NY/NJ BAYKEEPER EASTERN OYSTER REINTRODUCTION FEASIBILITY STUDY

PREPARED BY
RUTGERS CENTER FOR URBAN ENVIRONMENTAL SUSTAINABILITY (CUES)

FINAL REPORT
(2007 – 2013)

CUES
Center for Urban Environmental Sustainability

Year 7: 2013
YEAR END REPORT
FINAL Conservation Resources Inc. (CRI) Project Report

The purpose of this research is to provide NY/NJ Baykeeper with information related to their mission to restore oyster-reef based habitat within the Hudson-Raritan Estuary.

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EXECUTIVE SUMMARY

Beginning in the 1970's, the passage of environmental regulations has resulted in waters of the NY/NJ harbor estuary becoming progressively cleaner. The Harbor Estuary Program, in collaboration with the U.S. Army Corps of Engineers, US Environmental Protection Agency, US Fish & Wildlife, NOAA, the Port Authority of New York & New Jersey, Hudson River Foundation, and NY/NJ Baykeeper, completed a Draft Comprehensive Restoration Plan for the Hudson-Raritan Estuary (HRE), which includes the goal to reestablish 200 acres of Eastern Oyster (*Crassotrea virginica*) reef by 2020. However, due to both historic and/or current anthropogenic activities and contaminant inputs into the estuary, there are important issues to consider when identifying the ideal location(s) for oyster reintroduction projects in Raritan Bay and the HRE at large. ***The HRE oyster restoration goal is not to re-establish a commercial fishery in waters where contaminated sediments are still present.*** Reintroduction of the oyster is a mechanism to support continued improvements in water quality, to reestablish and enhance habitat conducive to the survival of other aquatic species, and to increase natural 'green' infrastructure that contributes to reduction in shoreline erosion and sediment stabilization.

In brackish estuaries of the northern US, subtidal oyster beds were commonly an important component of the benthic ecosystem. Oyster formation of dense aggregations provides structural heterogeneity and vertical topography, which create an ecologically complex habitat for all trophic levels. ***The ability to create this vertical habitat feature, unique to oysters among local shellfish, is the reason these animals are considered to be ecological engineers***, a characteristic not attributed to other local bivalve species.

The Hudson-Raritan Estuary has been identified as a "hot spot" with respect to relative sea level rise (SLR). Measurements taken at The Battery, NY, Sandy Hook and Atlantic City, NJ over the last 78 – 100 years indicate that sea levels in the region are rising at a rate of up to 4 mm/yr, a much faster increase than the predicted global average. This local rate of SLR is believed to be due to a confluence of three factors: overall ocean volume expansion due to warming and an increase in ice melt; local subsidence of the land caused by retreat of the last glacier; and changes in the Atlantic current circulation patterns. Raritan Bay is also subject to frequent tropical storms and periodic hurricanes. The devastation caused by SuperStorm Sandy is causing HRE communities to consider resiliency options that address sea level rise-induced flooding, storm surges, and shoreline erosion. Depending on site-specific conditions, the presence of an oyster reef system might contribute to shoreline protection, providing an alternative to "hard-edge" armoring of vulnerable shorelines.

In anthropogenically impacted urban estuaries such as the HRE, the absence of adult oyster populations, insufficient larval densities, altered hydrology and/or the presence of emerging and historic contaminants may overshadow physio-chemical factors required to support oyster reintroduction goals. Habitat selection is a critical issue in successful reintroductions, which often fail because 'apparently similar' habit is actually unsuitable.

KEYPORT HARBOR

Prior to implementing large scale oyster reintroduction activities, it is critical to understand the existing baseline conditions at a proposed reintroduction site. Two western Raritan Bay locations approximately 4.5 miles apart (the NJDEP-permitted Keyport Harbor and the non-NJDEP permitted Sayreville) were compared for their ability to support oyster over-winter survival. Although visually similar, the significantly higher survival and growth rates exhibited by oysters housed at the

Keyport site caused us to choose to focus reintroduction efforts on this location.

A ¼-acre oyster 'reef' was installed at Keyport Harbor in October, 2009 to test three alternative oyster support structures – Reefblk™, Reefball™, and a Rutgers designed 'Arch' structure. After one year *in situ*, oyster survival and growth appear to be greatest for spat-on-shell housed in the Reefblk™ structure. However, the research seed oysters were approaching the NJ 'market size' of 2.5", and in August, 2010 the NJDEP rescinded Baykeeper's required permits, causing the death of an estimated 30,000 oysters. Tissues of specimens sampled from this experiment were healthy and the oysters appeared to be capable of spawning. Marine community diversity associated with the oysters appeared to be much greater than adjacent waters without oysters. However, since the research time frame was less than one year, it would be premature to draw conclusions from the Keyport data.

OYSTER TISSUE ANALYSES

To determine overall oyster health and fitness live samples were obtained from the various Baykeeper oyster locations (Keyport Harbor, NYC Oyster Restoration Research Project (ORRP) sites, Baykeeper NYC oyster garden sites), and the specimens were analyzed. Oysters from Keyport Harbor, Soundview, Hastings on Hudson, and the Gowanus Canal were also analyzed to determine metal concentrations in the body soft tissues and shell. Total mean metal concentrations were lowest in the Keyport Harbor samples and highest in oysters from the New York locations. Based on the experiments conducted to date it is not possible to correlate oyster soft tissue damage with the presence of specific metal contaminants. However, the overall health and fitness of the oysters appears to be very site-specific.

Although oysters were able to survive and grow in the various locations we found that overall oyster health and fitness as evaluated by the number of lesions observed in soft tissues did not correlate to shell morphology (i.e. large shelled oysters could not automatically be assumed to be healthy).

REINTRODUCTION HABITAT MAPPING PROJECT

Survival and growth data, supported by soft tissue analyses, suggest that there are locations in the historically contaminated HRE where oysters can indeed survive and thrive. Potential oyster restoration sites identified in the CRP (2009) were determined by considering four characteristics required for oyster larvae survival: dissolved oxygen concentrations, total suspended solids, salinity, and bathymetry. However, our research indicates that HRE environmental stressors are quite site-specific, and so the macro-scale CRP (2009) map needs to be 'ground-truthed' at a more site-specific micro-scale. An extensive, but inexpensive survey of the HRE is needed to identify sites that could potentially contribute to meeting the CRP oyster restoration goals for the HRE. Twenty-three characteristics that could affect oyster survival were identified and approximately 30 miles along the NJ Raritan Bayshore and the southern shore of Staten Island were evaluated. Sites that achieved a high score in this evaluation were overlaid on NOAA bathymetry maps to determine the associated acreage in the 3 to 10 feet depth range at Mean Low Tide. This analysis indicates that the CRP (2009) goal of 200 acres of newly reintroduced oyster habitat is achievable, depending on support from the NJ/NY regulatory agencies.

A CUES-Baykeeper Oyster Restoration Site Selection Model was developed, which adds an *in situ* biological evaluation to the current physio-chemical parameters commonly employed to determine potential reintroduction sites.

NAVAL WEAPONS STATION EARLE (NWSE)

As an alternative to the NJDEP-prohibited Keyport oyster restoration site, the Baykeeper oyster setting facility and reintroduction research have been transferred to Naval Weapons Station Earle (NWSE) located in Middletown, NJ. Over-winter survival rates (2011-2012) were abnormally high (90% survival of the oysters recovered), and so the decision was made to expand the research footprint in two phases. The first phase is to recreate the 18 field research plots originally established in Keyport Harbor, and if successful, to expand oyster reintroduction up to the NJDEP-permitted 10-plus acre footprint between the NWSE piers. The proposed installation and data monitoring plan has been approved by the navy. After the navy sonar sweep of the area is complete, we anticipate installation of the field plots in summer, 2014.

PUBLICATIONS

The Baykeeper oyster restoration project has resulted in publications related to reintroduction of oysters into the Hudson-Raritan Estuary, including:

1. Eastern oysters (*Crassostrea virginica*) in the Hudson-Raritan Estuary: Restoration, research, and shellfishery Policy. B. Ravit, M. Comi, D. Mans, C. Lynn, F. Steimle, S. Walsh, R. Miskewitz, S. Quierolo. Environmental Practice. 2012.

2. Improving management support tools for reintroducing bivalve species in urban estuaries. B. Ravit, K. Cooper, B. Buckley, and M. Comi. Integrated Environmental Assessment and Management. *Submitted*.

3. 2011-2012: Raritan Bay Mapping Study: Pre-Superstorm Sandy. B. Ravit, M. Usarek-Witek, R. Lathrop, M. Comi., F. Steimle, M. Aquafreda. Submitted to USACE. 2013.

4. Citizen scientists, NGOs, and reintroduction of the eastern oyster in an urban estuary. Journal of Contemporary Water Research & Education. *In Preparation*.

We anticipate that the ongoing research at Naval Weapons Station Earle will continue to increase knowledge related to reintroduction of an 'ecologically extinct' bivalve to an urban estuary, and that this data will result in additional peer-reviewed publications.

CONCLUSIONS

Research conducted in the HRE between 2007 – 2013 indicates that there are viable locations, especially in Raritan Bay, where reintroduction of the Eastern Oyster may be successful. However, the long-term sustainability of these introductions will be quite site specific. Therefore, an inexpensive, but thorough, evaluation is necessary prior to investing large sums of money and time in order to ensure the locations selected will be successful.

I. HUDSON-RARITAN OYSTER REINTRODUCTION: BACKGROUND AND BENEFITS

NY/NJ HARBOR ESTUARY RESTORATION GOALS

Human activities occurring in the 19th and 20th centuries negatively affected the ecological integrity of numerous ecosystems in the New York/New Jersey (NY/NJ) region (Jackson 2001), including the once vast Hudson-Raritan Estuary (HRE) oyster fishery (McCay 1998). However, beginning in the 1970's, passage of environmental regulations has resulted in waters of the NY/NJ harbor estuary becoming progressively cleaner. As a result of these improvements in water quality it is now possible that aquatic species, which have been either 'ecologically extinct' from the region's coastline for decades or present in greatly reduced numbers, may now be restored in locations where they were historically present. The active reintroduction of aquatic species that were once abundant in waters of the HRE, particularly the Eastern Oyster (*Crassotrea virginica*), could potentially support further improvements in water quality, contribute to stabilization of coastal shorelines, and accelerate ecosystem level restoration processes.

NY/NJ Baykeeper has been the lead non-governmental organization (NGO) focusing on benthic habitat restoration, specifically reintroduction of the Eastern Oyster, in the HRE. In 2007 Baykeeper was awarded a Supplemental Environmental Penalty in compensation for environmental damages sustained in the Arthur Kill as the result of a Chevron oil spill. The funds were dedicated to oyster restoration in western Raritan Bay. The sustainable growth of oyster reefs could contribute to achieving Baykeeper's goal of Raritan Bay aquatic and benthic habitat restoration, providing estuarine habitat for native fin fish and benthic species, and stabilizing the Raritan Bay shoreline.

However, due to both historic and/or current anthropogenic activities and contaminant inputs into the estuary, there are important issues to consider with respect to human and ecosystem health when identifying potential location(s) for oyster reintroduction projects in the HRE.

The restoration of a variety of ecological systems within the HRE is conceptually supported by a wide range of Harbor Estuary Program (HEP) partners (Bain et al. 2005, TEC 2007), including Federal, State (NY and NJ), and NGOs. Partners in the HEP program that participated with NY and NJ in developing the draft Comprehensive Restoration Plan (CRP 2009) for the Hudson-Raritan Estuary include the US Army Corps of Engineers, US Environmental Protection Agency, US Fish & Wildlife Service, NOAA, the Port Authority of New York & New Jersey, Hudson River Foundation, and NY/NJ Baykeeper. Meeting the CRP (2009) restoration goals will provide critical habitat for native fin fish and benthic invertebrates, while contributing to continued improvements in water quality and the overall quality of human life within the HRE.

Large-scale oyster reintroduction is an important factor in achieving the long-term CRP restoration targets, and the plan calls for reestablishment of 200-acres of oyster reef (Fig. 1) by 2020 (CRP 2009). There is anecdotal evidence that small wild oyster populations are living in isolated locations within the HRE (T. Medley, *personal communication*). Although restoration of benthic habitat is viewed by various constituencies as an important goal, it is also one of the most difficult to achieve, due to both historic and ongoing contamination within the HRE (CARP 2007). ***However, these restoration goals are not to re-establish a commercial fishery in waters where contaminated sediments are still present.*** Establishment of oyster reefs is a mechanism that, if successful, would support continued improvements in water quality, reestablish and enhance habitat conducive to the survival of other aquatic species, and increase natural 'green' infrastructure that contributes to reduction in shoreline erosion.

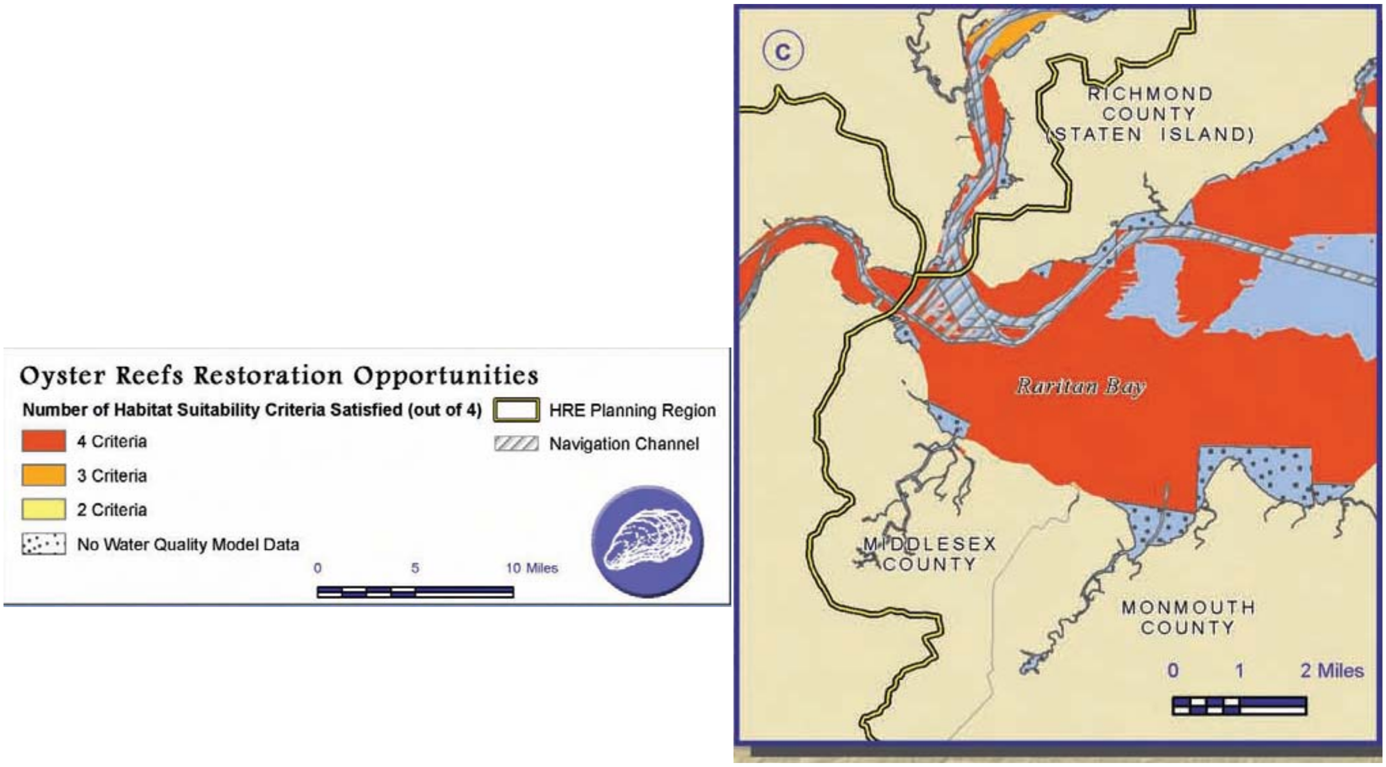


Figure 1. Hudson-Raritan Estuary potential oyster reintroduction locations based upon salinity, dissolved oxygen, turbidity, and bathymetric criteria (*map reproduced from USACE Draft Comprehensive Restoration Plan 2009*).

URBAN ESTUARY CHALLANGES

Typically oyster restoration initiatives are undertaken to support existing fishery resources. Management tools include enhancement of existing reefs by the addition of oysters, placement of hard substrate for larval settlement, and review of historic shellfish bed locations (Kennedy et al. 2011). It is common to employ GIS models (Barnes et al. 2007; Pollack et al. 2012) based on ranges of salinity, temperature, turbidity, dissolved oxygen, and bathymetry suitable for oyster larval survival and settlement. However, in anthropogenically impacted urban estuaries such as the HRE, the absence of adult oyster populations, insufficient larval density, altered hydrology and/or the presence of emerging and historic contaminants may overshadow physio-chemical factors necessary to achieve reintroduction objectives (Fig. 2).

Figure 2. Venn diagram illustrating the interaction of multiple environmental parameters that determine oyster reintroduction success in an urban estuary.

Habitat selection is a critical issue in successful animal reintroductions (Mihoub et al. 2009), which often fail because 'apparently similar' habit is actually unsuitable; identifying successful reintroduction sites can be challenging, often due to environmental changes since extinction of the target species (Hodder & Bullock 1997). Additionally, the original food webs may be missing or replaced with a food supply that does not meet the nutritional requirements of the reintroduced species. Under the non-natural conditions common in urban estuaries, sites available for restoration are typically limited (Simenstad et al. 2005).

HUDSON-RARITAN ESTUARY EASTERN OYSTER REINTRODUCTION

Since placing shell on Liberty Flats in 1999, NY/NJ Baykeeper has been the lead NGO focusing on oyster habitat reintroduction to the HRE. However, due to both historic and/or current anthropogenic activities and contaminant inputs to the HRE, there are important management questions to consider when identifying potential sites suitable for reintroduction activities. Although shellfish water regulatory designations do not take into account organic and metal contaminant loadings, due to the levels of fecal coliform bacteria in western portions of Raritan Bay, much of the Bay's waters are categorized by the NJDEP Bureau of Marine Water Monitoring as *"Special Restricted"* or *"Prohibited"* with respect to a commercial fishery. Shellfish surviving in "*Prohibited*" waters cannot be harvested for sale or human consumption, and so the purpose of reintroducing oysters in *"Prohibited"* waters is for habitat value only. However, creating a critical mass of adult oysters in Raritan Bay could potentially result in larval production sufficient to colonize existing substrate, both in the Bay and at other HRE locations that were historically colonized by spat produced in the Great Beds of Raritan Bay. The importance of a Raritan Bay population in restoring the Eastern Oyster to the HRE (Fig. 1) was documented in the CRP (2009); the long-term presence of significant acreage of Raritan Bay oyster reefs could potentially contribute to reestablishing and sustaining an oyster population in the HRE at large.

OYSTER REEF HABITAT VALUE

In brackish estuaries of the northern US, subtidal and intertidal oyster beds were commonly an important component of the benthic ecosystem (Bertness 1999). Oyster formation of dense aggregations provides structural heterogeneity and vertical topography, which create an ecologically complex habitat for all trophic levels (Harding & Mann 1999, 2001). ***The ability to create this vertical habitat feature, unique to oysters among HRE shellfish, is the reason these animals are considered to be ecological engineers.*** For this reason, the Eastern Oyster is commonly referred to as a "keystone species" (Coen et al. 2007), a characteristic not attributed to other local bivalve species.

Modern restoration efforts are now capitalizing on interconnections between multiple species living in tidal estuarine habitats, and the importance of establishing communities of species to achieve successful habitat development has been well documented (Harding & Mann 1999, Bertness 1999, Brumbaugh et al. 2006). It is estimated that the presence of bivalves can increase biomass and productivity of benthic invertebrate fish prey species by 20-fold (Steimle et al. 2002). This increased prey biomass can support an increase in fin fish and large crustacean biomass of up to 50 kg per square meter of oyster reef habitat (Peterson et al. 2003).

Oyster building of reefs can reduce water flow velocities, while providing organic material that serves as food for reef inhabitants (Kennish 2004). Oysters and other members of the community that grow on the oyster shell substrate (such as sponges) control overabundant micro-algae, which cause the algal blooms that lead to low dissolved oxygen levels in bottom waters. The microalgae and organic particulate matter the oysters remove from the water column are released as feces or psuedofeces (particles collected on its gills that the oyster does not use as food). These oyster waste products enhance the organic content of the adjacent soft bottom habitats, which contributes to the benthic community productivity that is in turn used by other non-reef fin fish and invertebrate species. This is an important bentho-pelagic coupling process that supports both fish and shellfish productivity. In addition to providing habitat for benthic organisms and transitory fish, oyster reefs serve as forage habitat for birds and mammals (Kennish 2004). An adult oyster is also capable of filtering prodigious amounts of water though its system. Unlike intertidal ribbed mussels (suggested by some regulators as an alternative to oysters) this filtering activity takes place continuously when oysters are placed in subtidal locations. This natural filtration process helps to reduce water turbidity, allowing light transmission through the water column and creating a habitat supportive of submerged sea grass growth (Bertness 1999), while improving overall water quality.

CHANGING CLIMATE AND RELATIVE SEA LEVEL RISE

The HRE has been identified as a "hot spot" with respect to relative sea level rise

(Gornitz et al. 2001; NRC 2007). Measurements taken at The Battery, Sandy Hook, and Atlantic City over the last 78 – 100 years indicate that sea levels in the region are rising at a rate of up to 4 mm/yr. This is a much faster rate than the predicted global average and is believed to the due to a confluence of three factors: overall ocean volume expansion due to warming and increased ice melt; local subsidence of the land caused by retreat of the last glacier; and changes in the Atlantic current circulation patterns (Kirshen et al. 2007).

Raritan Bay is also subject to frequent tropical storms and periodic hurricanes; it is estimated that approximately 3.7 million cubic yards of sand have been placed on Raritan Bay beaches for the purpose of flood protection (NRC 2007). The devastation inflicted by SuperStorm Sandy is now causing HRE coastal communities to consider resiliency options that address sea level rise-induced flooding, storm surges, and shoreline erosion. The USACE is currently conducting an analysis of post-Sandy shoreline conditions and potential actions to increase resilience to future extreme storm events. Depending on site-specific conditions, the presence of an oyster reef system could contribute to shoreline protection through reduction of wave energy, thus providing an alternative to "hard-edge" armoring of vulnerable shorelines (Piazza et al. 2005).

Studies were designed to yield data related to local environmental conditions, oyster survival and growth rates, and overall general oyster fitness in the hope that this data could contribute to Baykeeper's successful reintroduction of the Eastern Oyster to the HRE. The research described in this report was conducted between 2007 – 2013 (Table 1).

Table 1. Recap of CUES-Baykeeper oyster reintroduction research (2006 – 2013).

Date	Activity	Location	Description
Fall, 2007	Underwater Field Surveys	Sayreville, Keyport Harbor	Visual bottom surveys were conducted adjacent to Cheesequake Creek and on the site of Baykeeper's original Keyport Harbor reef.
Fall, 2007	Over-winter survival test	Sayreville, Keyport Harbor	Live juvenile seed oysters placed in cages at Sayreville and Keyport; recovered in Spring 2008
Summer, 2008	Acoustic Doppler Surveys	Sayreville, Keyport Harbor	Current activity and direction measured at Sayreville and Keyport using Acoustic Doppler equipment attached to the Baykeeper boat
Fall, 2008	Over-winter survival test	Sayreville, Keyport Harbor	Live juvenile seed oysters placed in cages at Sayreville and Keyport; recovered in Spring 2009
Fall, 2008	Sediment Survey	Keyport Harbor	Pre-winter storm survey of bottom topography
Spring, 2009	Sediment Survey	Keyport Harbor	Post-winter survey of bottom topography with no oysters/structures present
Fall, 2009	Sediment survey pre-installation	Keyport Harbor	Survey of field plot bottom topography pre-oyster installation
Fall, 2009	¼ acre oyster research field plot installed	Keyport Harbor	3 oyster support structures deployed in 18 field plots over a total area of ¼ acre in Keyport Harbor
Spring, 2010	Sediment Survey	Keyport Harbor	Survey of field plot bottom topography post-oyster installation
Summer, 2010	NJDEP revokes Baykeeper oyster permits	Keyport Harbor	Collected survival and growth data for support structures; soft tissue histopathology; shell and tissue metal analyses; biodiversity study prior to destruction of ~30,000 oysters
Summer, 2011	Raritan Bay Habitat Mapping	New Jersey Bayshore	Habitat data collected every 100 m over ~20 mi. from Keyport Harbor eastward to Sandy Hook
Summer, 2011-2013	Tissue Histopathology	Keyport Harbor, NYC ORRP sites	Dissection, visual examination, and analysis of oyster soft tissue health and reproductive fitness
Fall, 2011	Juvenile seed and SOS over-winter survival tests	Naval Weapons Station Earle	3,600 oysters placed in lantern nets attached to the working naval pier at NWSE
Summer, 2012	Raritan Bay Habitat Mapping	Staten Island, NY	Habitat data collected every 100 m over ~10 mi. from Keyport Harbor westward across Staten Island
Summer, 2013	Side Scan Sonar post-Sandy bottom Survey	Naval Weapons Station Earle	Determination of bottom condition post-Sandy between and adjacent to the two piers; waiting for navy to complete sonar sweep of research area
Summer, 2013	Rebuilding oyster setting facility	Naval Weapons Station Earle	One successful oyster set completed by Baykeeper; larval transport analysis
Fall, 2013	Placement of ~250,000 SOS *in situ*	Naval Weapons Station Earle	SOS in shell bags suspended from working pier platforms in subtidal position
Fall, 2013	Placement of Sonde continuous monitors	Naval Weapons Station Earle	Data recorders placed on inner and outer edge of working pier; probes for temperature, salinity, dissolved oxygen, pH, and chlorophyll

III. KEYPORT HARBOR RESEARCH

SITE SUITABLITY TESTS

Prior to implementing large scale oyster reintroduction efforts it is critical to understand the existing baseline conditions at a proposed reintroduction site. The initial research questions were related to determining whether the western portion of Raritan Bay would support the successful reintroduction of Eastern Oysters. Our research methodology was to contrast environmental conditions at a potential reintroduction location in western Raritan Bay (Sayreville) with conditions at the NJDEP-permitted Keyport Harbor (Fig. 3)and Navesink River restoration sites (**Note**: Baykeeper's permit for the Navsink River site was subsequently revoked due to high fecal coliform counts).

