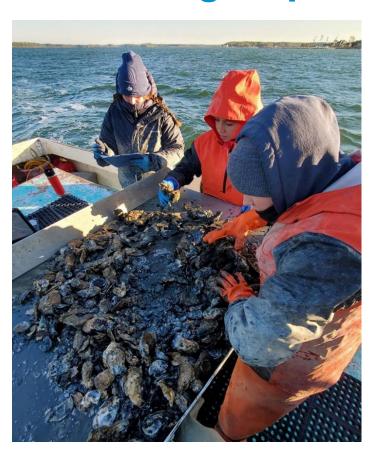


2024 Maryland Oyster Reef Monitoring Report



Analysis of Data from the '10 Tributaries' Sanctuary Oyster Restoration Initiative in Maryland



Data discussed in this report were collected in fall 2024. This report was produced by the NOAA Fisheries Office of Habitat Conservation's Restoration Center and NOAA Chesapeake Bay Office divisions, in partnership with the Maryland Oyster Restoration Interagency Workgroup of the Chesapeake Bay Program's Sustainable Fisheries Goal Implementation Team.

This report, past monitoring reports, tributary-specific oyster restoration plans ('blueprints'), and other oyster restoration technical documents produced by the Maryland Oyster Restoration Interagency Workgroup of the Chesapeake Bay Program's Sustainable Fisheries Goal Implementation Team are available at https://www.chesapeakebay.net/who/publications-archive/maryland and virginia oyster restoration interagency teams.

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Cover photo and above photo, credit: Oyster Recovery Partnership.

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Executive Summary

The <u>2014 Chesapeake Bay Watershed Agreement</u>¹ includes a goal to restore oyster populations in 10 Chesapeake Bay tributaries by 2025 (hereafter, the '10 Tributaries Initiative').

In Maryland, partners including the National Oceanic and Atmospheric Administration; U.S. Army Corps of Engineers, Baltimore District; Oyster Recovery Partnership; and the Maryland Department of Natural Resources are working to achieve this goal through the <u>Maryland Interagency Oyster Restoration Workgroup</u> (hereafter, 'the Workgroup'). The Workgroup is convened under the <u>Sustainable Fisheries Goal Implementation Team of the Chesapeake Bay Program</u> and is chaired by Stephanie Reynolds Westby (NOAA).

A set of <u>oyster restoration success criteria</u>, commonly known as the Chesapeake Bay Oyster Metrics², was developed prior to implementing restoration work in the 10 Tributaries. These guidelines recommend monitoring restored reefs three years and again six years after restoration. Success is evaluated at the six-year mark, with the three-year monitoring serving as an interim assessment. A subset of reefs in Little Choptank, St. Mary's, and Tred Avon rivers

were due for either three-year or six-year monitoring in fall 2024. The results are described in this report.

This report describes reef health relative to four reef-level success criteria set forth in the Oyster Metrics document: oyster density, oyster biomass, multiple year classes, and shell budget. See Discussion section for more information.

Data and analyses in this report can be used to inform what adaptive management measures, if any, should be taken on each of the monitored reefs. Results may also guide restoration in other tributaries.



Healthy oysters from the Little Choptank River Sanctuary sampled during fall oyster monitoring work. Credit: Oyster Recovery Partnership.

KEY MONITORING RESULTS

Key results from 2024:

13 six-year-old reefs (comprising 87 acres) were monitored in 2024. Of these:

- 100% met both the minimum threshold and target oyster density success criteria.
- 100% met both the threshold and target oyster biomass.
- 100% met the success criteria for shell budget and multiple year classes.

Key results from 2015 through 2024:

209 six-year-old reefs (comprising 767 acres) were monitored from 2015 through 2024. Of these:

- 99% met the threshold density and 84% met the target density.
- 99.5% met the threshold oyster biomass, and 89% met the higher, target biomass.
- 100% met the success criteria for multiple year classes.

Section 1: Background and Overview

1.1: Policy Drivers, Oyster Metrics Success Criteria, and Oyster Restoration Planning

The 2014 Chesapeake Bay Watershed Agreement¹ oyster outcome calls for restoring oyster populations in 10 Chesapeake Bay tributaries by 2025. The Chesapeake Bay Program's Sustainable Fisheries Goal Implementation Team (Fisheries GIT) is charged with working to achieve this goal. Driven by Executive Order 13508 (Chesapeake Bay Protection and Restoration) of 2009, some work toward tributary-scale oyster restoration was underway even before the 2014 Chesapeake Bay Watershed Agreement was signed. The Fisheries GIT had convened the Chesapeake Bay Oyster Metrics Workgroup, which, in its 2011 report "Restoration Goals, Quantitative Metrics and Assessment Protocols for Evaluating Success on Restored Oyster Reef Sanctuaries," (hereafter, 'Oyster Metrics') established Bay-wide, science-based, consensus success criteria for oyster restoration to be tracked three years, and again six years, following initial restoration (Table 1).

