Panel Recommendations on the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework and Nitrogen and Phosphorus Assimilation in Oyster Tissue Reduction Effectiveness for Oyster Aquaculture Practices

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Oyster BMP Expert Panel First Incremental Report

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Chesapeake Bay Program (CBP) Partnership Review:

Approved by Watershed Technical Work Group (WTWG):

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Acronyms in Document

BMP- Best Management Practice

CBP- Chesapeake Bay Program

TMDL- Total Maximum Daily Load

STAC- Scientific and Technical Advisory Committee

GIT- Goal Implementation Team

WQGIT- Water Quality Goal Implementation Team

UMCES- University of Maryland Center for Environmental Science

N- Nitrogen

NOAA- National Oceanographic and Atmospheric Administration

VIMS- Virginia Institute of Marine Science

TNC- The Nature Conservancy

CBF- Chesapeake Bay Foundation

EPA- Environmental Protection Agency

P- Phosphorus

PVC- Polyvinyl chloride

FARM model- Farm Aquaculture Resource Management

AFDW- Ash Free Dry Weight

DW- Dry Weight

WW- Wet Weight

SH- Shell Height

Key Definitions

Assimilation: The process where oysters incorporate the nitrogen and phosphorus from consumed algae into their tissue and shell.

Biodeposition: Organic matter (e.g., feces and pseudofeces from oysters) that is deposited on the bottom.

Burial: The process in which nutrients are trapped in the bottom sediment for long timescales.

Denitrification: The process that reduces nitrates or nitrites from organic matter to nitrogen gas, commonly by bacteria in the bottom sediment. Nitrogen gas ultimately escapes into the atmosphere.

Diploid oyster: Oysters containing two complete sets of chromosomes, one from each parent.

Hatchery-produced oyster: Oysters propagated outside their natural environment in private or State-run hatcheries.

Oyster aquaculture: Growing and harvesting oysters by the means of State-managed private leases.

Oyster sanctuary: Closed off area where harvest is prohibited in order to allow oyster populations to recover.

Oyster shell height: The longest distance (parallel to the long axis) between the hinge and lip of the oyster.

Public fishery: Managed fishery that is open to public fishing.

Quantile regression: Type of regression analysis that aims to estimate the conditional median or other quantiles of the response variable.

Spat-on-shell planting: Oyster larvae that have settled (attached) onto shell and have been placed in the water.

Substrate addition: The act of placing oyster substrate (e.g., shell, rock, concrete) on the bottom to enhance the recruitment of wild oyster larvae.

Suspended sediment: Sediment floating in the water column.

Triploid oyster: Oysters containing three homologous sets of chromosomes, typically a result of hybridizing a 2-set chromosome individual with a 4-set chromosome individual.

Wild oyster: Oysters that originated from their natural environment.

1.0 Introduction

Federal and State governments are investing millions of dollars annually in rebuilding the Chesapeake Bay's Eastern oyster (*Crassostrea virginica*) population for ecological benefits while concurrently building a robust oyster aquaculture industry. With scientific research demonstrating that oysters can contribute to the reduction of nutrients (nitrogen and phosphorus) and suspended sediment from the water column (Kellogg et al. 2013 and 2014a, Grizzle et al. 2008), there is growing interest in recognizing oyster practices as best management practices (BMPs) and crediting their nutrient and suspended sediment reduction effectiveness in the Chesapeake Bay Program (CBP) Partnership's model framework,[1] a tool used to assess whether appropriate progress towards water quality goals, established by the Total Maximum Daily Load (TMDL), is being made for the Chesapeake Bay (U.S. EPA 2010). As a result, the Chesapeake Bay Program (CBP) Partnership requested that an Oyster BMP Expert Panel be convened to develop recommendations for 1) a decision framework to determine the nutrient and suspended sediment reduction effectiveness of oyster practices as BMPs for application in the CBP Partnership's model framework and 2) the nitrogen, phosphorus, and suspended sediment reduction effectiveness of oyster practices based on existing science. The Oyster BMP Expert Panel (hereafter, "Panel") convened on September 30, 2015, has met monthly for a total of 15 meetings to date (see Appendix A for summary of the Panel's meetings and other activities and Appendix E for meeting minutes) and has had numerous e-mail and phone conversations to develop the recommendations found in this incremental report.

Oysters consume algae and other organic matter from the water column through filter feeding. A portion of the nutrients within that organic matter, including nitrogen (N) and phosphorus (P), are assimilated into the oyster's tissue and shell (Kellogg et al. 2013) and are thereby removed from the water column. Oysters further enhance nitrogen removal by creating conditions conducive to denitrification and burial of organic matter (Newell et al. 2005). Denitrification is the final step in a set of transformations that converts organic nitrogen to nitrogen gas, a form of nitrogen that cannot be used for growth by phytoplankton. When oyster waste is deposited onto the sediment surface, it can be buried, making the nitrogen and phosphorus it contains unavailable to the water column (Newell et al. 2005). In addition to filtering organic matter from the water column, oysters also remove inorganic matter in the form of suspended sediments and increase water clarity (Grizzle et al. 2008 and sources therein). These oyster-associated nutrient and suspended sediment reduction processes were used by the Panel to develop the individual reduction effectiveness crediting protocols for BMP application further described in Section 6.0.

Various oyster practices occur in Chesapeake Bay that either result in the eventual removal of the oysters (i.e., harvested oysters from private oyster aquaculture by the means of State-managed private leases or from the public fishery) or non-removal (e.g., protected and restored oysters from sanctuaries). Private oyster aquaculture practices are conducted within the bounds of private oyster leases (i.e., areas where public fishing is not allowed by the State) and use either hatchery-produced oysters (i.e., oysters propagated outside their

[1] Details of the CBP partnership's model framework can be found at http://www.chesapeakebay.net/groups/group/modeling_team.

natural environment in private or State-run hatcheries) or wild oysters (i.e., naturally-occurring oyster larvae or seed oysters). Oysters from aquaculture practices are either grown off the bottom in the water column in protective gear (e.g., floating rafts near the surface or cages near the bottom) or directly on the bottom without gear. Oysters grown off the bottom in gear are typically hatchery-produced diploid or triploid oysters. Hatchery-produced diploid oysters are similar to oysters that occur in the wild, but are selectively bred to exhibit faster growth and/or be resistant to common diseases (Rawson et al. 2010; Degremont et al. 2015). Hatchery-produced triploid oysters are created by manipulating chromosomes of diploid broodstocks to prevent reproduction, allowing faster growth compared to diploid oysters (Allen and Downing 1986). Triploids can also have greater disease resistance (Degremont et al. 2015). Aquaculturists growing oysters on the bottom without gear typically enhance their leases by placing substrate (e.g., shell, rock, concrete) suitable for recruitment of wild oyster larvae on the bottom (hereafter, "substrate addition") and/or diploid oyster larvae settled on oyster shell (hereafter, "spat-on-shell planting") from hatcheries or pubic grounds. If using wild oysters, they are typically moved from one location of the Bay and transplanted to a different area within the Bay. In some instances, lease holders do not enhance the bottom in any way. Instead, they take advantage of the less restrictive rules for harvest gear allowed within leased bottom to exploit wild oysters within the leased area.

Oyster practices that involve the public fishery includes the addition of hatchery-produced diploid oyster spat-on-shell, substrate addition, or both. These practices typically aim to enhance the oyster stock available for harvest. Another method that has been employed to increase harvestable oyster stock at public fishing grounds is to obtain wild seed oysters from one location, usually where oyster growth and/or survival is poor, and transplanting them to another location, usually where the conditions are better for growth and/or survival. While private oyster aquaculture and public fishery practices aim to increase the numbers or oysters available for harvest, oyster reef restoration practices aim to increase the number of oysters that will remain in the Bay within sanctuaries where harvest is not allowed. These practices include spat-on-shell planting, substrate addition, or both.

The Panel identified a total of 12 oyster practice categories after considering the oyster's fate, fisheries management approach, culture type, and activity (Table 1a). This first report only includes recommendations for private oyster aquaculture practices. Of the five private oyster aquaculture practice categories identified, the Panel felt that only the following three categories should undergo BMP consideration (further described in Section 5.0):

- Off-bottom private oyster aquaculture using hatchery-produced oysters
- On-bottom private oyster aquaculture using hatchery-produced oysters
- On-bottom private oyster aquaculture using substrate addition

The Panel's decision on whether an oyster practice category should be recommended for BMP consideration was based on whether the practice will enhance the overall production of new oysters. The Panelists agreed that "On-bottom private oyster aquaculture using transplanted wild oysters" and "Private oyster aquaculture with no activity" would not enhance overall new oyster production because oysters that are transplanted from

one location to another is more representative of transferring the production and no activity results in the same amount of oysters being present regardless whether a lease existed or not.

Table 1a. Chesapeake Bay oyster practices identified by the Panel based on oyster fate, fisheries management approach, oyster culture type, and activity.

Chesapeake Bay Oyster Practices												
Oyster Fate	Oysters removed (harvested) from Bay									Oysters remain in Bay		
Fisheries Management Approach	Private oyster aquaculture (water column and bottom leases)					Public fishery				Oyster reef restoration (sanctuaries)		
Oyster Culture Type	Hatchery-produced oysters		Wild oysters			Hatchery-produced oysters	Wild oysters			Hatchery-produced oysters	Wild oysters	
Activity	Hatchery-produced oysters grown off the bottom using some sort of gear (e.g., floating rafts near the surface or cages near the bottom)	Hatchery-produced oysters grown on the bottom using no gear	Moving wild oysters from one location to another.	Addition of substrate to the bottom to enhance recruitment of wild oyster larvae	None	Addition of hatchery-produced oysters (e.g. spat-on-shell)	Moving wild oyster from one location to another	Addition of substrate to enhance recruitment of wild larvae	None	Sanctuary creation followed by addition of hatchery-produced oysters	Sanctuary creation followed by addition of substrate	Sanctuary creation
Oyster Practice Title	Off-bottom private oyster aquaculture using hatchery-produced oysters	On-bottom private oyster aquaculture using hatchery-produced oysters	On-bottom private oyster aquaculture using transplanted wild oysters	On-bottom private oyster aquaculture using substrate addition	Private oyster aquaculture with no activity	On-bottom public fishery oyster production using hatchery-produced oysters	On-bottom public fishery oyster production using transplanted wild oysters	On-bottom public fishery oyster production using substrate addition	Public fishery with no activity	Active oyster reef restoration using hatchery-produced oysters	Active oyster reef restoration using wild oysters	Passive oyster reef restoration
***Panel Recommends for BMP Consideration**	Yes	Yes	No	Yes	No	TBD	TBD	TBD	TBD	TBD	TBD	TBD
Oyster Practice Category	A	B	C	D	E	F	G	H	I	J	K	L

* The Panel's recommendation on whether an oyster practice category should undergo BMP consideration was based on whether the practice enhances the production of new oysters. "Yes" indicates that the Panel endorses the use of the recommendations found in this report for those oyster practices because, in their opinion, these practices will enhance the production of new oysters, while "No" indicates the Panel's opinion that overall enhancement will not occur, and therefore, not endorsed. "TBD" indicates that the decision will be decided in a future report.

The Panel also identified eight oyster-associated nitrogen, phosphorus, and suspended sediment reduction effectiveness processes (further described in Section 6.0):

1. Nitrogen Assimilation in Oyster Tissue
2. Nitrogen Assimilation in Oyster Shell
3. Enhanced Denitrification Associated with Oysters
4. Phosphorus Assimilation in Oyster Tissue
5. Phosphorus Assimilation in Oyster Shell
6. Suspended Sediment Reduction Associated with Oysters
7. Enhanced Nitrogen Burial Associated with Oysters
8. Enhanced Phosphorus Burial Associated with Oysters

When paired with the oyster practice categories, this created 96 unique oyster practice category-reduction effectiveness crediting protocol combinations (hereafter, "practice-protocol combination") in which oysters could reduce nutrients and suspended sediments in Chesapeake Bay. The Panel decided they could move forward with recommending reduction effectiveness estimates for some of these combinations, while others they felt needed more deliberation due to various outstanding scientific and policy issues. In light of this, and the interest the CBP has expressed in considering the Panel's recommendations in the Chesapeake Bay TMDL 2017 midpoint assessment, the Panel decided to submit its recommendations incrementally, allowing the reduction effectiveness protocols for some practices to be applied in a timelier manner. This approach is in line with the Panel's recommended decision framework to determine the reduction effectiveness of oyster practices for BMP application (further described in Section 4.0). The Panel is following the procedures outlined in the CBP partnership's July 13, 2015 BMP Expert Review Protocol (CBP 2015). Appendix B describes this report's conformity with this protocol and additional information concerning BMP application.

The Panel's report schedule for these different combinations is presented in Table 1b, which highlights the combinations found in this report (1st), those tentatively scheduled for the next report (2nd), and those that are put on hold for Panel deliberation due to outstanding policy issues (i.e., legality of allowing nutrient sequestration and sediment deposition to be included in the reduction effectiveness estimate for a tidal in-water BMP). Oyster BMP policy issues are currently being discussed within the CBP Partnership Management Board.

Table 1b. The Panel's planned report schedule for the 96 oyster practice category-reduction effectiveness crediting protocol combinations. The recommendations found in this report are labeled "1st," the recommendations planned for the second incremental report are labeled "2nd," and recommendations that are labeled as "on hold" due to outstanding policy issues will likely be released in a third recommendation report.

	Private Oyster Aquaculture					Public Fishery				Oyster Reef Restoration		
Oyster Practice Category x Crediting Protocol	**A. Off-bottom private oyster aquaculture using hatchery-produced oysters**	**B. On-bottom private oyster aquaculture using hatchery-produced oysters**	**C. On-bottom private oyster aquaculture using transplanted wild oysters**	**D. On-bottom private oyster aquaculture using substrate addition**	**E. Private oyster aquaculture with no activity**	**F. On-bottom public fishery oyster production using hatchery-produced oysters**	**G. On-bottom public fishery oyster production using transplanted wild oysters**	**H. On-bottom public fishery oyster production using substrate addition**	**I. Public fishery with no activity**	**J. Active oyster reef restoration using hatchery-produced oysters**	**K. Active oyster reef restoration using wild oysters**	**L. Passive oyster reef restoration**
1. Nitrogen Assimilation in Oyster Tissue	**1st**	**1st**	**1st**	**1st**	**1st**	2nd	2nd	2nd	2nd	On hold	On hold	On hold
2. Nitrogen Assimilation in Oyster Shell	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	On hold	On hold	On hold
3. Enhanced Denitrification Associated with Oysters	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd
4. Phosphorus Assimilation in Oyster Tissue	**1st**	**1st**	**1st**	**1st**	**1st**	2nd	2nd	2nd	2nd	On hold	On hold	On hold
5. Phosphorus Assimilation in Oyster Shell	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	2nd	On hold	On hold	On hold
6. Suspended Sediment Reduction Associated with Oysters	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold
7. Enhanced Nitrogen Burial Associated with Oysters	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold
8. Enhanced Phosphorus Burial Associated with Oysters	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold	On hold

In summary, the Panel's recommendations found in this first incremental report include:

- A decision framework to determine the nutrient and suspended sediment reduction effectiveness of oyster practices, referred to as the "Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework" or simply "Decision Framework" throughout this report.

- The reduction effectiveness estimates for the "Nitrogen Assimilation in Oyster Tissue" and "Phosphorus Assimilation in Oyster Tissue" reduction effectiveness protocols for the following private oyster aquaculture-related oyster practices: Off-bottom private oyster aquaculture using hatchery-produced oysters, on-bottom private oyster aquaculture using hatchery-produced oysters, and on-bottom private oyster aquaculture using substrate addition.

Public/stakeholder engagement and outreach included the Panel hosting an open public stakeholder meeting on November 2, 2015, offering two review opportunities for CBP Partnership and public feedback on preliminary recommendations (February and April 2016), six open briefings to the Water Quality Goal Implementation Team (WQGIT) with notifications sent to interested parties, and several public presentations/webinars. Details of these engagement/outreach efforts are presented in Appendix A.

The CBP Water Quality Goal Implementation Team (WQGIT) determined that policy issues raised by the Panel and stakeholders were outside the purview of the Panel's charge and would be evaluated by the CBP Partnership Management Board. The CBP Partnership Management Board is working on resolving these policy issues in parallel to the Oyster BMP Expert Panel developing reduction effectiveness recommendations. It is the Panel's understanding that unresolved policy issues will not prevent a decision on the Panel's report since the Panel's recommendations focus on the scientific and technical aspects concerning the reduction effectiveness of oyster practices. The Panel is using the policy decisions from the Management Board to help prioritize which oyster practice category/reduction effectiveness crediting protocol combinations to focus on for each incremental report. The Panel has incorporated the relevant policy decisions from the June 15, 2016 Special Management Board Meeting[2] in this report. It is in the Panel's opinion that the recommendations found in this first incremental report are not influenced by any of the outstanding policy issues.

The Panel is asking the Partnership and the WQGIT, in coordination with the Fisheries and Habitat Goal Implementation Teams, to review and approve the recommendations found in this first incremental report. The Panel recommends that, once a reduction effectiveness crediting protocol is approved for a given oyster practice category (Table 1a), it can be implemented for the practices within that category (see Section 5.0 for oyster practice definitions and Section 6.0 for descriptions of the oyster-associated reduction processes the protocols are based on). The Panel also recommends allowing incremental determination, approval, and implementation of nitrogen, phosphorus, and suspended sediment reduction effectiveness estimates where science exists for the various oyster practices as detailed in its Oyster BMP Nutrient and Suspended Sediment

[2] Policy decisions from the June 15, 2016 Special Management Board meeting can be found at http://www.chesapeakebay.net/calendar/event/24109/

Reduction Effectiveness Determination Decision Framework (described in Section 4.0). The Panel felt that this Decision Framework would allow the most practical and adaptive strategy in implementing oyster practices as BMPs given the variety of oyster practices and the wide range in the amount of science available to evaluate the reduction effectiveness these practices.

2.0 Summary of Panel Recommendations in this Report

The Oyster BMP Expert Panel recommends that the CBP Partnership adopt a decision framework that would allow the incremental determination, approval, and implementation of the reduction effectiveness associated with oysters using individual nitrogen, phosphorus, and suspended sediment reduction effectiveness crediting protocols applied to different oyster practice categories (further described in Sections 4.0). The Panel's recommended oyster practice categories are based on grouping individual oyster practices that would have similar reduction effectiveness considerations (further described in Section 5.0). The Panel's recommended reduction effectiveness protocols are based on oyster-associated processes that reduce nitrogen, phosphorus, and suspended sediment (further described in Section 6.0). Table 2a summarized the Panel's reduction effectiveness determination status for the identified private oyster aquaculture practice-protocol combinations.

Table 2a. The reduction effectiveness determination status for the Panel's recommended oyster practice category-reduction effectiveness crediting protocol combinations for private oyster aquaculture categories. "#" indicates that the Panel has recommended a reduction effectiveness estimate that can be implemented once approved, "D" indicates that the Panel is still deliberating on this combination and will present recommendations in a future report, "X-Practice" indicates the combination wasn't or will not be assessed by the Panel because they felt it shouldn't be considered a BMP due to overall oyster production not being enhanced, and "? - Policy" indicates that there is an outstanding policy issue still being deliberated on by the CBP Partnership Management Board and that Panel deliberations are currently on hold until the policy issues are resolved.

	Private Oyster Aquaculture				
Oyster Practice Category x Crediting Protocol	**A. Off-bottom private oyster aquaculture using hatchery-produced oysters**	**B. On-bottom private oyster aquaculture using hatchery-produced oysters**	**C. On-bottom private oyster aquaculture using transplanted wild oysters**	**D. On-bottom private oyster aquaculture using substrate addition**	**E. Private oyster aquaculture with no activity**
1. Nitrogen Assimilation in Oyster Tissue	**#**	**#**	X-Practice	**#**	X-Practice
2. Nitrogen Assimilation in Oyster Shell	D	D	X-Practice	D	X-Practice
3. Enhanced Denitrification Associated with Oysters	D	D	X-Practice	D	X-Practice
4. Phosphorus Assimilation in Oyster Tissue	**#**	**#**	X-Practice	**#**	X-Practice
5. Phosphorus Assimilation in Oyster Shell	D	D	X-Practice	D	X-Practice
6. Suspended Sediment Reduction Associated with Oysters	? - Policy	? - Policy	X-Practice	? - Policy	X-Practice
7. Enhanced Nitrogen Burial Associated with Oysters	? - Policy	? - Policy	X-Practice	? - Policy	X-Practice
8. Enhanced Phosphorus Burial Associated with Oysters	? - Policy	? - Policy	X-Practice	? - Policy	X-Practice

For protocols where sufficient science exists to determine the reduction effectiveness for an oyster practice category, the Panel recommends that it be put forward for approval and implemented once approved, assuming there are no outstanding policy issues. For protocols where there isn't sufficient science, the Panel recommends that, once sufficient science is available, it is evaluated by an Expert Panel to determine the reduction effectiveness and put forward for approval. Once approved, the protocol's reduction effectiveness can be included with other approved protocols if the BMP implementer fulfills the qualifying conditions for each protocol they would like to use. For protocols that address the same pollutant, the reduction effectiveness would be added together for the total nitrogen, phosphorus, or suspended sediment reduction effectiveness.

The Panel recommends two options for the "Nitrogen Assimilation in Oyster Tissue" and "Phosphorus Assimilation in Oyster Tissue" protocols' reduction effectiveness estimates for endorsed private oyster aquaculture practices:

1. Default estimates for recommended practices regardless of location (Section 7.1).

2. Site-specific estimates developed by the BMP implementer, in coordination with the State and CBP, using the Panel's recommended methodology (Section 7.2).

The default reduction effectiveness estimates are based on using a regression equation to convert oyster shell height to tissue dry weight, applying the regression equation with the midpoints from recommended oyster size classes to determine the tissue dry weight, and then multiplying the tissue dry weight by the recommended % nitrogen and % phosphorus content in oyster tissue. The Panel's recommended default estimates for diploid and triploid oysters are presented in Table 2b. The method and rationale for the default estimates can be found in Section 7.1 and Appendix D.

Table 2b. Default nitrogen and phosphorus reduction effectiveness estimates in oyster tissue for diploid and triploid oysters. Nitrogen content is based on an average of 8.2% nitrogen in oyster tissue. Phosphorus content is based on an average of 0.9% nitrogen content in oyster tissue.

Default Estimates				
Oyster Size Class Range (inches)	Content in Oyster Tissue (g/oyster)			
	Diploid		Triploid	
	Nitrogen	Phosphorus	Nitrogen	Phosphorus
a. 2.0 - 2.49	0.05	0.01	0.06	0.01
b. 2.5 - 3.49	0.09	0.01	0.13	0.01
c. 3.5 - 4.49	0.15	0.02	0.26	0.03
d. 4.5 - 5.49	0.22	0.02	0.44	0.05
e. ≥ 5.5	0.31	0.03	0.67	0.07

The Panel also recommends that the States and the CBP Partnership adopt an approach that allows the BMP implementers (e.g., oyster aquaculturists) to establish site-specific nitrogen and phosphorus reduction effectiveness estimates for their practice. The Panel used a conservative approach to develop the default estimates, therefore, they likely underestimate the overall nitrogen and phosphorus reduction effectiveness. Site-specific estimates would offer an opportunity to refine the estimates to better reflect the nitrogen and phosphorus reduction effectiveness by that practice in the location the practice occurs. The Panel's recommended methodology to determine site-specific estimates is described in Section 7.2. It involves the BMP implementer working with the State and CBP to determine the representative oyster size classes and tissue biomass of their oysters.

The Panel recommends the following qualifying conditions to be applied to both the default and site-specific estimates (Section 8.0):

- Only includes oysters that are removed moving forward from the time the BMP is approved/implemented for reduction effectiveness credit in the TMDL. This baseline condition was proposed by the CBP Partnership Management Board and the Panel concurs with their decision.

- Oysters had to have been grown from initial sizes < 2.0 inches shell height.

- Oysters have to be alive when removed to count toward the reduction effectiveness.

The Panel's recommended application and verification guidelines can be found in Section 9.0. Briefly, the Panel identified three types of data that would be needed to apply the default reduction effectiveness estimates: type and total number of containers, average number of oysters in each container, and average size of oysters in each container type. The Panel also identified two ways in which oyster aquaculturists are packaging oysters, 1) variable sized oysters together in the same container and 2) uniform sized oysters in separate containers. The first approach is more relevant to on-bottom growers and the second approach is more relevant to off-bottom growers. The Panel's verification guidelines depend on the packaging approach. If packaging variable sized oysters in the same container then the implementer can only report in one oyster size class determined by the average shell height of 50 random oysters per two time periods that are 6 months apart. If packaging uniform sized oysters in separate containers then the implementer can report in multiple oyster size classes determined by the average shell height of 50 random oysters per two time periods that are 6 months apart for each size class that they are reporting in. For both packaging approaches, the Panel recommends that the number of oysters per container is determined by counting the oysters from 10 containers and using the average. In instances where verification measurements are missing, then the Panel recommends that the minimum legal size of oysters and State documented information specifying the average number of minimum legal sized oysters can be packaged in a specific container be used.
Examples on these approaches are presented in Section 9.4.

There are also instances where oysters are moved from their initial grow-out location to another location in the Bay. If the BMP implementer uses this strategy, then the Panel recommends that the reduction effectiveness of surviving oysters from the final grow-out location is partitioned to the different locations based on the size class when removed. The Panel suggests that the size class is verified by measuring the shell height of 50 random oysters when moved. If they oysters don't end up in different size classes, then the initial grow-out location will receive the credit.

The Panel recommends the following information to be reported if oyster are grown in one location or multiple locations:

If oysters are grown at one location

- Ploidy: Diploid or triploid oysters
- Type of aquaculture practice: Off-bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, On-Bottom Private Oyster Aquaculture using Hatchery-Produced Oysters, or On-Bottom Private Oyster Aquaculture Using Substrate Addition
- Reporting unit: Bushels, boxes, other container (indicate what type), or individuals
- Packaging type: Variable oyster sizes or uniform oyster sizes
- Central coordinates (latitude and longitude) of initial grow-out location
- Month/year removed from final grow-out location
- Number of containers of live oysters or individual oysters from final grow-out location
- Oyster count average for unit verification check (10 representative containers per two time periods from final grow-out location)
- Shell height average(s) for oyster size verification check (50 random oysters from 10 containers per two time periods from final grow-out location)

Additional reporting if oysters are grown at multiple locations

- Central coordinates (latitude and longitude) of any grow-out locations oysters are transferred to (if applicable)
- Month/year oysters are transferred
- Oyster size class category when placed at transfer location

The Panel's recommended estimates were developed to be reported annually based on removed alive oysters. For growers participating as a BMP, the Panel recommends that the State incorporates these components in the monthly reports to track the BMP. The Panel recommends that the estimates are re-evaluated every 5 years.

The technical requirements for reporting and simulating the private oyster aquaculture practices in the Phase 6 watershed model are described in Appendix F. Briefly, the pounds of nutrients reduced by these practices will be credited as a reduction to the edge-of-stream loads in the land-river segments adjacent to the practice location. If latitude and longitude is not submitted, then the practice benefits will be distributed amongst all land-river segments in the geography submitted.

3.0 Expert Panel Membership and Charge

3.1 Panel Membership

The Panel includes oyster scientists and practitioners from the East Coast region, including representatives from academia, non-profit organizations, and county, state, and federal agencies who have expertise in oyster biology/ecology, water quality, fishery management, and/or oyster practice implementation (Table 3a).

