NYC Environmental Protection

Grant 41931

Developing a Self-Sustaining Oyster Population in Jamaica Bay, New York City

Final Report and Project Deliverables

October 2020

U.S. Department of the Interior, March 3, 1849

National Fish and Wildlife Foundation, NFWF

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The project was located in the Northeastern end of Jamaica Bay, New York, at the Head of Bay. The successful establishment of a self-sustaining oyster population could create an oyster larvae source for Jamaica Bay and beyond and would benefit the Hudson Raritan Estuary as a whole, helping to attain the goals of the Comprehensive Restoration Plan (CRP) for oyster restoration and the Department's Jamaica Bay Watershed Protection Plan (JBWPP). Previous oyster restoration efforts focused on smaller scale projects - this project evaluated recruitment more on an ecological habitat scale as opposed to a smaller pilot project. Models from previous studies showed that the location has ideal conditions to promote oyster growth and recruitment due to supportive salinity, temperature, and dissolved oxygen levels, as well as slower tidal dispersion rate to increase larvae retention potential. The project provided an opportunity to study the potential of mid-scale oyster recruitment and growth in New York waters.

Executive Summary

The first step towards achieving this goal was to place a sufficient quantity of reproductively viable adult oysters in a floating longline "donor reef" situated in the vicinity of four individual beds constructed on soft sediment bottom from a combination of mixed mollusk shell and recycled porcelain. A secondary long-term project goal was to provide a source of natural oyster larval production within Jamaica Bay, where it is currently believed that oyster larvae are not present in the water column. The specific, quantitative performance indicator used to demonstrate project "success" was the occurrence of oyster larvae in the project area throughout the planned two-year monitoring program. The expected outcome was settlement of juvenile oysters ("spat") adhering to shells at the "receiver reef" located near the floating donor reef, eventually forming a self-sustaining reef complex. The Head of Bay region of Jamaica Bay was selected for project implementation because a longer larval retention period anticipated in this area was expected to increase the chance of fertilization of eggs released by the oysters and the slower current velocities in this area were expected to reduce the export of larvae to other areas within Jamaica Bay.

Six major project objectives were addressed throughout the study:

1. Initial (pre-construction) site suitability survey and selection
2. Donor reef design and construction
3. Receiver reef design and construction
4. Monitoring and maintenance of reefs
5. Assessment of ecosystem services
6. Assessment of results in the context of future projects

Site assessment and feasibility studies conducted in the project area prior to longline donor reef and receiver reef construction included bathymetric profiles and substrate characterization, along with a hydrodynamic survey, which was conducted from July – August 2016 using a stationary bottom-mounted Acoustic Doppler Current Profiler (ADCP). During construction (September 2016), a 650-foot floating longline was installed in the Head of Bay region of Jamaica Bay. At three foot intervals along the line, floating OysterGro™ cages were attached which housed reproductively viable adult oysters. The receiver reef installation consists of four individual 50 m x 10 m beds for a combined total of approximately 0.5 acres. This floating donor reef was stocked with approximately 35,200 adult oysters in September 2016, followed by an additional 9,300 adult oysters in November and December 2017. The four reef beds were constructed in October 2016 from seasoned surf clam shells *(Spisula solidissima)* or from a base of clam shells covered by a veneer of oyster shells or crushed porcelain. The new substrate material was designed to raise the bottom approximately 6" to 24." A high-resolution bathymetric profile of the receiver reef and surrounding seabed was conducted in November 2016 using a multi-beam echosounder (MBES) and acoustic backscattered intensity (side-scan sonar) to verify that the installation achieved the project design objectives. Subsequent annual qualitative dive survey assessments were used to evaluate changes in reef characteristics over time. The final dive survey to assess reef changes (August 2018) provided evidence of nominal subsidence and siltation on all the reef beds. This was further verified by a second MBES and side-scan sonar survey in October 2018. The reef materials still covered the majority of their designed

footprint and the surface veneer layer of material at each reef was still present.

Monitoring parameters, or performance indicators surveyed during the project included:

1. Growth, mortality, condition, incidence and intensity of disease and reproductive status of donor reef oysters – these parameters were monitored monthly throughout the duration of the project; oyster growth was determined by measurement of shell height (hinge to bill). The total number of live and dead oysters in subsamples from the OysterGro™ cages were counted and expressed as a ratio of live/dead. Subsamples of oysters from the donor reef were analyzed annually (October) for the protozoan oyster diseases MSX and Dermo.

2. Oyster density on receiver reef beds – Initially this parameter was assessed using patent tongs (2017) but was discontinued and replaced with diver-based excavations and video transects (2018-2019). These surveys did not document recruitment of oysters to the receiver reef during the study.

3. Plankton tows and spat collector monitoring - conducted weekly from late July 2018 through September 2018. This effort included analysis of plankton samples for oyster DNA presence/absence. Over the three-year period no oyster spat were observed on the spat collector stations; however oyster DNA was detected in plankton samples through late larval development stages.

4. Water filtration capacity (e.g., seston uptake) of the adult oysters in the floating donor reef, over "short-term" (i.e., minutes) to "long-term" (i.e., days) monitoring intervals using fluorometers and water quality datasondes. During 2017, a total of seven replicate short-term (<10 min duration) datasets were recorded. All showed consistent and substantial chlorophyll (CHL) depletion with overall removal rates ranging from 17.6% to 45.7%. Long-term deployment of datasondes and the acoustic doppler velocimeter (ADV) during 2017 yielded a total of ~160 separate measurements (~40 hours total; readings at 15-min intervals) of CHL concentrations, water quality parameters, and water flow speed deployed on the field flume apparatus. During 2018, a total of four short-term (6 to 35 min) datasets were recorded using handheld fluorometers showing an overall mean of 10.9% CHL removal. Long-term (28 hours) deployments in 2018 yielded a total of ~800 separate measurements of CHL concentrations upstream and downstream of the oyster bags in the field flume and CHL removal rates ranged from 18.5% to 22.5%. In sum, the donor reef provided temporary water filtration capacity comparable to natural and restored reefs in other areas, averaging (both years combined) about 30% removal of CHL from water passing over the oysters.

5. Benthic community assessment in the vicinity of the receiver reefs (2017, 2018) with comparison to pre-construction benthic communities (2016), to characterize habitat provision/substitution - the taxonomic composition of the macrofauna communities on the receiver reef differed substantially from that of the pre-construction soft-sediment macrofaunal communities. Taxa richness was higher on the receiver reef than on the pre-construction soft-sediments. With respect to habitat substitution, expected changes in benthic community composition occurred: the soft-sediment benthic infauna were replaced by an epifaunal community typical of those that occur on hard substrates. By the end of the monitoring program, the receiver reef were providing important new habitat for resident and transient macrofauna. Qualitative diver observations of macrofauna in the vicinity of the reef also indicated provision of habitat for a diversity of characteristic estuarine/marine species.

In the spring of 2018, project partners agreed to extend the term of the project and to maintain the donor reef for an additional 12 months. In November 2018, the donor reef cages and the line began to show signs of wear consistent with extended time in saltwater and sun exposure, and friction caused by the normal movement of the line related to wind and tide conditions, and a decision was made to remove the longline.Upon decommissioning in December 2018 and March 2019, approximately 30,000 live oysters from the donor reef were placed directly on two of the receiver reef beds at a density of approximately 50 oysters/m^2. These two reef beds were surveyed in July 2019 to provide a baseline assessment of planted density, to document growth and survival of the planted oysters, and to identify any new oyster spat recruitment.

Upon completion, major recognized accomplishments of the project included:

1. Construction of four new hard-substrate reef bases that will continue to provide new habitat for many species, and that are potentially available for settlement by oyster larvae;

2. Creation of oyster reef habitat from oysters planted on two of the hard-substrate reef bases providing a continuing source of larvae and increased habitat complexity;

3. Initial characterization of the plant and animal communities in the new habitat;

4. Temporary provision of water filtration, nutrient sequestration, and a natural source of oyster larvae by oysters and other organisms on the donor reef;

5. A test of the hypothesis that proximity to adult oysters enhances the recruitment potential for constructed reefs;

6. Provision of learning opportunities for New York Harbor School's Aquaculture, Diving, and Vessel Operations student teams and learning opportunities for Billion Oyster Project (BOP) and Natural Area Conservancy's volunteers and interns.

The project was designed to test the general hypothesis that proximity to a population of healthy adult oysters enhances the probability of recruitment to constructed reefs. This hypothesis was not supported by this study as there was no observed recruitment of oysters to the receiver reef or evidence of spat settlement in the surrounding area. Potential reasons for the lack of observed recruitment include:

1. Spatial scales of larval settlement/recruitment are not well understood and a much larger donor population (potentially orders of magnitude greater than that used for this study) may be required;

2. Oysters in the donor reef produced larvae and the plankton monitoring results provide evidence that a large number of oysters were surviving to late development stages but definitive evidence that the larvae are surviving to final developmental stages (just prior to settlement) is lacking. Future studies to elucidate larval viability though the final development stages would provide important insights into mortality rates and potential for recruitment;

3. The frequency and magnitude of plankton sampling was not sufficient to estimate the larval loss rate (mortality + emigration) from the Head of Bay. Higher resolution plankton monitoring for future efforts could provide a loss rate estimate to better scale the size of the donor population necessary to create a new self-sustaining population.

In summary, the present project resulted in new hard-bottom habitat that supports characteristic macrofauna associated with oyster reefs, and temporary water filtration by the oysters on the donor reef. The project also provided important new information and "Lessons Learned" relevant to future oyster restoration projects in Jamaica Bay. Monitoring of the longline donor reef demonstrated that juvenile and adult oysters grow and reproduce in the Bay. The OysterGro™ system was generally appropriate for the project, however as the oysters and the fouling community grew and increased the weight of the units, accessing the oysters inside them became challenging. Vessels and equipment used for the maintenance of gear need to be carefully considered and selected for the needs of the installation. The plankton DNA results indicated that large numbers of late development stage oyster larvae were present in the project area during the study, but extensive sampling using spat collectors indicated no successful recruitment. Surf clam shells (and to a lesser extent oyster shell) are prone to breakage with excessive handling, reducing the overall suitability of substrate for spat settlement. Project logistical designs should seek to reduce this handing as much as possible. To date, finding a source for shells that meet the state's origin and curing requirements remains a challenge to future restoration efforts. However, porcelain is more durable and represents a viable alternative substrate. Although the project did not result in the colonization of the receiver reef by live oysters, important information on how to proceed with future restoration efforts was obtained.

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1.0 PROJECT DESIGN AND PURPOSE

The New York City Department of Environmental Protection (DEP) is committed to researching innovative coastal restoration practices and improving water quality. The native eastern oyster (*Crassostrea virginica*) was once abundant in New York City waters, particularly in Jamaica Bay. The primary of the Developing a Self-Sustaining Oyster Population in Jamaica Bay, New York City was to evaluate the factors affecting oyster growth, survival and reproduction and to establish best practices for future oyster restoration efforts. The ultimate goal is to figure out how to establish a self-sustaining oyster population in Jamaica Bay. The first step towards achieving this goal was to place a sufficient quantity of reproductively viable adult oysters in a 650-ft floating longline donor reef where they were protected from predators and sedimentation, dramatically limiting mortality. The longline donor reef was situated in the immediate vicinity of four artificial "receiver" reef beds, and constructed from a combination of mixed mollusk shell and recycled crushed porcelain. A primary advantage of this approach to populating the constructed reef is that the density of adult oysters is much higher in floating or suspended enclosures than it would be on a natural or restored reef, which results in a higher fertilization rate during spawning events. A secondary long-term goal was to provide a source of natural oyster larval production within Jamaica Bay, where it is currently believed that oyster larvae are not present in the water column. Since oyster larvae tend to settle near adult conspecifics, the desired outcome was for the oyster reef to have spat recruitment and settlement from one another or from themselves. The specific, quantitative performance indicator used to demonstrate project "success" would be the occurrence of oyster larvae in the Head of Bay area throughout the planned two-year monitoring program. The expected outcome would be settlement of juvenile oysters ("spat") adhering to shells at the subtidal receiver reef located near the floating donor reef, eventually forming a self-sustaining reef complex, requiring no further outside inputs of energy or resources. Once self-sustainability is established, the donor reef and any mature oysters that have established on the receiver reef should spawn seasonally and provide new recruits. The longer retention period anticipated in the Head of Bay was expected to increase the chance of fertilization of eggs released by the oysters and the slower current velocities in this area were expected to reduce the export of larvae to other, less hospitable waters further west and outside of Jamaica Bay.

1.1 Site Selection and Planning Phase

In May 2007, the Hudson River Foundation (HRF) convened a working group comprised of scientists, regulators, restoration practitioners, and project sponsors to discuss the potential for conducting oyster restoration projects within the Hudson-Raritan Estuary (HRE), including Jamaica Bay. It was agreed that the potential benefits to the estuary from oyster restoration were numerous, but that oyster pilot projects were needed to demonstrate that oyster restoration was feasible. To further develop the idea of oyster pilot projects within Jamaica Bay, the DEP held a workshop in June 2009 and invited oyster restoration practitioners to discuss potential oyster pilot project ideas, focusing on project objectives, site selection, construction methods, and monitoring.

The workshop participants indicated that oyster restoration projects in the HRE should be compatible with local geography, existing aquatic and terrestrial habitats, land-use patterns and navigation features. Restoration projects should also seek to address the concerns and desires of the local community, including educational institutions, private advocacy groups, and nearby municipalities. Proposed oyster restoration efforts must also comply with federal, state, and local regulatory agency requirements and policies. Therefore, the site selection and planning process for the Head of Bay project involved extensive coordination with regulatory authorities and careful consideration of several critical siting factors where available, historical information on oyster populations/locations should be evaluated to provide geographic and ecological context.

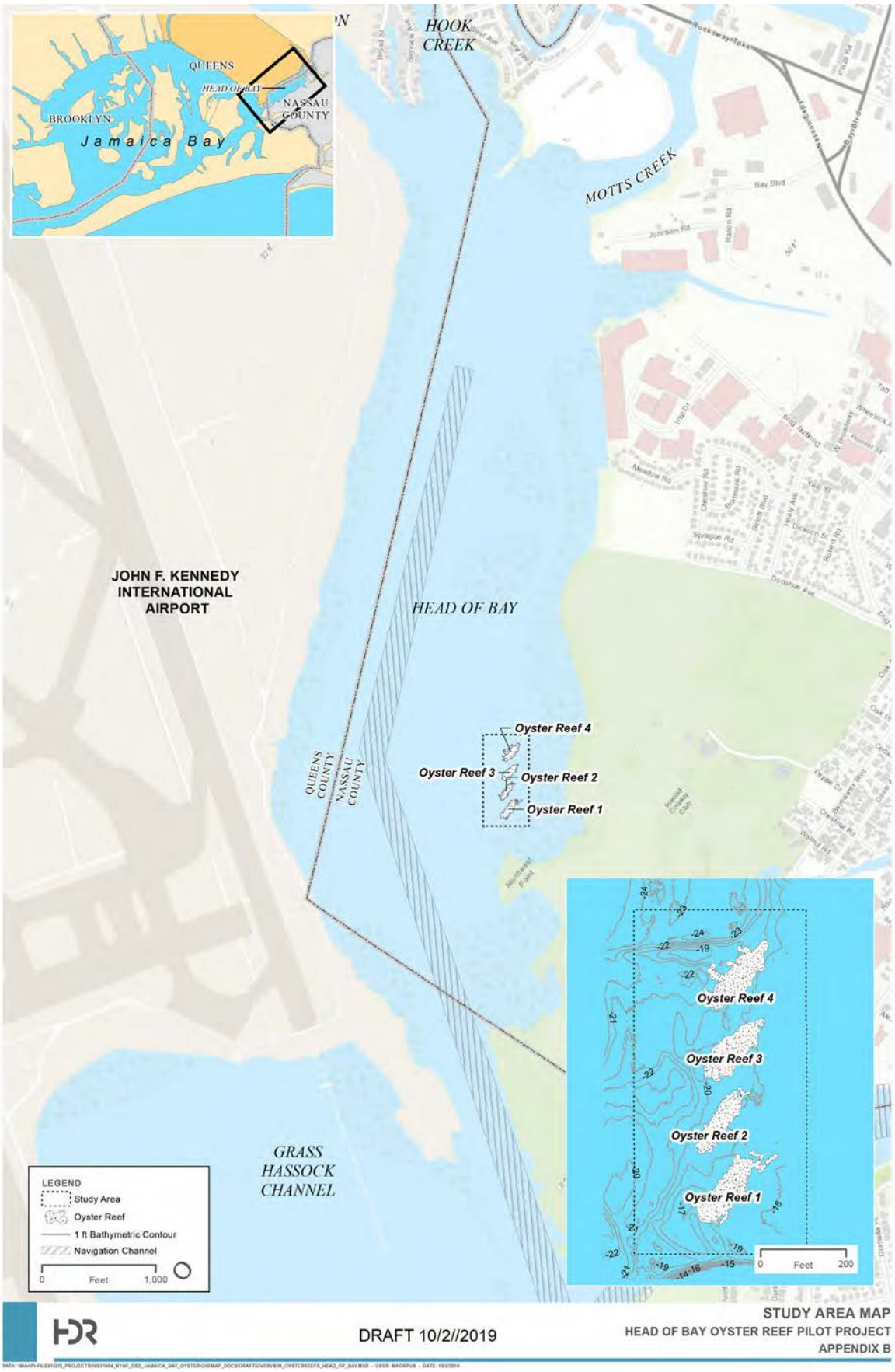


Figure 1.1 Project Site Map

Bathymetry — the height of constructed reefs should be maintained at least one foot below mean low water (MLW) to provide adequate tidal flow and oxygenation and at depths sufficient to discourage poaching (i.e., as deep as possible but well within the range of oyster life history requirements).

