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Effects of commercial clam aquaculture on biogeochemical cycling in shallow coastal ecosystems

Anna Elizabeth Murphy

*College of William and Mary - Virginia Institute of Marine Science*

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Effects of commercial clam aquaculture
on biogeochemical cycling in shallow coastal ecosystems

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the requirements for the Degree of
Doctor of Philosophy

By
Anna Elizabeth Murphy
2015
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

Anna E. Murphy

Approved, by the Committee, November 2015

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ABSTRACT.
As the bivalve aquaculture industry expands worldwide, there is growing interest in its use to mitigate coastal eutrophication, the increased supply of organic matter to an ecosystem. Bivalves influence eutrophication by exerting 'top-down' control on primary production through feeding while simultaneously influencing local 'bottom-up' effects by increasing nutrient recycling. Additionally, nitrogen (N) is removed via harvest and potentially enhanced denitrification (DNF); however, DNF competes for nitrate (NO$_3^-$) with dissimilatory nitrate reduction to ammonium (DNRA), an N retention process. Seasonal in situ flux measurements in Cherrystone Inlet, VA, demonstrated that clam aquaculture sediments are a source of ammonium (NH$_4^+$), derived from clam excretion and microbial mineralization of clam biodeposits. Macroalgae, which proliferate on predator-exclusion nets utilized by the US clam industry temporarily sequester this regenerated N. Clam cultivation influences eutrophication locally by providing N in excess of macroalgal N demand, facilitating increased macroalgal production. Experiments investigated the competition between DNF and DNRA within clam sediments. At clam beds in Cherrystone Inlet, DNRA was more favored over DNF than at uncultivated sediments, likely due to the availability of labile organic carbon supplied by clams, low nitrate availability, and sulfidic sediments. However, a comparative study across clam aquaculture sites in the Sacca di Goro, Italy, where Ruditapes philippinarum are cultured, and on the Eastern Shore, VA, where Mercenaria mercenaria are cultured, revealed that the competition between DNF and DNRA is highly dependent on the environment and particularly the relative availability of labile carbon to NO$_3^-$. DNF exceeded DNRA at sites in the Sacca di Goro with elevated water column NO$_3^-$, concurrent with high abundances of a burrowing amphipod (Corophium sp.) that promoted nitrification. DNRA exceeded DNF at the VA sites and in the eastern region of the Sacca di Goro, where clam biomass was high, water column NO$_3^-$ low, and sediments were generally reduced. Variability in rates across sites highlights the challenge in generalizing about the role of DNF in enhancing N removal across all clam aquaculture locations. An ecosystem-scale C and N budget was constructed for Cherrystone Inlet to understand the influence of clam cultivation on energy flow and eutrophication at a basin-wide scale. Although clam cultivation occupied only 3% of the Inlet's surface area, the clams filtered a volume equivalent to 7-44% of the system daily. Annually, N regeneration at the clam beds was ~3-fold higher than N removed by harvest. Due to the short water residence time, low watershed N load, and close vicinity of clam beds to the mouth of the Inlet, cultivated clams are likely subsidized by phytoplankton from the Chesapeake Bay. Thus, the N regenerated at the clam beds, which fuels macroalgal production would not be present in the system without facilitation by the cultured clams. This study demonstrates that although clams may dampen eutrophication by removing phytoplankton from the water column, high densities of clams can facilitate rapid N turnover through excretion and DNRA, fueling macroalgae, a form of eutrophication. The effect of clam aquaculture on N removal and subsequently organic matter supply is highly dependent on environmental conditions and clam cultivation practices, as well as the scale considered. At a large-scale (e.g. Chesapeake Bay) clam aquaculture is a net sink for N through harvest, however this study suggests that clam aquaculture may increase N and organic matter supply (i.e. macroalgae) on a basin-wide scale (e.g. Cherrystone Inlet).
Effects of commercial clam aquaculture on biogeochemical cycling in shallow coastal ecosystems
CHAPTER 1: INTRODUCTION
5.85 Motivation

Coastal and estuarine ecosystems play an important role in biogeochemical cycling and organic matter transformations as material is transported along the land-sea continuum. Due to their close proximity to land these highly productive regions are particularly vulnerable to anthropogenic activities, which may accelerate organic matter and nutrient delivery, posing risks of eutrophication (Nixon 2009). Aside from the conventionally considered human perturbations involving land-use, such as coastal development, land-based agriculture, and industrial wastewater effluent, a growing prevalence of in-water aquaculture practices has added a new route of anthropogenic disturbance to coastal waters. As the world’s wild fish stocks become depleted due to overfishing and disease, a reliance on aquaculture has become increasingly apparent and the industry has seen continuous expansion (FAO 2014). Cultivated bivalve production accounts for about 70% of total mariculture production (Cambell and Pauly 2013). In many nearshore ecosystems bivalve cultivation has become an important feature in the natural ecosystem. The interactions between aquaculture and the ecosystem are complex and highly dependent on the physiology and life history of the cultured species, the cultivation methods and management practices employed, and local physical and environmental conditions. As dependence on cultured fish as a food source intensifies globally, an understanding of the impacts in-water aquaculture activities have on nutrient cycling in coastal waters is needed to ensure sustainability and minimize negative consequences.

The continued expansion of the bivalve aquaculture industry globally, concurrent with increased coastal eutrophication, has prompted recent interest in the potential role
bivalves play in removing bioreactive nitrogen (N) from the aquatic system (Stadmark and Conley 2011; Bricker et al. 2014; Petersen et al. 2014). Bivalve cultivation is considered a bioextractive practice, as unlike finfish culture, bivalve grow-out does not require the input of organic matter or feed; juveniles bivalves feed on natural phytoplankton stock and are harvested upon reaching market-size. Thus, harvest is a net N sink from the aquatic environment as the N assimilated in the tissue of the organisms is physically removed from the system. Another potential means in which bivalve cultivation may facilitate N removal is by enhancing denitrification, the microbial process in which nitrate (NO$_3^-$) is reduced to N$_2$(g), a N-form not readily available for biological uptake. By delivering labile organic matter to sediments through biodeposition, suspension-feeding bivalves may promote denitrification (Kellogg et al. 2013, Smyth et al. 2013, Newell et al. 2002), which requires organic matter, anoxic conditions, and NO$_3^-$ supply (Seitzinger 1988). However, depending on environmental conditions, biodeposition may also promote dissimilatory nitrate reduction to ammonium (DNRA), which competes with denitrification and results in a recycling of N, retaining it in the system. It is important to understand the environmental drivers that dictate the competition between these two NO$_3^-$ respiration pathways as one removes N (denitrification) while the other recycles it (DNRA).

1.2 General clam cultivation methods

Hard clam cultivation in the US generally involves three phases: hatchery, nursery, and grow-out; details on _M. mercenaria_ aquaculture production methods are provided in Castagna and Kraeuter (1981) and Castagna (2001). Typically adult brood
stock clams are conditioned to spawn using gradual increases in temperature and food supply (i.e. cultured phytoplankton). Larval development occurs in the hatchery under controlled conditions (e.g. temperature, water quality, phytoplankton supply, etc.). After metamorphosis, juvenile clams are typically transferred to a land-based nursery operation where raw seawater continuously delivers food to the growing clams. Upon reaching a certain size (e.g. ~12-15mm in length on the Eastern Shore, VA), the clam seed is moved to leased grow-out sites. In VA, grow-out locations are typically shallow, relatively protected subtidal areas, leased to the growers by the state. Grow-out locations should have proper salinity and temperature requirements for the specific clam species, sufficient hydrologic flow to deliver ample food for the clams, and shallow water, which allows general maintenance and harvest to be conducted on foot. In VA, clams are planted directly in the sediments at densities of about 700-900 individuals m\(^{-2}\) and covered with predator exclusion nets. These plastic mesh nets are set flush to the sediment surface and provide protection from predators, for example blue crabs and cow-nose rays in VA. The nets are often fouled by macroalgae, particularly in the warm months, which can reduce water flow across the clam beds, reducing food and oxygen supply to the clams. Consequently, growers periodically remove the macroalgal mats using hydraulic brushes to prevent detrimental effects to the clams.

Cultivation of *Ruditapes philipinarum* in Sacca di Goro, Italy, differs from US methods of growing *M. mercenaria*. Clam growers in the Sacca di Goro obtain juvenile clams from regions just outside of the lagoon, where a natural set occurs. The juvenile clams are transported to leases inside the lagoon and planted at densities which vary from 100-500 individuals m\(^{-2}\), although densities up to 2,000 individuals m\(^{-2}\) is not uncommon.
R. philippinarum are non-native to the region and were introduced in 1986 for aquaculture production. Unlike cultivation in the US, predator-exclusion nets are not used. It typically takes 9-12 months for the clams to reach market size (~4 cm shell length).

1.3 Nitrate respiration pathways and bivalves

The competition for NOT between denitrification and DNRA is of ecological importance as denitrification removes bioavailable N from the aquatic ecosystem while DNRA recycles it back to NH$_4^+$. The competition between the two NO$_3^-$ respiration pathways is strongly controlled by the availability of the electron donor (typically labile organic carbon) relative to the electron acceptor (NO$_3^-$). Low organic carbon availability relative to NO$_3^-$ typically favors denitrification over DNRA as this pathway provides more free energy per mole of carbon oxidized than DNRA (Tiedje 1982). However, when NO$_3^-$ is limiting and there is ample labile organic carbon, DNRA often exceed denitrification as this pathway transfers more electrons, thus utilizing the limited NO$_3^-$ more efficiently than denitrification (Burgin and Hamilton 2007). A recent laboratory study demonstrated this theory (Hardison et al. 2015).

Whether N is removed through denitrification or retained through DNRA in bivalve-dominated systems depends upon numerous factors that ultimately affect the ratio of labile organic carbon to NO$_3^-$, such as the bivalve species, the ecological context (natural or aquaculture), the ambient water quality, and other physical characteristics of the system (e.g. residence time and depth). As summarized in Table 1-1, the overall effect of bivalves on NO$_3^-$ respiration rates is quite variable and highly dependent on the
environment and bivalve species. Additionally the majority of studies do not measure DNRA, making it difficult to determine the mechanisms by which bivalves influence NO$_3^-$ respiration rates.

5.85 Overall Objectives and General Approaches

1. To determine the effects of commercial hard clam (Mercenaria mercenaria) aquaculture on seasonal net benthic metabolism and nutrient fluxes, including the effects of macroalgae as an integral component of the ecosystem in Cherrystone Inlet, VA.

   **Approach:** Seasonal *in situ* flux measurements were conducted in the light and dark at a clam grow-out site in Cherrystone Inlet, VA with the following treatments: clam bed, clam bed with macroalgae, and bare sediment.

2. To characterize seasonal microbial N cycling processes at commercial hard clam aquaculture sediments with a focus on the competition between denitrification and DNRA compared to control uncultivated sediments.

   **Approach:** Seasonal N cycling rates were measured across a clam grow-out site in Cherrystone Inlet, VA. Denitrification and DNRA were measured using the isotope pairing technique in which $^{15}$NO$_3^-$ is added to intact sediment cores, the cores are incubated, and the production of $^{28}$N$_2$, $^{30}$N$_2$, and $^{15}$NH$_4^+$ are measured (Nielsen 1992, Risgaard-Petersen & Rysgaard, 1995). Gross microbial mineralization rates were measured using the isotope pool dilution method in which cores are spiked with $^{15}$NH$_4^+$, incubated and the dilution of the labeled $^{15}$NH$_4^+$ is used to calculate gross production of NH$_4^+$ (Wessel and Tietema 1992).
3. To quantify and scale C and N processes associated with large-scale hard clam aquaculture operations relative to other basin-wide ecosystem fluxes such as benthic and pelagic primary production for a shallow coastal ecosystem (Cherrystone Inlet, VA).

**Approach:** Clam physiological rates were modeled using equations provided in the literature, scaled to the clam population in Cherrystone Inlet, VA (estimated using aerial photography and field-collected data of clam densities and size), and compared to measured and modeled ecosystem processes such as phytoplankton production, benthic microalgal production, macroalgal production, and benthic respiration.

4. To investigate the effects of natural environmental gradients on altering N cycling processes (e.g. denitrification and DNRA) at commercial clam aquaculture sediments.

**Approach:** Isotope pairing technique and isotope pool dilution were used to measure denitrification, DNRA, and gross mineralization rates (as above in Objective 2) at five locations in the Sacca di Goro, Italy, and 2 locations on the Eastern Shore, VA.

1.4 Broader Implications

As suspension-feeders, bivalves play an important role in shuttling organic matter from the water column to the benthos through feeding and biodeposition. Consequently, high densities of bivalves associated with cultivation operations can significantly impact biogeochemical cycling of N and C in nearshore environments. Although, on a large-scale, bivalve cultivation is a net N sink from the coastal ecosystem through harvest, on a local-scale cultivation operations can significantly alter N availability and consequently C
production in shallow coastal environments. Quantifying the influence of bivalve cultivation on N and C cycling is necessary to ensure sustainability.
Literature Cited.


Turek KA, Hoellein TJ (2015) The invasive Asian clam (Corbicula fluminea) increases sediment denitrification and ammonium flux in 2 streams in the mollusk in the USA. Freshwater Science 34:472–484. Doi: 10.1086/680400

Table 1-1. A summary of previous studies that report nitrate respiration rates (denitrification (DNF) and DNRA) associated with bivalve-dominated systems, including both aquaculture and natural settings.

<table>
<thead>
<tr>
<th>Bivalve Species</th>
<th>Context</th>
<th>DNF</th>
<th>DNRA</th>
<th>DNF increased above Control Site?</th>
<th>DNRA increased above Control Site?</th>
<th>Location</th>
<th>Methods</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Corbicula fluminea</em>  (clam)</td>
<td>Natural/Invasive</td>
<td>~ 86.3 – 201.4</td>
<td>--</td>
<td>Yes, (only summer)</td>
<td>--</td>
<td>Freshwater streams (N. Branch Chicago River and Eagle Creek)</td>
<td>Homogenized sediment continuous-flow incubation; N2:Ar technique</td>
<td>(Turek and Hoellein 2015)</td>
</tr>
<tr>
<td><em>Tapes philipinarum</em>  (clam)</td>
<td>Aquaculture</td>
<td>~ 74 – 200</td>
<td>1 – 6</td>
<td>Yes in the summer, not in the winter</td>
<td>No</td>
<td>Eutrophic Lagoon (Sacca di Goro, Italy)</td>
<td>Whole core batch incubation; isotope pairing technique</td>
<td>(Nizzoli et al. 2006)</td>
</tr>
<tr>
<td><em>Austrovenus stutchburyi</em> (cockle)</td>
<td>Natural</td>
<td>0 – 30</td>
<td>--</td>
<td>No</td>
<td>--</td>
<td>Intertidal sandflats (New Zealand)</td>
<td>Slurry, acetylene inhibition technique</td>
<td>(Jones et al. 2011)</td>
</tr>
<tr>
<td><em>Mercenaria mercenaria</em> (clam)</td>
<td>Aquaculture</td>
<td>0.77 – 2.9</td>
<td>2.7 – 14.2</td>
<td>Only in fall</td>
<td>Yes, all seasons</td>
<td>Cherrystone Inlet, VA</td>
<td>Whole core batch incubation; isotope pairing technique</td>
<td>This study</td>
</tr>
<tr>
<td><em>Crassostrea virginica</em> (oyster)</td>
<td>Aquaculture</td>
<td>~ 0 – 65</td>
<td>--</td>
<td>No</td>
<td>--</td>
<td>Shallow mesohaline sediments below cages (St. Jerome Creek and Spencer Creek)</td>
<td>Whole core continuous-flow incubation; N2:Ar Technique</td>
<td>(Higgins et al. 2013)</td>
</tr>
<tr>
<td><em>Crassostrea virginica</em> (oyster)</td>
<td>Restored Reef</td>
<td>~ 250 – 1590</td>
<td>--</td>
<td>Yes</td>
<td>--</td>
<td>Mesohaline subtidal reef (Choptank River, MD)</td>
<td>Whole chamber batch incubations; N2:Ar Technique</td>
<td>(Kellogg et al. 2013)</td>
</tr>
<tr>
<td>Bivalve Species</td>
<td>Context</td>
<td>DNF</td>
<td>DNRA</td>
<td>DNF increased above Control Site?</td>
<td>DNRA increased above Control Site?</td>
<td>Location</td>
<td>Methods</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------</td>
<td>--------------</td>
<td>--------------</td>
<td>-----------------------------------</td>
<td>-------------------------------------</td>
<td>---------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Crassostrea virginica (oyster)</td>
<td>Restored Reef</td>
<td>~ 0 – 332</td>
<td>~ 0.8 – 104</td>
<td>Yes</td>
<td>Yes, in the summer</td>
<td>Intertidal sediments adjacent to reef (Bogue Sound, NC)</td>
<td>Whole core continuous-flow incubation; N2:Ar; then 15NO3 for potential DNRA measurements</td>
<td>(Smyth et al. 2013b)</td>
</tr>
<tr>
<td>Mytilus edulis (mussel)</td>
<td>Aquaculture</td>
<td>~ 9-144</td>
<td>--</td>
<td>1 of 3 sites increased above reference</td>
<td>--</td>
<td>Marine subtidal sediments below long lines (Skagerrak Strait, Sweden)</td>
<td>Whole core batch incubation; isotope pairing technique</td>
<td>(Carlsson et al. 2012)</td>
</tr>
<tr>
<td>Perna canaliculus (mussel)</td>
<td>Aquaculture</td>
<td>~ 8.3-15.3</td>
<td>--</td>
<td>No</td>
<td>--</td>
<td>Marine subtidal sediments, below long lines (Beatrix Bay, New Zealand)</td>
<td>Whole core batch incubation; isotope pairing technique</td>
<td>(Christensen et al. 2003)</td>
</tr>
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</table>

Table 1-1 (cont.)
CHAPTER 2: ENHANCED NUTRIENT REGENERATION AT COMMERCIAL HARD CLAM (MERCENARIA MERCENARIA) BEDS AND THE ROLE OF MACROALGAE

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Key words: Aquaculture, Clam, Shellfish, Macroalgae, Eutrophication, Nitrogen Cycling

Running page head: Clam aquaculture fuels macroalgae

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Abstract.

The bivalve aquaculture industry is expanding worldwide; sustainability requires improved understanding of its interactions with the natural environment. High densities of bivalves found in aquaculture can exert ‘top-down’ control on primary production through feeding while simultaneously influencing local ‘bottom-up’ effects on production by enhancing nutrient recycling. Thus bivalves may decrease or increase localized eutrophication (*sensu* Nixon), depending on environmental conditions and specific culture practices. This study investigates hard clam aquaculture influence on benthic nutrient regeneration and metabolism, seasonally using *in situ* incubations. Effects of macroalgae, which proliferate on predator-exclusion nets at cultivation sites, are also investigated. Ammonium (NH$_4^+$) and phosphate (SRP) effluxes averaged 154 and 100 times higher, respectively, at clam beds compared to reference sediments. Macroalgae decreased NH$_4^+$ efflux from clam beds by 20-77%, while having no significant effect on SRP. Nutrient release from clam beds to the water column supports macroalgal growth, supplying nitrogen in excess of macroalgal demand in spring and fall and 58% of demand in summer, suggesting N recycling in the benthos is sufficient to support macroalgal production. As a bio-extractive practice, clam aquaculture is a net sink for nutrients in aquatic systems. However, our data suggest clam cultivation may influence eutrophication locally by facilitating increased macroalgal production due to increased benthic nutrient recycling. Given the high capacity for macroalgae to temporarily sequester nutrients released from the clam beds, macroalgal harvest may be an effective means to negate these effects of the clams and remove unwanted nutrients from the ecosystem.
INTRODUCTION

Coastal and estuarine ecosystems remove, transform, and sequester nutrients and organic matter and provide diverse habitats and resources to commercially valuable fish and bivalve species. Due to their location along the land-sea continuum, these ecosystems are vulnerable to anthropogenic activities, which accelerate organic matter and nutrient delivery to the water, posing risks of eutrophication (Nixon 1995). Defined by Nixon (1995) as the increase in the rate of supply of organic matter to an ecosystem, eutrophication has become an increasingly pertinent global concern as it decreases ecosystem function and economic value (NRC 2000, Bricker et al. 2008). Eutrophication can be triggered by a number of factors including changes in grazer activity, increased nutrient input from the watershed or adjacent waters, and increased organic matter input. Eutrophication may be characterized by phytoplankton or macroalgal blooms. The dominance of these primary producers varies on both seasonal and annual time scales with a variety of natural and anthropogenic drivers (Nixon 2001, Valiela 1997).

Aside from land-based human perturbations, such as urban development, agriculture, and wastewater treatment, growth of in-water aquaculture represents an expanding anthropogenic perturbation to coastal waters. A decrease in wild shellfish populations, often attributed to eutrophic conditions resulting in habitat loss and increased disease prevalence, has increased reliance on cultured shellfish to meet the growing demand for seafood (FAO 2012). Shellfish aquaculture has become an important feature in many coastal waters worldwide and understanding its impact within the context of increasingly eutrophic waters is necessary to ensure its sustainability. The effect of bivalve aquaculture on nutrient cycling and eutrophication is currently the
subject of ongoing debate (Lindahl et al. 2005, Stadmark & Conley 2011, Rose et al. 2012). Depending on a variety of environmental factors, bivalves can exert ‘top-down’ (i.e. filter-feeding) and influence ‘bottom-up’ (i.e. increase nutrient recycling) control on primary production and thus may decrease or increase localized primary production, respectively.

Bivalve aquaculture, which does not require exogenous feed, may modulate eutrophication by removing phytoplankton (Gren et al. 2009, Bricker et al. 2014, Rose et al. 2014). Suspension feeding bivalves exert direct ‘top-down’ control on phytoplankton biomass through feeding, which reduces water column particulate organic matter (Cloern 1982, Officer et al. 1982, Cohen et al. 1984, Strayer et al. 1999). In a cultivation setting, the nutrients assimilated within the tissues of the bivalves are permanently removed from the aquatic system upon harvest. Additionally, denitrification, a microbial process that converts bioavailable nitrogen to N$_2$ gas, may be enhanced in aquaculture operations under certain environmental conditions (Kaspar et al. 1985, Carlsson et al. 2012). Nutrient removal by bivalves has been proposed as an approach to mitigate eutrophication (Lindahl et al. 2005, Rose et al. 2012, Petersen et al. 2014, Bricker et al. 2014); however, indirect bottom-up effects of high densities of bivalves must be considered.

Bivalves indirectly enhance primary production by increasing benthic nutrient fluxes to the water column (Doering et al. 1986, Bartoli et al. 2003). Commercial-scale bivalve aquaculture has been shown to reduce sediment and water quality and cause local nutrient enrichment (De Casabianca et al. 1997, Bartoli et al. 2001, Stadmark & Conley 2011). Bivalves are a direct source of nutrients to the water column through active
excretion of ammonium (NH$_4^+$), soluble reactive phosphorous (SRP), and dissolved organic nitrogen and carbon (DON, DOC) (Sma & Baggaley 1976, Magni et al. 2000). High clam biodeposition rates along with the gear used for cultivation (i.e. cages, nets, and racks) enhance sedimentation (Grenz et al. 1990, Spencer et al. 1997, Smaal et al. 2001), resulting in organically enriched sediments, increased microbial remineralization, reduced dissolved oxygen (DO), and sulfide accumulation causing fluxes of NH$_4^+$ and SRP out of sediments (Giles & Pilditch 2006, Nizzoli et al. 2007). Sulfidic and low oxygen conditions inhibit coupled nitrification-denitrification further enhancing NH$_4^+$ fluxes to the water column (Joye & Hollibaugh 1995, Heijs et al. 2000). Additionally, the aquaculture gear serves as hard substrate and may promote macrophyte attachment and growth, thus increasing local eutrophication by increasing organic C production.

Environmental factors, including hydrodynamics, residence time, temperature, light, and ambient nutrient concentrations, likely play a role in determining the extent to which bivalves facilitate or dampen eutrophication. Additionally, the particular bivalve species and the cultivation methods employed influence impacts on the environment, posing a challenge in generalizing across all bivalve aquaculture. Studies on the effects of cultivating epibenthic organisms, such as mussels and oysters, on sediment biogeochemistry have demonstrated increased nutrient regeneration in sediments (Hatcher et al. 1995, Gilbert et al. 1997, Chapelle et al. 2000, Christensen et al. 2003, Giles & Pilditch 2006). Few studies have investigated biogeochemical cycling within cultured clam beds and its impacts on eutrophication. Unlike oysters and mussels, which are typically grown suspended in the water column, clams are cultured within the sediment, and as a result their effects on sediment nutrient dynamics are quite different.
Through bioturbation, clams may directly increase advection/diffusion changing sediment DO and nutrient profiles and subsequently biogeochemical process rates (Nizzoli et al. 2006). However, use of predator exclusion nets, plastic mesh placed flush on the sediment surface, by clam growers on the U.S. east coast may reduce exchanges between overlying water and sediments. In addition, ephemeral macroalgae on the net surface affect DO, release POC and DOC to the sediments, and temporarily sequester nutrients sourced from the clam beds. Clam growers frequently sweep the nets of accumulated macroalgae, which are allowed to drift away and decompose releasing nutrients and potentially depleting DO in adjacent waters. Upon senescence due to density-dependent factors (i.e. self-shading) and/or environmental factors (i.e. increase in temperature), ephemeral macroalgae in the coastal bays of VA, degrade rapidly, releasing nutrients and decreasing DO (Hardison et al 2010, Tyler et al. 2001).

As aquaculture becomes more prevalent in coastal waters, an understanding of its interactions with the surrounding ecosystem, particularly with respect to nutrient cycling, is necessary to avoid creating eutrophic conditions. This study investigated the effects of hard clam (*Mercenaria mercenaria*, Linnaeus, 1785) aquaculture on benthic respiration and nutrient regeneration, specifically with regard to modifying fluxes to the water column and subsidizing macroalgal growth, seasonally. The study assessed the importance of macroalgae in modulating benthic net community production (NCP) and nutrient fluxes across the sediment water interface at the clam aquaculture sites. We hypothesized that clam beds are net heterotrophic, resulting in a release of nutrients to the water column, whereas uncultivated sediment sites are net autotrophic and a sink for
nutrients. Macroalgae will decrease nutrient effluxes at clam beds and shift community metabolism to net autotrophy (see Fig. 4).

METHODS

Site Description

Field experiments were conducted in May, July, and October 2012 in Cherrystone Inlet, a small tributary (5.7 km²) of Chesapeake Bay, located on the bayside of the eastern shore of Virginia, USA (Fig. 1). Average depth is less than 2 meters and approximately one third of its subtidal bottom is partitioned into private shellfish leases, most used to grow *Mercenaria mercenaria*, hard clams. Hard clam aquaculture on the east coast of the US typically involves planting hatchery-reared juvenile clams in sandy subtidal sediments, covering the beds with plastic predator exclusion nets, and mechanically harvesting the clams at market size (3-4 cm shell height) after about 2 years. The clams in Cherrystone are planted at 700-800 individuals m⁻², with an estimated standing stock of about 100 million cultured clams within the tributary (Condon 2005). The sampling sites for this study were located in the southern portion of the inlet close to the mouth that empties to Chesapeake Bay. The site experiences little salinity variation, which is driven largely by rain events. Macroalgae, including *Ulva lactuca* (Linnaeus, 1753), *Gracilaria* spp. (Greville, 1830), *Agardhiella tenera* (Kraft & M.J. Wynne, 1979), and *Cladophora* sp. (Kutzing, 1843), are present on the commercial clam bed nets throughout the year.

Experimental Design
During each season, fluxes were measured at three randomly selected clam beds and three bare sediment sites located in line with the clam beds, perpendicular to the shore. The clam beds consisted of mature, close to market size clams (approximately 3-4 cm shell height), and the nets had not been recently swept of macroalgae by the aquaculturists. *In situ* flux treatments included: clams plus macroalgae in the light (CML) and in the dark (CMD), clams without macroalgae in the light (CL) and dark (CD), bare sediments in the light (BL) and dark (BD) and water blanks in the light (WL) and dark (WD). One clam bed and one bare site were sampled each day, over 3 consecutive days. All treatments were conducted in triplicate each day, providing n=9 per season and treatment. As the bare sites were typically deeper than the clam sites, cores were elevated to the depth of the clam bed cores to ensure similar ambient light levels. On each clam bed, three sets of randomly-positioned blocks of four flux chambers, one for each clam treatment (CML, CMD, CL, and CD), were inserted through a hole in the predator exclusion net. At the bare site, three replicates of BL, BD, WL, and WD treatments were established. Opaque shade cloth covered the dark cores to prevent light penetration, verified by measuring the light under the cloth using a Li-Cor quantum deck sensor (LI-190SA, Li-Cor, Inc., Lincoln, NE, USA).

