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Invited feature

Use of oysters to mitigate eutrophication in coastal waters

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ABSTRACT

Enhancing populations of suspension feeding bivalves, particularly the eastern oyster, *Crassostrea virginica*, has been proposed as a means of mitigating eutrophication in coastal waters. Review of studies evaluating the effects of *C. virginica* on nitrogen (N) cycling found that oysters can have effects on water quality that vary by orders of magnitude among sites, seasons, and growing condition (e.g., oyster reefs, aquaculture). Nitrogen contained in phytoplankton consumed by oysters may be returned to the water column, assimilated into oyster tissue and shell, buried in the sediments, or returned to the atmosphere as dinitrogen gas, primarily via denitrification. Accurately quantifying oyster-related N removal requires detailed knowledge of these primary fates of N in coastal waters. A review of existing data demonstrated that the current state of knowledge is incomplete in many respects. Nitrogen assimilated into oyster tissue and shell per gram of dry weight was generally similar across sites and in oysters growing on reefs compared to aquaculture. Data on long-term burial of N associated with oyster reefs or aquaculture are lacking. When compared to suitable reference sites, denitrification rates were not consistently enhanced. Depending on environmental and oyster growing conditions, changes in denitrification rates varied by orders of magnitude among studies and did not always occur. Oyster aquaculture rarely enhanced denitrification. Unharvested oyster reefs frequently enhanced denitrification rates. Incorporating oysters into nutrient reduction strategies will require filling gaps in existing data to determine the extent to which relationships between N removal and environmental and/or growing conditions can be generalized.

Editors note

The widespread eutrophication of coastal waters clearly involves increased nitrogen loads, in most cases with nitrogen derived from human activities within contributing watersheds. This has prompted regulators, managers, and other stakeholders to set standards for water quality that involve lowering nitrogen loads to estuaries and other coastal waters. To carry out the proscribed lowering of nitrogen loads there are a number of options. In many instances and sites with high human density and intense activity, advanced wastewater treatment plants are unavoidable, but such environmental engineering approaches are costly. There are many coastal zones with lower population densities where other, less costly alternative strategies might be less expensive and more attuned to "green" approaches. In this issue Kellogg and many colleagues report the results of a workshop aimed at assessment of the potential of one such alternative, the use of shellfish as a way to intercept and remove nitrogen from within estuaries exposed to increased nitrogen loads from watersheds.

1. Introduction

Nitrogen (N) entering an estuary from the watershed and airshed stimulates phytoplankton growth and, in excess, can lead to

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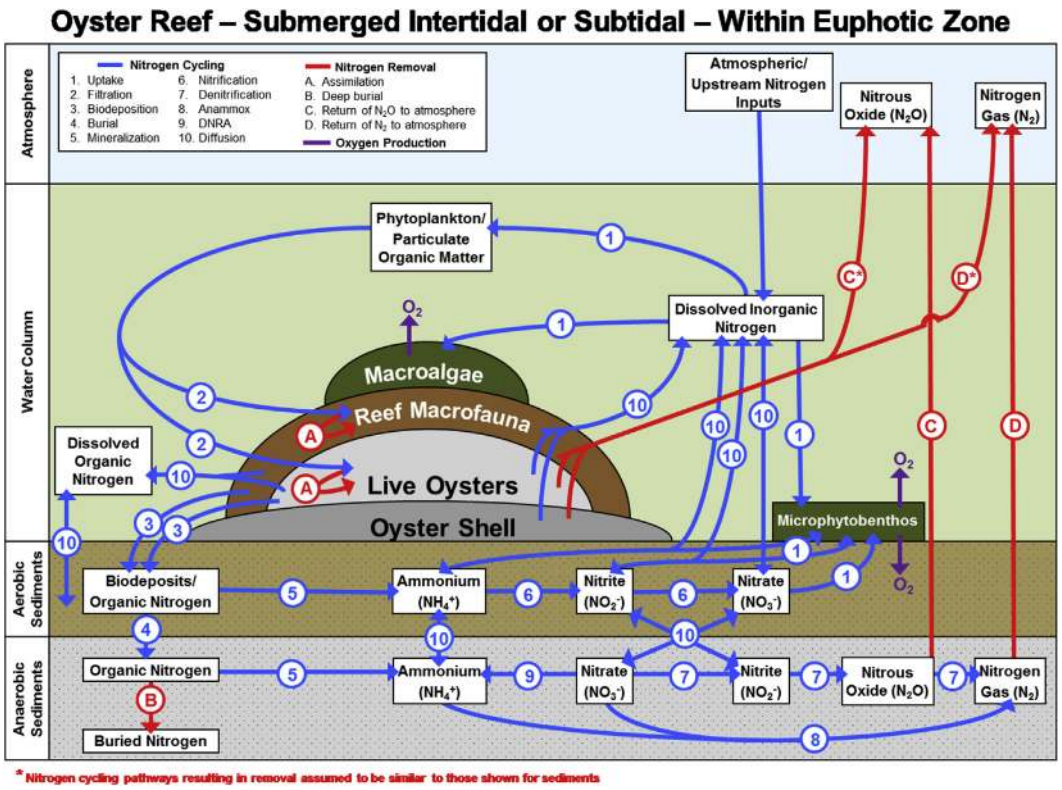

Fig. 1. Primary nitrogen cycling and nitrogen removal pathways for a shallow subtidal or submerged intertidal oyster reef in the euphotic zone.

eutrophication (Nixon, 1995; Valiela and Bowen, 2002; Kemp et al., 2005). Consequences of eutrophication include harmful algal blooms (Paerl, 1997; Glibert et al., 2005 and references therein), increased hypoxic events (Rabalais et al., 2010 and references therein) and loss of benthic habitats (Diaz and Rosenberg, 1995; Hauxwell et al., 2003; Diaz and Rosenberg, 2008 and references therein). Bivalve shellfish and other suspension feeding organisms remove a portion of the phytoplankton biomass from the water column as they feed (Fig. 1 [2]) thereby reducing turbidity and concentrations of particulate organic nitrogen (PON) in the water column (Kennedy and Newell, 1996; Newell, 2004; Newell and Koch, 2004; Grizzle et al., 2008; Dame, 2012 and references therein).

The amount of time that the nitrogen contained in phytoplankton and other particulate organic matter is removed from the water column varies from hours to permanent removal depending upon the fate of the N after consumption. Phytoplankton and other particles that are ingested but not digested will be deposited on the sediment surface as pseudofeces (Newell and Langdon, 1996). If particles are digested, some of the N will be returned quickly to the water column in the form of ammonium, urea and other nitrogenous waste products (Fig. 1 [10]); some will be assimilated into shell or soft tissue biomass (Fig. 1 [A]); and some will be deposited on the sediment surface as feces (Fig. 1 [3]; Newell et al., 2005). Nitrogen assimilated into tissue can be removed from the water column for years whereas that assimilated into shell may be removed for substantially longer periods of time. Nitrogen contained in biodeposits (feces and pseudofeces) can be consumed by deposit-feeding organisms, buried in the sediments for short or long periods of time (Fig. 1[B]), or decomposed to dissolved organic nitrogen followed by mineralization to ammonium (Fig. 1[5]; Newell et al., 2002; Giles and Pilditch, 2006; Dame, 2012 and references therein). Ammonium can diffuse into the water column or undergo a variety of transformations depending on local environmental and biological conditions. If aerobic environments exist in close proximity to anaerobic environments, ammonium will undergo nitrification (an aerobic process) followed by denitrification (an anaerobic process) leading to the production of dinitrogen gas (N_2), a form of N phytoplankton cannot utilize for growth (Fig. 1 [6–7]). Under anaerobic conditions anaerobic ammonium oxidation (anammox) can also produce N_2 gas (Fig. 1 [8]). Incomplete denitrification can produce N_2O which is not available to phytoplankton for growth but is a potent greenhouse gas (Fig. 1. [C]). Alternately, nitrate and/or nitrite can diffuse back to the water column and support phytoplankton growth (Fig. 1 [10]). In addition, nitrate can be converted back to ammonium through dissimilatory nitrate reduction (DNRA; Fig. 1 [9]). While all of these different processes may occur, three primary ways bivalves can remove N from the water column for substantial amounts of time are: 1) assimilation into animal tissue or shell (Songsangjinda et al., 2000; Higgins et al., 2011), 2) long-term burial in the sediments, and 3) conversion of bioavailable N to N_2 gas through the microbially-mediated coupling of nitrification-denitrification (Newell et al., 2002, 2005; Higgins et al., 2011; Piehler and Smyth, 2011; Smyth et al., 2013; Kellogg et al., 2013a).