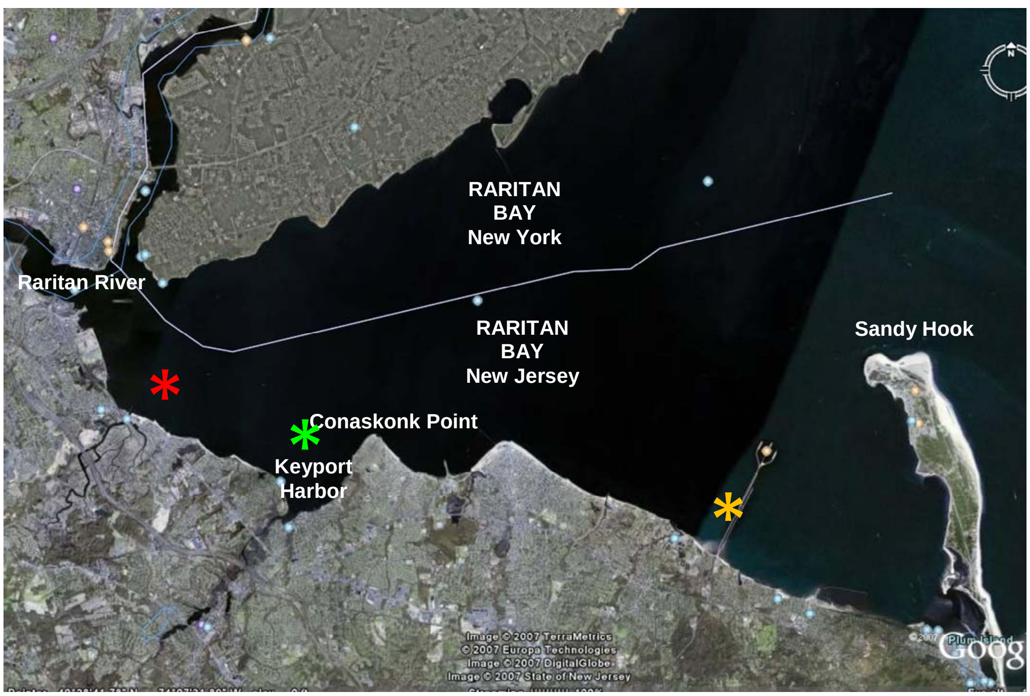


Figure 3. Approximate locations of the Keyport Harbor NJDEP permitted oyster reef (green) a potential oyster reef restoration site in the western portion of Raritan Bay (red), and Naval Weapons Station Earle (yellow).

A visual assessment of the bottom substrate and adjacent shoreline conditions at the Sayreville and Keyport Harbor sites was conducted on October 18, 2007 by Drs. B. Ravit and R. Miskewitz, C. Alderson (NOAA), and Andrew Willner (NY/NJ Baykeeper) on an incoming neap tide. The two sites were ~4.5 miles apart at the western end of Raritan Bay. The inspection revealed that the bottom composition at both locations was hard sand, littered with broken shells. A small number of live oysters were recovered from the Keyport Harbor location, but no live oysters were observed at the Sayreville site. The Keyport visual assessment revealed a bottom that was poorly colonized at the MLW 6′ contour. The only macro marine organisms observed were relatively low numbers of live oysters in combination with the broken clam shells at Keyport Harbor and high numbers of sea squirts at Sayreville. While the shorelines appeared to be similar (creek discharges, emergent *Spartina alterniflora* marshes forming), benthic grab samples were dissimilar (visible benthic fauna in Keyport, depauperate community in Sayreville).

Research was conducted to describe physical and ecological characteristics of the two western Raritan Bay sites, including a hydrologic assessment that mapped current and wave activity and a characterization of contaminant loadings. The site characterizations describe various water quality parameters, including dissolved oxygen, temperature, pH, salinity, nutrients and contaminants, and the presence of fecal coliform bacteria. The goal was to identify similarities and differences between potential reintroduction locations with respect to water quality parameters.

A. INITIAL SITE ASSESSMENTS

1. WATER QUALITY PARAMETERS

Water column samples were collected by Dr. Ravit and analyzed in NJDEP certified laboratories. The samples were collected from Sayreville and Keyport Harbor under dry conditions on an outgoing low tide on September 25, 2007; samples were collected from the Navesink River Baykeeper reef site under the same conditions on September 27, 2007. Water samples at all locations were obtained from a depth of approximately one meter by lowering a bucket into the water column from the deck of a boat and then transferring the water into sample collection containers. Oyster tissue samples at the Navesink Reef were obtained by tonging and transferring 3 live animals to the laboratory on ice. A subsequent set of water samples was collected on October 12, 2007 from Sayreville and Keyport Harbor to test for differences in fecal coliform levels under high flow conditions; the amount of rainfall recorded during the preceding storm was 1 - 2 inches in the Raritan Bay estuary (NOAA). These samples were collected from a depth of approximately one meter by walking into the water from the shore and placing collection bottles directly into the water column. After collection all samples were placed on ice and maintained at 4 ^{0}C until processed. The holding time for fecal coliform samples did not exceed 6 hours. Water column samples were analyzed for the presence of heavy metals at the Environmental & Occupational Health & Safety Institute (EOHSI), Rutgers University, New Brunswick, NJ; water and oyster tissue samples were analyzed for the presence of fecal coliform colony forming units (CFUs) at NJ Analytical Laboratories (NJDEP ID #11005); water samples were analyzed for nutrients (nitrogen, phosphorous) at the Rutgers EcoComplex, Bordentown, NJ. Dr. Ravit also obtained water monitoring data from NJDEP that characterizes Raritan Bay water quality parameters during the time period of 2000-2007 at 53 locations, including western portions of the Bay. The NJDEP data is from a minimum of 5 samples collected after heavy precipitation events that historically result in elevated coliform levels – a "worst case scenario" shellfish condition with respect to human health.

Concentrations of ammonia (Table 2) in Sayreville exceed the NJDEP water quality standards for saline estuaries; Keyport Harbor ammonia concentrations exceed the

NJDEP saline estuary water quality chronic standard (NJDEP 2006). Analysis of the NJDEP water quality data indicated that under certain high flow conditions fecal coliform contamination continues to negatively impact the water quality in the western portion of Raritan Bay.

Table 2. Water Quality Parameters (samples collected under low flow conditions).

Sample ID	COD	NH_4^+	NO_2^-	$NO_3^-+NO_2^-$	TKN	P_{Ortho}	P_{Total}
	O_2/L	mg/L	mg N/L	mg N/L	mg N/L	mg/L	mg/L
Sayreville	983	0.21	0.09	0.60	1.63	0.12	0.32
Keyport Harbor	1025	0.17	0.06	0.48	0.64	0.08	0.34
Navesink River	1009	ND	ND	ND	1.35	0.06	0.35
NJ Saline Coastal Water Standard (NJDEP 2006)	**N/A**	**0.115 acute 0.030 chronic**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**

2. HYDROLOGIC ASSESSMENT

The hydrologic conditions in Sayreville and Keyport Harbor were assessed to determine the effects wind and wave energies might have on oyster installations. Both current and wave forces can act in a positive manner by moving water through a reef and supplying food to the filter feeding oysters, or in a negative manner by destroying the physical structure of a reef. The conditions measured included current velocities in Sayreville and Keyport Harbor and wave heights in Keyport Harbor.

Current velocity was measured on August 29, 2007 by Dr. R. Miskewitz using an Acoustic Doppler Current Profiler (ADCP) (Fig. 4). The ADCP emits an acoustic signal into the water column, which then is reflected off the particles that are carried by the current. The Doppler shift of the signal as it returns to the instrument can then be used to calculate the speed and direction of the particles. An ADCP was side mounted to the Baykeeper vessel. Measurements were taken along two transects at both locations over a 13 hour period in order to assess currents at all stages of the 12.42 hour tidal cycle

(Fig. 4).

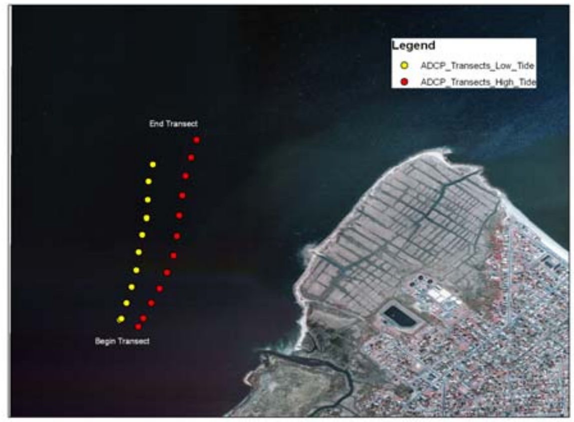


Figure 4. ADP Transects at a) Sayreville and b) Keyport Harbor taken on August 29, 2008.

The velocity transects showed variation in the magnitude and direction of the current flow, both over depth and across the transect. The average flow at Keyport Harbor was to the southwest at low tide and to the southeast at high tide (Fig. 4b). At the Sayreville location the average flow was northeast at both high and low tides (Fig. 4a). The measurements collected during the current sampling event revealed depth averaged mean current velocities of 7.5 cm per second at high tide, and 9 cm per second at low tide. These measurements were collected on a relatively calm day and most likely represent average conditions.

3. OVERWINTER SURVIVAL & GROWTH EXPERIMENT

Juvenile seed oysters were secured in June 2007 from J.M. Flowers (Oyster Bay, Long Island) and placed in the Navesink River at Bahr's landing docks for five months. On November 23, 2007, after measuring the length and height of each animal (Fig. 5), 250 oysters averaging 50 mm long were placed in a plastic basket; this density was the equivalent of 1,000 oysters/meter squared, considered a high density under natural conditions. The cages were secured shut with plastic ties and placed subtidally in Sayreville and Keyport Harbor using lengths of rebar that protruded through the enclosures, which were set into the hard sand bottom (Fig.6). The top of the each enclosure was at the water surface during mean low tide. Each enclosure was marked with a plastic buoy to identify its location.

Figure 5. Meredith & Andy Comi measuring oysters, November, 2007.

Figure 6. Meredith & Andy Comi placing oyster cage in Raritan Bay, November, 2007.

The oyster cages in Sayreville and Keyport Harbor were visually inspected during low tide in February, 2008. The cages were again inspected on June 3 (Keyport Harbor) and June 16 (Sayreville). During the June inspection, the cages were opened and the number of live versus dead oysters was determined. At the Keyport Harbor location, approximately 70% of the oysters were alive; at the Sayreville location only 3% of the oysters were found to be alive. Tissue analysis conducted by Dr. Pete Weis at UMDNJ revealed that live animals sampled in June from the Keyport location were ready to spawn; no eggs were observed in the 3 live oysters retrieved from the Sayreville location. Upon opening the oyster cages in June 2008, we observed a thriving benthic community at the Keyport site. This community consisted of crabs, shrimp, and snails, numbering in the hundreds. We did not observe significant numbers of marine organisms associated with the cage at the Sayreville site. This may be due to the fact that there were few surviving oysters at this location.

Test cages, each containing 100 juvenile seed oysters, were again placed in the Keyport Harbor and Sayreville locations in October, 2008 and the overwinter experiment repeated. The cages were retrieved during June, 2009 and the surviving and dead oysters were separated and measured to determine length. Over-winter survival at the Keyport Harbor reef was 65% and at the Sayreville location 53%. Although the Sayreville survival rate was greater than in the 2007-2008 winter experiment, the length of the Keyport live animals was almost 25% greater than the length of the Sayreville oysters. We did not attempt to determine what was affecting the mortality or growth at the Sayreville site, and recommended that Baykeeper's continuing restoration efforts be focused on the Keyport Harbor location where survival and growth were significantly higher.

KEYPORT HARBOR FIELD RESEARCH PLOTS

Based on the underwater field observations, the review of NJDEP water quality monitoring data (2000-2007), and live oyster over-winter survival experiments it was decided that Baykeeper would focus their reintroduction efforts on the original Keyport Harbor location. Although the data indicated that the Keyport Harbor site could be expanded, the wave, wind, and current energies in this location preclude merely placing shell piles on the bottom. The reintroduction as originally designed consisted of crushed shell and oysters piles placed in Keyport harbor by Baykeeper volunteers. The visual inspection determined that this structure was dispersed across the hard sand bottom and no longer viable as a self-sustaining oyster population. To provide the support require for oysters to accrete vertically, some form of engineered structure is needed. Such a support must be capable of withstanding the high energies generated during storm conditions, resist scouring and retain stability, and potentially provide a positive contribution to enhance sediment deposition on the shoreward side of the reef. The structures must be fabricated from easily obtainable and inexpensive materials, and should enhance oyster growth and survival, as well as the settlement of new spat.

The research plan for summer 2008 was to test four support structures at the Keyport Harbor site and to assess oyster growth and mortality associated with each structure. Baykeeper purchased support structures from two manufacturers (Reefblk™ and Reefballs®). Two non-proprietary structures were also designed to be tested for effectiveness under Keyport Harbor conditions (Rutgers "Arch" and Gaia "Lollipop"). However, the reef ball larval set failed and the lollipop structures proved to be too heavy for Baykeeper staff to handle. In September, 2008 it was decided to wait until the following year to conduct a test of the various support structures.

In summer 2009 Baykeeper obtained Eastern Oyster larvae from Horn Point Laboratories (Cambridge, MD) for setting in their Atlantic Highlands aquaculture facility (Fig. 7a). Reefballs™ were placed in the setting tanks and the set was judged to be successful (Fig. 7b). Eastern oyster larvae were also set in the Baykeeper aquaculture facility on cured surf clam shells housed in mesh bags (Fig. 8). The spat-on-shell and the Reefballs™ were removed from the setting tanks and placed in the "nursery" area of Raritan Bay adjacent to the facility (Fig. 9) to allow the larvae to grow to between 15 and 30 mm prior to placement on the Keyport Harbor research "reef" site. In addition to the set larvae, Baykeeper purchased juvenile seed Eastern Oysters approximately 25 mm in length that were spawned in Long Island Sound (Aeros Cultured Oyster Co., Southold, NY). These juvenile oysters were also placed in the Atlantic Highlands nursery area for acclimation to Raritan Bay prior to placement on the Keyport Harbor research site.

The objectives of the Keyport Harbor research were to:

1. Estimate long-term rates of oyster survival and growth at the Keyport Harbor site;
2. Identify the reef support structure(s) that:
    a. Supported the highest oyster survival rates;
    b. Supported the faster oyster growth rates;
    c. Resisted high energy during winter storm events;
    d. Facilitated sediment accretion on the shoreward side of the reef footprint; and to
3. Characterize the biodiversity associated with the presence of live oysters in Keyport Harbor.

In September, 2009 the individual seed oysters and spat set on shell (SOS) were enclosed in polypropylene mesh bags, which were attached to either a Reefblk™ or Arch support structure. A description of each structure tested follows.

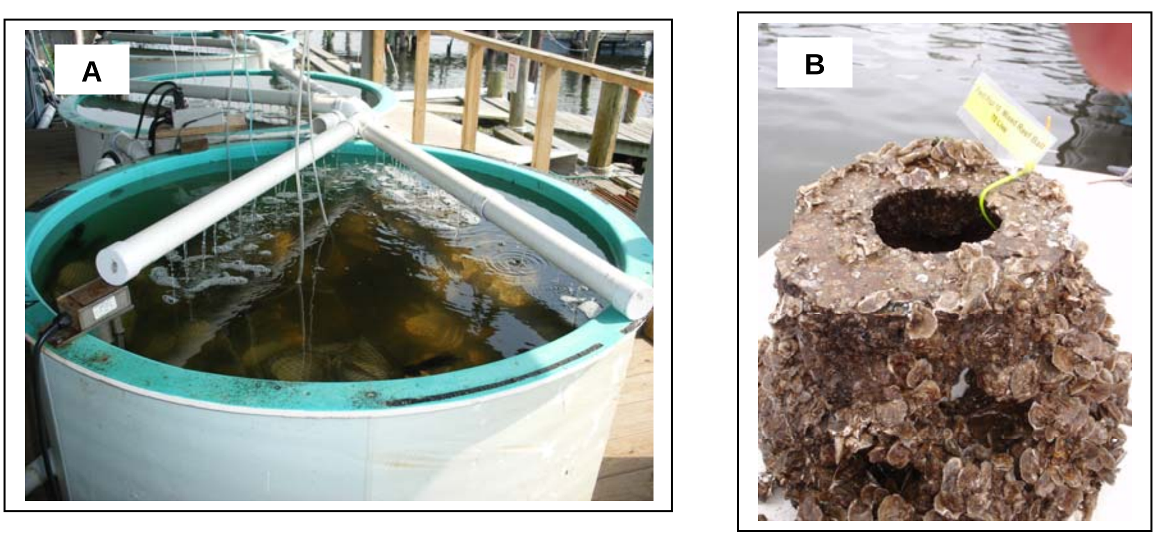


Figure 7. Baykeeper a) larvae setting tanks in the Atlantic Highland aquaculture facility and b) 2009 Reef Ball™ oyster set.

Figure 8. Setting oyster spat-on-shell in Baykeeper's aquaculture facility.

Figure 9. Baykeeper "nursery" in Atlantic Highlands, NJ.

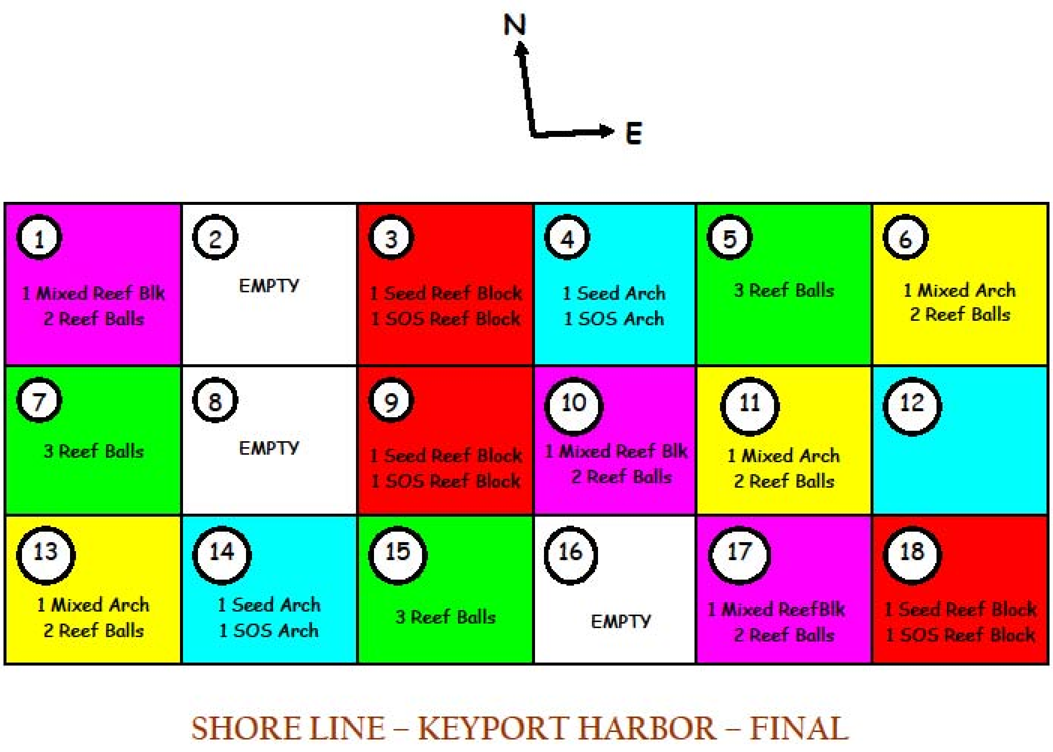


Figure 10. Random Block Experimental Design for Keyport Harbor Research Plots.

OYSTER (REEF) BALL DIMENSIONS

Reefballs™ are individual concrete semi-spherical units designed to emulate and create new oyster reef. The structure is formed using a fiberglass mold containing a central Polyform buoy surrounded by various sized inflatable balls to make holes. The structure is designed to contain holes that provide areas for the oysters to colonize, while being heavy enough to remain stable in a high energy environment.

The approximate dimensions were:

Width: 1.5 feet (0.46 m)

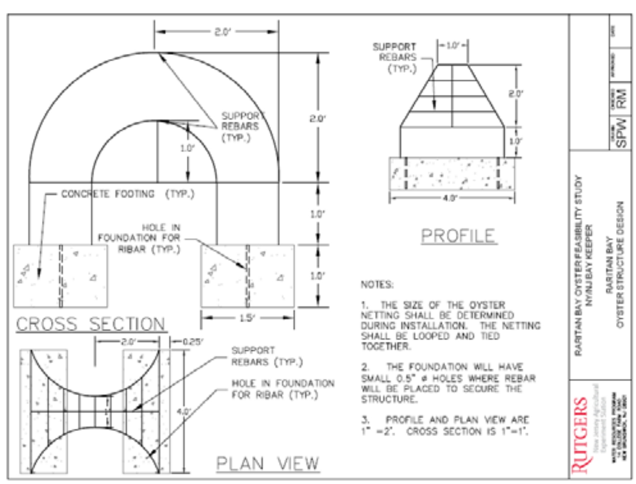
Height: 1 foot (0.30 m)

REEF BLOCK

Reefblks™ are prefabricated double framed rebar units that hold mesh bags filled with seed or SOS oysters. The structures emulate and create new artificial oyster reef. It is also possible that they may provide some immediate shoreline protection.

RUTGERS ARCH STRUCTURE

The Arch was designed to be a relatively inexpensive and simple framed rebar structure that supports mesh bags filled with seed or SOS oysters. The arch was designed to provide low resistance to water flows in the relatively high energy Raritan Bay system. The structures are low lying, but provide vertical relief from the substrate for the attached oysters.



Arch designed by Dr. R. Miskewitz and S. Walsh.

Each research plot contained either a single type of structure (two rebar structures or three Reefballs™), a mixture of one rebar structure with two adjacent Reefballs™, or was left empty (Fig. 10). The Arch and Reefblk™ structures were stabilized at each corner using rebar anchors driven at least 6″ into the sediment substrate. When two structures were installed in the same research plot, they were chained together to increase stability. Reefballs™ were placed in sets of two or three and chained together in order to prevent movement. Oysters were attached to the support structures in a density of 1,000 oysters/meter sq, a healthy natural density (Mann et al. 2009). Reefball™ density was not controllable since the set was random. In addition to the oysters attached to the large supports, small caged subsets of 250 oysters in the same density were attached to the top of the rebar support structures for retrieval during future monitoring events. Subset oysters were measured (length and height) prior to placement in the field plots. To obtain Reefball™ subsets, an area 2 ¼ meter sq. was marked on the face of the Reefball™ where live oysters were measured (length) and any dead shells were removed. Prior to placement in the research field plots, the following measurements were taken:

1. **Reefballs™**: A 10 cm x 10 cm area on the face of the Reefball™ was marked and the spat within this 100 cm^2 area were counted and measured. The heights of each live oyster were recorded (Day 0). This same area was again measured during the summer of 2010 when the oysters were destroyed. However, these measurements were obtained by volunteers under great duress, and so we do not have high confidence in the Reefball™ survival and growth data.

2. **Reefblk™ and Arch Structures**:
   A. <u>Oyster Seed</u>: Oysters were randomly selected and placed in ½″ net heavy plastic mesh bags. These bags were attached to the rebar support structures. From the original oysters selected, a subset of 250 oysters was randomly

selected and placed in a separate mesh bag, which was also attached to the rebar structure. The heights and widths of the randomly selected subset oysters were measured and recorded. The mean of these measurements describes the Day 0 size class. The subset oysters were re-measured during summer of 2010 prior to destruction. Oyster survival and growth rates associated with each structure were determined.

B. Oyster Spat-on-Shell: Cleaned and cured surf clam shells were placed in the Baykeeper larvae setting tanks and the spat randomly set on the clam shells. The mean number of oysters per shell is approximately 20-25, although there is variability between shells in the number of oysters set. After the oyster spat reached a height of at least 10 mm, the clam shells were placed in 1″ net heavy plastic mesh bags at approximately the same density as the oyster seed subset (500 oysters/m^2). These bags were attached to the rebar structures. A subset of randomly selected shells containing approximately 250 oyster spat was placed in a separate mesh bag, and this subset was also attached to the structure. The heights of the randomly selected subset oysters were measured and recorded. The mean of this measurement described the Day 0 size class. The subset oysters were re-measured during the summer of 2010 prior to destruction to determine oyster survival and growth rates associated with each structure.

3. ***Oyster Survival***: Using the Day 0 counts as the baseline for live oysters placed on the reef, we recorded the number of surviving oysters in July, 2010. Dead shells were measured and the number of dead oysters used to calculate annual mortality rates associated with each structure.

During the July and August, 2010 monitoring events the numbers of surviving and dead oysters attached to each rebar structure were recorded. The survival percentage was calculated based on the number of all oysters recovered (34% of the initial subset oyster population). We hypothesized that the loss of some juvenile oysters was a result of their falling through the mesh cages due to their small size. However, we were reluctant to use a smaller mesh size due to the potential decrease in water flows after cage fouling. To compare survival rates in the subset samples versus the larger Arch and Reefblk™ cages, 995 seed and SOS oysters were recovered from one Arch and one Reefblk™ cage. Oyster survivorship and growth rates were calculated for the large cages and compared to data collected from the subset attached to the same structure.