Table 3a. Experts participating in the Oyster BMP Expert Panel

Panelists	Affiliation	Expertise
Jeff Cornwell (Panel Chair)	U. of Maryland Center for Environmental Science (UMCES)	Oyster filter-feeding, nutrient cycling dynamics, modeling, sediment biogeochemistry, oyster ecology, population dynamics
Suzanne Bricker	NOAA, National Centers for Coastal Ocean Science	Nutrient-related water quality research, oyster and nutrient cycling modeling
Lynn Fegley	Maryland Department of Natural Resources, Fisheries Service	Fisheries management
Karen Hudson	Virginia Institute of Marine Science (VIMS)	Shellfish Aquaculture
Lisa Kellogg	Virginia Institute of Marine Science (VIMS)	Oyster reef ecology and restoration, oyster filter-feeding and nutrient cycling dynamics
Andy Lacatell	The Nature Conservancy (TNC)	Oyster restoration
Mark Luckenbach	Virginia Institute of Marine Science (VIMS)	Oyster ecology and restoration; interactions between shellfish aquaculture and the environment; land-use practices and water quality in tidal water environments
Chris Moore	Chesapeake Bay Foundation (CBF)	Fisheries and oyster restoration, oyster aquaculture, water quality, implementation of Chesapeake Bay TMDL, BMP review
Matt Parker	Sea Grant at U. of Maryland, Prince George's County Office	Oyster aquaculture, business planning
Ken Paynter	U. of Maryland Marine, Estuarine, Environmental Sciences/Chesapeake Bay Laboratory	Oyster restoration, oyster biology and population dynamics
Julie Rose	NOAA Northeast Fisheries Science Center, Milford Lab	Nutrient bioextraction, marine spatial planning for shellfish activities, aquaculture-environment interactions
Larry Sanford	U. of Maryland Center for Environmental Science (UMCES)	Coastal physical oceanography, sediment transport, oceanographic instrumentation
Bill Wolinski	Talbot County Department of Public Works	Watershed Implementation Plans, BMP implementation, water quality

Advisors	Affiliation	Expertise
Lew Linker	U.S. EPA Chesapeake Bay Program Office	Chesapeake Bay Modeling Team Representative
Jeff Sweeney	U.S. EPA Chesapeake Bay Program Office	Watershed Technical Workgroup (WTWG) Representative
Ed Ambrogio	U.S. EPA Region III	EPA Region 3 Representative
Lucinda Power	U.S. EPA Chesapeake Bay Program Office	Water Quality Goal Implementation Team Representative
Rich Batiuk	U.S. EPA Chesapeake Bay Program Office	BMP Verification Representative
Coordinators	**Affiliation**	**Expertise**
Julie Reichert-Nguyen	Oyster Recovery Partnership	Coordination and facilitation, Clean Water Act, TMDL program, water quality, fisheries science, climate change, ocean acidification
Ward Slacum	Oyster Recovery Partnership	Program management, oyster restoration, environmental monitoring, fisheries ecology
Emily French	Oyster Recovery Partnership	Seagrass ecology, water quality monitoring, oyster restoration
Guests	**Affiliation**	**Expertise**
Carl Cerco	US Army Corps of Engineers	Water Quality Modeling
Tom Schuler	Chesapeake Stormwater Network	Stormwater BMPs
Stephan Abel	Oyster Recovery Partnership	Implementation

3.2 Panel Charge

The Oyster BMP Expert Panel was charged with fulfilling three overall goals based on the Chesapeake Bay Program Partnership's Expert BMP Panel Review Protocol for nutrient (nitrogen and phosphorus) and sediment controls:

1. Reach a consensus on acceptable nutrient and suspended sediment reduction effectiveness estimates for oyster practices in Chesapeake Bay based on existing science.

2. Determine a methodology to update these estimates when new science becomes available.

3. Establish reduction effectiveness crediting and verification guidelines as it relates to their application in the CBP partnership's model framework used to inform the Chesapeake Bay TMDL.

To support the achievement of the above goals, the Oyster BMP Expert Panel is focusing on the following three charge items:

Charge Item 1: Identify and define oyster practices, including aquaculture operations and restoration activities for nutrient reduction BMP consideration. Evaluate whether existing science supports the evaluation of sediment reduction effectiveness.

Charge Item 2: Develop a reduction effectiveness crediting decision framework that will allow the incremental approval of nutrient and suspended sediment reduction effectiveness estimates based on oyster-associated processes (e.g., nitrogen and phosphorus assimilation in tissue, nitrogen and phosphorus assimilation in shell, nitrogen removal via denitrification) for various oyster practices.

Charge Item 3: Use the established reduction effectiveness decision framework from charge item 2 to propose reduction effectiveness estimates that are determined to have sufficient science to help inform the Chesapeake Bay TMDL 2017 Midpoint Assessment.

3.2.1 Key changes from the Oyster BMP Expert Panel Charge

- In the Panel charge,[3] the decision framework was referred to as the "pollutant removal crediting decision framework;" however, the Panel decided it would be better to refer to it as the "Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework," (hereafter, "Decision Framework") in order to make it clear that the framework is for determining the nitrogen, phosphorus, and suspended sediment reduction effectiveness of oyster practices and not decisions concerning other pollutants or how to implement nutrient trading credits.

- Initially, the Panel charge included in the timeline an incremental approval step for just the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Decision Framework. Because the Panel found the need to modify the decision steps in the framework as they developed reduction effectiveness estimates, the Panel determined that approval of the Decision Framework with the Panel's 1st set of recommended estimates would be more efficient than a stand-alone report on the Decision Framework. Thus, reduction effectiveness estimates presented in this report can be viewed as a test case for the application of the proposed decision framework. While there isn't a stand-alone report on the Decision Framework, the Panel did provide two review/comment opportunities on Decision Framework drafts during the Panel's updates to the Water Quality GIT (February and April 2016; see Appendix A for more information). The Panel felt it was important to have the Partnership and interested parties review and provide input on the Decision Framework early in the development process. Comments that were received were reviewed by the Panel and they made changes accordingly resulting in the decision framework presented in Section 4.0.

- Oyster practice titles and definitions have been refined from what was presented in the charge (see Table 1a in Section 1.0 and Table 5b in Section 5.0)

[3] The Oyster BMP Expert Panel Charge can be found at http://www.chesapeakebay.net/channel_files/23104/oyster_bmp_expert_panel_charge_final_9-14-15.pdf

4.0 Recommended Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework

This section describes the Panel's recommended decision framework to determine the nitrogen, phosphorus, and suspended sediment reduction effectiveness of oyster practices as BMPs for application in the model framework used to inform the Chesapeake Bay TMDL. The Panel felt it was important to develop and apply an agreed upon decision framework because there are no existing BMPs involving filter-feeders within the tidal waters of Chesapeake Bay. Any policy questions that were raised by the Panel were shared with the CBP Partnership Management Board for resolution. The Decision Framework the Panel is proposing is specific to determining the reduction effectiveness and the proper application of the reduction effectiveness in the CBP Partnership model framework used to inform the TMDL. Addressing policy issues (e.g. nutrient trading) is beyond the purview of the panel and not included in the Decision Framework. The Decision Framework is specific for oyster practices, but the Panel acknowledges that a similar framework could be developed for other filter-feeding organisms found in the Chesapeake Bay and its tributaries.

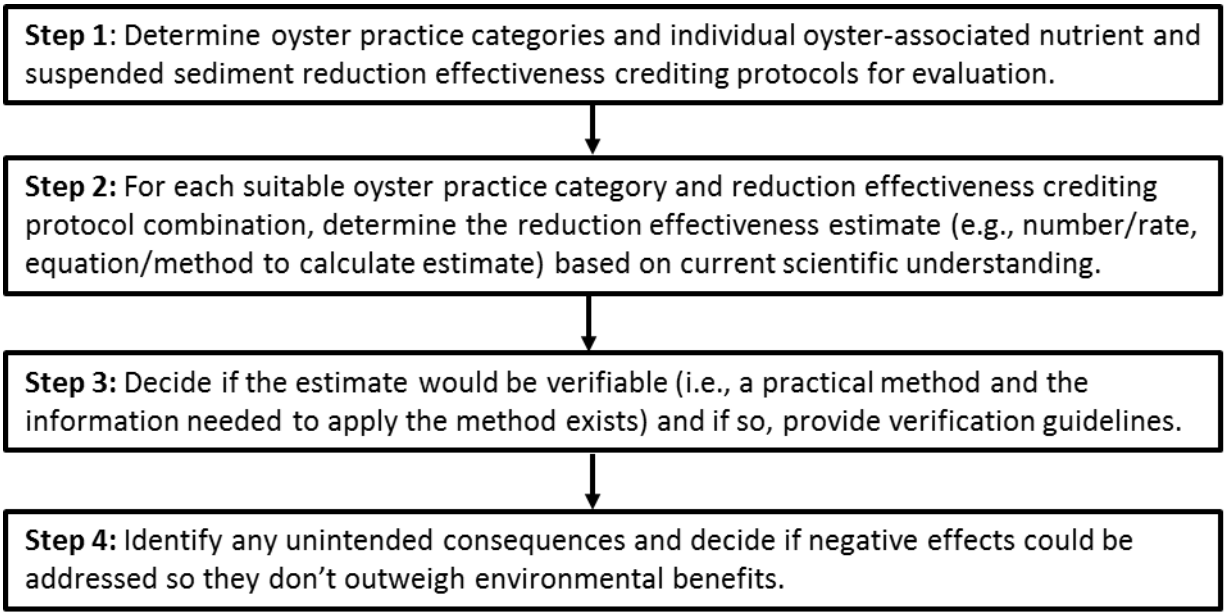
There were two public review/comment opportunities on the Panel's Decision Framework during the February 8 and April 25, 2016 WQGIT meetings (see Appendix A for more details). The Panel considered all the comments they received on the draft documents. The recommended Decision Framework presented in this section captures these considerations.

Overall, the Panel's recommended Decision Framework allows for the incremental determination, approval, and implementation of nitrogen, phosphorus, and suspended sediment effectiveness estimates based on available science for various oyster practices. The Panel agreed that the Decision Framework should consist of individual reduction effectiveness crediting protocols based on oyster-associated nitrogen, phosphorus, and suspended sediment reduction processes so that these protocols could be incrementally determined and applied for oyster practices where there is sufficient science to do so. The panel also built into the Decision Framework opportunities to identify knowledge gaps and/or additional data needed to determine reduction effectiveness, including a decision pathway where unknown estimates could be revisited when new science becomes available. The Panel's recommended Decision Framework is further described below.

4.1 Main Steps of the Decision Framework

The Panel identified four main steps for the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework (see Figure 4a). These steps are further described in their corresponding sections.

Figure 4a. Main steps for the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework.



The Panel's definitions of key criteria used in the Decision Framework are described below:

1. **Suitable for Consideration:** In the Panel's best professional judgement, the reduction process could occur in association with a particular oyster practice category.

2. **Sufficient Science:** In the Panel's best professional judgement, data of sufficient quality and scope exist and can be used to generate a reasonably constrained estimate of the reduction associated with a particular oyster practice category.

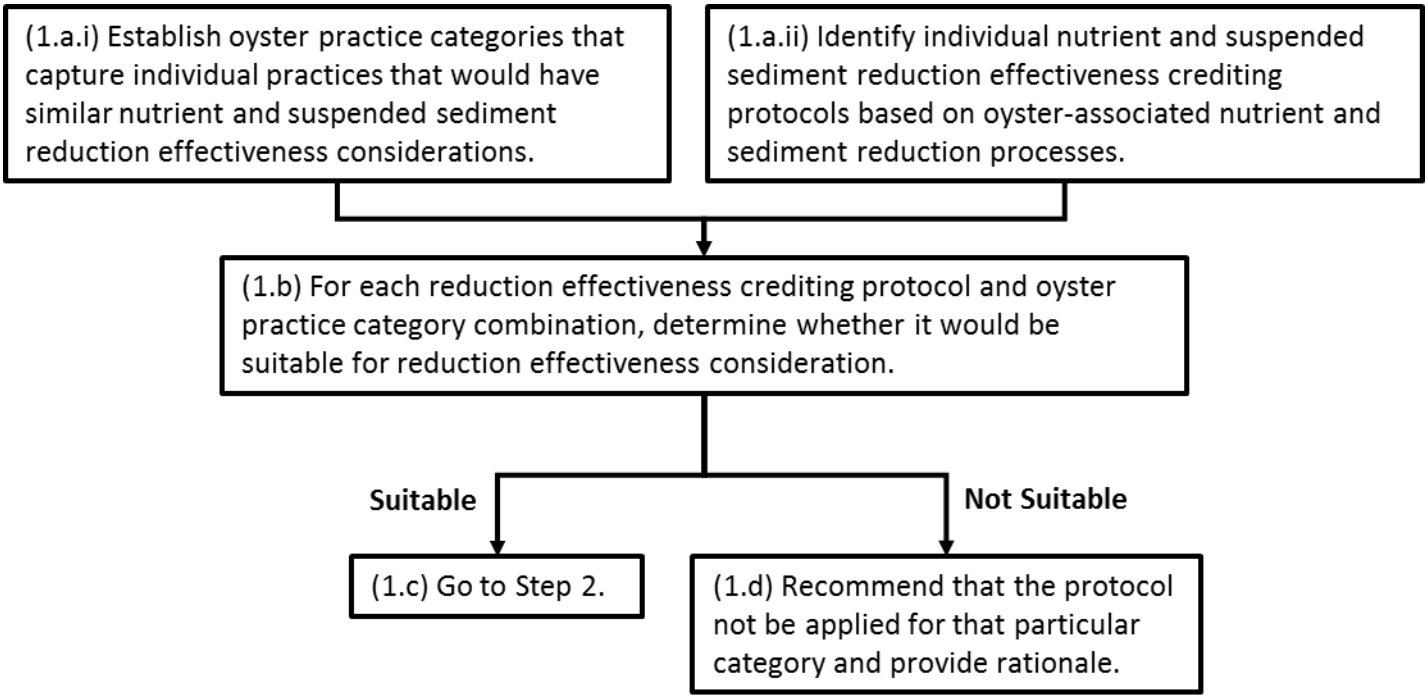
3. **Verifiable:** In the Panel's best professional judgement, a practical method exists, or could be created, to track reduction effectiveness if the BMP is implemented.

4. **Unintended Consequence:** Potential unexpected negative or positive effects on the environment resulting from the practice. Positive unintended consequences are referred to as "ancillary benefits" in this report to match the terminology found in the BMP Review Protocol (CBP 2015).

4.2 Step 1 Decision Points

The Panel's recommended Step 1 decision points for the Decision Framework are described in Figure 4b. During Panel discussions, it became clear that a wide variety of oyster-related practices are implemented in Chesapeake Bay and certain practices would likely require different reduction effectiveness considerations than others. The Panel agreed that grouping oyster practices, including cultivation (i.e., private oyster aquaculture practices and public fishery practices) and restoration (i.e., oyster reef restoration practices), into broad categories with similar reduction effectiveness considerations would be more efficient than assessing practices individually. Essentially, categorization of practices would allow a more focused evaluation of the data to determine the reduction effectiveness estimates and also simplify the establishment of reduction effectiveness crediting and verification guidelines because the practices in each category would involve similar decisions. This decision point is incorporated in Step 1 (see Figure 4b, box 1.a.i). Oyster practice categories that do not enhance new oyster production were not considered by the Panel (e.g. harvest of oysters from private leases to which neither substrate nor oysters have been added). The Panel proposes that endorsed categories for BMP consideration be thought of as separate BMPs so that an estimate that is established for one category can move forward through the BMP approval process.

Figure 4b. The Panel's recommended Step 1 decision points for the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework. The goal of this step is to determine the oyster practice categories and individual oyster-associated nutrient and suspended sediment reduction effectiveness crediting protocols for evaluation.



The Panel agreed that oysters can improve water quality because of their filter-feeding capabilities (described further in Section 6.0). The Panel decided that each oyster-associated process that reduces nitrogen, phosphorus, and suspended sediment should be developed as a separate reduction effectiveness crediting protocol (Figure 4b, 1.a.ii) that could be evaluated and applied individually. The Panel also agreed that reduction effectiveness of protocols involving the same pollutant (e.g., nitrogen assimilation in oyster tissue, enhanced denitrification associated with oysters, and enhanced nitrogen burial associated with oysters) could be added together to determine the total reduction effectiveness of the practice in a manner similar to the approved approach used by the Urban Stream Restoration BMP Expert Panel (Schuler and Stack 2014). The reduction effectiveness estimates for each protocol will be determined in Step 2 of the Decision Framework (see Section 4.3).

During discussions, the Panel agreed that there may be instances where a reduction effectiveness protocol wouldn't be suitable to consider with a particular oyster practice category because the oyster-associated process would not occur. For instance, protocols associated with enhanced burial of nitrogen and phosphorus may not be suitable to group with harvest-related oyster practice categories because disturbance from harvesting would likely prevent burial processes from happening (i.e., the conditions would never be suitable to support enhanced burial). As a result, the Panel incorporated this decision point into Step 1 of the Decision Framework (see Figure 4b, Box 1b). The Panel defined "suitable for reduction effectiveness consideration" as, "In the Panel's best professional judgement, the reduction process could occur in association with a particular oyster practice category." The Panel felt this decision point was important to evaluate early on in the Decision Framework to avoid spending time evaluating combinations where the reduction would not occur. It is important to note that this step aims to identify which potential crediting protocols should be evaluated for a particular oyster practice category and does not involve the decision whether there is sufficient science to determine the reduction effectiveness, which is built into Step 2 of the Decision Framework (see Section 4.3). Suitable combinations would move forward to Step 2 of the Decision Framework to determine the reduction effectiveness estimate (Figure 4b, Box 1c). The Panel decided that they would not evaluate any combinations that are determined to not be suitable for reduction effectiveness consideration (Figure 4b, Box 1d).

4.3 Step 2 Decision Points

The Panel's recommended Step 2 decision points for the Decision Framework are described in Figure 4.3. These decision points focus on determining the reduction effectiveness estimate for each suitable oyster practice category and reduction effectiveness crediting protocol combination that was identified in Step 1 (see Section 4.2).

The Panel decided that it would be important to begin with a decision point that asks whether there is sufficient scientific data to determine the reduction effectiveness estimate (Figure 4c, Box 2a). The Panel used their best professional judgement to answer this question. Specifically, they evaluated whether data of sufficient quality and scope existed to generate a reasonably constrained estimate of the reduction associated with a particular oyster practice category. If such data existed, then the Panel used it to determine the reduction effectiveness estimate (Figure 4c, Box 2b). The Panel also built into this decision the identification

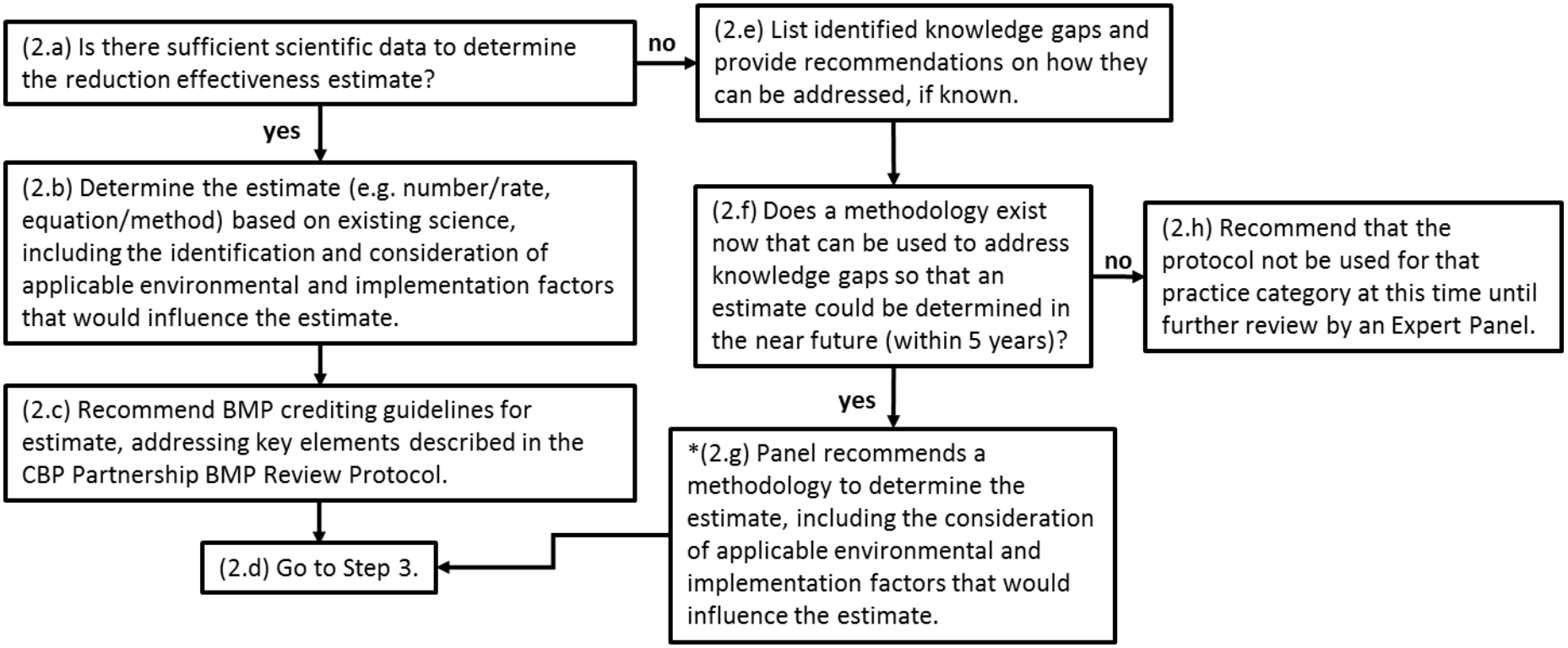
and consideration of any applicable factors that could influence the estimate, particularly environmental and implementation-related factors (e.g., genetic ploidy, seasonal effects, grow-out method/type of gear used). These considerations may result in multiple estimates within a protocol to account for these influencing factors.

The Panel included both "number/rate" and "equation/method" as examples of how the estimate may appear because the Panel recognized that for some of the reduction effectiveness protocols it would be feasible to recommend an exact number or rate that could be applied regardless of location (i.e., low variability in the data), while other protocols would be more influenced by site-specific conditions requiring a method for jurisdictions to use to calculate the estimate (i.e., high variability in the data; Figure 4c, Box 2b). In cases where the reduction effectiveness estimate for a protocol can be applied across multiple practice categories, the Panel recommends evaluating the crediting guidelines (Figure 4c, Box 2c) separately for the different practice categories because they may not be the same depending on how the practices are implemented. The Panel's recommended crediting guidelines are based on the key elements described in the CBP Partnership's Expert BMP Panel Review Protocol (CBP 2015) and includes the following:

- Guidelines on the environmental conditions (e.g., water chemistry, bottom substrate) needed for the estimate to be valid.
- Guidelines on crediting timeframe; cumulative or annual, temporal performance (i.e., lag time between establishment and full functioning, effectiveness of the practice over time), when the estimate should be re-evaluated.
- Guidelines on determining baseline conditions.
- Guidelines on where and how estimates could be incorporated into the Chesapeake Bay Modeling Framework, including the identification of guidelines for a credit duration for applicable crediting protocols for a given oyster practice category.

The Panel agreed that crediting guidelines developed by the Panel should focus on ensuring that the recommended reduction effectiveness is correctly applied and helping the CBP and jurisdictions make an informed decision. The Panel acknowledges that the final decisions concerning which reduction effectiveness estimates to pursue and how they will be implemented and verified is the responsibility of the CBP and jurisdictions.

Figure 4c. The Panel's recommended Step 2 decision points for the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework. The goal of this step is to determine the reduction effectiveness estimate for suitable oyster practice category-reduction effectiveness crediting protocol combinations identified in Step 1 (see Figure 4b).



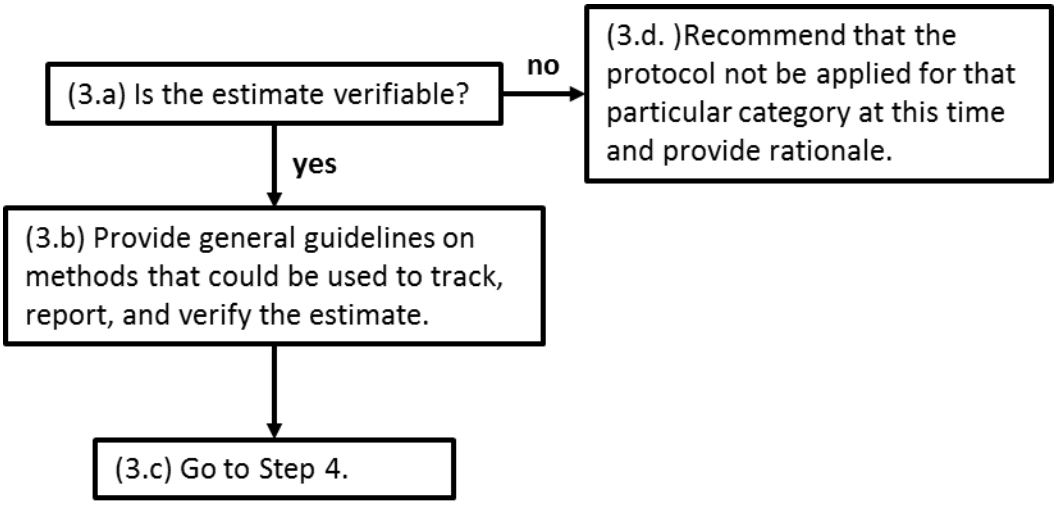
* If the recommended method is used to determine what the estimate would be within the 5 year timeframe, then the Panel suggests that the estimate is reviewed by at least 2 experts selected by the State and CBP before making an approval decision.

The Panel agreed that there should be a decision pathway indicating that sufficient data are not available to determine reduction effectiveness. In instances where data are currently insufficient, the Panel recommends listing these knowledge gaps along with recommendations on how to fill them (Figure 4c, Box 2e). For combinations where data are currently insufficient but the Panel is able to recommend methods for collecting sufficient data in the near future (within 5 years), the Panel agreed that, if the recommended methods were used, the estimate could be evaluated by a minimum of two experts (Figure 4c, Boxes 2f and 2g) selected by the State and CBP for review. If approved by the experts, it could then be submitted to the State or CBP for review and approval without convening a new expert panel. For cases where there isn't a clear method, the Panel recommends that the protocol be evaluated again by an Expert Panel. The Panel felt this distinction was important to make because there are cases where methods exist, but sufficient data have not been collected. The Panel felt it would not be necessary to convene a new Expert Panel to evaluate this information if an approved recommended methodology is in place.

4.4 Step 3 Decision Point

The Panel's recommended Step 3 decision point for the Decision Framework is described in Figure 4d. This decision point focuses on evaluating whether the reduction effectiveness estimate determined in Step 2 is verifiable (Figure 4d, Box 3a). The Panel added this decision point based on public comments that were received on a previous draft. Verifiable is defined by the Panel as, "In the Panel's best professional judgement, a practical method exists, or could be created, to track reduction effectiveness if the BMP is implemented."

Figure 4d. The Panel's recommended Step 3 decision point for the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework. The goal of this step is to evaluate whether the estimate would be verifiable (i.e., a practical method and the information needed to apply the method exists).

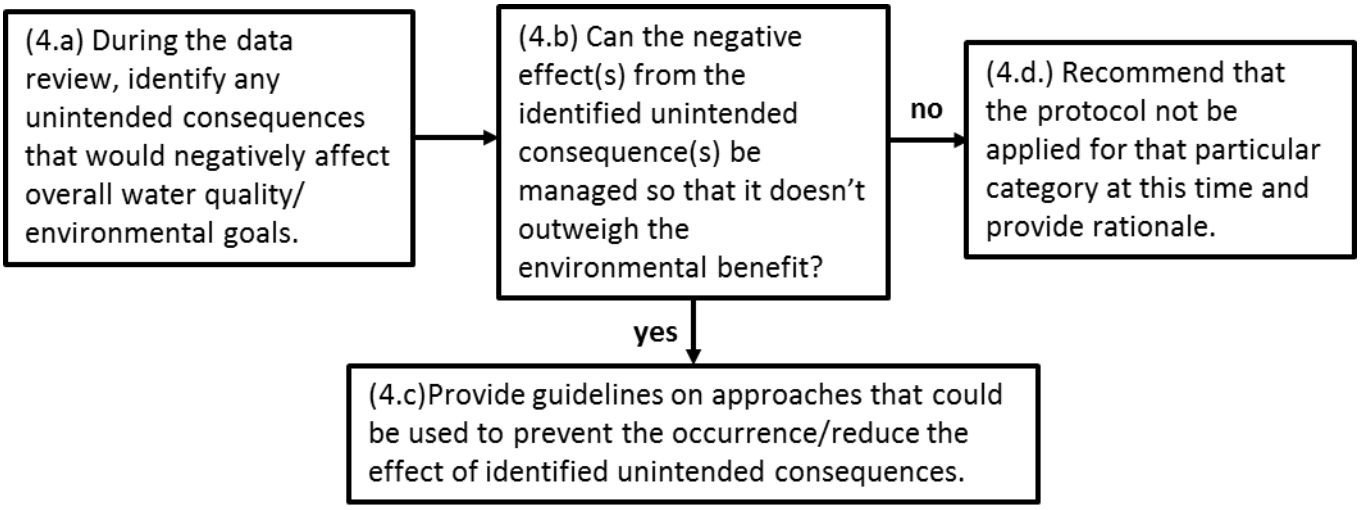


Per the CBP Partnership BMP Review Protocol (CBP 2015), the Panel also built into this step to provide general guidelines on methods that could be used to track, report, and verify the estimate (Figure 4d, 3b). The Panel agreed that these guidelines should focus on key variables that need to be measured and reported to ensure that the recommended estimate is being used correctly. If the Panel decides that the estimate is not verifiable, then they will recommend that the protocol not be applied for that particular category at this time (Figure 4d, 3d).

