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1 Inside Cover

CHESAPEAKE WATERSHED CESU W912HZ-18-SOI-0006

MONITORING OYSTER RESTORATION REEFS IN THE GREAT WICOMICO, PIANKATANK AND LYNNHAVEN RIVERS

FINAL REPORT: PART I - PIANKATANK AND LYNNHAVEN RIVERS*

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2 Summary

The Piankatank River harbors extensive subtidal bottom suitable for oyster restoration. In terms of overall restoration potential, there was no discernible spatial trend as most sites were deemed suitable for oyster restoration, and good sites were spread throughout the river.

The Piankatank River granite oyster reef was in excellent condition and already exceeds the five-year GIT metrics for adult oyster density and oyster biomass. Size frequency histograms indicated the presence of two year classes (2017 and 2018), with a high apparent survival rate (85.2 \pm 0.9%). This reef met the five-year target criteria by its second year with respect to adult oyster density (219.3 \pm 21.5 per m²) and dry mass (75.3 \pm 8.2 g DM per m^2). Mean live mussel density was also high on the granite reefs at 194.5 \pm 19.9 per m². At 3.2 (\pm 0.3) L per m², this reef is well on its way to meeting the target live oyster volume for restoration reefs, even though shell loss and burial are a lesser concern on alternative substrate reefs, as is evidenced by the total rock volume (82.9 \pm 3.9 L per m²). ROV video corroborated the high species diversity including shrimp, small fish, crabs, clams, snails, mussels, and sponges. Several predatory finfish species, often in schools, were on the reef, while crustaceans, including blue crabs, mud crabs, and shrimp, were walking and feeding along the reef surface. Given that this project is the first application of the subtidal granite reef 'row' design, the results are encouraging. A considerable number of oysters, mussels, and barnacles (the more sessile/sedentary filter-feeding species) were in reef crevices on rock surfaces that face inward. In addition, the animals in these interior reef spaces experienced lower overall siltation and fouling, which generally favors the survival of those individual organisms and increases the likelihood that subsequent larval encounters will result in higher recruitment to these relatively cleaner, more accessible surfaces. With the knowledge that much of the living reef is present within the interior of the granite reefs, assessments of reef performance that rely solely on underwater video or diver observation of the reef's exterior are not likely to be accurate and are thus not recommended.

Overall, the Lynnhaven River system performed exceedingly well. When corrected for a survey efficiency of 81%, total abundance in the system was 37.3 million adults and spat, with 15.4 million (\pm 1.7 SE) in Broad Bay, 21.7 million (\pm 2.7) in Linkhorn Bay and 132.2 thousand (\pm 160.0) in Lynnhaven

Bay. All three bays exceeded the GIT target for density, with Broad Bay at 190.4 oysters per m² (\pm 20.7), Linkhorn Bay at 200.2 oysters per m² (\pm 20.7), and Lynnhaven Bay at 59.8 oysters per m² (\pm 27.6). Broad Bay at 55.9 g DM per m² (\pm 6.2) and Linkhorn Bay at 88.4 g DM per m² (\pm 10.0) were above the GIT target for biomass, while Lynnhaven Bay at 22.8 g DM per m² (\pm 11.0) exceeded the GIT threshold, but not the target. Broad Bay reefs had higher spat (oysters smaller than 35.0 mm in shell length) densities than the other reef sites. All three Broad Bay reef sites greatly exceeded the GIT target for oyster density, while BB1 and BB2 exceeded the GIT target for biomass, but BB3 exceeded the GIT threshold but was short of the target. BB1 (6.4 \pm 0.8 L per m²) and BB2 (7.2 \pm 1.4 L per m²) met the target LOV for restoration reefs, while BB3 (4.6 \pm 0.5 L per m²) fell just short of the target. Five of the six Linkhorn Bay (LB) reef sites (LB1N, LB1S, LB2, LB3B, and LB4) surpassed the GIT target for restoration reefs based on adult oyster density; LB5 was the only site that did not meet the metric. Five of the six sites exceeded the GIT target for dry mass, whereas LB5 fell well below the threshold. Five of the six sites surpassed the target LOV, ranging from 5.5 (\pm 0.6) to 7.2 (\pm 1.8) L per m²; LB5 (1.6 \pm 0.8 L per m²) was the only site that did not meet that metric. In general, very few oysters were collected in the samples from the two Eastern Branch (EB) reefs (EB1 and EB2). Neither site met GIT targets for adult oyster density or dry mass, but EB 1 (16.2 \pm 10.1 adult oysters per m²; 18.5 \pm 8.9 g DM per m²) exceeded the GIT thresholds. Neither site (EB1: 3.5 ± 1.2 L per m^2 ; EB2: 0.0 ± 0.0 L per m^2) met the target LOV. Since their construction, Eastern Branch oyster reefs have contended with strong tidal currents which have displaced or buried base shells, which explains the poor performance and provides valuable information for future restoration reef siting.

3 Introduction

The native Eastern oyster Crassostrea virginica and its habitat have been severely depleted in Chesapeake Bay, as in many other regions of the world (Beck et al., 2011). Current populations in the Bay are estimated at approximately 1% (Wilberg et al., 2011) and while a limited recovery of the wild fishery is occurring at present, the overall recovery of the oyster stocks, fishery and associated reef habitat has been limited by poor habitat quality, low stock and continued low recruitment when compared to historical levels (Rothschild et al., 1994). An aggressive restoration effort was undertaken by the U.S. Army Corps of Engineers (USACE) as part of a larger commitment to restore the Chesapeake Bay ecosystem in response to the Executive Order by President Obama in 2009. For oysters, more specific goals were established with the 2014 Chesapeake Bay Agreement, which requires 10 tributary rivers be restored by 2025. The Chesapeake Bay Program Goal Implementation Team (GIT) established standard reef location, abundance and biomass metrics to be applied at reef sites to monitor their status and assess their success over time. The USACE is a member of the GIT and has adopted the GIT standard metrics to assess the status of constructed reefs.

A large-scale, multi-agency team involving both federal and state agencies as well as academia has been conducting large-scale oyster restoration projects in both Maryland and Virginia waters of the Bay and its tributaries. Tributaries were prioritized according to their chance for success of a largescale restoration project. Goals include: significant stock enhancement, expansion of oyster reef habitat, enhanced oyster recruitment, establishment of a network of sanctuary oyster reefs free from oyster fishing pressure, improvements to local ecology including secondary production, Submerged Aquatic Vegetation (SAV) expansion and water quality improvement, and enhancement of the oyster fishery in areas set aside for the fishery. As part of the Chesapeake Bay Native Oyster Recovery Project, the USACE constructed a subtidal granite reef at the Piankatank River (Figure 1) and subtidal shell reefs at the Lynnhaven River (Figure 2) and the Great Wicomico River (Figure 3). The Piankatank River lies south of the Great Wicomico River, which is the first major tributary on the western shore of the Bay south of the Potomac River. The Lynnhaven River is located within the City of Virginia Beach on the most southeastern shore of Chesapeake Bay near the confluence with the Atlantic Ocean.

