Evaluation of the Use of Shellfish as a Method of Nutrient Reduction in the Chesapeake Bay

A response to the request from the Management Board of the Chesapeake Bay Program

STAC Review Report
September 2013

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Table of Contents

STAC Panel Members……..... 2

Executive Summary……... 3

Background and Charge to the Review Panel…… 4

Review Approach……... 4

Panel Findings…… 5

Best Management Practices in Oyster Aquaculture……... 9

Inclusion of Oysters in Chesapeake Bay TMDL Implementation……. 10

Panel Responses to Specific Questions…….. 10

Literature Cited…….. 12

Appendix I Letter from Nicholas DiPasquale to STAC Requesting a Review…….. 14

Appendix II Shellfish Aquaculture: Ecosystem Effects, Benthic–Pelagic Coupling and Potential for Nutrient Trading…….. 16

Appendix III Quantifying Nitrogen Removal by Oysters: Workshop Report…….. 30

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Executive Summary

The CBP requested that STAC conduct a review of the relevant information on the potential use of shellfish as a method of nutrient reduction in Chesapeake Bay and advise the program specifically on how shellfish might be incorporated into nutrient reduction practices. STAC was also asked to address several questions related to (*i*) nutrient removal efficiencies by oysters, (*ii*) best management practices for oyster aquaculture and oyster reef restoration related to nutrient removal, and (*iii*) guidelines for crediting nutrient removal by oysters in Chesapeake Bay Total Maximum Daily Load (TMDL) implementation.

Drawing on a recent NOAA Chesapeake Bay Office sponsored workshop which brought together 32 scientists, resource managers, and restoration practitioners to review and evaluate the current data on this topic, STAC outlined a series of findings on these topics and addressed their implications for best management practices in oyster aquaculture and the inclusion of oysters in TMDL implementation.

The results of our review are summarized in six findings.

1. Nitrogen content of oyster soft tissue and shell can reasonably be estimated as 8.2% and 0.2% of dry weight, respectively.
2. Phosphorus content of oyster soft tissue and shell can reasonably be estimated as 1.07% and 0.06% of dry weight, respectively.
3. High variability in predicting oyster growth and survival in aquaculture necessitates that estimates of nutrient removal be based on actual harvest data (oyster dry weight) multiplied by the nutrient percentages above.
4. Burial rates for nutrients associated with biodeposits are not currently known.
5. Measured denitrification rates associated with oyster aquaculture have not revealed any enhancement above background levels.
6. Denitrification rates associated with oyster reefs typically exceed background levels, but are highly variable among locations and seasons.

The primary implications of these findings for the development of best management practices (BMPs) in oyster aquaculture related to nutrient reduction is the need for additional information related to practices or conditions that can lead to enhanced denitrification. Although enhanced denitrification has been observed in association with oyster reefs, the effect has been highly variable and it currently is not possible to provide reliable rates for inclusion in the TMDL implementation process without direct measurements on individual reefs.

1. Background and Charge to the Review Panel

STAC received a letter on January 8, 2013 from the Chesapeake Bay Program Director, Nicholas DiPasquale (Appendix I) requesting on behalf of the CBP Management Board that STAC conduct a review of a recent study by Mann and Newell (2012, Appendix II), as well as any other relevant information on the potential use of shellfish as a method of nutrient reduction in Chesapeake Bay. STAC was further asked to advise the program specifically on how shellfish might be incorporated into nutrient reduction practices and given a list of 12 specific questions and topics related to nutrient removal by oyster aquaculture and oyster reefs to consider in its review. The specific topics fell into three broad categories:

- Nutrient removal efficiencies by oysters;
- How BMPs for oyster aquaculture and oyster reefs might affect nutrient removal; and
- Guidelines for crediting nutrient removal by oysters in Chesapeake Bay TMDL implementation.

In considering this request, STAC noted that an effort funded by the NOAA Chesapeake Bay Office was already underway to review and summarize current data on nutrient removal by oysters. That effort, led by Drs. Lisa Kellogg and Mark Luckenbach (STAC member) from the Virginia Institute of Marine Science, was scheduled to bring together most of the scientists with published and ongoing research on the topic, along with resource management agency personnel and oyster restoration practitioners, in a workshop on January 10-11, 2013 to review and discuss the existing data. The focus of that group was specifically limited to reviewing and analyzing the existing data, and not on making policy recommendations or addressing the specific questions posed in the letter from the Chesapeake Bay Program Management Board. STAC concluded that it could best meet this request by establishing a panel to review the products of the NCBO workshop, along with the Mann and Newell report and any other available studies, and place the findings in the context of the BMP and TMDL implementation issues raised in the letter.

2. Review Approach

In reviewing the potential for oysters to remove nutrients for the Chesapeake Bay, the panel considered each of the major pathways depicted in Figure 1 in Newell and Mann (2012, Appendix II): bioaccumulation of nitrogen (N) and phosphorus (P) in soft tissue and shells, burial of N and P, and removal of N to the atmosphere via denitrification. We limited ourselves to data derived from studies with the native oyster, *Crassostrea virginica*, and to published studies or, in a few cases, to ongoing studies by authors who have previous publications on this

topic. As noted above, we relied heavily upon the review conducted by researchers actively engaged in this field (see workshop report, Kellogg et al. 2013b, Appendix III).

The panel also considered the available information on nutrient removal in the context of its applicability to oyster aquaculture and oyster reefs, either as sanctuary or harvested reefs. For the purpose of the panel's considerations, the definition of oyster aquaculture was limited to *intensive aquaculture*, which involves the grow-out of hatchery-produced oysters, and did not include *extensive aquaculture*, a term often applied of transplanting wild oysters to new areas for grow-out. The former practice places and removes new oysters in the Bay, the latter moves around wild individuals that are already there. Intensive culture of oysters in the Bay currently relies on one of three general grow-out approaches: (1) suspended culture in floating cages, (2) cages placed on the bottom, and (3) un-caged planting of juvenile oysters, termed *spat-on-shell*. Existing data were reviewed and recommendations made in the context of the particular grow-out approach to which they apply.

Assessing potential nutrient removal by oyster reefs required a reliance on data acquired from natural oyster reefs, restored oyster reefs, oyster reefs on private leases managed for harvest, and small reefs constructed as part of controlled experiments. Data from published and ongoing studies under these circumstances were collated and reviewed by the NCBO workshop (Kellogg et al. 2013b).

Consideration of BMPs that might affect nutrient removal by oysters and how nutrient removal by oysters might be incorporated into the Bay TMDL was limited to identifying guiding principles based upon the existing science; defining specific policy options were deemed outside the panel and STAC's expertise.

Panel Findings

The overriding finding by the panel is a dearth of data bearing directly on the issues under consideration. Newell and Mann (2012) included data from a total of three studies that directly measured bioaccumulation of N and P by oysters (Newell 2004, Grizzle and Ward 2011, Higgins et al. 2011); no studies are cited in that report with measurements of either burial rate of N and P or removal of N via denitrification. As summarized in the NCBO workshop report (Kellogg et al. 2013b), two additional published studies (Carmichael et al. 2012, Kellogg et al. 2013a) and one ongoing study (Dalrymple and Carmichael, in prep.) provide values for bioaccumulation of N and P by oysters. Two additional studies provide data on denitrification rates associated with suspended oyster aquaculture (Holyoke 2008, Higgins 2013), though no values are available on this process associated with other forms of oyster aquaculture. Finally, four completed studies (Piehler and Smyth 2011, Sisson et al. 2011, Smyth et al. 2013, Kellogg et al. 2013a) and two

additional studies (Kellogg et al. in prep and Kellogg et al. ongoing) provide data on denitrification rates associated with oyster reefs.

Finding 1: Average nitrogen content in oysters, though somewhat variable, can reasonably be estimated as 8.2% of soft tissue dry weight and 0.21% of shell dry weight.

Rationale: Five studies, one from Great Bay, NH, one from Cape Cod, MA and three from Chesapeake Bay, found average values for nitrogen as a percent of dry weight in soft tissues ranging from 7.28 – 11.8 (Great Bay: Grizzle and Ward 2011, Cape Cod: Carmichael et al. 2012, Chesapeake Bay: Newell 2004 , Higgins et al. 2011, Kellogg et al. 2013a). The same three studies from Chesapeake Bay found average values for nitrogen as a percent of dry weight in shell ranging from 0.19 – 0.3. These studies included oysters grown in suspended aquaculture and on natural and restored reefs, oysters of varying sizes, and oysters collected in different seasons, thereby adding some robustness to the combined datasets. A note of caution is added by the results of an ongoing study in Mobile Bay for which nitrogen comprises 12% of the dry weight biomass of oyster soft tissue (Dalrymple and Carmichael in prep.). As noted by Newell and Mann (2012), seasonal variation in nutrient content of oyster tissues has not been well documented for oysters in Chesapeake Bay, and future studies of seasonally-specific nutrient content values for oysters could provide an improvement over current average values. Nevertheless, these values provide a reasonable starting point for estimating the amount of nitrogen in oysters on a weight-specific basis.

Finding 2: Average phosphorus content in oysters can be reasonably estimated as 1.07% of soft tissue dry weight and 0.06% of shell dry weight.

Rationale: The three studies cited above from Chesapeake Bay found average values for phosphorus as a percent of dry weight in soft tissues ranging from 0.80 – 1.60 and in shells ranging from 0.04 – 0.10. Again, these values provide a good starting point for estimating phosphorus content in oysters, and future research could provide seasonally adjusted values.

Finding 3: Estimating total nutrient removal attributable to bioaccumulation in cultured oysters in advance of harvesting for the purpose of providing BMP efficiencies is limited by large uncertainties in predicting oyster production. Reliable estimates of N and P removal from the harvest of cultured oysters can be based upon the biomass of oysters actually harvested.

Rationale: Production of oysters in an aquaculture operation is a function not only of the numbers of oysters planted, but also of their growth and survival rates. Both of these rates can be highly variable both spatially and temporally. Estimated farm production capacity may be used for planning purposes, but actual nutrient reduction credit should be applied only to those oysters that are harvested. Since cultured oysters in Chesapeake Bay generally require >1 year to reach harvest size, protocols would need to be established for determining the timeframe for crediting nutrient removal.

Finding 4: Burial rates of nutrients associated with oyster biodeposits have not been quantified and cannot at this time be assigned values for nutrient reduction.

Rationale: Oysters enhance the rate of nutrient flux from the water column to the bottom as a result of the deposition of feces and pseudofeces; however, as pointed out in Newell and Mann (2012), the fate of these nutrients once they reach the bottom "…is variable, site specific, and cannot be consistently estimated as a constant for all situations." In particular for burial of nutrients, no data currently exist for reliably estimating these rates associated with either oyster aquaculture or oyster reefs. Moreover, resuspension of shallow buried nutrients may be associated with episodic events, such as harvesting and storms, but these rates have also not been quantified.

Finding 5: Denitrification rates at sites with suspended oyster aquaculture have not been observed to be elevated relative to comparable sites without aquaculture.

Rationale: The potential exists for the enhanced delivery of nitrogen to the bottom in oyster biodeposits to drive enhanced denitrification. However, the two studies that have investigated this issue (Holyoke 2008, Higgins et al. 2013) for suspended aquaculture have not observed increased rates of denitrification compared to reference areas without oyster aquaculture. These studies were conducted over a range of environmental conditions. While both studies measure enhanced deposition of N under suspended culture, they did not observe an increase in denitrification rates. No studies are available on the effects of either on-bottom cage culture or spat-on-shell grow-out approaches on rates of denitrification. Newell and Mann (2012) point out that the effect of oyster aquaculture on denitrification rate is likely to vary with environmental conditions and culture practices; however, these relationships have not yet been quantified.

Finding 6: Denitrification rates measured for oyster reefs typically exceed background levels in adjacent non-structured environments, with most, but not all, reefs exhibiting rates of denitrification that are 1.5- to 14-fold increases above

reference sites. However, several factors including oyster biomass in combination with tidal exposure, depth relative to the euphotic zone, and other unknown environmental factors affect these rates in ways that have not yet been fully quantified.

Rationale: Several completed studies (intertidal reefs in NC: Piehler and Smyth 2011, Smyth et al. 2013; intertidal reefs in VA: Sisson et al. 2011, subtidal reef in MD: Kellogg et al. 2013b) and two ongoing studies by Kellogg et al. (subtidal reefs in VA and intertidal reefs in VA) have measured denitrification rates associated with natural, restored and experimental oyster reefs. A meta-analysis of these studies reveals that (1) both absolute rates of denitrification and enhancement of denitrification above background vary substantially with season, (2) denitrification rates are higher and enhancement above background is greater for subtidal reefs compared to intertidal reefs, (3) there is a general, but weak, pattern of increasing denitrification rates and enhancement with increasing oyster biomass, and (4) substantial variance in the data remains unexplained (see Figure 1 and Kellogg et al. 2013b, Appendix III).

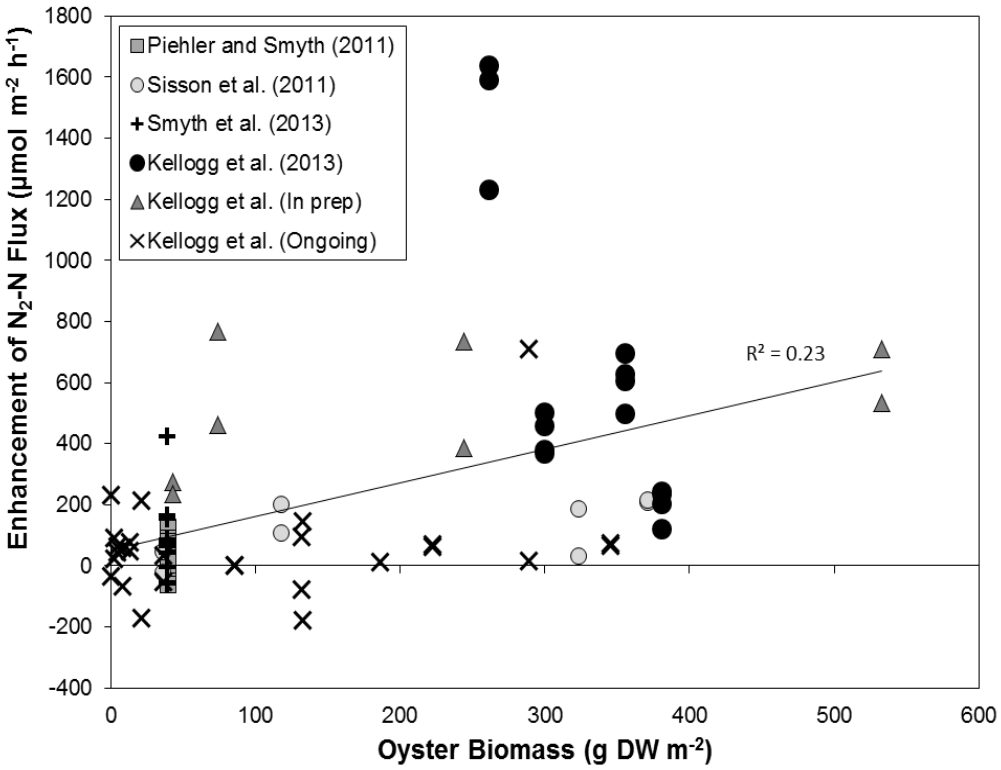

Figure1. Enhancement of denitrification rates in relation to oyster biomass density. Biomass density for Piehler and Smyth (2011) and Smyth et al. (2013) are approximate and based on estimated adult oyster density at study sites combined with size and biomass distribution data from Kellogg et al. (Ongoing). [Figure taken from Kellogg et al. 2013a].

Best Management Practices in Oyster Aquaculture

Limited data on the effects of oyster aquaculture on nutrient fluxes preclude the development of a full suite of BMPs at this time. However, a few basic issues are highlighted here in the hopes that they will drive future research and provide a basis for moving toward BMPs for nutrient management in oyster aquaculture.

First, it is apparent that oyster aquaculture, whether suspended, on-bottom cage, or un-caged spat-on-shell bottom culture, increases the movement of organic nitrogen compounds from the water column to bottom through the feeding and biodeposition actions of the oysters. Results from the few studies on suspended oyster culture notwithstanding, oyster aquaculture has the potential to enhance denitrification above background levels for soft-bottom benthic habitats. The fact that the two studies conducted to date in Chesapeake Bay, which have measured nitrogen fluxes associated with oyster culture (Holyoke 2008, Higgins et al. 2013), did not observe enhanced denitrification may reflect one or more of the following conditions: (1) local environmental conditions (e.g., reduced oxygen or high iron concentrations), (2) inadequate microbial community development (i.e., insufficient time or conditions for the development of nitrifying and denitrifying microbial communities), or (3) insufficient surface area providing contact between oxic and anoxic conditions required to support nitrifying and denitrifying communities, respectively. These uncertainties suggest the need for research in this area in support of BMP development.