Oyster growth and survival were analyzed using two-factor Analysis of Variances (ANOVAs) (STRUCTURE factor: Reefball™ vs. Reefblk™ vs. Arch, and TYPE factor: Seed vs. Spat-on-Shell vs. Set; N=7,330 observations). All Summary Statistics and ANOVAS were conducted using SAS GLM (SAS Software, Version 9.2). Relative growth rates were calculated using the following formula (Hunt 1990): $G = (Log_e N_2 - Log_e N_1)/t$ where G = the mean rate of increase over the time interval; N = the average length of oysters in mm; t = time (320 days). Captured species diversity (N = 20 sampling events) was compared by means of the Shannon Index of Diversity using the following formula (Magurran 1988): $H = -\Sigma p_i ln(p_i)$ where H = the sample diversity; p_i = the proportion of the number of a single species to the total number of individuals in the sample; and ln = Log_e. One-way ANOVA was conducted to test community diversity differences between samples. Post hoc means were tested using Tukey's HSD method.

The support structure and the type of juvenile oyster produced significant differences in survival patterns (Fig. 11). Significant differences in oyster growth were observed among the structures and between the types of juvenile oysters (Table 3). Live Arch oysters were 22% larger than Reefblk™ oysters; dead Arch oysters were also significantly larger than oysters housed on the other structures. Seed oysters (alive and dead) were significantly longer than SOS or Reefball™ set oysters. Lengths of dead oysters did not differ among the type of juvenile. No interaction affecting length was seen between the structure and the type of juvenile oyster in the subset samples. Conversely, two-factorial ANOVA of oyster lengths in the larger cages showed an interaction between the structure and the type of juvenile for both live ($F = 20.30$, $p < 0.0001$) and dead ($F = 22.96$, $p < 0.0001$) oysters. Live oysters were significantly larger than dead animals (mean Arch oyster length 46 mm, mean Reefblk™ oyster length 51 mm versus mean dead length of 36 mm). Mean length of seed oysters housed on Reefblk™ was 48 mm versus a length of 36-38 mm for Arch seed oysters and SOS on both structures.

The proportion of surviving oysters was greater in the subset samples than in the two larger cages that we had time to sample. The difference seen in the Reefblk™ comparison may be due to the fact that the smaller subset bags were attached to the top of the structure, and so were less subject to sedimentation impacts (Powers et al. 2009) than the larger cage that came into contact with the bottom substrate. The SOS exhibited the highest survival rate (approximately 40%) and the spat set on the Reefball™ exhibited the lowest (15%); the highest structure survival rate (almost 60%) was associated with the Reefblk™ support (Table 3).

Differences in survival associated with the various structures within the ¼-acre research site suggest that the specific support structure(s) chosen for a given location might positively affect oyster survival rates. Based on the limited results of this study, the most successful restoration approach in Keyport Harbor could be SOS housed in

Reefblk™ structures. However with only one year of data, it would be premature to draw this conclusion, and additional longer-term research is needed to characterize

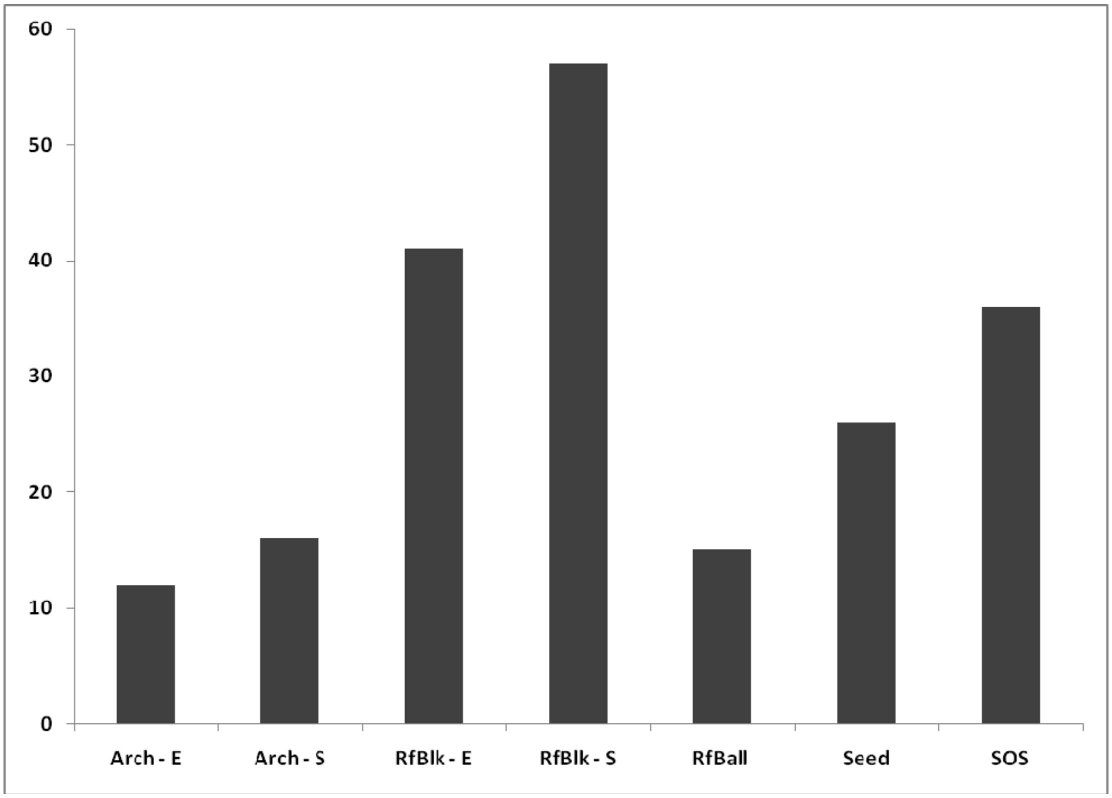


Figure 11. Proportion of living Eastern Oysters recovered from all subsets after one year in Keyport Harbor, NJ (September 2009 – August 2010). Cage types = Arch, Reefblk™ and Reefball™. Oyster types = Seed, Spat set on Shell (SOS), and Spat set directly on Reefballs™. S = subset sample; E = large cage sample (one each of an Arch and Reefblk™ large cage).

oyster survival and spawning patterns (Hawkins et al. 2002), and to observe whether new oyster larvae produced ultimately set on the adult oyster shells in this location. It is interesting to note the larger length of the oysters housed in the Arch structure. It is possible that an increased water flow through this structure versus the other two supports brought the oysters into greater contact with food sources. However, their larger size did not result in higher survival rates. Further investigation that combines desirable features of both the Arch and Reefblk™ structures might further improve both growth and survival rates.

High mortality observed with the Reefballs™ may be due to sedimentation. We did note large numbers of dead animals at the base of the Reefballs™ and sediment was observed inside the dead shells. Although the Keyport substrate is gravel and hard sand, this site experiences high turbidity during storm events and sedimentation is known to be a major obstacle in restoring oyster populations (Powers et al. 2009). We note that the monitoring of the Reefball™ structures was done by Baykeeper volunteers, and occurred concurrently with destruction of the oysters in the research plots. Therefore, we have low confidence in the accuracy of this data and believe this structure needs to be retested.

Table 3. Mean length (plus/minus Std Err) attained by oysters after eleven months in Keyport Harbor. Oysters were housed in Reefblk™, Reef Ball™, or Arch structural supports. Letters indicate statistically significant differences in length.

	# Individuals		Length		Height	
Structure	**Live**	**Dead**	**Live**	**Dead**	**Live**	**Dead**
Arch	256	1282	45.1±0.69[a]	39.5±0.31[A]	14.7±0.25 [c]	12.4±0.10
Reef Block	472	358	37.2±0.49[b]	31.6±0.52[B]	12.6±0.25 [d]	12.6±.034
Reef Ball	18	99	40.8±1.84[a,b]	28.4±0.88[B]	NA	NA
Type						
Seed	330	925	46.9±0.55[e]	43.77±0.31[D]	13.7±0.19	12.4±0.10
Spat-on-Shell	398	715	34.2±0.46[f]	30.0±0.31[E]	NA	NA
Set on Reef Ball	18	99	40.8±1.84[g]	28.4±0.88[E]	NA	NA

Live *Two-Factorial ANOVA* $F_4 = 88.20$ $p < 0.0001$

LENGTH			HEIGHT		
Structure	F = 16.27	*p* <0.0001	Structure	F = 16.27	*p* <0.0001
Type	F = 217.08	*p* <0.0001	Type	F = 217.08	*p* > 0.0001

Dead *Two-Factorial ANOVA* $F_4 = 266.04$ $p < 0.0001$

Structure	F = 7.70	*p* = 0.0056
Type	F = 436.83	*p* <0.0001

TISSUE ANALYSES

Histopathology

To qualitatively assess the health of the year old Keyport oysters, 10 adult oysters were retrieved in June, 2010 prior to spawning and 10 were retrieved post-spawning on the day the research plots were destroyed. The oysters were weighed, shucked, and individual shell and wet body weights were measured (Table 4). The oysters were preserved in 10% formalin and subsequently transferred to a 70% ethanol solution. The oysters were then placed in a casing, which was immersed in a paraffin bath at a temperature of 135^{o} F (Reichert Histostat Rotary Microtome). After removal from the bath the paraffin was allowed to solidify. The sample was then sliced into cross sections 6 µm thin, placed on a microscope slide, and baked at 60^{o} C for 30 min. to remove any paraffin from the slide. The slide was then stained with lithium carbonate, which colored the oyster tissues dark blue, allowing us to observe and evaluate the condition of various soft tissues, including the mantle, gill, digestive, and reproductive systems. A minimum of four slides were prepared for each sample.

Lesion Severity scores are commonly used to determine the degree of tissue damage (Ray 1954; Ford & Tripp 1986). Tissue lesions, hyperplasia (an abnormal increase in the number of cells), infiltration of macrophages, parasites, dysmorphic (misshapen) cell and/or tissue structures were scored using a 3-point rating system where:

1 = normal appearance of oyster cell/tissue structure
2 = presence of some visible degree of oyster cell/tissue abnormality
3 = oysters cell/tissue appearance highly abnormal

Lesion Severity scores for 20 randomly selected adult Eastern Oysters (approximately 40 - 130 mm in length) were calculated for each tissue type based on this visual histopathology evaluation system (Table 5). The typical 50:50 sex ratio of females to males (Morton 1991) was observed in the Keyport oysters sampled. Oysters collected in June were ripe and females appeared to be ready to spawn. The 10 oysters sampled post-spawning two months later (August 2010) after the summer growth period exhibited a more than double mean wet body weight and a mean total weight almost three-fold greater (Table 4) than oysters sampled pre-spawning.

Table 4. Physical Characteristics of One-Year Old Keyport Harbor Eastern Oysters

	Pre-Spawn	**Post-Spawn**
Parameter	**June_2010**	**August_ 2010**
Length (cm)	4.7 ± 0.27	6.1 ± 0.38
Height (cm)	1.3 ± 0.7	1.8 ± 0.10
Width (cm)	3.6 ± 0.19	4.5 ± 0.19
Wet Body Wt (g)	2.8 ± 0.51	6.1 ± 0.75
Shell Thickness (mm)	2.0 ± 0.20	3.4 ± 0.30
Shell Wt (g)	7.9 ± 1.20	23.0 ± 2.28
Total Wt (g)	11.9 ± 1.90	32.0 ± 3.36

In general, the Keyport Harbor oysters' mantle, gill, and gonadal tissues appeared to be normal. The shell gland, responsible for formation of the shell at its outer edges, also appeared to be normal, although some minor localized edema was observed. Digestive particles were present in the stomach and gastrointestinal tract of all samples and the digestive gland appeared to be normal. The histopathology evaluation suggests that the year old adult Keyport Harbor oysters able to survive in Raritan Bay appeared to be healthy, ecologically fit, and capable of successful reproduction.

Table 5. Lesion Severity Scores for Year Old Keyport Harbor Oysters

Gonad	1.25 + 0.105
Gill	1.58 + 0.178
Labial Palps	1.33 + 0.333
Digestive Gland	1.28 + 0.109
Mantle	1.53 + 0.118
Abductor Muscle	1.00 + 0.000
Kidney	1.50+ 0.136
Connective Tissue	1.11 + 0.072
Heart	1.67 + 0.211

A score of 1 = normal appearance and a score of 2 = some visible degree of abnormality.

METAL ANALYSES

Composited subsamples taken from the oysters evaluated for histopathology were analyzed at the Rutgers Environmental & Occupational Health Sciences Institute (EOHSI) to determine soft tissue and shell metal concentrations. The shell and body tissues were separately digested using a MarsX microwave sample digester (CEM Corp, Matthews, N.C.). Between 90-260 mg of air-dried sample was reacted with 0.5 ml of nitric acid (EMD Omni Trace, ultra high purity). The samples were microwaved repeatedly (300W, 75% power, 5 minutes at a time) until no further digestion occurred. Another 0.25 ml of nitric acid was added and the samples were microwaved repeatedly (300W, 100% power, up to 10 min). When no further digestion occurred, another 0.75 ml of nitric acid was added and the samples were sonicated 1 hr, microwaved a final time (300W, 100% power, 20 min), cooled to room temperature, and diluted to 30 ml (Milli-Q ultra pure de-ionized water of 18.2 megaohms, Millipore Corp.) The samples were centrifuged, a few ml of supernatant was removed and re-centrifuged prior to five-fold dilution with 5% nitric acid. Samples were analyzed for metals with an inductive coupled plasma mass spectrometer (X5, Thermo Electron Corp.) using a multi-element scan. This method required significant modification from the laboratory's previous microwave tissue digests (Buckley et al. 2003; Xie et al. 2007). The stepwise addition of nitric acid allowed for complete digestion while keeping the sample volume low.

Although oyster soft tissue metal concentrations have been reported for individual metals, our analysis included twenty-four metals, and so was more extensive than most studies (Table 6). The total body metal concentration averaged 0.4% of body weight. Only three soft tissue metal concentrations (aluminum, barium, titanium) were higher than values reported from other urbanized estuaries; in general, the soft tissue metals were lower than those observed in other studies.

Conversely, shell metal concentrations were higher than soft tissue concentrations for eleven metals (aluminum, barium, cobalt, iron, lithium, magnesium, manganese, nickel, strontium, titanium, vanadium), indicating the oysters' ability to successfully transfer metals to the shell. Off loading of metals into the shell, as seen in the Keyport oysters, protects the animal from potentially harmful or lethal effects of metal exposure.

Table 6. One-Year-Old Keyport Harbor Eastern Oyster Shell and Soft Tissue Metal Concentrations.

Metal (PPM)	Keyport Oysters		Literature Values		
	Shell	Soft Tissue	Shell	Soft Tissue	Reference
Aluminum	194 ± 47.2	140 ± 8		6 - 101	Sadig & Alam 1989
Arsenic	0.6 ± 0.06	1.5 ± 0.01		5 - 21 0.5 3 - 43	Volety 2008 Elston et al. 2005 NOAA 1987
Barium	7.0 ± 0.5	2.5 ± 0.10		<0.7	Sadig & Alam 1989
Cadmium	0	1.9 ± 0.02		3 - 16 0.7 - 1.4 2.2 1.8 - 16	Frazier 1975 Volety 2008 Guzman-Garcia et al. 2009 Hayes et al. 1998
Chromium	0.5 ± 0.09	2.2 ± 0.25		3 -6 6 1 0.1 - 5	Volety 2008 Guzman-Garcia et al. 2009 Elston et al. 2005 NOAA 1987
Cobalt	0.8= ± 0.07	0.3 ± 0.00[r]			
Copper	2.3 ± 0.10	60 ± 0.3	160	50 - 225 500 98 - 376 56 - 212 31 15 - 1,603	Frazier 1975 Frazier 1976 Volety 2008 Hayes et al. 1998 Elston et al. 2005 NOAA 1987
Gallium	0.3 ± 0.03	0.2 ± 0.01			
Iron	402 ± 56.2	139 ± 3	19	200 - 500 600 319 - 628	Frazier 1975 Frazier 1976 Volety 2008
Lead	1.2 ± 0.16	1.8 ± 0.02[x]		0.7 - 1.3 6 1.4 - 15	Volety 2008 Guzman-Garcia et al. 2009 Hayes et al. 1998
Lithium	1.8 ± 0.13[h]	0.2 ± 0.01[z]			
Magnesium	2,346 + 110	485 ± 13[bb]			
Manganese	208 ± 18.1	7.1 ± 0.10	330 - 520 505	5 - 30 114 19 - 25	Frazier 1975 Frazier 1976 Volety 2008

Table 6 (continued).

Metal (PPM)	Keyport Oysters		Literature Values		
	Shell	Soft Tissue	Shell	Soft Tissue	Reference
Nickel	5.0 ± 0.3	1.8 ± 0.03		0.6 - 1.4 0.9 0.6 - 13	Volety 2008 Elston et al. 2005 NOAA 1987
Potassium	0	1,234 ± 9			
Rubidium	0.3 ± 0.08	0.6 ± 0.02			
Selenium	0.5 ± 0.03	1.7 ± 0.23		1 - 1.8 0.4 0.9 - 5.7	Volety 2008 Elston et al. 2005 NOAA 1987
Silver	0	2.8 ± 0.04		1.2 - 1.6 0.5 0.3 - 7	Volety 2008 Elston et al. 2005 NOAA 1987
Strontium	1,386 ± 76	12.2 ± 0.58		9 - 53	Sadig & Alam 1989
Titanium	43 ± 1.0	17.6 ± 0.80		0.9 - 3	Sadig & Alam 1989
Uranium	0.1 ± 0.01	0.2 ± 0.00		2 - 2.5	Akyil & Yusof 2007
Vanadium	0.8 ± 0.14	0.4 ± 0.01	0.5 - 1.4 (shell/soft tissue)		Blotcky et al. 1979
Zinc	0	1,886 + 51	2500	1,800 - 5,000 1,495 - 5,669 1,806 - 2,902 478 300 - 13,000	Frazier 1975, 1976 Volety 2008 Hayes et al. 1998 Elston et al. 2005 NOAA 1987

KEYPORT BIODIVERSITY

To test whether the presence of the oysters and/or the rebar structures had an effect on the Keyport Harbor marine community, fish traps (small mesh shrimp and minnow trap and larger mesh semi-oval fish trap (Fig. 12), Memphis Net & Twine Co.) were placed in pairs, either: 1) adjacent to the three support structures; 2) in empty research plots; or 3) outside the eastern and western edges of the research footprint. These traps were selected to test whether the density of motile marine organisms was greater in the research plots containing oysters. After 24 hours, the traps were retrieved and the captured animals identified at the genus and/or species level by F. Steimle and M. Comi. A Shannon Diversity Index score for the various structures was calculated based on the data collected during four sampling events which occurred between July 21, 2010 and July 29, 2010. Two additional sampling events (September 8 – 9, 2010) were conducted after removal of the research animals to test the effect of only empty structures with no oysters present on marine community composition.

Figure 12. Large mesh fish trap.

Due to the small number of sampling events, no statistically significant differences in the Shannon Index of Diversity among the various structures were observed. However, the decrease in Shannon Diversity Scores when oysters were not present was particularly noticeable for the Arch and Reefblk™ structures; the number of individuals captured when the oysters were present was 2 to 3-fold greater than the numbers after removal of the oysters (Table 7). Conversely, the empty plot diversity score actually increased after oyster removal. The increased biodiversity associated with the presence of the oysters was very encouraging and certainly warrants further research. We note that this limited sampling timeframe did not capture biodiversity impacts related to oyster presence on spring and fall transitory species.

The crustaceans associated with the oysters are important fish prey, and during all sampling events with oysters present fin fish were captured (Fig. 13). The two eel species captured represent fisheries in decline in this region, and due to their complex life cycle patterns, the eel's use of estuaries and adequate measures to protect them are not well understood. The American Eel is currently being evaluated by USFWS for inclusion on the Federal Threatened & Endangered List. We hypothesize that the catch data suggests the presence of oysters created and enhanced habitat structural complexity, positively affecting prey density and abundances relative to higher trophic levels in the marine food web.

Figure 13. July 9, 2010 diversity sampling with large mesh fish trap.

Table 7. Species and number of individuals observed in Keyport Harbor, NJ during 4 sampling events (July 21, 2010 – July 29, 2010) *with* oysters present and 2 sampling events after oysters were removed (September 8-9, 2010).

Species (common name)	Latin name	Reefblk™ With	Arch With	Reef Ball™ With	Empty With	Reefblk™ Without	Arch Without	Empty Without
American eel	*Anguilla rostrata*	1	2	1	-	-	1	-
Blue Claw Crab	*Callinectes sapidus*	17	7	8	7	4	2	4
Conger Eel	*Conger oceanus*	-	1	-	1	-	-	-
Ctenophore	*Phylum Ctenophora*	50	55	34	20	10	10	15
Grass Shrimp	*Palaemonetes*	156	208	232	66	18	38	41
Hermit Crab	*Pagurus longicarpus*	12	13	22	20	23	15	19
Mud Crab	*Neopanopeus*	11	9	13	2	15	-	4
Mud Snail	*Ilyanassa obsoleta*	159	205	113	48	-	27	103
Oyster Drill	*Urosalpinx cinerea*	1	1	1	-	-	-	-
Pipefish	*Syngnathus fuscus*	1	2	2	-	-	1	-
Spider Crab	*Libinia emarginata*	3	-	-	1	3	12	7
Spotfin Butterfly Fish	*Chaetodon ocellatus*	-	-	-	-	1	-	-
Tautog	*Tautoga onitis*	-	-	-	-	1	-	-
Toad Fish	*Opsanus tau*	-	1	1	1	-	-	-
Total	**All Species**	**411**	**504**	**427**	**166**	**75**	**106**	**193**
	Shannon Diversity Score	**2.92**	**2.52**	**2.82**	**1.99**	**2.41**	**1.85**	**2.33**

3. SEDIMENTATION

To determine whether the presence of oysters and their support structures influenced sediment deposition patterns, bottom elevations were determined using laser surveying equipment (CST/Berger Dual Beam Rotary Laser, Watseka, IL, USA) and standard surveying methods (Lindeburg 1992). Sediment elevations at the four corners of the individual plots were recorded during low tide in the fall of 2008. The surveys were tied into NAD83 survey datum using the USGS benchmark located at the end of Walnut Street in Keyport, NJ. Using the same procedure, the reef was again surveyed during the summer of 2009 prior to installation of the research oysters and their support structures. These two datasets provided a baseline for changes in Keyport Harbor sediment elevations over the winter of 2008 prior to the installation of the oyster research plots. The measurements were repeated in June, 2010 after the research oysters and their support structures were in place for ten months to determine if: 1) sediment elevation patterns differed with oysters present, and 2) the presence of the oysters/structures increased scouring. Sediment elevation heights outside the research plot footprint on the western and eastern sides served as "NO OYSTER" controls. Maps were generated by entering the survey data into ArcMap. A raster image of the surface was created using the Natural Neighbor toolbox function in ArcMap.

A comparison of changes in the topography of the reef footprint suggests that after the 2009 winter storm season the presence of the oysters and their cages might have contributed to increased sediment stability (Fig. 14). While more seasonal data needs to be collected to determine if these initial results are repeatable, the presence of the oysters and their housing structures did not appear to increase sediment scouring.

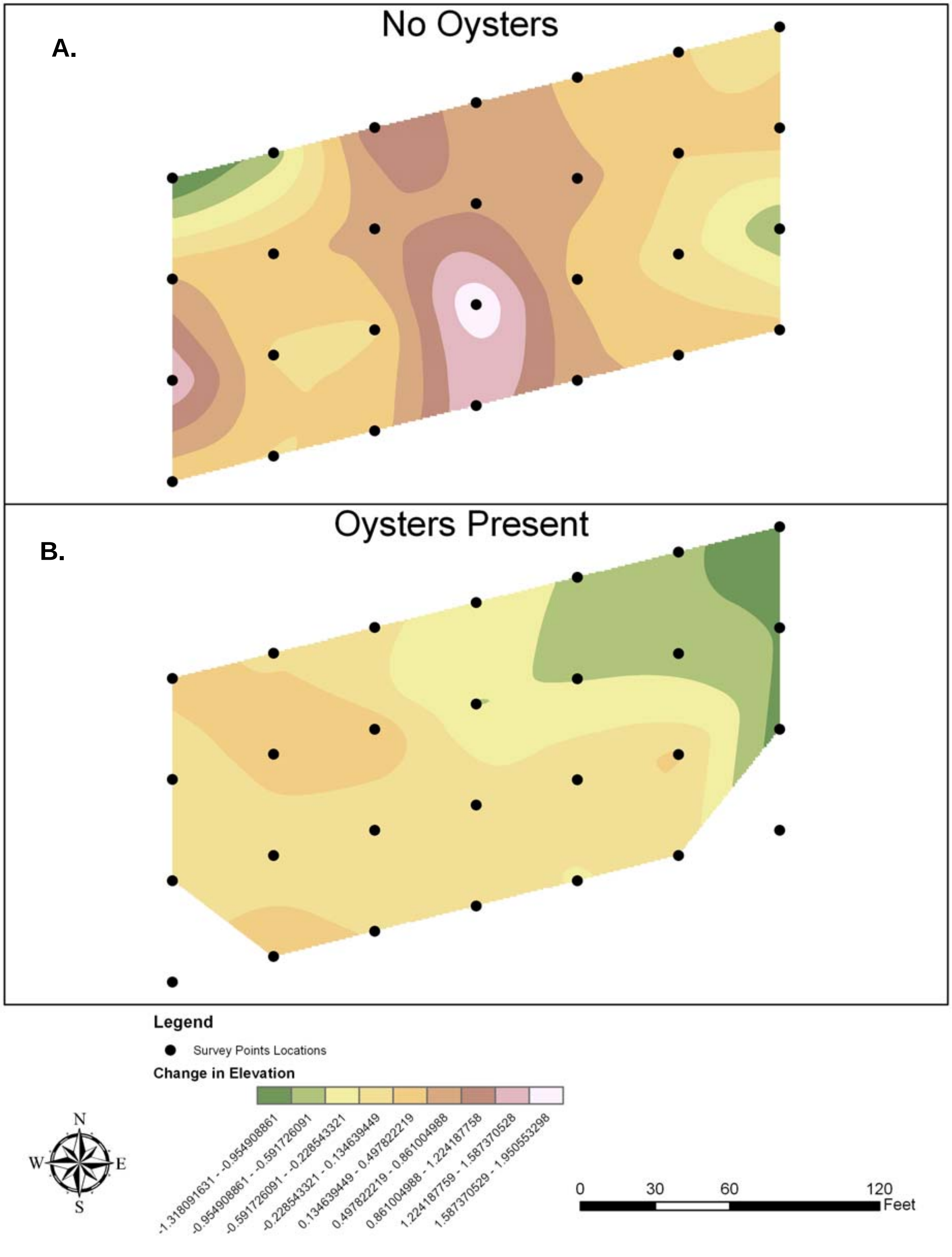


Figure 14. Keyport Harbor, NJ over-winter change in sediment topography: A) with no oysters or structures present (2008-2009), and B) with oysters present (2009-2010).