The amount of N removed from or recycled in a system ultimately will depend on complex interactions between biological, geochemical and physical variables. Assimilation and biodeposition rates, for example, depend heavily upon filtration and ingestion rates that are influenced by temperature, salinity, tidal regime, water residence time, and the abundance of phytoplankton and other particulates in the water column (Newell and Langdon, 1996; Cranford et al., 2011). Whether biodeposits are resuspended or buried will depend on the local hydrodynamic regime. The proportion of N in biodeposits that is returned to the atmosphere as N_2 gas versus remineralized will be influenced by a variety of factors

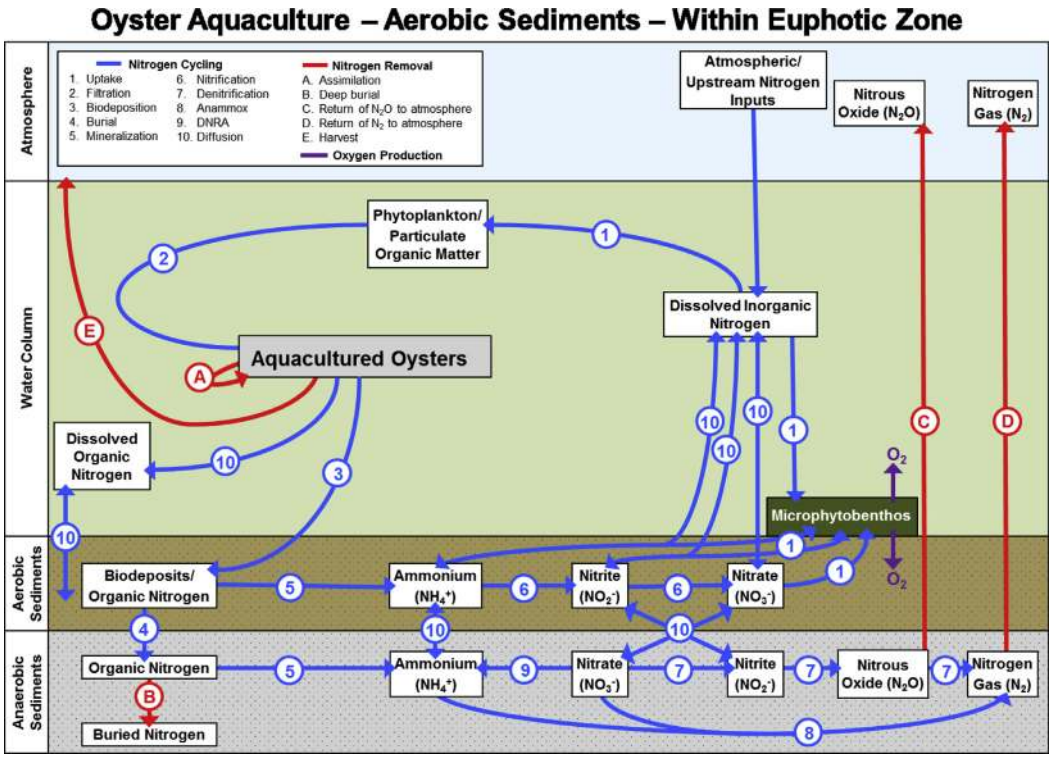

Fig. 2. Primary nitrogen cycling and nitrogen removal pathways for intensive oyster aquaculture occurring over aerobic sediments within the euphotic zone.

including dissolved oxygen concentration and redox zonation in the sediment (Cornwell et al., 1999; Joye and Anderson, 2008 and references therein), sediment geochemistry (Sündback et al., 1991; Joye and Hollibaugh, 1995), water column nutrient concentrations, effects of the benthic macrofaunal community (Pelegri et al., 1994, Nizzoli et al., 2007), microbial community abundance and composition (Fulweiler et al., 2013), and the presence or absence of microphytobenthos and macroalage that can alter both the availability of dissolved inorganic nitrogen and oxygen concentrations (Fig. 1; Thouzeau et al., 2007). Even within the same system, N removal pathways are expected to differ between natural or restored oyster populations growing on the bottom (Fig. 1) and those growing in an above-bottom aquaculture setting (Fig. 2). Nitrogen removal pathways are expected to be further modified by light availability, oxygen concentrations in the sediments, and aerial exposure (Appendix A, Figs. A1–A4). Few studies have attempted to integrate the suite of complex datasets on the mechanisms and mediating factors essential to providing

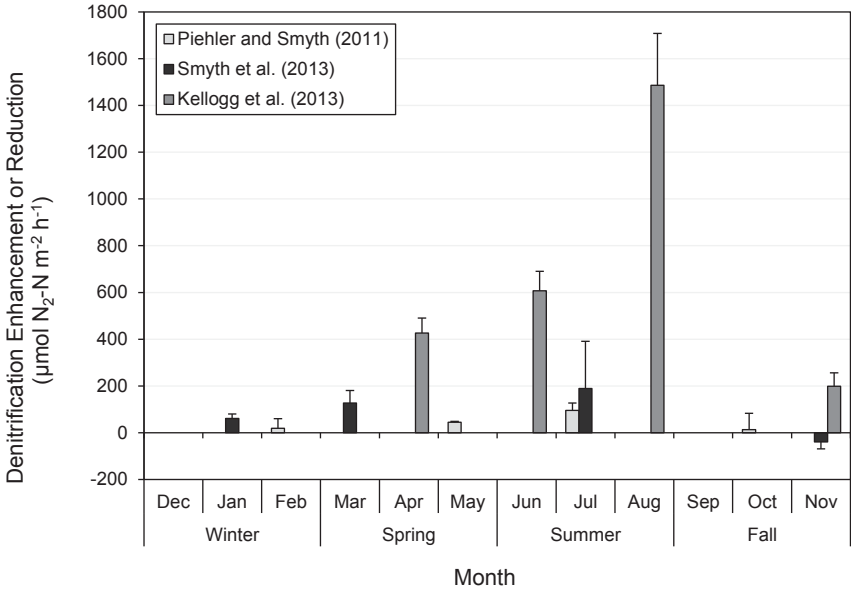

Fig. 3. Seasonal patterns of denitrification enhancement/reduction for natural intertidal oyster reefs in Bogue Sound, NC (Piehler and Smyth, 2011; Smyth et al., 2013) and restored oyster reefs in Choptank River, MD (Kellogg et al., 2013a) relative to reference sites. Error bars represent standard deviation.

researchers and coastal policymakers with clear guidance regarding the potential effectiveness of shellfish-based N removal strategies across sites and growing conditions (Carmichael et al., 2012).

The potential for wild (Cloern, 1982; Officer et al., 1982; Dame et al., 1984) and cultivated (Smaal et al., 2001; Lindahl et al., 2005; Lindahl, 2011) populations of suspension-feeding bivalves to alter water quality through top-down control of phytoplankton, biodeposition of suspended sediments, and alteration of nutrient dynamics has long been recognized. These effects have led several authors to suggest that enhanced populations of suspension-feeding bivalves could mitigate eutrophication in coastal waters (Officer et al., 1982; Newell, 2004; Lindahl et al., 2005; Cerco and Noel, 2007; Bricker et al., 2014; Rose et al., 2014). Others have expressed concern that this approach could have negligible positive effects or even negative effects (Dame et al., 1992; Newell, 2004; Pomeroy et al., 2006; Fulford et al., 2010; Burkholder and Shumway, 2011; Carmichael et al., 2012). Nitrogen budgets developed thus far for oysters, mussels and clams from wild and cultured populations over a range of environments (e.g., Jordan and Valiela, 1982; Dame et al., 1984; Mazouni, 2004; Nizzoli et al., 2006; Burkholder and Shumway, 2011 and references therein) reveal that the portion of N consumed that is returned to the environment (as DIN and biodeposits) varies widely, but generally exceeds the amount incorporated into shellfish biomass. A recent review examined the potential use of bivalves either to remove particles from the water column or remediate N loads to coastal waters and found that at least 30 studies since 1980 have assessed some aspect of the bioremediation potential of at least 16 different species of bivalves from around the world (Carmichael et al., 2012). This review found that, although these studies suggest that bivalves can reduce local particle concentrations by 30–45%, reported removal of N is much lower, ranging from <1% to 15% of total annual N loads, with a maximum of 25% of daily loads.