Second, it is important to bear in mind that through the same processes of feeding and biodeposition, oyster aquaculture also has the potential to reduce nitrification (and hence coupled nitrification-denitrification) and degrade local bottom conditions. High rates of biodeposition associated with intensive suspended culture of mussels in areas with low flushing rates have been observed to result in the localized depletion of oxygen in near bottom and pore waters (e.g., Chamberlain et al. 2001, Hargrave et al. 2008). This depletion of oxygen can inhibit nitrification, thereby shutting down the coupled nitrification-denitrification processes. Similar situations have not been widely observed for suspended oyster culture, but would be expected under conditions that lead to a large build-up of organic matter on the bottom (i.e., low flushing rates and high oyster production). The development of simple modeling tools that provide site-specific guidance on oyster stocking densities and overall farm scale to avoid localized nutrient enrichment will be an important step in implementing BMPs for oyster aquaculture that avoid these negative effects and maximize the positive nutrient removal benefits.

Third, there is a lack of data on the effects of other grow-out methods (e.g., oysters grown in bottom cages and spat-on-shell grown on the bottom without cages) on denitrification rates. Though the latter approach bears some resemblance to an oyster reef, we caution against

applying rates measured on natural or sanctuary reefs to spat-on-shell aquaculture because of differences in age class structure and harvesting effects.

Inclusion of Oysters in Chesapeake Bay TMDL Implementation

In the case of nutrients removed in the harvest of cultured oysters, subject to the caveats listed in the BMP section above, the Panel concurs with the Newell and Mann report that nutrient removal can reasonably be estimated using existing relationships as a percentage of dry weight biomass harvested annually.

Incorporation of nitrogen removal via denitrification into TMDL implementation plans is currently unsupported both for oyster aquaculture and for oyster reef restoration. Further research on factors affecting denitrification rates involving a greater breadth of aquaculture approaches and oyster reefs under varying environmental conditions is likely to clarify the conditions under which enhancement of denitrification associated with oysters can provide substantial water quality benefits. When reliable estimates of nitrogen removal via enhanced denitrification do become available, with appropriate temporal and spatial accuracy, this process can be modified within the estuarine model to refine the nitrogen processing capacity of the system.

Panel Responses to Specific Questions

The charge letter from the Bay Program listed 12 specific topics and questions for the STAC to address in its review (Appendix I). These fall into three general topic areas and the Panel has grouped these together in providing the responses below.

<u>Nutrient removal efficiencies</u>

What are the nutrient removal efficiencies associated with oyster aquaculture and oyster reefs based on current science? (Questions 1, 4, 6, 7, 9, 12)

Nutrient removal efficiency in the sense that it is generally applied to terrestrial BMPs cannot readily be assigned to the role of oysters in nutrient removal from estuarine and coastal waters. Oysters capture phytoplankton above 6 μm in diameter with near 100% efficiency in the water that they pump through their siphons, but estimating how much water within a region actually gets pumped through the siphons of a particular group of oysters requires site-specific, high resolution, 3-D hydrodynamic models. Once filtered and ingested, reasonable estimates can be made for the percentage of nutrients that are incorporated into oyster tissues, though this may vary seasonally. Nutrients deposited to the bottom in oyster biodeposits may be removed via burial and denitrification. As described above, we do not have estimates of burial rates and

denitrification is highly variable depending on environmental conditions and the resident microbial community. Under some conditions much of the nitrogen in oyster biodeposits returns to the water column where it can fuel further phytoplankton growth. With these constraints in mind, it is evident that reliable, comprehensive, nutrient removal efficiencies cannot be assigned to oysters at this time, although partial nutrient reductions can be based on harvested amounts of tissue and shell.

Oyster BMP's

How can oysters (cultured, wild, or restored) be used as in situ BMPs? Is our current knowledge of this representative throughout the Bay? How do environmental conditions affect the BMP efficiencies? (Questions 2, 3, 7, 9)

Most of the answers to these questions are provided in the response to the previous question. Simply put, assigning nutrient removal efficiencies to oysters in the sense of a typical terrestrial-based BMP is both impractical and illogical. However, BMPs in aquaculture which manage stocking densities to avoid localized eutrophication can be employed to avoid negative effects of excessive organic loading on nutrient fluxes. Further, it may be the case that oyster reefs that are harvested can be managed in a way that maximizes the positive benefits of nutrient burial, denitrification, and nutrient removal via harvest, while minimizing the presumed negative effects of resuspension of nutrients during harvesting, but none of this has been adequately quantified.

In the one scenario for which sufficient data exist at present to recommend quantifiable nutrient reduction associated with oysters—nutrient removal via bioaccumulation in harvested cultured oysters—it is important to ensure that (1) the estimates are based on the actual amount of soft tissue and shell biomass harvested, (2) that shells are not returned to the water, and (3) that farming activity does not reduce background levels of nitrogen removal via denitrification through the over-enrichment of bottom sediments with oyster biodeposits.

Guidance for TMDL Implementation

When can nutrient reductions be counted towards Chesapeake Bay TMDL (annually, at harvest, by season)? (Question 10)

Currently, nutrient reductions from properly located oyster aquaculture could be calculated based on harvested amount. Future refinements could be added to account for seasonal nutrient-biomass variations, once those data are available and relationships have been developed.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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JAN – 8 2013

Dr. Chris Pyke, Chair
Chesapeake Bay Program Scientific and
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Dear Dr. Pyke:

The Chesapeake Bay Program (CBP), pursuant to a discussion by the CBP Management Board during its November 14, 2012 meeting, hereby requests the Scientific and Technical Advisory Committee's (STAC) review the recent study "Shellfish Aquaculture: Ecosystem Effects, Benthic-Pelagic Coupling and Potential for Nutrient Trading" (June 21, 2012; Roger Mann and Roger Newell) as well as other relevant studies, on the use of shellfish as a method of nutrient reduction and advise the program on how this can be incorporated into nutrient reduction practices.

The Mann and Newell shellfish aquaculture study was developed to analyze the nutrient assimilation of shellfish and their potential to assist in the Chesapeake Bay restoration activities. Shellfish are filter feeders with documented potential ability to remove nutrient and suspended sediment pollution from the Bay. The study compiles and analyzes available information on nutrient assimilation and provides a calculation for nutrient reduction and can serve as an introduction to a state-of-the-science review.

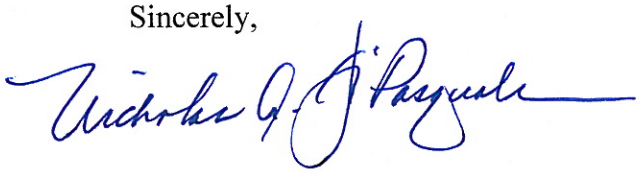
Specifically, we ask that STAC consider the following during its review:

1. The nutrient removal efficiencies of nitrogen by oyster aquaculture and oyster reefs as described in the current state-of-science.
2. The current science support using oyster aquaculture, current Chesapeake oyster beds including oyster sanctuaries or oyster reefs as an *in situ* best management practice in the Chesapeake Bay.
3. The current oyster aquaculture BMP research is representative of all Chesapeake Bay watersheds. (If not, how so?)
4. What would be the most appropriate nitrogen removal efficiency based upon the current science.
5. What are your recommended baseline conditions for an oyster aquaculture, current Chesapeake oyster beds including oyster sanctuaries and for a natural reef site? Conditions may include oyster density, water flow, depth, salinity, use of diploid or triploid populations, in-column or on-bottom reef/caged cultures, a reef component, etc.

6. How should nutrient efficiencies be calculated based on best available data? Which site-specific factors (e.g. salinity, temperature, water flow, depositional load, depth, and bottom/benthic conditions) should be considered in the calculations?
7. Should BMP efficiencies be based on nutrient assimilation, biogeochemistry (denitrification and burial processes), or both? How might this differ based on aquaculture type (on-bottom reef or caged versus in-column).
8. How would on-bottom reef, on-bottom caged, and in-column aquaculture nutrient removal differ from one another? How would these differences impact BMP efficiencies?
9. How will efficiencies change seasonally? How will fluctuations in filtration rate due to low/high temperatures or algae concentrations impact BMP efficiencies?
10. When can nutrient reductions be counted towards the Chesapeake TMDL (annually, at harvest, by season)?
11. Should tissue sampling, water quality data or other methods be used to verify reductions? If so, what would be the measuring protocols?
12. Taking into account the above questions, what are STAC's recommended nutrient reduction efficiencies for oyster aquaculture? How might those efficiencies translate for oyster restoration projects?

As always, we appreciate the role of STAC in serving as an independent review body to the Chesapeake Bay program and we look forward to your response.

Sincerely,



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Shellfish Aquaculture: Ecosystem Effects, Benthic–Pelagic Coupling and Potential for Nutrient Trading

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A Report Prepared for the Secretary of Natural Resources, Commonwealth of Virginia.

June 21, 2012

Summary
The processes connecting dissolved inorganic nitrogen (N) and phosphorus (P) uptake by phytoplankton from the water column and incorporation with carbon fixed by photosynthesis into organic molecules that are an essential food source for shellfish are briefly reviewed. The fate of N and P bound in shellfish biomass and voided as biodeposits are described and discussed in the context of nutrient trading. Nitrogen and P that are incorporated in tissue and shell of harvested bivalves can reliably be quantified, thereby allowing shellfish farmers in Virginia to participate in nitrogen trading should such a market become established. In 2010, the most recent year for which data are available, there were 16.9 million eastern oysters (*Crassostrea virginica*) harvested from aquaculture farms in Virginia. We estimate that this level of harvest will have removed from Chesapeake Bay a total of 2,197 Kg of N and 338 kg of P in oyster tissue and shell combined.

The fate of organic N and P ingested by bivalves but not assimilated into tissue and shell is more difficult to predict because key biogeochemical processes are influenced by season, location, and farm management practices. Although the bacterially mediated process of denitrification can achieve permanent nitrogen removal from the aquatic system to the atmosphere, this is likely to occur only in well-managed shellfish aquaculture sites that have been placed in suitable locations. At the present time there are no methods to reliably quantify and predict such nitrogen removal on a large scale and on an ongoing basis. Current research efforts are attempting to quantify the magnitude of nitrogen loss via denitrification in shellfish farms. In all probability, site-specific monitoring will be required to quantify such nitrogen removal if the intent is to include denitrification in nutrient trading; however, sufficient information is available to encourage farm Best Management Practices that will promote nitrogen loss via denitrification, thereby gaining ecosystem benefits.

Introduction
Shellfish harvesting from the Chesapeake Bay was an important food source for native people prior to the arrival of European colonists. The more recent history of shellfish harvest, especially oyster harvest, through the 19th and 20th century, is that of an industry that considered the resource limitless; however, the cumulative impacts of disease, overharvest, and environmental degradation resulted in a drastically depleted oyster stock by the end of the 20th century. Consequently, there has been a forced transition from a wild fishery exploiting public oyster bars to intensive oyster aquaculture which is now a rapidly expanding industry in the Chesapeake Bay (Murray and Hudson 2011). In concert with selective breeding for disease tolerance and ploidy manipulation that is ongoing at the Virginia Institute of Marine Science, the intensive culture of oysters' proffers hope as a major element for the resurgence of the native oyster fishery. The development of Pacific oyster culture on the U.S west coast over the last five decades provides an example of the scale of production that might be achieved in the mid-Atlantic with the native eastern oyster. The extant intensive efforts in shellfish culture in many Asian and European countries suggest that there exists enormous growth potential for the oyster aquaculture industry.

Many economic benefits are associated with developing shellfish aquaculture in Chesapeake Bay. The Bay is highly productive, with excess nutrient inputs from point and non-point

anthropogenic sources that stimulate high levels of phytoplankton primary production that is the food source for shellfish. Aquaculture can provide both employment in rural areas and a continuous supply of seafood that will reduce dependence on foreign imports. But there are ecological consequences associated with greatly enhancing stocks of bivalves in intensive aquaculture farms. These consequences can be beneficial if managed correctly or highly adverse if aquaculture farms are not sited and managed correctly (Newell 2004, Shumway 2011). Potential ecosystems benefits have been promoted, but these must be viewed with caution in that they are rarely accompanied by consideration of the impacts of intensive culture in situations with less than ideal management. Of recent interest is the possible role of intensive shellfish culture in nutrient reduction in the Bay's receiving waters, that is, the use of shellfish culture as a component of nutrient trading directed at overall improvement of Bay water quality. A balanced discussion is required.

Connecting shellfish production with the source and fates of nutrients: how does this fit with nutrient trading?

The objective of this review is to briefly summarize the ecosystem effects of shellfish aquaculture, predominantly the culture of oysters suspended near the water surface in floats or held in cages on the bottom, in the Chesapeake Bay. Additional details and supporting literature are provided in Newell (2004), Newell et al. (2005), Shumway (2011), and Dame (2012). We specifically identify areas where lack of information limits the accurate estimation of associated ecosystem benefits at the present time. We focus on the role of shellfish in connecting water column processes (the pelagic zone) where nutrients are central to the production of single cell plankton upon which oysters feed, and processes at the sediment water interface and surface sediment layers (the benthic zone). Nutrients, nitrogen N and phosphorus P, from terrestrial point and non-point sources flow into the Bay as dissolved inorganic nitrogen and phosphorus (DIN and DIP).

The first question to be addressed is how inorganic nutrients result in phytoplankton growth, and how oyster feeding on this phytoplankton may improve water quality. The entire process is summarized in a series of graphics (Figure 1). The dissolved inorganic nitrogen (DIN = ammonium [NH_4^+] + nitrite [NO_2^-] + nitrate [NO_3^-]) and phosphorus (DIP = phosphate [PO_4^{3-}]), together considered as the DIN and DIP pool, are taken up by the phytoplankton and incorporated as part of their growth and cell multiplication process to form particulate organic nitrogen (PON) and particulate organic phosphorus (POP), collectively termed particulate organic matter (PON + POP = POM). The production of POM is driven by photosynthesis and dependent on the availability of specific wavelengths of light (photosynthetic active radiation or PAR) passing through the water column. Turbidity reduces light penetration and PAR, and diminishes photosynthesis. Production of phytoplankton results in removal of dissolved nitrogen and phosphorus from the water column (i.e., the oysters themselves do not directly remove the inorganic nutrients).