KEYPORT HARBOR RESEARCH ENDED

Approximately 50,000 oysters were placed in Keyport Harbor on September 16, 2009. On June 10, 2010, the New Jersey Department of Environmental Protection revoked the decade-old permit that allowed Baykeeper to place oysters at this site in Raritan Bay. An estimated 30,000 healthy oysters were destroyed on August 9, 2010. Due to the short time interval allowed to remove the oysters, Reefballs™ were retrieved on the day the research plots were destroyed. While volunteers attempted to collect subset data from the Reefball™ structures, given the circumstances and time constraints, we believe the Reefball™ portion of the data set to be less accurate than the data collected from the rebar support structure subsets.

Typically oyster research is conducted at sites where existing populations are present and often threatened. ***This study is unique in that it took place at research locations where oysters are currently ecologically extinct.*** The success of the first and largest oyster restoration research attempted to-date within the Hudson-Raritan Estuary is evidenced by the decision of the NJDEP to rescind the project permit as the oysters began to reach New Jersey's market size of 2.5 inches. In addition to the oyster's growth and survival, healthy gametes signifying the survivors' ability to spawn are also indicative of the project's success. Although the experiment was of very short duration (eleven months), the fact that oysters were beginning to approach market size and that their tissues appeared to be healthy indicate a majority of the animals could reach market size in the three year timeframe that is considered typical of mid-Atlantic waters.

IV. NYC OYSTER RESEARCH

Oyster Restoration Research Project (ORRP) Histopathology

To qualitatively assess the health of year old oysters placed in NYC locations, year old adult animals were retrieved on July 19-20, 2011 from the Hastings, Soundview, and Staten Island ORRP research sites and from a Gowanus Canal site. In the Rutgers University laboratories the oysters were weighed, shucked, and individual shell and wet body weights were measured. The oysters were preserved in 10% formalin and subsequently transferred to a 70% ethanol solution. The oysters were then placed in a casing, which was immersed in a paraffin bath at a temperature of 135^{o} F (Reichert Histostat Rotary Microtome). After removal from the bath the paraffin was allowed to solidify. The sample was then sliced into cross sections 6 μm thin, placed on a microscope slide, and baked at 60^{o} C for 30 min. to remove any paraffin from the slide. The slide was then stained with lithium carbonate, which colored the oyster tissues dark blue, allowing us to observe and evaluate the condition of various soft tissues, including the mantle, gill, digestive, and reproductive systems. A minimum of four slides were prepared for each sample except those from Staten Island Raritan Bay, where a limited number of live samples were recovered (N=3).

Lesion Severity scores for 20 randomly selected adult Eastern Oysters (approximately 70 mm in length) were calculated for each tissue type based on the visual histopathology evaluation system (Table 8). The typical 50:50 sex ratio of females to males (Morton 1991) was not observed in oysters sampled from the Staten Island shore of Raritan Bay (100% male, N=3) or Soundview (research 100% male, N=5; native 80% male, N=6). Conversely, oysters from Hastings-on-Hudson were 50% female (N=6).

However, we caution that these results were from a small number of samples, and so should be considered within a very limited context.

Table 8. NY Oyster Histopathology Observations

Location	Date of Collection	N	General Comments on Histological Findings
Hudson River (Hastings, NY)	7/19/11	10	• Of the oysters with gonadal tissue (N=8) 50% were female. Both male and female gonads were fully developed and ready to spawn. • The oysters were heavily parasitized with "Dermo"; 8/10 had visible spores in the mantle, gill or connective tissue. • The damage was evident in all of these tissues with macrophage infiltration and loss of glycogen rich connective tissue. • There was an increase in goblet cells lining both the water tubes of the gills and the mantle epithelium. This condition indicates some type of chronic irritation.
Soundview ***Native***	7/20/2011	6	• Two out of the six oysters were female (33%). Three (1 Female and 2 Male) of the six oysters were fully developed and ready to spawn. One male and one female appeared to have spawned and one male had delayed sperm development. • "Dermo" was not evident in the slides. • There was low to moderate goblet cell hyperplasia along the mantle epithelium and the digestive gland ducts. • There were minor/moderate structural abnormalities in a few oysters involving the gills and shell gland, but these were relatively minor.
Soundview ***Research***	7/20/2011	10	• Eight of nine oysters were male in this group. There was no gonad tissue for one oyster present on the slide. The single female and two of the eight male oysters were ready to spawn, while the other six were immature • "Dermo" was evident in one oyster • The oysters did display some minor goblet cell proliferation along the water tubes of the gill and the digestive gland ducts, but they were much less in prevalence and severity than the native oysters. • In the majority of the tissues examined no significant lesion was observed in this group.

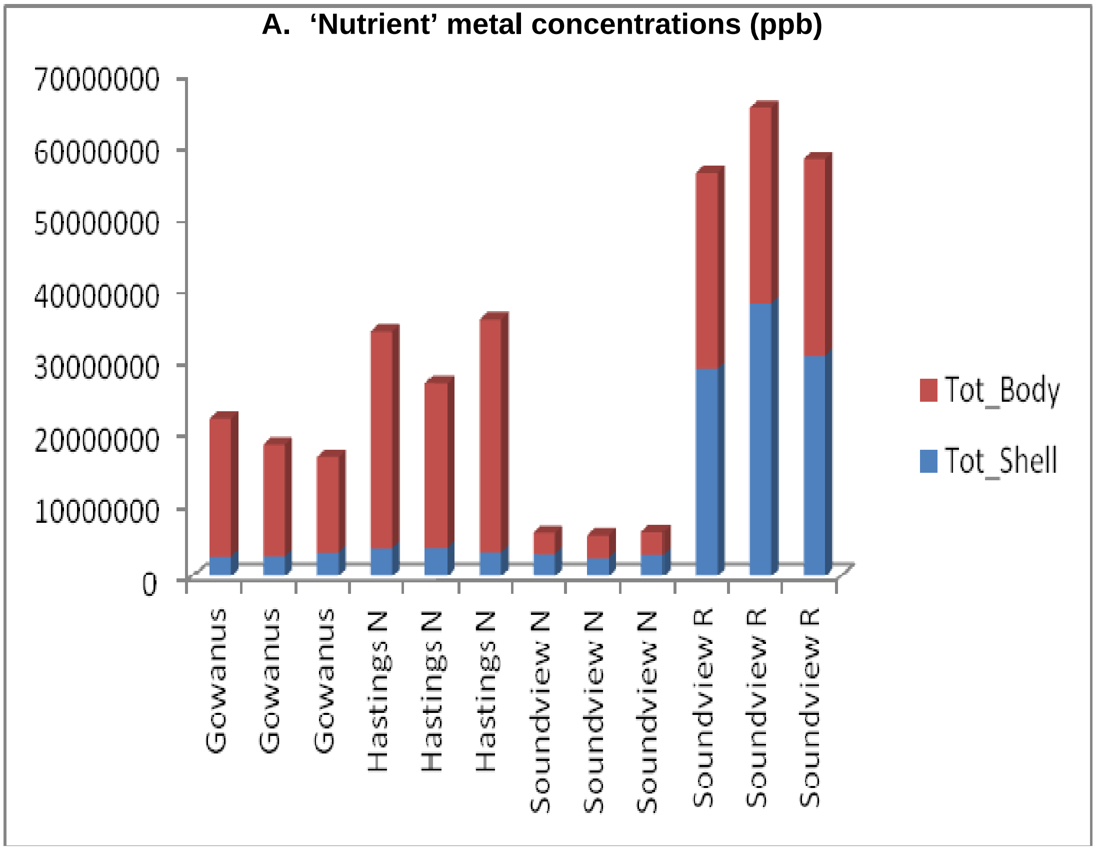
Dominance of males does follow the sex ratio trend observed in the previously sampled Hackensack research oysters (Ravit et al., 2013). Based on this histopathologic evaluation, the healthiest oysters would be the Soundview Research followed by Soundview Native, and the least healthy would be the Hudson River (Hastings, NY) oysters. Although the Hastings oysters appeared able to spawn, the survival of the adults due to "*Dermo*" infections would likely limit the population's growth and sustainability.

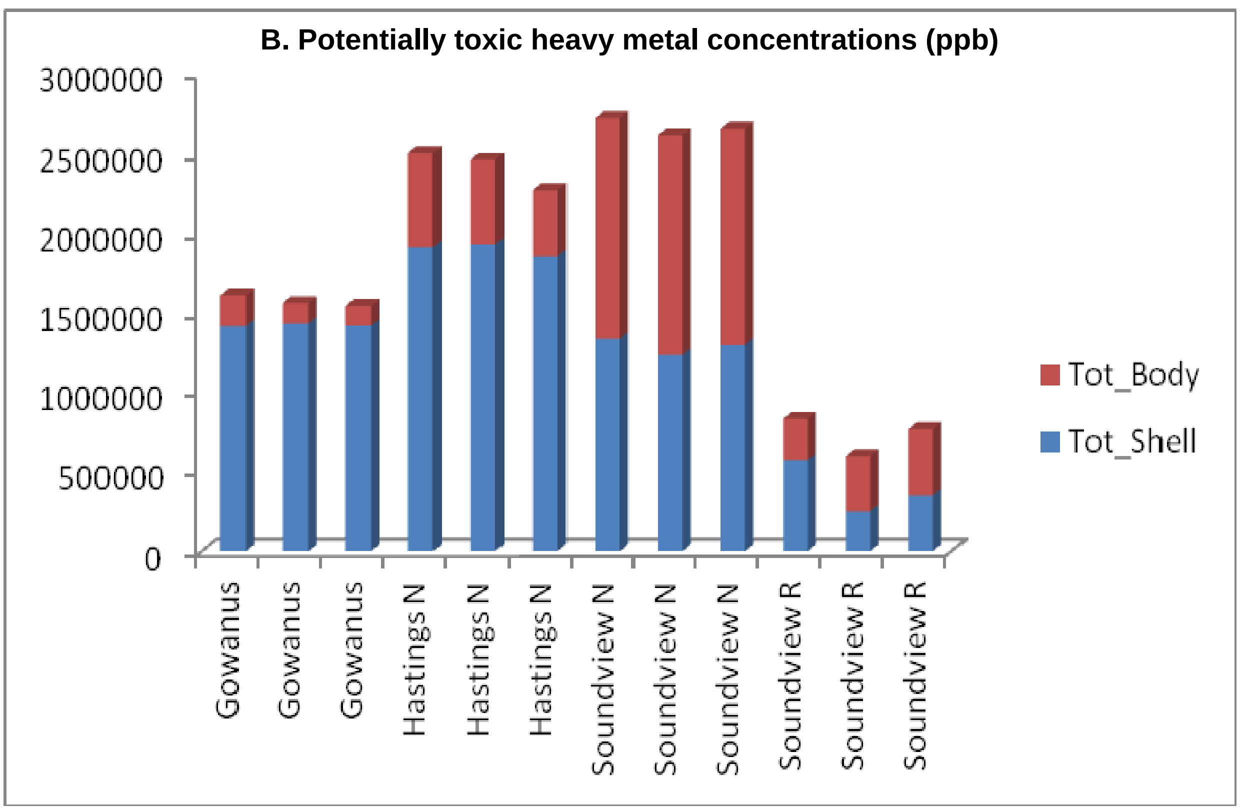
ORRP Oyster Metal Analysis

Composited subsamples taken from the oysters evaluated for histopathology were analyzed at the Rutgers Environmental & Occupational Health Sciences Institute (EOHSI) to determine soft tissue and shell metal concentrations. The shell and body tissues were separately digested using a MarsX microwave sample digester (CEM Corp, Matthews, N.C.). Between 90-260 mg of air-dried sample was reacted with 0.5 ml of nitric acid (EMD Omni Trace, ultra high purity). The samples were microwaved repeatedly (300W, 75% power, 5 minutes at a time) until no further digestion occurred. Another 0.25 ml of nitric acid was added and the samples were microwaved repeatedly (300W, 100% power, up to 10 min). When no further digestion occurred, another 0.75 ml of nitric acid was added and the samples were sonicated 1 hr, microwaved a final time (300W, 100% power, 20 min), cooled to room temperature, and diluted to 30 ml (Milli-Q ultra pure de-ionized water of 18.2 megaohms, Millipore Corp.) The samples were centrifuged, a few ml of supernatant was removed and re-centrifuged prior to five-fold dilution with 5% nitric acid. Samples were analyzed for metals with an inductive coupled plasma mass spectrometer (X5, Thermo Electron Corp.) using a multi-element scan. Although oyster soft tissue metal concentrations have been reported for individual nutrient metals and heavy metals of concern, our analysis of twenty-four metals (Cu, Zn, K, Mg, Mn, As, Ag, Cd, Cr, Rb, Se, U, Ti, Sr, Co, Li, Fe, V, Al, Pb, Ni, Bi, Ga, Cs) included soft tissue and shell concentrations and so was more extensive than most studies.

Two-Factorial Analysis of Variance (ANOVA) was conducted to test differences in metal uptake between the various research sites and between soft body and shell tissues (SITE Factor Gowanus, Hastings, Soundview Native, Soundview Research; TISSUE Factor Body vs. Shell). Oyster metal uptake varied by site, and interestingly, varied in native oyster populations versus research seed oysters at the Soundview location (Fig. 15, Table 9). With the exception of strontium, the Soundview native oysters had significantly lower concentrations of the metals tested, particularly in their soft body tissues, versus metal concentrations in the research seed oysters placed at this location. Sr body concentrations were almost thirty times greater (an order of magnitude) in native Soundview oysters than in the research oysters sampled (15b, Table 9). There is no long term data to determine whether restoration oysters placed at a specific location will over time exhibit tissue metal concentrations that more closely mirror concentrations found in a native population, although other research suggests this might be the case (Frazer 1975, 1976). It is also not known if native oysters have evolved metabolic defenses to mitigate the presence of historic heavy metal contaminants.

Oysters from the three NYC sites and two NJ sites were compared to determine total mean metal concentrations in shells and soft body tissue (Fig. 16). Hastings and Soundview oysters exhibited the highest mean total soft tissue metal concentrations (25,000 PPM or greater), followed by Gowanus (~15,000 PPM), Hackensack (~10,000 PPM) and Keyport (<5,000 PPM) specimens. Shell mean metal concentrations exhibited less variation, ranging from a low of ~3,300 PPM (Hackensack) to a high of ~5,500 PPM (Hastings). However, based on the research to date it is not possible to know if/how these metals might be affecting the oysters' overall health or reproductive abilities.


A. 'Nutrient' metal concentrations (ppb)
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
Gowanus
Gowanus
Gowanus
Hastings N
Hastings N
Hastings N
Soundview N
Soundview N
Soundview N
Soundview R
Soundview R
Soundview R
Tot_Body
Tot_Shell


B. Potentially toxic heavy metal concentrations (ppb)
3000000
2500000
2000000
1500000
1000000
500000
0
Gowanus
Gowanus
Gowanus
Hastings N
Hastings N
Hastings N
Soundview N
Soundview N
Soundview N
Soundview R
Soundview R
Soundview R
Tot_Body
Tot_Shell

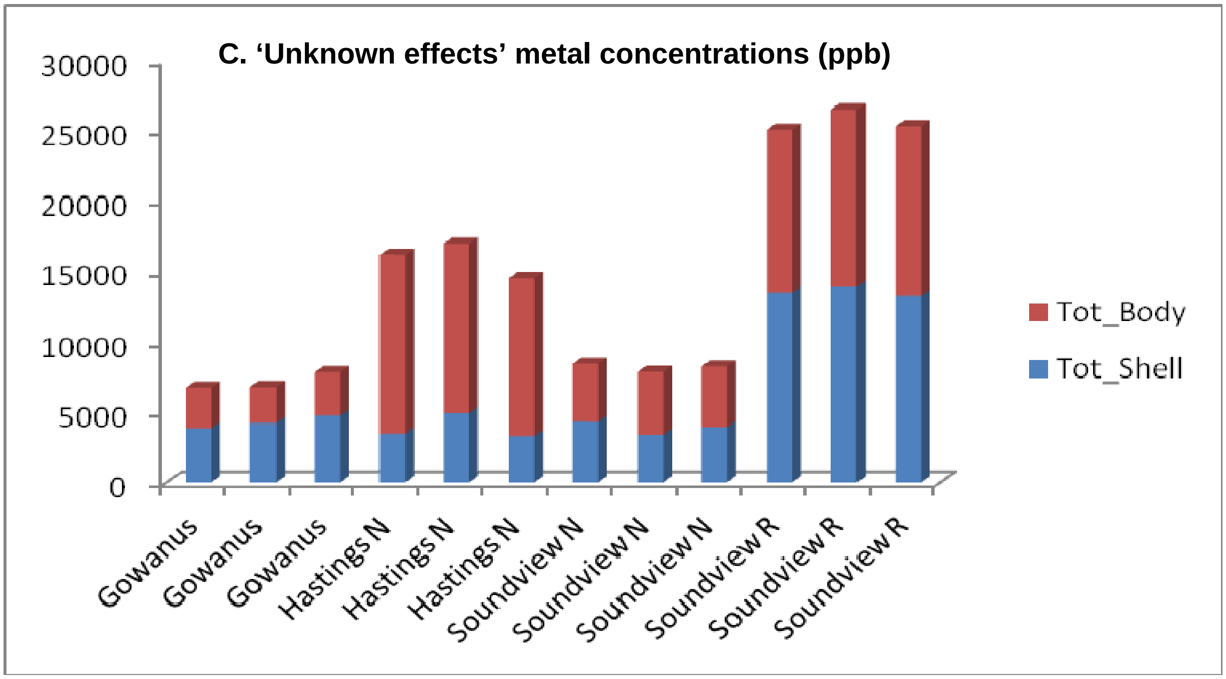


Figure 15. Total PPB metal concentrations of: a) seven 'Nutrient' metals (Cu, Fe, K, Mg, Mn, Se, Z); b) nine potentially toxic Heavy Metals (Ag, Al, As, Cd, Cr, Ni, Pb, Sr, Ti); and c) eight metals of unknown effect (Bi, Co, Cs, Ga, Li, U, V) observed in three replicate samples from the Gowanus Canal, Hastings-on-Hudson and Soundview research sites. Note order of magnitude differences in Y axis concentrations.

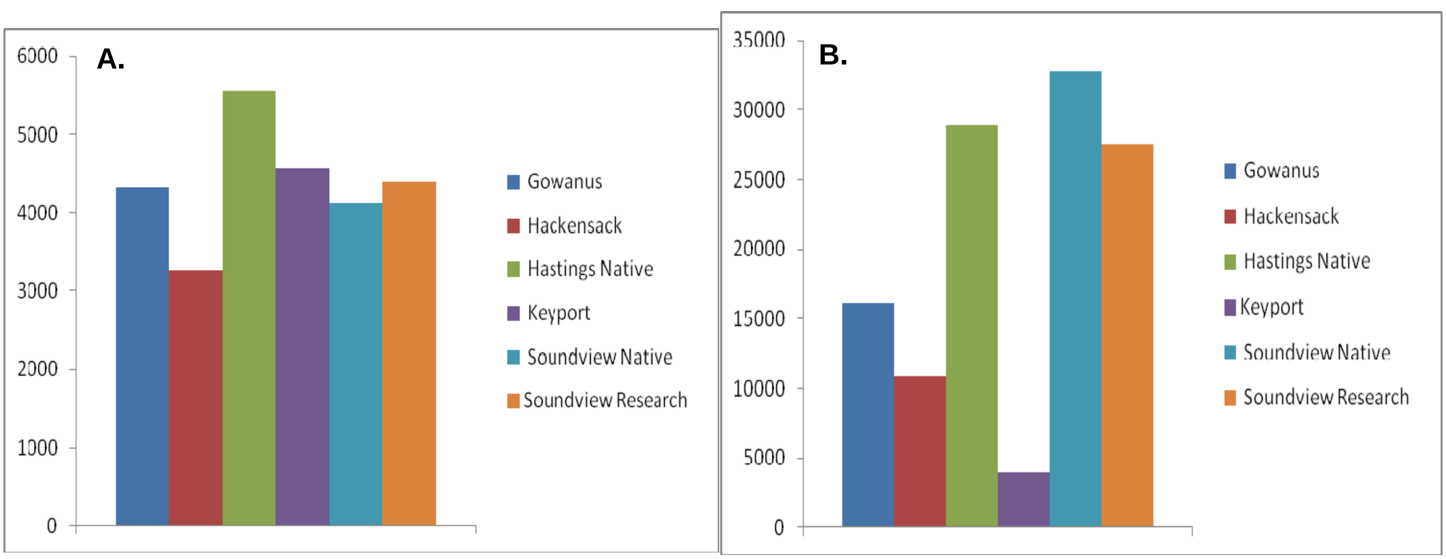


Figure 16. Mean total metal concentrations (PPM) in: a) shell and b) soft body tissues observed in oysters sampled from two NJ (Keyport, Hackensack) and three NY (Gowanus Hastings, Soundview) locations. Note the order of magnitude difference in the Y axes.

Table 9. Metal concentrations (ppb) in Eastern Oyster shell and body tissue from NYC restoration test locations. N= Native population; R= Research seed oysters.

Location	Nutrients		Heavy Metals		Non-Essential?	
	Tot_Shell	Tot_Body	Tot_Shell	Tot_Body	Tot_Shell	Tot_Body
Gowanus R	2709686	19210204	1413807	192345	3834	2898
Gowanus R	2747868	15382692	1430592	127874	4191	2580
Gowanus R	3235529	13217083	1415958	123559	4706	3089
Hastings N	3835293	30140703	1913771	595120	3457	12711
Hastings N	3858218	22859817	1929148	537715	4873	12075
Hastings N	3270037	32328737	1855338	418786	3297	11172
Soundview N	3060209	2868468	1334382	1395292	4265	4136
Soundview N	2532601	3058341	1231823	1389679	3383	4434
Soundview N	2940633	3101717	1294250	1368302	3904	4299
Soundview R	28694547	27265881	563336	264249	13481	11556
Soundview R	37728356	27289995	253183	335771	13858	12617
Soundview R	30648914	27146144	342029	419676	13261	12022

NYC Oyster Histopathology (2013)

Oysters sampled from three Baykeeper oyster gardening sites at the mouth of the Arthur Kill (Tottenville) and Richmond County Yacht Club (Staten Island), and Canarsie, Brooklyn (Fig. 17) were analyzed to determine the health of gardened oyster at these locations. Measurements included oyster length, width, height, total weight, body weight, shell weight, shell thickness, number of lesions observed on the whole animal and on the cellular level, and growth rates. Using this numerical data, as well as visual observations of the individual oysters sampled, we concluded that oysters placed at the Canarsie, Brooklyn site appeared to be the healthiest. An exact comparison could not be made between the Richmond County oysters and those from the two other sites due to age differences (two years old versus one year old adult oysters, respectively).

Figure 17. Three Baykeeper New York City oyster gardening sites and Soundview reintroduction site.

The oyster gardening sites at Tottenville , Richmond County Yacht Club, and Canarsie were sampled on 6/27, 6/28, and 7/2, respectively. Fourteen live specimens were collected from the Tottenville and Canarsie sites and fifteen specimens were collected from the Richmond County Yacht club, of which five were kept alive in the lab for other experimental purposes. In total, 38 oysters were processed. Each oyster was cleaned of barnacles, mud, and other fouling before being weighed. Specimen length (from hinge to lip), width (ventrally/dorsally across the greatest distance), and height (across the two valves) were measured. Each oyster was then shucked, retaining as much fluid as possible, and the soft tissue and shell weighed separately. Each organism was transected on a slight diagonal from posterior to anterior and the two halves preserved in formalin. Shells were measured for thickness and any abnormalities of the shell or soft tissues were noted. After at least 48 hours soft tissues were transferred to 70% ethanol and sliced for preparing histopathology slides. Special attention was paid to include sections of the gills and mantle edge; other tissues of interest included kidneys, digestive organs, gonads, and the heart.

Shell thickness was calculated using Frazier's (1976) formula for shell surface area (SA):

$SA=(2.5h)^{1.56}$ where h= shell height (hinge to lip)

Surface area was then used to calculate the shell thickness in grams per square centimeter by dividing shell weight by surface area.

Oyster growth rates were calculated by dividing change in shell length by time in days. Change in shell length was determined by subtracting the average shell length of the seed oysters as reported on the Baykeeper website (3/4", or 19.05 mm) from the shell length of the sampled oysters. The time in days was the number of days since the seed oysters were released to the gardeners until the day of sample collection. Differences between oysters collected from the three sites are shown in the following graphs (Figs. 18-26). Morphological measurements (total weight, shell weight, body weight, and width), exhibited a significant difference between oysters collected from Richmond County and the other sites because Richmond County oysters were a year older than the Tottenville and Canarsie oysters. Length and shell thickness of the Tottenville oysters were significantly shorter and thicker, respectively, than the Canarsie oysters. The number of lesions on each oyster varied within each site, but the Richmond County oysters had more lesions than the Canarsie oysters ($p < .05$). Growth rates of the Canarsie oysters were significantly higher ($p < .005$) than at the other two sites.