4.5 Step 4 Decision Point

The Panel's recommended Step 4 decision point for the Decision Framework is described in Figure 4e. This decision point focuses on identifying any unintended consequences associated with the practice-protocol combination (Figure 4e, Boxes 4a-4d). The Panel defines unintended consequences as, "potential unexpected negative or positive effects on the environment resulting from the practice." For identified negative unintended consequences, the Panel will evaluate whether they can be managed so that it doesn't outweigh the environmental benefit (Figure 4e, Box 4b). For manageable negative unintended consequences, the Panel agreed that they would provide guidelines on approaches that could be used to prevent or reduce the negative effect. Positive unintended consequences are referred to as "ancillary benefits" in this report to match the terminology found in the BMP Review Protocol (CBP 2015).

Figure 4e. The Panel's recommended Step 4 decision points for the Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Determination Decision Framework. The goal of this step is to identify any negative unintended consequences and decide if they could be addressed so they don't outweigh environmental benefits.



5.0 Private Oyster Aquaculture Practices Defined

5.1 Private Oyster Aquaculture Practice Categories

This section defines the private oyster aquaculture practice categories that the Panel recommends for BMP reduction effectiveness consideration. The Panel first categorized all oyster practices that occur in the Chesapeake Bay by oyster fate (i.e., removed or remains in the Bay), fishery management approach (i.e., private leases, public fishery, sanctuaries), oyster culture type (hatchery-produced, wild) and activity (e.g., off-bottom, on-bottom) (Table 1a). The goal of the categories were to group individual practices with similar reduction effectiveness and implementation considerations. From there, the Panel determined which categories they would recommend for BMP consideration. Out of the five private oyster aquaculture practice categories (Table 5a) that the Panel identified, they decided to recommend three of the categories for BMP consideration (Table 5b). They based their recommendation on the oyster practice categories enhancing new oyster production. Definitions of the recommended oyster practice categories for BMP consideration can be found in Table 5b.

Table 5a. Identification and grouping structure of identified private oyster aquaculture practices in Chesapeake Bay. Definitions of categories recommended for BMP consideration are found in Table 5b.

Chesapeake Bay Oyster Practices					
Oyster Fate	Oysters removed (harvested) from Bay				
Fisheries Management Approach	Private oyster aquaculture (water column and bottom leases)				
Oyster Culture Type	Hatchery-produced oysters		Wild oysters		
Activity	Hatchery-produced oysters grown off the bottom using some sort of gear (e.g., floating rafts near the surface or cages near the bottom)	Hatchery-produced oysters grown on the bottom using no gear	Moving wild oysters from one location to another.	Addition of substrate to the bottom to enhance recruitment of wild oyster larvae	None
Oyster Practice Title	Off-bottom private oyster aquaculture using hatchery-produced oysters	On-bottom private oyster aquaculture using hatchery-produced oysters	On-bottom private oyster aquaculture using transplanted wild oysters	On-bottom private oyster aquaculture using substrate addition	Private oyster aquaculture with no activity
***Panel Recommends for BMP Consideration**	Yes	Yes	No	Yes	No
Oyster Practice Category	A	B	C	D	E

* The Panel's recommendation on whether an oyster practice category should undergo BMP consideration was based on whether the practice enhances the production of new oysters. “Yes” indicates that the Panel endorses the use of the recommendations found in this report for those oyster practices because, in their opinion, these practices will enhance the production of new oysters, while "No" indicates the Panel's opinion that overall enhancement will not occur, and therefore, not endorsed.

Table 5b. Private oyster aquaculture practice categories recommended for BMP consideration and their definitions.

Category	Oyster Practice Title	Definition
A	Off-bottom private oyster aquaculture using hatchery-produced oysters	Hatchery-produced oysters grown off the bottom in the water column using sort of gear (e.g., floating rafts near the surface or cages near the bottom) in a private oyster aquaculture designated area where public fishing does not occur (e.g., water column leases) for eventual removal from the water.
B	On-bottom private oyster aquaculture using hatchery-produced oysters	Hatchery-produced oysters (e.g., spat-on-shell) grown directly on bottom using no gear in a private oyster aquaculture designated area where public fishing does not occur (e.g., bottom leases) for eventual removal from the water.
D	On-bottom private oyster aquaculture using substrate addition	Planting oyster shells or alternative substrate, such as granite, directly on the bottom to attract recruitment of natural (wild) oysters in private oyster aquaculture designated areas where public fishing does not occur (e.g., bottom leases) for eventual removal from the water.

5.2 Representative Oyster Practices for the Endorsed Private Oyster Aquaculture Categories

The representative oyster practices that occur in Chesapeake Bay that fall under each of the endorsed private oyster aquaculture practice categories defined in Table 5b are described below.

5.2.1 Category A: Off-Bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters

Representative off-bottom private oyster aquaculture methods using hatchery-produced oysters involve rearing hatchery-produced cultchless oysters or spat-on-shell oysters in rafts, cages, bags, trays, nets or suspended on lines above the sediment surface. Oysters are typically reared in the gear for over a year until they reach market size (76 mm) and then harvested for consumption. Examples include:

- Raft culture—Rafts use floatation devices (e.g., buoys, PVC, foam) to suspend plastic mesh bags on the water surface or cages just below the surface of the water. Oysters are typically submerged at the surface using rafts. Rafts are frequently monitored and cleaned, and the oysters are sorted for size and transferred between containers as they get larger. Oysters are typically removed from the containers once they reach market size.

- Cage culture—Oyster cages are constructed with metal or plastic mesh surrounding a rigid metal frame that sits on the seafloor. Oysters remain suspended off the bottom because the frame of the cages are designed to touch the bottom and keep oysters several inches above the sediment surface. Like rafts, cages are frequently monitored and cleaned, and the oysters are sorted for size and transferred between cages as they grow. The oysters typically remain in the cages until they reach market size.

In some cases, oysters are moved from one water column lease to another. Reasons for moving oysters include poor water quality (e.g., moving oysters from polluted waters to approved waters for harvesting or to change the taste profile (e.g., moving oysters to a more salty location).

5.2.2 Category B: On-Bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters

Representative on-bottom private oyster aquaculture methods include planting hatchery-produced oyster spat-on-shell directly on the bottom (no gear) for eventual removal from the water when oysters reach market size and can be harvested for consumption. Oysters typically require over two years to reach minimum market size, but oysters may be left in the water beyond this size. This practice may also involve moving the oysters from one bottom lease to another for eventual removal from the water. Reasons for moving oysters include poor water quality (e.g., moving oysters from polluted waters to approved waters for harvesting or to change the taste profile (e.g., moving oysters to a more salty location).

5.2.4 Category D: On-Bottom Private Oyster Aquaculture Using Substrate Addition

Representative on-bottom private oyster aquaculture using substrate addition methods involve planting oyster shell or an alternative substrate, such as granite, directly on the bottom (no gear) to attract recruitment of natural (wild) oysters for eventual removal from the water. Oysters produced using this practice are treated and harvested similarly to on-bottom, hatchery-produced cultured oysters.

6.0 Oyster-Associated Processes for Reduction Effectiveness Crediting Protocols

The Panel discussed the various oyster-associated nitrogen, phosphorus and suspended sediment reduction processes and currently identified the following eight individual reduction effectiveness crediting protocols:

1. Nitrogen Assimilation in Oyster Tissue
2. Nitrogen Assimilation in Oyster Shell
3. Enhanced Denitrification Associated with Oysters
4. Phosphorus Assimilation in Oyster Tissue
5. Phosphorus Assimilation in Oyster Shell
6. Suspended Sediment Reduction Associated with Oysters
7. Enhanced Nitrogen Burial Associated with Oysters
8. Enhanced Phosphorus Burial Associated with Oysters

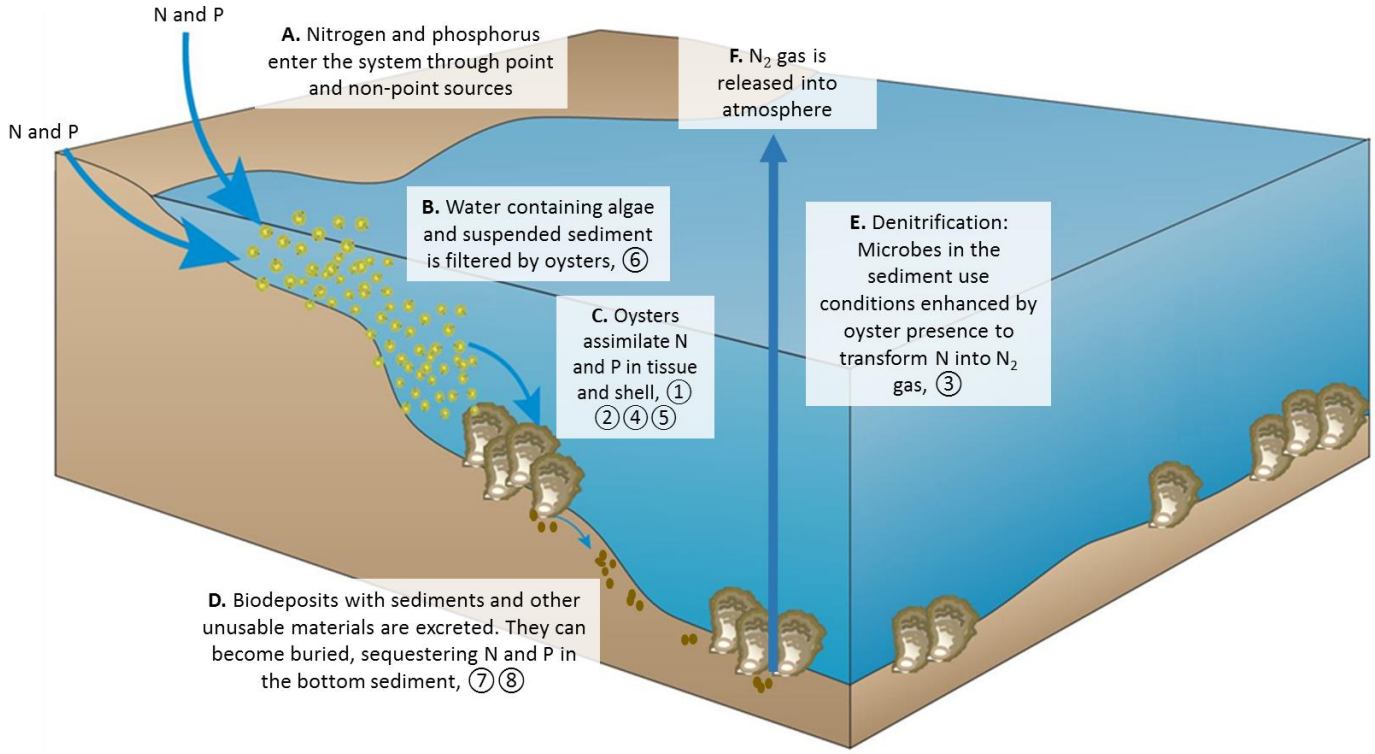
When algae or other organic matter are consumed by oysters through filter-feeding, the nitrogen and phosphorus within are assimilated in the oyster's tissue and shell. These nutrients are therefore unavailable to

water column processes for a range of timescales dependent on whether oyster tissue or shell is considered (Kellogg et al. 2013). Oyster shells, whether buried in the bottom sediment or dissolved back into the water column, represent sequestration of nitrogen and phosphorus over long periods of time; while nutrients sequestered in tissue may be cycled back into the environment on a shorter timescale (Kellogg et al. 2013, Newell 2004 and sources therein). Oysters removed from the water for consumption or other purposes also remove the nutrients sequestered within.

Denitrification, which involves microbial transformation of biologically available nitrogen to N_2 gas, is an additional means by which nitrogen is removed from the system, and it is enhanced by the presence of oysters. Oysters enhance the formation of a heterogeneous sediment surface with oxic and anoxic sediments in close proximity and supply carbon in the form of biodeposits to the sediment surface, two characteristics that are necessary for denitrification to occur (Smyth et al. 2015 and sources therein, Gutierrez and Jones 2006, Kellogg et al. 2013). Once nitrogen is transformed to N_2 gas, it travels through the water column and escapes into the atmosphere.

The oyster filter feeding process removes inorganic particles, such as suspended sediments, and organic particles, such as algae containing nitrogen and phosphorus, from the water column, increasing water clarity (Grizzle et al. 2008). Like oyster shells, biodeposits may become buried (Newell 2004), which serves to remove nutrients from the water column for long time scales. Figure 6a depicts these N, P, and suspended reduction processes associated with oysters.

Figure 6a. Oyster-associated processes that reduce nitrogen (N), phosphorus (P), and suspended sediment. The numbers in circles correspond with the reduction effectiveness crediting protocols.



The Panel concurred that Protocols 1 and 4, involving nitrogen and phosphorus assimilation in oyster tissue, have sufficient data to recommend reduction effectiveness estimates for oyster aquaculture practices (see Section 7.0). The Panel also believes that there may be sufficient data to recommend estimates for nitrogen and phosphorus assimilation in shell (Protocols 2 and 5), but have decided to include these recommendations in the second incremental report because it is unclear at present how to address the issue of shells from harvested oysters being eventually returned to the Bay. Because oyster aquaculture and reef restoration practices rely heavily on oyster shell and with oyster shell being a limited resource, any decrease of shell being returned to the Bay could have unintended negative impacts on these practices.

The Panel agreed that Protocols 3, 7, and 8 would require more in depth discussion given the variability in denitrification data and the complexity of quantifying the enhanced burial of nutrients associated with an increase in oysters. The term “enhanced” and “associated with oysters” were added to Protocols 3, 7, and 8 because the Panel wanted to be clear that the oysters are not directly carrying out denitrification or burial, but instead enhance these processes by increasing the movement of organic particulate matter from the water column to the bottom through filtering and increasing the habitat area (via reef structures) for other contributing organisms to populate. The Panel is mindful that denitrification-related reduction effectiveness recommendations will have to adequately address variability. The Panel also recognizes that burial rates of

nitrogen and phosphorus will depend on sedimentological and physical characteristics of sites and requires demonstrable long term sequestration for consideration of whether Protocols 7 and 8 should be applied to certain oyster practice categories. The Panel plans to include recommendations on the reduction effectiveness from enhanced denitrification associated with oysters in the second incremental report. Even though there is likely less information on enhanced burial of nutrients associated with oysters, the Panel still felt strongly that these protocols should be included because of their potential in reducing nutrients from the water column. However, the Panel agreed to put enhanced nitrogen and phosphorus burial protocols on hold until the CBP Management Board determines whether crediting burial from oyster practices would be legal since the pollutants are technically still in the Bay.

The Panel had an in depth conversation concerning how suspended sediment could be incorporated into a crediting protocol (protocol 6) since suspended sediment would only be removed from the water column and deposited on the bottom by the oysters and not removed from the Bay. The Panel decided that it would be important for the CBP Partnership Management Board to first evaluate the policy/legal issue of this (i.e., can removal from the water column followed by deposition on the bottom be incorporated in the reduction effectiveness credit?). The Panel is putting the evaluation of this protocol on hold until the Management Board reviews pertinent policy issues.

7.0 Reduction Effectiveness Estimates for Nitrogen and Phosphorus Assimilated in Oyster Tissue

This section describes the Panel's recommendations concerning the reduction effectiveness estimates for the "Nitrogen Assimilation in Oyster Tissue" and the "Phosphorus Assimilation in Oyster Tissue" reduction effectiveness crediting protocols specific to *C. virginica* oysters when they are removed from the water via private oyster aquaculture practices. The Panel is endorsing these recommendations only for practices in the following oyster practice categories (see description of practices in Section 5.0):

- Off-bottom private oyster aquaculture using hatchery-produced oysters
- On-bottom private oyster aquaculture using hatchery-produced oysters
- On-bottom private oyster aquaculture using substrate addition

Concerning assimilation of nitrogen and phosphorus in oyster tissue, the Panel is cognizant of the fact that these recommendations could also apply to removed oysters from public fishery practice categories (refer to Table 1a), since from a biological perspective, they are assimilating nitrogen and phosphorus in their tissue that the default estimates could apply to. However, the Panel felt that the practices in these categories would benefit from more discussion given that they could be influenced by existing oysters in the public fishery, therefore, making it difficult to distinguish the reduction effectiveness of the practice itself (i.e., increase of new oysters as a result of the practice). On the other hand, the endorsed practices are minimally or not at all influenced by existing oysters given that designated bottom for private oyster aquaculture are in areas with minimal oyster populations; therefore, oysters that are grown and removed from these practices reflect the reduction effectiveness from the practice itself.

The Panel is recommending two options for the "Nitrogen Assimilation in Oyster Tissue" and "Phosphorus Assimilation in Oyster Tissue" protocols' reduction effectiveness estimates:

1. Default estimates for recommended practices regardless of location (Section 7.1).

2. Site-specific estimates developed by the BMP implementer, in coordination with the State and CBP, using the Panel's recommended methodology (Section 7.2).

The Panel reasoned that option 1 could be applied as long as the BMP implementer meets the qualifying conditions described in Section 8.0. Option 2 can be pursued if the BMP implementer decides they want to develop a site-specific estimate (see Section 7.2) for their practice. Because the Panel's default estimates are intentionally conservative (see Section 7.1), the Panel felt it was important to give BMP implementers the option to develop site-specific estimates that might have higher values than default estimates. The same qualifying conditions apply to all estimates.

Default reduction effectiveness estimates for assimilated nitrogen and phosphorus are described in detail in Section 7.1. While models exists that can be used to estimate growth and assimilation (e.g. FARM model, Appendix C), the Panel agreed that the reduction effectiveness estimates should be developed using an empirical approach. Default estimates are calculated using regressions based on existing data to convert oyster shell height to oyster biomass in terms of soft tissue dry weight of shell height midpoints and multiplying by the percent nitrogen and phosphorus content in dry tissue. Separate regressions were developed for diploid and triploid oysters due to clear differences in the shell height to tissue dry weight regression curves for these two types of oysters. The shell height midpoints are based on oyster size class ranges recommended by the Panel. The percent nitrogen and phosphorus content in oyster tissue were derived using averages from existing data from the Atlantic Coast of the U.S.

7.1 Option 1: Default Reduction Effectiveness Estimates for Nitrogen and Phosphorus Assimilated in Oyster Tissue

The Panel decided that there was sufficient empirical data to establish conservative default reduction effectiveness estimates for nitrogen and phosphorus assimilated in the soft tissue of diploid and triploid oysters using the following three-step process:

Step 1: Determine the oyster shell height to tissue dry weight regression equations for diploid and triploid oysters

This step involves the analysis of empirical data to establish regression equations to convert shell height to soft tissue dry weight (further described in Section 7.1.1). Refer to Figure 7.1 for the shell height measurement location on an oyster shell.

Step 2: Establish oyster size class ranges for the shell height midpoints that will be used to calculate the oyster soft tissue dry weight

This step involves establishing oyster size class ranges and using the midpoint of those ranges in the regression equations from Step 1 to calculate the soft tissue dry weight needed for Step 3 (further described in Section 7.1.2).

Step 3: Establish and apply the percent nitrogen and phosphorus content in oyster tissue to determine the reduction effectiveness estimates

This step involves multiplying the oyster soft tissue dry weights from Step 2 by the established percent nitrogen and phosphorus content in the oyster tissue (further described in Sections 7.1.3) to determine the reduction effectiveness estimates for the different oyster size class ranges (see Section 7.1.4).

Although similar to the approach recommended by STAC (STAC 2013 and 2014), the Panel's estimates explicitly consider variability resulting from ploidy (i.e., diploid and triploid), culture location (i.e., off-bottom in water column and on-bottom), season of harvest, and locations with different environmental conditions (e.g. salinity). In addition, the Panel also established estimates for different oyster shell height size class categories instead of one default estimate based on a 76 mm (3 inch) oyster.

7.1.1 Step 1: Oyster Shell Height to Tissue Dry Weight Regression Equations

The first step of the default nitrogen and phosphorus assimilated in oyster tissue reduction effectiveness estimates was to identify appropriate regression curves for the relationship between oyster shell height (Fig. 7a) and soft tissue biomass in terms of dry weight based upon actual shell height and tissue dry weight measurements from individual oysters.

Figure 7a. The measurement location for shell height. Shell height is the longest distance (parallel to the long axis) between the hinge and lip of the oyster. Note that shell height is also referred to as oyster shell length in some studies.

In reviewing the existing scientific literature from the Chesapeake Bay region, the Panel found that the shell height to biomass regressions highly variable (Table 7a), likely because the individual studies focused on different characteristics that could influence oyster growth (i.e., subtidal versus intertidal reefs, low versus high salinity environmental conditions) and/or used different biomass metrics (i.e., tissue dry weight versus tissue ash-free dry weight). Because incineration of samples is a required step in determining the ash-free dry weight and nitrogen content cannot be derived from an incinerated sample, it is not feasible to determine both ash-free dry weight and nitrogen content for the same individual. ***Therefore, the Panel concluded that only datasets from within the Chesapeake Bay watershed that included individual oyster shell heights and corresponding biomass in terms of tissue dry weight were suitable for inclusion in the compiled dataset used to determine default estimates for nitrogen and phosphorus contained in oysters.***

Table 7a. Summary of shell height to soft tissue dry weight biomass regression models for the Eastern Oyster grown in a variety of locations and conditions near or within the Chesapeake Bay. DW=dry tissue weight (g), AFDW=ash-free dry tissue weight (g), WW=wet weight (g), and SH=shell height (mm).

Ploidy	Location	Habitat/Culture Method	Equation	R^2	Reference
Diploid	Great Wicomico, Rappahannock and Piankatank Rivers, VA	Subtidal reefs - Spring	$AFDW=0.00004 \cdot SH^{2.4257}$	0.8	Ross & Luckenbach unpubl. data
		Subtidal reefs - Summer	$AFDW=0.00007 \cdot SH^{2.1704}$	0.7	
		Subtidal reefs - Fall	$AFDW=0.00001 \cdot SH^{2.6497}$	0.9	
		Subtidal reefs - All seasons	$AFDW=0.00002 \cdot SH^{2.5988}$	0.9	
	James River, VA	Subtidal reefs	$DW=0.000423 \cdot SH^{1.7475}$	-	Mann & Evans 1998
	Lynnhaven Inlet, VA	Intertidal Reefs	$AFDW=0.0003 \cdot SH^{1.9352}$	0.8	Ross & Luckenbach unpubl. data
		Subtidal Reefs	$AFDW=0.00003 \cdot SH^{2.3465}$	0.7	
		All Reefs	$AFDW=0.00006 \cdot SH^{2.2809}$	0.7	
	Spencer's Creek, VA	Floating Caged Aquaculture	TL (mm) = -35.5408 + (0.955 * shell DW (g))	0.8	Higgins et al. 2011
	St. Jerome Creek, MD				
	Upper Chesapeake Bay, MD	Subtidal reefs - Natural and Restored	$DW=0.00003 \cdot SH^{2.35}$	0.7	Liddel 2008
	West River, MD	Floating Tray	$WW=0.000068 \cdot SH^{2.49}$	0.9	Paynter & DiMichele 1990
Triploid	Chincoteague Bay	Floating Aquaculture	$AFDW=0.00003 \cdot SH^{2.3952}$	0.8	Ross & Luckenbach unpubl. data

The Panel sent out requests to various researchers for oyster data within Chesapeake Bay and its tributaries that included both oyster shell height and soft tissue dry weight measurements that captured different sizes, ploidy, culture methods, season, and locations with different environmental conditions. For inclusion in the Panel's compiled dataset, the Panel required that data be of high to medium quality and suitability based upon study location, data collection methods, data quality, and data age. After closely examining data from unpublished studies and studies older than five years to data from recent, peer-reviewed datasets, the Panel concluded that the data warranted inclusion because there was no indication that these data were outliers. From their analysis, the Panel observed two very distinct shell height to tissue dry weight regression curves when comparing the diploid and triploid datasets and agreed that ploidy was likely driving differences in biomass and that these datasets would be sufficient to develop separate conservative oyster shell height to soft tissue dry weight regression equations for diploid and triploid oysters. The data used to establish the diploid and triploid regression equations are described in Section 7.1.1.1 and the method used, considerations, and recommended equations are described in Section 7.1.1.2.

7.1.1.1 Description of Data used to derive the Oyster Shell Height to Tissue Dry Weight Regression Equations for Diploid and Triploid Oysters

The Panel ultimately included data on a total of 5,750 diploid oysters collected between 1998 and 2015 from seven data sources (four published and three unpublished) and 1,066 triploid oysters collected between 2005 and 2007 from one published data source. Data were collected using standard methods, were from studies within the Chesapeake Bay and its tributaries, included a large range of shell heights (diploid shell heights: minimum = 13.5 mm, 0.53 inches, maximum = 184 mm, 7.24 inches; triploid shell heights: minimum = 10.2 mm, 0.40 inches, maximum = 139 mm, 5.47 inches), and included measurements from a variety of culture methods, seasons, and habitat locations (Figure 7b, Table 7b, and Table 7c). Appendix D includes a summary of the studies used in the diploid and triploid regression analyses, description of data not used in the analyses, and description of other potential data sources that could be pursued to expand the compiled dataset.

Figure 7b. General locations where oysters in the compiled diploid and triploid datasets were grown grouped by their approximate location in the CBP Bay Program Bay (U.S. EPA 2004) and salinity characteristics based on the U.S. Army Corp. of Engineers salinity gradient maps (mesohaline =5-18 ppt, polyhaline = 19-30 ppt; http://www.nab.usace.army.mil/Missions/Environmental/Oyster-Restoration/Oyster-Master-Plan/).