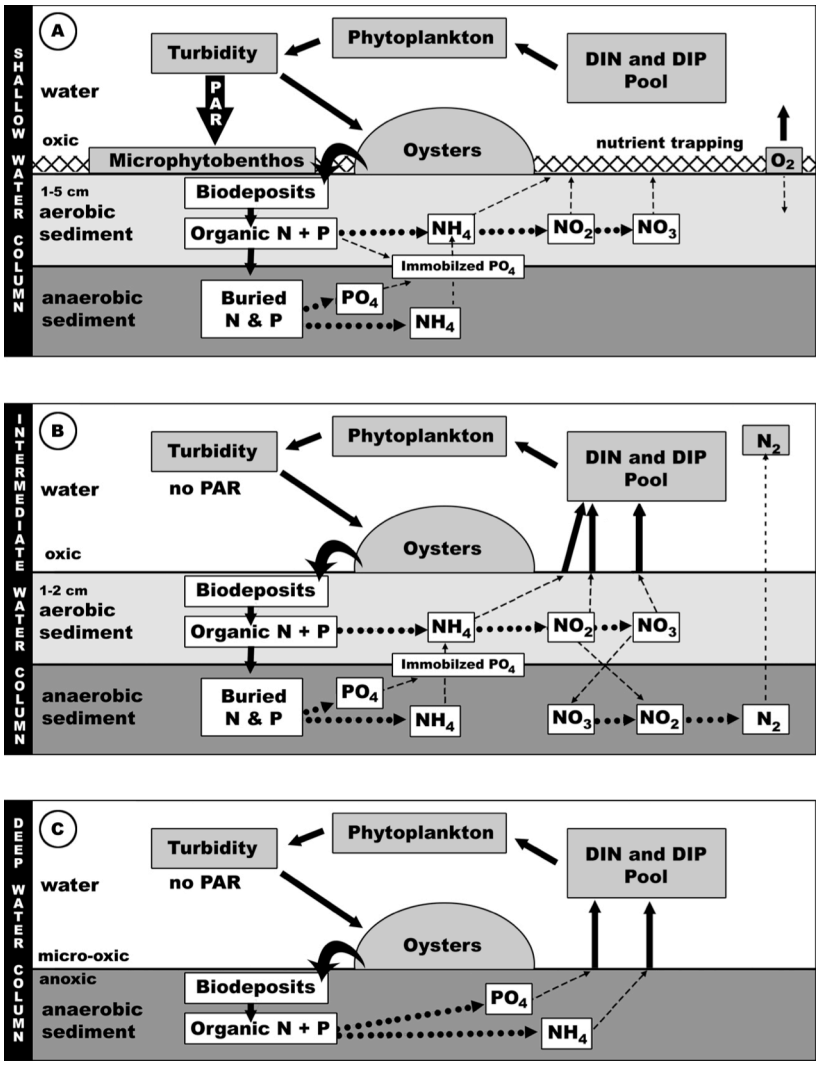

Figure 1. Role of eastern oysters in removing phytoplankton from the water column and transferring undigested particulate material as biodeposits to the sediment surface. Illustrated are benthic-pelagic scenarios for three locations (Panels A, B, and C) with different levels of photosynthetic active radiation (PAR) and dissolved oxygen concentrations. From surficial aerobic sediments (middle panel) N and P are released to the water column. The microbially mediated process of nitrification in the aerobic surface sediments coupled to denitrification within the underlying anaerobic sediments causes N to be lost from biodeposits as N_2 gas. N not regenerated is buried in accumulating sediments and P is immobilized in the aerobic sediments. In contrast, little nutrient regeneration into the water column takes place in locations with sufficient light to support active microphytobenthos that absorb regenerated N and P at the sediment surface (upper panel). Coupled nitrification-denitrification is also reduced because the microphytobenthos out-compete bacteria for NO_2 NO_3, and NH_4. In locations where the sediments are anoxic (lower panel) nitrification is inhibited and all N and P is regenerated from the sediments as NH_4 and PO_4. Some burial of N occurs but P sorption is precluded. Solid lines indicate transfer of materials; dashed lines indicate diffusion of materials; dotted lines indicate microbially mediated reactions. From Newell et al. (2005)

Nitrogen and Phosphorus in Harvested Oysters

Phytoplankton (PON + POP) are filtered from the water column by the oysters and after ingestion and digestion these nutrients are available to support the oyster's metabolism and growth. Nitrogen, and to a lesser amount phosphorus, are required to synthesize proteins used to build tissue as the oyster grows from the small, newly metamorphosed juvenile (= spat or seed) to a large market-size individual. Some of these proteins are also required to form an organic matrix along the margin of the shell that serves as a framework for the deposition of calcium carbonate as the growing oyster enlarges it shell (Carriker 1996). By the time oysters have grown to market size they have incorporated appreciable amounts of N and P into tissue and shell and so these nutrients are removed from Chesapeake Bay when the oysters are harvested.

In order to allow the process of nutrient removal by oyster harvest to be used for nutrient trading credits it is necessary to develop equations to estimate the amount of N and P removed based on the numbers and size of harvested oysters. Also, in order to have credibility, it is necessary to assess the variation in oyster nutrient content that can stem from seasonal differences in oyster tissue composition and site specific variation in oyster growth.

Shell

Oysters grown in aquaculture floats in Chesapeake Bay reach market size of 3-inch shell height almost twice as fast as oysters grown on natural oyster bottom (= reefs). This faster growth is attributable to high water flow though oyster floats that serves to provide the oysters with large amount of phytoplankton and avoid food depletion. Palmer (1981) hypothesized for bivalves in general that faster-growing individuals have thinner shells than slower-growing individuals because calcium carbonate deposition in molluscs is a rate-limited rather than an energy-limited process. This has been found to be true for oyster grown in floats in Chesapeake Bay which have thinner and lighter shells compared with oysters growing more slowly on-bottom (Paynter and Dimichele 1990, Newell et al. 2005, Higgins et al. 2011). For example, Newell et al. (2005) reported that the shell of a market size oyster grown on bottom weighed ~150 g (Table 1), which is about 5 times greater than the ~ 30 g of a similarly sized oyster grown in floats (Higgins et al. 2011). Therefore, the relative contribution of the shell to removal of N and P will typically be less in oysters grown in floating cages than for oysters grown on natural oyster bottom.

Tissue

There are seasonal differences in protein (and hence nitrogen) content in oyster tissue (meats) when expressed as percentage of total dry weight which are caused by relative changes in the amount of non-nitrogen containing glycogen that the female oyster uses as a nutrient reserve (Thompson et al. 1996). (We only need consider here the situation for females since the eastern oyster is protandric and hence market size oysters are predominately female). As the oyster builds glycogen reserves to a maximum in early winter this effectively reduces the percentage of the total weight attributable to protein in the harvested oyster meats. In the spring, these glycogen reserves are used to synthesize lipids for yolk reserves in eggs, which are eventually spawned in early summer. As a consequence of this annual reproductive cycle, female protein content varies from a maximum of ~45% immediately post-spawning (June) to ~ 35% in early winter (November) when oysters are "fattest" (Thompson et al. 1996). This 10% seasonal

variation in percent protein content must be considered when applying equations relating N and P tissue content to oyster size that have been generated from data obtained at only one season.

The most comprehensive study of Nitrogen and Phosphorus incorporation in tissues of oysters in Chesapeake Bay was undertaken by Higgins et al. (2011) at two sites near the mouth of the Potomac River. They grew oysters in aquaculture floats from seed to various shell sizes over a period of up to three years. In April and May prior to spawning, oysters of different shell sizes were collected for analysis of tissue and shell N and P content They found quite a narrow range of 7.3% to 8.2% for N and 0.8% for P in tissue, and 0.17% to 0.26% N and 0.04% for P in shell (Table 1). These percent composition data are similar to that reported by Newell (2004) for older oysters collected from a natural oyster bar.

Table 1. Literature data for Nitrogen and Phosphorous content in tissue and shell of oysters from Chesapeake Bay grown on bottom or in floating cage culture. DW = dry weight

			tissue		shell		whole oyster	
Shell size (mm)	Shell DW (g)	Tissue DW (g)	%N	%P	%N	%P	Total N (g)	Total P (g)
Wild oysters collected from natural oyster reefs (Newell 2004)								
76	150	1.0	7.0	0.8	0.3	0.1	0.52	0.16
Cultivated oysters grown in floating cages (Higgins et al. 2011)								
43.6	4.8	0.20	8.15	0.83	0.18	0.04	0.025	0.003
64.8	24.3	0.80	8.06	0.83	0.19	0.04	0.112	0.016
85.5	37.6	1.58	7.28	0.82	0.17	0.04	0.176	0.026
117.8	71.9	3.00	7.37	0.82	0.26	0.04	0.394	0.050

Spatial variability

Oyster growth can vary widely among aquaculture sites within an estuary, probably in most cases largely due to variations in food supply and other environmental factors, such as salinity (Brown et al., 1998; Brown et al., 2005a). At six sites in the Great Bay estuarine system in New Hampshire, Grizzle and Ward (2011) studied nitrogen assimilation over a 3-month period by oysters grown in bags suspended 10-20 cm off the bottom. They reported that among the six sites the mean percent nitrogen content in tissue ranged from 4.7% to 10.6% content. This was much wider variation than observed by Higgins et al (2011) for their two aquaculture farm sites in Chesapeake Bay. Grizzle and Ward (2011) offered no explanation for their findings. It is

possible that this high spatial variability was an artifact stemming from their short three-month study period. Oysters from the sites with better growing conditions will have accumulated larger glycogen stores, thereby effectively reducing percent protein content. In contrast, oysters held at sites with less favorable conditions for growth would have less available energy to build glycogen reserves and hence protein would constitute a greater fraction of the animals total tissue weight. But if the oysters had been grown from spat to market size this longer growth period may have reduced this spatial variability in tissue composition.

Initial Estimates of N and P removal by Oyster Aquaculture

Higgins et al. (2011) developed from their floating cage-grown oyster data the following predictive equations relating total nitrogen (TN in g) and phosphorus (TP in g) contained in oyster tissue and shell to individual maximum shell size (TL in mm) (Figure 2).

$$TN = e^{(-14.1569 + 2.7994 \times \ln(TL))} \quad (R^2 = 0.76;\ SE = 0.47) \quad [1]$$

$$TP = e^{(-15.6926 + 2.7061 \times \ln(TL))} \quad (R^2 = 0.78;\ SE = 0.44) \quad [2]$$

Higgins et al. (2011) used these equations to estimate that, at harvest, the total nutrient content of one market-sized oyster (maximum shell size of 76 mm) is 0.13 g N and 0.02 g P. This is lower than the 0.5 g N and 0.16 g P reported by Newell et al. (2005) in comparably sized oysters harvested from oyster bottom due to the greater amount of N and P in the considerably heavier shell (Table 1).

We used values from Higgins et al (2011) for N and P in an individual oyster to estimate the total nutrients removed in 2010 by the aquaculture production of oysters in Virginia. The production of oysters from aquaculture farms in Virginia has been collated by Murray and Hudson (2011) who performed a detailed survey of annual harvest by all known producers (Figure 3). Their survey did not differentiate between oysters grown off-bottom in floating cages, on bottom in cages, or on cultch planted directly on-bottom. For the purpose of our calculation we assumed that the values of N and P measured for oysters grown in floating cages (Table 1) applies to all oysters grown to 3-inch market shell size in these various different ways. During 2010, the most recent year for which data are available there were 16.9 million oysters harvested. This level of harvest will have removed from Chesapeake Bay an estimated total of 2,197 Kg of N and 338 kg of P.

Benefits of Using Oysters in Nutrient Trading

The use of oyster aquaculture as a means to remove nutrients is a unique solution to helping attain water quality improvements in Chesapeake Bay because it offers the only opportunity to reduce nutrients after they have entered a receiving body of water (Newell 2004, Newell et al. 2005). This may be especially important in ameliorating the effects of non-point source inputs that are the most difficult to regulate and control. On a per-unit area basis, oyster aquaculture removes a relatively large quantity of nutrients from receiving waters compared with implementing agricultural Best Management Practices (BMP), such as cover crops (Higgins et al. 2011). In addition it should be noted that oyster aquaculture results in substantial revenues

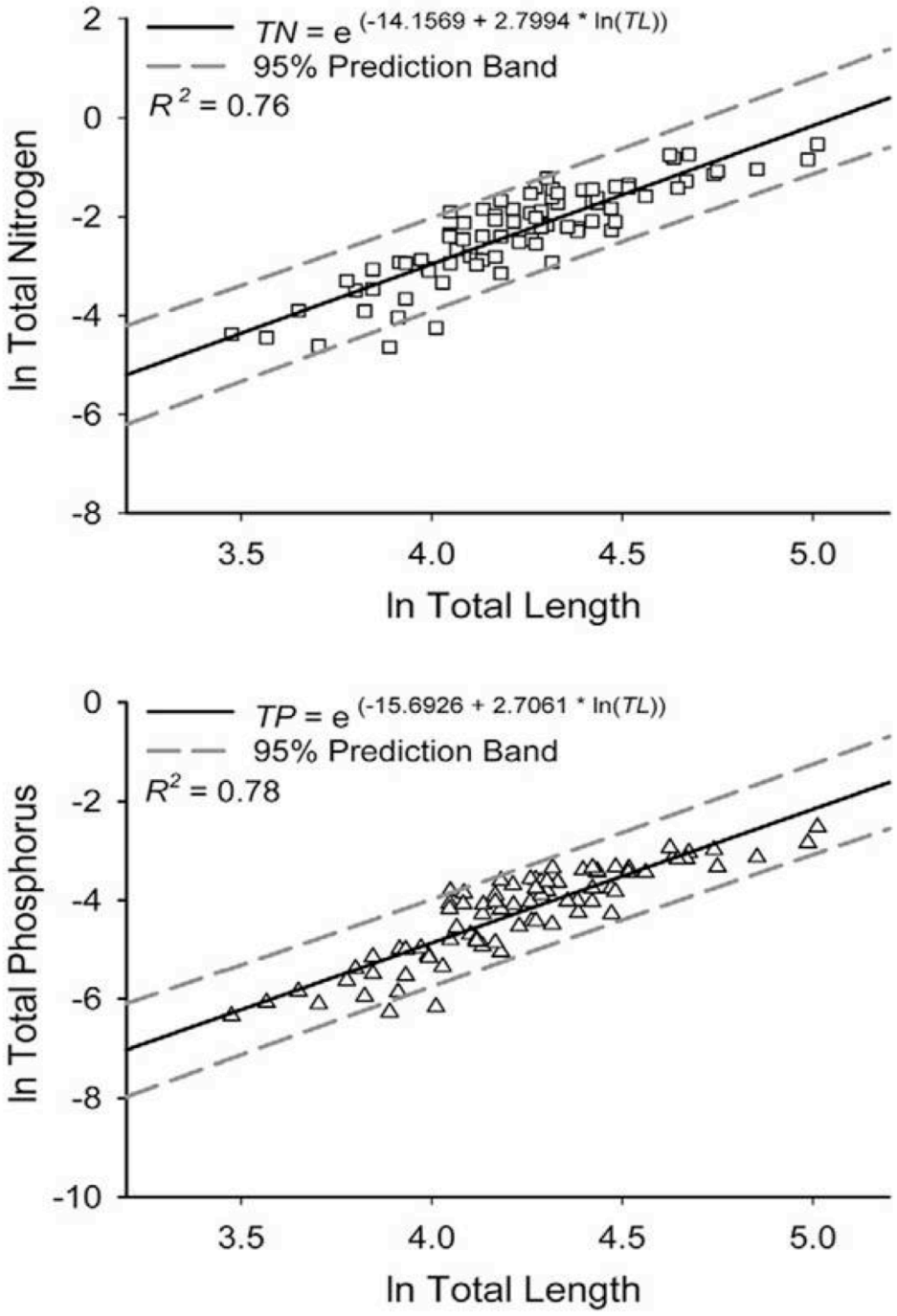

Figure 2. Linear regressions of $\log_e$–transformed total nutrient content [g total nitrogen (TN; squares) and g total phosphorous (TP; triangles)] against $\log_e$–transformed maximum shell size (TL; mm) for eastern oysters cultivated in floats in Chesapeake Bay. (From Higgins et al. 2011)

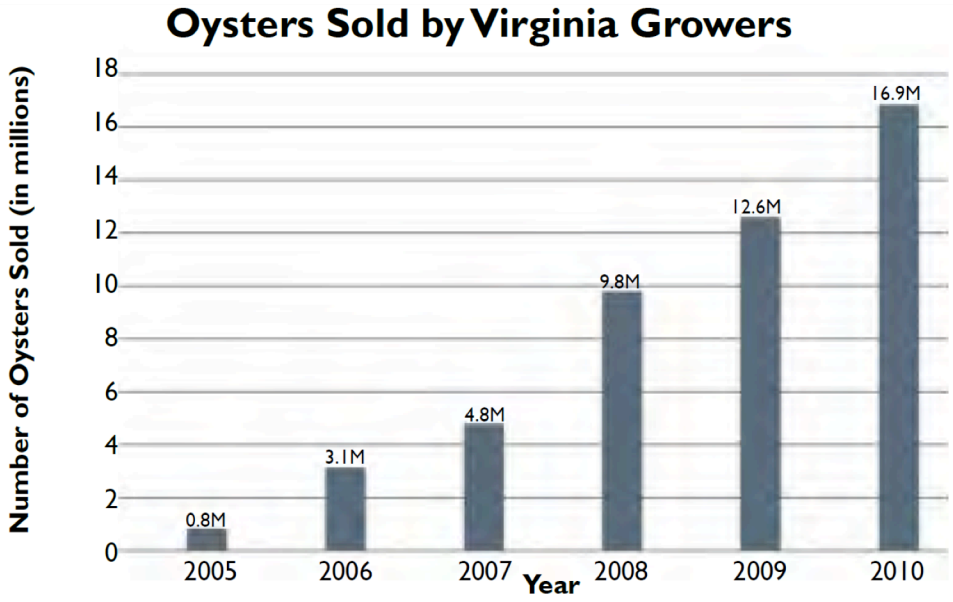

Figure 3. Total aggregate annual production of eastern oysters from aquaculture farms in Virginia. (From Murray and Hudson 2011).

from oyster sales and ancillary economic value in economically depressed rural communities. The additional revenue provided to farms from Nutrient Trading may be sufficient to allow these farms to be economically viable over the long-term (Newell 2004)

A requirement for any nutrient-trading program is the ability to document the exact amount of nutrient removed. This can be done accurately for oysters based on periodic farm inspections to check the number and size of oysters being grown and from records of numbers and size of oysters harvested and sold. Such clear quantifiable benefits are in contrast to agricultural BMP's where the amount of nutrient removal attributable to a specific practice implemented at a particular location is based on highly extrapolated data (Higgins et al. 2011).