In the U. S., coastal policymakers, environmental organizations, scientists, and the general public have increasingly embraced the notion that enhancing populations of the eastern oyster, *Crassostrea virginica*, through restoration and/or aquaculture, can reduce effects of eutrophication while also providing other valuable ecosystem services (Newell, 1988; Jackson et al., 2001; Newell, 2004; Coen et al., 2007, 2011; Grabowski et al., 2012; Rose et al., 2014. Bricker et al., 2014 and references therein). Nutrient reduction programs in Chesapeake Bay and elsewhere in the U.S. are employing a Total Maximum Daily Load (TMDL) approach toward setting nutrient reduction targets (US EPA, 2013) and, in conjunction, several states have developed nutrient trading programs (Branosky et al., 2011). In many areas, including Chesapeake Bay, significant reductions in nutrient loads have been achieved (Chesapeake Bay Program, 2014), primarily by reducing point sources from wastewater treatment facilities and implementing relatively inexpensive agricultural best management practices (BMPs). Additional reductions in N loadings to meet mandated requirements will be increasingly expensive, with most of the administrative and financial responsibility for meeting these reductions falling on local governments (US EPA, 2003; World Resources Institute, 2009). Among local and state authorities, this burden has generated considerable interest in alternative, less costly options for meeting water quality goals (e.g., Commonwealth of Virginia, 2012). Public interest in using oysters to improve water quality has increased largely due to restoration and management activities of environmental groups (e.g., The Nature Conservancy, Chesapeake Bay Foundation, North Carolina Coastal Federation) and an increasing number of articles in the public media, some of which raise unrealistic expectations that planting shellfish will “rescue” embayments from environmental catastrophe (e.g., Tuohy, 2011). In combination, these factors have enhanced interest in the water quality benefits of oyster reef restoration and aquaculture.

In the Chesapeake Bay region of the U.S. mid-Atlantic coast, enhancement of oyster (*Crassostrea virginica*) populations through oyster reef restoration and/or oyster aquaculture has been proposed as an alternate BMP to meet TMDL allocations and is currently under consideration by management agencies tasked with approving Watershed Implementation Plans (e.g. Commonwealth of Virginia, (2012)). Inclusion of shellfish aquaculture in nutrient trading markets also has been proposed (Shabman and Stephenson, 2007; Stephenson et al., 2010a; Newell and Mann, 2012; Rose et al., 2014). The need to make decisions on these issues served as the impetus for a workshop supported by the Chesapeake Bay Office of the National Oceanic and Atmospheric Administration (NOAA, Kellogg et al., 2013b). The workshop brought together 30 scientists, policymakers, and restoration practitioners to review the state of knowledge regarding the ability of oysters to improve water quality by removing N (Appendix B). This paper summarizes the meta-analysis of existing data resulting from that workshop. Specifically, this work identifies the extent to which relationships between oysters and N removal in relation to environmental or oyster growing conditions have been sufficiently studied to support application to management strategies, recommends how data can be used in the context of management and policy needs, and highlights key gaps in knowledge that limit robust policy recommendations at this time.

2. Approach

To define the state of knowledge regarding oyster-related water quality improvements, studies that directly measured values of N removal by assimilation into oyster soft tissue and shell, long-term burial of biodeposits in sediments, and transformation to N_2 (hereafter denitrification) were reviewed. Studies conducted only in the laboratory were excluded in favor of ones that focused on field studies that collected samples at oyster reef (natural, restored, or experimental) and aquaculture sites (actual or experimental). In cases where published works did not pass this initial screening procedure, no additional screening was performed.

Assimilated N was defined as the N contained in soft tissue and/or shell of an oyster at the time of sampling and was generally calculated by measuring the % N in tissue or shell and multiplying by the dry weight of that material. Because oysters of the same shell height can differ significantly in shell morphology, soft tissue biomass, and shell mass as a result of a variety of interacting factors (Galtsoff, 1964; Carriker, 1996 and references therein), the review was focused on studies that measured % N in tissue and/or shell. For inclusion in the review, studies had to report sampling location and include sufficient replication to allow calculation of variance for measurements of % N (i.e. $n \geq 3$). The total amount of N in the tissue and shell of an individual oyster and the rate at which it is assimilated is a function of oyster size, the relative proportions of tissue and shell, and growth rates. The total amount of N assimilated by oysters per unit area is a function of oyster abundance per unit area, population structure, and rates of recruitment and mortality. Assimilation at the scale of an estuary is constrained by the amount of bottom suitable for the growth and survival of oysters. Because most of these factors vary widely in space and time, the present review focused only on the % N in tissue and shell, leaving calculations of assimilation per unit area and estimation of assimilation rate to site-specific assessments (e.g., Higgins et al., 2011; Carmichael et al., 2012).

Long-term N burial was defined as retention of particulate N beneath the taphonomically active zone (Powell et al., 2012). The review focused on burial of N held in oyster shell and oyster biodeposits because they were expected to be the primary means by which particulate N was increased on the sediment surface and subject to burial. For inclusion in the review, studies had to directly measure long-term burial at an oyster reef or oyster aquaculture site, as well as at an appropriate reference site without oysters.

For the purposes of the review, denitrification was defined as the net flux of N_2 gas from the benthos to the overlying water column. While recognizing that N_2 can be produced by pathways other than coupled nitrification-denitrification (e.g., annamox), this definition was selected for its direct relevance to management applications. Based on consensus of workshop participants and the best available literature (Groffman et al., 2006 and references therein), the review focused on studies that directly measured denitrification in terms of N fluxes to the overlying water using membrane inlet mass spectrometry (MIMS) and that compared sites with oysters (reefs or aquaculture) to reference sites. Net effect of oysters on denitrification was calculated by subtracting the mean denitrification rate at the reference site from the denitrification rates measured at the oyster growing site. Positive values indicated enhancement of denitrification rates whereas negative values indicated reduction in denitrification rates at the oyster site compared to the control site. Because appropriate conversion of these values to longer time periods or the greater spatial scales depends heavily on site-specific factors such as hours of aerial exposure and proportion of estuarine area suitable for oyster growth and survival, the present review focused only on comparing enhancement or reduction in denitrification in terms of μmol N_2–N m^{-2} h^{-1}, leaving calculation of effects at greater temporal and spatial scales to site-specific assessments (e.g., Piehler and Smyth, 2011; Higgins et al., 2013; Smyth et al., 2013; Kellogg et al., 2013a).

One sample *t*-tests were used to determine if the presence of oysters significantly enhanced or reduced denitrification rates by testing against a hypothesized mean of zero ($\alpha = 0.05$). Data were transformed as needed to meet the assumption of normality. In cases where transformed data failed normality testing but violations of normality were small ($p \geq 0.01$), the one-sample *t*-test was assumed to be robust with respect to violation of this assumption and the test was continued. Factors influencing the effect of oysters on denitrification rate were compared using ANOVA ($\alpha = 0.05$) using data transformed as needed to meet assumptions of normality and/or equal variance. In cases where data were resistant to transformation or transformed data failed normality testing but where violations of normality were small ($p \geq 0.01$), ANOVA was assumed to be robust to these violations and the test was continued. In all other cases, data resistant to transformation were compared using Kruskal–Wallis one-way ANOVA on ranks.

3. Results

No study was found to have collected sufficient data from a single site to estimate the combined effects of assimilation, long-term burial, and denitrification on net N removal associated with *Crassostrea virginica*. Complementary data on assimilation and denitrification for the same location existed for one restored reef site and two aquaculture sites in Chesapeake Bay in the mid-Atlantic region (Higgins et al., 2011, 2013; Kellogg et al., 2013a). All other studies focused on a single mechanism of N removal.

The greatest number of measurements have been published for assimilation of N into oyster soft tissue (10 sites, Table 1). The majority of work has been conducted in the New England region at five sites in Cape Cod, Massachusetts. Three locations, all within Chesapeake Bay, have been studied in the mid-Atlantic and two locations, both within Mobile Bay, have been studied on the Gulf

Table 1
Summary of studies included in review including locations, growth setting and environmental conditions. DNF: denitrification, PAR: photosynthetically active radiation.