Ambient macroalgal biomass was obtained by randomly tossing a ring sampler (0.014 m²) five times on clam nets near the flux incubation nets, to avoid disturbing these sites. Macroalgal wet weight was scaled to the size of the flux chambers, and the appropriate amount of macroalgae was added to the cores. Ambient macroalgal biomass and community composition at the clam farm varied seasonally, and the macroalgae placed in the experimental cores reflected this seasonality.
Benthic Metabolism and Nutrient Flux Measurements

Seasonal *in situ* flux incubations were conducted on ebbing tides on days with minimal cloud cover to allow adequate light levels during the experiments. *In situ* incubations minimized disturbance to the sediments and clams and ensured clams were not starved of food prior to the experiments. Flux chambers, clear acrylic cores (13.3 cm ID by 40 cm tall), were inserted into the sediment to a depth resulting in a 20 cm water column, allowed to equilibrate for an hour, then capped, excluding air bubbles. Central motors were used to power small magnetic stir bars suspended below each core cap to prevent gradients from developing. Half of the flux chambers were covered with opaque fabric to prevent light penetration and obtain respiration values under dark conditions. Water blanks, cores filled with ambient water, were sampled to distinguish water column from sediment processes. Overlying water in each of the chambers was sampled hourly over a 4-hour incubation period. Dissolved oxygen (DO) was measured by pulling the sample with a syringe into an airtight chamber containing a Hach LDO101 Luminescent DO sensor (Hach Co., Loveland, CO, USA). Dissolved inorganic carbon (DIC) samples, stored in 8-ml hungate tubes (Bellco Glass Inc., Vineland, NJ, USA), were preserved with 15 µL saturated mercuric chloride and kept cold under water until analyzed within one month of collection using a Li-Cor 6252 infrared gas analyzer (Neubauer & Anderson 2003). Samples collected concurrently with the DO and DIC samples were filtered and frozen until analyzed for DIN, DIP, DOC, DON, and chlorophyll *a* (as described below).
Flux Calculations

Hourly fluxes for each analyte were calculated as:

\[ \text{Flux} = \frac{(m \cdot V)}{A} \]  

(1)

where \( m \) is equal to the slope of the linear regression of concentration (\( \mu M \) or \( mM \)) versus time (hours); \( V \) is equal to the volume of water in the flux chamber (L); and \( A \) is the sediment surface area within the chamber (m\(^2\)). A flux from the sediment to the water column is positive while a flux to the sediment from the water column is a negative value.

Water blank fluxes were subtracted from the whole core fluxes to obtain a benthic community flux. Benthic metabolism (DIC based in mmol C m\(^{-2}\) day\(^{-1}\)) and daily nutrient fluxes were calculated as follows:

\[ R = F_d \times 24 \text{h} \]  

(2)

\[ \text{GPP} = h_l \times (F_l - F_d) \]  

(3)

\[ \text{NCP} = \text{GPP} + R \]  

(4)

Daily nutrient flux = \( (F_l \times h_l) + (F_d \times h_d) \)  

(5)

where \( R \) is community respiration, GPP is gross primary production, NCP is net benthic community production, \( F_d \) and \( F_l \) are hourly fluxes in the dark and light, respectively.
(mmol m$^{-2}$ hr$^{-1}$), $h_d$ and $h_l$ are the number of hours of dark and light in a day, which varied seasonally. When NCP is negative, GPP exceeds R and the system is net autotrophic with net uptake of DIC. Net heterotrophy and thus net release of DIC is represented by a positive NCP.

*Clam and Macropical Measurements*

Upon completion of the flux measurements, all clams were removed from each chamber and the ash-free dry weight (DW) determined by the difference in dry weight prior to and after combusting at 500°C for 5 hours. All macroalgae were removed from the CML and CMD chambers and the DW determined for each species present. A subset of dried macroalgal tissue samples from the dominant species each season were stored in the freezer until analyzed on a Carlo Erba (Thermo Electron Corp. Flash EA 1112 Series) elemental analyzer for POC (samples were acidified prior to analysis) and total nitrogen content.

*Estimating Clam Excretion*

To estimate the contribution of clam metabolism (i.e. excretion) to the net NH$_4^+$ flux measurements at the clam sites, we used an equation derived by Sma & Baggaley (1976):

\[
\log_{10} E = 0.94\log_{10} DW + 1.33
\]  

(6)
where E is equal to the excretion rate (µmol NH$_4^+$ ind$^{-1}$ d$^{-1}$) and DW is the tissue dry weight (g) of an individual clam. Sma & Baggaley (1976) measured the production of NH$_4^+$ from individual *M. mercenaria* in a laboratory setting where the clams were starved prior to static incubations during which the clams were fed cultured algae. After calculating excretion for the individual clams in each core we summed these rates per core and scaled to per m$^2$ to compare to the net benthic NH$_4^+$ flux measurements.

*Estimating Macroalgal Growth Rate and Nitrogen Demand*

The importance of benthic nutrient regeneration at the clam beds in meeting the macroalgal N demand was assessed using estimated macroalgal growth rates and nutrient content in the macroalgal tissue. Macroalgal production rates were not directly measured in this study but were estimated by subtracting the NCP (mmol C m$^{-2}$ d$^{-1}$) of the CM treatment from the C treatment. The average total N and organic C content of each macroalgal species retrieved from the cores after the incubations during each season were used to generate a weighted average N and C content for the macroalgal community during each season as:

\[
N \text{ or } C \text{ content} = \sum W_i X_i \tag{7}
\]

where $W_i$ is equal to the proportion of species $i$ relative to the total macroalgal biomass and $X_i$ is equal to the tissue N or C content of species $i$ (g C g DW$^{-1}$ or g N g DW$^{-1}$). Estimated production rates were converted to growth rates by dividing them by the species-weighted average macroalgal C content. Macroalgal N demands were then
calculated by multiplying growth rate by the species-weighted average N content of the macroalgal tissue. The following equations summarize our calculations:

\[ MP = NCP_C - NCP_{CM} \]  \hspace{5cm} (8)

\[ MG = MP / C \text{ content} \]  \hspace{5cm} (9)

\[ MN = MG \times N \text{ content} \times (1 \text{ mol} / 14 \text{ g}) \times (1000 \text{ mmol} / 1 \text{ mol}) \]  \hspace{5cm} (10)

where \( MP \), \( MG \), and \( MN \) refer to macroalgal production rate (g C m\(^{-2}\) d\(^{-1}\)), growth rate (g DW m\(^{-2}\) d\(^{-1}\)), and N demand (mmol N m\(^{-2}\) d\(^{-1}\)), respectively; \( NCP_C \) and \( NCP_{CM} \) are the net community production in the clam only treatment and the clam plus macroalgae treatment (g C m\(^{-2}\) d\(^{-1}\)), respectively; and C content and N content are the species-weighted average organic C and total N of the macroalgal community during each season (g C g DW\(^{-1}\) or g N g DW\(^{-1}\)) (see equation 7).

**Water Quality and Sediment Parameters**

Triplicate sediment cores were collected at each clam and bare flux location, seasonally, sub-sectioned horizontally at 0-1 cm and 1-5 cm, and analyzed for porosity, as loss of wet weight after drying at 70°C, and organic matter, as loss on ignition after combustion at 500°C for 5 hours. Dried subsamples were acidified and analyzed on a Carlo Erba (Thermo Electron Corp. Flash EA 1112 Series) elemental analyzer for POC and total nitrogen content. Triplicate water column and porewater samples were
collected at each flux location, filtered (0.45 μm Whatman polyethersulfone (PES)) and frozen until analysis for DIN (NO$_3^-$, NO$_2^-$, and NH$_4^+$) (Liao 2001, Smith & Bogren 2001), SRP (Knappe & Bogren 2001), and DON (Koroleff 1983) on a Lachat QuikChem 8000 automated ion analyzer (Lachat Instruments Milwaukee, WI, USA; detection limits for NO$_3^-$, NH$_4^+$, and PO$_4^{3-}$ are 0.20, 0.36, and 0.16 μM, respectively). Porewater was collected at 5-7cm below the sediment surface using a stainless steel push-point sampler (MHE Products, East Tawas, MI, USA), and also analyzed for hydrogen sulfide (Cline 1969). Water column samples were filtered (0.7 μm GF/F) and extracted for chlorophyll $a$ (chl $a$) and phaeophytin analysis as described by Anderson et al. (2013). Salinity, temperature, DO, chl $a$, and turbidity were monitored continuously throughout each 3-day experiment using a YSI model 6600 datasonde (YSI, Inc., Yellow Springs, OH, USA) mounted on a rebar frame, with the sensors 5 cm above the sediment surface. Incident light and underwater photosynthetically active radiation (PAR) were monitored continuously throughout each 3-day experiment using a Li-Cor quantum deck sensor (LI-190SA) and underwater quantum sensor (LI-192SA; Li-Cor, Inc., Lincoln, NE, USA).

**Statistical Analysis**

Daily metabolic rates and descriptive measurements (i.e. porewater nutrients, sediment organic matter, etc.) were analyzed using mixed-effect models with season and treatment as independent fixed factors and the location within the farm as a random factor. Effects due to location within the farm could not be distinguished from those due to the day incubations were conducted (e.g. differences in ambient light), however they were not a focus of this study. Post hoc tests were performed using Tukey’s HSD. When
significant interactions were observed, post hoc tests were conducted to determine differences across seasons within treatments as well as across treatments within seasons. When necessary, data were transformed using Box-Cox to meet assumptions. Linear regressions were used to assess the relationship of clam biomass to NH$_4^+$ and DIC fluxes. The stoichiometric relationship between NH$_4^+$ and DIC fluxes was assessed for each treatment using linear regression, where the C:N ratio is equal to the slope of the regression. A significance value of $p < 0.05$ was used for all statistical tests, which were conducted in Rstudio software (version 0.98.484).

RESULTS

Ambient Environmental Conditions

Water temperature ranged from 18.5°C in October to 29.3°C in July, with an intermediate temperature of 20.8°C in May. Salinity varied seasonally, increasing from 20.3 ppt in the spring to 25.3 ppt in the fall (Table 1) with no diel tidal variation observed. Water and sediment quality parameters are provided in Table 2. Water column NO$_x$ remained low at both the clam and bare sites during all seasons, with no significant effect of site or season. Water column NH$_4^+$ was slightly higher than NO$_x$ and was significantly higher at the clam bed sites (1-2µM) than the bare sites (0.3-1µM) despite their close proximity. Porewater DIN, dominated by NH$_4^+$, and SRP were significantly higher at the clam bed sites compared to the bare sediments in May and October, with no significant differences in July. Although not significant and highly variable, porewater sulfide concentrations tended to be higher at the clam beds than the bare sites. The sediment organic matter content and C:N were similar between the clam and bare sediments.
**Clam and Macroalgal Biomass**

The experimental design controlled for clam size by targeting locations within the lease with clams close to market size. Shell lengths averaged 39.1 mm (± 6.2 standard deviation (s.d.)) and clam biomass averaged 263.7 g m\(^{-2}\) (± 103.1 s.d.; ash-free DW), with no significant difference in size across seasons or treatments (Table 1). However, clam beds sampled in October had significantly higher clam biomass than the other months, due to higher densities as opposed to larger individuals (Table 1, p<0.05).

Typically dominated by *Gracilaria* spp., macroalgal biomass was highest in July (123.8 ±14.9 g DW m\(^{-2}\)) with the lowest biomass in May (24.1 ±9.5 g DW m\(^{-2}\)) and a biomass of 52.8 ±11.9 g DW m\(^{-2}\) in October (Table 3). Macroalgal biomass varied seasonally but was also likely influenced by the frequency at which the aquaculturists swept the nets. The effects of sweeping on macroalgal biomass and sediment biogeochemistry were not a focus of this study.

**Nutrient Fluxes**

Net daily fluxes are shown in Figure 2. Net NH\(_4^+\) efflux was observed for all treatments during all months except in the bare treatment (B) in July (Fig. 2A). NH\(_4^+\) fluxes were significantly affected by season and treatment as indicated by the significant interaction term (Table 4). Clam beds (C) had significantly higher net NH\(_4^+\) efflux rates, ranging from 13.5 to 18.6 mmol N m\(^{-2}\) d\(^{-1}\), than B (-0.38 to 0.16 mmol N m\(^{-2}\) d\(^{-1}\)) during all months. The presence of macroalgae resulted in decreases of 32%, 77%, and 20% in daily NH\(_4^+\) effluxes at the clam beds in May, July, and October, respectively; with
effluxes in May and July in CM significantly lower than in C. When macroalgal biomass was highest (July), the NH$_4^+$ efflux in CM (4.1 mmol N m$^{-2}$ d$^{-1}$) was significantly lower compared with May (12.6 mmol N m$^{-2}$ d$^{-1}$) and October (10.7 mmol N m$^{-2}$ d$^{-1}$). NH$_4^+$ effluxes in CM were significantly higher than in B, with average fluxes of 16.6 and -0.06 mmol N m$^{-2}$ d$^{-1}$, respectively.

Generally, NO$_x$ fluxes were variable but typically low and positive at all sites during all seasons (Fig. 2B). NO$_x$ fluxes were affected by treatment and season, as indicated by the significant interaction (Table 4). Within season, there was no significant treatment effect in July, while in May, B had significantly higher NO$_x$ rates than CM and in October B had significantly lower NO$_x$ rates than both C and CM. Within treatments, no significant seasonal effect was observed in either the C or CM treatments with average net NO$_x$ rates of 170 μmol N m$^{-2}$ d$^{-1}$ and 160 μmol N m$^{-2}$ d$^{-1}$, respectively. The B treatment had significantly lower NO$_x$ flux rates in October (-64 umol N m$^{-2}$ d$^{-1}$) compared with B sites in May and July.

The clam beds and bare sediments typically released DON to the water column (Fig. 2C). Treatment and month had significant effects on DON fluxes with a significant interaction (Table 4). There was no difference across treatments in July or October. However, in May the DON flux in B (2.8 mmol N m$^{-2}$ d$^{-1}$) was significantly higher than in CM (-1.7 mmol N m$^{-2}$ d$^{-1}$). Within the B and C treatments, there was no effect of season. Within the CM treatment, May was significantly less than October, while July was not different than May or October.

Net effluxes of SRP in the C and CM treatments were significantly greater than in B during all seasons (Fig. 2D). However, the presence of macroalgae had no significant
effect on the clam bed SRP flux. Seasonal trends were exhibited in the C and CM treatments with a significantly lower net SRP efflux in October, averaging 369.3 μmol P m$^{-2}$ d$^{-1}$ compared with May and July, which averaged 1221.7 and 943.8 μmol P m$^{-2}$ d$^{-1}$, respectively. The B treatment, which showed no seasonal variation, had negligible SRP flux rates.

DOC was typically released from sediments at all treatments and during all seasons (Fig. 2E). There was no significant effect of season or treatment on DOC fluxes, with no significant interaction (Table 4). DOC effluxes were generally higher in the clam treatments compared to the bare sediments, although not significantly.

*Benthic Metabolism*

NCP in the C treatment was net heterotrophic during all seasons and significantly different from the net autotrophic bare sediment sites (Fig. 2F). The presence of macroalgae significantly decreased NCP at the clam beds during all seasons, shifting it towards net autotrophy, which in our calculations is represented by a negative value. The NCP in the CM treatment was not significantly different than the B treatment. Therefore, the presence of macroalgae negated the influence of clams on the net benthic metabolism. No seasonal variation in B was observed with an average NCP of -51.0 mmol C m$^{-2}$ d$^{-1}$. The C treatment was significantly more heterotrophic in May (311.2 mmol C m$^{-2}$ d$^{-1}$) than July and October, which averaged 159.3 mmol C m$^{-2}$ d$^{-1}$. The CM treatment was slightly net heterotrophic during May and October (average of 28 mmol C m$^{-2}$ d$^{-1}$) and shifted to net autotrophic in July (-190 mmol C m$^{-2}$ d$^{-1}$). Similar to the NH$_4^+$ flux, the seasonal NCP trends observed in the CM treatment were likely a result of higher
macroalgal biomass added to the cores in the summer, when macroalgal standing stock biomass was highest on the nets (Table 3).

**Variation of NH$_4^+$ and DIC Fluxes with Clam Biomass**

Estimated clam NH$_4^+$ excretion rates (using Sma & Baggaley 1976 equation) ranged from 233.6 (± 66.6 s.d.) µmol N m$^{-2}$ hr$^{-1}$ in July to 542.8 (± 225.0 s.d.) µmol N m$^{-2}$ hr$^{-1}$ in May and an intermediate of 410.5 (± 136.2 s.d.) µmol N m$^{-2}$ hr$^{-1}$ in October. Estimated clam excretion accounted for an average of 66%, 40%, and 83% of the hourly flux rates of NH$_4^+$ in the clam only treatments in May, July, and October, respectively.

When all three seasons were analyzed together, NH$_4^+$ and DIC fluxes were positively correlated with clam biomass (ash-free DW core$^{-1}$) (p=0.001, R$^2$=0.20; p=0.004, R$^2$=0.16, respectively) and DO fluxes were negatively correlated with clam biomass (p=0.005, R$^2$=0.15) (Fig. 3, Table 5). However, in July NH$_4^+$, DIC, and DO fluxes were not significantly correlated with clam biomass. Additionally, in October, DIC flux was not significantly correlated with clam biomass. Notably, clam biomass varied little across samples, as beds were planted at relatively constant densities and only sites with clams close to market size were sampled.

**Flux Stoichiometry**

The ratio between DIC and NH$_4^+$ fluxes is a metric used to infer about the characteristics and fate of the organic matter being remineralized as well as the relative importance of phototrophic and denitrifying activity. A low C:N ratio may indicate high N release and/or the remineralization of highly labile organic matter, with a low C:N
signature. Alternatively, a high C:N ratio suggests denitrification and/or N immobilization by phototrophic and/or bacterial uptake. Linear regression analyses of DIC fluxes as a function of NH$_4^+$ fluxes were used to obtain the C:N of the fluxes for each treatment (i.e. C:N = the slope). The C:N at the clam bed (9.9) was lower than in the clam with macroalgae (23.7) and bare treatments (66.1) (Table 6). In the light the C:N increased in clam treatments with and without macroalgae. However, the C:N was higher in the dark than light at the bare sites.

**Macroalgal growth rate and nitrogen demand**

Estimated macroalgal production rates were 3.38, 4.26, and 1.53 g C m$^{-2}$ d$^{-1}$ in May, July, and October, respectively. Using the species-weighted average percent carbon of the macroalgal tissue collected from the cores after the incubations each month (Table 3), macroalgal growth rates were estimated to be 12.36, 13.24, 4.56 g DW m$^{-2}$ d$^{-1}$ in May, July, and October, respectively. Macroalgal N demands were estimated as 17.84, 30.42, and 11.04 mmol N m$^{-2}$ d$^{-1}$ in May, July, and October, respectively. The sediment NH$_4^+$ fluxes as percent of macroalgal N demand in May, July, and October were 105%, 58%, and 122%, respectively.

**DISCUSSION**

Although clam aquaculture is a growing industry worldwide, there are currently few studies on its effects on nutrient cycling and subsequent influences on autotrophic production (as reviewed in Burkholder & Shumway 2011). Clam aquaculture sediments are sites of high metabolic activity, significantly enhancing nutrient release to the water
column, while drawing down DO. Bivalve aquaculture does not require organic matter addition (i.e. feed) and is therefore a bio-extractive activity and overall a net nutrient sink. However, our data suggest high densities of bivalves significantly alter local nutrient supply and enhance macroalgal production. The macroalgae serve an important ecological function in temporarily sequestering nutrients released from the cultivated clam beds. But without proper management, this increased organic matter, may lead to adverse conditions in the estuary; upon senescence, microbial degradation of the macroalgae may decrease oxygen and release nutrients. Implementing macroalgal harvest practices concurrent with clam harvest (i.e. an integrated multi-trophic aquaculture (IMTA) program) would eliminate the potentially detrimental impacts of excess macroalgal material in the system.

Cultivated Clam Beds Alter Benthic Metabolism and Nutrient Supply

Clams directly alter the local environment through their respiration and excretion. We estimated clam excretion to account for between 40-83% of the total NH$_4^+$ efflux at the clam beds. Others have similarly observed that bivalve excretion can significantly increase net sediment nutrient effluxes (Magni et al. 2000, Hiwatari et al. 2002, Gibbs et al. 2005). Despite high excretion rates, the clam beds did not alter DON fluxes relative to bare sediments, likely because the majority of _M. mercenaria_ excretion is DIN rather than DON (Hammen 1980). Clam respiration is a large component of benthic community metabolism as clam biomass is significantly correlated with DO and DIC fluxes when data from all seasons were grouped. However, in the summer and fall, anaerobic microbial respiration was likely driving DIC fluxes, as these fluxes were not tightly
coupled to clam biomass. Additionally, DO fluxes in the summer were not strongly correlated with clam biomass, which may be due to the narrow range in biomass sampled as only market-size clam beds were targeted in this study and cultivated clams are planted at relatively constant densities. Despite high respiration rates, clam beds did not contribute to hypoxic conditions in the water column; nighttime DO at the clam beds was only slightly lower than concentrations observed at the control sites (see appendix).

Clams indirectly affect benthic nutrient fluxes and respiration by fueling microbial mineralization of biodeposits. Increased nutrient effluxes and sediment oxygen demand have been attributed to bivalves enriching sediments with biodeposits (e.g. Nizzoli et al. 2006, Smyth et al. 2013). The bulk sediment organic C to total N ratio in Cherrystone is relatively low (6.6-7.6) compared to nearby systems such as Hog Island Bay, VA that averages 13.3 (Anderson et al. 2003). In Cherrystone the low sediment C:N is likely due to delivery of N-rich, phytoplankton-derived clam biodeposits to the sediments. Bivalve biodeposits are typically labile and readily remineralized in sediments (Giles & Pilditch 2006, Carlsson et al. 2010). Although biodeposit mineralization can cause decreased DO and sulfide accumulation in the porewater, clam bioturbation can oxygenate the sediments, increasing rates of nutrient transformations and transport across the sediment-water interface (Aller 1982, Kristensen & Blackburn 1987, Kristensen 2000). However in an aquaculture setting bioturbation may be limited due to the high planting density, which may cause constricting conditions.

At our sites, clam aquaculture decreased the relative proportion of DIC to NH$_4^+$ fluxes compared to uncultivated bare sites, further highlighting that clam beds are a source of regenerated nutrients to the water column. At the net autotrophic bare sites, the
high DIC:NH$_4^+$ of the fluxes and increased uptake of N in the light versus dark incubations indicates N immobilization in the benthos by microphytobenthos (MPB); alternatively N may be removed by denitrification. At the clam beds low DIC release relative to NH$_4^+$ reflects the high rate of N recycling in the benthos and suggests low rates of denitrification and/or a lack of MPB uptake. High sulfide accumulation in the clam bed porewater may inhibit nitrification coupled to denitrification (Joye & Hollibaugh 1995), further enhancing NH$_4^+$ release to the water column. However, release of NO$_x$, albeit at low rates, at both the clam and bare sites suggests that nitrification may be occurring at low rates. Another potential source of NO$_x$ at the clam beds is subterranean groundwater discharge through the sandy sediments (Reay et al. 1992, Stanhope et al. 2009), which could be captured in this in situ experiment. Benthic chlorophyll, typically lower at the clam beds than the bare sites, suggests less MPB, potentially due to shading by the nets and macroalgal mats or due to restricted exchange and increased grazing by clams under the nets (Sauriau and Kang 2000, Cognie et al. 2001, Secrist 2013). When macroalgae were included at the clam beds, the DIC:NH$_4^+$ of the fluxes increased to 23.7, with a higher ratio in the light compared to the dark, indicating the significant role macroalgae play in modulating N released from the clam beds.

_Cultivated Clam Beds Support Macroalgal Production_

The dominant macroalga on clam nets in Cherrystone Inlet, the ephemeral, opportunistic _Gracilaria_ spp., have a high capacity to intercept nutrients sourced from the clam sediments; benthic NH$_4^+$ efflux was reduced by 20-77% and SRP efflux up to 43%. Other studies have similarly reported that macroalgae effectively assimilate nutrients
fluxing from sediments, temporarily sequestering them in their tissue (i.e. Sundbäck et al. 2003, McGlathery et al. 2003, Hardison et al. 2011). As macroalgae are not long-lived, this nutrient storage is only temporary and macroalgal biomass rapidly decays upon senescence, releasing inorganic and organic nutrients back to the water column (Tyler et al. 2001, Hardison et al. 2010).

By enhancing nutrient recycling, clam beds in Cherrystone serve as an important internal source of nutrients to primary producers within the system. Others have similarly found bivalves to greatly influence the availability of sediment derived nutrients to benthic and pelagic producers (Doering et al. 1986, Asmus & Asmus 1991, Giles & Pilditch 2006). In natural clam-dominated systems, nutrient fluxes from M. mercenaria beds can exceed phytoplankton net demand for N and P (Murphy & Kremer 1985). In Cherrystone, we found that the clam aquaculture sediments provided 58-122% of the macroalgal N demand. The percent of macroalgal N demand supplied by the benthos exceeds estimates reported in nearby systems (27-75%; Tyler et al. 2003), which rely more on external nutrient loading.

The relative importance of clam aquaculture as an internal nutrient source to the system was assessed by comparing the total NH$_4^+$ released from all the clam beds in Cherrystone Inlet to the external watershed load, estimated at 30,269 kg N yr$^{-1}$ (Kuschner & Brush, in prep). Although according to the Virginia Marine Resources Commission the entire embayment is leased for shellfish aquaculture, only some of the leases are active. Provided the actual coverage of clam aquaculture in Cherrystone, estimated using aerial photographs taken in 2012 and GIS delineation (Fig. 1), is between 181,008 and 476,048 m$^2$ (Emery, in prep), we found that the total NH$_4^+$ released from clam operations
(without macroalgal uptake) is 37-98% that of the N load from the watershed. Therefore, this considerable amount of N recycled in the benthos fuels autotrophic production. Not only do cultivated clam beds provide nutrients, the shallow nets serve as a convenient structure for macroalgal attachment in close proximity to the nutrient source, allowing them to outcompete other primary producers (i.e. phytoplankton, benthic microalgae, and submerged aquatic vegetation) for nutrients, light, and space.

The 'bottom-up' influence of clam aquaculture on macroalgal production is certainly site-specific and dependent on a number of environmental factors such as external nutrient loading, residence time, depth, etc. For example, nutrient regeneration facilitated by bivalve aquaculture is likely more consequential, in pristine systems with low external nutrient loading, such as Cherrystone, compared to systems where allochthonous sources dominate. Additionally, the source of phytoplankton filtered by cultivated bivalves determines whether the nutrients regenerated in the benthos represent those already existing in the system or originating outside the system. If the residence time of the system is short, the particulate nutrients (i.e. phytoplankton) subsidizing bivalve growth are likely sourced from outside the system (e.g. the Chesapeake Bay) and delivered by incoming tides. If bivalves feed primarily on externally produced phytoplankton, the bivalves facilitate the regeneration of nutrients that would not be present if cultivation were not there.

**Macroalgae have a High Capacity to Sequester N**

Unlike natural systems, macroalgal biomass on cultivated clam nets is controlled by aquaculture management practices, specifically the frequency of net sweeping. Nets
are regularly cleaned to prevent detrimental effects on the clams due to decreased water flow as macroalgae accumulate. If the aquaculturists sweep the nets often enough to prevent density-dependent limitations of macroalgal production and N uptake (i.e. self-shading, competition for nutrients, etc.), it can be assumed that the macroalgae grow at optimal rates given the water temperature. Based on our estimates of seasonal macroalgal N demand, the number of clam beds (approximately 700, each 72 m²) within the studied farm, and assuming negligible N uptake in the winter months, macroalgae have the capacity to assimilate approximately 3,652 kg N yr⁻¹ on this single farm, an amount equivalent to 116% of the annual NH₄⁺ released from the clam operation if no macroalgae were on the nets (3,158 kg N yr⁻¹) and assuming negligible release in the winter months from the clam sediments. Notably, macroalgal production rates and hence N uptake rates are likely overestimated as macroalgal C exudation, which could range from 0.5 to 40% of the total C fixed (e.g. Khailov & Burlakova 1969, Tyler & McGlathery 2006), was not included in the calculation. Additionally losses due to grazing and detachment/floating away were not taken into account. Nonetheless, given this high ability of macroalgae to intercept and temporarily sequester nutrients from the clam beds, harvesting the macroalgae could remove a significant amount of N from the system, decreasing the local nutrient input of the clam operation.

Though IMTA has generally been used to refer to a cultivation approach in which a fed species (finfish) is grown in combination with an organic extractive species (bivalves) and an inorganic extractive species (macroalgae) (Troell et al. 2009), to the extent that cultured clams in Cherrystone Inlet are serving to concentrate nutrients from a broader area, many of the same principles should apply. To develop an efficient IMTA
program in which clams and macroalgae are harvested concurrently, additional research is needed to determine how macroalgal growth rates differ across the farm and due to aquaculture management practices (i.e. net cleaning). The assumption that the macroalgae grow at optimal rates on the clam nets is most certainly an overly simplified reality, which neglects density-dependent effects on growth and N uptake. Macroalgal growth rates and N demands are variable and strongly dictated by the frequency in which the nets are swept. After the nets are cleaned, growth rates will initially be low when little macroalgal biomass is present, and then increase as biomass accumulates. However, as the macroalgal mats become thick, self-shading will result in decreased growth rates and N demands. More accurate measurements of macroalgal function and nutrient uptake are required to develop best management practices within an IMTA framework; specifically the frequency of sweeping that optimizes macroalgal nutrient sequestration while minimizing negative effects of dense macroalgal mats on clam growth.