Validation Studies

Once oysters reared in aquaculture farms start to be used in nutrient trading schemes in Virginia it will be necessary to perform a study of the seasonal variation in total nitrogen and phosphorous content of market size oyster tissue and shell at one farm site. These data can then be used to determine if the average values (Table 1 and Figure 2) reported by Higgins et al. (2011) for oysters harvested in April and May can be applied to oyster harvested at other times of the year. If the seasonal variations observed are large then appropriate seasonal values must be developed to estimate N and P removal associated with oysters harvested at various times of the year.

For each aquaculture farm site that becomes involved in nutrient trading it will be important to take samples of the oysters being marketed to establish the relationships between shell size, tissue dry weight, and percent N and P content. These data can then be used to build a larger data set for all of Virginia to estimate the among-farm variation in these fundamental relationships. If this variability is small then the same size and seasonal conversion factors can be applied to harvest from all farms. If the site and seasonal specific differences are large among farms then location specific conversion factors will be required.

The Role of Oyster Aquaculture in Enhancing Sedimentary Nutrient Removal

A proportion of the ingested PON and POP is passed through the oyster gut and ends up on the sediment surface as feces (biodeposits). In oysters, but not clams, the possibility also exists that phytoplankton is filtered but not ingested. When the oyster gills become clogged, they are cleaned by a rapid shell closure action and the material is simply ejected onto the bottom in a mucous bound mass called pseudofeces (which are also part of biodeposits). The fate of nitrogen and phosphorus in both the fecal (ingested and processed) and pseudofecal (filtered but not ingested) biodeposits is variable, site specific, and cannot be consistently estimated as a constant for all situations.

There is a significant seasonal element to shellfish feeding because the animal's overall activity is strongly controlled by water temperature. This activity response varies with species. Oysters in Chesapeake Bay feed actively and consume phytoplankton when water temperature exceeds 8^0C, with maximum feeding activity occurring when temperatures are between ~20 and 28^0C. Hard clams feed maximally when temperatures are lower with decreased feeding when temperatures exceed 20^0C. Thus the processes of nutrient removal discussed herein for oyster and clams and illustrated in Figure 1 are only important for ~6 month period each year.

There are several fates for the residual organic material and nutrients in biodeposits (Figure 1). Any particulate material that is deposited to the sediment surface is subject to degradation by bacterial and metazoa. The details of the process are particularly dependent on local conditions of light (PAR) and oxygen. Both the community of animals that feed on organic detritus (detritivores) and bacteria (aerobic and anaerobic bacteria that function in the presence and absence of oxygen, respectively) can degrade organic biodeposits in the sediments and regenerate DIN and DIP to the water column where it can support additional phytoplankton production.

Where oxygen is present in the surface sediments the bacterial metabolism of PON results in the release of ammonium ions. In the continuing presence of oxygen (aerobic or oxic sediments) bacteria oxidize ammonium first to nitrite, and then nitrate. The process of oxidation of ammonium to nitrate is termed nitrification. A portion of these dissolved inorganic forms (DIN) can be returned to the water column and a portion can diffuse deeper into the sediments to reach anoxic sediments where all oxygen has been depleted by aerobic bacterial respiration. When nitrite and nitrate enter this anaerobic zone then, in a process called denitrification, anaerobic bacteria can use the oxygen from these molecules to sustain their metabolism. The end result is that these molecules are reduced, resulting in the production of nitrogen gas [N_2] which is

released to the atmosphere and NOT retained in the system. Such denitrification is an important mechanism whereby natural bacterial processes found in sediments and marshes can lead to the net removal of nitrogen from the estuary. This is the same process that is harnessed in modern waste water treatment facilities to enhance nitrogen removal. This two-layer, aerobic over anaerobic sediment processing of organic material is illustrated in Box B in Figure 1. Note that in the scenarios illustrated in both Boxes A and B in Figure 1 phosphate is generally immobilized by binding with iron.

In shallower areas (<~ 3 m) there is often sufficient light (a region termed the euphotic zone where PAR can support photosynthesis) to allow benthic microalgae (also termed microphytobenthos) to grow at the sediment water interface. During daylight these microalgae can intercept a large proportion of inorganic nutrients before they are released back up into the water column. This is the scenario illustrated in Box A of Figure 1. These benthic microalgae are a crucial food resource for many mobile and sessile benthic animals. However, if the DIN is returned to the water column then it again becomes available for uptake by pelagic phytoplankton and the process starts all over for a second cycle. So there is an option for the DIN to end up as oyster tissue on the second, third and so on cycles. In this scenario only the nitrogen removed in harvested oysters or clams can be consistently estimated for a possible nutrient credit.

In sheltered locations, tidal and wave generated water currents may not be sufficient to widely disperse the biodeposits generated from shellfish aquaculture farms. In such locations, often characterized by fine grain "muddy" bottoms, then the accumulation of deposits may overwhelm the normal sediment biogeochemical processes. This excess organic material results in stimulation of such intense aerobic bacterial respiration that it exceeds the resupply of oxygen from the overlying water. In such situations even the surface layers of the sediment become anoxic and the resulting accumulation of hydrogen sulfide kills any remaining benthic infaunal organisms, including nitrifying bacteria. This is the scenario illustrated in Box C of Figure 1. The remaining anaerobic bacteria continue to metabolize the residual PON in the biodeposits but only ammonium is released due to the absence of the nitrifying bacteria. The PON and POP are released to the water column as dissolved inorganic forms that can again support phytoplankton production. This is a highly adverse environmental situation requiring immediate changes in farm management practices including reducing the stocking density of animals in the floats, reducing the number of floats or relocating the floats and allowing the area to recover (Newell 2004).

The practical outcome of the above discussion is that at the present time the pathways of processing nitrogen and phosphorus from biodeposits cannot be uniformly predicted for a shellfish population of defined size for a defined time period. The pathways of processing are both location and seasonally specific, and subject to modification by short-term physical disturbance by wind, tide and even storm events. When assessing a nutrient trading value for a shellfish farm the inclusion of calculations describing the fate of biodeposit nitrogen and phosphorus are highly variable – precisely what we do not need for a trading situation and a discussion with a federal agency requiring a precise budgeting approach.

A final note is worthy of consideration with respect to the location of oyster farms in shallow waters and seasonal processes in deeper waters of the Chesapeake Bay. In the summer months the waters of the Bay stratify, that is warmer and less dense water overlays deeper, colder water of higher density. There is generally insufficient wind energy to mix the water column so stable stratification characterizes the water column throughout the summer months, usually with the deeper water becoming depleted in oxygen over the time course of the summer. The depth of greatest density stratification is termed the pycnocline. When bivalves feed in the shallows and deposit PON to aerobic sediments (boxes A and B in Figure 2) this particulate material is prevented from being deposited to sediments beneath the pycnocline, which are generally anaerobic in summer months (as in Box C in Figure 1). In the absence of bivalve feeding, senescent phytoplankton sink and can be advected to the deeper channels where bottom waters are often anaerobic. In such conditions, nitrifying bacteria (that require oxygen) cannot survive and hence coupled processes illustrated in Boxes A and B are precluded. Thus, in such a situation all nitrogen is bacterially remineralized as ammonium (Box C in Figure 2) and available to support further phytoplankton production when it becomes mixed into waters above the euphotic zone. In properly located sites, cultured shellfish populations may facilitate deposition of particulate material to aerobic sediments, and hence form and important control mechanism of particulate organic regeneration, by altering the location where this material is processed (compare Boxes A and B with Box C). When natural oyster stocks were highly abundant in Chesapeake Bay this may once have been a very important ecosystem function. But today, natural stocks are so depleted, and oyster aquaculture not yet extensive, that such processes are very modest in the overall nitrogen cycling in Chesapeake Bay. Continued expansion of shellfish culture would, however, present the opportunity for positive ecosystem effects to become locally important.

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Quantifying Nitrogen Removal by Oysters

Workshop Report

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*Participant joined by phone

Executive Summary

The NOAA Chesapeake Bay Office sponsored a workshop with the goal of evaluating the current state of knowledge related to the capacity for oysters to remove nitrogen from coastal waters. The workshop, which was held at Virginia Institute of Marine Sciences Eastern Shore Lab in Wachapreague, VA, on January 10 – 11, brought together a total of 30 resource management agency personnel, restoration practitioners and scientists with expertise in the field to (1) identify the best available values, or ranges of values, for nitrogen removal by oysters primarily focusing on denitrification and bioassimilation, (2) discuss the uncertainty associated with these estimates, (3) identify the research needed to fill data gaps and (4) discuss minimum requirements for studies to accurately measure nitrogen removal rates associated with oysters.

The workshop included five presentations by scientists detailing their field studies that have measured nitrogen dynamics associated with oyster reefs and oyster aquaculture in Chesapeake Bay and elsewhere. The range of observed values for nitrogen (1) assimilated in oyster soft tissue and shell and (2) removed from the system via coupled nitrification/denitrification processes were summarized. Workshop participants engaged in fruitful discussions about the sources of variation in the observed values, the generality of the findings, and the conditions under which it is appropriate to apply the few estimates that currently exist.

The overarching finding of the workshop was that our current state of knowledge on the effects of oysters, both on reefs and in aquaculture, on nitrogen dynamics is incomplete in many respects. Removal of particulate nitrogen from the water column via filtration, incorporation into oyster tissues and biodeposition of nitrogen are all relatively straightforward to quantify, though the rates vary with environmental conditions and oyster growth. Determining the portion of that nitrogen that is returned to the atmosphere or sequestered for a significant period of time is less straightforward. No published rates exist for burial of nitrogen associated with oyster reefs or oyster aquaculture. Four separate studies conducted in a total of 14 different tributaries and sub-estuaries from Cape Cod, MA, Great Bay, NH and Chesapeake Bay provide very similar estimates of amount of nitrogen as a percent of dry weight found in soft tissue (8.22 ± 0.89% SD) of the Eastern Oyster, *Crassostrea virginica*, across a range of conditions, including subtidal reefs, floating aquaculture, high and low flow regimes, and varying degrees of eutrophication. Two of these studies, both conducted in Chesapeake Bay, also measured the nitrogen content of oyster shell. They provide similar estimates (0.19% and 0.21%) of the amount of nitrogen as a percent of oyster shell dry weight. The consistency of these values suggests that reasonably accurate estimates can be made of the amount of nitrogen removed via oyster harvest. This finding is tempered, however, by the results of one study in Mobile Bay, AL which found that nitrogen comprised 11.8% of oyster soft tissue dry weight, suggesting that the percentage of nitrogen in oyster tissue can vary by location. We also caution that estimating potential nitrogen removal via oyster harvest is subject to a much greater uncertainty related to the variance associated with oyster growth and survival rates, necessitating precise measures of oyster abundance and biomass at harvest. In addition, the ratio of oyster tissue to oyster shell dry weight varies widely with location, growing conditions and physiological status of oysters, with aquacultured oysters tending to have higher tissue to shell ratios than wild oysters.

Data collected thus far on denitrification associated with either intensive oyster aquaculture or oyster reefs show much greater variability than data on nitrogen bioassimilation. To date, no study has shown significant net annual enhancement of denitrification associated with intensive oyster aquaculture. At present, we recommend assigning a value of zero for nitrogen removal via denitrification associated with aquacultured oysters. However, we note that the only existing data come from studies of the sediments beneath oysters growing in aquaculture floats. As additional data become available for other types of intensive aquaculture and/or for nitrogen dynamics within aquaculture floats, this recommendation should be reviewed and revised as needed.

Although data on denitrification associated with oyster reefs suggest that they significantly enhance net annual denitrification rates over those at reference sites, the degree of enhancement is highly variable. Denitrification rates vary with season, tidal regime, oyster biomass density and other unidentified factors. Additional studies are needed to better understand the sources of this variation prior to assigning a value to the nitrogen removal capacity of oyster reefs attributable to denitrification. Although it is not possible at present to provide generalized relationships for estimating the enhancement in denitrification associated with oyster reefs under varying conditions, reliable methods do exist for measuring these rates, interest in clarifying these relationships is growing and new research is underway.

TABLE OF CONTENTS

Workshop participants i
Executive Summary ii
Background 1
Purpose of the Workshop 3
Workshop Scope and Structure 3
Summary and Meta-Analysis of Existing Data 5
Gaps in Existing Data 18
Methodology Recommendations for Future Studies 20
Recommendations for Application of Existing Data 21
Literature Cited 23
Appendix 27

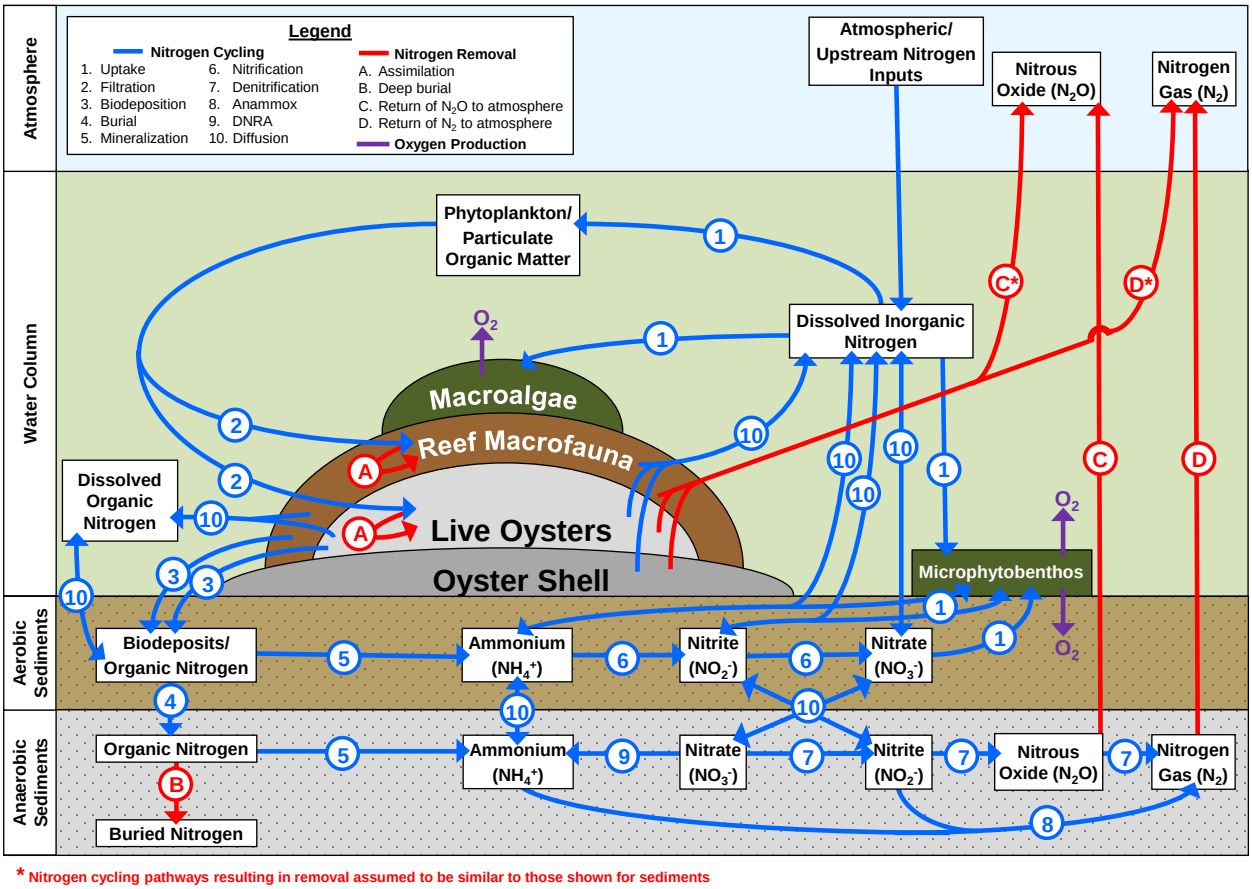
Background

It has long been recognized that suspension-feeding bivalves can alter water quality, through top-down control of phytoplankton, biodeposition of suspended sediments and alteration of nutrient dynamics (Officer et al. 1982; Newell 1988, 2004; Newell et al. 2002, 2005; Newell and Koch 2004). By calculating that the summertime filtration capacity of the oyster population of Chesapeake Bay in late 19th Century would have allowed them to filter a volume of water equivalent to the entire Bay in 3 – 6 days, Newell (1988) sparked public interest in the role of oysters in controlling water quality, providing much of the impetus for numerous restoration programs by environmental groups and government agencies (Brumbaugh and Coen 2009, Kennedy et al. 2011). It also generated considerable scientific debate about the capacity of oysters, either historical or restored populations, to affect phytoplankton dynamics at a bay-wide or tributary level (e.g., Pomeroy et al. 2006, 2007, Coen et al. 2007, Newell et al. 2007, Fulford et al. 2007). In its focus on the ability of oysters to filter phytoplankton from the water column, this debate has generally overlooked the fact that far greater uncertainty about the role of oysters in improving water quality surrounds the fate of the nitrogen contained in phytoplankton filtered by oysters.