USACE reefs in the Great Wicomico River were sampled in 2006-2007, and again in six different years spanning 2008 through 2017. USACE reefs in the Lynnhaven River were sampled in 2011, and again in three different years from 2013 through 2017. We established the protocol for effective and efficient sampling of restoration reefs with patent tong gear; validated the method with underwater remotely operated vehicle (ROV) video observations, and determined the efficiency of patent tong gear (Schulte et al., 2018). Suitability of restoration sites in the Piankatank River where USACE constructed the granite reef to be monitored was determined in 2011, and a Habitat Suitability Index was generated for oyster reef restoration in the Great Wicomico River (Theuerkauf and Lipcius, 2016); this index is also applicable to the Lynnhaven and Piankatank Rivers.

4 Objectives

In this report, we assessed the performance of reefs constructed by USACE in the Piankatank and Lynnhaven Rivers, evaluated bottom habitat suitability of potential restoration sites in the Piankatank River, and summarized prior results on metapopulation connectivity through hydrodynamic modeling in the Piankatank and Lynnhaven Rivers. Monitoring objectives included assessing abundance and biomass, oyster demographics (live and dead) including age classes, and accretion rates on the restored reefs. All work was performed in accordance with applicable local, state, and federal regulations.

The period of performance was 15 September 2018 through 14 April 2020. The original period of performance was extended due to circumstances beyond the control of the investigators. Surveys were conducted in Fall and Winter 2018, and Spring and Summer 2019. Additional video surveys were to be conducted Winter 2020, but the COVID-19 pandemic precluded their completion, though the investigators intend to conduct the video surveys and report on them as an addendum to this report. As required by the contract, specific tributary sampling plans were reviewed with USACE personnel prior to the actual surveys, and adjustments made to suit USACE needs.

5 Piankatank River Bottom Survey

5.1 Methods

The objectives of this project were to (i) evaluate bottom restoration suitability of substrate (via auger), (ii) evaluate all biogenic shell-bottom areas (via patent tong) for restoration suitability and determine if relict reefs are present, and (iii) gather baseline data (via patent tong) on a subset of established reefs for future comparative investigations, at feasible locations within the Piankatank River (Figure 4). Feasible area (total = 262 acres) criteria included: (i) areas one acre or greater, (ii) water depth 6 - 16 ft, (iii) hard base sediment excluding shell or mud dominant bottom, and (iv) within safe distance from maintained navigation channels, navigation aids, private docks, lease boundaries and Virginia Oyster Stock Assessment Replenishment Archive sampling sites. Physical variables (water clarity, temperature, salinity, dissolved oxygen) were measured and underwater video was recorded using GoPro cameras at each suitable area (n = 30) from December 5 - 12, 2018. Within each area, adaptive site selection approximately proportional to site area ensured equal sample coverage in spatial extent (Figure 5). A 10-m hand auger was deployed at each site (n = 113) to evaluate depth, substrate stability and sediment composition. All sites were assessed in situ and assigned a restoration potential index from poor to exceptional (0 - 11).

5.2 Results

Physical variables during sampling were well within the dissolved oxygen (10.8 - 16.2 mg/L), thermal (5.5 - 9.0 °C) and salinity (7.8 - 8.9) tolerances of the Eastern oyster (Theuerkauf and Lipcius, 2016). Water depth ranged from 4.0 - 19.0 ft and Secchi depth ranged from 4.0 - 9.9 ft. Of the three areas with 4-ft Secchi depth readings, two were 4 ft deep while the last was taken late afternoon when solar altitude would be an issue and thus may not be indicative of turbidity.

Most samples were muddy sand (n = 75), followed by sand (n = 25) and then mud (n = 7), with remaining samples consisting of variable combinations of mud, sand and shell (n = 6). Visual site assessment from the video footage confirmed sediment type and that there were neither structures nor sessile epibenthic communities that would warrant further investigation. Most sites (n = 80) received an index score of 10 and are considered very good, followed

by exceptional (index = 11, n = 8), and poor (index = 0, n = 8), with the rest considered marginal (index = 1 - 9, n = 15) or not evaluated (n = 2). These results were mapped as both an area average (Figure 6) and by sampling site (Figure 7).

Restoration potential differed by substrate (Figure 8), with all mud sites scoring poorly and all sand sites scoring exceptionally. The two exceptional areas warrant extra attention when planning restoration sites. In addition to stable bottom, their mid-river location may be advantageous as potential source populations when considering metapopulation dynamics throughout the river.

5.3 Conclusions

In terms of overall restoration potential, there was no discernible spatial trend as most sites were deemed suitable for oyster restoration, and good sites were spread throughout the sampling area. This study indicates the value of *in situ* measurement of submerged bottom stability as an essential part of planning and placement of oyster restoration reefs.

6 Piankatank River Granite Reef Survey

6.1 Methods

Field Survey

. To both qualitatively and quantitatively sample the granite oyster restoration reefs (Figure 9) in the Piankatank River (Figures 10 and 11), various techniques were employed. Using coordinates supplied by the USACE for the survey area, one site within each randomly selected 10 x 10 m² grid (Figure 12), the captain navigated a 24-ft aluminum Sea Ark class boat with a davit crane to each set of designated coordinates using a Garmin 76 GPS (Figure 13). The vessel was then anchored (additional anchors were kept on board and deployed when wind/current/wave conditions called for it) and the weighted, reinforced sample basket (equipped with a rope and buoy) was released to settle on the sampling site. Basic water quality data were collected using YSI, Hydrolab, or similar equipment prior to oyster sampling, including temperature, salinity, and dissolved oxygen (Table 1).

Next, a team of two certified SCUBA divers entered the river from the

vessel, with a third safety diver remaining on board to assist the active divers if the need arose. As the divers descended and approached the reef surface, they released a 0.25-m^2 (0.5 m x 0.5 m) weighted PVC quadrat; releasing it prior to reaching the bottom eliminated any potential sampling bias. To ensure the safety of the divers, the vessel motored away from the site to a distance of roughly 25 to 35 m, while the certified site and safety health officer (SSHO) and dive supervisor kept a fixed eye on the divers' air bubbles (a proxy for locating the divers' location underwater).

From within the sample quadrat, which was excavated to a depth of 15 to 20 cm, each granite rock was removed and placed into the sample basket until all rocks were removed within the quadrat, including all stone and live shell material. At that point, the divers returned to the surface. Once both divers surfaced, the captain navigated the vessel at no-wake speed towards the divers to allow a crew member to pass the cable to the divers to hook up onto the sample basket. The crew aboard the vessel then awaited the divers to signal the 'all clear' to raise the sample basket using the davit crane; during this process, the divers were clear of the area to ensure their safety as the basket was lifted. Once aboard the vessel, the basket was emptied into large sampling trays. At this point, photographs of the rocks were taken with a GPS-enabled digital camera. Each photograph contained a dry erase board indicating the sample site number, the date, and any other relevant descriptors. Lastly, the rocks were loaded into large PVC bushel baskets with a labeled sample bag to ensure correct identification during the next stage of sample processing.

Quantitative sampling of these rocks (Figure 14) was destructive, labor-intensive, and time-consuming. Given ambient water (and wind) temperatures in January and February, the divers wore dry suits and thick gloves, but immersion time still needed to be limited to 120 min for their safety. Thus, the team elected to process all samples on land, either aboard CNU's utility trailer or on campus. Metal scrapers were used to remove all oysters, mussels, barnacles, and other epifauna on the rocks; all sample material was sealed in Ziploc bags marked with the same identifying information as on the dry erase board. All samples were then stored in freezers. Lastly, the volume (to the nearest 0.1 L) of each rock was measured using water displacement, by placing the stone into a bucket marked in 0.1-L increments and measuring the amount of water displaced.

