MONITORING AND EVALUATING THE SUCCESS OF THE ST. MARY'S RIVER OYSTER REEF PROJECT

by

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1 Abstract

Oysters are a keystone species in the Chesapeake Bay ecosystem that reduce pollutants, including sediment, nitrogen, and phosphorous, through filtration. The goal of the St. Mary's River Oyster Reef Project was to restore a five-acre oyster reef within the St. Mary's River Shellfish Sanctuary. After nearly a decade of restoration, this study aims to evaluate the success of these restoration efforts. In this study, we assessed the success of the reef project using four major criteria as defined by the Oyster Metrics Workgroup in 2011: percent coverage, oyster density, oyster biomass, and presence of multiple year classes. Sampling was conducted during August 2022 and April 2023. The St. Mary's River Oyster Reef Project met and exceeded the target values for all four criteria, indicating that restoration efforts have been successful. Calculated values for oyster density, oyster biomass, and percent coverage were all at least twice the target value indicating that according to the criteria outlined by the Oyster Metrics Workgroup, the St. Mary's River Oyster Reef Project is successful. The restored reef is not only providing ecosystem services to the St. Mary's River but also serving as a living classroom to the adjacent St. Mary's College of Maryland campus.

2 Definitions

For the purposes of this study, the following terms are defined in the following ways.

Alternate Substrate- Concrete rubble, large stones, or reef balls

Flat Bottom- Areas without concrete rubble, reef balls, or stone piles

 \mathbf{m}^2 - abbreviation for square meter, an area measurement

Oyster Density- Number of oysters per square meter

Oyster Biomass- Estimated oyster tissue dry weight (g) per square meter

Percent Coverage- Percentage of the project area containing oysters

Reef Ball- A concrete structure designed for suitable substrate for reef formation

Spat- Post-settlement juvenile oysters; defined as oysters less than 10 mm in all

portions of this study with the exception of Figure 6

Year Classification used to determine oyster age groups in relation to the year

they were spawned

3 Introduction

The St. Mary's River is a tributary of the lower Potomac River with a drainage area of about 45,000 acres and a state-designated shellfish sanctuary in the upper portion of the River exceeding 1,300 acres (Maryland Department of the Environment [MDE], 2014). The river is listed as "impaired" under section 303d of the Clean Water Act due to low pH in first through fourth order streams, the presence of fecal coliform, and high nitrogen and phosphorus (Maryland Department of Natural Resources [MD DNR], 2012). Human activities in the watershed, such as increasing impervious surface area and overfishing, present environmental challenges including nutrient pollution and depletion of natural stock.

However, the St. Mary's River is listed as a Tier 1 tributary, indicating its suitability for oyster restoration (U.S. Army Corps of Engineers [USACE], 2012). The once-prevalent Eastern oyster (*Crassostrea virginica*) provides a variety of

ecosystem services, including
water filtration, nutrient uptake,
and substrate and refuge for
other organisms, making oyster
restoration highly beneficial to
the river ecosystem. The St.
Mary's River Oyster Reef
Project, the five-acre oyster
restoration site addressed in this
report, is adjacent to St. Mary's

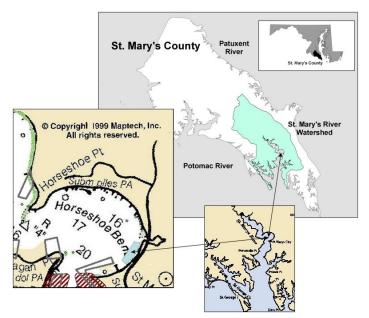


Figure 1. Location of five-acre study site, the St. Mary's River Oyster Reef Project.

College of Maryland (SMCM) and is located in an area commonly referred to as Horseshoe Bend (Figure 1). The project was established in 2012 by the St. Mary's River Watershed Association (SMRWA), Leonardtown Rotary, and SMCM. Goals included restoring a healthy oyster reef in the St. Mary's River Shellfish Sanctuary.