Substrate — Sediments in the vicinity of a proposed reef site should be resistant to periodic re-suspension by passing vessels or wind/wave events and must be able to support the weight of shell mounds, and any underlying rock or artificial substrate used, in addition to spat on shell or seed oysters. Potential sources of sedimentation in the vicinity of the reef(s) should be identified and determinations made to establish that the rate of sedimentation is less than the anticipated rate of vertical reef accretion.

Salinity — Oysters are tolerant of a range of salinities, however, growth/survival is diminished at sustained salinities below 7.5 ppt. Oysters will not feed or grow in waters of less than 5 ppt or above 32 ppt. An optimal salinity range (12-27 ppt) ensures production of gametes and rapid larval growth while maintaining protection from oyster predators (common in higher salinity waters) and disease.

Tidal Hydrodynamics — Tidal circulation patterns determine whether the area may act as a source or sink for larvae, help reduce or eliminate episodic hypoxia, and gently scour fine silt, feces and pseudofeces that may foul an oyster reef in quiescent waters. Areas with higher current flows promote food delivery and waste removal.

1.2 Hydrodynamic and Larval Transport Model Development

In 2012, HDR Engineering developed a larval transport model to inform the site selection process for oyster restoration in Jamaica Bay (HDR-HydroQual 2012). The objective of the model was to determine optimal locations for placement of constructed oyster reefs throughout the bay. A secondary project objective was to identify potential environmental constraints to the success of oyster restoration in the bay. Model segmentation was based on an existing model developed by HDR Engineering – the Jamaica Bay Eutrophication Model (JEM). The hydrodynamic model uses a series of differential equations that are numerically solved to predict current speed and direction and vertical stratification. The particle tracking model uses the predictions obtained from the hydrodynamic model to move particles and cue their behavior to simulate the transport of individual oyster larvae. The eutrophication model also uses the predictions of current speed/direction and vertical stratification, as well as predicted salinity and temperature, from the hydrodynamic model, together with differential equations which relate temperature, light, and nutrients to phytoplankton biomass and dissolved oxygen. These equations are also solved numerically and predict the concentrations of dissolved oxygen over space and time. These dissolved oxygen concentrations are then used in the particle tracking model to determine oyster larvae survival as affected by exposure to low concentrations of dissolved oxygen.

The model simulated the release of 47,500 larvae from a total of 19 individual reef locations within Jamaica Bay, with a predicted settlement and recruitment rate of approximately 16%. The differences between reefs varied considerably. Reefs located in the southeastern portion of the Bay had the highest settlement success, between 18 and almost 30%, while most other reefs had settlement success of less than 12%. In addition to informing the restoration design and final site selection in the Head of Bay area (southeastern Jamaica Bay; Figure 1.1), the larval transport model identified significant challenges to oyster restoration in Jamaica Bay, including high flushing rates, and the potential for high mortality of newly settled oyster spat as a result of episodic hypoxia throughout the bay. When the potential for hypoxic conditions in the vicinity of simulated reefs was considered in the model runs, bay-wide average settlement success declined from 16.0% to 10.1%, about a 37% reduction. The reefs showing the greatest reduction in settlement were, generally, located in the eastern portion of the bay and at the mouths of some of the tributaries, while reefs showing the smallest reductions in settlement were, generally, located in the central and western portions of the bay.

2.0 BACKGROUND AND IMPORTANCE OF RESTORATION

The eastern oyster (*Crassostrea virginica*) forms three-dimensional reefs that historically were a dominant feature of many U.S. Atlantic and Gulf estuaries, but oyster populations (and associated reefs) have declined substantially in most estuaries (Kurlansky 2006; Levinton and Waldman 2006; Beck et al. 2011). As a result, most coastal states have some type of oyster restoration program. A major impetus for oyster restoration in some areas, including the New York Harbor region, has been restoration of the ecosystem services oysters provide (Coen and Luckenbach 2000; Coen et al. 2007; Grizzle et al. 2013; Baggett et al. 2014; Lodge et al. 2015). Most of the oyster reef restoration projects in the region have focused on potential improvements in water quality that oysters and other filter-feeding species on the reef provide, and characterization of the habitat that oyster reefs provide for many species of fish and invertebrates. For this project, chlorophyll depletion rates were used as an indicator of nutrient sequestration and water quality improvements, and taxonomic richness of the invertebrate and macrofauna communities were used as indicators of habitat creation.

2.1 Oyster History in New York - New Jersey Harbor

At the time of European settlement, oyster beds were present throughout the Hudson-Raritan Estuary. Principal concentrations were along the Brooklyn and Queens shorelines in the East River, in Jamaica Bay, and along the Manhattan shoreline of the Hudson and East Rivers. Oyster beds occurred in the Hudson River as far north as Stony Point, and also along the Raritan Bay shoreline in the vicinity of Keyport, NJ. Oysters grew in the Raritan and Hackensack Rivers, and on reefs surrounding Staten Island, City Island, Liberty Island and Ellis Island (Mackenzie 1996). It is well-documented that indigenous peoples consumed oysters, as evidenced by the numerous shell middens left behind throughout present-day New York City. Evidence of shellfish harvesting by the native Jameco Indian tribes could be seen in eastern Jamaica Bay as late as the 1890s. Large mounds of discarded oyster shell were located in Inwood, Hog Island, and Bayswater (the Bayswater mounds being the largest) (Bellot 1917).

Local laws governing the over-exploitation and degradation of oyster beds were enacted in New York City during colonial times. In 1658, the then-Dutch colony of New Netherland enacted legislation regulating take of oysters on Manhattan Island and in the East River. In nearby Great South Bay limits on the number of vessels engaging in the harvest of oysters were set forth in 1679 (Kirby and Miller 2005). By the mid-18th Century raw sewage was entering the waters of NY/NJ Harbor adjacent to Manhattan Island. Shoreline modifications and fill represented a direct impact to native oyster beds. Overharvesting of natural oyster populations was so prevalent that by the early 19th Century, the oyster industry of Jamaica Bay was primarily based on seed stock brought in from other estuaries to the north and south of New York City, including Delaware and Chesapeake Bays (see review in Kirby 2004). Nonetheless, by 1880, New York City's oyster beds, whether farmed or wild, were producing 700 million oysters each year (Kurlansky 2006). However, by the early 20th century, the relationship between oyster consumption in New York City and the periodic outbreak of diseases such as cholera and typhoid was apparent. Temporary closures of New York City oyster beds occurred in 1915 and in 1921. By 1925, the Jamaica Bay oyster fishery was closed permanently (Franz 1982).

2.2 Ecosystem Services Provided by Oyster Reefs

Important ecosystem functions attributed to oyster reefs include maintenance of water quality, nutrient processing, shoreline stabilization, and the provision of nursery habitat for many estuarine-dependent finfish and shellfish species. Oysters remove nitrogen (ammonium and total nitrogen) as well as particulate phosphorus from the water column at a relatively high rate, indicating that oyster reefs may be an important mechanism in the recycling of N and P in temperate estuaries (Dame et al. 1989). Research on the water filtration capabilities of restored and natural oyster reefs initially focused on laboratory-derived rates and theoretical extension to the field (e.g., Newell 1988, Cerco and Noel 2007). More recently, methods for field measurement of whole-reef filtration have been developed and used in several geographic areas (reviewed by Dame 2012 and Bayne 2017). It has been argued that field methods, in general, represent a

more ecologically realistic approach than laboratory-derived filtration measurements (Powell et al., 1992; Dame and Libes 1993; Harsh and Luckenbach 1999; Grizzle et al. 2006; 2008). Moreover, they include the ecological complexities such as additional filter-feeding species and other organisms that typically live on reefs and cannot be simulated in the laboratory. Previous oyster reef restoration projects in the region have characterized various ecosystem services provided by the constructed/restored reefs, including water filtration and habitat provision (Mass and Ruzicka 2008, 2009; Grizzle et al. 2013; Peterson and Kulp 2013). The present study yielded reef filtration data similar to previous studies in other areas (Grizzle et al. 2008; Milbrandt et al. 2016) and the NY Harbor region (Lodge et al. 2014). Although much remains to be learned about how oyster filtration affects nutrient cycling and long-term water quality changes in ecosystems (Fulford et al. 2007; Coen et al. 2007), the present study confirms the capabilities of oysters and other filter feeding species that typically co-occur with them to process substantial quantities of water on a daily basis.

Oyster reefs provide valuable habitat for a variety of organisms, including many commercially important fishery species such as summer flounder (*Paralichthys dentatus*) and blue crab (*Callinectes sapidus*). Grabowski et al. (2005) reported that restored oyster reefs enhance the presence of infaunal and epifaunal invertebrates, including polychaetes, nemerteans, anemones, crustaceans and mollusks. The hard structure of restored oyster reefs, placed in intertidal areas, as well as in deeper subtidal waters can moderate wave climate and potentially reduce shoreline erosion from storm events and more chronic, anthropogenic activities (i.e., vessel wakes). In the Northeast and mid-Atlantic regions, oyster beds/reefs often occur seaward of salt marshes and may enhance/supplement the ability of marshes to stabilize shorelines and moderate wave energy.

2.3 Past Restoration Efforts in the Hudson-Raritan Estuary

Interest in oyster habitat restoration and community engagement using small "Oyster Research Stations" or the "ORS Program" (typically referred to as "oyster gardening" elsewhere) has increased in the New York/New Jersey Metropolitan area. BOP's ORS Program allows users to assess growth and mortality of small populations of oysters throughout NY Harbor, but the program is not designed to provide or produce information about oyster larvae distribution or attempt to direct participants to areas where oyster growth and survival may be ideal. Research underway by academic institutions, natural resource agencies and NGOs in the NY/NJ Harbor Estuary, including Jamaica Bay, may yield important information on environmental conditions necessary for oyster reproduction, survival, and growth, potentially improving the likelihood of successful oyster restoration in the near future.

NY/NJ Baykeeper Program's Oyster Restoration Project - The NY/NJ Baykeeper Program implemented several oyster restoration projects in the Hudson Raritan Estuary. These included the Keyport Reef, the Navesink Reef, and the Liberty Island Reef. The most successful of these was the Keyport Reef, encompassing 0.5 acres, created in 2001 by placing 10,000 bushels of crushed clam and oyster shell on the Raritan Bay seafloor. In collaboration with the Baykeeper Program, Rutgers University investigators identified environmental conditions conducive to growth and survival of oysters in the HRE. Study objectives include determination of growth and survival rates, incidence of disease and analysis of tissues to assess contaminant levels. Growth was greater in the subtidal zone relative to the intertidal zone; however, a high incidence of shell-thinning was observed among caged oysters. Dermo and MSX were isolated from 50-60% of test oysters and elevated levels of copper and zinc were found in shell and soft tissue samples from lower Hackensack River oysters.

NYCDPR (NRG) Oyster Reef Restoration - In 2006, the New York City Department of Parks and Recreation, Natural Resource Group (NRG) began an oyster restoration pilot study at the confluence of the Bronx and East Rivers, near Soundview Park, in the Bronx. This is an area where researchers from CUNY had documented the presence of a small population of live oysters. Objectives of the NRG study were to monitor two demonstration reefs built using surf clam shells, and constructed in sequence in 2006 and 2007, for spat settlement, sediment deposition, benthic/epiphytic invertebrate community development, and finfish utilization. After the first year of monitoring the constructed reef it was found that oyster spat were present in the area, although settlement was very limited, and not sufficient to sustain a population on the constructed reefs. However, the reefs were shown to enhance habitat for benthic and epibenthic organisms, as well as for resident and transient finfish (Mass and Ruzicka 2008). This project was conducted in partnership with several local community organizations and educational institutions, and the construction of a 3rd demonstration reef was

completed in 2009. Shortly thereafter, this location was incorporated into HRF/USACE's Oyster Restoration Research Program (ORRP) and underwent additional expansion of reef substrate and supplemental placement of spat-on-shell oysters.

DEP Ecosystem Restoration Pilot Projects in Jamaica Bay, Phase 1- In 2010, DEP, in partnership with Cornell University's Cooperative Extension Service undertook two small oyster pilot projects within Jamaica Bay– the design and construction of a 150 sq. ft. oyster reef constructed from a shell base with a veneer of loose spat-on-shell surrounded by shell bags at Dubos Point, Queens, and in Gerritsen Creek, Brooklyn, the placement of an array of 12 concrete "Reef Balls" remote-set with oysters. These oyster pilot projects were undertaken to evaluate uncertainties associated with the climate and environmental conditions within the bay, to determine if oysters could, in fact, survive in Jamaica Bay, and if they did survive, determine how they might be affected by disease and whether or not they would reproduce. Preliminary results indicated that adequate environmental conditions for oyster growth and survival occurred within Jamaica Bay, as surviving oysters continued to grow until the conclusion of monitoring in 2014. In addition, a diverse assemblage of fish and macrocrustaceans was observed at both pilot-scale reefs. However, high observed rates of epiphyte colonization and sediment deposition on both of the reefs represented issues of concern, and recruitment was not observed at these initial pilot-scale sites.

Oyster Restoration Research Program (ORRP) – In 2009, HRF and the USACE-New York District led a partnership of more than 30 foundations, not-for-profits, and state and city agencies in creating and conducting research at a series of oyster reef research sites in the HRE. The objective of the Oyster Restoration Research Project (ORRP) was to determine where oysters might flourish in the HRE and develop methods best suited for scaling up to large-scale oyster reef restoration. In 2010, experimental oyster reefs (~15' x 30', 450 ft^2 or ~42 m^2) were constructed at five sites: Bay Ridge Flats, Governors Island, Hastings-on-Hudson, Soundview Park and Staten Island near Great Kills Harbor. Reef construction consisted of placement of rock bases followed by a thin mollusk (mostly surf clams) shell veneer. Remotely set spat-on-shell oysters were then spread by hand over the surface of each reef. The constructed reefs were monitored to assess oyster survival and growth, ecosystem services provision, and techniques best suited for future restoration efforts within the HRE. Overall, among all five sites the Soundview reef yielded the best overall development patterns indicating the best prospects for successful restoration efforts utilizing similar reef construction techniques which rely on high natural recruitment and lower energy environments. A second phase of the program involved the establishment of several additional acres of oyster reef habitat at Soundview Park (Grizzle et al. 2013; Lodge et al 2015).