Study	Region	Growth setting	Site	Salinity	Assimilation		DNF	Other environmental information
					Tissue	Shell		
Intensive aquaculture								
Carmichael et al. (2012)	New England	Aquaculture cages 6 cm above bottom	Sage Lot Pond, Cape Cod, MA	28	x			N load: 14×10^{-4} kg N m^{-2} y^{-1}
			Wild Harbor, Cape Cod, MA	26	x			N load: 65×10^{-4} kg N m^{-2} y^{-1}
			Green Pond, Cape Cod, MA	28	x			N load: 178×10^{-4} kg N m^{-2} y^{-1}
			Snug Harbor, Cape Cod, MA	25	x			N load: 236×10^{-4} kg N m^{-2} y^{-1}
			Childs River, Cape Cod, MA	26–27	x			N load: 601×10^{-4} kg N m^{-2} y^{-1}
Holyoke (2008)	Mid-Atlantic	Floating aquaculture cages	Lowry Cove, LaTrappe Creek, MD	13.25 ± 0.96			x	PAR: ~70–80 μmol m^{-2} s^{-1}
			Mainstem, LaTrappe Creek, MD	6.75 ± 1.50			x	PAR: ~70–80 μmol m^{-2} s^{-1}
			Pier, LaTrappe Creek, MD	5.50 ± 1.29			x	PAR: 5–25 μmol m^{-2} s^{-1}
Higgins et al. (2011, 2013)	Mid-Atlantic	Floating aquaculture cages	Spencer's Creek, VA	5–15	x	x	x	Low flow, high sedimentation
			St. Jerome Creek, MD	12–15	x	x	x	High flow, low sedimentation
Dalrymple (2013 and unpub. data)	Gulf of Mexico	Aquaculture cages ~10–20 cm above bottom	Mobile Bay, AL (2 sites)		x	x		
Oyster reefs								
Kellogg et al. (2013a and unpub. data)	Mid-Atlantic	Restored oyster reef	Choptank River, MD	7.0–11.6	x	x	x	
Sisson et al. (2011)	Mid-Atlantic	Extensive oyster aquaculture site	Humes Marsh, Lynnhaven River, VA	29.4			x	
Piehler and Smyth (2011)	Mid-Atlantic	Natural oyster reef	Bogue Sound, NC	27–36			x	
Smyth et al. (2013)	Mid-Atlantic	Natural oyster reef	Bogue Sound, NC	29–32			x	

Table 2
Summary of nutrient assimilation data meeting requirements for inclusion in review (see text for detailed requirements).

Source	Shell height (mm)	Oyster density (ind. m^{-2})	Site	Growing location	*N*	Tissue % N g^{-1} DW ± SE (range)	Shell % N g^{-1} DW ± SE (range)
Carmichael et al. (2012)	8–68	447	Sage Lot Pond, Cape Cod, MA	6 cm above bottom	160	8.47 ± 0.09	
			Wild Harbor, Cape Cod, MA	6 cm above bottom	160	8.95 ± 0.16	
			Green Pond, Cape Cod, MA	6 cm above bottom	160	8.04 ± 0.24	
			Snug Harbor, Cape Cod, MA	6 cm above bottom	160	9.19 ± 0.15	
			Childs River, Cape Cod, MA	6 cm above bottom	160	8.37 ± 0.27	
Kellogg et al. (2013a and unpub. data)	114 ± 1.5	131	Choptank River, MD	On bottom	15	9.27 ± 0.35 (8.58–9.71)	
				On bottom	16		Live: 0.21 ± 0.05 (0.16–0.30)
				On bottom	15		Aged: 0.15 ± 0.01 (0.13–0.17)
Higgins et al. (2011)	44–118	400	Spencer's Creek, VA	Upper water column	47	8.10 ± 0.13 (5.80–9.97)	0.20 ± 0.01 (0.11–0.39)
			St. Jerome Creek, MD	Upper water column	37	7.37 ± 0.19 (5.43–10.36)	0.20 ± 0.02 (0.11–0.48)
Dalrymple (2013 and unpub. data)	42–98	309		~10–20 cm above bottom	108	11.8 ± 0.1 (9.10–13.54)	
					12		Juvenile: 0.46 ± 0.01
					12		Adult: 0.26 ± 0.01

Coast. No data have been published for intertidal reefs and no studies evaluated seasonal patterns in N concentration.

To date, there have been no published studies of long-term N burial for samples collected from oyster reefs or oyster aquaculture. Denitrification rates associated with oyster aquaculture have been measured as part of two studies (Holyoke, 2008; Higgins et al., 2013) encompassing a total of five sites, all in the mid-Atlantic within Chesapeake Bay (Table 1). Denitrification rates associated with oyster reefs have been measured as part of four studies (Piehler and Smyth, 2011; Sisson et al., 2011; Smyth et al., 2013; Kellogg et al., 2013a). Two studies focus on the same reef complex in Bogue Sound, North Carolina (Piehler and Smyth, 2011; Smyth et al., 2013). Two additional studies focus on locations in Chesapeake Bay: one subtidal site in Maryland (Kellogg et al., 2013) and one intertidal site in Virginia (Sisson et al., 2011).

3.1. Assimilation

Mean values for N assimilated in oyster soft tissue at individual sites ranged from 7.4 to 11.8% of soft tissue biomass, with the highest values measured at Mobile Bay, Alabama (Table 2). Averaging across the eight sites on the U. S. Atlantic coast yielded a mean value of 8.5 ± 0.6% N g^{-1} dry weight (DW) of soft tissue biomass for the region (Table 2), lower than values measured thus far from the Gulf Coast. Two studies have examined the % N in oyster tissue for different size classes of oysters (Higgins et al., 2011; Dalrymple, 2013). Neither found significant differences but data from Higgins et al. (2011) show a tendency for the % N in tissue to decrease with increasing oyster size.

Mean values for % N assimilated in the shells of living adult oysters collected at four sites ranged from 0.20% to 0.26% N g^{-1} DW with the highest value again coming from the Gulf Coast (Table 2). Among the three sites in Chesapeake Bay, there was little variation in oyster shell N content despite differences in growing conditions. Greater variation was observed between size classes within site, but patterns differed among sites. Higgins et al. (2011) observed similar % N (0.17–0.19) across three size classes but somewhat higher values (0.26% N g^{-1} DW) in their largest size class. Dalrymple (2013), working in the Gulf of Mexico, found comparable % N in adult oyster shell (0.26% N g^{-1} DW) but values were higher in juveniles (0.46% N g^{-1} DW). Lower N content in aged shell from these same regions (0.15% N g^{-1} DW, shell age >7 years, Kellogg, Chesapeake Bay unpublished data; 0.05% N g^{-1} DW, shell age 820–2500 BP, Darrow and Carmichael Gulf of Mexico, unpublished data) suggested that the N content of shell declines through time.

3.2. Denitrification

Six studies encompassing nine sites used the MIMS approach to compare net denitrification rates at a site with oysters to a reference site without oysters, allowing calculation of the net effect of oysters on denitrification rates (Table 1). Methods used to measure exchanges across the sediment–water interface varied in area of substratum from 32 cm^2 (Holyoke, 2008; Piehler and Smyth, 2011; Higgins et al., 2013; Smyth et al., 2013) to 1000 cm^2 (Sisson et al., 2011; Kellogg et al., 2013a) and in materials collected for incubation; samples included sediments and associated infauna (Holyoke, 2008; Piehler and Smyth, 2011; Higgins et al., 2013; Smyth et al., 2013), or intact sections of oyster reef with oysters, sediments, infauna and reef-associated macrofauna (Sisson et al., 2011; Kellogg et al., 2013a). Incubation techniques included sealed chamber (a.k.a. "batch style"; Holyoke, 2008; Sisson et al., 2011; Kellogg et al., 2013) and continuous flow incubations (Piehler and Smyth, 2011; Higgins et al., 2013; Smyth et al., 2013) and were performed under both dark conditions only (Piehler and Smyth, 2011; Higgins et al., 2013; Smyth et al., 2013; Kellogg et al., 2013a) and under both dark and light conditions (Holyoke, 2008; Sisson et al., 2011).