The qualitative aspect of this reef survey involved the recording of underwater video. Ultimately, we decided that underwater video at this site needed to be recorded on non-sampling days, given the rigor and complexity of the sampling process, as well as general exposure limits and concerns for the divers. Also, the conditions necessary to collect high-quality underwater footage in the turbid waters of Chesapeake Bay required careful selection of field days with calm weather. Once the forecast called for such conditions, the captain and crew proceeded with field days dedicated to underwater video. Upon arrival at the Piankatank River granite reef, a Deeptrekker brand remotely-operated vehicle (ROV) capable of streaming live video was deployed, with all video recorded by a Dell XFR Toughbook computer. The selected approach was to arrive near slack tide and navigate along fixed transects. The most successful method was to submerge the ROV, direct it via remote control to hover just above the bottom, and then drift with the current, powering the ROV as needed. This method allowed the camera to capture video with minimal sediment and biological disturbance, as well as avoiding entanglement of the ROV's tether around the vessel's propeller. Each video file was checked on site to verify its integrity.

Laboratory Processing

. Each sample was thawed and rinsed over a 1-mm sieve, enabling the removal of any excess mud and fine solids. The contents of each bag were sorted into three categories: live oysters, dead bottom valves, and dead top valves. Unlike shell reef samples, the physical act of scraping oysters from hard, jagged and uneven surfaces can affect the integrity of oysters' shells, especially bottom valves (those directly adhered to the rock surface) of dead oysters. As a result, it was challenging to identify all dead oysters and to avoid overestimating dead oyster density. Consequently, oysters were scraped off the surface with great care and subsequently sorted with the knowledge that the top valves of most small oysters, predominantly spat, were all that remained intact from each rock. All live oysters, dead bottom valves, and the top valves of disarticulated (where the two valves have been separated at the umbo) dead oysters were measured using digital calipers. Any additional organisms in the samples were identified, set aside in aluminum weigh boats, and counted. Live oyster volume (LOV) was determined in the lab with graduated (premarked and accurate to 0.5 L) 20-L buckets or smaller graduated cylinders (accurate to 0.1 L or 0.01 L), where appropriate. LOV included live oysters, dead bottom valves, and dead top valves. Concrete and rock substrates do not produce 'dead oyster volume', also known as base shell reef material, since shells were not deployed as part of this reef construction.

Oyster dry mass (DM = Dry Weight [DW]) and Ash-Free Dry Mass (AFDM) were estimated using oyster biomass models (simple linear regressions of log DM/AFDM vs. log shell height) from similar reef environments in the Great Wicomico River [Note: processing the oysters from this survey for oyster biomass and condition was not funded as part of this contract.]. The equations were selected from Great Wicomico River oyster reef sites that closely resembled the Piankatank River granite reef in relation to salinity, depth, and reef age (DM: y = 2.4556x - 4.8795, n = 257, $r^2 = 0.87$; AFDM: y = 2.4821x - 4.977, n = 257, $r^2 = 0.86$). Should funding be identified to conduct the laboratory processing of the Piankatank River granite reef oysters at a later date, a subset of oysters has been selected from reef samples and remains in freezer storage for future analysis.

6.2 Results

The Chesapeake Bay Program Goal Implementation Team (GIT) has determined that the thresholds for oyster restoration reefs are 15 adult oysters per m² bottom area (BA) and 15 g dry oyster tissue mass (DM) per m² BA, while the targets are 50 adult oysters per m² BA and 50 g DM per m² BA. An adult oyster was classified as any live oyster over 35.0 mm in shell length (= shell height).

Size frequency histograms for the Piankatank River (Figure 15) indicated the presence of two year classes (2017 and 2018), with a high apparent survival rate (85.2 \pm 0.9%). Mean oyster shell length was 39.7 (\pm 0.7) mm for live oysters and 22.0 (\pm 1.1) mm for dead oysters. This reef thus met the five-year target criteria (Table 2) by its second year with respect to adult oyster density (219.3 \pm 21.5 per m²; Figure 16) and dry mass (75.3 \pm 8.2 g DM per m²; Figure 17). Mean live mussel density was also high on the granite reefs at 194.5 \pm 19.9 per m² (Figure 18). At 3.2 (\pm 0.3) L per m², this reef is well on its way to meeting the target live oyster volume (LOV) for restoration reefs (Figure 19), even though shell loss and burial are a lesser concern on alternative substrate reefs, as is evidenced by the total rock volume (82.9 \pm 3.9 L per m², Figure 20). Lastly, the ROV video corroborated the high species diversity noted in the laboratory (including shrimp, small fish, crabs,

clams, snails, mussels, and sponges). Several predatory finfish species, often in schools, were on the reef, while crustaceans, including blue crabs, mud crabs, and shrimp, were walking and feeding along the reef surface.

6.3 Discussion

The Piankatank River granite oyster reef is in excellent condition and already exceeds the five-year GIT metrics for adult oyster density and oyster biomass. Given that this project is the first application of the subtidal granite reef 'row' design, the results are encouraging. One noteworthy observation typical of three-dimensional reef structures is that a considerable number of oysters, mussels, and barnacles (the more sessile/sedentary filter-feeding species) were present within the reef crevices on rock surfaces that face inward. In addition, the animals in these interior reef spaces experienced lower overall siltation and fouling (a generally cleaner appearance) which generally favors the survival of those individual organisms and increases the likelihood that subsequent larval encounters will result in higher recruitment rates to these relatively cleaner, more accessible surfaces. With the knowledge that much of the living reef is present within the interior of the granite reefs, proposed considerations to rely solely on cursory underwater video or diver observation of the reef's exterior to make quantitative population estimates are not recommended.

7 Lynnhaven River Shell Reef Survey

7.1 Methods

Field Survey

. The Lynnhaven River oyster reef survey (delineated by the Norfolk District of the USACE (Figures 21 and 22) was focused on 11 constructed oyster shell reefs in Broad Bay (3), Linkhorn Bay (6), and the Eastern Branch (2) of the Lynnhaven River. In total, 80 samples were collected: 31 in Broad Bay, 44 in Linkhorn Bay, and 5 on the Eastern Branch reef sites. Bottom conditions ranged across the full spectrum of sediment types, including mud, mud-clay mix, sandy mud, muddy sand, sand-shell mix, sparse shell, and thick cohesive shell. Basic water quality data were collected at each reef site using YSI, Hydrolab, or similar equipment before all oyster sampling, including measurements of temperature, salinity, and dissolved oxygen (Table

3).

To obtain subtidal bottom samples across the diverse bottom conditions, a commercial 'deadrise' vessel containing an oyster patent tong was employed (Figure 23). The captain navigated the vessel to each set of designated coordinates using a Garmin 76 GPS. Upon reaching each sample site, a large chain anchor was lowered to keep the vessel on site. The captain then lowered the patent tong to the sediment/reef surface and manipulated the tongs to ensure a deep, full grab; each grab sampled approximately one square meter of reef/river bottom (Figures 23 and 24). Upon raising the sample to the surface and placement on a sorting table, but prior to any processing, a photograph was taken of the sample with a dry-erase board displaying the site information located behind, above, or adjacent to the sample. Then, the best third of the sample (a continuous sample portion extending across seven patent tong teeth) was retained, cleaned of most sediment, placed in pre-labeled, sealable freezer bags, and stored in a large cooler for subsequent processing in the lab at Christopher Newport University.