Figure 18. Oyster shell length (hinge to lip) from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.

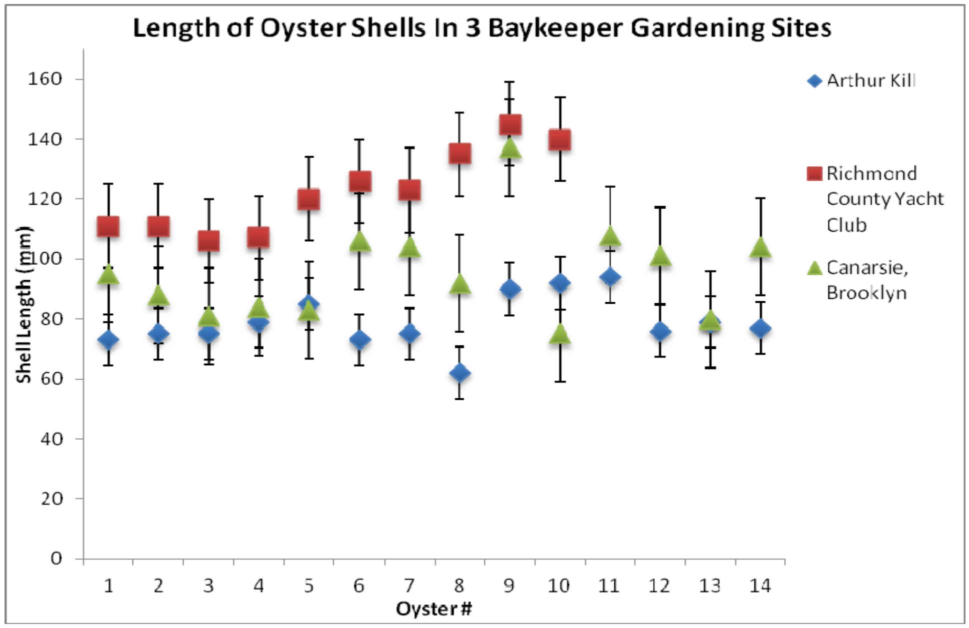


Figure 19. Oyster shell width (laterally across one valve) from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.

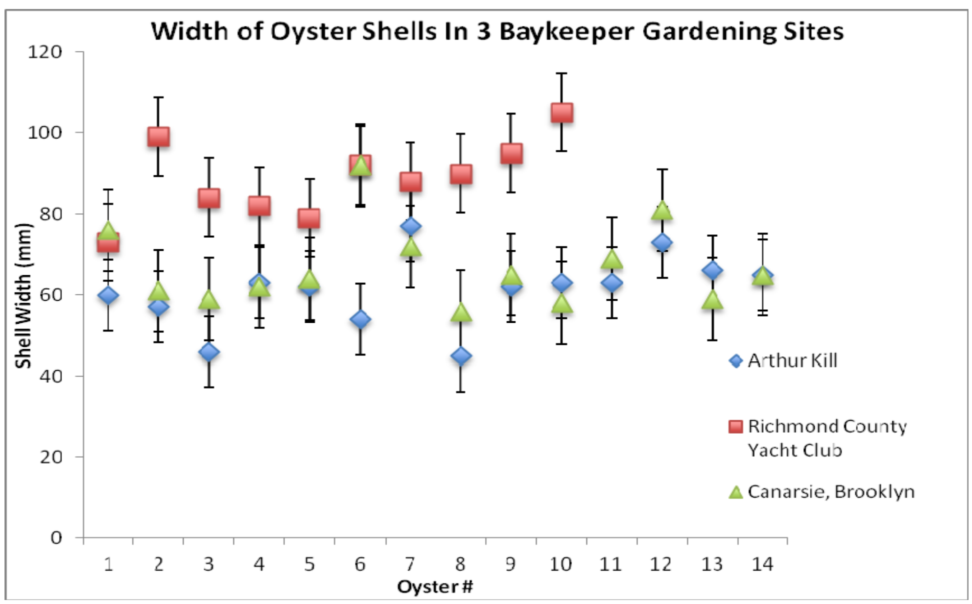


Figure 20. Oyster shell height (across both valves) from Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.

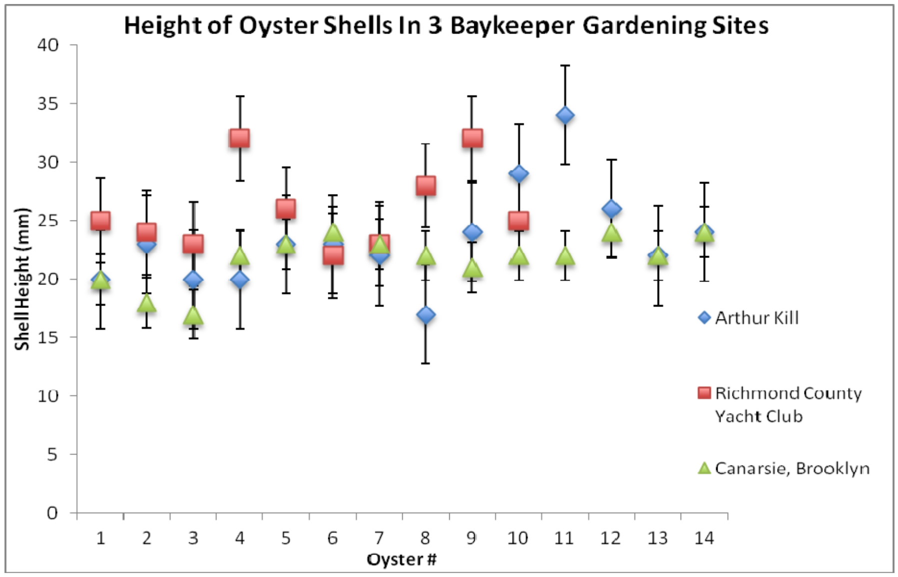


Figure 21. Total weight of oysters from Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.

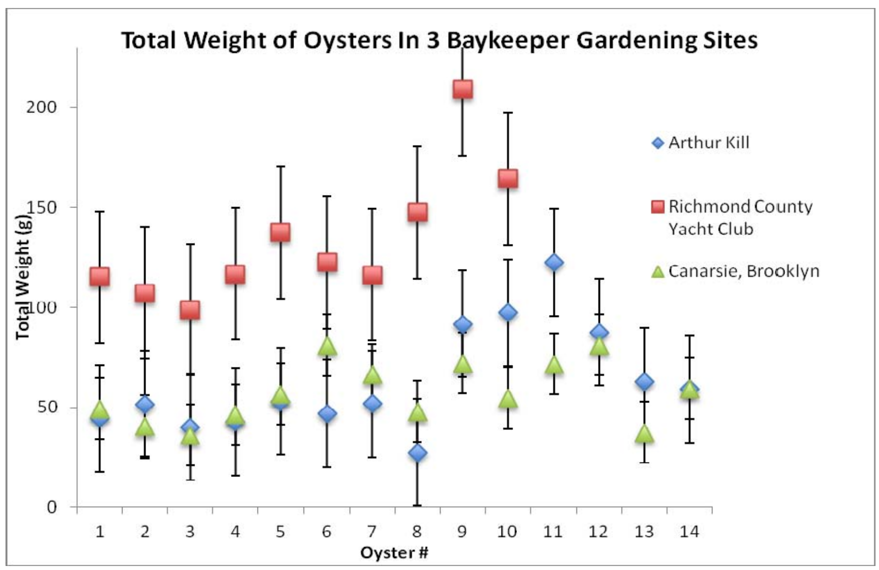


Figure 22. Soft tissue weight of oysters from Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.

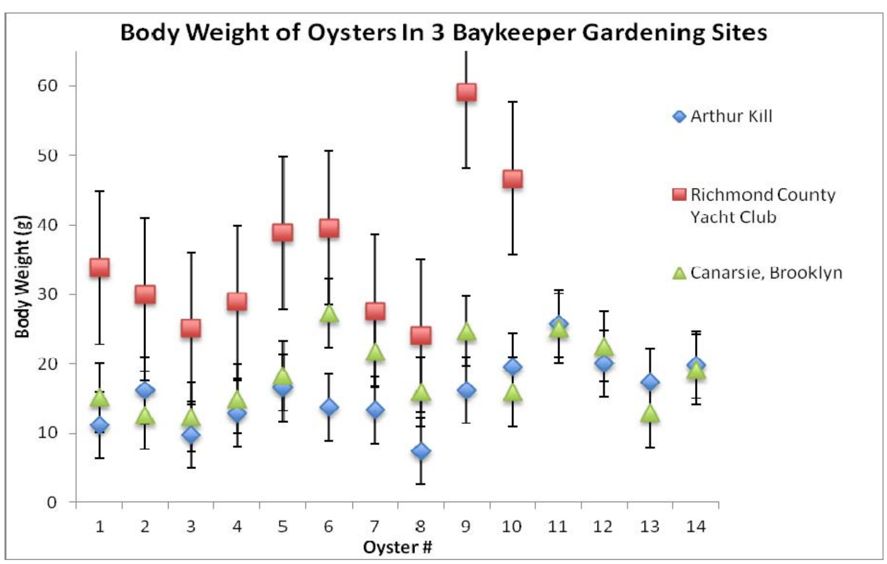


Figure 23. Shell weight of oysters from Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.

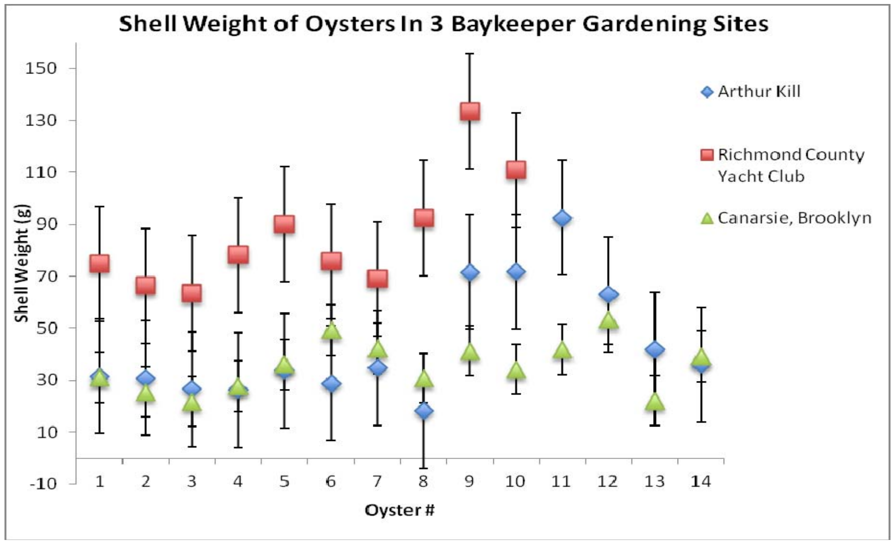


Figure 24. Shell thickness of oysters from Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.

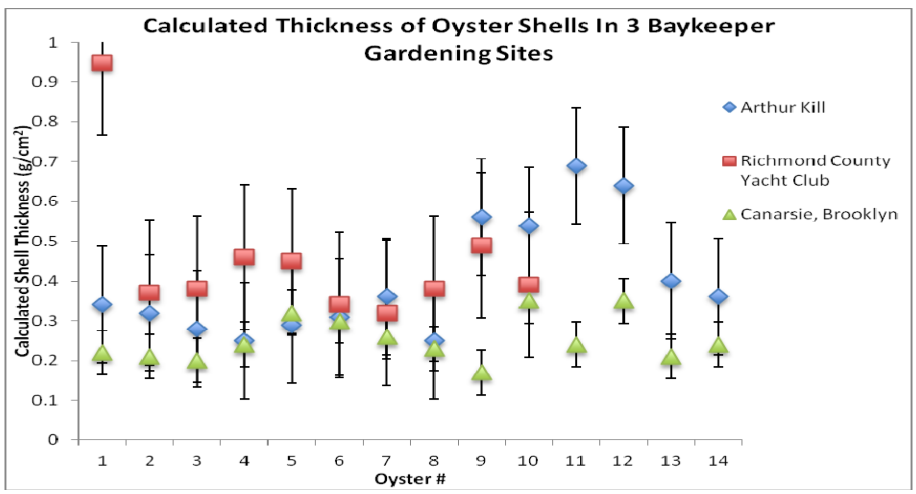
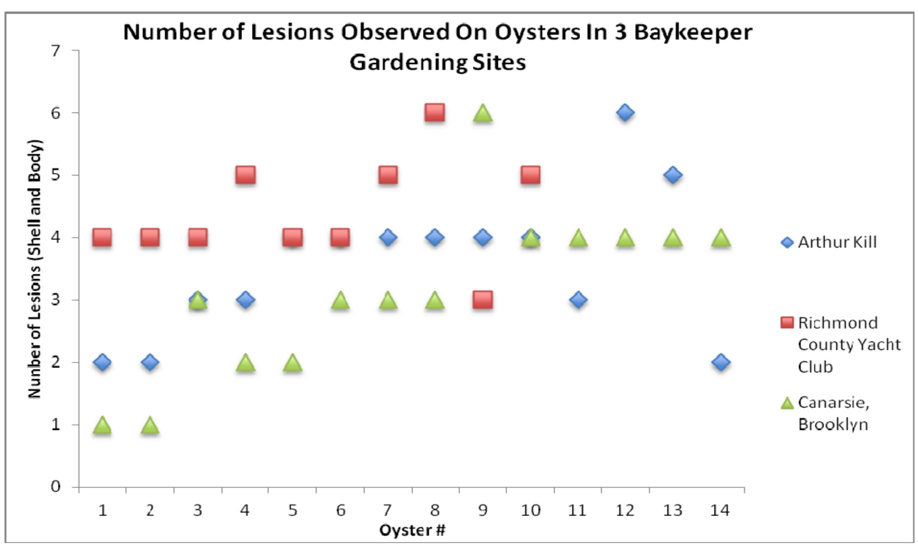


Figure 25. Shell and soft tissue lesions observed in oysters from Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.



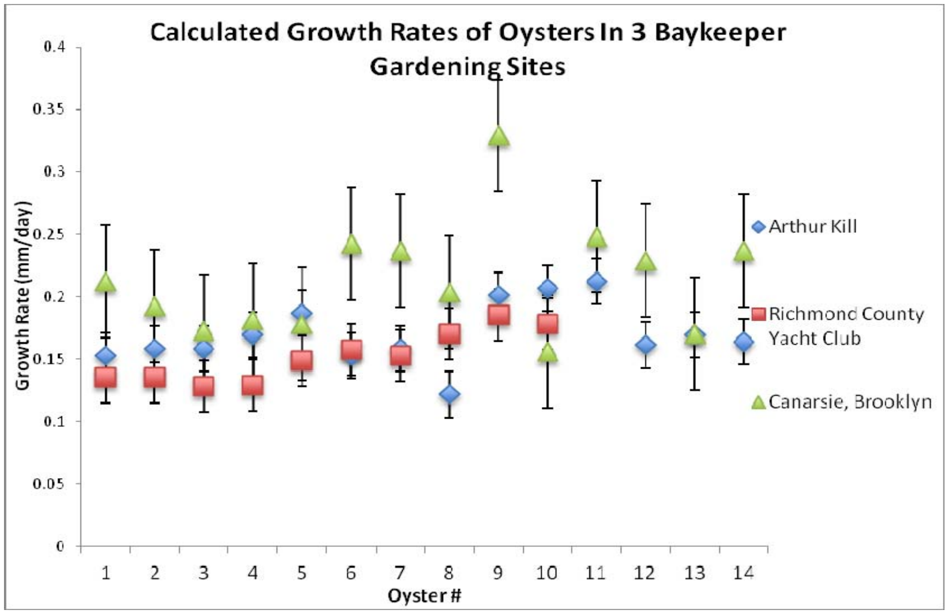


Figure 26. Growth rates of oysters from Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Error bars are ± 1 standard deviation.

The number of lesions observed in soft tissues and shells are shown in Figure 27 and Table 10. A high diversity in the type of body lesions was observed in oysters from Canarsie and a high occurrence of a pale mantle lining was seen in Richmond County oysters. There was also a high incidence of chalky white deposits and yellowing in shell lesions in specimens from all sites. Canarsie oysters had the lowest percentage (50%) of yellow shell discolorations, while all of the Arthur Kill and Richmond County oysters exhibited yellowing. Approximately 90% of the Richmond County specimens exhibited mud blisters compared to 20% to 30%of specimens from the other sites.

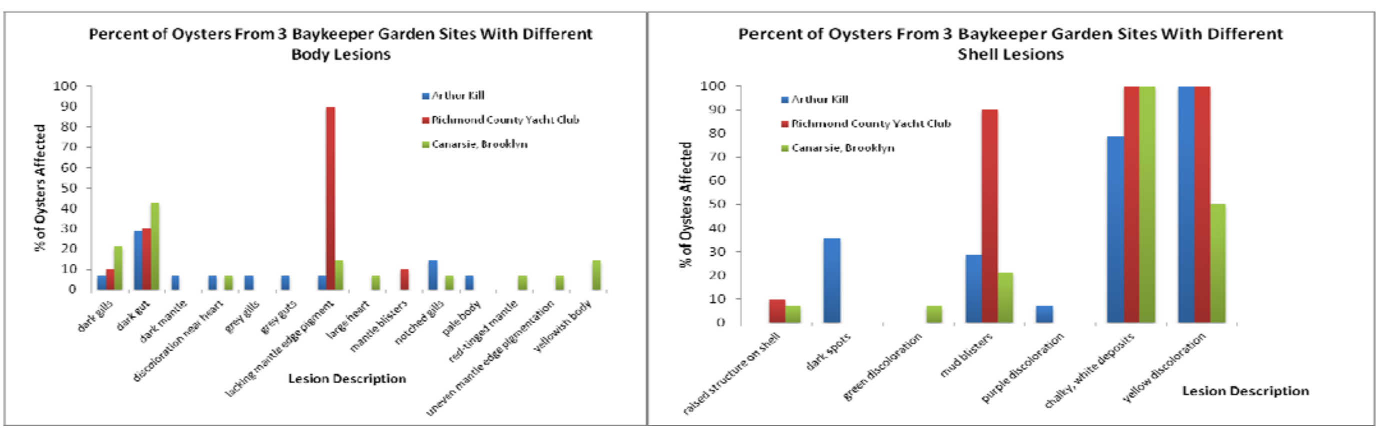


Figure 27. Percentage of oysters from three Baykeeper gardening sites with: a) body and b) shell lesions. Richmond County oysters were two years old; oysters from the other sites were one year old.

Comparisons were made to assess the relationship between shell morphological factors (length, width, height) and oyster weight. As an indicator of overall shell size irrespective of age, the length of the shell was multiplied by height and width; shell weight, body weight, and shell thickness were then compared with the shell size (Figs. 28-33). The number of lesions was also compared with shell size (Fig. 34). Shell weight and body weight exhibited a strong correlation with shell size (R^2 values of .821 and .835, respectively), but shell thickness and the number of lesions did not show a significant correlation with shell size (Fig. 35).

Figure 28. Shell weight versus overall shell size of oysters from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old.

Figure 29. Regression analysis of shell weight versus overall shell size of oysters from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Sample size = 38 oysters.

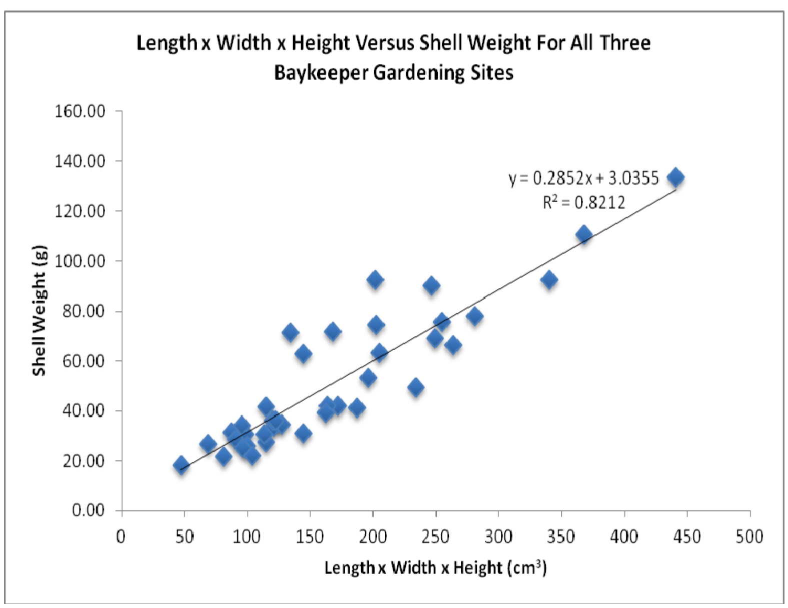


Figure 30. Body weight versus overall shell size of oysters from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old.

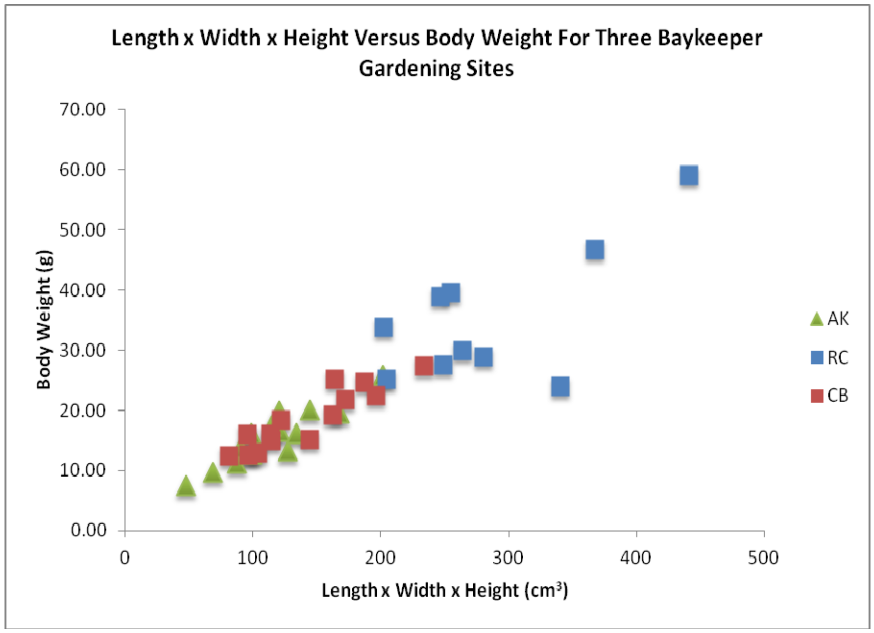


Figure 31. Regression analysis of body weight versus overall shell size of oysters from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Sample size = 38 oysters.

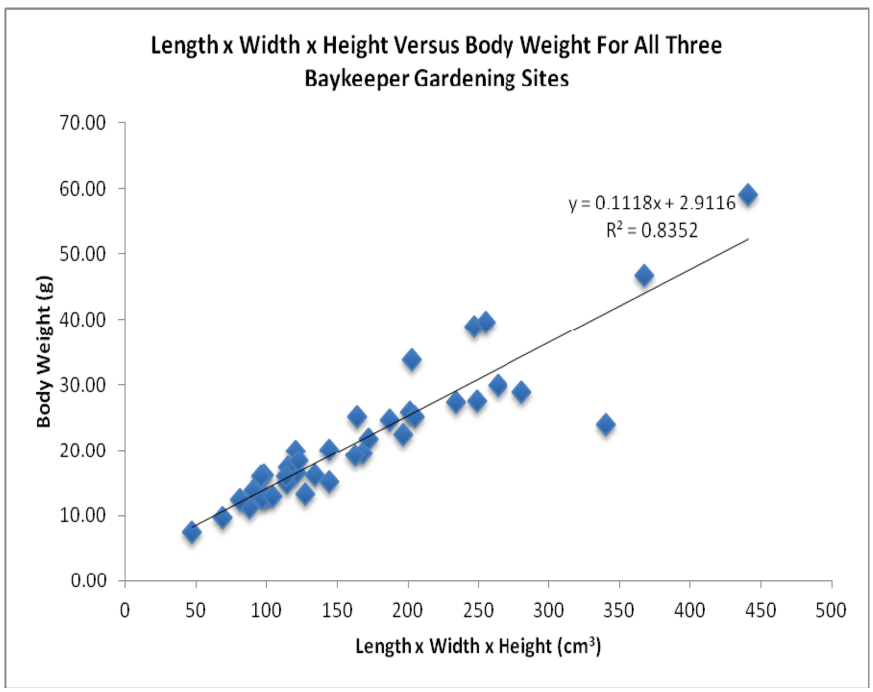


Figure 32. Shell thickness versus overall shell size of oysters from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old.