Macroalgal harvest would benefit the ecosystem by decreasing the risk of eutrophic conditions, particularly when macroalgae senesce and are mineralized. However, a successful IMTA in Cherrystone would require economic sustainability. In the US macroalgae have a limited market, making economic viability a challenge. Developing a market for the macroalgae, which is a naturally mixed species community, would likely be the largest challenge. However, possibilities exist; for example using the material as fertilizer on agriculture fields, as a carbon source for producing biofuel (Wei et al. 2013), or as feed for poultry farms (Abudabos et al. 2013)). Clearly, many challenges would need to be addressed prior to implementing IMTA, however the potential ecological benefit of harvesting the macroalgae should serve as motivation.
Conclusions

Our data suggest bivalve aquaculture may promote local primary production by recycling nutrients to the water column. The extensive clam cultivation operations in Cherrystone Inlet serve a significant role in nutrient cycling, altering the dominant primary producers. The clams filter particulate nutrients (i.e. phytoplankton) from the water column; a portion of this material is transformed to dissolved nutrients and subsequently to particulate nutrients again, but now in the form of macroalgae (Fig. 4). Although macroalgae temporarily sequester nutrients from the clam sediments, common management practice is to clean the macroalgae off the predator-exclusion nets, allowing it to drift away. The fate of this macroalgae is likely microbial decomposition, which releases the sequestered nutrients back to the water column and may lead to hypoxic conditions in the system. If macroalgae were harvested, a considerable amount of aquaculture-facilitated recycled N would be removed from the system. The potential ecological benefit in establishing an IMTA system in which both clams and macroalgae are harvested should be further assessed.

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greatly improved this manuscript. We also thank the 3 anonymous reviewers for their constructive comments. This work was supported by Virginia Sea Grant (NA10OAR4170085, # R/71515W), The National Science Foundation GK12 Fellowship (NSF DGE-0840804), and NSF Virginia Coast Reserve Long Term Ecological Research (LTER 0080381, 0621014). This manuscript is contribution No. XXXX from the Virginia Institute of Marine Science, College of William and Mary.
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Emery K (in prep) Master's thesis, University of Virginia, Charlottesville, VA


Kuschner M, Brush M (in prep) Master's thesis, Virginia Institute of Marine Science, Gloucester Point, VA


Table 2-1. Seasonal site characteristics, clam biomass, density, and shell length observed at the clam beds where flux incubations were conducted. Means and (standard error) are shown for all parameters.

<table>
<thead>
<tr>
<th>Month</th>
<th>Salinity</th>
<th>Temperature (°C)</th>
<th>Chlorophyll a (ug l⁻¹)</th>
<th>Dissolved Oxygen (mg l⁻¹)</th>
<th>Kₐ (m⁻¹)</th>
<th>Clam Biomass (ash free DW g m⁻²)</th>
<th>Clam Density (individuals m⁻²)</th>
<th>Mean Clam Size (shell length, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>20.3 (0.03)</td>
<td>20.8 (0.08)</td>
<td>5.19 (0.09)</td>
<td>8.42 (0.11)</td>
<td>1.48 (0.03)</td>
<td>242.9 (19.0)</td>
<td>821 (82.5)</td>
<td>40.3 (0.3)</td>
</tr>
<tr>
<td>July</td>
<td>22.2 (0.05)</td>
<td>29.3 (0.13)</td>
<td>4.41 (0.08)</td>
<td>7.18 (0.16)</td>
<td>2.16 (0.09)</td>
<td>228.9 (16.6)</td>
<td>790 (47.7)</td>
<td>39.8 (0.4)</td>
</tr>
<tr>
<td>October</td>
<td>25.3 (0.01)</td>
<td>18.5 (0.06)</td>
<td>2.05 (0.07)</td>
<td>8.19 (0.05)</td>
<td>1.43 (0.03)</td>
<td>319.27 (18.8)</td>
<td>999 (70.5)</td>
<td>40.4 (0.3)</td>
</tr>
</tbody>
</table>
Table 2-2. Seasonal sediment and water quality characteristics of the bare and clam sites, including porewater (PW) and water column (WC) nutrients (μM), PW sulfide (μM), benthic chlorophyll and phaeophytin (μg cm⁻²), sediment organic matter (%), and sediment organic carbon to total nitrogen ratio (C:N).

<table>
<thead>
<tr>
<th></th>
<th>MAY</th>
<th>JULY</th>
<th>OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PW NH₄⁺</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>20.95 (5.4)*</td>
<td>56.3 (6.5)</td>
<td>46.15 (4.5)*</td>
</tr>
<tr>
<td>Clam</td>
<td>118.7 (25.2)</td>
<td>66.1 (14.3)</td>
<td>113.2 (14.2)</td>
</tr>
<tr>
<td><strong>WC NH₄⁺</strong></td>
<td>Bare†</td>
<td>Clam†</td>
<td>Bare†</td>
</tr>
<tr>
<td>Bare</td>
<td>0.31 (0.17)</td>
<td>0.56 (0.10)</td>
<td>0.94 (0.20)</td>
</tr>
<tr>
<td>Clam</td>
<td>0.99 (0.15)</td>
<td>1.98 (0.36)</td>
<td>1.12 (0.05)</td>
</tr>
<tr>
<td><strong>PW NO₃⁻</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>0.75 (0.11)</td>
<td>0.34 (0.09)</td>
<td>1.16 (0.56)</td>
</tr>
<tr>
<td>Clam</td>
<td>0.37 (0.04)</td>
<td>0.39 (0.09)</td>
<td>0.39 (0.07)</td>
</tr>
<tr>
<td><strong>WC NO₃⁻</strong></td>
<td>Bare†</td>
<td>Clam†</td>
<td>Bare†</td>
</tr>
<tr>
<td>Bare</td>
<td>0.24 (0.04)</td>
<td>0.12 (0.05)</td>
<td>0.11 (0.03)</td>
</tr>
<tr>
<td>Clam</td>
<td>0.11 (0.02)</td>
<td>0.12 (0.05)</td>
<td>0.11 (0.03)</td>
</tr>
<tr>
<td><strong>PW PO₄³⁻</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>1.32 (0.39)*</td>
<td>4.12 (0.40)</td>
<td>3.02 (0.44)*</td>
</tr>
<tr>
<td>Clam</td>
<td>8.5 (1.25)</td>
<td>5.9 (1.53)</td>
<td>7.8 (1.23)</td>
</tr>
<tr>
<td><strong>WC PO₄³⁻</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>0.06 (0.004)</td>
<td>0.08 (0.01)*</td>
<td>0.11 (0.01)</td>
</tr>
<tr>
<td>Clam</td>
<td>0.04 (0.004)*</td>
<td>0.15 (0.02)</td>
<td>0.11 (0.01)</td>
</tr>
<tr>
<td><strong>PW Sulfide</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>NS¹</td>
<td>91.9 (24.0)</td>
<td>116.9 (23.2)</td>
</tr>
<tr>
<td>Clam</td>
<td>NS¹</td>
<td>127.6 (13.0)</td>
<td>206.8 (63.5)</td>
</tr>
<tr>
<td><strong>Benthic Chl a</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>3.50 (0.34)</td>
<td>5.62 (0.42)</td>
<td>4.78 (0.38)</td>
</tr>
<tr>
<td>Clam</td>
<td>1.31 (0.28)</td>
<td>3.09 (0.18)*</td>
<td>3.40 (0.13)*</td>
</tr>
<tr>
<td><strong>Benthic Phaeophytin</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>1.31 (0.11)*</td>
<td>3.09 (0.25)*</td>
<td>3.40 (0.50)*</td>
</tr>
<tr>
<td>Clam</td>
<td>5.53 (0.38)</td>
<td>6.98 (0.40)</td>
<td>6.75 (0.38)</td>
</tr>
<tr>
<td><strong>Sediment OM</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>0.99 (0.13)</td>
<td>0.84 (0.09)*</td>
<td>0.90 (0.11)</td>
</tr>
<tr>
<td>Clam</td>
<td>1.09 (0.19)</td>
<td>1.87 (0.28)</td>
<td>1.02 (0.13)</td>
</tr>
<tr>
<td><strong>Sediment C:N</strong></td>
<td>Bare</td>
<td>Clam</td>
<td>Bare</td>
</tr>
<tr>
<td>Bare</td>
<td>7.55 (0.37)</td>
<td>7.40 (0.17)</td>
<td>7.34 (0.16)</td>
</tr>
<tr>
<td>Clam</td>
<td>6.63 (0.46)</td>
<td>7.39 (0.13)</td>
<td>7.03 (0.09)</td>
</tr>
</tbody>
</table>

Standard errors reported in parentheses.

¹ NS, no sample; sulfide samples were not collected in May

* denotes significant difference between treatments within each month (post hoc results, implies significant interactions were observed between month and season)
† denotes significant difference between treatments across all months (implies no significant interactions were observed)
Table 2-3. Mean biomass (g DW m⁻²) and (standard error) of macroalgae by species retrieved from the CML and CMD cores after the incubations during each season. The species within the experimental cores reflects the ambient species composition found in situ during each month. Mean percent total N and organic C content of each species per month. Standard error is included in parenthesis. NS denotes no subsamples were collected for CN analysis.

<table>
<thead>
<tr>
<th>Species</th>
<th>May Biomass</th>
<th>% N</th>
<th>% C</th>
<th>July Biomass</th>
<th>% N</th>
<th>% C</th>
<th>October Biomass</th>
<th>% N</th>
<th>% C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ulva lactuca</em></td>
<td>0.52 (0.2)</td>
<td>2.44 (0.43)</td>
<td>27.08 (2.5)</td>
<td>1.54 (0.5)</td>
<td>NS</td>
<td>NS</td>
<td>0</td>
<td>41.1 (4.9)</td>
<td>3.53 (0.3)</td>
</tr>
<tr>
<td><em>Gracilaria</em></td>
<td>10.35 (2.0)</td>
<td>1.51 (0.04)</td>
<td>27.84 (0.9)</td>
<td>84.1 (6.8)</td>
<td>3.40 (0.3)</td>
<td>33.96 (2.3)</td>
<td>8.88 (2.9)</td>
<td>2.76 (0.4)</td>
<td>29.42 (1.1)</td>
</tr>
<tr>
<td><em>Agardhiella</em></td>
<td>0</td>
<td>0</td>
<td>NS</td>
<td>38.17 (6.7)</td>
<td>2.80 (0.2)</td>
<td>28.28 (0.8)</td>
<td>NS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Other</em></td>
<td>0.57 (0.1)</td>
<td>NS</td>
<td>NS</td>
<td>0</td>
<td>NS</td>
<td>NS</td>
<td>52.8 (2.8)</td>
<td>3.39</td>
<td>33.69</td>
</tr>
</tbody>
</table>

Species-weighted mean of macroalgal community

<table>
<thead>
<tr>
<th></th>
<th>May % N</th>
<th>% C</th>
<th>July % N</th>
<th>% C</th>
<th>October % N</th>
<th>% C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.02</td>
<td>27.32</td>
<td>3.22</td>
<td>32.19</td>
<td>3.39</td>
<td>33.69</td>
</tr>
</tbody>
</table>

* ‘Other’ species in May were predominantly *Cladophora* sp.

NS, no sample
Table 2-4. Statistical parameters from the mixed-effect models with Treatment and Month as fixed factors on daily flux rates, including F-statistics (F Stat), degrees of freedom (DF) and p value for each test. Summaries of the post hoc test results are provided when a significant interaction was observed. NA: No significant interaction term was observed for the DOC fluxes. n.s: B=C=CM.

<table>
<thead>
<tr>
<th>Response</th>
<th>Factors</th>
<th>F Stat</th>
<th>DF</th>
<th>p value</th>
<th>Post hoc Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Month within Treatment</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>Treatment</td>
<td>220.99</td>
<td>2</td>
<td>&lt;0.001</td>
<td>C: n.s.</td>
</tr>
<tr>
<td></td>
<td>Month</td>
<td>5.47</td>
<td>2</td>
<td>0.044</td>
<td>CM: J &lt; M = O</td>
</tr>
<tr>
<td></td>
<td>Treatment * Month</td>
<td>5.17</td>
<td>4</td>
<td>0.001</td>
<td>B: n.s.</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>Treatment</td>
<td>0.60</td>
<td>2</td>
<td>0.55</td>
<td>C: n.s.</td>
</tr>
<tr>
<td></td>
<td>Month</td>
<td>0.13</td>
<td>2</td>
<td>0.88</td>
<td>CM: J &lt; M = O</td>
</tr>
<tr>
<td></td>
<td>Treatment * Month</td>
<td>4.07</td>
<td>4</td>
<td>0.005</td>
<td>B: n.s.</td>
</tr>
<tr>
<td>DON</td>
<td>Treatment</td>
<td>0.45</td>
<td>2</td>
<td>0.64</td>
<td>C: n.s.</td>
</tr>
<tr>
<td></td>
<td>Month</td>
<td>0.76</td>
<td>2</td>
<td>0.51</td>
<td>CM: M &lt; O</td>
</tr>
<tr>
<td></td>
<td>Treatment * Month</td>
<td>4.42</td>
<td>4</td>
<td>0.003</td>
<td>B: n.s.</td>
</tr>
<tr>
<td>SRP</td>
<td>Treatment</td>
<td>122.40</td>
<td>2</td>
<td>&lt;0.001</td>
<td>C: J = M &gt; O</td>
</tr>
<tr>
<td></td>
<td>Month</td>
<td>4.17</td>
<td>2</td>
<td>0.07</td>
<td>CM: M &gt; O</td>
</tr>
<tr>
<td></td>
<td>Treatment * Month</td>
<td>4.67</td>
<td>4</td>
<td>0.002</td>
<td>B: n.s.</td>
</tr>
<tr>
<td>DOC</td>
<td>Treatment</td>
<td>2.77</td>
<td>2</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month</td>
<td>2.86</td>
<td>2</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment * Month</td>
<td>1.36</td>
<td>4</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>NCP</td>
<td>Treatment</td>
<td>52.60</td>
<td>2</td>
<td>&lt;0.001</td>
<td>C: J = O &lt; M</td>
</tr>
<tr>
<td></td>
<td>Month</td>
<td>6.30</td>
<td>2</td>
<td>0.033</td>
<td>CM: J &lt; M = O</td>
</tr>
<tr>
<td></td>
<td>Treatment * Month</td>
<td>4.50</td>
<td>4</td>
<td>0.003</td>
<td>B: n.s.</td>
</tr>
</tbody>
</table>
Table 2-5. Regression statistics of hourly fluxes of ammonium (NH₄⁺), dissolved inorganic carbon (DIC), and dissolved oxygen (DO) as a function of clam biomass (g ash-free DW m⁻²). Only clam treatments (light and dark) were included in the analyses. Figure 3 provides graphical representation of the data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Month</th>
<th>Slope</th>
<th>R²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺ (mmol N m⁻² hr⁻¹)</td>
<td>May</td>
<td>0.002</td>
<td>0.42</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>0.001</td>
<td>0.04</td>
<td>0.533</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>0.002</td>
<td>0.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>0.001</td>
<td>0.13</td>
<td>0.008</td>
</tr>
<tr>
<td>DIC (mmol C m⁻² hr⁻¹)</td>
<td>May</td>
<td>0.071</td>
<td>0.44</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>0.024</td>
<td>0.10</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>0.008</td>
<td>0.03</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>0.033</td>
<td>0.15</td>
<td>0.005</td>
</tr>
<tr>
<td>DO (mmol O m⁻² hr⁻¹)</td>
<td>May</td>
<td>-0.014</td>
<td>0.31</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>-0.002</td>
<td>0.01</td>
<td>0.822</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>-0.011</td>
<td>0.29</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>-0.011</td>
<td>0.13</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Table 2-6. Linear regression estimates of the relative proportion (slope) of dissolved inorganic carbon (DIC) to ammonium (NH$_4^+$) fluxes on a net daily basis, as well as in the dark and in the light for all treatments: clam only ®, clam plus macroalgae (CM), and control sediment (B).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Slope</th>
<th>$R^2$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>9.9</td>
<td>0.23</td>
<td>0.007</td>
</tr>
<tr>
<td>CM</td>
<td>23.7</td>
<td>0.56</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>66.1</td>
<td>0.16</td>
<td>0.022</td>
</tr>
<tr>
<td>Dark</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>8.3</td>
<td>0.07</td>
<td>0.098</td>
</tr>
<tr>
<td>CM</td>
<td>10.3</td>
<td>0.29</td>
<td>0.002</td>
</tr>
<tr>
<td>B</td>
<td>39.9</td>
<td>0.10</td>
<td>0.062</td>
</tr>
<tr>
<td>Light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>13.1</td>
<td>0.27</td>
<td>0.003</td>
</tr>
<tr>
<td>CM</td>
<td>30.1</td>
<td>0.47</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>26.7</td>
<td>-0.02</td>
<td>0.466</td>
</tr>
</tbody>
</table>
Figure 2-1. Cherrystone Inlet, Chesapeake Bay, USA.

Aerial photograph of Cherrystone Inlet taken in 2012. Black polygons delineate active clam aquaculture operations.
Figure 2-2 A – E. Net daily flux measurements.

Net daily flux rates of ammonium (NH$_4^+$) (A), nitrate+nitrite (NO$_x$) (B), dissolved organic nitrogen (DON) (C), soluble reactive phosphorous (SRP) (D), dissolved organic carbon (DOC) (E), and benthic community production, calculated using DIC fluxes (F), for all treatments including clam only (Clam), clam plus macroalgae (Clam+Macro), and control sediment (Bare), in May (gray), July (white), and October (black).
Figure 2-3. Hourly flux rates as a function of clam biomass.

Hourly (light and dark) ammonium (NH$_4^+$) (A), dissolved inorganic carbon (DIC) (B), and dissolved oxygen (DO) (C) fluxes as a function of clam biomass (ash-free DW g m$^{-2}$), in May (gray), July (white), and October (black). Analyses included clam dark and clam light treatments only. The solid line is the regression including all months. Slopes and regression statistics are provided in Table 3.
A. $y = 0.0013x + 0.35$
$R^2 = 0.15$

B. $y = 0.033x + 0.19$
$R^2 = 0.16$

C. $y = -0.011x - 3.24$
$R^2 = 0.15$
Figure 2-4. A conceptual model illustrating the net annual fluxes of NH$_4^+$, NO$_x^-$ (mol N m$^{-2}$ yr$^{-1}$) and NCP (mol C m$^{-2}$ yr$^{-1}$); a positive flux represents net heterotrophy; a negative flux net autotrophy.
Appendix A.

Continuous dissolved oxygen in the bottom water at the clam bed (black) and bare, uncultivated (gray) sites during each 3-day experiment. Shaded boxes represent nighttime hours. DO probes were placed approximately 5 cm from the sediment-water interface and continuously monitored over the 3-day period.
CHAPTER 3: MICROBIAL NITROGEN PROCESSING IN COMMERCIAL HARD CLAM
(MERCENARIA MERCENARIA) AQUACULTURE SEDIMENTS: NITROGEN REMOVAL VERSUS
RECYCLING PATHWAYS

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Keywords: Denitrification, DNRA, Clam, Eutrophication, Aquaculture, Mineralization,
nirS, nrfA, Nitrogen cycling

Running Title: Nitrogen cycling in clam aquaculture sediment

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ABSTRACT

As commercial bivalve aquaculture expands worldwide, an understanding of its role in nutrient cycling is necessary to ensure ecological sustainability and determine the potential of using bivalves for nutrient mitigation. Whereas several studies, primarily of epifaunal bivalves, have assessed denitrification, few have considered nutrient regeneration processes such as dissimilatory nitrate reduction to ammonium (DNRA), which competes with denitrification for nitrate and results in retention rather than loss of nitrogen. To our knowledge this study is the first to characterize sediment nitrogen cycling including mineralization, DNRA, and denitrification within U.S. clam aquaculture sediments and to compare with nearby uncultivated sediments, seasonally. Clam aquaculture significantly increased sediment ammonium and phosphate effluxes relative to uncultivated sediments. Both DNRA and denitrification were significantly enhanced at clam beds compared to uncultivated sediments in July and November, while in May only DNRA was increased. The relative proportion of DNRA to denitrification was significantly higher at the clam beds compared to uncultivated sediments, demonstrating DNRA is favored likely due to a ready supply of labile organic carbon, low nitrate, and sulfidic conditions. Functional gene abundances, \textit{nrfA} (DNRA) and \textit{nirS} (denitrification) followed similar patterns to nitrate respiration rates with highest \textit{nrfA} abundances at the clam sediments and similar \textit{nirS} abundance across seasons and sediment type. Ultimately clam sediments were found to be a significant source of nutrients to the water column whereas uncultivated sediments retained \textit{NH}_4^+ produced by microbial mineralization. Thus, clam cultivation may promote local primary production by facilitating nutrient regeneration in the sediments.
INTRODUCTION

Global aquaculture production more than doubled from 2000 to 2012 (FAO 2014) with bivalve production accounting for about 70% of total mariculture production (Campbell and Pauly 2013). The continued growth of the bivalve aquaculture industry globally, concurrent with increased coastal eutrophication, has prompted recent interest in the potential role bivalves may play in removing bioreactive nitrogen (N) (Stadmark and Conley 2011; Petersen et al. 2014; Bricker et al. 2014). Sediments associated with high densities of suspension feeding bivalves are often characterized as having high rates of denitrification, the microbially mediated removal of bioreactive nitrogen (N), relative to local reference sediments (Newell 2004; Kellogg et al. 2013; Smyth et al. 2013). Due to their impressive capacity to remove particulates from the water column and potentially enhance denitrification, increasing bivalve populations through either restoration or aquaculture has been proposed as an effective in-water strategy to reduce N in aquatic environments and subsequently mitigate eutrophication (Lindahl et al. 2005; Rose et al. 2014). However, by delivering organic matter to anaerobic sediments through filtration and biodeposition, bivalves may also create favorable conditions for dissimilatory nitrate reduction to ammonium (DNRA), the recycling of nitrate (NO$_3^-$) back to ammonium (NH$_4^+$). Thus the affect of bivalves on the partitioning of NO$_3^-$ between the two competing pathways will dictate the degree to which bivalves facilitate microbial N removal.

The competition for NO$_3^-$ between denitrification and DNRA, is of significant ecological importance due to the outcomes of the processes: N removal versus retention, respectively. Denitrification occurs widely in coastal anoxic sediments where both
organic matter and NO₃⁻ are available (Seitzinger et al. 2006); DNRA has been less studied and its distribution across aquatic systems is not fully understood (Burgin and Hamilton 2007; Giblin et al. 2013). Further, the factors that control the partitioning of NO₃⁻ between denitrification and DNRA are complex and not well defined across all systems. However, environmental factors such as NO₃⁻ supply, sulfide concentrations, and organic carbon quality have been shown to affect NO₃⁻ respiration rates and determine the dominant pathway (i.e. denitrification or DNRA) (Magni et al. 2000; Hiwatari et al. 2002; Gibbs et al. 2005; Burgin and Hamilton 2007; Algar and Vallino 2014).

The relative importance of denitrification and DNRA in bivalve-dominated systems is likely to differ depending on environmental factors, the physiology and behavior of the bivalve species, and the ecological setting (i.e. natural or aquaculture). Few studies have fully characterized N cycling rates within bivalve aquaculture systems, with many neglecting to consider recycling processes including DNRA, microbial mineralization, and direct bivalve excretion (as reviewed in Burkholder and Shumway 2011). Furthermore, the majority of previous studies have focused on epifaunal bivalves (i.e. oysters and mussels) with few considering the effects of infaunal bivalve species on NO₃⁻ respiration rates (but see (Nizzoli et al. 2006). In fact, this is the first study to our knowledge to characterize N cycling rates at a U.S. commercial clam aquaculture site.

Clam activities such as biodeposition, bioturbation, and excretion likely influence the dominant NO₃⁻ respiration pathway by altering NO₃⁻ and dissolved oxygen (DO) supply, sulfide concentrations, and organic carbon quality. For example, nitrification, a two-step aerobic process in which NH₄⁺ is oxidized to NO₂⁻ and NO₃⁻, may be enhanced
by clam bioturbation and excretion, which supply DO and NH$_4^+$, respectively to the sediments (Hammen 1980; Henriksen et al. 1983; Nizzoli et al. 2006). Nitrification is often tightly coupled to NO$_3^-$ reduction pathways in estuarine sediments, serving as an important NO$_3^-$ source (Seitzinger et al. 2006); thus, by potentially increasing nitrification, clams may enhance denitrification and/or DNRA. Alternatively, clam biodeposition may suppress nitrification by fueling microbial mineralization and increasing sediment oxygen demand (SOD), resulting in reduced sediments with high sulfide. Low DO and sulfide accumulation may inhibit nitrification (Joye and Hollibaugh 1995; Giles and Pilditch 2006; Carlsson et al. 2010), causing NO$_3^-$ limitation, which concurrent with high organic carbon concentrations, may favor DNRA over denitrification (Aller 1982; Kristensen and Blackburn 1987; Tiedje 1988; Kristensen 2000; Algar and Vallino 2014). Additionally, sulfide may directly inhibit the last step in denitrification, the conversion of N$_2$O to N$_2$ (Joye and Hollibaugh 1995; Brunet and Garcia-Gil 1996), while enhancing chemoautotrophic DNRA, in which sulfide rather than organic matter serves as the electron donor during NO$_3^-$ respiration. Thus, high densities of clams associated with aquaculture will likely have significant and complex effects on NO$_3^-$ respiration, affecting both the rates and the dominant pathway.

The overall objective of our study was to determine the effects of clam aquaculture on sediment nutrient dynamics including rates of N removal and N recycling. Specifically, we were interested in determining the relative importance of DNRA versus denitrification in clam beds compared to nearby uncultivated sediments. The functional genes encoding nitrite reductase, cytochrome cd nitrite reductase (nirS) and cytochrome C reductase (nrfA), were selected to quantify abundance of denitrifying and DNRA.
communities, respectively. The relationships between \( \textit{nirS} \) and \( \textit{nrfA} \) gene abundances to rates of denitrification and DNRA were investigated. Our experimental design aimed to capture a range of sediment conditions (i.e. porewater sulfide and sediment organic content) across a clam aquaculture lease to account for variability due to clam size-class and time since clams were planted. We hypothesized that by delivering labile organic matter to the sediments, clam cultivation will enhance overall nitrate respiration rates above control sediments; however, DNRA will be dominant over denitrification, which will be reflected in higher \( \textit{nrfA} \) abundances than \( \textit{nirS} \) abundances.

METHODS

*Site Description*

Located on the bayside of the Eastern Shore of Virginia, Cherrystone Inlet is a shallow tributary of Chesapeake Bay (Figure 1). Hard clams (*Mercenaria mercenaria*) are cultured in the shallow subtidal regions of the estuary (<1m, MLW). Approximately 145 million cultivated clams inhabit the private shellfish leases across the 5.6 km\(^2\) embayment at any given time. Juvenile clams (8-15 mm), reared in land-based hatcheries and nurseries, are planted directly in the sediments. A plastic net, set flush to the sediment surface, is used to protect the clams from natural predators. After about 2 years, market-sized clams are mechanically harvested from the sediments (Castagna and Kraeuter 1981).

*Sampling Design*
The sampling design aimed to capture a range of sediment conditions varying both seasonally and spatially across a leased area. Each clam bed (approximately 4 m x 18 m) consists of approximately 50,000 clams of a homogenous size-class, as the clams within each bed are all planted at the same time. As a result, we anticipated that the clam beds across the lease would have varying levels of organic matter enrichment as well as porewater sulfide and nutrient concentrations, depending on clam metabolism and length of time clams had occupied the space. In May and July 2013, 16 randomly selected clam beds and 4 uncultivated sites and in November 2013, 7 randomly selected clam beds and 3 uncultivated sites were sampled. Uncultivated sites were located adjacent to, approximately 20 m, from the clam beds.

* Sediment and Water Column Characteristics *

At each clam bed and uncultivated site sampled, porewater was collected at 5-7 cm below the sediment surface using a stainless steel push-point sampler (MHE Products, East Tawas, MI, USA) for nutrient and hydrogen sulfide analysis. Porewater sulfide samples were immediately fixed in zinc acetate, filtered, and stored until analysis on a spectrophotometer within a week of collection (Cline 1969). Triplicate water column grab samples, collected over the clam beds and uncultivated sites, were filtered (0.45 uM Whatman polyethersulfone (PES)) and frozen until analysis for dissolved inorganic nitrogen (DIN) (including NO₃⁻, NO₂⁻, and NH₄⁺) (Liao 2001) and soluble reactive phosphorus (SRP) (Knapel and Bogren 2001) on a Lachat QuikChem 8000 automated ion analyzer (Lachat Instruments Milwaukee, WI, USA).
A sediment core (2.2 cm i.d.) was collected at each clam bed and uncultivated site, sub-sectioned horizontally at 0-2 cm and 2-5 cm and analyzed for porosity, as loss of wet weight after drying at 70°C, and organic content, as loss on ignition after combustion at 500°C for 5 hours. Prior to combustion, subsamples of dried sediments were acidified and analyzed on a Carlo Erba elemental analyzer (Thermo Electron Corp. Flash EA 1112 Series) for organic C and total N content.