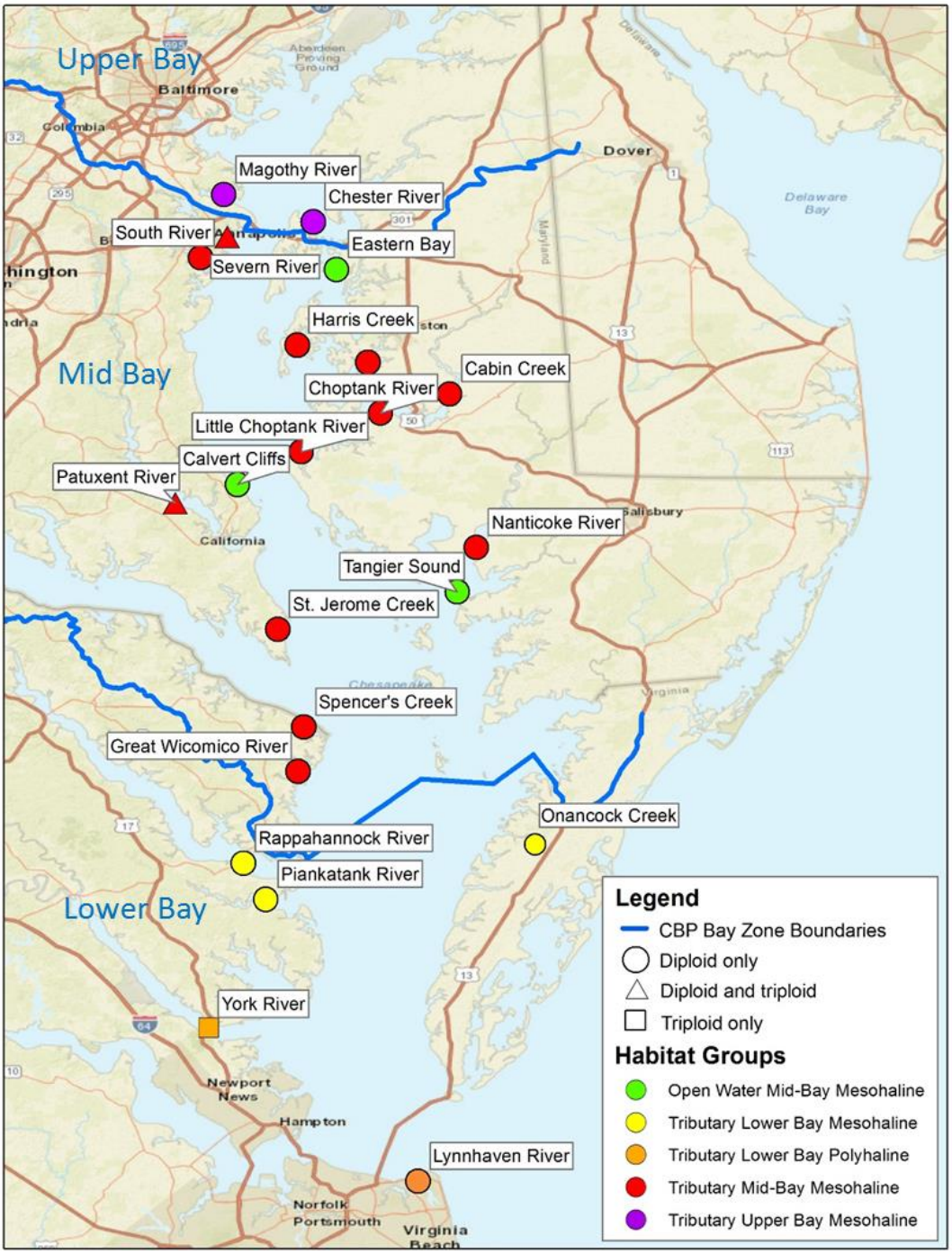


Table 7b. Summary of diploid oyster data (n = 5,750 individual oysters) and corresponding growth influencing factors (i.e., culture method, location oysters were grown, and year and season removed) from the different data sources used in the diploid regression analysis to determine the equation to convert shell height (mm) to soft tissue dry weight (g). Additional literature/data review information on these data sources can be found in Appendix D.

Data Sources	Related Culture Method	Locations	Year Oysters Removed	Season Oysters Removed	% Total Oysters Used in Regression Analysis
Higgins unpubl. data	Bottom Oyster Planting	Choptank River, MD	2008	Spring	0.16
		Lynnhaven River, VA			0.31
Higgins et al. 2011	Off Bottom in Water Column	Spencer's Creek, VA	2008	Spring	0.80
		St. Jerome Creek, MD			0.66
Kellogg unpubl. data	Bottom Oyster Planting	Harris Creek, MD	2015	Fall	2.80
				Winter	2.87
				Spring	2.92
				Summer	9.83
	Off Bottom in Water Column and Bottom Oyster Planting Combination	Onancock Creek, VA	2012	Spring	1.90
				Summer	0.02
Kellogg et al. 2013	Bottom Oyster Planting	Choptank River, MD	2009	Fall	0.97
			2010	Spring	0.85
				Summer	1.91
Luckenbach and Ross 2009 (Part 1 of Report)	Bottom Oyster Planting	Great Wicomico River, VA	2004, 2005	Fall	2.50
			2004	Spring	0.57
			2005	Summer	3.77
		Lynnhaven River, VA	2005	Fall	1.62
				Summer	2.07
		Piankatank River, VA	2004	Fall	0.71
			2004, 2005	Spring	0.87
				Summer	1.41
		Rappahannock River, VA	2004	Fall	0.35
			2004, 2005	Spring	1.72
				Summer	0.54

Luckenbach and Ross 2009 (Part 3 of Report)	Bottom Oyster Planting	Lynnhaven River, VA	2005, 2006	Spring	14.24
			2006	Winter	2.26
Paynter unpubl. data found in Liddel 2008	Bottom Oyster Planting	Cabin Creek, MD	1998	Fall	0.17
			2000	Spring	0.10
			1998	Summer	0.26
		Calvert cliffs, MD	1998	Fall	0.43
			1999	Spring	0.09
			1998, 1999	Summer	0.83
		Chester River, MD	2001, 2002, 2004	Fall	2.37
			2002	Spring	1.88
			2001, 2002, 2004	Summer	2.38
		Choptank River, MD	2000, 2002	Fall	3.20
			2001, 2002	Winter	1.67
			2001, 2002, 2004	Summer	5.77
		Eastern Bay, MD	2001	Fall	0.49
			2002	Winter	0.68
			2004	Spring	0.21
			2001, 2002	Summer	2.09
		Little Choptank River, MD	2004	Summer	0.43
		Magothy River, MD	2001, 2002	Fall	1.15
			2002	Spring	0.47
			2001, 2002	Summer	0.87
		Nanticoke River, MD	2001, 2002	Fall	0.21
			2002	Summer	0.26
		Patuxent River, MD	2001	Fall	0.31
				Spring	1.37
			2000, 2001, 2002	Summer	4.59
		Severn River, MD	2001, 2002	Fall	1.39
			2001	Winter	0.35
			2002	Spring	0.17
			2001, 2002	Summer	1.51
		South River, MD	2001, 2002	Fall	0.43
			2002	Spring	0.26
				Summer	0.24
		Tangier Sound, MD	2000, 2001, 2002	Fall	2.00
			2001, 2002	Summer	2.14
		Tred Avon River, MD	2000	Fall	0.26
			2001	Summer	0.30

Table 7c. Summary of triploid oyster data (n = 1,066 individual oysters) and corresponding growth influencing factors (i.e., culture method, location oysters were grown, and year and season removed) from the different data sources used in the triploid regression analysis to determine the equation to convert shell height (mm) to soft tissue dry weight (g). Additional literature/data review information on these data sources can be found in Appendix D.

Data Sources	Related Culture Method	Locations	Year Oysters Removed	Season Oysters Removed	% Total Oysters Used in Regression Analysis
Kingsley-Smith et al. 2009	Off Bottom (cages near bottom; experiment was designed to be representative of oysters on the bottom)	Patuxent River, MD	2006, 2007	Fall	11.16
			2005	Winter	5.53
			2006, 2007	Spring	11.16
				Summer	11.26
		Severn River, MD	2006, 2007	Fall	14.07
			2005	Winter	5.63
			2006, 2007	Spring	11.26
				Summer	9.47
		York River, VA	2005, 2006	Fall	9.47
			2006	Spring	5.63
				Summer	5.35

7.1.1.2 Recommended Regression Equations to Convert Shell Height to Tissue Dry Weight for Diploid and Triploid Oysters

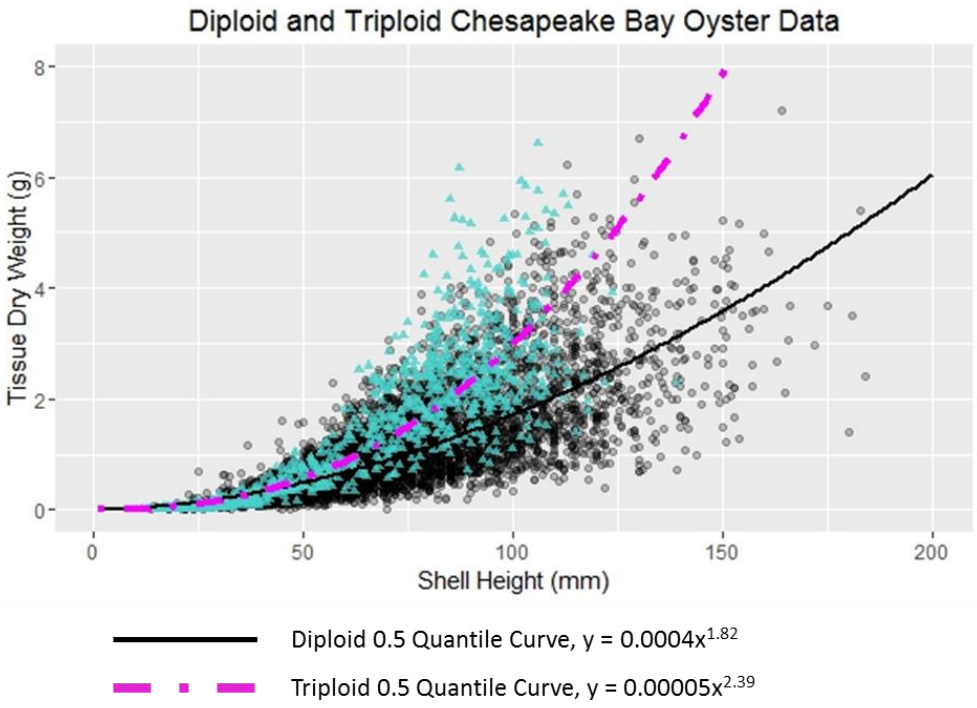
Given that there was a range of values for dry weight observed across the range of oyster shell heights (Figures 7c, 7d and 7e), the panel used the quantile regression statistical method (Koenker and Bassett, 1978) that was not sensitive to the presence of outliers, as the appropriate technique to derive and select conservative equations that could be used to convert shell height to soft tissue dry weight using the datasets from Table 7b (for diploid equation) and Table 7c (for triploid equation). Quantile regression is a commonly used statistical method that is employed across a variety of disciplines to explore relationships between two variables of interest (Yu et al. 2003). Regression quantiles represent a series of planes that contain an increasing proportion of sample observations (Cade and Noon, 2003). When the 0.5 quantile is calculated, this corresponds to the median of the dataset; i.e., 50% of the y values lie above and 50% of the y values lie below each specified x value. It is possible to calculate both linear (lrq) and nonlinear quantile regressions (nlrq) using the R statistical package quantreg (Koenker 2006; Koenker 2016), which was necessary since the relationship between oyster dry weight and shell length has been shown to be a power function. Overall, quantile regression was favored by the Panel because it better addresses datasets where high variability exists, which is the case for converting oyster shell height to soft tissue dry weight.

The Panel recommends using the 0.5 quantile oyster shell height (mm) to soft tissue dry weight (g) regression equations to derive default nitrogen and phosphorus reduction effectiveness estimates.

The 0.5 quantile was calculated for the entire dataset based on the equation $y = ax^b$, using the nlrq function and starting values for *a* and *b* based on mean estimates of the power function. Analyses indicated that differences in ploidy resulted in clear differences in the relationship between oyster shell height and oyster soft tissue dry weight, warranting the use of separate regression equations for diploid and triploid oysters (Figure 7c).

As a result, the Panel recommends that the corresponding 0.5 quantile regression equations be used to derive separate estimates from diploid and triploid oysters.

Figure 7c. Shell height to tissue dry weight 0.5 quantile regression curves for diploid and triploid oysters. Refer to Tables 7b and 7c for data that was used to develop the curves.



The following is the Panel's recommended 0.5 quantile regression equation for diploid oysters (Figure 7d):

$$y = 0.0004x^{1.82}$$

where x equals the oyster shell height in millimeters and y equals the soft tissue dry weight in grams.

The following is the recommended 0.5 quantile regression equation for triploid oysters (Figure 7e):

$$y = 0.00005x^{2.39}$$

where x equals the oyster shell height in millimeters and y equals the soft tissue dry weight in grams.

Figure 7d. The Panel's recommended equation to convert diploid oyster shell height in millimeters (x-variable) to tissue dry weight in grams (y-variable) based on the 0.5 quantile regression curve ($y = ax^b$; n = 5,750 oysters). Table 7b summarizes this data by data source, culture method, location, and date and season oysters were removed/harvested from Chesapeake Bay and its tributaries. The solid red line depicts the 50th quantile and the dashed red lines represents the error terms *a* and *b*.

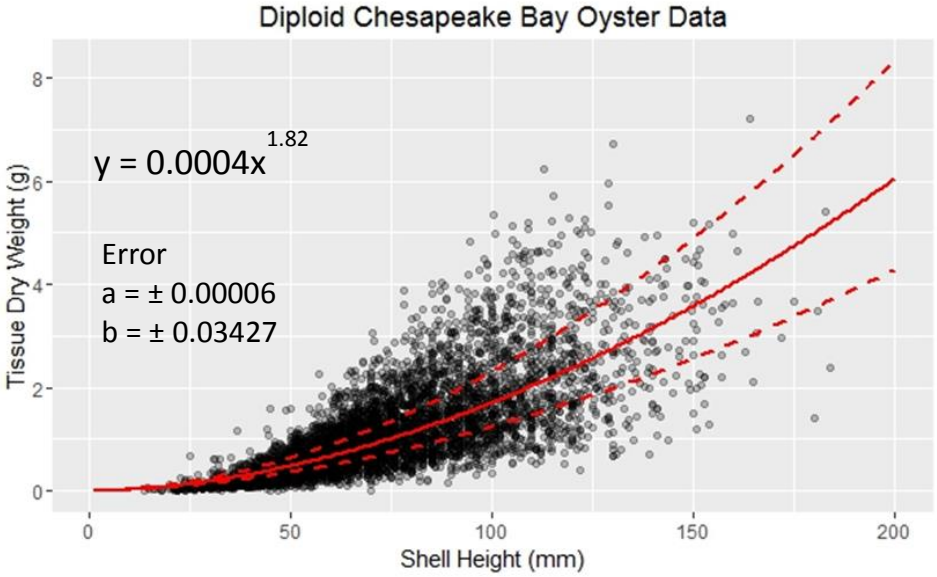
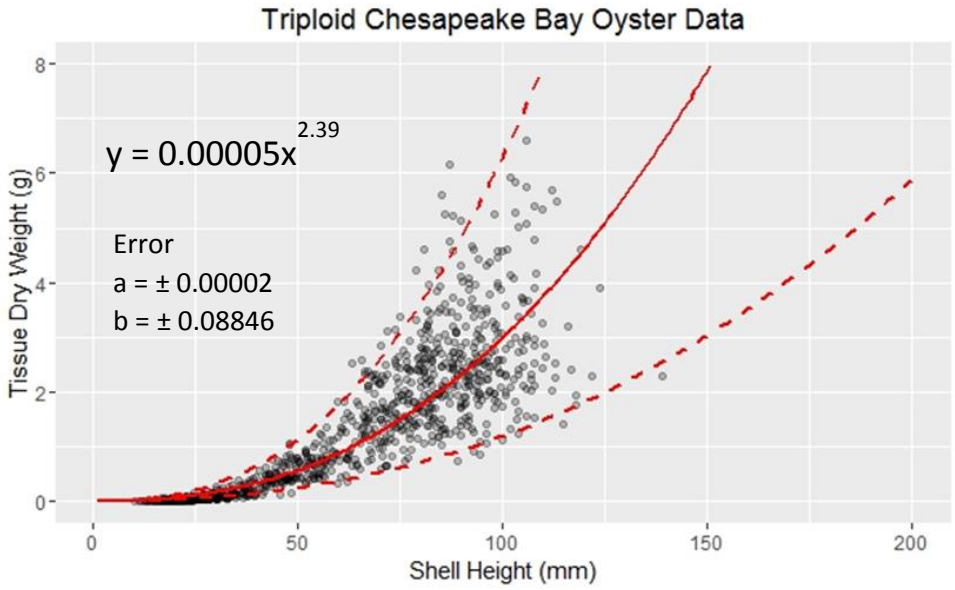


Figure 7e. The Panel's recommended equation to convert triploid oyster shell height in millimeters (x-variable) to tissue dry weight in grams (y-variable) based on the 0.5 quantile regression curve ($y = ax^b$; n = 1066 oysters). Table 7c summarizes this data by data source, culture method, location, and date and season oysters were removed/harvested from Chesapeake Bay and its tributaries. The solid red line depicts the 50th quantile and the dashed red lines represents the error terms *a* and *b*.



In addition to ploidy, the Panel also considered other factors that could influence growth, including differences in culture method, season of harvest and locations with different environmental conditions. In all cases, the Panel found that either there was not sufficient evidence that the factors significantly altered shell height to tissue dry weight relationships or that there were insufficient data to create robust regressions for all levels of a particular factor. Thus, at this time, the Panel does not recommend developing separate equations for these factors but recognizes that additional equations may be warranted if additional data are incorporated into the compiled dataset. In the meantime, the Panel agreed that the 0.5 quantile regression curves of the current diploid and triploid complied datasets allow for conservative reduction effectiveness estimates. Table 7d briefly summarizes the Panel's rationale for why the 0.5 quantile regression equations would support conservative estimates for nitrogen and phosphorus assimilated in oyster tissue. Details of these analyses and considerations are described in Appendix D.

Table 7d. Rationale for why the Panel concluded that the 0.5 quantile shell height to tissue dry weight regression equations would be conservative. For season and location factors, results were similar except the triploid data did not include all the habitat groups evaluated. For culture method, conclusions are based on the diploid dataset because only the off-bottom culture method was available for the triploid data. The Panel's evaluation leading to these conclusions is presented in Appendix D.

Factor	Conservative Estimate Rationale for 0.5 Quantile Regression Equation
Ploidy	Triploid oysters clearly exhibited higher biomass per mm shell height than diploid oysters and appeared to be driving the biomass differences observed when the two datasets were used together, warranting separate reduction effectiveness estimates for diploid and triploid oysters. The 50^{th} quantile regression equations were chosen by the Panel because it represents the median of the data, allowing for an even likelihood of underestimating or overestimating the biomass. The Panel felt this was the best quantile to develop the default reduction effectiveness estimates because it best captures the variability in the data.
Culture Method	Oyster data for the culture methods that were evaluated (i.e., off-bottom, on-bottom, and combination of both) either skewed above or were similar to the 0.5 quantile curve of the entire dataset, suggesting that the recommended equations will more likely underestimate the tissue dry weight and hence, the reduction effectiveness.
Season	The fall, winter, and spring oyster data skewed above the 0.5 quantile curve of the entire dataset, while the summer data skewed slightly below. Given that 3 seasons skewed above the recommended 0.5 quantile curves and the reduction effectiveness is based on annual reporting with growers typically harvesting year round, the Panel felt that the reduction effectiveness would balance out (i.e., any potential overestimation would be negated by instances of underestimation).
Location/Environmental Condition	Sampling locations were grouped by location within the Bay (Upper, Mid, Lower; tributary or open-Bay) and by salinity (mesohaline and polyhaline). Oyster data for four of the five habitat groups skewed above or were similar to the 0.5 quantile curve of the entire dataset, suggesting a greater chance to underestimate the reduction effectiveness. The remaining habitat group was only slightly below the 0.5 quantile curve of the entire dataset.

7.1.2 Step 2: Oyster Size Class Range Midpoints to Determine the Oyster Soft Tissue Dry Weight

The second step of the default nitrogen and phosphorus assimilated in oyster tissue reduction effectiveness estimates involves the establishment of oyster shell height midpoints for different oyster size class ranges to calculate the oyster soft tissue biomass that will be used with the regression equations from Step 1. The Panel opted to use this midpoint size class approach because it is more realistic in regards to implementing the BMP than requiring growers to measure all removed oysters (typically more than 1,000,000 oysters a year per grower). The oyster size class ranges are based on midpoints that reflect what is typically reported (e.g., harvested oysters are usually reported by whole numbers for the shell height in inches).

Table 7e outlines the Panel's recommended oyster size class categories and midpoints for determining the oyster soft tissue biomass using regression equations from Step 1 (see Section 7.1.1).

Table 7e. Recommended oyster size class ranges and midpoints in inches and millimeters (mm). Note that the shell height midpoints in millimeters would be the input into the diploid and triploid regression equations from Step 1 (see Section 7.1.1) to determine the oyster soft tissue dry weight biomass.

Oyster Size Class Range (Shell Height in inches)	Oyster Size Class Range (Shell Height in mm)	Approximate Shell Height Midpoint (in inches)	Shell Height Midpoint to Use with Regression Equation (Shell Height in mm)
a. 2.0 - 2.49	~50 - 63	2.25	57
b. 2.5 - 3.49	~64 - 88	3.0	76
c. 3.5 - 4.49	~89 - 114	4.0	102
d. 4.5 - 5.49	~115 - 139	5.0	127
e. ≥ 5.5*	≥ 140	6.0	152

*Midpoint based on 5.5-6.49 range

The oyster soft tissue biomass associated with each oyster size class range is determined by using the shell height midpoints in millimeters of each oyster size class range with the diploid and triploid regression equations from Step 1 (see Section 7.1.1). Table 7.6 shows the results of these calculations. The resulting biomass is needed to determine the total nitrogen and phosphorus content in the oyster tissue.

Table 7f. The diploid and triploid oyster tissue dry weight values for the default reduction effectiveness estimates. The oyster size class range shell height midpoints in millimeters from Table 7e were used with the diploid and triploid oyster shell height (mm) to soft tissue dry weight (g) regression equations to calculate the soft tissue biomass.

Oyster Size Class Range (Shell Height in inches)	Diploid Tissue Dry Weight from Midpoint (g/oyster)*	Triploid Tissue Dry Weight from Midpoint (g/oyster)**
a. 2.0 - 2.49	0.63	0.79
b. 2.5 - 3.49	1.06	1.56
c. 3.5 - 4.49	1.81	3.16
d. 4.5 - 5.49	2.70	5.33
e. ≥ 5.5	3.74	8.20

*Diploid 0.5 quantile regression equation: oyster tissue dry weight (g) = 0.0004 * Shell Height (mm)$^{1.82}$

**Triploid 0.5 quantile regression equation: oyster tissue dry weight (g) = 0.00005 * Shell Height (mm) $^{2.39}$

The Panel decided that the above oyster size class ranges and associated midpoints would conservatively reflect the oyster soft tissue dry weight biomass of oysters being harvested. Oyster aquaculture harvest sizes vary according to the individual aquaculturists' marketing strategy. Often cultured oysters are marketed by specific size-classes. For example, "cocktail" or "petite"= 2 - 3 inches shell height, "standards" or "market" = 3.0 - 4.0 inches shell height, "extra large" = 4.0 - 5.0 inches shell height, jumbos > 5 inches shell height (see http://www.pangeashellfish.com/blog/the-culling-process-oyster-grades-and-sizes). However, the names and size ranges do vary between growers and even among different "brands" of oysters within a single aquaculture company (see http://www.ballardfish.com/products/oysters for sizes marketed by Ballard Fish & Oyster Co.). In contrast to oyster aquaculture, the wild fishery minimum size at harvest is set by regulation (3 inch, or 76 mm, shell height in VA and MD). While stock enhancement practices are not being considered yet due to ongoing Panel deliberation, these size class ranges would also be suitable for these practices.

7.1.3 Step 3: Percent Nitrogen and Phosphorus Content in Oyster Tissue

The third step of the reduction effectiveness estimates involve using percent nitrogen and phosphorus content in oyster tissue to determine how much nitrogen and phosphorus is contained in the oyster soft tissue dry weight (calculated by multiplying the tissue dry weight by the percent nitrogen and phosphorus content values). The panel conducted a literature review of studies that measured the percent nitrogen and phosphorus content in oyster tissue of C. *virginica* and concluded that the nitrogen and phosphorus content in oyster tissue was well constrained in estuaries along the Atlantic Coast of the United States. The Panel reasoned that the variation in total nitrogen and phosphorus content in tissue is driven more by biomass than the percent content (similar to conclusions found in Carmichael et al. 2012); therefore, they felt it would be

appropriate to use the average percent content from the Atlantic coast studies. However, the Panel agreed that the average should be more weighted by Chesapeake Bay sites and therefore used the site-specific averages for studies within Chesapeake Bay and the overall average (sites combined) for studies outside of Chesapeake Bay.

The majority of studies found reported % nitrogen dry weight tissue content for diploid oysters. Only 1 study (Reitsma et al. 2014) included measurements from triploid oysters from off bottom aquaculture in Cape Cod, MA. There were also a few studies that reported the % phosphorus content in tissue for diploid oysters, but the Panel were not able to find any studies on triploid oysters. Given that the nitrogen and phosphorus content in oyster soft tissue is well constrained and large changes in the total nitrogen and phosphorus in oyster tissue appear to be driven by the oyster's biomass and not the % nitrogen and % phosphorus content in tissue (see Section 7.1.1; Carmichael et al. 2012), the Panel concluded that the average percent nitrogen and phosphorus content values from diploid oysters can be applied to triploid oysters.

The Panel recommends an average percent nitrogen content in oyster tissue of 8.2% and an average percent phosphorus content of 0.9%. Studies used to develop the recommended nitrogen content in oyster tissue of 8.2% and phosphorus content of 0.9% are further discussed in Sections 7.1.3.1 and 7.1.3.2, respectively. Appendix D includes a summary of the literature review.

7.1.3.1 Recommended Percent Nitrogen Content in Oyster Tissue

The Panel reviewed the literature and found eight published and three unpublished studies that measured and reported the percent nitrogen content in oyster tissue (summaries can be found in Appendix D). The average nitrogen percent content from these studies ranged from 5.64 in Fox Point, Great Bay, NH (Grizzle and Ward 2011) to 11.8 in Mobile Bay, AL (Dalrymple and Carmichael 2015). Even though the nitrogen content in oyster tissue, expressed as a percentage of dry weight, is relatively well constrained for estimate development purposes, the Panel agreed that it would be most conservative to use the average percent nitrogen content in oyster tissue from only the Atlantic Coast studies, resulting in the exclusion of one study in Mobile Bay, AL (Dalrymple and Carmichael 2015). The Panel also decided to omit Grizzle et al. 2016 and Newell and Mann 2012 because Grizzle et al. 2016 only reported size averages and not site averages (site averages would have to be estimated from a graph) and Newell and Mann 2012 didn't report the sample size or how the measurements were obtained; however, the Panel noted that the % nitrogen content was comparable to the other studies (~7.8% and 7.0%, respectively). The Panel also left out unpublished Chesapeake data from Higgins from the Choptank River in MD and Lynnhaven River in Virginia because the data has not been fully analyzed yet. However, preliminary analyses show that the oysters in the Choptank River would have a % nitrogen mean in tissue of 8.2% (n = 9 oysters) and in the Lynnhaven River, 8.8% (n = 18 oysters). These values are comparable to the values used to determine the average % nitrogen content in tissue for the reduction effectiveness estimates.

The Panel recommends a % nitrogen content in oyster tissue of 8.2% to be used to calculate the nitrogen assimilation in oyster tissue reduction effectiveness estimates. This number is based on 5 published studies and 1 unpublished study on diploid oysters from Atlantic Coast estuaries found in Table 7g. These studies are further described in Appendix D. These studies capture varying culture methods (off bottom and on bottom), seasons, and environmental conditions. Data gaps include no data on triploid oysters or during the winter season. Concerning triploid oysters, the Panel did find a Sea Grant funded study that measured the nitrogen content of triploid oysters grown off bottom in Cape Cod, MA (Reitsma et al. 2014). Their results showed that the average % nitrogen in tissue was 8.5%, which is comparable and within the range of the diploid measurements.

Based on the six studies included in analyses (Table 7g; Appendix D), ***the Panel recommends a % nitrogen content in oyster tissue of 8.2% to be used to calculate the nitrogen assimilation in oyster tissue reduction effectiveness estimates.*** These studies capture a variety of culture methods (off bottom and on bottom), seasons, and environmental conditions. They do not include any data on triploid oysters or on oysters collected during winter. Concerning triploid oysters, the Panel did find a Sea Grant funded study that measured the nitrogen content of triploid oysters grown off bottom in Cape Cod, MA (Reitsma et al. 2014). Their results showed that the average % nitrogen in tissue was 8.5%, which is comparable and within the range of the diploid measurements.