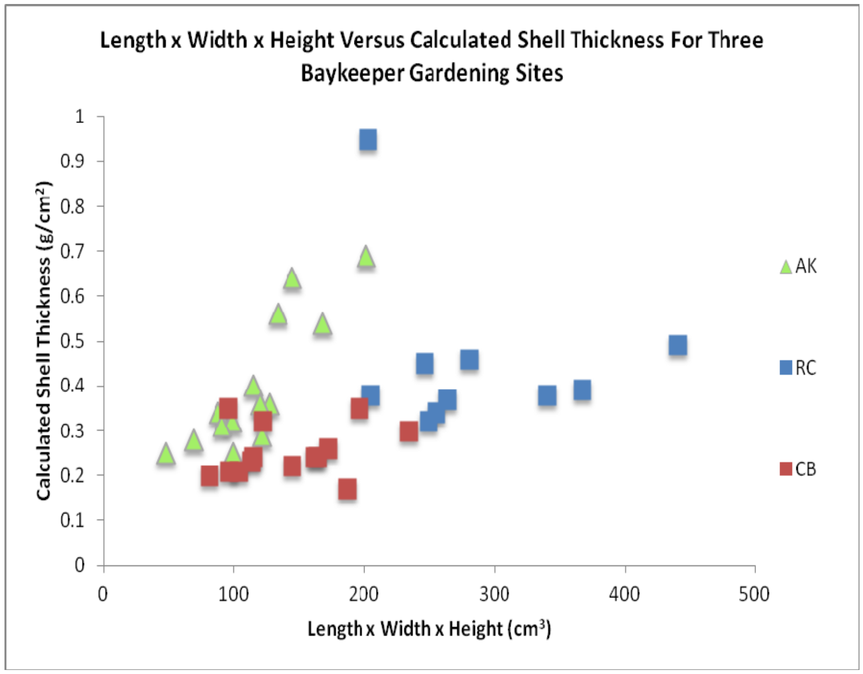


Figure 33. Regression analysis of shell thickness versus overall shell size of oysters from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Sample size = 38 oysters.

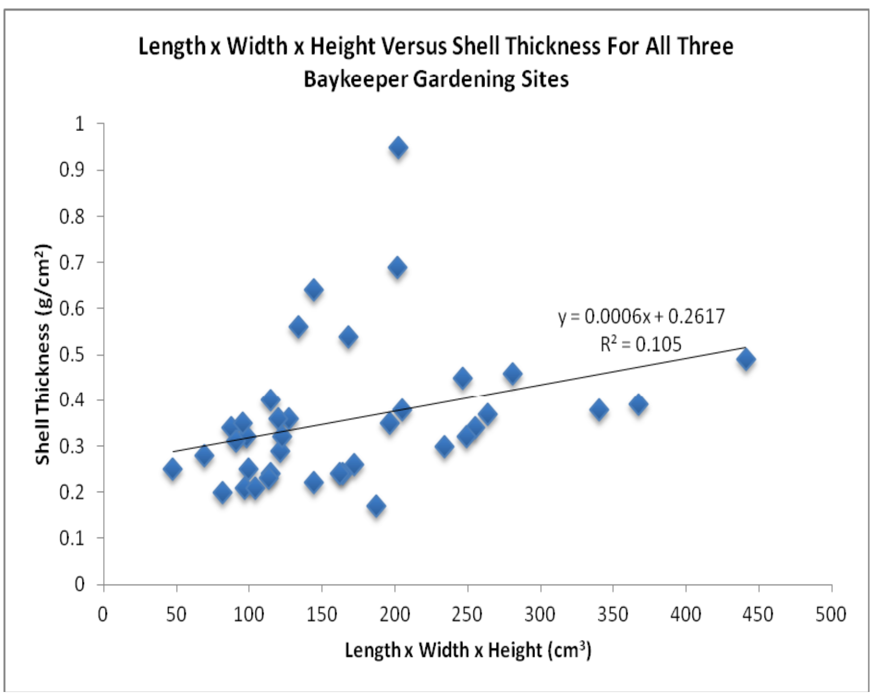


Figure 34. Shell lesions versus overall shell size of oysters from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old.

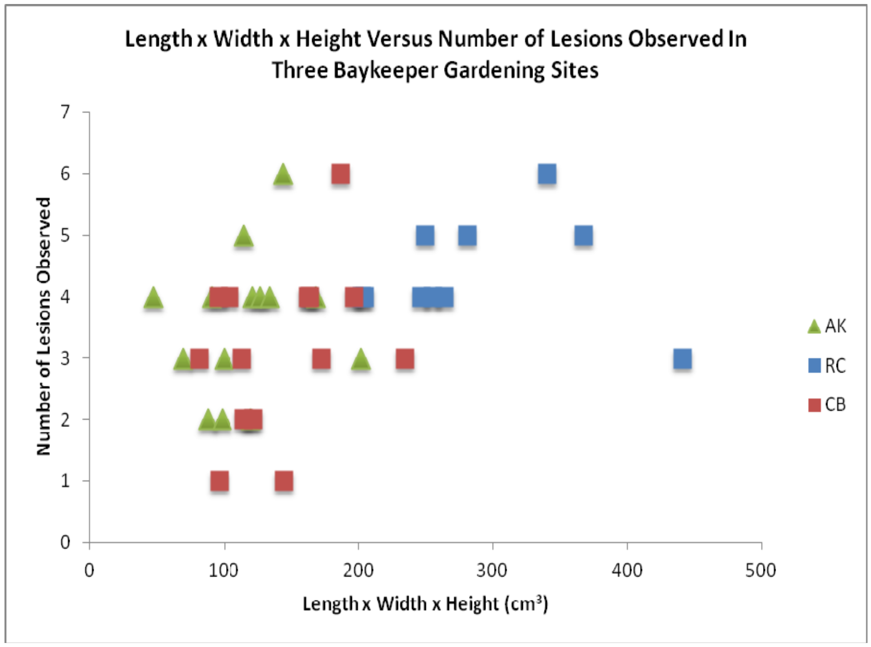


Figure 35. Regression analysis of shell lesions versus overall shell size of oysters from three Baykeeper gardening sites. Richmond County oysters were two years old; oysters from the other sites were one year old. Sample size = 38 oysters.

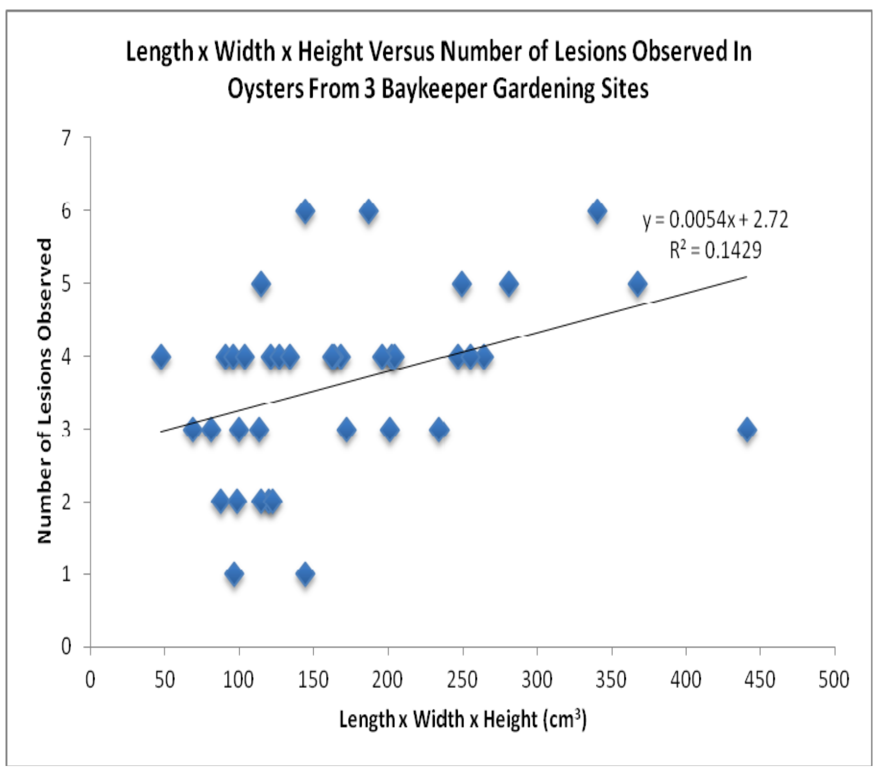


Table 10: Summary of oyster histopathology from Baykeeper gardening sites.

Site	Tissue	NSL	Abnormal	NP	Female	Male	% Female
Tottenville	**Epithelium/Mantel**	5	8	1			
(Arthur Kill)	**Connective**	7	7	-			
N = 14	**Heart**	4	2	8			
	Gastrointestinal	10	1	3			
	Labial Palps	4	4	6			
	Digestive	11	3	-			
	Kidney	3	2	9			
	Gills	6	7	1			
	Gonads	13	1	-	6	8	43%
Richmond County	**Epithelium/Mantel**	2	7	1			
N = 10	**Connective**	2	7	1			
	Heart	6	1	3			
	Gastrointestinal	6	2	2			
	Labial Palps	-	-	10			
	Digestive	7	2	1			
	Kidney	2	3	8			
	Gills	4	5	1			
	Gonads	4	6	-	5	4	50%
Canarsie	**Epithelium/Mantel**	10	3	-			
N = 14	**Connective**	11	1	2			
	Heart	12	-	2			
	Gastrointestinal	9	-	5			
	Labial Palps	4	-	10			
	Digestive	14	-	-			
	Kidney	5	-	9			
	Gills	11	-	3			
	Gonads	14	-	-	8	6	57%

NSL = No Significant Lesions; Abnormal = Lesions present; NP = Organ not present on the slide.

Oysters sampled from Canarsie exhibited the lowest number of lesions; Richmond County oysters exhibited the highest number of lesions per specimen (Fig. 36 and Table 10). Percentage of females ranged from 43% to 57% at Tottenville and Canarsie, respectively. The main abnormality exhibited by the Canarsie oysters was their significantly thinner shells.

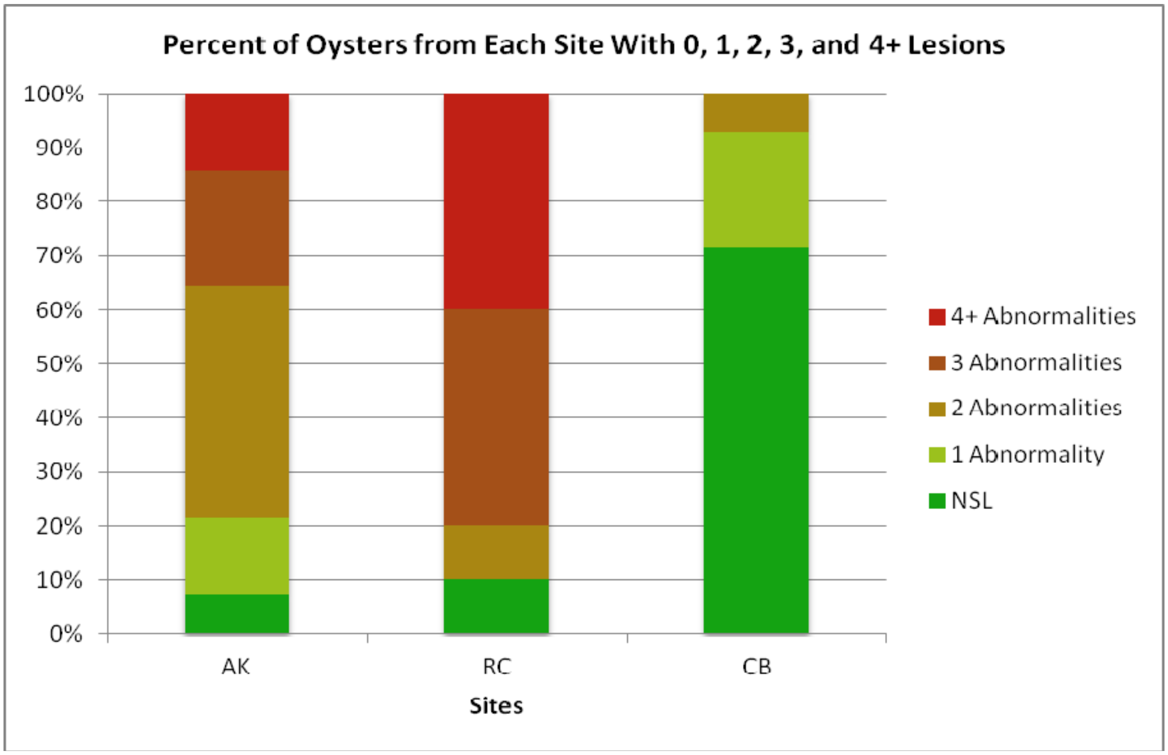


Figure 36. Proportion of oyster that exhibited no significant lesions (NSL), 1 – 4 lesions. Richmond County oysters were two years old; oysters from the other sites were one year old. Sample size = 38 oysters. AK = Arthur Kill/Tottenville (N = 14); RC = Richmond County Yacht Club (N = 10); CB = Canarsie, Brooklyn (N = 14).

The physical measurements indicate that shell morphological measurements are not indicative of tissue or reproductive health. It is not possible to comment on whether the Arthur Kill oysters exhibited more lesions because they were *in situ* for a year longer than oysters from the other sites. We do note that SuperStorm Sandy caused extensive damage to the Richmond County Yacht Club and extensive sediment dredging was required during the rebuilding process, which could have exposed oysters at this location to resuspended sediments. The Richmond County Yacht Club also docks a large number of boats with motors, which could result in exposure of the oysters to associated pollution. Concentrations of hydrocarbons, metals, solvents, and surfactants are higher in marinas due to normal boat activity, and it is certainly possible that these pollutants could have had an effect on the oysters' growth and development (http://www.epa.gov/owow/nps/mmsp/index.html).

The data suggest that some physical characteristics, such as shell weight and body weight are correlated with shell size. However, it also appears that ***overall oyster size is not highly correlated with the health or fitness of the oyster***, and so evaluating tissue histopathology is a necessary tool in assessing the suitability of a reintroduction site with respect to long-term oyster population sustainability.

RUTGERS-NY/NJ BAYKEEPER 'CITIZEN-SCIENTIST' SITE SELECTION MODEL

Implementing an oyster restoration project is expensive, with costs ranging from $200,000 to $2,000,000 per acre. USACE restorations cost billions and NOAA places the cost at $1.5 million per mile of constructed oyster reef[1]. From a management point of view the question becomes where should a reintroduction(s) be located in order to achieve long-term sustainability and maximize costly reintroduction efforts? In an estuary lacking a significant oyster population, where there is a high uncertainty of reintroduction success, relying solely on management methods used to maintain an existing fishery is problematic.

Although overall water quality in the HRE is improving, the presence of historic environmental contaminants can have site-specific affects on successful reintroduction of Eastern Oysters. HRE environmental constraints can diminish successful restoration

[1] http://www.habitat.noaa.gov/pdf/tnc_oyster_economics_factsheet.pdf

outcomes and it is important to test proposed oyster reintroduction sites using low-cost screening techniques prior to implementation of large-scale and expensive restoration activities. We propose the ***Rutgers-Baykeeper Site Selection Model*** for pre-testing of sites (Fig. 37) to identify locations with a higher likelihood of oyster survival and reproductive fitness. This model could increase the possibility of long-term restoration success, while lowering restoration costs. The first step in evaluating a proposed HRE restoration site is to place juvenile oysters in locations where physio-chemical parameters are within a range that supports oyster survival to determine over-winter survivorship rates. This initial screening can be done easily and cheaply by utilizing 'citizen scientist' volunteers. If over-winter survivorship meets pre-set targets at a given location, histopathology analyses of year old oysters should be conducted to determine the animals' overall health and reproductive fitness. Based on the results of this rapid and inexpensive screening, expanded restoration activities can commence at HRE locations where survival rates are high and oyster health appears normal. Conversely, at locations where survivorship is low and/or oysters exhibit abnormal histopathology, restoration activities should be re-directed to another location, and research to identify the source(s) of environmental stressors that would inhibit successful oyster reintroduction should be conducted.

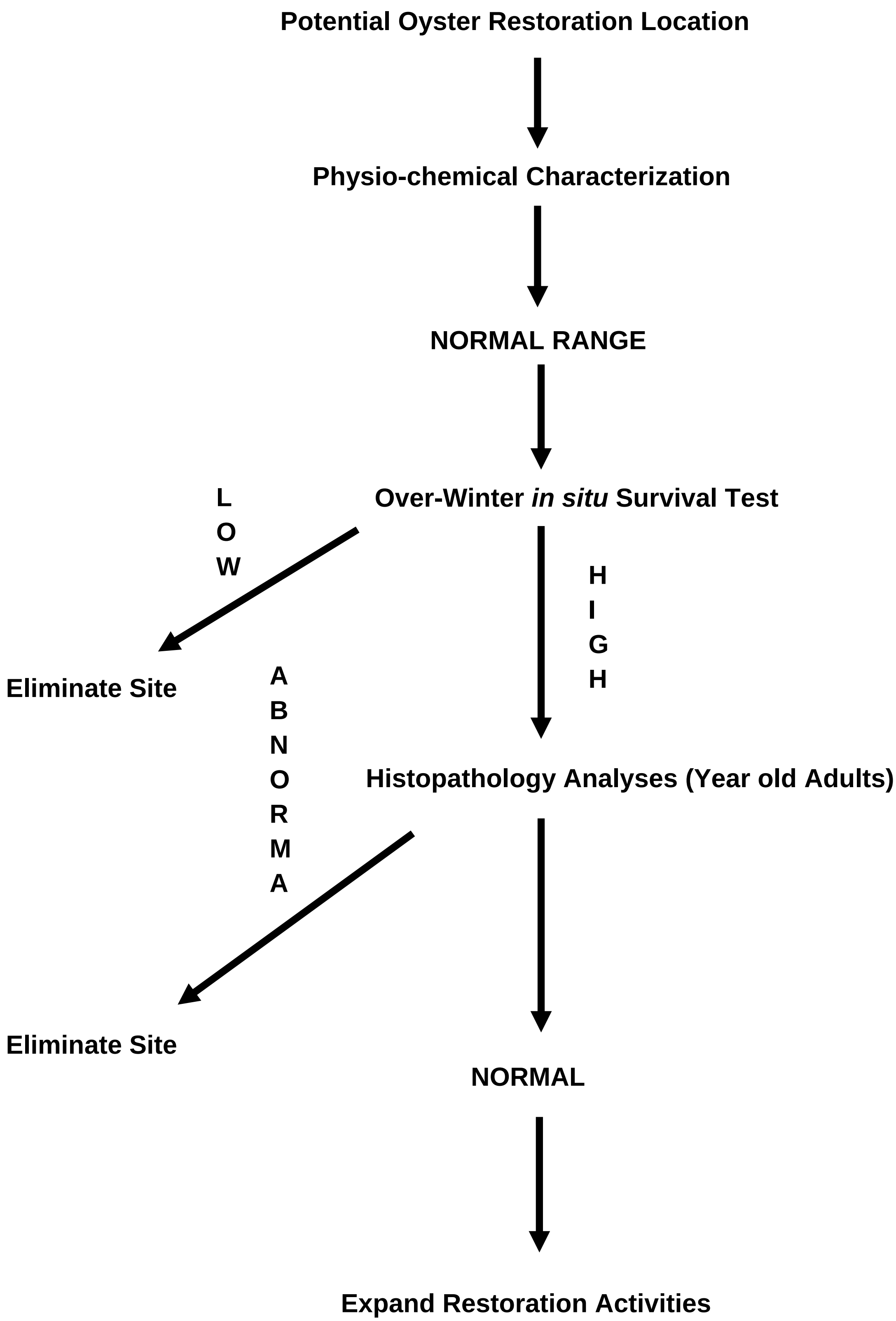


Figure 37. Schematic representation of the proposed Rutgers-Baykeeper Site Screening Model to identify HRE sites with high potential for long-term oyster restoration success.

V. RARITAN BAY HABITAT MAPPING PROJECT

In an effort to further refine the CRP (2009) map of potential oyster reintroduction sites, during the summers of 2011 and 2012, NY/NJ Baykeeper Oyster Program Staff and Rutgers University scientists lead groups of 'citizen-scientist' volunteers in an effort to survey the NJ Raritan Bayshore (2011) and the southern shore of Staten Island, NY (2012). The goal of the Mapping Project was to document environmental parameters that could serve as positive or negative indicators when evaluating the potential of a specific location to support reintroduction of the Eastern Oyster into Raritan Bay by fine-tuning the oyster restoration maps (Fig. 1) included in the CRP (2009). These original maps were developed based on only four (4) criteria known to be significant in the survival of spawned oyster eggs and larvae (salinity, dissolved oxygen, turbidity, bathymetry).

Sampling sites were located approximately 100 meters apart, beginning at Keyport Harbor municipal dock and extending eastward to the northern tip of Sandy Hook and westward along the southern shoreline of Staten Island. Twenty-three (23) habitat parameters (Table 11) were selected and evaluated at sampling locations covering approximately 30 miles along NJ's and NY's Raritan Bayshore (Fig. 37). At each sampling location water column and substrate data were collected approximately 12" below the water surface in 3 ft. of water during a time period between two hours before to two hours after mean low tide. Shoreline observations were made at the location which was at a direct right angle to the water column sampling point and digital photos were taken of all shoreline data collection points. The collected data were uploaded and stored in hand-held Magellen Arc GIS units. The data were downloaded at the Rutgers Center for Remote Sensing and Spatial Analysis (CRSSA), where data analysis and mapping was completed.

Table 11. Environmental factors employed to create the Raritan Bay Oyster Restoration Survey Map.

Factor	Rank	Criteria	Score	Comments
Bulkhead	Negative	Presence	-1	Non-natural
Oyster Drill	Negative	Presence	-1	Potential predator
Armored	Negative	Presence	-2	Potential attractive nuisance
Roadway	Negative	Presence	-3	Runoff potential toxic effects
Soft clam Live	Negative	Presence	-4	Existing species
Substrate Silt	Negative	Presence	-4	Potential to smother oysters
Ulva mats	Negative	Presence	-4	Potential to smother oysters
Water Salinity	Negative	Below 10	-4	Too fresh
Hard clam	Negative	Presence	-5	Existing species
High Energy	Negative	Presence	-5	Unstable conditions for accretion
Substrate Soupy Mud	Negative	Presence	-5	Potential to smother oysters
Dissolved oxygen	Negative	Below 4	-10	Summer conditions
Blue Mussel Live	Positive	Presence	1	Indication of food & water quality
Oyster Dead	Positive	Presence	1	Substrate
Rock	Positive	Presence	1	Substrate
Sand Beach	Positive	Presence	1	Natural shoreline
Gravel	Positive	Presence	2	Potential substrate
Eel Grass	Positive	Present at shore	3	Good water quality
Spartina	Positive	Presence	4	Natural, lower energy
Water Salinity	Positive	Above 10	4	
Dissolved oxygen	Positive	Above 4	5	Summer conditions
Firm Hard Sand	Positive	Presence	5	
Live Oysters	Positive	Presence	5	Supportive conditions for survival
Oyster spat	Positive	Presence	5	Food & Water Quality supportive of oysters
Dock	Neutral	Presence		
Mussel Bed	Neutral	Present at shore		
Phragmites	Neutral			Shoreline
Razor clam Live	Neutral			
Red Seaweed	Neutral			
Riprap	Neutral	Presence		May be positive (substrate) or negative
Shell Hash	Neutral	Presence		
Water Temperature	Neutral			Summer sampling

An Oyster Restoration Index Score (ORIS) based on the environmental data was calculated for each sampling location. The final score was a compilation of the favorable and unfavorable scores (ranked +1 through +5; ranked -1 through -5 and -10 for dissolved oxygen below 4 mg/L, respectively) associated with each attribute sampled. A map was generated using the ORIS (Fig. 38), which shows the overall ranking for each sampling location. A link to an interactive version of the map can be found on at http://cues.rutgers.edu/benthic/index.html. The interactive map allows the user to connect to photographs documenting the shoreline condition and to view all the data collected at any specific location. These maps are a first attempt to eliminate sites that appear to be unsuitable for oyster reintroduction at this time, and to focus attention toward locations that may have a greater chance of supporting long-term oyster restoration success. A report describing this study and the data collected was submitted to the USACE in December, 2013 to aide in their post-Sandy evaluation research.

Using the locations where the overall ORIS was positive, we employed a NOAA bathymetry map to calculate the adjacent subtidal acreage in a depth range of 3 – 10 ft (Figure 39). These maps were created to address the question of whether the goal of 200-acres of reestablished oyster reef is feasible under existing HRE conditions. Using the criteria described, we estimate that over 1,000 subtidal acres could be considered for future oyster restoration evaluation. ***We emphasize that these Maps are a starting point only, and further scientific vetting is required before any of these high scoring locations could be considered as appropriate for long-term oyster reintroduction activities.***

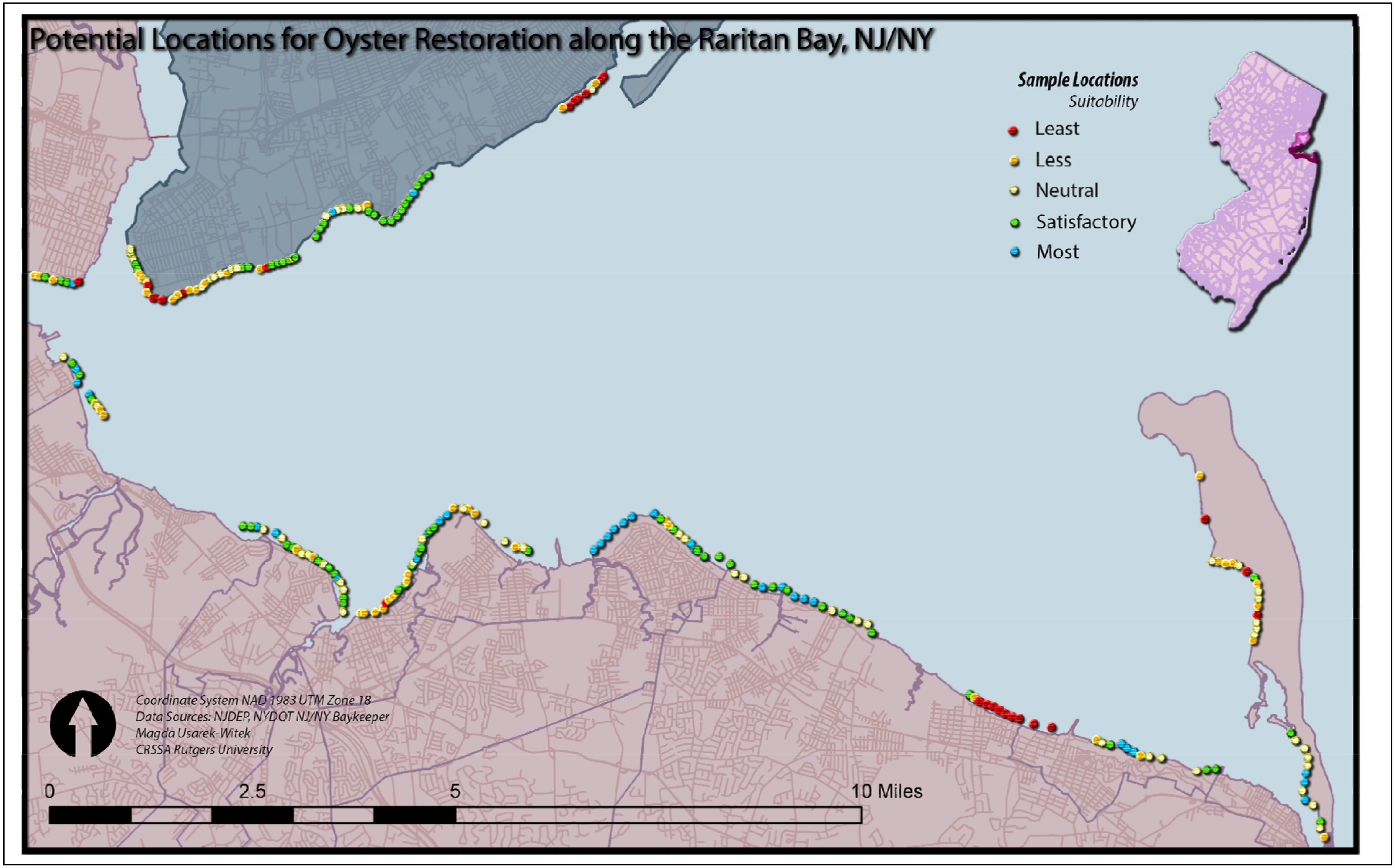


Figure 38. Oyster Restoration Index Score Map (ORIS).

