2) have generally low chlorophyll *a* values, below 10 µg/L (NYCDEP 2008) and DO is the lowest of any area throughout the harbor. Summer DO averaged 5.6 mg/L and 4.8 mg/L for surface and bottom waters (NYCDEP 2008).

East River, Queens (ERQ) at MacNeil Park:

MacNeil Park is located directly across the East River from Soundview Park. This is the site of a pilot oyster reef project by James Cervino of Pace University where oyster spat were placed on an experimental reef. Upon attending the launch of his project on July 1, 2007, hundreds of wild oysters were easily found attached to rock immediately west of the site. Salinity here is +/- 23 ppt, which is favorable to oyster survival and growth. Chlorophyll *a* and DO concentrations are the same as described above for ERB.

Long Island Sound (LIS) at Orchard Beach Lagoon:

Orchard Beach is also located in the Western Long Island Sound portion of the estuary. David Kunstler of NYC Parks & Recreation identified the location of living oysters in Orchard Beach Lagoon. During a visit on September 14, 2006, several oysters were also found scattered in and around a jetty at Orchard Beach. Summer average surface DO values here as measured at NYCDEP's Station E10 met or exceeded 5.0 mg/L, but average bottom waters were below the 5.0 mg/L NYCDEC standard for bathing (NYCDEP 2008). The frequency and extent of hypoxia are significantly worse traveling northeasterly from the East River into this portion of the Western Long Island Sound (NYCDEP 2004). There were eight instances of hypoxia (DO < 3.0 mg/L) at stations E8 and E10 which are closest to this site from July 14th to September 15th 2008 (NYCDEP 2008). The stations of this region generally have low chlorophyll *a* averages (< 10 µg/L) with the E10 station being the exception averaging 12.7 µg/L in 2008 (NYCDEP 2008).

Hackensack River (HKS):

The Hackensack River empties into Newark Bay in the Inner Harbor portion of the estuary. Oysters had been reported to be in the area by members of the Hackensack Riverkeeper organization and were reported in the 2001-2003 Meadowlands Environmental Research Institute (MERI) Fish Survey. Oysters were discovered at Laurel Hill County Park in Secaucus, NJ on September 9, 2007, attached to rock along the shoreline. The Hackensack River is bounded only by New Jersey, so is therefore excluded from the NYCDEP Harbor Water Quality Survey. However, the New Jersey

Harbor Discharges Group (NJHDG) prepared a report in consort with NYCDEP in 2006 and reported the 2006 summer average DO for both surface and bottom waters at their Station 15, just south of the location, was 4.00 to 4.79 and 3.00 to 3.99 at their Station 14 north of the site (NJHDG 2006). The seasonal salinity here remains below 10 ppt according to data from the MERI Amtrak Bridge Station sampling location.

Figure 2-3. Exposed oysters at low tide attached to rocks in the Hackensack River (HKS), Secaucus, NJ.

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Hudson River at Palisades Interstate Park (HDS):

Palisades Interstate Park runs along the New Jersey side of the Hudson River from Alpine, NJ, south to Fort Lee, NJ, near the George Washington Bridge. A settlement of spat was first discovered here on September 29, 2007, and small adult oysters were found in subsequent visits. In this portion of the Hudson River, summer DO reached a record high average of 7.4 mg/L and 6.6 mg/L for surface and bottom waters in 2008 (NYCDEP 2008). Chlorophyll *a* values average less than 15 µg/L (NYCDEP 2008). The salinity here can be as low as 2 ppt and can increase to 19 ppt,

with values generally being below 12 ppt (George Washington Bridge Station of the Hudson River Environmental Conditions Observing System of Stevens Institute of Technology) for the last 6 months of 2009.

Other Investigated Locations:

Several live oysters were also confirmed to be attached to Pier 40 in lower Manhattan, the home of the non-profit organization, the River Project. However, access to them was limited and permission could not be given by the River Project for their use. Two live oysters were also found at Fort Tilden in Jamaica Bay; however, this was in a marina location where in 2001 students of Kingsborough Community College and Brooklyn College lowered several hundred oysters in cages as part of an experimental oyster restoration project. Wild oysters could not be found in a suitable oyster habitat area of rocks and other bivalves just adjacent to the marina. Therefore the conclusion was that the two oysters found were non-wild oysters remaining or having descended from the abandoned project.

The New Jersey shorelines of Raritan Bay were extensively searched for the presence of oysters with no result other than an occasional unattached shell. Frank Steimle, formerly of the National Marine Fisheries Service, stated that in 1995, he had found recently dead oysters, both valves still attached, in the Arthur Kill on the south side of Tufts Point in Woodbridge, NJ. However, this area is highly industrialized and several attempts to access the shoreline were unsuccessful due to private property, fences and warnings of contamination. Waterfront access at the Alvin P. Williams Memorial Park in Woodbridge was entirely fenced with warnings posted along the entire stretch of the

park. Further south in Perth Amboy, NJ, public access near the marina area of Front Street was completely bulkheaded with no available shoreline to search. Other than a small area that could be reached at the Carteret Waterfront Park due to the existing boat launch there, access to the Arthur Kill was limited to the Staten Island side of the river. However still, in Tottenville, NY, Staten Island, numerous oyster shells were found broken and eroded, washing up with the waves, but no live oysters were found.

John Vargo, editor and publisher of Boating on the Hudson & Beyond and commercial fisherman near the Tappan Zee Bridge stated that oysters were being pulled up in fisherman nets there. Upon exploring Palisades Interstate Park near that area of the Hudson River, small oysters were found, but none as far north as Piermont Pier. Oysters may indeed exist subtidally in this area of the Hudson River, as this is the same area where live oysters were occasionally recovered during sampling for an archaeological study of fossil oyster beds, but their does not appear to be a strong intertidal population that can be accessed by the shoreline.

DISCUSSION

Oysters can be found in both the intertidal and subtidal zones of estuaries. In warmer regions like South Carolina, approximately 95% of oysters are intertidal, (Willson & Burnett, 2000) however intertidal populations are not as common in the northern part of the range of *C. viginica* due to ice exposure during the winter. This survey was limited to intertidal areas where oyster survival is already at a disadvantage, so it is not surprising that general abundance was low and individual oysters were found as opposed to intertidal reefs. However, there are no known living subtidal reefs in the estuary and with the lack of sophisticated underwater equipment; the shorelines provide an easily accessible area for a visual survey.

The five identified locations are geographically separated throughout the estuary. With the exception of the LIS location, all areas contained large rocks, or in the case of ERB also tires and other debris, jutting out of the sandy bottoms to provide a source of attachment for the oysters. However, oysters were also found attached to small rocks, each other or other bivalves, mostly mussels. For as many live oysters that could be found, there were just as many or more dead oysters.

Habitat at the LIS location was not of a kind considered ideal for oysters. Though oysters could be found attached to rock in the upper intertidal areas, many oysters were found both dead and alive, attached only to a small pebble, sinking into the sandy bottom at the end of Orchard Beach Lagoon. These oysters were visibly larger than oysters found at any other location. How they have managed to survive from year to year and grow to such a large size without being submerged in the sand is unknown. It is possible that dredging or other human disturbance in the area has caused a suspension of silt that

is now settling at the end of the lagoon, smothering the oysters in the lower intertidal areas.

The HDS location had the lowest visual abundance of oysters. With the exception of spat, 10 to 20 oysters, mostly juveniles, could only be found at each of the four site visits. The ERQ location had the highest visual abundance of oysters, with multiple oysters easily found on the same rock or within arm's reach of the same area. Here the exposed beach area at low tide was the widest of all locations.

Many non-profit and New York State agencies began oyster restoration programs without the knowledge of where natural oyster larvae were traveling from or where growth was favored. In 2001, the NY/NJ Baykeeper implemented three oyster restoration projects in the estuary: the Keyport reef, Navesink reef, and Liberty Island reef. Their most successful project was the Keyport reef; however, in 2008 it could no longer be found, having been dispersed by tides and wave action. It was a half-acre oyster habitat created with the deposit of 10,000 bushels of crushed clam and oyster shell (NY/NJ Baykeeper 2005). Ten thousand live oysters were then added to the reef by Baykeeper's oyster gardeners. The Baykeeper planned to add more habitat and oysters each year from Baykeeper's Oyster Gardening Program and from the Remote Setting Program (NY/NJ Baykeeper 2005).

Other groups conducting oyster restoration projects include the New York City Department of Environmental Protection, the Hudson River Foundation and the New York City Department of Parks and Recreation's Natural Resources Group. The River Project on Pier 26 in lower Manhattan has documented spat attached to the pilings underneath their pier, but found that they do not survive through the winter. They have

begun to deposit shell underneath the pier with the hope that the spat will attach and survive when submerged year round.

The waters of the HRE are divided between New York and New Jersey and until recently the two States had opposing rules governing oyster restoration with New York limiting projects and New Jersey being less restrictive. However, in June of 2010, the New Jersey Department of Environmental Protection under a new administration banned all oyster restoration involving the placement of live oysters in restricted waters. Those organizations and researchers with active oyster restoration projects in New Jersey waters were to remove the oysters placed. The concern in both States is for the health of the general public if the oysters are illegally accessed and consumed. With the introduction of live oysters being restricted, the future of oyster restoration in the HRE may be limited to encouraging the settlement of existing wild larvae by placing suitable habitat for attachment. The most ideal location for such placement would be in an area where wild oysters are thriving.

Five locations in which wild oysters can be found in the HRE have been identified. They exist in areas that exhibit varying salinity, DO, phytoplankton levels and environmental contamination from one another. The effects of these environmental factors on shell growth, biomass weight, parasite abundance and metals uptake were unknown. In addition, it was unknown as to whether the oysters were genetically isolated from one another or if they constitute one population.

The objectives of this study were to: (1) determine where oyster growth is favored in the estuary and the contributing ecological factors, (2) determine if two parasite diseases, MSX and Dermo, are present in the oysters, (3) identify local metal

contamination that may be affecting oyster health (4) determine if the populations are genetically isolated from one another and from the general Atlantic Coast population, and (5) provide recommendations for oyster restoration efforts in the HRE.

Chapter 3: Oyster Growth and Condition Index

INTRODUCTION

The oyster shell serves as an exoskeleton, providing protection from mechanical impacts and predators, serving as support to the internal organs and preventing collapse of the mantle cavity. The left valve is larger and more concave than the right and the oyster usually attaches to a hard substrate by its left valve. Bottom types, salinity, temperature, current velocity, turbidity, direct sunlight, calcium concentration and chemical pollution all contribute to shell morphology (Galtsoff 1964; Medcof & Kerswill 1965; Frazier 1976; Palmer & Carriker 1979; Seilacher et al. 1985; Carriker 1996). Shells of oysters grown under unfavorable conditions are often thin and fragile. In a tributary of Chesapeake Bay contaminated by manganese, iron, zinc, copper and cadmium, young oysters developed significantly thinner valves than control oysters over a period of a year (Frazier 1976; Carriker 1996).

Oyster shells continue to grow throughout the life cycle, though more slowly with age (Carriker 1996). The ligamental area grows incrementally in a ventral direction, secreting external layers that consist of convex and concave bands. These bands have proven to show representation of growth biannually, with concave bands precipitated in colder months and convex bands precipitated in the warmer months as determined by oxygen and carbon isotope profiles of *C. virginica* by both Andrus and Crowe (2000) in the Altamaha Sound, GA and Kirby and Jackson (2004) in the Chesapeake Bay. Life span can be estimated by counting the number of annually formed, skeletal growth ligamental increments in the chondrophore (Figure 3-1) of each left valve (Kirby et al. 1998).

Growth is more rapid in warm waters, such as the Gulf of Mexico, where a marketable oyster can be grown in 2 years, whereas in northern waters like the Long Island Sound, it can take 4 or 5 years to achieve this same height (Shumway 1996). Harvest size restrictions vary among states, but a minimum size limit of 76 mm is required in most (Coen & Luckenbach 2000). Environmental factors such as food supply, temperature, substratum, pH, salinity, light, depth, turbidity, and population density (crowding) are known to influence growth in bivalves. Oysters can tolerate a range in levels of salinity, but growth is stunted at low salinity levels of 7.5 ppt and is almost nonexistent at 5 ppt (Chanley 1958). Ten ppt is the minimum salinity needed to grow with 12-27 ppt being the range for normal growth in juveniles and adults (Chanley 1958).

Changes in phytoplankton food supply have been shown to affect the growth and survival of living *Crassostrea* (Brown & Hartwick 1988; Lenihan et al. 1996; Lenihan 1999, Kirby & Jackson 2004). Increased concentrations of phytoplankton favor the growth of oysters however, when conditions become hypoxic, oyster survival and growth could be jeopardized. In a study of three centuries of oysters in the Chesapeake Bay, it was found that both shell and soft tissue growth increased significantly during a eutrophied period between 1760 and 1860, and slowed between 1860 and 2000 during hypoxic estuarine conditions (Kirby & Miller 2005).

Air exposure has also been found to inhibit growth in oysters with those that remain submerged in the subtidal zone having faster growth than those in the intertidal. One study showed that those exposed 20% of the time grow twice as fast as those exposed 60% of the time (Stanley & Sellers 1986). Upon air exposure in the intertidal

zone, oysters tightly close their valves and cease feeding. In addition, the exposure to higher air than water temperatures causes them to allocate more energy to metabolic maintenance, therefore reducing the amount available for growth (Bartol et al. 1999). However, increased survivorship has been correlated with low intertidal zones because oysters there do not experience the high rates of predation, sedimentation and fouling that occurs at subtidal depths nor do they encounter the severe atmospheric extremes and respiratory stresses associated with higher intertidal zones (Bartol et al. 1999).

The Condition Index (CI) measured as dry meat weight / dry shell weight x 100 (Lucas & Beninger 1985) is used to measure the physiological condition of an oyster. It is widely used as a measure of health in studies involving the effects of environmental contamination in oysters. It has also been found to be related to *P. marinus* and MSX infection, though other studies fail to show that the CI of infected oysters differ from that of uninfected oysters (Chu et al. 1993). High values of CI are due to the storage of large quantities of glycogen in the oyster (Shumway 1996), which is lost during periods of stress, under such conditions as high temperature, low salinity and disease (Riedel et al. 1998). The CI varies with reproductive cycle as oysters shed gametes during the spawning season (May-October) (Volety et al. 2003). It has also been shown to be influenced by salinity, with locations with the largest freshwater inflow having lower condition indices (Volety 2008).

The small populations of oysters that have been discovered in this study exist in geographically separate areas of the HRE. These locations exhibit different levels of salinity, DO, phytoplankton and environmental contamination. An oyster stressed either by its water quality or by disease has less energy for growth, therefore a comparison of

oyster condition index should be indicative of oyster health and the influence of environmental and contaminant stress in each of the given areas (Volety 2008). A sample of Delaware Bay oysters was used as a standard for comparison to the HRE oysters since it was expected that growth and CI would be greater in oysters from a subtidal reef of harvestable waters exhibiting low levels of contamination. Of the HRE locations, it was anticipated that growth would be greatest at the higher salinity locations of ERB, ERQ and LIS.

Figure 3-1. Left valve of an oyster shell depicting the chondrophore area and annually secreted ligamental increments.

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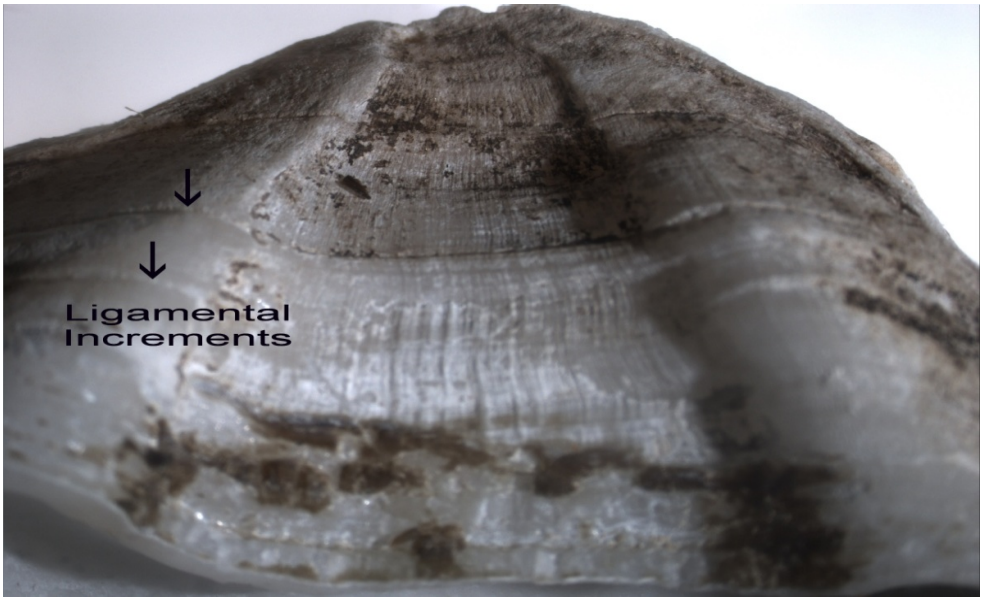

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METHODS

Condition Index

Collections were made in 2007-2009 at the five previously identified locations of populations of oysters in the HRE: Hackensack River (HKS), Hudson River (HDS), East River, Bronx (ERB), East River, Queens (ERQ) and Long Island Sound (LIS). All oysters collected were used in the CI analysis with the exception of spat or those juveniles with a shell height of 35 mm or less, where the shell was too delicate to be detached from its substrate and weighed without being destroyed. Oysters were also obtained and measured from a sample of Delaware Bay (DB) oysters, from a reef known as Shell Rock, as a comparison to oyster condition in harvestable, uncontaminated waters. The Delaware Bay oysters were obtained courtesy of the Rutgers Haskins Shellfish Laboratory in May of 2007.

Oysters were shucked open and the wet meat was separated and weighed. The shell was allowed to dry and then weighed. The CI was determined by dry meat weight (assuming 80% water content by weight) / dry shell weight x 100 (Lucas & Beninger 1985).

Growth

Shell height (mm), the maximum distance from hinge to growth edge, and width (mm) of the left valve of each shell were measured and recorded. Life span was estimated by counting the number of skeletal growth ligamental increments in the chondrophore of each left valve (Kirby et. al. 1998). Annual growth (mm/yr) was averaged by the total length divided by the lifespan.

All oysters with undamaged shells collected from each location in 2007-2009 and of age 2+ years were used in the growth analysis. The estimated age of all oysters collected at HDS was 0-1 year and therefore this location was excluded from the annual growth analysis.

Statistical analysis

Statistical tests for differences in CI and growth at each location were conducted in JMP statistical software using a one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. Results were deemed significant at $P < 0.05$.