The Panel also found in Grizzle et al. 2016 results indicating that there is a substantial decrease in soft tissue % nitrogen content as an oyster ages in Great Bay, NH (from fitted model: ~65 oyster shell height = 8.3%, ~76 mm oyster shell height = 7.9%, and ~102 mm shell height = 5.6%). The Panel was not able to find any similar conclusions in any of the Chesapeake Bay studies. Since the estimates are based on conservative biomass determinations and average percent content, the Panel recommends that same average of 8.2% nitrogen content in tissue be used the different size class ranges. However, it may be beneficial to explore potential differences in % nitrogen content in oyster tissue of various oyster size classes in Chesapeake Bay in future studies.

Table 7g. Studies used to establish the recommended average % nitrogen content in oyster tissue. SH = shell height, N = number of oysters analyzed, SE = standard error, and SD = standard deviation. Oysters evaluated in these studies were diploid. Studies are further summarized in Appendix D.

Source	Culture Method	Study Site and Environmental Conditions	N	% Nitrogen Mean	% Nitrogen Range
Carmichael et al. (2012)	Off Bottom Hatchery-Produced Oysters (cages 6 cm off bottom) SH = mean of 8.2 ± 0.2 mm at start of study to maximum of ~68 mm at end of study Growing Period: June-October 2003	Cape Cod, MA (5 sites total) Salinity Ranges = 25-28 N load Ranges =14 x 10^{-4}-601 x 10^{-4} kg N m-2 y-1	800	8.6 ± 0.2 SE	N/A
Grizzle and Ward (2011)[a]	Off Bottom (cages ~10-20 cm off bottom) SH = 7.8-55.6 mm Growing Period: April-November 2010	Great Bay, NH (6 sites total) Sites represent a range of ambient nutrient concentrations, water flow conditions, and locations with the estuary	108	7.28 ±0.49 SE	3.0-14.01
Higgins et al. (2011)	Off Bottom Hatchery-Produced Oysters (floating aquaculture cages) Mean SH = ~32-128 mm (from raw data) Growth Period: November 2006, August 2007 to October 2009	Spencer's Creek, VA Salinity = 5-15 Low flow, high sedimentation	47	8.1 ± 0.13 SE	5.80-9.97
	Off Bottom Hatchery-Produced Oysters (floating aquaculture cages) SH = ~57-150 mm (from raw data) Growth Period: May-July 2007 to October 2009	St. Jerome Creek, MD Salinity = 12-15 High flow, low sedimentation	37	7.37 ± 0.19 SE	5.43-10.36

Kellogg et al. (2013)	On Bottom Hatchery-Produced Oysters (restored subtidal oyster reef) Mean SH = 114 mm Growth Period: October 2009-August 2010	Choptank River, MD Salinity = 7.0-11.6	15[b]	9.27 ± 0.60 SD	8.58-9.71
Kellogg unpubl. data	On Bottom (intertidal reef) SH = 53-122 mm Time Period Removed: April, June, July, August, and October 2012; April and July, 2013	Hillcrest Oyster Sanctuary, Mockhorn Bay, VA	9[c]	8.13 ± 0.27 SE	7.8-7.92[e]
Sebastino et al. (2015)[d]	Off Bottom Hatchery-Produced Oysters (cages 1 m depth) Mean SH Range = 65-82 mm Growth Period: Spawned and settled in summer of 2009 and 2010; transplanted to sites June 2010 and 2011 and removed during July, August, and October 2010 and 2011	Jamaica Bay and Great South Bay, NY	N/A	8.94	N/A

[a]Values calculated using raw data provided in report appendix

[b]Three samples composed of five individuals per sample

[c]Three samples composed of 3 individuals

[d]Three sites were sampled within each bay, but results in Table 1 & 2 were aggregated data by bay.

[e]Range of aggregate averages

7.1.3.2 Recommended Percent Phosphorus Content in Oyster Tissue

The Panel reviewed the literature and found 3 published and 3 unpublished studies that reported the percent phosphorus content in oyster tissue (literature review summaries can be found in Appendix D). The average from these studies ranged from 0.62% (Hillcrest Oyster Sanctuary, Mockhorn Bay, VA; Kellogg unpubl. data) to 1.26% (Choptank River, MD; Kellogg et al. 2013). The Panel agreed to use the same data inclusion (i.e., Atlantic Coast studies) and averaging approach (i.e., site-specific averages for studies within Chesapeake Bay and the overall average of combined sites for studies outside of Chesapeake Bay) as for percent nitrogen content. Using the same rationale as that used for nitrogen, the Panel decided to omit the data from Newell and Mann 2012 and from Higgins from the Choptank River in MD and Lynnhaven River in Virginia. However, the reported

% phosphorus content in Newell and Mann 2012 of 0.8% and the preliminary analyses from Higgins of 1% for oysters (n = 9) in the Choptank River and 0.81% (n = 18 oysters) in the Lynnhaven River are comparable to the values used to determine the average % phosphorus content in tissue for the reduction effectiveness estimates.

The Panel recommends a phosphorus content value of 0.9% of dry tissue weight be used to calculate the phosphorus assimilation in oyster tissue reduction effectiveness estimates. This number is based on two published studies and one unpublished study of diploid oysters from Atlantic Coast estuaries found in Table 7h. These studies are further described in Appendix D. These studies capture a variety of culture methods (off bottom and on bottom), seasons, and environmental conditions. They do not include any data on triploid oysters or on oysters collected during winter. However, since the % phosphorus values are so well constrained and the % nitrogen value of triploid oysters was comparable to the % nitrogen content in triploid oysters, the Panel felt that the recommended % phosphorus content based on diploid oysters could also be applied to triploid oysters.

Table 7h. Studies used to establish the recommended average % phosphorus content in oyster tissue. SH = shell height, N = number of oysters analyzed, SE = standard error, and SD = standard deviation. Oysters evaluated in these studies were diploid. Studies are further summarized in Appendix D.

Source	Culture Method	Study Site and Environmental Conditions	N	% Phosphorus Mean	% Phosphorus Range
Higgins et al. (2011)	Off Bottom Hatchery-Produced Oysters (floating aquaculture cages) Mean SH = ~32-128 mm (from raw data) Growth Period: November 2006, August 2007 to October 2009	Spencer's Creek, VA Salinity = 5-15 Low flow, high sedimentation	47	0.83 ± 0.01 SE	0.60-1.05
	Off Bottom Hatchery-Produced Oysters (floating aquaculture cages) SH = ~57-150 mm (from raw data) Growth Period: May-July 2007 to October 2009	St. Jerome Creek, MD Salinity = 12-15 High flow, low sedimentation	37	0.82 ± 0.02 SE	0.53-1.07
Kellogg et al. (2013)	On Bottom Hatchery-Produced Oysters (restored subtidal oyster reef) Mean SH = 114 mm Growth Period: October 2009-August 2010	Choptank River, MD Salinity = 7.0-11.6	3[a]	1.26 ± 0.18 SD	N/A
Kellogg unpubl. data	On Bottom (intertidal reef) SH = 53-122 mm Time Period Removed: April, June, July, August, and October 2012; April and July, 2013	Hillcrest Oyster Sanctuary, Mockhorn Bay, VA	9[b]	0.62 ± 0.02 SE	0.59-0.67

[a]Three samples composed of five individuals per sample

[b]Three samples composed of 3 individuals

7.1.4 Default Reduction Effectiveness Estimates for Nitrogen and Phosphorus Content in Oyster Tissue for Diploid and Triploid Oysters

The Panel's recommended default diploid and triploid nitrogen and phosphorus reduction effectiveness estimates for endorsed practices when qualifying conditions are met are shown in Table 7i and 7j, respectively. These estimates were derived using the biomass from the oyster size class midpoints from Step 2 (Section 7.1.2; Table 7f) and multiplying by the recommended average percent nitrogen (8.2%) and phosphorus (0.9%) contents in oyster tissue from Step 3 (Section 7.1.3).

Table 7i. Nitrogen and phosphorus assimilated in oyster tissue reduction effectiveness estimates for diploid oysters.

Oyster Size Class Range (Shell Height in Inches)	Default Diploid N Content (g/oyster)	Default Diploid P Content (g/oyster)
a. 2.0 - 2.49	0.05	0.01
b. 2.5 - 3.49	0.09	0.01
c. 3.5 - 4.49	0.15	0.02
d. 4.5 - 5.49	0.22	0.02
e. ≥ 5.5	0.31	0.03

Table 7j. Nitrogen and phosphorus assimilated in oyster tissue reduction effectiveness estimates for triploid oysters.

Oyster Size Class Range (Shell Height in Inches)	Default Triploid N Content (g/oyster)	Default Triploid P Content (g/oyster)
a. 2.0 - 2.49	0.06	0.01
b. 2.5 - 3.49	0.13	0.01
c. 3.5 - 4.49	0.26	0.03
d. 4.5 - 5.49	0.44	0.05
e. ≥ 5.5	0.67	0.07

7.2 Option 2: Methodology for a Site-Specific Reduction Effectiveness Estimate for an Individual Practice

In addition to the default estimates, ***the Panel recommends that the States and the CBP Partnership adopt an approach that allows the BMP implementers to pursue the development and implementation of site-specific nitrogen and phosphorus reduction effectiveness estimates***. The Panel acknowledges that, because the default reduction effectiveness estimates for nitrogen and phosphorus assimilation in oyster tissue are conservative, they will underestimate the total nitrogen and/or phosphorus removed in many cases and, much more rarely, overestimate it. Overall, the Panel felt that site-specific estimates would best represent the reduction effectiveness of an individual practice at a particular location. The Panel recommends the methodology described below to establish a site-specific estimate for their practice.

- The implementer of the oyster practice will first work with the State who will work with CBP to define their practice-specific oyster size class categories if using different categories than the default estimate and two timeframes set by the State to reflect seasonal differences.
- Once approved by the State and CBP, the operation will have 50 random oysters per size class per season analyzed to determine the average tissue dry weight.
- The BMP implementer sends the samples to a lab that uses standardized methods to acquire the tissue dry weight in grams (e.g., tissue heated at 60°C until samples reach constant weight; Holme and McIntyre 1984; Mo and Neilson 1994).
- The average tissue dry weight for each size class will be multiplied by the default 8.2% nitrogen content and 0.9% phosphorus content in oyster tissue to determine the site-specific reduction effectiveness estimates.
- The site-specific reduction effectiveness estimates are reviewed by at least two experts selected by either the State or CBP before making an approval decision.
- Once approved, the estimate is good for 5 years and then should be re-evaluated.

The Panel conducted a sensitivity analysis to determine how many oysters should be measured per oyster size class. Diploid data from one habitat group (tributary mid-bay mesohaline) and from aquaculture practices representative of on-bottom oyster growth were selected based on yielding the largest data set (n = 3,016 oysters). Data were then divided into the default size classes in inches: 2.0-2.49; 2.5-3.49; 3.5-4.49; 4.5-5.49; 5.5-6.49. Using the R command "sample()," forty random subsamples were taken of each size class: 10 with n=25, 10 with n=50, 10 with n=75 and 10 with n=100. There was only enough data to do this for four of the five size classes, as the 5.5-6.49 class only had 42 total data points. The 0.5 quantile regressions for each subset were generated using the power function $y = ax^b$.

The average and distribution of each of the coefficients were examined, as well as the error terms, for each of the sample sizes. The error associated with *a* did not consistently change with increasing sample size and so was not used in this analysis. The error associated with *b* decreased the most when the sample size increased

from 25 to 50 (see Table 7k). Further increased sample sizes did yield smaller error terms, as would be expected, but the largest gains were from increasing from 25 to 50 samples. Based on this analysis, the panel recommends n=50 per size class as a reasonable approach to minimizing both error and effort required to seek site-specific reduction effectiveness estimates. The panel also notes that, while the data used for these analyses encompass a range of locations, they are still likely much more variable than would be expected from a gear-intensive aquaculture operation with market pressures to produce a consistent product.

Table 7k. Error terms associated with coefficient *b* as the size of the subsample was increased.

Default Oyster Size Class	n = 25	n = 50	n = 75	n = 100
2.0 – 2.49	2.28	1.47	1.10	0.99
2.5 – 3.49	1.60	1.01	0.89	0.88
3.5 – 4.49	1.84	1.29	1.07	0.85
4.5 – 5.49	2.30	1.65	1.32	1.00

Since the method to determine oyster tissue dry weight is well established, the Panel felt there wouldn't be a need to convene a new expert panel to review the site-specific estimates. However, they did agree that the site-specific estimates should be reviewed by at least two experts to ensure that the method was followed correctly. Also, the Panel opted for the implementer to measure the biomass of their oysters and use the default percent nitrogen and phosphorus content instead of analyzing the percent nitrogen and phosphorus content in the tissue because, overall, the Panel saw that converting shell height to oyster biomass had much greater variability than the percent nitrogen and phosphorus content and felt that the more costly chemical analyses (approximately $30.00 per oyster) would not be reasonable for the BMP implementers. The Panel recommends that the refinement of the nitrogen and phosphorus percent contents in oyster tissue be done through research and not by the BMP implementers.

8.0 Qualifying Conditions

The Panel agreed that the qualifying conditions described below would apply to both the default and the site-specific estimates:

- Only includes oysters that are removed moving forward from the time the BMP is approved/implemented for reduction effectiveness credit in the TMDL. This baseline condition was proposed by the CBP Partnership Management Board and the Panel concurs with their decision.

- Oysters had to have been grown from initial sizes < 2.0 inches shell height.

- Oysters have to be alive when removed to count toward the reduction effectiveness.

9.0 Recommended Application and Verification Guidelines for the Nitrogen and Phosphorus Assimilation in Oyster Tissue Protocols for Private Oyster Aquaculture Practices

In Chesapeake Bay, commercial fishermen and aquaculturists are required to quantify and report monthly oyster harvest to their State management agency. Harvest is reported according to how oysters are packaged and sold which includes units of bushels, counts of oysters in boxes, or individual oysters (http://dnr2.maryland.gov/fisheries/Pages/aquaculture/harvest-reporting.aspx; http://mrc.virginia.gov/regulations/FR610.shtm;).

For aquaculture, the unit of sale can vary depending on the method of harvest or how an individual business markets and sells its product. Oysters packaged in bushel baskets will typically have a range of shell heights and will most likely have been harvested directly from the bottom (on-bottom oyster aquaculture). Off-bottom aquaculture is done in cages and harvesters typically grade the product so that batches of oysters of similar size can be harvested together and marketed. Cage cultured oysters are generally packaged and sold in boxes or as individuals.

The common elements among these practices is that oysters are actively culled to a specific size by the processor to ensure they are legal or to group them uniformly for sale and they are placed in a standard type of container (e.g., bushel baskets, boxes or other standard container). Therefore, the most reasonable expectation for oyster aquaculture reduction effectiveness crediting is to report the number and sizes of oysters harvested using methods similar to State reporting requirements.

The Panel identified three types of data that would be needed to apply the nutrient reduction effectiveness estimates:

1. *Types and total numbers of containers*- The total number and type of container used to package oysters needs to be documented annually.
2. *Average number of oysters in each container type*- The average number of oysters in a container is needed to apply the nutrient reduction effectiveness estimates to the number of oysters removed on an annual basis. The Panel recommends that the average number of oysters in a container is quantified by counting and documenting the total number of oysters in 10 containers. Oyster counts should be conducted during the two times a year when oysters are measured (see below).
3. *The average size of oysters in each container type*- The Panel recommends that the average size of oysters in containers be quantified by measuring and documenting the shell heights of 50 randomly selected oysters measured from representative containers (depends on packaging method described below). The Panel recommends that shell heights be measured two times a year to address any seasonal variability in biomass for similar shell heights. The Panel suggests that measurements are

taken 6 months apart based on timeframes set by the State to reflect any changes in minimum harvest sizes.

The total number of containers is multiplied by the average number of oysters in a container to determine the total number of oysters eligible for reduction credit. Using these values, the nitrogen and phosphorus reduction effectiveness estimates will be applied to the oyster size category where the average shell height of all measured oysters falls.

There are two different ways in which aquaculturists currently package their oysters for reporting:

1. Oysters of variable shell heights are packaged together in the same container.
2. Oysters of uniform size are packaged in separate containers.

The representative containers in which oyster shell height measurements are made depends on which packaging method is used, further described below:

Oysters of variable shell heights are packaged together in the same container- Twice a year, 50 oysters are randomly selected from at least 10 containers for shell height measurements. The average shell height of all measurements is used to verify which oyster size class range estimate to use to determine the nutrient reduction effectiveness. This approach would most likely apply to on-bottom growers who typically report in bushels. Growers using this method will only be able to report in one size class category (unless oysters were moved; if so, then they will partition the credit to the appropriate locations based on recommendations under, "Movement of Oysters" below).

Oysters of uniform size are packaged together in separate containers- Twice a year, 50 oysters are randomly selected from at least 10 containers for each oyster size class range that the implementer is reporting in for shell height measurements. The average shell height of all measurements for that particular size class is used to verify which oyster size class range estimate to use to determine the nutrient reduction effectiveness. This approach would most likely be more relevant to off-bottom growers who package oysters in boxes at specific sizes. Growers using this method will be able to report in multiple oyster size class categories.

9.1 Movement of Oysters

There are instances where oysters are moved from their initial grow-out location to another location in the Bay or elsewhere. Reasons for moving the oysters include, but are not limited to, changing the taste by moving oysters to an area with higher salinity (e.g., oysters that are moved from Chesapeake Bay to Chincoteague Bay) or having to move the oysters because of water quality problems in the initial grow-out location (e.g., the initial grow-out location is closed due to bacteria concentrations).

The Panel recommends the following guidelines to apply the reduction effectiveness estimates if oysters are moved from the initial grow-out location:

- If locations are in different water segments: Partition the credit by their size class when removed from grow-out location based on the surviving oysters from the last grow-out location.
    - Verification Guideline: When moved, 50 random oysters are measured following the same guidelines described above based on how they're packaged.
    - The average shell height from the measured oysters is used to determine what size class they are in before being transplanted into the new location.
    - Example:
        - Diploid oysters are removed from Location 1 and moved to Location 2—50 random oysters were measured and the average shell height = 2.3 inches
        - 1,000,000 diploid oysters are removed from Location 2 for harvest—50 random oysters were measured and the average shell height = 3.5 inches
        - Location 1 N reduction credit = 1,000,000*0.05 grams = **50,000 grams**
        - Location 2 N reduction credit = 1,000,000*0.15 grams = 150,000 grams minus the 50,000 grams partitioned to Location 1 = **100,000 grams**
- If oysters end up in the same size class for the multiple locations, then the 1st location will receive the credit.

9.2 Reporting

Summarized below are the reporting components for the nitrogen and phosphorus assimilation in oyster tissue reduction effectiveness crediting protocols.

If oysters are grown at one location:

- Ploidy: Diploid or triploid oysters
- Type of aquaculture practice: Off-bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, On-Bottom Private Oyster Aquaculture using Hatchery-Produced Oysters, or On-Bottom Private Oyster Aquaculture Using Substrate Addition
- Reporting unit: Bushels, boxes, other container (indicate what type), or individuals
- Packaging type: Variable oyster sizes or uniform oyster sizes
- Central coordinates (latitude and longitude) of initial grow-out location
- Month/year removed from final grow-out location
- Number of containers of live oysters or individual oysters from final grow-out location
- Oyster count average for unit verification check (10 representative containers per two time periods from final grow-out location)

- Shell height average(s) for oyster size verification check (50 random oysters from 10 containers per two time periods from final grow-out location)

Additional reporting if oysters are grown at multiple locations:

- Central coordinates (latitude and longitude) of any grow-out locations oysters are transferred to (if applicable)
- Month/year oysters are transferred
- Oyster size class category when placed at transfer location

The Panel's recommended estimates were developed to be reported annually based on removed alive oysters. For growers participating as a BMP, the Panel recommends that the State incorporates these components in the monthly reports to track the BMP. The Panel reasoned that the State would compile the monthly information and provide an annual report of the reduction effectiveness to the CBP.

9.3 Default Approach to Deal with Missing Information

The Panel recommends that if average oyster shell heights and average numbers of oysters in containers are not known then a default approach where the minimum legal size of oysters and State documented information specifying the average number of minimum legal sized oysters can be packaged in a specific container is used. For example, in Maryland, the minimum legal oyster size is 2" (~50 mm) during parts of the year and 3" (~76 mm) during other parts. Maryland defines a bushel for 3-inch oysters as 300 individuals. Therefore, if there are missing measurements and the minimum is 3 inches and the grower is reporting in bushels for diploid oysters, all bushels would be multiplied by 300 and individual oysters would be assigned to the 2.5-3.49 oyster size class range and the corresponding diploid estimates for nitrogen and phosphorus assimilation in oyster tissue would be applied (Table 7i).

If ploidy is missing, then the Panel recommends applying the diploid estimates.

9.4 Application and Verification Examples

Two examples are given below. One for an on-bottom grower who uses diploid oysters and packages variable sized oysters in bushel baskets and one for an off-bottom grower that uses triploid oysters and packages uniform sized oysters in boxes. These examples are specific to oysters being grown in one location.

9.4.1 On-Bottom Example (Oysters Grown in One Location)

Reported Information

Presented in Table 9a is an example of reported information a grower needs to submit in order to calculate the reduction effectiveness. In practice, there will likely be multiple reports since growers typically harvest and report their harvest monthly. The number of containers with live oysters reported from these monthly reports would be added together to determine the total number to use in the annual reduction effectiveness calculations. The reported verification information (Table 9b) is used to determine the amount of oysters per container and the oyster size class category the oysters belong in, both needed to calculate the reduction effectiveness credit.

Table 9a. Example of reported information for on-bottom private oyster aquaculture when variable sized oysters are packaged together.

On-Bottom Private Oyster Aquaculture-Variable Oyster Sizes per Packaged Container	
Reporting Component	**Information provided by grower**
Ploidy	Diploid
Practice Title	On-Bottom Private Oyster Aquaculture using Hatchery-Produced Oysters
Reporting unit	Bushels
Packaging type	Variable oyster sizes
Central coordinates of initial grow-out location	37° 36.444, -76° 25.411
Central coordinates of final grow-out location	37° 36.444, -76° 25.411
Month/Year removed from final grow-out location*	January-December 2016
Number of containers with live oysters	10,000

*In practice, monthly reports that are sent to the State would have to be compiled to determine the total number of containers with live oysters harvested in the year.

Table 9b. Example of reported verification information for on-bottom private oyster aquaculture when variable sized oysters are packaged together.

Oyster Count for Unit Verification Check			
Time Period	**Date Measured**	**Container #**	**Oyster Count**
Time Period 1	**3/21/2016**	1	98
		2	112
		3	120
		4	156
		5	150
		6	149
		7	160
		8	98
		9	101
		10	105
Time Period 2	**9/22/2016**	1	100
		2	110
		3	120
		4	125
		5	180
		6	155
		7	150
		8	150
		9	170
		10	145
Average			**132**
Average Shell Height for Oyster Size Class Verification Check			
Time Period	**Date Measured**	**Container #**	**Average Shell Height (inches)***
Time Period 1	**3/21/2016**	1	3.25
		2	3.5
		3	3.5
		4	4.25
		5	3
		6	3.5
		7	2.5
		8	3.5
		9	3
		10	3.5
Time Period 2	**9/22/2016**	1	3
		2	3.5
		3	3.25
		4	3.5
		5	3
		6	3.5
		7	2.5
		8	3.5
		9	3.5
		10	3.75
Average (n = 100 oysters, 50 oysters per time period)			**3.33**

*Example shows the average of five random oysters per container. In practice, growers should report the measurements for all oysters.

Calculations

Number of individual oysters = 10,000 bushels multiplied by 132 oysters per bushel from unit verification check = 1.32 million oysters

Diploid estimates of 0.09 g N content and 0.01 g P content would be applied because the average from the shell height verification check was 3.3 inches falling into the 2.5-3.49 oyster size class. The total nitrogen and phosphorus removal would be calculated as follows using estimates from Table 7i:

Nitrogen (N)		g N Removed	
1,320,000 x 0.09 g N oyster^{-1}		118,800	
	Total	118,800	= 118.8 kg N removed

Phosphorus (P)		g P Removed	
1,320,000 x 0.01 g P oyster^{-1}		13,200	
	Total	13,200	= 13.2 kg P removed

9.4.2 Off-Bottom Example (Oysters Grown in One Location)

Reported Information:

Presented in Table 9c is an example of reported information a grower needs to submit in order to calculate the reduction effectiveness for an off-bottom private oyster aquaculture practice that packages uniform sized oysters per container. In practice, there will likely be multiple reports since growers typically harvest and report their harvest monthly. The number of containers with live oysters for each oyster size class reported from these monthly reports would be added together to determine the total number for each oyster size class to use in the annual reduction effectiveness calculations. The reported verification information (Table 9d) is used to determine the amount of oysters per container and the oyster size class categories the oysters belong in, both needed to calculate the reduction effectiveness credit.

Table 9c. Example of reported information for off-bottom private oyster aquaculture when uniform sized oysters are packaged together per container.

Off-Bottom Private Oyster Aquaculture-Uniform Oyster Sizes per Packaged Container	
Reporting Component	**Information provided by grower**
Ploidy	Triploid
Practice Title	Off-bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters
Reporting unit	Boxes
Packaging type	Uniform oyster sizes
Central coordinates of initial grow-out location	37° 36.444, -76° 25.411
Central coordinates of final grow-out location	37° 36.444, -76° 25.411
Month/Year removed from final grow-out location	January-December 2016
Containers with live oysters*	
Oyster Size Class (inches)	**Container Count**
2.0 - 2.49	0
2.5 - 3.49	5,000
3.5 - 4.49	5,000
4.5 - 5.49	0
≥ 5.5	0
Total	**10,000**

*In practice, monthly reports that are sent to the State would have to be compiled to determine the total number of containers with live oysters harvested in the year.

Table 9d. Example of reported verification information for off-bottom private oyster aquaculture when uniform sized oysters are packaged together per container.

Oyster Count for Unit Verification Check				
Oyster Size Class (inches)			2.5-3.49	3.5-4.49
Time Period	Date Measured	Container #	Oyster Count	Oyster Count
Time Period 1	3/21/2016	1	99	95
		2	100	101
		3	101	100
		4	100	100
		5	105	105
		6	102	98
		7	100	99
		8	100	100
		9	100	100
		10	99	100
Time Period 2	9/22/2016	1	100	100
		2	98	100
		3	101	103
		4	100	101
		5	100	102
		6	98	98
		7	100	99
		8	102	95
		9	100	101
		10	103	105
Average			**100**	**100**
Average Shell Height for Oyster Size Class Verification Check				
Oyster Size Class (inches)			2.5-3.49	3.5-4.49
Time Period	Date Measured	Container #	*Average Shell Height (inches)	*Average Shell Height (inches)
Time Period 1	3/21/2016	1	3.25	4.25
		2	3.5	4.5
		3	3.5	4.5
		4	4.25	3.25
		5	3	4
		6	3.5	4.5
		7	2.5	3.5
		8	3.5	4.5
		9	3	4
		10	3.5	4.5

Time Period 2	**9/22/2016**	1	3	4
		2	3.5	4.5
		3	3.25	4.25
		4	3.5	4.5
		5	3	4
		6	3.5	4.5
		7	2.5	4.25
		8	3.5	4.25
		9	3.5	4.5
		10	3.75	4.75
Average (n = 100 oysters, 50 oysters per time period)			**3.33**	**4.25**

*Example shows the average of five random oysters per container. In practice, growers should report the measurements for all oysters for each size class they are seeking credit for.