Surface sediment samples (0-3 cm) were collected at a subset of clam beds (n=7, 6, 6 in May, July, and November, respectively) and uncultivated sites (n=3, 2, 3 in May, July, and November, respectively) using a small core (2.2 cm i.d.) for gene abundance analysis. Molecular samples were placed in liquid nitrogen in the field and stored at -80°C upon return to the lab until DNA extraction and molecular analysis were performed (see below for details).

**Benthic Metabolism and Nutrient Flux Measurements**

At each clam bed and uncultivated site three sediment cores (9.5 cm i.d., with approximately 10 cm overlying water and 8 cm sediment depth) were collected for determinations of benthic metabolism, nutrient fluxes and N cycling rates. Cores were not treated as replicates, but were used to conduct concurrent incubations in the light and dark (paired cores) and for measurement of N cycling by isotope-pairing (with a T₀ core; see details below). Cores were transported to the Virginia Institute of Marine Science Eastern Shore Laboratory (VIMS ESL) in Wachapreague, VA, placed in a water bath with continuously flowing water maintained at ambient conditions of the sampling location, and allowed to equilibrate overnight. Continuous flowing water was used to
provide continued supply of oxygen and phytoplankton to the clams and sediments overnight. Battery-operated central spinners powered magnetic stir bars suspended in each core to prevent gradient formation.

The following day, one core from each site was illuminated while the other two were kept dark (dark core and T₀ core). The T₀ cores were capped but not sampled during the initial flux incubation. Cores were capped with lids that contained an inflow and outflow port, with no air bubbles, and the overlying water was sampled through the outflow port approximately hourly for 3-4 hours for NH₄⁺, SRP, NOₓ⁻ (combined NO₂ and NO₃) and dissolved inorganic carbon (DIC). Samples collected for NH₄⁺, SRP and NOₓ⁻ were immediately filtered (0.45 μM Whatman polyethersulfone (PES)) and frozen until analysis (as described above). DIC samples were placed in 8 ml hungate tubes, pre-spiked with 15 μl saturated mercuric chloride, and stored cold underwater until analysis using a Li-Cor 6252 infrared gas analyzer within a month of collection as described by (Neubauer and Anderson 2003). During the incubation a Hach LDO101 Luminescent DO sensor (Hach Co., Loveland, CO, USA), secured in the lids of 12 randomly selected cores, continuously monitored DO in real time to determine the duration of the isotope pairing incubation which followed (necessary to keep DO above 70% of the original concentration; (Dalsgaard et al. 2000)).

Hourly and daily fluxes for each analyte were calculated as:

Hourly Flux = \((m \times V)/A\) \hspace{1cm} (1)

Daily Flux = \((F_i \times h_i) + (F_d \times h_d)\) \hspace{1cm} (2)
where \( m \) is equal to the slope of the linear regression of concentration (\( \mu \text{M} \) or \( \text{mM} \)) versus time (hours); \( V \) is equal to the volume of water in the flux chamber (liters); \( A \) is the sediment surface area within the chamber (\( m^2 \)); \( F_d \) and \( F_l \) are hourly fluxes in the dark and light, respectively (mmol \( m^{-2} \text{ hr}^{-1} \)), \( h_d \) and \( h_l \) are the number of hours of dark and light in a day, which varied by season. A flux from the sediment to the water column is positive (production) while a flux to the sediment from the water column is a negative value (consumption).

**Denitrification and DNRA Rate Measurements**

After the flux incubation, the sediment cores were uncapped and allowed to re-equitrate in the oxygenated water bath for at least an hour. Water level was dropped to just below the lip of the cores and each core was spiked with \( ^{15}\text{NO}_3^- \) (98.9 atm%) to obtain a final concentration of approximately 100 \( \mu \text{M} \). After spiking, a water sample was collected from each core and analyzed on the Lachat for total \( \text{NO}_3^- \) (\( ^{14+15}\text{NO}_3^- \)). The cores were left uncapped and each gently bubbled for about an hour to allow \( ^{15}\text{NO}_3^- \) to diffuse to the zone of active denitrification and DNRA in the sediments. The diffusion rate for \( ^{15}\text{NO}_3^- \) to reach the anoxic zone, estimated using calculations based on Fick's law, described in the NICE handbook (Dalsgaard et al. 2000) and the DO penetration depth of approximately 2 mm obtained using an oxygen microsensor and micromanipulator (OX100, Unisense, Aarhus N, Denmark) (A.E. Murphy, unpubl.), was approximately 16 minutes. At the completion of the pre-incubation period, the \( T_0 \) core from each site was sampled to account for any \( ^{29}\text{N}_2 \) and \( ^{30}\text{N}_2 \) produced prior to sealing the cores (see below
for details on post-incubation-sampling). The remaining light and dark cores from each site were capped and incubated for 2-4 hours depending on the sediment oxygen demand determined in the previous flux incubation allowing DO to drop no more than 70% of the initial concentration.

At the end of the incubation each core was uncapped, gently homogenized, and sampled for $^{29,30}\text{N}_2$ and extracted $^{15}\text{NH}_4^+$ (see below). After, all clams were removed from each core counted, measured, and the tissue ash-free dry weight (DW) determined by the difference in DW prior to and after combusting at 500°C for 5 h. Samples were collected for $^{29}\text{N}_2$ and $^{30}\text{N}_2$ by siphoning the slurry into a 12 ml exetainer vial and preserving the sample with 100 μl of 7M ZnCl₂. Samples were analyzed within a month on a membrane inlet mass spectrometer (MIMS) (Kana et al. 1994). For sediment $^{15}\text{NH}_4^+$ analysis, approximately 120 ml of the core slurry was collected in a whirlpak bag with potassium chloride (KCl) (final concentration of 2M), shaken for one hour, centrifuged, filtered (0.45 μM Whatman polyethersulfone (PES)), and stored frozen until diffusion. Samples were diffused and trapped for analysis of $^{15}\text{NH}_4^+$ enrichment and concentration using a method modified from Brooks (1989). Water samples were placed in specimen cups; an acidified (25μl of 2.5M sulfuric acid) GFF filter (1cm, i.d.), threaded onto a stainless steel wire, was suspended on the lip of the cup; magnesium oxide was added and the samples were allowed to diffuse for 2 weeks, after which samples were encapsulated in tin capsules and analyzed on an EA-IRMS at the University of California Davis Stable Isotope Facility.

Denitrification rates were calculated as described by (Nielsen 1992) as follows:
\[ D_{15} = p29 + 2p30 \]  
\[ D_{14} = D_{15} \times (p29/2p30) \]

where \( D_{15} \) represents denitrification of the added \(^{15}\text{NO}_3^-\); \( p29 \) and \( p30 \) are equal to the rates of production of \(^{29}\text{N}_2\) and \(^{30}\text{N}_2\), respectively, and \( D_{14} \) is the denitrification rate of ambient \(^{14}\text{NO}_3^-\). Direct denitrification of \( \text{NO}_3^- \) from the water column, \( (D_w) \), and coupled denitrification \( (D_n) \) were calculated as (Nielson 1992):

\[ D_w = \left( ^{14}\text{NO}_3^- / ^{15}\text{NO}_3^- \right) \times D_{15} \]  
\[ D_n = D_{14} - D_w \]

where \(^{14}\text{NO}_3^-\) is equal to the ambient unlabeled \( \text{NO}_3^- \) concentration (\( \mu\text{M} \)) and \(^{15}\text{NO}_3^-\) is equal to the isotopically-labeled \( \text{NO}_3^- \) concentration at the start of the incubation.

Actual DNRA rates were calculated according to Risgaard-Petersen & Rysgaard (1995) as:

\[ \text{DNRA}_t = p^{15}\text{NH}_4^+ \times (D_{14}/D_{15}) \]

where \( p^{15}\text{NH}_4^+ \) is equal to the production of \(^{15}\text{NH}_4^+\). This assumes that DNRA occurs in the same sediment horizon as denitrification (Rysgaard et al. 1993). DNRA coupled to nitrification \( (\text{DNRA}_n) \) and direct from water column \( \text{NO}_3^- \) \( (\text{DNRA}_w) \) were calculated as:
Gross ammonification measurements

Gross ammonification rates, which include NH\textsubscript{4}\textsuperscript{+} production from organic matter mineralization and some contribution from DNRA and heterotrophic N fixation, were measured using the isotope pool dilution method (Anderson et al. 1997). Two paired cores (5.7 cm i.d, with approximately 5 cm overlying water and 5 cm sediment depth) were collected at each sampling site, transported to the laboratory, and placed in a water bath filled with site water. Prior to collection in the field, clams were carefully removed from the area to obtain sediments void of clams in order to measure microbial ammonification independent of the contribution of clam excretion. Cores were uncapped and held underwater overnight in the dark with gentle mixing and aeration. The following day the sediments were homogenously spiked with \textsuperscript{15}N-NH\textsubscript{4}\textsuperscript{+} (3.6 ml of [NH\textsubscript{4}]\textsubscript{2}SO\textsubscript{4}, 30 at.\%, 10 mM) by injecting 100 μl of the stock solution into 36 silicone-filled holes through the vertical sediment column. Prior to sacrificing, the cores were sectioned horizontally 0-2 cm and 2-5 cm, although rates did not differ between the two horizons and therefore only rates associated with the top 2 cm are reported. One of the paired cores from each site, T\textsubscript{0}, was immediately sacrificed after spiking by shaking in 2M KCl for an hour; the extractant was filtered and frozen until analysis. The remaining core from each site, T\textsubscript{r}, was capped and incubated for 24 hours in the dark at in situ temperatures. After

\begin{equation}
\text{DNRA}_w = \left(\frac{1^{14}\text{NO}_3^-}{1^{15}\text{NO}_3^-}\right) \cdot p^{15}\text{NH}_4^+ \tag{8}
\end{equation}

\begin{equation}
\text{DNRA}_n = \text{DNRA}_r - \text{DNRA}_w \tag{9}
\end{equation}
the incubation, the Tf cores were extracted. NH$_4^+$ in the extracts was trapped, diffused, and analyzed as described above for the DNRA samples. Rates of gross ammonification were calculated using a model described by Wessel and Tietema (Wessel and Tietema 1992) as

$$\text{Ammonification} = \frac{\ln (T_{f_{\text{atm}}} - k) / (T_{0_{\text{atm}}} - k)}{\ln [\text{NH}_4^+ T_f] / [\text{NH}_4^+ T_0]} \cdot \frac{[\text{NH}_4^+ T_0] - [\text{NH}_4^+ T_f]}{\text{time}}$$

where $T_{f_{\text{atm}}}$ and $T_{0_{\text{atm}}}$ refers to the $^{15}$NH$_4^+$ enrichment of the $T_f$ and $T_0$ cores; $k$ is equal to natural abundance of $^{15}$NH$_4^+$ expressed as atom %; $[\text{NH}_4^+ T_f]$ and $[\text{NH}_4^+ T_0]$ are the concentrations of NH$_4^+$ in the $T_f$ and $T_0$ cores, and time is the incubation time.

**DNA Extraction and Quantitative PCR**

Sediment DNA was extracted from homogenized surface sediments (0-3cm) using the PowerSoil DNA Kit (Mo-Bio Laboratories, Inc., Carlsbad, CA), following the manufacturer’s protocol with the following modifications: 0.5g of wet sediment was used and Thermo Savant Fast Prep FP 120 Cell Disrupter (Qbiogene Inc. Carlsbad, CA) was used for cell disruption. Sediment DNA concentration was measured using Qubit double-stranded DNA High Sensitivity assay kit and a Qubit fluorometer according to the manufacturer’s protocol (Life Technologies, Grand Island, NY). Samples were subsequently diluted to a concentration of 1ng µL$^{-1}$.

Quantitative PCR (qPCR) assays were carried out to quantify the abundance of genes responsible for denitrification (nir$S$) and DNRA (nrf$A$). The nir$S$ primers used
were NIRS1F and NIRS-Q-R (Braker et al. 1998; Mosier and Francis 2010) and the nrfA primers were NRFAF2 and NRFA1R (Mohan et al. 2004; Welsh et al. 2014). Each qPCR incubation mixture (total volume 20 µl) contained Go-Taq qPCR Master Mix (Promega Corporation, Madison, WI), the primers (0.5 uM), and sediment DNA (3 ng). The nrfA mixture also contained 0.5 µl of MgCl for amplification optimization. Preparation of qPCR standards and PCR cycling were previously reported in (Song et al. 2014) and Lisa et al. (submitted). All qPCR analyses were conducted in triplicate. PCR specificity and primer dimer were assessed using dissociation curves. The R² values for the standard curves were 0.986 and 0.997 for nirS and nrfA, respectively.

Clam Respiration and Excretion Estimates

Clam respiration rates were estimated using an equation reported by Hofmann et al. 2006 and adjusted by Wiseman 2010 using data collected in Cherrystone Inlet, VA (Condon 2005); the estimate takes into account clam biomass per m² and temperature. Clam excretion rates, primarily composed of NH₄⁺ (Hammen 1980), were estimated stoichiometrically. The ratio of C respired to nitrogen excreted is dependent on the bivalve’s rate of catabolism and the composition of the food source (Bayne 1976). The respiration to excretion ratio was estimated at 7.0, which is the theoretical minimum signifying protein catabolism (Mayzaud & Conover 1988, Dame 2012). This is may overestimate excretion as a higher respiration to excretion ratio may occur if carbohydrate and lipid catabolism is significant. Nonetheless this ratio provides a reasonable estimate for excretion (Dame 2012).
**Statistical Analysis**

To determine whether significant differences existed between the uncultivated sediments and the clam sediments, the increase or decrease of a clam bed measurement relative to the mean uncultivated site for each season was calculated as

\[
\Delta r = C_{is} - U_s
\]  

(7)

where the \(C_{is}\) is the response measurement at clam bed \(i\) during season \(s\), and \(U_s\) is the mean response measurement at the uncultivated sediments for each season \((s)\). T-tests were conducted to determine if the mean \(\Delta r\) was significantly different from zero. This approach was used to assess sediment characteristics (e.g. porewater nutrients, sulfide, sediment organic matter) as well as rate measurements (e.g. net fluxes, DNRA, denitrification rates). If the mean \(\Delta r\) is significantly different than zero \((p < 0.05)\), this implies that sediments exposed to clam aquaculture behave differently than uncultivated sediments (Kellogg et al. 2014). Linear regression analyses were used to investigate the relationships between clam metrics (including size, biomass, and density) and sediment characteristics (porewater sulfide, nutrients, and organic content). Linear regressions were also used to assess the relationship between functional gene abundances and rates of denitrification and DNRA. To investigate the potential mechanisms driving the proportion of DNRA relative to denitrification linear regressions were conducted for the ratio of DNRA/denitrification against porewater sulfide concentrations and gross ammonification measurements. A significance value of \(p < 0.05\) was used for all
statistical tests, which were conducted in R studio software (version 0.98.1091 and R version 3.0.2)

RESULTS

*Environmental Characteristics*

Water temperatures ranged from 12°C in November to 25°C in July, with an intermediate of 17°C in May. Salinity did not vary across seasons, with an average of 23.5 (Table 1). Water column nutrients were generally low across all seasons and sites. Despite the close proximity of the clam and control sites, water column SRP and NH$_4^+$ were significantly higher above the clam beds compared to the control sites in May and July; water column NO$_x^-$ was significantly higher above the clam beds than the control sites in May (Table 2).

Sampling sites included a range of clam sizes, with shell lengths ranging from 11.8 to 58.1 mm (Table 1). Clam density ranged widely (46.9 – 3333.3 individuals m$^{-2}$) and was dependent on clam size; higher densities were observed at recently planted clam beds with smaller individuals while lower densities occurred at clam beds planted 1-2 years prior to sampling, which had larger individuals.

Within the clam beds, no significant relationships were observed between clam metrics (size, density, or biomass) and porewater nutrients, sediment organic content, or sulfide concentrations. However, mean porewater NH$_4^+$, SRP, and sulfide concentrations were generally higher in clam compared to uncultivated sediments during summer and fall (Table 3). In spring, mean porewater NH$_4^+$, SRP, and sulfide, were all significantly higher at the uncultivated control sites than the clam beds.
Sediment organic content was low and similar between the clam and uncultivated sediments, ranging from 0.74 to 1.30% (Table 3). However, in July clam beds had significantly higher organic content than the uncultivated sediments, 1.30 and 0.82%, respectively. Sediment C:N was similar at the clam and uncultivated sediments across all seasons, ranging from 6.8 in the spring to 7.6 in the fall.

Nutrient Fluxes

Daily NH$_4^+$ fluxes were significantly higher at clam compared to uncultivated sediments during all months, with the highest efflux rates (23.3 ± 2.8 mmol m$^{-2}$ d$^{-1}$) from clam beds in the July (Figure 2A and Table 4). Clam excretion was estimated to contribute approximately 42, 21, and 38% of the daily NH$_4^+$ flux in May, July, and November, respectively (Figure 2A). Whereas a net release of NH$_4^+$ from the clam sediments to the water column was observed during all months, a net uptake occurred in the uncultivated sediments in July and November with a small release in May. Increased NH$_4^+$ fluxes from clam beds relative to those from uncultivated sediments on average ranged from 2.74 mmol N m$^{-2}$ d$^{-1}$ in May to 24.4 mmol N m$^{-2}$ d$^{-1}$ in July (Table 4).

SRP fluxes followed similar trends to those of NH$_4^+$ with net effluxes occurring at the clam sediments and net uptake in the uncultivated sediments. SRP fluxes were significantly higher in the clam sediments than the uncultivated sites except in May (Figure 2B, Table 4).

NO$_x^-$ fluxes were generally low relative to the NH$_4^+$ fluxes and highly variable across sites and seasons, with similar rates at clam and uncultivated sediments. In both sediment types there was net uptake of NO$_x^-$ in May and November, averaging -0.29 ±
0.13 mmol m\(^{-2}\) d\(^{-1}\) and -0.14 ± 0.03 mmol m\(^{-2}\) d\(^{-1}\), respectively and a net efflux in July with a mean of 0.74 ± 0.31 mmol m\(^{-2}\) d\(^{-1}\) (Figure 2C). There was no significant effect of clams on NO\(_x\) fluxes (Table 4).

DON fluxes were highly variable with no net flux at the uncultivated sediments during any of the months (Figure 2D). In July, clam sediments were a net sink for DON averaging -3.7 ± 0.74 mmol m\(^{-2}\) d\(^{-1}\), while a highly variable net release of DON to the water column was observed at the uncultivated sediments (2.6 ± 0.30 mmol m\(^{-2}\) d\(^{-1}\)).

**Benthic Metabolism**

Seasonal variability of daily SOD and DIC fluxes was observed at both the clam and uncultivated sediments, with higher metabolic rates measured in the summer. DO uptake and DIC release was observed at all sites with significantly higher SOD at clam beds compared to uncultivated sites (Figure 2E and F, Table 4). DIC release was generally higher at clam sites than uncultivated sediments but the difference was only significant in July. Estimated clam respiration contributed 15, 26, and 15% of SOD fluxes in May, July, and November, respectively (Figure 2E). The respiratory quotient (RQ), which is equal to the net DIC flux divided by SOD, was 1.2, 2.0, and 0.9 at the clam beds in May, July, and November, while at the uncultivated sediments RQ was estimated as 1.0, 1.6, and 2.3 in May, July, and November.

**Ammonification Rates**

Seasonal variation was apparent in gross ammonification with highest rates measured in July followed by May and lowest rates in November. Ammonification rates
at clam and uncultivated sediments were not significantly different in May or November averaging 1.53 and 0.49 mmol N m$^{-2}$ d$^{-1}$, respectively (Figure 3); however in July, clam beds had significantly higher rates of ammonification than uncultivated sediments (Table 4). It is important to note that as described in the methods, excretion of NH$_4^+$ by clams did not contribute to the measured ammonification rates but did contribute to the net NH$_4^+$ fluxes (Table 2).

*Nitrate Respiration Rates*

Total nitrate respiration rates (denitrification plus DNRA) varied seasonally and were significantly higher at the clam beds than uncultivated sediments in July and November, with no significant difference in May (Figure 4). At both the clam and uncultivated sediments more than 96%, 95%, and 99% of denitrification and DNRA rates were coupled to nitrification in May, July, and November, respectively. Generally denitrification rates were lower than DNRA rates during all seasons and at both the clam and uncultivated sediments (Figure 4).

DNRA rates were significantly higher at the clam beds compared to uncultivated sediments during all seasons (Figure 4, Table 4). Overall, across all seasons, clam beds enhanced DNRA rates above the control sediments by a mean of 151.3 μmol m$^{-2}$ d$^{-1}$ (Table 4). Denitrification rates were significantly higher at the clam beds than the uncultivated sediments in July and November, with overall average rates of 42.8 and 19.6 μmol m$^{-2}$ d$^{-1}$, respectively (Figure 4, Table 4). However, in May clam and uncultivated sediments had similar denitrification rates, averaging 73.0 μmol m$^{-2}$ d$^{-1}$. 

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DNRA and denitrification rates were positively correlated with each other at both the clam beds and uncultivated sediments (Figure 5). However, the relative proportion of DNRA to denitrification (i.e. the slope of the regression) was significantly higher at the clam beds than the uncultivated sediments (Table 4). A positive relationship between the relative proportion of DNRA to denitrification and gross ammonification rates, a proxy for organic C quality and availability, was observed (Figure 6A). Additionally, DNRA relative to denitrification generally increased with increasing porewater sulfide when all seasonal data were pooled (Figure 6B).

Functional Gene Abundances

Abundances of *nirS*, encoding cytochrome cd nitrite reductase in denitrification, were similar between the clam and uncultivated sediments throughout all seasons with no seasonal variation (Table 3). However, abundances of *nrfA*, which encodes for cytochrome C nitrite reductase in DNRA, were significantly higher at the clam beds compared to the uncultivated sediments during all months sampled. At the clam beds, *nrfA* abundances were an order of magnitude higher than at the uncultivated sediments, with highest mean *nrfA* abundance observed at the clam site in July ($3.56 \times 10^8$ gene copies g sed$^{-1}$). A strong relationship between functional gene abundances and process rates was observed with *nirS* and *nrfA* abundances and denitrification and DNRA rates, respectively (Figure 7).

DISCUSSION
This study demonstrates that clam aquaculture significantly affects sediment N cycling rates, favoring retention rather than removal of N in shallow coastal ecosystems. Similar findings were reported in a previous study at this site, which measured *in situ* fluxes of nutrients and metabolism in clam beds of close to market size individuals (~40 mm shell length) and included a clam plus macroalgae treatment (Murphy et al. 2015). The present study builds on these findings by directly quantifying benthic microbial processes contributing to the benthic N cycling (e.g. DNRA and denitrification). This study found generally lower net NH$_4^+$, SRP, and metabolic fluxes than Murphy et al. (2015), likely because sampling included sediments from clam beds with varying clam sizes (11.8 – 58.1 mm shell length), not just large individuals, which impacted the contribution of clam metabolism to overall benthic rates. Additionally, Murphy et al. (2015) reports net autotrophy at the uncultivated sediments while this study showed slightly heterotrophic control sediments, potentially due to the greater availability of light in the field than in the lab. By sampling intensively across a leased area, the present study captured the natural variability in metabolism and N transformation rates due to season and spatial differences in sediment properties as related to time-since planted and clam size. As a result we were able to scale our results across the farm to construct an annual sediment N budget for clam and uncultivated sediments (Figure 8). On an annual scale, clam aquaculture had little affect on denitrification but facilitated increased nutrient regeneration in the benthos through enhanced DNRA, microbial mineralization, and clam excretion. Increased benthic nutrient recycling processes resulted in elevated nutrient release from the clam sediments to the water column, which may serve as an important subsidy for local primary production in the ecosystem (Murphy et al. 2015).
The sediment N budgets (Figure 8) highlight the major difference between the clam and uncultivated sediments: clam sediments are a net source of regenerated nutrients to the water column while uncultivated sediments are a net sink for DIN. Although microbial ammonification rates were only slightly higher at the clam sediments, the fate of the NH$_4^+$ produced in the benthos differed between the two sediment types. During all seasons, NH$_4^+$ was released from the clam beds while either little release or uptake occurred in uncultivated sediments. High DIN efflux has previously been observed in infaunal bivalve-dominated sediments, particularly when bivalves are included in the incubations at both natural (e.g. Doering et al. 1987; Sandwell et al. 2009; Jones et al. 2011) and aquaculture settings (e.g. Bartoli et al. 2001; Nizzoli et al. 2006). This NH$_4^+$ is sourced from microbial mineralization of organic matter, DNRA, and clam excretion. Even after clam excretion was subtracted from the net NH$_4^+$ flux, our data showed little to no retention of microbial-derived DIN in the clam sediments perhaps due to reduced benthic microalgal (BMA) activity.

Previous studies in shallow coastal bays located on the Eastern Shore of VA show that benthic microalgae (BMA) can take up much of the nitrogen produced by sediment microbial mineralization, provided that sufficient light is available (Anderson et al. 2003). Although benthic chl a concentrations were similar between the clam beds and uncultivated sediments, the sources are likely different. In fact, Secrist (2013) found that the bulk sediment chl a biomass at Cherrystone clam beds was composed mainly of detrital macroalgal material as opposed to BMA (i.e. pennate diatoms). At the clam sites the predator-exclusion nets, which sit flush on the sediment surface and are colonized by thick macroalgal mats (Murphy et al. 2015), cause significant shading, decreasing BMA
biomass (Secrist 2013). At the uncultivated sites the majority of the NH$_4^+$ produced was retained in the benthos (Figure 8) indicating active BMA. In our study system calculated BMA N demand in the uncultivated sediments, not accounting for C exudation (as described in Anderson et al. 2003), could account for uptake of all measured mineralized N.

Concurrent with net DIN release, clam aquaculture sediments generally had higher porewater nutrients, sulfide, and organic content than control sites, indicative of highly reduced conditions with limited oxygen penetration, similar to results of other studies that have characterized sediments associated with bivalve aquaculture (e.g. Mazouni et al. 1996; Christensen et al. 2003). Organically enriched sediments, a result of clam biodeposition, leads to high microbial respiration, particularly in the summer when high temperatures increase clam and microbial metabolism. Although infaunal clams are often reported to be important sediment bioturbators that deliver oxygen (and other solutes) to the sediments (Welsh 2003), bioturbation is likely limited in the cultivated clam beds due to high clam densities and predator exclusion nets, which may limit movement and water exchange, further promoting sulfide accumulation and oxygen depletion. Surprisingly during the spring, the uncultivated sediments had higher porewater nutrients, sulfide, and organic content than the clam sediments, concurrent with elevated mineralization and NO$_3^-$ respiration rates. A likely explanation is that these sediments, adjacent to clam operations, experienced periodic pulses of organic matter deposition caused by aquaculture practices (e.g. sweeping the predator-exclusion nets of macroalgae and hydraulic clam harvesting).
Clam cultivation in Cherrystone Inlet tended to have enhanced DNRA and denitrification compared to uncultivated sediments, although rates were low overall compared to other rates, such as clam excretion and gross ammonification (Figure 8). DNRA was more dominant at the clam beds than the uncultivated sediments as reflected in the relative proportion of DNRA to denitrification, which was significantly higher at the clam beds than the uncultivated sediments. The contribution of DNRA to total NO$_3^-$ respiration, which averaged 82% in the clam sediments, is on the very high-end compared to other estuarine systems, which typically range from 0 to 60% (Tobias et al. 2001; Burgin and Hamilton 2007; Koop-Jakobsen and Giblin 2010). These trends were corroborated with observed functional gene abundances in Cherrystone sediments; nrfA was significantly higher in the clam sediments, whereas nirS abundances were similar across sediment types and months. Despite the fact that nrfA is known to be present in diverse genera of bacteria, capable of a variety of metabolic pathways (Mohan et al. 2004), in Cherrystone sediments nrfA abundance was strongly correlated with DRNA rates. This strong relationship indicates that the abundance of DNRA bacteria may be an important microbial control on the process and, thus, serve as a genetic proxy for DNRA potential (Song et al. 2014). A number of environmental factors may explain why clam aquaculture favors DNRA and nrfA abundance over denitrification and nirS abundance.