Subsequently, underwater video collection was completed on board a 24 foot aluminum research vessel (Sea Ark) using a Deeptrekker remotely-operated vehicle (ROV) and a Dell XFR Toughbook computer.

Laboratory Processing

. Laboratory processing was required because (i) spat cannot be sampled accurately in the field without a lengthy examination onboard the project vessel, and (ii) it is more cost-efficient to use the vessel time to sample, rather than both sample and process the material. Each sample was thawed and rinsed over a 1-mm sieve, enabling the removal of any excess mud and fine solids. The remainder of the sample was then partitioned into three separate bags. The first bag contained all live oysters, the second bag contained all of the dead shell and base shell material, and the third bag contained smaller shell fragments (often with initially-undetected spat), heavy sediment/solids, and other organisms present in the sample. Each bag was labelled with the corresponding site and sample ID. The bags containing live material (bags 1 and 3) were stored in a chest freezer. The remaining bag (bag 2) was placed in a cold storage room until sample analysis was conducted.

In the next sample processing stage, the first and third bags were removed from the freezer, thawed, and placed in separate sorting trays. Similarly, the second bag was removed from cold storage and dumped into its own sorting tray. The contents of each bag were sorted into four categories: live oysters, dead bottom valves, dead top valves, and base reef material. The live oysters and dead bottom valves were measured using digital calipers. For the dead oysters, only the bottom valves were measured to avoid overestimation of dead oyster density. Additional organisms in the samples were identified, set aside in aluminum weigh boats, and subsequently counted. Live oyster volume (LOV) was determined in the lab with graduated (premarked and accurate to 0.5 L) 20-L buckets or smaller graduated cylinders (accurate to 0.1 L or 0.01L), where appropriate. LOV included live oysters, dead bottom valves, and dead top valves. Dead oyster volume (DOV), containing all of the base reef material, was determined using the same water displacement procedure.

Oyster dry mass (DM = Dry Weight [DW]) and Ash-Free Dry Mass (AFDM) were estimated using oyster biomass models (simple linear regressions of log DM/AFDM versus log shell height) from a combination of previous data from the Lynnhaven River system (Lipcius et al., 2015) and similar reef environments in the Elizabeth River. [Note: Processing the oysters from this survey for oyster biomass and condition was not funded as part of this contract.] The equations were for Linkhorn Bay: logDM = 2.1782x - 4.286 and logAFDM = 2.1943x - 4.433, where $logath{x} = logath{y} = logath{y}$

7.2 Results

The Chesapeake Bay Program Goal Implementation Team (GIT) has determined that the thresholds for oyster restoration reefs are 15 adult oysters per m² bottom area (BA) and 15 g dry oyster tissue mass (DM) per m² BA, while the targets are 50 adult oysters per m² BA and 50 g DM per m² BA. An adult oyster was classified as any live oyster over 35.0 mm in shell length (= shell height). And, though a specific value for sustainable oyster volume has not been formally adopted by the GIT, we offer 5.0 L of shell volume as sufficient for oyster reef sustainability (Schulte et al., 2009).

Overall, the Lynnhaven River system performed exceedingly well (Figure 25). Total abundance in the system was 37.3 million adults and spat, with 15.4 million (\pm 1.7 SE) in Broad Bay, 21.7 million (\pm 2.7) in Linkhorn Bay and 132.2 thousand (\pm 160.0) in Lynnhaven Bay - Eastern Branch reef 1 (Figure 25A). [Note: We excluded Eastern Branch reef 2 as it had no live oysters.] All three bays exceeded the GIT target for density (Figure 25B), with Broad Bay at 190.4 oysters per m² (\pm 20.7), Linkhorn Bay at 200.2 oysters per m² (\pm 20.7), and Lynnhaven Bay at 59.8 oysters per m² (\pm 27.6). Broad Bay at 55.9 g DM per m² (\pm 6.2) and Linkhorn Bay at 88.4 g DM per m² (\pm 10.0) were above the GIT target for biomass, while Lynnhaven Bay at 22.8 g DM per m² (\pm 11.0) exceeded the GIT threshold, but not the target (Figure 25C).

The preceding values were corrected for a survey efficiency of 81% (Schulte et al., 2018), whereas the results below are presented as the uncorrected estimates to portray the actual data. Note that the uncorrected values are underestimates of the true values because they have not been corrected for survey efficiency.

7.2.1 Broad Bay

Size frequency histograms for Broad Bay (Figure 26)) were skewed left, indicating a relatively higher number of oyster spat. Broad Bay reefs had higher spat (oysters smaller than 35.0 mm in shell length) densities than the other reef sites. The most frequently observed adult shell length, live or dead, was approximately 60.0 mm. All three Broad Bay reef sites, Broad Bay 1 – BB1 ($104.1 \pm 20.2 \text{ per m}^2$), Broad Bay 2 – BB2 ($115.2 \pm 6.1 \text{ per m}^2$), and Broad Bay 3 – BB3 ($69.2 \pm 10.4 \text{ per m}^2$), met the sustainable criteria in regards to adult oyster density (Figure 27). In terms of dry mass, BB1 ($60.2 \pm 11.1 \text{ g}$ DM per m²) and BB2 ($55.4 \pm 10.3 \text{ g}$ DM per m²) could be classified as sustainable (Figure ??); BB3 ($35.7 \pm 5.3 \text{ g}$ DM per m²), although not considered sustainable for that metric, was still well above the minimum threshold for dry mass to be classified as a restoration reef. BB1 ($6.4 \pm 0.8 \text{ L}$ per m²) and BB2 ($7.2 \pm 1.4 \text{ L}$ per m²) met the target LOV for restoration reefs (Figure 29), while BB3 ($4.6 \pm 0.5 \text{ L}$ per m²) fell just short of the target.

7.2.2 Linkhorn Bay

Size-frequency histograms for Linkhorn Bay (Figure 30) had a more normal distribution than the ones generated for Broad Bay (Figure 30). There were fewer spat present, both live and dead. The most frequent adult oyster size was between 70 and 80 mm. Five of the six Linkhorn Bay (LB) reef sites (LB1N, LB1S, LB2, LB3B, and LB4) surpassed the sustainable mark for restoration reefs based on adult oyster density, ranging from 61.4 (\pm 23.6) to 162.1 (\pm 7.6) per m² (Figure 27). LB5 (5.1 \pm 5.1 per m²) was the only site that did not meet that metric. Four of the six sites could be classified as sustainable based on dry mass, ranging from 71.6 (\pm 9.2) to 97.8 (\pm 38.0) g DM per m² (Figure ??). Regarding the two sites that did not meet that metric, LB1S (41.1 \pm 16.9 g DM per m²) fell just short of the sustainable mark, while LB5 (2.9 \pm 2.9 g DM per m²) fell well below the minimum threshold for restoration reefs. Five of the six sites surpassed the target LOV, ranging from 5.5 (\pm 0.6) to 7.2 (\pm 1.8) L per m²; LB5 (1.6 \pm 0.8 L per m²) was the only site that did not meet that metric (Figure 29).