3.0 PROJECT GOALS AND OBJECTIVES

As discussed above there is an ongoing long-term effort with the overarching goal of re-building oyster populations in the New York - New Jersey Harbor region. The present project builds upon earlier work in Jamaica Bay and elsewhere in the Harbor aimed at reaching this goal by restoring self-sustaining oyster populations throughout the region. The extended time (2 weeks or more) of the oyster's larval stages results in the potential for wide dispersal. However, there is no quantitative understanding of the spatial scales involved in larval dispersal and subsequent recruitment (Kennedy 1996; Eckert 2016). In their recent review, Coen and Humphries (2017) discuss proximity to natural reefs and oyster recruitment potential as two of eleven factors potentially important in selecting sites for oyster restoration projects, but there is minimal knowledge of the spatial scales involved (also see Lipcius et al. 2017). Previous assessments of the long-term (>10 year in some cases) success of multiple oyster reef restoration projects in New Hampshire found that the most successful (i.e. highest densities and multiple year classes) projects were constructed within 1 km of a healthy reef (Eckert 2016; Grizzle and Ward 2016). In sum, previous research strongly suggests that reef restoration success may in large part be determined by how close the constructed reef is to its "donor" larval source population. The present project was in part designed to test this notion as well as quantify the habitat provisioning and water filtration capabilities of the experimental reef. The following six major objectives were addressed:

Objective 1: Initial (pre-construction) site suitability survey and selection

Objective 2: Donor reef design and construction

Objective 3: Receiver reef design and construction

Objective 4: Monitoring and maintenance of reefs

Objective 5: Assessment of ecosystem services

Objective 6: Assessment of results in the context of future projects

4.0 METHODS

4.1 Site Survey - Hydrodynamics

In order to characterize baseline conditions at the potential donor reef and receiver reef locations, a hydrodynamic survey using a bottom-mounted Acoustic Doppler Current Profiler (ADCP) was conducted at two locations in the Head of Bay between 7 July 2016 and 5 August 2016. The first deployment (7 July through 21 July) was located in the middle of the study area, while the second deployment (21 July through 5 August) was conducted in the southwest portion of the study area. During both deployments, the ADCP was set to record current profiles in 20 cm depth increments every 20 minutes (Appendix B2).

4.2 Site Survey - Bathymetry and Bottom Type

The major bottom types at the study site were mapped to provide pre-project baseline information on sediment types and data to support decisions for locating the four receiver reef beds. Samples were taken using a Van Veen grab that excavated 0.04 m^2 surface area to characterize the major bottom types in the project area (Figure 4.1). Each sample location was marked by GPS coordinates and the retained sediments inspected in the field and assigned to one of three classes: soft sediments (mud/sand), mixed sediments (sand/gravel), and hard sediments (gravel/rock). A map was produced using ArcGIS software to show the spatial arrangement of the bottom types (Pre-Project Appendix A).

Figure 4.1 Deployment of Van Veen grab used to take pre-construction sediment and benthos samples at project site.

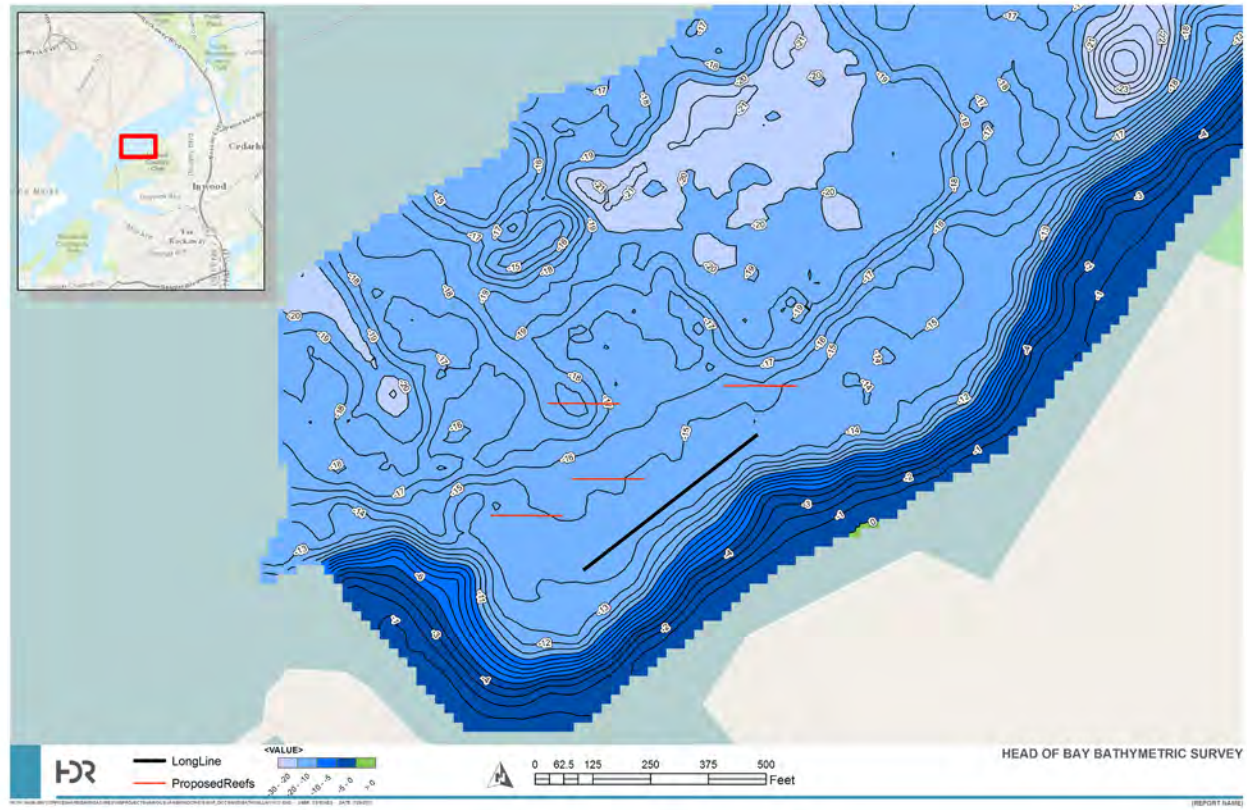


Figure 4.2 Bathymetric profile.

The bathymetry of the study site was mapped to provide information on project depths and slopes. The bathymetry data aided decisions for locating the receiver reefs and logistical considerations for reef construction (Figure 4.2 and Appendix B3).

4.3 Site Selection

The bathymetry, bottom type, and current speed of the sites were evaluated in the selection of the location for the reef beds. Bathymetry and bottom type were relatively consistent between the two primary sites evaluated therfore current speed was the dominant consideration. Measurements associated with constructed reefs in the southeastern United States indicate that the theoretical optimum water speed for oyster growth and survival is between 3 and 6 cm/s for low-profile reefs (approximately one meter height).

Figure 4.3 The OysterGro Donor Reef.

Below this range, nutrient availability declines and parasitism increases, while above this range recruitment may decline (Lenihan 1999). While both of the surveyed locations in the Head of Bay had bottom-averaged velocities primarily below 6 cm/s, the second deployment location suggested that a shoreline feature extending out into Head of Bay at the southwestern edge of the study area may offer some shelter from high current velocities by deflecting the currents to the north and west. This hydrodynamic feature was thought to offer the potential for increased larval settlement and recruitment and was ultimately chosen as the site to construct the reef.

4.4 Donor reef design and construction

The donor reef consisted of a 650-foot longline (Figure 4.3). Four 400-lb mushroom style mooring anchors were selected as the ground tackle after sediment characterization revealed soft sediment throughout much of the project site. Each anchor was followed with 30 feet of 1/2-inch, galvanized chain. The weight of chain served to soften shocks on the anchor system caused by waves or currents and to increase the holding power of the ground tackle system. From the chain, 1/2-inch, polypropylene line was used as an anchor rode. This line ran to floats at the extreme ends of the longline. The longline itself was constructed of 1/2-inch, sinking, polypropylene line.

One hundred and five OysterGro™ units consisting of 12-gauge, vinyl-coated, wire mesh cages supported by high-density, polyethylene, air-filled floats were attached to the line. The units measured 41" x 31" x 9". The floats that suspended the units were constructed of rigid, UV resistant marine plastic.

Each cage had six separate compartments for storing oysters. Soft, plastic mesh ADPI bags held the oysters within each compartment.

The goal was to stock the donor reef with up to 40,000 live adults as allowed by the project permit. The first cohort was supplied by Island Creek Oysters (Duxbury, Massachusetts) and installed on September 1, 2016 with 35,200 adults. To supplement this installation, a second cohort (supplied by Merry Oysters, also in Duxbury, Massachusetts) was installed on October 13, 2017 with 9,000 adults.

The units were attached to the longline of the donor reef using short bridles of 3/8-inch, sinking, polypropylene line. All together the longline consisted

of 650 feet at the surface and an additional 100 feet of subsurface line, chain and anchors. The donor reef was fully assembled prior to the arrival of live oysters which were added after assembly.

4.5 Receiver Reef Design and Construction

The oyster reef installation consists of four 50 meters (m) x 10 m subtidal beds with a total 0.5 acres (0.2 hectare) in combined area. The beds are less than 1 m in height and sit at depths between 4.5 m and 6 m. Each of the four beds is constructed with a base layer of cured Atlantic surf clam (*Spisula solidissima*) shell. One half of each bed is covered with additional clam shell and one half is covered with either cured oyster shell or cured porcelain. For example, "Bed 1A" is constructed entirely of clam shell while "Bed 1B" is composed of clam shell topped with porcelain (Figure 4.4).

In total, the 0.5-acre reef required 700 cubic yards of clam shells, 100 cubic yards of oyster shells and 180 cubic yards of porcelain (Figure 4.5). All material used for the reef beds were "cured" following procedures established by New York State Department of Environmental Conservation (NYSDEC) which requires the materials to remain out of water in piles of less than three feet for a minimum of 6 months in temperatures above 40 degrees (NYSDEC, unpublished memorandum). The NYSDEC limited the acceptable clam shell material to clams harvested from NY waters or from areas north of NY. The oyster shells were collected from NYC restaurants under the BOP's shell collection program. The porcelain materials were collected by DEP under a program to replace school bathroom fixtures with high-efficiency water-saving models (Figure 4.6).

4.5.1 Construction Methods

Prior reef bed construction efforts in the HRE (see Grizzle et al 2013, Lodge et al. 2015) have utilized front-end loaders, cranes and high-pressure hoses to deploy the substrate materials. For this project, a novel approach was designed by the project team and their consultant Ken's Marine Service (KMS) to facilitate a more precise deployment of the materials. KMS adapted a 15 cubic yard salt spreader with a "V" trough and a 24" wide conveyor belt that fed a spinning spreader disk. The width of the material application was controlled by a baffle adjustment resulting in an application range of 4' to 40'. The application depth was controlled by a combination of adjusting the conveyor belt speed, the discharge width and the speed that the spreader was moved (Figure 4.7). The construction method proved to be highly effective for the placement of the oyster and clam shell materials but was not utilized for deploying the porcelain materials because large and rigid pieces were jamming the conveyor belt and spreader disk. The team switched to using front-end loaders to deploy the porcelain materials directly from barges positioned over the reef beds. Utilizing the two methods the team was able to meet the project's goals for material placement of greater than 75% overall spatial coverage within each of the four defined reef perimeters, shell mounds ranging from 6" to 2' in height and 95% of the material within the defined perimeters (Figure 4.8).

4.6 Monitoring and Maintenance of Reefs

The donor reef was maintained in order to ensure the integrity and security of the gear and the safety of the oysters within, and to ensure that the oysters had adequate water flow by removing marine fouling organisms through desiccation.

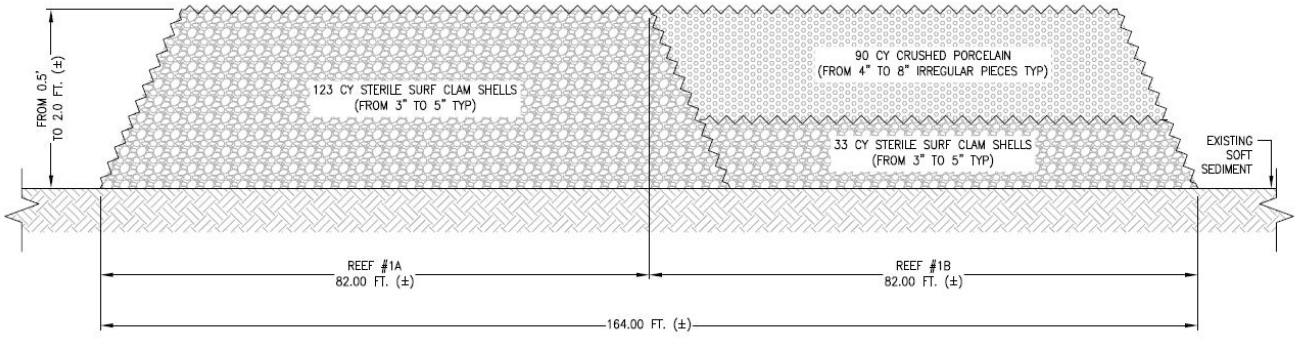


Figure 4.4 Side profile view of receiver reef bed #1 illustrating material composition of one of the reef beds.

Figure 4.5 Receiver reef materials: seasoned clam shells, oyster shells and crushed porcelain

Figure 4.6 Porcelain Material "curing" prior to placement

Figure 4.7 Picture of placement materials by the spreader.

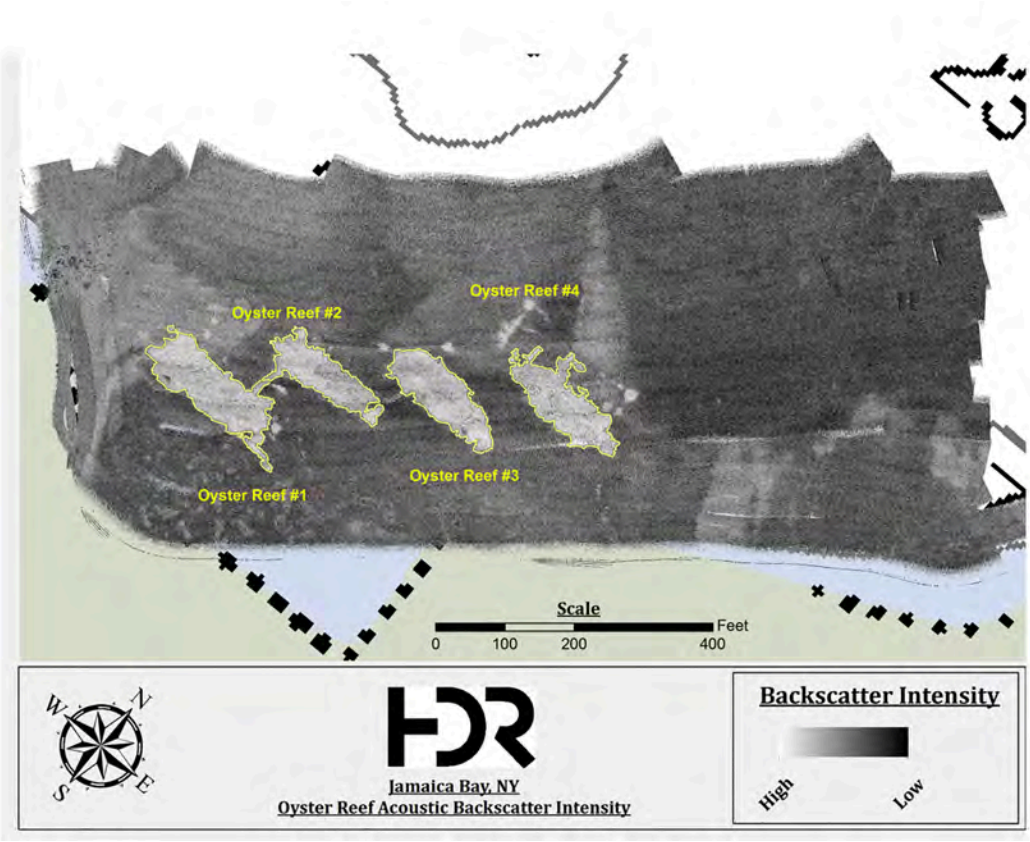


Figure 4.8 Materials placement within defined parameters

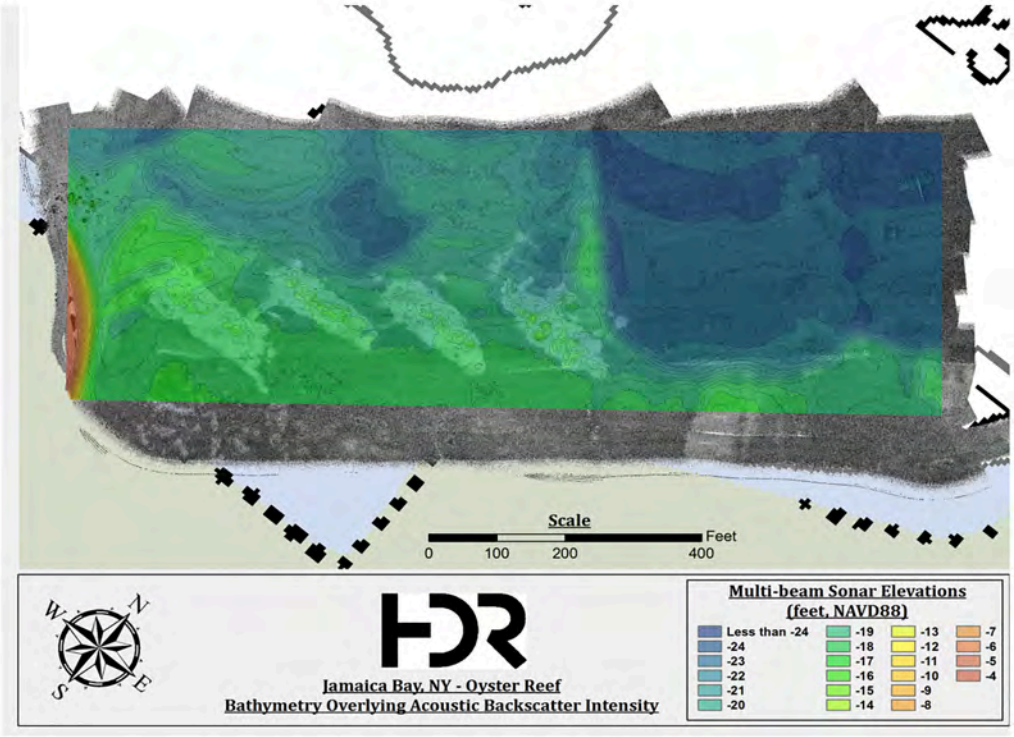


Figure 4.9 HDR image from post-construction bathymetry.

