Appendix A. Summary of Oyster BMP Expert Panel Activities

A.1 External Updates and Activities

A summary of external BMP Panel activities with relevant stakeholders is listed below. All updates to the CBP Goal Implementation Teams (GITs) were also made available to stakeholders and other interested parties.

April 4, 2017 – Oyster BMP Presentation, Interstate Seafood Seminar, Harrisburg, PA

May 8, 2017 – Water Quality GIT (WQGIT): The Panel provided an update on BMP report progress and sought feedback on draft recommendations. *Meeting materials and presentation:* https://www.chesapeakebay.net/what/event/water-quality-goal-team-conference-call-may-8th-2017

May 22, 2017 – The Panel hosted an open meeting to solicit feedback on draft recommendations. *Meeting materials and summary of discussion:* https://www.chesapeakebay.net/what/event/oyster-bmp-expert-panel-open-feedback-meeting

November 5-9, 2017 – Oyster BMP presentation(s), Coastal and Estuarine Research Federation biennial meeting, Providence, RI

November 27, 2017 – WQGIT: The Panel provided an update on draft recommendations in this report. *Meeting materials and presentation:* https://www.chesapeakebay.net/what/event/water-quality-goal-implementation-team-november-27-conference-call

December 18, 2017 – Fisheries GIT: The Panel provided an update on draft recommendations in this report. *Meeting materials and presentation:* https://www.chesapeakebay.net/what/event/december-2017-full-sustainable-fisheries-git-meeting

December 20, 2017 – CBP Trading and Offsets Workgroup: The Panel provided an update on the BMP report status and recommendations. *Meeting materials:* https://www.chesapeakebay.net/what/event/trading-and-offsets-workgroup-conference-call-december-2017

February 1, 2018 – CBP Partnership: The Panel provided a written update summarizing the oyster practice-protocol combinations that are covered in this report. *Update:* https://oysterrecovery.org/wp-content/uploads/2015/10/Update-on-Oyster-BMP-Expert-Panel-2nd-Report_2-1-18_Final.pdf

February 23, 2018 – Citizen Advisory Committee: The Panel provided an overview of report structure and an update on draft recommendations in this report. *Meeting materials and presentation:*

https://www.chesapeakebay.net/what/event/citizens-advisory-committee-quarterly-meeting-february-2018

March 29, 2018 – Oyster BMP presentation, Interstate Seafood Seminar, Rehoboth Beach, DE

June 27, 2018 – Panel members met with EPA to discuss how the licensed oyster harvest using hatchery produced oysters practice would qualify as a BMP.

July 24, 2018 – Oyster BMP presentation, Mussel Restoration Discussion and Luncheon hosted by Chesapeake Bay Foundation & VCU Rice Rivers Center, Charles City, VA

December 18, 2018 – Fisheries GIT: The Panel presented an update on draft recommendations in this report. *Meeting materials and presentation:* https://www.chesapeakebay.net/what/event/sustainable-fisheries-december-2018-biannual-full-git-meeting

July 8, 2019 – WQGIT: The Panel presented an update on draft recommendations in this report. The Panel also discussed draft recommendations to be used for watershed management planning scenarios while the Panel's recommendations are still being developed.
Meeting materials and presentation: https://www.chesapeakebay.net/what/event/water-quality-goal-implementation-team-conference-call-july-8-2019

January 8, 2020 – Fisheries GIT: The Panel provided an update on BMP report status. *Meeting materials and presentation:* https://www.chesapeakebay.net/what/event/winter-2019-2020-sustainable-fisheries-git-biannual-meeting

March 5-6, 2020 –CBP Scientific and Technical Advisory Committee, Incorporating Freshwater Mussels in the Chesapeake Bay Partnership Workshop, Annapolis, MD: The Panel discussed components of the oyster BMP that could be considered for freshwater mussels. *Meeting materials:* https://www.chesapeake.org/stac/events/incorporating-freshwater-mussels-in-the-chesapeake-bay-partnership/

May 1, 2020 – Oyster BMP denitrification data presentation, Choose Clean Water Coalition webinar, virtual

April 25, 2022 – WQGIT: The Panel gave a brief verbal update on the BMP report status. *Meeting materials:* https://www.chesapeakebay.net/what/event/water-quality-goal-implementation-team-conference-call-april-25-2022

July 21, 2022 – Fisheries GIT: The Panel presented an updated on report status and an overview of the draft oyster BMP recommendations. *Meeting materials and presentation:* https://www.chesapeakebay.net/what/event/fisheries-git-summer-meeting-july-2022

October 26, 2022 – Oyster BMP presentation, CBF Oyster Alliance Steering Committee meeting, virtual.

February 7 & 14, 2023 – Public webinars to describe the Panel's recommendations in this report during the open comment and feedback period. Webinars will include Q&A.
Report and Webinar 1: https://www.chesapeakebay.net/what/event/oyster-bmp-expert-panel-recommendation-roll-out-webinar-part-1-oyster-reef-enhanced-denitrification-protocols
Webinar 2: https://www.chesapeakebay.net/what/event/oyster-bmp-expert-panel-recommendation-roll-out-webinar-part-2-oyster-assimilation-protocol

A.2 Panel Meetings

The Panel met on the following dates to discuss items described in this report. Both in-person and remote attendance options were available. Meetings were 2 hours unless stated otherwise. Panel meeting minutes are listed in Appendix L. During the COVID-19 pandemic, most Panel discussion was conducted via email. Periods of significant email discussion are also listed below.

December 8, 2016 – The Panel discussed how to calculate reduction estimates for the shell assimilation protocols

January 19, 2017 – The Panel discussed how to calculate reduction estimates for the denitrification protocol

February 16, 2017 – The Panel discussed how to calculate reduction estimates for the denitrification protocol

March 16, 2017 – The Panel reviewed the denitrification literature and discussed shell dissolution considerations for the shell assimilation protocols

April 20, 2017 – The Panel outlined materials on denitrification and shell assimilation protocols for the May 8, 2017 WQGIT update

May 18, 2017 – The Panel finalized the logistics and content for the May 22, 2017 open feedback meeting

May 22, 2017 (*closed session, 1.5 hrs*) – The Panel reviewed feedback from the open May 22, 20217 meeting

June 15, 2017 – The Panel discussed and defined the oyster practices included in this report

August 17, 2017 – The Panel discussed and defined oyster practices in this report, reviewed new data relevant to the denitrification and shell assimilation protocols

September 19, 2017 – The Panel discussed how to calculate reduction estimates for multiple oyster practice-protocol combinations in this report

October 19, 2017 – The Panel reviewed draft denitrification text and discussed which oyster practices should be recommended for BMP consideration

November 16, 2017 – The Panel discussed how to calculate reduction estimates for multiple oyster practice-protocol combinations in this report

January 4, 2018 – The Panel discussed how to calculate reduction estimates for the denitrification protocol, and which oyster practices to recommend for BMP consideration

January 18, 2018 – The Panel reached consensus on practice-protocol practices to be included in this report, and discussed how to calculate reduction estimates for the denitrification and assimilation protocols

February 15, 2018 – The Panel discussed how to verify nutrient reduction associated with restoration-assimilation and restoration-denitrification protocols

March 15, 2018 – The Panel discussed how to verify nutrient reduction associated with restoration-assimilation and restoration-denitrification protocols

May 17, 2018 – The Panel continued to develop recommendations for practice-protocol combinations in this report

June 8, 2018 – The Panel continued to develop recommendations for practice-protocol combinations in this report

June 28, 2018 – The Panel continued to develop recommendations for practice-protocol combinations in this report

August 16, 2018 – The Panel continued to develop recommendations for practice-protocol combinations in this report

October 18, 2018 – The Panel continued to develop recommendations for practice-protocol combinations in this report

November 7, 2018 (*1 hr*) – The Panel reviewed definitions and recommendations for the harvest-assimilation protocols

April 18, 2019 (*3 hrs*) – The Panel reviewed technical applications for the proposed recommendations and discussed revisions to the report

May 10, 2019 (*3 hrs*) – The Panel discussed revisions to the report related to the harvest- and restoration-assimilation protocols

June 21, 2019 (*2.5 hrs*) – The Panel continued to develop recommendations for practice-protocol combinations in this report

July 24, 2019 (*2.5 hrs*) – The Panel continued to develop recommendations for practice-protocol combinations in this report

September 5, 2019 (*2.5 hrs*) – The Panel discussed revisions to the denitrification protocol recommendations

July 29, 2020 (*2.5 hrs*) – The Panel reviewed recommendations for the denitrification protocol

October 2020 (*email*) – The Panel refined recommendations for the denitrification protocol (created lookup tables, Appendix G)

June-July 2021 (*email*) – The Panel reviewed recommendations and draft chapters for the restoration-denitrification and restoration-assimilation protocols

December 15, 2021 (*3 hrs*) – The Panel discussed revisions to the draft recommendations and reviewed new data relevant to the denitrification protocol

January 2022 (*email*) – The Panel revised the restoration-denitrification and restoration-assimilation protocols (focus: baseline approaches)

May 2021 (*email*) – The Panel refined terms and definitions in this report and refined the restoration-assimilation and restoration-denitrification qualifying conditions (focus: substrate categories)

July 2022 (*email*) – The Panel refined recommendations for the harvest-assimilation protocols (focus: default spat survival rate)

October 6, 2022 – The Panel reviewed final major report revisions and approved the report

Appendix B. Conformity with the CBP Partnership BMP Review Protocol

The CBP Partnership Expert Panel BMP review protocol established by the Water Quality Goal Implementation Team outlines the expectations for the content of expert panel reports. This appendix references the specific chapters within the report where the Panel addressed the requested protocol criteria.

1. **Identity and expertise of panel members:** Subchapter 3.1

2. **Practice name or title:** Chapter 1.0

The Panel is recommending three oyster practices and 5 oyster protocols (12 total practice-protocol combinations) for BMP consideration. These fall into three BMP categories:

- Harvest-Assimilation: Nitrogen and phosphorus assimilation in tissue of live oysters (**Protocols 1 & 4**) from endorsed licensed harvest using hatchery-produced oysters (**Practice F**).
- Restoration-Assimilation: Nitrogen and phosphorus assimilation in tissue (**Protocols 1 & 4**) and shell (**Protocols 2 & 5**) of live oysters from oyster reefs restored using hatchery produced oysters (**Practice J**) and substrate addition (**Practice K**).
- Restoration-Denitrification: Enhanced denitrification associated with oysters (**Protocol 3**) from oyster reefs restored using hatchery produced oysters (**Practice J**) and substrate addition (**Practice K**).

3. **Detailed definition of the practices:**
   Practice F. Licensed oyster harvest using hatchery produced oysters: Subchapter 5.2
   Practice J. Oyster reef restoration using hatchery produced oysters: Subchapter 5.3
   Practice K. Oyster reef restoration using substrate addition: Subchapter 5.3

4. **Recommended reduction effectiveness estimates for each practice:**
   Harvest-Assimilation: Subchapter 6.3
   Restoration-Assimilation: Subchapter 7.3
   Restoration-Denitrification: Subchapter 8.3, Appendix G

5. **Justification of selected effectiveness estimates:**
   Harvest-Assimilation: Subchapter 6.2, Appendix D
   Restoration-Assimilation: Subchapter 7.2, Appendix E
   Restoration-Denitrification: Subchapter 8.2, Appendix E, Appendix F

6. **List of references used:** Chapter 11.0

7. **Detailed discussion on how each reference was considered:**

Harvest-Assimilation: First report, Subchapter 6.1, Appendix H
Restoration-Assimilation: Subchapter 7.1, Appendix E
Restoration-Denitrification: Subchapter 8.1, Appendix E, Appendix F, Appendix I

8. **Land uses to which BMP is applied**: Not applicable. This is a tidal in-water BMP. The Phase 6 Model estimates nutrient loads in shoreline segments that can be reduced by shoreline and tidal water practices. Credit for the pounds of nutrients reduced by the oyster practices will go to the shoreline segments closest to the practice location. If geographic coordinates are not submitted, then the credit will be distributed amongst all shoreline segments in the reported geographic area (Appendix K).

9. **Load sources that the BMP will address and potential interactions with other practices**: The CBP Partnership Management Board decided during the Oyster BMP Policy Issues Special Session on June 15, 2016 that oyster BMPs will not be credited to a specific source. Instead, reduction credit will go toward total nonpoint source load allocation.

10. **Description of pre-BMP and post-BMP circumstances and individual practice baseline:**
    Harvest-Assimilation: Subchapter 6.4
    Restoration-Assimilation: Subchapter 7.4
    Restoration-Denitrification: Subchapter 8.4

11. **Conditions under which the BMP works/does not work:**
    Harvest-assimilation: Subchapter 6.2.2, Subchapter 6.5
    Restoration-assimilation: Subchapter 7.2.2, Subchapter 7.5
    Restoration-denitrification: Subchapter 8.2.2, Subchapter 8.5

12. **Temporal performance of BMP including lag times between establishment and full functioning:**
    Harvest-assimilation: Subchapter 6.2.4, Subchapter 6.4
    Restoration-assimilation: Subchapter 7.2.7, Subchapter 7.4
    Restoration-denitrification: Subchapter 8.2.8, Subchapter 8.4

13. **Unit of measure:** Pounds of nitrogen and/or phosphorus removed

14. **Locations in Chesapeake Bay watershed where the practice applies:** Tidal segments in Chesapeake Bay watershed where the qualifying conditions are met.

15. **Useful life of the BMP:**

Harvest-assimilation: 5 years after enhancement (Subchapter 6.2.4)
Restoration-assimilation: lifetime of BMP site, as long as biomass is accumulating (Subchapter 7.2.7)
Restoration-denitrification: lifetime of BMP site (Subchapter 8.2.8)

16. **Cumulative or annual practice:**
    Harvest-assimilation: Annual (Subchapter 6.2.4)
    Restoration-assimilation: Annual (Subchapter 7.2.7)
    Restoration-denitrification: Annual (Subchapter 8.2.8)

17. **Description of how BMP will be tracked and reported:**
    Harvest-assimilation: Subchapter 6.6
    Restoration-assimilation: Subchapter 7.6
    Restoration-denitrification: Subchapter 8.6

18. **Ancillary benefits, unintended consequences, double counting:**
    Harvest-assimilation: Subchapter 6.7, Subchapter 6.8
    Restoration-assimilation: Subchapter 7.7, Subchapter 7.8, Chapter 9.0
    Restoration-denitrification: Subchapter 8.7, Subchapter 8.8, Chapter 9.0

19. **Timeline for a re-evaluation of the panel recommendations:** 5 years; if new science becomes available, follow the established re-evaluation procedures for existing estimates in the CBP Partnership Expert Panel BMP Review Protocol.

20. **Outstanding issues:** None

Appendix C. EPA Legal Opinion

Recognizing Pollutant Reductions via In-situ Oyster Filtration Under the Clean Water Act

The Chesapeake Bay Program Partnership's (Partnership) Oyster BMP Expert Panel posed the question "Can in-situ, permanent removal of sediment, nitrogen, and phosphorus pollutants from the estuarine water column via oyster filtration be recognized and credited as pollutant removal under the Clean Water Act?". The U.S. Environmental Protection Agency[1] (EPA) prepared the following response to this specific question.

The use of term "credited" in this context is assumed by EPA to mean the acceptance of a certain best management practice (BMP), treatment or technology to count toward achievement of a Chesapeake Bay watershed jurisdiction's pollutant reduction goals based on application through the Chesapeake Bay Program Partnership's suite of modeling tools. The use of term "credited" was not assumed by EPA to refer to water quality offsets or trading.

EPA recognizes that the Oyster BMP Expert Panel has concluded in its first report, approved by the Partnership in December 2016, and will possibly further conclude in forthcoming panel reports, that there is scientific and technical support for in-situ oyster filtration, in the form of aquaculture or oyster reef restoration, as a Partnership-approved BMP that results in the permanent removal of pollutants—nitrogen, phosphorus, and sediment—from the water column. EPA further assumed that this involves native oyster species only and does not contemplate introduction of non-native oyster species.

Having established those assumptions, EPA sees nothing in the Clean Water Act or its implementing regulations that would prevent a Partnership-approved BMP from qualifying for nitrogen, phosphorus or sediment pollutant reductions simply because it is physically located within the water column instead of outside the water column. EPA notes that there are at least a few existing examples of in-situ BMPs that have been documented as achieving water quality improvements through pollutant reductions and are recognized as accepted BMPs. These BMPs include the floating wetland BMP already approved by the Partnership[2], as well as the Anacostia River Trash Trap Program and Baltimore Water Wheel Trash Interceptor, both of which are described in EPA's December 2016 Aquatic Trash Prevention National Great Practices Compendium[3]. All of these BMPs are physically located within the water body and are recognized as achieving pollutant reductions.

[1] Prepared by the U.S. Environmental Protection Agency Region 3's Office of Regional Counsel and Chesapeake Bay Program Office, in consultation with the Agency's Office of General Counsel, and provided to the Chesapeake Bay Program Partnership's Oyster BMP Expert Panel on January 4, 2018.
[2] https://www.chesapeakebay.net/who/group/bmp_expert_panels
[3] https://www.epa.gov/sites/production/files/2017-02/documents/aquatic_trash_prevention_national_great_practices_compendium_december_2016.pdf

Appendix D. Calculation of a Default Spat Survival Rate for Harvest-Assimilation Protocols (Ch 6)

Oyster enhancement activities for harvest-assimilation protocols occur in areas that likely have pre-existing oyster populations. Therefore, the Panel concluded it was necessary to try to prevent crediting oysters that are unlikely to have resulted from the enhancement activity. The Panel's first step was to determine a maximum harvest allowance based on the number of hatchery-produced oysters planted and a survival rate from time of planting to time of harvest (see Subchapter 6.2.3). The Panel generated a default survival rate using a subset of oyster reef restoration monitoring data collected from Harris Creek, MD three years after planting (NOAA 2016, 2017). These data were deemed suitable because:

- Monitoring data came from areas in Maryland relatively close to where licensed oyster harvest using hatchery produced oysters currently occurs
- The materials and methods used for restoration in the subset of data analyzed were similar to the materials and methods commonly used for licensed oyster harvest using hatchery produced oysters
- Monitoring data were collected three years after planting. Harvest from areas supplemented with hatchery produced oysters typically occurs two to three years after planting. Use of a spat survivorship from three years after planting should lead to a relatively conservative survival estimate, thereby preventing over-crediting.

The subset of data used to calculate spat survivorship were selected following these criteria:

- Reefs must have been treated with spat on shell only (hatchery produced oysters)
- Data were only eligible for analysis if they were from 3 years after planting

Using these criteria, the Panel selected a total of 15 reefs for analysis that were planted in 2012 and monitored in 2015 and planted in 2013 and monitored in 2016 (Table D-1).

Since it can take two to three years for oysters to grow to harvest size, it is unlikely that monitored oysters in the 'spat' size class had originated from the planted hatchery-produced oysters. To account for this, the Panel used the percent spat reported at monitoring to estimate an adult oyster density (Table D-1). The percent spat was multiplied by oyster density, and then the resulting value was subtracted from oyster density for each reef.

The spat survival rate from planting to harvest size was calculated by dividing the density of adult oysters by the density of planted oysters (which was first converted to spat m^{-2} by multiplying by 4046.86 m^2 per acre) (Table D-1). Spat survival was then weighted to account for the acreage of each reef. Survival was multiplied by reef area to estimate the resulting area over which oysters had survived from planting to 3 years (Table D-1, % Survival x Area). The resulting areas were summed and divided by the 122.25 total acres that were planted with spat on shell.

Oyster survival from planting of spat on shell to three years ranged from 0.07% to 4.49% with an average of 2.58% (Table D-1). Weighting of data to account for the acreage of each reef resulted in a weighted average survivorship of 2.91%.

Table D-1 Planting and 3-year post-planting data from a subset of restored oyster reefs in Harris Creek, MD that were used to calculate a default spat survival rate. Text highlighted in gray was calculated by the Panel. Unhighlighted text was reported in NOAA (2016, 2017). Proportion spat (%) is the percent of the sampled oysters that were < 40 mm shell height. Adult oyster density is the density of oysters minus the density of spat. % Survival x area was used to calculate a weighted average survival rate based on reef area.

Reported Reef ID	Restoration Type	Year Planted	Year Monitored	Area (acres)	Reported spat planted (mil.)	Planted spat density (mil. acre^{-1})	Planted spat density (m^{-2})	Reported oyster density (m^{-2})	Proportion spat (%)	Adult oyster density (m^{-2})	Survival rate (%)	% Survival x Area (acres)
Reef 03	Spat on shell only	2012	2015	6.56	29.50	4.49	1,111	32.86	7	30.56	2.75	0.18
Reef 04	Spat on shell only	2012	2015	11.24	44.30	3.94	974	38.96	13	33.90	3.48	0.39
Reef 05	Spat on shell only	2012	2015	15.65	62.24	3.98	983	47.15	12	41.49	4.22	0.66
Reef 07	Spat on shell only	2012	2015	10.95	50.06	4.57	1,130	29.95	5	28.45	2.52	0.28
Reef 08	Spat on shell only	2012	2015	7.34	48.65	6.63	1,639	24.11	6	22.66	1.38	0.10
Reef 09	Spat on shell only	2012	2015	12.29	47.23	3.84	950	32.18	11	28.64	3.02	0.37
Reef 10	Spat on shell only	2012	2015	10.88	52.09	4.79	1,183	58.10	12	51.13	4.32	0.47
Reef 11	Spat on shell only	2012	2015	6.53	28.19	4.32	1,067	20.39	6	19.17	1.80	0.12
Reef 12	Spat on shell only	2012	2015	7.83	31.10	3.97	981	16.53	3	16.03	1.63	0.13
Reef H42	Spat on shell only	2013	2016	5.63	49.58	8.80	2,175	41.84	6	39.33	1.81	0.10
Reef H43	Spat on shell only	2013	2016	4.52	19.10	4.22	1,044	43.34	15	36.84	3.53	0.16
Reef H44	Spat on shell only	2013	2016	2.58	16.42	6.35	1,570	43.09	29	30.59	1.95	0.05
Reef H45	Spat on shell only	2013	2016	3.08	52.51	17.03	4,207	3.04	4	3.00	0.07	0.002
Reef H46	Spat on shell only	2013	2016	7.95	46.00	5.79	1,431	25.06	19	24.23	1.69	0.13
Reef H47	Spat on shell only	2013	2016	9.21	40.85	4.44	1,097	50.88	14	49.23	4.49	0.41
										Average survival rate		***2.58%***
										Average survival rate weighted by reef area		***2.91%***

D.1 References

NOAA (National Oceanic and Atmospheric Administration) (2016) Analysis of monitoring data from Harris Creek Sanctuary oyster reefs: data on the first 102 acres/12 reefs restored. https://dnr.maryland.gov/fisheries/documents/2015_oyster_monitoring_report.pdf (accessed 30 July 2022)

NOAA (2017) 2016 Oyster reef monitoring report: analysis of data from large-scale sanctuary oyster restoration projects in Maryland. https://www.chesapeakebay.net/what/publications/2016-oyster-reef-monitoring-report (accessed 22 Nov 2022)

Appendix E. Supporting Literature Review and Data Analyses for the Restoration-Assimilation (Ch 7) and Restoration-Denitrification (Ch 8) Protocols

This appendix provides details on (1) the percent nitrogen and phosphorus content in oyster biomass and (2) the Panel's default 50th quantile regression equations for estimating oyster biomass. The default equations convert oyster shell height to tissue and shell dry weight, and should be used for cases specified in Chapters 7 and 8 to determine the reduction effectiveness of the restoration-assimilation and restoration-denitrification protocols (Table E-1).

Table E-1. Percent N and P content and the Panel's recommended default quantile regression equations for the restoration-assimilation and restoration-denitrification protocols.

Parameter	Percent N	Percent P	0.50 Quantile Regression Equation
Tissue	8.2	0.9	$y = 0.00037x^{1.83359}$
Shell	0.2	0.04	$y = 0.00147x^{2.3964}$

E.1 Percent Nitrogen and Phosphorus Content

The Panel reviewed the existing scientific literature to estimate the content of nitrogen and phosphorus in oyster tissue and shell as a percentage of dry weight. A review of the data for oyster tissue is described in the Panel's first report (Reichert-Nguyen et al. 2016).