In 2012, SMRWA applied for Maryland Wetlands and Army Corps of Engineers permits to authorize the use of the five-acre project site for oyster restoration utilizing alternate substrates. During the winter of 2012-2013, Chris Tanner and Robert Paul (both marine biologists at SMCM) were contracted to write a plan for restoration on the northern 2.5 acres. In January 2013, implementation of the reef project began by deploying alternate substrates including reef balls and aged and cleaned concrete rubble.

The five-acre St. Mary's River Oyster Reef Project, referred to from here on as the study site, has two distinct areas. Initially, the southern area was planted with only oyster shells and spat (flat bottom), and on the northern end, alternate substrate (concrete rubble, rocks, and reef balls) covers much of the bottom. However, in 2018, SMRWA began adding alternate substrate (reef balls) to the southern area. Over the past ten years, 8,000 bushels of spat-on-shell, 2,644 reef balls, and 250 tons of concrete rubble have been added to the study site.

In the summer of 2017 after four years of restoration, SMRWA and SMCM partnered to conduct a pilot survey to evaluate the success of the study site. SMCM students measured the oyster density, biomass, and age structure on seventeen alternate substrate sites within the five-acre reef site. Additional restoration has occurred annually on the five-acre reef site.

Criteria and Goals

The goal of this study is to evaluate the success of oyster restoration in the five-acre study site. With funding from the National Fish & Wildlife Foundation's Small Watershed Grant program (2021), a Quality Assurance Protection Plan (QAPP) was written and approved in 2022 by the Environmental Protection Agency. Following this Plan, we examined four different metrics outlined by the Oyster Metrics Workgroup (2011; Table 1).

Table 1. Summary of restoration success criteria as defined by the 2011 Oyster Metrics Workgroup.

Metric	1. <u>Percent</u> <u>Coverage</u>	2. <u>Oyster</u> <u>Density</u>	3. <u>Oyster</u> <u>Biomass</u>	4. Presence of multiple year class of live oysters
Minimum	None	15 oysters/m ² covering at least 30% of the target restoration	weight/m ² covering at least 30% of the target restoration	-
Target	30% coverage with spat-on-shell, alternate substrates	50 oysters/m ² covering at least 30% of the target restoration	50 grams dry weight/m ² covering at least 30% of the target restoration	2 year classes present

4 Materials and Methods

Preliminary Methods Determination

In 2017, a pilot study found that scraping oysters off alternate substrate caused some mortality. To avoid the mortality associated with scraping, we conducted a preliminary study to compare two methods: retrieval (scraping) and in situ (visual). For the *in situ* method, a diver (the primary sampler) utilizing a surface air compressor visually counted the oysters within a 0.25 m² quadrat placed on a reef ball or concrete substrate. The observed number of oysters was reported to a data recorder on the boat. The primary sampler then returned to the location of the quadrat and scraped the oysters off of the alternative substrate and placed them in a basket. When all oysters were removed from inside the quadrat, the basket was brought onboard the boat, and the oysters were counted. The two counting methods, in situ and retrieval, were repeated for five separate sites. Only five oysters were killed during the scraping. An average of 91 more oysters (per m²) were counted with the retrieval method than with the visual in situ method (Figure 2). Therefore, we concluded that retrieval methods would be necessary for an accurate assessment of oyster reef density on alternate substrate.

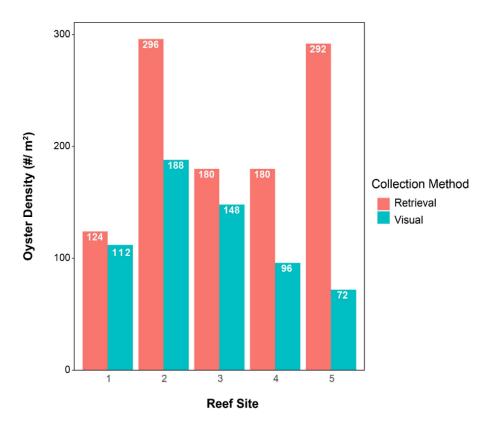


Figure 2. Comparison of oyster density counts (#/m²) using *in situ* methods (visual) and retrieval methods. On average there were 91 less oysters/m² when assessed visually than when assessed using the retrieval method.