Calculations

Number of individual oysters in the 2.5-3.49 size class = 5,000 boxes multiplied by 100 oysters per box from verification check = 500,000 oysters

Number of individual oysters in the 3.5-4.49 size class = 5,000 boxes multiplied by 100 oysters per box from verification check = 500,000 oysters

Triploid estimates of 0.13 N and 0.01 P tissue content would be applied for the 2.5-3.49 oyster size class and 0.26 N and 0.03 P tissue content for the 3.5-4.49 oyster size class. The total nitrogen and phosphorus removal would be calculated as follows:

Nitrogen (N)		g N Removed	
500,000 x 0.13 g N oyster^{-1}		65,000	
500,000 x 0.26 g N oyster^{-1}		130,000	
	Total	195,000	= 195 kg N removed

Phosphorus (P)		g P Removed	
500,000 x 0.01 g P oyster^{-1}		5,000	
500,000 x 0.03 g P oyster^{-1}		15,000	
	Total	20,000	= 20 kg P removed

10.0 Unintended Negative Consequences

There are consequences for each and every action made within the Chesapeake Bay watershed and aquatic ecosystem, including effects on economic activity, competition for utilization of estuarine resources, and water quality. The removal of nitrogen and phosphorus in oyster tissue from harvest by off-bottom and on-bottom private oyster aquaculture is the primary focus of this discussion. Any unintended consequences associated with reduction effectiveness protocols other than nitrogen and phosphorus assimilation in oyster tissue for private oyster aquaculture practices are not discussed here and will be evaluated in a future incremental report when those estimates are developed. This includes concerns that have been raised about permanent removal of oyster shell, a precious resource for oyster aquaculture and restoration practices. Moreover, any concerns about disease, aesthetic changes in waterfront views, competition with wild harvest, etc. are part of the broader issues with private oyster aquaculture, and this BMP would not change these concerns in any way.

The Panel identified two potential unintended negative consequences of bivalve aquaculture regarding Chesapeake Bay water and sediment quality. These are outlined in the Table 10a and discussed afterward.

Table 10a. Identified negative unintended consequences concerning nitrogen and phosphorus assimilation for aquaculture practices.

Issue	Select Relevant Studies
Biodeposition by bivalves leads to increased nutrient releases from sediment	Choptank River: (Testa et al. 2015) St. Jerome Cr., Spencer's Cr., Chesapeake Bay: (Higgins et al. 2013)
Loss or change of benthic biota through excessive organic matter loading	Puget Sound fish farm: (Weston 1990) New Zealand mussel farm: (Christensen et al. 2003)

The excessive loading of organic matter to sediments can have negative consequences to water quality, including the highly efficient return of nitrogen (mainly ammonium) and phosphorus (soluble reactive phosphorus) to the water column, rather than sequestration of phosphorus in solid phase deposits (Hartzell et al. 2010, Li et al. 2015) or removal of nitrogen through microbial denitrification (Jenkins and Kemp 1984, Cornwell et al. 1999). In the case of soluble reactive phosphorus, the loss of phosphorus-binding iron oxide minerals via conversion to iron sulfide minerals (O'Keefe 2007, Jordan et al. 2008) is enhanced by high rates of sediment metabolism and the consequent anaerobic conditions close to the sediment-water interface. For nitrogen, increased rates of sediment metabolism result in lower oxygen penetration depths in sediments, decreasing rates of nitrification – which needs oxygen, and minimizing denitrification because that process requires nitrate (Cornwell et al. 1999, Testa et al. 2013).

Biogeochemical unintended consequences generally arise because of the deposition of biodeposits to the sediment surface. The positive benefits of increased denitrification that are observed with oysters grown on the bottom (Kellogg et al. 2013) may result from the complex physical and biological structure within a reef, whereas the lower benthic complexity associated with off bottom aquaculture may result in biodeposits being

overloaded onto sediments and a deleterious effect on beneficial sediment processes such as phosphorus burial and denitrification. Location may be a prime determinant in the fate of nutrients, with observations in the Choptank River that show physical redistribution of biodeposits leads to relatively good sediment quality (Testa et al. 2015). In contrast, the location of floating oyster farms in locations in the mesohaline Chesapeake Bay with lower physical forcing and initial poor sediment quality resulted in negative effects of aquaculture on sediment nutrient balances (Higgins et al. 2013). Holyoke observed modest changes in sediment nutrient fluxes in La Trappe Creek, but an increase in iron sulfide mineral formation (Holyoke 2008). For oysters planted on the bottom, enhanced denitrification is commonly observed and negative consequences appear minimal (Piehler and Smyth 2011, Kellogg et al. 2013, Smyth et al. 2013, Kellogg et al. 2014). The return of aquaculture nitrogen and phosphorus in forms that can grow algae is partially mitigated by the nature of oyster filtration, in which water column particulates, both algae and detritus, are brought to the bottom of the estuary. During such removal, the part of the decomposition and nutrient release that would have occurred in the water column in now transferred to the sediment; water column nutrient remineralization is completely efficient at growing algae.

Less is known about changes in the biomass and structure of benthic communities located underneath water column culture. There has been concern for such changes under finfish pens (Weston 1990) and some observation under mussel farms suggest community changes (Christensen et al. 2003). On bottom oyster communities tend to be diverse and abundant (Kellogg et al. 2013).

In conclusion, on-bottom culture appears to have few unintended negative consequences, with a general enhancement of nutrient removal likely. Off-bottom oyster culture in the water column may result in over-enrichment of sediments and elevated fluxes of fixed nitrogen and phosphorus relative to control sites. However, there is still a benefit to removing nitrogen and phosphorus from the water column where remineralization is more efficient in sustaining elevated algal biomass. The Panel felt that these unintended consequences can be managed for off-bottom aquaculture operations. Because the impact of excessive organic matter loading in estuarine sediment are visually identifiable, representative clear cores of sediment, collected by any means (example presented in Owens et al. 2016), would yield the information necessary to identify changes compared to control areas. Heavily loaded sediments are jet black because of the predominance of iron sulfide minerals at the sediment surface.

11.0 Ancillary Benefits

Oysters provide valuable ecosystem services to the coastal water bodies where they reside. In addition to the nutrient reduction benefits detailed in this report, oyster reefs provide habitat for an abundance of marine species, and enhance water quality through filtration. The presence of oysters and shellfish may provide more light availability to seagrasses directly by filtering water, and indirectly by providing refuge for grazing animals which eat algae from seagrass leaves (Newell and Koch 2004, Orth and van Montfrans 1984). A Chesapeake Bay modeling study shows that when oysters filter seston out of the water column, light penetration in the Bay has the potential to increase, which could contribute to increased seagrass growth and depth limit (Newell and Koch 2004). An additional study that assessed the influence of a 10% increase of oyster populations in the Bay determined that the greatest potential benefit would be an increase in SAV through water clarity (Cerco and Noel 2007). Because seagrasses are a refuge for marine animals and enhance water clarity (Orth et al. 2006 and sources therein), the relationship between oyster presence and seagrass establishment could be synergistic.

There is also a demonstrated connection between oyster reef presence and more abundant and rich marine life when compared to areas with no reef structure (Coen et al. 1999, Tolley and Volety 2005). Through ongoing research, details are emerging that suggest restored oyster reefs and oyster aquaculture systems may provide similar or a portion of the marine organismal habitat that natural oyster reefs provide. Dealteris et al. 2004 and O'Beirn et al. 2004 highlighted the diverse and abundant marine species associated with rack-and-bag and floating gear used for Eastern oyster aquaculture in a Rhode Island estuary and a coastal bay of Virginia, respectively. Tallman and Forrester (2007) showed that in Narragansett Bay, Rhode Island, near bottom Eastern oyster aquaculture cages provided valuable habitat for finfishes. Peer-reviewed literature on aquaculture and seagrass presence is still emerging; several North American studies highlight concerns about direct competition for space between oyster aquaculture gear and seagrass, or disturbances associated with harvest in the aquaculture areas (Dumbauld et al. 2009, Tallis et al. 2009).

12.0 Conclusions and Future Research

Using the decision framework described in Section 4.0 the Panel made the following conclusions:

- Nitrogen and phosphorus assimilation in oyster tissue protocols are suitable for consideration and sufficient science exists to provide reduction effectiveness estimates for private oyster aquaculture practices in Off-bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, On-Bottom Private Oyster Aquaculture using Hatchery-Produced Oysters, and On-Bottom Private Oyster Aquaculture Using Substrate Addition categories.

- The Panel agreed that the estimates are verifiable by conducting oyster shell height and oyster count verification checks using the guidelines described in Section 9.0.

- Potential unintended negative consequences were identified for Off-bottom Private Oyster Aquaculture Using Hatchery-Produced Oyster, but the Panel felt these are manageable by monitoring the condition of the sediment.

Future research suggestions to refine the estimates presented in this report are listed below:

- **Studies that work directly with aquaculturists to understand how aquaculture techniques influence oyster growth.**

The default shell height to tissue dry weight regression equations were strongly influenced by data from oysters grown by researchers or as part of oyster reef restoration efforts. Since aquaculturists use techniques to enhance oyster growth, the default regression equations likely underestimate the reduction effectiveness.

- **Studies that evaluate seasonal differences for off bottom aquaculture.**

The default estimate only included data from the spring. While the Panel is not concerned about this for the default estimate because oysters are usually at their smallest biomass at that time resulting in a conservative estimate, it would be beneficial to have a better understanding of these differences to refine the estimate to be more representative across all seasons for off bottom aquaculture.

- **Studies that evaluate the % nitrogen and phosphorus contents in tissue for triploid oysters.**

There was only one study found that measured the % nitrogen in tissue for triploid oysters (Reitsma et al. 2014) and no studies for the % phosphorus content. Such studies could help refine the estimate and provide confirmation that the average % nitrogen and % phosphorus contents in tissue are similar between diploid and triploid oysters. Preferably, studies should focus on triploid and diploid oysters grown using the same culture methods at the same location. Overall, the Panel is not concerned with applying the average % nitrogen and % phosphorus contents in tissue from diploid oysters for the default triploid oyster reduction effectiveness estimates because of evidence that triploid oysters likely have similar percent contents in tissue as diploids (i.e., percent nitrogen value reported by Reitsma et al. 2014 of 8.5% is comparable to the recommendation of 8.2%) and the fact that biomass appears to have a bigger role in influencing the total nitrogen and phosphorus in the tissue.

- **Studies that evaluate potential differences in the % nitrogen and % phosphorus content between different oyster size classes.**

The Panel found in Grizzle et al. 2016 results that suggest that the % nitrogen content in tissue may decrease as the oyster ages. While this study was conducted in Great Bay, NH where environmental conditions may affect oyster growth differently than in Chesapeake Bay, the Panel felt it would be beneficial to examine potential differences in the % nitrogen and % phosphorus content among different oyster size classes to better understand any variability that may exist.

- **Studies that evaluate the amount of nitrogen and phosphorus removal resulting from removal of bio-fouling materials from aquaculture cages.**

The Panel found that the extent of the scale and management of bio-fouling in aquaculture operations has been reported in numerous publications (reviewed by Fitridge et al. 2012). Specific best management practices for shellfish aquaculture operations addressing bio-fouling are addressed in a State of Maryland publication, Maryland Aquaculture Coordinating Council (2007). Given that bio-fouling already has a need to be managed because of the negative impact it has on growing the oysters, the Panel discussed the potential of an added nitrogen and phosphorus removal benefit if the bio-fouling is managed in such a way where it is not being washed back into the Bay. The amount of nitrogen and phosphorus removal associated with bio- fouling control operations would be expectedly straightforward to determine by weighing and analyzing the nitrogen and phosphorus content in the organic matter.

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Appendix A: Summary of Oyster BMP Expert Panel Activities

Updates to the Water Quality Goal Implementation Team (WQGIT)

April 13, 2015—The Oyster Recovery Partnership (ORP) presented rationale for convening an Oyster BMP Expert Panel during the Chesapeake Bay Program (CBP) Partnership's Water Quality Goal Implementation Team (WQGIT) meeting. The WQGIT approved ORP to convene and coordinate the Oyster BMP Expert Panel. Meeting materials and minutes are located here.

August 5, 2015—Panel coordinators submitted a draft of the Panel's charge and membership recommendations to the CBP Partnership for review and comment.

September 14, 2015—Panel coordinators presented response to comments on the Panel's membership and charge that was sent for CBP Partnership review on August 5, 2015. The WQGIT approved the revised Panel's membership and charge. Meeting materials and minutes are located here.

February 8, 2016—The Panel chair and coordinators updated the CBP Partnership on the draft Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Decision Framework during their WQGIT meeting. The briefing paper and presentation can be found here. The CBP Partnership review and comment period on the framework paper was from February 1 to 15, 2016.

April 25, 2016—The Panel coordinator updated the CBP Partnership on their responses to public comments concerning the draft Oyster BMP Nutrient and Suspended Sediment Reduction Effectiveness Decision Framework and status of the Panel's data review. The responses to comments and presentation can be found here. The Panel also offered another 2 week review and comment period from April 25 to May 13, 2016 on the modified Decision Framework and the Panel's preliminary decisions from the data review.

August 22, 2016—The Panel coordinator and Chair updated the CBP Partnership on their 1st set of recommendations concerning nitrogen assimilation in oyster tissue for water column and designated bottom oyster planting aquaculture practices. Meeting materials and minutes are located here.

Panel Meetings

Panel meetings were 2 hours long unless indicated otherwise. Both in person and remote attendance options were made available to the panelists.

September 30, 2015—The Panel convened its first meeting. The agenda included panelist introductions and an overview of the CBP's Partnership BMP Expert Panel Review Protocol, panel charge, and panel member roles and expectations.

October 26, 2015—Agenda included a presentation on the STAC Review findings from their report, "Evaluation of the Use of Shellfish as a Method of Nutrient Reduction in the Chesapeake Bay" and discussions on oyster practice definitions and considerations for a decision framework for determining the nutrient (nitrogen and phosphorus) and suspended sediment reduction effectiveness of oyster practices.

November 2, 2015—Agenda included a presentation by Tom Schueler from the Chesapeake Stormwater Network on their stream restoration crediting decision framework and discussion on the oyster practice category descriptions and what steps should be included in the oyster BMP nutrient and suspended sediment reduction effectiveness decision framework.

November 19, 2015—Agenda included finalizing the oyster practice category descriptions.

December 14, 2015—Agenda included a more in depth discussion on the steps for the oyster BMP nutrient and suspended sediment reduction effectiveness decision framework.

January 7, 2016—Agenda included finalizing the decision points for the oyster BMP nutrient and suspended sediment reduction effectiveness decision framework.

February 24, 2016 (5-hour meeting)—The Panel's data review workshop. This workshop included data review sessions led by panelists on nitrogen and phosphorus assimilation in oyster tissue and shell, enhanced denitrification associated with oysters, enhanced nitrogen and phosphorus burial associated with oysters, and suspended sediment reduction associated with oysters. The Panel also reviewed the public comments on the draft oyster BMP nutrient and suspended sediment reduction effectiveness decision framework.

March 16, 2016 (3-hour meeting)—Agenda included presentation by Carl Cerco on the Oyster Sub-Model and the Panel discussed definitions to clarify terms used in the oyster BMP nutrient and suspended sediment reduction effectiveness decision framework.

April 14, 2016—Agenda included discussing which methods/papers should be considered in developing the reduction effectiveness estimates for the nitrogen assimilation-related protocols for water column and bottom oyster planting aquaculture practices.

May 19, 2016—Agenda included discussing the empirical approach (converting shell height to dry weight and applying a known % nitrogen content) to determine the nitrogen assimilation in oysters.

June 16, 2016—Agenda included reviewing data analysis results for the oyster shell height to tissue dry weight quantile regression, deciding on a % nitrogen content in oyster tissue from available studies, and discussing

outstanding report items concerning qualifying conditions and timeline for re-evaluation of the Panel's recommended estimates.

July 14, 2016—Agenda included discussing acquired triploid oyster data and determining the oyster size classes that would be used when applying the shell height to tissue dry weight quantile regression.

July 25, 2016 (1.5 hour meeting)—Agenda included finalizing outstanding items for the 1st recommendation report.

August 18, 2016—Agenda included reviewing the 1st recommendation report.

September 15, 2016—Agenda included reviewing Panel comments on the 1st recommendation report and reaching consensus.

Presentations/Webinars

November 2, 2015—The Panel hosted a public stakeholder meeting and webinar. Around 60 people participated, including 5 stakeholder groups who presented information related to how oyster practices could be implemented as BMPs to reduce nutrient pollution. More information on the public stakeholder meeting can be found on the CBP calendar webpage.

November 16, 2015—Panel coordinators briefed the Fisheries Goal Implementation Team on the Panel objectives and status. The briefing presentation can be found here.

November 19, 2015—Panel coordinators briefed the Citizen Advisory Committee on the Panel objectives and status.

January 16, 2016—Panel coordinators presented information about the Panel during the Maryland Watermen Association Trade Show in Ocean City, MD.

March 18, 2016—Panel coordinators presented information about the Panel during the "Conference on New Ideas to Accelerate Chesapeake Bay Restoration" hosted by the University of Maryland Center for Environmental Science. The presentation can be found here.

Other

June 15, 2016—Special CBP Partnership Management Board meeting to provide policy recommendations on issues raised by the Oyster BMP Expert Panel and stakeholders. Meeting materials and decisions can be found here.

Appendix B: Conformity with the CBP Partnership BMP Review Protocol

The BMP review protocol established by the Water Quality Goal Implementation Team (WQGIT 2015) outlines the expectations for the content of expert panel reports. This appendix references the specific sections within the report where the panel addressed the requested protocol criteria. These items are specific to nitrogen and phosphorus assimilation in oyster tissue of removed oysters for Off-Bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, On-Bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, and On-bottom Private Oyster Aquaculture Using Substrate Addition.

1. **Identity and expertise of panel members:** See Section 3.0.

2. **Practice name or title:** Off-Bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, On-Bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, and On-bottom Private Oyster Aquaculture Using Substrate Addition

3. **Detailed definition of the practice:** Section 5.0. The recommendations apply to all practices in the Off-Bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, On-Bottom Private Oyster Aquaculture Using Hatchery-Produced Oysters, and On-bottom Private Oyster Aquaculture Using Substrate Addition.

4. **Recommended nitrogen reduction effectiveness estimate pertaining to nitrogen and phosphorus assimilation in oyster tissue of removed oysters:** See Section 7.0.

5. **Justification of selected effectiveness estimates:** See Section 7.0 and Appendix D.

6. **List of references used:** See Section 7.0

7. **Detailed discussion on how each reference was considered:** See Section 7.0 and Appendix D.

8. **Land uses to which BMP is applied**: Not applicable. This is a tidal in-water BMP. The Phase 6 Model will have an estimated nutrient load in shoreline segments that can be reduced by shoreline and tidal water practices. The pounds of nutrients reduced by this practice will be credited as a reduction to the nutrient loads in the nearest shoreline segments to the practice location. If latitude and longitude are not submitted, then the practice benefits will be distributed amongst all shoreline segments in the geography submitted.

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 sector. Instead reduction credit will go toward total nonpoint source load allocation.

10. **Description of pre-BMP and post-BMP circumstances and individual practice baseline:** The CBP Partnership Management Board decided during the Oyster BMP policy issues special session on June 15, 2016 that when crediting the TMDL based on removal of oysters (aquaculture), only include oysters that are removed moving forward from the time the BMP is approved/implemented.

11. **Conditions under which the BMP works/not works:** See Section 8.0.

12. **Temporal performance of BMP including lag times between establishment and full functioning:** Removed oysters that are ≥ 2" can receive reduction effectiveness credit for the nitrogen and phosphorus assimilation in oyster tissue protocols.

13. **Unit of measure:** Grams of nitrogen and phosphorus in oyster tissue per oyster. Growers may report in bushels, boxes, or individuals. See Section 9.0 for application and verification guidelines to determine how many individual oysters are in the reported unit. See Appendix F for appropriate reporting units to the Bay program.

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

15. **Useful life of the BMP:** Remains useful until oysters die. As long as oysters are alive they are assimilating N and P in their tissue.

16. **Cumulative or annual practice:** Annual based on removed alive oysters.

17. **Description of how BMP will be tracked and reported:** See Section 9.0.

18. **Ancillary benefits, unintended consequences, double counting:** Potential unintended consequences are outlined in Section 10.0 and ancillary benefits in Section 11.0.

19. **Timeline for a re-evaluation of the panel recommendations.** 5 years.

20. **Outstanding issues:** None

Appendix C: Review of Approaches to Determine the Reduction Effectiveness Estimate for Nitrogen and Phosphorus Assimilation in Oyster Tissue

The Panel reviewed two approaches in the scientific literature that could be used to determine the reduction effectiveness estimate for nitrogen (N) and phosphorus (P) assimilated in oyster tissue:

1. Empirical Approach (STAC 2013, Higgins et al. 2011)—This approach uses an established shell height (also referred to as shell length in some studies) to tissue dry weight regression equation and analyzed percent nitrogen and phosphorus content to calculate the total nitrogen and phosphorus assimilated in the oyster tissue.
    a. Converts shell height in millimeters to tissue dry weight in grams.
    b. Similar approach as recommendations presented by STAC during July 9 and August 7, 2014 Watershed Technical Workgroup meetings.

2. FARM Model Approach (Ferreira et al. 2007 and 2009, Bricker et al. 2014 and 2015, Rose et al. 2015)—Crediting N removal based on model estimates of potential farm-level oyster production via filtration/mass balance.
    a. The FARM Model framework combines physical and biogeochemical models, shellfish growth models, and screening models at the farm scale to determine shellfish production and assess water quality changes on account of shellfish cultivation.
    b. Applicable to suspended culture from rafts or longlines and bottom culture.

STAC Review Summary

The Chesapeake Bay Program's Management Board requested the Scientific and Technical Advisory Committee (STAC) to review the paper, "Shellfish Aquaculture: Ecosystem Effects, Benthic-Pelagic Coupling and Potential for Nutrient Trading" by Newell and Mann (2012) and other relevant studies related to the use of shellfish as a method of nutrient reduction and advise how it could be applied in the Chesapeake Bay TMDL watershed model. STAC leveraged the review conducted by the 2013 workshop, "Quantifying Nitrogen Removal by Oysters" and made the following conclusions:

- Nitrogen content of oyster soft tissue and shell can reasonably be estimated as 8.2% and 0.2% of dry weight, respectively.
- Phosphorus content of oyster soft tissue and shell can reasonably be estimated as 1.07% and 0.06% of dry weight, respectively.
- Due to variability in predicting oyster growth and survival, nutrient removal BMP efficiencies should be based on actual harvest data (oyster dry weight) multiplied by the nutrient percentages above.
- Nutrient removal rates for shell only apply to shell which is not returned to the Bay.
- Burial rates for nutrients associated with biodeposits are not currently known.

- Measured denitrification rates associated with oyster aquaculture have not revealed any enhancement above background levels.
- Denitrification rates associated with oyster reefs typically exceed background levels, but are highly variable among locations and seasons.
- Lack of data on other grow-out methods (e.g., oyster grown in cages near the bottom and cage-less, spat-on-shell grown on the bottom) on denitrification rates.
- Oyster aquaculture has the potential to reduce nitrification (and hence coupled nitrification-denitrification) if rates of biodeposition by the oysters coupled with low flushing rates cause oxygen depletion. Modeling tools that provide site-specific guidance on oyster stocking densities should be developed to avoid this negative effect.

FARM Model Review Summary

The Panel reviewed the FARM model (Ferreira et al. 2007 and 2009, Bricker et al. 2014 and 2015, Rose et al. 2015), but agreed that there were existing limitations that wouldn't allow its use in determining the nitrogen reduction effectiveness at this time. The Panel planned to run a mock experiment to compare the nitrogen reduction in oyster tissue of harvested oysters determined by the FARM model versus the empirical approach recommended by STAC (2013 and 2014) but found that the present configuration of the model has limitations preventing such a comparison, including that it does not separate out the nitrogen assimilated in oyster tissue and shell or include oyster sizes related to the modeled biomass.

During their review of the model, the Panel also found that site-specific model calibration and nitrogen removal validation would be needed given that the FARM model was developed primarily as a tool to estimate harvestable biomass in certain areas outside of Chesapeake Bay and only provides an estimate of nitrogen removal that has not yet been validated with actual measurements.

Panel's Overall Conclusions

Empirical Approach:

1. Straightforward for tissue if harvest is reported (well established methods exist).
2. Sufficient shell height and tissue dry weight data exists to determine conservative nitrogen and phosphorus reduction effectiveness estimates related to oyster tissue.
3. The Panel is in the process of evaluating available empirical data to determine whether estimates can be determined for N and P assimilated in oyster shell (results will be presented in a different report).
4. If sufficient data exists, N and P reduction effectiveness in shell could be determined using this approach, but verification would be difficult due to not knowing where the shell ends up and what the degradation rate would be (i.e., release of N and P back in the water)

FARM Model Approach:

1. Not knowing the # of oysters per size class associated with the biomass and N removal numbers from the FARM model made it not possible to directly compare the amount of N assimilated between the STAC empirical (0.177 as the estimate of N content per market size, 76 mm, oyster) and FARM model approaches.
2. N content of particulate matter is considered as Redfield ratio with FARM model calculations done via carbon and then converted to nitrogen. The Panel is unsure if this would be representative of the location that is being simulated. The Panel felt N validation is needed.
3. Site-specific model calibration would be needed.
4. The Panel views this as a potential management tool; however, in the context of a method for BMP reduction effectiveness crediting, verification would likely be challenging; model limitations would need to be addressed and refined model re-evaluated by an expert panel.

Overall, panelists agreed that the empirical approach could be applied now for nitrogen and phosphorus assimilated in oyster tissue of removed (harvested) oysters. Due to current limitations of the FARM model, the Panel did not feel this method could be implemented for BMP reduction effectiveness determination at this time. In summary, the Panel concluded that the FARM model could be used as a management tool if calibrated to the location's growing conditions to assist with planning by allowing for a better understanding of the potential harvestable biomass, but for use in determining the reduction effectiveness for BMP application, the model's limitation concerning nitrogen validation would have to be addressed and the updated model re-evaluated by an expert panel. Panelists also expressed that the modeling approach, at best, would only estimate oyster growth, nitrogen and phosphorus assimilation, and harvestable biomass from an aquaculture operation and not actual removal; therefore, the number of oysters harvested would still have to be quantified to account for the actual biomass removed from the water. Given that growers already have to monitor oyster sizes and report numbers of oysters harvested, the empirical approach is a straight-forward method to calculate the amount of nitrogen and phosphorus in the tissue of removed oysters.

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Appendix D: Literature Review and Supplemental Analyses

Shell Height to Tissue Dry Weight Regression Analysis

This section provide additional details on the recommended 50th quantile regression equations to convert shell height to tissue dry weight, including literature/data reviewed, description of data removed from the analysis, the Panel's considerations concerning various oyster growth influencing factors, data limitations, and potential other sources that may have additional data that could be used to supplement the regression.

Literature/Data Review

The Panel considered the studies/data summarized below for the shell height to tissue dry weight regression analysis.

Ploidy	**Data Source**	**Study/Data Purpose**	**Growing Conditions**	**How were measurements obtained?**	**Study Conclusions**
Diploid	Higgins unpubl. data	Measurements included shell height and tissue dry weight	Reef	Similar to Higgins et al. 2011	None-unpublished
	Higgins et al. 2011	To evaluate the effectiveness of removal of excess nutrients from the Chesapeake Bay via bioassimilation of nutrients into oyster tissue and shell using oyster aquaculture.	Floating aquaculture cages Oysters per cage = 200 Cage area = 0.5 m^{-2} Mean SH = 44 – 118 mm	Oysters were opened, tissue and shell were separated and dried at 60°C for a period of time needed to dehydrate completely (greater than or equal to 7 days)	Model simulations showed greater nutrient reductions on basin rather than the bay-wide scale.
	Kellogg unpubl. data	Measurements included shell height and tissue dry weight.	Subtidal/restored reef	Similar to Kellogg et al. 2013	None-unpublished
	Kellogg et al. 2013	To evaluate water column removal of nutrients via assimilation by a restored oyster reef compared to an unrestored reef, as well as to measure oxygen and nitrogen fluxes on both types of reefs.	Restored reef	All organisms in the study were dried to a constant weight at 60°C and weighed to the nearest 0.1 mg.	The standing stock of nutrients was greater on the restored reef than the unrestored reef.