As federal, state and local governments seek ways to meet new water quality improvement goals for the Bay, there is growing interest in incorporating the effects of public oyster restoration projects and private aquaculture into Bay water quality models, load reduction strategies and nutrient trading markets. Doing so requires an understanding of the effects of oysters on the removal of nitrogen, the primary pollutant of concern.

Nitrogen entering an estuary from the watershed and airshed stimulates phytoplankton growth, excesses of which can result in eutrophication and oxygen-depleted dead zones (Kemp et al. 2005). Benthic grazers, including oysters, remove a portion of the phytoplankton from the water column and facilitate the transformation of nitrogen into other forms, some of which do not support phytoplankton growth (Fig. 1 and Appendix Fig. A1-A5). Importantly, there are three primary means by which nitrogen can be removed from the water column for a significant amount of time: 1) conversion to a gaseous form with subsequent return to the atmosphere, 2) conversion to animal tissue or shell and 3) deep burial in sediments. The primary pathway of conversion from organic nitrogen to gaseous forms on oyster reefs is thought to be microbially-

mediated coupling of nitrification-denitrification, although the role of anammox and release of nitrous oxide have yet to be elucidated. Bioassimilation of nitrogen into the tissues of oysters, other grazers and higher trophic levels represents a more or less ephemeral pool of nitrogen within the system. However, bioassimilation into oyster shell may represent a means of longer term or permanent sequestration if shells are removed from the estuary or deeply buried. Harvesting oysters removes the bioassimilated nitrogen from the estuary, but as seen in the case of oysters in the Chesapeake Bay, overharvesting leads to population decline and system degradation. Burial of oyster biodeposits and shell can remove nitrogen, but the timescale of this removal has yet to be estimated and is likely to vary widely among sites with the majority of biodeposits being buried at some sites and being remineralized at others.



* Nitrogen cycling pathways resulting in removal assumed to be similar to those shown for sediments

Figure 1. Primary nitrogen cycling and nitrogen removal pathways for a shallow subtidal or submerged intertidal oyster reef in the euphotic zone.

The potential for shellfish aquaculture to participate in nutrient trading markets has been suggested in the scientific literature (e.g., Newell 2004, Lindhal et al. 2005, Shabman and Stevenson 2007) and is currently being considered by resource management agencies in both

Virginia and Maryland. Ongoing publically- and privately-funded oyster restoration efforts are, in part, based upon the expectation of water quality benefits and oyster restoration is being considered by some local governments as a Best Management Practice (BMP) to meet their Total Maximum Daily Load (TMDL) allocations (e.g., VA Beach: http://www.vbgov.com/ government/offices/eso/ Documents/tmdl-local-strategy.pdf). Recognition of the need for well-supported, consensus-based values for the nutrient removal capacity of oysters in support of these considerations served as the impetus for the workshop.

Purpose of the Workshop

The NOAA Chesapeake Bay Office sponsored the workshop with the goal of evaluating the current state of knowledge related to the capacity for oysters to remove nitrogen from coastal waters. The workshop, which was held at Virginia Institute of Marine Sciences Eastern Shore Lab in Wachapreague, VA, on January 10 – 11, 2013, brought together a total of 30 scientists, resource management agency personnel and restoration practitioners with expertise in the field to (1) identify the best available values, or ranges of values, for nitrogen removal by oysters primarily focusing on denitrification and bioassimilation, (2) discuss the uncertainty associated with these estimates, (3) identify the research needed to fill data gaps and (4) discuss minimum requirements for studies to accurately measure nitrogen removal rates associated with oysters.

Workshop Scope and Structure

Prior to the workshop a literature review was conducted and relevant publications made available to all participants. This initial review included data on nutrient content in numerous bivalve species, including oysters (*Crassostrea gigas, C. virginica, Pinctada imbricata*) and mussels (*Mytilis edulis, M. galloprovincialis*, *Perna canaliculus* and *Geukensia demissa*) (reviewed in Carmichael et al. 2012), and denitrification rates measured in large-scale aquaculture of clams (*Tapes philippinarum*) in the Saca di Goro lagoon in Italy (Nizolli et al. 2011). After reviewing these data the decision was made that the workshop would best address its charge by focusing only on those data obtained for the Eastern oyster *Crassostrea virginica*. The workshop included five presentations by scientists (Brown, Carmichael, Cornwell, Kellogg and Phieler) detailing their past and on-going field studies that have measured nitrogen dynamics associated with *C. virginica* in Chesapeake Bay and elsewhere. The range of observed values for

nitrogen (1) assimilated in oyster soft tissue and shell and (2) removed from the system via coupled nitrification/denitrification processes were summarized. Workshop participants engaged in fruitful discussions about the sources of variation in the observed values, the generality of the findings, and the conditions under which it is appropriate to apply the few estimates that currently exist to oyster-related nutrient removal in other locations or under other conditions.

Through these discussions three additional limitations were placed on the scope and the eventual conclusions and recommendations from the workshop. Two pertained to the definition of aquaculture under consideration and one to policy recommendations. With regard to oyster aquaculture, definitions and approaches commonly used in the Chesapeake Bay vary (Table 1). Though frequently considered aquaculture, *extensive aquaculture* practices, which rely on the recruitment of wild oysters onto planted shell or the transplantation of wild juvenile oysters are fundamentally extensions of wild fishery practices and are not included in our recommendations related to aquaculture. Further, though *spat-on-shell aquaculture* shares some techniques with restoration approaches used in recruitment-limited areas, it varies sufficiently in harvest practices and population age structure that data on enhanced denitrification rates derived from sanctuary reefs constructed in this manner are not applicable to this form of aquaculture. Finally, though our discussions frequently turned to policy implications of the findings, we concluded that the groups' expertise lay primarily outside that arena and limited our conclusions and recommendations to interpreting the data and determining how they can appropriately be used.

Table 1. Summary of aquaculture practices in the Chesapeake Bay.

Type	**Definition**	**Approach**	**Description**
Extensive	Cultivation of natural wild stocks	Shell planting	Oyster shell placed on the bottom to attract recruitment of wild oysters
		Seed relay	Transplant wild juvenile oysters from natural reefs to private leases
Intensive	Cultivation of hatchery-produced oysters	Spat-on-shell bottom aquaculture	Hatchery-produced larvae settled onto shell then planted on bottom leases
		Bottom cage or rack-and-bag aquaculture	Cultchless oysters raised in protective cages or bags near the bottom
		Suspended aquaculture	Cultchless oysters reared in floating cages near the surface

Summary and Meta-Analysis of Existing Data

A preliminary review of the published literature on the role of oysters and oyster reefs in nitrogen cycling quickly made it clear that few published data exist. One of the workshop goals was to gather scientists actively working in this field to learn more about ongoing projects and unpublished data. The studies described below span the range from completed works resulting in published peer-reviewed papers to ongoing projects that are still gathering data.

Nitrogen assimilation

Summary - We define assimilated nitrogen as the nitrogen contained in the soft tissue and/or shell of an oyster at the time of sampling. All nitrogen data considered as part of our review were reported as a percentage of the total dry weight of the material sampled. Each study is described briefly below and data are summarized in Tables 2 and 3.

Newell (2004) reported the nitrogen content of the soft tissue (7.0% N) and shells (0.3% N) of wild oysters from a natural reef in Chesapeake Bay. Because information on study site characteristics, the number of oysters analyzed and associated variance of nitrogen content are not provided, we have not included these data in Tables 2 or 3.

Higgins et al. (2011) measured the nitrogen content of the soft tissue and shell of individual oysters grown in floating aquaculture cages at two sites in Chesapeake Bay: Spencer's Creek, VA and St. Jerome Creek, MD. They found no significant differences in the percentage of nitrogen in oyster shell or tissue between sites (Tables 2 and 3). Using data on the nitrogen content of a range of oyster size classes they found that total nitrogen content of an aquacultured oyster in their study could be predicted based on its total length (i.e. shell height) using the equation:

$$TN = e^{(-14.1569+2.7994*\ln(TL))}$$

where: TN = total nitrogen content (g)

TL = total oyster shell height (mm)

Higgins et al. noted that this equation results in a total predicted nitrogen content for a harvest-sized (76.2 mm) aquacultured oyster that is one-fourth of that previously estimated for wild oysters in Chesapeake Bay (Newell 2004) and attributed the difference to a combination of the lower shell weight and the lower shell nutrient content of aquacultured oysters.

Carmichael et al. (2012) reported original measurements of the nitrogen content of oyster soft tissue and reviewed previously published studies that included measurements of the nitrogen in the soft tissue of several bivalve species. For their own study, Carmichael et al. (2012) deployed oysters at five locations near Cape Cod, MA that spanned a range of nitrogen loading levels and measured the nitrogen content of oyster soft tissue. They found no significant differences among sites (Table 2) and used the mean value (8.6% N) for all subsequent calculations.

Kellogg et al. (2013) measured the nitrogen content of the tissues and shells of oysters collected from a restored subtidal oyster reef in the Choptank River, MD (Tables 2 and 3). Each sample contained material from multiple oysters (n = 3-6). Oyster tissue nitrogen content as a percent of dry weight (9.27% N) was slightly higher than that reported by Higgins et al. (2011) but shell nitrogen content (0.21% N) was very similar. This same study sampled oyster shells used as part of the site preparation prior to restoration. These shells, assumed to be >7 years old, contained less nitrogen (0.15% N) than shells from live oysters at the same site (Table 3, Kellogg unpublished data).

Dalrymple and Carmichael measured the percentage of nitrogen in both the tissues and shells of juvenile and adult oysters (Dalrymple 2013; Dalrymple and Carmichael, In prep) and of diploid and triploid oysters (Dalrymple 2013; Dalrymple et al., In prep) held at two sites in Mobile Bay, Alabama. Sites were sampled nine times at two week intervals. Tissue nitrogen content did not differ with ploidy or site within Mobile Bay, but values (11.8% N) in soft tissue were higher than those observed in studies along the Atlantic Coast. Collection of data on oyster shell nitrogen content is complete and will be available in the near future.

Grizzle and Ward (2011) reported data on nitrogen content of two size classes of cultured oysters from six locations in Great Bay, NH. They observed significant differences in soft tissue nitrogen content across the six sites with mean values ranging from 5.64 to 9.27%. Although Grizzle and Ward characterized these data as preliminary, we chose to include them in our analysis because many of their observed values fall within the range observed in other studies (Table 3).

Table 2. Nitrogen content of oyster tissue as a percentage of dry weight. N = number of oysters sampled, SH = Shell height, * All values calculated using raw data provided in report appendix.

Source	Growing Conditions	Study Site and Environmental Conditions	% Nitrogen Mean	% Nitrogen Range	N
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	8.10 ± 0.13 SE	5.80 – 9.97	47
		St. Jerome Creek, MD Salinity = 12 – 15 High flow, low sedimentation	7.37 ± 0.19 SE	5.43 – 10.36	37
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	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	N/A	160
		Wild Harbor, Cape Cod, MA Salinity = 26 N load = 65 x 10^{-4} kg N m^{-2} y^{-1}	8.95 ± 0.16 SE	N/A	160
		Green Pond, Cape Cod, MA Salinity = 28 N load = 178 x 10^{-4}kg N $m^{-2}y^{-1}$	8.04 ± 0.24 SE	N/A	160
		Snug Harbor, Cape Cod, MA Salinity = 25 N load = 236 x 10^{-4}kg N $m^{-2}y^{-1}$	9.19 ± 0.15 SE	N/A	160
		Childs River, Cape Cod, MA Salinity = 26-27 N load = 601 x 10^{-4}kg N $m^{-2}y^{-1}$	8.37 ± 0.27 SE	N/A	160
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]
Dalrymple and Carmichael (In prep)	Cages ~10-20 cm off bottom Cage area = 0.65 m^{-2} Mean juvenile SH = 42mm Mean adult SH = 98mm	Mobile Bay, AL 2 study sites	11.8 ± 0.1 SE	9.10 – 13.54	108
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
		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

[a] All values calculated using raw data provided in report appendix

[b] Three samples composed of five individuals per sample

Table 3. Nitrogen content of shell as a percentage of dry weight. SH = Shell height, N = number of oysters sampled.

Source	Growing Conditions	Study Site and Environmental Conditions	% Nitrogen Average	% Nitrogen Range	N
Higgins et al. (2011)	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	0.20 ± 0.01 SE	0.11 – 0.39	47
		St. Jerome Creek, MD Salinity = 12 – 15 High flow, low sedimentation	0.20 ± 0.02 SE	0.11 – 0.48	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	0.21 ± 0.08 SD	0.16-0.30	16[a]
Kellogg et al. (Unpublished data)	Restored oyster reef Aged shell (presumed to be >7 years old)	Choptank River, MD Salinity = 7.0-11.6	0.15 ± 0.02 SD	0.13-0.17	15[b]

[a] Three samples composed of four to six individuals per sample

[b] Three samples composed of five individuals per sample

Meta-analysis - The studies included in our meta-analysis measured nitrogen content of oyster tissue at a total of 16 sites. Six of these sites were in close proximity to one another in New Hampshire, another five in Cape Cod, three sites were in Chesapeake Bay and two sites were in Mobile Bay. Mean values for soft tissue at individual sites range from 7.20 to 11.8% N with the highest values measured at Mobile Bay. Averaging across studies conducted on the Atlantic Coast yielded a mean oyster tissue nitrogen content of 8.22 ± 0.89% N. Although differences in the nitrogen content of oyster tissue across sites on the Atlantic Coast appears to be relatively small, it should be noted that no studies have been conducted on the Atlantic coast of the United States south of Chesapeake Bay and none of these studies have explicitly considered the impact of oyster reproductive condition on nitrogen content.

The two recent studies measured the nitrogen content of the shells of living oysters from three sites in Chesapeake Bay produced similar estimates of 0.20 and 0.21% N (Higgins et al. 2011 and Kellogg et al. 2013, respectively; Table 3). Lower nitrogen content in aged shell suggests that the nitrogen content of shell declines through time (Kellogg, unpublished data; Carmichael, unpublished data).

Although variation in the percentage of nitrogen in the tissues and shell of aquacultured oysters versus those collected from natural or restored oyster reefs is relatively small, the ratio of oyster tissue dry weight to oyster shell dry weight varies widely. The significantly higher ratio of tissue dry weight to shell dry weight for aquacultured oysters likely reflects differences in growing conditions.

Denitrification

Summary **-** Few studies have been conducted that directly measure the impacts of oysters on denitrification rates. Below are brief summaries of studies published to date and descriptions of ongoing work. To facilitate comparisons across studies, only data resulting from studies using membrane inlet mass spectrometery (MIMS) to assess net fluxes of N_2 gas in the water column are included below, although Higgins et al. (2013) also made measurements using ^{15}N. All values have been converted to the same units ($\mu mol\ N_2\text{-}N\ m^{-2}\ h^{-1}$) and reported in terms of enhancement of denitrification over that measured at an appropriate reference site. For the purposes of this review, we define enhancement of denitrification as a net increase in flux of nitrogen gas from the sediments into the water column as compared to a reference site. Data from sites without an appropriate reference site nearby have been excluded.

Laboratory Experiments

Newell et al. (2002) simulated the effects of oyster biodeposition on estuarine nitrogen dynamics in the laboratory. Under aerobic conditions in the absence of light, they found that ~20% of the nitrogen in simulated bivalve biodeposits (pelletized *Thallasosira pseudonana*) was converted to nitrogen gas and returned to the atmosphere via denitrification. Under anoxic conditions, organic nitrogen was returned to the water column as ammonium. Under oxic conditions with sufficient light, a benthic algal and cyanobacterial community developed that both absorbed inorganic nitrogen and fixed nitrogen. Many subsequent estimates of the influence of oysters on nitrogen cycling and related modeling efforts have been based upon the results of this laboratory simulation of the effects of oyster biodeposition.