RESULTS

Annual shell growth was not significantly different amongst most locations but growth (mm/yr) of DB oysters was significantly less than ERQ oysters (Figure 3-3). All CI values fell within the range of values for *C. virginica* obtained from other studies using the same methods (Volety 2008; Chu et al. 1993). Of the HRE samples, the LIS oysters averaged the largest in size and weight of those sampled (Table 3-1) and had a significantly higher ($P < 0.05$) CI than all other locations other than HDS (Table 3-2). The HDS oysters were the smallest in size of all locations. The CI was lowest and was not significantly different ($P > 0.05$) among the ERB, ERQ and the HKS locations. The Delaware Bay samples exhibited a significantly lower average CI ($P <0.0005$) than any of the HRE sample sites but also had the heaviest shells per shell height (Figure 3-10).

The Condition Index was independent of the size of the oyster, but the trend for the LIS, ERB, HKS and DB locations was decreased CI with increasing size (Figures 3-4 to 3-9).

DISCUSSION

Annual shell growth did not differ significantly among all the HRE populations (Figure 3-3). There are many factors that affect oyster growth but water temperatures and salinity are among the most important. Water temperatures may vary slightly at each of the locations at any given time but variations should not be enough to affect oyster growth. Low salinity is unfavorable to oyster growth. The two low salinity sites are HKS and HDS, of which the HDS location was excluded due to the small size of oysters there. However, average growth at HKS was greater than all locations except at ERQ though the difference was not significant (Table 3-3).

It was expected that DB oysters would have greater annual growth than HRE oysters being raised in a subtidal reef of harvestable waters. However, annual growth was not significantly greater in the DB sample than the HRE samples and growth at the ERQ location was significantly more than that of DB. Most ERQ oysters analyzed were no more than 2 years old and oysters have the greatest shell growth in the earlier years of life, so the significant growth difference may have been due to the inclusion of the mostly young oysters at this location. With the exception of HKS, all oysters used in the analyses were collected during the May-October spawning season. Those from HKS were collected during unseasonably warm weather in January and February of 2008. Since these did not lose mass due to spawning as would be expected of the other

locations, a false high CI could have resulted. These oysters were also found to be heavily diseased and surviving in low salinity waters which could negatively affect condition. However, the CI of oysters collected from HKS did not differ from the higher salinity locations in the East River.

The CI for the LIS, ERB, HKS and DB samples decreased with increasing age (shell height) however, this trend was not seen for the ERQ and HDS samples and may have been due to the lack of larger individuals, above 90 mm in height, where the greatest decrease in CI was shown at LIS, ERB and DB (Figures 3-4 to 3.9). The lack of larger individuals at the HDS site in particular, may have positively skewed the CI, since oysters do grow more rapidly in the earliest stages of their life.

It was anticipated that the DB oysters would have higher condition indices than all HRE locations since these are consumable oysters living subtidally, in low levels of environmental contamination and bred for meat yield. The oysters sampled were from the Shell Rock Bed in the Upper Central region of Delaware Bay which has a salinity range of 9.0-16.5 ppt. The cause for the Delaware Bay oysters to have a statistically significant lower average condition index (3.06 +/- 1.06) was not due to low meat weight, averaged at 10.84 g, but was due to their heavy shells. The Delaware Bay oysters had the heaviest shells per shell height of any HRE location (Figure 3-10) lowering the meat weight to shell weight ratio. Condition indices, using the same calculation methods, reported for *Crassostrea virginica* for the James River, Virginia, also exhibited condition indices in this low range (Chu et al. 1993). Therefore, using the CI as a measure of health may be useful only when comparing oysters in the same estuary to exclude factors

such as water temperatures, water quality, genetics, and ecological pressures that may be contributing to shell thickness and weight in any given area.

The lighter and thinner shells of HRE oysters were not impacting the internal meat weight. The cause for thinner shells may be attributed to stress from the environment. Water contamination considerably inhibits mollusk growth. The shell of *M. galloprovincialis* proved 30-50% heavier as compared to mollusks of the same length from locations polluted with urban waste (Shadrin & Lezhnev 1990; Alyakrinskaya 2005). The dissolving effect of acidic environments on the shell of marine mollusks is also well known (Alyakrinskaya 2005). Though the thinner shells of HRE oysters are not affecting meat weight, the thicker and heavier the shell, the more reliable it is in serving the primary function of protection. Mollusks with solid shell can better survive exposure to waves, predators, freezing, heating, drying and other unfavorable conditions (Alyakrinskaya 2005).

The LIS location had notably the highest average condition indices. Here the largest, hence oldest, oysters can also be found. The average height of the LIS sample measured 84.7 mm whereas the average height from the other HRE locations ranged from 45.6 - 64.4 mm. The largest oysters from LIS tended to have a lower CI, so it cannot be concluded that the larger size caused increased CI. There are limited data on the meat to shell ratio of older *C. virginica* because most oysters are harvested before their true growth potential can be shown. However, it is known that shell excretion slows with age; therefore the same may be concluded for the internal shell volume. The lower condition indices of larger oysters therefore is not reflective of less fit individuals, but achievement of maximum meat growth potential in relation to their shell. Even with the larger oysters

included in the LIS sample, its average CI remains the highest of all other locations. This is because the average meat weight (g) here was 33% - 42% more than the same sized (50-70 mm) individuals from the East River and HKS locations. All oysters from each site, including the LIS location, were collected from the intertidal zone in areas that are at least exposed during low tide at and around the full and new moon of the month, with a tide chart prediction height of -0.8 to 0.4 feet. Therefore, less air exposure and more time for feeding cannot be attributed to their larger size.

Increased levels of phytoplankton in the water column may have contributed to the large and heavy oysters found at LIS. Levels of phytoplankton measured here in 2008 were 12.7 µg/L at the NYCDEP's E10 station closest to the site (NYCDEP 2008). There may be limited flushing at the end of Orchard Beach Lagoon where the oysters are found, allowing phytoplankton to remain abundant for feeding. Regular measurements of chlorophyll *a* directly at the location would confirm this. The majority of oysters observed at the LIS site are much larger than what is commonly documented of oysters, but only because there is limited research on oysters from non-harvestable waters like the HRE, where they are allowed to reach their maximum growth potential. The largest *C. virginica* found in North Carolina was noted at a height of 21.2 cm by Porter (1975) and the largest oyster found by Galtsoff (1964) was found in Maine measuring 20.6 cm high, 9.7 cm long (Carriker 1996). Of 53 oysters collected, the largest at the LIS location measured 17.0 cm high and 9.1 cm long, not much smaller than the largest found by Galtsoff. It is quite possible that a living oyster still exists at the site that exceeds those reported. The oysters found at the other sampling locations (ERB, ERQ, HKS and HDS)

are either not surviving to achieve their potential growth or are more recent settlements that have not yet had time to reach their growth potential.

Using CI as a measure of health proved to be problematic in this study for two reasons. First, the Delaware Bay oysters appeared to be of lower condition than the HRE oysters due to a significantly heavier shell. The CI is assuming that that all the animals taken into consideration exhibit the same potential for both shell and biomass growth. Different ecological pressures may have caused the shells of oysters in DB to be thicker and heavier whereas in the HRE they are thinner and lighter. The CI of oysters from the two estuaries is not comparable for this reason. Second, average CI was calculated over a span of age classes. Oysters are known to grow at a faster rate in the earlier stages of their life and this study showed that CI decreases with the size (age) of an oyster. Only juvenile oysters were available to be measured at the HDS location resulting in a significantly higher CI when compared to a broader age range of oysters at the other locations. Therefore, the CI may only be an affective measure of health when comparing the same age class of oysters in the same estuary.

Nevertheless, the health, as assessed by the CI, of HRE oysters does not reflect an animal that is struggling to survive. Despite a thin external shell, internal meat weight was not affected and there was no evidence of external shell damage that would be indicative of predation by crabs, drills or boring sponges. There is a long history of pollution and neglect of the HRE oyster population, but the growth and CI of existing wild oysters are supportive to the potential for oysters to persist in the Hudson River Estuary.

Figure 3-2. Largest oyster found at Long Island Sound (LIS), Orchard Beach Lagoon: Age: 8, measuring 17.0 (H) X 9.1 (L) cm. Dermo intensity = 3.0.

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Table 3-1. Mean Condition Index, size (height in mm) wet meat weight (g) and dry shell weight (g) at sampled locations.

Location	n	Condition Index Mean +/- SD	Height (mm)	Wet meat weight (g) (range in parenthesis)	Shell weight (g)
HKS	31	4.80 +/- 1.21 (2.95 to 7.34)	64.30 (44.8 to 93.8)	6.80 (1.76 to 16.18)	29.30 (2.40 to 67.5)
ERB	39	4.98 +/- 1.84 (1.62 to 9.13)	64.39 (35.0 to 111.1)	5.53 (1.10 to 13.67)	26.49 (4.32 to 68.10)
ERQ	71	4.80 +/- 0.98 (2.80 to 8.10)	59.67 (41.2 to 93.2)	4.51 (1.50 to 15.86)	18.60 (5.30 to 33.10)
LIS	47	5.87 +/- 1.59 (1.98 to 8.64)	84.70 (42.8 to 170.2)	14.10 (4.66 to 37.20)	60.00 (13.15 to 261.5)
HDS	11	5.56 +/- 0.64 (4.68 to 6.72)	45.60 (39.5 to 50.5)	2.25 (1.46 to 3.09)	8.13 (5.54 to 10.71)
DB	20	3.06 +/- 1.06 (1.49 to 4.93)	81.49 (60.8 to 110.68)	10.84 (5.51 to 23.65)	80.24 (20.86 to 167.47)

Table 3-2. ANOVA results of condition indices at sampled locations.

Location 1	**Location 2**	**p-Value**
LIS	DB	0.0000*
HDS	DB	<.0001*
ERB	DB	<.0001*
HKS	DB	<.0001*
ERQ	DB	<.0001*
LIS	ERQ	0.0002*
LIS	HKS	0.0060*
LIS	ERB	0.0252*
HDS	ERQ	0.4663
HDS	HKS	0.5627
HDS	ERB	0.7942
LIS	HDS	0.9812
ERB	ERQ	0.9788
ERB	HKS	0.9917
HKS	ERQ	0.9999

Table 3-3. Growth (mm/yr) of *Crassostrea virginica* at sampled HRE locations and Delaware Bay.

Location	N	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
DB	31	22.78	4.26	0.76	21.21	24.34
ERB	15	22.32	3.93	1.01	20.14	24.50
ERQ	55	25.66	3.67	0.49	24.67	26.65
HKS	27	25.49	5.18	0.99	23.44	27.54
LIS	22	24.84	4.71	1.00	22.75	26.93

Figure 3-3. ANOVA of growth (mm/yr) by population. Growth at ERQ and DB are significantly different ($P = 0.0263$). All other locations $P > 0.05$.

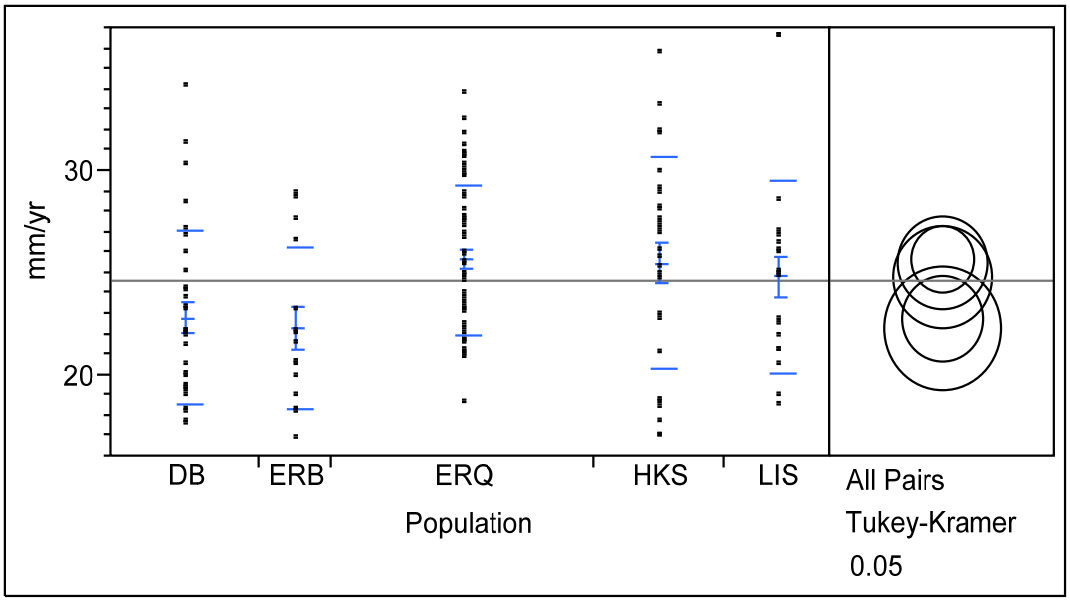


Figure 3-4. Condition Index to shell height (mm) at Long Island Sound (LIS).

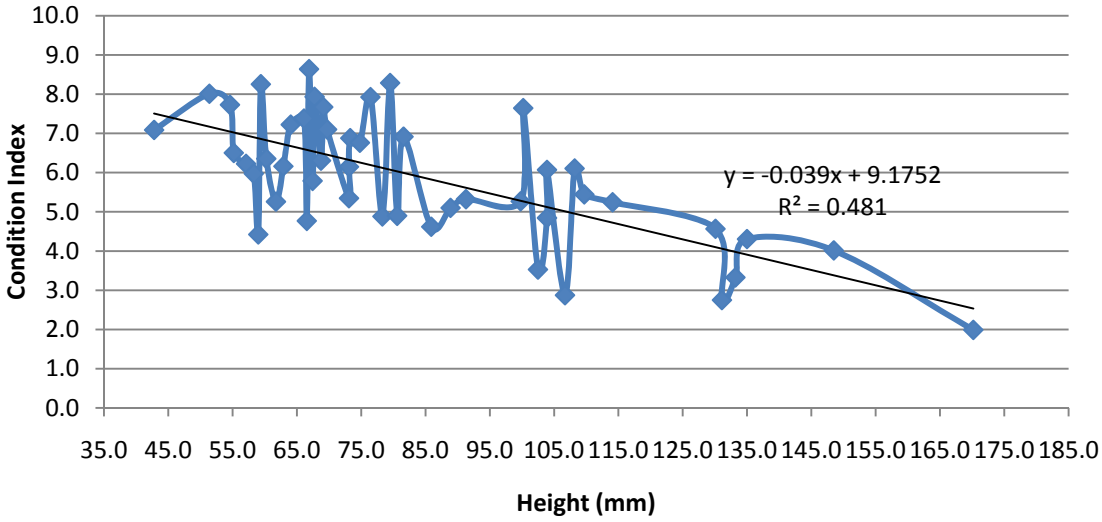


Figure 3-5. Condition Index to shell height (mm) at East River Bronx (ERB).

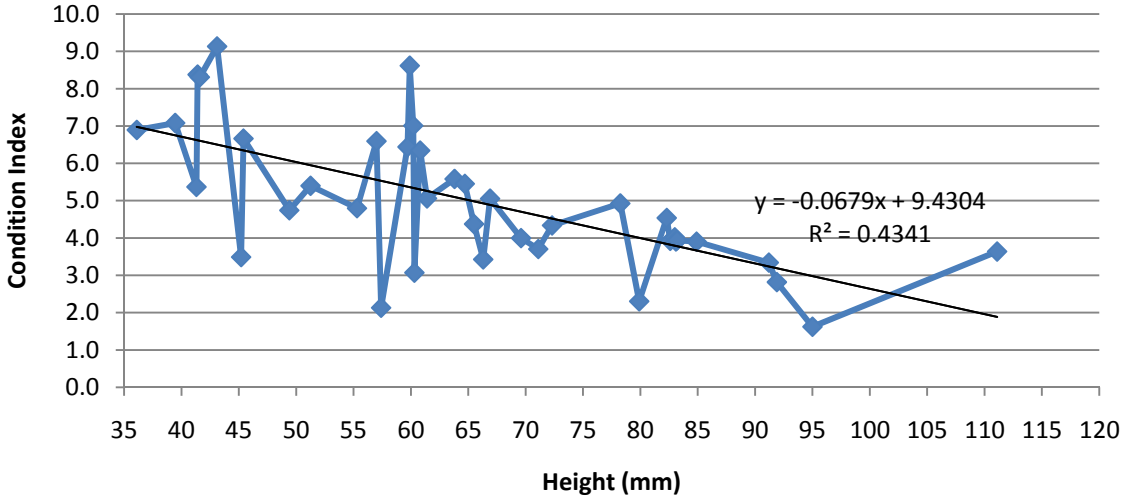


Figure 3-6. Condition Index to shell height (mm) at East River Queens (ERQ).

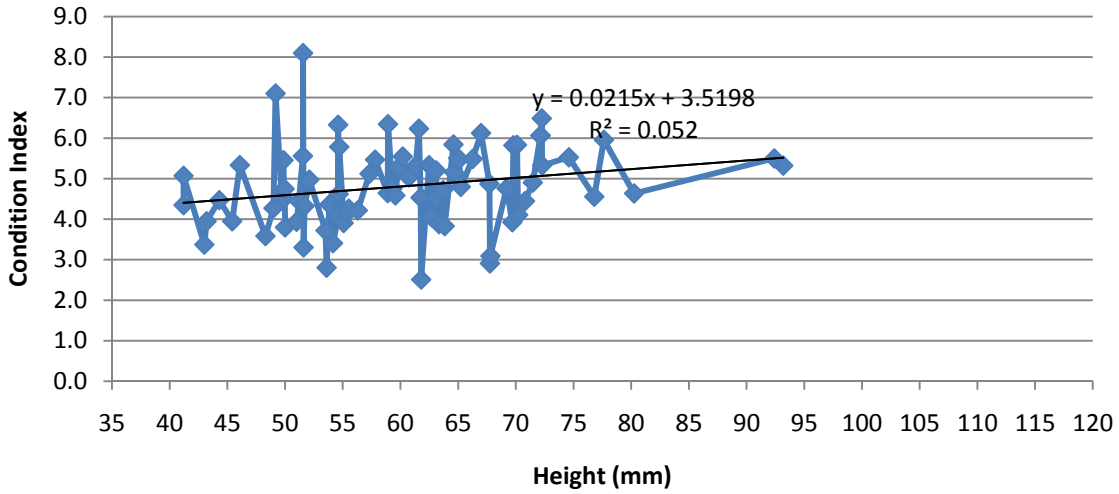


Figure 3-7. Condition Index to shell height (mm) at Hackensack River (HKS).