Both DNRA and denitrification depend on concentrations of available electron donors (typically organic carbon) and the electron acceptor, NO$_3^-$ (as reviewed in Seitzinger 2006; Burgin and Hamilton 2007). In fact, the relative importance of DNRA and denitrification is often correlated with the ratio of available carbon relative to NO$_3^-$ concentrations (Tiedje 1982, Ferrón et al. 2009, Burgin and Hamilton 2007; Algar and
Denitrification tends to dominate in systems with low labile carbon relative to NO$_3^-$, as this process generates more free energy per mole of C oxidized than DNRA. However, in a system with high labile carbon relative to nitrate, DNRA may be more important as this process utilizes NO$_3^-$ more efficiently than denitrification (i.e. transfers more electrons) (Tiedje 1988).

The availability of labile organic carbon, delivered as clam biodeposits to sediments, in Cherrystone Inlet is likely high. Despite the fact that clam beds had similar porewater DOC, sediment organic matter, and sediment C:N as the uncultivated sediments, these measurements are of bulk sediment C and may not provide insight into C quality. Previous laboratory studies have shown that bivalve biodeposits degrade quickly (Giles and Pilditch 2006; Carlsson et al. 2010; Jansen et al. 2012). Additionally, ammonification rates, which may serve as an indicator of C quality and availability, were strongly positively correlated with the proportion of DNRA to denitrification (Figure 6A).

At the same time, low water column NO$_3^-$ concentration indicates that both sediment denitrification and DNRA are strongly reliant on nitrification for NO$_3^-$ supply, as our data show that the majority of both DNRA and denitrification is coupled to nitrification ($D_n$ and DNRA$_n$). However, NO$_x$ produced by nitrification is low in both clam and uncultivated sediments, calculated as 100.6 and 73.0 mmol N m$^{-2}$ yr$^{-1}$, respectively (Figure 8). Nitrification is generally inhibited by low DO and high sulfide (Joye and Hollibaugh 1995). Thus, both DNRA and denitrification in these systems are regulated by oxygen penetration depth and sulfide concentrations, particularly in warm summer months when clam biodeposition and microbial respiration rates are high. Sulfide has been shown to inhibit nitrification by up to 75% at 60 µM and completely at 100 µM
(Joye and Hollibaugh 1995). Although one might expect complete inhibition of nitrification at the porewater sulfide concentrations observed in clam sediments in July, we did see a small efflux of NO$_3^-$ suggesting incomplete inhibition. Additionally, the sulfide measurements were collected across a bulk sediment horizon, with the sampler window centered approximately 5-7cm from the sediment surface, and, therefore, may not reflect the sulfide concentrations at the zone of nitrification, which is likely within the top few millimeters of the sediments.

In addition to suppressing nitrification, sulfide may directly enhance DNRA relative to denitrification. Chemolithotrophic DNRA bacteria are capable of oxidizing reduced forms of sulfur, including free sulfide and elemental sulfur, while reducing NO$_3^-$ to NH$_4^+$ (Brunet and Garcia-Gil 1996; Otte et al. 1999). Additionally the final two steps in denitrification (i.e. NO to N$_2$O and N$_2$O to N$_2$) may be directly inhibited under sulfidic conditions (Brunet and Garcia-Gil 1996; Burgin and Hamilton 2007). Thus the highly sulfidic sediments associated with clam aquaculture promote DNRA over denitrification (Figure 6B).

Although clam cultivation in Cherrystone Inlet tends to favor DNRA, the overall effect of bivalves on NO$_3^-$ respiration rates is quite variable and highly dependent on the environment and type of bivalve. Some studies report denitrification enhancement in bivalve-dominated sediments compared to reference locations (Kellogg et al. 2013; Smyth et al. 2013; Turek and Hoellein 2015) while other studies show no difference in denitrification across sediment types (Christensen et al. 2003; Jones et al. 2011; Higgins et al. 2013), and still other studies report spatial and/or temporal variability on the effects of bivalves on sediment denitrification (Nizzoli et al. 2006; Carlsson et al. 2012).
Ultimately, whether N is removed or retained in bivalve dominated systems depends upon numerous factors such as the bivalve species, the ecological context (natural or aquaculture), the ambient water quality, and other physical characteristics of the system (e.g. residence time, depth, etc). Cherrystone Inlet clam aquaculture has lower rates of denitrification than those observed in other bivalve studies, including those in nearby tributaries of the Chesapeake Bay (e.g. Higgins et al. 2013; Kellogg et al. 2013), although these focused on oyster-dominated systems. DNRA rates in clam beds in Cherrystone are comparable to previously reported rates associated with infaunal clams (Nizzoli et al. 2006). Most similar studies did not measure DNRA and therefore the ability to determine the mechanisms by which bivalves influence NO$_3^-$ respiration in these studies is limited.

To place the enhanced DIN regeneration at the clam beds into context, we compared it to an estimate of the amount of N removed from the system via clam harvest, assuming an average harvested clam to be 45.7 mm in length (littleneck size; 0.87 g DW, of which 13.1% is N (A.E. unpubl.) and two years to reach market size. Thus, at a density of 700 individuals m$^{-2}$, approximately 2.85 mol N m$^{-2}$ yr$^{-1}$ is removed through harvest, comparable to the estimated annual NH$_4^+$ regenerated from the sediments (2.88 mol N m$^{-2}$ yr$^{-1}$) (Figure 8). Although requiring higher spatial and temporal resolution, this exercise demonstrates the importance of considering N regeneration processes when estimating the total N removed from a bivalve cultivation system, particularly in systems where this enhanced N recycling may promote local eutrophication. For example, in Cherrystone Inlet the fate of these regenerated nutrients has been shown to promote macroalgal production (Murphy et al. 2015).
Clam aquaculture is a growing industry on the Eastern Shore of VA (Emery 2015) and globally (FAO 2014). As this coastal anthropogenic activity expands, an understanding of how it alters ecosystem functioning such as benthic nutrient cycling is necessary to prevent overexploitation and ecosystem degradation. Numerous studies have suggested suspension-feeding bivalves may promote denitrification and thus serve an important function in reducing bioavailable N and subsequently eutrophication (e.g. Rose et al. 2014). However, this study, which is, to our knowledge, the first to measure sediment N cycling processes associated with clam aquaculture in the U.S., highlights the importance of variables such as NO$_3^-$ availability, organic matter lability, and sulfide accumulation in determining the relative importance of nitrogen removal versus retention in bivalve aquaculture systems.
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Analysis.


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Table 3-1. Environmental conditions in Cherrystone Inlet during sampling. Clam metrics including clam lengths (mm), biomass (ash free DW, g m\(^{-2}\)), and densities (ind m\(^{-2}\))

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
<th>Shell Length</th>
<th>Biomass</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>17</td>
<td>24.5</td>
<td>11.8 - 58.1</td>
<td>3.5 - 419.8</td>
<td>46.9 - 1126.8</td>
</tr>
<tr>
<td>July</td>
<td>25</td>
<td>23.0</td>
<td>21.0 - 48.8</td>
<td>23.1 - 539.8</td>
<td>140.8 - 2441.3</td>
</tr>
<tr>
<td>November</td>
<td>12</td>
<td>23.0</td>
<td>15.3 - 54.0</td>
<td>76.3 - 497.2</td>
<td>328.6 - 3333.3</td>
</tr>
</tbody>
</table>
Table 3-2. Water column nutrient concentrations (µM) at the clam site and uncultivated site. Standard errors are provided in parentheses. * indicates significantly higher concentrations above the clam beds compared to uncultivated sites within each month.

<table>
<thead>
<tr>
<th>Month</th>
<th>Site</th>
<th>NO$_x^-$</th>
<th>NH$_4^+$</th>
<th>SRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>Clam</td>
<td>*0.46 (0.04)</td>
<td>*0.48 (0.05)</td>
<td>*0.09 (0.01)</td>
</tr>
<tr>
<td></td>
<td>Uncultivated</td>
<td>0.04 (0.003)</td>
<td>0.18 (0.01)</td>
<td>0.07 (0.001)</td>
</tr>
<tr>
<td>July</td>
<td>Clam</td>
<td>0.22 (0.01)</td>
<td>*4.04 (0.35)</td>
<td>*0.26 (0.01)</td>
</tr>
<tr>
<td></td>
<td>Uncultivated</td>
<td>0.19 (0.04)</td>
<td>0.48 (0.17)</td>
<td>0.10 (0.01)</td>
</tr>
<tr>
<td>November</td>
<td>Clam</td>
<td>0.05 (0.01)</td>
<td>0.96 (0.19)</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td></td>
<td>Uncultivated</td>
<td>0.06 (0.01)</td>
<td>1.41 (0.05)</td>
<td>0.02 (0.01)</td>
</tr>
</tbody>
</table>
Table 3-3. Mean sediment characteristics at uncultivated and clam sediments seasonally including porewater DOC (µM), nutrients (µM), and sulfide (µM), percent sediment organic matter (SOM), molar C to N ratio in the surface 0-2cm sediment horizon (C:N), benthic chlorophyll (B. Chla) and phaeophytin (B. Phaeo) (µg cm⁻²), nirS and nrfA gene abundances (copy number g sediment⁻¹). N refers to the number of sites sampled. Standard errors are in parentheses. * denotes significant difference between uncultivated and clam sediments within each month.

<table>
<thead>
<tr>
<th>Site</th>
<th>Uncultivated</th>
<th>Clam</th>
<th></th>
<th>Uncultivated</th>
<th>Clam</th>
<th></th>
<th>Uncultivated</th>
<th>Clam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
<td></td>
<td></td>
<td>July</td>
<td></td>
<td></td>
<td>November</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>16</td>
<td></td>
<td>4</td>
<td>16</td>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>DOC</td>
<td>371.6 (51.8)</td>
<td>404.2 (51.6)</td>
<td></td>
<td>607.8 (372.2)</td>
<td>393.3 (101.4)</td>
<td></td>
<td>226.3 (14.2)</td>
<td>287.8 (19.3)</td>
</tr>
<tr>
<td>NO₃</td>
<td>0.1 (0.01)</td>
<td>0.3 (0.02)</td>
<td></td>
<td>0.3 (0.02)</td>
<td>0.3 (0.02)</td>
<td></td>
<td>0.1 (0.01)</td>
<td>0.5 (0.2)</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>107.4 (17.0)</td>
<td>47.9 (4.0)</td>
<td></td>
<td>47.9 (4.0)</td>
<td>59.3 (4.7)</td>
<td></td>
<td>37.2 (9.3)</td>
<td>64.7 (16.5)</td>
</tr>
<tr>
<td>SRP</td>
<td>7.3 (0.8)</td>
<td>2.9 (0.4)</td>
<td></td>
<td>6.3 (1.2)</td>
<td>141.1 (50.8)</td>
<td></td>
<td>9.0 (0.6)</td>
<td>5.0 (1.5)</td>
</tr>
<tr>
<td>Sulfide</td>
<td>241.6 (36.1)</td>
<td>147.8 (10.2)</td>
<td></td>
<td>101.2 (21.0)</td>
<td>141.1 (50.8)</td>
<td></td>
<td>13.5 (13.5)</td>
<td>38.0 (16.3)</td>
</tr>
<tr>
<td>SOM</td>
<td>1.2 (0.2)</td>
<td>0.8 (0.1)</td>
<td></td>
<td>0.8 (0.1)</td>
<td>1.3 (0.1)</td>
<td></td>
<td>0.7 (0.1)</td>
<td>1.1 (0.3)</td>
</tr>
<tr>
<td>C:N</td>
<td>6.8 (0.3)</td>
<td>7.4 (0.5)</td>
<td></td>
<td>7.2 (0.1)</td>
<td>7.6 (1.7)</td>
<td></td>
<td>7.6 (1.7)</td>
<td>7.2 (0.6)</td>
</tr>
<tr>
<td>B. Chla</td>
<td>2.7 (0.4)</td>
<td>2.0 (0.7)</td>
<td></td>
<td>4.2 (0.5)</td>
<td>3.7 (0.43)</td>
<td></td>
<td>3.7 (0.43)</td>
<td>4.3 (0.38)</td>
</tr>
<tr>
<td>B. Phaeo</td>
<td>1.7 (0.5)</td>
<td>4.0 (2.3)</td>
<td></td>
<td>8.2 (0.6)</td>
<td>3.7 (0.31)</td>
<td></td>
<td>7.4 (1.23)</td>
<td></td>
</tr>
<tr>
<td>nirS</td>
<td>3.69x10⁷ (7.1x10⁶)</td>
<td>3.65x10⁷ (6.3x10⁶)</td>
<td></td>
<td>2.67x10⁷ (2.9x10⁶)</td>
<td>4.21x10⁷ (9.7x10⁶)</td>
<td></td>
<td>2.33x10⁷ (2.7x10⁶)</td>
<td>2.44x10⁷ (5.2x10⁶)</td>
</tr>
<tr>
<td>nrfA</td>
<td>1.07x10⁸ (1.6x10⁷)</td>
<td>*2.24x10⁸ (3.5x10⁷)</td>
<td></td>
<td>3.81x10⁷ (5.0x10⁶)</td>
<td>*3.56x10⁸ (1.2x10⁹)</td>
<td></td>
<td>4.43x10⁷ (1.6x10⁷)</td>
<td>1.16x10⁸ (3.1x10⁷)</td>
</tr>
</tbody>
</table>
Table 3-4. The mean difference between the clam beds and the average uncultivated sediments for each season as well as overall (across all seasons; All) of ammonium (NH₄⁺), nitrate+nitrite (NOₓ⁻), phosphate (SRP), dissolved organic nitrogen (DON), dissolved inorganic carbon (DIC) and sediment oxygen demand (SOD), denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and gross ammonification (mmol m⁻² d⁻¹). Additionally, the mean difference in the ratio of DNRA relative to denitrification (DNRA:DNF) between the clam beds and uncultivated sites for each month (unit-less). A positive value represents a higher measurement at the clam beds relative to the uncultivated sediments while a negative value reflects a lower rate. Bold text denotes values are significantly different than zero (t-test; alpha = 0.05).

<table>
<thead>
<tr>
<th>Response</th>
<th>May</th>
<th>July</th>
<th>November</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺</td>
<td>2.74</td>
<td>24.42</td>
<td>6.36</td>
<td>11.96</td>
</tr>
<tr>
<td>NOₓ⁻</td>
<td>-0.24</td>
<td>-0.36</td>
<td>0.01</td>
<td>-0.06</td>
</tr>
<tr>
<td>SRP</td>
<td>-0.04</td>
<td>0.79</td>
<td>0.28</td>
<td>0.34</td>
</tr>
<tr>
<td>DON</td>
<td>-0.78</td>
<td>-6.25</td>
<td>0.55</td>
<td>-2.79</td>
</tr>
<tr>
<td>DIC</td>
<td>52.74</td>
<td>159.53</td>
<td>31.69</td>
<td>92.05</td>
</tr>
<tr>
<td>SOD</td>
<td>34.92</td>
<td>65.52</td>
<td>65.65</td>
<td>52.99</td>
</tr>
<tr>
<td>Denitrification</td>
<td>-0.010</td>
<td>0.020</td>
<td>0.014</td>
<td>0.006</td>
</tr>
<tr>
<td>DNRA</td>
<td>0.108</td>
<td>0.260</td>
<td>0.043</td>
<td>0.151</td>
</tr>
<tr>
<td>Ammonification</td>
<td>-0.18</td>
<td>0.76</td>
<td>0.36</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Figure 3-1. Cherrystone Inlet, Chesapeake Bay, USA.

Aerial photograph of Cherrystone Inlet taken in 2012, black polygons delineate active clam aquaculture operations.
Figure 3-2. Seasonal mean daily fluxes of ammonium (NH$_4^+$) (a), phosphate (SRP) (b), nitrate+nitrite (NO$_x^-$) (c), dissolved organic nitrogen (DON) (d), dissolved oxygen (DO) (e), and dissolved inorganic carbon (DIC) (f) at uncultivated sediments (white) (n=4 in May and July, n=3 in November) and clam beds (gray) (n=16 in May and July, n=7 in November). Dotted lines in (a), (e), and (f) represent estimated clam metabolic contribution to the net fluxes. Error bars represent standard errors.
Figure 3-3. Gross ammonification rates at uncultivated (white) and clam sediments (gray) seasonally. Error bars represent standard errors. * denotes clam sediments are significantly higher than uncultivated sediment within each month.
Figure 3-4. Nitrate respiration rates in May, July, and November 2013 at uncultivated sediments (white) and clam sediments (gray). Crosshatched bars represent denitrification and the remainder is DNRA. Error bars are standard errors. Uncultivated sediments, n=4 in May and July, n=3 in November. Clam sediments, n=16 in May and July, n=7 in November. * and † denotes DNRA and denitrification, respectively are significantly higher at the clam sediments relative to the uncultivated sites within each month.
Figure 3-5. Relationship of DNRA and denitrification (mmol m$^{-2}$ d$^{-1}$) at clam sediments (black symbols) and uncultivated sediments (open symbols). Regression statistics include all seasons within each sediment type; dashed line is linear regression of clam beds and solid line is linear regression of uncultivated sediment. May, July, and November samples are shown as diamonds, squares, and triangles, respectively.

$y = 4.22x + 0.06$
$R^2 = 0.61$
$p < 0.001$

$y = 2.67x - 0.002$
$R^2 = 0.89$
$p < 0.001$
Figure 3-6. The relative importance of DNRA to denitrification as a function of gross ammonification (mmol N m$^{-2}$ d$^{-1}$) (a) and porewater sulfide (uM) (b). Solid lines are regressions of the average values for each season, including both sediment types. Clam data are shown in the black symbols while uncultivated sites are represented by open symbols. Diamond, squares, and triangles, represent May, July, and November, respectively. Error bars are standard errors.

(a) \[ y = 2.14x + 1.63 \] \[ R^2 = 0.43 \]

(b) \[ y = 0.01x + 3.73 \] \[ R^2 = 0.11 \]
Figure 3-7. Nitrate respiration rates (DNRA (squares) and denitrification (circles)) as a function of log-transformed *nrfA* and *nirS* gene abundance, respectively. Solid lines is linear regression of denitrification and *nirS*; dashed line is linear regression of DNRA and *nrfA*.
Figure 3-8. Annual microbial N cycling rates (mmol N m$^{-2}$ yr$^{-1}$) within the sediments/porewater at the uncultivated and clam sediments, including ammonification (AMN), nitrification (NIT), denitrification (DNF), DNRA (DNR), net fluxes of NH$_4^+$ and NO$_x^-$ (FLX), and immobilization of NH$_4^+$ into microbial and benthic microalgal biomass (IMM). Solid arrows represent processes directly measured in this study while dashed lines represent calculated rates. Vertical arrows show exchanges between the sediment and water column. Nitrification was estimated as rates of DNRA + denitrification + net NO$_x^-$ flux; immobilization of NH$_4^+$ into microbial biomass was estimated as gross ammonification + net NH$_4^+$ flux. *clam excretion rates were estimated as described in the text and subtracted from the net NH$_4^+$ flux to determine the amount contributed by microbial processes at the clam sediments (2142); the discrepancy between this number and gross ammonification may be due to excretion by other infaunal organisms.
CHAPTER 4. BIOGEOCHEMICAL RESPONSES TO CLAM AQUACULTURE: SACCA DI GORO, ITALY AND EASTERN SHORE, VA, USA

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ABSTRACT

As bivalve aquaculture expands globally, an understanding of how it alters the fate of nitrogen (N) in coastal environments is important to maintain sustainability and minimize impacts on sediment and water quality. The effect of aquaculture on biogeochemical cycling, and specifically its impact on the partitioning of nitrate between denitrification and DNRA, is strongly influenced by local environmental conditions and specific culture techniques. This study compares clam aquaculture effects on sediment N cycling, including net ammonium ($NH_4^+$) and nitrate plus nitrite ($NO_x^-$) fluxes, mineralization, denitrification, and dissimilatory nitrate reduction to ammonium (DNRA), between two cultivation settings: the Sacca di Goro, Italy ($Ruditapes philippinarum$) and the Eastern Shore, VA, USA ($Mercenaria mercenaria$). Within both settings a number of sites were sampled to capture a range of environmental conditions (e.g. salinity, water column $NO_x^-$, benthic infaunal community). On a local scale, clams significantly increased $NH_4^+$ fluxes and sediment oxygen demand, and had little to no significant effect on $NO_x^-$ fluxes, denitrification, or DNRA. Differences in measured physiological rates between the two species revealed that per unit of clams cultivated, $R. philippinarum$ consumes approximately 6 times more oxygen and regenerates approximately 5 times more $NH_4^+$ than $M. mercenaria$. Highest denitrification rates were measured in the western region of the Sacca di Goro where water column $NO_x^-$ was elevated concurrent with high abundances of the burrowing amphipod $Corophium$ and low clam biomass. At these sites, denitrification exceeded DNRA likely due to the high availability of $NO_x^-$ both from the water column and nitrification stimulated by $Corophium$ bioirrigation. In the eastern region of the Sacca di Goro and at the US sites DNRA exceeded
denitrification; at these sites clam biomass was high, water column NO$_x^-$ was low, and sediments were generally reduced. This study demonstrates the importance of environmental conditions on N cycling rates within the context of clam cultivation.
INTRODUCTION

Denitrification, the microbial-mediated removal of bioreactive N from the aquatic environment through the reduction of NO$_3^-$ to N$_2$(g), is an important natural buffer in reducing coastal eutrophication caused by excess N inputs (Seitzinger 1988). Controls on denitrification rates are complex, involving numerous factors including oxygen, NO$_3^-$ and organic carbon supply (Megonigal et al. 2004, Seitzinger et al. 2006). Additionally, dissimilatory nitrate reduction to ammonium (DNRA) has been increasingly recognized as an important competitor with denitrification (Burgin & Hamilton 2007, Giblin et al. 2013). The partitioning of NO$_3^-$ between denitrification and DNRA is of particular ecological importance as DNRA retains bioavailable N in the system while denitrification removes it.

Denitrification and fermentative DNRA are both sensitive to changes in O$_2$, NO$_3^-$, and organic carbon, and the relative availability of each often dictates the dominant pathway. Generally, denitrification is favored over DNRA as it yields more free energy per mole of carbon oxidized (Tiedje 1988). However, DNRA may exceed denitrification when NO$_3^-$ availability is low and organic carbon is high, as DNRA utilizes the oxidizer (NO$_3^-$) more efficiently (i.e. transfers more electrons per NO$_3^-$ reduced) than denitrification (Tiedje 1988, Burgin & Hamilton 2007, Giblin et al. 2013). This theory has been supported by both manipulative experiments and modeling studies (e.g. Algar & Vallino 2014, Hardison et al. 2015).

NO$_3^-$ supply in coastal systems is strongly driven by direct inputs from the watershed as well as nitrification, the oxidation of ammonium to NO$_3^-$. Estuaries are generally characterized as having increasing salinity and decreasing NO$_3^-$ moving
downstream, particularly in systems with high freshwater input and watershed NO$_3^-$ loading. In addition to direct inputs of NO$_3^-$ from the watershed, nitrification activity also varies along the salinity gradient. Nitrification rates are generally inversely related to salinity with higher rates at lower salinity (Rysgaard et al. 1999, Bernhard et al. 2007). Additionally, higher salinity often correlates with higher sulfide concentrations due to increased sulfate reduction rates, which inhibit nitrification (Joye & Hollibaugh 1995).

Benthic infaunal communities may alter organic carbon and NO$_3^-$ supply, both directly and indirectly influencing N cycling rates and benthic metabolism (as reviewed in Laverock et al. 2011). Through bioturbation and bioirrigation, infauna transport particles and solutes (e.g. NO$_x^-$, DO) through sediments, substantially changing redox gradients (Aller 1982, Kristensen et al. 1985). Infaunal activity can also alter the architecture of the sediments, causing heterogenous redox gradients, and affecting redox sensitive microbial processes such as nitrification and denitrification (Stief 2013 and references therein). Additionally, through feeding and biodeposition, benthic infauna, specifically suspension-feeders, actively deliver organic matter to the sediments from the water column. Biodeposition fuels microbial decomposition pathways and enhances microbial respiration and oxygen demand. Finally, benthic infauna excrete dissolved inorganic and organic N, increasing benthic N fluxes to the water column and providing substrate (e.g. NH$_4^+$) for microbial processes such as nitrification.

The use of coastal ecosystems for clam aquaculture, a growing industry worldwide (FAO 2014), may substantially alter the natural benthic infaunal community structure and function and have implications to N cycling rates. Specific cultivation methods depend on the clam species and environmental conditions, but all practices
generally involve growing artificially high densities of clams in sandy sediments, feeding on ambient phytoplankton. High clam densities can significantly affect biogeochemical cycling as outlined above (e.g. bioturbation, biodeposition, excretion), with effects varying depending on environmental conditions (e.g. nutrient concentrations, salinity, etc) and interactions with other infauna.

The effect of bivalve cultivation on biogeochemical cycling is of increasing interest, particularly with respect to denitrification. Numerous studies have reported bivalves enhance denitrification by delivering organic matter to anoxic sediments (Newell et al. 2002, Kellogg et al. 2013, Smyth et al. 2013). Implementing bivalve aquaculture as a means to promote N removal and mitigate coastal eutrophication has become a recent topic of debate (e.g. (Stadmark & Conley 2011, Rose et al. 2012). The overall objective of this study was to investigate benthic N cycling changes along a salinity gradient in coastal areas where mollusk cultivation is dominant. We sought to determine the role clams and environmental conditions play in nutrient regeneration processes, with a focus on the competition between denitrification and DNRA (N removal vs N recycling). The study also compared N cycling responses to two different clam species.

METHODS

Study Sites

The Sacca di Goro is a lagoon system of the Po River Delta, Italy. Approximately 26 km² with an average depth of 1.5 m, the lagoon hosts a substantial clam aquaculture industry, with about 1/3 of the area occupied by clam cultivation. The system is generally
divided into three areas based on hydrologic characteristics; the eastern portion is shallow and characterized by low energy and slow water exchange, the central region is tidal influenced, while the western portion is riverine dominated with freshwater flow from the Po di Volano. The lagoon, particularly the eastern region, experiences periodic dystrophic events, particularly in the early summer when macroalgae bloom. Drastic changes to the hydrodynamics of the system have been made over the past 20 years to improve water flow and alleviate dystrophic events, including channel construction along the southern sand spit to increase flow to the Adriatic Sea and dredging of internal canals (Viaroli et al. 2006). The manila clam, *Tapes philippinarum*, is farmed in privately leased portions of the lagoon at densities ranging from 100 to >2000 ind. m\(^{-2}\). Growers collect juvenile clams at the mouth and directly outside the lagoon, transport them to individual leases within the lagoon, and after approximately 9 months, hydraulically harvest the market-sized clams.

Cherrystone Inlet, located on the Chesapeake Bay-side of the Delmarva Peninsula, Virginia, USA, is a small shallow embayment (~6 km\(^2\), mean 1m depth), with little freshwater discharge. Smith Island Bay is the southern-most lagoon, located on the seaside of the Delmarva Peninsula and is protected by a barrier island. The hard clam *Mercenaria mercenaria* is cultivated in privately owned leases in the subtidal regions of Cherrystone Inlet and Smith Island Bay. At both US sites, clams are sourced from land-based hatcheries and nurseries and planted in the environment at ~8-15 mm in shell length. Growers set plastic mesh nets over the clam beds, flush to the sediment surface, to protect their product from natural predation. Macroalgal blooms occur on the predator-exclusion nets; periodically growers sweep the nets of the macroalgae to prevent
smothering the clams. After about two years the market-sized clams are hydraulically harvested.

Benthic Metabolism and Nutrient Flux Measurements

In June 2013, five sites were sampled in the Sacca di Goro along a salinity gradient (Figure 1A). In July 2013, one site in Cherrystone Inlet and one site in Smith Island Bay were sampled (Figure 1B). At each site, sediment cores were collected (US sites, 9.5cm i.d.; Italy sites, 8 cm i.d.) for determination of benthic metabolism, nutrient fluxes, and N cycling rates, including denitrification and DNRA. In the Sacca di Goro, 12 cores were randomly collected at each site, six for light and six for dark incubations.

In Cherrystone, a total of 40 cores were collected randomly across the largest leased area in the system, 20 for light and 20 for dark incubations. In Smith Island Bay, 12 cores were collected, again, half for light and half for dark incubations.

Sediment cores from Cherrystone and Smith Island were transported back to Virginia Institute of Marine Science, Eastern Shore Laboratory (VIMS ESL) in Wachapreague VA, while cores collected in the Sacca di Goro were transported back to the University of Parma. Cores were placed in water baths with site-specific salinity and temperature and allowed to equilibrate overnight. The following day, half the cores were illuminated while the other half remained dark. All cores were sealed and the overlying water was measured for changes in oxygen, NH$_4^+$, and NO$_x^-$. Water column nutrient samples were immediately filtered (0.45 um) and stored frozen until analysis. For the US sites (Cherrystone and Smith Island), oxygen was measured using Hach LDO101 Luminescent DO sensors (Hach Co., Loveland, CO, USA) secured in the lids of the cores.
While for the Sacca di Goro incubations, a polargraphic microsensor (50um tip; Unisense, DK) connected to an amperometer (PA2000, Unisense, DK) was used to measure DO concentrations. Samples collected during the incubation were stored in 12ml exetainers (Labco Inc.) and preserved with ZnCl prior to analysis. Hourly fluxes for each analyte (mmol m\(^{-2}\) hr\(^{-1}\)) were calculated as the change in concentration over time multiplied by the core water volume and divided by the core surface area. Fluxes from the sediment to the water column are represented by positive values (production), while fluxes to the sediment from the water column are negative (consumption). Additional details on flux measurements can be found in Murphy et al. (in review) and Nizzoli et al. 2006.