Aquaculture

Only two studies addressing the effects of intensive aquaculture of *Crassostrea virginica* on nitrogen dynamics currently exist. In both cases nitrogen fluxes from sediments into the water column were measured beneath oysters in suspended aquaculture and at adjacent sites without oyster cultivation that served as reference sites. No data currently exist on nitrogen dynamics associated with other forms of intensive oyster aquaculture (see Table 1).

Holyoke (2008) studied the impact of oysters growing in aquaculture floats on nitrogen dynamics in underlying sediments during four sampling periods at each of three sites in La

Trappe Creek, MD. Impacts were highly variable across sampling periods and under both light and dark conditions. Of the 24 estimates of denitrification rates produced by this study (12 under light conditions and 12 under dark conditions), half indicate reduced denitrification at the aquaculture site compared to the reference site. Of the 12 estimates that indicate enhanced denitrification, only three rates were >50 µmol N_2-N m^{-2} h^{-1} (>0.7 mg N_2-N m^{-2} h^{-1}).

Higgins et al. (2013) studied the impacts of oysters growing in aquaculture floats on nitrogen dynamics in underlying sediments. Studies were conducted at a low flow, high sedimentation site in Spencer's Creek, VA and a high flow, low sedimentation site in St. Jerome Creek, MD. In addition to an oyster treatment, which included oysters in floats at common aquaculture densities, this study included a "biodeposit fence" treatment that prevented dispersal of biodeposits, concentrating them immediately beneath the floats. To facilitate comparisons with other studies, our meta-analysis below only considers the data from their oyster treatment and reference sites. Similar to Holyoke (2008), this study found both positive and negative impacts on denitrification. Denitrification rates at St. Jerome Creek were reduced compared to the reference site in May but enhanced in August. The effect of oysters on denitrification ranged from a reduction of -59.2 µmol N_2-N m^{-2} h^{-1} (0.8 mg N_2-N m^{-2} h^{-1}) to an enhancement of 95.0 µmol N_2-N m^{-2} h^{-1} (1.3 mg N_2-N m^{-2} h^{-1}) relative to reference sites (Table 4).

Table 4. Effects of intensive oyster aquaculture on denitrification (DNF) rates calculated as the flux of nitrogen gas from the sediments to the atmosphere at the aquaculture site minus the flux at a reference site. Positive values indicate enhanced DNF rates and negative values reduced DNF rates relative to the reference site. LTC = La Trappe Creek.

Source	Growing Conditions	Study Site and Environmental Conditions	Month(s) Sampled	Incubation Type	Average Effect on DNF (µmol N_2-N m^{-2} h^{-1})
Holyoke (2008)	Floating cages Oyster density = 184-216 m^{-2}	Lowry Cove, LTC, MD Salinity = 13.25 ± 0.96 PAR: ~70-80 µmol m^{-2} s^{-1}	Jul. Aug. Sep.	Light	-36.1 – 25.2
				Dark	-86.1 – 343.5
		Mainstem, LTC, MD Salinity = 6.75 ± 1.50 PAR: ~70-80 µmol m^{-2} s^{-1}	Jul. Aug. Sep.	Light	-7.1 – 117.6
				Dark	-103.1 – 48.0
		Pier, LTC, MD Salinity = 5.50 ± 1.29 PAR: 5-25 µmol m^{-2} s^{-1}	May Jun. Jul.	Light	-118.6 – 6.0
				Dark	-146.3 – -32.6
Higgins et al. (2013)	Floating cages 200 oysters per bag Cage area = 0.5 m^{-2} Max. oyster density = 286 m^{-2}	Spencer's Creek, VA Salinity = 5-15 Low flow, high sedimentation	Aug.	Dark	70.8
		St. Jerome Creek, MD Salinity = 12-15 High flow, low sedimentation	May Aug.	Dark	-59.2 – 95.0

Oyster Reefs

Estimates of denitrification rates associated with oyster reefs have been made in five recently completed studies and one on-going study (Table 5). The study sites spanned a wide range of conditions from intertidal to subtidal below the euphotic zone and had salinities ranging from 7 to 36. The sites included natural intertidal reefs, reefs formed from shell plantings and wild recruitment on leased bottom (i.e. extensive aquaculture), a restored reef produced by multiple year classes of spat-on-shell addition to a sanctuary area, and replicated, experimental reefs constructed at varying densities. Some of these studies were conducted in a single season, whereas others span a greater portion of the year. Reference sites included nearby soft sediment areas and unrestored reefs.

Piehler and Smyth (2011) collected sediment cores from within and adjacent to natural intertidal oyster reefs in Bogue Sound, NC during four sampling periods distributed throughout the year. Denitrification rates were higher for the oyster reef than for the control site with an annual enhancement rate of 2.7 g N m^{-2} y^{-1}(22.0 μmol N_2-N m^{-2} h^{-1}). In both spring and summer, all samples indicated enhanced denitrification rates at the oyster reef site. In winter and fall, results were mixed with some samples demonstrating enhanced rates and others showing reduced rates.

Sisson et al. (2011) measured denitrification rates on intertidal oyster reefs of varying density in the Lynnhaven River, VA during a single sampling period. These reefs were the result of wild oyster settlement onto shell plantings on a privately-held lease and thus fall into the fishing practice sometimes referred to as extensive aquaculture (see discussion on pg. 4). Samples encompassed a range of oyster biomass densities (35 – 218 g tissue DW m^{-2}). With the exception of the lowest oyster density under dark conditions, all samples indicated enhanced denitrification rates and there was a tendency for denitrification rates to increase with increasing oyster biomass density.

Smyth et al. (2013) used the same methods and sites as those used by Piehler and Smyth (2011). These studies indicated net enhancement (3.2 g N m^{-2} y^{-1} = 26.1 μmol N_2-N m^{-2} h^{-1}) of annual denitrification rates associated with oyster reef. Similar to the previous study, they found enhancement for all samples collected in spring and summer. In contrast to previous work, all samples collected in winter indicated enhanced denitrification compared to the reference site whereas all samples collected in fall indicated reduced denitrification rates.

Kellogg et al. (2013) compared nitrogen dynamics at a mature, subtidal restored oyster reef to a nearby reference site that was suitable for oyster reef restoration in the Choptank River, MD. Both sites lay beneath the euphotic zone. Rates of denitrification at the restored site were enhanced over those at the reference site during all seasons. The degree of enhancement varied between seasons with greatest enhancement in August (1486 μmol N_2-N m^{-2} h^{-1} = 20.8 mg N_2-N m^{-2} h^{-1}). Net annual enhancement was estimated at 55.6 g m^{-2} y^{-1} (453.1 μmol N_2-N m^{-2} h^{-1}) for this site.

Kellogg et al. (In prep) used experimental reefs to examine the relationship between oyster biomass density and denitrification rates at a shallow subtidal site in Onancock Creek, VA. Although studies had been planned to encompass four seasons, a die-off of >99% of the oysters limited sampling to April. Results from the single sampling period indicate a significant positive relationship between oyster biomass density and denitrification rates. However, the relationship appears to be non-linear with limited increases in denitrification per unit oyster biomass above ~100 g tissue dry weight m^{-2}.

Kellogg et al. (Ongoing) are using experimental reefs to examine the relationship between oyster biomass density and denitrification rates at an intertidal site at the Hillcrest Oyster Sanctuary in the Virginia Coast Reserve near Oyster, VA. Thus far, studies indicate that there may be a positive relationship between oyster biomass density and denitrification rate. However, preliminary data suggest that this relationship may be more variable for intertidal reefs than for subtidal reefs, change with season and require relatively high oyster biomass density to enhance denitrification rates.

Table 5. Effects of oyster reefs on denitrification rates. Calculations as in Table 4.

Source	Incubation Chamber Contents and Reef Characteristics	Study Site and Environmental Conditions	Month(s) Sampled	Incubation Type	Average Effect on DNF (μmol N_2-N m^{-2} h^{-1})
Piehler and Smyth (2011)	Sediments collected within and adjacent to natural oyster reefs Intertidal Oyster biomass density = ~39 g DW m^{-2}	Bogue Sound, NC Salinity = 27 – 36 Temp. = 11 – 24 ºC	February May July October	Dark	-58.4 – 128
Sisson et al. (2011)	Intact section (0.1 m^2) of reef from extensive oyster aquaculture site Intertidal Oyster biomass density = 35.4 – 217.8 g DW m^{-2}	Humes Marsh, Lynnhaven River, VA Salinity = 29.4 Intertidal	October	Light	48 – 217.8
				Dark	-21.0 – 209.5
Smyth et al. (2013)	Sediments collected within and adjacent to natural oyster reefs Intertidal reef Oyster biomass density = ~39 g DW m^{-2}	Bogue Sound, NC Salinity = 29 – 32 Temp. = 3 – 30 ºC	January March July November	Dark	-58.4 – 422.5
Kellogg et al. (2013)	Intact section (0.1 m^2) of experimentally restored oyster reef Subtidal Oyster biomass density = 262 – 382 g DW m^{-2}	Choptank River, MD Salinity = 7.0-11.6 Temp. = 13.5 – 27.4 ºC	April June August November	Dark	199.2 – 1486.4
Kellogg et al. (In prep)	Intact section (0.1 m^2) of experimental oyster reef treatments Subtidal Oyster biomass density = 42.8 – 533.0 g DW m^{-2}	Onancock Creek, VA Salinity = 16 Temp. = 14 ºC	April	Light	235.3 – 533.5*
				Dark	273.9 – 767.8*
Kellogg et al. (Ongoing study)	Intact section (0.1 m^2) of experimental oyster reef treatments Intertidal Oyster biomass density = 0.0 – 345.2 g DW m^{-2}	Hillcrest Oyster Sanctuary, Oyster, VA Salinity = 32 - 34 Temp. = 18.0 – 26.5 ºC	August October	Light	-178.3 – 329.7*
				Dark	0 – 709.5*

* Study included a "shell only" treatment created by placing a layer of oyster shell at the site without adding live oysters. Because oyster biomass density was zero for the entire experimental plot, resulting data have not been included in the table.

Meta-analysis – A total of eight studies have measured oyster-associated denitrification rates in the field. The geographic range of these measurements is narrow, including five sites in Maryland, four sites in Virginia and one site in North Carolina. To date, measurements have been made at five aquaculture sites and five oyster reef sites.

Aquaculture

The two existing studies from suspended oyster aquaculture in Chesapeake Bay provide little evidence for significant net annual increase in denitrification. Although there is a slight trend towards increasing rates of denitrification later in the year (Fig. 2), this pattern explains very little of the overall variation. Denitrification rates have not been assessed for either the material within aquaculture floats or for any other type of intensive aquaculture.

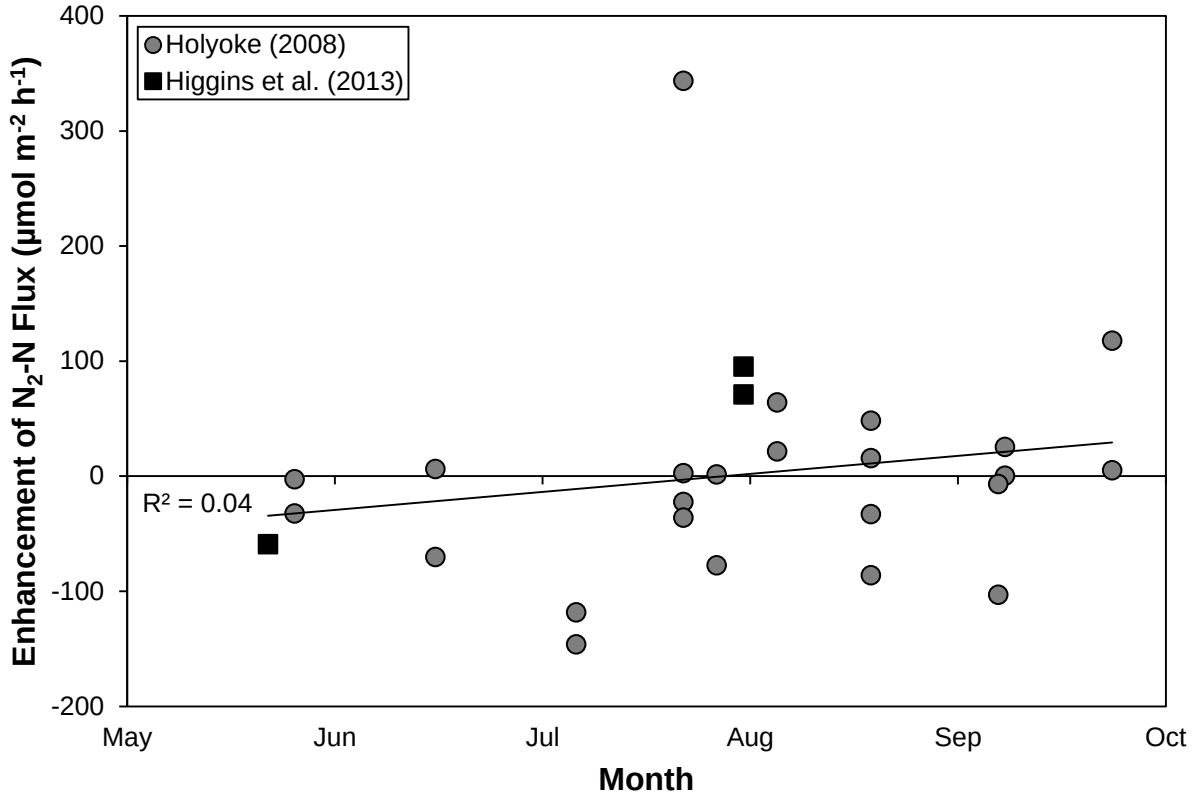


Figure 2. Seasonal denitrification rates for intensive aquaculture. Data generated by MIMS from sediment cores collected beneath oysters in growing in aquaculture floats. Holyoke (2008) data include both light and dark incubations. Higgins et al. (2012) data include only dark incubations.

Oyster Reefs

At present, five completed studies and one ongoing study have measured denitrification rates associated with oyster reefs with varied results. Across all studies, seasons and oyster biomass levels, the degree to which denitrification is enhanced over nearby reference sites varies by four orders of magnitude and includes both positive (an increase relative to the reference site) and negative values (a decrease relative to the reference site, Fig. 3). Within individual studies, enhancement of denitrification often varies by three orders of magnitude and sometimes includes both positive and negative values. Although we suggest possible sources of variation in denitrification rates below, we emphasize that these apparent patterns are based on very few data points.

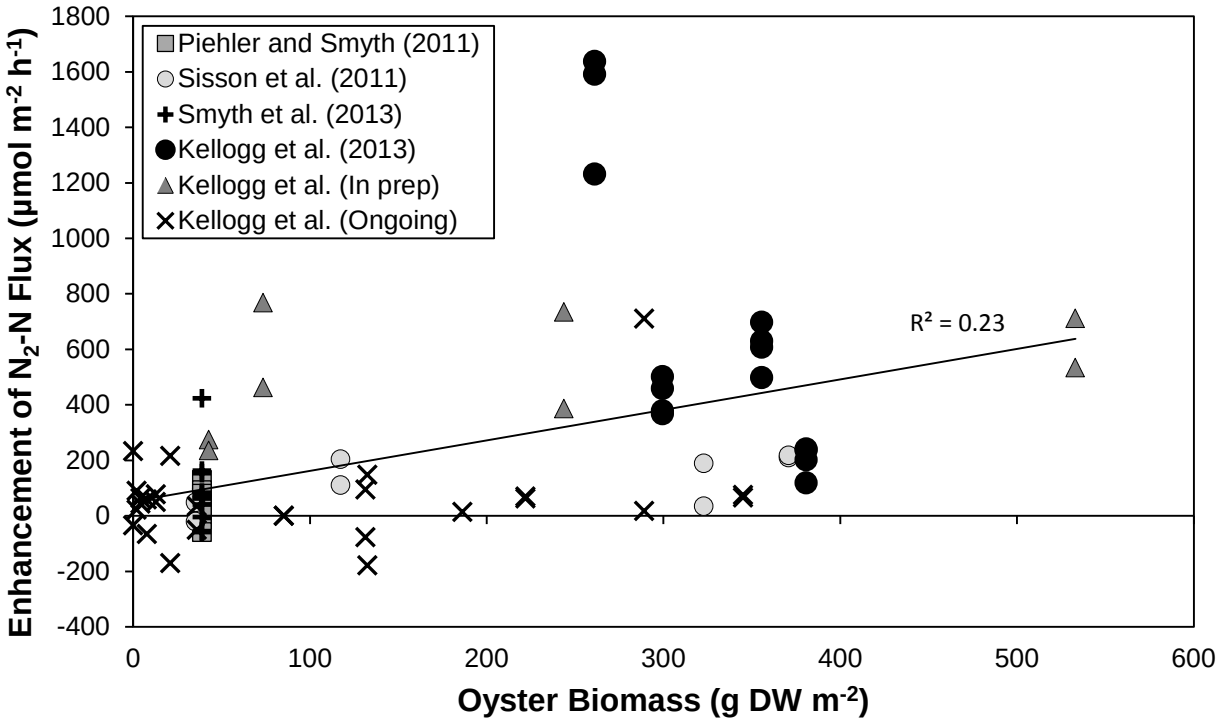


Figure 3. Enhancement of denitrification rates in relation to oyster biomass density. Biomass density for Piehler and Smyth (2011) and Smyth et al. (2013) are approximate and based on estimated adult oyster density at the study site combined with size and biomass distribution data from Kellogg et al. (Ongoing).