Oyster Density

We utilized a random selection method to determine sites for oyster density sampling. The five-acre restoration area was divided into 3x3 meter grids using QGIS

3.28.1-1. This grid was converted into polygons, and we obtained the centroid of each

polygon using geometry
functions in QGIS. Then, we
randomly selected 5% of the
centroids to serve as our study
sites for density measurements.

A Garmin

GPSMap276Cx was used to locate the sampling site (point in the center of the 3x3 m grid).

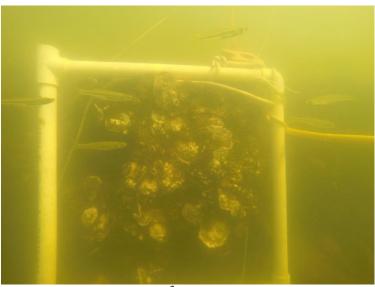


Photo 1. Quadrat (0.25 m²) deployed underwater on a reef ball.

Once we were within 9 m of

the sampling site (our GPS's accuracy), we threw a 0.25 m² quadrat and a basket for collecting oyster shells overboard. On flat bottom sites, the primary sampler traveled to the quadrat and basket and collected oysters within the quadrat to a depth of 15 cm below the surface. On alternate substrate sites (Photo 1), the primary sampler scraped the oysters within the quadrat from all surfaces of the alternate substrate and placed them in the basket. In all sampling locations, the basket was brought to the surface, and the number of live and dead oysters over 10 mm were counted and recorded (Photo 2). Our procedures regarding spat (oysters less than 10 mm) will be discussed later. In 2022, sampling took place between August 9th and 23rd; in 2023, sampling took place between April 5th and 26th.

The number of live and dead oysters collected from within the 0.25 m² quadrat were multiplied by four to determine the number of oysters per m².

Observations made by the samplers, including oxygenation of the sediment and vertical growth, were also recorded.



Photo 2. Oysters retrieved for density measurements.

Estimating spat counts in density measurements

We chose to exclude oysters under 10 mm from our field counting for oyster density for two reasons. The first is that in 2022, sampling was conducted in late summer. Oysters in the Chesapeake Bay generally spawn annually from May to August (Maryland Fish Facts: Shellfish - Eastern Oyster, n.d.). Therefore, oysters less than 10 mm in summer of 2022 were presumably from the summer spawn of 2022. In 2023, sampling was conducted in the spring, and therefore, we did not capture the summer 2023 spawn in our samples. Our results would not have been comparable between years, so we did not count spat during density measurements to ensure

sampling was consistent across seasons. The second reason is that during age structure analysis, we found many oysters less than 10 mm. With limited funding and staff time, we decided to estimate the number of spat post-sampling rather than count each individually. We estimated the average number of spat per oyster shell from our age structure measurements and multiplied by the number of adult oysters per m² to determine total spat per m². The number of live adult oysters per m² and the estimated spat per m² were added to estimate live oysters per m².

Age Structure

Bagget et al. (2014) recommended a sample size of 250 oysters per reef for oyster size-frequency distributions. We initially measured 250 total oysters from two alternate substrate locations (n=100) and three flat bottom (n=150) locations, and separated them into 5 mm bin size classes. The alternative substrate and flat bottom age structure distributions were skewed differently, so we increased our sample size to 500 total oysters—250 from alternate substrate and 250 from flat bottom. Oysters were retrieved from ten sampling locations (Figure 3). The number of spat (<10 mm) were counted on each oyster, and all other oysters were measured to the nearest mm. The oysters were separated into 5 mm size bins and into three year classes. All year class sampling was completed in August 2022.

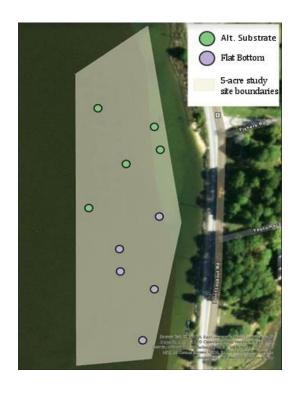


Figure 3. Map of sampling locations for age structure across the five-acre study site.