*Denitrification and DNRA Rate Measurements*

After the initial flux incubation, all cores were uncapped and allowed to equilibrate in freshly replaced water within the water bath for at least one hour; the light cores remained illuminated and the dark cores remained dark. The isotope pairing technique was used to measure denitrification (Nielsen 1992) and DNRA (Risgaard-Petersen & Rysgaard 1995). Complete details are described in Nizzoli et al. 2006 and Murphy et al. (in review). Briefly, the water bath level was dropped to just below the core top; \(^1\!5\!)\!\ NO_3^-\ (98.9 \, \text{atm}\%)\ was\ added\ to\ the\ overlying\ water\ of\ each\ core;\ a\ water\ column\ sample\ was\ collected\ from\ each\ core\ immediately\ before\ and\ after\ \(^1\!5\!)\!\ NO_3^-\ addition\ to\ determine\ the\ \(^15\!\!N\)-enrichment\ of\ the\ nitrate\ pool\ and\ the\ cores\ were\ sealed.\ Incubations\ typically\ lasted\ 3-4\ hours,\ depending\ on\ the\ specific\ sediment\ oxygen demand\ determined\ in\ the\ previous\ incubation\ (see\ above),\ allowing\ DO\ to\ drop\ no\ more
than 70% of the initial concentration (Dalsgaard et al. 2000). After the incubation, each core was gently homogenized, and sampled for $^{29,30}N_2$ and extracted $^{15}NH_4^+$. 

Dissolved $^{29,30}N_2$ gas samples were collected by siphoning the slurry into 12ml exetainer vials (Labco, Inc) without headspace and preserving the sample with 100 μl of saturated ZnCl. Samples were analyzed within a month on a membrane inlet mass spectrometer (MIMS) (Kana et al. 1994). Denitrification rates were calculated using the production of $^{29}N_2$ (p29) and $^{30}N_2$ (p30), assuming a binomial distribution of the production of $^{28,29,30}N_2$ (Nielsen 1992) as follows:

$$D_{15} = p29 + 2p30 \quad (3)$$

$$D_{14} = D_{15} \times (p29/2p30) \quad (4)$$

where $D_{15}$ represents denitrification of the added $^{15}NO_3^-$ and $D_{14}$ is the denitrification rate of $^{14}NO_3^-$. Direct denitrification of $NO_3^-$ from the water column, ($D_w$), and coupled denitrification ($D_n$) were calculated as described by Nielsen (1992):

$$D_w = (^{14}NO_3^- / ^{15}NO_3^-) \times D_{15} \quad (5)$$

$$D_n = D_{14} - D_w \quad (6)$$

where $^{14}NO_3^-$ is equal to the ambient unlabeled $NO_3^-$ concentration (μM) and $^{15}NO_3^-$ is equal to the isotopically-labeled $NO_3^-$ concentration at the start of the incubation.
Previous manipulation experiments in which denitrification rates were measured with varying concentrations of added $^{15}\text{NO}_3^-$, demonstrated that in both the eutrophic Sacca di Goro Lagoon and Cherrystone Inlet, anammox contributes to a negligible amount of $\text{N}_2$ relative to denitrification. Thus, the assumptions upon which the isotope pairing technique are based were met and the equations are valid for these systems (Nielsen 1992).

The slurried cores were also sampled for $^{15}\text{NH}_4^+$ production to calculate ambient DNRA rates. Approximately 200ml of slurry was placed in potassium chloride (KCl) for a final concentration of 2M. Samples were shaken for 1 hour, filtered (0.2 μm membrane filters), and frozen until they were diffused and trapped for analysis of $^{15}\text{NH}_4^+$ enrichment and concentration using a method modified from Brooks (1989). Water samples were placed in specimen cups; an acidified (25μl of 2.5M sulfuric acid) GFF filter (1cm, i.d.), threaded onto a stainless steel wire, was suspended on the lip of the cup; magnesium oxide was added and the samples were allowed to diffuse for 2 weeks, after which samples were encapsulated in tin capsules and analyzed on an EA-IRMS at the University of California Davis Stable Isotope Facility.

DNRA rates of the ambient $^{14}\text{NO}_3^-$ ($\text{DNRA}_t$) were calculated according to Risgaard-Petersen & Rysgaard (1995) as:

$$\text{DNRA}_t = P^{15}\text{NH}_4^+ \times (D_{14}/D_{15})$$

(7)
where $p^{15}\text{NH}_4^+$ is equal to the production of $^{15}\text{NH}_4^+$. This assumes that DNRA occurs in the same sediment horizon as denitrification, resulting in the same proportional use of $^{14}\text{NO}_3^-$ and $^{15}\text{NO}_3^-$ as denitrification (Rysgaard et al. 1993).

**Clam Respiration and Excretion Rate Measurements**

After the ‘whole-sediment’ incubations, all cores were sieved and the clams from each core were placed back into the core tubes for flux incubation without sediment. As described above, cores were capped and samples were collected for changes in DO, $\text{NH}_4^+$, $\text{NO}_3^-$, and SRP over time. After the incubations all clams were measured (shell length) and dry weight (DW) and ash-free DW (loss on ignition) were obtained.

**Gross Microbial Ammonification Rates**

Additional cores were collected at each site for gross ammonification rate measurements using the isotope pool dilution technique (Anderson et al. 1997). Details on the method are provided in Murphy et al. (in review). Briefly, paired cores (5.7cm i.d, with approximately 5 cm overlying water and 5 cm sediment depth) were collected at each sampling site, carefully avoiding inclusion of clams in order to measure microbial ammonification independent of clam excretion. To attribute the ammonification measured using this method entirely to microbial processes assumes negligible contribution of $\text{NH}_4^+$ from the infaunal community. Cores were transported to the laboratory, placed in site water, and held overnight uncapped with gentle mixing and aeration. The following day the sediments were homogenously spiked with $^{15}\text{N-NH}_4^+$ (3.6ml of $[\text{NH}_4]_2\text{SO}_4$, 30 at.%, 10mM). One paired core, $T_0$, was immediately sacrificed...
after spiking by shaking in 2M KCl for an hour; the extractant was filtered and frozen
until analysis. The $T_f$ cores were capped and incubated for 24 hours in the dark at in situ
temperatures, after which the cores were processed the same as $T_0$ cores above. $\text{NH}_4^+$
was processed and analyzed as described above for the DNRA samples (diffusion
methods modified by Brooks 1989). Rates of gross ammonification were calculated using
a model described by (Wessel & Tietema 1992) as

$$\text{Ammonification} = \frac{\ln \left( \frac{T_{a\text{m}^\%} - k}{T_{0\text{m}^\%} - k} \right)}{\ln \left( \frac{[\text{NH}_4^+ T_f]}{[\text{NH}_4^+ T_0]} \right)} \times \frac{[\text{NH}_4^+ T_0] - [\text{NH}_4^+ T_f]}{\text{time}}$$

where $T_{f\text{m}^\%}$ and $T_{0\text{m}^\%}$ refer to the $^{15}\text{NH}_4^+$ enrichment of the $T_f$ and $T_0$ cores; $k$ is equal
to natural abundance of $^{15}\text{NH}_4^+$ expressed as atom %; $[\text{NH}_4^+ T_f]$ and $[\text{NH}_4^+ T_0]$ are the
concentrations of $\text{NH}_4^+$ in the $T_f$ and $T_0$ cores, respectively, and time is the incubation
time.

**Denitrification Efficiency Calculation**

Denitrification efficiency, the percent of mineralized nitrogen removed via
denitrification, was calculated as:

$$\text{Denitrification Efficiency (\%)} = \frac{D_{14}}{NO_2^-+\text{NH}_4^++D_{14}} \times 100$$
where \( D_{14} \) is denitrification, and \( NO_3^- \) and \( NH_4^+ \) represent the positive fluxes of these nutrients (effluxes).

**Statistical Analyses**

To determine the local effects of clams on the fluxes of \( NH_4^+ \), \( NO_3^- \), and DO as well as denitrification and DNRA rates, linear regressions with clam biomass were conducted within each site. Additional regressions were conducted to determine the relationship of oxygen consumption and \( NH_4^+ \) release with clam biomass in the clam-only incubations to obtain the direct contribution of clam metabolism to the whole sediment fluxes. The clam contribution, calculated using the slope of the regressions (mmol \( O_2 \) g DW\(^{-1}\) or \( \mu \)mol \( NH_4^+ \) g DW\(^{-1}\)) and the clam biomass present (g DW m\(^{-2}\)) in each core, was subtracted from the whole-sediment \( NH_4^+ \) and DO fluxes to obtain a ‘sediment-only’ flux rate, which represented the microbial contribution (plus any other infaunal organisms) to the flux. The effect of light within each site on these calculated ‘sediment-only’ fluxes was determined using ANOVA.

Regional differences in flux rates, denitrification, DNRA, mineralization rates and environmental variables across sites were initially explored using principle components analysis (PCA). The effect of mineralization rates (proxy for carbon availability) relative to water column \( NO_3^- \), sediment organic matter, clam biomass, and clam species on the proportion of denitrification relative to DNRA (DNF:DNRA) across the seven sites were assessed using general linear models. Maximum likelihood values were used to determine Akaike’s information criterion corrected for small sample size (\( AIC_c \)) (Burnham and Anderson 2002). \( AIC_c \), which penalizes for increasing number of
estimated parameters in a model, infers the model’s explanatory power and was used to rank the models according to goodness of fit. The model with the smallest AICc represents the ‘best model’. AICc differences ($\Delta_i$) relative to the smallest AICc value were calculated, such that the model with the minimum AICc values had a $\Delta = 0$. Model averaging was used to generate a composite of the top best performing models, which were considered as those with $\Delta_i < 4$. A comparison between observed DNF:DNRA and predicted DNF:DNRA using the averaged model was used to assess the predictive power of the variables included in the averaged model.

All statistical analyses were conducted in R studio, 0.99.447.

RESULTS

Environmental Characteristics and Clam Measurements

Across the sites salinity ranged from 10 to 33, while temperature was relatively consistent with slightly lower temperatures at the Sacca di Goro sites (20-21 °C) than Cherrystone Inlet (25°C) and Smith Island Bay (27°C) (Table 1). Water column NOx$^-$ was significantly inversely correlated with salinity ($R^2 = 0.74$, $p = 0.01$), with the highest concentration at Goro-10 (54 µM) and lowest concentration at Cherrystone Inlet (0.2 µM). Water column NH$_4^+$ ranged from 0.88 µM at Smith Island Bay to 38.4 µM at Goro-16, with no significant relationship with salinity. Sediment organic matter (0-2 cm sediment horizon) was highest at Goro-15 (2.38) and lowest at Goro-16 (0.92), but was generally similar across sites.

Average clam densities in the Sacca di Goro ranged from 365 to 2089 individuals m$^{-2}$ while average densities in Cherrystone Inlet and Smith Island Bay were 630 and 258
individuals m$^{-2}$, respectively. In the Sacca di Goro, clam density increased with salinity ($R^2 = 0.90, p = 0.01$), although clam densities at the US sites (i.e. Cherrystone Inlet and Smith Island Bay) did not fit this trend. Average clam biomass ranged from 82.9 to 553 g DW m$^{-2}$, and generally increased with increasing salinity across all sites (Table 1). Clams were generally larger at the US sites, averaging 35.5 and 43.9 mm (shell length) at Cherrystone Inlet and Smith Island Bay, respectively, compared to the Italy sites, which ranged from 24.5 to 32.5 mm.

Direct contribution of clams to benthic fluxes

Clam-only incubations revealed the direct contribution of clam physiological rates (i.e. respiration and excretion) to benthic sediment oxygen demand and NH$_4^+$ fluxes. After subtracting the calculated clam contribution from the ‘whole sediment’ net benthic O$_2$ and NH$_4^+$ fluxes, it was possible to infer about benthic microbial processes (e.g. photosynthesis and nitrification) and the influence of other dominant infauna on these rates.

In the clam-only incubations DO consumption and NH$_4^+$ production were significantly positively correlated with clam biomass (g DW m$^{-2}$) at all sites (Table 2). A clear difference was detected between the physiological rates of the two species of infaunal clams; T. philippinarum (Sacca di Goro sites) had on average higher rates of respiration (0.03 mmol O$_2$ g DW$^{-1}$ hr$^{-1}$) and NH$_4^+$ excretion (3.8 umol NH$_4^+$ g DW$^{-1}$ hr$^{-1}$) compared to M. mercenaria (Cherrystone Inlet and Smith Island Bay) respiration and excretion, which averaged 0.006 mmol O$_2$ g DW$^{-1}$ hr$^{-1}$ and 0.8 umol NH$_4^+$ g DW$^{-1}$ hr$^{-1}$, respectively.
Clam biomass was not significantly correlated with NO\textsubscript{x}\textsuperscript{-} fluxes in the clam-only incubations, suggesting no direct effect of clams on net NO\textsubscript{x}\textsuperscript{-} fluxes (data not shown).

*Benthic Nutrient and DO Fluxes*

Sediment NH\textsubscript{4}\textsuperscript{+} fluxes (‘whole core’ rates including clams) were generally out of the sediments at all sites in the light and dark, with the exception of sites up-estuary in the Sacca di Goro (Goro-10, Goro-13, and Goro-15), which tended to portray no net flux of NH\textsubscript{4}\textsuperscript{+} in the light (Figure 2A). Within each site, net sediment NH\textsubscript{4}\textsuperscript{+} fluxes were significantly, positively correlated with clam biomass, except at Goro-15 and Smith Island Bay (Table 3).

Clam excretion, calculated using data obtained from the clam-only incubations (see above), was subtracted from the ‘whole-core’ NH\textsubscript{4}\textsuperscript{+} fluxes to obtain ‘sediment only’ estimates (Figure 2B). After accounting for clam excretion, ‘sediment-only’ NH\textsubscript{4}\textsuperscript{+} fluxes in the light were typically into the sediments, except at Cherrystone Inlet (23) (Figure 2B). In the dark, ‘sediment-only’ NH\textsubscript{4} fluxes were out of the sediments at Goro-13, Goro-15, Cherrystone Inlet and Smith Island Bay, while an influx was observed at Goro-10, Goro-16, and Goro-21.

NO\textsubscript{x}\textsuperscript{-} fluxes were negligible at the high salinity sites (Smith Island Bay, Cherrystone Inlet, and Goro-21); sediments were a net sink of NO\textsubscript{x}\textsuperscript{-} at the mid-salinity site (Goro-16), and shifted to a net source of NO\textsubscript{x}\textsuperscript{-} to the water column at the low salinity sites (Goro-10 and Goro-13) (Figure 3). NO\textsubscript{x}\textsuperscript{-} fluxes across the sites were significantly inversely related to salinity ($R^2 = 0.24$, $p <0.001$). Within each site there was no significant relationship between sediment NO\textsubscript{x}\textsuperscript{-} fluxes and clam biomass, except at Smith
Island Bay where NO$_x$ fluxes significantly decreased with increasing clam biomass (Table 3). Light significantly decreased NO$_x$ fluxes at Goro-10 and Goro-13, significantly increased NO$_x$ fluxes at Goro-15, and had no effect on NO$_x$ within each of the remaining sites.

All sites were net heterotrophic, with significant DO consumption, with highest uptake at Goro-15 (Figure 4A). ‘Whole core’ sediment oxygen demand was significantly correlated with clam biomass, except at Goro-10, where the relationship was not significant (Table 3). The contribution of clam respiration, which was obtained using data from the ‘clam-only’ incubations (see above), was subtracted from the ‘whole-sediment’ DO fluxes to provide insight into differences between light and dark incubations due to microbial processes and any infauna other than the clams. Within each site, the estimated ‘sediment only’ DO consumption was significantly lower in the light at Goro-16 and Cherrystone Inlet (Figure 4B). The contribution of clam respiration to the dark benthic DO fluxes ranged from 26% to 176% (Figure 4B).

**Gross Mineralization Rates**

Gross microbial mineralization rates (note: collected cores did not contain clams) were highest up estuary in the Sacca di Goro, ranging from 8.1 mmol m$^{-2}$ d$^{-1}$ to 11.6 mmol m$^{-2}$ d$^{-1}$ at Goro-10 and Goro-15, respectively (Figure 5). Rates were significantly lower at the higher salinity sites (Goro-16, Goro-21, and Cherrystone Inlet) (Figure 5). Note: a significant relationship between amphipod density and gross mineralization rates is expected, although data is not yet available.
Denitrification and DNRA

Average denitrification rates ranged from 1.6 (± 0.2; SE) μmol m⁻² hr⁻¹ at Cherrystone Inlet to 259.1 (± 54.1; SE) μmol m⁻² hr⁻¹ at Goro-10. The percent of total denitrification that was coupled to nitrification (Dn) ranged from 31% to >99% at Goro-16 and Smith Island Bay, respectively. Despite the high water column Nox concentrations at the low salinity sites (Goro-10, Goro-13, and Goro-15), the percent denitrification coupled to nitrification was relatively high (>50%). While at Goro-16, which also had high water column Nox, the percent coupled was only ~31% (Figure 6A, Table 4). Total denitrification significantly increased with clam biomass at Goro-13 and Smith Island Bay, while having no significant relationship at the other sites (Table 3). Denitrification efficiency was generally low across all sites, ranging from 9% in Cherrystone Inlet to 31% at Goro-15 (Table 4).

DNRA rates ranged from 8.2 μmol m⁻² hr⁻¹ at Goro-13 to 87.7 μmol m⁻² hr⁻¹ at Goro-16 (Figure 6B). The percent of total DNRA that was coupled to nitrification (DNRAₙ) varied similarly as Dn across sites, ranging from 31% at Goro-16 to 98% at Smith Island Bay (Figure 6B, Table 4). Total DNRA significantly increased with clam biomass at Goro-10, Goro-13, and Cherrystone Inlet (Table 3).

The ratio of denitrification relative to DNRA (DNF:DNRA) was highest at Goro-13, averaging 14.2 and lowest at Smith Island Bay, averaging 0.1. Denitrification exceeded DNRA at Goro-10, Goro-15, Goro-13, while DNRA was higher than denitrification at Goro-21, Cherrystone Inlet, and Smith Island Bay. At Goro-16 the ratio of denitrification to DNRA was about 1 (Table 4).
The best performing models are provided in Table 5 and include four models that incorporate all four predictor variables (clam biomass, MIN:water column NO$_x^-$, SOM, and clam species). The ratio of mineralization rates relative to water column NO$_x^-$ was the most important variable in the weighted model, with clam species being least important. Variable estimates are provided in Table 6. The weighted model describes DNF:DNRA increasing with decreasing MIN:water column NO$_x^-$ and decreasing clam biomass. The model generally underestimated DNF:DNRA with a regression of predicted versus observed values having a slope of 0.41 (p<0.001) and an R$^2$ of 0.54 (Figure 7).

General trends across sites

The PCA (Figure 8) highlights the environmental gradients across the sites, with strong inverse relationship between salinity and water column NO$_x^-$. The study sites tended to separate into three general groups. Goro-10, Goro-13, and Goro-15 clustered together and were associated with high denitrification relative to DNRA and high water column NO$_x^-$. Goro-16 was separate from the other sites and strongly associated with high DNRA, SOD, and clam biomass. Cherrystone Inlet and Goro-21 clustered closely and were associated with high mineralization rates relative to water column NO$_x^-$, high salinity, and high NH$_4^+$ fluxes.

DISCUSSION

This study demonstrates the important influence of environmental factors (i.e. water column NO$_x^-$, benthic infaunal community) on benthic N cycling. Within the
context of these environmental conditions, clam cultivation can have variable effects on 
N processing, which is dependent on specific cultivation practices (e.g. densities planted, 
predator-exclusion nets), the clam species cultured, and local processes, including 
interactions with other dominant macrofauna species and microbial metabolism.

*Clam bioenergetics directly affect NH$_4^+$ and DO fluxes*

A clear difference in metabolic rates was observed between the two species of 
clams in the study; *R. philippinarum* in the Sacca di Goro had 5.8 (±1.3) times higher 
respiration and 4.8 (±1.3) times higher excretion rates than *M. mercenaria* at the US sites. 
Additionally, the ratio of oxygen consumed to NH$_4^+$ excreted was higher for *R. 
philippinarum*, averaging 8.2, than *M. mercenaria*, which averaged 6.8. These differences 
could be due to intrinsic species-specific physiological and/or behavioral differences, 
size/age differences, and/or variation in food sources between the regions. These results 
indicate that per unit of clams cultivated, *R. philippinarum* consumes approximately 6 
times more oxygen and regenerates approximately 5 times more NH$_4^+$ than *M. 
mercenaria*. These results may suggest *R. philippinarum* also has higher filtration rates 
than *M. mercenaria*, and depending on food availability, which will vary by location, 
may deliver more organic carbon to the sediments. The methods used to estimate clam 
respiration and excretion in this study assumes clams behave similarly when removed 
from the sediment as they do *in situ*. However, our rates reflect reasonable 
approximations, as they are similar to previously reported rates for *M. mercenaria* (Sma 
& Baggaley 1976, Hofmann et al. 2006) and *R. philippinarum* (Magni & Montani 2005, 
Han et al. 2008) measured at similar temperatures.
Clams had a direct effect on DO and NH$_4^+$ sediment fluxes within each site, however the relative importance of clam metabolism to total benthic respiration varied across sites depending on clam biomass present and other processes affecting DO and NH$_4^+$ at each site (e.g. microbial and other infauna metabolism). Clam respiration accounted for a high percentage of the dark DO consumption at the down-estuary sites in the Sacca di Goro (i.e. Goro-16 and Goro-21), where clam biomass was high and mineralization and sediment organic matter were low relative to the other Sacca di Goro sites, causing lower background sediment oxygen demand from microbial respiration. While in the up-estuary sites of the Sacca di Goro (i.e. Goro-10, Goro-13, and Goro-15) and the US sites, clam respiration accounted for <50% of total dark DO consumption, suggesting other oxygen consuming processes were important. As discussed below, Goro-10, Goro-13, and Goro-15 had high abundances of the burrowing amphipod Corophium sp. (~400-20,000 ind m$^{-2}$) as well as high nitrification (approximated as NO$_x^-$ efflux plus coupled denitrification and coupled DNRA) and mineralization, which likely account for more of the oxygen consumption than clam respiration. At the US sites, despite high clam biomass, the contribution of clams to DO consumption and NH$_4^+$ production was low (~18%). These sites were highly reduced with high sulfide concentrations (Murphy, in review, Murphy, unpublished data), suggesting microbial mineralization and the re-oxidation of reduced compounds such as sulfide may consume the majority of the oxygen. Additionally, at Cherrystone Inlet, after subtracting out the clam contribution, a net NH$_4^+$ release was observed both in the light and dark suggesting low uptake by benthic microalgae, as described in Murphy et al. (in review). The highly reduced sediment conditions are likely attributable to the use of predator exclusion nets,
which restrict water flow, promote macroalgal recruitment, and cause sediment shading (Murphy et al. 2015).

**Locally, clams have little effect on denitrification, DNRA, and NO$_x^-$ fluxes**

It was expected that by depositing organic matter to the sediment surface, clams would increase nitrate respiration rates as demonstrated in other studies (Nizzoli et al. 2006, Kellogg et al. 2013, Welsh et al. 2015). However, within each site, clam biomass had little to no effect on denitrification, DNRA, or net NO$_x^-$ fluxes (Table 3); when the relationship was significant, the magnitude (i.e. regression slope) was low, generally an order of magnitude lower than clam effects on NH$_4^+$ and DO fluxes. This suggests that on a local scale, factors other than organic carbon supply are more important in regulating these processes (e.g. sulfide, NO$_x^-$ supply, O$_2$ conditions, bioturbation by burrowing macrofauna). Also, the large variability in the regression estimates indicates that our approach (i.e. random field sampling and core incubations) may not adequately depict how clams affect small-scale sediment heterogeneity. Therefore, our results cannot exclude a local stimulation or inhibition of clam biomass on denitrification or DNRA within each site. However it is still apparent that if present, the effect of clams on denitrification and DNRA is much less than that on DO and NH$_4^+$.

**Factors controlling N fluxes differ depending on location**

Benthic N cycling rates varied across sites due to a number of interacting variables. General environmental gradients were observed across the study sites with salinity inversely correlated with water column NO$_x^-$ and positively correlated with clam
biomass. The seven sites sampled in this study can be generally partitioned into three groups based on observed N flux trends and specifically, the ratio of denitrification to DNRA (Table 7, Figure 8): (1) three sites located in the western portion of the Sacca di Goro where denitrification exceeded DNRA (Goro-10, Goro-13, and Goro-15), (2) three higher salinity sites where DNRA exceeded denitrification (Goro-21, Cherrystone Inlet, and Smith Island Bay), and (3) one site located in the central portion of the Sacca di Goro where denitrification and DNRA were similar (Goro-16). Across these sites different factors appeared to control the major N cycling rates (summarized in Table 7).

**Western Sacca di Goro**

The three sites in the western region of the Sacca di Goro (Goro-10, Goro-13, and Goro-15) where denitrification exceeded DNRA were characterized with higher water column NO\textsubscript{x}\textsuperscript{+}, NO\textsubscript{x}\textsuperscript{−} effluxes, mineralization rates, and denitrification rates, relative to the other sites. The benthic infauna community was also quite different than the other sites, with lower clam biomass and very high abundances of the amphipod *Corophium* sp. (~400-20,000 individual m\textsuperscript{−2}) (side note: actual densities will be available soon, counts have not yet been completed). These amphipods, which create shallow ‘U’-shaped burrows, are important drivers in N cycling processes; *Corophium* enhance oxygen consumption, nitrification, and denitrification (Henriksen et al. 1983, Pelegri et al. 1994).

The interaction of *Corophium* and *T. philippinarum* likely enhanced nitrification, explaining the elevated NO\textsubscript{x}\textsuperscript{+} effluxes and the high percentage of denitrification and DNRA coupled to nitrification (~55-64%), despite the ample NO\textsubscript{x}\textsuperscript{−} in the water column. The clams and amphipods provide nitrifiers with NH\textsubscript{4}\textsuperscript{+} through excretion, while the
amphipods create extensive oxic niches within their burrows, creating favorable conditions for nitrification (Pelegri et al. 1994, Middelburg et al. 1996, Kristensen 2000). The amphipods also likely enhanced mineralization and sediment oxygen demand; as bioturbators they increase the oxic surface area promoting aerobic microbial respiration and also directly contribute to these rates through NH$_4^+$ excretion and respiration. Amphipod-sourced NH$_4^+$ was unavoidably captured in the mineralization core incubations in this study, elevating these rates above solely microbial mineralization.

Despite the high NO$_x^-$ both from the water column and sediment nitrification at these western Sacca di Goro sites, denitrification, although higher than the other sites and dominant over DNRA, remained low relative to the NO$_x^-$ flux, resulting in low denitrification efficiency (~25%). Excess nitrification over denitrification may be a result of low carbon availability and/or lack of anoxic conditions. Although high mineralization rates and sediment oxygen demand observed at these sites would suggest the presence of labile C (Middelburg et al. 1996), these rates may be confounded by Corophium excretion and respiration. It is more likely that the nitrate produced in the oxic burrow walls has a short residence time in the sediments as the amphipods continuously bioirrigate and flush the burrows, increasing the diffusion of NO$_x^-$ out of the oxic sediment horizon into both the water column and anoxic sediment (Mermillod-Blondin and Rosenberg 2006).

**Central Sacca di Goro**

The study site in the central portion of the Sacca di Goro (Goro-16) where the ratio of denitrification to DNRA was ~1 had high water column NO$_x^-$, no Corophium
present and the highest clam biomass relative to the other sites, likely due to clam farming management practices. The DIN flux was dominated by NH$_4^+$ efflux, which was entirely attributed to clam excretion (Figure 2B). After subtracting the direct contribution of clam metabolism, a net uptake of NH$_4^+$ and a relatively low sediment oxygen demand was observed at this site. As a result of high clam excretion rates associated with the high clam biomass, denitrification efficiency was low (~13%). Although not likely a significant portion of the overall NH$_4^+$ efflux, DNRA rates were the highest at this site, likely due to increased organic matter delivery to the sediments from high clam biodeposition. Additionally, sulfide accumulation in this area has previously been reported (Viaroli et al. 2006), which may promote DNRA over denitrification (Burgin & Hamilton 2007). A strong competition between denitrification and DNRA is apparent at this site due to high water column nitrate, continuous supply of organic carbon from the clams, and potentially high sulfide concentrations.