Reef-level success criteria	Biological Metrics Structural Metrics	Oyster Density: Minimum threshold = 15 oysters per m ² over 30% of the reef area Target = 50 oysters per m ² over 30% of the reef area				
		Oyster Biomass: Minimum threshold = 15 grams dry weight per m ² over 30% of the reef area Target = 50 grams dry weight per m ² over 30% of the reef area				
		Multiple Year Classes: Presence of multiple year classes on the reef, as defined by oysters in at least two of the following size classes: market (>76 mm); small (40-75mm); spat (<40mm)				
		Shell Budget: Stable or increasing shell volume on the reef				
		Reef Footprint: Stable or increasing reef footprint compared to baseline				
		Reef Height: Stable or increasing reef height compared to baseline				
Tributary-level success criteria	A minimum of 50% of currently restorable area within a given tributary, which constitutes at least 8% of historic oyster habitat, meets the reef-level success criteria					

Table 1: Oyster Metrics reef-level and tributary-level success criteria. Success relative to these criteria are determined when monitoring occurs six years post restoration.

Once these success criteria were adopted, the Fisheries GIT convened <u>interagency workgroups in Maryland</u> and <u>Virginia</u> to plan and coordinate restoration work in each state. In Maryland, the Workgroup is chaired by the National Oceanic and Atmospheric Administration (NOAA) and includes members from the Maryland Department of Natural Resources (DNR), Oyster Recovery Partnership (ORP), and the U.S. Army Corps of Engineers (USACE), Baltimore District (USACE). The Workgroup developed oyster restoration tributary plans (also known as "blueprints") for Harris Creek³, Little Choptank River⁴, Tred Avon River⁵, upper St. Marys River⁶, and Manokin River⁷, in consultation with a group of consulting scientists and the public.

1.2: Overview of Report Content

Reefs are monitored at three and six years after restoration, per recommendations from the Oyster Metrics Workgroup and each river's tributary plan. In 2024, a subset of restored reefs in Little Choptank River, St. Marys River and Tred Avon River had matured to three or six years and were monitored in the fall. Summarized monitoring results for these reefs are included in this report (Table 2), as well as a summary of cumulative monitoring results for the entire project, 2015–2024 (Table 3). In additional, sentinel reefs (restored sites that are monitored annually) in Tred Avon were monitored in fall 2024. Data on these reefs are in Appendices A1, A2, and A3.

This report describes success relative to four of the six reef-level Oyster Metrics criteria: oyster density, oyster biomass, multiple year classes, and shell budget. In the earlier years of monitoring under the 10 Tributaries Initiative, all six Oyster Metrics success criteria were measured (the previously listed four metrics, plus reef height and reef footprint). However, over the course of nearly a decade of monitoring, all reefs (100%) passed the reef height and reef footprint metrics. This information, combined with a requisite halt in sonar work during the pandemic years and the resource-intensive nature of measuring these parameters using sonar equipment, has led NOAA to adapt to measuring these parameters only on reefs that, per the other, more sensitive, metrics, are showing signs of potential poor reef health. This revised protocol is consistent with the adaptive management recommended in the original Oyster Metrics document. (See Discussion section for full description.)

Past monitoring reports are available from the Chesapeake Bay Program's <u>Maryland and Virginia Oyster Restoration</u> Interagency Teams Publications page.

In addition to Oyster Metrics success criteria monitoring, oyster disease data is also collected by DNR, and is available in <u>DNR's annual Fall Survey Report</u>. Water-quality data is available at DNR's <u>Eyes on the Bay website</u> and on the NOAA Chesapeake Bay Interpretive Buoy System website.

1.3: Funding and Acknowledgements

Monitoring data for the biological success metrics (oyster density, oyster biomass, multiple year classes, and shell budget) were collected, managed, and analyzed by ORP, Specialty Underwater Services, and contracted commercial watermen, with assistance from Workgroup partners.

This was funded by a \$117,500 NOAA contract to ORP, and a \$106,969 USACE programmatic agreement to ORP.

This report was drafted by NOAA, with guidance from the Workgroup. Results of these analyses will be used to document the success or failure of restoration relative to the Oyster Metrics criteria, to guide adaptive management of these reefs, and to inform future oyster restoration efforts. Technical review of this report was provided by scientific experts and Workgroup members, per NOAA research communications guidelines.

Section 2: Overview of Monitoring

2.1: Monitoring Synopsis

In fall 2024, the following were monitored (Figures 2, 3, and 4):

- Three-year-old reefs: 12 in the St. Marys River and 34 in the Tred Avon River
- Six-year-old reefs: 10 in the Little Choptank River and three in the Tred Avon River

Data used to determine success relative to the four measured metrics (oyster density, oyster biomass, multiple year classes, and shell volume) were collected at the same time, using a stratified random survey design. Methods used to select sampling sites, analyze samples, and assess success relative to each metric were identical for all reefs. See Appendix B for full monitoring methods.