Monitoring activities were undertaken to 1) quantify changes in the installed oyster cohorts in the floating donor reef, 2) to assess temporary water quality improvements resulting from the presence of the donor reef, 3) to quantify the functional habitat of the installed beds, and 4) to survey the subtidal oyster beds for new oyster recruitment.

4.6.1 Receiver Reef Construction Assessment

Following the construction of the receiver reef, a high-resolution hydrographic survey was completed in November 2016 and then repeated in October 2018 using a multi-beam echosounder (MBES) to measure the oyster reef elevations (bathymetry) and acoustic backscattered intensity (side-scan sonar) of the reef and surrounding seabed (Appendix B3). The post-construction hydrographic surveys yielded a full-surface, high-resolution dataset with a vertical accuracy within 0.1 to 0.2 feet for comparison between surveys and for comparison of as-built conditions to specified design criteria.

4.6.2 Receiver Reef Development

Development of the receiver reef would be dependent on successful recruitment to the constructed reef beds, presumably from donor reef oysters but potentially from wild oysters in the region. Therefore, the overall "recruitment potential" of eastern Jamaica Bay was characterized by three methods: direct sampling of the receiver reef in Head of Bay, deployment of spat collectors and collection of plankton samples in eastern Jamaica Bay. The reef bases were sampled using three different methods: patent tongs, diver-excavated quadrats, diver deployed video transects and trays filled with reef base materials.

4.6.2.1 Patent Tong Sampling (2017)

Six replicate patent tong (0.1 m^2) samples were collected per reef (24 samples total) on October 19, 2017 (Figure 4.10). All substrate material (shell and porcelain) collected was examined in the field for live oysters and returned to the water after examination. This sampling methodology was discontinued after 2017 and replaced by the diver-based excavations and video transects.

4.6.2.2 Diver Excavation and Video Transects (2018-2019)

In October 2018, diver excavation quadrat sampling and video transects were used to monitor the four receiver oyster reef beds for oyster spat recruitment. At each of the four reef beds, eight haphazardly tossed 0.25-meter quadrat samples were collected and brought to the surface for examination. For the video transects, divers swam along east-west transect lines and recorded high-resolution video. The video files were reviewed in the lab to identify and measure any oysters found.

Figure 4.10. Patent tongs used in 2017 to take quantitative samples of the constructed reef bases.

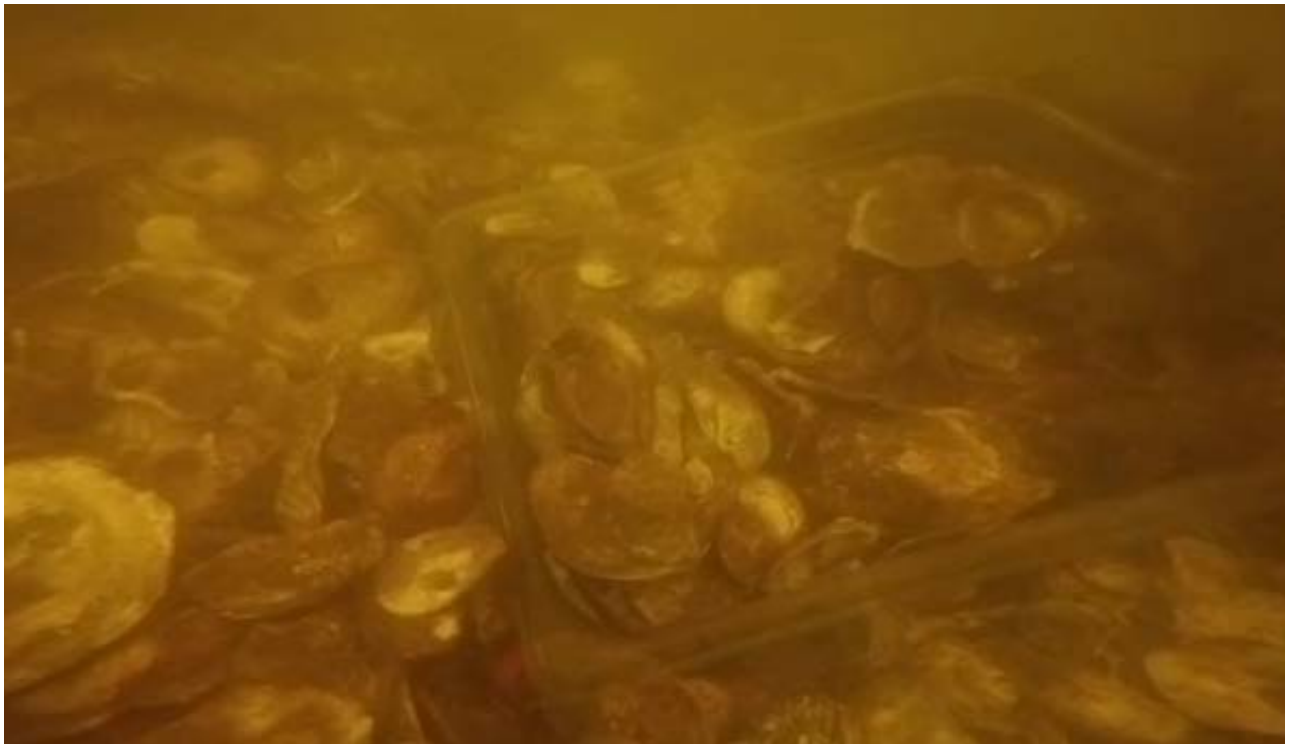
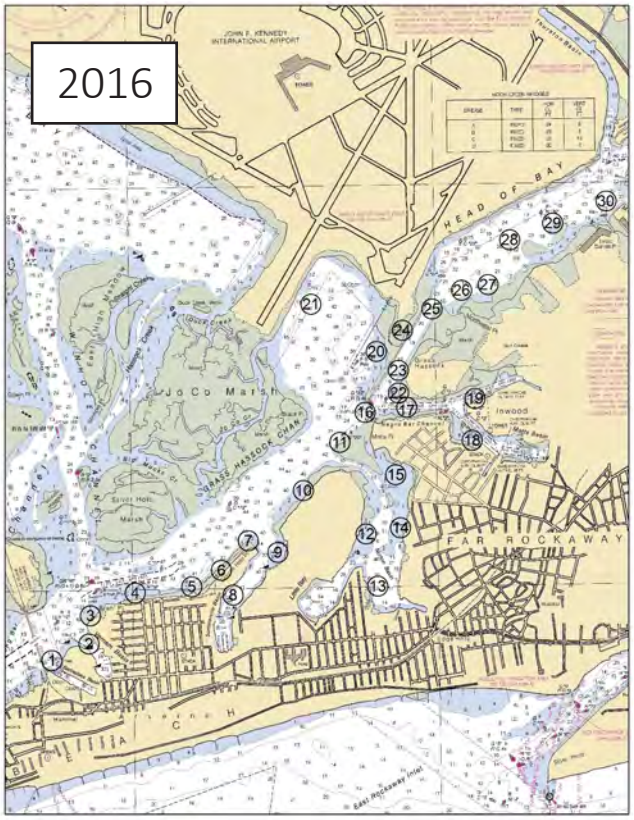
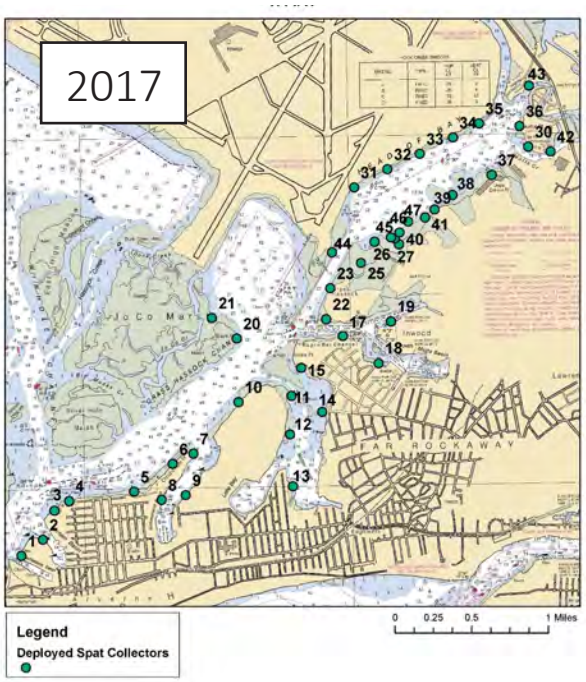


Figure 4.11 Underwater picture of benthic trays on a clam shell reef bed.

Figure 4.12 Divers retrieving trays containing materials used to construct reef bases and used to characterize biotic communities that developed on the constructed oyster reefs.





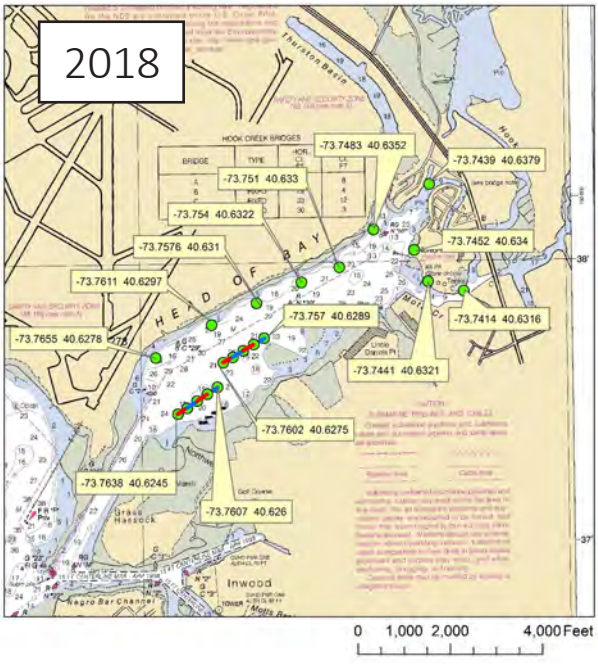


Figure. 4.13 (Above) Spat Collector and Sampling Locations

Figure. 4.14 (Left) Plankton Sampling near Oyster Donor Reef

4.6.2.3 Benthic Community Assessment (2018-2019)

A total of 24 plastic trays (0.1 m^2 surface area) were filled with one of the materials (clam shell, oyster shell, or porcelain chips) used to construct the four reef bases and deployed on August 1, 2017 (6 trays per reef) approximately one year after base construction by nestling the trays into the top of each reef base (Figure 4.11).

A total of 15 of the trays were retrieved on August 14 – 15, 2018 by divers (Figure 4.12). The contents of each tray were washed on a 4-mm mesh sieve. The organisms were separated from the base material, placed into a labeled plastic bag, and refrigerated until processed in the laboratory. Processing consisted of sorting the organisms into major taxa under a 3x lens, identifying them to the lowest taxonomic level practical using standard keys (Weis 1995, Pollack 1998), and counting and weighing (wet weight to nearest 0.1 g).

4.6.2.4 Spat Settlement and Plankton Monitoring

Spat collectors (Figure 4.13) were deployed at multiple sites throughout eastern Jamaica Bay during 2016, 2017, and 2018. The design and the deployment methods for this project's spat collectors were similar to those used for the Tappan Zee project (AKRF 2016a, AKRF 2016b) on which oyster spat successfully recruited. Each spat collector consisted of a 28" long x 4" diameter segment of corrugated plastic drainpipe and had a total outer surface area of approximately 0.25 m^2. Two spat collectors were strung together horizontally, anchored by a concrete block, held upright in the water column by submerged floats, and deployed at each site. The number of collectors, their deployed locations and the method of retrieval was adapted over the course of the study to address several concerns: 1) The lack of observed spat recruitment, 2) the loss of collectors – to theft/vandalism or natural forces and 3) the difficulty of retrieval for monitoring. In all cases, the spat collectors were deployed at a minimum depth of 2 feet below mean low water (MLW) to ensure that the collectors were always submerged and not impacted by boats. During each successful retrieval event, one of the spat collectors was scraped clean. This was done to ensure that one of the spat collectors was returned with a clean surface area for oyster settlement while avoiding the possibility of removing any recently attached but unseen oysters from the other collector. Monitoring of the spat collectors occurred monthly from June through September in 2016, 2017 and 2018. During each monitoring event a minimum of 20 collectors were retrieved and examined for the presence of spat.

Plankton tows were conducted weekly by HRF and Cornell Cooperative Extension (CCE) from late July 2018 through September 2018 (Figure 4.14). After the first three plankton samples were collected and preserved whole in late June, the subsequent 18 samples (3 at each weekly collection) were split into>180 µm and 64-180 µm fractions by washing plankton through 180 µm and then 64 µm sieves before preserving each size fraction separately. One of the June samples was fractionated in the lab, making for a total of 40 samples genetically analyzed. Early D-stage *C. virginica* larvae are approximately 70 µm in size and pediveligers competent to settle are about 320 µm, so these methods separated the larval size distribution roughly in half.

4.6.2.5 Plankton DNA Analysis

Cornell University analyzed the plankton material for eastern oyster (*Crassostrea virginica*) DNA presence/absence. Each sample fraction was washed with distilled water through a 40 µm sieve to remove ethanol, then all material was transferred to PowerBead tubes for the initial stage of DNA extraction using Qiagen PowerSoil kits following the manufacturer's protocol. Bulk plankton DNA was expected to be a diverse mix from many species, with a potential for *C. virginica* to represent a tiny fraction. Thus, DNA was analyzed at full concentration, and also at 1:10 dilution to test for PCR inhibition.

To amplify *C. virginica* DNA and be certain that no other bivalve was inadvertently being amplified (false positive), the lab designed PCR primers that were predicted to be specific to *C. virginica* at a portion of the cytochrome oxidase I (COI) gene. The lab designed the primers from an alignment of mtCOI sequences from *C. virginica* and several other bivalve species: *Mya arenaria, Gemma gemma, Argopecten irradians, Mytilus edulis, Mulinia lateralis, Geukensia demissa, Mercenaria mercenaria, Rangia cuneata, Spisula solidissima solidissima* and *S. solidissima similis*. Species specificity was predicted when the 3' end of oligonucleotide primers matched *C. virginica* COI sequence but had at least 2 nucleotide differences with all other bivalves. After preliminary testing on reference material for all bivalves listed above, one pair of primers was selected for use in the plankton assay: Cv_ssCOI-3F, 5'-TTTAGGTCTCTTATTCGCTGAAGT-3' and Cv_ssCOI-2R, 5'- AAAGCATTTAATCGAGG-GAACTG-3'. This primer pair was then jointly optimized for species specificity and strong amplification.

All plankton DNA extracts were initially tested using PCR reactions consisting of: 0.48U Invitrogen recombinant Taq, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.5 mg/mL bovine serum albumen, 0.2 mM each dNTP, and 0.4 mM each primer, in a 20 µl total reaction volume. Each plankton sample was tested using 1 µl concentrated DNA stock and 1 µl of a 1:10 dilution of DNA, the latter to test for PCR inhibition. PCR conditions were 95°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 60 seconds, 72°C for 60 seconds, followed by a final extension of 72°C for 5 minutes. All experiments included a negative (water substituted for template) and positive control.