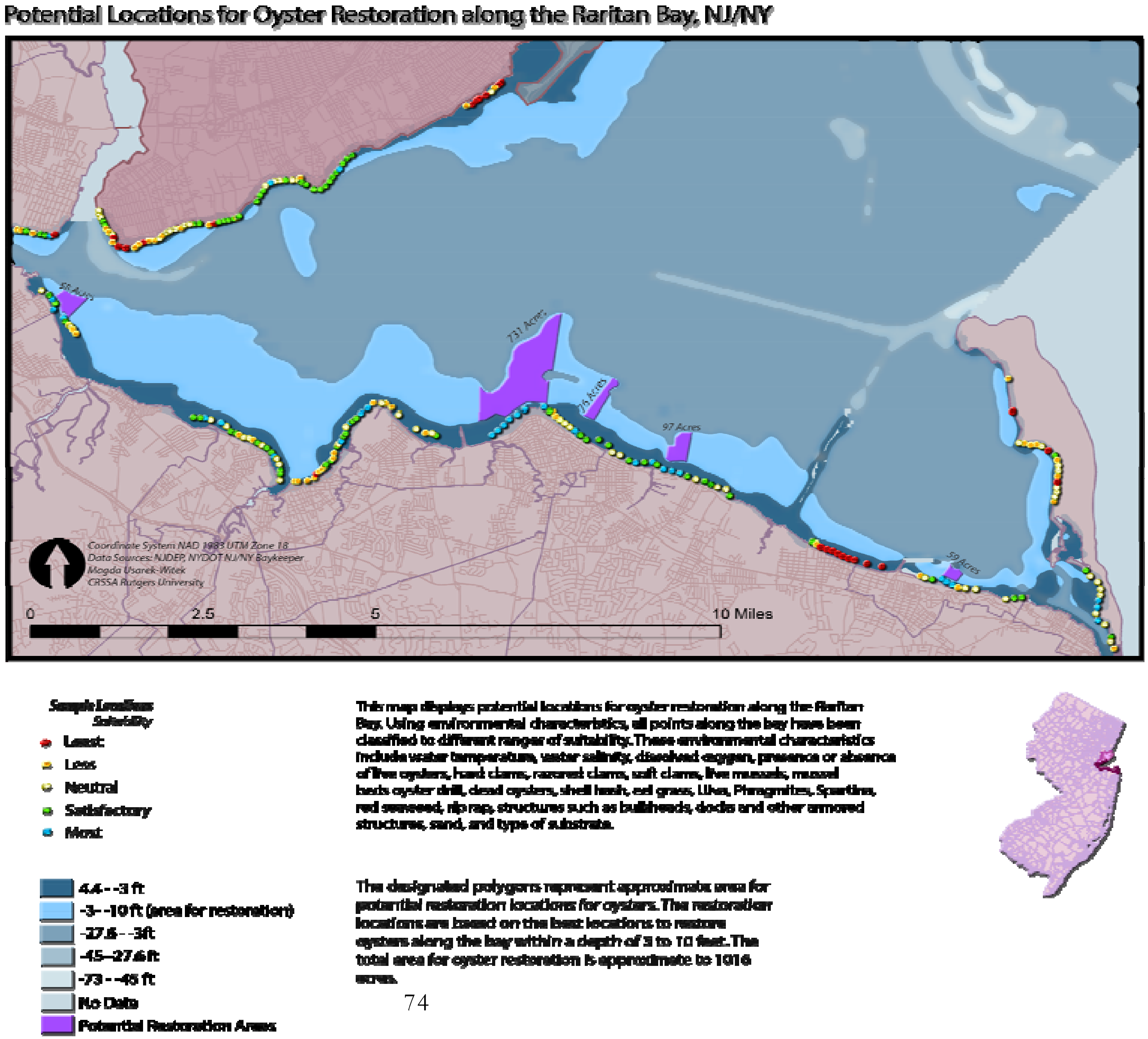


Figure 39. Potential subtidal acreage (3 to 10 ft. depth range) adjacent to high scoring ORIS sampling sites.

VI. NAVAL WEAPONS STATION EARLE

EARLE PROJECT OVERVIEW

As an alternative to the prohibited Keyport oyster restoration site, the NJDEP agreed in September, 2011 to issue the required permits for Baykeeper to resume oyster research within the confines of the Naval Weapons Station Earle (NWSE) pier complex. NWSE waters are patrolled 24/7, and this location offers a unique opportunity to continue HRE oyster reintroduction research under conditions that are protective of human health. This NGO-military model will be the first of its kind in the HRE and offers a way forward to continue to expand beneficial habitat restoration initiatives while addressing health issues considered important by various regulatory agencies. The goal was to replicate Keyport Harbor research in a safe and secure Raritan Bay site which could sustainably support these living marine resources. The NWSE project is an important component of the Baykeeper's long-term HRE benthic habitat/near shore restoration strategy. In order to re-establish Eastern Oyster populations that become self- sustaining natural systems it will be necessary to construct reefs in multiple sites throughout the HRE. The data gained at NWSE will be invaluable in evaluating the potential for successful reintroduction at other locations.

To determine Eastern Oyster over-winter survival at NWSE, 18 lantern nets containing a total of 3,600 animals were attached to six locations on the NWSE working pier. Live juvenile oysters were measured (length, height) prior to their placement in Earle waters on October 1, 2011. Five-tier lantern nets (Fig. 40), each containing 200 juvenile oysters (100 SOS; 100 seed), were placed subtidally and then attached directly to the naval pier at 6 locations (3 on the western Raritan Bay side of the pier and 3 on the eastern Sandy Hook Bay side of the pier). Three lantern nets at each location were anchored to a cinder block resting on the bottom substrate.

We report the results of these research installations, but include the *caveat* that the oysters only spent approximately one year *in situ* at NWSE prior to Hurricane Sandy destroying many of the research cages. The oyster survival rates must be viewed with caution because a large number of oysters were lost due to lantern net damage and loss of the net cages. It appeared some predator(s) was able to damage the lantern nets, and so we were only able to recover 25% of the animals placed at NWSE in September 2011 (902 out of 3,600 original oysters). We hypothesized that the physical loss of 75%of the animals was the result of predation and the lack of protection afforded by the lantern nets. We note that the off-the-shelf lantern nets were selected due to the limited amount of time we had to set up the initial research field samples after the Baykeeper permit application was approved. We recommend that this net not be employed again in the HRE system for research purposes.

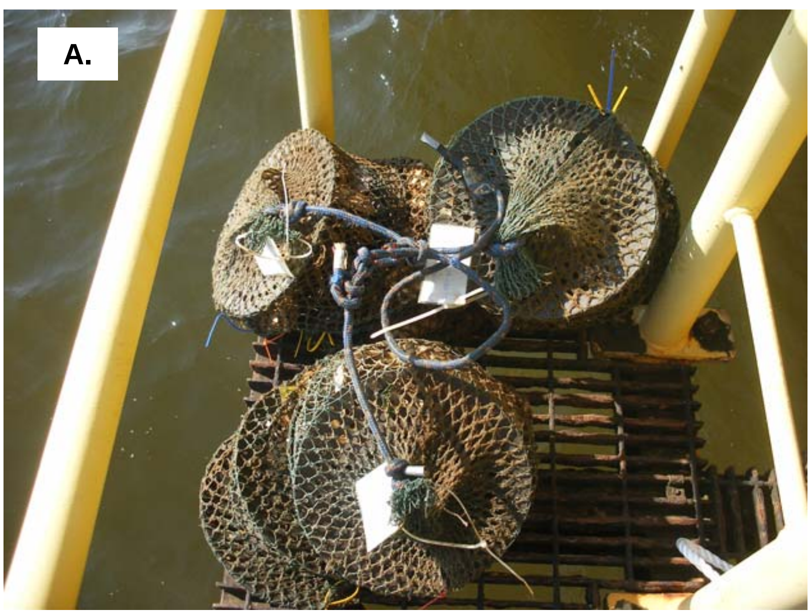


Figure 40. Three nets attached to the Earle naval pier (a); and five-tier lantern net containing 200 Eastern Oysters (b).

Two monitoring events were conducted in June and October, 2012. During both events the numbers and sizes of surviving and dead oysters were recorded. Survival percentages were calculated based on the number of total oysters recovered rather than the number of oysters that were originally placed at NWSE. During the July monitoring event any damaged nets were removed and their oysters consolidated into plastic containers for greater security. Undamaged lantern nets remained in place. Due to the lantern net damage, most oysters did not remain in their original positions on the net shelves.

Over-winter survival for the 675 recovered oysters was very high – 90% were found to be alive. We note that this is not a normal survival rate and may be due to an inaccurate count due to the low sample recovery or the result of an abnormally mild winter (Fig. 41).

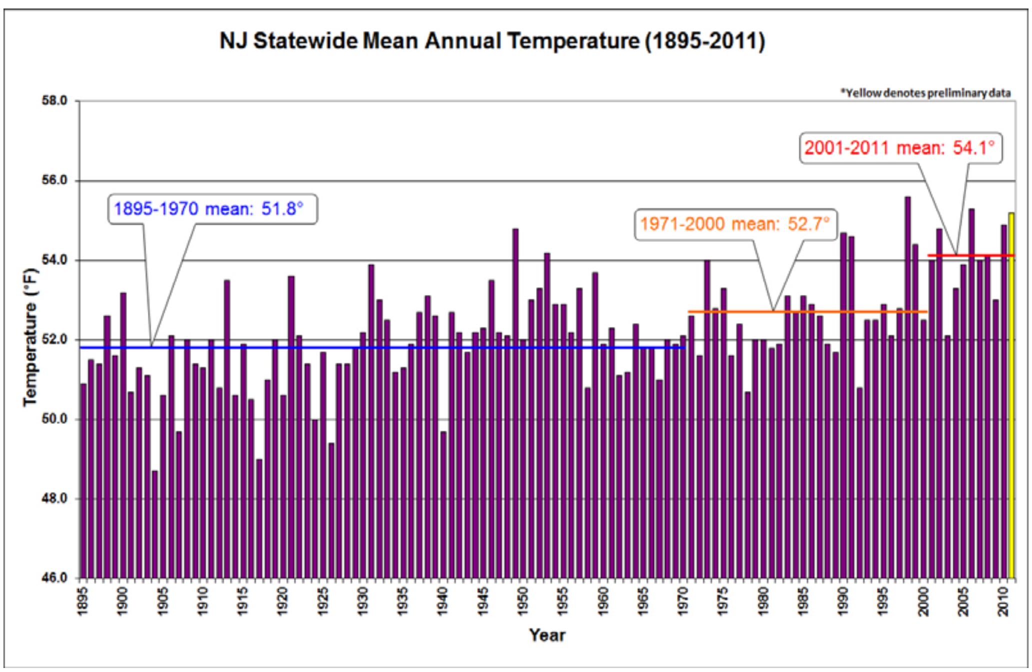


Figure 41. New Jersey 2011 temperatures averaged some of the warmest since 1895. http://climate.rutgers.edu/stateclim_v1/images/nj_temp.jpg

During the July, 2012 monitoring event live oysters were on average 30% longer than dead animals; the lengths of live and dead oysters in October were similar (Fig. 42). The greater length of the live July animals suggests that mortality occurred relatively early after the winter ended, prior to the growth period that occurs when feeding rates increase. Seed oysters were approximately 40% longer than SOS in July and 30% longer in October.

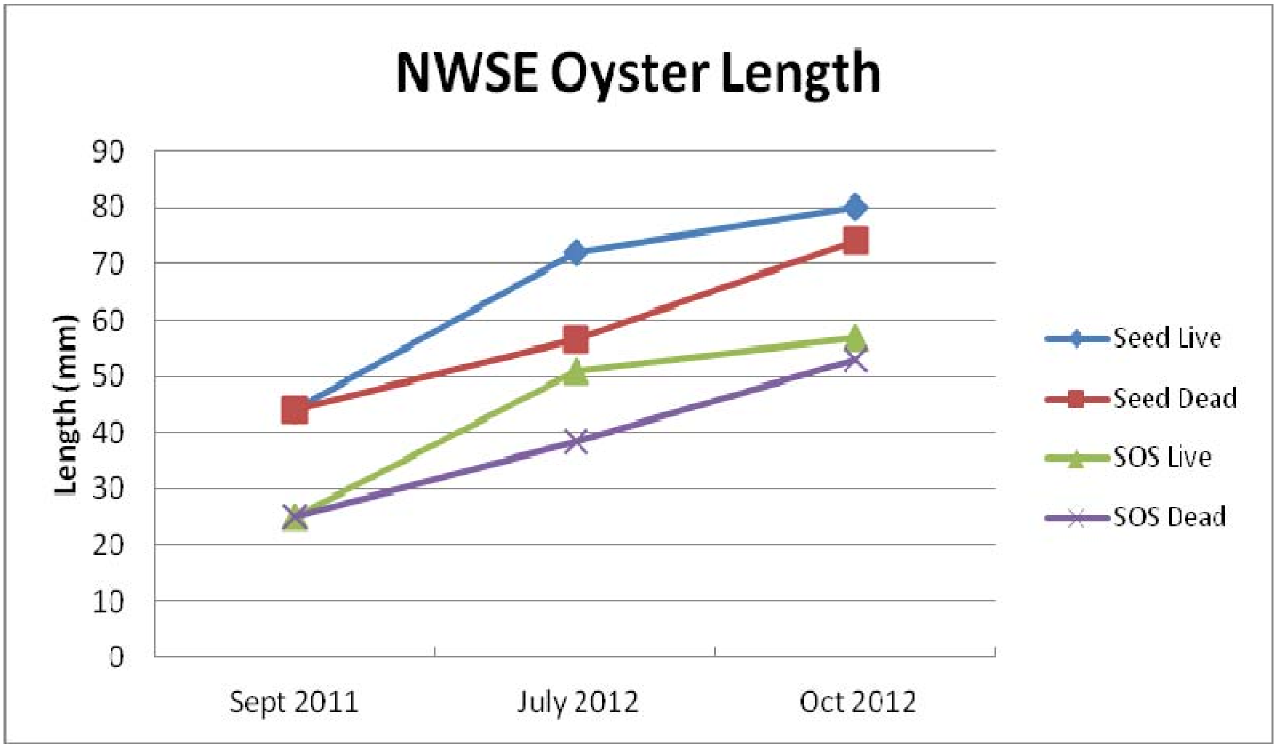


Figure 42. Eastern Oyster mean lengths at time of placement (Sept. 2011), and lengths observed during the July and October, 2012, monitoring events.

During the July monitoring event 50 dead animals were removed from the cages and the 625 live oysters were placed back in the water (Table 12). During the October monitoring event only 373 animals were recovered (60%). Some loss was again due to damage of the lantern nets. We also lost the three lantern nets attached to location #5, Platform R-6, in the middle position on the eastern Sandy Hook Bay side of the pier. The survival rate of recovered oysters was much lower (35%) than that observed in July. This lower rate could have resulted because less fit animals that would normally die off over the winter survived under a milder temperature regime, but then died later in their first year of life. The lower survival rate could also be inaccurate due to the 252 oysters lost (40%). To obtain accurate survival rates at NWSE secure aquaculture cages and longer term monitoring of the oysters housed in those cages is needed. A survey by NWSE personal post-Hurricane Sandy (October 29-30) and post a nor'easter (Nov 7-8, 2012) found that four of the six pier locations no longer had oyster cages attached.

TABLE 12. Recovered Live and Dead NWSE Oysters – 2012

Date	Type	Live	Dead	# Lost
Jul-12	Seed	407	34	1359
Jul-12	SOS	218	16	1566
Oct-12	Seed	51	119	237
Oct-12	SOS	78	125	15

NWSE Biodiversity

Fin fish species found inside the oyster enclosures included juvenile Black Sea Bass, Eel, Cunner, Cling Fish, and Freckled Goby. Invertebrate marine species included Grass Shrimp, Oyster Drills, Spider Crabs, Mud Crabs, Blue Mussels, Barnacles, Red Beard Sponge, Slipper Shells, Blood Worms, Asian Shore Crabs, and Blue Claw Crabs. Empty lantern nets did not exhibit comparable biodiversity and were relatively empty of other marine species.

Baykeeper decided that the overwinter NWSE survival rate warranted additional research and that restoration efforts at NWSE should be expanded to a ¼-acre research footprint containing 18 plots. It was recommended by Earle staff that the expanded research should be conducted between the working and non-working piers (Fig. 43). This location ensures that Baykeeper activities do not impinge on the naval mission, while at the same time providing a location that would be completely inaccessible to poaching activity. Baykeeper received final approval in late 2012 for the required NJDEP permits to install Phases II and III, consisting of a ¼-ace research footprint followed by expansion of oyster reintroduction up to the permitted 10-plus acres. However, the Baykeeper Atlantic Highlands facility was destroyed by SuperStorm Sandy, and so Summer 2013 was devoted to reassembling the setting tank facility at NWSE. The Navy also required that the research area be swept with side scan sonar post-Sandy in order to detect any unexploded ordinance prior to Baykeeper and Rutgers personnel entering the water. This navy scan is now scheduled for completion in spring 2014.

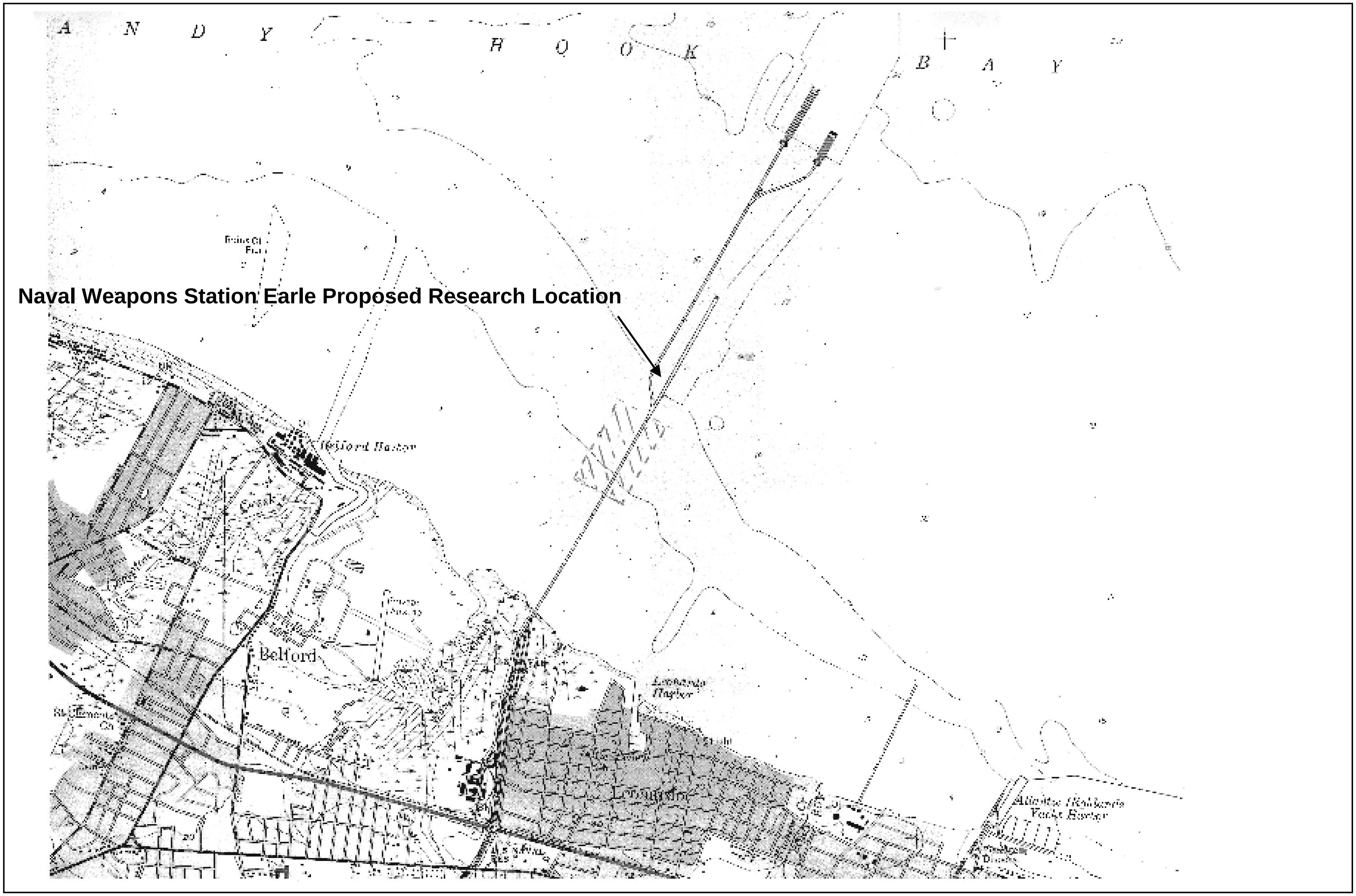


Figure 43. Proposed naval restoration locations at Naval Weapons Station Earle.

OYSTER REEF RESTORATION RESEARCH – PHASE II (2014-2017)

The nest phase of the NWSE research (regulated by NJDEP Scientific Collection, Tideland, and Waterfront Development Permits) will include the testing of three different oyster enclosures to determine which supports the highest oyster survival and growth rates. The structures will be placed subtidally so 2 – 3 ft. of water will cover the oysters during the lowest portion of the tidal cycle. We have found this positioning to be beneficial for oyster survival and subtidal placement also reduces the possibility of facilitating illegal poaching activity (although based on the naval patrols protecting the working pier, poaching is a very highly unlikely occurrence at NWSE). We will initially test 3 support structures to determine their potential to withstand winter storm energies encountered in Raritan Bay waters and support oyster survival and growth: Reefblk™ triangular rebar structures, Reef Ball™ concrete supports, and heavy duty wire mesh cages (Figure 44). SOS oyster densities of ~1,000 oysters per meter sq (Mann et al. 2009) will be housed in heavy duty polypropylene aquaculture mesh cages affixed to rebar support structures (Reefblk™); set directly onto concrete Reef Balls™; and placed into heavy duty wire mesh cages atop a layer of cured clam shell ~2 ft deep. The research field plots will cover the ¼-acre footprint, and will mirror the Keyport Harbor random block design (Figure 45). As requested by the NWSE staff, the test cages will not be anchored directly into the bottom sediments. Therefore, we will need to attach the rebar and metal cages to the naval piers in order to eliminate the possibility of movement of the test structures. The Reef Balls™ will be placed in between the metal structures. Based on over-winter survival and growth rates, one of these three structures will be chosen to support the NWSE oyster research reef population.

OYSTER (REEF) BALL DIMENSIONS

Reefballs™ are individual concrete semi-spherical units designed to emulate and create oyster reef. The structure is formed using a fiberglass mold containing a central Polyform buoy surrounded by various sized inflatable balls to make holes. The structure is designed to contain holes that provide areas for the oysters to colonize, and be heavy enough that it will not move in a high energy environment.

REEF BLOCK™

Reefblks™ are prefabricated double framed steel units which hold polypropylene aquaculture cages filled with juvenile oysters. The structures will be attached to the pier supports. It is hypothesized that they may provide some immediate shoreline protection. The triangular structure is 3 ft on each side.

METAL MESH CAGE

The navy will supply the wire metal cage, which will contain a minimal 3 ft bottom layer of cured surf clams. Oyster eyed larvae spat will be set on cured surf clams and allow to grow out to a minimum length of 10 mm. The spat-on-shell (SOS) will then be placed on top of the surf clam base.