7.2.3 Eastern Branch

In general, very few oysters were collected in the samples from the two Eastern Branch (EB) reefs (EB1 and EB 2) (Figure 31). No live oysters were reported in either of the EB 2 samples. Neither site met the sustainable criteria in regards to adult oyster density or dry mass (Figures 27 and ??). EB 1 (16.2 \pm 10.1 adult oysters per m²; 18.5 \pm 8.9 g DM per m²) barely met the minimum threshold for restoration reefs for both metrics. Neither site (EB1: 3.5 \pm 1.2 L per m²; EB2: 0.0 \pm 0.0 L per m²) met the target LOV (Figure 29).

7.3 Discussion

7.3.1 Broad Bay

Broad Bay had a much higher proportion of spat than other sites in the survey. There are two potential reasons for this observation. First, Broad Bay has a sandy bottom substrate, which is a firm, stable substrate for the spat-on-shell reefs that were deployed there over 10 y ago. The substrate is harder, which decreases the risk of reef subsidence over time; this feature also makes the reefs less susceptible to the effects of siltation. Second, the hydrodynamics of the Lynnhaven River watershed, including tidal effects (ebb

and flood cycles) are such that Broad Bay is self-sustaining in larval settlement (Lipcius et al., 2015), meaning that a substantial fraction of gametes released by oysters in Broad Bay will return to Broad Bay. Larval settlement does not guarantee survival, however, as is evidenced by the high frequency of dead juvenile oysters in the size-frequency histogram. The effects of intraspecific competition may explain why adult oysters from the Broad Bay reefs (which have higher overall oyster densities) are, on average, smaller than adult oysters at the Linkhorn Bay and Eastern Branch reefs.

Dry mass was estimated using equations from another water body that closely resembles Broad Bay in terms of depth and salinity. However, these calculated values were generated from models of 5+ year old reefs, not 10+ year old reefs – thus, the values generated from these linear regression models may be underestimates, which could explain why BB3 is currently not classified as sustainable by that metric. BB3 was slightly below the target LOV for restoration reefs, which may be the result of the higher density of spat and smaller oysters, but lower density of larger, adult oysters at the site. Broad Bay samples with higher oyster density also had greater species diversity, including shrimp, small fish, crabs, clams, snails, mussels, and sponges.

7.3.2 Linkhorn Bay

Bottom substrate at Linkhorn Bay is a mix of sand and mud, which can be a suitable substrate for construction of oyster shell reefs depending on the ratio. A harder substrate is desirable for loose shell reefs so that they do not sink into the soft bottom. The substrate in LB5 was mostly mud, which explains its low performance. Shell placed over 10 y ago has likely subsided or covered by silt over time, inhibiting new recruitment and precluding growth and survival of larvae settling there. There was a fairly uniform distribution of shell lengths, both live and dead, across all of the Linkhorn Bay reefs, which indicates regular recruitment and conditions suitable for growth and survival into adulthood. The oysters that settled there were capable of growing and thriving for multiple years. Larger (wider and deeper) oysters have more tissue and, thus, produce greater dry mass. Larger oysters also have larger shells that displace more water, explaining Linkhorn Bay's higher LOV values. Linkhorn Bay reefs exhibited a similar pattern to Broad Bay reefs with respect to secondary production, providing habitat for reef-associated species.

7.3.3 Eastern Branch

Since its construction, Eastern Branch oyster reefs have contended with strong tidal currents which have displaced or buried base shells. EB1 had very few oysters, while EB2 had no live oysters in either of the two samples. The two reefs sampled in Eastern Branch were not classifiable as sustainable by any metric. Species diversity in each sample was much lower than the Linkhorn Bay and Broad Bay reefs. Although salinity at the Eastern Branch reef sites likely supports a suite of phytoplankton species suitable for filtration by oysters of all sizes, and the sandy bottom substrate is firm enough to support oyster shell reefs, the packed nature of the sand and the high tidal current velocity reflect suboptimal conditions for the production of sustainable shell reefs. Artificial three-dimensional reefs, such as prefabricated concrete or rock reefs, would likely fare better.

8 Tables

Table 1: Water quality data collected over the course of the Piankatank River granite oyster reef survey, including temperature, salinity, and dissolved oxygen.

Reef Site	Date Sampled	Temperature	Salinity	Dissolved Oxygen
		$^{\circ}\mathrm{C}$		mg per L
47	2/7/2019	1.1	17.9	8.7
671	5/2/2019	14.4	16.4	6.3

Table 2: Oyster reef biomass over time on reefs (dry weight in g m^{-2}). A sustained population level of 50 g dry weight m^{-2} needs to be maintained over time as evidence that a reef is viable.

Year	Oyster Biomass	Non-Oyster Biomass	Total Biomass
0	5	3	8
1	10	6	16
2	20	12	32
3	30	18	48
4	40	24	64
5	50	30	80

Table 3: Water quality data collected over the course of the Lynnhaven River oyster-shell reef survey, including temperature, salinity, and dissolved oxygen.

Reef Site	Date Sampled	Temperature	Salinity	Dissolved Oxygen
		$^{\circ}\mathrm{C}$		mg per L
Broad Bay reef 2	3/17/2019	5.6	18.8	6.6
Linkhorn Bay reef 5	3/18/2019	5.8	14.3	5.9
Linkhorn Bay reef 2	3/19/2019	5.8	16.7	6.1
Eastern Branch reef 1	5/4/2019	16.1	21.6	5.5

9 Figures

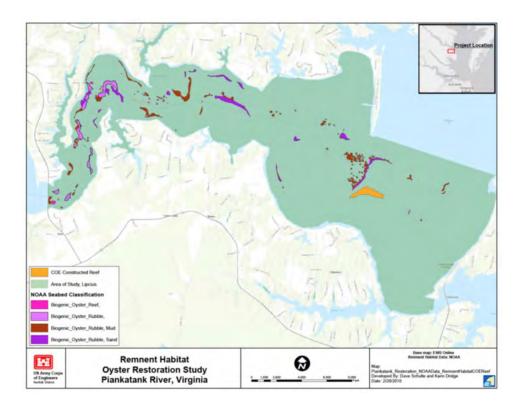


Figure 1: Piankatank River showing USACE reef and potential restoration sites.

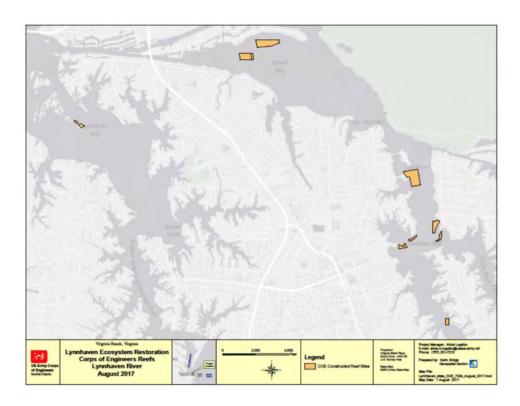


Figure 2: Lynnhaven River showing USACE reefs.

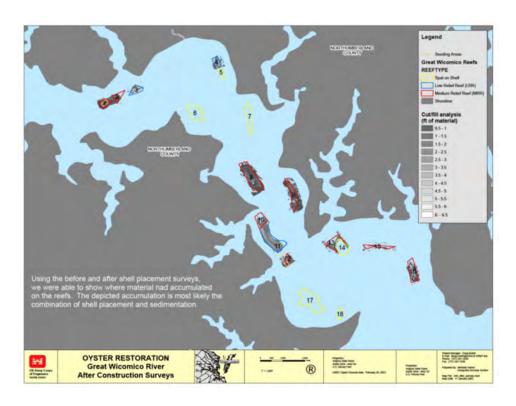


Figure 3: Great Wicomico River showing USACE reefs.