Biomass

We estimated oyster biomass using the following allometric formula from Jordan et al. (2002). Jordan et al. (2002) measured 450 oysters from 45 sites (10 oysters per site) in the Maryland portion of the Chesapeake Bay for both size (mm) and dry weight (g). They compared the dry tissue weight (g) to the shell height (mm) and developed the following formula (Equation 1):

 $\log_{10}(dry\ tissue\ weight) = 2.06[\log_{10}(shell\ height)] - 3.76$ Equation 1

We used the shell height of oysters (>10 mm) to estimate their biomass using Equation 1. Then, we averaged the biomasses (n = 500) for these oysters. We multiplied the average biomass by the live adult oyster (>10 mm) density (per m^2) for each density measurement. This calculation provided an approximation of the biomass (dry tissue weight in grams) per m^2 at each site where density was calculated.

Percent Coverage

Percent coverage was calculated using the total number of sites sampled for oyster density. The number of sites with zero live or dead oysters was divided by the total number of sites to determine the percentage of sites with zero oysters. Similar methods were used to determine the percentage of sites with greater than zero and greater than fifty oysters per m².

5 Results

Age Structure

A total of 500 live oysters greater than 10 mm were measured to the nearest mm. We counted 502 spat (oysters less than 10 mm) that settled on the 250 oysters measured on hard substrate and 560 spat that settled on the 250 oysters measured on flat bottom. Age structure among oysters retrieved from flat bottom was more evenly distributed than oysters retrieved from hard substrate (Figure 4 and Figure 5). The

distribution of oyster sizes on hard substrate skewed right: more oysters were from smaller size bins. On flat bottom, the highest number of oysters fell within the 96-100 mm size bin (n = 18; Figure 4). The mean oyster shell height fell within the 75-80 mm size bin. On hard substrate, the highest number of oysters fell within the 41-45 mm (n=25) and 46-50 mm (n=25) size bins (Figure 5). The average oyster fell within the 65-70 mm size bin. Oysters under 20 mm and over 145 mm were present at both sites. NOAA Fisheries defines three-year classes in their 2021 Maryland Oyster Monitoring Report: spat (<40 mm), small (40-75 mm), and market (>75 mm). Oysters in all three year classes were present on both hard substrate and flat bottom. The fewest oysters (n=95) were present in the small year class on flat bottom, and the most oysters (n=647) were present in the spat year class on hard substrate (Figure 6).

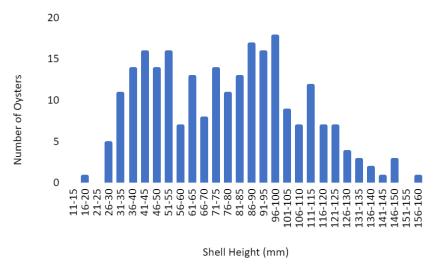


Figure 4. Size distribution (shell height in mm) of oysters (n=250) in adult 5 mm bin size classes on flat bottom.

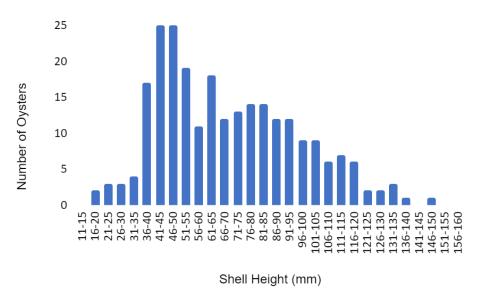


Figure 5. Size distribution (shell height in mm) of oysters (n= 250) in adult 5 mm bin size classes on alternate substrate.

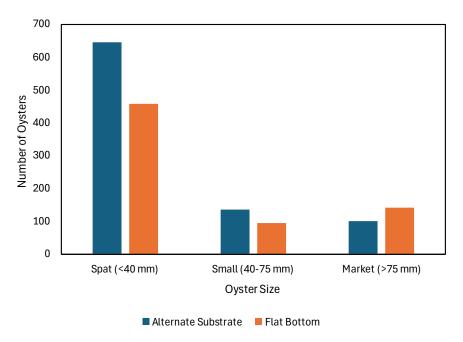


Figure 6. Size distribution (shell height in mm) of oysters (n=1,581) in year classes as defined by NOAA Fisheries on alternate substrate and flat bottom. This graph includes oysters less than 10 mm.