**Eastern Sacca di Goro and US Sites**

The higher salinity sites, which spanned the two countries, (Goro-21, Cherrystone Inlet and Smith Island Bay) were characterized as having low water column NO$_x^-$, high NH$_4^+$ effluxes and a denitrification to DNRA ratio <1. These sites had very low to negligible NO$_x^-$ fluxes compared to the other sites indicating low nitrification rates, and notably no *Corphium* were present. Goro-21 likely had an active benthic microalgal community, as DO production and NH$_4^+$ uptake was observed after clam metabolism was subtracted from the whole sediment fluxes (Figures 2B, 4B). Benthic microalgae may
compete with DNRA and denitrification for NO$_x^-$ (Risgaard-Petersen 2003), further limiting the availability of NO$_x^-$ for microbial respiration at this site. To the contrary, at the US sites (Cherrystone Inlet and Smith Island Bay) a net release of NH$_4^+$ and DO consumption occurred even after clam metabolism was removed, suggesting limited nitrification and benthic microalgal activity. Additionally, these sites typically have high sulfide concentrations (Murphy et al. 2015) Smyth et al. in prep), which may inhibit nitrification (Joye & Hollibaugh 1995). The use of predator exclusion nets at the US sites may reduce benthic microalgal activity by causing sediment shading and create reduced conditions by decreasing advective flow across the sediments. In general across these three sites, denitrification and DNRA were likely limited by NO$_x^-$ availability due to low water column NO$_x^-$, limited nitrification, and benthic microalgal competition (Goro-21).

**General controls on partitioning of NO$_x^-$ between denitrification and DNRA across sites**

The weighted model obtained from mineralization rates relative to water column NO$_x^-$ (MIN: WC Nox), clam biomass, sediment organic matter, and clam species, generated values that generally underestimated denitrification relative to DNRA when compared to observed values (Figure 7). This is likely because the model does not account for *Corophium* biomass (side note: data will be added when they become available; when presence/absence of *Corophium* or porewater sulfide concentrations are included as a predictors in the model they generate a better performing model (slope=0.90, R$^2$=0.80). As explained above, *Corophium*, may significantly increase the availability of NO$_x^-$ by promoting nitrification. As a result the sites with higher *Corophium* densities typically have higher denitrification:DNRA ratios than the model.
predicts. Nonetheless, the predictor estimates provide insight into the relative influence each factor has on the partitioning of NO$_x$ between denitrification and DNRA and generally support our *a priori* hypotheses. For example, we hypothesized that the ratio of labile carbon to NO$_x$ availability would have a negative influence on denitrification:DNRA (Tiedje 1988, Hardison et al. 2015, Algar and Vallino 2014).

Assuming higher organic C availability would result in high N mineralization, N mineralization rates were used as a proxy for labile C availability. The model demonstrates that as mineralization:water column NO$_x$ increases, NO$_x$ respiration shifts toward DNRA and DNF:DNRA decreases. Additionally clam biomass was shown to have a negative effect on DNF:DNRA, which is likely due to clam biodeposition delivering organic carbon to the sediment surface.
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Table 4-1. Environmental characteristics and clam population data at each site. n.d., no data collected.

<table>
<thead>
<tr>
<th>Site</th>
<th>Salinity</th>
<th>Temp. (°C)</th>
<th>$\text{NO}_x^-$ (μM)</th>
<th>$\text{NH}_4^+$ (μM)</th>
<th>Clam density (ind m$^{-2}$)</th>
<th>Clam biomass (g DW m$^{-2}$)</th>
<th>Clam shell length (mm)</th>
<th>Sediment Organic Matter (0-2cm)</th>
<th>Porewater Sulfide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goro-10</td>
<td>10</td>
<td>20</td>
<td>53.98 (3.43)</td>
<td>19.11 (1.45)</td>
<td>398 (139)</td>
<td>82.9 (31.7)</td>
<td>28.0 (0.79)</td>
<td>1.36 (0.06)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Goro-13</td>
<td>13</td>
<td>21</td>
<td>33.96 (1.13)</td>
<td>8.50 (0.41)</td>
<td>365 (117)</td>
<td>87.1 (26.0)</td>
<td>28.0 (1.03)</td>
<td>1.74 (0.05)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Goro-15</td>
<td>15</td>
<td>21</td>
<td>40.04 (0.66)</td>
<td>9.51 (0.36)</td>
<td>1161 (268)</td>
<td>188.9 (40.6)</td>
<td>25.8 (0.44)</td>
<td>2.38 (0.35)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Goro-16</td>
<td>16</td>
<td>20</td>
<td>34.84 (0.59)</td>
<td>38.4 (2.32)</td>
<td>1127 (193)</td>
<td>553.0 (103.4)</td>
<td>32.5 (0.64)</td>
<td>0.92 (0.08)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Goro-21</td>
<td>21</td>
<td>20</td>
<td>1.07 (0.03)</td>
<td>18.43 (1.06)</td>
<td>2089 (478)</td>
<td>316.9 (64.4)</td>
<td>24.5 (0.35)</td>
<td>1.62 (0.09)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Cherrystone</td>
<td>23</td>
<td>25</td>
<td>0.20 (0.02)</td>
<td>2.10 (0.55)</td>
<td>630 (102)</td>
<td>192.4 (27.8)</td>
<td>35.5 (1.81)</td>
<td>1.21 (0.11)</td>
<td>246.5 (92.6)</td>
</tr>
<tr>
<td>Smith Island</td>
<td>33</td>
<td>27</td>
<td>0.25 (0.03)</td>
<td>0.88 (0.27)</td>
<td>258 (95)</td>
<td>192.4 (84.9)</td>
<td>43.9 (2.02)</td>
<td>1.50 (0.15)</td>
<td>647.0 (282.6)</td>
</tr>
</tbody>
</table>

146
<table>
<thead>
<tr>
<th></th>
<th>Goro-10</th>
<th>Goro-13</th>
<th>Goro-15</th>
<th>Goro-16</th>
<th>Goro-21</th>
<th>Cherrystone</th>
<th>Smith Island</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiration : Excretion</strong></td>
<td>6.9</td>
<td>10.4</td>
<td>10.0</td>
<td>7.4</td>
<td>7.7</td>
<td>6.9</td>
<td>7.1</td>
</tr>
<tr>
<td><strong>DO (mmol gDW$^{-1}$ hr$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-0.036</td>
<td>-0.038</td>
<td>-0.029</td>
<td>-0.020</td>
<td>-0.033</td>
<td>-0.005</td>
<td>-0.007</td>
</tr>
<tr>
<td>SE</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.005</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>pvalue</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>NH4 (umol gDW$^{-1}$ hr$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>5.22</td>
<td>3.69</td>
<td>2.91</td>
<td>2.70</td>
<td>4.26</td>
<td>0.78</td>
<td>0.97</td>
</tr>
<tr>
<td>SE</td>
<td>0.78</td>
<td>0.33</td>
<td>0.31</td>
<td>0.86</td>
<td>0.48</td>
<td>0.10</td>
<td>0.54</td>
</tr>
<tr>
<td>pvalue</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.145</td>
</tr>
</tbody>
</table>

Table 4-2. Statistical results from linear regressions of DO and NH4 fluxes as functions of clam biomass (g DW m$^{-2}$) during the clam-only incubations (no sediments). Slopes represent change in DO (mmol) or NH4 (umol) g DW$^{-1}$ hr$^{-1}$. 

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Table 4-3. Statistical values for linear regressions conducted within each site of response variables, including NH$_4^+$, NO$_x^-$, and DO fluxes, and denitrification (DNF) and DNRA rates, measured in the ‘whole sediment’ incubations as a function of clam biomass (g DW m$^{-2}$). Both light and dark data included in analyses.

<table>
<thead>
<tr>
<th></th>
<th>Goro-10</th>
<th>Goro-13</th>
<th>Goro-15</th>
<th>Goro-16</th>
<th>Goro-21</th>
<th>Cherry-23</th>
<th>Smith-33</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>40</td>
<td>12</td>
</tr>
<tr>
<td><strong>NH$_4^+$</strong> (umol m$^{-2}$ hr$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>1.87</td>
<td>8.95</td>
<td>3.31</td>
<td>0.94</td>
<td>3.55</td>
<td>1.55</td>
<td>0.60</td>
</tr>
<tr>
<td>SE</td>
<td>0.62</td>
<td>1.97</td>
<td>2.63</td>
<td>0.25</td>
<td>1.22</td>
<td>0.30</td>
<td>0.32</td>
</tr>
<tr>
<td>pvalue</td>
<td>0.013</td>
<td>0.001</td>
<td>0.238</td>
<td>0.00</td>
<td>0.02</td>
<td>9.10E-06</td>
<td>0.1</td>
</tr>
<tr>
<td>R$^2$</td>
<td>0.43</td>
<td>0.64</td>
<td>0.05</td>
<td>0.54</td>
<td>0.41</td>
<td>0.40</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>NO$_x^-$</strong> (umol m$^{-2}$ hr$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-1.07</td>
<td>1.39</td>
<td>-0.58</td>
<td>-0.30</td>
<td>-0.01</td>
<td>-0.01</td>
<td>-0.01</td>
</tr>
<tr>
<td>SE</td>
<td>2.34</td>
<td>1.38</td>
<td>0.90</td>
<td>0.20</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>pvalue</td>
<td>0.66</td>
<td>0.34</td>
<td>0.53</td>
<td>0.17</td>
<td>0.67</td>
<td>0.82</td>
<td>0.03</td>
</tr>
<tr>
<td>R$^2$</td>
<td>-0.08</td>
<td>-0.08</td>
<td>-0.06</td>
<td>0.09</td>
<td>-0.08</td>
<td>-0.03</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>DO</strong> (mmol m$^{-2}$ hr$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>-0.01</td>
<td>-0.05</td>
<td>-0.03</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>SE</td>
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<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>pvalue</td>
<td>0.45</td>
<td>0.001</td>
<td>0.03</td>
<td>0.0002</td>
<td>2.5E-05</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>R$^2$</td>
<td>-0.04</td>
<td>0.65</td>
<td>0.34</td>
<td>0.74</td>
<td>0.83</td>
<td>0.08</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>DNF</strong> (umol m$^{-2}$ hr$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.56</td>
<td>0.38</td>
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<td>0.04</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>SE</td>
<td>0.81</td>
<td>0.10</td>
<td>0.11</td>
<td>0.06</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>pvalue</td>
<td>0.51</td>
<td>0.003</td>
<td>0.07</td>
<td>0.57</td>
<td>0.11</td>
<td>0.56</td>
<td>0.05</td>
</tr>
<tr>
<td>R$^2$</td>
<td>-0.06</td>
<td>0.55</td>
<td>0.22</td>
<td>-0.09</td>
<td>0.16</td>
<td>-0.02</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>DNRA</strong> (umol m$^{-2}$ hr$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.12</td>
<td>0.03</td>
<td>0.01</td>
<td>-0.03</td>
<td>-0.04</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>SE</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
<td>0.07</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>pvalue</td>
<td>0.001</td>
<td>0.01</td>
<td>0.28</td>
<td>0.71</td>
<td>0.14</td>
<td>0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>R$^2$</td>
<td>0.62</td>
<td>0.47</td>
<td>0.02</td>
<td>-0.12</td>
<td>0.12</td>
<td>0.06</td>
<td>-0.10</td>
</tr>
</tbody>
</table>
Table 4-4. A summary of the percent of denitrification and DNRA coupled to nitrification ($D_n$ and $DNRA_n$), denitrification efficiency (DNF efficiency), relative proportion of denitrification to DNRA (DNF:DNRA), and mineralization rates relative to water column Nox- concentrations (MIN : Nox) at each site. n.d. no data collected.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>% Dn</th>
<th>%DNRA</th>
<th>DNF Efficiency</th>
<th>DNF:DNRA</th>
<th>MIN : Nox</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>56%</td>
<td>55%</td>
<td>18%</td>
<td>8.0</td>
<td>5.9</td>
</tr>
<tr>
<td>13</td>
<td>63%</td>
<td>63%</td>
<td>25%</td>
<td>14.2</td>
<td>10.3</td>
</tr>
<tr>
<td>15</td>
<td>61%</td>
<td>60%</td>
<td>31%</td>
<td>9.4</td>
<td>10.0</td>
</tr>
<tr>
<td>16</td>
<td>31%</td>
<td>31%</td>
<td>13%</td>
<td>1.0</td>
<td>6.3</td>
</tr>
<tr>
<td>21</td>
<td>77%</td>
<td>80%</td>
<td>12%</td>
<td>0.6</td>
<td>132.5</td>
</tr>
<tr>
<td>23</td>
<td>95%</td>
<td>95%</td>
<td>9%</td>
<td>0.2</td>
<td>498.2</td>
</tr>
<tr>
<td>33</td>
<td>100%</td>
<td>98%</td>
<td>21%</td>
<td>0.1</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
Table 4-5. A summary of the best performing models based on an Akaike’s information criterion corrected for small sample size (AICc) $\Delta < 4$; four models were included out of a total of 32 models from the universal model DNF:DNRA $\sim$ Clam Biomass + Mineralization Rates/WC Nox (MIN:WCNOx) + sediment organic matter (SOM) + Species. $k$ = number of estimated parameters including the intercept and error. $AIC_c =$ corrected Akaike’s information criterion, $\Delta_i = AIC_c$ differences relative to the smallest AICc value, $w_i =$ Akaike weights.

<table>
<thead>
<tr>
<th>Model #</th>
<th>k</th>
<th>LogLik</th>
<th>Clam Biomass</th>
<th>MIN:WC NOx</th>
<th>SOM</th>
<th>Species</th>
<th>AICc</th>
<th>$\Delta_i$</th>
<th>$w_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1234</td>
<td>6</td>
<td>-74.7</td>
<td>$\beta 1$</td>
<td>$\beta 2$</td>
<td>$\beta 3$</td>
<td>$\beta 4$</td>
<td>162.8</td>
<td>0</td>
<td>0.40</td>
</tr>
<tr>
<td>123</td>
<td>5</td>
<td>-75.7</td>
<td>$\beta 1$</td>
<td>$\beta 2$</td>
<td>$\beta 3$</td>
<td></td>
<td>162.8</td>
<td>0.01</td>
<td>0.40</td>
</tr>
<tr>
<td>124</td>
<td>5</td>
<td>-76.9</td>
<td>$\beta 1$</td>
<td>$\beta 2$</td>
<td>$\beta 3$</td>
<td>$\beta 4$</td>
<td>165.1</td>
<td>2.29</td>
<td>0.13</td>
</tr>
<tr>
<td>23</td>
<td>4</td>
<td>-78.7</td>
<td>$\beta 2$</td>
<td>$\beta 3$</td>
<td></td>
<td></td>
<td>166.3</td>
<td>3.50</td>
<td>0.07</td>
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</table>
Table 4-6. Averaged model estimates for the 4 measured predictors including mineralization rates relative to water column NO$_x$ (MIN: WC NO$_x$), clam biomass, sediment organic matter (SOM), and species on the response DNF:DNRA. $\beta_j$ = estimate; SE = standard error.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta_j$</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
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<tr>
<td>Intercept</td>
<td>0.26</td>
<td>0.52</td>
<td>0.63</td>
</tr>
<tr>
<td>MIN:WC Nox</td>
<td>-0.60</td>
<td>0.19</td>
<td>0.001</td>
</tr>
<tr>
<td>Clam Biomass</td>
<td>-0.19</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>SOM</td>
<td>1.07</td>
<td>0.46</td>
<td>0.02</td>
</tr>
<tr>
<td>Species (Tapes)</td>
<td>1.20</td>
<td>0.73</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Table 4-7. A summary of the general characteristics that influence N cycling associated with each site.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Western Sacca di Goro</th>
<th>Central Sacca di Goro</th>
<th>Eastern Sacca di Goro + US Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site IDs</strong></td>
<td>Goro-10, Goro-13, Goro-15</td>
<td>Goro-16</td>
<td>Goro-21, Cherrystone, Smith</td>
</tr>
<tr>
<td>Salinity</td>
<td>10-15</td>
<td>16</td>
<td>21-33</td>
</tr>
<tr>
<td>Water Column NO₃⁻</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Amphipod presence</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Clam Species</td>
<td>Tapes</td>
<td>Tapes</td>
<td>Tapes/Merc</td>
</tr>
<tr>
<td>Clam Biomass</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>DNF : DNRA</td>
<td>&gt;1</td>
<td>~1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>DNF Efficiency including clam excretion</td>
<td>~25%</td>
<td>13%</td>
<td>~14%</td>
</tr>
<tr>
<td>DNF Efficiency without clam excretion</td>
<td>~30%</td>
<td>86%</td>
<td>~30%</td>
</tr>
<tr>
<td>Mineralization : Water Column NO₃⁻</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>
**Figure 4-1.** Study sites in the Sacca di Goro, Italy (A) and the Eastern Shore, VA, USA (B) (note: higher resolution available.)
Figure 4-2. Net NH$_4^+$ fluxes from the whole sediment core incubations in the light (white) and dark (gray) (A). The NH$_4^+$ fluxes attributed to direct clam excretion (solid bars) and microbial processes plus other infaunal (hatched bars) in the light (white) and dark (gray) (B) (see text for details). Sites are organized by salinity. Error bars are standard errors.
Figure 4-3. Whole-sediment net NO\textsubscript{x} fluxes in the light (white) and dark (gray). Sites are organized by salinity. Error bars are standard errors. Inset shows sites Goro-21, Cherrystone Inlet (23) and Smith Island (33) on smaller scale.
Figure 4-4. DO fluxes from the whole sediment core incubations in the light (white) and dark (gray) (A). The DO fluxes attributed to direct clam respiration (solid) and microbial processes plus other infaunal (hatched bars) in the light (white) and dark (gray) (B) (see text for details). Data are organized by salinity. Error bars are standard errors.
Figure 4-5. Mineralization rates (mmol N m\(^{-2}\) d\(^{-1}\)) at each site, organized by salinity. n.d. = no data; mineralization rates were not measured at Smith Island Bay (salinity = 33).
Figure 4-6. Denitrification (DNF) rates (A) and DNRA rates (B), in the light (white) and dark (gray), including the portion coupled to nitrification, $D_n$ and $DNRA_n$ (dotted) and direct ($NO_x^-$ from the water column), $D_w$ and $DNRA_w$ (solid). Error bars are standard errors.
Figure 4-7. Predicted DNF:DNRA values \((y)\) derived from the weighted model (predictor estimates provided in Table 5) as a function of observed DNF:DNRA values \((x)\). Dashed line represents the linear regression, \(y = 0.41x + 1.06\) \((R^2 = 0.54, p < 0.001)\).
Figure 4-8. Principle components analysis (PCA) grouped by site and showing relationships among all variables including Nox fluxes, NH4 fluxes, sediment oxygen demand (SOD), denitrification (DNF), DNRA, ratio of denitrification to DNRA (DNF.DNRA), water column Nox (WC.NoX), sediment organic matter (SOM), salinity, mineralization rates relative to water column Nox (MIN..Nox), and clam biomass (Biomass). Note: Smith Island data were not included in the analysis, as mineralization rates were not measured at this site.
CHAPTER 5: QUANTIFYING THE EFFECTS OF COMMERCIAL CLAM AQUACULTURE ON C AND N CYCLING: AN INTEGRATED ECOSYSTEM APPROACH

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4Woods Hole Oceanographic Institute, Woods Hole, MA

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ABSTRACT

As commercial bivalve cultivation expands globally, an understanding of its interactions with the environment is necessary to avoid overexploitation, which could lead to both negative ecological (e.g. local eutrophication) and economic (e.g. decreased production) consequences. Additionally, increased interest in using the bioextractive practice as mitigation for eutrophication, requires a comprehensive understanding of the net carbon and nitrogen (N) budgets associated with cultivation on an ecosystem scale. This study aimed to quantify C and N processes related to commercial hard clam (Mercenaria mercenaria) aquaculture in a shallow coastal environment (Cherrystone Inlet, VA) where the industry has rapidly increased over the past two decades. Clam physiological rates were compared with other basin-wide ecosystem fluxes including primary production, benthic nutrient regeneration, and respiration. Although clam beds occupy only 3% of the ecosystem’s surface area, the clam standing stock filtered 7-44% of the system’s volume daily, an annual average of 92% of the phytoplankton production, creating a significant flux of particulate C and N to the sediments. Annually, N regeneration and C respired at clam beds, resulting from clam and microbial metabolism, were ~3-fold and ~1.5-fold higher, respectively, than particulate N and C removed through harvest. Due to the short water residence time, the relatively low watershed load, and the close vicinity of clam beds to the mouth of the Inlet, cultivated clams are subsidized by phytoplankton sourced from the Chesapeake Bay. Consequently, the increased N mineralization associated with clam cultivation is ‘new’ N as it would not be present in the system without bivalve facilitation. Additionally, high respiration rates may result in decreased pH and low oxygen conditions. Macroalgae that are subsequently fueled by the enhanced N regeneration can be considered local eutrophication. This synthesis demonstrates the importance of considering the ecological context of bivalve
aquaculture when assessing effects on the local environment, through particle removal, which significantly shifts C and N cycling.
INTRODUCTION

Suspension feeding bivalves can significantly shift energy flow through an ecosystem, particularly in a high-density aquaculture setting. As shellfish mariculture expands globally (FAO 2014), an understanding of the magnitude by which these operations alter fluxes of carbon and nitrogen (N) in nearshore marine ecosystems is needed to ensure ecological as well as economic sustainability. Further, there has been growing interest in using the bioextractive processes associated with bivalve cultivation for nutrient trading (Stadmark and Conley 2011; Bricker and others 2014; Petersen and others 2014; Rose and others 2014) and carbon sequestration programs (Filgueira and others 2015). However, few studies have investigated the net influence of bivalve cultivation on an ecosystem scale, integrating the direct and indirect ecological feedbacks associated with operations.

As suspension feeders, bivalves directly affect phytoplankton, and their metabolism influences ecosystem respiration and nutrient availability. By consuming phytoplankton and detrital material from the water column, bivalves may exert a strong 'top-down’ control on phytoplankton primary production (Cloern 1982; Officer and others 1982; Cohen and others 1984; Strayer and others 1999). A portion of this organic material is assimilated and respired by the bivalves; the rest is released as biodeposits to the sediments. As heterotrophic organisms, bivalves release dissolved inorganic carbon (DIC) (Chauvaud and others 2003; Mistri and Munari 2012), and dissolved inorganic nitrogen (DIN) through respiration and excretion, respectively. However, actively growing bivalves also sequester C and N in their tissue and shell (Newell 2004; Tang and others 2011; Beseres Pollack and others 2013).

Bivalves also facilitate indirect ecological feedbacks in an ecosystem (as reviewed in Newell 2004). By delivering labile organic matter in the form of biodeposits to sediments,
bivalves fuel microbial processes (Mirto and others 2000; Giles and Pilditch 2006). Microbial mineralization of biodeposits transforms particulate organic matter into dissolved organic and inorganic nutrients and carbon. Sediments associated with bivalve aquaculture are often organically enriched with elevated porewater nutrient concentrations (Mesnager and others 2007; Metzger and others 2007). The dissolved nutrients regenerated from biodeposits may be released to the water column, in addition to the nutrients sourced from bivalve excretion, and serve as an important substrate for primary producers (e.g. Doering and others 1987; Souchu and others 2001; Murphy and others 2015). Thus bivalves may indirectly facilitate ‘bottom up’ control on primary production.

Local physical environmental characteristics, specifically water residence time, depth, and nutrient loading are important factors that dictate the degree to which bivalves directly and indirectly alter C and N cycling. For example, if an aquaculture operation is located in a shallow photic system, the dominant primary producer fueled by the bivalve operation may be benthic microalgae, submerged aquatic vegetation, and/or macroalgae, as opposed to pelagic phytoplankton. This has direct consequences on bivalve growth, as these producers are typically not considered available for bivalve consumption (although, see Hondula and Pace 2014; Emery and others 2015). Further, bivalve aquaculture located in a highly flushed system with a short residence time is likely subsidized by phytoplankton produced outside of the immediate ecosystem, causing significant alterations to the energy budget within the system (Guyondet and others 2013; Filgueira and others 2014).

The majority of relevant studies have focused on organismal scale impacts neglecting the consideration of context-dependent ecological feedbacks, such as the support of additional production through nutrient regeneration. These, often complex, trophic interactions and indirect
effects driven by bivalve aquaculture are important components in assessing the net ecosystem effects of mariculture on C and N cycling. Additionally, these interactions must be explored within the context of local variability as basin-scale characteristics affect how bivalves interact with the environment.

The objective of this study was to quantify hard clam (*Mercenaria mercenaria*) aquaculture C and N processes relative to other basin-wide ecosystem fluxes for a shallow coastal ecosystem, Cherrystone Inlet, VA. We compare clam feeding, respiration, excretion, egestion, and shell production to ecosystem processes such as benthic and pelagic primary production and benthic microbial metabolism, including denitrification rates. We incorporate an ecosystem framework accounting for the trophic interactions of the clams, phytoplankton, macroalgae, and microbial community, while including clam harvest as a C and N loss from the aquatic system. We hypothesized that the high densities of clams in Cherrystone Inlet would significantly alter the magnitude of C and N fluxes within the system given their capacity to filter a large volume of the tidal prism.

**METHODS**

*Site Description and Clam Cultivation Practices*

Cherrystone Inlet is a small tidal embayment (5.7 km²) on the western shore of the Delmarva (Delaware-Maryland-Virginia) peninsula in Virginia (Figure 1). The Inlet averages 1 m in depth and is characterized by shallow flanking shoals with a narrow channel that leads to the Chesapeake Bay (Reay and others 1995). Although the entire embayment is leased for shellfish production (Virginia Marine Resources Commission), active leases only exist in the shallow subtidal portions of the bay (Emery 2015), with the majority of this space occupied by
hard clam (*Mercenaria mercenaria*) mariculture (Figure 1). The volume of the embayment is 6.2x10^6 m^3 with a tidal prism of 4.5x10^6 m^3 (Kuschner 2015) and an average hydrologic residence time of 2-3 days (Herman and others 2007).

Typical clam cultivation practices in the US involve planting hatchery-reared juveniles in subtidal sediments. Since all clams within a bed are planted concurrently, each clam bed (4x18m) consists of a homogenous size-class. Growers place a plastic mesh net, set flush to the sediment surface, over each clam bed to protect the clams from natural predators (e.g. blue crabs, cow-nose rays). Use of a predator-exclusion net is a common clam cultivation practice throughout the US (Castagna 2001). In the warmer months, macroalgae typically foul the nets and proliferate, fueled by nutrient regeneration associated with the clams and microbial processes in the sediments (Bendell 2015; Murphy and others 2015). The macroalgae significantly reduce flow across the sediment water interface (Adams and others 2011) and are swept off the nets by the aquaculturists approximately monthly to prevent detrimental effects to the clams (T. Rapine, Cherrystone Aquafarms, pers. comm.).

**Water Quality**

Dataflow surface water quality mapping surveys, which were conducted throughout the Inlet in March 2011, and May, July, and October 2012, measured surface water temperature, pH, chlorophyll *a* (chl *a*), turbidity, and dissolved oxygen (DO). The dataflow system is equipped with a YSI 6600 datasonde, which is calibrated prior to and after each survey, a Garmin global positioning system, and data acquisition system (Madden and Day 1992). During each survey, surface water samples were collected for extractable chl *a* analysis (Shoaf and Lium 1976) at 6 randomly selected stations across the embayment. Regressions were made between *in situ* YSI
chl a measurements versus extractable chl a samples to calibrate the data. Chl a data were visualized using ArcGIS 10.2 and the inverse distance weighting tool (IDW) to create a spatially interpolated map of chl a over the entire Inlet, during the four months sampled. A zonal statistics tool was used to compare chl a in water directly over the clam beds with the rest of the Inlet, to determine to effect of clams on proximal chl a concentrations.

*Clam Aquaculture Spatial Coverage*

The standing stock of clams in Cherrystone Inlet was estimated using aerial image analysis. Clam beds are readily visible in photographs taken at low tide for the annual Submerged Aquatic Vegetation Survey conducted by the Virginia Institute of Marine Science (VIMS) (Orth and others 2010). The clam beds (72 m²), covered with anti-predator netting, appear as black rectangles in the images. Active clam leases were delineated in ArcGIS (ESRI) using photographs from 2001 and 2003 – 2012 and combined with prior estimates of clam aquaculture coverage for 1990-1997 (Woods 2001). A linear regression of the number of active clam beds and time was used to analyze industry trends from 1990-2012 and estimate the expansion over time in Cherrystone Inlet.

The standing stock of clams in the system in 2012 was estimated by multiplying surface area of clam beds, obtained from aerial photographs in 2012, by the clam density data collected in the field (see below). The surface area estimate included only the active clam beds in the photographs and represents a conservative estimate of clam coverage that excludes the space between clam beds and inactive beds.