Large-scale restoration work under the 10 Tributaries Initiative has also occurred in Harris Creek and Manokin rivers. All Harris Creek restored reefs are now more than six years old, which is the monitoring time frame recommended in Oyster Metrics. Results from restoration work here have been tallied as part of Table 3, where cumulative results are described. Restored reefs in the Manokin River are not yet three years old, so have not yet been monitored per Oyster Metrics recommendations.

2.1: Location of Monitored Reefs

All three-year-old and six-year-old reefs monitored in 2024 are represented on the maps that follow.

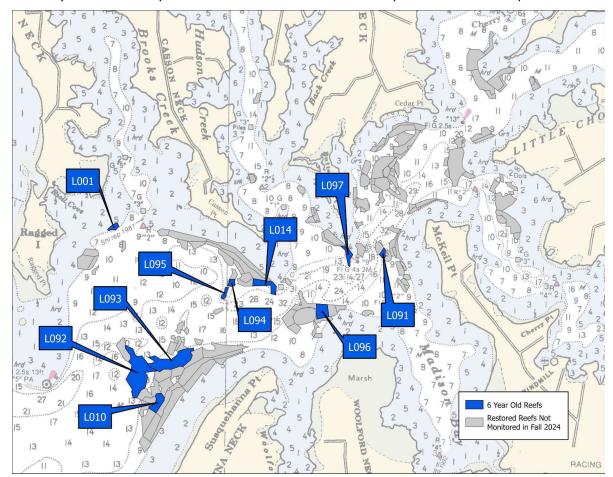


Figure 2: Locations of Little Choptank River reefs monitored in fall 2024. 10 six-year-old reefs were monitored.

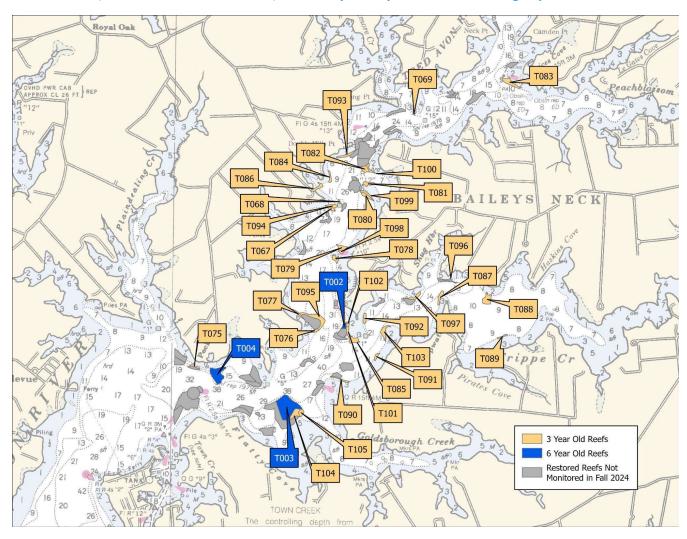


Figure 3: Locations of Tred Avon River reefs monitored in fall 2024. 34 three-year-old reefs and three six-year-old reefs were monitored.

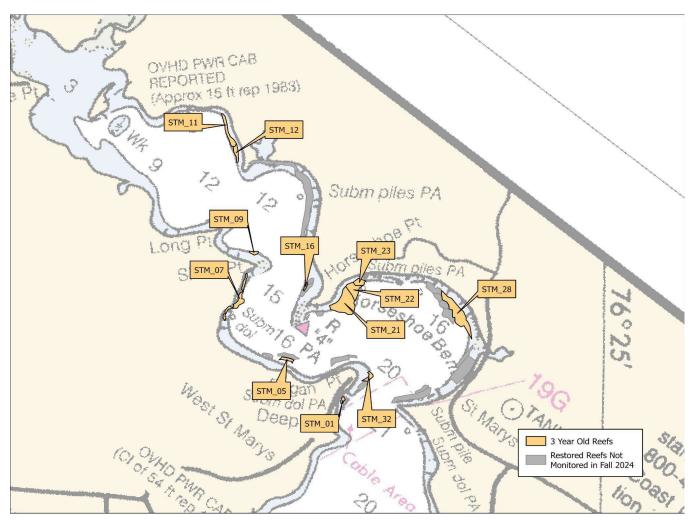


Figure 4: Locations of St. Marys River reefs monitored in fall 2024. 12 three-year-old reefs were monitored.

Section 3: Results Summary

3.1: Summary of Fall 2024 Monitoring Results

A summary of results is depicted in Table 2. The table shows the percent of three-year-old and six-year-old reefs in each tributary, and across all tributaries, that met each Oyster Metrics success criteria.