PCR product was run alongside a DNA size standard and visualized after electrophoresis in a 2% agarose gel. For samples with a band at the expected size (about 180 bp), the remaining PCR product was sent for Sanger sequencing in two aliquots: one using the Cv_ssCOI-2R primer and one using an internal forward primer CvCOIspec-L (5'-GCTAAATTTTTAGAGC CTGTTGTG-3'). The amount of PCR product sent for sequencing ranged from 2.5-5 µl, depending on the brightness of the band. Resulting sequences were compared to sequences in Genbank using BLAST to determine species ID. If all the top hits (most similar database sequences) for both the forward and reverse strand were *C. virginica* then the sample was considered positive for this species. Samples that did not show bands in the initial PCR were retried up to six times using the same conditions, except that only the concentrated stocks were used, and 40 cycles were used instead of 35. Any positive bands were sequenced as above.

4.6.3 Donor Reef Maintenance and Assessment

The OysterGro™ donor reef was installed on September 1, 2016 with an expected project term of two seasons. The donor reef was visited for inspection, maintenance, and/or monitoring 16 times in 2016, 27 times in 2017, 28 times in 2018, and four times in 2019. Standard inspection activities included counting OysterGros™ and making a visual assessment of the line to look for worn or loose sections and attachment points. Maintenance activities included pulling the donor reef to shore for desiccating, and in 2018, power washing. Maintenance activities also include replacing worn line sections, re-aligning the line between each cage's pontoon set, draining flooded pontoons, replacement of cracked caps, and replacement of worn or missing tags. Monitoring activities included removing bags of oysters from OysterGro™ cages for monitoring growth and mortality (performed at the BOP lab on Governors Island).

4.6.3.1 Oyster Growth and Mortality

Adult oysters in the donor reef OysterGro™ cages were monitored for growth and mortality approximately once per month from May through December of each year. During each sampling, 30 adult oysters were haphazardly sampled for shell height measurement from one bag for each cohort. Oyster shell height was measured using calipers. The total number of living and dead oysters from each bag was also recorded in order to determine the proportion mortality for each subset of oysters.

4.6.3.2 Disease

Stony Brook University Marine Animal Disease Laboratory (SBU MADL) performed disease diagnostics for the project. Dermo diagnosis was made using FTM (fluid thioglycollate medium) on mantle and rectal tissues. MSX diagnosis was made using histopathology on formalin-fixed sections. SBU MADL also provided data on shell height, condition index, stage of gonad development, and sex ratio (see below). Samples from June were assumed to best represent the reproductive status of oysters, and samples from October represented the maximum disease loads.

4.6.3.3 Reproductive Status

Reproductive status of the donor reef oysters were assessed by SBU MADL. The tally of oysters from each sex and in each reproductive stage was provided and ranking was assigned from the following 10 stages:

I: Inactive (no gonadal activity, resting)

G1: Gametogenic I stage; gametogenesis has begun; no ripe gametes visible

G2: Gametogenic II stage; first ripe gametes appeared; gonad developed to about one-third of its final size

D1: Developing I stage; Gonad increased in mass to about half the full ripe condition; each follicle contains about equal proportions of ripe and developing gametes

D2: Developing II stage; Gametogenesis still progressing; follicles mainly contain ripe gametes

R: Ripe stage; Gonad fully ripe, early stages of gametogenesis rare; follicles distended with ripe gametes; ova compacted into polygonal configurations; sperm with visible tails

S1: Spawning stage I; Active emission of gametes has begun; gametes density reduced

S2: Spawning stage II; Gonad about half empty

S3: Spawning stage III; Gonadal area reduced; follicles about one-third full of ripe gametes

S4: Spawning stage IV; Only residual gametes remain; some may be undergoing cytolysis

Undetermined: these are oysters where gonad tissue was rudimentary (completely undeveloped as is the case in very young oysters) or absent

Gonad conditioning was also assessed on oysters from the Head of Bay donor reef between August 10, 2017 and October 23, 2017. Oysters were held dry and refrigerated until they could be processed. Shell heights of all oysters were measured prior to shucking. After shucking, the conditions of the gonads were assessed via a qualitative scoring system (Table A3). The gonad assessment system scores the oyster's gonads based on four criteria (venation, plumpness, extent of gonads, and color), with a score of 0 indicating "no gonads present" and a score of 4 indicating "ripe and ready to spawn."

4.6.3.4 Condition Index

The condition index was calculated following the method of Filgueira et al. (2013) method (CI = (dry meat weight / dry shell weight) x 100). The condition index of oysters was measured in spring and fall of each year. Condition index was calculated as (dry mean weight / dry shell weight) x 100. In 2018, the condition index was measured each month from May through August to track changes in results that may be triggered by spawning events.

4.6.4 Decommissioning of Donor Reef

In the spring of 2018, project partners agreed to extend the term of the project and to maintain the donor reef for an additional 12 months.

In November 2018, the donor reef cages and the line began to show signs of wear consistent with extended time in saltwater and sun exposure, friction caused by the normal movement of the line related to wind and tide conditions in Head of Bay. Many of the cages had begun to rust as a result of damage caused by repeated contact with each other. Some PVC-coated pontoon wires were snapped due to rust build-up. Several repairs were made to the longline, several partially sunken units were hoisted and drained, and cracked caps were replaced. Communication with OysterGro™ revealed that other users of cages manufactured during the same time period as the ones used by this project had experienced similar problems with the equipment and that OysterGro™ has since modified the design and materials to increase the longevity of the gear (Ben Lord, OysterGro™, pers. comm.). BOP recommended dismantling the line and placing the oysters on-bottom over the center two reef beds.

To eliminate the risk of losing the gear and oysters as the line and gear continued to deteriorate, the donor reef oysters were placed on the bottom on December 14th, 17th and 19th of 2018 and March 13-14, 2019. This provided the additional benefit of increasing the chances of reproductive success by increasing the density of the oysters, resulting in a population that more closely resembles a natural oyster reef.
Long-term, the new aggregated arrangement will likely also result in enhanced water filtration impacts and habitat value. Movement of the oysters over winter when predation activity is low likely also minimized mortality.

Donor Reef decommission activities were completed using two vessels (two 47' ex-USCG buoy tenders) in the following manner:

The outside corners of the two center reef beds (2 and 3) were located using coordinates confirmed during reef bed installation and previous monitoring dives. Sonar was used to confirm position over the two target beds and the approximate center of each bed was marked with a float.

Individual OysterGro™ cages were raised using an onboard crane. The oysters within each OysterGro™ cage were removed and placed into holding containers. A vessel was then positioned over the approximate center end of each of the two center reef beds and crews distributed the oysters both by using shovels and by hoisting larger containers of oysters with the crane and pouring them as the tender moved along the centerline of each bed. Oysters were deployed over the length of the center area of each 500-meter square reef in an approximately 300-meter square rectangle with an expected average distribution of approximately 50 oysters per meter square.

Figure 4.15. Temporary field flume to measure long-term (~40 hours) water filtration by oysters in eight bags on the long line. Note Sontek ADV current meter and Manta datasonde mounted vertically on left end of flume.

Approximately 24,000 oysters from the Island Creek cohort and 6,200 oysters from the Merry Oysters cohort were combined for distribution. Cohorts were combined as they were removed from the bags for mixed distribution on the two reefs. Approximately half the oysters were deployed on Reef 3 in December 2018 and half on Reef 2 in March 2019.

4.6.4.1 Density of Planted Oysters

Diver surveys of the two receiver oyster reef beds that received oysters from the "donor reef" (Reef 2 and 3) were conducted in July 2019 to provide a baseline assessment of planted density, to document growth and survival of the planted oysters, and to identify any new oyster spat recruitment. Divers started at the western edge of each reef and swam 20 ft. east along the east-west transect line. At the first 20 ft. interval, a 0.25-meter quadrat was haphazardly tossed south of the line. The next sample was taken from the quadrat tossed north of the line from the second 20 ft. interval. The divers continued in this manner until reaching the end of each reef. The divers excavated all surface material within the quadrat and placed it in a mesh bag for processing on the surface. The number of oysters collected from each quadrat and the shell

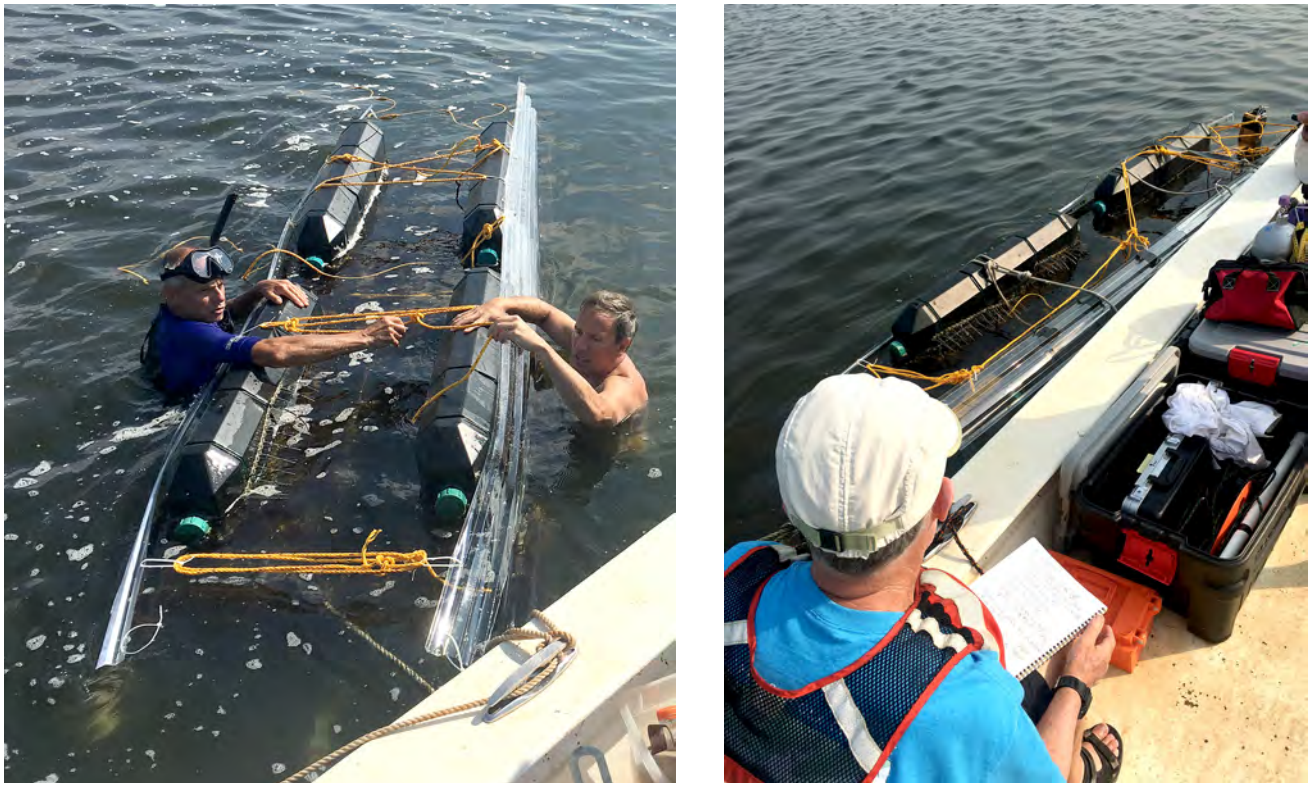


Figure 4.16. Left: Constructing temporary field flume (August 15, 2018) used to measure water filtration by oysters in bags. Right: Field flume anchored in position near channel; note one of two Manta datasondes mounted vertically on each end of flume is visible.

height was recorded. Divers collected 8 samples from Reef 2 and 7 samples from Reef 3.

During operations, divers reported high densities of oysters in patchy distribution throughout both deployment areas and an absence of oysters outside of the areas (off-reef), so a second method for assessing distribution was devised in the field. For this, a pair of divers swam the length of Reef 3 and noted the density of oysters at a distance of approximately 4' both to the north and to the south of the center line. In this way, divers recorded two observations eight feet apart every 10' for a total of 32 observation points on Reef 3. Density was described as either 'none', 'few', 'many', or 'abundant.'

4.6.4.2 Size, Proportion Mortality, and Pathology of Planted Oysters

2018 - In December 2018, 6 Island Creek and 4 Merry Oyster bags were monitored prior to deployment on the reef. The average height of living oysters and the proportion mortality for each bag was recorded.

2019 - In July 2019, oysters deployed on the receiver reef were monitored. The height of all oysters and the proportional mortality for each quadrat sample was recorded. Lab results are pending.

4.7 Assessment of Ecosystem Services

The assessment of ecosystem services focused on the provision of new oyster reef habitat of the receiver reef and water filtration capacity of the donor reef.

4.7.1 Water Filtration

The water filtration capacity of the oysters on the donor reef were quantified in 2017 and 2018. Handheld fluorometers (calibrated to measure chlorophyll *a* [CHL] concentrations) were used to quantify seston uptake over short-term (<10 min) intervals following the methods in Grizzle et al. (2006, 2008). Datasondes with fluorometer probes were also deployed for up to 40 hours upstream and downstream of the longlines to quantify longer-term seston removal. Water filtration (based on differences between average up-current and down-current CHL measurements) were calculated using the equation below which yields the overall (%) decrease (or increase when negative) in CHL as water flows over the oysters. It should be noted that the upstream vs. downstream protocol assumes a well-mixed water column and no horizontal transport of seston into or out of the sampling area.

$$\% \text{ CHL removal} = (C_1 - C_2) / C_1 \times 100$$

Where C_1=upstream CHL concentration (µg/L) and C_2=downstream CHL concentration (µg/L)

During 2017, the handheld fluorometers were used to make multiple measurements (from August 22 – 24) of CHL concentrations very close to the oyster bags in several locations along the longline compared to a meter or so away from the longline. The close measurements were assumed to represent filtered water and the outside unfiltered water, but no water flow speed and direction measurements were made. Longer-term measurements of water filtration were recorded using a temporary "field flume" similar in principle to Dame's (2012) benthic ecosystem tunnel (BEST) apparatus which constrains water flow so it is unidirectional (also see Comeau et al. 2010). The field flume consisted of two sheets of flexible roofing material wrapped around the bottoms of two of the oyster bags and floats that had been removed from the longline. The design was such that water flow could only enter or exit from each end (Figure 4.15). A datasonde and acoustic doppler velocimeter (ADV) were placed on one end and a second datasonde was placed on the other end. The sensors of all three instruments were positioned at the same height in the water column as the oyster bags (~15 cm below the water surface). The field flume was anchored from the end with a datasonde and ADV so that end would always be "upstream" as the apparatus moved with the prevailing water currents. The datasondes and ADV were set to record at 15-min intervals, and the apparatus was deployed from August 22 - 24, 2017.

During 2018, all fluorometry measurements were made using a field flume similar in design to the model used in 2017 (Figure 4.16). The field flume consisted of sheets of flexible roofing material wrapped around the bottoms and sides of two of the oyster bags and floats that had been removed from the longline.

A Manta datasonde was placed on each end. The datasonde sensors were positioned and all water flow measurements were made at the same height in the water column as the oyster bags (15 - 20 cm below the water surface). The field flume was anchored from both ends near the main channel and just west of the longline. The datasondes had sensors for chlorophyll *a* (CHL), salinity, temperature (°C), and dissolved oxygen (DO) and were set to record at 2-min intervals. Data were recorded for approximately 28 hours, beginning at 10:38 am on August 15 and ending at 2:46 pm on August 16, 2018. Handheld fluorometers (Seapoint Sensors probes with Dataron recorders calibrated to

measure CHL) also were used to quantify CHL removal over short-term (<1 hour) intervals. A handheld YSI water quality meter was used to measure water parameters sporadically and a Marsh-McBirney electromagnetic current meter was used to measure water flow speeds at multiple positions in and around the field flume in order to characterize water flow patterns and speeds. Releases of rhodamine dye (Kingscote FWT Red 50) were also made sporadically to visualize flow patterns in and around the flume.

4.7.2 Habitat Provision and Substitution

The purpose of this task was twofold: to characterize the ecosystem service of habitat provision provided by the receiver reef, and to assess differences between the benthic communities that existed before the receiver reef was constructed and the communities that existed on the reef after 2 years (i.e., to assess habitat substitution).

Habitat provision was assessed by characterization of the macrofauna extracted from the 24 plastic trays filled with reef base materials (see details in "Receiver Reef Development" section above). Habitat substitution was assessed by comparing the macrofauna collected in the plastic trays with macrofauna collected by the Van Veen grab samples that were taken before the reef bases were constructed (see details in "Site survey: benthic community" section above).