Figure 44. Descriptions of the Baykeeper NWSE Oyster Support Structures

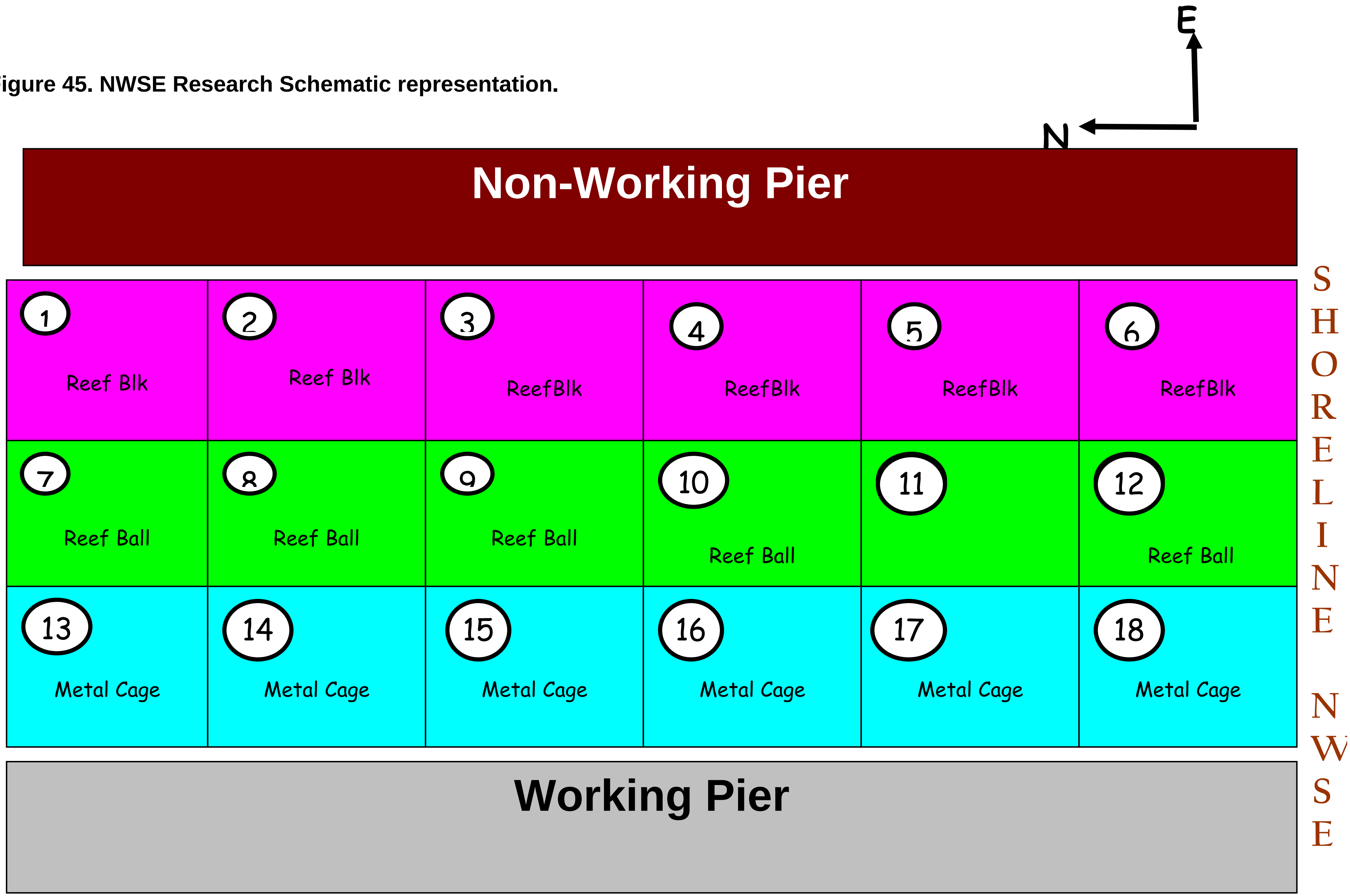
To evaluate each structure, oyster subsets will be measured prior to placement at NWSE during late summer 2014, and subsequently monitored during the summer of 2015 when the subsets will be recovered from the field plots, the animals re-measured, and live animals returned to the research plots. The 2015 monitoring will be done pre- and post-spawning (June and September). In addition to monitoring the oysters, we will utilize temporary placement of fish traps to determine marine biodiversity associated with the oyster research footprint. A set of two fish traps will be placed next to each type of support structure and left in place for 24 hours. Upon recovery, the species and number of each individuals collected in the traps will be documented. This sampling will minimally occur at least once during the fall, summer, winter, and spring seasons.

To obtain water quality physiochemical parameters two continuous YSI Sonde probes (salinity, temperature, pH, dissolved oxygen, turbidity, chlorophyll) will be installed. Two Sonde installations were tested in October, 2013 – January, 2014 (Table 13). One probe appears to be working properly, but the second probe did not record data. Temperature and pH ranges were typical of a mid-Atlantic estuary during the late fall/winter time period. One very low dissolved oxygen event was recorded (10//18/13), but this was the first day that the probe was in place and could therefore be an inaccurate reading. Turbidity exhibited wide fluctuations and was often high.

Table 13. Minimum, Maximum, and Mean NWSE Water Quality Parameters 10/18/13-1/4/14.

Date	Temp (°C)	Dissolved O_2 (mg/L)	pH	Turbidity (NTU)	Chl (mg/L)
10/18-11/21/13	5.6-22.6	1.98-19.8	6.2-7.8	0-238	0.3-150
Mean	11.4	9.6	7.1	4.8	8.1
11/21/13-1/4/14	-1 to 8	10-19	6.4-7.6	1-902	0.8-66
Mean	4.3	14	7.1	9	17

Figure 45. NWSE Research Schematic representation.



PROPOSED NWSE MONITORING WORK PLAN

TASK 1.0
ENVIRONMENTAL COMPLIANCE

NY/NJ Baykeeper will be responsible for Scientific Collecting Permits, Tidelands, and Waterfront Development applications to NJDEP. Installation and monitoring activities would be conducted on the Earle research site during the months of May through October, subject to approval of the permits and in coordination with a monitoring schedule approved by Earle.

TASK 2.0
WATER QUALITY

Permanently installed YSI Sonde probes (a minimum of two attached at pier interior and exterior locations) will measure dissolved oxygen, salinity, pH, turbidity, chlorophyll, and temperature.

TASK 3.0
REEF BIODIVERSITY

Biodiversity associated with the research plots and adjacent to the research area will be monitored using various sizes of crab and fish traps. Proposed sampling devices and sampling schedule will be submitted to the Naval Weapons Station Earle for pre-approval prior to commencement of research activities. The traps will be placed at the research site during the May – October period for 24 hour intervals. Species collected in the traps will be identified and this data will be used to characterize marine biodiversity associated with the various support structures and the presence/absence of live oysters.

TASK 4.0
OYSTER GROWTH & SURVIVORSHIP

The goal of the Earle Oyster Reef Restoration Project is to determine restoration methods that support long-term vertical accretion of oyster reef habitat. Oyster growth, survivability, spat settlement, and associated marine community composition will be monitored for a minimum two year period to determine the ability of Eastern Oysters to survive and thrive in this location and to assess the effect of the oysters' presence on the local marine community. The Earle monitoring program will follow the methods used in our Keyport Harbor study. Attached to the structures will be retrievable 0.1 m^2 baskets housing randomly selected subsets of 100 oysters. These baskets will be monitored to determine oyster growth, mortality, and marine species associated with the reef. These baskets and the overall structures will also be monitored to determine the degree (if any) of new spat recruitment. Oyster tissues will be evaluated annually to determine histopathology (Dr. Keith Cooper, Rutgers toxicology lab) and tested at the Rutgers Haskins Shellfish Laboratories to determine the intensity of *MSX* and *Dermo* infection(s). Under the direction of Baykeeper staff and Rutgers University researchers, volunteers may be involved in the monitoring process (measuring, recording data, visual inspections). Volunteers will participate in the NY/NJ Baykeeper oyster training workshop prior to joining the monitoring team.

TASK 5.0
PROJECT MONITORING & REPORTING

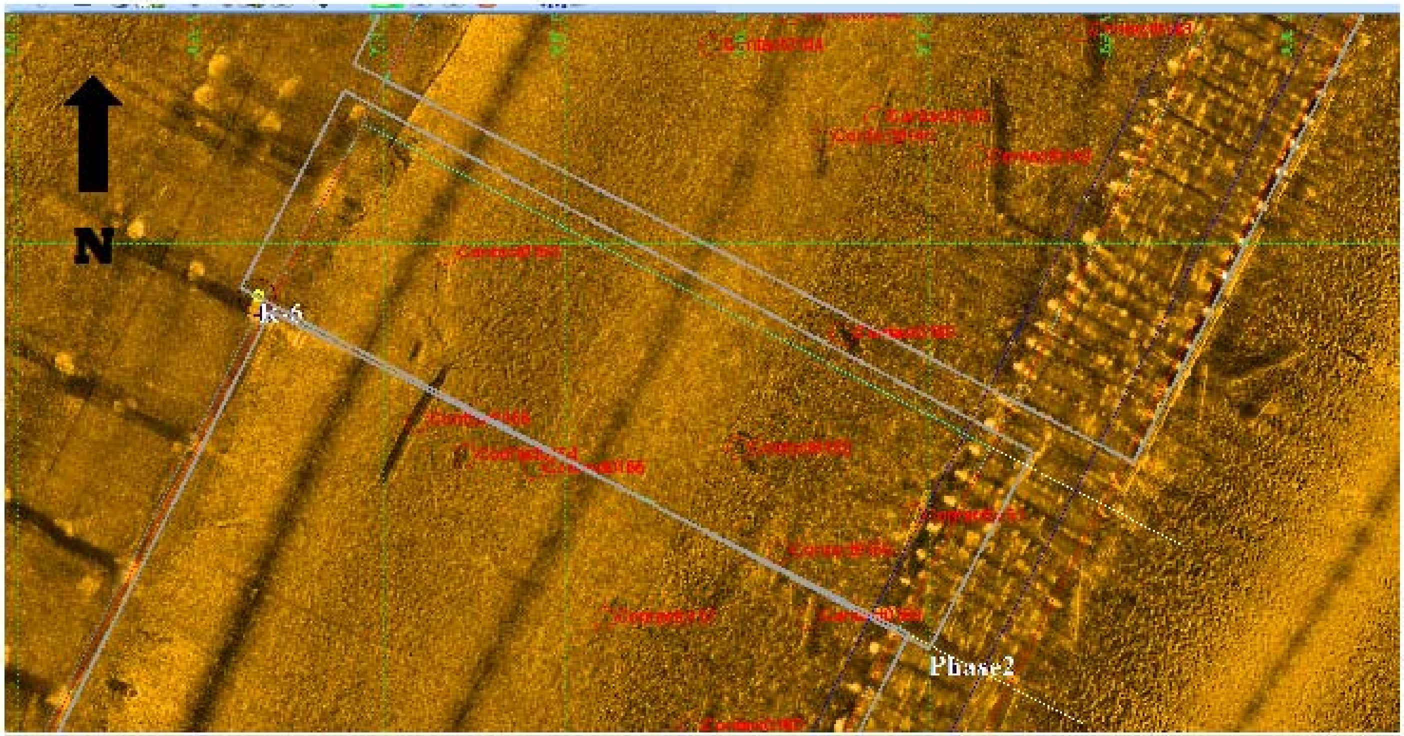
A monitoring report will be compiled at the conclusion of each year's monitoring activities. Data collected will be housed on the Rutgers University server (www.cues.rutgers.edu) and will be available to all project partners. The results of this restoration project will be communicated to the general public, regulatory agencies, and academic researchers through meeting presentations and papers submitted to restoration publications and peer-review journals. A project overview will be available through the Rutgers University website.

ADDITIONAL NWSE AREAS OF RESEARCH

Larval settlement on the adult oysters placed at NWSE is a critical component of long-term population sustainability. An initial mapping of potential NWSE larval transport patterns was completed based upon existing Raritan Bay current and flow data obtained from Dr. Bob Chant, Rutgers Institute of Marine and Coastal Sciences. This analysis indicates that fertilized larvae produced by adult oysters located at Earle could potentially remain in the general vicinity of the Earle piers over the 2 – 3 week period the larvae remain in the water column. Should this larval transport projection be correct, it would indicate the strong possibility of the larvae attaching to the adult oysters at the Earle piers. This model prediction will be field verified if the 2015 year old adults spawn. We note that the research seed oysters recovered from the Richmond County Yacht Club location were observed to spawn while being held in the Rutgers laboratories, and so are hopeful that Earle oysters will also be able to spawn after a year *in situ*.

A further area of research should be to attempt to determine the effects that placement of oysters/cage enclosures have on sediment and shoreline stability; research initiatives that address this question should be introduced as part of future NWSE data collection activities. The substrate between the working and non-working piers and adjacent to the pier area where the oyster research field plots will be installed was surveyed (Figure 46) by James Nickels (Monmouth University) in July, 2013, post-SuperStorm Sandy. Debris from Sandy-inflicted pier damage was observed in the general area where the research plots will be located, but this debris was not extensive; debris appeared denser adjacent to the wooden non-working pier (eastern side of the field plots). We had anticipated that this survey would provide substrate detail prior to research cage installations in fall, 2013. However, the navy has not yet completed their own sonar survey and therefore the cages will not be installed until summer, 2014. Since the substrate could be affected by winter storms, the results of the 2013 survey should not be used as a baseline comparison of conditions pre-oyster installation.

Additional NWSE research may include installation of oysters in Ware Creek, located west of the pier on naval property, and in front of the eroding sand beach adjacent to the creek. This project has been proposed as part of a larger Ware Creek wetland restoration effort and funding is currently pending.



Bay Keeper Earle NWS Side Scan Survey 7/23/13
Phase 2

Figure 46. Side scan sonar survey of the substrate under and adjacent to the NWSE pier where research plots will be installed.

VII. BAYKEEPER OYSTER RESTORATION: CONCLUSIONS

The survival and histopathology data obtained from research Eastern Oysters placed in Keyport Harbor, at Baykeeper oyster gardening sites, and at NWSE Earle for approximately one year suggest that there are indeed locations within the HRE where oysters could potentially survive. However, it is NOT known how many of these locations exist or where they are located, and so a large-scale. An inexpensive survey and testing of potential restoration sites is need in order to meet the Oyster Restoration targets set out in the Draft Hudson-Raritan Estuary Comprehensive Restoration Plan. Based on the Raritan Bayshore habitat survey, it appears that there are locations in the Bay that show promise of being able to support oyster populations, and that if successful, these sites could indeed contribute to meeting the CRP (2009) goal of reestablishing 200 acres of oyster reef in the short term and 5,000 acres by 2050. Keyport Harbor and preliminary NWSE data suggest that achieving the CRP oyster restoration targets would indeed be supportive of increased biodiversity in the HRE.

This report highlights the amount of data that is not yet known, but which is needed to ensure successful HRE oyster reintroduction, as well as the great need for further basic (and inexpensive) research prior to investing large amounts of restoration dollars. Tissue abnormalities were not correlated with oyster size or weight, illustrating the need for a biological *in situ* biomarker to aide in determining overall oyster health and fitness at a specific reintroduction location. The CUES-Baykeeper Site Selection Model provides a basis for such an analysis.

The Earle collaboration demonstrates that there is an opportunity to conduct oyster research in the HRE under conditions that do not increase the potential for illegal poaching activity. Identification of other secure sites in NY and NJ should be undertaken and conversations with the site owners should be initiated. Once owner and regulatory consent is obtained, the Site Selection Model should be followed and the process of testing over-winter survival at other secure locations should begin. This approach may include initial site selection in heavily patrolled waters (such as Earle) or in locations where ***all*** harvesting of shellfish is prohibited, which would make regulatory control and oversight much simpler. We also note that no further Raritan Bay restoration research can occur without the cooperation of the NJDEP or NYDEC. Conducting oyster restoration research activities at Naval Weapons Station Earle should address regulatory fears that poaching would occur. This location supports the continuation of ongoing long-term research, while addressing the regulatory management issues associated with oyster reintroduction.

It is our hope that further Baykeeper oyster reintroduction research will elucidate the actions required to ultimately reestablish a sustainable Eastern Oyster population in the Hudson-Raritan Estuary.

REFERENCES CITED

1. Akyil, S., and A.M. Yusof. 2007. The distribution of uranium and thorium in samples taken from different polluted marine environments. Journal of Hazardous Materials 144:564-569.
2. Bain, M., D. Suszkowski, J. Lodge, and X. Lijie. 2005. Setting Targets for Restoration of the Hudson-Raritan Estuary. Report of an Interdisciplinary Workshop. Hudson River Foundation. New York, NY.
3. Barnes, T.K., Volety, A.K., Chartier, K., Mazzotti, F.J., Pearlstine, L. 2007. A habitat suitability index model for the eastern oyster (*Crassostrea virginica*), a tool for restoration of the Caloosahatchee estuary, Florida. Jour Shellfish Res 26(4):949-959.
4. Bertness, M. D. 1999. The Ecology of Atlantic Shorelines. Sinauer Associates, Inc. Sunderland MA.
5. Blotcky, A.J., C. Falcone, V.A. Medina, and E.P. Rack. 1979. Determination of trace-level vanadium in marine biological samples by chemical neutron activation analysis. Analytic Chemistry 51:178-182.
6. Brumbaugh, R.D., Beck, M.W., Coen, L.D., Craig, L., Hicks, P. 2006. A Practitioner's Guide to the Design and Monitoring of Shellfish Restoration Projects: An Ecosystem Services Approach. The Nature Conservancy. Arlington, VA.
7. Buckley, B., Johnson, W., Fischer, E., Tu Q.and Heintz, M. 2003. Measurement of Heavy Metals in Biological and Environmental Matrices Using Microwave Extraction, Inductively Coupled Plasma Mass Spectrometry and Ion Chromatography for Assessing Potential Risk to Human Health, (Chinese) Journal of Environmental and Occupational Medicine, 20(6): 418-421.
8. CARP. 2007. http://www.carpweb.org/main.html. Downloaded 3/31/11.
9. Coen, L.D., R.D. Brumbaugh, D. Bushek, R. Grizzle, M.W. Luckenbach, M.H. Posey, S.P. Powers, and S.G. Tolley. 2007. Ecosystem services related to oyster restoration. Marine Ecology Progress Series 341:303-307.

10. CRP. 2009. Draft Hudson-Raritan Estuary Comprehensive Restoration Plan. http://www.nan.usace.army.mil/harbor/index.php?crp. Downloaded 9/28/10.
11. Elston, R., E.W. Cake Jr., K. Humphrey, W.C. Isphording, and J.E. Rensel. 2005. Dioxin and heavy-metal contamination of shellfish and sediments in St. Louis Bay, Mississippi and adjacent marine waters. Journal of Shellfish Research 24:227-241.
12. Ford, S.E., Tripp, M.R. 1996. Diseases and Defense Mechanisms. In Kennedy, V.S., Newell, R.I.E., Eble, A.F. (eds) The Eastern Oyster: *Crassostrea virginica*. p. 581-660.
13. Frazier, J.M. 1975. The dynamics of metals in the American Oyster, *Crassostrea virginica*. I. Seasonal Effects. Chesapeake Science 16(3):162-171.
14. Frazier, J.M. 1976. The dynamics of metals in the American Oyster, *Crassostrea virginica*. II. Environmental effects. Chesapeake Science 17(3): 188-197.
15. Gornitz, V., Couch, S., Hartig, E.K. 2001. Impacts of sea level rise in the New York City metropolitan area. Global and Planetary Changes 32:61-88.
16. Guzman-Garcia, X., A.V. Botello, L. Martinez-Tabche, and H. Gonzalez-Marquez. 2009. Effects of heavy metals on the oyster (*Crassostrea virginica*) at Mandinga Lagoon, Veracruz, Mexico. Rev. Biol. Trop. 57:955-962.
17. Harding, J.M. and R. Mann. 1999. Fish species richness in relation to restored oyster reefs. Bulletin of Marine Science 65(1):289-300.
18. Harding, J.M. and R. Mann. 2001. Oyster reefs as fish habitat: Opportunistic use of restored reefs by transient fishes. Journal of Shellfish Research 20(3):951-959.
19. Hawkins, S.J., Gibbs, P.E., Pope, N.D., Burt, G.R., Chesman, B.S., Bray, S., Proud, S.V., Spence, S.K., Southward, A.J., Langston, W.J. 2002. Recovery of polluted ecosystems: the case for long-term studies. Marine Ecology Progress Series54:215-222.
20. Hayes, W.J., I.J. Anderson, M.Z. Gaffor, and J. Hurtado. 1998. Trace metals in oysters and sediments of Botany Bay, Sydney. Science of the Total Environment 212:39-47.
21. Hodder, K.H. Bullock, J.M. 1997. Translocations of native species in the UK: Implications for biodiversity. Jour App Ecol 34:547-565.

22. Jackson, J.B.C. 2001. What was natural in the coastal oceans? Proceedings of the National Academy of Sciences. 98(10):5411-5418.
23. Kennedy, V.S., Breitburg, D.L., Christman, M.C., Luckenbach, M.W., Paynter, K., Kramer, J., Sillner, K.G., Dew-Baxter, J., Keller, C., Mann, R. 2011. Lessons learned from efforts to restore oyster populations in Maryland and Virginia, 1999 to 2007. Jour Shellfish Res 30(3):719-731.
24. Kennish, M.J. 2004. Estuarine Research, Monitoring, and Resource Protection. CRC Press, Boca Raton, FL.
25. Kimbrough, K.L., W.E. Johnson, G.G. Lauenstein, J.D. Christensen, and D.A. Apeti. 2008. An Assessment of two decades of contaminant monitoring in the nation's coastal zone. Silver Spring, MD. NOAA Technical Memorandum MOS NCCOS 74. 105 pp.
26. Kirshen, P., Watson, C., Douglas, E., Gontz, A., Lee, J., Tian, Y. 2007. Coastal flooding in the northeastern United States due to climate change. Mitigation and Adaptation Strategies for Global Change as part of the Special Issue entitled: "Northeast United States Climate Change Impact Assessment".
27. Lindeburg, M.R. 1992. Civil Engineering Reference Manual. 6th Ed. Professional Publications, Inc. Belmont, CA.
28. Mann, R., M. Southworth, J.M. Harding, and J.A. Wesson. 2009. Population studies of the native eastern oyster, *Crassostrea virginica* (Gmelin, 1791) in the James River, Virginia, U.S.A. Journal of Shellfish Research 28(2):193-220.
29. McCay, B. J. 1998. Oyster Wars and the Public Trust: Property, Law, and Ecology in New Jersey History. University of Arizona Press. Tucson, AZ.
30. Mihoub, J-B., P. Le Gouar, Sarrazin, F. 2009. Breeding habitat selection behaviours in heterogeneous environments: implications for modeling reintroduction. Oikos 118:663-674.
31. Morton, B. 1991. Do the bivalvia demonstrate environment-specific sexual strategies? A Hong Kong model. Journal of the Zoological Society of London 223:131-142.
32. NJDEP. 2006. Surface Water Quality Standards (N.J.A.C. 7:9B). Trenton, NJ.

33. NOAA. 1987.
34. NRC. 2007. Coastal Sensitivity to Sea Level Rise: A Focus on the Mid-Atlantic Region. http://www.epa.gov/climatechange/effects/coastal/pdfs/CCSP_chapter8.pdf. Downloaded 3/6/2012.
35. Peterson, C.H., Grabowski, J.H., Powers, S.P. 2003. Estimated enhancement of fish production resulting from restoring oyster reef habitat: quantitative valuation. Marine Ecology-Progress Series 264:249-264.
36. Piazza, B.P., Banks, P.D., La Peyre, M.K. 2005. The potential for created oyster shell reefs as a sustainable shoreline protection strategy in Louisiana. Restoration Ecology 13(3):499-506.
37. Pollack, J.B., Cleveland, A., Palmer, T.A., Reisinger, A.S., Montagna, P.A. 2012. A restoration suitability index model for eastern oyster (*Crassostrea virginica*) in the Mission-Aransas estuary, TX, USA. PlosOne 7:e40839.
38. Powers, S.P., C.H. Peterson, J.H. Grabowski, and H.S. Lenihan. 2009. Success of constructed oyster reefs in no-harvest sanctuaries: implications for restoration. Marine Ecology Progress Series 389:159-170.
39. Ravit, B., Cooper, K., S., Buckley, B., Comi, M. 2013. Improving management support tools for reintroducing bivalve species in urban estuaries. Integrated Environmental Assessment & Management. *Submitted.*
40. Ray, S.M. 1954. Biological studies of Dermocystidium marinum, a fungus parasite of oysters. Rice Inst. Pam. Spec. Issue Nov. 1954. Monograph Biol. 114p.
41. Sadig, M., and I. Alam. 1989. Metal concentrations in Pearl Oyster, *Pinctada radiate*, collected from Saudi Arabian coast of the Arabian Gulf. Bulletin of Environmental Contamination & Toxicology 42:111-118.
42. Simenstad, C., C. Tanner, C. Crandell, J. White, Cordell, J. 2005. Challenges of Habitat Restoration in a Heavily Urbanized Estuary: Evaluating the Investment. Jour Coastal Res Special Issue 40:6-23.

43. Steimle, F., Foster, K., Kropp, R., Conlin, B. 2002. Benthic macrofauna productivity enhancement by an artificial reef in Delaware Bay, USA. Journal of Marine Science 59:S1200-S105.
44. TEC. 2007. Target Ecosystem Characteristics for the Hudson Raritan Estuary: Technical Guidance for Developing a Comprehensive Restoration Plan. A report to the Port Authority of New York and New Jersey. New York, NY.
45. Volety, A.K. 2008. Effects of salinity, heavy metals and pesticides on health and physiology of oysters in the Caloosahatchee Estuary, Florida. Ecotoxicology 17:579-590.
46. Xie, R., Johnson, W., Rodriguez,, L., Gounder, M., Hall, G. and Buckley, B. 2007. A Study Of The Interactions Between Carboplatin And Blood Plasma Proteins Using Size Exclusion Chromatography Coupled To Inductively Coupled Plasma Mass Spectrometry, Anal. and Bioanal. Chem. 387:2815–2822.