The data reviewed for oyster shell were for *Crassostrea virginica* in the Chesapeake Bay, Mid-Atlantic, and Northeast US (Table E-2, E-3). All data were for diploid oysters, except for Reitsma et al. (2017), which included measurements for both diploid and triploid oysters. When not specified, data were assumed to be for diploid oysters.

Based on the data reviewed from the sources in Table E-2, the Panel recommends a mean nitrogen content in oyster shell of 0.2%. Based on the data reviewed from the sources in Table E-3, the Panel recommends a mean phosphorus content in oyster shell of 0.04%.

Table E-2. Nitrogen content of shell as a percentage of dry weight. SH = Shell Height, N = number of oysters sampled.

Source	Oyster Information	Location(s)	% Nitrogen mean	% Nitrogen Range	N	% Nitrogen Mean for BMP Use
Grizzle et al. 2016	Cages 10 cm off bottom using hatchery-produced oysters Initial SH of "small" oysters = 19-42 mm (0.3 years old) 1-yr olds = 200 individuals Sampling Period: August 2010-November 2012	Adams Point, Great Bay, NH (AP)	0.10	0.03 - 0.24	10	0.11
		Bellamy River, Great Bay, NH (BMY)	0.11	0.05-0.16	4	
		Oyster River, Great Bay, NHG (GSS)	0.09	0.06-0.14	10	
		Little Bay, Great Bay, NH (LBO)	0.08	0.02-0.18	10	
		Nannie Island, Great Bay, NH (NI)	0.14[a]	0.04-0.14	10	
		Squamscott River, Great Bay, NH (SQR)	0.14[a]	0.09-0.23	10	
Higgins et al. 2011	Off bottom floating aquaculture cages using hatchery-produced diploid oysters Mean SH=~32-128mm (from raw data) Sampling Period: November 2006, August 2007 to October 2009	Spencer's Creek, VA Salinity=5-15 Low flow, high sedimentation	0.20 ± 0.01SE	0.11-0.39	47	0.20
	Off bottom floating aquaculture cages using hatchery-produced diploid oysters SH=~57-158mm (from raw data) Sampling Period: May-July 2007 to October 2009	St. Jerome Creek, MD Salinity=12-15 High flow, low sedimentation	0.20 ± 0.02SE	0.11-0.48	37	0.20
Kellogg et al. 2013	On bottom hatchery-produced diploid oysters (restored subtidal oyster reef) Mean SH=144mm Sampling Period: October 2009-August 2010	Choptank River, MD Salinity=7.0-11.6	0.21 ± 0.08SD	0.16-0.30	16[b]	0.21
Reitsma et al. 2017	Wild on bottom diploid oysters; Mean SH=82.7mm; represents 4 sites Sampling Period: June 2012, October 2012	Cape Cod, MA (12 sites; see Table 1 in study)[c]	0.26	Spring: 0.23-0.40 Fall: 0.15-0.29	32	0.26
	Cultured on bottom using hatchery-produced diploid oysters Mean SH=84.9mm; represents 6 sites Sampling Period: June 2012, October 2012	Cape Cod, MA (12 sites; see Table 1 in study)[c]	0.26	Spring: 0.15-0.45 Fall: 0.15-0.33	48	
	Cultured off bottom using hatchery-produced diploid oysters Mean SH=83.1mm; represents 9 sites Sampling Period: June 2012, October 2012	Cape Cod, MA (12 sites; see Table 1 in study)[c]	0.21	Spring: 0.12-0.33 Fall: 0.14-0.37	64	

Table E-2 (continued). Nitrogen content of shell as a percentage of dry weight. SH = Shell Height, N = number of oysters sampled.

Reitsma et al. 2017 (continued)	Cultured off bottom using hatchery-produced triploid oysters Mean SH=86.5 mm; represents one site and age of oysters likely much younger: fall triploid samples were ~6 months versus rest of oysters that were 1+ years in age)	Cape Cod, MA (12 sites; see Table 1 in study)[c]	0.32	N/A	8	0.26
Sebastiano et al. 2015[d]	Off bottom cages using hatchery-produced oysters (1 m depth) Mean SH Range=65-82mm Sampling Period: July, August, and October 2010 and 2011	Jamaica Bay, NY	0.20 ± 0.07SE	N/A	10	0.2
		Great South Bay, NY	0.19 ± 0.07SE	N/A	10	
					Average for BMP	**0.2**

[a]Grizzle et al. 2016 Fig. 10 refers to data from November 2012 and sample sizes in Table 1. To match sample size, the Panel also included Jan 2013 data sent by Grizzle used to derive graph (n = 29 small, 25 large); numbers look slightly off (NI and SQR both equal 0.14% instead of SQR having a higher percent).
[b]Three samples composed of 4-6 individuals
[c]Reitsma et al. 2017 included several sites within and near Cape Cod: Cape Cod Bay (n=3 sites), Atlantic Ocean (n=2 sites), Nantucket Sound (n=4 sites), and Buzzards Bay (n=3 sites); % N content data was aggregated based on culture method.
[d]Three sites were sampled within each bay, but results in Table 1 & 2 aggregated data by bay.

Table E-3. Phosphorus content of shell as a percentage of dry weight. SH = Shell Height, N = number of oysters sampled.

Source	Oyster Information	Location(s)	% Phosphorus Mean (Study)	% Phosphorus Range	N	% Phosphorus Mean for BMP Use
Higgins et al. (2011)	Off bottom floating aquaculture cages using hatchery-produced diploid oysters Mean SH=~32-128 mm (from raw data) Sampling Period: November 2006, August 2007 to October 2009	Spencer's Creek, VA Salinity = 5-15 Low flow, high sedimentation	0.04 ± 0.00	0.03-0.05	47	0.04
	Off bottom floating aquaculture cages using hatchery-produced diploid oysters SH=~57-150 mm (from raw data) Sampling Period: May - July 2007 to October 2009	St. Jerome Creek, MD Salinity = 12-15 High flow, low sedimentation	0.04 ± 0.00	0.03-0.05	37	0.04
Kellogg et al. (2013)	On bottom hatchery-produced diploid oysters (restored subtidal oyster reef) Mean SH=114mm Sampling Period: October 2009-August 2010	Choptank River, MD Salinity = 7.0 - 11.6	0.04 ± 0.01	N/A	16[a]	0.04
				Average for BMP		**0.04**

[a]Three samples composed of 4-6 individuals

E.2 Quantile Regression Analyses

The quantile regression approach was used to establish regression equations to convert measured oyster shell heights to oyster tissue and shell dry weights for BMP use. The Panel established a positive relationship between oyster shell height and tissue dry weight in their first report (Reichert-Nguyen et al. 2016) and Higgins et al. (2011) established a positive relationship between oyster shell height and shell dry weight. The regressions varied greatly between these two studies; therefore, the Panel decided to generate new equations using a dataset of oysters from across the Chesapeake Bay to establish regression equations that could be applied Chesapeake Bay-wide for BMP use.

Quantile regression is a statistical approach that estimates the conditional median (50^{th} or 0.50) or other quantiles (e.g., 25^{th}, 75^{th}) of the response variable (Yu et al. 2003). This approach is not as sensitive to outliers as regression approaches that use the data mean. In a BMP context, this could help account for potential differences in factors that influence oyster growth (e.g., ploidy, culture method, season, habitat type), and minimize overestimation of nutrient reduction. The Panel decided this approach would best represent the data given the variability in oyster shell heights and growth forms across the Chesapeake Bay.

The Panel specifically applied the 50^{th} quantile (median) regression framework to a Chesapeake Bay-wide dataset. Most of the data included in the 50^{th} quantile regression analysis were from subtidal reefs. The Panel included data from one study conducted in intertidal reefs in northern Virginia. The oyster shell height and biomass relationships fit the power function:

$$y = ax^b$$

where y is tissue or shell dry weight in grams and x is the shell height in millimeters. The Panel used the R statistical package quantreg (Koenker 2006; Koenker 2016) to generate nonlinear quantile regressions (nlrq). The starting values for coefficients *a* and *b* were based on mean estimates of the power function.

The resulting 50^{th} quantile of the Chesapeake Bay-wide dataset was compared with 50^{th} quantiles generated from a series of sub-datasets to determine whether the season (i.e., Spring, Summer, Fall, and Winter) and habitat (i.e., mesohaline and polyhaline environments located in the upper, mid, and lower regions of Chesapeake Bay) from which oysters were collected influenced the resulting regression equations. Analyses involving ploidy and culture method were not needed since oyster reef restoration practices only involve diploid oysters.

In cases where the 50^{th} quantile regression generated from a sub-dataset was lower than the 50^{th} quantile regression generated from the Chesapeake Bay-wide dataset, the Panel conducted a sensitivity analysis to evaluate whether a lower quantile (< 0.50) should be applied to the Chesapeake Bay-wide dataset. Applying a lower quantile would prevent overestimating oyster biomass, which would prevent overestimating the total nitrogen and phosphorus reduction. If the 50^{th} quantile regression generated from a sub-dataset was near or above the 50^{th} quantile regression generated from the entire dataset, the Bay-wide regression would result in no change or an underestimate of biomass and nutrient reduction. In these cases, the Panel concluded that

the 50th quantile of the Chesapeake Bay-wide dataset was appropriate to use with data collected anywhere in the Bay.

E.2.1 Oyster Tissue Biomass

The Chesapeake Bay-wide dataset used to establish the default shell height to tissue dry weight regression equation included a total of 6,888 oysters from eight studies (three from peer-reviewed publications, two studies presented in a report, and one unpublished source) from 22 locations (Figure E-1, Table E-4).

Figure E-1. Sampling locations and assigned habitat groups for diploid oysters used in the shell height to tissue dry weight regression analyses.

Table E-4. Data used to generate shell height to tissue dry weight regression.

Data Source	Habitat/Oyster Information	Location	Data Age (Year Oysters Removed)	Season Oysters Removed	Percent of Total Oysters used in Regression Analysis
Higgins unpubl. data	Subtidal reefs; wild oysters	Choptank River, MD	2008	Spring	0.13
	Reefs; wild oysters	Lynnhaven River, VA			0.26
Kellogg unpubl. data	Subtidal reefs; mix of wild and hatchery-produced oysters	Harris Creek, MD	2015	Fall	2.34
				Winter	2.40
				Spring	2.44
				Summer	8.2
Kellogg et al. 2013	Subtidal reefs; hatchery-produced oysters	Choptank River, MD	2009	Fall	0.81
			2010	Spring	0.71
				Summer	1.6
Luckenbach and Ross 2009 (Part 1 of Report)	Subtidal patch reefs; mix of wild and hatchery-produced oysters	Great Wicomico River, VA	2004, 2005	Fall	2.09
			2004	Spring	0.48
			2005	Summer	3.15
		Lynnhaven River, VA	2005	Fall	1.35
				Summer	1.73
		Piankatank River, VA	2004	Fall	0.6
			2004, 2005	Spring	0.73
				Summer	1.18
		Rappahannock River, VA	2004	Fall	0.29
			2004, 2005	Spring	1.44
				Summer	0.45
Luckenbach and Ross 2009 (Part 3 of Report)	Restored and existing oyster reefs on intertidal structures, intertidal patch reefs, and subtidal reefs; wild oysters	Lynnhaven River, VA	2005, 2006	Spring	11.89
			2006	Winter	1.89
Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015	Subtidal reefs (public grounds); wild oysters	Great Wicomico River, VA	2010, 2011, 2012	Fall	2.13
		James River, VA	2010, 2011, 2012	Fall	5.73
		Mobjack Bay, VA	2010, 2012	Fall	0.73
		Piankatank River, VA	2010, 2011, 2012	Fall	1.8
		Pocomoke Sound, VA	2010, 2011, 2012	Fall	0.71
			2010, 2011, 2012	Winter	0.36
		Rappahannock River, VA	2010, 2011, 2012	Fall*	4.99
		Tangier Sound, VA	2011, 2012	Fall	0.73
		York River, VA	2010, 2011	Fall	2.15

Table E-4 (continued). Data used to generate shell height to tissue dry weight regression.

Paynter unpubl. data found in Liddel 2008	Subtidal reefs; hatchery-produced oysters	Cabin Creek, MD	1998	Fall	0.15
			2000	Spring	0.09
			1998	Summer	0.22
		Calvert Cliffs, MD	1998	Fall	0.36
			1999	Spring	0.07
			1998, 1999	Summer	0.7
		Chester River, MD	2001, 2002, 2004	Fall	1.97
			2002	Spring	1.57
			2001, 2002, 2004	Summer	1.99
		Choptank River, MD	2000, 2002	Fall	2.67
			2001, 2002	Winter	1.39
			2001, 2002, 2004	Summer	4.82
		Eastern Bay, MD	2001	Fall	0.41
			2002	Winter	0.57
			2004	Spring	0.17
			2001, 2002	Summer	1.74
		Little Choptank River, MD	2004	Summer	0.36
		Magothy River, MD	2001, 2002	Fall	0.96
			2002	Spring	0.39
			2001, 2002	Summer	0.73
		Nanticoke River, MD	2001, 2002	Fall	0.17
			2002	Summer	0.22
		Patuxent River, MD	2001	Fall	0.26
				Spring	1.15
			2000, 2001, 2002	Summer	3.83
		Severn River, MD	2001, 2002	Fall	1.16
			2001	Winter	0.29
			2002	Spring	0.15
			2001, 2002	Summer	1.26
		South River, MD	2001, 2002	Fall	0.36
			2002	Spring	0.22
				Summer	0.2
		Tangier Sound, MD	2000, 2001, 2002	Fall	1.67
			2001, 2002	Summer	1.79
		Tred Avon River, MD	2000	Fall	0.22
			2001	Summer	0.25

*25 oysters not labeled with a season. Assumed oysters were removed in the fall

The 50th quantile regression for oyster tissue dry weight using the Chesapeake Bay-wide dataset (n=6,888 oysters) is: **$y = 0.00037 \times^{1.83359}$** (Figure D-2).

The sections below describe the results of the comparisons between the Bay-wide regression and regressions generated from sub-datasets designated by season and oyster habitat (Table E-5). The goal was for the Bay-wide regression to minimize overestimating oyster tissue biomass and therefore minimize over crediting nitrogen and phosphorus reduction.

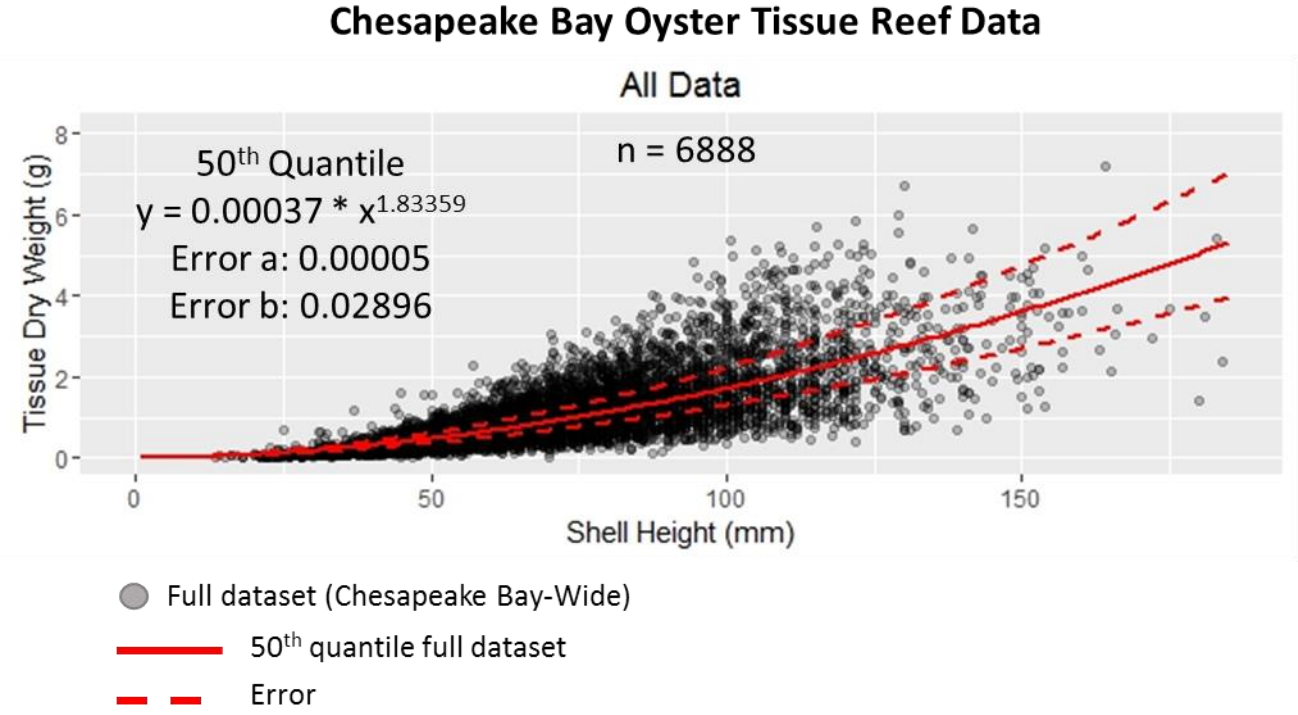

Figure E-2. Oyster shell height to tissue dry weight 50th quantile regression using oysters included in the full Chesapeake Bay-wide dataset.

Table E-5. Regression and error terms associated with each of the 50th quantile regression analyses for tissue biomass. Open Water Lower Bay Polyhaline habitat did not have enough tissue data to generate a quantile regression.

Oyster Data	# of Oysters	a	b	Error a	Error b
Full Dataset					
Chesapeake Bay-wide	6888	0.00037	1.83359	0.00005	0.02896
Sub-Datasets					
Fall	2511	0.00029	1.89203	0.00006	0.04508
Winter	475	0.00014	2.04735	0.00007	0.10898
Spring	1507	0.00041	1.86954	0.00008	0.04522
Summer	2370	0.00029	1.84454	0.00004	0.03217
Tributary Upper Bay Mesohaline	524	0.00043	1.80483	0.00019	0.09888
Tributary Mid-Bay Mesohaline	3163	0.00024	1.90115	0.00005	0.04556
Tributary Lower Bay Mesohaline	790	0.00016	2.08482	0.00006	0.08291
Tributary Lower Bay Polyhaline	1722	0.00028	1.92701	0.0001	0.08752
Open Water Mid-Bay Mesohaline	639	0.00003	2.44470	0.00002	0.14148
Intertidal	130	0.00043	1.91395	0.00028	0.15908

E.2.1.1 Tissue Seasonal Considerations

The Panel evaluated whether the Bay-wide tissue regression was sensitive to the season in which oysters were collected. The 50th quantile tissue regression of the fall, winter, and spring sub-datasets were in line or slightly above the 50th quantile tissue regression of the Bay-wide dataset (Figure E-3). This suggests that the Chesapeake Bay-wide 50th quantile tissue regression equation does not overestimate tissue biomass or over credit nitrogen and phosphorus reduction, and is appropriate for estimating the reduction across these seasons. For the spring sub-dataset, tissue biomass and the nitrogen and phosphorus reduction are likely underestimated for oysters larger than ~60 mm shell height (SH).

The summer sub-dataset fell below the 50th quantile tissue regression curve of the Bay-wide dataset for oysters > 75 mm SH (Figure E-3). There may be a greater chance that tissue biomass, and therefore nitrogen and phosphorus reduction, may be overestimated relative to other seasons if oysters are monitored in the summer.

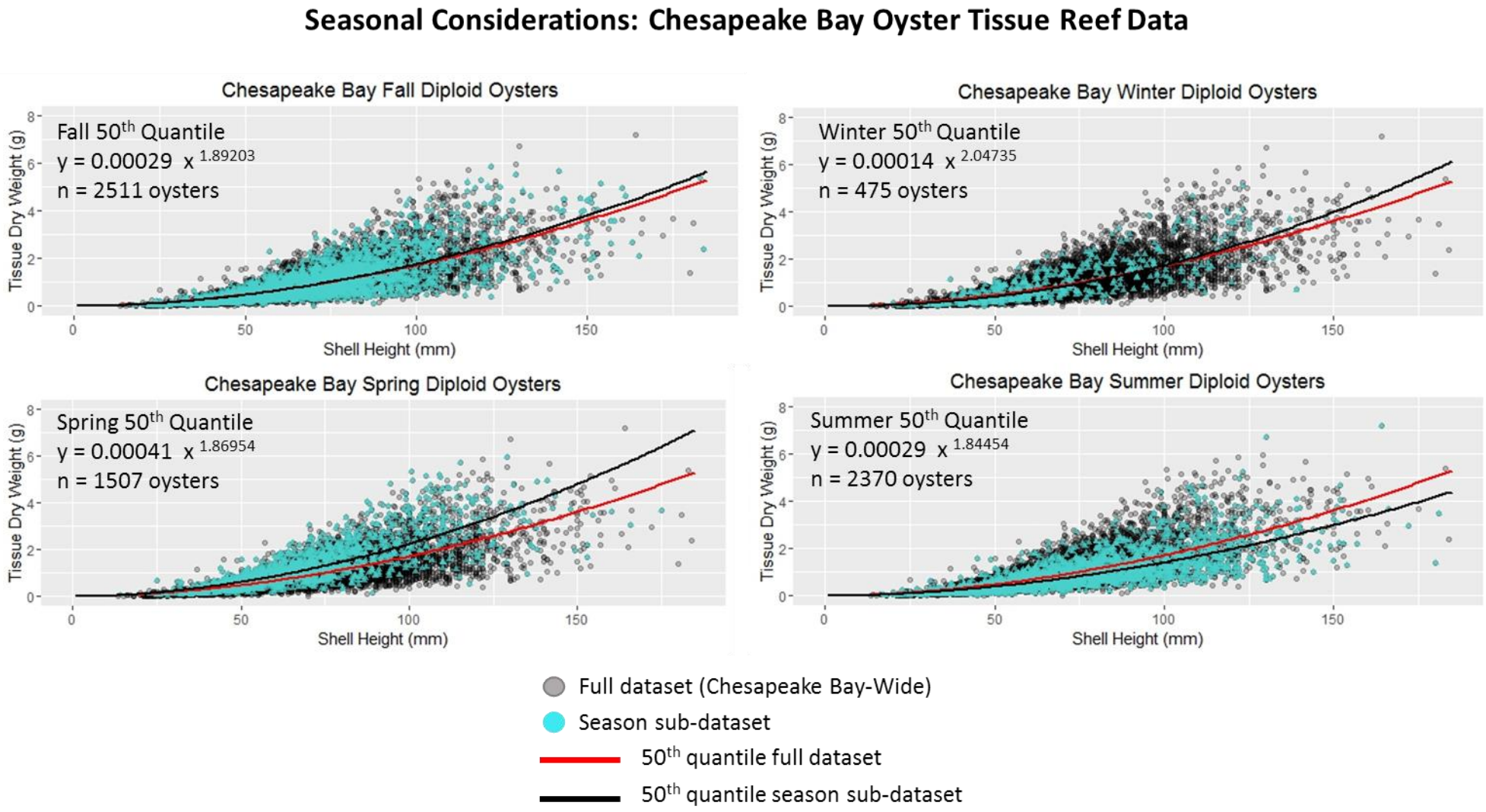

Figure E-3. Oyster tissue 50th quantile regression by season (turquoise dots, solid black line) evaluating potential seasonal differences in tissue dry weight compared to the full, Chesapeake Bay-wide dataset (gray dots, solid red line).

The Panel conducted a sensitivity analysis comparing the nitrogen reduction estimates from using the Bay-wide tissue regression versus the tissue regression for a subset of data from oysters sampled in the summer to evaluate whether the potential overestimation warranted generating a tissue regression equation for the Bay-wide dataset using a lower (< 0.50) quantile. The Panel calculated oyster tissue biomass across a range of size classes using both the Bay-wide and sub-dataset tissue regressions. Oyster tissue biomass was then multiplied by the mean percent nitrogen content in oyster tissue (8.2%) to estimate the total nitrogen reduced (Table E-6). The Panel only performed this sensitivity analysis for nitrogen since it has the greater percent content in oyster tissue compared to phosphorus. The proportion of the data (6,888 oysters) in each size class was used to estimate the potential nitrogen reduction overestimates for a site with one million oysters.