5.0 RESULTS

5.1 Site Survey - Hydrodynamics

Results of the first deployment showed a general bi-directionality of the prevailing currents in the study area with typical currents on an ebb and flood tide approximately 4-8 cm/s in the near-bottom zone (i.e. in the bottom 3 meters of the water column) (Figure 5.1). Surface currents in the upper 4 meters of the water column were generally stronger (8-12 cm/s) but were less uniform in direction. Predominant current direction was southwest for ebb tide and northeast for flood tide (Appendix B2).

5.2 Donor Reef Oyster Development

The assessment of donor reef development focused on the growth, mortality, oyster condition and prevalence of disease (MSX and Dermo).

5.2.1 Growth

Oyster shell heights for both cohorts are presented in Figures 5.2 and 5.3 to give a general sense of seasonal growth over time. The growth curve of the Island Creek oyster measurements is somewhat irregular due to the variability of the initial sizes of oysters which were contributed by multiple Duxbury, Massachusetts area growers. Both cohorts grew slowly but steadily between May and November of each year, as expected for adult oysters, with the younger Merry Island cohort gaining approximately 24 mm in height between December 2017 and December 2018 and the older Island Creek cohort gaining approximately 19 mm between September 2016 and December 2018.

2019 - On July 16, 2019, the average size of live oysters from the combined cohorts that were collected from Reef 2 and 3 was 132 mm and 144 mm respectively. The average total height of all live oysters was 139 mm. This combined average demonstrates an expected healthy measure of growth for adult oysters placed on-bottom between the months of May and July of 2019.

The growth of oysters while in the donor reef and continued growth after placement on the receiver reef show that Head of Bay can serve as a productive growing environment for adult oysters.

5.2.2 Density of Planted Oysters

Quadrat Surveys: Reef 2 had an average of 5 total (live plus dead) donor reef specimens and 3 live oysters per meter square. Reef 3 had an average of 7 total (live plus dead) donor reef specimens and 4 live oysters per meter square. Figure 5.1.3 shows a representative sample taken from reef bed #2.

Diver Observation Surveys: Eight observation points were scored as 'none', sixteen as 'few', six as 'many', and six as 'abundant'. The relative diver-reported densities align approximately with the densities of the areas sampled with quadrats (Figure 5.4). The diver observations provide greater spatial coverage than the quadrat samples. Together, the two surveys support the assumption that oysters likely came to rest on-bottom in relatively patchy, high-density aggregations within the targeted areas and remained on the reef between December 2018 and July 2019.

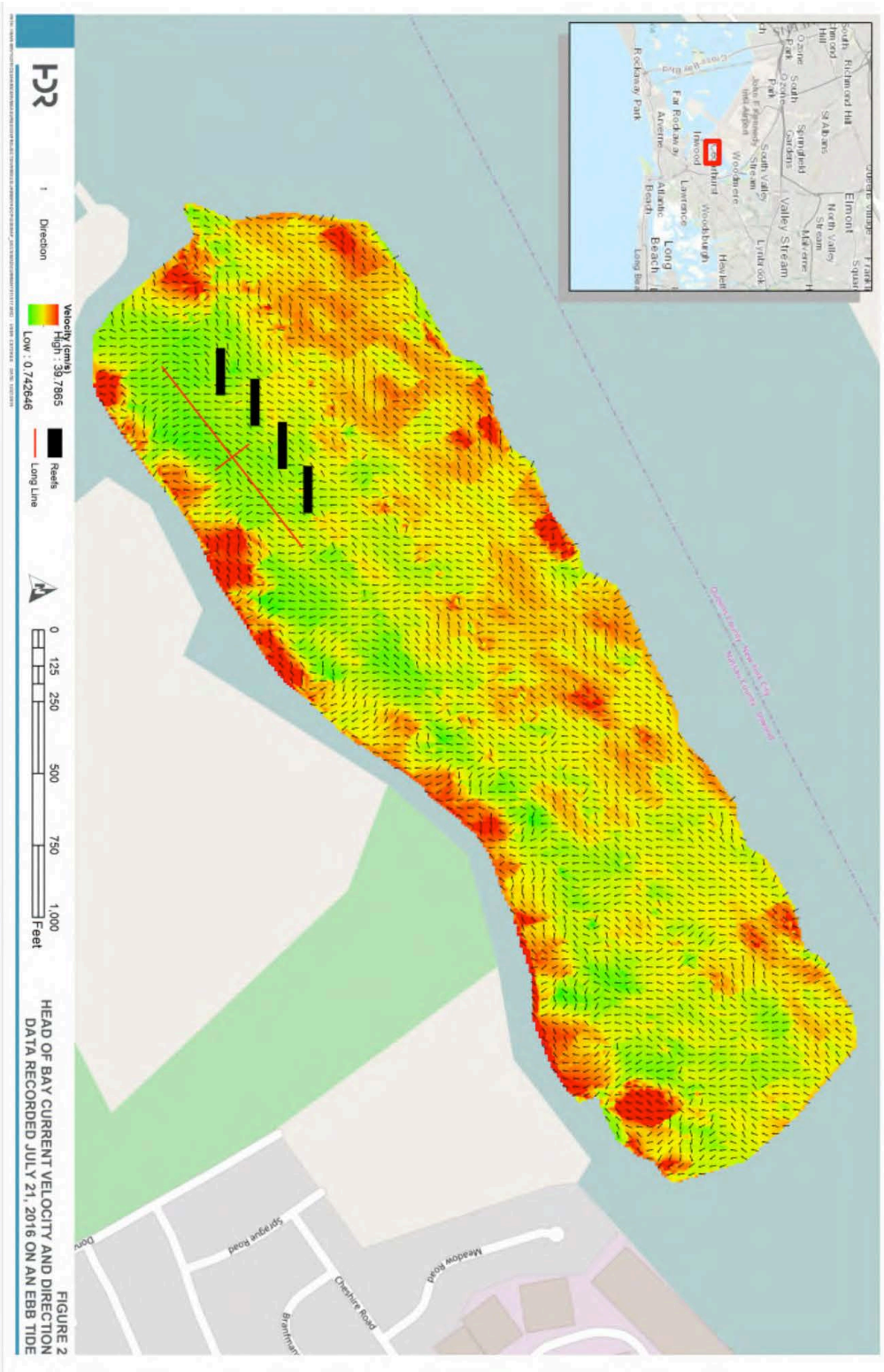


Figure 5.1. ADCP measurements of current velocity and direction.

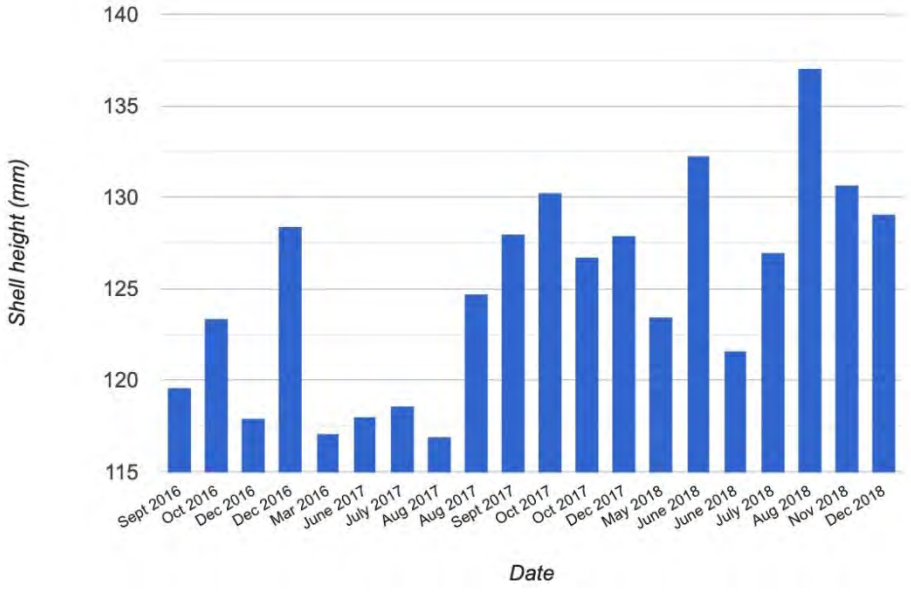


Figure 5.2 Shell height, Island Creek cohort.

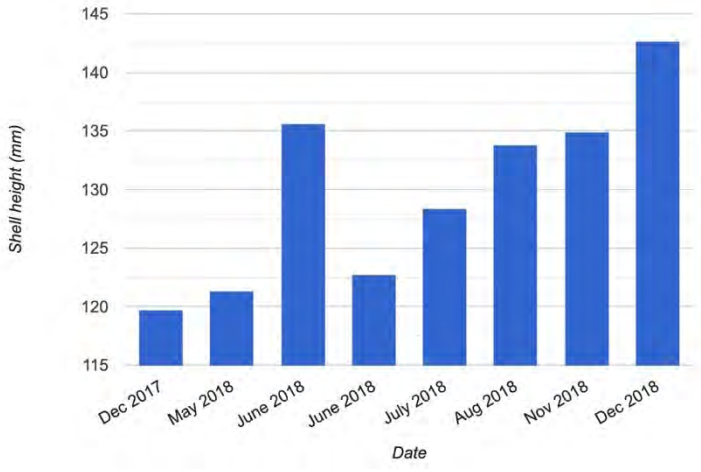


Figure 5.3 Shell height, Merry Island cohort.

While density results from the quadrat survey were lower than expected, it is likely that the eight 0.25 meter square samples collected per reef were too few and too small to characterize the distribution of the oysters across the large reef areas given the method of deployment. Diver observation is a suitable method for recording a greater number of sampling points in a shorter amount of time, however frequent low visibility presents a challenge to using this method.

5.2.3 Mortality

The total number of live and dead oysters in the sampling bags were counted during most sampling events (but not all). The proportion mortality is presented in Figures 5.5 and 5.6.

5.2.4 Gonad Development

On all four dates a portion of oysters received a score of 4, indicating that some oysters were ripe and ready to spawn (Table 5.1, Figure 5.7). August 10, 2017 and September 11, 2017 had the highest proportion of oysters received a score of 4 with 27% and 30% respectively. Across all sampling dates, very few oysters received a score of 0, representing 9% or less of the oysters sampled.

5.2.5 Condition Index

The physiological condition of an oyster can be measured by its condition index—the ratio of meat weight to shell weight or meat weight to shell volume. The condition index (CI) of an individual oyster will change during the year. Factors influencing CI include the density of filter feeders (not just oysters), gonadal condition, disease prevalence and intensity, presence of parasites such as pea crabs and changes in temperature, salinity, and food availability. In general, a higher condition index indicates a fuller shell cavity and suggests a healthier oyster. (Figure 5.8)

5.2.6 Disease

Oyster diseases have caused mass mortalities of both wild and cultivated populations for over 50 years in our region. Two protozoan parasites, commonly known as Dermo and MSX, negatively affect overall health, growth, reproduction and survival of oysters. While Dermo is directly transmitted from an infected oyster to a naïve one, MSX is not. There is a suspected alternate host necessary to transmit MSX—this host has not been identified despite decades of research. See Table 5.2 for tabular results of these diseases from both cohorts.

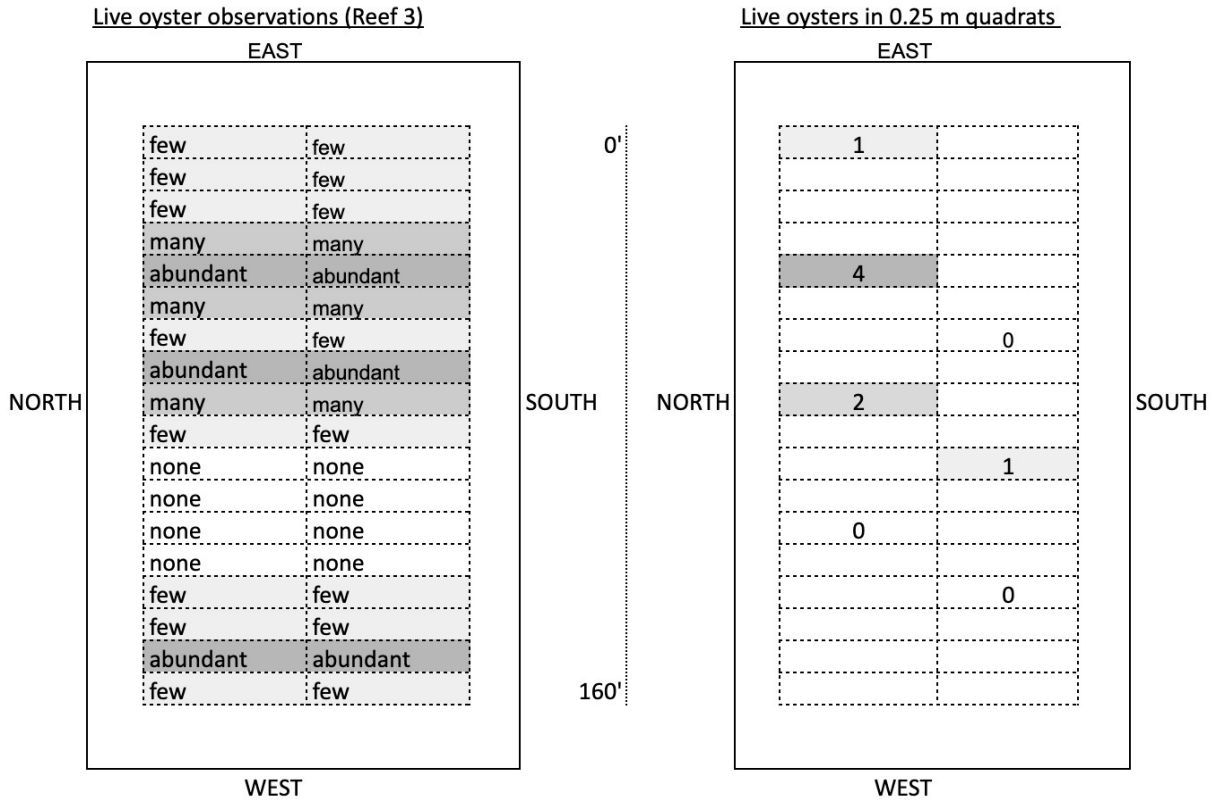


Figure 5.4 Diagrams representing density of oysters planted on Reef 3 using diver observations (left) and 0.25 meter square quadrats (right).

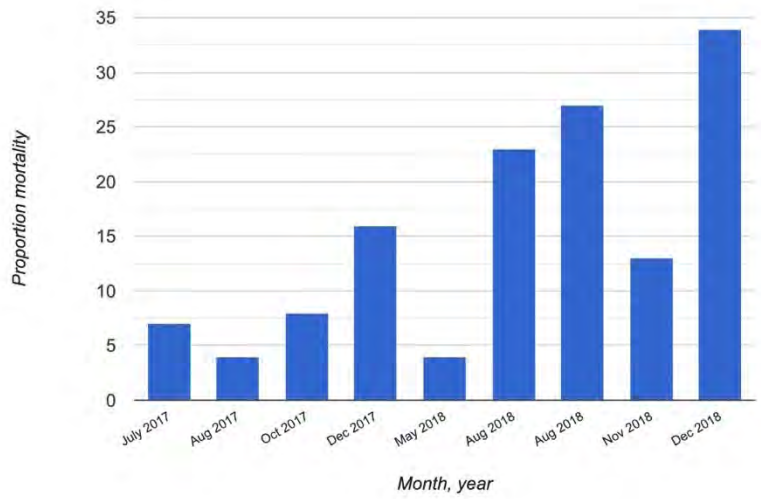


Figure 5.5 Proportion mortality of Island Creek cohort over time. Note: oysters were sampled both during the first and last weeks of August.