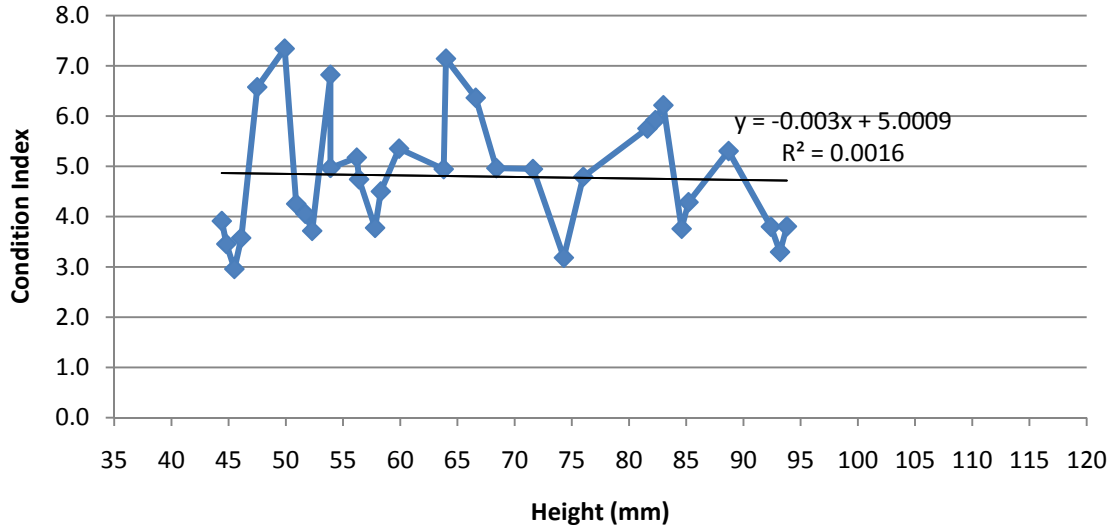


Figure 3-8. Condition Index to shell height (mm) at Hudson River (HDS), Alpine, NJ.

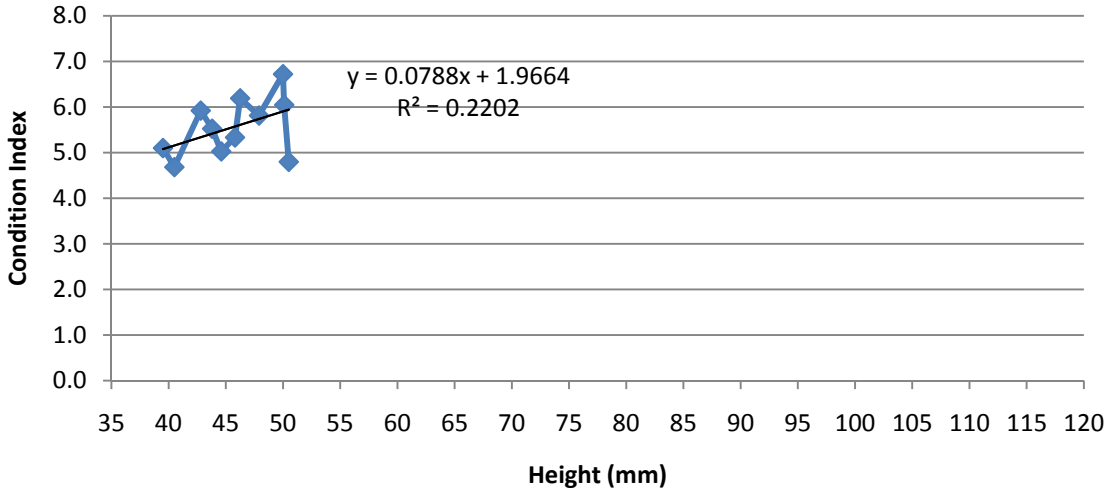


Figure 3-9. Condition Index to shell height (mm) at Delaware Bay (DB).

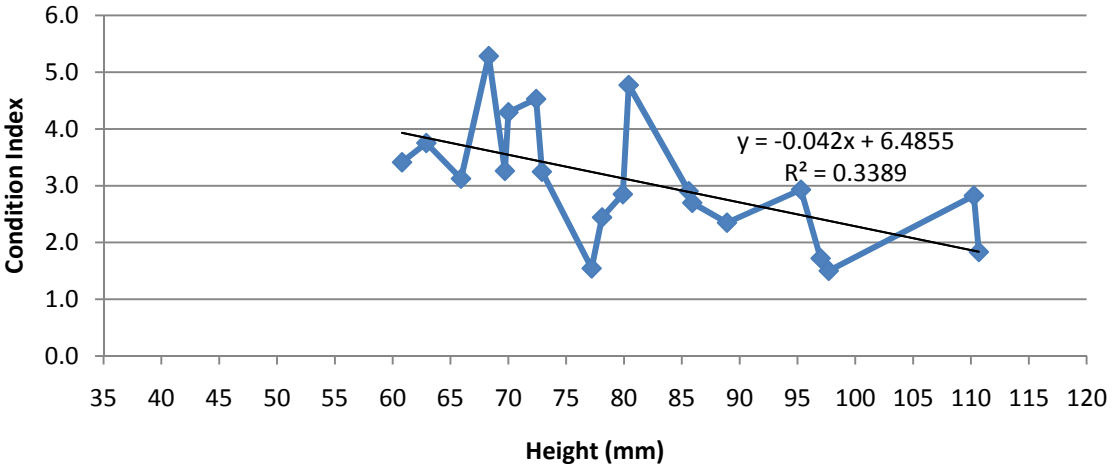
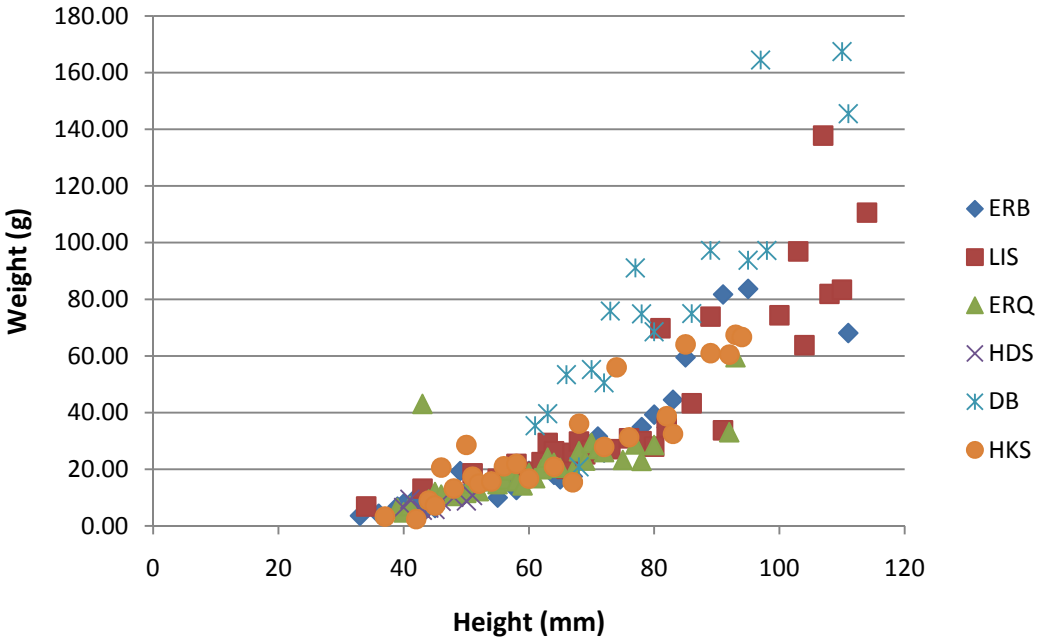


Figure 3-10. Oyster shell weight at shell heights. Delaware Bay (DB) oysters have significantly heavier shells per shell height than the HRE samples ($P < 0.0001$).



Chapter 4: Prevalence of Oyster Diseases, MSX and Dermo, in the Hudson River Estuary

INTRODUCTION

Two protozoan parasites are endemic in *C. virginica* throughout much of its range, *Perkinsus marinus,* better known as Dermo disease and *Haplosporidium nelsoni*, MSX disease (Coen & Luckenbach 2000). The two diseases, along with over-fishing and habitat destruction, are among the leading causes for the collapse of oyster fisheries in the mid-Atlantic region and are hindering efforts in oyster restoration and aquaculture (Yu & Guo 2006).

Dermo was first documented in the late 1940s in the Gulf of Mexico, was briefly observed in Delaware Bay in the mid 1950s after the importation of infected seed oysters from the Chesapeake Bay and then returned there in 1990 with abnormally high winter temperatures (Ford & Tripp 1996). Since 1991 it has also been found in Connecticut, New York, Massachusetts and Maine (VIMS 2005). Transmission of Dermo is direct from oyster to oyster by dead and dying oysters releasing infective stages back into the water column to infect other oysters. Oysters are exposed as they feed; causing the initial infection to be found in the gill, but the parasite first attacks the digestive system (Babb 2005). Dermo is most prevalent in oysters exposed to conditions of high temperature and high salinity (Shumway 1996). It proliferates above 20˚C, and rapidly multiplies, spreads and kills oysters at temperatures above 25° C. Reduced prevalence of *P. marinus* in low salinity waters has been a consistent finding of field studies (Ford & Tripp 1996) although the pathogen has shown to be tolerant of salinities as low as 3 ppt (Chu et al. 1993). Moderately to heavily infected oysters usually exhibit a reduction in growth rate, poor condition and reduced reproductive capacity (VIMS 2005).

MSX disease was first documented in 1957 in Delaware Bay and named MSX for multinucleated sphere X (unknown). Its range is now from Maine to Florida. It is caused by the protozoan *Haplosporidium nelsoni,* which is a spore-forming protozoan and its mode of transmission is unknown (VIMS 2005). The inability to transmit the parasite in laboratory settings has led researchers to believe that an intermediate host exists. The parasite is usually detected first in the gill and then enters the blood stream to infect other tissue. It interferes with respiration and feeding and eventually leads to death (Babb 2005). Effects of the disease include decreased condition, reduced reproductive capacity, and mortality, which occur primarily in the late summer and fall. Death of highly susceptible oysters is so rapid that it often occurs without loss of soft tissue condition (Ford et al. 1988, Ford & Tripp 1996). However, more tolerant oysters, living months to years after infection, have shown clear reduction of CI correlated with infection intensity (Ford & Tripp 1996). Other studies (Chu et al. 1993) report that the CI of infected oysters did not differ from that of uninfected oysters.

As with Dermo, temperature and salinity play an important role in regulating MSX. Infections are acquired at temperatures above 20˚ C and salinity at and above 15 ppt (VIMS 2005). Between 5° C and 20° C the pathogen proliferates more rapidly than the oyster can control it and above 20° C susceptible oysters are killed (Ford & Tripp 1996). The notable absence of the parasite from the upper reaches of Delaware and Chesapeake Bays, led researchers to believe that low salinity limited the pathogen. This was confirmed in field studies in the 1980s where oysters moved from high salinity (>20 ppt) to low salinities of 10 ppt or less were free from infection within 2 weeks (Ford &

Tripp 1996). Therefore, *H. nelsoni* has been demonstrated to be purged from an oyster if moved to areas with low salinity levels.

There is evidence that some oysters are genetically resistant or tolerant to the two diseases and selective breeding programs are in place in Delaware Bay and Chesapeake Bay (Yu & Guo 2006). The absence of the diseases or tolerances by oyster populations is especially important to know when constructing an oyster restoration project near an already established area. The genetic mixing of populations could cause disease to spread.

The prevalence of the two parasites in Hudson River Estuary was locally investigated by the NY/NJ Baykeeper in samples of non-native oysters from their Keyport Oyster Restoration Reef in 2005. Eighteen of the 25 oysters tested were infected with Dermo and 1 of 25 oysters was infected with MSX (NY/NJ Baykeeper 2005). These oysters were supplied by Frank. M. Flower & Sons, Inc. in Long Island Sound and were bred to be disease resistant/tolerant. Non-native oysters placed in experimental research cages by Rutgers University in the Hackensack River near the HKS sample location in 2008, were also tested for MSX and Dermo disease. These oysters were also supplied by Frank. M. Flower & Sons Inc., but bred for rapid growth. It was found that 100% of the oysters were infected with Dermo disease and 60% with MSX disease (Rutgers University 2008).

No other published data on disease prevalence exists for the HRE. An extensive study of the prevalence of *P. marinus* was conducted along the Connecticut shoreline of Long Island Sound in 1996 and 1997 (Karolus et al. 2000) The closest sampling location to the present study was at Greenwich, CT, in the Western Long Island Sound. There

samples were taken from shallow waters, 2.5 m or less deep. At the Greenwich location, *P. marinus* prevalence ranged from 80-100% in both 1996 and 1997 with infection intensities as rated using the Mackin scale (Table 4-1) averaging 2.1 in 1996 and 1.1 in 1997 (Karolus et al. 2000). The Mackin scale is used to measure the level of infection intensity of *P. marinus* and ranges from 0-5 with increasing levels of infection.

Both MSX and Dermo disease were anticipated to be present in the HRE wild oyster populations identified in this study. A greater influence of Dermo disease than MSX disease was expected since it is a more recent occurrence and oysters of nearby estuaries appear to have recovered from the initial outbreak of MSX disease. Both diseases are expected to be more prevalent in the higher salinity locations (ERB, ERQ, LIS).

METHODS

Oysters were collected from the Hackensack River (HKS) September 1, 2008 and from the Hudson River (HDS) on September 21, 2009 and sent whole to the Rutgers Haskins Shellfish Research Lab (RHSRL) for dissection and disease analysis. Due to the low abundance of oysters and permit restrictions of numbers collected at New York City Department of Parks & Recreation locations, the entire oyster could not be sacrificed and sent to RHSRL for testing at those locations. Therefore, oysters were collected from ERB, ERQ and LIS during August 16-18, 2007 and dissected in the lab prior to being sent to the RHSRL. A portion of adductor muscle and gill tissue was extracted and saved for genetic and metal analyses. For analysis of *P. marinus*, a portion of mantle and rectum from each oyster was placed in a tube pre-prepared by RHSRL containing Ray's

Fluid Thiogycollate Medium (RFTM). Then a cross section containing visceral mass, mantle and gill was placed in a tissue cassette and deposited in Davidson's fixative (33% ethyl alcohol, 22% formalin (37-39%), 11.5% glacial acetic acid and water) for MSX testing. The samples were sent to RHSRL where they were stained and microscopically examined for disease infection with the *Perkinsus* samples being incubated for a total of 5 days from the date of the time fixed before examination. Due to handling, some samples were not preserved in the shipping process.

The RHSRL conducts the most common method for *P. marinus* detection, the RFTM assay, which involves culturing oyster tissue in fluid thioglycollate medium, to enlarge *P. marinus* present in the tissue (Ray 1952). The tissue is then stained, which is absorbed by the thick walls of the hypnospores, giving the parasite a blue-black appearance under a light microscope. The number or coverage of enlarged hypnospores is used to estimate infection intensity as rated using Mackin's (1962) semi-quantitative scale Table 4-1. The disease progression of MSX is measured on a scale of 1-4 with the infection intensity described as outlined in Table 4-2. The RHSRL provides a report that includes sample number, sex, and the *Perkinsus* and MSX score and intensity (Table 4-3).

Appropriate timing of the collections was imperative because infection prevalence and intensities increase from late spring through mid fall (Andrews & Hewatt 1957; Krantz & Jordan 1996; McCollough 2007). Existing infections become latent during winter and spring (McCollough et al. 2007) and are often undetectable by standard RFTM assays of tissue subsamples (Ray 1952; McCollough et al. 2007).

Differences in the frequency of occurrence of the two diseases between each population were determined using the chi-square statistic of a 2 X 2 contingency table of disease presence and location.

Table 4-1. Mackin semi-quantitative scale for infection intensity of *Perkinsus marinus*.

Letter Designation	**Infection Intensity**	**Numerical Value**	**Description**
N	Negative	0.00	No hypnospores present
VL	Very Light	0.33	1-10 hypnospores
L-		0.67	11-74 hypnospores
L	Light	1.00	75-125 hypnospores
L+		1.33	>125 hyponospores but much less than 25% of tissue is hypnospores
LM-		1.67	<25% of tissue is hypnospores
LM	Light/moderate	2.00	25% of tissue is hypnospores
LM+		2.33	>25% but much less than 50% of tissue is hypnospores
M-		2.67	>25% but >50% if tissue is hypnospores
M	Moderate	3.00	50% of tissue is hypnospores
M+		3.33	>50% but much less than 75% of tissue is hypnospores
MH-		3.67	>50% but >75% if tissue is hypnospores
MH	Moderately heavy	4.00	75% of tissue is hypnospores
MH+		4.33	>75% but much less than 100% of tissue is hypnospores
H-	Heavy	4.67	>75% of tissue is hypnospores but some oyster tissue is still visible
H		5.00	Nearly 100% of tissue is hypnospores

Table 4-2. MSX disease testing scale.

Score	Description
1	Rare infection
2	epithelial infection
3	systemic infection
4	advanced infection
Range	
Rare	less than 10 MSX plasmodia
Very light	10-100 plasmodia
Light	>100 plasmodia
Moderate	1-5 plasmodia per field of 100X view
Heavy	more than 5 plasmodia per field of 100X view

RESULTS

The RHSRL provides a report that contains the sample number, level of Dermo and MSX infection intensity and sex of the oyster if determined. The results from the testing at each location are summarized in Table 4-3.

The Hackensack River (HKS) had the highest level of MSX disease infected oysters with 100% of the 20 samples analyzed testing positive for the disease with 55% having systemic or advanced infections (Table 4-3). In addition, more than half of these oysters were also infected with Dermo disease. Of the 30 HKS samples sent for Dermo testing, 18 tested positive for the disease. MSX disease was not detected at all at HDS, in only one sample from the East River locations (advanced infection) and detected in only two oysters at the LIS location (rare infections). Dermo disease had a low presence at HDS, but was detected in 15 of 23 oysters from the LIS location and 8 of 38 from the East River populations. The frequency of Dermo disease was statistically the same between the HKS and LIS collections but both had significantly higher frequencies than

the other locations (Table 4-3). The frequency of MSX disease was statistically greater at HKS than any other location.

Both male and female oysters were present at all locations with the exception of HDS where the sex of most oysters could not be determined.

Figure 4-1. Disease prevalence (% infected oysters) of *Perkinsus marinus* in oysters from the Hudson River Estuary.