3.2.1. Aquaculture

Three of the reviewed studies measured denitrification rates associated with oyster aquaculture, two focused on intensive aquaculture (Holyoke, 2008; Higgins et al., 2013) and one on extensive aquaculture (Sisson et al., 2011). The extensive aquaculture site relied on natural recruitment to shell substrate added on the bottom with no subsequent maintenance, such that the resulting structure was representative of common oyster reef restoration techniques used in the area. For this reason, we considered these data along with oyster reef data from other studies. In contrast, oysters at the intensive aquaculture sites were produced in a hatchery and subject to frequent maintenance. Both studies (Holyoke, 2008; Higgins et al., 2013) were conducted in Chesapeake Bay between early summer and fall at sites spanning a similar range in salinity and both compared N fluxes from sediments underneath aquaculture floats to adjacent reference sites without oyster cultivation. Out of the 14 sets of incubations, the value of the mean change in denitrification rate was negative for eight of the incubations and positive for six, but only one of these values (Higgins et al., 2013; August) differed significantly from zero (Table 3). In Higgins et al. (2013), there were significant differences in the effect of oysters on denitrification rates among months ($p = 0.023$) but the same was not true for Holyoke (2008),

Table 3
Summary of aquaculture denitrification data that met requirements for inclusion in review (see text for detailed requirements). Effect on denitrification rates was calculated as the rate at the aquaculture site minus the rate at the control site, with positive values indicating enhancement and negative ones indicating reduction in net denitrification rate. *P*-values give the results of single sample *t*-tests against a mean of zero. Values indicative of significant enhancement or reduction are indicated by an asterisk. "Pooled" analyses included data from both light and dark incubations.

Data source	Month	Incubation type	*N*	Denitrification enhancement or reduction (μmol N_2-N m^{-2} h^{-1}; mean ± SD)	*p*-value
Holyoke (2008)	May	Dark	1	−32.6	–
		Light	1	−2.7	–
		Pooled	2	−17.6 ± 21.1	0.447
	June	Dark	1	−70.3	–
		Light	1	6.0	–
		Pooled	2	−32.2 ± 54.0	0.554
	July	Dark	4	24.3 ± 218.8	0.839
		Light	4	−37.7 ± 56.8	0.277
		Pooled	8	−6.7 ± 151.6	0.580
	Aug	Dark	3	8.6 ± 82.4	0.873
		Light	3	1.3 ± 29.8	0.947
		Pooled	6	5.0 ± 55.6	0.836
	Sept	Dark	3	−32.6 ± 61.1	0.453
		Light	3	45.2 ± 64.7	0.350
		Pooled	6	6.3 ± 70.6	0.835
	Pooled	Dark	12	−6.45 ± 126.5	0.518
		Light	12	−0.7 ± 53.7	0.967
		Pooled	24	−3.6 ± 95.1	0.473
Higgins et al. (2013)	May	Dark	3	−58.9 ± 99.0	0.411
	Aug	Dark	6	82.8 ± 52.0	0.011*

Table 4
Summary of oyster reef denitrification data that met requirements for inclusion in review (see text for detailed requirements). Effect on denitrification rates was calculated as the rate at the aquaculture site minus the rate at the control site, with positive values indicating enhancement and negative ones indicating reduction in net denitrification rate. *P*-values give the results of single sample *t*-tests against a mean of zero. Values indicative of significant enhancement or reduction are indicated by an asterisk. For incubation type, "pooled" analyses included data from both light and dark incubations. For data source, "pooled" data indicate that analyses were conducted on data pooled across studies within season.

Data source	Sample type	Month	Incubation type	*N*	Denitrification enhancement or reduction (μmol N_2–N m^{-2} h^{-1}; mean ± SD)	*p*-value
Kellogg et al. (2013a)	Reef	Apr	Dark	4	426.5 ± 63.9	<0.001*
		Jun	Dark	4	607.7 ± 82.4	<0.001*
		Aug	Dark	3	1486.4 ± 222.0	0.007*
		Nov	Dark	4	199.2 ± 56.7	0.006*
Sisson et al. (2011)	Reef	Oct	Dark	4	83.3 ± 99.8	0.194
			Light	4	165.0 ± 78.5	0.025*
			Pooled	8	124.1 ± 93.9	0.007*
Piehler and Smyth (2011)	Sediments	Feb	Dark	3	18.8 ± 41.2	0.511
		May	Dark	3	44.9 ± 3.9	0.003*
		July	Dark	3	95.8 ± 30.9	0.033*
		Oct	Dark	3	13.5 ± 69.1	0.767
Smyth et al. (2013)	Sediments	Jan	Dark	3	61.0 ± 18.8	0.030*
		Mar	Dark	3	126.9 ± 54.0	0.055
		Jul	Dark	3	188.9 ± 202.6	0.248
		Nov	Dark	3	−39.4 ± 30.0	0.151
Pooled - Piehler and Smyth (2011) and Smyth et al. (2013)	Sediments	Jan/Feb	Dark	6	39.9 ± 36.8	0.045*
		Mar/May	Dark	6	85.9 ± 56.5	0.004*
		JulS	Dark	6	142.3 ± 139.3	0.007*
		Oct/Nov	Dark	6	−12.9 ± 55.8	0.594

regardless of whether dark incubations ($p = 0.854$), light incubations ($p = 0.194$), or both types of incubations were included in analyses ($p = 0.596$). There were no significant differences between these two studies in the effect of oysters on denitrification rates, regardless of whether all data from both studies were compared ($p = 0.370$) or whether only data from the most similar set of incubations (August only, dark incubations only) were compared ($p = 0.135$).

Holyoke (2008) was the only aquaculture study included in the review that considered the effect of light on denitrification rates and ran both dark and light incubations. The presence or absence of light did not significantly alter the effect of oysters on denitrification rates ($p = 0.636$). None of the aquaculture studies included in this review collected data across a broad enough range of seasons to allow estimation of annual effects.

3.2.2. Oyster reefs

Four studies encompassing a wide range of salinities (7–36) measured denitrification rates associated with oyster reefs (Piehler and Smyth, 2011; Sisson et al., 2011; Smyth et al., 2013; Kellogg et al., 2013a). Three studies examined intertidal reefs (Piehler and Smyth, 2011; Sisson et al., 2011; Smyth et al., 2013) and one focused on a subtidal reef below the euphotic zone (Kellogg et al., 2013a). Study sites included a natural intertidal reef, a restored reef and an extensive aquaculture site (described above).

In general, oyster reefs enhanced denitrification rates but the effect of oyster reefs on denitrification varied widely both within and among studies. Of the 14 incubations included in analyses, mean values for 13 were positive and eight of these were significantly greater than zero, indicating significant enhancement in denitrification rates (Table 4). Across all studies, seasons, and oyster biomass levels, the net effect on denitrification varied by up to three orders of magnitude and sometimes included both positive and negative values.

Three studies estimated the effect of oyster reefs on denitrification rates at four time points during a single year (Table 4, Piehler and Smyth, 2011; Kellogg et al., 2013a; Smyth et al., 2013). Sampling month had a significant effect on the enhancement of denitrification rates in Kellogg et al. (2013a; $p < 0.001$) and *post-hoc* testing indicated that enhancement differed significantly among all sampling months and was always significantly greater than zero. Smyth et al. (2013) data also showed a significant effect of sampling month ($p = 0.009$) with a significant difference in the effect of oyster reefs on denitrification rates in November versus all other sampling months. However, the mean enhancement in denitrification rates was significantly greater than zero only during the January sampling period (Table 4). Sampling month had no effect on enhancement/reduction in denitrification rates in Piehler and Smyth (2011; $p = 0.157$) but mean enhancement was significantly greater than zero in both May and July (Table 4).