Three completed studies have estimated annual enhancement of denitrification rates by oyster reefs compared to reference sites (Table 6). Each of these studies measured denitrification rates a four time points within a single year and extrapolated these values to produce annual rates. For the purposes of this review, we define annual enhancement of denitrification as the net annual increase in N_2-N flux from the sediments to the atmosphere. For the two studies of

intertidal reefs in North Carolina, annual enhancement ranged from 2.7 to 3.2 g N_2-N m^{-2} y^{-1} (22.0 to 26.1 µmol N_2-N m^{-2} h^{-1}). The one study of a subtidal reef in Maryland estimated annual enhancement at 55.6 g m^{-2} y^{-1}(453.1 µmol N_2-N m^{-2} h^{-1}). An ongoing study of experimental intertidal oyster reefs in the Virginia coastal bays will produce estimates of annual denitrification enhancement for that site.

Table 6. Estimated annual enhancement of denitrification rates by oyster reefs. See Table 5 for details of each study. DNF = denitrification.

Source	Number of Sampling Periods	Annual DNF Enhancement (g N_2-N m^{-2} y^{-1})	Method Used to Calculate Annual Rate
Piehler and Smyth (2011)	4	2.7	Each seasonal rate applied to three months of the year, adjusted for hours submerged per day in the dark
Smyth et al. (2013)	4	3.2	Each seasonal rate applied to three months of the year; adjusted for hours submerged per day in the dark.
Kellogg et al. (2013)	4	55.6	Values from each sampling period applied to two months of the year. Assumed no denitrification in other four months of year.

The three completed studies that measured denitrification in multiple seasons show a significant effect of season within site (Fig. 4). Highest rates are observed in summer, lower rates in spring and lowest rates in fall or winter. This pattern is likely driven by a combination of water temperature and the supply of organic material.

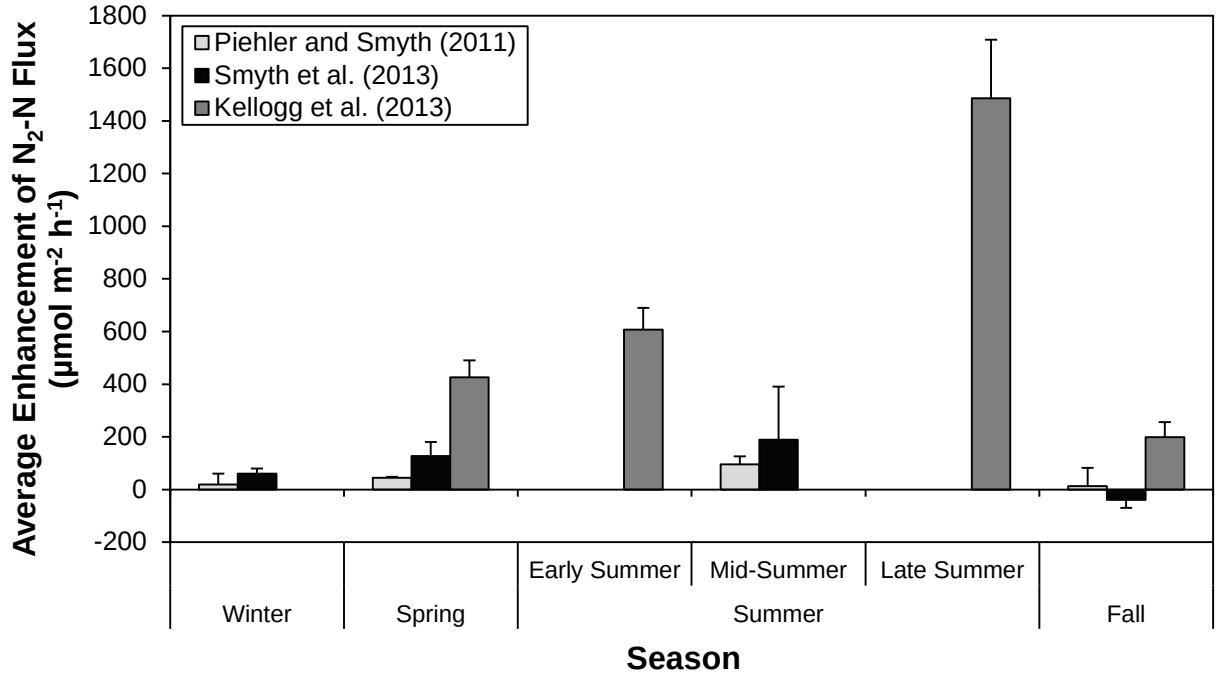


Figure 4. Seasonal patterns of denitrification enhancement relative to reference sites. Error bars represent standard deviaiton.

All three studies in Virginia were explicitly designed to examine the relationship between oyster biomass density and enhancement of denitrification. These studies suggest that there is a positive but non-linear relationship between oyster soft tissue biomass and denitrification rates within a site. However, the exact nature of this relationship appears to vary widely among sites and among seasons within site (Fig. 5).

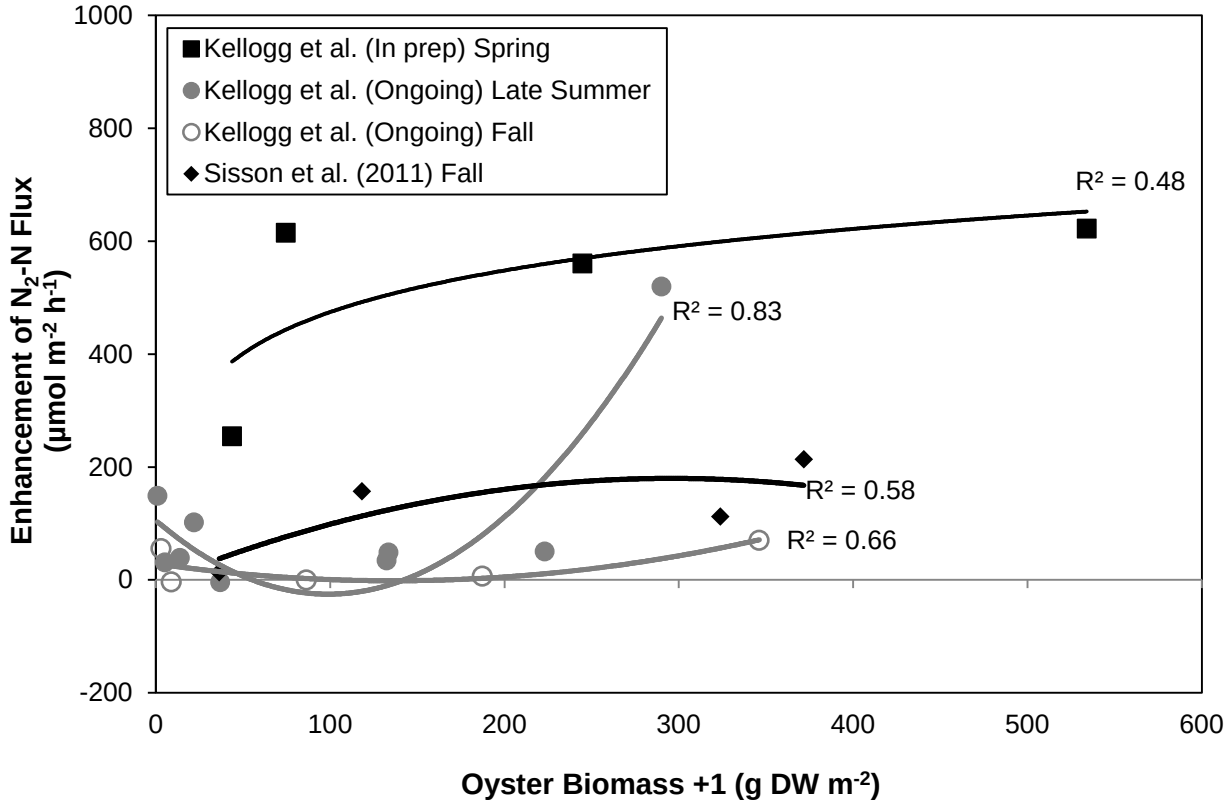


Figure 5. Enhancement of denitrification in relation to oyster biomass density. Biomass data have been +1 transformed to allow fitting of a logrithmic function. Enhancement rates are the average of the enhancement under light and dark conditions.

The two studies of intertidal reefs in North Carolina measured denitrification rates for sediments collected within or adjacent to oyster reefs. The other four studies in the Chesapeake Bay region (two subtidal and two intertidal) measured denitrification rates from intact sections of oyster reef that included sediments, oysters and the associated macrofaunal community. Research by Kellogg et al. (unpublished data) using samples from a subtidal restored reef in Maryland found significant levels of nitrification and denitrification associated with oyster clumps in the absence of underlying sediments, suggesting that fluxes measured for oyster reef sediments alone could underestimate actual denitrification rates.

Gaps in Existing Data

Our literature review along with the workshop presentations and discussions make it clear that few data on the impacts of oysters on nitrogen cycling exist. Below we list some of the primary gaps in existing data. This is not a comprehensive list, but rather highlights gaps in our current knowledge that significantly limit our ability to assign values for nitrogen removal to oysters growing in aquaculture or reef settings.

Nitrogen assimilation

1. Impact of reproductive state on the nitrogen content of oyster soft tissue: No published data exist for seasonal patterns in reproductive state on nitrogen content.
2. Percentage of nitrogen in the soft tissues and shells of intertidal oysters: All published data come from oysters growing either in aquaculture cages or on subtidal reefs.
3. Geographic variation in percentage of nitrogen in oyster soft tissue and shell: At present, data for oyster shell have been gathered at three sites in Chesapeake Bay and will be available soon for Mobile Bay, AL. Data on oyster tissue have been gathered in Chesapeake Bay, Cape Cod, MA, Mobile Bay, AL and Great Bay, NH. More data are needed to understand and define the importance of regional differences in relation to environmental conditions.

Denitrification

4. Denitrification rates for common forms of aquaculture: Data exist for the sediments underlying aquaculture floats but no data exist for the material inside aquaculture cages or for other types of intensive aquaculture (e.g. bottom cage and spat-on-shell culture techniques).
5. Factors controlling the relationship between oyster biomass density and denitrification rates: Recently completed and ongoing studies find positive relationships between oyster biomass density and denitrification but these relationships are non-linear and vary between sites and seasons. Research is needed to clarify the roles of biotic and abiotic factors which affect the relationship between oyster biomass density and denitrification rates.

Other nitrogen removal processes

6. Nitrogen removal via burial of biodeposits or shells: To date, no data have been published for nitrogen burial rates associated with oysters. Quantifying burial rates of both shell and

biodeposits under a variety of environmental conditions will establish the conditions, if any, under which these pathways lead to significant removal of nitrogen from the system.

7. Nitrogen removal via anammox or nitrous oxide release: At present, no published data exist to determine whether nitrous oxide release represents a significant mechanism for the return of nitrogen to the atmosphere. Although data on net fluxes of nitrogen gas (collected using MIMS) exist for both oyster reefs and oyster aquaculture, the relative roles of denitrification and anammox in producing nitrogen gas cannot be distinguished using these data alone. A better understanding relative importance of each pathway leading to the return of nitrogen to the atmosphere is needed.

Methodology Recommendations for Future Studies

Nitrogen assimilation

1. Nitrogen content should be reported as a percentage of dry weight for oyster tissues and shell.

Denitrification

2. For the purposes of estimating water quality benefits, measurement of denitrification rates as the net flux of di-nitrogen gas in the water column using membrane inlet mass spectrometry is the most appropriate method available at present. However, we note that this method does not allow identification of the process by which nitrogen gas is generated (e.g. microbially mediated denitrification versus anammox) and that other techniques (e.g. stable isotope analyses) or a combination of techniques are more suited to identifying the role of various potential nitrogen cycling pathways.
3. All studies seeking to assess the impacts of oyster aquaculture, oyster reef restoration or similar management actions should include a nearby reference site with similar physical and environmental conditions to allow calculation of resulting denitrification enhancement. Because oysters only survive in areas not prone to extensive periods of anoxia, consideration of the impacts of concentrating biodeposits in these areas versus alternate fates including deposition in deep channels prone to anoxia where denitrification is unlikely is also warranted.
4. Whenever feasible, reef materials should be incorporated into samples intended to estimate denitrification rates for oyster reefs.

Recommendations for Application of Existing Data

Nitrogen assimilation

1. Because oyster growth and survival can vary widely spatially and temporally, as well as with grow-out method, accurate estimates of the removal of assimilated nitrogen via harvest of cultured oysters will require collecting site-specific data on the tissue and shell biomass of oysters harvested.
2. Because length to dry weight relationships can vary significantly with environmental conditions, food quality, oyster reproductive state and oyster health, the most accurate estimates of nitrogen assimilation will be derived using length to biomass relationships from the oyster population of interest at the time of interest. Data from other locations or sampling periods should be extrapolated with caution, especially for older animals.
3. Variation in the amount of nitrogen (as a percentage of dry weight) in oyster tissue and shell is relatively low for a range of sites and environmental conditions along the mid-Atlantic and northeast coasts of the United States. We recommend using the mean of existing values (8.22% N in oyster tissue and 0.20% N in oyster shell) when making estimates for oysters from these regions only. However, we caution that these estimates should be revised as additional data become available.

Denitrification

4. To date, no studies show significant net annual enhancement of denitrification associated with intensive oyster aquaculture. At present, we recommend assigning a value of zero for nitrogen removal via denitrification associated with aquacultured oysters. However, we note that the only data that exist at present come from studies of the sediments beneath oysters growing in aquaculture floats. As data become available for other types of intensive aquaculture and/or for nitrogen dynamics within aquaculture floats, this recommendation should be reviewed and revised as needed.
5. Studies estimating annual enhancement of denitrification rates associated with oyster reefs suggest that they significantly enhance denitrification rates relative to appropriate reference sites. However, rates of enhancement vary with season, tidal regime, oyster biomass density and other unidentified factors. We recommend conducting additional

studies to better understand the sources of this variation prior to assigning values to the nitrogen removal capacity of oyster reefs attributable to denitrification.

6. Although it is not possible at present to provide generalized relationships for estimating the enhancement in denitrification associated with oyster reefs under varying conditions, reliable methods do exist for measuring these rates and applying them to the regions within which the measurements are made.