Diploid	Luckenbach and Ross 2009 (Part 1 of Report)	To monitor larval settlement on clean substrate placed in four tributaries, and to evaluate substrate quality on restoration reefs.	Subtidal patch reefs	Oysters were frozen, removed from the freezer, shell height measured to the nearest 0.1 mm, thawed, shucked, placed into aluminum pans and placed in a 90°C drying oven for a 48 hour minimum or until a constant weight was achieved.	Settlement onset and rate varied between tributaries and years measured.
	Luckenbach and Ross 2009 (Part 3 of Report)	To assess the stock of both restored and natural populations oysters in the Lynnhaven River prior to an Army Corps of Engineers oyster restoration project.	Restored and existing oyster reefs on bulkheads, in intertidal patch reefs, marsh, riprap, subtidal bottom (not discrete patches)	Oysters were frozen, removed from the freezer, shell height measured to the nearest 0.1 mm, thawed, shucked, placed into aluminum pans and placed in a 90°C drying oven for a 48 hour minimum or until a constant weight was achieved.	Restoration reefs were mapped. Patterns of oyster cover were analyzed according to dominant shoreline types.
	Paynter unpubl. data found in Liddel 2008	These data were originally collected as monitoring data of restoration reefs, then used in Liddel (2008) for modeling purposes.	Restored oyster reefs	Oyster tissue was dried in a drying oven at 60°C for 72 h and weighed. Clump height was measured.	None-unpublished

Triploid	Kingsley-Smith et al. 2009	To evaluate the growth and survival of triploid Eastern Oysters and Asian oysters at four sites with different salinities, tidal regimes, depths, predation intensities and disease pressures.	On-bottom cages	Tissue dry weight was determined by drying all soft tissue at 40°C to a constant weight for 1-7 days depending upon oyster size.	Oyster growth and survival varied significantly with site and species.

Data Removed from Analysis

The Panel decided to not use shell height and tissue dry weight data from sites that were outside of the Chesapeake Bay and its tributaries because of the variability that exists concerning oyster biomass between oysters with the same shell heights. This resulted in the exclusion of 2,429 oysters from four different data sources and four different locations:

Date Source	**Location**	**Number of Oysters**
Kellogg unpubl. data	Hillcrest Sanctuary in Mockhorn Bay	1232
Kingsley-Smith et al. 2009	Machipongo River	393
Paynter unpubl. data found in Liddel 2008	Chincoteague Bay	448
O'Beirn et al. 2004	Waters near Chincoteague Bay	356
Total		**2,429**

The Panel also removed the data below due to missing shell height or tissue dry weight measurements or because of quality concerns (e.g., dry weight greater than wet weight, zero or negative weights, note from researcher indicating the sample was compromised, obvious data entry error):

Data Source	Location	Number of Oysters
Higgins et al. 2011	n/a	1
	St. Jerome Creek	1
	Spencer's Creek	1
Kellogg et al. 2013	Choptank River	4
Kellogg unpubl. data	Harris Creek	2
Kellogg-Hillcrest	Mockhorn Bay	1
Kingsley-Smith et al. 2009	Machipongo River	10
	Patuxent River	1
	Severn River	2
	York River	7
Paynter unpubl. data found in Liddel 2008	Choptank River	2
	Eastern Bay	1
	Patuxent River	5
	South River	2
	Tangier Sound	2
Luckenbach and Ross 2009 (Part 3 of Report)	Lynnhaven River	2
Luckenbach and Ross 2009 (Part 1 of Report)	Great Wicomico River	1
	Lynnhaven River	5
	Rappahannock River	1
Ross unpubl. data	Rappahannock River	133
Total		**184**

The Ross unpubl. data from Rappahannock was removed in its entirety because there appeared to be a data entry error that resulted in the data being an order of magnitude greater than all the other Rappahannock River data points.

Evaluation of Influencing Factors on Oyster Growth

The Panel evaluated individual aspects of the data in order to confirm that the 50th quantile would conservatively address factors that could influence oyster growth and resulting tissue biomass, including ploidy (i.e., diploid and triploid), culture method (i.e., off-bottom, referred to as "water column," on-bottom, and combination of both), season (Fall, Winter, Spring, Summer), and environmental condition scenarios based on different habitat groups (i.e., location in the Bay: Upper, Mid, Lower, tributary or open Bay; salinity characteristics: mesohaline and polyhaline). Differences in ploidy appeared to have the largest impact on oyster growth resulting in the Panel recommending the use of separate regression equations for triploid and diploid oysters. The diploid data is presented for the seasonal and environmental condition considerations. The triploid data is not presented here, but it had similar trends as the diploid data for available scenarios (four seasons—Fall, Winter, Spring, Summer, and two habitat groups—Chesapeake Bay Tributary Mid-Bay Mesohaline and Chesapeake Bay Tributary Lower-Bay Polyhaline). For culture method considerations, only the diploid data could be evaluated because the triploid data were from one study that used the same culture method at each site. However, since the trends were similar for the two available habitat groups and also for the four different seasons, the Panel felt confident that the missing triploid scenarios would also be similar. Overall, the Panel felt that the driving factor influencing tissue biomass is the differences in ploidy, likely due to triploid oysters not having to expend energy on reproduction.

The 0.5 quantiles for subsets of the data corresponding to different ploidy, geographic location, and season were calculated.

The table below shows the equation terms and associated error for the different curves.

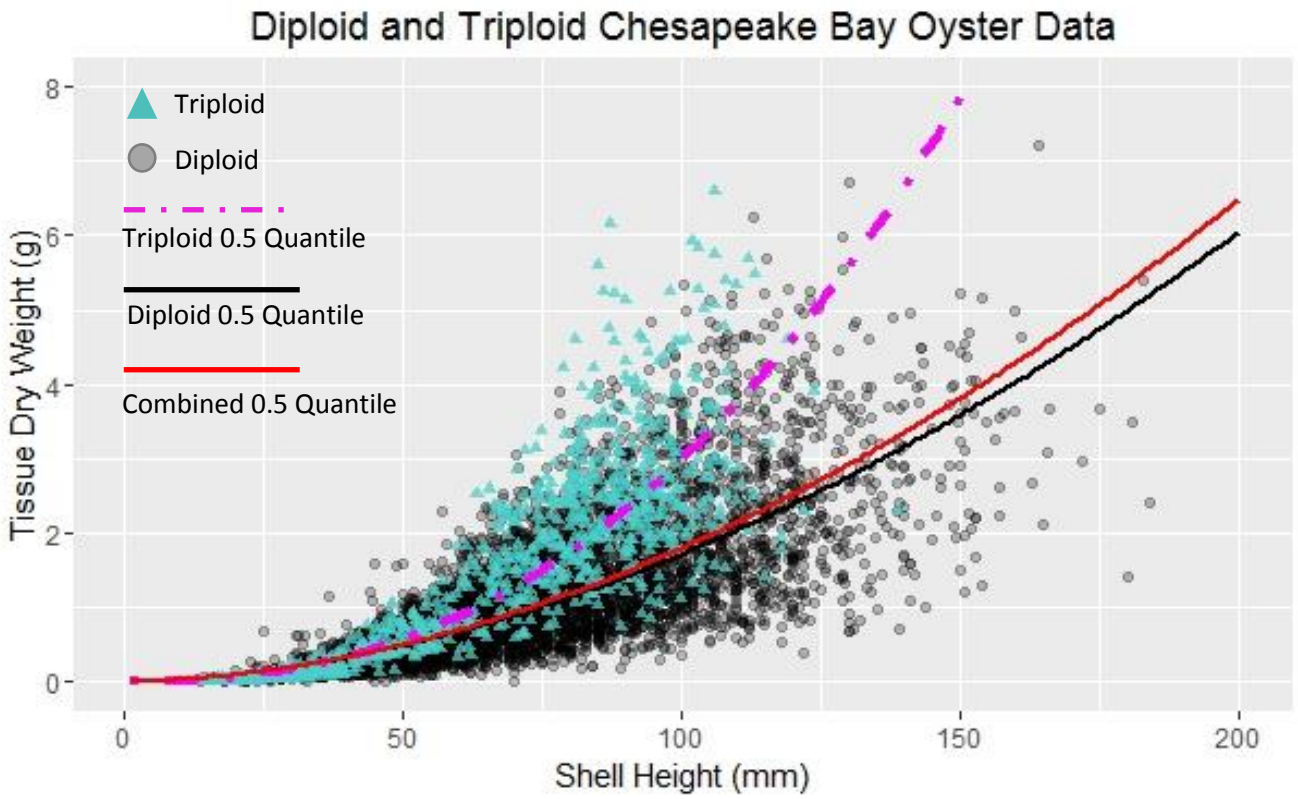
Equation: $y = ax^b$ **y = tissue dry weight (g), x = shell height (mm)**						
Oyster Data	**Number of Oysters**	**Quantile**	**a**	**b**	**Error a**	**Error b**
All data	6816	50	0.00036	1.84958	0.00004	0.02616
Diploid only	5750	50	0.00040	1.81627	0.00006	0.03427
Triploid only	1066	50	0.00005	2.38812	0.00002	0.08846
Tributary Mid-Bay Mesohaline	3948	50	0.00027	1.89815	0.00004	0.03869
Open Water Mid-Bay Mesohaline	515	50	0.00003	2.40561	0.00001	0.12084
Tributary Lower Bay Mesohaline	432	50	0.00026	2.00597	0.00016	0.15426
Tributary Lower Bay Polyhaline	1397	50	0.00008	2.2407	0.00003	0.07461
Tributary Upper Bay Mesohaline	524	50	0.00043	1.80438	0.00017	0.08703
Winter	569	50	0.00005	2.25556	0.00003	0.11467
Spring	1999	50	0.00031	1.94123	0.00007	0.05564
Summer	2649	50	0.00036	1.80421	0.00004	0.02776
Fall	1599	50	0.00037	1.86002	0.00009	0.05919
Water Column	1150	50	0.00005	2.36601	0.00003	0.11302
Bottom Oyster Planting	5556	50	0.0004	1.8112	0.00005	0.02909
Water Column and Bottom Oyster Planting	110	50	0.0001	2.29765	0.00009	0.22416

The Panel's evaluation on each of the growth-influencing factors are further described below.

Ploidy Considerations

Research suggests that triploid oysters grow faster and have more biomass than diploid oysters (Degremont et al. 2012). Therefore, the Panel evaluated and compared the 50th quantile regression curves using the combined triploid and diploid data (n = 6816 oysters), diploid only data (n = 5750 oysters), and triploid only data (n = 1066 oysters).

The figure below depicts the different ploidy curves in relation to one another.



The Panel chose the 50th quantile curve because it captures the median of the dataset where 50% of the data falls above the curve and 50% of the data falls below the curve. The Panel felt this would be most appropriate for a default estimate because it evenly distributes the likelihood of underestimating or overestimating the biomass associated with a certain shell height.

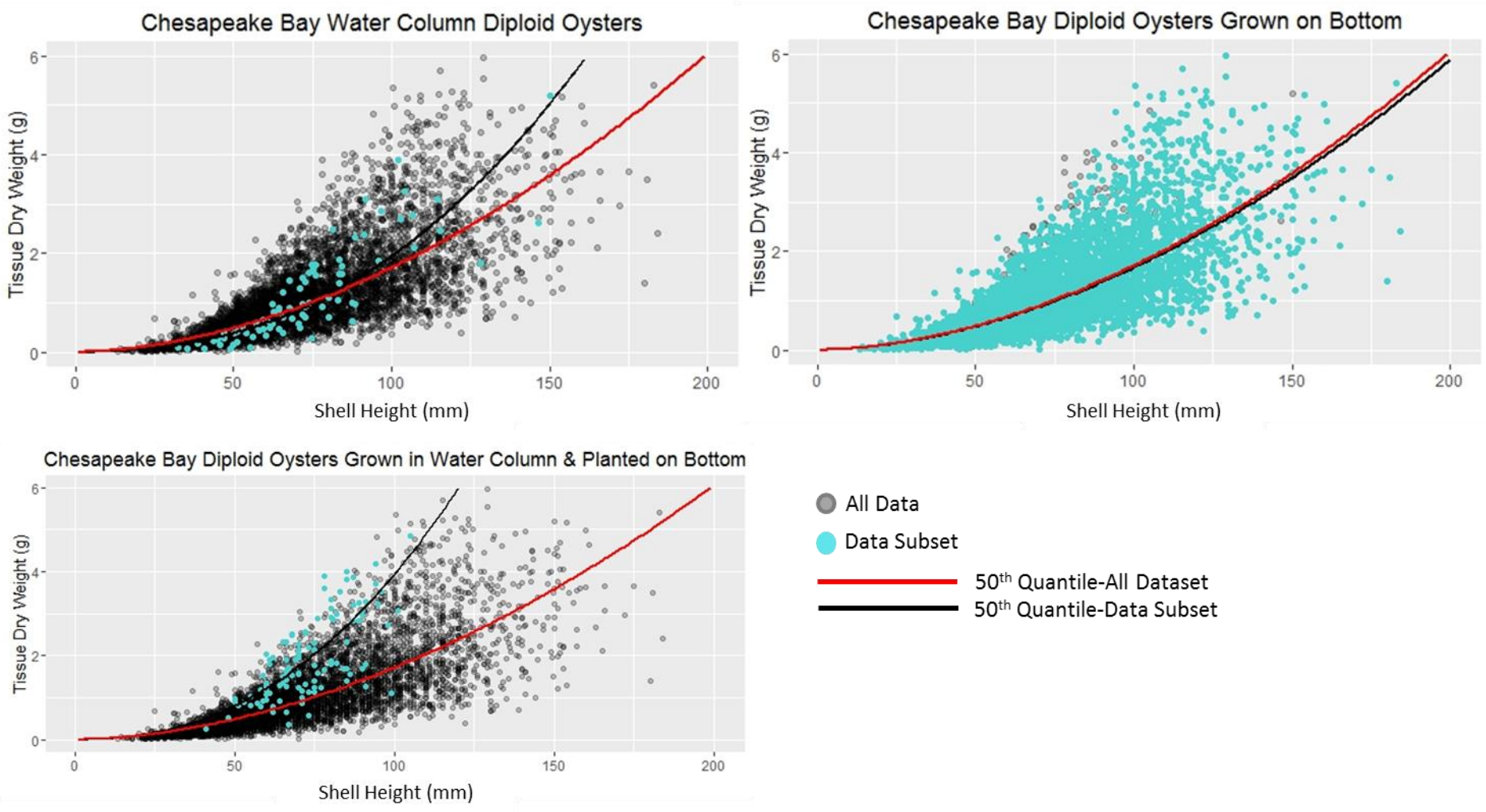
The triploid only 50th quantile curve was clearly distinct and much steeper than the combined diploid and triploid curve and the diploid only curve. The diploid only curve matches the combined diploid and triploid curve to around 75 mm shell height, and slightly deviates below the combined curve around shell heights greater than 75 mm. The Panel felt that the much steeper triploid curve warranted separate reduction

effectiveness estimates for triploid oysters because the data shows that triploid oysters have greater tissue biomass than diploid oysters. Therefore, the triploid only and the diploid only regression equations were used to develop separate reduction effectiveness estimates for triploid and diploid oysters.

Culture Method Considerations

Research has shown that oysters grown off-bottom in the water column tend to have more tissue biomass at smaller shell heights than oysters grown directly on the bottom (Kinsley-Smith et al. 2009). Therefore, the Panel evaluated the data based on the culture method used to grow the oysters. The diploid dataset included 3 main oyster culture methods—off-bottom in the water column (oysters grown in cages near the bottom or floating rafts near the surface), on-bottom (planting hatchery-produced oysters or sampling wild oysters from existing or restored reefs), and a combination of off-bottom water column and on-bottom oyster practices (oysters were grown to 50 mm in cages and then planted on the bottom). The Panel plotted these different categories as an overlay over the entire dataset and considered their location in relation to the 50th quantile curve of the entire dataset. These plots are shown below.

Oyster Culture Method Considerations



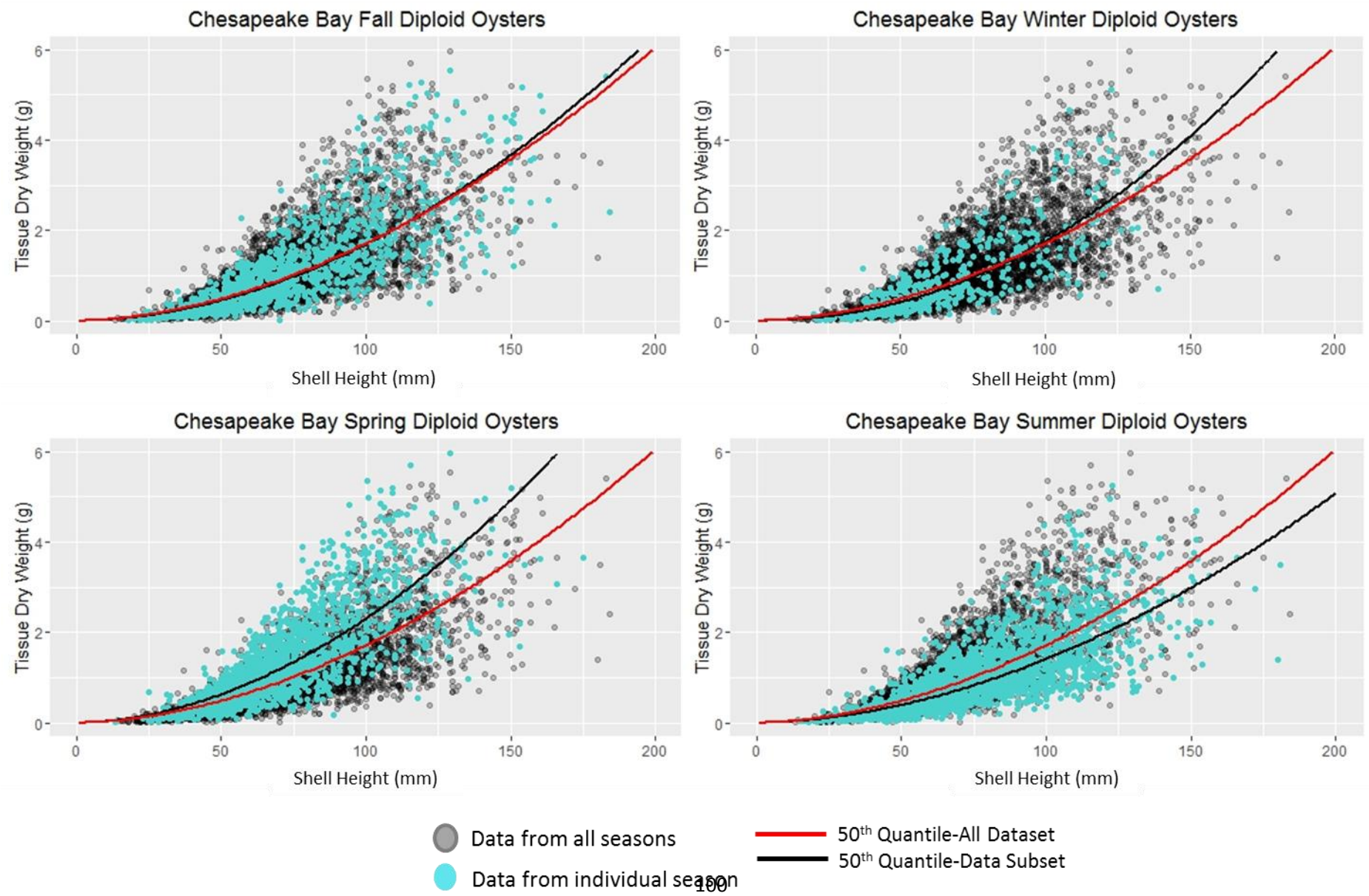
The water column (i.e., off-bottom) and the combination of water column and on-bottom culture methods skewed above the 50th quantile curve of the entire dataset (i.e., smaller shell sizes have greater biomass), with the latter having a much steeper curve than the 50th quantile of the entire dataset. Since this culture method and the water column culture method produced steeper curves, the Panel decided that the 50th quantile regression equation will more likely underestimate the tissue dry weight, and hence, the nitrogen content, supporting that it will result in a conservative reduction effectiveness estimate.

The on-bottom data skewed slightly below the 50th quantile curve; however, since the majority of the data is from this category, the regression curve itself is influenced by it, meaning that greater shell sizes have less biomass (i.e., the curve is more shallow than steep), supporting a conservative estimate. Additionally, aquaculturists use techniques for optimal oyster growth, unlike the data in this category which includes wild oysters and research grown oysters, suggesting that in real-world application as a BMP, the data will likely fall more so above the 50th quantile curve of the entire dataset.

Seasonal Considerations:

Research has shown that there could be seasonal differences in oyster soft tissue biomass due to differences in food availability (Lenihan et al. 1996) and reproductive cycle (e.g., tissue lost during spawning) (Kennedy et al. 1996). The Panel evaluated potential seasonal differences by plotting the four different seasons (fall, winter, spring, and summer) as an overlay over the entire dataset and considering their location in relation to the 50th quantile curve of the entire dataset. These plots are shown below.

Seasonal Considerations



The spring oyster data skewed above the 50th quantile curve of the entire dataset and the summer oyster data skewed below. The spread of the fall and winter oyster data is evenly distributed above and below the 50th quantile curve to the approximate shell height of 125 mm and 100 mm, respectively, producing similar curves as the 50th quantile curve of the entire dataset. Above these shell heights, the data subset curves are slightly above the entire dataset curves. If only removing oysters during the spring, there may be a greater chance in underestimating the biomass and if removing only during the summer, a greater chance in overestimating the biomass. Given that 3 seasons skewed above the recommended 0.5 quantile curves and the reduction effectiveness is based on annual reporting with growers typically harvesting year round, the Panel felt that the reduction effectiveness would balance out (i.e., any potential overestimation would be negated by instances of underestimation).

Environmental Condition Considerations:

The Panel considered potential environmental condition differences on oyster growth by grouping the data by location within the Bay (Upper, Mid, Lower), including whether the oysters were grown in tributaries or in open-Bay waters, and by their salinity characteristics (i.e., mesohaline, polyhaline). The Bay locations and the salinity regimes were defined using the Chesapeake Bay Program Bay-wide segmentation scheme (U.S. EPA 2004) and spatially-explicit salinity gradient maps from the U.S. Army Corp. of Engineers (http://www.nab.usace.army.mil/Missions/Environmental/Oyster-Restoration/Oyster-Master-Plan/), respectively. These raster salinity maps were generated in support of the U.S. Army Corp. of Engineers Oyster Restoration Master Plan and derived from interpolated spring and summer water quality samples collected between 2001 and 2006.

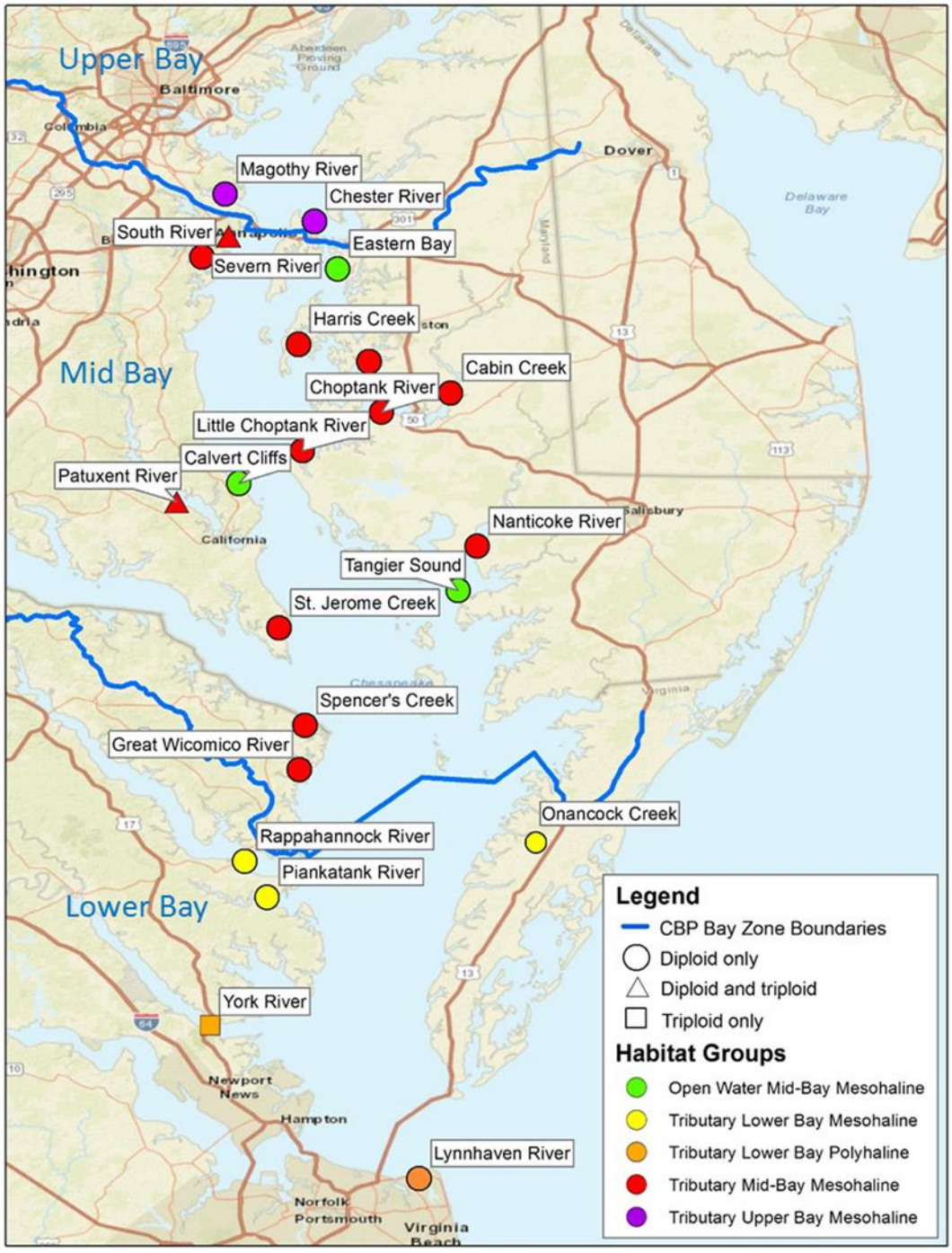
This strategy resulted in five main habitat groups:

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

The dataset used to develop the regression equations included oysters collected at 22 general sampling locations distributed throughout the Chesapeake Bay and its tributaries. Raster maps and the geographic coordinates of each sampling location 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.

The map below depicts the sampling locations and assigned habitat groups for the diploid and triploid oysters used in the shell height to tissue dry weight regression analysis.