Because Piehler and Smyth (2011) and Smyth et al. (2013) studied the same intertidal oyster reef complex in North Carolina on the Atlantic coast of the United States and used identical methods, data were pooled to increase the power of the analyses. Pooled data indicated significant enhancement in denitrification in winter, spring and summer (Table 4). A two-way ANOVA with data source and season as factors found no interaction between factors ($p = 0.213$), no effect of data source ($p = 0.498$), and a significant effect of season ($p = 0.002$). *Post-hoc* testing indicated that the effect of oyster reefs on denitrification rates was significantly lower in fall than in winter ($p = 0.023$), spring ($p = 0.006$), or summer ($p = 0.002$). Comparison among the three studies that collected seasonal data indicated that values from Kellogg et al. (2013a) are significantly higher than those from Piehler and Smyth (2011) and

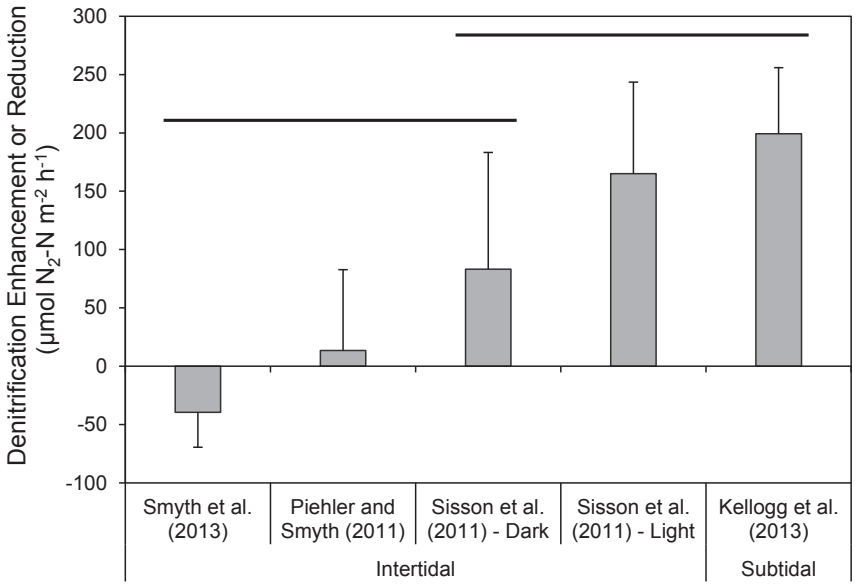

Fig. 4. Comparison of enhancement/reduction in fall denitrification rates for oyster reefs. Bars indicate means that are not significantly different from one another.

Smyth et al. (2013), which do not differ significantly from one another.

In contrast to the three seasonal studies, Sisson et al. (2011) collected data in a single season but was the only oyster reef study included in the review that considered the effect of light on denitrification rates and utilized both dark and light incubations. Sisson et al. (2011) sampled an intertidal oyster reef in October and found that presence or absence of light did not significantly alter the effect of oyster reefs on denitrification rates ($p = 0.246$). Data from incubations conducted in the light indicated significant enhancement in denitrification whereas data from dark incubations were not different from zero (Table 4). Pooled data from both dark and light incubations indicated significant enhancement in denitrification.

Because all four studies (Piehler and Smyth, 2011; Sisson et al., 2011; Smyth et al., 2013; Kellogg et al., 2013a) collected data in the fall (October or November), these data were used to determine whether there were significant differences among studies within sampling season. One-way ANOVA indicated that values from both Kellogg et al. (2013a) study and the light incubations from Sisson et al. (2011) were significantly greater than those from Piehler and Smyth (2011) and Smyth et al. (2013, Fig. 4). Values for Sisson et al. (2011) dark incubations were not significantly different from any other incubations.

3.2.3. *Oyster reefs versus oyster aquaculture*

Only one of the 14 sets of incubations from aquaculture sites included in the review showed a significant enhancement in denitrification associated with oyster aquaculture compared to eight of the 14 sets from oyster reefs (Tables 3 and 4). Although a significantly greater proportion of oyster reef incubations demonstrated enhancement in denitrification rates ($p = 0.015$), samples from aquaculture sites were concentrated in the summer months whereas those from reef sites were more evenly distributed throughout the year. To gain a better understanding of the role of oysters in aquaculture versus oyster reefs, a one-way ANOVA was used to compare incubation results across studies within late summer (July and August), the sampling period with the greatest number of incubations. To enhance comparability of results among studies, data were further restricted to include only dark incubations. Results indicated that enhancement in Kellogg et al. (2013a) was significantly greater than all other studies (Fig. 5). There were no significant differences between the other two oyster reef studies (Piehler and Smyth, 2011; Smyth et al., 2013) and the aquaculture studies (Holyoke, 2008; Higgins et al., 2013). Data were compared again after removing Kellogg et al. (2013a) from analyses and pooling data within oyster growth setting (i.e., aquaculture or oyster reef). Although mean enhancement values were greater for oyster reefs, they were not significantly different from those for aquaculture ($p = 0.135$).

4. Discussion

The current state of knowledge about the effects of oysters (on reefs or in aquaculture) on N dynamics is incomplete in many respects. No studies of oyster-based N removal have incorporated field data on all three primary mechanisms of N removal: assimilation, long-term burial, and denitrification. Until robust models of N removal based on comprehensive data collected from a broad range of oyster growth settings and environmental scenarios have been developed, accurate estimates of net annual N removal associated with oyster reefs or oyster aquaculture will require direct sampling of all three primary removal mechanisms at the location of interest.

4.1. *Assimilation*

Assimilation of N into soft tissues and shell was the most easily quantified mechanism of potential N removal and the one for which most data are available. Percent N in oyster tissue and shell for adult oysters fell within relatively narrow ranges, not an unexpected result given physiological constraints on oyster growth and survival. Limited variation in %N content of adult oyster tissue and shell for samples from the mid-Atlantic and New England regions suggests that average values (soft tissue: 8.5% N g^{-1} DW, shell: 0.2% N g^{-1} DW) can reasonably be used for adult oysters from these regions in lieu of site-specific measurements. However, there is reason to believe that these values may not encompass the entire range of appropriate values. Both tissue samples and juvenile oyster shell samples from the Gulf Coast have higher average N content

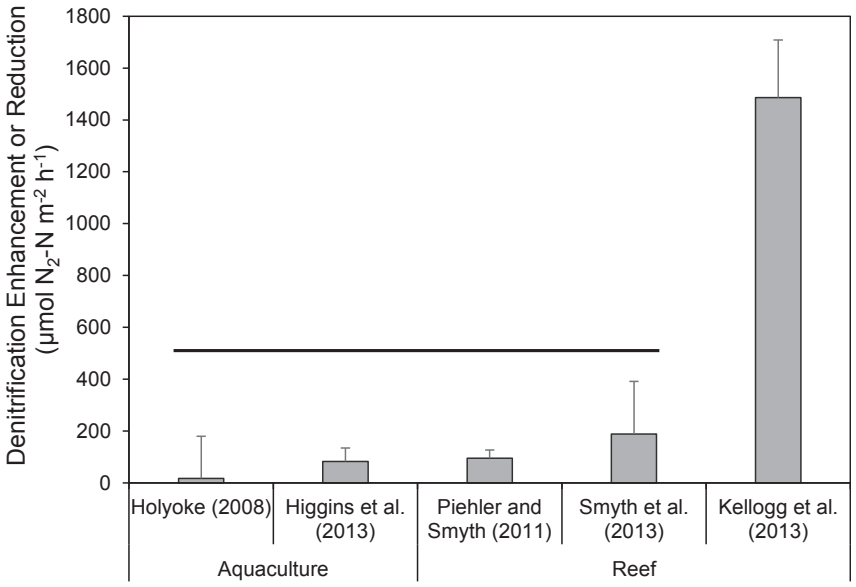

Fig. 5. Comparison of enhancement/reduction late summer (July/August) denitrification across all oyster reef and aquaculture studies that collected data during this time period.

than those from the Mid-Atlantic, suggesting that greater variance may be observed if the geographic range of studies was expanded. Studies of the amount of N in the shell of oysters of different size classes have yielded equivocal trends thus far and, at present, the factors driving these differences are unclear. Controlled studies are needed to assess oyster nitrogen content in relation to a much broader range of growing conditions (e.g., intertidal versus subtidal reefs, salinity, food quality), genetic history (e.g., wild oysters versus selectively-bred strains), ploidy, oyster health, and physiological state.