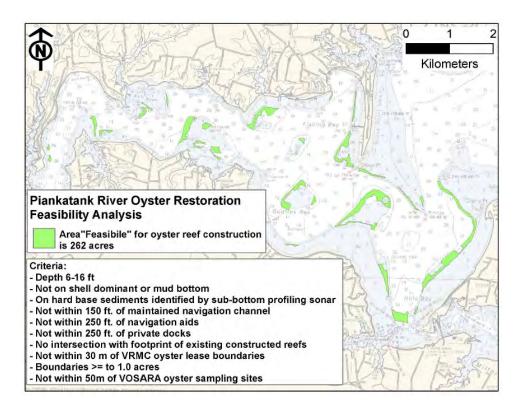


Figure 4: Potential restoration and sampling sites (green) in the Piankatank River.

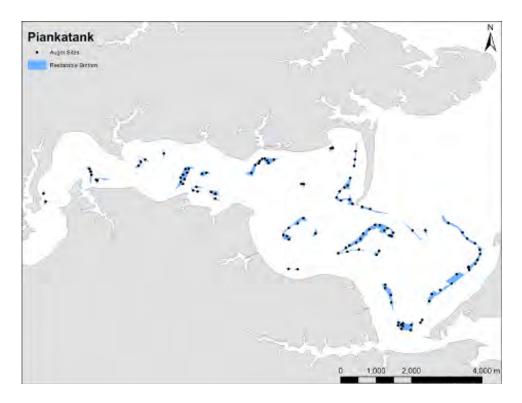


Figure 5: Actual sampling points in potential restoration sites in the Piankatank River.

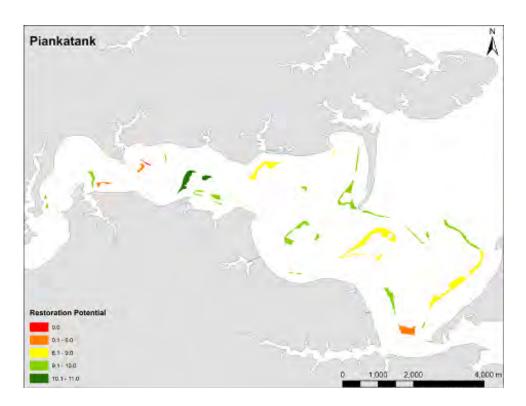


Figure 6: Area-based averages of restoration potential (poor = 0, exceptional = 11).

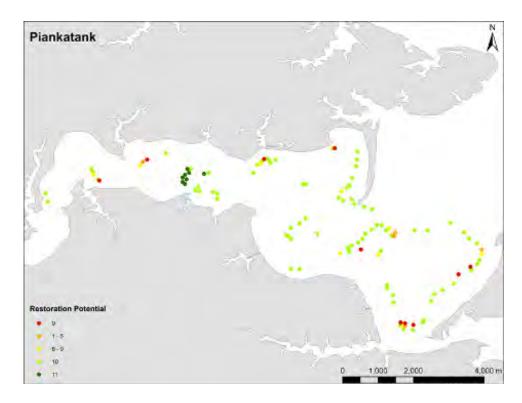


Figure 7: Site-specific restoration potential (poor = 0, exceptional = 11).

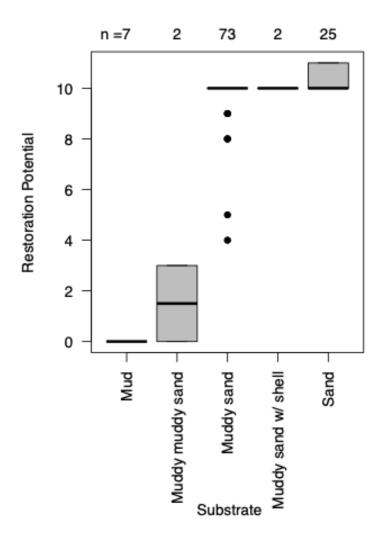


Figure 8: Restoration potential as a function of substrate.

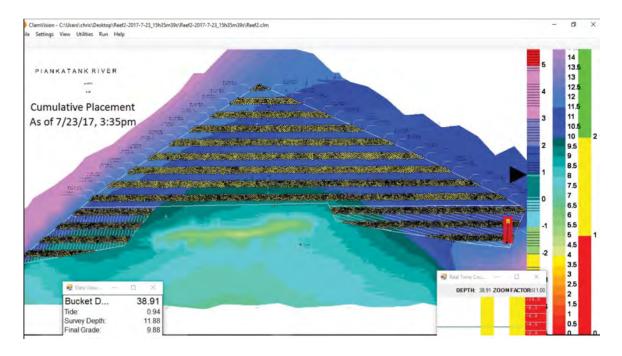


Figure 9: Piankatank River granite oyster reef post-construction.

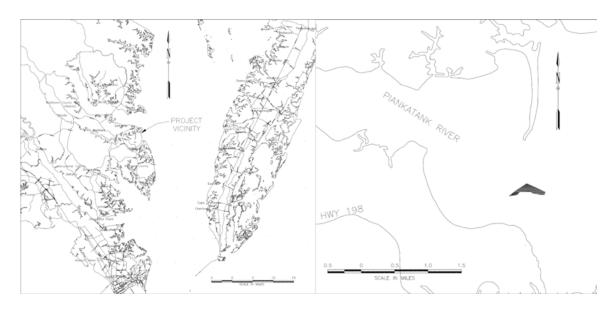


Figure 10: Location of Piankatank River granite oyster reef.

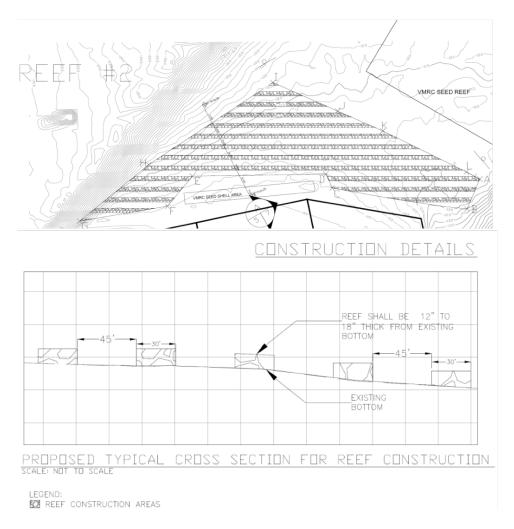


Figure 11: Construction schematic for Piankatank River granite oyster reef.

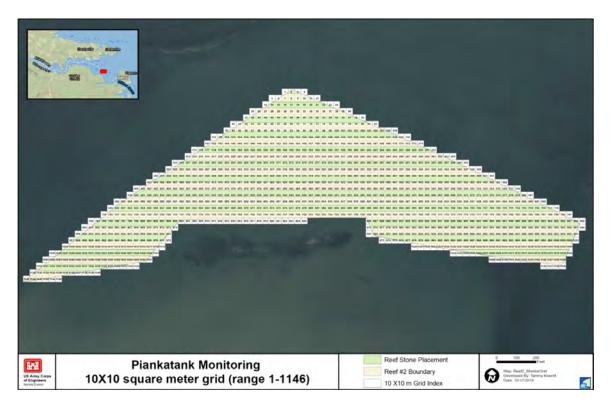


Figure 12: Piankatank River granite oyster reef gridded sampling map (randomly-selected sampling points were supplied by the USACE at the start of the contract).