			Oyster Density		Oyster Biomass		Multiple Year Classes	Shell Budget	
Reef Type	Tributary	# of reefs monitored in fall 2022	% of reefs meeting minimum threshold	% of reefs meeting target	% of reefs meeting minimum threshold	% of reefs meeting target	% with multiple year classes present	% with stable/ increasing shell budget	
	Little Choptank	No 3-year-old reefs in Little Choptank in Fall 2024							
3 Year Old	St. Marys	12	92%	92%	92%	67%	100%	TBD	
	Tred Avon	34	97%	88%	97%	97%	100%	TBD	
	All Tribs Combined	46	96%	89%	96%	89%	100%	тво	
	Little Choptank	10	100%	100%	100%	100%	100%	100%	
6 Year Old	St. Marys	No 6-year-old reefs in St. Marys in Fall 2024							
	Tred Avon	3	100%	100%	100%	100%	100%	100%	
	All Tribs Combined	13	100%	100%	100%	100%	100%	100%	

Table 2: Percents of three-year-old and six-year-old reefs monitored in fall 2024 that met each Oyster Metrics success criterion. In 2024, only St. Marys River and Tred Avon had three-year-old reefs monitored, and only Little Choptank and Tred Avon had six-year-old reefs monitored. Three-year-old reefs have the designation 'TBD' for shell budget, as shell volume must be measured at least one more time to determine if there is a stable, increasing, or decreasing shell volume trend (shell budget).

3.1: Summary of Cumulative Results, 2015–2024

Reef monitoring under the 10 Tributaries Initiative started in 2015. Summary results from fall 2015 through fall 2024 are depicted in Table 3. The table shows the percent of three-year-old and six-year-old reefs in each tributary, and across all tributaries combined, that met each oyster metric throughout the monitoring years. Figures 5 and 6 are graphic representations of the percent of reefs from 2015 through 2024 that met oyster density and biomass minimum threshold and target metrics.

			Oyster Density		Oyster Biomass		Multiple Year Classes	Shell Budget
Reef Type	Tributary	# of reefs monitored in fall 2015–2023	% of reefs meeting minimum threshold	% of reefs meeting target	% of reefs meeting minimum threshold	% of reefs target	% with multiple year classes present	% with stable/ increasing shell budget
	Harris Creek	90 (348 acres)	98%	79%	98%	74%	100%	TBD
	Little Choptank	104 (358 acres)	98%	88%	98%	83%	100%	TBD
3-year-old	Tred Avon	68 (131 acres)	93%	54%	96%	76%	100%	TBD
	St. Marys	12 (29 acres)	92%	92%	92%	67%	100%	TBD
	All Tribs Combined	274 (866 acres)	96%	77%	97%	78%	100%	TBD
6-year-old	Harris Creek	90 (348 acres)	99%	79%	99%	81%	100%	98%
	Little Choptank	92 (339 acres)	100%	91%	100%	97%	100%	86%
	Tred Avon	27 (80 acres)	96%	74%	100%	93%	100%	96%
	All Tribs Combined	209 (767 acres)	99%	84%	99.5%	89%	100%	92%

Table 3: Percents of three-year-old and six-year-old reefs monitored from 2015 through 2024 that met each Oyster Metrics success criterion. Three-year-old reefs have the designation 'TBD' for shell budget, as shell volume must be measured at least one more time to determine if there is a stable, increasing, or decreasing shell volume trend (shell budget). Some results may differ slightly from previous reports due to corrections made to previous minor calculation errors.

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Figure 5: Cumulative percentages of three- and six-year-old reefs meeting the oyster density threshold and target success criteria 2015–2024.

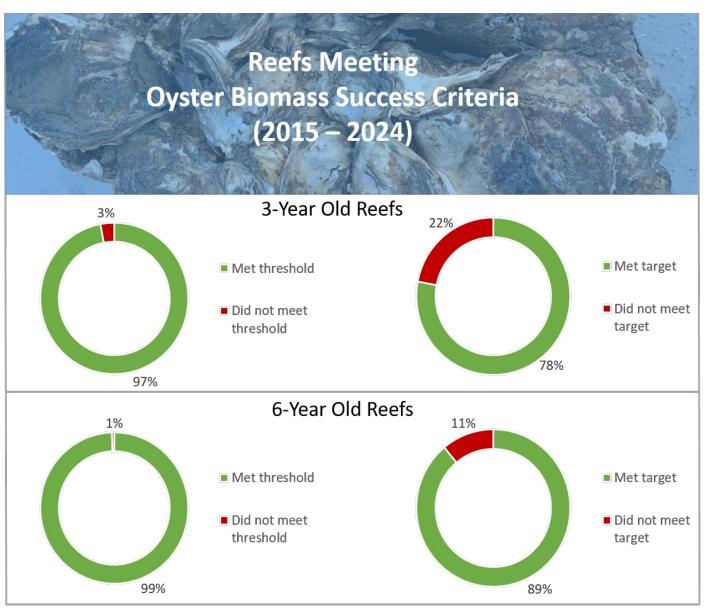


Figure 6: Cumulative percentages of three- and six-year-old reefs meeting the oyster biomass threshold and target success criteria 2015–2024.