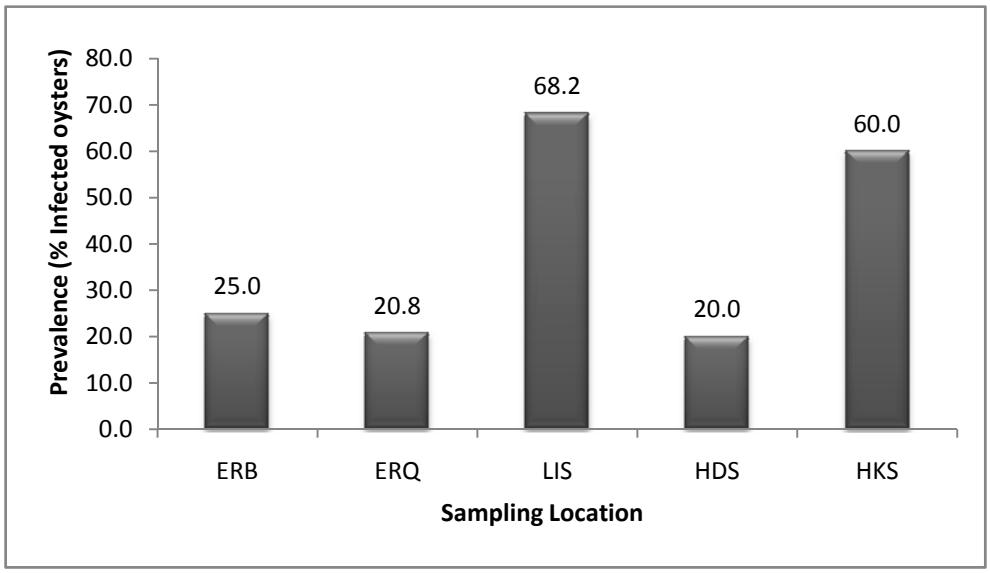


Figure 4-2. Average intesity of *Perkinsus marinus* infected oysters from the Hudson River Estuary (range is 0-5 on Mackin Scale).

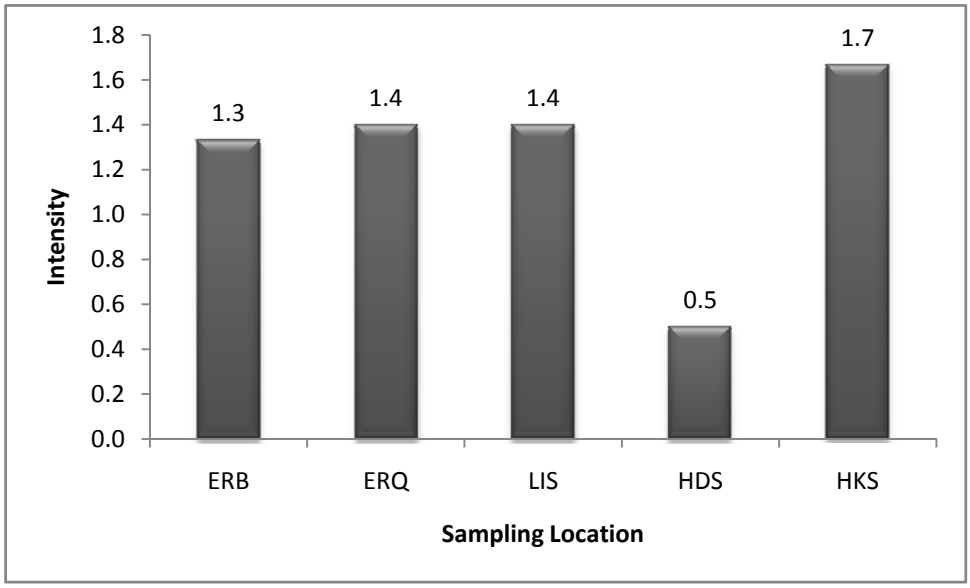
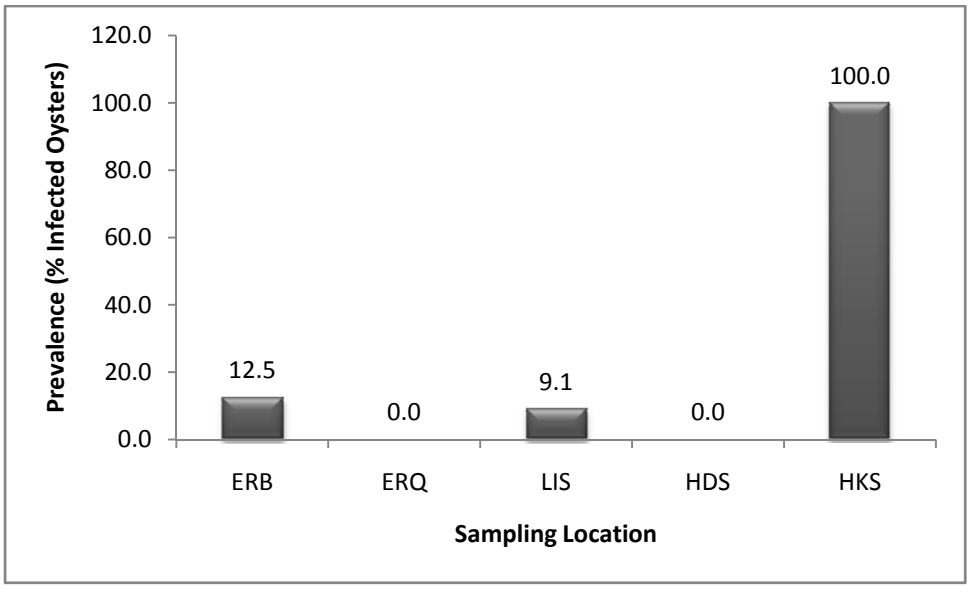


Table 4-3. Population comparison of Dermo disease frequency of oysters in the Hudson River Estuary using chi-square analysis.

Population Comparison	χ^2	$\chi^2_{0.05,1}$	Conclusion
HKS & LIS	0.099	3.841	HKS = LIS
HKS & ERB	5.717	3.841	HKS ≠ ERB
HKS & ERQ	10.044	3.841	HKS ≠ ERQ
HKS & HDS	4.921	3.841	HKS ≠ HDS
LIS & ERB	7.674	3.841	LIS ≠ ERB
LIS & ERQ	12.487	3.841	LIS ≠ ERQ
LIS & HDS	6.471	3.841	LIS ≠ HDS
ERB & ERQ	0.020	3.841	ERB = ERQ
ERB & HDS	0.024	3.841	ERB = HDS
ERQ & HDS	0.830	3.841	ERQ = HDS

Figure 4-3. Disease prevalence (% infected oysters) of *Haplosporidium nelsoni* (MSX disease) in oysters in the Hudson River Estuary.



DISCUSSION

It was anticipated that levels of Dermo disease would be more prevalent than that of MSX disease, as oysters have recovered from the initial outbreak of MSX in other estuaries, but are still heavily impacted by Dermo. For example, in Delaware Bay, following a major bay-wide MSX epizootic, outbreak, in the mid 1980s, most of the oyster population there appears to have become resistant to MSX and recent monitoring in 2008 shows that MSX continues to be of insignificant presence (Powell & Ashton-Alcox 2009). In this study of the Hudson River Estuary, most locations exhibited a low presence of MSX with the exception of the Hackensack River where 100% of the oysters tested were infected. Results of the MSX testing at the HKS site were particularly surprising due to the low salinity levels here. It is well documented that *H. nelsoni* is not tolerant of low salinity and management practices in Delaware Bay and Chesapeake Bay include moving oysters to lower salinity to prevent MSX outbreaks or to purge the oyster of the disease. The parasite shows reduced survivability at salinities between 5 and 15 and zero survival below 5 ppt (Ford & Haskin 1988; Paraso et al. 1999; Hofmann et al 2001).

Salinity is well documented by the New Jersey Meadowlands Commission, Meadowlands Environmental Research Institute (MERI) for several stations along the Hackensack River and salinity averages 9 ppt at their Station 4 Amtrak Bridge, just south of the study location. The fact that MSX disease is not only present here, but thriving, raises questions about the environmental constraints of *H. nelsoni*. Transmission of MSX disease is not direct from oyster to oyster as Dermo disease is. The HKS location may be providing suitable environmental requirements of the unknown second host that is

allowing the disease to persist. High *H. nelsoni* intensities may also be due to other environmental stressors weakening the oysters allowing for disease to take over where it would not have in a healthy system. It also suggests that another environmental factor, other than high salinity, is the determining factor for the proliferation of the parasite.

A modeling study of MSX disease in Chesapeake Bay showed temperature, especially cold winters, to be the most important factor controlling the presence and abundance of MSX disease (Hofmann et al 2001). Prolonged cold, especially at temperatures below 5° C, debilitates *H. nelsoni* and increases its susceptibility to hemocyte attack the following spring (Hofmann et al. 2001). Winter temperatures along the east coast of the United States have been increasing and a continued winter temperature warming trend will allow MSX to remain in areas where it is already established as well as facilitate its northward movement. Some of the patchy appearances of *H. nelsoni* epizootics in the northeast United States occurred during a marked warming period with water temperatures in the relatively shallow areas becoming nearly as warm in the summer as more mid-Atlantic locations (Hofmann et al. 2001).

Monthly water temperatures of the Hackensack River for 2009-2010 as provided by MERI are consistent with stations of the lower Hudson River as monitored by The Center of Maritime Systems of Stevens Institute of Technology, achieving lows near freezing 0 °C in the winter months and upwards to 22° C in the warmer months. However, there have been fluctuations in the Hackensack River water temperatures reported by MERI in the past due to industrial discharges, particularly, that of a local power plant, PSE&G's Bergen Generating Station. But in 1995, they ceased using the Hackensack River water as coolant and returning the higher temperature water to the

River (Bragin et al. 2005). Significant improvements in the fish community in the upper and middle portion of the River were found in a comparative study by MERI of years 2001-2003 from 1987-1988 which they attributed to the change. However, the river water is still used in industrial cooling applications in the southern reaches of the river near Kearny, NJ and no improvement in the fish community were seen there in 2001-2003 (Bragin et al. 2005).

Monthly year round monitoring of water temperatures directly at the HKS location along with monthly testing of MSX disease would confirm if warm winter water temperatures are allowing *H. nelsoni* to thrive. The prevalence and intensity of MSX disease in oysters follows a well established seasonal progression where infections occur in late May – early June, reach maximum prevalence and intensity in the fall and level off. A decrease in the late winter occurs due to mortality of heavily infected oysters as well as mortality of the parasites exposed to low winter temperatures (Hofmann et al 2001). If winter temperatures are not low enough to cause mortality of the parasites, the parasite would continue to thrive to the next season and would help explain the 100% disease prevalence of MSX at this location in 2008.

The HKS location also showed a 60% prevalence of Dermo disease, with an average infection intensity of 1.7, the highest of any sampled location. Oysters from the East River, both ERB and ERQ, appear to be tolerating the diseases as most oysters were not infected with MSX and with the exception of two oysters, levels of Dermo disease did not exceed a rating of 0.5, which corresponds to a very light infection on the Mackin scale. The frequency of Dermo disease was statistically the same at LIS as for HKS (Table 4-3). However, it should be noted that the LIS location has the oldest oysters and

the higher rates of Dermo disease found here may be a reflection of the time allowed for the disease to infect the oyster. Dermo disease has a tendency to manifest in older year classes, typically those that have attained some size refuge to predation and are desirable for harvest. Due to disease mortality, large oysters are rarely seen in Chesapeake Bay, or most other estuaries, even in regions rarely subjected to commercial harvest (Mann & Powell 2007). The two large oysters at the LIS location that showed a level 3 infection were estimated to be 8 years old and did not have lower condition indices than others sampled from the same location. Therefore, it can be concluded that though Dermo disease testing resulted in 68.2% prevalence here, the oysters are surviving the infections.

Oysters at HDS had the lowest levels of disease prevalence, as would be expected of a low salinity area. The oysters here are generally small and immature (age 1 and under) where only one oyster, the largest from the set sampled, could be sexed. Small juvenile *C. virginica* are less likely to become infected by *P. marinus* than adults (Ray 1954; Albright et al. 2007), so despite low salinity, the true explanation for such a low disease presence here could be due to the immaturity of the oysters and lack of time for the disease to proliferate. However, with the low level of disease here, disease can be ruled out as a contributing factor to the lack of larger, hence older, individuals at this location.

The testing of disease for this study of the HRE was conducted in 2007-2009. During this time, Delaware Bay was experiencing an epizootic of Dermo disease where by September of 2008, nearly 100% of oysters there tested positive for the disease with average infection intensity near 3.0 on the Mackin scale (Powell & Ashton-Alcox 2009). The mean prevalence of Dermo disease in the oyster beds of Delaware Bay from 1990-

2008 was 80% by September with an average infection intensity of 2.0 on the Mackin scale (Powell & Ashton-Alcox 2009). In comparison, though no long term trending of Dermo disease in the HRE exists, the results of this study show a lower prevalence of Dermo disease at all sampled locations with 42.7% of oysters in total from all HRE locations testing positive for the disease throughout the three sampling years with an average infection intensity of 1.5.

Delaware Bay has experienced a 7-year cycle of multiyear epizootic occurrences of Dermo disease since the onset in 1990 (Powell & Ashton-Alcox 2009). Whether the HRE has experienced the same pattern is unknown, however it does not appear that there was an epizootic occurring during the same time period of 2007-2009 as was in Delaware Bay. Annual sampling of the HRE for both MSX and Dermo disease is needed to determine trends and if oysters at any given location are more susceptible or resistant to the two oyster diseases. The mid-Atlantic oyster industry could benefit from future studies of HRE oysters if they are found to be a disease resistant strain. But with the lack of a local oyster industry and abundance of oyster reefs, further studies of MSX and Dermo disease in the Hudson River Estuary will likely be limited.

Table 4-4. Rutgers Haskins Shellfish Research Lab Report Summary (2007, 2008, 2009) of Dermo and MSX testing at HDS, HKS, ERB, ERQ and LIS.

Location	Sample No.	*Perkinsus* score	MSX score	MSX Intensity	Sex
East River, Bronx (ERB)	1	0	0		Male
	2	0.5	0		Female
	3	3	no sample		
	4	0	0		indeterminant
	5	0.5	0		indeterminant
	6	0	0		Female
	7	0	0		Female
	8	0	no sample		
	9	0	0		Male
	10	0	4	heavy	indeterminant
	11	0	no sample		
	12	0	no sample		
Long Island Sound (LIS)	1	3	0		indeterminant
	2	3	0		female
	3	0	0		indeterminant
	4	1	0		indeterminant
	5	0.5	0		indeterminant
	6	0	0		indeterminant
	7	2	1	rare	indeterminant
	8	0.5	0		female
	9	0.5	0		female
	10	2	0		indeterminant
	11	0	0		male
	12	2	0		indeterminant
	13	no sample			
	14	0	0		indeterminant
	15	0	0		female
	16	1	0		indeterminant
	17	0	0		indeterminant
	18	1	1	rare	indeterminant
	19	1	0		indeterminant
	20	0.5	0		female
	21	1	0		indeterminant
	22	2	0		indeterminant
	23	0	0		female

Table 4-4 (continued).

Location	Sample No.	*Perkinsus* score	MSX score	MSX Intensity	Sex
East River, Queens (ERQ)	1	0	0		female
	2	5	0		male
	3	0	0		hermaphrodite
	4	0	0		female
	5	0	0		female
	6	0	0		indeterminant
	7	0	0		male
	8	0	0		female
	9	0	0		female
	10	0.5	0		indeterminant
	11	0	0		male
	12	0	0		male
	13	0	0		female
	14	0	0		female
	15	0	0		female
	16	0.5	0		female
	17	0	no sample		
	18	no sample			
	19	no sample			
	20	0	0		male
	21	0.5	0		male
	22	0	0		indeterminant
	23	0	0		female
	24	0	0		female
	25	0.5	0		indeterminant
	26	0	0		female
	27	no sample	0		female
Hackensack River (HKS)	1	4	2	light	male
	2	3	2	very light	indeterminant
	3	2	4	moderate	female
	4	0	2	light	indeterminant
	5	1	1	light	female
	6	0			
	7	0.5			
	8	2			
	9	0	4	moderate	female
	10	1	2	light	indeterminant

Table 4-4 (continued).

Location	Sample No.	*Perkinsus* score	MSX score	MSX Intensity	Sex
Hackensack River (HKS) cont'd	11	2	4	moderate	female
	12	0	4		male
	13	0	3	light	indeterminant
	14	3			
	15	1			
	16	0			
	17	0.5			
	18	0	4	moderate	female
	19	1	2	light	male
	20	0	4	heavy	indeterminant
	21	2	2	very light	female
	22	0	4	moderate	male
	23	1			
	24	2			
	25	0			
	26	0	3	moderate	female
	27	0	3	light	female
	28	2	2	light	female
	29	2	1	rare	indeterminant
	30	1	3	light	female
Hudson River (HDS)	1	0	0		female
	2	0	0		indeterminant
	3	0.5	0		indeterminant
	4	0	0		indeterminant
	5	0	0		indeterminant
	6	0.5	0		indeterminant
	7	0	0		indeterminant
	8	0	0		indeterminant
	9	0	0		indeterminant
	10	0	0		indeterminant
	11	0	0		indeterminant
	12	0	0		indeterminant
	13	0.5	0		indeterminant
	14	0	0		indeterminant
	15	0	0		indeterminant

Chapter 5: Bioaccumulation of Metals in Oysters of the Hudson River Estuary

INTRODUCTION

Although many metals are released naturally into the environment through weathering and leaching processes, human activities have modified the budgets of these elements in estuaries and coastal waters such that fish and shellfish that inhabit them pose a threat to human consumers (Karouna-Renier et al. 2007). Trace elements that exist naturally include copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), vanadium (Va), and zinc (Zn), all of which are essential elements in living organisms. Other metals, such as cadmium (Cd), arsenic (As), chromium (Cr) and lead (Pb) are toxic at low concentrations and considered heavy metals (Sajwan et al. 2008). Anthropogenic sources of metals include fossil fuel and waste burning, mining and ore processing, foundries and smelters, chemical production, agriculture, piping, and sewage disposal. Transport of metals to coastal and estuarine water occurs primarily from runoff and atmospheric deposition (Kimbrough et al. 2008).

The New York / New Jersey Harbor is best known for high levels of PCB and dioxin contamination, but the sediments are also among the nation's most contaminated with trace metals (Bopp et al. 2006). Major heavy metal sources to the Hackensack River include the Berry's Creek Superfund site (Goeller 1989; Bopp et al 2006). In the upper Hudson River, significant sources of zinc have been related to pulp and paper plant operations and lead, chromium, and cadmium have been associated with discharges of pigment manufacturing at a Hercules/Ciba-Geigy Plant (Bopp et al. 2006).