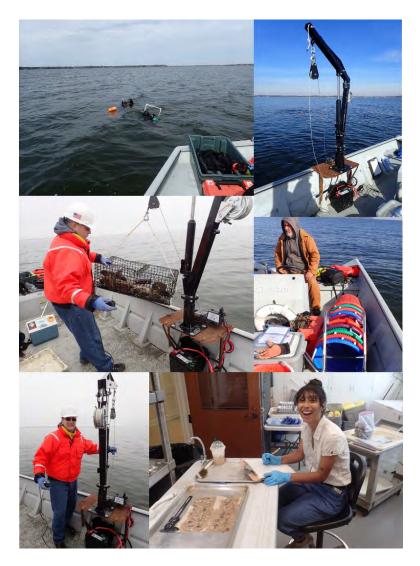


Figure 13: Photographs of Captain Darryl Nixon, his aluminum research vessel with davit crane rig, the dive team, the field crew and laboratory technician during the Piankatank River granite oyster reef survey.



Figure 14: Photographs of granite oyster reef samples with oyster clusters on rocks.

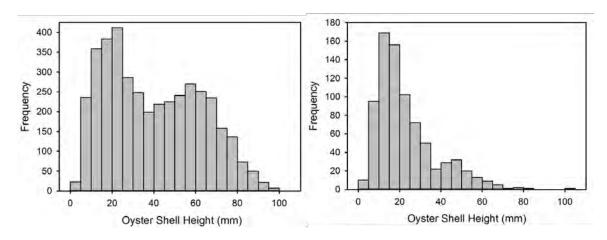


Figure 15: Oyster population size structure from the Piankatank River granite reefs, including (A) live and (B) dead oysters on shell reef samples. Note the different scales for live and dead oysters.

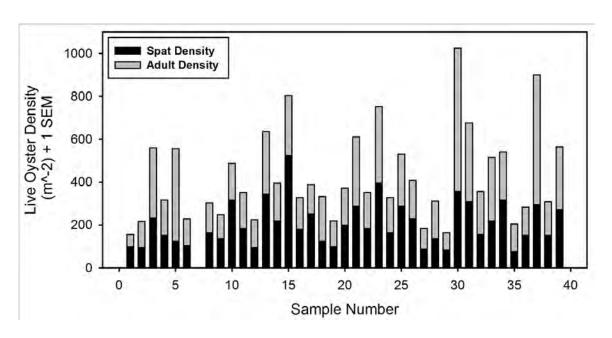


Figure 16: Live oyster density by granite reef sample.

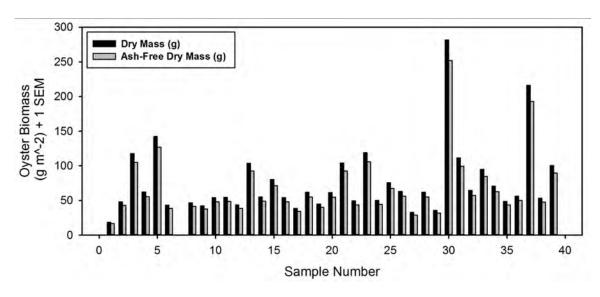


Figure 17: Oyster biomass (g DM/AFDM $\rm m^{-2}$; AFDM = Ash-Free Dry Mass) by granite reef sample, with biomass estimated from representative regression models from the 2009 Great Wicomico River Oyster Reef Survey (Schulte et al. unpublished data).

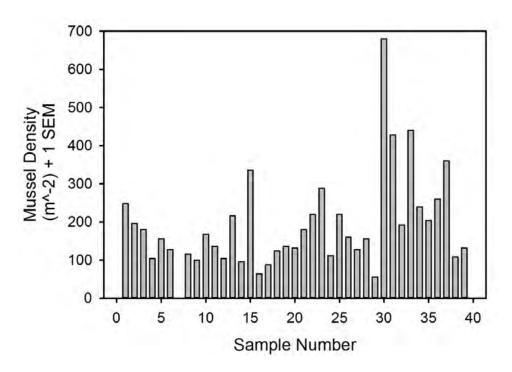


Figure 18: Live mussel density by granite reef sample.

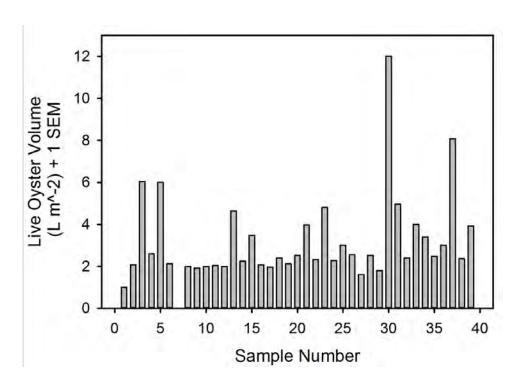


Figure 19: Live oyster shell volume by granite reef sample.

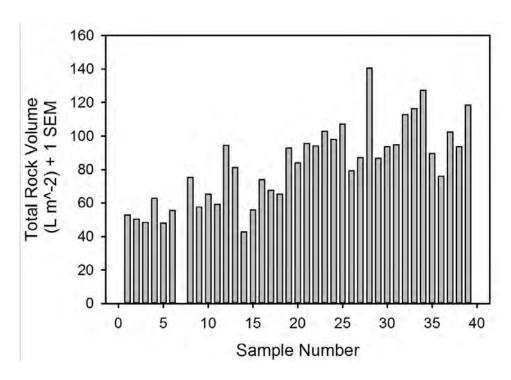
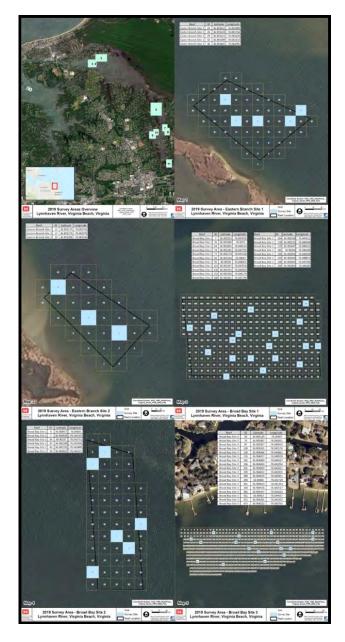


Figure 20: Total rock volume by granite reef sample.



 $\label{eq:Figure 21: Eastern Branch and Broad Bay oyster reef maps with randomly-selected sampling points.}$

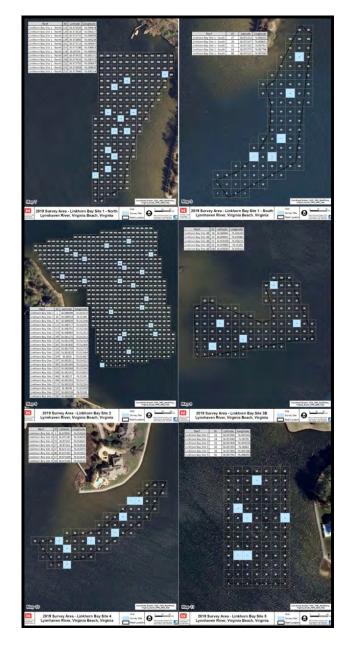


Figure 22: Linkhorn Bay oyster reef maps with randomly-selected sampling points.