Section 4: Discussion

4.1: Overall Trends

The trends observed in previous monitoring years generally continued in 2024, with a large majority of restored reefs meeting the Oyster Metrics success criteria measured.

Standout points include:

- Across all tributaries combined, on the six-year-old reefs (the point at which, per Oyster Metrics, a reef can be considered successfully restored):
 - Among those monitored in 2024, 100% met the targets for both oyster density and biomass, and 100% had a stable or increasing shell budget.
 - Among those monitored from 2015 through 2024, 99% met the minimum threshold for oyster density, and 84% met the higher target level for oyster density.
- 100% of all reefs monitored met the success criteria for multiple year classes. See Figure 7 for a visual representation of the relative proportion of large (market-size) oysters on each reef monitored in 2024.

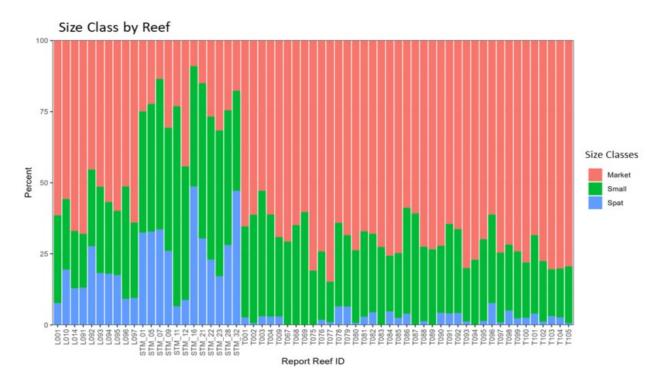


Figure 7: Percentage of spat (< 40 mm), small (40 - 75 mm), and market (≥76 mm) oysters on each reef monitored in 2024. The large proportion of market-size oysters (red) is consistent with the finding that 100% of reefs monitored in 2024 meet the ideal, target biomass, per Oyster Metrics. The Little Choptank reefs (those starting with 'L' along the X axis) are six-year-old reefs; Tred Avon ('T') reefs are a mix of three-year-old and six-year-old reefs; and St. Marys ('STM') reefs are three years old. Spat (blue shading) found on all these reefs are likely natural recruitment, as the most recent hatchery plantings were three or more years ago, and those oysters would have grown beyond this size. Tred Avon typically receives a lower natural spat set than Little Choptank and St. Marys rivers, which is evident in the low spat numbers in the graph. More information about each reef can be found in Appendix A, referenced by reef number (along X axis).

4.2: Monitoring for Reef Height and Reef Footprint Success Criteria

This report describes restoration success relative to four of the six Oyster Metrics criteria (oyster density, oyster biomass, multiple year classes, and shell budget, collectively known as the 'biological metrics'). In the earlier years of monitoring under the 10 Tributaries Initiative, all six Oyster Metrics success criteria were measured (the four biological metrics, plus reef height and reef footprint). However, in more than a decade of measuring reef height and reef footprint, not a single reef has failed these criteria.

NOAA has been the sole partner monitoring reef height and footprint, and has surveyed 134 reefs, comprising 511 acres, with multibeam sonar, at 0.25-meter resolution. Each of these reefs has been monitored at least twice since 2015, as required by Oyster Metrics. Through this work, NOAA has learned that 100% of the reefs monitored, including both restored and untreated reefs (control/reference reefs), met the reef height and footprint metrics. This information, combined with a requisite halt in sonar work during the pandemic years and the resource-intensive nature of measuring these parameters using sonar equipment, led NOAA to adapt to measuring these parameters only on reefs that, per the biological metrics—which appear to be more sensitive—show signs of potential poor reef health.

This revised protocol is consistent with the adaptive management approach recommended in the original Oyster Metrics document. Specifically, the Oyster Metrics document recognizes that "...future research will inform oyster restoration practices, and strongly encourages the use of sound adaptive management practices. We expect that, as the state of knowledge advances, targets and approaches outlined here will evolve." No reefs failed the biological metrics in 2024; therefore, per this revised protocol, no reefs were monitored for reef height or footprint.

4.3: Use of Multiple Gear Types

As in previous years, two different types of gear (hydraulic patent tongs and divers) were used to collect oyster samples, as each was more suited to different substrate types. (See Appendix B, Table B1, for which reef types were monitored with which gear). This was done to maximize the efficiency of restoration monitoring. Earlier field comparisons⁸ on harvested natural oyster reefs revealed no difference between oyster densities estimated using divers and those estimated using patent tongs. However, a 2020 study⁹ in Harris Creek on restored reefs found that oyster densities estimated by divers were, on average, 3.35 times higher than those estimated using patent tongs, indicating that patent tongs resulted in statistically significantly fewer oysters.

In 2024, new reinforced patent tongs were used (See Appendix B for full methods) that likely sampled more efficiently, and may reduce the difference between patent tong and dive sampling. However, because different gear types were used for sampling, and research^{8, 9} results varied, it may not be appropriate to use data in this report to compare the efficacy of different reef treatment types. For clarity and consistency, results in this report are given without the diver-based correction factor on reefs monitored using patent tongs. However, the authors recognize that these results may be conservative.