As filter feeders, oysters bioaccumulate these contaminants in their tissue. The early developmental stages of bivalve mollusks are most sensitive to metal toxicity

(Roesijadi 1996), but most metals, even at high concentrations, will not have a detrimental effect on the oyster. However, human consumption of an oyster in a heavily contaminated area can be dangerous. Large doses of nickel (Ni) can cause bronchitis and long-term exposure can result in cancer. Elevated levels of tin (Sn) in humans leads to liver damage, kidney damage and cancer. Arsenic (As) is also linked to cancer and can also be toxic to fish, birds and plants at high concentrations (Kimbrough et al. 2008). Copper accumulated in oysters can be toxic to humans (Chang 1962; O'Shaghnessy 1966; Pringle et al. 1968); however, the tissue of oysters with high levels of Cu has a characteristic green coloration and an unpleasant taste (Roosenburg 1969; Han & Hung 1990; Rieldel et al. 1998) and is therefore usually not consumed.

Oysters can tolerate high concentrations of Cu and Zn in particular with little effect (Shuster & Pringle 1969; Roesijadi 1996) because they possess specialized cells, the granular hemocytes, that are known to selectively uptake Cu and Zn, making these elements the most abundant in oyster tissue (Sajwan et al. 2008). Metal concentrations as high as 13,000 ug Cu g^{-1} and 25,000 µg Zn g dry $weight^{-1}$ have been observed in granular hemocytes of a related oyster specie, *Ostrea edulis* (George et al. 1978; Roesijadi 1996). Iron and calcium have also been detected in granular hemocytes but at much lower concentrations (Thomson et al. 1985; Roesijadi 1996).

Salinity has been reported to be a controlling factor of the bioaccumulation of Cu, with salinity having an inverse relationship with Cu concentrations in bivalves (Wright & Zamuda 1987; Sanders et al. 1991; Riedel et al. 1995). Higher concentrations in low salinity water could be due to an enhanced supply from fresh water and the desorption of Cu from particulate matter during initial mixing of fresh water and salt water (Knezovich

1994; Jiann & Presley 1997). Oysters are also known to take up Ag and Cd from solution more rapidly at lower salinities (Engel at al. 1981; Wright & Zamuda 1987; Abbe & Sanders 1990; Amiard-Triquet et al. 1991; Riedel et al. 1998). Freshwater discharges near the upper portions of estuaries also contribute to higher Cu, Ag and Cd in oysters at lower salinities.

Ag, Cu, Zn and Cd concentrations in oysters are normally an order of magnitude higher than that in sediment (Jiann & Presley 1997). The concentrations of Pb and Cr in oysters are significantly lower than that in sediment and the physiology of the oyster allows it to greatly discriminate against Pb and Cr, and for Cu and Zn, which are essential to life (Huanxin et al. 2000). In general, concentrations of Fe and Pb in oysters are about 1-5% of the concentration in sediments (Jiann & Presley 1997). Fe and Mn are both abundant in sediment, but also deposited quickly and are strongly bound in sediment under estuarine conditions so they are not readily available to oysters (Huanxin et al. 2000).

Of all metals, cadmium (Cd) has shown the most detrimental effect on oyster survival and health. It has no known biological function in animals and can interfere with various physiological processes in organisms from invertebrates to mammals (Lannig et al. 2006). It is known that Cd is toxic to fish, salmonid species and juveniles are especially sensitive and chronic exposure can result in reduction of growth (Kimbrough et al. 2000). In marine bivalves, Cd causes oxidative cellular damage by interfering with the antioxidant defense systems and increases cellular oxygen demand which stresses the organism (Lannig et al. 2006). In oysters, cadmium at 100 and 200 $\mu g\ L^{-1}$ has been shown in laboratory experiments to cause significant mortalities, general emaciation of

tissues and inhibition of shell growth during 20 weeks of exposure (Shuster & Pringle 1969; Roesijadi 1996).

There is no current data on the levels of metal bio-accumulation in oysters of the Hudson River Estuary. The National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) "Mussel Watch" program has been measuring chemical contamination by analyzing mussels (*Mytilus* species and *Dreissena* species) and oysters (*Crassostrea virginica*) from the East and Gulf Coast since 1986. The data are used to establish trends in more than 140 chemical contaminants of nearly 300 monitoring sites for use in assessing the impact of emerging contamination or environmental progress of site remediation and management decisions (Kimbrough et al. 2008). For the New York/New Jersey Harbor area and Long Island Sound, NOAA has test data from mussels only. It is known that different bivalve species accumulate different metal concentrations when exposed to the same environment (Riedel et al. 1995). Oysters have a greater affinity for zinc, copper and silver while mussels are better able to accumulate lead and chromium (Kimbrough et al. 2008), so their data on mussels cannot be used in substitute for oysters. In addition, their local sampling locations include Jamaica Bay, Raritan Bay and Long Island Sound, but no sampling is done in the Hudson, Hackensack or East Rivers.

NOAA's NS&T program has established National summary data with Low, Medium and High classification values for select metals based on concentrations typical of oysters collected on the East and Gulf Coasts (Table 5-1). In addition, NOAA has three sampling locations in Delaware Bay where oysters are tested: Ben Davis Point

Shoal (DBBD), Arnolds Point Shoal (DBAP) and Hope Creek (DBHC). The results of their 2004-2005 data are shown in Table 5-2.

The previously identified locations of oysters in the Hudson River Estuary; Hackensack River (HKS), Hudson River (HDS), East River, Bronx (ERB), East River Queens (ERQ) and Long Island Sound (LIS) are exposed to different sources of metal pollution. It was expected that the HRE oysters living in contaminated waters would exhibit higher metal concentrations than that of DB oysters where they are harvested and must not exceed Food and Drug Administration (FDA) action levels. It was also anticipated that the low salinity locations (HKS and HDS) would exhibit the highest metal concentrations due to their upstream location closest to freshwater inputs and to also have particularly higher concentrations of Cu and Cd, which are known to be more available for uptake in low salinity.

METHODS

Oysters at each location were tested for 13 metals: As, Cd, Cr, Co, Cu, Fe, Pb, Mo, Mn, Ni, Se, V and Zn. In addition, oysters from the harvestable waters of Delaware Bay and an additional reference group from the Narrow River, a tidal inlet also known as the Pettaquanscott River, in southern Rhode Island were also tested. This data along with NOAA's National summary data (Table 5-3) were used for comparison with the results of the HRE oysters.

With the exception of LIS, all oysters used in the analysis were from one seasonal collection from each study location (HDS, HKS, ERB, ERQ, DB, RI) noted in Table 5-3. Gill tissue was extracted, weighed and placed in 2-ml capacity screw cap vessels. One

ml of hydrogen peroxide (30%) and 1 ml nitric acid (69-71%) was added to each vessel and caps were tightly sealed. Samples were then placed in a microwave digester. Once digested, samples were transferred to 30-ml capacity polypropylene bottles and diluted to 10 ml. The samples were run through a Perkin Elmer Optical Emissions Spectrometer, Optima 5300 DV for As, Cd, Cr, Co, Cu, Fe, Pb, Mo, Mn, Ni, Se, V and Zn. Results in µg/L were converted to mg/kg dry weight by µg/L * (ml final volume/ (g wet sample * 0.20)) / 1000 µg/mg. To test precision, a duplicate sample was run which resulted in a relative percentage difference range of 1.1 – 16.3 %, with most metals having less than a 10% difference.

Significant differences between sampling locations and metal contamination were analyzed by performing a one-way ANOVA for each metal followed by a multiple comparisons test (Tukey-Kramer). Results were deemed significant at $P < 0.05$. All analyses were conducted using JMP statistical software.

RESULTS

Average concentrations (ppm) of the metals tested at each location are displayed in Table 5-3 and ANOVA results noting significant differences in select metal concentrations between locations are shown in Table 5-4. Lead in the Hackensack River (HKS) was the only contaminant to exceed the FDA action level for consumption (Table 5-3). Lead in the Hackensack and Hudson were both well above the "High" value established by the NS&T Program (Table 5-1). In addition to Pb, Zn and Cu were significantly higher ($P < 0.05$) in the Hackensack than any other location. HKS and

HDS, which are the lowest salinity sites, also had significantly more As and Cd than any other location.

ERB and the second sampling of Long Island Sound (LIS2), both conducted in July of 2009, had significantly more Cr than any other location. Nickel in the East River and LIS2 were higher than the "High" classification range established under the Mussel Watch program (Table 5-1). Levels of Cr and Ni varied significantly over two sampling times in LIS (LIS1 and LIS2) across a two year period (Table 5-4).

DISCUSSION

Metal concentrations in oysters at each site were compared using mean data which could give very different information than data derived from individual oysters. Oysters in poorer physiological condition could have higher metal levels (Riedel et al. 1998). In addition, variation in metal concentration can exist temporally as is shown by the large differences in contaminant concentrations of LIS oysters sampled two years apart. Metals can be purged from oysters within days if the water quality changes. For example, the rate of Cd loss in oysters once moved to non-contaminated waters has a reported half-life of 110 days (Zaroogian 1979; Roesijadi 1996) and 200 days (Van Dolah et al. 1987; Roesijadi 1996) and it can even turn over more quickly in gill tissue with a half-life of 35 days (Roesijadi & Klerks 1989; Roesijadi 1996). Therefore, the averages shown in Table 5-3 give a picture of what metals may have been influencing a particular area at a given time, but in order to establish a true baseline for metal contamination, samples would have to be taken monthly or seasonally for several years. A rise in metal content could also be due to the loss of tissue during spawning (Haven

1962; Galtsoff 1964; Abbe & Sanders 1988; Riedel et al. 1998). With the exception of HKS, all oysters were collected during the spawning season. However, even without the loss of tissue from spawning, HKS oysters exhibited the highest levels of metals overall.

Arsenic was highest at the low salinity sites of HKS (8.93 ppm) and HDS (8.91 ppm), but these levels are considered "Low" by NOAA's National summary data classification,. Zinc was highest at HKS (8406.67 ppm) and ERB (3699.67 ppm) but these levels are classified as "Medium" and all other locations fell into the "Low" classification. Copper at all locations was also in the "Low" to "Medium" range with the exception of HKS (724.29 ppm) which is considered "High".

Average Pb in oysters at HKS (9.84 ppm) exceeded the FDA action level of 1.7 ppm wet weight (8.5 ppm dry weight) with 10 of the 18 samples actually exceeding the standard. Lead in oysters is a small fraction of what it is in sediment and it is possible that concentrations detected are from fine sediment particles digested with the tissue in the process. However, it is unlikely that this happened in error of 10 samples from the same location and not for any of the other locations. Levels of Pb also exceeded the "High" value established by the NS&T Program at HKS and HDS. Low salinity and higher river flows of fresh water runoff at these locations most likely contributed to the high levels of lead. Lead occurs naturally in the Earth's crust, but high levels in the environment are linked to anthropogenic activities such as manufacturing processes, paint and pigment, solder, ammunition, plumbing, incineration and fossil fuel burning (Kimbrough et al. 2008).

Levels of Ni were substantially higher in both East River locations (ERB: 17.56 ppm; ERQ: 7.42 ppm) and LIS2 (22.09 ppm) than the national "High" level of 4.9 ppm

dry weight established under the NS&T program, although the program did report a maximum Ni concentration of 10 ppm wet weight (50 ppm dry weight) measured in Puget Sound, Washington State. Sampling at ERB and LIS2 were both conducted in July of 2009, and Ni levels likely reflect a local discharge during that time. High levels of Cr at these locations (ERB: 29.42 ppm; LIS2: 34.71 ppm) during this time also support a contaminated discharge with Cr levels of only 0.36 ppm detected at LIS1 in August of 2007. National trends in Cr are not published in NOAA's NS&T program report, but in comparison with the other Hudson River sampling locations and Delaware Bay (3.92 ppm) and RI (0.68 ppm), the levels of Cr at ERB and LIS2 are significantly higher. Determining the source of Ni and Cr pollution at these locations could be a subject for further study. Chromium and Ni values remain well below the FDA action level for consumption, 65 ppm and 400 ppm dry weight respectively, but more frequent monitoring of the locations are needed to determine their true ranges.

Cadmium was highest at the low salinity sites of HKS (9.02 ppm) and HDS (8.81 ppm), but levels remained in the lower "High" range of the NS & T program and were less than two of NOAA's sampling locations in Delaware Bay. Cadmium is known to affect oyster survival, but the levels found in the one-time sampling of the HRE do not appear to be of concern. In a study of Cd exposure and increased temperatures, there was a 96% survival rate of oysters at 20° with Cd accumulation near 200 ppm after 40 days of exposure (Lannig et al. 2006). Cadmium levels detected in oysters of the HRE were a fraction of this. However, continued monitoring of Cd would be needed to determine if cadmium reaches levels that could affect oyster survival.

Since the estuary is highly contaminated with many organic contaminants as well as metals, it is difficult to point to a particular pollutant or pollutants as the cause for a given problem (Wirgin et al. 2006). Metals will continue to inhibit fisheries in the HRE, but there are other contaminant problems that supersede those of metals contamination. With the exception of cadmium, metals are not known to inhibit the survival of oysters. Instead oyster uptake of metals from seawater and sediment allows them to be used as indicators of marsh and estuary health. They can be used to identify hotspots of chemical contamination and monitor trends in environmental cleanups.

The Hackensack River oysters had the highest levels of metals tested in this study, and may be the least ideal location for oyster restoration if accidental human consumption of the oysters is of concern. However, it cannot be proven that a particular metal or group of metals in any of the study locations are negatively impacting the oysters.

Table 5-1. NOAA National Status and Trends Mussel Watch Program (2004-2005), National summary classification of test levels of select metals (ppm dry weight).

Contaminant	Low	Medium	High
Arsenic (As)	3-11	12-22	23-57
Cadmium (Cd)	0-3	4-6	7-15
Copper (Cu)	7-211	212-636	637-1660
Lead (Pb)	0.1-0.5	0.6-0.9	1.0-2.2
Nickel (Ni)	0.7-1.6	1.7-2.5	2.6-4.9
Tin (Sn)	0.0-0.2	0.3-0.6	0.7-1.9
Zinc (Zn)	99-3260	3261-9165	9166-18950

Table 5-2. NOAA National Status & Trends results from three testing locations in Delaware Bay: Ben Davis Point Shoal (DBBD), Arnolds Point Shoal (DBAP) and Hope Creek (DBHC) (2004-2005) (ppm dry weight).

Location	As	Cd	Cu	Ni	Pb	Sn	Zn
DBBD	8.1	5.4	498	4	1.3	0.39	9165
DBAP	5.1	15	1660	4.9	1.4	0.28	18950
DBHC	4.3	20	857	4.4	0.72	0.17	11500

Table 5-3. Hudson River Estuary, Delaware Bay and Narrow River, RI metals testing results (ppm dry weight).

Location	Date	n	As	Cd	Cr	Co	Cu	Fe	Pb	Mo	Mn	Ni	Se	V	Zn
HKS	Feb-08	18	8.93	9.02	2.46	0.71	724.29	500.75	9.84	0.10	46.36	1.63	1.25	6.92	8406.67
ERB	Jul-09	12	0.75	0.70	29.42	ND	272.93	522.78	0.86	1.84	16.34	17.56	0.67	8.13	3699.67
LIS1	Aug-07	10	ND	0.18	0.36	ND	93.60	274.67	0.93	ND	8.85	ND	0.41	7.19	2354.15
LIS2	Jul-09	4	0.37	0.93	34.71	ND	145.99	514.08	1.52	1.34	18.97	22.09	0.45	9.30	2026.26
HDS	May-09	8	8.91	8.81	0.76	0.12	434.42	313.03	4.92	0.14	16.72	0.44	0.39	4.13	2470.83
ERQ	Aug-07	24	ND	ND	ND	NA	77.97	189.41	ND	NA	12.09	7.42	7.76	ND	1052.85
RI	Oct-08	19	0.58	0.65	0.68	0.13	28.49	177.41	ND	0.18	6.27	0.52	0.76	8.00	1771.50
DE	May-07	12	2.77	2.84	3.92	0.20	175.98	161.40	1.49	0.60	8.65	4.36	0.40	4.32	2691.58
FDA action level (wet weight) dry weight			(86) 516	(4) 20	(13) 65				(1.7) 8.5			(80) 400			

Table 5-4. ANOVA results of select metals detected from the Hudson River Estuary, Delaware Bay and Narrow River, RI.

Arsenic				**Cadmium**				
HKS	A			HKS	A			
DB		B		HDS	A			
RI			C	DB		B		
ERB			C	RI			C	
LIS2		B	C	ERB			C	
ERQ			C	LIS2		B	C	
LIS1			C	LIS1			C	
				ERQ			C	
Chromium				**Copper**				
LIS2	A			HKS	A			
ERB		B		HDS		B		
HKS			C	ERB		B	C	D
DB			C	DB		B	C	
HDS			C	LIS2		B	C	D
RI			C	LIS1			C	D
LIS1			C	ERQ			C	D
ERQ			C	RI				D
Iron				**Lead**				
ERB	A			HKS	A			
LIS2	A	B		HDS		B		
HKS	A			DB		B	C	
HDS	A	B		ERB		B	C	
LIS1	A	B		LIS2		B	C	
DB	A	B		LIS1		B	C	
ERQ		B		ERQ			C	
RI		B		RI			C	
Nickel				**Zinc**				
LIS2	A			HKS	A			
ERB		B		DB		B		
DB			C	ERB		B	C	
HKS			C	HDS		B	C	
ERQ			C	LIS1		B	C	
HDS			C	LIS2		B	C	
RI			C	RI		B	C	
LIS1			C	ERQ			C	

Chapter 6: Genetic Population Structure of Oysters in the Hudson River Estuary

INTRODUCTION

Low abundances of oysters exist in the Hudson River Estuary attached to rock and other hard substrate in geographically separated intertidal zones previously identified in this study (Figure 2-1). The HKS location is associated with tidal flows from Newark Bay, the HDS is located upriver of Raritan Bay and the other three populations (ERB, ERQ and LIS) are associated mostly with the Long Island Sound, which has limited flow with the Hudson River and Raritan Bay. The extent of oysters living subtidally between these areas is unknown. Surviving larvae of *C. virginica* travel with the tides for 2-3 weeks before setting, so it is unknown as to whether oysters living in a given area are reproducing with those of another area. There is the potential that oysters of the HRE genetically constitute one population especially if oysters exist continuously in subtidal areas not identified in this study. It is expected, however, that gene flow will be limited based on geographic separation and the low numbers of oysters found in each location.