The Bay-wide 50th quantile tissue equation overestimated nitrogen reduction by 33 lbs per one million oysters compared to the tissue sub-dataset for summer alone. This estimate is within the margin of error associated with the 50th quantile of the Baywide default tissue equation (Table E-5). Therefore, the Panel agreed that the potential nutrient reduction overestimation in the summer season could be negligible, and these data should be included with the full dataset.

Table E-6. Sensitivity analysis performed on the summer dataset to consider potential overstimulation.

Oyster Size Class Category	Average Difference (lbs N)	Proportion of Dataset (n=6888)	Proportion of 1 million (# of oysters)	Difference in lbs N per 1 million oysters
< 2.0	0.00001	0.2	200000	2
2.0 - 2.49	0.00002	0.2	200000	4
2.5 - 3.49	0.00003	0.35	350000	12
3.5 - 4.49	0.00006	0.2	200000	11
4.5 - 5.49	0.00008	0.04	40000	3
5.5 - 7.49	0.00013	0.01	10000	1
			Total difference (lbs)	**33**

E.2.1.2 Tissue Habitat Considerations

The Panel evaluated whether the Bay-wide tissue regression was sensitive to the habitat in which oysters were collected. Habitat was characterized by geographic location within the Bay (upper, mid, lower), reef context (within a tributary vs. in open water), and salinity regime (mesohaline, polyhaline). The Chesapeake Bay locations and the salinity regimes were defined using the Chesapeake Bay Program bay-wide segmentation scheme (https://www.chesapeakebay.net/what/maps/20chesapeake-bay-2003-segmentation-scheme-codes) and spatially-explicit salinity gradient maps from the U.S. Army Corps of Engineers (http://www.nab.usace.army.mil/Missions/Environmental/Oyster-Restoration/Oyster-Master-Plan/). These raster salinity maps were generated in support of the U.S. Army Corps of Engineers Oyster Restoration Master Plan and derived from interpolated spring and summer water quality samples collected between 2001 and 2006.

This strategy resulted in six main habitat groups:

- Tributary Upper Bay Mesohaline
- Tributary Mid-Bay Mesohaline
- Tributary Lower Bay Mesohaline
- Tributary Lower Bay Polyhaline
- Open Water Mid Bay Mesohaline
- Open Water Lower Bay Polyhaline

The dataset used to develop the shell height to tissue regression equation included oysters collected at 22 general sampling locations distributed throughout the Chesapeake Bay and its tributaries (Figure E-1). Raster maps and the geographic coordinates of each sampled reef were plotted in a geographic information system (GIS) and the spring and summer salinity for each location were documented. Dominant salinity regimes were assigned based on the range of spring and summer salinities (Figure E-1).

Most (65%) of the oysters were collected from Tributary Mesohaline habitats in Maryland, followed by Tributary Polyhaline habitats (25%). A small portion of the data were from Open Water Mesohaline (9.28%) and Polyhaline (0.73%) environments (Table E-7). Intertidal oysters made up 2% (n=130) of the sample size used to calculate the Bay-wide tissue regression.

Table E-7. Summary of the percent of the oyster tissue data within each habitat group and data sources (references). All oysters are diploid. Data from Table E-4.

Habitat Group	General Sampling Location	Percent of Oyster Data (n=6888)	References
Tributary Upper Bay Mesohaline	Chester River	5.53	Paynter unpubl. data found in Liddel 2008
	Magothy River	2.08	Paynter unpubl. data found in Liddel 2008
Tributary Mid-Bay Mesohaline	Cabin Creek	0.45	Paynter unpubl. data found in Liddel 2008
	Choptank River	12.14	Higgins unpubl. data Kellogg et al. 2013 Paynter unpubl. data found in Liddel 2008
	Great Wicomico River	7.85	Luckenbach and Ross 2009
	Harris Creek	15.37	Kellogg unpubl. data
	Little Choptank River	0.36	Paynter unpubl. data found in Liddel 2008
	Nanticoke River	0.39	Paynter unpubl. data found in Liddel 2008
	Patuxent River	5.24	Paynter unpubl. data found in Liddel 2008
	Severn River	2.86	Paynter unpubl. data found in Liddel 2008
	South River	0.78	Paynter unpubl. data found in Liddel 2008
	Tred Avon River	0.46	Paynter unpubl. data found in Liddel 2008
Tributary Lower Bay Mesohaline	Piankatank River	4.3	Luckenbach and Ross 2009, Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
	Rappahannock River	7.17	Luckenbach and Ross 2009, Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
Tributary Lower Bay Polyhaline	Lynnhaven River	17.12	Higgins unpubl. data Luckenbach & Ross 2009
	James River	5.73	Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
	York River	2.15	Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
Open Water Mid-Bay Mesohaline	Calvert Cliffs	1.13	Paynter unpubl. data found in Liddel 2008
	Eastern Bay	2.89	Paynter unpubl. data found in Liddel 2008
	Pocomoke Sound	1.07	Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
	Tangier Sound	4.18	Paynter unpubl. data found in Liddel 2008, Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
Open Water Lower Bay Polyhaline	Mobjack Bay	0.73	Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015

For most of the reviewed habitats, the 50th quantile tissue regression of each sub-dataset was above the 50th quantile tissue regression of the Bay-wide dataset, which suggests that the 50th quantile tissue regression equation from the Bay-wide dataset does not overestimate the nitrogen and phosphorus reduction (Figure E-4). The 50th quantile tissue regression of the Tributary Mid-Bay Mesohaline dataset was slightly below the 50th quantile tissue regression of the Bay-wide dataset for oysters > 75 mm SH (Figure E-4). However, this is within the margin of error of the tissue regression for the Bay-wide dataset (Table E-5) and does not significantly overestimate the nitrogen reduction.

Data from only one intertidal location were included in the Panel's Chesapeake Bay-wide tissue dataset (Luckenbach and Ross 2009 [Part 3]; 2% of oyster tissue data). The 50th quantile tissue regression of the sub-dataset was above the 50th quantile tissue regression of the Bay-wide dataset (Figure E-5). If this relatively small dataset is representative of intertidal oysters across the Chesapeake Bay, then the Bay-wide tissue regression likely underestimates oyster tissue biomass and under credits nutrient removal. Given the large error and small sample size for the intertidal data (Table E-5), the Panel agreed that the Bay-wide tissue regression equation could be used to estimate oyster tissue biomass for oysters on both subtidal and intertidal reefs. Because of the sparsity of data, the Panel recommends re-evaluating this relationship as more data become available from intertidal reefs in other locations and seasons.

The Panel concluded that the 50th quantile tissue regression equation of the full, Bay-wide dataset can be used for all habitats and seasons.

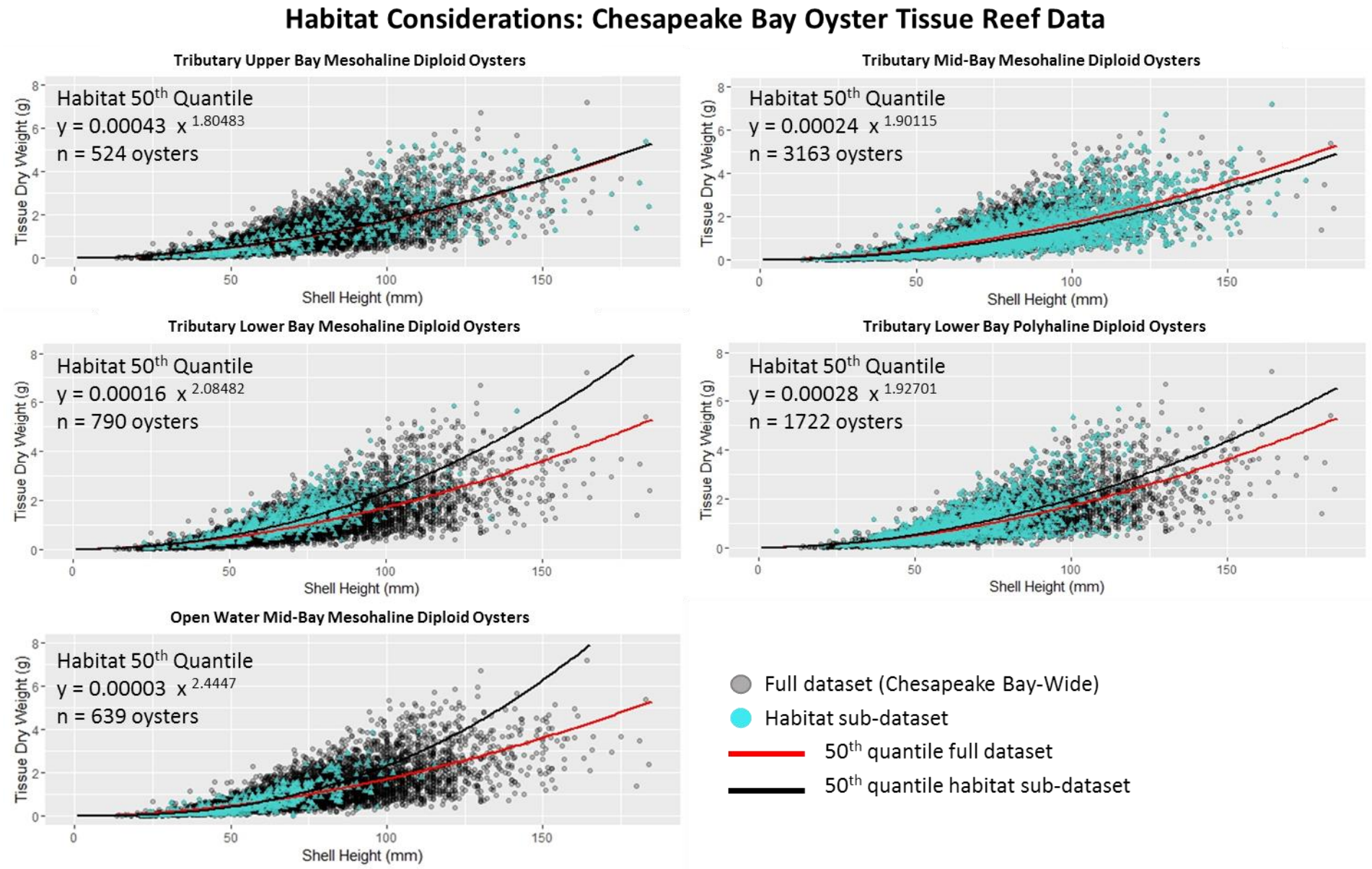

Figure E-4. Oyster tissue 50th quantile regression by habitat (turquoise dots, solid black line) evaluating potential habitat differences in tissue dry weight compared to the full, Chesapeake Bay-wide dataset (gray dots, solid red line).

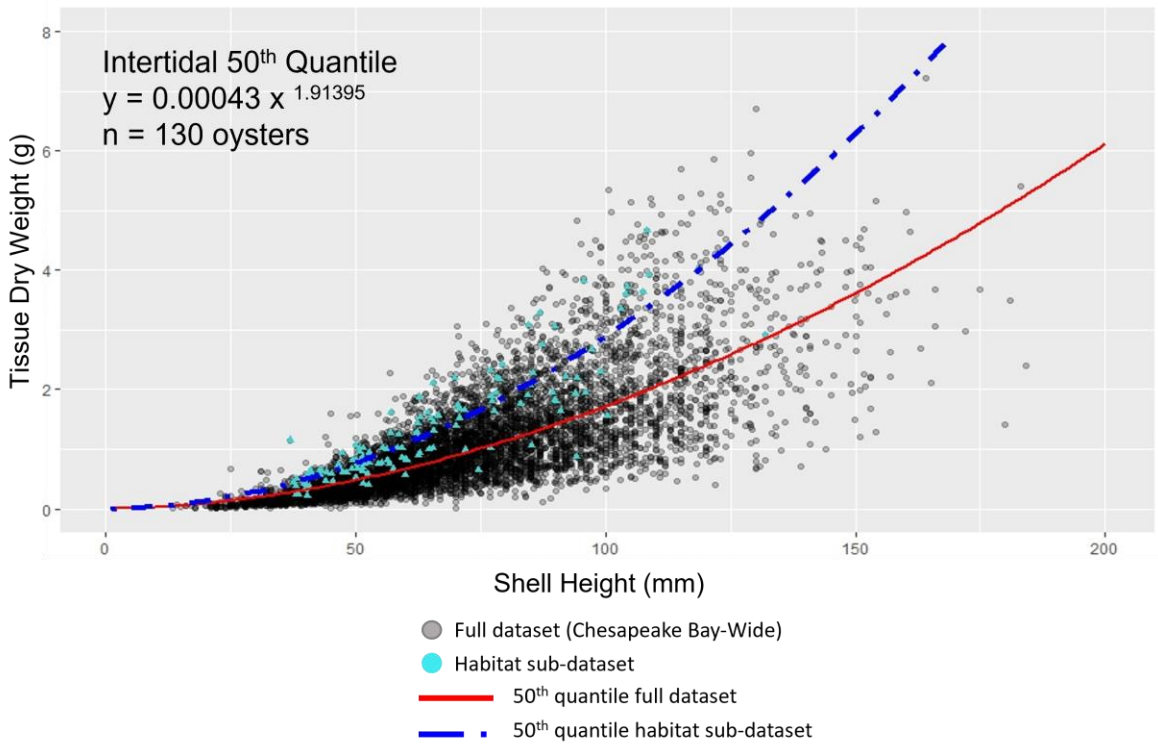

Figure E-5. Oyster tissue 50th quantile regression for oysters growing on intertidal reefs (turquoise triangles, blue dashed line) compared to the full Chesapeake Bay-wide dataset (gray dots, red line).

E.2.2 Oyster Shell Biomass

The Chesapeake Bay-wide dataset used to establish the shell height to shell dry weight regression equation included a total of 4,296 oysters from five studies (one peer-reviewed publication, two studies presented in a report, and two unpublished data sources) from 11 locations (Figure E-6, Table E-8). Some data used to generate the shell height to tissue dry weight equation were not included in this analysis since a number of those studies reported only tissue dry weight and not shell dry weight. Oyster samples from aquaculture sites were also removed.

Figure E-6. Sampling locations and assigned habitat groups for diploid oysters used in the shell height to shell dry weight regression analyses.

Table E-8. Data for the shell height to shell dry weight regression analyses.

Data Sources	Habitat/Oyster Information	Locations	Data Age (Year Oysters Removed)	Season Oysters Removed	Percent of Total Oysters used in Regression Analysis
Higgins unpubl. data	Subtidal reefs; wild oysters	Choptank River, MD	2008	Spring	0.21
	Reefs; wild oysters	Lynnhaven River, VA			0.42
Kellogg unpubl. data	Subtidal reefs; mix of wild and hatchery-produced oysters	Harris Creek, MD	2015	Fall	3.75
				Winter	3.84
				Spring	3.91
				Summer	13.20
Luckenbach and Ross 2009 (Part 1 of Report)	Subtidal patch reefs; mix of wild and hatchery-produced oysters	Great Wicomico River, VA	2004, 2005	Fall	3.35
			2004	Spring	0.77
			2005	Summer	5.05
		Lynnhaven River, VA	2005	Fall	2.16
				Summer	2.77
		Piankatank River, VA	2004	Fall	0.95
			2004, 2005	Spring	1.16
				Summer	1.89
		Rappahannock River, VA	2004	Fall	0.47
			2004, 2005	Spring	2.30
				Summer	0.72
Luckenbach and Ross 2009 (Part 3 of Report)	Restored and existing oyster reefs on bulkheads, intertidal patch reefs, marsh, riprap, subtidal bottom (not discrete patches); wild oysters	Lynnhaven River, VA	2005, 2006	Spring	19.04
			2006	Winter	3.03
Mann, Southworth and Wesson unpubl. data found in Powell et al. 2015	Subtidal reefs (public grounds); wild oysters	Great Wicomico River, VA	2010,2011, 2012	Fall	3.42
		James River, VA	2010,2011, 2012	Fall	9.19
		Mobjack Bay, VA	2010, 2012	Fall	1.16
		Piankatank River, VA	2010, 2011, 2012	Fall	2.89
		Pocomoke Sound, VA	2010, 2011, 2012	Fall	1.14
			2010, 2011, 2012	Winter	0.58
		Rappahannock River, VA	2010, 2011, 2012	Fall*	8.01
		Tangier Sound, VA	2011, 2012	Fall	1.16
		York River, VA	2010, 2011	Fall	3.45

*25 oysters not labeled with a season (assumed oysters were removed in the fall)

The 50th quantile regression for oyster shell dry weight using the Chesapeake Bay-wide dataset (n=4,296 oysters) is: **$y = 0.00147 \times^{2.3964}$** (Figure E-7).

The sections below describe the results of the comparisons between the Bay-wide shell regression and regressions generated from sub-datasets designated by season and oyster habitat (Table E-9). The goal was for the Bay-wide shell regression to minimize overestimating oyster shell biomass and therefore minimize over crediting nitrogen and phosphorus reduction.

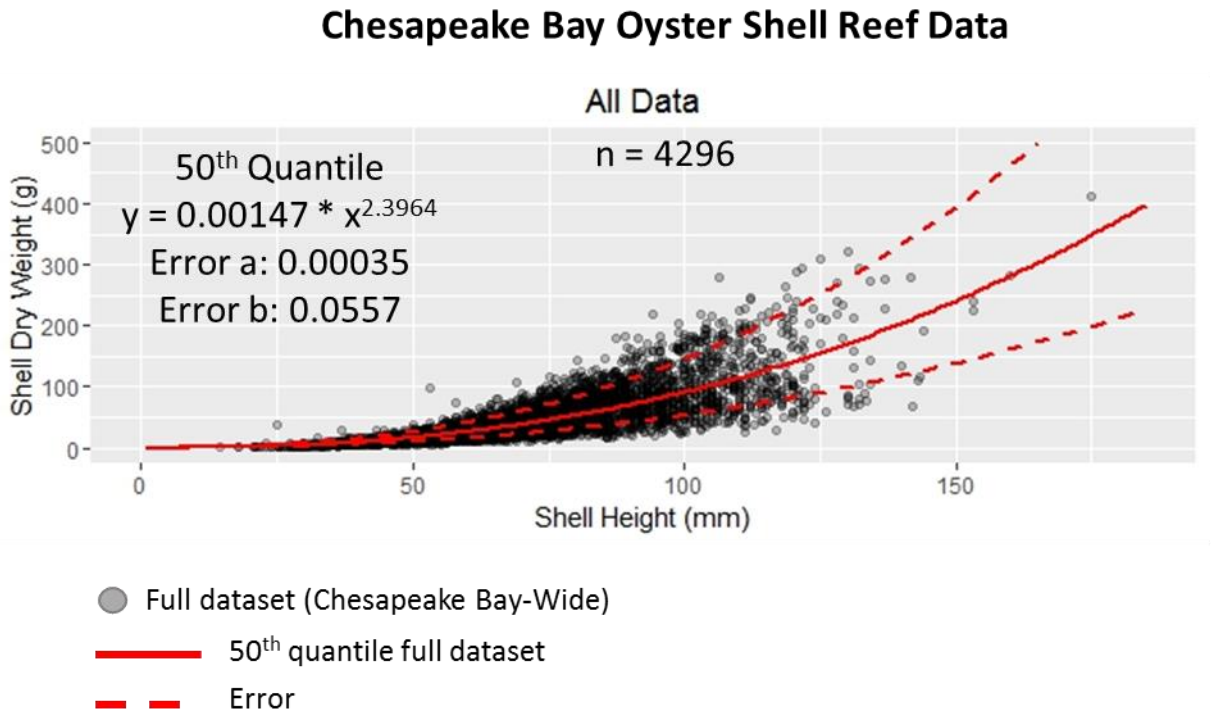

Figure E-7. Oyster shell height to shell dry weight 50th quantile regression using the full Chesapeake Bay-wide dataset (n=4,296 oysters).

Table E-9. Regression and error terms associated with each of the 50th quantile regression analyses for shell biomass. Open Water Lower Bay Polyhaline habitat did not have enough shell data to generate a quantile regression.

Oyster Data	# of Oysters	a	b	Error a	Error b
Full Dataset					
Chesapeake Bay-wide	4296	0.00147	2.3964	0.00035	0.0557
Sub-Datasets					
Fall	1741	0.00176	2.40328	0.00045	0.05928
Winter	320	0.00756	2.00003	0.00395	0.12503
Spring	1195	0.00026	2.73590	0.00007	0.06134
Summer	1015	0.00413	2.15238	0.0015	0.08283
Tributary Mid-Bay Mesohaline	1611	0.00251	2.27161	0.00083	0.0765
Tributary Lower Bay Mesohaline	790	0.00084	2.59788	0.00029	0.08208
Tributary Lower Bay Polyhaline	1721	0.00084	2.46481	0.00034	0.09904
Open Water Mid-Bay Mesohaline	124	0.00033	2.82404	0.0002	0.14097
Intertidal	130	0.00105	2.50074	0.00087	0.20242

E.2.2.1 Shell Seasonal Considerations

The Panel evaluated whether the Bay-wide shell regression was sensitive to the season in which oysters were collected. The summer and winter 50th quantile shell regression were below the 50th quantile shell regression of the Bay-wide dataset for oysters > 100 mm SH (Figure E-8). The Panel agreed the resulting overestimate of nitrogen and phosphorus reduction was negligible and within the margin of error of the full Bay-wide dataset (Table E-9). The fall 50th quantile shell regression was slightly above the 50th quantile shell regression of the Bay-wide dataset (Figure E-8). The Panel agreed this was also within the margin of error of the full dataset and would not significantly underestimate the nitrogen and phosphorus reduction. **The Panel concluded that the 50th quantile regression equation of the full shell dataset can be used to estimate shell biomass across seasons.**

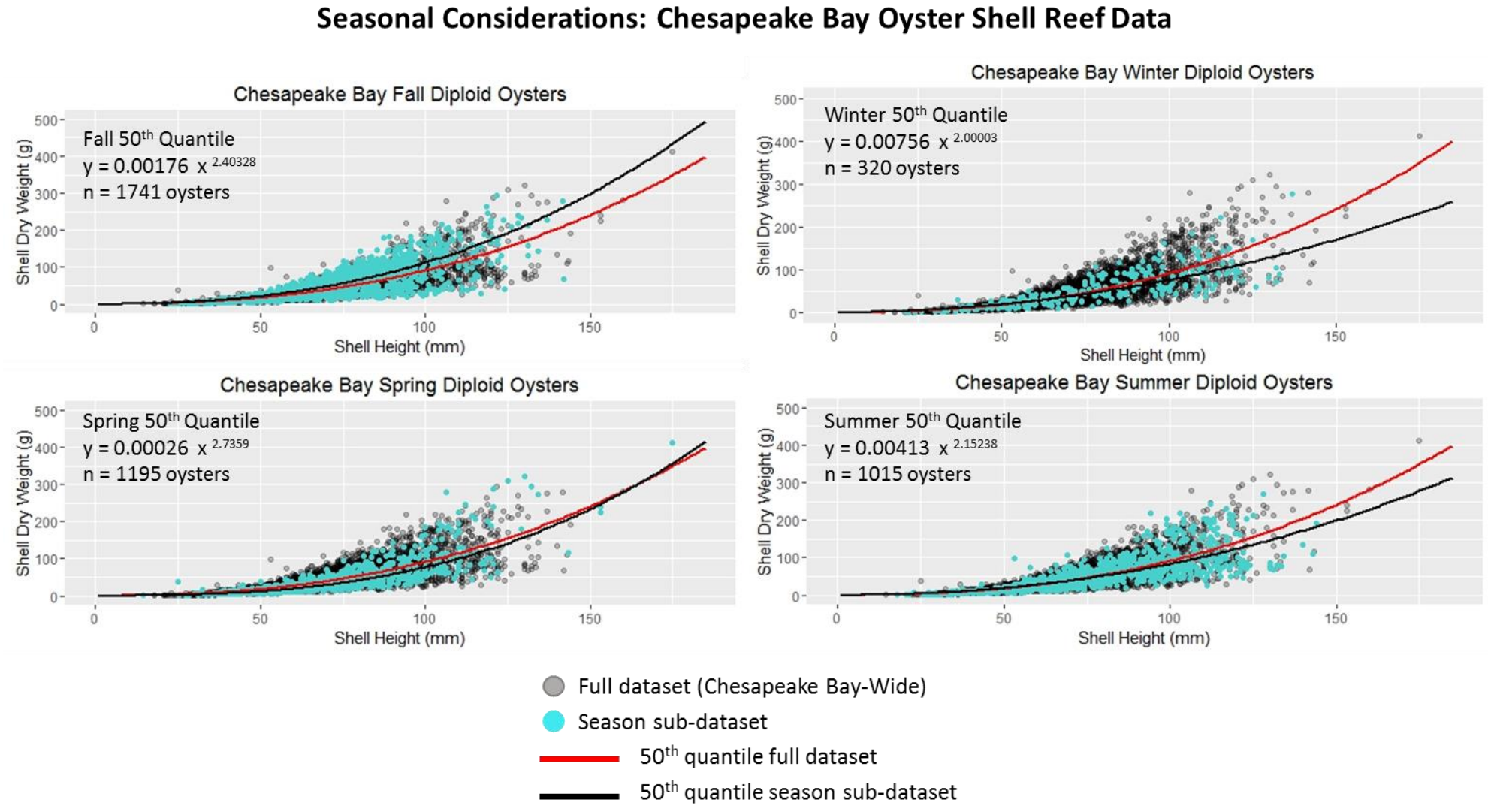

Figure E-8. Oyster tissue 50th quantile regression analyses by season (turquoise dots, solid black line) evaluating potential seasonal differences in shell dry weight compared to the full dataset (gray dots, solid red line).