4.4: Elevated Natural Spat Sets in Maryland in Recent Years

In 2020–2024, Maryland saw natural oyster spatsets above the long-term, statewide average. This included particularly abundant spatsets in the St. Marys River in 2022 and a high spatset in Tred Avon River in 2023. The Tred Avon has historically been a lower-natural-recruitment area than any of the other 10 tributaries slated for restoration. These trends bode well for the longer-term success of the restored reefs. In 2024, the St. Marys Sanctuary had an above average spatset, while the other four sanctuaries had regional spatsets below their 20-year average.

4.5: Factors Influencing Future Reef Health

Although the information in this report looks promising for the long-term sustainability of reefs in Harris Creek, Little Choptank River, Tred Avon River, and St. Marys River, several factors could affect continued success. These include future water-quality issues (e.g., low salinity, low dissolved oxygen levels), oyster disease, funding for future monitoring and adaptive management, and poaching (illegal oyster harvesting). Data and analyses in this report can be used to understand the success or failure of restored reefs within a six-year time frame, and to inform future restoration and adaptive management. The monitoring undertaken three years post restoration is considered an adaptive management checkpoint. Information from this interval is used by restoration partners to determine whether a reef requires the second seed planting called for in each river's tributary plan, and if unsuccessful reefs should receive other management actions.

Section 5: Definitions

Some words defined here are used only in appendices, not in the report itself.

Biological metrics: Four of the six Oyster Metrics success criteria, including oyster density, oyster biomass, multiple year classes, and shell budget.

Fall 2024 monitoring: Monitoring undertaken on restored reefs that turned three or six years old in fall 2024. Monitoring was also done annually on sentinel reefs in the Tred Avon River.

Fossil shell: Consolidated fossil oyster shell material from Florida used as a base to construct reefs. This is oyster shell cemented into a fossilized limestone, and is a true fossil, mined from 30 to 40 feet under dry land, as opposed to the Chesapeake Bay dredged shell.

Mixed shell: A mixture of scallop, whelk, and clam shell from seafood processing plants. The shell is double-cracked before being imported into Maryland to ensure a clean product. This process results in the shell being largely fragmented.

Oyster gardening reef: A reef planted with oysters from various community-based oyster programs, where volunteers grow oysters in cages hanging from docks.

Oyster Metrics: Success criteria for restored oyster reefs targeted for restoration under the 2014 Chesapeake Bay Watershed Agreement. These are defined in the report "Restoration Goals, Quantitative Metrics and Assessment Protocols for Evaluating Success on Restored Oyster Reef Sanctuaries." See Table 1 for description of the six reeflevel criteria.

Premet reefs: Reefs that were assumed to have met the Oyster Metrics density target criteria (50+ oysters per m²) when surveyed prior to commencement of large-scale restoration efforts, and therefore did not initially receive further restoration treatment. However, the prerestoration data on some reefs was at an insufficient resolution to determine definitively whether or not the reefs met the density target. Thus, it is an assumption that the reefs in fact met the density success metric at that time, but it is not certain. These reefs are monitored every three years, as are other reefs, to determine appropriate adaptive management needs.

Reef restoration treatment: The particular method used to restore a reef. See Appendix B, Table 1, for description of reef treatment types.

Second-year-class seeding: A second planting of spat-on-shell some reefs receive approximately four years after initial restoration. This is intended to ensure that each reef has at least two year classes, which is an Oyster Metrics criteria. It can also help ensure that reefs meet the oyster density and biomass criteria. Second-year-class seedings are called for in each river's oyster restoration tributary plan. If a reef shows higher-than-expected oyster density when monitored three years post restoration, and a second year class is present, a second-year-class seeding may not be required.

Seed-only reefs: Reefs treated only with hatchery-produced oyster seed (spat-on-shell). No base reef-building substrate was added prior to seeding. This treatment was generally used on reefs where the prerestoration population was 5 oysters per m² or greater, but less than 50 oysters per m² (see Harris Creek Tributary Plan², Little Choptank Tributary Plan³, and Tred Avon Tributary Plan⁴ for detailed description of how the Workgroup determined treatment type for each reef). See Appendix B, Table 1, for reef treatment type relative to other treatment types.

Sentinel reefs: A subset of the restored reefs that are monitored annually (rather than only three years and six years after restoration, which is the standard for other restored reefs). See Appendix B, Table 1, for reef treatment type relative to other treatment types.

Six-year-old reef: Reef that received restoration treatment six years prior, and—per Oyster Metrics and tributary plans—was monitored three years post restoration and again six years post restoration.

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Spat-on-shell: Hatchery-produced juvenile oysters attached to the shells of dead oysters. Shell typically comes from shucking houses.