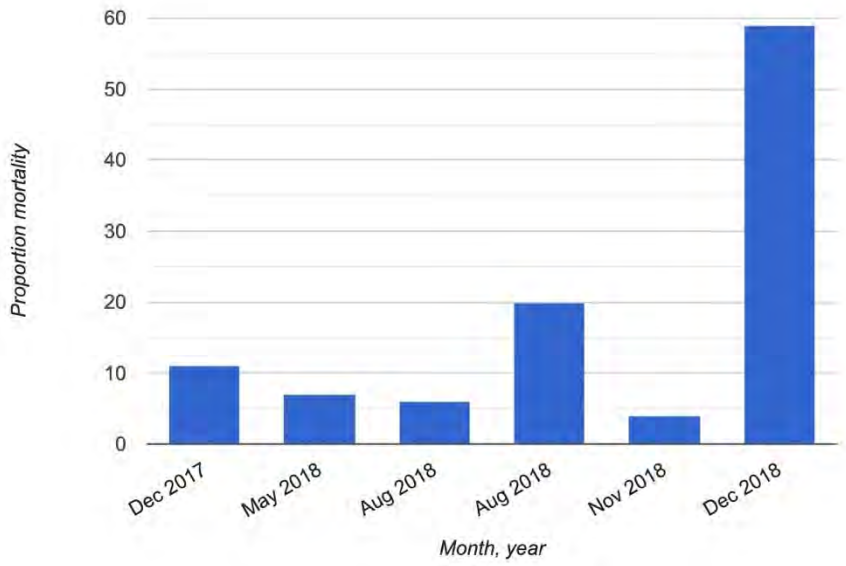


Figure 5.6 Proportion mortality of Merry Oysters cohort over time

Dermo infections usually takes two to three years to intensify and become fatal to an oyster. From the initial Head of Bay sample, Dermo (*Perkinsus marinus*) was found in 23% of the Island Creek oysters. Prevalence of Dermo in the Merry Oyster cohort reached a maximum of 10% in the August 28, 2018 sample.

MSX (*Haplosporidium nelsoni*) affects young-of-the-year animals at a higher prevalence than adults. In the first sample from the donor reef, taken on October 4, 2016, MSX was observed in one individual (3.3% of sample) from the Island Creek cohort. Only one subsequent sample showed one oyster (3.3%) with a moderate MSX intensity; that sample was taken on August 28, 2018 and was from the Merry Oysters cohort.

In general, MSX was not epizootic and did not appear to cause excessive mortalities nor reduced condition indices. The prevalence and intensity of Dermo found in this study (medium prevalence, low intensity) was similar to levels found in most east coast farmed oysters. Dermo is likely to have caused some of the mortality observed throughout the study and most, if not all, of the mortality that occurred in December, 2018.

5.3 Receiver Reef Assessment - Reef Area/Shape and Reef Height

The receiver reef was designed as four separate reef beds each approximately 10 meters by 50 meters in size for a total acreage of approximately 0.5 acres. The new substrate material was designed to raise the bottom approximately 6" to 24." The installation methodology proved effective and the base materials were placed within the designed footprints at the required elevation above the existing bottom (Appendix B3). A qualitative diver survey assessment and a high-resolution multi-beam and side scan sonar survey conducted after the initial construction confirmed the installation achieved the project design objectives. Subsequent annual qualitative dive survey assessments were used to evaluate changes in reef characteristics over time. The final qualitative dive survey conducted in August 2018 provided evidence of nominal subsidence and siltation on all the reef beds. The reef materials still cover the majority of their designed footprint and the upper veneer layer of material at each reef was still present.

5.4 Functional Assessment of Receiver Reef Beds

5.4.1 Macrofaunal Community Assessment

Total invertebrate community densities in the plastic trays filled with reef base materials ranged from 3 to 112 individuals/0.1 m^2 and taxonomic richness ranged from 2 to 8 taxa/0.1 m^2, and there were no apparent differences among the four reef beds or three reef base materials (clam shells, oyster shells and porcelain) (Table 5.3). In addition to the data from the trays, divers who retrieved the trays reported useful information on the fish, invertebrates, and macroalgae that were observed on and around the reef and were not collected in the trays (Zoë Greenberg, BOP, pers. comm., September 2018): pipefish, gobies, flounders, sea robins, striped bass, *Ulva* (sea lettuce), and two species of sponges. In sum, the data from the trays and diver observations confirmed that the constructed reef (i.e., the "receiver reef beds") provided habitat for fish, invertebrates, and macroalgae.

The taxonomic composition of the macrofauna communities on the receiver reef differed substantially from that of the pre-construction soft-sediment macrofaunal communities (**Appendix G and Pre-Project Appendix C**). As expected, the pre-construction communities consisted of species typical of soft sediments in estuaries in the region compared to species that mainly occur on rocky bottoms. For example, the numerically dominant pre-construction species were infaunal polychaetes (e.g., *Nereis succinea*) and bivalve mollusks (e.g., the hard clam *Mercenaria mercenaria*), compared to slipper shells (*Crepidula* spp.) and xanthid crabs (e.g., *Panopeus herbstii*) that typically occur as part of the fouling community on hard substrates. Total community densities were somewhat higher on the pre-construction soft sediments (25 to 378 individuals/0.1 m^2 compared to 3 to 112 individuals/0.1 m^2) than on the receiver reef, but taxonomic richness was higher on the receiver reef (2 to 8 taxa/0.1 m^2) than on the pre-construction soft-sediments (1 to 6 taxa/0.1 m^2). Thus, with respect to habitat substitution, expected changes in benthic community composition occurred: the soft-sediment benthic infauna were replaced by an epifaunal community typical of those that occur on hard substrates.

5.4.2 Water Filtration

During 2017, a total of seven replicate short-term (<10 min duration) datasets were recorded on August 23, 2017, with three in different areas along the eastern end of the longline on a flooding tide and four on ebb tide flows on the western end of the longline (Table 5.4). All seven short-term datasets showed consistent and substantial CHL depletion with overall removal rates ranging from 17.6% to 45.7%.

	Date			
score	8/10/2017	8/24/2017	9/11/2017	10/23/2017
0	2	2	1	0
1	2	7	2	0
2	3	12	10	14
3	9	8	8	11
4	6	5	9	5
TOTAL	22	34	30	30

Table 5.1. Gonad Assessments, 2017

Long-term deployment of datasondes and the ADV during 2017 yielded a total of ~160 separate measurements (~40 hours total; readings at 15-min intervals) of CHL concentrations, water quality parameters, and water flow speed deployed on the field flume apparatus. Initial inspection of the overall dataset clearly indicated that the "upstream" CHL concentrations were usually greater than "downstream" (Figure 5.9); the overall means were 24.0 µg/L upstream and 18.7 µg/L downstream, indicating an average 32.1% CHL removal (uptake) over the 40-hour measurement period. However, there were several periods lasting up to a few hours when downstream CHL was greater than upstream (indicating CHL export).

The ADV measurements of water flow during the 40-hour period showed wide variability in speed and direction. All three sensors were fixed so that "upstream" could be determined regardless of instrument orientation, and only one had a net flow that was positive: 1.3 cm/s from an oblique angle entering the flume. The other two indicated average negative ("downstream") flows of ~3 cm/s. These data confirm the minimal flow speeds and variable direction for water currents in the general area, but also the probable effects of wind waves that persisted during most of the 40-hour measurement period.

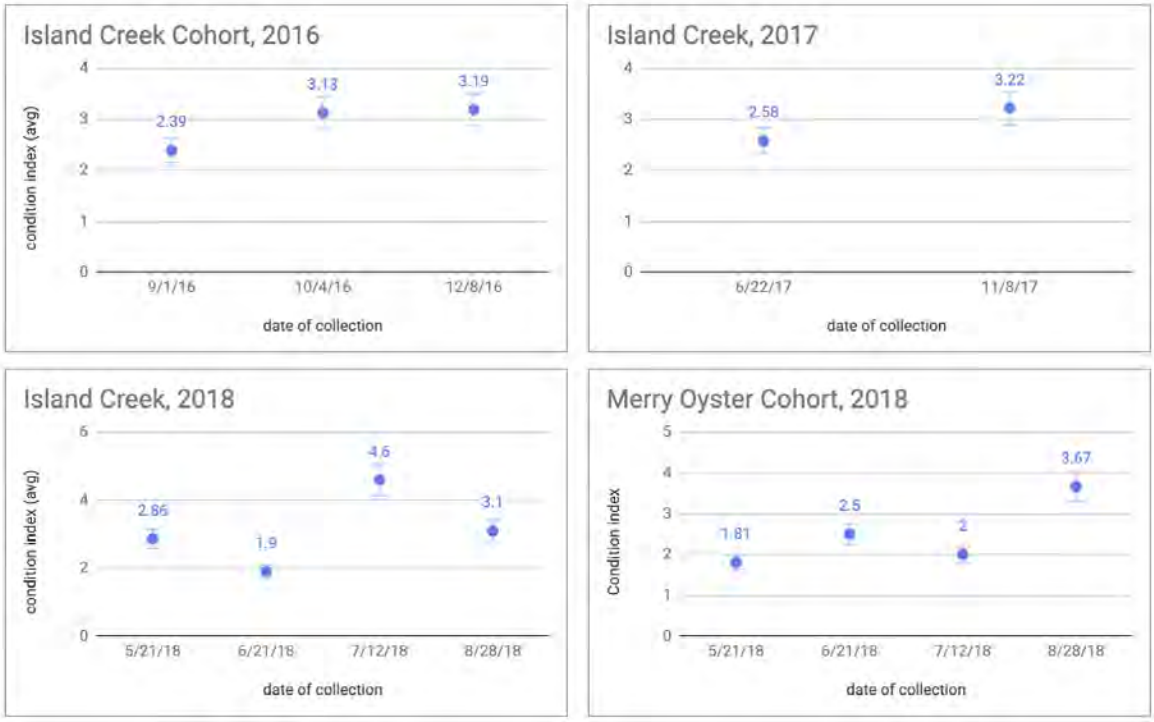


Figure 5.8. Condition Index (avg ± 1 SE) of Oysters

Dermo / MSX		Island Creek	Merry Oysters
Date of Collection	Collection Site	Percent Prevalence	
Dermo			
5/10/16	Duxbury MA	0	-
10/4/16	HOB	23.3	-
6/22/17	HOB	44	-
11/8/17	HOB	20	-
8/14/17	Duxbury MA	-	0
8/28/18	HOB	23	10
MSX			
5/10/16	Duxbury MA	0	-
10/4/16	HOB	3.3	-
6/22/17	HOB	0	-
8/14/17	Duxbury MA	-	0
11/8/17	HOB	0	-
6/21/18	HOB	0	0
7/12/18	HOB	0	0
8/28/18	HOB	0	3.3
"-": not tested			

Table 5.2 Disease prevalence by date and cohort

	oyster shell	clam shell	porcelain
Invertebrate density	27.3	71.5	50.4
(1 SE)	6.8	33.1	22.5
Taxonomic richness	5	3.7	5.8
(1 SE)	0.8	2.2	2.6

Table 5.3 Means of resident invertebrate density (#/0.1 m^2) and taxonomic richness (taxa/0.1 m^2) by substrate type: oyster shell, clam shell, porcelain

Outside Longline (CHL, µg/l)	Inside Longline (CHL, µg/l)	% REMOVAL	Measurement Duration (min)	Notes
10.0	8.0	20.6	3.7	Eastern end of longline
11.1	8.1	26.6	6.5	Eastern end of longline
11.3	9.2	18.8	6.5	Bags containing spat only
11.0	7.9	28.7	8.0	Western end of longline
12.9	9.4	26.8	7.0	Western end of longline
13.3	10.9	17.6	7.0	Western end of longline
15.8	8.6	45.7	7.0	Western end of longline
	MEAN:	**26.4**		

Table 5.4 Summary of means for short-term measurements of CHL concentrations inside and outside of the floating oyster bags on the longline.

Date	Measurement Duration (min)	CHL upstream (µg/L)	CHL downstream (µg/L)	% CHL Removal	DO (mg/L)	Temp (°C)	Salinity
8/15/2018	6	12.5	11.2	10.4	7.1	27.2	24.3
8/16/2018	27	10.4	7.8	25.0	4.7	26.0	24.0
8/16/2018	35	10.6	9.4	11.3	4.1	25.9	26.1
8/16/2018	20	15.1	15.6	-3.3	7.1	26.8	24.0
			MEAN:	**10.9**			

Table 5.5 2018 means for short-term measurements of CHL concentrations using handheld fluorometers upstream and downstream of oysters in field flume, and water quality measurements upstream.

Date	Time	Position	Flow speed (cm/s)	Salinity	Temp (°C)	DO (mg/L)
8/15/2018	11:10 - 11:30am	upstream (sonde #1)	2	24.6	25.8	3.4
		downstream (sonde #2)	1			
		across top of bags	4			
	3:35 - 3:50pm	upstream (sonde #2)	9	24.3	27.2	7.1
		downstream (sonde #1)	2			
		across top of bags	19			
8/16/2018	09:15 - 09:30am	upstream (sonde #2)	(nd)	24.0	26.0	4.7
		downstream (sonde #1)	1			
		across top of bags	2			
	11:38 - 11:50am	upstream (sonde #2)	(nd)	26.1	25.9	4.1
		downstream (sonde #1)	2			
		across top of bags	5			
		along side of flume	14			
	12:05 - 12:15pm	upstream (sonde #1)	3	23.9	26.9	5
		downstream (sonde #2)	(nd)			
		across top of bags	(nd)			
	2:30 - 2:50pm	upstream (sonde #2)	2	24.0	26.8	7.1
		downstream (sonde #1)	1			
		across top of bags	4			
		along side of flume	5			

Table 5.6 Summary of water flow speed and water quality perameters measured by handheld instruments during the 28-hour field flume deployment.

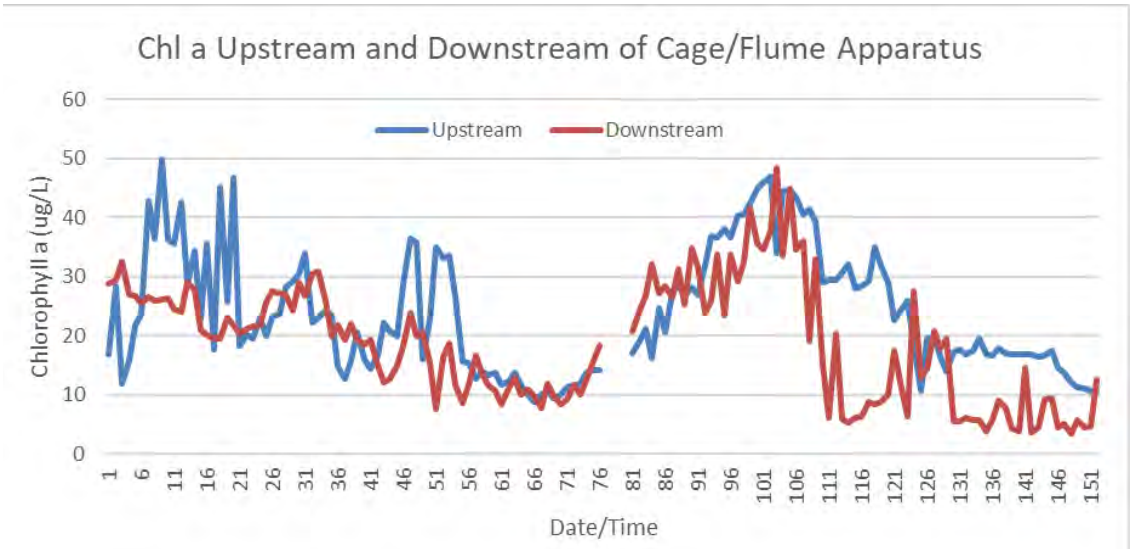


Figure 5.9 Time series (~40 hours total) of CHL measurements by two datasondes at 15-min intervals deployed on field flume.

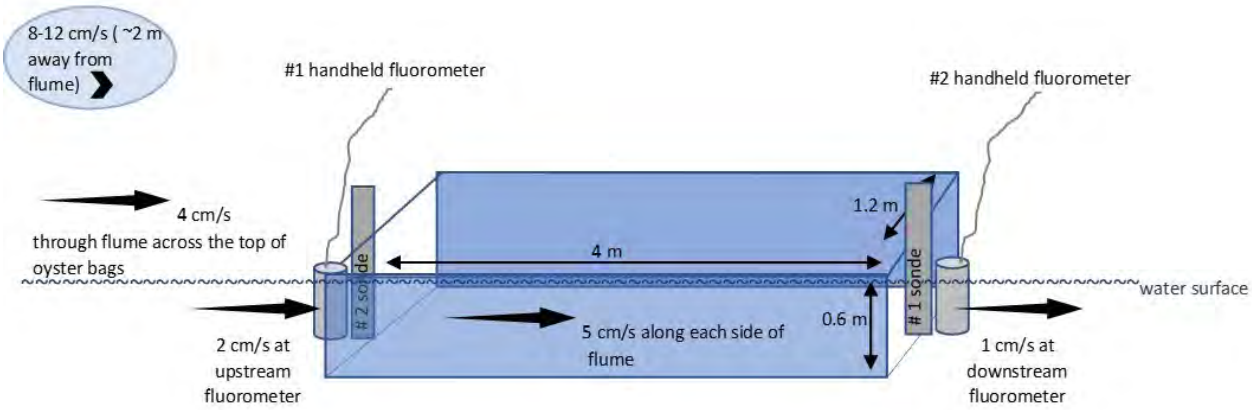


Figure 5.10 Schematic illustrating water flow speed patterns in and around the flume based on measurements made from 2:30 – 2:50 pm on August 16, 2018 (see Table 5.6).