E.2.2.2 Shell Habitat Considerations

The Panel used the same approach as for oyster tissue to determine whether the Bay-wide shell regression was sensitive to oyster habitat. The shell dataset included the following five habitat groups:

- Tributary Mid-Bay Mesohaline
- Tributary Lower Bay Mesohaline
- Tributary Lower Bay Polyhaline
- Open Water Mid Bay Mesohaline
- Open Water Lower Bay Polyhaline

Table E-10. Percentage of oyster shell data within each habitat group and data sources (references). Data from Table E-8.

Oyster Restoration-Tissue Assimilation Habitat Analysis			
Habitat Group	**General Sampling Location**	**Percent of Oyster Data (n=4296)**	**References**
Tributary Mid-Bay Mesohaline	Choptank River	0.21	Higgins unpubl. data, Kellogg et al. 2013, Paynter unpubl. data found in Liddel 2008
	Great Wicomico River	12.59	Luckenbach and Ross 2009, Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
	Harris Creek	24.70	Kellogg unpubl. data
Tributary Lower Bay Mesohaline	Piankatank River	6.89	Luckenbach and Ross 2009, Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
	Rappahannock River	11.5	Luckenbach and Ross 2009, Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
Tributary Lower Bay Polyhaline	James River	9.19	Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
	Lynnhaven River	27.42	Higgins unpubl. data, Luckenbach and Ross 2009
	York River	3.45	Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
Open Water Mid-Bay Mesohaline	Pocomoke Sound	1.72	Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
	Tangier Sound	1.16	Paynter unpubl. data found in Liddel 2008, Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015
Open Water Lower Bay Polyhaline	Mobjack Bay	1.16	Mann, Southworth, and Wesson unpubl. data found in Powell et al. 2015

The dataset used to develop the shell height to shell dry weight regression equation included oysters collected at 11 general sampling locations distributed throughout the Chesapeake Bay and its tributaries (Figure E-6). Raster maps and the geographic coordinates of each sampled reef were plotted in a geographic information system (GIS) and the spring and summer salinity for each location were documented. Dominant salinity regimes were assigned based on the range of spring and summer salinities (Figure E-6).

Most of the oysters were collected from Tributary Mesohaline (56%) habitats in Maryland, followed by Tributary Polyhaline habitats (40%). A small portion of the data are from Open Water Mesohaline (3%) and Polyhaline (1%) environments (Table E-10). Intertidal oysters made up 3% (n=130) of the sample size used to calculate the Bay-wide regression for shell.

The Open Water Mid-Bay Mesohaline and Tributary Lower Bay Mesohaline 50th quantile shell regression was greater than the 50th quantile shell regression of the full Bay-wide dataset (Figure E-9). This suggests that the 50th quantile regression equation from the Bay-wide dataset does not overestimate oyster shell biomass and therefore the nitrogen and phosphorus reduction. Instead, the reduction is likely underestimated at these habitat types for oysters > 75mm SH.

The Tributary Lower Bay Polyhaline shell regression was slightly below the 50th quantile shell regression of the Bay-wide dataset for oysters > 75mm SH (Figure E-9). The Panel conducted a sensitivity analysis following the same process as for oyster tissue in summer (Section E.2.1.1, Table E-6). The sensitivity analysis estimated that the Tributary Lower Bay Polyhaline regression overestimated nitrogen reduction by ~50 lbs of nitrogen per one million oysters. The Panel considered this difference to be negligible from a reduction effectiveness standpoint. The Panel agreed that this overestimation was within the margin of error associated with the 50th quantile of the Bay-wide default shell equation and that these data should be included with the full dataset.

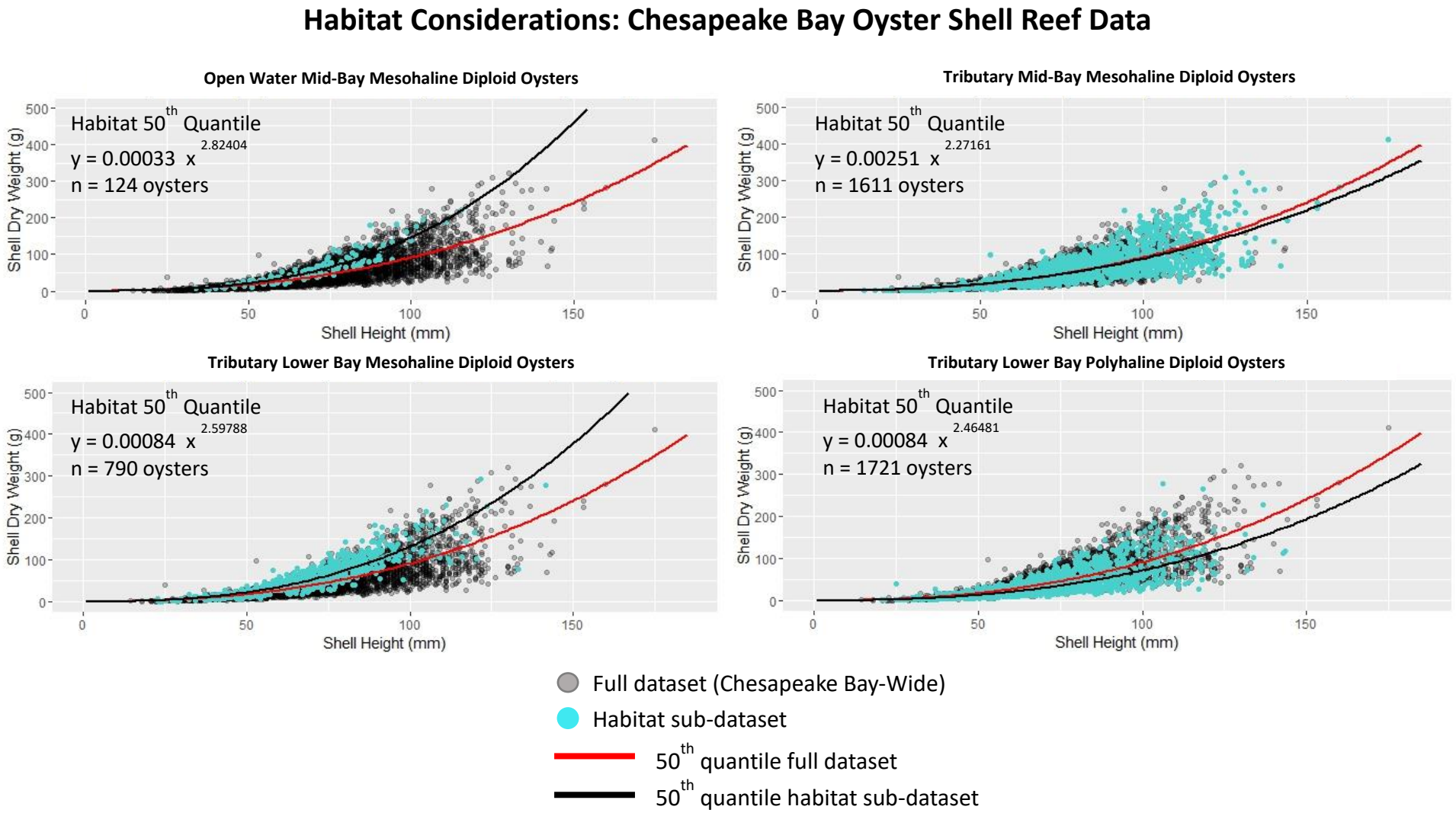

Figure E-9. Overlays of oyster shell heights and corresponding shell dry weight associated with four habitat groups (differentiated by location and salinity (turquoise dots, solid black line) added to 50th quantile regression plots using the full dataset (gray dots, solid red line).

Data from only one intertidal location were included in the Panel's Chesapeake Bay-wide dataset (Luckenbach and Ross 2009 [Part 3]; 3% of oyster shell data). The intertidal shell regression was slightly above the 50th quantile shell regression and within the margin of error of the Bay-wide dataset, suggesting that the 50th quantile shell regression equation for the full dataset does not overestimate the nitrogen and phosphorus reduction for oysters growing on intertidal reefs (Figure E-10). The Panel agreed that the shell regression equations could be used to estimate oyster biomass for oysters on both subtidal and intertidal reefs. Because of the sparsity of data, the Panel recommends re-evaluating this relationship as more data become available from intertidal reefs in other locations and seasons.

The Panel concluded that the 50th quantile shell regression equation of the full dataset can be used for all habitats.

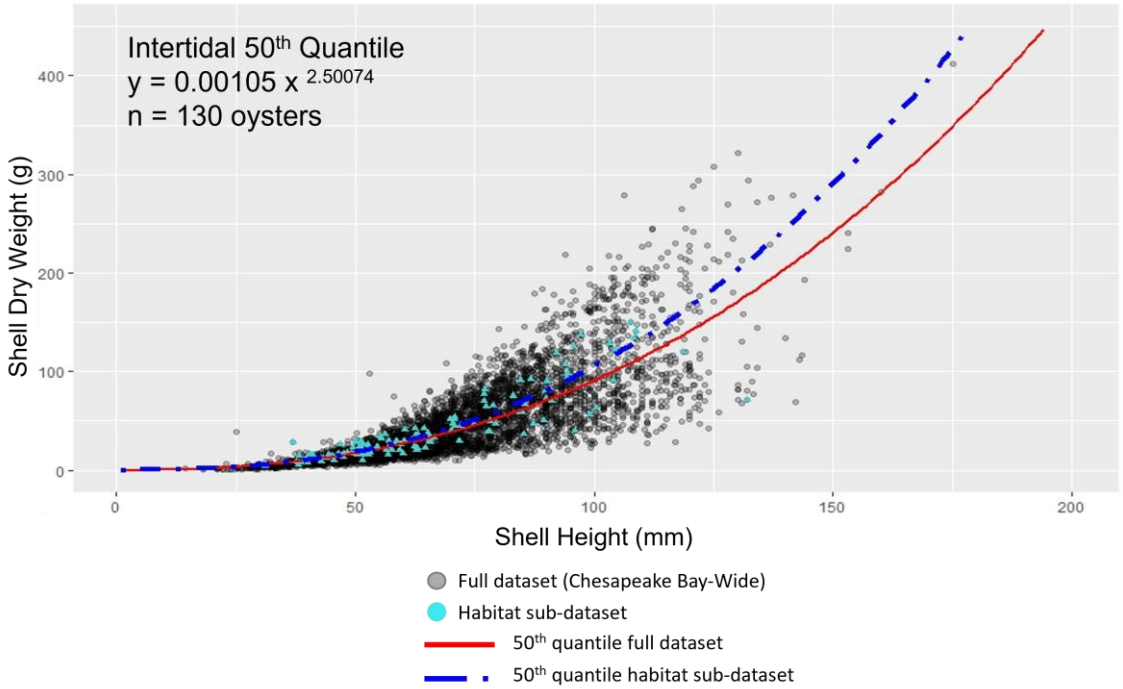

Figure E-10. Oyster shell 50th quantile regression for oysters growing on intertidal reefs (turquoise triangles, blue dashed line) compared to the full Chesapeake Bay-wide dataset (gray dots, red line).

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Appendix F. Panel Criteria for Data Used to Estimate Enhanced Denitrification and Future Study Considerations for Restoration-Denitrification Protocols (Ch 8)

The Panel recognized that the inherent variability in denitrification rates and the range of methods used to assess them could introduce significant errors into the evaluation of denitrification enhancement associated with oyster reef restoration. As a result, the Panel established a set of criteria that must be met for data to be included in the dataset used to determine enhanced denitrification estimates for BMP use. The Panel based these criteria on a combination of the best available scientific information and the applicability of measurements in the context of BMP crediting. The questions the Panel considered to develop the criteria are described below. Each question is followed by the criteria (and supporting rationale) for inclusion or exclusion of data based on that question.

The Panel believes that it is feasible to develop denitrification reduction estimates for restored reef types and settings for which data are currently insufficient (e.g., restoration using large substrates or restoration of intertidal reefs). Developing crediting for these situations or refining the current crediting recommendations for subtidal reefs will require collecting additional denitrification data from restored reefs. Considering this and the extensive discussions the Panel held to determine whether data was suitable for inclusion in current recommendations, each of the criteria below is also followed by guidance for future efforts to measure restored reef denitrification rates for BMP use. Some guidance (e.g., data needed for QA/QC) also applies to denitrification data needed for developing BMPs for other oyster practices (e.g., aquaculture). Some guidance (e.g., sampling design considerations) will apply in concept, but the details will need to be revised to fit the oyster practice of interest. For example, gear type, gear location in the water column, and farm practices (such as oyster maintenance and harvest) will have to be considered when developing denitrification sampling designs for oyster aquaculture practices. The Panel recommends that all denitrification sampling designs be developed in consultation with expert(s) knowledgeable in the oyster practice of interest, denitrification experiments, and local habitat characteristics.

F.1 Are the samples used to determine denitrification rates representative of the site?

F.1.1 Panel Criteria

To date, most studies assessing denitrification rates on restored oyster reefs sample the reef using cores or trays. Core samples are taken using a relatively small diameter tube to collect sediments from within or adjacent to an oyster reef. The core diameter is small enough to exclude intact oyster clumps and associated fauna from the sample. Tray samples are taken from trays that are large enough to hold multiple clumps of oysters and embedded within the reef matrix. The tray is filled with reef material including oysters, shell, sediment and associated fauna.

Several studies to date have demonstrated significant nitrogen cycling associated with oysters, oyster clumps and associated fauna (Caffrey et al. 2016, Arfken et al. 2017, Jackson et al. 2018). In a study directly comparing denitrification rates measured using core samples versus tray samples from the same reef, Kellogg et al. (in

revision) found that core samples significantly underestimated reef denitrification rates. Based on these studies, the Panel concluded that oyster reef sampling approaches that include the entire reef matrix (i.e., sediment, oysters, shell, associated fauna), such as the tray approach, provide a better estimate of total enhanced denitrification associated with oyster restoration. The Panel also concluded that cores containing only sediments can be used to assess baseline denitrification values on unrestored areas, or areas with no pre-existing oysters.

F.1.2 Panel Guidance for Future Studies

Future studies that measure denitrification rates on restored oyster reefs should collect samples that are representative of the entire habitat of interest (e.g., using trays). Specifically, the Panel recommends that the entire reef matrix (reef materials, underlying sediment, and associated organisms) be collected to estimate denitrification rates at the reef scale. To date, studies that have taken samples representative of the entire reef habitat used divers to collect samples from the field and incubated them in the lab (e.g., Kellogg et al. 2013) or incubated samples in situ (e.g., Humphries et al. 2016). These are currently the only published methods using sampling trays to capture denitrification within the reef matrix.

These approaches have been applied on subtidal reefs and intertidal reefs. Presently, data are too sparse to develop enhanced denitrification estimates for intertidal reefs, but, once more data are available, the Panel agreed that this can be done. For intertidal reefs, care should be taken when scaling measured hourly denitrification rates to annual rates because enhanced denitrification can only be credited for the times that the restored intertidal reef is submerged.

The ex-situ approach for measuring denitrification was developed to minimize concerns that chambers or domes used for in situ, on-bottom incubations would not seal due to the irregularity of reef topography. If not properly sealed, water in the incubation chamber will be diluted by exchanging with water outside the chamber, which will result in an inaccurate denitrification measurement. Installation of semi-permanent "rings" into the substrate have been used to attach chambers with promising results (Humphries et al. 2016). However, using semi-permanent rings for in situ denitrification measurements limit studies to fixed sites.

An ongoing study funded by the Chesapeake Bay Trust is examining the efficacy of using of chambers that can be lowered to the bottom on restored oyster reefs to measure denitrification. A tracer can be used to determine the rate of chamber dilution in combination with time course measurements of oxygen and N_2. Denitrification rates can then be determined through a flux/dilution model. Potential advantages of this approach include being able to conduct rapid measurement with lower effort and measuring more accurate denitrification rates by minimizing bottom disturbance. However, remotely measuring denitrification from the surface does not allow for determining oyster biomass, which is necessary for calibrating the relationship between biomass and enhanced denitrification. Therefore, the Panel recommends that denitrification measurements be made at sites with known oyster biomass.

Measuring denitrification rates associated with most large substrates will likely require developing new methods for incubating samples as well as careful thought about how measured values will be scaled up to the entire restoration site. For large substrates that are deployed as individual units (i.e., spaced far enough apart that the biogeochemical processes of adjacent units are unlikely to interact with one another), the appropriate sampling unit is likely the individual structure (see Subchapter 7.6.2.2). Measuring denitrification rates associated with these units (in or ex-situ) could likely be achieved by building incubation chambers large enough to handle these units. Using this approach and measuring the biomass of oysters on each unit would contribute to developing regressions of denitrification rates against oyster biomass per unit that could be implemented similar to the approach for subtidal oyster reefs described in this report. In this case, verification would require assessing mean oyster biomass per structural unit rather than per unit area of substratum (Subchapter 7.6.2.2). Scaling up to entire restoration site would then require multiplying by the total number of structures rather that the area of the site (Subchapter 7.6.2.2). Because the designs of large substrates vary widely, it is unlikely that measurements from one type of structure will be applicable to another.

Measuring denitrification associated with arrays of structures (e.g., many Oyster Castles® adjacent to and stacked on top of each other) will be more challenging because the position of the element within the array may determine its influence on local fluxes. Measuring denitrification rates associated with arrays of large substrates may require developing new techniques that allow measurement of denitrification rates in the field. For both arrays and individual large substrates, separate regressions for subtidal and intertidal settings will likely be needed.

Regardless of the type of restored reef for which denitrification data are collected, the design of denitrification sampling regimes should consider that both denitrification rates and oyster biomass can change significantly over time. Seasonal changes in denitrification rates are well documented, emphasizing that data collected in one season cannot reasonably be extrapolated to a different season. The meta-analysis conducted by the Panel also underscores that denitrification rates for reefs at one level of oyster tissue biomass cannot accurately be extrapolated to other oyster biomass levels unless sufficient data are available to conduct regression analyses. Without denitrification data for a range of oyster biomass levels, it may not be feasible to determine an appropriate nitrogen reduction credit if oyster biomass declines or increases significantly at a specific reef/ BMP site. The Panel recommends that sampling regimes should be developed in consultation with expert(s) knowledgeable in oyster reef restoration, denitrification experiments, and local habitat characteristics.

F.2 Are the characteristics of the reef site described in sufficient detail (e.g., oyster biomass, habitat characteristics, etc.) to determine the types of reefs to which the data can reasonably be extrapolated in a BMP context?

F.2.1 Panel Criteria

Oyster reef denitrification is supported by the feeding activity of oysters followed by microbial processing of the resulting biodeposits. Biodeposit production is generally expected to increase with increasing oyster abundance and/or biomass until food becomes limiting. Therefore, reef samples collected for measuring

denitrification should also include a description of the oysters and/or an oyster tissue biomass per unit area. This will also be important to assess whether rates measured at one site can reasonably be extrapolated to another. Studies in Chesapeake Bay have demonstrated that increases in oyster biomass per unit area can lead to increases in denitrification rates (Sisson et al. 2011, Kellogg et al. 2014a, Kellogg et al. 2014b, Cornwell et al. 2016).

The environmental setting of the oyster reef is also important. Intertidal oyster reefs are exposed to air during low tides for a portion of the day; therefore, denitrification rates collected from subtidal reefs should not be extrapolated to intertidal reefs, and vice-versa. The Panel agreed that only those studies that assessed the biomass of oysters within the sample used for denitrification measurements and that included a sufficient description of reef characteristics should be included in data analyses.

F.2.2 Panel Guidance for Future Studies

Studies seeking to assess denitrification enhancement on restored oyster reefs should include sufficient data to allow for the direct comparison of denitrification rates from one site to rates measured at other sites. At a minimum, data should include information on the types and amounts of materials used for restoration, the location of the restoration site, the position of the restoration site relative to local tidal regimes (i.e., is the site subtidal or intertidal), and mean oyster tissue biomass before and after restoration. Oyster tissue biomass information should be collected and reported using the units most meaningful for the type of materials used for restoration. For small substrates, the most appropriate unit for reporting will likely be oyster tissue biomass per unit substratum area (i.e., g oyster tissue dry weight m^{-2}). For large substrates, the most appropriate unit will likely be oyster tissue biomass per structural unit (i.e., g oyster tissue dry weight $unit^{-1}$). Oyster biomass should be calculated from oysters in each sample used for denitrification measurements. Additional data, such as sediment characteristics (e.g., organic content), ambient conditions of dissolved nitrogen, and fouling community structure, among others, may also be used to compare denitrification rates between sites.

F.3 Of the many analytical approaches used to measure denitrification, which are most appropriate for use in a BMP context?

F.3.1 Panel Criteria

Existing literature included both indirect and direct denitrification assessment techniques. Indirect techniques that have been used to measure microbial denitrification include N_2 depuration/flux (Seitzinger et al. 1993), acetylene block (Joye et al. 1996), and overall mass balances to determine rates (Cornwell et al. 1999). These techniques have limitations since they generally produce *potential* rates. The Panel agreed that studies using these methods should not be applied to determine denitrification rates for BMP use. Instead, the Panel agreed to only use studies that employ one of the two methods that adequately capture *areal* denitrification rates: N_2:Ar gas ratios (e.g., Kana et al. 1994, Kellogg et al. 2013) and ^{15}N isotope pairing (e.g., Nielsen 1992). Overall, the field of biogeochemistry has coalesced around these two techniques for measuring benthic denitrification in estuarine environments.

F.3.2 Panel Guidance for Future Studies

To date, only the N_2:Ar gas ratio approach has been used to measure areal denitrification rates for restored oyster reefs. Although use of ^{15}N isotope pairing techniques is theoretically feasible, use of this method requires complete mixing of the labeled nitrogen throughout the incubated sample. Given the difficulties of achieving complete mixing in samples that contain impervious structures (e.g., oyster shells, large substrates), studies using this approach will need to demonstrate that complete mixing has been achieved for associated measurements to be considered valid. The Panel recognized that new methods for accurately measuring N_2 fluxes are likely to be developed, especially for large substrates, and noted that these should be considered on a case by case basis to determine if they are suitable for use in the context of BMP crediting.

F.4 Are "batch" and "flow-through" incubation techniques equally appropriate for measuring denitrification rates on restored oyster reefs?