Oyster Density

All but one of the 122 sampling sites were on flat bottom; the remaining site was located on a reef ball. A total of 122 locations were sampled—58 in August 2022 and 64 in April 2023. Oyster density varied across these sites (Figure 7). Across all bottom types there were an average of 75 adult live oysters/m² and 10 adult dead oysters/m². Using the estimated average spat per oyster (2.2 spat per oyster on flat bottom and 2.0 spat per oyster on alternate substrate), we calculated a total of 165.3 spat per m², resulting in an average of 240.7 live oysters per m² (Figure 8). These numbers varied across bottom type. For example, density on shell bottom was more than twice as high as density on both mud and sand bottoms (shell: 486.4 oysters per

m², mud: 221.9 oysters per m², sand: 196.7 oysters per m²). There were seven randomly selected density sampling sites on shell bottom, and fifty-six density sampling sites on mud bottom.

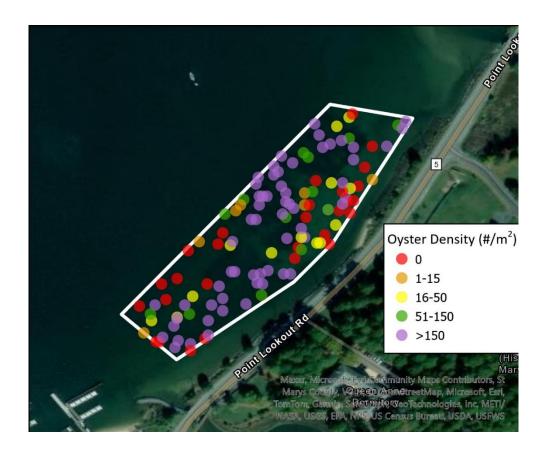


Figure 7. Map of the oyster density (oysters/m²) at each of the 122 density sampling sites.

No live oysters were found at twenty-eight (23.0%) of the 122 density sampling sites. Oysters were present at the remaining 77.0 % of sites. Of the sample sites, 60.7 % had greater than 50 oysters per m², which is more than twice the target value of 30 % coverage.

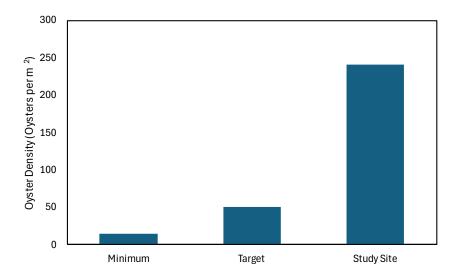


Figure 8. Comparison of the minimum, target, and calculated average oyster densities (oysters/m²) for the five-acre study site.

Biomass

Using the formula from Jordan et al. (2002) and our age structure measurements, we calculated an average biomass per adult oyster of 1.4 g. After multiplying by the oyster density measurements, we found an average biomass of 107.0 g/m^2 across all study sites (Figure 9). Because biomass calculations were derived from the oyster density results, the data are similar for these two metrics. The same 28 sites with no live oysters also had no biomass, and the 65 sites with greater than 50 adult oysters/m² also had greater than 50 g/m². A total of 53.28 % of the study sites had greater than 50 g/m^2 , which is well above the target value of 30 % coverage.

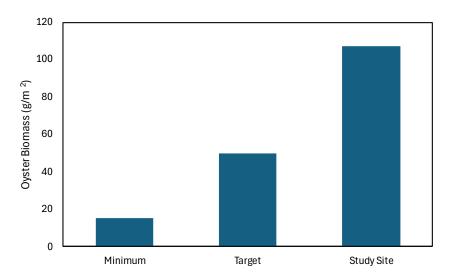


Figure 9. Comparison of the minimum, target, and calculated average oyster biomass values (g/m^2) for the five-acre study site.

6 Discussion

The success of the St. Mary's River Oyster Reef Project was evaluated using four criteria as outlined by the Oyster Metrics Workgroup (2011) and specified in the 2014 Bay Agreement. The first criterion used to determine oyster reef success was percent coverage. According to the Oyster Metrics Workgroup, the target value for percent coverage is the presence of oysters at a density of at least 15 oysters/m² on over 30 % of bottom coverage of the study area. We calculated oysters were present at this density on over 77.05 % of the study area, indicating that the first criterion of success was met at the St. Mary's River five-acre study site.