Establishing a baseline of the genetic structure of HRE oysters will help in understanding how to restore them to the estuary. The genetics of the Eastern oyster has been linked to survival, disease resistance, and adaptability in different estuary environments. It was found by Dittman et al. (1998) that oyster strains with origins in Long Island Sound were significantly larger over several generations than oyster strains from Delaware Bay and Chesapeake Bay when raised in the same environment (Dittman et al. 1998). In addition, oyster strains with Long Island origin initiated gonadal development and began spawning about one month earlier than oysters from Delaware Bay when raised in the same environment, even after six generations (23 years) (Barber

et al. 1991). These oysters were not analyzed to distinguish them genetically, but the studies imply that there may be genetic differences that cause oysters to maintain their growth and reproductive timing when brought into a different environment.

Selective breeding has been used to increase genetic resistance to MSX and Dermo disease. So far, strong resistance to MSX and moderate resistance to Dermo are obtained after 4-5 generations of mass selection (Guo et al. 2008). Selective breeding has been practiced without extensive knowledge of the genetics behind the disease resistant trait. Recent studies have found that the microsatellite marker *Cvi2i23*, which was a locus chosen for this study, is the most strongly affected marker showing significant frequency shifts after disease-caused mortalities (Guo et al. 2008).

Mitochondrial DNA gene sequences and microsatellite markers (nuclear DNA) have been used in previous genetic studies of *C. virginica*. With few exceptions, animal mitochondrial DNA is a closed circular molecule, typically 15-20 kilobases in length, and composed of about 37 genes coding for 22 tRNAs, 2 rRNAs, and 13 mRNAs specifying proteins involved in electron transport and oxidative phosphorylation (Avise 1994). Mitochondrial DNA normally evolves rapidly, which is why it is favorable to use in analyses of population structure. For *C. virginica*, the cytochrome oxidase Subunit III (CO3) and NADH dehydrogenase Subunit IV (ND4) regions have been found to be most polymorphic (Milbury and Gaffney 2005).

Microsatellite markers, tandem arrays of short nucleotide repeats, are useful in genetic analyses because they are often polymorphic, relatively easy to detect and have been presumed to be dispersed fairly evenly throughout the genome and to be selectively neutral (Reece et al. 2004). Microsatellites are typically one to four and sometimes six

nucleotides in length and can consist of ten to a hundred repeats. Indels generated by slipping and mispairing of DNA strands at replication and by unequal crossing over alter the repeat number. Individual alleles of these loci differ from one another by the number of tandemly repeated copies (Altukhov & Salmenkova 2002). Microsatellite primers for *C. virginica* have previously been developed by Reece et al. (2004) and Carlsson et al. (2006), of which six were chosen for this study.

Microsatellite and mitochondrial DNA markers were used to determine the genetic divergence of HRE oysters from one another, a southern population from Delaware Bay and a northern population from a tidal inlet in Rhode Island. The wild oysters found today may be recent reestablishments or descendents of those of the large oyster industry that once existed in the early 1900's. The analysis can help determine if the HRE populations are isolated, bottlenecked groups at risk for local extinction or if the genetics reflect that of a larger population. It was expected that due to the low abundance of oysters at each of the HRE locations that genetic diversity would be low and gene flow would be limited especially from the HDS and HKS locations due to the large geographic separation. It was also anticipated that there would be significant genetic differentiation of the HRE oysters from those from Delaware Bay and Rhode Island.

METHODS

Oysters used in the genetic analysis were collected once from the Narrow River, Rhode Island in October, 2008 and the Shell Rock Bed of Delaware Bay in May, 2007 and over multiple sampling events in years 2007 to 2009 from each of the HRE locations.

Sample size was limited due to yearly permit collection restrictions and availability of oysters at each location.

Oysters were shucked and a portion of adductor muscle was cut and excised and stored at -80°C. In the case of juvenile oysters where the biomass of the adductor muscle was minimal, the entire soft tissue was excised and stored. DNA was extracted using a DNeasy tissue kit (Quagen Inc.).

Mitochondrial DNA

Mitochondrial DNA haplotypes were assayed from cytochrome oxidase Subunit III (CO3) and NADH dehydrogenase Subunit IV (ND4). Polymerase chain reactions (PCR) were conducted with 2.5 µl deoxynucleoside triphosphate (dNTPs), 1.0 µl each primer, 0.05 *Taq* polymerase (Fisher), 2.5 µl polymerase buffer 1 µl template DNA and deionized water for a total volume of 25 µl. PCR amplification of CO3 and ND4, followed Milbury (2003) (Table 6.2). After PCR amplification, 5 µl of PCR product was placed in an agarose gel and size was determined by comparison to a one-kilobase ladder. PCR products were cleaned with AMPure and cycle sequenced. The cycle sequence product was cleaned by alcohol precipitation and final product was re-suspended in 8 µl of injection solution. The amplified fragments were sequenced on an ABI 3730XL sequencer.

Data Analysis

Sequences were edited in Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI) and aligned in Genious software (Drummond et. al 2009). Gene diversity analyses were performed with the software package DnaSP Version 5 (Librado & Rozas 2009). For each location, the gene diversity statistics at the haplotype and nucleotide levels were

computed. Tajima's (1989) D-test, Fu and Li's F and Fu and Lis's D tests were also performed in DnaSP Version 5 to test for deviations from neutral molecular evolution. Phylogenetic analyses of the mtDNA sequences were prepared using MEGA 6.0 software.

Analysis of molecular variance (AMOVA) was performed to test population structure of *C. virginica* using ARLEQUIN version 3.1 (Excoffier et al. 2005). The calculation of significance ($P < 0.05$) of pairwise comparisons of the F_{ST} analogue φ_{ST} among localities was based on 1000 permutations of the data matrix. The F_{ST} indices were estimated using haplotype frequencies and the significance levels of pairwise F_{ST} were determined by means of 10,000 permutations of haplotypes between the populations.

Microsatellites

Oysters were genotyped for six microsatellite loci, *Cvi1g3*, *Cvi1i24b*, *Cvi2g14*, *Cvi2i4* and *Cvi2i23* previously developed by Reece et al. (2004) and *Cvi5* developed by Carlsson et al. (2006). The primers are listed in Table 6.1 with annealing temperatures. These primers were chosen for genetic comparison across all study areas and for additional comparison purposes with Rhode Island and Delaware Bay stock.

A total volume of 25 µl consisting of 2 µl of dNTPs, 0.2 µl forward primer, 1.0 µl reverse primers, 1 µl of DNA, 0.1 µl of *Taq* polymerase (Fisher), 2.5 µl polymerase buffer, 0.9 µl fluorescently labeled primer (FAM) and deionized water was prepared for PCR. The PCR was programmed as outlined in Rose et al. (2006). After amplification, 8.8 µl formamide and 0.2 µl LIZ 600 size standard was combined with 1 µl PCR product

for fragment analysis. PCR products were electrophoresed with an Applied Biosystems ABI 3730XL sequencer.

Data Analysis

Allele sizes were estimated using GeneMapper® software. Peaks greater than 1000 relative fluorescent units were scored. Repeats of PCR were performed on samples where no peak was present. If electropherogram peaks were still absent then the locus was considered nonamplifying.

Microsatellite results were exported from GeneMapper® to Microsoft Excel. Using the Excel Microsatellite Toolkit results were inputted to GENEPOP. The tests for departure from Hardy-Weinberg equilibrium (HWE) were performed by calculations of Wright's F_{IS} according to Weir and Cockerham (1984) using GENEPOP Version 3.3 (Raymond & Rousset 1995). Dememorization number, number of batches and number of iterations per batch were set at 1000, 100 and 1000 respectively. The genetic differentiation between populations (F_{ST}) was analyzed with analysis of molecular variance (AMOVA; Excoffier et al. 1992; Huff et al. 1993) in GenAlEx Version 6 (Peakall & Smouse 2006). Nei's genetic distance matrices among populations were also computed in GenAlEx Version 6 and used to construct an unweighted pair-group method with arithmetic averages (UPGMA) tree with the NEIGHBOR, CONSENSE and DRAWGRAM programs in the PHYLIP package (Version 3.69; Felenstein 2004). The effective population size was calculated using the linkage disequilibrium method (Hill 1981) in NeEstimator version 1.3 (Ovendun et al. 2007).

Table 6-1. Microsatellite markers and annealing temperatures (T_A in °C).

Locus	Repeat sequence	Primers (5' to 3')	T_A
*Cvi*lg3	(TAA)2TA(TAA)	F: CATAAAGTTAATGCTTC R: ATAGCGAGTTGAGGAACC	51
*Cvi*li24b	(CA)10	F: TGACACCTCCCCACACTGTTG R: TCCTTAGAAAACCCATAGCAATGC	52
*Cvi*2g14	(TC)4TT(TC)20	F: GTCCTAACTACATTTATCACAC R: TTCTTCTTACAAAACAGACTG	52
*Cvi*2i4	(GATT)24	F: AATAATACAAAATCCAGTAGC R: CCAATCAAATCTCACTAAAG	47
*Cvi*2i23	(GTTT)7	F: TAACACAAAGCCAACATCGCC R: AAGTAAAAGACGGTCAAAGGGTCC	51.5
Cvi5	(CT)19	F: ATAAAAGTCCATTCGTAAGC R: AGATTTGAAGTATTGCTATCG	47

Table 6-2. PCR and sequencing primers and conditions used to amplify two mitochondrial gene regions of *C. virginica* in the sampled sites.

mtDNA Region	PCR Primers	PCR amplicon (bp)	Cycling conditions	Sequence product (bp)
CO3	Forward: ATTTAGTTGATCCTAGGCCTTGACC Reverse: CCCACAAAACAACAGCCCGCAAGT	635	94°C 2:00; 35 cycles: 94°C 0:45 57°C 0:45 72°C 0:45; 72°C 5:00	598
ND4	Forward: AAATAGGTTAGGGGGACTCAGC Reverse: GGAACCAGAAAAATCTCGACC	790	94°C 2:00; 35 cycles: 94°C 0:45 54°C 2:00 72°C 0:45; 72°C 5:00	668

RESULTS

Mitochondrial DNA

The number of haplotypes, number of polymorphic sites, the values of nucleotide diversity (π) and haplotype diversity (h) within each population are presented in Table 6-3. The CO3 gene was sequenced for 598-base pairs among 121 specimens. Forty six polymorphic sites and 50 haplotypes were detected. The haplotype diversity, which estimates the probability that two randomly chosen individuals from the population will have different haplotypes, was high in each locality along with the sequence divergence between the haplotypes (0.263%-0.456%). The samples from ERQ showed the lowest number of haplotypes (No = 6) and haplotype diversity (h = 0.648). Samples from HKS showed the largest number of haplotypes (No = 17) but moderate haplotype diversity (h = 0.802) and the lowest sequence divergence between the haplotypes (π = 0.00263). Samples from LIS showed the highest haplotype diversity (h = 0.884) and nucleotide diversity (π = 0.00456).

For the ND4 region, haplotype diversity was again high within each locality. The sequence divergence (0.201%-0.352) was also high, but less than that of the CO3 region. The samples from ERQ again showed the lowest number of haplotypes (No = 3), polymorphic sites (No = 6) and haplotype diversity (h = 0.600), but also had the smallest sample size (n = 6). The HKS location again showed the lowest nucleotide diversity (π = 0.00201). ERB and HDS had much higher haplotype diversity and the LIS location had much lower haplotype and nucleotide diversity than had previously been shown by the CO3 region.

For the CO3 region, only haplotype 1 (46.3% in total) was shared among the six populations (Table 6-4). Haplotype 3 (HKS, ERQ, LIS and DB) and haplotype 15 (HDS, ERB, LIS and DB) were shared among four populations, haplotype 23 (HKS, HDS, DB) among three, haplotype 9 (HDS, LIS) haplotype 16 (ERB, LIS) and haplotype 35 (HKS, ERQ) among two. The others were population specific haplotypes. Each population had their own characteristic 'private' alleles ranging from thirteen at HKS to three at ERQ.

The ND4 gene was sequenced for 668-base pair among 97 specimens, 44 polymorphic sites and 41 haplotypes were detected (Table 6-5). Haplotype 3 (43.3%) was the most common and detected in all six populations. Haplotype 4 (HKS, HDS, ERB, DB) was shared among four populations, haplotype 5, haplotype 7 and haplotype 31 were all detected in HKS and HDS only, haplotype 9 (HDS, LIS), haplotype 10 (HKS, DB) and haplotype 29 (LIS, DB) were presented in two populations. All other haplotypes were population specific. Each population again had their own private alleles with HKS again having the highest number (n = 10), and ERQ having the fewest, (n = 2).

Results from Tajima's D, Fu and Li's F* and Fu and Li's D* are shown in Table 6-6. Tajima's D, which measures the disparity between the number of segregating sites and the pairwise genetic distance, resulted in negative values for both the CO3 and ND4 genes for each location and the overall population. For the CO3 gene, Tajima's D for HKS and ERB along with the total population was highly significantly different from zero ($P<0.01$) and location HDS was significantly different ($P<0.05$) from zero. For the ND4 gene, Tajima's D for HKS and HDS were significantly different from zero and the total population was highly significantly different.

For the CO3 region, the Fu and Li's D* and Fu and Li's F* statistical tests both deviated highly significantly from the neutral molecular evolution at ERB and for the total population and deviated significantly at HDS and LIS pointing to an excess of rare haplotypes. Fu and Li's F* test was significant only for HKS. The Fu's F-test was devised specifically to detect population expansion and is more sensitive to the presence of singetons in the samples. For the ND4 region, the Fu and Li's D* and Fu and Li's F* statistical tests both deviated significantly at HKS and DB and was highly significant at HDS and the total population.

The AMOVA analysis of *C. virginica* based on haplotype frequencies for the CO3 region revealed that 96% of the genetic variation occurred within the population, whereas 4% of the genetic variation occurred among populations. AMOVA results for the ND4 region revealed 99% genetic variation within and only 1% among populations (Table 6-7). The average fixation indices for the CO3 region (φ_{ST}= 0.04023, *P*=0.009) departed significantly from zero, indicating genetic structures exist in the *C. virginica* populations; however, the fixation indices for the ND4 region (φ_{ST}= 0.00790, *P*=0.216) were not significant.

Minimal population genetic structuring within the six localities was revealed by F_{ST} analyses (Table 6-9). According to the F_{ST} values derived from the haplotype frequencies, genetic differentiation was found with both the CO3 and ND4 regions between LIS and DB. CO3 region analysis showed additional genetic differentiation between HKS and both HDS and DB. The ND4 region analysis revealed additional differentiation between HKS and LIS.

A neighbor-joining (NJ) tree of all the haplotypes was constructed using both the *C. virginica* mtDNA CO3 and ND4 gene sequences with K2P distances implemented in MEGA. However, the topology of both the unrooted NJ trees revealed that the haplotypes were not phylogeographically structured (detailed data not shown).

Microsatellite

The number of alleles per locus varied greatly among loci, ranging from 4 at locus *Cvi1g3* to 25 at locus *Cvi2g14*. The observed and expected heterozygosity (H_o and H_e) varied among loci and to a lesser extent among populations (Table 6-10). Significant Hardy-Weinberg deviations were found in 33 of the 49 cases (7 loci x 7 populations) with the F_{IS} estimator found to be significantly different from zero ($P < 0.001$). The deviation was more locus-specific than population specific, although the RI location did not deviate from HWE at locus *Cvi2i4* and both RI and ERB at locus *Cvi1g3* while the other populations did. Most significant deviations were deficient for heterozygotes, with the exception of locus *Cvi5* which revealed significant heterozygote excess at the LIS location.

There was significant genetic differentiation among the seven populations (Table 6-11). Pairwise F_{ST} statistics from all six microsatellite loci show significant genetic differentiation from DB to at least one other HRE population (HDS, HKS, ERQ, ERB or LIS), suggesting that the greatest genetic difference with the HRE populations is from the Delaware Bay population. The RI oysters showed genetic differentiation from DB and all HRE populations at either locus *Cvi5*, *Cvi2i23* or *Cvi2g14,* with the exception of ERQ. Within the HRE populations, loci *Cvi2i23* and *Cvi2g14* showed the most significant genetic differentiation results with differences detected between the Hudson

River (HDS) and both LIS and ERB, and the Hackensack River (HKS) with both LIS and ERB. The East River, Queens (ERQ) and East River, Bronx (ERB) showed significant differentiation at loci *Cvi2i23*, *Cvi24b* and *Cvi2g14*. ERQ and LIS were significantly different at loci *Cvi1g3* in addition to *Cvi2i23* and *Cvi2g14*.

The effective population size (Table 6-12) of Delaware Bay oysters (N_e = 395) exceeded that of any HRE location. Of the HRE locations, ERQ had the largest Ne (N_e = 242) and ERB had the least (N_e = 38).

DISCUSSION

Oysters collected from the HRE populations are from intertidal areas where oysters are found scattered and in low abundance. It was expected that they would exhibit a reduced number of haplotypes and polymorphic sites relative to abundant oyster reefs of Delaware Bay. However there were not a reduced number of polymorphic sites or haplotypes relative to the number of individuals sampled in any of the HRE populations. In addition, 3 of 6 intermediate haplotypes of the CO3 region and 3 of 7 intermediate haplotypes of the ND4 region were shared with the Delaware Bay population indicating a lesser degree of genetic differentiation of HRE oysters and Delaware Bay. The negative value of Tajima's D for all populations also supports this observation. A negative Tajima's D signifies an excess of low frequency polymorphisms, indicating population size expansion and/or positive selection. A high frequency of polymorphisms, represented by a positive Tajima's D indicates a decrease in population size and/or balancing selection. Isolation of the HRE populations is not supported by Tajima's D. However, each population does exhibit private alleles that

could be used as indicators of stock identification or to monitor recruitment. Such a study was done in Chesapeake Bay where the successful reproduction of Louisiana oysters, which can be distinguished from Atlantic oysters by single-nucleotide polymorphisms in their mitochondrial DNA, was monitored by detecting the Gulf Coast haplotype in newly recruited oysters (Milbury et al. 2004).

F_{ST} analysis for microsatellite loci (Table 6-11) revealed much more genetic differentiation between localities than the mtDNA revealed (Table 6-9). Populations that were found by mtDNA analysis to have genetic differentiation agreed with the microsatellite results, with the exception of the CO3 region analysis, indicating that HKS and HDS populations were significantly different. For mtDNA analysis a disproportionately high sample number was analyzed from HKS than the other HRE locations due to an abundance of oysters at HKS and minimal permit collection restrictions. Therefore, the significant F_{ST} values for HKS in the mtDNA analysis may have been affected by the sample size.