F.4.1 Panel Criteria

Two time-course incubation methods are generally used for obtaining denitrification measurements: "batch" and "flow-through." In batch incubations, the sample is sealed in a water-tight and gas-tight chamber and multiple water samples are collected over the time course of the incubation via sampling ports. The concentrations of N_2 in the water samples are then regressed against time to determine the change in concentration per unit time. In contrast, flow-through incubations involve continual flow of water through the incubation chamber and assess denitrification rates based on the flow rate and the difference in concentration of di-nitrogen gas in the water flowing into the chamber and the water flowing out of the chamber. This technique requires the concentrations of gases and nutrients to be at steady state prior to the start of the incubation which, for sediment cores, takes 17-24 hours. Although flow-through chamber incubations of oyster reef samples are theoretically possible, none have been successfully conducted to date. Flow through incubations of oyster reef samples will require overcoming challenges associated with providing a flow of temperature-equilibrated water sufficient to maintain necessary oxygen concentrations without disturbing the sediment-water interface of the sample. A pilot study by Kellogg et al. (2013) that repeatedly incubated reef samples over a two-day period observed changes in nitrogen dynamics over time. These results suggest that denitrification rates are more likely to deviate from field conditions the longer samples are held in the laboratory. The long equilibration time surpasses the time frame for dark or light time periods, and the flow-through approach is generally limited to dark-only incubations. For these reasons, the Panel only considered measurements made using batch incubation techniques.

F.4.2 Panel Guidance for Future Studies

To date, only batch incubations have been used to assess denitrification rates for oyster reef samples that include representative samples of the habitat. However, this does not mean that other approaches could not be used or developed. Any measurements using new techniques will need to demonstrate that the assumptions of the technique have been met. For samples brought into the laboratory, care needs to be taken to ensure that laboratory methods do not lead to significant changes in nitrogen dynamics that would not be observed under field conditions. The most recent studies of denitrification on restored oyster reefs (e.g., Cornwell et al. 2019) aerate samples during transport and begin incubation at field salinity and temperatures

within an hour of reaching the laboratory to ensure minimal deviations from field conditions. Regardless of the method used, data should be collected to demonstrate that oxygen concentrations were sufficient (i.e., dissolved oxygen concentrations ≥4 mg L^{-1} during incubations) and temperature was constant (±1 °C) throughout the incubation. Because it is difficult to accurately measure denitrification rates for complex substrates, such as an oyster reef, sampling methods should be selected and/or developed in consultation with expert(s) knowledgeable in measuring denitrification in these environments.

F.5 Are incubations under both light and dark conditions necessary for accurate determination of denitrification rates?

F.5.1 Panel Criteria

Oyster reefs in Chesapeake Bay occur across depths ranging from intertidal to deep subtidal reefs below the euphotic zone (the portion of the water column with sufficient light to support photosynthesis). For soft sediments, it is well-known that denitrification can be reduced by the presence of photosynthetic organisms (e.g., macroalgae and benthic microalgae) that compete with microbes for the substrates needed to support denitrification. In this context, the Panel considered whether studies of oyster reef denitrification that did not include incubations under both light and dark conditions were appropriate for inclusion in the dataset used to develop default denitrification rates. The Panel concluded that studies of reefs in the euphotic zone should include both light and dark incubations but that studies of reefs below the euphotic zone did not need to include an incubation under light conditions.

F.5.2 Panel Guidance for Future Studies

Where sufficient light reaches the bottom to support photosynthesis (i.e., where ≥ 2% of incident sunlight reaches the bottom or benthic algae are present), both dark and light incubations are necessary for extrapolating measured hourly denitrification rates to a daily rate. If sufficient light does not reach the bottom, then only dark incubations are required, and data can be extrapolated to the entire day. In some cases, accurate measurement of denitrification rates is not feasible in the presence of light because photosynthesis that occurs during the incubation leads to the formation of bubbles in the incubation chamber. The presence of bubbles in the incubation chamber alters gas fluxes in ways that would not normally be observed under field conditions. If significant bubble formation occurs during light incubations or if light incubations are not conducted for sites where light is sufficient to allow benthic photosynthesis, then only data from dark incubations can be used. In this situation, denitrification during daytime hours should be assumed to be zero and denitrification measurements from dark incubations should be multiplied by the number of dark hours in the day to determine daily denitrification rates.

F.6 Are additional data reported that can be used to assess the quality of the denitrification data?

F.6.1 Panel Criteria

Studies that include data on other nitrogen fluxes (e.g., ammonium fluxes, nitrate or combined nitrate/nitrite fluxes) and oxygen fluxes are preferred because these data can be helpful in verifying the quality of the data

collected. The ratio of oxygen (or dissolved inorganic carbon [DIC]) to the sum of all nitrogen fluxes should be reasonable based on oxygen and total nitrogen (O_2:N) stoichiometry and the amount of ammonium that is nitrified (Froelich et al. 1979). Because denitrification rates are difficult to measure, the Panel decided that reporting these fluxes is valuable for verifying the quality of the data incorporated into a BMP.

F.6.2 Panel Guidance for Future Studies

Accurately measuring denitrification is difficult and mistakes can easily be made. Thus, the Panel suggests that additional data be collected that can be used to verify the quality of the denitrification measurements. At a minimum, sufficient data should be collected to confirm that temperature remained constant (±1 °C) throughout incubations, that oxygen concentrations did not fall below 4 mg L^{-1}, and that total nitrogen concentration (sum NO_3^-, NH_4^+, and N_2-N gas) increased linearly as oxygen concentration decreased. Ideally, sufficient data should be collected to allow for the calculation of stoichiometric ratios for C:N:P based on fluxes to determine if the ratios are similar to those expected based on the Redfield ratio of 106C:16N:1P for marine algae (see Kellogg et al. 2013 for an example). If O_2 is measured but CO_2 is not, a flux ratio of 1:1 for O_2:CO_2 can be assumed, which will allow oxygen fluxes to be used for these calculations. If the calculated stoichiometric ratio is similar to the Redfield ratio, it is likely that the majority of carbon (or oxygen), nitrogen, and phosphorus fluxes have been accounted for in flux measurements. Significant deviation from the Redfield ratio would suggest that significant fluxes may not have been captured and/or that mistakes were made during sample collection or incubation. If this is the case, the resulting data should be examined carefully and used with caution or rejected as possibly erroneous.

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Appendix G: Lookup Table for Annual Denitrification Enhancement (Ch 8)

Table G.1. Lookup table developed by Panel to determine annual nitrogen removal attributed to enhanced denitrification on subtidal restored oyster reefs. Refer to text for definitions and survey methods for determining baseline and post-restoration biomass. See Subchapter 8.6.2 for examples illustrating how to use the lookup table.

Enhanced Nitrogen Removal (lbs acre^{-1} yr^{-1})				Post-restoration Oyster Tissue Biomass Range (g DW m-2)																			
				0 - 14.9	15 - 24.9	25 - 34.9	35 - 44.9	45 - 54.9	55 - 64.9	65 - 74.9	75 - 84.9	85 - 94.9	95 - 104.9	105 - 114.9	115 - 124.9	125 - 134.9	135 - 144.9	145 - 154.9	155 - 174.9	175 - 194.9	195 - 214.9	215 - 234.9	235 - 254.9	255 - 274.9
				Annual DNF Rate for Midpoint of Biomass Rage (lbs. acre^{-1} yr^{-1})																			
				132	161	184	206	229	252	275	298	301	305	308	311	315	318	322	327	334	341	348	355	359
Baseline Oyster Tissue Biomass Range (g DW m^{-2})	0 - 14.9	Annual DNF Rate for Midpoint of Biomass Range (lbs. acre^{-1} yr^{-1})	132		29	51	74	97	120	143	165	169	172	176	179	183	186	190	195	202	209	215	222	226
	15 - 24.9		161			23	46	68	91	114	137	140	144	147	151	154	158	161	166	173	180	187	194	198
	25 - 34.9		184				23	46	68	91	114	118	121	124	128	131	135	138	143	150	157	164	171	175
	35 - 44.9		206					23	46	68	91	95	98	102	105	109	112	115	121	128	134	141	148	152
	45 - 54.9		229						23	46	68	72	75	79	82	86	89	93	98	105	112	119	125	129
	55 - 64.9		252							23	46	49	53	56	59	63	66	70	75	82	89	96	103	107
	65 - 74.9		275								23	26	30	33	37	40	44	47	52	59	66	73	80	84
	75 - 84.9		298									3	7	10	14	17	21	24	29	36	43	50	57	61
	85 - 94.9		301										3	7	10	14	17	21	26	33	40	47	54	57
	95 - 104.9		305											3	7	10	14	17	22	29	36	43	50	54
	105 - 114.9		308												3	7	10	14	19	26	33	40	47	51
	115 - 124.9		311													3	7	10	16	22	29	36	43	47
	125 - 134.9		315														3	7	12	19	26	33	40	44
	135 - 144.9		318															3	9	16	22	29	36	40
	145 - 154.9		322																5	12	19	26	33	37
	155 - 174.9		327																	7	14	21	28	32
	175 - 194.9		334																		7	14	21	25
	195 - 214.9		341																			7	14	18
	215 - 234.9		348																				7	11
	235 - 254.9		355																					4
	255 - 274.9		359																					

Table G.1. (continued)

Enhanced Nitrogen Removal (lbs acre^{-1} yr^{-1})				Post-restoration Oyster Tissue Biomass Range (g DW m^{-2})															
				275 - 294.9	295 - 314.9	315 - 334.9	335 - 354.9	355 - 374.9	375 - 394.9	395 - 414.9	415 - 434.9	435 - 454.9	455 - 474.9	475 - 494.9	495 - 514.9	515 - 534.9	535 - 554.9	555 - 574.9	575 - 594.9
				Annual DNF Rate for Midpoint of Biomass Rage (lbs. acre^{-1} yr^{-1})															
				353	348	343	338	332	327	322	317	312	306	301	296	291	285	280	275
Baseline Oyster Tissue Biomass Range (g DW m^{-2})	0 - 14.9	Annual DNF Rate for Midpoint of Biomass Range (lbs. acre^{-1} yr^{-1})	132	221	216	211	205	200	195	190	185	179	174	169	164	158	153	148	143
	15 - 24.9		161	193	187	182	177	172	166	161	156	151	146	140	135	130	125	119	114
	25 - 34.9		184	170	165	159	154	149	144	138	133	128	123	118	112	107	102	97	91
	35 - 44.9		206	147	142	136	131	126	121	116	110	105	100	95	89	84	79	74	69
	45 - 54.9		229	124	119	114	108	103	98	93	88	82	77	72	67	61	56	51	46
	55 - 64.9		252	101	96	91	86	80	75	70	65	60	54	49	44	39	33	28	23
	65 - 74.9		275	78	73	68	63	58	52	47	42	37	31	26	21	16	11	5	
	75 - 84.9		298	56	50	45	40	35	30	24	19	14	9	3					
	85 - 94.9		301	52	47	42	37	31	26	21	16	10	5						
	95 - 104.9		305	49	44	38	33	28	23	17	12	7	2						
	105 - 114.9		308	45	40	35	30	24	19	14	9	4							
	115 - 124.9		311	42	37	31	26	21	16	11	5								
	125 - 134.9		315	38	33	28	23	18	12	7	2								
	135 - 144.9		318	35	30	24	19	14	9	4									
	145 - 154.9		322	31	26	21	16	11	5										
	155 - 174.9		327	26	21	16	11	5											
	175 - 194.9		334	19	14	9	4												
	195 - 214.9		341	12	7	2													
	215 - 234.9		348	6															
	235 - 254.9		355																
	255 - 274.9		359																

Appendix H: Informational Recommendations on the Harvest-Assimilation Protocols

H.1 Preliminary Re-evaluation of Default Estimates for Nitrogen and Phosphorus Content in Tissue of Oysters Removed via Licensed Oyster Harvest

The licensed oyster harvest Practice F (licensed oyster harvest using hatchery-produced oysters) being submitted for approval in this report only credits diploid oysters that are grown directly on the bottom in harvest areas without gear. Some members of the Panel had concerns that the diploid shell height to tissue dry weight quantile regression equation from the first report (Reichert-Nguyen et al. 2016) would not be appropriate to estimate nitrogen and phosphorus reduction resulting from this practice since it included data from oysters that were grown off the bottom in gear (e.g., cages, floating bags). The Panel did a preliminary re-evaluation of these data by ploidy and culture method using the same quantile regression approach described in Reichert-Nguyen et al. (2016) to examine whether culture method significantly altered the reduction estimates. This preliminary re-evaluation included additional data from Cubillo et al. (2018) and Mann, Southworth, and Wesson (unpubl.) that were provided after the approval of the first report. Data from Cubillo et al. (2018) provided additional shell height and tissue dry weight measurements for both diploid (n = 420) and triploid (n = 2,328) oysters grown in gear from oyster aquaculture farms located in Chesapeake Bay, Chester River, Honga River, and Potomac River in Maryland (Table H-1). Data from Mann, Southworth, and Wesson (unpubl.) provided additional shell height and tissue dry weight measurements for diploid oysters (n = 1,332) grown directly on reefs from eight locations in Virginia (Table H-1, summarized in Appendix E). These data were added to the dataset used to determine the diploid and triploid shell height to tissue dry weight regression equations from the Panel's first report (Reichert-Nguyen et al. 2016).

Table H-1. Comparison of sample sizes from datasets used in the Panel's first report and this report by ploidy and culture method.

Category Based on Ploidy and Culture Method	First Report Sample Size	Updated Sample Size
Triploid, With Gear	1066	3394
Diploid, With Gear	84	504
Diploid, Without Gear	5556	6888

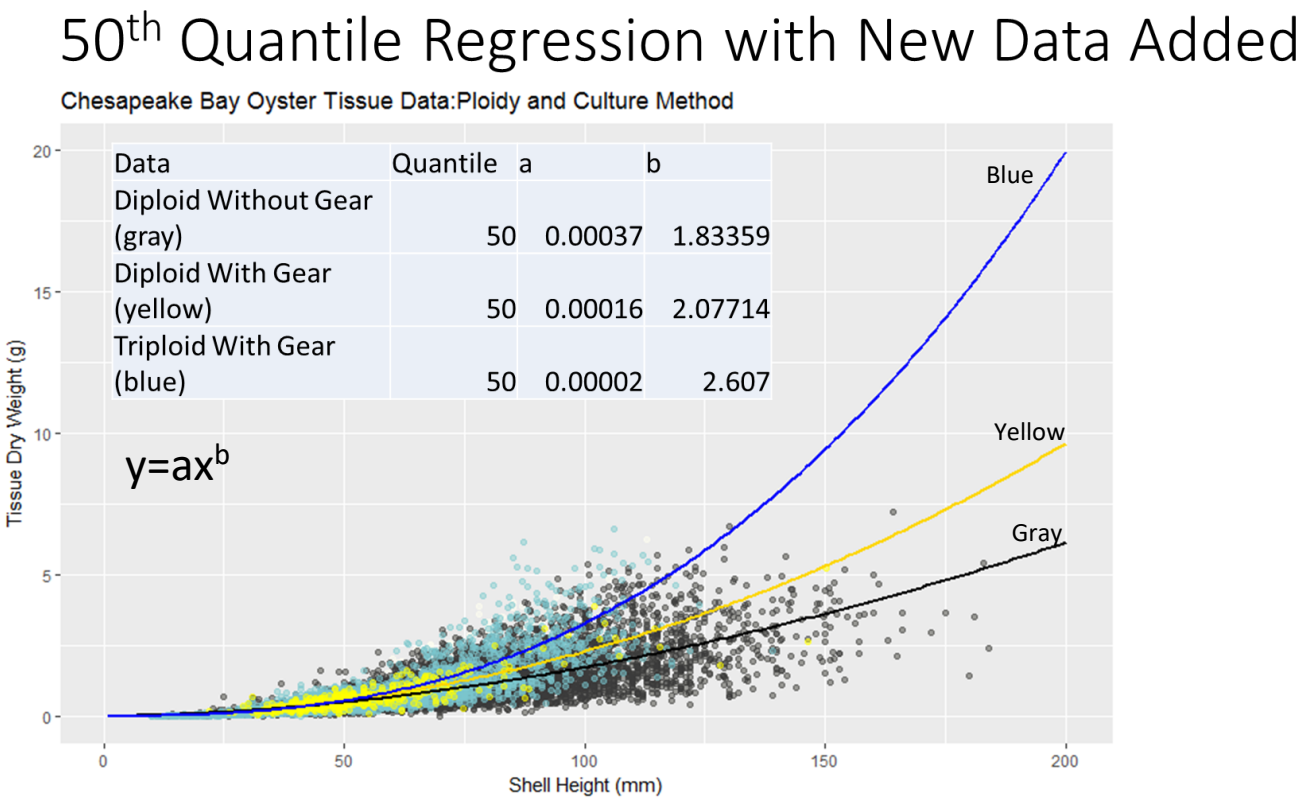


Figure H-1. Oyster shell height to tissue dry weight 50th quantile regression with additional data on ploidy and culture method: diploid without gear (gray), diploid with gear (yellow), triploid with gear (blue).

The quantile regression analysis using the additional data described in Table H-1 generated three distinct curves for oysters greater than 50 mm (~ 2 inches) shell height. Triploid oysters had greater tissue biomass than diploid oysters grown with or without gear (Figure H-1). Diploid oysters grown in gear had greater tissue biomass than diploid oysters grown without gear (Figure H-1). Estimates of nitrogen and phosphorus removed from harvested tissue were generated from these three equations and compared with estimates from the first report (Table H-2).

The tissue contents for diploid oysters grown without gear were unchanged relative to the contents generated using oysters grown with and without gear in the first report (Table H-2). The Panel agreed that the decrease in nitrogen by 22 lbs per million oysters in the largest size class (6-inch midpoint) was not a substantial change. Moreover, oysters this large are not typically harvested, which reduces the likelihood that total nitrogen reduction from harvest would be overestimated by using the approved equation. As a result, the Panel concluded that the reduction estimates for diploid oysters grown with and without gear from Reichert-Nguyen et al. (2016) would be appropriate to use for licensed oyster harvest practices.

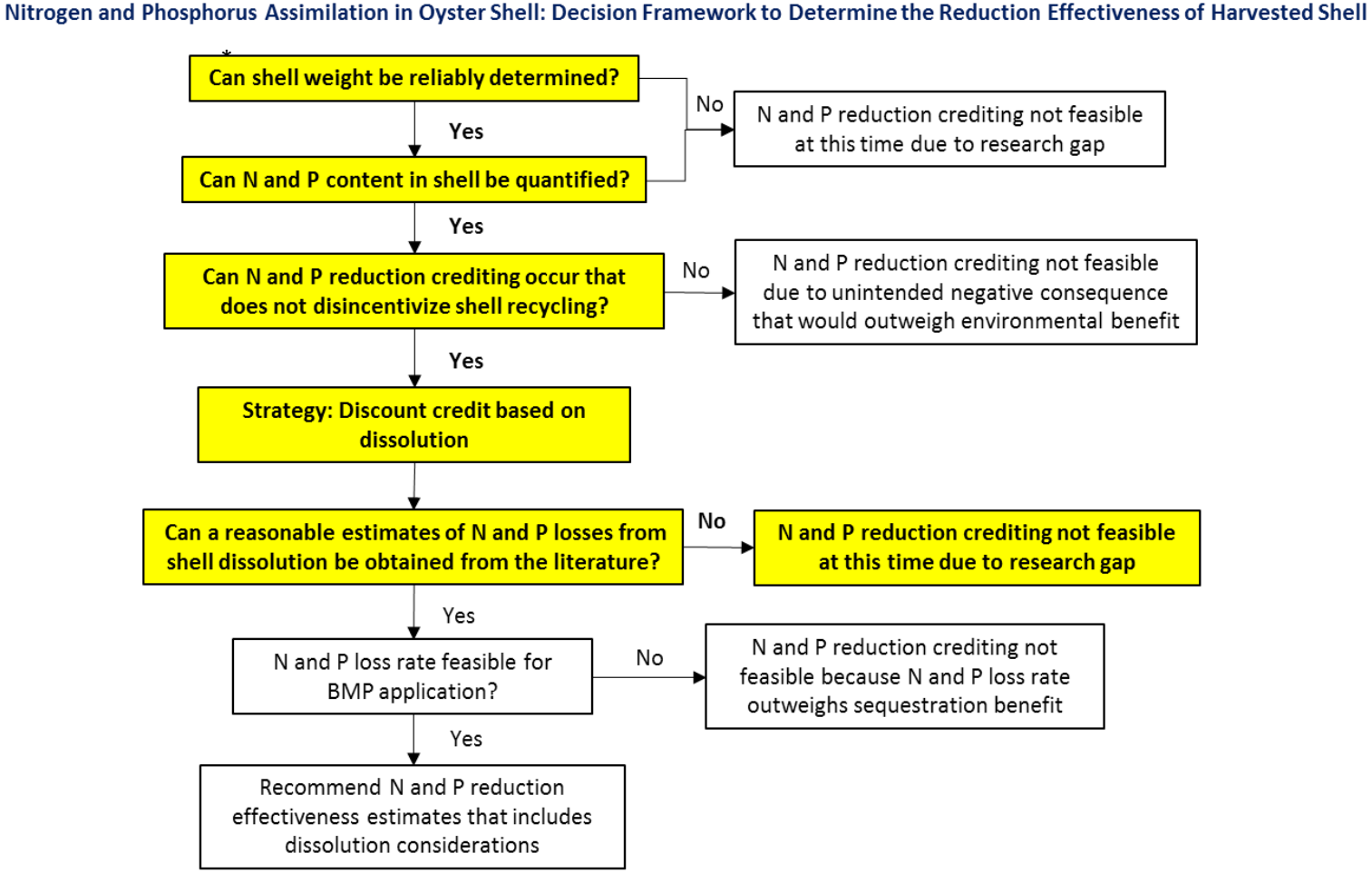
The reduction estimates for diploid and triploid oysters grown in gear increased substantially relative to the approved estimates in the first report (Table H-2). While not relevant for the licensed oyster harvest practices, the Panel recommends that the approved estimates are re-evaluated for the private oyster aquaculture practices that use gear and oysters with different ploidy.

Table H-2. Change in oyster tissue N & P reduction estimates (lb per million oysters) relative to approved reductions from Reichert-Ngyuen et al. (2016) for different combinations of ploidy and culture type when adding new data.

Size Class Midpoint (in)	Size Class Midpoint (mm)	Diploid				Triploid	
		Without Gear		With Gear		With Gear	
		Nitrogen	Phosphorus	Nitrogen	Phosphorus	Nitrogen	Phosphorus
2.25	57	0	0	+22	0	0	0
3	76	0	0	+45	0	0	0
4	102	0	0	+110	0	+44	0
5	127	0	0	+198	+22	+132	0
6	152	-22	0	+309	+44	+287	+44

H.2 Literature Review and Recommended Reduction Effectiveness Determination Framework for Nitrogen and Phosphorus Assimilated in Shell of Harvested Oysters

This section describes the Panel's recommended decision framework and strategies to determine the reduction estimates for nitrogen (Protocol 2) and phosphorus (Protocol 5) assimilated into shell of harvested oysters. This framework would be applied for private oyster aquaculture or licensed oyster harvest practices. Oyster shell is a limited resource and the successful implementation of oyster practices to enhance oyster recruitment, survival, and growth is dependent on the availability of shell. Therefore, the Panel agreed that recommendations for implementing these practice-protocol combinations should not negatively impact oyster practices that rely on the return of harvested shell to the Bay by unintentionally incentivizing the collection of shell to receive credit. Such an unintended consequence would outweigh any water quality benefit. The Panel proposes implementing the decision framework in Figure H-2 to allow for nutrient reduction credit from assimilation in the shell of harvested oysters while not disincentivizing shell recycling.



*Bolded yellow decision boxes indicate the current decision pathway. Overall, research is lacking to assign a dissolution rate regarding shell returned to the Chesapeake Bay.

N = Nitrogen, P = Phosphorus

Figure H-2. The Panel's recommended decision framework to determine the reduction effectiveness of harvested shell.

The Panel agreed that shell dry weight and the amount of nitrogen and phosphorus assimilated in shell can be reliably quantified with existing data. Since percent nitrogen and phosphorus content in oyster shell is well constrained (Appendix E; Table E-2 & E-3), the Panel agreed that the values applied for the restoration-shell assimilation protocols could be applied to aquaculture-shell assimilation protocols.

To convert shell height to shell dry weight, a similar strategy applied in Appendix E could be implemented to develop Bay-wide shell quantile regression equations for harvested oysters. Separate quantile regression equations may be needed for diploid and triploid oysters (following tissue regression equations in Reichert-Nguyen et al. 2016). Data currently exist for oysters harvested from private oyster aquaculture (Higgins et al. 2011, Cubillo et al. 2018) and licensed oyster harvest practices (Powell et al. 2015) that could contribute to generating these regressions.