Stone substrate reefs: Reefs constructed using a type of stone that is geologically classified as amphibolite. The stone was graded to fit through a 6-inch mesh screen. These reefs were then seeded with spat-on-shell. See Appendix B, Table 1, for reef treatment type relative to other treatment types.

Stone reefs topped with mixed shell: Reefs constructed from a stone base, then capped with mixed shell and seeded with spat-on-shell. See Appendix B, Table 1, for reef treatment type relative to other treatment types.

Stone reefs topped with fossil shell: Reefs constructed from a stone base, then capped with fossil shell and seeded with spat-on-shell. See Appendix B, Table 1, for reef treatment type relative to other treatment types.

Substrate + seed reefs: Reefs treated with reef-building substrate, generally to a height of 6 inches to 1 foot above the surrounding soft bottom. Substrate was either mixed shell, fossil shell, stone, or a combination. Substrate placement was followed by planting with hatchery-produced spat-on-shell. Substrate + seed treatment type was typically used where pre restoration oyster populations were below 5 oysters per m², or where sonar surveys found no evidence of shell. See Appendix B, Table 1, for reef treatment type relative to other treatment types.

Three-year-old reef: Reef that received restoration treatment three years prior, and—per Oyster Metrics and tributary plans—was monitored three years post restoration.

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Appendix A: Downloadable Table of Summary Data by Reef, and Length-Frequency Histogram for Each Reef

2024 data is broken into three tables:

- Table A1: 2024 Summary data by reef
- Table A2: 2024 Length-frequency histograms for reefs monitored using divers
- Table A3: 2024 Length-frequency histograms for reefs monitored using patent tongs

To access Appendix A, click on this link to download an Excel file:

https://www.chesapeakebay.net/what/publications/2024-appendix-a-table-of-summary-data-by-reef-final

Appendix B: Monitoring Methods

This section describes methods for determining success relative to biological Oyster Metrics criteria (oyster density, oyster biomass, multiple year classes, shell budget).

Survey Design

A stratified random sampling survey is used to collect biological data on restored reefs within a given tributary. Each reef is its own stratum, and a random number of sample points are assigned based on reef size, reducing relative error among samples. The number of samples collected at each reef is optimized for data precision and accuracy for each gear type used¹. For reefs sampled using patent tongs, the number of samples increased with reef size and averaged 2.5 samples per acre. For reefs sampled using divers, five samples were collected per reef, averaging 1.5 samples per acre. ArcGIS Pro is used to generate sampling points for each reef. All reefs that are due for monitoring are compiled into a shapefile, and sampling locations are generated within the area of the reef that was planted with spat on shell. This ensures that sample points are created within the area that received oysters.

Field Collection

Data to determine success relative to the four biological metrics (oyster density, oyster biomass, multiple year classes, and shell volume) were collected at the same time. Data collection occurred from October through December of 2024. Sampling is conducted during daylight hours. Navigation to sampling locations and sample coordinate documentation is done using a differential global positioning system (DGPS) attached to a laptop with ArcGIS 10.8.2 used as the navigational program. The vessel navigates as closely as possible to the designated random points, and a waypoint (virtual GPS marker) is created at the location of each sample.

As in previous years, two different types of gear were used to collect samples, depending on reef substrate type (Table B1). Divers were used to collect samples from reefs with substrate materials that were not amenable to patent tong sampling (stone and fossil shell substrate reefs; see definitions in Section 5 of the report). Patent tongs were used to collect samples from all other reef types (seed only, mixed-shell base, and pre-met reefs; see definitions in Section 5 of the report).

Because two different gear types were used for sampling, and the relative sampling efficiencies of those gears can vary^{2,3}, oyster density and biomass data may not be directly comparable between reef treatment types. For both diver and patent tong data, oyster density and oyster biomass information were standardized based on area sampled.

Hydraulic patent tongs are a specialized commercial fishing gear used to harvest oysters in the Chesapeake Bay. The patent tong design functions much like a benthic grab, collecting oysters and underlying substrate from a known fixed area of the bottom. The tongs used in 2024 were reinforced by welding heavy-gauge steel teeth onto the frame. These sampled an area equal to 1 m² of the seafloor. The patent tongs were suspended from a boom over one side of the vessel and deployed to the bottom at each sampling location. A DGPS antenna was positioned adjacent to the location where the patent tongs were deployed, and a waypoint with the geographic coordinates of each sample location was documented.