The second criterion of the Oyster Metrics Workgroup was oyster density: a minimum density of 15 oysters/m² and a target density of 50 oysters/m² covering at

least 30 % of the target restoration. Oyster density metrics traditionally include first year settlement (spat), but due to the large amount of spat (2.0 per oyster on the reef ball and 2.2 per oyster on the shell bottom) and differing sampling times, we did not include individual spat counting in our density counts. Spat were instead estimated using the average spat per shell and used to determine total oysters per m². Across all bottom types surveyed there were an average of 240.7 oysters/m², which is over four times the target density. We calculated that 60.7 % of sites had an oyster density greater than 50 oysters/m². Therefore, the second criterion, oyster density, has also been met at the St. Mary's River five-acre study site.

Oyster density is a commonly measured indicator of restoration success and can be highly variable between reefs and regions. The oyster density and biomass in the St. Mary's River five-acre study site is similar to other restored reefs in the Chesapeake Bay. In 2017, oyster density on restored reefs in Virginia's Great Wicomico, Lynnhaven, and Lafayette Rivers averaged between 98.83 and 308.51 oysters/m², and biomass averaged between 53.91 and 75.14 g/m² (Bruce et al., 2021). Density and biomass in the St. Mary's River (240.7 oysters/m² and 107.0 g/m²) indicate that our five-acre study site is performing well within the context of Chesapeake Bay restored oyster reefs.

Restoration activity also impacts resultant oyster density, particularly for projects utilizing alternate substrates. A restoration project in the Tappan Zee region of the Hudson River reported a high average oyster density (1,976 oysters/m²) only two years post-construction (AKRF Inc. et al., 2021), which is substantially higher

than the density measured in this study. However, the Tappan Zee reefs were restored entirely with alternate substrate types—reef balls and gabions—while the St. Mary's River Oyster Reef Project, in addition to alternate substrates, was partially restored with shell on flat bottom, spat-on-shell on flat bottom, or no treatment. Of the 122 sampling sites where density was measured in the five-acre study area, only one was located on alternate substrate—a chance result from the random methodology of selecting sampling sites. That one sampling site was located on a reef ball and was among the highest density of all the sites (1,200 oysters/m²), which is comparable in oyster density to the Tappan Zee reefs. This suggests that alternate substrate restoration allows for higher oyster densities, most likely due to providing greater surface area for colonization. Although only one of the randomly selected sampling sites for oyster density was located on alternate substrate, our five-acre study site still exceeded the target threshold for density. The average density would be substantially higher had more measurements been taken from alternate substrates.

The third criterion by which we evaluated reef success involves biomass, with a minimum of 15 grams dry weight/ m^2 and a target of 50 grams dry weight/ m^2 covering at least 30 % of the target restoration. We found that the average biomass of the reef was 107.0 g/m^2 and that 53.3 % of the five-acre study site had an approximate biomass greater than 50 g/m^2 . This indicates that this criterion has been met.

The fourth criterion regarding reef success used in this study was the presence of multiple year classes. Year classes were defined by NOAA Fisheries in their 2021

Maryland Oyster Monitoring Report as market (>76 mm), small (40-75 mm), and spat (<40 mm). In the St. Mary's oyster reef, a large number of oysters were present from each year class. Of the 500 oysters sampled for age structure (250 from both flat bottom and alternate substrate). The number of individuals per year class ranged from 95 in the 'small' year class on flat bottom to 647 in the 'spat' year class on alternate substrate. Therefore, multiple year classes were present, and this criterion has been met.

7 Conclusions and Future

Based on our evaluation of the St. Mary's River oyster reef, all four target criteria (percent coverage, oyster density, oyster biomass, and presence of multiple year classes) have been met and exceeded in the study site, the St. Mary's River Oyster Reef Project. In the spring of 2024, additional alternate substrates will be deployed in the few areas where additional restoration efforts will benefit the overall project. At this time, no restoration beyond spring 2024 is planned for this site. With its proximity to St. Mary's College of Maryland, the site will continue to serve the marine science, environmental studies, and other programs as a living classroom.

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