The Panel agreed that including a step that discounts the reduction credit based on shell dissolution (1) accounts for returning shell to the Bay and (2) reduces risk to shell recycling programs. The dissolution value will be based on relevant research (Table H-3). This approach assumes that 100% of harvested shell will be returned to the Chesapeake Bay to create no incentive to keep shell on land. This assumption can be adjusted once more is known about the fate of shell (e.g., dissolution rates, burial).

The Panel reviewed the literature to assess whether there was enough information to estimate nitrogen and phosphorus loss from shell dissolution. The Panel found several studies that examined shell loss rates and decay (Table H-3), but only one study directly measured shell dissolution. These studies measured carbonate loss only; nitrogen and phosphorus loss were assumed to be proportional to carbonate loss. The Panel concluded that there is not currently enough information to determine the fate of harvested shell once it is returned to the Bay. Spatial variability in environmental conditions and geochemical processes could alter the mechanisms of shell loss, burial, and dissolution in different locations; therefore, additional research is required to understand the long-term storage of nitrogen and phosphorus in harvested shell before the reduction effectiveness for private oyster aquaculture and licensed oyster harvest practices can be determined.

Table H-3. Summary of shell dissolution results and conclusions from literature review.

Study Group	Summary of Findings	Conclusions
Annual shell loss rates from field studies in Delaware Bay and James River	Powell et al. 2006 Delaware Bay: Half-life of shell added in a given year ranges from 2-10 years. Intermediate salinities have shortest half-lives. Avg shell loss rate per year ranged from 5-37%. Mann et al. 2009 James River: Most shell loss rates are >20% per year for high and medium relief reefs, with many between 30-50% per year; suggests shell half lives of less than or equal to 3 yrs for high and medium relief reefs	In these two well-managed study areas, sedimentation rates are very low, so these rates were assumed to be a result of dissolution. In other areas, sedimentation could be significant such that it limits shell production and changes dissolution rates.
Instantaneous shell decay rates based on field studies	Jordan-Cooley et al. 2011; DePiper et al. 2016: instantaneous rate of shell decay 0.5-0.9 per year based on Smith et al. 2005: determined how long it took shell to reach various degraded conditions. Wilberg et al. 2011: instantaneous decay rates of 0.45 per year for market size and 0.52 per year for small size oysters based on Christmas et al. 1997: determined time-since-death required for oyster disarticulation.	
Shell dissolution rates lab study	Waldbusser et al. 2011: Weathered shell degraded from 0.06-0.15% per day depending on pH. Fresh shell degraded faster than weathered or dredged shell. Shell dissolution generally increased with increasing pH.	Only study directly measuring dissolution. Concern that lab conditions do not adequately account for ecosystem processes.

H.3 References

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Wilberg MJ, Livings ME, Barkman JS, Morris BT, Robinson JM (2011) Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. Mar Ecol Prog Ser 436: 131-141

Appendix I: Research Gaps for Applying Denitrification Protocols to Oyster Harvest and Restoration Practices Using Large Substrates

This appendix provides a synopsis of information currently available for developing BMP recommendations for harvest- and aquaculture-denitrification protocols. The Panel agreed that private oyster aquaculture and licensed oyster harvest practices may have the potential to remove nitrogen via microbial denitrification; however, the Panel agreed that data were too sparse at this time to develop BMP recommendations for these practices in this report. This appendix also provides considerations to apply restoration-denitrification protocols to reefs restored using large substrates (e.g., ReefBalls™, Oyster Castles®).

I.1 Literature Review of Enhanced Denitrification for Oyster Aquaculture and Licensed Oyster Harvest Practices

A review of the current peer-reviewed literature indicates that few denitrification studies have been conducted in areas where oysters are harvested. Three studies evaluated denitrification associated with off-bottom private oyster aquaculture practices. Two studies estimated denitrification rates associated with on-bottom aquaculture practices.

I.1.1 Off-Bottom Aquaculture Studies

Currently in the Chesapeake region, there are only three water column aquaculture studies that are useful for consideration of an oyster harvest denitrification BMP:

- **Higgins et al. (2013)** assessed denitrification rates in floating rafts within St. Jerome Creek, MD and Spencer's Creek. VA. St. Jerome Creek was open with a sandy bottom, while Spencer Creek was a very shallow, low-energy site in a cove. Sampling took place four times in St. Jerome Creek and three times in Spencer Creek. Denitrification was measured from homogenized sediment with ^{15}N amendments, and therefore should be regarded as "potential rates" that have a tenuous connection to areal rates. Denitrification rates were also estimated using the N_2:Ar approach with intact cores, with rates generally similar to the ^{15}N potential rates. Most oyster N_2 flux rates (cores at site) were 25-65 µmol N m^{-2} h^{-1}. The rates in the aquaculture area were lower than those observed at control sites and the effects of benthic microalgae were not evaluated. Considerable effort was devoted to determining the oyster biodeposition rates, with variable total nitrogen (sum of all forms) flux rates below the floats (70-590 µmol N m^{-2} h^{-1}). Intact core releases of ammonium were considerably higher under floats than in control areas.
- **Testa et al. (2015)** assessed denitrification rates and benthic fluxes of nitrogen in floating aquaculture cages at Marinetics Inc. oyster farm (now the Choptank Oyster Company) on LeCompte Bay on the Choptank River, MD. The specific study site is located at Castle Haven Point in a relatively high energy environment. Denitrification and nutrient fluxes were measured in sediments under the floats (farm site) and at two control sites (near farm and reference sites). Fluxes were measured using the N_2:Ar approach under both dark and light conditions four times throughout the growing season. In addition, sedimentation rates were measured directly under floats, at the sediment surface, and at control sites.

Average denitrification rates at the farm, near-farm, and reference sites were 55.8 ± 20.8, 72.8 ± 21.4, and 56.6 ± 16.1 μmol N m^{-2} h^{-1}, respectively. Average ammonium flux rates at the farm, near-farm, and reference sites were 394, 127, and 21.8 μmol N m^{-2} h^{-1}, respectively. Thus, in the footprint of the floats, decreased denitrification and increased ammonium effluxes suggest the sediments are overloaded with biodeposits. However, if the whole system is examined, the sediment nitrogen cycling rates at the site are inconsistent with the N biodeposition rates. A mass balance indicates that the downward flux of nitrogen biodeposits (3,714 μmol N m^{-2} h^{-1}) is much larger than the net deposition to footprint sediments, suggesting a net export of almost 90% of biodeposits. If the exported biodeposits are denitrified at rates similar to those measured in nearby sediments, the net denitrification associated with aquaculture may be positive instead of negative (Figure I-1).

- **Lunstrum et al. (2018)** examined denitrification and benthic fluxes of nitrogen and oxygen in a "rack and bag" oyster farm in Cherrystone Inlet of the Virginia Eastern Shore. Nutrient incubations were carried out similar to those in Testa et al. (2015), with measurement sequentially made using 1) the N_2:Ar approach for denitrification and 2) the ^{15}N isotope pairing approach, using $^{15}NO_3^-$ additions. In addition, the $^{15}NH_4^+$ generated was used as a measure of DNRA (dissimilatory nitrate reduction to ammonium), a process that competes with denitrification. Maximum aquaculture denitrification rates were very low (19.2 μmol N m^{-2} h^{-1}) and were dwarfed by the high efflux of ammonium (maximium 900 μmol N m^{-2} h^{-1}). Thus, at this site denitrification was a minor process in the footprint of the aquaculture farm.

Combined, these three studies suggest that the increase in ammonium efflux associated with oyster aquaculture is proportionately much larger than any increases in denitrification. This suggests that the sediment conditions beneath off-bottom aquaculture leases may not always be conducive to enhancing denitrification (Burkholder & Shumway 2011), and that the potential consequences of enhanced ammonium may negate any enhancements in denitrification within the aquaculture lease footprint.

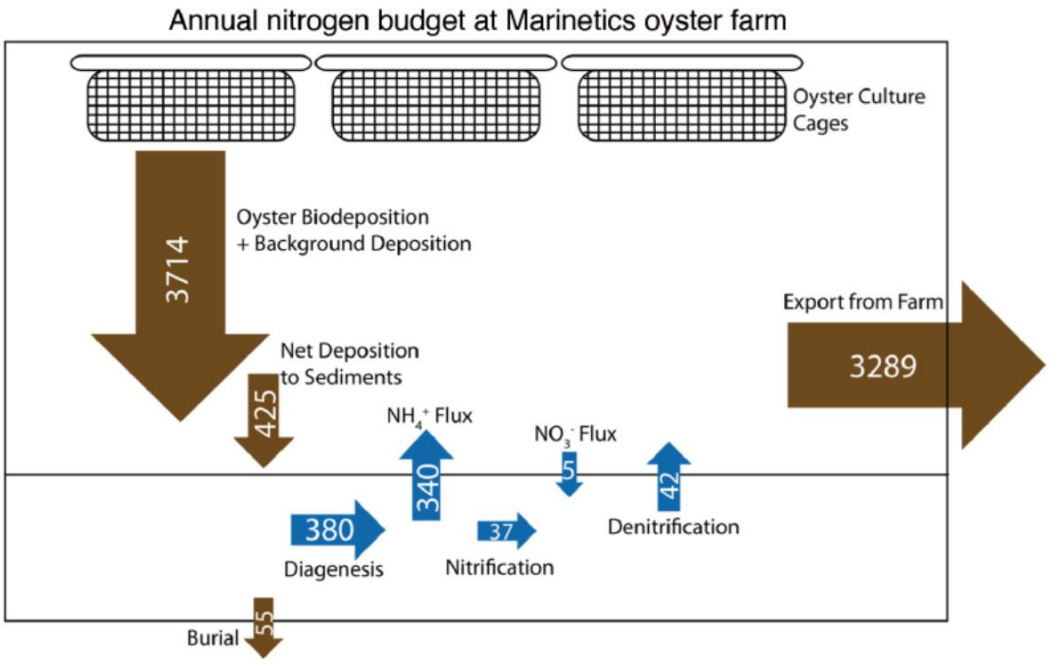

Figure I-1. Annual nitrogen balance at Marinetics oyster farm from Testa et al. (2015). All rates are µmol $m^{-2}h^{-1}$. These are modeled rates with a strong correspondence to measured rates.

I.1.2 On-Bottom Aquaculture and Licensed Oyster Harvest Studies

The Panel identified two studies that assessed denitrification rates on on-bottom aquaculture:

- **Humphries et al. (2016)** assessed denitrification rates on restored oyster reefs and on-bottom aquaculture areas in a shallow estuary in Rhode Island. The measurements were made with bottom chambers fixed to a ring inserted into the substrate and used the N_2:Ar approach. Restored oyster reefs and on-bottom oyster aquaculture both greatly increased the rates of denitrification relative to bare sediments and sediments only containing cultch. The aquaculture denitrification rates (mean ± S.E. 346 ± 169 µmol m^{-2} h^{-1}) were 14 times higher than rates on bare sediment.
- **Jackson et al. (2018, 2019)** assessed denitrification rates in sediment cores from an on-bottom oyster aquaculture lease that was planted with spat on shell in the Nanticoke River, MD. The N_2:Ar approach was used to measure denitrification. Rates were elevated in spring and fall relative to sediment that was not planted, with rates in the spring occurring at 4 times the rate on bare sediment (Jackson 2019). Since denitrification was measured in sediment cores rather than within the reef itself, rates may be conservative (Jackson et al. 2018).

Collectively, these studies suggest that on-bottom aquaculture enhances denitrification rates relative to un-treated sediments. The timing of sampling will be especially important as denitrification rates may vary based on when and how often oyster harvest and replenishment (planting shell or spat on shell) occur.

No studies were identified that assess denitrification rates on reefs in harvest areas.

I.2 Research Gaps and Considerations Associated with Enhanced Denitrification for Oyster Aquaculture and Licensed Oyster Harvest Practices

Through their literature review, the Panel concluded that estimates of enhanced denitrification cannot be made for practices where oysters are harvested (private oyster aquaculture, licensed oyster harvest) at this time because data is lacking that demonstrates an enhancement in denitrification and nitrogen reduction. The Panel also found that there is no research on the influence of harvest on denitrification rates.

Broadly, the Panel recommends additional studies that (1) better define temporal changes (over 1-2 years) in the biogeochemistry in areas that received spat on shell or shell additions and (2) examine post-harvest denitrification and nutrient balances. Control sites should be carefully selected to contain similar sediment characteristics as those beneath or around an aquaculture site or harvest area. Collecting baseline data on denitrification rates prior to the addition of shell or spat on shell would also be useful for estimating denitrification enhancement.

The following sections describe specific research needs and considerations for different culture types.

I.2.1 Off-Bottom Aquaculture Considerations

There is limited understanding on how denitrification associated with off-bottom oyster aquaculture results in a reduction of nutrients from the water column. Since oysters are near the surface, less disturbance of benthic sediments and biodeposits may occur which may reduce the likelihood that sequestered nitrogen is resuspended. However, the Panel agreed that oyster biodeposits are more likely to be swept away by water currents before reaching the seafloor than retained. It is possible that the export of biodeposits may result in off-site denitrification enhancements. The advection of biodeposits may also occur in oyster reef restoration settings but has not yet been assessed.

Specific questions for future research include:

- Can the proportion of off-site deposition and remineralization of oyster biodeposits be estimated?
- Can an estimate of oyster biodeposit export, denitrification rates, and denitrification efficiency in surrounding areas be used to estimate enhanced denitrification associated with oyster practices?
- What is the fate of the remineralized nitrogen within oyster floats and cages and does denitrification occur in close association with the oysters?

To assess the complete effects of off-bottom aquaculture on denitrification rates, study designs should consider areas beyond the lease site (Figure I-2). It is possible that net denitrification occurs if biodeposits are dispersed to the surrounding ecosystem. An understanding of all fates of seston is required to quantify the export of biodeposits. Specifically, studies should:

- Determine sediment denitrification and other N cycling processes in the aquaculture footprint, areas potentially subject to biodeposit deposition, and control areas (Process 3A, 3B in Figure I-2).

- Measurement of water column respiration and/or water column nitrogen remineralization (Process 4, Figure I-2).
- Biodeposit downward flux as a measure of biodeposit input to the ecosystem (Process 1A).
- Estimation of biodeposit export (Process 4A). This can be accomplished by direct measurement of biodeposit export using current meters and seston filtration; there are no studies published on this approach. Alternatively, modeling remineralization of N in the footprint to back-calculate net deposition/retention can result in an export calculation by difference (Process 1A minus the net deposition predicted by process 2A).
- An understanding of direct nutrient processing and release to the water column from the floats/bags may be valuable for determining the net water quality value.

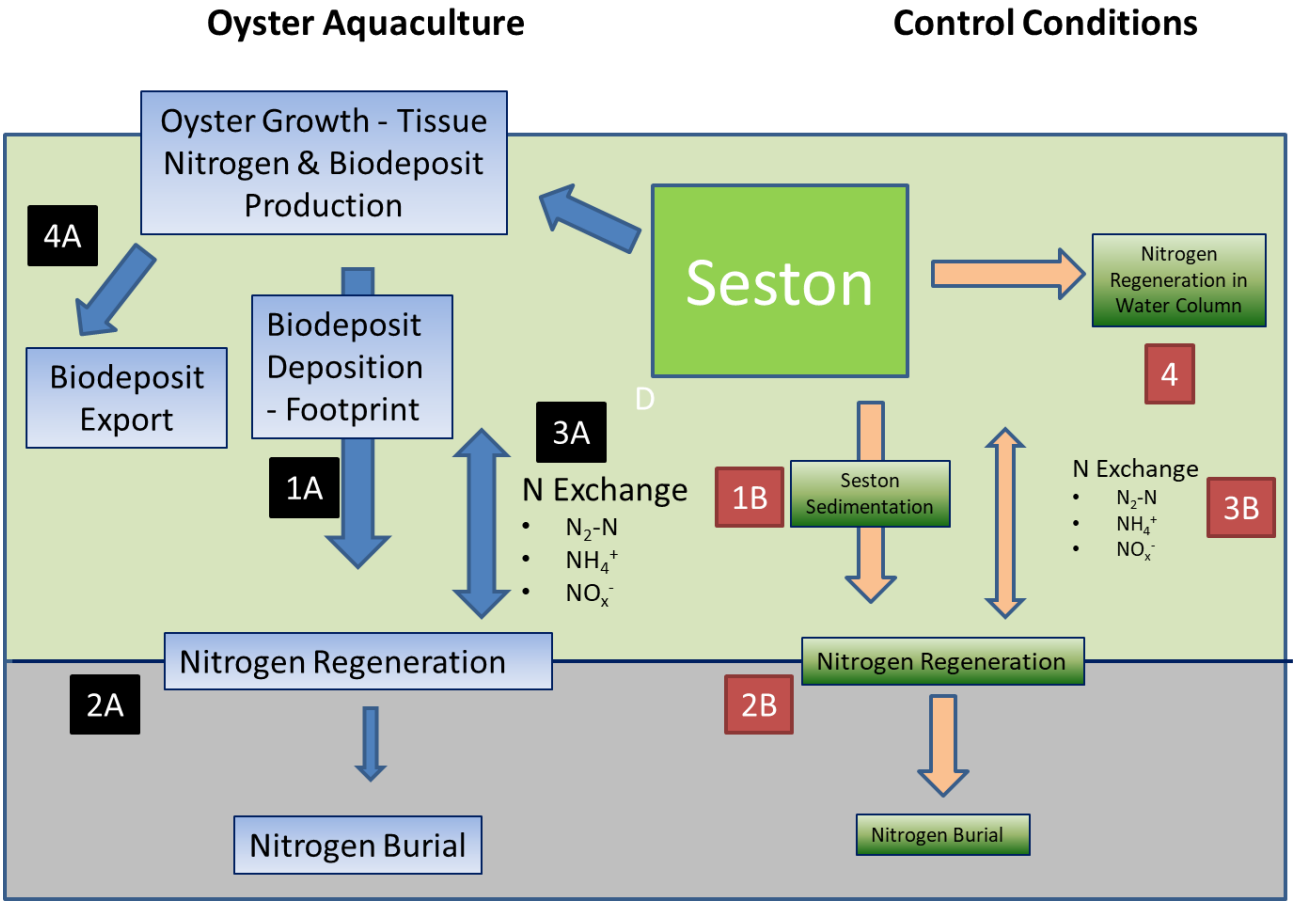

Figure I-2. Elements of measurement needed for estimating net biogeochemical nitrogen removal from the ecosystem. Prior to the addition of oysters, N is regenerated in the water column (4) and sediments (2B) after deposition (1B), leading to the exchange of dissolved N, leading to denitrification (N_2-N) and N burial. The addition of oysters increases the downward flux of N as biodeposits (1A, 4A), leading to N regeneration and production of N_2-N and other N species. The net value of denitrification from adding oysters is the denitrification that is observed in the footprint of the oyster float plus the denitrification of biodeposits exported elsewhere, minus the denitrification that would have occurred without oyster additions.

I.2.2 On-Bottom Aquaculture and Licensed Oyster Harvest Considerations

No information exists on the denitrification rates associated with on-bottom oyster aquaculture or in harvest areas. The act of harvesting oysters may disrupt biogeochemical processes with negative or positive consequences for microbial denitrification. For future studies, the timing of sampling will be important as denitrification rates may vary based on when and how often oysters are harvested or planted. For licensed oyster harvest, a time series of denitrification rates from the planting of spat on shell or shell through harvest, and for a period (1-2 years) after harvest would be useful for assessing the impact of harvest on denitrification. Since on-bottom aquaculture and licensed oyster harvest practices typically occur on reefs rather than in gear, approaches (e.g., Humphries et al. 2016) that sample the entire benthic community (sediments, oysters, other fouling organisms) may be useful for estimating realistic denitrification rates.

For on-bottom aquaculture practices, assessments of oyster biomass will be key for estimating enhanced denitrification. The Panel recommends that nitrogen reduction credit associated with denitrification for aquaculture practices only be allowed prior to harvest (Reichert-Nguyen et al. 2016).

Specific research questions include:

- How soon after oyster deployment does enhanced denitrification start and what is the short-term trajectory of denitrification as oysters mature?
- What is the effect of harvest on the denitrification process? Are initial benefits minimized by harvest-related disturbance?
- Are there minimum rates of enhanced denitrification that can be broadly applied to on-bottom aquaculture?
- Are biodeposits exported beyond oyster communities, resulting in off-site denitrification?

I.3 Denitrification Associated with Oyster Restoration Practices Using Large Substrates

Large structures for the creation of fish habitat and for shoreline protection can also enhance oysters in certain areas and under certain conditions. For example, concrete structures such as ReefBalls™ have been deployed by the Coastal Conservation Association (https://www.ccamd.org/living-reef-action-campaign/) and the Chesapeake Bay Foundation (https://www.baltimoresun.com/news/environment/bs-md-reef-balls-20170621-story.html) to enhance fish habitat. In several cases, post-deployment monitoring identified that oysters had settled on these structures. In some cases, concrete structures such as Oyster Castles®(inter-locking concrete blocks) have been inoculated with oyster spat prior to deployment (https://blog.nwf.org/2020/11/greening-the-grey-to-grow-more-oysters-in-the-bay/).

There are no published data on nutrient removal associated with oyster ReefBalls™ or Oyster Castles® but efforts are being made to determine their value in oyster-related denitrification. Preliminary results from unpublished research (Cornwell, Owens, Colden, Gray unpubl.) show elevated rates of denitrification on these large substrates relative to reefs restored with small substrates (Figure I-3). Additional measurements of denitrification rates in different settings would be needed to 1) suggest a standard methodology for measuring denitrification rates on these substrates and 2) identify whether rates are related to oyster biomass. In

addition, a comparison of the ex-situ approach used by Cornwell, Owen, Colden, Gray (unpubl.) to in-situ methodology would provide added information about scaling up incubation of individual units to deployments on larger structures.

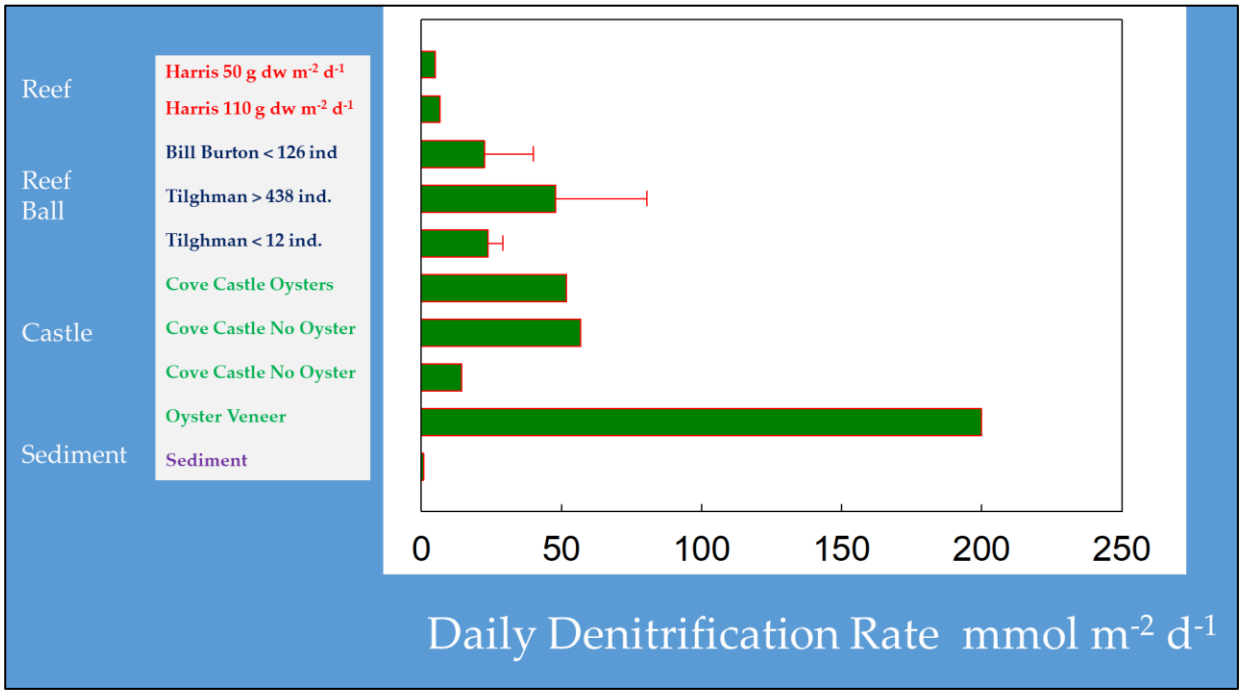

Figure I-3. Daily denitrification rate per unit area on different restoration substrates and in different locations in Chesapeake Bay. Harris = oyster reef restoration using hatchery produced oysters in Harris Creek, MD; Bill Burton and Tilghman = restoration using ReefBalls™ in Choptank River and Tilghman Island, MD; Cove = restoration using Oyster Castles© in Choptank River, MD; Oyster Veneer = restoration using Oyster Castles© and rock in Choptank River, MD; Sediment = no restoration, muddy bottom in Choptank River, MD. Data from Cornwell, Owens, Colden, Gray unpubl. data.