Sampling locations and habitat groups of oyster data used in shell height to tissue dry weight regression analysis

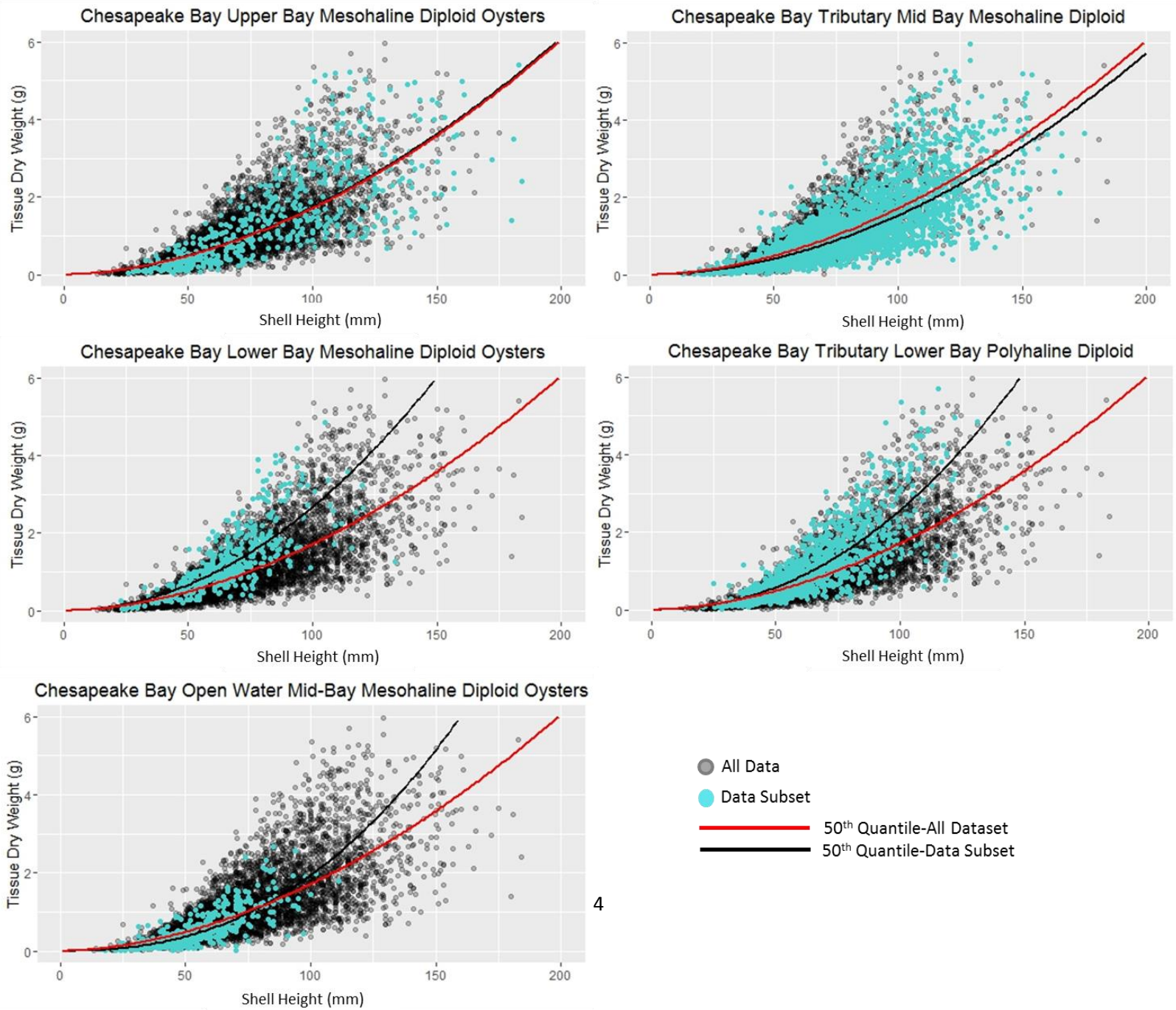


Most of the oysters were collected from riverine mesohaline habitats in Maryland, but 8% and 20% were from open-Bay mesohaline and tributary polyhaline portions of the Bay, respectively. The number of individual oysters associated with each habitat group ranged between 25 and 1179. The table below further summarizes the number of sites and oysters within each habitat group. References refer to the studies the oyster data came from.

Diploid				
Habitat Group	**General Sampling Location**	**Sites (#)**	**Oysters (#)**	**References**
Open Water Mid-Bay Mesohaline	Calvert Cliffs	1	78	Paynter unpubl. data found in Liddel 2008
	Eastern Bay	9	199	Paynter unpubl. data found in Liddel 2008
	Tangier Sound	5	238	Paynter unpubl. data found in Liddel 2008
Tributary Upper Bay Mesohaline	Chester River	23	381	Paynter unpubl. data found in Liddel 2008
	Magothy River	4	143	Paynter unpubl. data found in Liddel 2008
Tributary Mid-Bay Mesohaline	Cabin Creek	1	31	Paynter unpubl. data found in Liddel 2008
	Choptank River	22	836	Higgins unpubl. data, Kellogg et al. 2013, Paynter unpubl. data found in Liddel 2008
	Great Wicomico River	2	394	Luckenbach and Ross 2009
	Harris Creek	8	1059	Kellogg unpubl. data
	Little Choptank River	2	25	Paynter unpubl. data found in Liddel 2008
	Nanticoke River	1	27	Paynter unpubl. data found in Liddel 2008
	Patuxent River	14+	361	Paynter unpubl. data found in Liddel 2008
	Severn River	6+	197	Paynter unpubl. data found in Liddel 2008
	South River	1	54	Paynter unpubl. data found in Liddel 2008
	Spencer's Creek	1	46	Higgins et al. 2011
	St. Jerome Creek	1	38	Higgins et al. 2011
	Tred Avon River	2	32	Paynter unpubl. data found in Liddel 2008
Tributary Lower Bay Mesohaline	Onancock Creek	1	110	Kellogg unpubl. data
	Piankatank River	2	172	Luckenbach and Ross 2009
	Rappahannock River	2	150	Luckenbach and Ross 2009
Tributary Lower Bay Polyhaline	Lynnhaven River	24	1179	Higgins unpubl. data, Luckenbach and Ross 2009
Triploid				
Tributary Mid-Bay Mesohaline	Patuxent River	1	417	Kingsley-Smith et al. 2009
	Severn River	1	431	Kingsley-Smith et al. 2009
Tributary Lower Bay Polyhaline	York River	1	218	Kingsley-Smith et al. 2009

Overlays of oyster shell heights and corresponding tissue biomass associated with the habitat groups were added to the quantile regression plots to evaluate whether distinct differences were noticeable. The diploid plots are shown below.

Environmental Condition Considerations



Oyster data from the polyhaline tributary and mesohaline tributary and open-Bay locations in the Lower Bay skewed above the 50th quantile curve of the entire dataset (i.e., smaller oyster sizes exhibited greater biomass), suggesting a greater chance to underestimate the reduction effectiveness. Oyster data from the mesohaline tributary locations in the mid-Bay zone skewed slightly below, while the mesohaline upper-Bay tributary curve overlapped the curve of the entire dataset. Given that four of the five habitat group curves either matched or were steeper than the 50th quantile curve of the entire dataset and the tributary mesohaline curve was only slightly below, the Panel felt that the 50th quantile equation of the entire dataset would produce conservative default reduction effectiveness estimates.

Data Limitations

The tables below summarizes the number of oysters available for the shell height to tissue dry weight regression analysis based on the different factor scenarios:

Diploid	**Season Removed (# of Oysters)**				
Representative Culture Method	**Fall**	**Winter**	**Spring**	**Summer**	**Total**
Off-Bottom Water Column	0	0	84	0	**84**
On-Bottom Oyster Planting	1229	450	1507	2370	**5556**
Water Column and Bottom Oyster Planting Combination	0	0	109	1	**110**
Total	**1229**	**450**	**1700**	**2371**	**5750**

Triploid	**Season Removed (# of Oysters)**				
Representative Culture Method	**Fall**	**Winter**	**Spring**	**Summer**	**Total**
Off-bottom Water Column	370	119	299	278	**1066**
On- Bottom Oyster Planting	0	0	0	0	**0**
Water Column and Bottom Oyster Planting Combination	0	0	0	0	**0**
Total	**370**	**119**	**299**	**278**	**1066**

Diploid oyster data were limited for the off-bottom culture method within all seasons, with data only being available in the spring, when compared to the on-bottom culture method. On the other, triploid oyster data were limited for the on-bottom culture method, with no data being available in any season, when compared to the off-bottom culture method. These scenarios would benefit from additional research.

The combined water column and bottom oyster planting culture method was unique to the research project and does not typically occur as an aquaculture technique. However, given that the data produced a much steeper curve than the recommended 50th quantile curve (see culture method considerations above), it may be worthwhile to investigate if this difference is being driven by the culture method or by the season the oysters are removed. The current dataset is not sufficient to explore this because it only has limited spring diploid oyster data.

Other Potential Data Sources

The 2010-2012 Chesapeake data from the Virginia portion of the Bay used in Powell et al. 2015 is another large dataset that includes shell height and dry tissue weight measurements from 19 oyster grounds. This dataset could be mined to fill in data gaps; however, since the data was representative of diploid on-bottom culture (collections were from subtidal reefs) from the spring (oysters were removed during April and May), the Panel did not feel it would greatly change the results of the regression analysis. Supporting this conclusion is the scaling exponent range (*b*) presented in Powell et al. 2015 of 1.61-2.75. The scaling exponent of the recommended diploid regression equation is 1.82, which fall within this range.

Percent Nitrogen and Phosphorus Content in Oyster Tissue Literature Review

Nitrogen Content Literature Review

Summary of studies that were reviewed by the Panel that included information on the nitrogen content of oyster tissue as a percentage of dry weight are presented in the tables below. N = number of oysters sampled, SH = Shell Height. All oysters were *Crassostrea virginica*. All studies were on diploid oysters, except for Reistma et al. 2014, which included measurements for both diploid and triploid oysters (only triploid values are reported in table below).

Studies used for the estimate								
Source	**Growing Conditions**	**Study Site**	**% Nitrogen Mean**	**% Nitrogen Range**	**N**	**Study Purpose**	**How were N content measurements obtained?**	**Study Conclusions**
Carmichael et al. (2012)	Cages 6 cm off bottom Oysters per cage = 67 Cage area = 0.15 m^{-2} SH = 8.2 ± 0.2 mm at start of study Maximum SH ~68 mm at end of study Growing season: June-October 2003	Sage Lot Pond, Cape Cod, MA Salinity = 28 N load =14 x 10-4 kg N m-2 y-1	8.47 ± 0.09 SE		160	To evaluate N removal by oyster tissue assimilation by examining removal from land-derived N sources in five different Cape Cod estuaries. The sampling design aimed to capture spatial and temporal variation in growth, survival, and N content during the growing season.	Obtained empirical, estuary-specific N content of oyster tissue only. Dried samples were ground to a powder, and combusted during stable isotope analysis. Researchers estimated time they would reach harvestable size (76.2 mm shell height), and used regression analysis to extrapolate the soft tissue N content when oysters did not reach harvestable size within the study season (page 1137).	The data on N content and time to reach harvest size from this study was consistent with previous studies measuring the same variables in N-enriched estuaries. This suggests food resources are not limited, and that N removal in a given estuary to be relative to N load. N content did not differ among estuaries; however, the significant difference in relative dry mass at harvest size resulted in different N content per oyster and therefore, different N removal capacities via tissue assimilation.
		Wild Harbor, Cape Cod, MA Salinity = 26 N load = 65 x 10-4 kg N m-2 y-1	8.95 ± 0.16 SE		160			
		Green Pond, Cape Cod, MA Salinity = 28 N load = 178 x 10-4kg N m-2y-1	8.04 ± 0.24 SE		160			
		Snug Harbor, Cape Cod, MA Salinity = 25 N load = 236 x 10-4kg N m-2y-1	9.19 ± 0.15 SE		160			
		Childs River, Cape Cod, MA Salinity = 26-27 N load = 601 x 10-4kg N m-2y-1	8.37 ± 0.27 SE		160			

Grizzle and Ward (2011)[a]	Cages ~10-20 cm off bottom Oyster density per cage: "Seed" = 1,000 indiv. 1-yr olds= 200 indiv.	Adams Point, Great Bay, NH	7.20 ± 1.61 SD	5.20 - 9.56	10	Carbon and nitrogen removal was evaluated at six sites in the Great Bay estuarine system in NH in order to determine the bioextraction potential by oysters.	Obtained empirical, site-specific N content of oyster tissue only, as well as growth data. Tissue was dried, homogenized (using a blender) and N content was determined by using an elemental analyzer. Shell height and soft tissue dry weight were used as a proxy for growth. N removal variations were assessed by comparing final %N content. To obtain a whole-oyster N content for the two larger size classes evaluated in this study, Higgins et al. 2011 shell N content data were used (pages 8 and 11).	Significance testing yielded lower C and N assimilation values at one site compared to the others, as well as higher C and N assimilation at one site compared to the others.
		Bellamy River, Great Bay, NH	6.63 ± 2.13 SD	3.00 - 9.87	10			
		Oyster River, Great Bay, NH	7.55 ± 2.14 SD	3.23 - 9.55	9			
		Fox Point, Great Bay, NH	5.64 ± 1.70 SD	3.85 - 9.07	10			
		Nannie Island, Great Bay, NH	7.39 ± 2.07 SD	3.70 - 10.66	10			
		Squamscott R., Great Bay, NH	9.27 ± 2.38 SD	5.13 - 14.01	10			
Higgins et al. (2011)	Floating aquaculture cages Oysters per cage = 200 Cage area = 0.5 m^{-2} Mean SH = 44 – 118 mm	Spencer's Creek, VA Salinity = 5 – 15 Low flow, high sedimentation Growth Period: November 2006, August 2007 to October 2009	8.10 ± 0.13 SE	5.80 – 9.97	47	To evaluate the effectiveness of removal of excess nutrients from the Chesapeake Bay via bioassimilation of nutrients into oyster tissue and shell using oyster aquaculture.	Obtained empirical N content of oyster tissue by grinding up dehydrated tissue into a fine powder, combusting and use of an elemental analyzer (page 273). Nutrient contents of tissue were recorded as percent of dry weight.	Model simulations showed greater nutrient reductions on basin rather than the bay-wide scale.
		St. Jerome Creek, MD Salinity = 12 – 15 High flow, low sedimentation Growth Period: May-July 2007 to October 2009	7.37 ± 0.19 SE	5.43 – 10.36	37			
Kellogg et al. (2013)	Restored oyster reef Oyster density = 131 m^{-2} Mean SH = 114 mm	Choptank River, MD Salinity = 7.0-11.6 Subtidal reef	9.27 ± 0.60 SD	8.58 – 9.71	15[b]	To evaluate water column removal of nutrients via assimilation by a restored oyster reef compared to an unrestored reef, as well as to measure oxygen and nitrogen fluxes on both types of reefs.	Obtained empirical N content of oyster tissue data. Dried samples were ground to a fine power and N was analyzed with an automated CHN analyzer. The total amount of nitrogen assimilated was determined by multiplying the total dry weight of oysters by its percentage of N (Page 7). Shell and tissue were analyzed separately when the oyster was greater than 10 mm in shell height.	The standing stock of nutrients was greater on the restored reef than the unrestored reef.
Kellogg unpubl. data	Wild oysters from intertidal reef Oyster sizes ranged from 53 to 122 mm	Hillcrest Oyster Sanctuary, Mockhorn Bay, VA	8.13	7.8 - 8.67	9[c]	Included %N content measurements in oyster tissue.	Similar to Kellogg et al. 2013	None-unpublished

Sebastino et al. (2015)[d]	Cages 1 m depth Cage area: 0.4 m^2 Oysters per cage: 600	Jamaica Bay, NY	8.93 ± 0.03 SE			The potential sequestration of nutrients by oysters was evaluated in a high-nitrogen input estuary and a medium-nitrogen input estuary in New York state.	Obtained empirical N content of oyster tissue (page 575). Tissue N content was determined by grinding and homogenizing the dry sample and combustng it using an elemental analyzer, and standardizing to aspartic acid samples. Regression analysis was used to determine an equation from which tissue %N could be predicted from a given dry tissue mass after one season of growth.	Oysters in the high-N input estuary (Jamaica Bay) provided less removal of the total N input in that area than the oysters at the medium-N input estuary removed of the total N input to that estuary (Great South Bay). Oysters from Jamaica Bay would not be able to be sold as food.
		Great South Bay, NY	8.94 ± 0.03 SE					

[a]Values calculated using raw data provided in report appendix

[b]Three samples composed of five individuals per sample

[c]Three samples composed of 3 individuals

[d]Three sites were sampled within each bay, but results in Table 1 & 2 were aggregated data by bay.

Studies not used for the estimate								
Source	**Growing Conditions**	**Study Site and Environmental Conditions**	**% Nitrogen Mean**	**% Nitrogen Range**	**N**	**Study Purpose**	**How were N content measurements obtained?**	**Study Conclusions**
Dalrymple and Carmichael (2015)[a]	Cages 10-20 cm off bottom Cage area: 0.65 m^{-2} Oysters per cage Juvenile: 600 Adult: 200 Mean juvenile SH = 42 mm Mean adult SH = 98 mm	Mobile Bay, AL	11.8 ± 0.01 SE	9.10-13.54	108	To understand how nitrogen removal by oysters is affected by ontogeny	Obtained empirical N content of oyster tissue via combustion during stable isotope analysis. For some oyster samples, only the adductor muscle N content was measured, and a correction factor was applied from the relationship between adductor muscle and whole tissue N (page 208).	Juvenile oyster assimilated N while adult oysters lost mass and returned N to the estuary. However, the percentage of N in soft tissues did not differ between age classes.
Grizzle et al. (2016)[b]	Cages 10 cm off bottom Oyster density per cage: "Seed" = 1000 individuals 1-yr olds = 200 individuals	Oyster River, Great Bay, NH	~8.3			To quantify the relationships among various factors (oyster size, seasonality, nutrient content in ambient water) on the growth, morphometrics, and percent N and C content of farmed Eastern oysters.	Obtained empirical, site-specific N content of oyster tissue (page 2). Tissue was dried, homogenized, and analyzed using a CHN/O elemental analyzer.	A quadratic polynomial fit showed a 63 mm oyster would be reached after 2 years and a 76 mm oyster would be reached after 3 years. Significant differences in growth rates and nutrient content existed between the sites.
		Little Bay, Great Bay, NH	~6.9					
		Adams Point, Great Bay, NH	~7.8					
		Bellamy River, Great Bay, NH	~7.6					
		Squamscott River, Great Bay, NH	~8.7					
		Nannie Island, Great Bay, NH	~7.4					
Higgins unpubl. data	Reef	Choptank River, MD	8.2	5.48-10.6	9	%N, %P, and %C content in oyster tissue were measured.	Similar to Higgins et al. 2011	None-unpublished
		Lynnhaven River, VA	8.8	6.99-10.52	18			
Newell, R. I. E. and Mann, R. 2012	See Note[c]	See Note[c]	7	See Note[c]	See Note[c]	See Note[c]	See Note[c]	See Note[c]

Reitsma et al. 2014	On bottom and off bottom Mean SH = 83.8 mm	Cape Cod, MA (10 sites; aggregated data presented)	8.5[d]			To test the % N and %C content of C. virginica and M. mercenaria from coastal waters in Cape Cod, MA	%N and %C analysis was done on dried ground soft tissue with gut intact using standard laboratory methods.	Local oysters had an average of 0.69% N by total dry weight (~0.22N/animal). Values did vary by season and to a lesser extent by location or grow-out method. Biggest driver of N content differences among similar size cohorts was the tissue mass as opposed to the %N content in tissue.

[a]Figure 10 reports additional unpublished data from Mobile Bay, AL, and Grand Bay, MS. The mean nitrogen tissue content for all Gulf of Mexico sites was 10.62 ± 0.17 SE

[b]Average %N estimated from Figure 9 in study

[c]The table provided in this review did not have a range or number of samples, and did not explicitly state that the %N was a mean. This publication references: Newell, R.I.E. 2004. Ecosystem Influences of Natural and Cultivated Populations of Suspension feeding Bivalve Molluscs: a Review. Journal of Shellfish Research. 23:51-61, but this paper only had a sentence about the data, and does not contain any of the information above, and also has no information about location, growing conditions, or how N measurements were.

Phosphorus Content Literature Review

Summary of studies that were reviewed by the Panel that included information on the phosphorus content of oyster tissue as a percentage of dry weight are presented in the tables below. N = number of oysters sampled, SH = Shell Height. All oysters were *Crassostrea virginica*. All studies were assumed to be on diploid oysters.

Studies used for estimate								
Source	**Growing Conditions**	**Study Site**	**% Phosphorus Mean**	**% Phosphorus Range**	**N**	**Study Purpose**	**How were P content measurements obtained?**	**Study Conclusions**
Higgins et al. (2011)	Cultivated oysters; grown in floating cages, 200 oysters per bag Bag size = 100 cm L x 50 cm W x 8 cm D	Spencer's Creek, VA Salinity = 5 – 15 Low flow, high sedimentation	0.83 ± 0.01	0.60–1.05	47	To evaluate the effectiveness of removal of excess nutrients from the Chesapeake Bay via bioassimilation of nutrients into oyster tissue and shell using oyster aquaculture.	Tissue was dehydrated and ground into a fine powder. Phosphorus was then measured by using USEPA method SW 846-3051/6010B, which involves acid digestion followed by inductively coupled plasma–atomic emission spectrometry.	Assuming no mortality, at a density of 286 oysters m^{-2} harvest size (76 mm shell height) phosphorus removal rate can be as high as 54 kg TP ha^{-1}
		St. Jerome Creek, MD Salinity = 12 – 15 High flow, low sedimentation	0.82 ± 0.02	0.53–1.07	37			
Kellogg et al. (2013)	1 restored oyster reef and one adjacent unrestored control reef were used as sites for the experiments. 4 X4 m experimental plots were on each one. Presumably oysters were both hatchery and wild oysters (see next column). At restored site, oyster density was increased to ~100 oysters/ m2 by taking oysters from the surrounding areas and placing them in the plots.	One restored site and one control site in Choptank River, MD.	1.26±0.18	NA	3	To evaluate water column removal of nutrients via assimilation by a restored oyster reef compared to an unrestored reef, as well as to measure oxygen and nitrogen fluxes on both types of reefs.	Samples were ground into a fine powder using a mortar and pestle prior to nutrient analyses. Phosphorus was analyzed by extraction of P from combusted sampled using 1 N HCl followed by colorimetric analyses (Aspila et al. 1976). For oysters >10mm in shell height, shells and soft tissue were analyzed separately.	The standing stock of nutrients was greater on the restored reef than the unrestored reef.
Kellogg unpubl. data	Wild oysters from intertidal reef Oyster sizes ranged from 53 to 122 mm	Hillcrest Oyster Sanctuary, Mockhorn Bay, VA	8.13	7.8 - 8.67	9[a]	Included %P content measurements in oyster tissue.	Similar to Kellogg et al. 2013	None-unpublished

[a]Three samples composed of 3 individuals

Studies not used for the estimate								
Source	**Growing Conditions**	**Study Site**	**% Phosphorus Mean**	**% Phosphorus Range**	**N**	**Study Purpose**	**How were P content measurements obtained?**	**Study Conclusions**
Higgins unpubl. data	Reef	Choptank River, MD	1	0.79-1.25	9	%N, %P, and %C content in oyster tissue were measured.	Similar to Higgins et al. 2011	None-unpublished
		Lynnhaven River, VA	0.81	0.67-1.03	18			
Newell, R. I. E. and Mann, R. 2012	See Note[a]	See Note[a]	0.80	See Note[a]	See Note[a]	See Note[a]	See Note[a]	See Note[a]

[a]The table provided in this review did not have a range or number of samples, and did not explicitly state that the %P was a mean. This publication references: Newell, R.I.E. 2004. Ecosystem Influences of Natural and Cultivated Populations of Suspension feeding Bivalve Molluscs: a Review. Journal of Shellfish Research. 23:51-61, but this paper only had a sentence about the data, and does not contain any of the information above, and also has no information about location, growing conditions, or how N measurements were.

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Appendix E: Panel Meeting Minutes

To be included in final report.

Appendix F: Technical Requirements for Reporting and Simulating Oyster Aquaculture BMPs in the Phase 6 Watershed Model

Background: In June, 2013 the Water Quality Goal Implementation Team (WQGIT) agreed that each BMP expert panel would work with CBPO staff and the Watershed Technical Workgroup (WTWG) to develop a technical appendix for each expert report. The purpose of the technical appendix is to describe how the expert panel's recommendations will be integrated into the modeling tools including NEIEN, Scenario Builder and the Watershed Model.

Q1: What types of oyster aquaculture practices will be available for credit for nutrient reductions in the Phase 6 Model?

A1: The expert panel recommended pound reduction credits for nutrients assimilated in harvested oyster tissue for the following types of aquaculture operations:

- Off-bottom private oyster aquaculture using hatchery-produced oysters
- On-bottom private oyster aquaculture using hatchery-produced oysters
- On-bottom private oyster aquaculture using substrate addition

The reduction credits may be applied for any of these types of operations based upon the size and type of oyster harvested.

Q2: What are the reduction credits for these oyster aquaculture practices?

A2: The expert panel recommended that all three types of oyster aquaculture practices will receive the same pound reduction credits, which will vary based upon the average size and type of oyster harvested at an operation. The table below provides the new BMP names and lbs of nutrient reduction related to maximum size and type of oysters harvested.

Table 1. Nutrient Reductions per 1,000,000 Oysters Harvested by BMP

BMP Name	Lbs N Reduced/1,000,000 Oysters Harvested	Lbs P Reduced/1,000,000 Oysters Harvested
Diploid Oyster Aquaculture 2.25 Inches	110	22
Diploid Oyster Aquaculture 3.0 Inches	198	22
Diploid Oyster Aquaculture 4.0 Inches	331	44
Diploid Oyster Aquaculture 5.0 Inches	485	44
Diploid Oyster Aquaculture Greater 6.0 Inches	683	66
Triploid Oyster Aquaculture 2.25 Inches	132	22
Triploid Oyster Aquaculture 3.0 Inches	287	22
Triploid Oyster Aquaculture 4.0 Inches	573	66
Triploid Oyster Aquaculture 5.0 Inches	970	110
Triploid Oyster Aquaculture Greater than 6.0 Inches	1,477	154
Site-Specific Monitored Oyster Aquaculture	NA	NA

Q3: What credit may be given if an operation or state does not know the type or average size of oysters harvested?

A3: If the type or average size is not known, then the diploid estimate will be used based on the State's minimum legal harvest size. For example, if the minimum legal harvest size is 3 inches then the State should submit this for credit under the "Diploid Oyster Aquaculture 3.0 Inches" BMP. States are expected to describe the minimum legal harvest requirements in their Quality Assurance Project Plan (QAPP).

Q4: How would an operation or state receive credit for the "Site-specific Monitored Oyster Aquaculture" practice?

A4: An operator will need to provide the state with the average tissue dry weight of subsample of 50 oysters per oyster size class category within two seasons that are at least six months apart. These dry tissue estimates can then be multiplied by a default nitrogen content of 8.2% and a default phosphorus content of 0.9%, and aggregated to determine the total nutrients reduced by the harvested oysters.

Q5: What should a state report to NEIEN to receive credit for the diploid or triploid oyster practices?

A5: States should report the following parameters to NEIEN:

- *BMP Name:* Select from list in Table 1 above.
- *Measurement Name:* Oysters Harvested
- *Land Use:* NA
- *Geographic Location:* Approved NEIEN geographies: Latitude, Longitude; County; County (CBWS Only); Hydrologic Unit Code (HUC12, HUC10, HUC8, HUC6, HUC4); State (CBWS Only)
- *Date of Implementation:* Year oysters were harvested.

Q6: What should a state report to NEIEN to receive credit for the sit-specific monitored practice?

A6: States should report the following parameters to NEIEN:

- *BMP Name:* Site-Specific Monitored Oyster Aquaculture
- *Measurement Name(s):* Oysters Harvested; Lbs TN; Lbs TP
- *Land Use:* NA
- *Geographic Location:* Approved NEIEN geographies: Latitude, Longitude; County; County (CBWS Only); Hydrologic Unit Code (HUC12, HUC10, HUC8, HUC6, HUC4); State (CBWS Only)
- *Date of Implementation:* Year oysters were harvested.

Q7: How will the practice be credited in the Phase 6 Watershed Model?

A7: The Phase 6 Model will have an estimated nutrient load in shoreline segments that can be reduced by shoreline and tidal water practices. The pounds of nutrients reduced by this practice will be credited as a reduction to the nutrient loads in the nearest shoreline segments to the practice location. If latitude and longitude are not submitted, then the practice benefits will be distributed amongst all shoreline segments in the geography submitted.

Q8: Can this practice be submitted in non-tidal waters?

A8: No. This practice is only eligible in tidal waters.

Q9: Is this an annual practice?

A9: Yes. States must report the number of oysters harvested or pounds reduced annually.

Q10: How should a state report the practice if oysters are relocated to a new location within the reporting year?

A10: The expert panel recommended that states calculate and report the nutrient reductions for oysters grown at two different locations using the site-specific monitored practice. An example of this approach is provided below, and is described in more detail in Section 9.1 of the report.

Site 1 Situation: 1,000,000 diploid oysters harvested from final location were initially grown at site 1 to 2.25 inches

Site 1 Calculation and reporting: 1,000,000 X 0.05 g N = 50,000 g N (or 110 lbs)

Site 2 Situation: 1,000,000 diploid oysters harvested from final location were grown to 3.5 inches.

Site 2 Calculation and reporting: 1,000,000 X 0.15 g N = 150,000 g N – 50,000 g N from site 1 = 100,000 g N (or 220 lbs)