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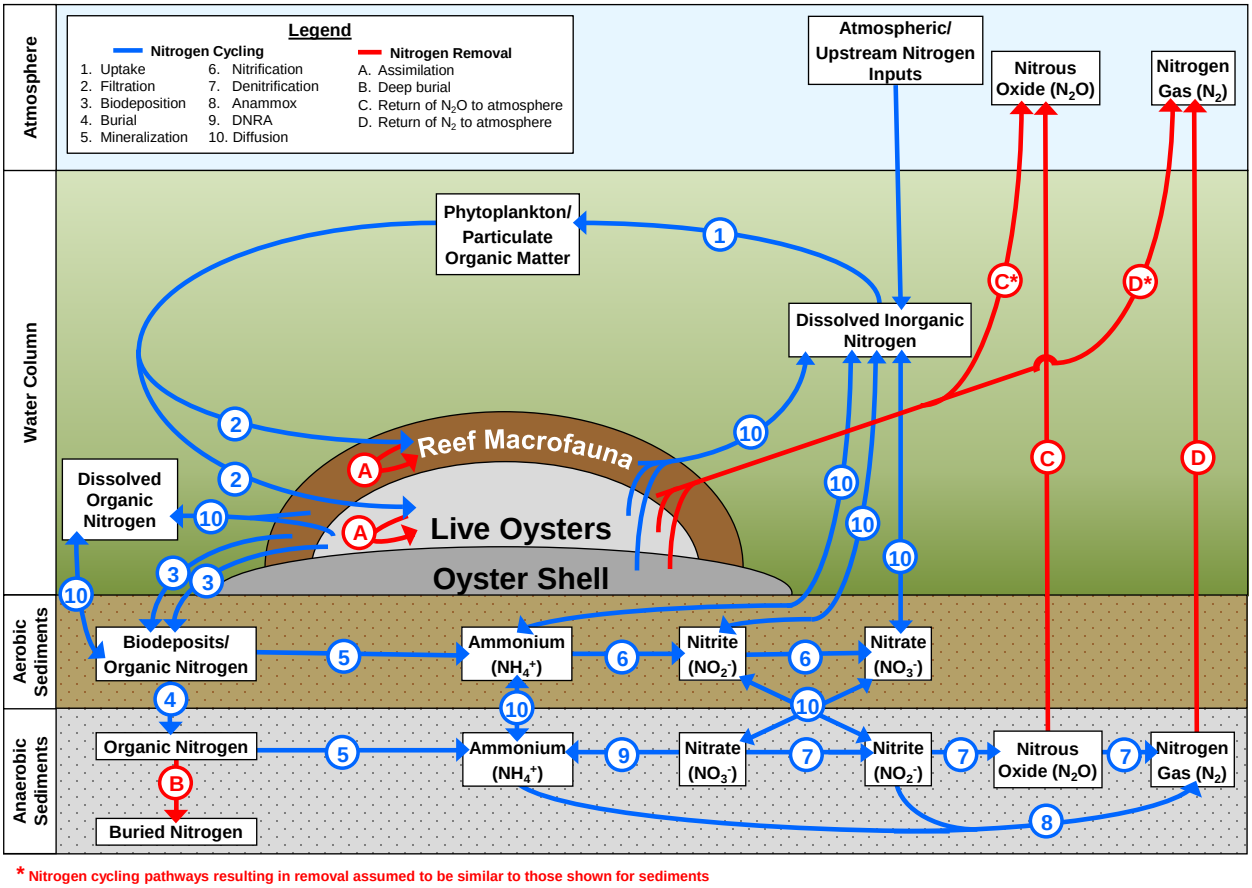
Appendix



* Nitrogen cycling pathways resulting in removal assumed to be similar to those shown for sediments

Figure A1. Primary nitrogen cycling and nitrogen removal pathways for a subtidal reef below the euphotic zone.

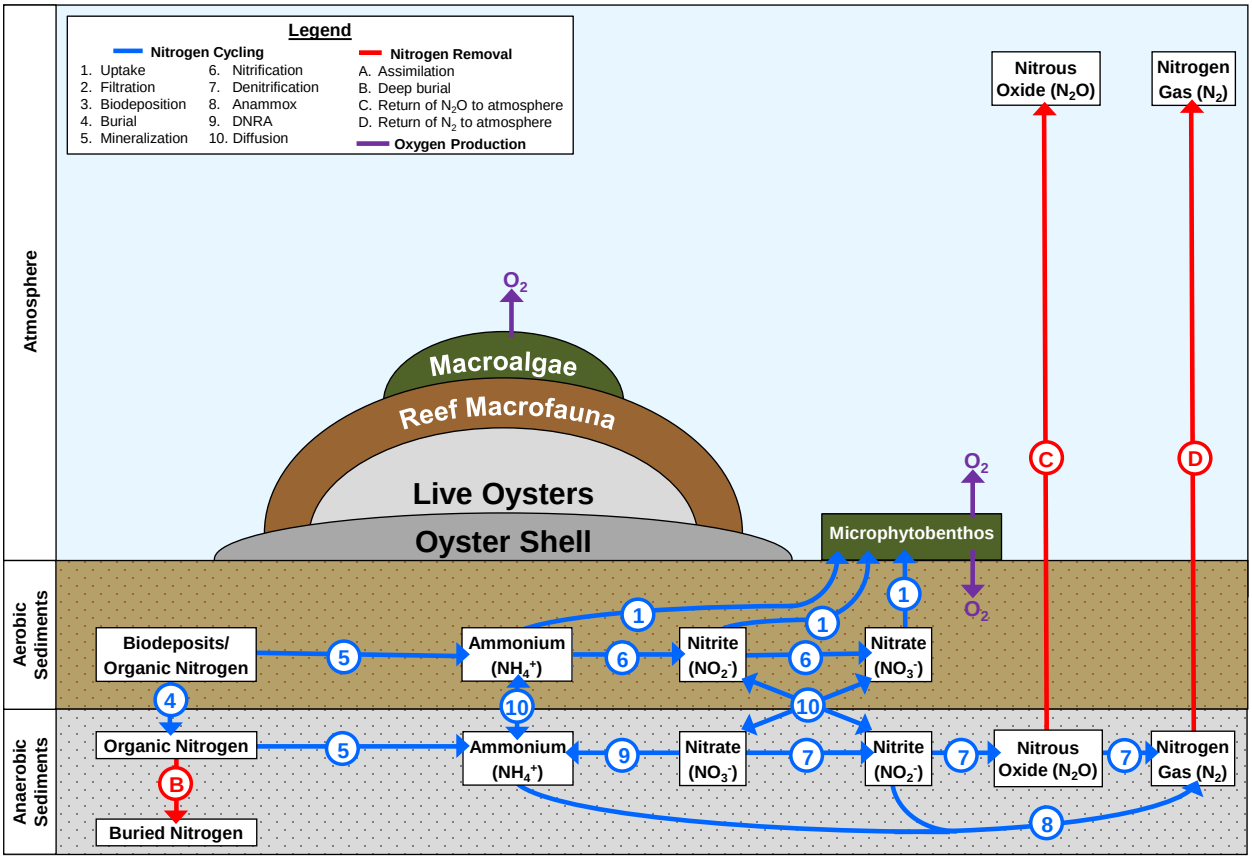


Figure A2. Primary nitrogen cycling and nitrogen removal pathways for an aerially exposed intertidal oyster reef.

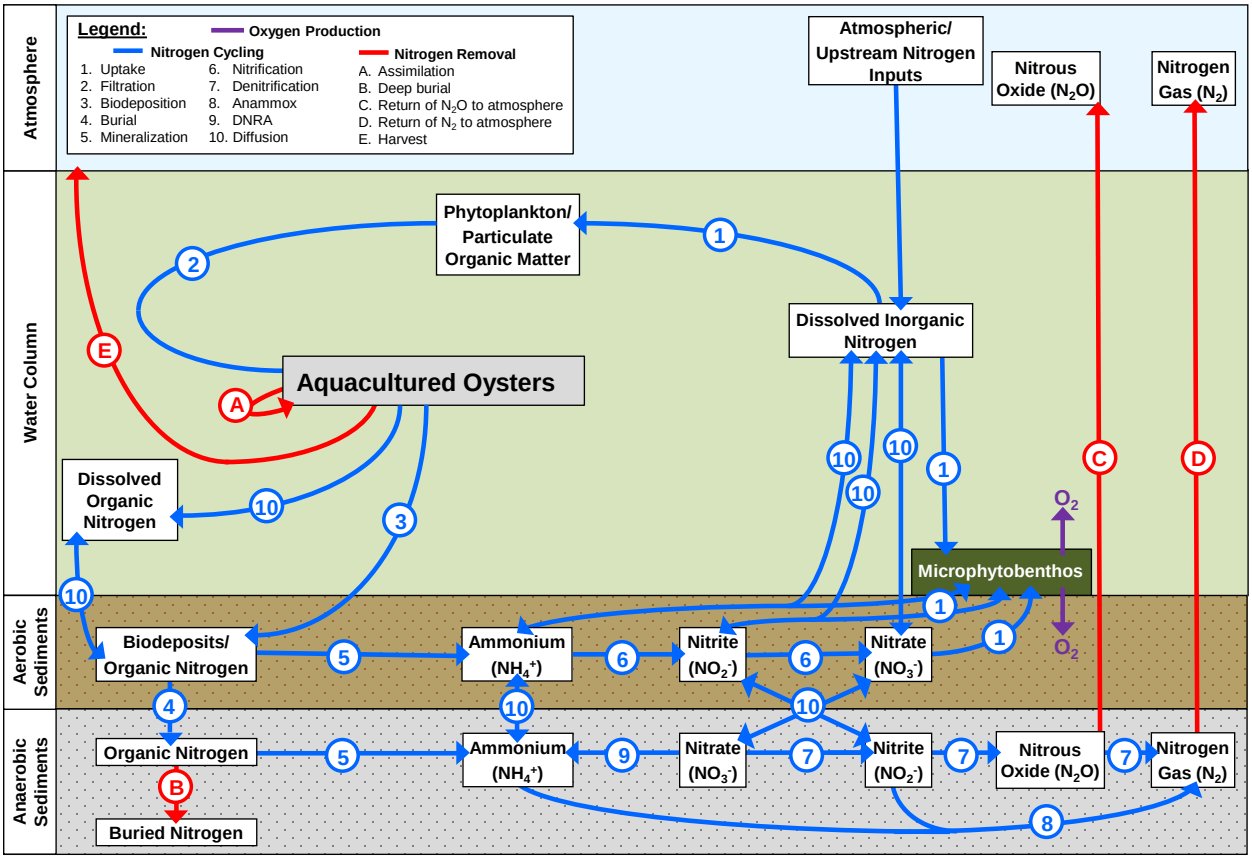


Figure A3. Primary nitrogen cycling and nitrogen removal pathways for intensive oyster aquaculture occurring over aerobic sediments within the euphotic zone.

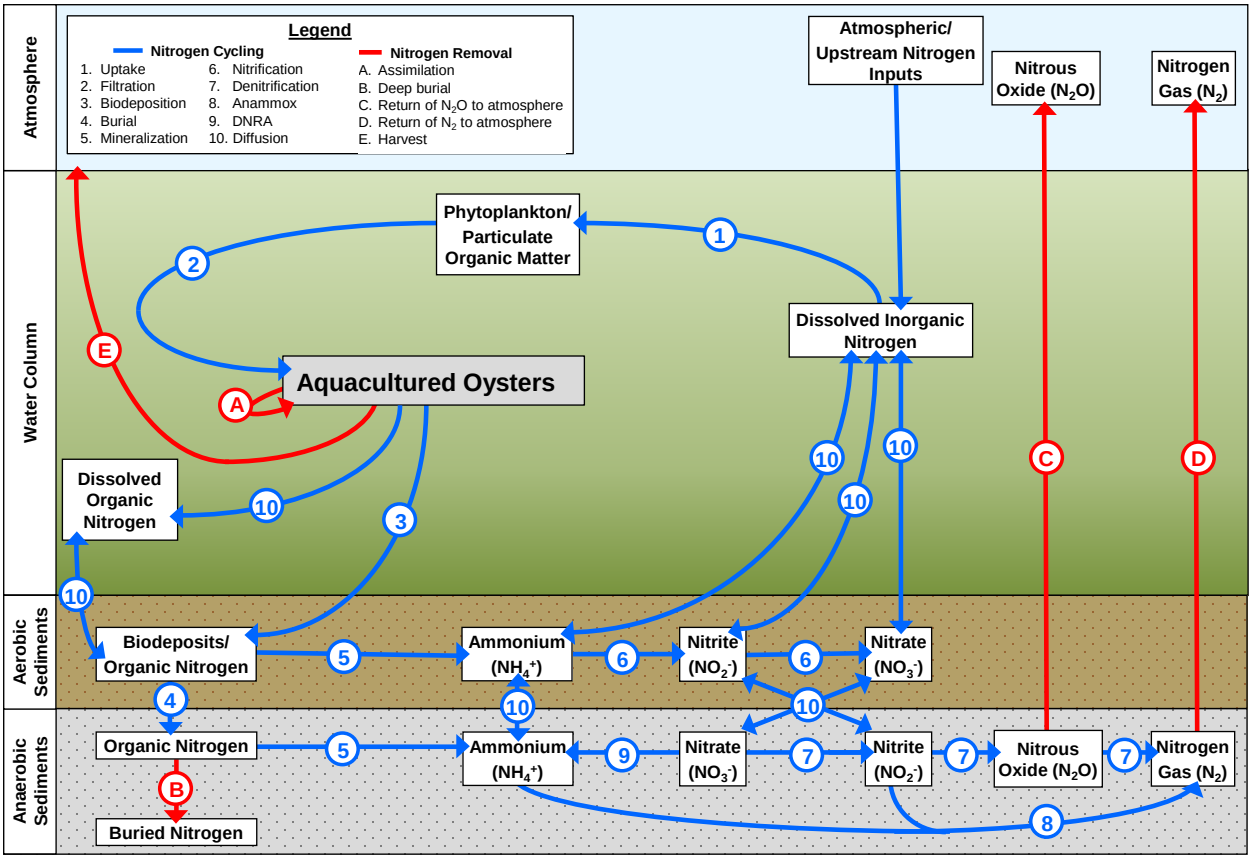


Figure A4. Primary nitrogen cycling and nitrogen removal pathways for intensive oyster aquaculture occurring over aerobic sediments beneath the euphotic zone.

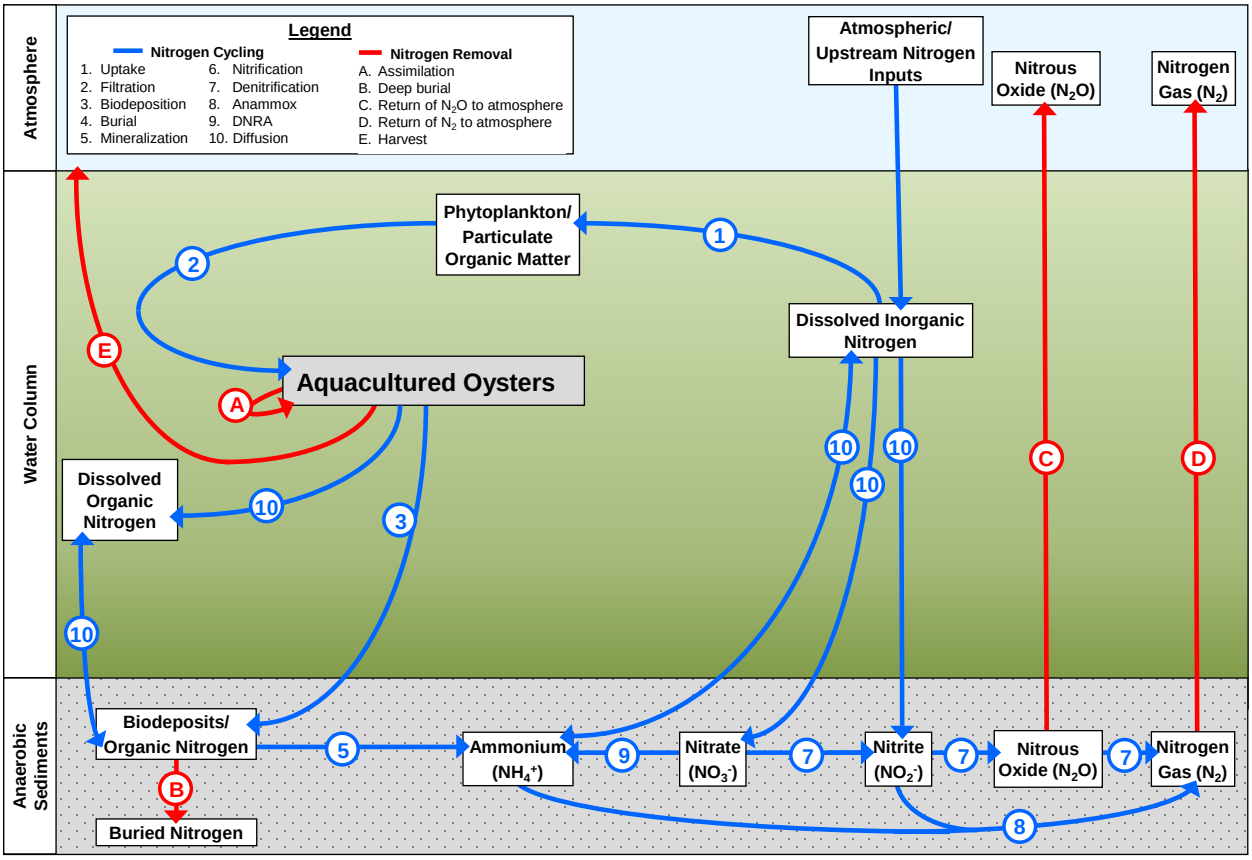


Figure A5. Primary nitrogen cycling and nitrogen removal pathways for intensive oyster aquaculture occurring over anaerobic sediments beneath the euphotic zone.