I.4 References

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Appendix J. Chesapeake Bay Program Modeling Integration on Assessment of Oyster Influence on Chesapeake Water Quality

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Suggested Citation:

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J.1 Introduction

Bivalve filter feeders were introduced in the Chesapeake Bay Model as part of the Tributary Refinements phase (Cerco et al. 2002). The initial representation included two freshwater species, *Corbicula flumenea* and *Rangia cuneata*, and one saltwater species, *Macoma balthica*. Subsequently, native oysters, *Crassostrea virginica*, were substituted for *Macoma* to investigate the potential impact of a tenfold increase in native oyster population (Cerco & Noel 2007). Oysters were included in the 2010 model version but received limited attention. Their activity was not explicitly incorporated into the 2010 TMDL. Oysters are the subject of renewed attention because of increases in the natural population in sanctuaries and the tremendous growth of the aquaculture industry. Nutrient removal credits associated with oyster restoration and with aquaculture will be included in future nutrient management plans. Renewed management attention demands a corresponding renewal of the oyster module in the 2017 Chesapeake Bay Water Quality and Sediment Transport Model (WQSTM). Representation of the freshwater bivalves is unchanged from the previous model version (Cerco & Noel 2010).

J.2 Oyster Model Basics

The revised oyster module considers four populations. The influence of the four populations on Chesapeake water quality are made by an integrated approach by the Oyster BMP Expert Panel and the WQSTM so that double counting of the influence is avoided. The populations are:

- Natural populations on public reefs and subject to harvest – simulated by the WQSTM.
- Natural populations in sanctuaries and not subject to harvest – simulated by the WQSTM with oyster harvest mortality set to zero.
- Natural populations in sanctuaries, not subject to harvest, and with rebuilt/enhanced oyster habitat– estimated by Oyster BMP Expert Panel procedures and approaches.
- Aquaculture operations - estimated by Oyster BMP Expert Panel procedures and approaches for nutrients in soft tissue harvested and by the WQSTM for biogeochemical processes resulting from oyster aquaculture biomass.

Application of the WQSTM to each population requires resolution of the following issues:

- Location

- Biomass
- Model parameterization

J.2.1 Mass-Balance Equation

The fundamental mass-balance equation for the filter feeders is shown in equation 1:

$$\frac{dO}{dt} = \propto \cdot Fr \cdot POC \cdot IF \cdot (1 - RF) \cdot O - BM \cdot O - \beta \cdot O - H \cdot O \quad (1)$$

where:

O = oyster density (g C m^{-2})
α = assimilation efficiency ($0 < \alpha < 1$)
Fr = filtration rate (m^3 g^{-1} C d^{-1})
POC = particulate organic carbon (g m^{-3})
IF = ingestion fraction ($0 < IF < 1$)
RF = respiration fraction ($0 < RF < 1$)
BM = basal metabolism (d^{-1})
β = mortality (d^{-1})
H = harvest rate (d^{-1})

Parameter values in the governing equation are largely as described by Cerco & Noel (2007).

Oyster reefs occupy small fractions of model computational cells, which average 1 km by 1 km in extent. The "foraging arena" concept was introduced in the model to represent the limited encounters between predators and prey induced by the small fraction of each computational cell occupied by reefs (Cerco & Noel 2010). We found, however, that the computed biomass of oysters, in g C, was excessive when the computed density, in g C m^{-2}, was multiplied by the cell area. We also found that the potential impact of oysters on prey was exaggerated despite the foraging arena. Consequently, the concept of "coverage" is introduced in the WQSTM. Coverage is the fraction of cell area occupied by oyster reefs. Biomass is computed as the product of density, cell area, and fraction of cell covered by reefs. Corrections for coverage are also introduced into the mass-balance equations for mass transfers between oysters and their surroundings.

J.2.2 Modifications for Aquaculture

Oyster density in each cell, as computed by equation 1, varies spatially and temporally depending on local conditions. Aquaculture operations, including year-round planting and harvesting, tend to reduce the intraannual and interannual oscillations that occur in natural oyster beds. The spatial distribution of oyster biomass depends on the location of aquaculture operations. Water quality managers wish to explore the impacts of varying levels of aquaculture activity, which has led to a model representation in which oyster density in each cell is a specified constant value. Setting dO/dt = 0 in equation 1 leads to the representation in equation 2:

$$IF = \frac{BM + \beta + H}{\alpha \cdot Fr \cdot POC \cdot (1 - RF)} \quad (2)$$

The ingestion fraction becomes a variable rather than a parameter, as in equation 1. Employment of the variable ingestion fraction in the balance of the model formulations results in a constant oyster density, which is specified at model initiation.

In the event the rate of biomass loss (represented in the numerator on the right-hand side of equation 1) exceeds food intake (represented in the denominator), the computed ingestion fraction will exceed unity. That situation is physically impossible, although the model will operate under those conditions. Consistent computation of an ingestion fraction greater than unity indicates that oysters cannot persist at the specified density under modelled conditions. Either sufficient food resources are unavailable or losses from respiration, mortality, and harvest exceed sustainable levels.

J.3 Location

J.3.1 Natural Population

More than 8,000 oyster bars were located as part of a 2008 study of oyster restoration alternatives (MD DNR 2008). Bar locations were mapped to the model grid and consolidated by cell (Figure J-1). The total bar area in each cell was employed to compute coverage (see section J.2, *Oyster Model Basics*). Oyster bars occurred in 2,068 of the 11,064 model surface cells. Coverage for those 2,068 cells ranged from less than 0.01 percent to 100 percent. The median coverage was 5 percent and was less than 10 percent for the vast majority of cells with oyster bars.

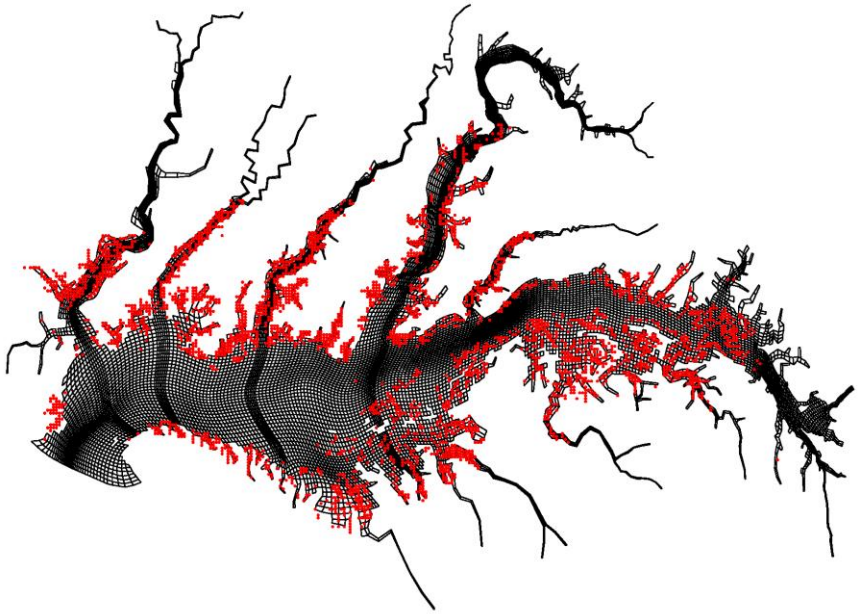

Figure J-1. Location of natural oyster bars mapped to model grid.

J.3.2 Sanctuaries

Locations of oyster sanctuaries in Maryland were obtained by the project sponsor and mapped to the model grid. Considerable overlap occurred between the location of reefs determined in 2008 and the present location of sanctuaries. In the event a natural bar and a sanctuary were coincident, we assumed the bar is presently a sanctuary.

J.3.3 Aquaculture

Locating aquaculture operations presented a considerable challenge. Although various state agencies have that information, they cannot release anything considered proprietary. For the state of Maryland, we were provided with the aquaculture harvest totals by county for the years 2014–2016 collected by Maryland Department of Natural Resources (J. Reichert-Nguyen, Oyster Recovery Partnership, pers. comm., December 21, 2016). We created a map of potential model aquaculture cells within those counties by assuming aquaculture is restricted to water less than 12 feet deep and with salinity greater than 7 parts per thousand (ppt) (Figure J-2). The depth constraint was based on assumptions regarding accessibility. The salinity constraint was determined by Cerco & Noel (2007) as the minimum required for a healthy natural population.

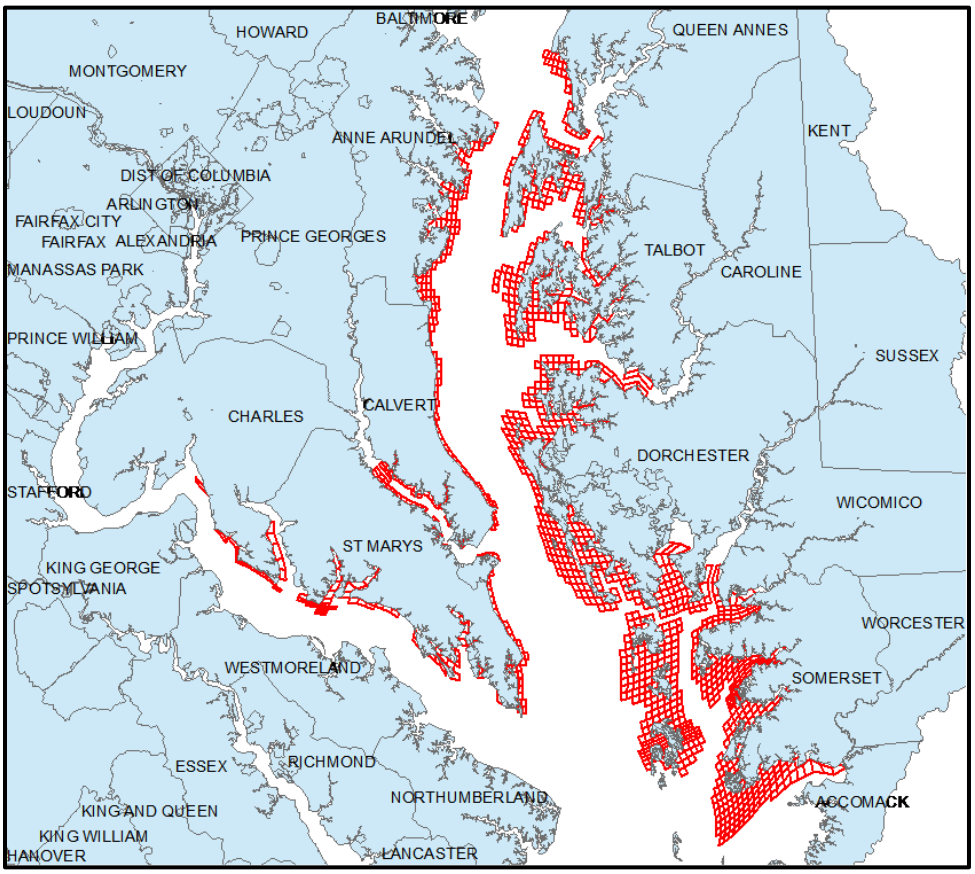

Figure J-2. Potential aquaculture cells in Maryland. Criteria are depth ≤ 12 feet and salinity > 7 ppt.

A geographic information system file was available that mapped private lease areas in Virginia. That map was superimposed on the model grid to indicate cells that contain leases (Figure J-3). Potential aquaculture cells were then limited to lease areas less than 12 feet deep and with greater than 7 ppt salinity.

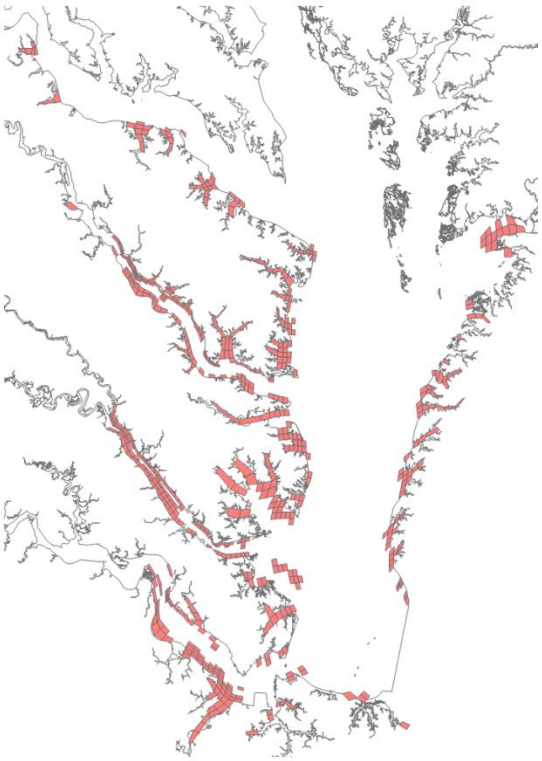

Figure J-3. Potential aquaculture cells in Virginia. Cells shown include private lease areas and meet the criteria depth ≤ 12 feet and salinity > 7 ppt.

As noted in, Section J.2 *Oyster Model Basics*, it is possible to assign aquaculture to a cell that cannot support the specified level of activity. We minimized this possibility through a "self-locating" process. An exploratory model run was conducted in which oysters were assigned to all potential aquaculture cells. They were modelled as a natural population that was allowed to thrive or perish according to ambient conditions. We restricted aquaculture cells to those that supported a density of 10 mg C m^{-2} (Figure J-4).

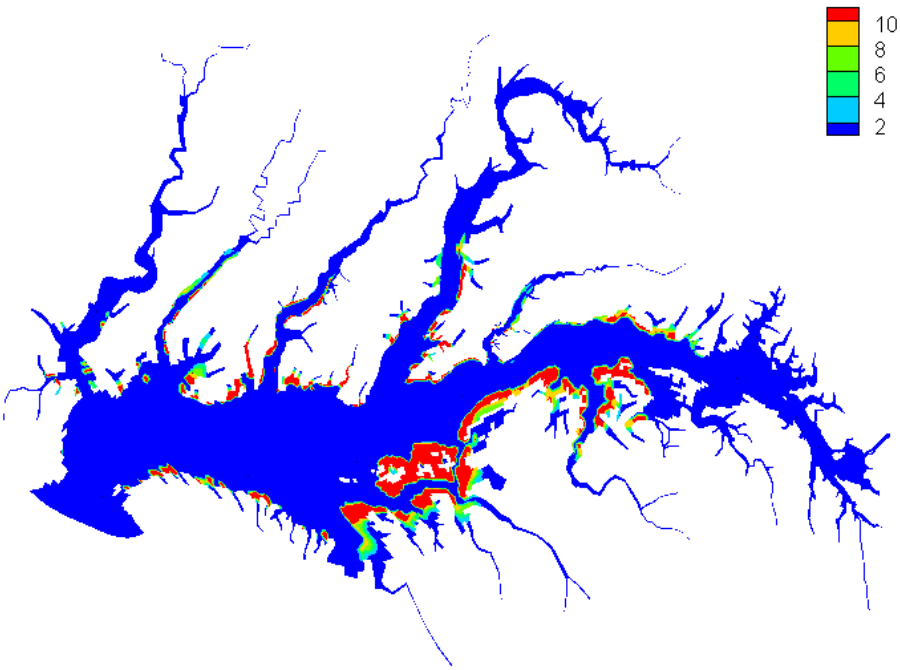

Figure J-4. Self-location of aquaculture cells. Aquaculture is restricted to areas capable of supporting a density of 10 mg C m^{-2}.

J.4 Biomass

J.4.1 Natural Population and Sanctuaries

The primary data source for the population on oyster bars is the website for the Chesapeake Bay Oyster Population Estimate (CBOPE) project, which is maintained by the Virginia Institute of Marine Science (VIMS) (VIMS 2017). The CBOPE project was conducted to monitor progress towards a tenfold increase in Chesapeake Bay oyster population called for in the Chesapeake 2000 Agreement. The site reports various categories of standing stock and harvest for Virginia (1994–2008) and Maryland (1994–2002). The state totals are reported for various basins within each state in various years. Major population categories include the following:

- Fishery-Independent Data—Collected during annual patent tong surveys in Virginia and annual dredge surveys in Maryland.
- Fishery-Dependent Data–Public/Commercial—Based on annual oyster landings reported to the Virginia Marine Resources Commission (VMRC) and the Maryland Department of Natural Resources (MDNR).
- Fishery-Dependent Data–Private Fishery—Based on reports by private leaseholders to VMRC and MDNR.

The total population in each state was considered to be the sum of the fishery-independent data plus the amount removed in public and private landings (Table J-1). For Virginia, the private landings were adjusted to remove aquaculture activities from 2005 onward. The landings, adjusted for aquaculture, were tracked separately to assist in parameter assignment of the harvest rate in equation 1.

Table J-1. Reef biomass and harvest

Year	VA Biomass (kg DW)	VA Harvest (kg DW)	VA Harvested Fraction	MD Biomass (kg DW)	MD Harvest (kg DW)	MD Harvested Fraction
1994	512560	23548	0.046	411614	21614	0.053
1995	511522	7519	0.015	512930	51930	0.101
1996	681933	9923	0.015	561680	70680	0.126
1997	471609	8606	0.018	631470	68470	0.108
1998	581486	20475	0.035	721221	122221	0.169
1999	582623	9615	0.017	736000	147000	0.200
2000	657979	9753	0.015	720555	129555	0.180
2001	698260	13246	0.019	698568	138568	0.198
2002	561166	18215	0.032	184000	40000	0.217
2003	575272	9997	0.017			
2004	734962	33864	0.046			
2005	993351	71780	0.072			
2006	819680	37747	0.046			
2007	651726	29950	0.046			
2008	1039207	28039	0.027			

J.4.1.1 Assignment to Basins

CBOPE reporting by basins was sporadic and the state data could not be reliably split by basin over the reporting period. Based on alternative data sources, 13 basins were defined (Table J-2). The Virginia basins were defined to coincide with harvest data provided by the VMRC (J. Wesson, department head, Conservation and Replenishment, pers. comm., December 30, 2016). The Virginia population was split into basins in proportion to the total public harvest taken in each basin. The Maryland basins were defined to coincide with a 1994–2006 population estimate (Greenhawk & O'Connell 2007). The Maryland population was split into basins according to the proportions in the estimate.

Table J-2. Basin fractions of total reef biomass

VA Basin	Fraction	MD Basin	Fraction
Chesapeake	0.294	Chester	0.151
James	0.354	Eastern Bay	0.076
York	0.082	Choptank	0.118
Rappahannock	0.262	Little Choptank	0.026
Potomac	0.007	Tangier Sound	0.136
		Potomac	0.074
		Patuxent	0.037
		Chesapeake	0.371

J.4.2 Aquaculture

The aquaculture biomass was difficult to estimate because of the proprietary nature of the data on operations. In addition, necessary information was obtained through personal communication and sources were not always in agreement. The original source for Virginia aquaculture biomass was a summary of surveys conducted by VIMS (Hudson & Murray 2016). The surveys reflect the number of oysters sold through Virginia aquaculture operations for the years 2005–2015. The surveys risk underreporting the sales because of a lack of response from some operators. Alternatively, the surveys risk overestimating the sales since operations on the Atlantic side of the Delmarva Peninsula are included. Nevertheless, the survey report is the primary citable source for Virginia aquaculture data.

The number of oysters sold was converted to dry tissue weight using the factor for market-size oysters of 2.1 g DW per oyster (Cerco & Noel 2007). Converting the harvest to standing stock required consideration of aquaculture practices and grow-out period from seed to harvest. Aquaculture practices can be broadly divided into "cage culture" and "bottom culture." We were advised that roughly 80 percent of aquaculture in Virginia is conducted in cages and 20 percent is conducted on bottom. We were further advised that the grow-out period for cage culture is 2 years while the grow-out period for bottom culture is 3 years (M. Parker, University of Maryland Extension, pers. comm., February 16, 2017). Assuming linear growth and continuous planting and harvest, the standing stock of oysters in cages is 1.5 times the annual harvest. The standing stock of oysters on bottom is twice the annual harvest. Combining these factors indicates the biomass of aquaculture oysters in Virginia is 1.6 times the annual harvest (Table J-3).

Table J-3. Aquaculture biomass and harvest

Year	VA Biomass (kg DW)	VA Harvest (kg DW)	MD Biomass (kg DW)	MD Harvest (kg DW)
2005	3398	2124		
2006	11892	7433		
2007	16989	10618		
2008	25483	15927		
2009	32279	20174		
2010	56063	35039		
2011	79847	49904		
2012	93438	58399		
2013	103631	64770		
2014	134211	83882	40905	21529
2015	118921	74326	60612	31901
2016			64550	33974
2025	508032	317520	241315	127008

Note: Data for the year 2025 are projections employed in management scenarios as detailed in subsection J.6.1.1 *Estimate of Aquaculture Activity through 2025*.

Data for Maryland aquaculture originated with the MDNR and was provided through the Oyster Recovery Partnership (J. Reichert-Nguyen, Oyster Recovery Partnership, pers. comm., December 21, 2016). The original data consisted of the number of bushels harvested for the years 2014–2016. Statewide totals as well as data

for some counties were provided. Bushels were converted to number of oysters using the factor of 300 oysters per bushel provided along with the data. The number of oysters was subsequently converted to dry tissue weight using the factor of 2.1 g DW per oyster for market-size oysters (Cerco & Noel 2007). We were advised that, in Maryland, roughly 80 percent of aquaculture is conducted on the bottom while 20 percent is conducted in cages. Those proportions are the inverse of operations in Virginia. Using the grow-out periods quoted previously, the Maryland aquaculture standing stock is 1.9 times the annual harvest (Table J-3).

J.4.2.1 Assignment to Basins

Data on private landings for major basins in Virginia were provided by the VMRC (J. Wesson, department head, Conservation and Replenishment, pers. comm., December 30, 2016). The Virginia aquaculture biomass was assigned to basins according to the fraction of the total private landings in each basin (Table J-4).

Maryland aquaculture biomass was assigned to counties in proportion to the fraction of the total harvest represented by each county (Table J-4). Data were not available for all individual counties, however, so fractions were assigned to those counties according to surface area.

Table J-4. Basin fractions of aquaculture biomass

VA Basin	Fraction	MD Basin	Fraction
Chesapeake	0.293	Anne Arundel	0.022
James	0.360	Calvert	0.030
York	0.128	Dorchester	0.475
Rappahannock	0.050	St. Mary's	0.215
Potomac	0.170	Somerset	0.025
		Talbot	0.072
		Wicomico	0.162

J.5 Model Parameterization

J.5.1 Model Calibration to Reef Population

The fundamental parameter values for the oyster module are adapted from the 2005 study of the impact of a tenfold increase in natural oyster population (Cerco & Noel 2007). Values of two parameters, mortality and harvest (equation 1), are newly assigned in the 2017 model to match current biomass data. First the harvest is assigned to calculate values representative of data, then the mortality is assigned to obtain representative biomasses. Harvest values range from 1.23×10^{-4} to 6.75×10^{-4} d^{-1} in the months from October through April. Harvest is zero otherwise. The seasonal assignment reflects that harvest from natural reefs is minimal during spawning season. Mortality ranges from 0.025 to 0.05 d^{-1} in the months from June to October. Mortality is zero otherwise. The seasonal assignment reflects the influence of temperature on predators and disease organisms.

The reef biomass data reflect annual surveys (fishery-independent data) combined with annual summaries of oyster landings (fishery-dependent data). They are compared to annual-average biomass computed by the model. Comparison of computations and observations (e.g., Figures J-5 and J-6) indicates the model largely reflects the regional biomasses, although interannual variations in the observations are not reproduced.