Total pairwise F_{ST} and φ_{PT} statistics for all six microsatellite loci combined detected significant differentiation of ERB and LIS from all other populations except one another. These two populations are located in the Upper East River/ Western Long Island Sound portion of the estuary where there is limited net flushing into the Hudson River/Raritan Bay likely inhibiting genetic mixing with the other Hudson and Hackensack River locations. It was expected that the ERQ location would be genetically similar to ERB which are across from one another in the East River, but instead significant genetic differentiation was found. The ERQ location also exhibited the highest effective population size of all HRE locations (Table 6-12), while the ERB

location had the least, suggesting that the ERB oysters are bottlenecked. The hydrodynamics of the East River may be preventing larvae from mixing horizontally between shores. Water circulates in the upper East River with the net movement of mostly surface water to the Long Island Sound and a westward net movement of mostly bottom water toward New York Harbor. It was found that water moves from Long Island Sound to New York Harbor at a rate of about 310 m^3 s^{-1} and toward Long Island Sound at a rate of about 260 m^3 s^{-1}(Blumberg & Pritchard 1997). The ERB and LIS locations are both on the northern shores of the East River and entering into the western Long Island Sound while the ERQ location is on the southern side. Larvae originating from an oyster population near a shoreline and traveling with the upper East River currents are likely to remain on the same side of the river. In addition, the hydrodynamics of Orchard Beach Lagoon, where the LIS population resides, may be limiting the flushing of larvae into the main waters of Long Island Sound. The oysters were found living at the very end of the lagoon where there is no outlet and larvae may become trapped here and forced to settle. If this could be proven it would provide reasoning for how this population is maintaining itself in an area where seston is accumulating.

The RI location was also significantly different from all except HKS; and DB differed significantly from all except HDS and ERQ. The RI and DB oysters are separated from the HRE oysters by approximately 210 km of ocean and bays, so it is not surprising that significant genetic differences were detected. Only a small sample size of oysters from RI was available (n= 22) so the lack of significant genetic differences from HKS may have been due to low sample size.

AMOVA analysis revealed insignificant genetic structure for the ND4 region and significant but weak genetic structure for the CO3 region (96% within populations and 4% among populations) and microsatellite analysis (98% within populations and 2% among populations) (Table 6-7 and Table 6-8). The microsatellite analysis included an additional group of Rhode Island oysters. The AMOVA results of this study are similar to that of other studies of *C. virginica* and other wild marine species with high larval dispersal. AMOVA of microsatellite data from wild Delaware Bay oysters with a strain subject to long-term selection for disease resistance with original lines from Delaware Bay resulted in variance of 96% within populations and 4% among populations (Yu & Guo 2005). The disease resistant strain had been bred for disease resistance since the early 1960s. In the same study, AMOVA results of microsatellite data of disease resistant strains originating from the Northeast, Delaware Bay, Long Island Sound and Connecticut resulted in variance of 83% within and 17% among populations, which are a result of selective inbreeding and the origin of the parent stock. In a study of the genetic structure of natural populations of the larvae dispersing California black abalone (*Haliotis cracherodii*), using mitochondrial cytochrome oxidase subunit one (COI) DNA sequences, the highest among group variation was 6%, of eleven locations spanning 750 km (Gruenthal & Burton 2008). Similarly, a genetic analysis using mtDNA 16s rRNA sequences of the swimming crab, *Portunus trituberculatus*, resulted in 93.7% variation within populations and 6.3% among six localities along the coast of the East China Sea (Xu et al. 2009).

HWE was not observed in 4 out of 6 microsatellite loci. Heterozygote deficits at microsatellite loci for wild bivalve populations are well known (Reece et al 2004, Rose et

al 2006, Carlsson et al. 2006, Galindo-Sanchez et al. 2008). Inbreeding, aneuploidy, and null alleles have been used to explain this phenomenon. Inbreeding would result in fewer heterozygous individuals, but this view is discounted due to the reproductive biology of the oyster, which should ensure random mating (Gaffney 1996). Another theory is the occurrence of aneuploidy, the lack of particular chromosomes or fragments of, resulting in hemizygous individuals, which would also be scored as homozygous (Gaffney 1996). The most common explanation is an abundance of null heterozygote alleles, which would be scored as homozygotes because they carry only one active allele. They are present when certain alleles fail to amplify, resulting in heterozygous genotypes that appear to be homozygous (Chakraborty et al. 1992). The high level of nuclear DNA polymorphism observed in oysters, particularly in noncoding regions, makes it difficult to design PCR primers that will reliably amplify the target region in all individuals (Hedgecock et al. 2004). Null alleles may elevate perceived levels of genetic divergence; however, the two loci in HWE (*Cvi5* and *Cvi2g14*) along with *Cvi2i23*, displayed the most significant pairwise values of F_{ST}. In addition, the AMOVA analysis was rerun using only *Cvi5* and *Cvi2g14* loci and resulted in variance of 97% within populations and 2% among populations, only in a 1% difference from the result of all six loci included. Hence the inclusion of four loci with high frequencies of null alleles did not significantly impact the results. *Cvi5* and *Cvi2g14* have also been found in other studies to remain in HWE (Carlsson et al. 2006; Hare et al. 2006). In addition, *Cvi5* was successfully used to show significant genotypic disequilibrium in Chesapeake Bay in comparing wild and hatchery stock (Carlsson et al. 2006).

Cvi5 for the LIS location was the only marker to deviate from HWE in the direction of heterozygote excess. Singh and Zouros (1978) found a positive relationship between multilocus heterozygosity and growth rate in the Eastern oyster. In addition, Zouros et al. (1983) also found higher survival rates of more heterozygous individuals from two weeks to three years of age. Growth was not significantly different among any of the HRE locations however the condition index of oysters at the LIS location was significantly higher. Whether the difference is related to the genetics of the LIS oysters cannot be determined but the heterozygote excess at this locus was noted.

The effective population size (N_e) of each location (Table 6-12) correlated with the number of oysters observed during times of collection. N_e was highest for the DB population and then the ERQ and HKS locations followed, where oysters were easily seen and collected. Locations where they appeared more spaced like the ERB and HDS locations had the smallest N_e. Oysters were easily found at the LIS location at the inner end of Orchard Beach Lagoon, but very few were found at the other end of the lagoon toward Long Island Sound suggesting that they are a limited population, as was reflected by the low N_e.

C. virginica is a marine species spawning eggs and sperm with resultant larvae having high potential for dispersal which could result in little intraspecific population structuring over large geographic areas. The fixation of the polymorphic alleles observed in our study requires time. It is known that there is little genetic variation in the entire Eastern oyster population at the allozyme level (Gaffney 1996). However, there is support for an Atlantic and Gulf Coast split in the area known to marine biogeographers as the demarcation zone, where other marine species have also been known to diverge.

The Atlantic mid-coast of Florida is a long-recognized transitional zone between warm-temperature and tropical faunal assemblages, with many marine species exhibiting northern or southern distributional limits in this area (Reeb & Avise 1990). In addition to this southern split, allelic frequencies vary in populations north and south of Cape Cod to Corpus Christi, therefore, there are at least four genetically distinct Eastern oyster populations: eastern Canada, Cape Cod to Corpus Christi, Laguna Madre, and southern Gulf of Mexico (Gaffney 1996).

Marine bivalves that have been bottlenecked would be expected to show genetic divergence and a decrease in heterozygosity in comparison with the main population. However, there is a lack of information available on the genetic effects of bottlenecking for marine bivalves due to their widespread geographic distribution and gene flow associated with mobile larvae. Carlton and Mann (1996) state that two populations of *C. virginica* from British Columbia and Oahu that have been isolated from parental Atlantic stocks since the 1930s and 1940s would be of particular interest to study for the effects of bottlenecks on population fitness and for comparison to gene flow from the mainland Atlantic, Gulf Coast and Caribbean islands in maintaining observed levels of genetic heterozygosity.

For highly differentiated populations, population structure can be statistically detected even with small sample sizes. However, this is generally not the case for marine populations with high gene flow (Ruzzante 1998; Rose et al 2006). Sample sizes of this study were limited to the oysters that were available at each location and to permit restrictions in sample collections due to the low abundance of oysters. A larger data set of samples may have provided somewhat different results.

Nevertheless, the microsatellite analysis did detect genetic differentiation, particularly separating the LIS and ERB locations from the others and individual loci detected significant differentiation of DB and RI from the HRE populations. Both microsatellites and mtDNA have been used successfully to distinguish wild and aquaculture populations in other estuaries such as Chesapeake Bay (Carlsson et al. 2006; Hare et al. 2006). However, in a study of gene flow of geographically separated local oyster populations in Chesapeake Bay little genetic differentiation was revealed by F_{ST} (Rose et al 2006). Therefore, the discovery of significant genetic differentiation in population pairs of wild oysters in the HRE indicates that there is limited gene flow occurring among some populations. However, there were not a reduced number of alleles as shown by microsatellite analysis or reduced number of haplotypes and polymorphic sites as shown by mtDNA analysis than the known larger population of Delaware. This suggests that the abundance of oysters in the HRE may be greater than initially thought.

Subsequent to the collection of oysters in this study, hatchery oysters were introduced at the ERB, ERQ and HKS locations. Oyster restoration projects often use larvae and juvenile oysters acquired from oyster nurseries from other estuaries such as Delaware Bay, Chesapeake Bay and Eastern Long Island Sound that are bred for growth or disease resistance. The large number of oyster transplantation programs in the Chesapeake, along with the extent of larval dispersal are explanations for the lack of genetic variation found there. The genetics underlying the wild populations of the HRE will change with time if more oysters are introduced. Introducing disease resistant strains for the potential of reestablishing a larger oyster stock outweighs the concern of genetic

effects in estuaries like Chesapeake Bay but there is still caution in doing so. Introducing disease-tolerant strains of *C. virginica* that have been inbred could cause them to have reduced fitness when exposed to natural environments as compared with their wild counterparts. In addition, hatchery strains may be subjected to specific selective forces at the hatchery, which may be very different from those encountered on natural restoration sites (Carlsson et al. 2006). The consequences of inbreeding depression could occur, lowering average viability or fecundity. Millions of dollars that are spent on oyster restoration with an inbred stock could result in a failed project. In addition, in the long term, population supplementation with inbred stocks can cause the genetic health of wild populations to deteriorate (Waples & Do 1994; Wang and Ryman 2001; Hare et al. 2006).

Unlike Delaware Bay and Chesapeake Bay, the oysters of the HRE are not a harvested fishery where depletions of stocks can have devastating effects on local industry. The interest in reestablishing the Eastern oyster to the estuary is strictly ecological. However, the oysters that do exist naturally have survived through unique ecological pressures, including poor water quality that may make them more genetically fit for survival than inbred nursery stock. The results of this study have shown that the genetic structure of the sampled HRE populations is different from Delaware Bay and Rhode Island oyster populations. To ensure survival, oyster restoration may benefit in the long term by attempting to recruit the settlement of natural larvae from these populations as opposed to transplanting non-native stock.

Table 6-3. Summary of genetic diversity indices of *Crassostrea virginica* by mitochondrial DNA gene and geographic location.

Location	N	No. haplotypes	No. private haplotypes	No. polymorphic sites	Haplotype diversity (*h*)	Nucleotide diversity (π)
COIII region						
HKS	31	17	13	17	0.802	0.00263
HDS	21	11	7	15	0.738	0.00355
ERQ	15	6	3	10	0.648	0.00289
ERB	13	7	4	10	0.731	0.00268
LIS	20	13	8	17	0.884	0.00456
DB	21	12	8	12	0.857	0.00315
Total	121	50	43	46	0.783	0.00319
ND4 region						
HKS	34	16	10	15	0.752	0.00201
HDS	18	13	7	16	0.902	0.00304
ERQ	6	3	2	6	0.600	0.00295
ERB	9	7	5	10	0.917	0.00352
LIS	17	7	4	9	0.765	0.00210
DB	13	9	5	10	0.910	0.00272
Total	98	41	33	44	0.809	0.00251

Table 6-4. The haplotype frequencies of CO3 gene in *Crassostrea virginica from six* populations.

Haplotype	Haplotype frequency						
	HKS	HDS	ERQ	ERB	LIS	DB	Total
H1	14	11	9	7	7	8	56
H2	-	1	-	-	-	-	1
H3	1	-	2	-	1	2	6
H4	-	-	1	-	-	-	1
H5	-	-	1	-	-	-	1
H6	-	-	-	-	1	-	1
H7	-	-	-	-	1	-	1
H8	-	-	-	-	1	-	1
H9	-	1	-	-	1	-	2
H10	-	-	-	-	1	-	1
H11	-	-	-	-	1	-	1
H12	-	-	-	-	1	-	1
H13	-	-	-	-	1	-	1
H14	-	-	-	-	1	-	1
H15	-	1	-	1	2	1	5
H16	-	-	-	1	1	-	2
H17	-	-	-	1	-	-	1
H18	-	-	-	1	-	-	1
H19	-	-	-	1	-	-	1
H20	-	-	-	1	-	-	1
H21	-	1	-	-	-	-	1
H22	-	1	-	-	-	-	1
H23	1	1	-	-	-	2	4
H24	-	1	-	-	-	-	1
H25	-	1	-	-	-	-	1
H26	1	-	-	-	-	-	1
H27	1	-	-	-	-	-	1
H28	1	-	-	-	-	-	1
H29	1	-	-	-	-	-	1
H30	1	-	-	-	-	-	1
H31	1	-	-	-	-	-	1
H32	2	-	-	-	-	-	2
H33	1	-	-	-	-	-	1
H34	1	-	-	-	-	-	1
H35	1	-	1	-	-	-	2
H36	1	-	-	-	-	-	1

Table 6-4 (continued).

H37	1	-	-	-	-	-	1
H39	1	-	-	-	-	-	1
H40	-	-	1	-	-	-	1
H41	-	-	-	-	-	1	1
H42	-	-	-	-	-	1	1
H43	-	-	-	-	-	1	1
H44	-	-	-	-	-	1	1
H45	-	-	-	-	-	1	1
H46	-	-	-	-	-	1	1
H47	-	-	-	-	-	1	1
H48	-	-	-	-	-	1	1
H49	-	1	-	-	-	-	1
H50	-	1	-	-	-	-	1

Table 6-5. The haplotype frequencies of ND4 gene in *Crassostrea virginica* from six populations.

Haplotype	Haplotype frequency						
	HKS	HDS	ERQ	ERB	LIS	DB	Total
H1	-	1	-	-	-	-	1
H2	-	1	-	-	-	-	1
H3	17	6	4	3	8	4	42
H4	1	1	-	1	-	1	4
H5	1	1	-	-	-	-	2
H6	-	1	-	-	-	-	1
H7	1	1	-	-	-	-	2
H8	-	1	-	-	-	-	1
H9	-	1	-	-	1	-	2
H10	3	-	-	-	-	2	5
H11	1	-	-	-	-	-	1
H12	1	-	-	-	-	-	1
H13	1	-	-	-	-	-	1
H14	1	-	-	-	-	-	1
H15	1	-	-	-	-	-	1
H16	1	-	-	-	-	-	1
H17	1	-	-	-	-	-	1
H18	1	-	-	-	-	-	1
H19	1	-	-	-	-	-	1
H20	-	-	1	-	-	-	1
H21	-	-	-	-	1	-	1
H22	-	-	-	-	2	-	2
H23	-	-	-	-	1	-	1
H24	-	-	-	-	-	1	1
H25	-	-	-	-	-	1	1
H26	-	-	-	-	-	1	1
H27	-	-	-	-	-	1	1
H28	-	-	-	-	-	1	1
H29	-	-	-	-	3	1	4
H30	-	1	-	-	-	-	1
H31	1	1	-	-	-	-	2
H32	-	1	-	-	-	-	1
H33	-	1	-	-	-	-	1
H34	1	-	-	-	-	-	1
H35	-	-	1	-	-	-	1
H36	-	-	-	-	1	-	1

Table 6-5 (continued).

H37	-	-	-	1	-	-	1
H38	-	-	-	1	-	-	1
H39	-	-	-	1	-	-	1
H40	-	-	-	1	-	-	1
H41	-	-	-	1	-	-	1

Table 6-6. Results of neutrality tests for CO3 and ND4 genes in *Crassostrea virginica* from six populations.

Location	Tajima's D	Fu and Li's D	Fu and Li's F
CO3 region			
HKS	-2.1899**	-2.3793	-2.7253*
HDS	-1.8554*	-2.6480*	-2.8077*
ERQ	-1.7317	-1.5947	-1.8720
ERB	-2.0877**	-2.6599**	-2.8611**
LIS	-1.6986	-2.4409*	-2.5832*
DB	-1.7839	-1.6448	-1.9584
Total	-2.4846**	-6.2029**	-5.5918**
ND4 Region			
HKS	-2.0628*	-2.5523*	-2.8174*
HDS	-2.1282*	-2.8139**	-3.0295**
ERQ	-1.3673	-1.3999	-1.4897
ERB	-1.6327	-1.7309	-1.9074
LIS	-1.6731	-2.0429	-2.2350
DB	-1.7063	-2.2512*	-2.4033*
Total	-2.5293***	-6.2070**	-5.6749**

*Significant differentiation (P<0.05)
**Highly significant differentiation (P<0.01)

Table 6-7. AMOVA results using two mitochondrial DNA regions from six populations of *C. virginica*.

COIII					
Locations		%	d.f.	Variance components	phi statistics	*P*
DB, ERQ, ERB, LIS, HDS, HKS					
	among pops.	4	5	0.09282	0.04023	0.009
	within pops.	96	115	2.21452		
ND4					
Locations					
DB, ERQ, ERB, LIS, HDS, HKS					
	among pops.	1	5	0.00668	0.00790	0.216
	within pops.	99	92	0.83821		

Table 6-8. AMOVA of phi statistics using six microsatellite loci from seven populations of *C. virginica*.

Locations		%	phi statistics	*P*
DB, ERQ, ERB, LIS, HDS, HKS, RI				
	among pops.	2	0.020	0.001
	within pops.	98		

Table 6-9. F_{ST} (below diagonal) and *P* values (above diagonal) from mitochondrial DNA data. Significance level = 0.05.

	HDS	**ERQ**	**LIS**	**ERB**	**HKS**	**DB**
COIII Region						
HDS		0.9369	0.3964	0.5135	**0.0180**	0.0631
ERQ	-0.0333		0.5225	0.9730	0.0991	0.2703
LIS	0.0035	-0.0080		0.5495	0.1351	**0.0000**
ERB	-0.0107	-0.0380	-0.0169		0.1622	0.2252
HKS	**0.0644**	0.0327	0.0204	0.0345		**0.0000**
DB	0.0347	0.0142	**0.0952**	0.0263	**0.1840**	
ND4 Region						
HDS		0.9369	0.3153	0.7117	0.7297	0.2703
ERQ	-0.0452		0.1532	0.8739	0.5225	0.1982
LIS	0.0064	0.0509		0.1532	**0.0451**	**0.0090**
ERB	-0.0166	-0.0443	0.0295		0.1532	0.1532
HKS	-0.0117	-0.0116	**0.0318**	0.0036		0.2252
DB	0.0077	0.0171	**0.0565**	-0.0109	0.0159	

Table 6-10. Per-locus number of individuals (*n*), number of alleles (*a*), allele size range in base pairs (*as)*, expected heterozygsity (*He*), observed heterozygosity (*Ho*) and F_{IS} (f).