Due to observed variations in the % N in oyster tissue and shell, reliable estimates of the amount of N sequestered in oysters will require site-specific measurements of soft tissue and shell dry weights, or the development of robust models informed by site-specific data. The naturally occurring plasticity of oyster shell morphology and its influence on length to soft tissue biomass relationships has been documented in relation to a variety of environmental parameters including bottom substrate, turbidity, salinity, food availability, pollution, and calcium concentrations (Galtsoff, 1964; Carriker, 1996 and citations therein). Oysters grown in aquaculture conditions (either suspended in the water column or elevated above the bottom) frequently exhibit thinner shells and higher tissue dry weight to total weight ratios than oysters growing on the bottom, an observation supported by comparing the percentage of total dry weight held in tissue between the aquacultured oysters studied by Higgins et al. (2011; 3.3–4.2%) to those from restored reefs studied by Kellogg et al. (2013a; 1.7%).

The proportion of N assimilated into oyster tissue and shell that can be considered removed for the purposes of water quality improvement depends upon the fate of the oysters and the timescale of interest. Determining the amount of N removed via the harvest of cultured oysters is straightforward. For all practical purposes, once harvested and consumed by humans, the N in oyster tissue can be considered permanently removed from the system. Although the potential exists for some small portion of the nitrogen held in an oyster to be returned to the water body of interest after passing through a waste treatment facility, the net effect of humans consuming protein from a food source that requires neither supplemental food nor fertilizer likely has net positive effects on water quality. The majority of nitrogen contained in oyster shell is removed by harvest. Although maximum short-term removal would be achieved by retaining oyster shell on land, net long-term removal should be enhanced by returning shells to areas where they can serve as settlement substrate for future generations of oysters that would in turn assimilate nitrogen in their shells and tissues. Although some nitrogen from returned shells will be released into the water column via bioerosion and diagenesis, the limited data collected on the N content of aged oyster shell suggest that shells retain much of their nitrogen for extended periods of time. Additional studies are needed to clarify the net effect of returning shell to the water body and the factors controlling its persistence or degradation.

For restored oyster reefs, assimilation of N into the tissues of oysters, other suspension feeders and higher trophic levels represents a more or less ephemeral pool of N within the system depending upon the material in which that nitrogen is sequestered. Harvesting oysters from reefs would remove assimilated nitrogen from the estuary, but, as seen in the case of oysters in the Chesapeake Bay, overharvesting leads to population decline, loss of ecosystem services, and system degradation (Beck et al., 2011; Wilberg et al., 2011). The occurrence of shell from oysters and other reef associated organisms in the fossil record provides evidence that, at least under some conditions, oyster shell can persist for long periods of time (Waldbusser et al., 2011). While we are not aware of any studies detailing N taphonomy in *Crassostrea* shells, the presence of amino acids in subfossil oyster shell confirms the presence of N in buried shells for centuries after oyster death (Surge et al., 2003). Although additional data are clearly needed, preliminary data suggest that oyster shell N does decline over time but that the rate of that decline is relatively slow (Kellogg et al. unpublished data, Chesapeake Bay; Darrow and Carmichael, unpublished data, Gulf of Mexico).

Although the time span of sequestration of N in the tissues of individual oyster reef organisms is relatively short compared to shell, when the entire community is considered as a standing stock of nitrogen, the amount of nitrogen is not trivial. By definition, a healthy oyster reef has significant numbers of live oysters and will provide habitat for other organisms, all of which contain nitrogen in their tissues (Kellogg et al., 2013a). As a reef grows, the standing stock of N held in the tissue of oysters and reef-associated

organisms will increase. Additional research is needed to fully quantify the scale, time span, and trajectories of N retention in relation to reef type (e.g., intertidal versus subtidal), reef age, environmental setting, and other factors that control macrofaunal communities on oyster reefs.

4.2. Long-term burial

At present, no data exist for long-term N burial associated with oyster reefs or oyster aquaculture. A study utilizing two oyster aquaculture sites differing in hydrodynamic regime assessed initial retention of particulate nitrogen from biodeposits in the sediments and found significant differences between sites despite similar scale and method of aquaculture, suggesting that long-term burial rates are likely site-specific (Stephenson et al., 2010b). Long-term burial rates also are likely to depend heavily on aquaculture practices (e.g., stocking density, growth setting, maintenance protocols, and harvest techniques) and how those practices influence deposition rates, sediment biogeochemistry, and sediment resuspension. Studies assessing the effects of these and other factors influencing long-term burial rates associated with oyster aquaculture are needed.

Although long-term N burial rates also are unavailable for oyster reefs, both the growth pattern of oyster reefs over ecological time scales and the persistence of fossil oyster shell over geologic times scales demonstrate the capacity for unharvested oyster reefs to bury nitrogen associated with shells. Similar to oyster aquaculture, long-term burial of a portion of the nitrogen contained in oyster biodeposits is a potential means of nitrogen removal. In contrast to aquaculture of oysters in floats, healthy oyster reefs likely provide sufficient physical structure to reduce the amount of buried particulate nitrogen that becomes resuspended. Studies of long-term burial of both oyster shell and biodeposits are needed to assess rates and the physical, biological and environmental conditions that control those rates.

4.3. Denitrification

Studies of denitrification associated with oyster reefs and oyster aquaculture raise a variety of questions about the factors controlling these rates and thus far suggest that oyster reefs generally enhance denitrification rates whereas oysters growing in intensive aquaculture settings generally do not. However, data for oyster aquaculture are limited both in season (all studies have been conducted between May and September) and in the types of aquaculture examined to date (all data are from sediments underlying aquaculture floats). Additional measurements from material inside aquaculture floats and from other types of oyster aquaculture (e.g., bottom cage and on-bottom spat-on-shell culture techniques) are needed. Forms of aquaculture that more closely resemble natural reefs (e.g., on-bottom spat on shell) and that are maintained and harvested with minimal disturbance to sediments could result in denitrification rates more similar to those observed for oyster reefs, an assertion that is supported by the limited data collected from one extensive aquaculture site (Sisson et al., 2011).

Research is needed to clarify the effects of biotic and abiotic factors in determining denitrification rates for oyster reefs. Although reefs in the euphotic zone are expected to have lower denitrification rates during daylight hours due to the presence of benthic algae that both compete for some forms of N and produce oxygen that can inhibit denitrification, preliminary results have suggested that interactions between light penetration depth and denitrification rates are not straightforward (Sisson et al., 2011). Other factors likely to influence denitrification rates on oyster reefs include: tidal regime, temperature, phytoplankton concentrations, sediment characteristics, water residence time, salinity, and microbial community structure.

Because the net N_2 flux to the water column is the most direct method available for estimating effects on water quality, this review was restricted to studies that collected samples from the field, incubated samples in the laboratory, and measured fluxes using MIMS. The focus of this review on N_2 production did not allow assessment of the relative importance of different N cycling pathways. Other techniques (e.g., stable isotope analyses) or a combination of techniques are more suited to enhancing our understanding of how oysters alter individual nitrogen cycling pathways (Groffman et al., 2006; Higgins et al., 2013). Even within the constraints of this review, there was considerable variation in methods across studies. Until controlled experiments are conducted, the influence of these methodological differences on measured rates will remain unclear.

In addition to variation in methods, the scale and scope of data collected thus far are worth noting. To date, studies have employed approaches that determine areal rates of denitrification. While this net flux approach provides needed data for managers to assess water quality improvements attributable to oysters at specific locations, a key step in the development of predictive models will be identification of the factors controlling microbial nitrification and denitrification within oyster reefs and aquaculture settings. A more thorough understanding of processes leading to N losses via N_2–N efflux should better inform scientific and management models that could in turn guide oyster reef restoration strategies and assist in developing BMPs for aquaculture to achieve maximum water quality benefits.

4.4. Oyster reefs versus aquaculture

For the two aquaculture sites, available data suggest that floating oyster aquaculture at these sites likely resulted in net enhancement of N removal. Data collected thus far have indicated that rates of N assimilation into oysters destined for harvest were likely sufficient to result in net nitrogen removal even though aquaculture rarely enhanced denitrification rates. However, sufficient seasonal data have not been collected to estimate annual denitrification rates, so this conclusion is tentative at best. In the absence of data on long-term burial rates, it is not possible to quantify net N removal or even state with confidence that the net effect is positive. Thus, based on the two sites for which data are currently available, net N removal is likely, but it is unclear whether this result is broadly applicable to other aquaculture sites. Effects of oyster aquaculture on net N removal likely depend heavily on aquaculture practices including site selection, type of gear used (e.g., floating cages, cages near the bottom, or on-bottom aquaculture), maintenance practices, and harvest practices.