Figure 23: Photographs of Captain Carol Melvin Smith and his patent tong rig.



 $Figure\ 24:\ Photographs\ of\ organisms\ collected\ during\ the\ Lynnhaven\ River\ Oyster\ Reef\ Survey, including\ oysters,\ sponges,\ blue\ crabs,\ and\ oyster\ toadfish.$

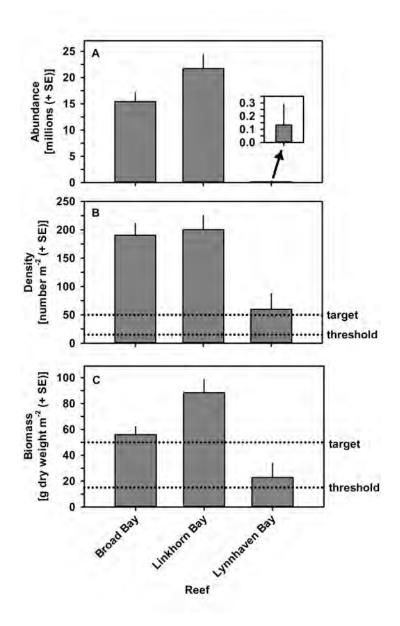


Figure 25: Oyster abundance (A), density (B), and biomass (C) of adults and spat in each of the three bays of the Lynnhaven River system. The Lynnhaven Bay data are only for Eastern Branch reef 1. The data were corrected for survey efficiency (81% Schulte et al. (2018)).

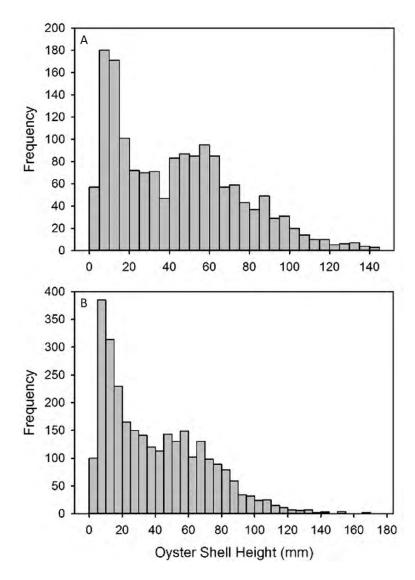


Figure 26: Oyster population size structure from Broad Bay, including (A) live and (B) dead oysters on shell reef samples. Note the different scales for live and dead oysters (See Appendix 1 for oyster population size structure for each Broad Bay reef).

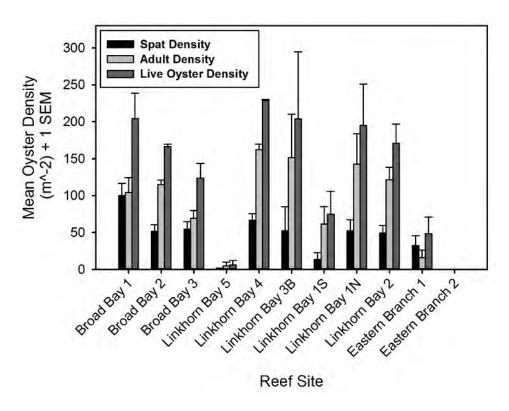


Figure 27: Mean live oyster density (per $m^2 + 1$ SE) by reef site.

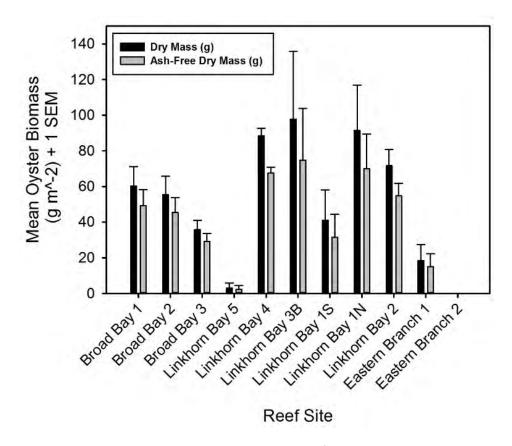


Figure 28: Mean oyster biomass (g DM/AFDM per $m^2 + 1$ SEM; AFDM = Ash-Free Dry Mass) by reef site, with biomass estimated from representative regression models from the 2018 Craney Island Eastward Expansion Oyster Mitigation Reef Survey (Burke and Lipcius 2019).

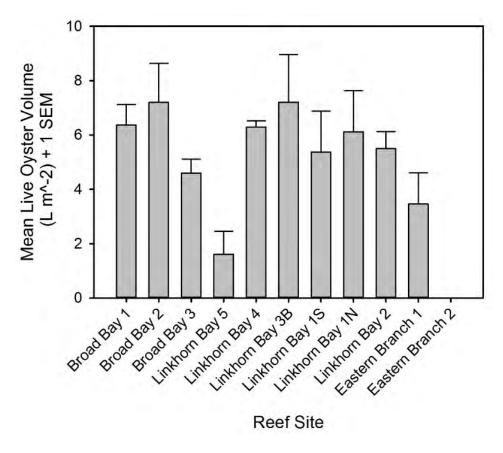


Figure 29: Mean live oyster shell volume (L per $m^2 + 1$ SEM) by reef site.

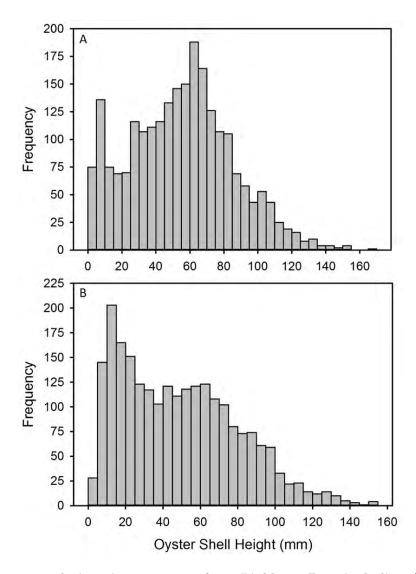


Figure 30: Oyster population size structure from Linkhorn Bay, including (A) live and (B) dead oysters on shell reef samples. Note the different scales for live and dead oysters (See Appendix 1 for oyster population size structure for each Linkhorn Bay reef).

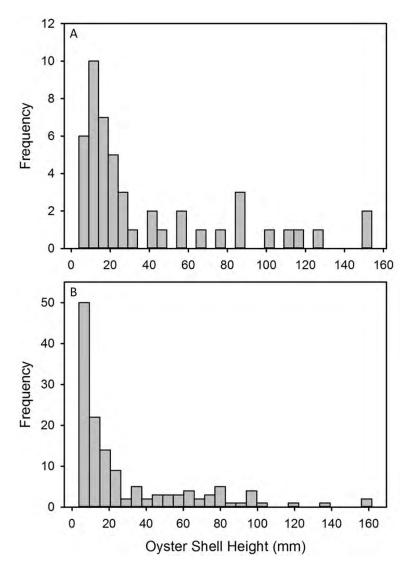


Figure 31: Oyster population size structure from Eastern Branch, including (A) live and (B) dead oysters on shell reef samples.

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