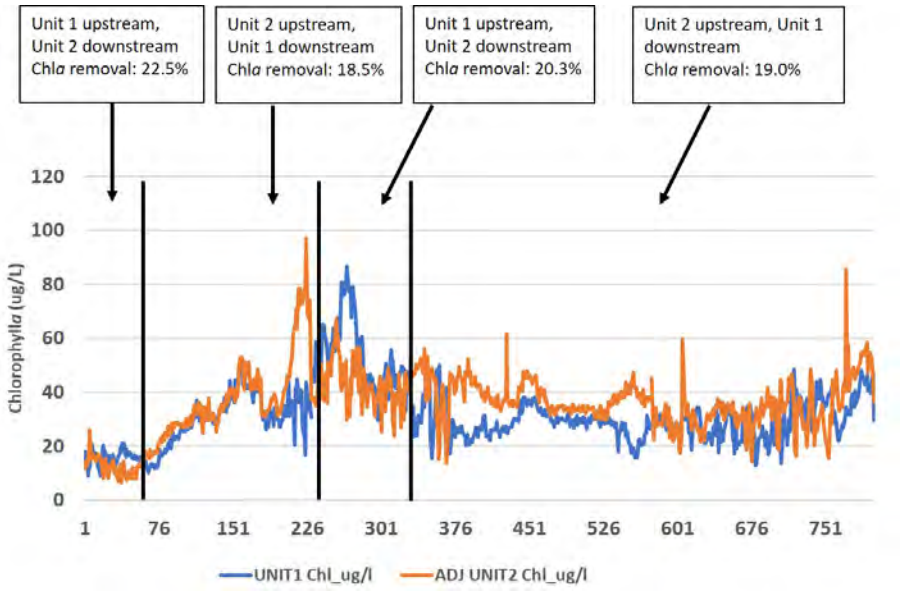


Figure 5.11 Time series (28 hours) of CHL measurements by two datasondes at 2-minute intervals deployed on the field flume during August 15 – 16, 2018.

total by week	# samples analyzed	% positive	total positive	>180 micron
6/27	4	50	2	0
7/12	6	50	3	1
7/26	6	50	3	1
8/2	6	50	3	0
8/14	6	67	4	2
8/28	6	33	2	0
9/6	6	0	0	0

Table 5.7 *Crassostrea virginica* presence/absence assay results by sample week, including 2 PCR positive results that were not confirmed by sequencing.

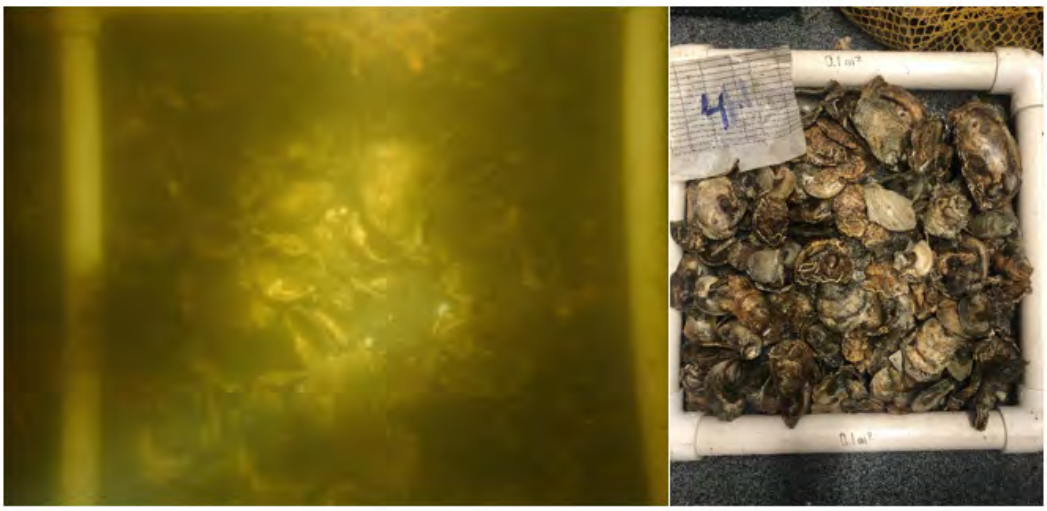


Figure 5.13 Underwater photo of quadrat prior to excavation (left) and after (right).

Additional water parameters that were measured by the datasondes and are known to affect oyster feeding rates included temperature, salinity, and dissolved oxygen. Salinity ranged from 22.9 ppt to 27.2 ppt; water temperature ranged from 24.6 °C to 26.8 °C; and dissolved oxygen 5.0 mg/L to 12.9 mg/L.

During 2018, a total of four replicate short-term (6 to 35 min) datasets were recorded using handheld fluorometers on August 15 - 16, showing an overall mean of 10.9% CHL removal (Table 5.5).

Water flow speeds were also characterized around the field flume in order to confirm flow direction for calculation of CHL removal rates, and to provide information on how the donor reef affected ambient flows. Overall, the bags appeared to behave almost as a solid object, with flow speeds substantially increased over the tops of the bags and greatly reduced behind them (Table 5.6, Figure 5.10).

During 2018, 28-hour deployment of datasondes yielded a total of ~800 separate measurements of CHL concentrations upstream and downstream of the oyster bags in the field flume (Figure 5.11). The overall dataset could be divided into four separate measurement intervals based on observed (or likely) changes in flow direction associated with tidal flows, as discussed above (Figure 5.9). CHL removal rates ranged from 18.5% to 22.5% when calculated on a per interval basis. However, inspection of the data plots (Figure 5.11) indicates substantial variability in CHL removal rates within each interval. Water quality parameters measured by the datasondes ranged from 24.6 to 26.2 for salinity, 24.9 to 29.1 °C, and 2.6 to 9.0 mg/L DO (compare with handheld YSI measurement in Tables 5.5 and 5.6).

5.4.3 Spat Settlement and Plankton Monitoring

Over the three-year period no spat were observed on the collectors. Potential explanations for the lack of observed recruitment are reviewed in the discussion section below.

The results of the plankton study confirm that oysters transplanted to the "donor reef" in 2016 produced larvae during the expected reproductive season of 2018. Overall more than three times as many small-fraction samples produced a positive result (13) than large-fraction (4), indicating that young larvae smaller than 180 µm were more abundant in the areas sampled. Half of the large-size larvae were sampled in July, as early as July 12, and the other half were found in the August 14 sample (Table 5.7). The higher frequency of small-size larvae among the samples is to be expected from normal processes of larval mortality (which often is substantial even under ideal conditions in culture) and emigration. Positive assay results for the large-size fraction suggest that at least some larvae are growing and persisting in Jamaica Bay for over a week (this size class was reported to occur anywhere from 7 to 18 days after spawning in different locations (Kennedy 1996).

6.0 DISCUSSION

Major accomplishments of the project include: (1) construction of four new hard-substrate reef bases that will continue to provide new habitat for many species, and that are potentially available for settlement by oyster larvae; (2) initial characterization of the plant and animal communities in the new habitat; (3) temporary provision of water filtration and nutrient sequestration by oysters and other organisms on the donor reef; and(4) a test of the hypothesis that proximity to adult oysters enhances the recruitment potential for constructed reefs.

6.1 Creation of New, Stable Hard-Substrate Habitat

A qualitative diver survey assessment and a high-resolution multi-beam and side scan sonar survey conducted after the initial construction confirmed the installation achieved the project design objectives. Subsequent annual qualitative dive survey assessments reported that each of the reef beds were still intact with only limited areas of subsidence and siltation observed. Additionally, on the beds with an applied veneer layer, the reef surface remains covered by the oyster shell or porcelain materials.

6.2 Water Filtration Capacity

The donor reef provided temporary water filtration capacity comparable to natural and restored reefs in other areas, averaging (both years combined) about 30% CHL removal of the water passing over the oysters in the bags. For example, Cressman et al. (2003) measured CHL removal of 10 to 25% over their constructed reefs, and previous measurements on natural and restored intertidal oyster reefs in other areas showed CHL removal up to 62% (Grizzle et al. 2006, 2008; Milbrandt et al. 2016). It should also be noted that many of the bags holding the oysters had been colonized by sea squirts and other filter feeding species that contributed to the measured filtration rates, as has been noted in other areas (Byers et al. 2014; Milbrandt et al. 2016). Although it was beyond the scope of the present project to quantify, there was probably substantial nutrient sequestration by the oysters on the donor reef. Previous studies involving oysters of the size used in the present study have quantified nutrient (mainly nitrogen) cycling and sequestration rates by both farmed oysters (briefly reviewed by Grizzle et al. 2016) and wild reefs (Piehler and Smyth 2011; Kellogg et al. 2013; Hollein and Zarnoch 2014; Hollein et al. 2015). Finally, recent research has noted that although small oyster restoration projects such as the present project will not have effects that are significant at the whole ecosystem level, important ecological effects should be expected on smaller spatial scales (Buzzelli et al. 2013; Grizzle et al. 2018).

6.3 Macrofaunal Community Composition

There was wide variability in total community density (30 to 1,120/m^2) and taxonomic richness (3 to 8 taxa/0.10 m^2) of invertebrates that had colonized the reef bases, and the dominant taxa included those typical of epibenthic communities (e.g., *Crepidula* spp., several xanthid crab species) in the region and constructed oyster reefs in other areas of the HRE (Cerrato 2006; Mass and Ruzicka 2008, 2009; Peterson and Kulp 2013; Lodge et al. 2015, 2017). The qualitative diver observations of fish and invertebrates that were on and around the new reef bases also indicated that the new reef bases provided good habitat for a diversity of plant and animal species.

The new animal communities that colonized the reef bases were different in several respects from the pre-construction infaunal benthos. As noted above, they differed with respect to dominant species, with the receiver reef being dominated numerically by species typical of hard-bottom areas. Also, taxonomic richness was higher on the receiver reef (2 to 8 taxa/0.1 m^2) than on the pre-construction soft sediments (1 to 6 taxa/0.1 m^2). The new benthic communities, however, did not have greater total densities than the soft-sediment communities they replaced, thus differing from trends reported in previous studies in the HRE (Grizzle et al. 2013) and other areas (Shervette et al. 2011; Wong et al. 2011). In sum, these data confirm that the new receiver reef bases at the end of the monitoring period were providing important new habitat for a wide diversity of fish and invertebrates species.

6.4 Creating New, Self-Sustaining Oyster Populations

This project was designed in part to test the general hypothesis that proximity to a population of healthy adult oysters enhances the probability of recruitment to constructed reefs. This hypothesis was not supported by this study as there was no observed recruitment of oysters to the receiver reef or evidence of spat settlement in the surrounding area. It should be emphasized that the present study does not suggest that proximity to a healthy reef (population of adult oysters) is unimportant in siting reef restoration projects. Rather, the finding that no oyster recruitment was observed on any of the spat collectors in the Head of Bay area, suggests that the oysters held on the donor reef did not produce larvae that successfully recruited to the benthos in the study area.

We discuss a few plausible reasons and potential follow up studies below.

1. Because it can be assumed that a healthy population of adult oysters is needed to provide potential recruits (oyster larvae) within the general area of a constructed reef, part of the answer is that the spatial scales involved are not well understood and a much larger donor population (potentially orders of magnitude larger than 40,000 adults maintained for this study) is needed before recruitment would be observed.

2. The donor reef and plankton monitoring results provide solid evidence that oysters in the donor reef were producing larvae and that the larvae were surviving until late reproductive stages, but we don't have definitive evidence that the larvae are reaching the final development stages. Future studies that fractionate samples into the final development stage before settling would provide important insights into larval viability and mortality.

3. The number of plankton samples and the frequency of the monitoring is not sufficient to estimate the larval loss rate (mortality + emigration) from the Head of Bay. Higher resolution plankton monitoring as described above could provide a loss rate estimate which could be compared to studies in other areas and help us understand the size of the donor population that may be necessary to create a new self-sustaining population.

4. The spatial arrangement of the oysters on the donor reef may also be involved. Regardless of the reason(s) for the observed lack of recruitment to the receiver reef, it would be useful to continue to monitor the reef bases. This is particularly recommended because the oysters in the bags on the longline were deposited directly onto the constructed reef bases in the final year of the project, which might result in successful recruitment in the future.

5. Another possible reason for the lack of oyster set on the beds or on the spat collectors is that diseases (primarily MSX and Dermo), parasites (e.g. ciliates, pea crabs) and/or adverse environmental conditions (e.g. salinity, dissolved oxygen and temperature extremes) can lead to a reduction in fecundity. This could include egg (and to a lesser extent, sperm) quantity and quality that could lead to fewer, poor quality larvae that might not survive to set. Additionally, the timing of the infection can have an effect on severity of the impact.

6.5 Lessons Learned

1. Finding a source for shells that meet the state's origin and curing requirements remains a significant challenge to oyster restoration projects in New York. Curing requirements necessitate planning and securing shells up to a year in advance of a project's start date. This is often problematic from a funding perspective and can result in significant delays to a project's start date.

2. Surf clam shells and oyster shells are relatively durable, but if handled multiple times from source to application, as done in this project, the size of the shell pieces will be greatly reduced. Smaller size shell pieces are expected to be a less desirable substrate for spat settlement. Project logistical designs should seek to reduce this handing as much as possible. Hard clam shell is more durable than surf clam shell but large quantities of the former are less readily available than surf clam shell. Oyster shells are less available but are more

durable than surf clam shells. Importing shell from outside of New York City is possible, however sources are generally scarce and becoming more so in recent years, and the expense of importing shell by truck or barge is substantial. Oyster recycling programs should be encouraged and supported. Porcelain isn't as prone to breaking and from that perspective may be an attractive alternative substrate.

3. Use of the conveyor belt and salt spreader to place the reef base material was generally efficient and precise when deploying the clam and oyster shells. As constructed and implemented the conveyor belt and salt spreader did not work well with the larger and much harder porcelain material causing frequent jams and inconsistent application of the material. It is possible that some small adjustments or design alterations could have achieved a workable method but these changes were only nominally tested during this project.

4. Commercial-scale aquaculture installations require substantial maintenance and monitoring to ensure that the equipment is secure, cleared of fouling and in proper working order. During cold and windy conditions, the distance of the installation from the project team's home base was sometimes a limiting factor to the ability to access the installation for maintenance. Future projects should consider siting similar installations in

Figure 6.2 Community Engagement with the project (upper left: The Nature Conservancy, NY State Parks, and BOP demonstrate oyster filtration at an HOB project event in Bayswater Point State Park; upper right: volunteers from Bloomberg learn about the project and how to survey for wild oyster recruitment with BOP and NY State Park staff; lower left: Merry Oyster staff loading oysters being donated to the project; lower right: Natural Areas Conservancy summer interns unloading OysterGro™ units.

locations that are accessible year-round either from shore or in closer proximity to the crew's home port.

5. The OysterGro™ aquaculture system was generally appropriate for the project, however as the oysters and the fouling community grew and increased the weight of the units, turning them over for desiccation and accessing the oysters inside them became challenging. Vessels and vessel equipment used for the maintenance of gear, and the strength and height of the crew need to be carefully considered and selected for the needs of the installation.

6.6 Ecological Importance of the Research and Next Steps

The present project resulted in ecologically important new hard-bottom habitat that supports many species of resident invertebrates and transient fish, and temporary water filtration by the oysters on the donor reef and a short-term source of natural oyster larval production within Jamaica Bay. The present project also provided important new information relevant to future oyster restoration projects in Jamaica Bay. The oyster data from the donor reef demonstrated that juvenile and adult oysters grow and reproduce in the Bay. The plankton DNA results indicated that oyster larvae were present in the general restoration area during the study, but extensive sampling using spat collectors indicated no successful recruitment. Taken together, these data suggest that oyster restoration may well be possible in Jamaica Bay, but more research is needed. Future research also might include testing different donor reef designs that are based on theoretical considerations of water flow, oyster arrangement, and potential fertilization success. Oyster restoration is a new and rapidly developing field of study and practice. Each project represents to some extent an experiment. The present project did not result in the anticipated results of new receiver reef populated by live oysters, but it did provide potentially important information for how to proceed.

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