The correlation (R^2) between computed annual-average biomass in Maryland basins and observed biomass is 0.62 and is highly significant ($p < 0.01$) (Figure J-7). The correlation between computed and observed biomass in Virginia is lower ($R^2 = 0.47$) but remains significant nonetheless (Figure J-8).

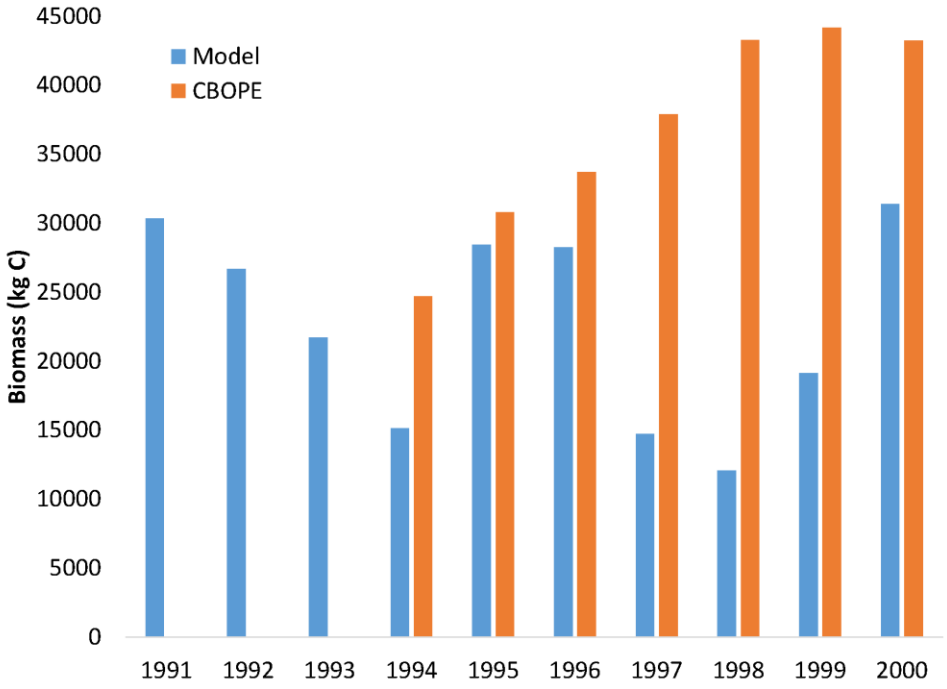

Figure J-5. Computed (annual average) and observed oyster biomass in the Choptank River, MD.

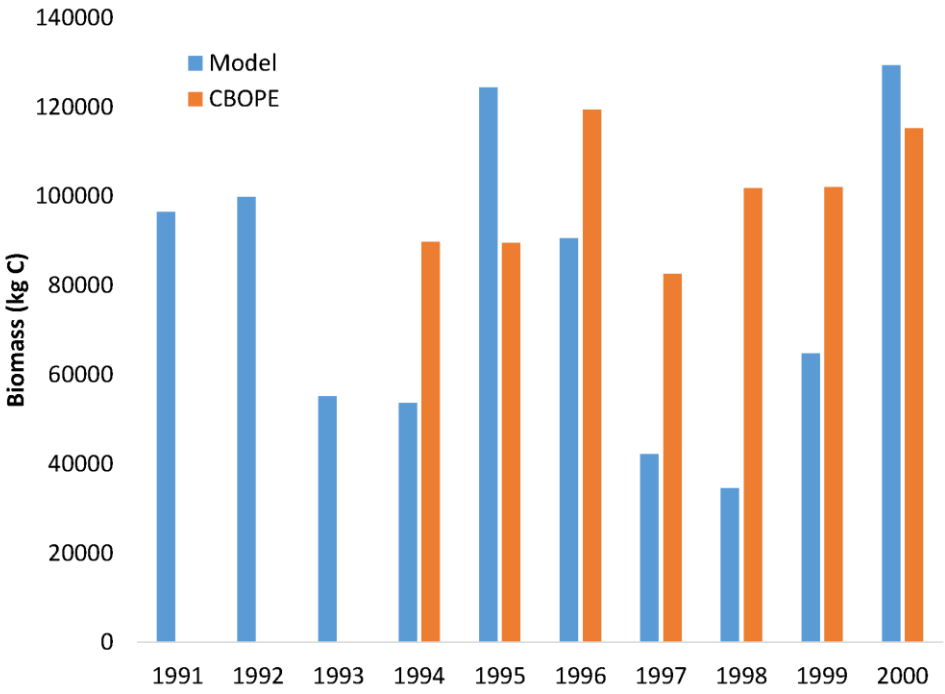

Figure J-6. Computed (annual average) and observed oyster biomass in the James River, VA.

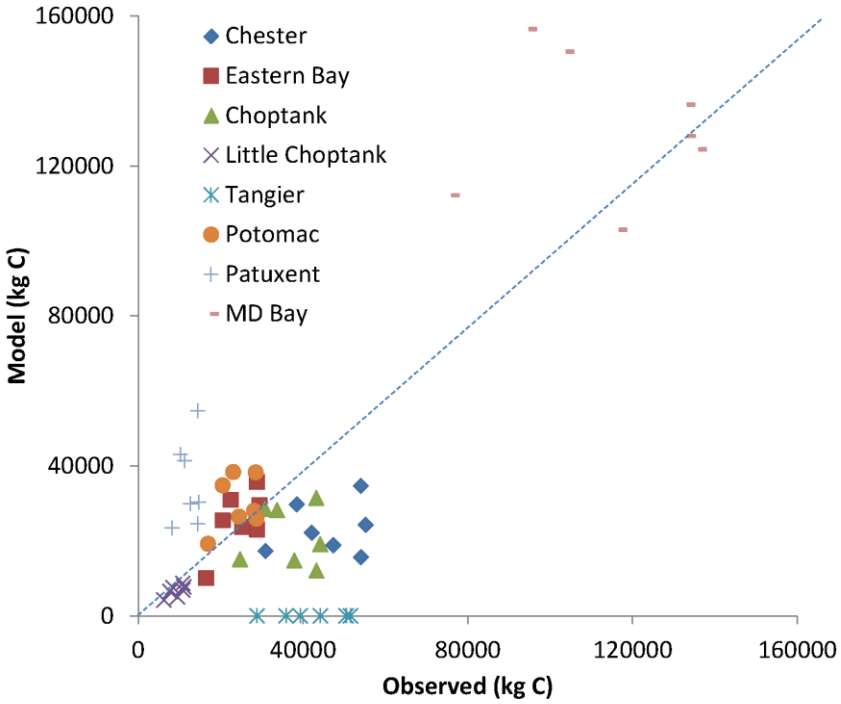

Figure J-7. Computed vs. observed biomass for Maryland basins designated in Table J-2. Computed values are annual averages for 1994–2000. Observations are derived from CBOPE.

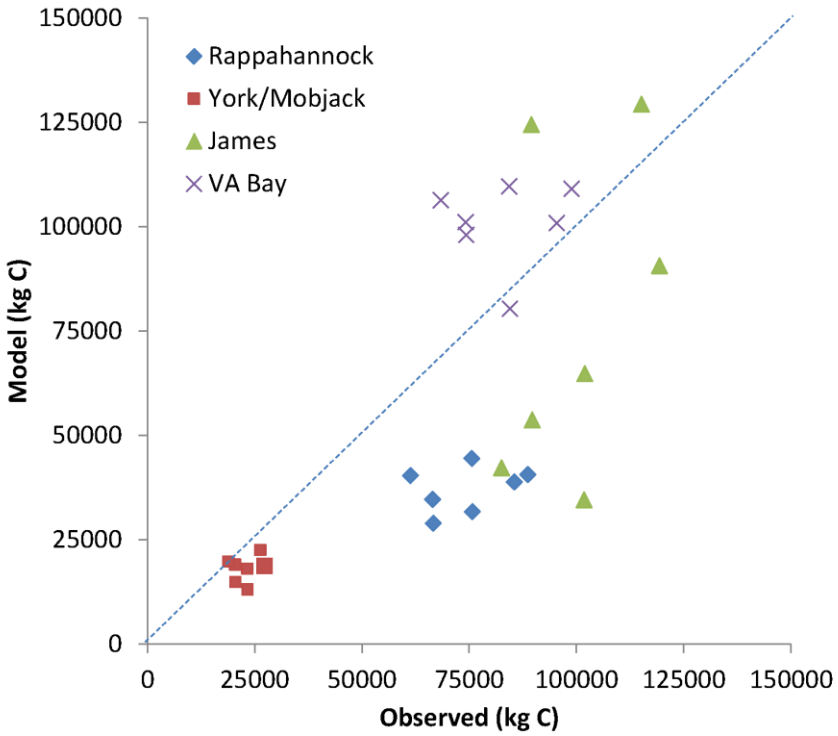

Figure J-8. Computed vs. observed biomass for Virginia basins designated in Table J-2. Computed values are annual averages for 1994–2000. Observations are derived from CBOPE.

J.6 Management Considerations

J.6.1 Oyster Aquaculture

The Virginia aquaculture biomass was negligible, compared to the reef biomass, through the WQSTM calibration and verification years, 1991–2000 and 2002–2011 (Figure J-9). Aquaculture in Maryland was nonexistent during those periods. Consequently, the aquaculture feature of the oyster module was not implemented in the calibration or verification periods spanning 1991 to 2011. Aquaculture activities are growing rapidly, however, in both Virginia (Figure J-9) and Maryland (Figure J-10) and nutrient removal through aquaculture is now considered a best management practice (Reichert-Nguyen et al. 2016). Consequently, aquaculture is implemented in various Chesapeake Bay Model scenarios for 2025 conditions.

J.6.1.1 Estimate of Aquaculture Activity through 2025

We were provided with 2025 projections of aquaculture activity by state (O. Devereux, Devereux Consulting, pers. comm., December 8, 2017). Data included the number of oysters harvested and the nitrogen and phosphorus content of individual oyster soft tissue. Since the WQSTM quantifies oysters as carbon, the total nitrogen removed was multiplied by the WQSTM oyster carbon-to-nitrogen ratio of 6 g C g^{-1} N to convert the projected harvest to model carbon units. Harvest was converted to standing-stock biomass as described in subsection, *Aquaculture*, under section, *Biomass*.

The aquaculture biomass obtained from the harvest was distributed to model cells capable of supporting aquaculture in each state. The projected harvest and biomass were subsequently converted to dry weight for

comparison with previously computed values for the years 2005–2016 (Table J-3). The 2025 projections are much higher than the most recent data but are consistent with extrapolations from present trends.

The 2025 "full buildout" oyster aquaculture estimates are the approximate maximum biomass of Maryland aquaculture oysters because of constraints in available shallow waters of suitable salinities and water quality. For scenario years of 2025 and beyond, the 2025 full buildout biomass estimates are used. For Progress Scenario years before 2025, Virginia and Maryland provide the annual estimates of aquaculture oyster harvest through the Chesapeake Assessment and Scenario Tool (CAST) (CBPO 2017), which is then used to represent the influence of aquaculture on water quality.

J.6.1.2 Implementing Aquaculture in the WQSTM

CAST operates by reducing the watershed loads to appropriate cells of the WQSTM to account for nutrient removal by harvest and consumption of oyster soft tissue (Reichert-Nguyen et al. 2016). (Nitrogen and phosphorus content of harvested shells are uncounted and assumed to be net zero because shells from oyster aquaculture are commonly collected and replanted on aquaculture grounds with new oyster spat.) Therefore, in the WQSTM, the harvest of aquaculture oysters is specified as zero (equations 1 and 2) to prevent "double counting" of nutrient removal in both watershed loads and through algorithms in the oyster module. However, oyster functions of particle filtration and nutrient recycling to the water and sediments remain in operation for simulation of oyster aquaculture. Consequently, aquaculture in scenarios provides potential benefits in water clarity and enhanced nutrient burial and denitrification as well as the reductions of oyster soft tissue nitrogen and phosphorus represented in CAST as per the guidance of the Oyster BMP Expert Panel.

J.6.1.3 Sensitivity Scenario

The overall influence of oyster aquaculture at 2025 full buildout biomass is estimated to increase spring and summer bottom dissolved oxygen (DO) by more than 0.05 mg/l in CB4MH and CB5MH segments (Figure J-11) (Modeling Workgroup 2018). Improvement in bottom DO from the WQSTM oyster simulated processes of particle filtration and nutrient cycling is more than twice that of nutrient removal by oyster aquaculture harvest alone.

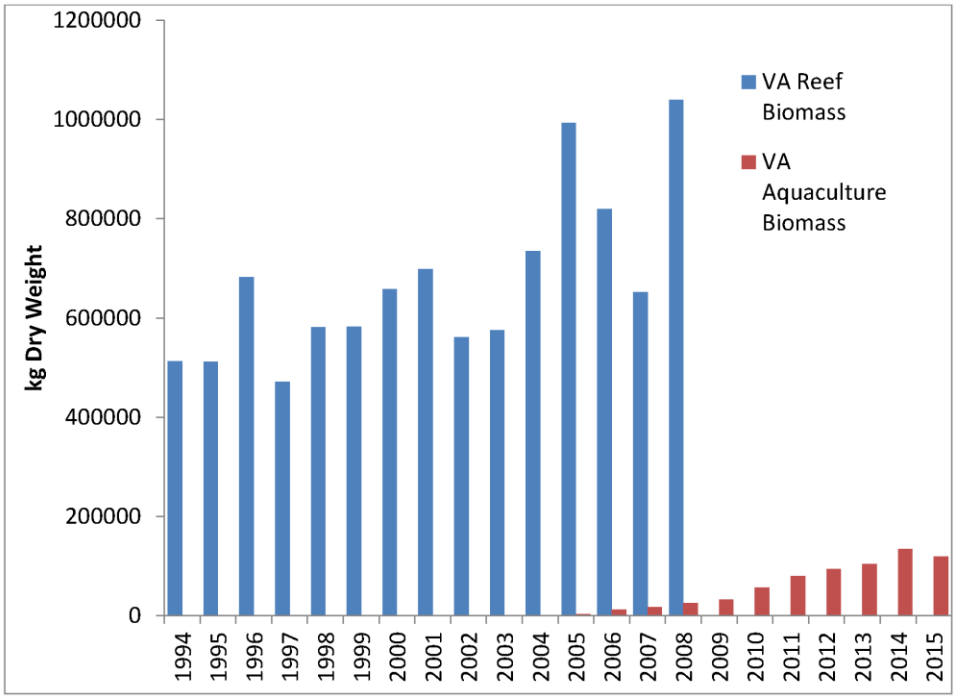

Figure J-9. Virginia natural reef and aquaculture biomass 1994–2015.

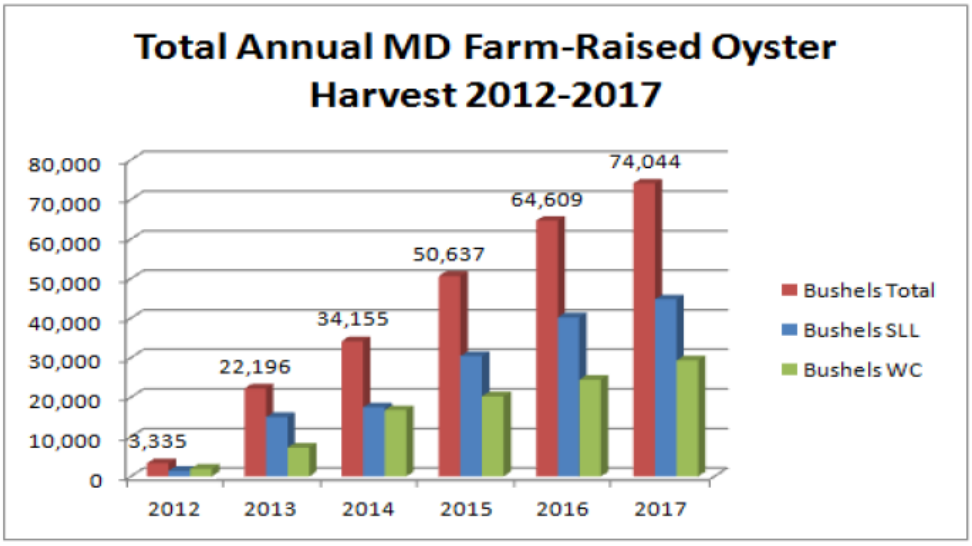

Figure J-10. Annual harvest of aquaculture (farm-raised) oysters in Maryland 2012–2017. SLL = submerged land lease; WC = water column lease (*Source*: Roscher 2019)

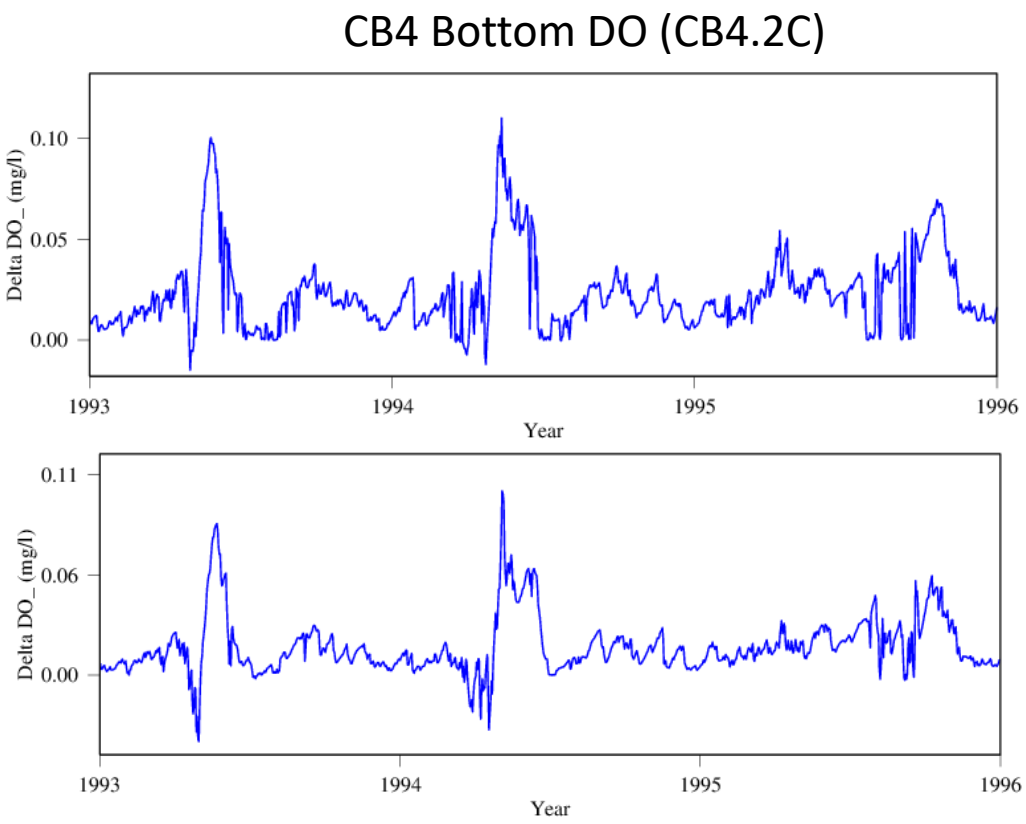

Figure J-11. Influence of oyster aquaculture at 2025 full buildout biomass on bottom cell DO in CB4MH and CB5MH. Delta DO > 0 indicates an increase in DO (*Source*: Modeling Workgroup 2018).

J.7 Nutrient Credits for Oyster Habitat Restoration

By 2025, 10 areas of the Chesapeake Bay will have an extensively restored bottom along with oyster spat planting. The 10 areas of existing or planned specific, large, and intensive restoration of oyster habitat are shown in Figure J-12. Nutrient removal associated with restoration in those areas is equated to load reductions in CAST (CBPO 2019). The credits for restoration are 81 pounds of nitrogen and 4 pounds of phosphorus per acre of restored oyster sanctuary habitat (CBPO 2019). The credits account for nutrient assimilation into oyster tissue and enhanced sediment denitrification. To avoid double counting of nutrient removal in the oyster module and in CAST, the oyster module is disabled in the areas for which specific information on restoration site size and location is available.

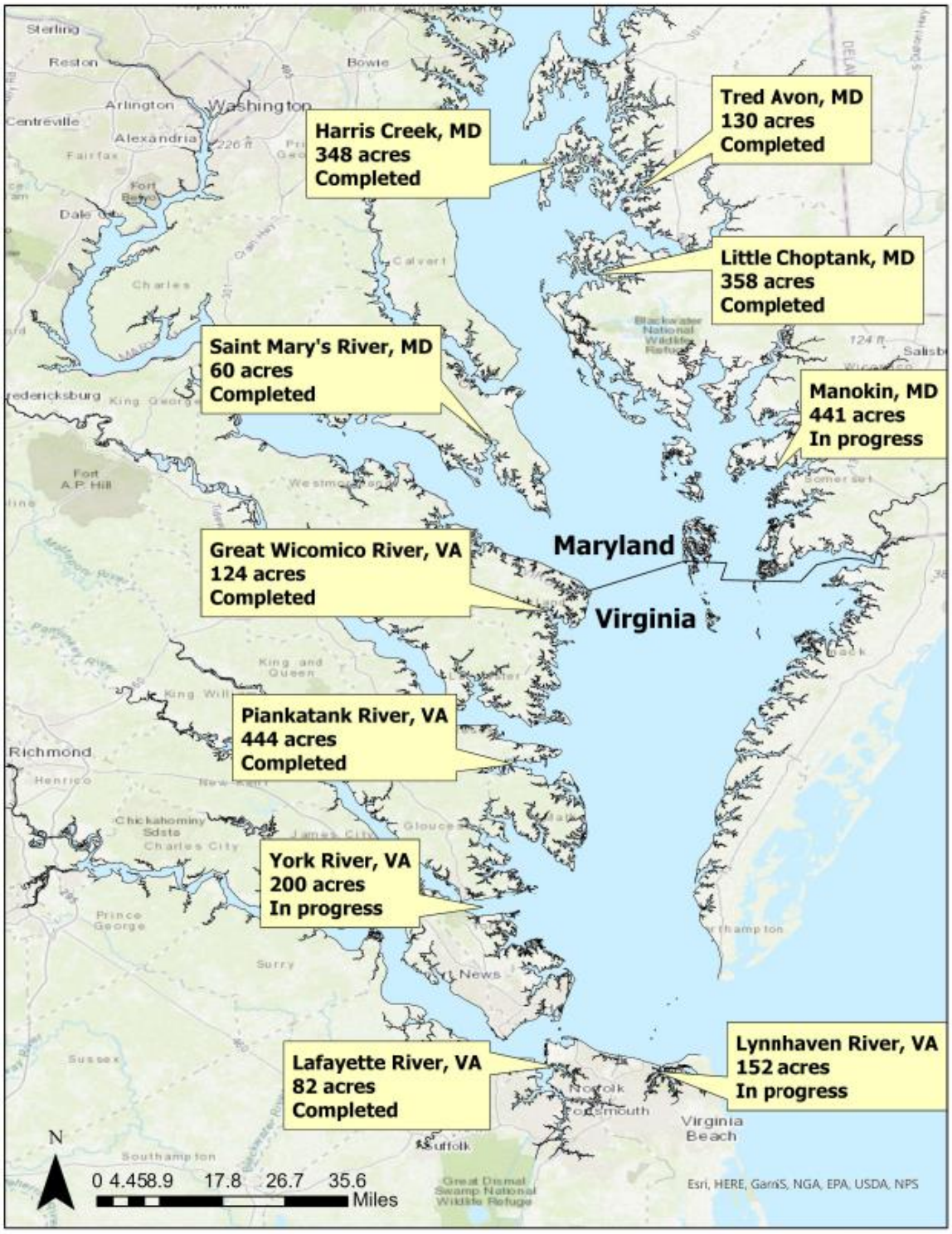

Figure J-12. Location of 10 areas of large-scale oyster habitat restoration to be completed by 2025. Restoration status as of September 2022.

J.8 References

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Appendix K. Technical Requirements for Reporting and Simulating Oyster BMPs in the Phase 6 Watershed Model

[Draft text in a separate document – will be released during review period]