	Reef-building substrate	Substrate		Seed oysters planted	Gear type used to collect biological
				•	
Treatment Type	added?	Material	Cap Material	onto reef?	metrics data
				Yes	
Seed only	No	None	None	(spat-on-shell)	Patent tongs
		Mixed shell			
		(clam, conch,		Yes	
Mixed shell	Yes	whelk)	None	(spat-on-shell)	Patent tongs
				Yes	
Fossil shell	Yes	Fossil shell	None	(spat-on-shell)	Divers
Oyster gardening				Yes	
reef	No	None	None	(spat-on-shell)	Patent tongs
		Amphibolite		Yes	
Stone	Yes	(stone)	None	(spat-on-shell)	Divers
			Mixed shell		
Stone topped		Amphibolite	(clam, conch,	Yes	
with mixed shell	Yes	(stone)	whelk)	(spat-on-shell)	Divers
Stone topped		Amphibolite		Yes	
with fossil shell	Yes	(stone)	Fossil shell	(spat-on-shell)	Divers

Table B1: Description of treatments used to restore reefs in Harris Creek, Little Choptank River, and Tred Avon rivers. Also listed is the gear type used to monitor each reef treatment type for the biological metrics (oyster density, oyster biomass, multiple year classes, and shell volume). See Section 5 in the report for full definitions.

Sample Processing

In each sample, all oysters are counted and identified as live or dead, and a minimum of 30 live oysters are measured for each sample. Oyster clumps, the number of oysters associated with a clump, and the substrate type that oysters are attached to are documented. The shell height and total count of dead (old box) and recently dead (gapers) oysters are documented from each sample. The percent of the sample covered by tunicates or mussels and the percent of the sample that is black (anoxic shell) is documented for each sample. Additionally, field crews measure oyster and shell volume to the nearest half liter using graduated buckets.

Surface and bottom water temperature, dissolved oxygen, pH, and salinity are collected during each sampling event using a YSI Pro-Plus water quality sonde (YSI Corporation, Yellow Springs, Ohio). Other environmental and station-specific variables collected at each site include sample number, date and time, weather information, Yates Bar name, vessel name, and staff conducting the monitoring.

Data Entry and Analysis

All data were entered into a Microsoft Access database. QA/QC protocols were used to review data for nonsensical values and typos. Oyster lengths and counts were used to derive density and biomass estimates for each reef.

Graphs were made to visually display size class information and proportion of live to dead oysters at the reef level. Additionally, all sample locations were plotted in ArcGIS to ensure that samples were collected on the reef footprint. Methods for analyzing data per each Oyster Metrics success criterion follow.

Oyster Density

- Oyster Metrics success criteria: Minimum threshold = 15 oysters per m² over 30% of the reef area; Target = 50 oysters per m² over 30% of the reef area.
- Method: Oyster density was calculated as the number of individual live oysters collected in the area of a
 patent-tong grab or diver quadrat standardized to a square meter. Total counts of live oysters or other
 variables (e.g., oyster size class, shell volume) were averaged over all samples collected at the individual
 reef. To meet the Oyster Metrics threshold or target, at least 30% of the samples collected must meet the
 specified densities.

Oyster Biomass

- Oyster Metrics success criteria: Minimum threshold = 15 grams dry weight per m² over 30% of the reef area; Target = 50 grams dry weight per m² over 30% of the reef area.
- Method: Oyster biomass per m² was calculated from the size of individual live oysters within each sample, using the regression⁴: W=((10^((log10(L)*2.06)-3.76))), where W = dry tissue weight in g and L = shell height in mm. Biomass was then summed for the entire sample and standardized to a square meter. The biomass value was scaled based on the number of oysters measured out of the total number of oysters counted. The same approach as oyster density (above) was employed, in which at least 30% of samples collected had to meet the threshold or target to demonstrate restoration success.

Multiple Year Classes

- Oyster Metrics success criterion: Presence of two or more year classes of live oysters.
- Method: Year-class presence was approximated by examining length frequency data of all oyster heights
 measured at each reef. For simplicity, a reef was determined to have multiple year classes when oysters
 from at least two standard size class categories (market: >76 mm; small: 40–75 mm; spat: <40mm) were
 present. There was no differentiation between hatchery-produced oysters and natural oysters.

Shell Budget

- Oyster Metrics success criterion: Neutral or positive shell budget on the reef.
- Method: The volumes of sampled shell and oysters were measured with graduated buckets and standardized to square meter based on the area sampled by patent tong or diver quadrat. Field measurements of shell resources included total shell volume, total live oyster volume, and the percent of black (buried) shell in a sample. Surface shell estimates were calculated as the percent of the total sampled shell volume that was not considered black shell, as shown below:

Surface shell volume=Total shell volume-(Total shell volume*Percent Black Shell)

For patent tong sampled reefs, changes to the shell budget at individual reefs were analyzed by comparing surface shell volume data from three-year monitoring to surface shell volume from six-year monitoring. For diver sampled reefs, calculating shell volume was conducted similarly but using oyster volume instead of surface shell volume. The Workgroup found that previous volume estimates for stone sites did not involve excavating the entire dive quadrat. Therefore, members of the Workgroup concluded that oyster volume would be a truer representation of volume. To determine if the shell budget was increasing or stable, two-way t-tests were conducted for each reef (three-year vs six-year volume, significance P<0.05). Sites that did not have significant differences between measurements at three-year monitoring and measurements at six-year monitoring were concluded to have a stable shell budget. Sites with significant increases in shell budget were also concluded to have met the metric.

Appendix B References

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