Location		*Cvi2g14*	*Cvi24b*	*Cvi2i23*	*Cvi2i4*	*Cvi1g3*	*Cvi5*
Delaware Bay (DB)	*n*	66	69	61	51	51	41
	a	25	16	20	17	8	11
	as	201-251	102-174	373-471	335-411	251-278	136-158
	Private						
	a	1	2	3	0	2	0
	He	0.9486	0.8873	0.8362	0.9297	0.6168	0.8982
	Ho	0.8939	0.3768	0.3934	0.6863	0.2353	0.9268
	FIS	0.0581	**0.5771**	**0.5316**	**0.2638**	**0.6209**	-0.0323
Hudson River (HDS)	*n*	38	35	35	30	23	24
	a	22	14	10	16	5	13
	as	203-247	108-140	375-455	335-415	266-278	134-170
	Private						
	a	0	1	0	1	0	0
	He	0.9442	0.8737	0.8195	0.9339	0.5517	0.914
	Ho	0.9211	0.6857	0.2571	0.7333	0.3043	1
	FIS	0.0248	**0.2176**	**0.6893**	**0.2177**	**0.4539**	-0.0963
Hackensack River (HKS)	*n*	59	50	48	53	38	45
	a	24	15	12	19	5	13
	as	201-247	102-142	373-447	335-407	257-275	134-160
	Private						
	a	0	0	2	1	0	0
	He	0.9532	0.8828	0.7855	0.9172	0.5442	0.9039
	Ho	0.8983	0.58	0.3542	0.6981	0.3947	1
	FIS	0.0581	**0.3453**	**0.5518**	**0.2406**	**0.2773**	-0.1077
East River, Queens (ERQ)	*n*	69	67	57	66	50	45
	a	22	15	16	15	7	13
	as	205-247	108-154	371-471	335-403	251-278	130-162
	Private						
	a	0	1	2	0	0	0
	He	0.951	0.8841	0.8413	0.9076	0.5782	0.8984
	Ho	0.913	0.5373	0.386	0.5909	0.26	0.8667
	FIS	0.0402	**0.394**	**0.5435**	**0.3507**	**0.5528**	0.0357

Table 6-10 (continued).

East River, Bronx	*n*	37	37	22	39	33	29
(ERB)	*a*	24	11	8	18	5	13
	as	201-253	108-144	383-467	343-435	257-278	128-160
	Private a	1	1	0	2	0	1
	He	0.9508	0.87	0.7928	0.9171	0.5361	0.9008
	Ho	0.9189	0.5676	0.3636	0.7179	0.3939	0.931
	FIS	0.0339	**0.3508**	**0.5472**	**0.2194**	0.2682	-0.0342
Long Island Sound	*n*	34	43	24	44	24	40
(LIS)	*a*	20	15	10	18	4	16
	as	203-249	102-142	375-467	335-419	269-278	130-170
	Private a	0	0	0	0	0	0
	He	0.9421	0.8731	0.8041	0.9261	0.5328	0.9209
	Ho	0.9706	0.5116	0.4176	0.7727	0.2083	0.95
	FIS	-0.0308	**0.4169**	**0.4872**	**0.1672**	**0.6141**	**-0.032**
Rhode Island	*n*	22	19	14	20	16	22
(RI)	*a*	18	14	7	13	6	16
	as	205-249	102-142	375-455	335-407	251-275	134-176
	Private a	0	0	0	0	0	1
	He	0.9471	0.9189	0.8519	0.9244	0.7581	0.9154
	Ho	0.8182	0.5263	0.1429	0.8	0.5625	0.9545
	FIS	**0.139**	**0.434**	**0.8375**	0.1376	0.2643	-0.0438

Table 6-11. Value of Φ_{PT} (above diagonal) and F_{ST} (below diagonal) from microsatellite data.

	DB	ERQ	RI	HDS	HKS	LIS	ERB
Cvi5							
DB	*0.000*	0.000	***0.159***	0.000	0.029	0.039	0.000
ERQ	0.000	*0.000*	**0.134**	0.000	0.017	0.018	0.000
RI	***0.105***	***0.088***	*0.000*	***0.141***	**0.057**	**0.058**	***0.165***
HDS	0.000	0.000	***0.086***	*0.000*	0.014	0.021	0.000
HKS	**0.018**	0.011	***0.032***	0.008	*0.000*	0.000	0.026
LIS	***0.025***	0.012	***0.033***	0.013	0.000	*0.000*	0.023
ERB	0.000	0.000	***0.106***	0.000	0.016	0.015	*0.000*
Cvi1g3							
DB	*0.000*	0.000	0.000	0.000	0.000	0.042	0.005
ERQ	0.000	*0.000*	0.000	0.000	0.000	0.039	0.012
RI	0.000	0.000	*0.000*	0.001	0.000	0.041	0.000
HDS	0.000	0.000	0.001	*0.000*	0.000	0.000	0.012
HKS	0.000	0.000	0.000	0.000	*0.000*	0.020	0.000
LIS	***0.038***	**0.035**	0.036	0.000	0.017	*0.000*	0.035
ERB	0.004	0.010	0.000	0.010	0.000	0.031	*0.000*
Cvi2i4							
DB	*0.000*	**0.031**	0.022	0.000	**0.029**	0.012	0.002
ERQ	***0.023***	*0.000*	0.000	0.010	0.000	0.000	0.004
RI	0.015	0.000	*0.000*	0.002	0.000	0.000	0.001
HDS	0.000	0.007	0.002	*0.000*	0.009	0.000	0.000
HKS	**0.020**	0.000	0.000	0.006	*0.000*	0.000	0.008
LIS	0.009	0.000	0.000	0.000	0.000	*0.000*	0.000
ERB	0.002	0.003	0.001	0.000	0.005	0.000	*0.000*
Cvi2i23							
DB	*0.000*	0.000	0.024	0.007	0.000	**0.101**	***0.112***
ERQ	0.000	*0.000*	0.003	0.019	3.000	0.068	***0.077***
RI	0.020	0.003	*0.000*	0.040	0.022	0.005	0.015
HDS	0.006	0.016	**0.036**	*0.000*	0.012	***0.131***	***0.142***
HKS	0.000	0.003	0.019	0.010	*0.000*	***0.091***	***0.102***
LIS	***0.085***	***0.058***	0.004	***0.115***	***0.078***	*0.000*	0.000
ERB	***0.095***	***0.066***	0.013	***0.126***	***0.087***	0.000	*0.000*

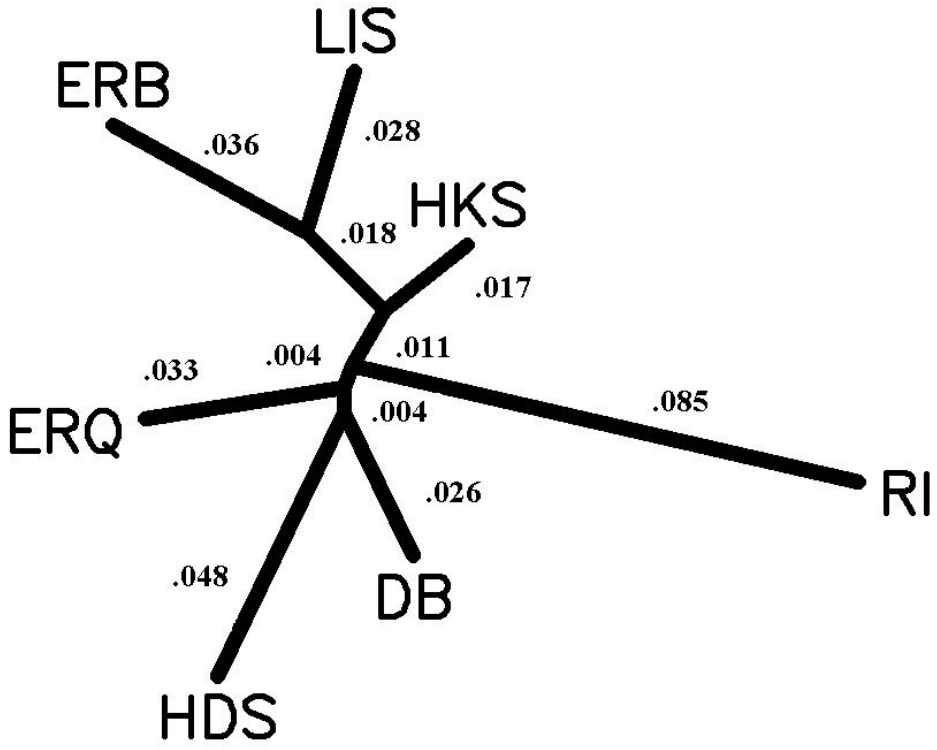
Table 6-11 (continued).

Cvi24b	**DB**	**ERQ**	**RI**	**HDS**	**HKS**	**LIS**	**ERB**
DB	*0.000*	0.001	0.000	0.009	0.009	**0.026**	**0.029**
ERQ	***0.001***	*0.000*	0.000	0.000	0.000	0.012	0.016
RI	0.000	0.000	*0.000*	0.000	0.000	0.000	0.000
HDS	0.007	0.000	0.000	*0.000*	0.000	0.000	0.005
HKS	0.007	0.000	0.000	0.000	*0.000*	0.000	0.000
LIS	***0.020***	0.009	0.000	0.000	0.000	*0.000*	0.000
ERB	0.023	**0.012**	0.000	0.003	0.000	0.000	*0.000*
Cvi2g14							
DB	*0.000*	0.004	0.004	0.000	0.000	***0.058***	0.022
ERQ	0.002	*0.000*	0.000	0.010	0.000	***0.073***	**0.031**
RI	0.003	0.000	*0.000*	0.000	0.000	**0.099**	0.052
HDS	0.000	0.005	0.000	*0.000*	0.003	**0.076**	**0.043**
HKS	0.000	0.000	0.000	0.002	*0.000*	***0.097***	***0.051***
LIS	***0.035***	***0.044***	***0.063***	***0.046***	***0.059***	*0.000*	0.000
ERB	0.013	***0.018***	**0.032**	***0.025***	***0.030***	0.000	*0.000*
Total							
DB	*0.000*	0.003	**0.030**	0.000	0.008	***0.050***	***0.032***
ERQ	0.003	*0.000*	0.015	0.004	0.001	***0.036***	***0.024***
RI	***0.021***	**0.010**	*0.000*	**0.030**	0.002	**0.028**	**0.036**
HDS	0.000	0.003	***0.020***	*0.000*	0.003	***0.042***	***0.036***
HKS	**0.006**	0.001	0.002	0.002	*0.000*	***0.036***	***0.031***
LIS	***0.035***	***0.025***	***0.020***	***0.029***	***0.024***	*0.000*	0.005
ERB	***0.023***	***0.017***	***0.025***	***0.025***	***0.021***	0.004	*0.000*

Table 6-12. Effective population size estimated from the deviation of expected compared to observed degree of linkage disequilibrium.

			95% CI	
Location	n	N_e	Lower	Upper
DB	73	395	171	Infinity
HDS	39	80	50	186
HKS	60	129	85	249
ERQ	73	242	134	982
ERB	48	38	28	56
LIS	52	52	37	80
RI	22	61	35	182

Figure 6-1. Unrooted phylogenetic tree inferred by Fitch-Margoliash distance matrix method from six microsatellite loci.



Chapter 7: Conclusion and Recommendations

The lower Hudson River Estuary once supported a large oyster industry that mainly because of water pollution has ceased to exist. Though most of the HRE waters are still contaminated and restricted from commercial fishing, water quality has improved over the past decades. Environmental awareness in recent years has drawn attention to cleaning up the harbor. Local environmental groups as well as State agencies have publicized the idea of oyster restoration and the benefits that it may bring to the estuary. This coupled with the role that the oyster has played in the history of New York that was brought to the public's attention in 2006 by Mark Kurlansky's book, The Big Oyster: History on the Half Shell, has caused an unprecedented local interest in oysters.

This study has documented five locations where wild oysters can be found in the estuary. They include four different water bodies, the Hackensack River (HKS), Hudson River (HDS), East River (ERB, ERQ) and western Long Island Sound (LIS) where water quality parameters including salinity, DO, phytoplankton levels and environmental contamination vary. Difference in shell growth, condition index, parasite abundance and metal uptake at each location were assessed.

Growth and condition index, a measure of health by ratio of biomass to shell weight, were measured at each location and compared with oysters obtained from Delaware Bay, where oysters are harvested and expected to be in good condition. Average annual growth rates of oysters in the HRE meet or exceed the rates found in the sample of Delaware Bay oysters. The condition index statistically showed that oysters from the LIS location have greater condition. The analysis unexpectedly showed that Delaware Bay oysters have significantly heavier shells than that of the HRE oysters,

which negatively skewed the condition index of the Delaware Bay samples making them falsely appear less fit than the HRE samples. Lighter and thinner shells of HRE oysters are not inhibiting the growth of meat weight, but thicker and heavier shells are desirable for protection. The thinner shells are subjecting the oysters to increased exposure to waves, predators, freezing, heating, drying and other unfavorable conditions.

MSX and Dermo disease analyses showed that the HKS location is the most heavily diseased, particularly with MSX disease where 100% of the oysters sampled were found to be infected. Disease at this location is likely contributing to survival by the lack of older individuals found there. The other four locations appear to be tolerating disease with less prevalence and lower infection intensities. The overall prevalence of Dermo disease in the entire HRE was less than that of the recent epizootic of Delaware Bay.

It was concluded that metals may not be affecting oyster survival in any of the sampled HRE locations. However, samples were taken once and there is a need for more monitoring to test this assumption. All metals tested were within acceptable consumption levels with the exception of Pb in the Hackensack River. Consumption of oysters and other shellfish is prohibited in all areas of the estuary where samples were taken, so the health risks associated with the metal concentrations are limited.

Analysis of both mitochondrial and microsatellite markers showed that the HRE oysters do not have low genetic diversity as would be expected of a small population. There was not a decreased level of heterozygosity, number of alleles, haplotypes or polymorphic sites in relation to the larger population of Delaware Bay and an additional outgroup of Rhode Island oysters. Unique haplotypes were identified, but this was most likely the result of small sample size. Significant but weak genetic differentiation was

found and was comparable with other studies of *C. virginica* along with other marine invertebrates. The microsatellite analysis detected genetic differentiation, particularly separating the LIS and ERB populations from the other sampling sites, and individual loci detected significant differentiation of DB and RI from the HRE populations.

The New York State Department of Environmental Conservation (NYDEC) is currently limiting their support to small-scale restoration projects overseen by NY government agencies. Their stand has been that the restoration of oyster reefs will create a potential "attractive nuisance" requiring the expenditure of already limited resources for enforcement and monitoring, to prevent the removal and consumption of oysters by the general public (Yozzo, et al. 2004). The deposit of hundreds of thousands of live oysters on an artificially created reef anywhere in the estuary is could tempt poaching and the illegal sale of the oysters which could cause illness if consumed. New Jersey recently followed suit (June of 2010), with the Department of Environmental Protection under a new administration banning existing and future research-related gardening of commercial shellfish in waters classified as contaminated. These include portions of Raritan Bay, the Arthur Kill, Hackensack River and Hudson River. The intent is to minimize potential negative impacts to New Jersey's shellfish industry in non-prohibited waters that could result in public illness due to consumption of shellfish raised in prohibited waters for research of educational projects. Their concern may not be an overly cautious one. Despite the governmental and scientific concern for the safety of fish, there is often a disconnect between scientific knowledge, fish advisories and the actions of the public (Burger et al. 1999).

Restoration efforts that include placing shell to encourage the settlement of naturally existing larvae are not addressed in the ban. Therefore, the future of oyster restoration may be limited to encouraging the expansion of existing wild populations. This study identifies several locations where wild oysters can be found, but an in depth survey of the entire estuary to map the locations of oysters both intertidally and subtidally is needed. Habitat in the form of shell or artificial reefs can then be placed in the water near populated wild oysters and recruitment monitored.

The US Army Corp of Engineers and the Port Authority of NY/NJ in partnership with the New York New Jersey Harbor Estuary Program developed the Hudson-Raritan Estuary Comprehensive Restoration Plan (CRP) as a master plan to ecosystem goals that could be used by all organizations associated with ecosystem restoration in the estuary including local government agencies, universities and non-profit organizations. Establishing oyster reefs are one of eleven Target Ecosystem Characteristics (TECs) that have been developed in the plan. Their goal is to create 500 acres (2 kilometers) of self-sustaining and naturally expanding oyster reef habitat in the HRE across 10 to 20 sites by 2015 and by 2050 to have 5,000 acres (20 kilometers) of established oyster reef habitat. They have identified that there are sizable areas that could provide oyster reef habitat in the East River, particularly at Flushing Bay, Westchester Cove and near the Bronx River. They have developed an estuary-wide map depicting areas most suitable for oyster reef habitat based on four parameters: salinity, DO, Total Suspended Solids and bathymetry. These four parameters were met for the upper East River in the waters surrounding the ERB and ERQ locations and three of the four parameters were met near the LIS location.

Their other recommended areas of focus for oyster restoration include Jamaica Bay and areas in and around Raritan Bay.

Focusing oyster restoration efforts in the East River and western Long Island Sound is supported by this study. This is the region where the majority of wild oysters were found living and analyses of their growth, condition and disease prevalence all support continued survival. Salinity in the Hackensack River and upper Hudson River is too low and efforts downriver in Raritan Bay have proved unsuccessful by the NY/NJ Baykeepers due to wave action. Oysters were not identified in Jamaica Bay and the Arthur Kill and the lack of their presence suggests that the ecological conditions are not favorable to support a population. Subtidal oysters could exist in any portion of the estuary or intertidal areas could have been overlooked, but oyster restoration efforts may benefit from focusing on areas that currently have a known wild oyster population. Organizations such as the Hackensack Riverkeeper have good intentions with wanting oysters to live in their local waters, but oysters are not likely to thrive in the current conditions of the Hackensack. Besides low salinity, metal contamination and disease prevalence was the highest at this location and most oysters here are small and not surviving to the next year. The upper East River was shown to be the most promising location for oyster restoration in this study.

The CRP acknowledges New York and New Jersey governmental concerns and restrictions on oyster restoration, but does not address how that may interfere with meeting their target goals. But even if the idea of oyster restoration is abandoned due to the efforts in government in limiting the existence of oysters in contaminated waters of the HRE, this study supports that wild oysters will continue to persist. With time and

the help of habitat supplementation, they could potentially make their own natural recovery.

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