Data from the one study of a restored subtidal reef in Chesapeake Bay that measured both denitrification and assimilation (standing stock) of N indicated that both were enhanced in all seasons when compared to a nearby reference site (Kellogg et al., 2013a). In this case, annual assimilation rates cannot be calculated because oysters were three to seven years old at the time of sampling, but the assimilation rate was positive over that period of time as evidenced by the existence of a thriving oyster reef at a site that had previously had extremely low oyster density ($<<1$ individual m^{-2}). Combined with the expectation that at least some oyster shell will be buried long-term, successfully restored oyster reefs protected from harvest should result in net N removal. However, placing estimates on net annual N removal across the range of environmental conditions, reef growth forms and oyster biomass densities will require additional data.

4.5. Scaling up

Determining the potential for oysters to remove N from an estuary requires more than accurate estimates of assimilation, burial and denitrification. As a first step, these values must be scaled up to appropriate temporal and spatial scales. For oysters grown in intensive aquaculture, the appropriate timescale is the amount of nitrogen assimilated in a growth cycle (i.e. from the time juveniles are place in the field until they are harvested). Based on their sites in Chesapeake Bay, Higgins et al. (2011) estimate that one million aquacultured oysters with 76 mm shell height would contain 132 kg N in their tissues and shells. Extrapolating from data collected over a 112-day deployment during which oysters grew to ~61% of harvest size, Carmichael et al. (2012) predict that the tissue of one million Cape Cod oysters of the same size would contain between 200 and 400 kg N, depending upon the estuary in which they were reared. The differences between estuaries within Carmichael et al. (2012) and between that study and Higgins et al. (2011) emphasize the need for site-specific data. In most cases, the appropriate timescale for oyster reefs is the annual increase (or decrease) in standing stock of N per unit area of reef, with care taken to ensure that data are collected in the same season each year. To date, no studies have reported this value.

Because the effects of oysters on denitrification vary widely with season, annual estimates will rely heavily upon accurate seasonal data and appropriate extrapolation of these values. To date, no studies of oyster aquaculture have collected sufficient data to extrapolate annual rates. Three studies of oyster reefs (Piehler and Smyth, 2011; Smyth et al., 2013; Kellogg et al., 2013a) measured seasonal denitrification rates and also calculated annual denitrification rates for both an oyster reef site and a site without oysters (Table 5). All calculated annual rates were positive suggesting net enhancement of denitrification. Estimated annual enhancement was an order of magnitude higher for the subtidal restored reef in Maryland (Kellogg et al., 2013a) than for the natural intertidal reef in North Carolina (Piehler and Smyth, 2011; Smyth et al., 2013), again highlighting the need for site-specific data.

Accurately increasing spatial scale to that of an estuary requires determining the proportion of that estuary that could potentially support oyster aquaculture or oyster reef restoration, a task that can be quite difficult. To estimate the amount of substratum suitable for oyster reef restoration in the Choptank River, MD, Kellogg et al. (2013a) first reduced previous estimates of the amount of suitable bottom by 39% based on the results of side-scan sonar surveys, then reduced it by another 56% based upon subsequent diver surveys. These calculations were only made possible by the existence of three overlapping datasets for the Choptank River, something that will not be available for most estuaries. Even after the amount of substratum suitable for aquaculture or restoration is determined, it is necessary to consider the amount of substratum that one might reasonably expect to be committed to this activity over other competing activities, as task that ventures into the realm of public policy.

The potential effects of oysters in an estuary must be considered in the context of N loads and the likely fate of N contained in phytoplankton if it is not consumed by oysters. Estimates for the proportion of the nitrogen load to an estuary that could potentially be removed by oysters varied widely between the studies reviewed. Higgins et al. (2011) estimated that an order of magnitude increase in oyster aquaculture production in Chesapeake Bay would remove less than 0.1% of the N load. For Cape Cod, Carmichael et al. (2012) estimated that oyster aquaculture or oyster reef restoration could reasonably be expected to remove no more than 15% of the N load to an estuary. For restored oyster reefs in the Choptank River, Kellogg et al. (2013a) estimated that restoring all suitable bottom with dense populations of adult oyster could result in enhanced denitrification that would offset ~48% of the external N inputs and that restoring only 23% would offset an amount equivalent to recently mandated nitrogen reductions for that system. Kellogg et al. (2013a) also note the importance of considering the likely fate of N contained in phytoplankton not consumed by oysters in this system. Highest rates of denitrification were recorded for the restored oyster reef in late summer, a time when denitrification rates in deeper waters often decline dramatically as a result of reduced oxygen concentrations and a much greater portion of particulate organic nitrogen is expected to be returned to the system as ammonium (Newell et al., 2005).

Assessing whether oysters are a viable means of mitigating eutrophication in a particular estuarine environment will rely heavily on site-specific considerations, some of which lie outside the realm of scientific research (e.g., costs and viability of alternate options). Additional research can fill the gaps in knowledge about factors controlling net N removal per unit area and ultimately should allow for reasonably accurate estimates of nitrogen removal associated with oyster aquaculture and oyster reef restoration. However, these estimates will rely heavily on the design of the aquaculture or restoration project, its environmental setting, the goal of the project, and the viability and expense of other options for preventing entry or removing nitrogen from a particular estuary.

Table 5
Estimated annual enhancement of denitrification rates at oyster reefs compared to reference sites, from studies reported in Table 1. DNF = denitrification. Values in parentheses are the annual enhancement rate converted to an hourly rate to aid in comparison to other reported values.

Source	Number of sampling periods	Annual denitrification enhancement gN m^{-2} y^{-1} (μmol N_2–N m^{-2} h^{-1})	Method used to calculate annual rate
Piehler and Smyth (2011)	4	2.7 (22.0)	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 (26.1)	Each seasonal rate applied to three months of the year; adjusted for hours submerged per day in the dark.
Kellogg et al. (2013a)	4	55.6 (453.1)	Values from each sampling period applied to two months of the year. Assumed no denitrification in other four months of year.

5. Conclusions

Despite the narrow focus of this review on *Crassostrea virginica* growing in U.S. coastal waters, the results should serve as a framework for considering the potential use of any bivalve species to ameliorate eutrophic conditions. Regardless of species or location, assessment of net N removal depends on gathering information on all primary N fluxes in the system, recognizing that consumption of the nitrogen contained in phytoplankton is not equivalent to nitrogen removal and understanding that the addition of bivalves can have negative impacts on some nitrogen removal pathways relative to reference sites. This review has clearly demonstrated that bivalve-associated N fluxes can vary by orders of magnitude both within and among sites, highlighting the need for

caution and verification when applying existing rates to new locations. Development and use of reliable shellfish-based nutrient reduction strategies will require filling key data gaps, particularly by collecting data specific to the location and grow-out methods in use at the time data are needed. Values used to estimate shellfish-based N assimilation or removal from coastal waters, including N content, growth metrics, biodeposition rates, long-term N burial rates, or denitrification should be revised as additional, location-specific data become available. Furthermore, even after reliable and sufficient condition-specific data are collected, application of those data to any nutrient reduction strategy will require addressing a number of public policy issues. For example, it is not intuitive how "in water" removal of N by oysters will be incorporated into TMDL targets primarily focused on reducing inputs from land. Although it has been demonstrated that oysters can assimilate land-derived anthropogenic N loads (Carmichael et al., 2012), the spatial and temporal scale on which oysters and other shellfish may remediate these loads (which may occur upstream, during pulse runoff events, or in seasons when oysters are not actively growing) is not well resolved. Some data suggest that oysters and other shellfish may be most successful at managing anthropogenic N loads in estuaries where loads are relatively low and quality habitat is abundant, conditions not always attainable in coastal waters (Carmichael et al., 2012). Implementation of aquaculture-based N removal strategies also will make local governments reliant upon ongoing harvest by members of the private aquaculture industry to meet TMDL or other management targets, which may not be feasible. Considerable thought will need to be given to integrating knowledge of the N removal capacity of harvested stocks and unharvested oyster reefs into water quality and fishery management plans. Recognizing the limits of current knowledge and addressing the data gaps outlined here is one of the first steps towards informing these important policy decisions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2014.09.025.

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