*Clam Population Size Class Distribution*
Clam population and size distribution data, including density, mean shell lengths and biomass, were obtained from sediment cores collected seasonally in 2013 across the largest lease in Cherrystone Inlet. Triplicate sediment cores (9.5 cm inside diameter) were collected from 16, 16, and 7 randomly selected clam beds in May, July, and November, respectively. In each core, clams were counted, measured, and dry weights and ash-free dry weights were obtained. A subset of dried clam tissue samples was analyzed for total nitrogen and organic carbon content on a Carlo-Erba elemental analyzer (Thermo Electron Corp. Flash EA 1112 Series).

An estimate of the total number of clams in the Inlet was obtained by multiplying the average clam density (individuals m⁻²) by the total areal extent of the clam beds in the Inlet (see “Clam Aquaculture Spatial Coverage”). Clams were binned according to industry-designated size categories as seed, button, littleneck, and middleneck. The mean shell length for each category was determined by measuring the shell lengths of clams harvested and sold in these size categories. The mid-point between the means of each size class served as the breakpoint from one size class to the next in order to obtain a range for each size class. Button clams were designated as 25.3 to 42.1 mm, littlenecks were 42.2 to 50.9 mm, and middlenecks were 60.0 mm and greater. Seed clams were designated as averaging 12 mm (T. Rapine, Cherrystone Aquafarms, pers. comm.) and ranged from 0 to 25.25 mm. The total number of clams within each size class was estimated using the size frequency distribution of the field survey data. For example, the total number of littleneck clams within the Inlet was calculated as the percentage of clams that were between 42.2 to 50.9 mm from the field data multiplied by the total number of clams in the Inlet.
The relationship between shell length and soft tissue dry weight of a subset of clams collected in the field (n = 159) was used to estimate the biomass ($DW_{\text{tissue}}; g$ DW individual$^{-1}$) for each size class (Supplementary Figure S1) as

$$DW_{\text{tissue}} = 0.0009L^2 - 0.028L + 0.266 \quad (1)$$

where $L$ is equal to shell length (mm). Biomass was scaled to the total population of each size class and summed to obtain the total biomass of clams within the Inlet. The total C and N in the soft tissue of the clam standing stock was obtained by multiplying total tissue dry weight by the average percent organic C (37.8%) and total N (13.05%) obtained from the subsamples collected in Cherrystone. A similar estimation was used to determine the C and N removed annually through harvest (see below).

Shell dry weights ($DW_{\text{shell}}; g$ DW individual$^{-1}$) for each size class were estimated using an equation derived from Wiseman (2010) as

$$DW_{\text{shell}} = 0.0002L^{2.93} \quad (2)$$

where $L$ is shell length (mm). $DW_{\text{shell}}$ were converted to inorganic and organic carbon assuming shells are predominantly composed of calcium carbonate (95% by mass), of which 12% is carbon and 1.9% is organic carbon (Price and others 1976; Doering and others 1987; Bouillon and others 2011). The total C in the tissue and shell were summed to estimate the total C stored in the Inlet's living clam population.
The total number of individuals within each size class harvested from Cherrystone Inlet in 2012 was obtained from local growers and used to calculate the amount of C and N removed annually in clam tissue and shell material using the relationships described above.

*Clam Physiological Rates*

Clam physiological rates were calculated using equations from the literature and scaled to the seasonal standing stock clam population in Cherrystone Inlet in 2012, seasonally. Specific equations are summarized in Supplementary Table S1 and briefly described below. Physiological rates were calculated for each of the four size classes (i.e. seed, button, littleneck, and middleneck) using measured environmental data from each season (winter, spring, summer, and fall) and scaled to seasonal and annual rates.

Filtration rates (FR, ml ind\(^{-1}\) d\(^{-1}\)) were estimated using equations from Hofmann et al. 2006 and modified by Wiseman 2010 in which the maximum filtration rate (FR\(_{\text{max}}\), ml ind\(^{-1}\) d\(^{-1}\)), originally derived from an equation by Doering and Oviatt (1986), was adjusted by dimensionless functions to account for the effects of temperature and salinity. Additionally, the predator exclusion nets in Cherrystone Inlet were found to reduce filtration rates by 35% (Condon 2005); therefore, our rates were reduced to account for this local cultivation effect.

Filtration rates were converted to ingestion rates (g PN and g PC day\(^{-1}\)) using the mean seasonal chl \(\alpha\) concentrations across the Inlet measured during the dataflow surveys and the ratios of PN and PC to chl \(\alpha\) (7.03 g N g chl \(\alpha\)\(^{-1}\) and 57.21 g C g chl \(\alpha\)\(^{-1}\), respectively) obtained using water column data collected by the VA Department of Environmental Quality directly outside of Cherrystone Inlet (monitoring station CB 7.3).
Clam respiration rates were estimated using an equation from Hofmann and others (2006) adjusted by Wiseman 2010 using data collected in Cherrystone (Condon 2005). Rates of clam excretion, primarily composed of NH$_4^+$ (Hammen 1980), were calculated stochiometrically (Mayzaud and Conover 1988), using a respiration to excretion ratio for *M. mercenaria* of 7.83. This ratio was obtained in an experiment conducted in July 2013, in which Cherrystone clams of varying sizes were incubated in closed chambers without sediments and fluxes of oxygen and NH$_4^+$ were measured (Supplementary Data, Figure S2).

Egestion rates were estimated using an assimilation efficiency fixed at 75%. Although assimilation efficiency is dependent on seston quality and quantity (Secrist 2013, Turner et al. 1988, Bass et al. 1990), this estimate is reasonable for Cherrystone Inlet based on findings from a clam growth model specific to Cherrystone Inlet where this assimilation rate produced realistic weights and growth rates (Kuschner 2015) and is supported by literature findings for *M. mercenaria* (Tenore and others 1973).

**Primary Production**

Seasonal macroalgal, benthic microalgal, and phytoplankton net production rates and N demands were estimated using data collected in Cherrystone and reported in previous studies (Reay and others 1995; Murphy and others 2015) Kuschner 2015).

The macroalgal community on the predator exclusion nets is typically dominated by *Gracilaria* spp., intermixed with *Ulva lactuca* and *Agardhiella* spp. (Murphy and others 2015). Murphy et al. (2015) provides seasonal benthic in situ flux data from 2012 at clam beds in Cherrystone Inlet with and without the addition of macroalgae. These data were used to calculate macroalgal production and N uptake rates; details on the approach are provided in
Murphy et al. (2015). Macroalgal production and N demand rates were scaled to the estuary by multiplying by the total areal coverage of clam nets. Self-shading effects on macroalgal production and N demand were not considered important as aquaculturists sweep the nets frequently, maintaining a biomass (24-124 g DW m$^{-2}$; (Murphy and others 2015)) generally lower than when self-shading becomes significant (>100 g DW m$^{-2}$; (McGlathery and others 2001)).

Benthic metabolic rates in sediments outside of the cultivation areas reported by both Reay and others (1995) and Murphy and others (2015) were used to estimate benthic microalgal production and N demands. Reay and others (1995) reports seasonal in situ flux measurements in 1990-1991, prior to the expansion of clam beds in the system (Figure 3). Therefore, these measurements may be considered an estimate of baseline benthic metabolism and nutrient fluxes that have not yet been altered by clam aquaculture. Conversely, the sediment fluxes reported by Murphy and others (2015) in control areas outside of the clam beds have presumably been affected by the two decades of clam aquaculture in the system. These two studies, which were conducted using similar methods, provide an opportunity to compare benthic processes before and after clam cultivation was established. Benthic microalgal production rates were estimated using the benthic gross primary production rates reported in Reay and others (1995) and Murphy and others (2015) ('Bare' treatment) and adjusting for autotrophic respiration, assumed to be 10% of production (Cloern 1987). As rates were measured during peak irradiance hours during the day, values were adjusted using a P-I curve model described by (Pinckney and Zingmark 1993), which accounts for diel light variation (measured in the field) on benthic microalgal production. Production rates were converted to N demand using a molar C/N ratio of benthic microalgae of 9.0 (Sundbäck and others 2000). These rates were scaled to the estuary by
multiplying by the total surface area of the Inlet, assuming the majority of the sediments are
photic. This assumption is reasonable as the average depth of the system is 1m and Reay and
others (1995) report the photic zone, in which >1% of the surface irradiance reaches the sediment
surface, to be from 0.7m in the summer to 6.1m in the winter.

Phytoplankton production rates were obtained from an ecosystem model described by
Kuschner (2015). Briefly, a reduced complexity water quality box-model relevant to shallow
coastal systems (Lake and Brush 2015) was adapted to Cherrystone Inlet using
forcing data specific to the system (e.g. temperature, salinity, TSS, photosynthetically active
radiation (PAR)). The phytoplankton primary production rates were modeled using empirical
relationships that previous studies have demonstrated are applicable across temperate estuaries
(Brush and others 2002; Brush and Brawley 2009). Model calibration was conducted by
Kuschner (2015) using water quality data collected by the CBP (VA DEQ) at three monitoring
stations (C-1, C-2, and C-3) within Cherrystone Inlet during 2001-2002.

Benthic Microbial Respiration and N Mineralization

Microbial respiration and N mineralization rates were estimated at the clam cultivation
sediments and control sediments (i.e. outside the cultivation areas) using seasonal in situ benthic
flux data from 2012 (Murphy and others 2015). The microbial contribution to N remineralization and respiration at the clam beds was estimated by subtracting the calculated clam excretion and respiration rates from the dissolved N fluxes and benthic respiration at the clam beds (Murphy and others 2015); these values were scaled to the surface area of the clam beds in Cherrystone in 2012. Respiration and dissolved N flux rates at the uncultivated
sediments, reported by Murphy and others (2015), were scaled to the surface area of the embayment minus the clam bed area.

RESULTS

*Environmental Characteristics – 2012*

In 2012, water temperature ranged from an average of 7.1°C in the winter to 27.5°C in the summer. Salinity varied little across the year, with slightly lower salinity in the spring and summer than the fall and winter months (Table 1). Chl $a$ concentrations were highest in the spring at 15.2 $\mu$g l$^{-1}$ and lowest in the winter at 1.8 $\mu$g l$^{-1}$. POC and PN varied similarly throughout the year with lowest values in the winter and highest values in the spring and summer (Table 1).

The dataflow surveys provide a spatial snapshot of water quality parameters across the Inlet seasonally in 2012. There was little difference between chl $a$, pH, or DO measurements in areas close to the clam cultivation operations versus regions away from the operations indicating a well-mixed system (Supplementary Table S2). Turbidity was typically lower in the regions with clam beds compared to uncultivated regions (data not shown). Chl $a$ varied seasonally, with highest concentrations observed in the summer. Typically higher chl $a$ was found in the upper regions of the estuary, particularly in the summer (Figure 2).

*Clam standing stock population and harvest*

There were 2,514 clam beds in Cherrystone Inlet in 2012. Overall clam aquaculture coverage in Cherrystone Inlet has significantly increased since 1989 (Figure 3, $R^2 = 0.77$, $p < 0.001$) with an annual clam bed growth rate of about 104 beds per year. Clam standing stock,
total biomass, and harvest information from 2012 are provided in Table 2. In 2012, the clam biomass to water volume ratio in Cherrystone was 12.5 g DW m\(^{-3}\). The total C and N contained within the clam standing stock was 208.3 Mg C and 10.5 Mg N. About 20% of the clam population is harvested annually, equating to about 45% of the total C and N contained in clam standing stock biomass, since the larger clams are harvested (Table 2). Approximately 95.8 Mg C and 4.7 Mg N is removed from the aquatic system annually through clam harvest (Figure 6).

Clam Physiological Rates-2012

Total filtration rates of the clam population in Cherrystone ranged from 0.43\(\times 10^6\) m\(^3\) day\(^{-1}\) in the winter months to 2.76\(\times 10^6\) m\(^3\) day\(^{-1}\) in the fall months with intermediate rates in the spring and summer (Table 3). With this filtration capacity, the number of days it took the cultivated clam population to filter the whole system ranged from 2.3 to 14.5 days (Table 3); between 20 and 124% of the tidal exchange was filtered each day (2 tides per 24 hr).

Table 4 summarizes the seasonal clam physiological rates (ingestion, respiration, excretion, egestion, and assimilation). Similar to filtration rates, clam ingestion rates also varied seasonally; however, despite highest filtration rates in the fall, highest ingestion rates occurred in the summer months when water column PC and PN concentrations were elevated (Table 1). Highest ingestion rates, observed in the summer, were 1905.5 kg C day\(^{-1}\) and 287.6 kg N day\(^{-1}\), while lowest rates, measured in the winter, were 43.9 kg C day\(^{-1}\) and 6.6 kg N day\(^{-1}\) (Table 4).

Clam respiration rates ranged from 59.2 kg C day\(^{-1}\) in the winter to 462.6 kg C day\(^{-1}\) in the summer. Similarly excretion rates were highest in the summer at 59.1 kg N day\(^{-1}\) and lowest in the winter at 7.6 kg N day\(^{-1}\) (Table 4). On an annual basis approximately 28% of the assimilated C was respired by the clams and released as DIC while approximately 21% of the assimilated N
was excreted as NH$_4^+$. Our clam energetics model set biodeposition rates at a constant of 25% of ingested C and N as assimilation was assumed to be 75%. Therefore egestion followed similar seasonal trends as ingestion, with rates ranging from 11.0 kg C day$^{-1}$ in the winter to 476.4 kg C day$^{-1}$ in the summer.

**Primary Production**

Phytoplankton net production rates ranged from 0.08 to 0.41 gC m$^{-2}$ d$^{-1}$ throughout the Inlet, with an annual net production rate of 77.7 gC m$^{-2}$ yr$^{-1}$ (Kuschner 2015, M. Brush, pers. comm.). Phytoplankton turnover time (i.e. biomass : production) in the Inlet thus ranged from 1.1 to 3.2 days, with an annual average of 2.2 days (Table 5). Scaled to the entire system, phytoplankton production rates ranged from 0.48 Mg C d$^{-1}$ in the winter to 2.3 Mg C d$^{-1}$ in the summer (Figure 4) with an overall annual production of 437.6 MgC yr$^{-1}$ (Table 6). Clams were estimated to ingest between 9 and 131% of the net phytoplankton production depending on the season, with an annual average of 92% (Figure 4, Table 6).

Macroalgal net production rates on the clam cultivation nets ranged seasonally from 1.5 to 4.3 gC m$^{-2}$ d$^{-1}$, with an annual production rate of 825.3 gC m$^{-2}$ yr$^{-1}$, assuming zero macroalgal production in the winter (no winter data available). Scaled to the total surface area of the clam nets in the Inlet, macroalgal net production rates ranged from 0.28 Mg C d$^{-1}$ in the fall to 0.77 Mg C d$^{-1}$ in the summer (Figure 4), with an overall annual production of 149.4 Mg C yr$^{-1}$ (Table 6).

Historical BMA net production rates, estimated using Reay and others (1995) data collected in 1990-1991, ranged from 0.06 to 0.20 gC m$^{-2}$ d$^{-1}$, with an annual production of 53.0 gC m$^{-2}$ yr$^{-1}$. These rates were lower than BMA net production rates obtained in 2012 (Murphy
and others 2015), which ranged between 0.81 and 1.04 gC m$^{-2}$ d$^{-1}$ with an annual production of 230.2 gC m$^{-2}$ yr$^{-1}$, although winter data was not available for this dataset. Scaled to the entire ecosystem, assuming the sediments are photic throughout the year (Reay and others 1995), total BMA production rates were 298.2 MgC yr$^{-1}$ in 1990-1991 and 1295.6 MgC yr$^{-1}$ in 2012.

Benthic Respiration and N Remineralization Rates

At the uncultivated sediments (area outside of the clam beds), respiration, scaled to the surface area of the Inlet, ranged from 1.3 MgC d$^{-1}$ in spring to 3.1 MgC d$^{-1}$ in fall, with rate of 1.4 Mg C d$^{-1}$ in the summer (Figure 5A). Total clam bed respiration rates, including both clam and microbial respiration, scaled to the surface area of the clam beds, ranged from 0.37 MgC d$^{-1}$ in the fall to 0.65 MgC d$^{-1}$ in the spring and 0.43 MgC d$^{-1}$ in the summer (winter data not available). Total clam bed respiration rates averaged 31% of the uncultivated benthic respiration rates, despite clam bed surface area accounting for only 3% of the total system surface area.

Annual C and N Budget

Annual fluxes of C and N associated with clam aquaculture are of a similar magnitude to primary production and microbial respiration, and clam harvest results in significant C and N losses (Figure 6). Clam ingestion rates of PC and PN are approximately 90% of the annual net phytoplankton production. Of the PC and PN ingested by the clams, 25% is egested as biodeposits into the sediments. About half the egested PC and PN is respired and remineralized by microbial processes; the remainder is either buried or resuspended and transported away from the shallow clam beds. Of the C and N that are assimilated by the clams, 15-30% is subsequently respired and excreted by the clams. Annual harvest accounts for 21% and 8% of

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the PC and PN ingested by the clams, respectively. Notably, as reported above only 20% of the clam population is harvested each year, therefore the remaining assimilated C and N in the clam standing stock remains in the system, and also accounts for mortality and reproductive output.

DISCUSSION

This synthesis study demonstrates that high densities of clams in Cherrystone Inlet significantly alter C and N cycling in the system. Although the clam cultivation aerial footprint only occupies 3% of the total surface area of the embayment, the cultured clam population has the capacity to filter a significant portion of the water column daily (7-44%), creating a large flux of PC and PN from the water column to the sediments. By shifting energy from the water column to the benthos, clam cultivation significantly alters fundamental ecosystem processes such as ecosystem primary production, respiration, and nutrient cycling (Peterson and Heck 1999, Grizzle et al. 2001). A comparison between rate processes (i.e. respiration, net community production, and NH$_4^+$ flux) at the clam bed sediments and uncultivated sediments reveals that even after scaling to the entire estuary, despite the relatively small surface area of the clam beds, these operations are strongly influencing benthic rates on an ecosystem scale (Figures 4, 5). As clam cultivation continues to expand in this tributary, as well as worldwide, an understanding of its interactions with the environment, specifically with respect to energy flow and nitrogen cycling, is necessary to avoid overexploitation, which could lead to both negative ecological (e.g. local eutrophication) and economic (e.g. decreased production due to carry capacity issues) consequences.

_Budget Uncertainties_
A number of uncertainties are inherent in our estimates that scale to the entire system, particularly with respect to spatial and temporal environmental variability. However, the objective of this study was not to estimate uncertainty using rigorous statistical approaches, but rather to compare the relative magnitudes of ecosystem C and N fluxes. While a more precise budget would need to encompass estimate uncertainty, our analysis indicates at a first order that clam fluxes at an intensive cultivation site are large relative to key ecosystem fluxes.

Despite uncertainties, our estimates fall within reasonable range of previous studies in Cherrystone Inlet (Luckenbach and Wang 2004) Condon 2005). Luckenbach and Wang (2004) reported that Cherrystone Inlet’s clam standing stock filtered approximately 28% of the tidal exchange, requiring an average 10 days to filter the system, while Condon (2005) estimated 10-82% of the total Inlet is filtered daily by the clam population. These rates are comparable to our estimates that the clams filtered 10-62% of the tidal exchange, 7-44% of the total Inlet daily, requiring 2.3-14.5 days to filter the entire system, depending on the season. Modest differences between our findings and those reported by Luckenbach and Wang (2004) and Condon (2005) likely stem from using different volume, surface area, and tidal prism data. We used data from Kuschner 2015, while Luckenbach and Wang (2004) and Condon (2005) utilize a larger spatial footprint for Cherrystone obtained from Kuo and others (1998), which includes a broad area outside the mouth of the estuary, periodically enclosed by a dynamic spit of sand.

A more significant difference in our results is the estimate of 5 MgN yr\(^{-1}\) for harvested clam PN. Luckenbach and Wang (2004) derived a much larger estimate of 18 MgN yr\(^{-1}\). This discrepancy is likely due to differences in assumed harvested clam sizes. The average harvested clam size used by Luckenbach and Wang (2004) was 60 mm in height or approximately 73 mm shell length, which is much larger than our harvested clam size, which ranged from 38.5 to 56.1
mm shell length (button to middleneck sizes). We obtained our values directly from the growers. However, our estimated PN harvested annually (5 MgN yr$^{-1}$) was very close to that estimated by Condon (2005), which ranged from 2.4 to 5.5 MgN yr$^{-1}$.

**Clam Grazing, Internal Phytoplankton Production, and External Inputs**

In Cherrystone Inlet, the cultured clam population has a strong effect on phytoplankton biomass as the clams annually graze an average of 92% of the estimated phytoplankton production in the system. Theoretically, if the time it takes the bivalve community to filter the entire system (total clearance time) is approximately equal to the phytoplankton turnover time and shorter than the system residence time, the bivalve population will control phytoplankton biomass (Dame and Prins 1998), as occurs in San Francisco Bay (Cloern 1982).

In Cherrystone, the clam clearance time (~2.3-14.5 days) is generally greater than the phytoplankton turnover time (~1.1-3.2 days) and the system’s residence time (~2-3 days), implying that internal production may be sufficient to support current clam production. However, this conclusion is an oversimplification since it assumes that clams have continuous access to the internally produced phytoplankton, which is not likely in Cherrystone where the majority of clam beds are located close to the mouth of the Inlet (Figure 1), whereas, chl $a$ concentrations are highest up-estuary (Figure 2). Spatial variation in food supply may be due to clam grazing activity, depleting chl $a$ in adjacent waters; however, in a system like Cherrystone Inlet, with a short residence time (~2-3 days) and high tidal forcing, advection is likely the dominant process controlling chl $a$ spatial distribution (as reviewed in (Prins and others 1998). Thus the clam population, particularly in the outer portion of the Inlet, is likely fueled by phytoplankton delivered from the Chesapeake Bay on incoming tides. Additionally, dissolved nutrient input
from the Chesapeake Bay supports a portion of the internal phytoplankton production (Figure 6). A modeling exercise by Kuschner 2015 corroborates this hypothesis, demonstrating that if exchange with Chesapeake Bay is removed from the model, clam growth is reduced by approximately 40%, implying that the incoming tide provides an important food subsidy for the cultured clams.

Although bivalves may be considered a natural control for eutrophication due to their removal of phytoplankton (Officer and others 1982), bivalves may also promote local eutrophication (sensu Nixon 1995) indirectly by stimulating primary production by BMA, macroalgae, and/or seagrass (as reviewed in Newell 2004; Dumbauld and others 2009). In Cherrystone Inlet, clam cultivation may promote BMA and macroalgal production by alleviating nutrient and/or light limitation altering benthic fluxes. A comparison between benthic rate measurements collected prior to the rapid expansion of clam cultivation in Cherrystone Inlet in 1990-1991 (Reay and others 1995) with data collected in 2012 (Murphy and others 2015) revealed BMA production in sediments outside of the cultivation area has increased by about 4-fold. This increase in benthic primary production may be due to a general decrease in light attenuation in the system, potentially a direct effect of clam filtration activity. Between 1990-91 and 2012 average seasonal light attenuation decreased by an average of 46% (Supplemental Figure S3) (Reay and others 1995; Murphy and others 2015), which may be attributed to the increased clam cultivation in the system. By clearing particulates from the water column, bivalves increase light penetration depth and have been shown to enhance benthic primary production including BMA and submerged aquatic vegetation (Peterson and Heck 2001, Newell et al. 2002, Newell and Koch 2004). Additionally, over time the active drawdown of particulates
to the sediments by clam feeding may enrich the benthic compartment with organic matter and nutrients, fueling BMA production (Newell and others 2002).

High macroalgal production on the shallow predator exclusion nets associated with the cultivation operations is comparable to production rates of BMA and phytoplankton even when scaled to the entire ecosystem (Figure 4). The clam cultivation operations provide both a shallow hard substrate for macroalgal attachment as well as a nutrient source for macroalgal growth (Powers and others 2007; Murphy and others 2015). As highlighted in Figure 6, the clam beds are a large source of NH4+ to the water column due to both clam excretion and microbial N mineralization. This increase in N regeneration is sufficient to meet the entire N demand of the macroalgae proliferating on the nets (Murphy and others 2015).

Implications of N removal

The concept that grazers may control eutrophication (i.e. the increase supply of organic matter in a system, Nixon 1995) directly by exerting top-down control on phytoplankton is not new (e.g. (Cloern 1982; Officer and others 1982; Dame and Prins 1998; Prins and others 1998; Smaal and Prins, 1993). However, recently the debate has shifted to include the effects of bivalves on N removal and thus, indirectly, eutrophication control. Bivalve aquaculture has been suggested as an effective means to mitigate nutrient pollution and reduce eutrophication risk (Lindahl and others 2005; Bricker and others 2014; Rose and others 2014), since cultivation methods do not require feed input and upon harvest, N sequestered in the bivalve tissue and shell is removed from the aquatic environment. However, this removal term should be assessed relative to nutrient regeneration to determine the overall effect of intensive bivalve culture on the ecosystem N budget.
In Cherrystone Inlet inorganic N regenerated in the clam sediments by both clam metabolism (i.e. excretion) and microbial mineralization of clam biodeposits is ~3-fold higher than the removal of N via harvest on an annual basis (approximately 14 vs. 5 Mg N yr\(^{-1}\); Figure 6). The origin of this regenerated N, which fluxes from the clam sediments, dictates the overall impact of clam cultivation on the ecosystem N budget. For example, if the dominant food source for the clams is produced internally then N regeneration facilitated by the clams, is a recycling of particulate N to dissolved N that was already in the system (i.e. no net effect). However, if the PN filtered by the clams is drawn from outside the system, the regenerated N facilitated by the clams is considered ‘new’ to the ecosystem, and would not be delivered to the sediments in the absence of clam cultivation. As discussed above, the origin of food (PN and PC) for the clams (i.e. internal vs. external) is dependent on the time the total clearance time of the clam population relative to the hydrologic residence time and internal phytoplankton production turnover time (Dame and Prins 1998). Due to the short residence time, the high clam biomass to water volume ratio (12 kg m\(^{-3}\)), and the distribution of clam cultivation in outer portions of the embayment, the system is likely functioning as a ‘feedlot’ as reviewed in Dame 2011 (Dame 2011). Every incoming tide brings more Chesapeake Bay-sourced-phytoplankton for the clams, which filter this material and facilitate its transformation to dissolved inorganic forms. Subsequently, inorganic nitrogen fluxes from the sediments and fuels macroalgal growth, a form of local eutrophication.

Another N removal process that is often associated with bivalve aquaculture is denitrification, the microbial process that reduces NO\(_3^-\) to N\(_2\). Although certainly site-specific, numerous studies have reported elevated denitrification rates in bivalve-dominated sediments, facilitated by the increased organic matter deposition through bivalve egestion (Newell and
others 2002; Kellogg and others 2014). For comparison to other fluxes, the seasonal
denitrification rates at clam beds in Cherrystone Inlet reported by Murphy et al. (in review) are
included in our annual N budget (Figure 6). Denitrification is small compared to N regeneration
rates and N removal through harvest, only accounting for 0.05% of the particulate N filtered by
the clams and 0.2% of the particulate N egested by the clams.

**Implications of Carbon Removal**

Bivalve filter feeders can greatly alter the flow of carbon through a system, especially
when cultivated in large numbers (Dame and Prins 1998; Dumbauld and others 2009; Tang and
others 2011; Filgueira and others 2014; 2015). Our estimates of carbon fluxes in Cherrystone
Inlet associated with clam aquaculture highlight several of these pathways (Figure 6). A large
amount of carbon is removed from the water column through ingestion and either assimilated
into the clam tissue, respired, or transferred to the benthos as feces and/or pseudofeces. As
described above, the transfer of organic matter to the sediments can lead to improved water
clarity and stimulated benthic algal production. However, there are also ecological implications
for the respired and assimilated carbon.

The respired carbon is released to the local environment as DIC. An additional source of
CO$_2$ from clams results from the calcification process where 1 molecule of CO$_2$ is produced for
each molecule of CaCO$_3$ produced (Frankignoulle and Canon 1994; Hily and others 2013).
Significant CO$_2$ production has been observed in large bivalve populations (Chauvaud and others
2003; Mistri and Munari 2012), yet there are possible abatement factors to this CO$_2$ production
including stimulating the production of macroalgae (Murphy and others 2015) and the use of 5-
37% of respired CO$_2$ as the inorganic carbon source in the shell building process (Gillikin and
others 2007). Depending on system characteristics, a subsequent loss of CO$_2$ through gas transfer to the atmosphere is possible and would represent a loss from the system. This may occur naturally depending on the CO$_2$ saturation state of the system, but is potentially enhanced through shellfish aquaculture (Chauvaud and others 2003; Mistri and Munari 2012).

A second loss of carbon from the system occurs through harvest of the assimilated carbon in shell and tissue material (Tang and others 2011). While clam tissue is likely consumed and respired on a short time scale, the fate of shell material is not as certain. In some cases shells are returned to coastal systems for aquaculture or restoration purposes (Piazza and others 2005). However, shells are also largely disposed of on land, representing a potentially long-term carbon sink (NRC 2010). Also, consistent withdrawals of calcium carbonate through harvests can reduce alkalinity thereby increasing the potential for acidification of the system (Waldbusser and others 2013). Given the impacts of aquaculture estimated in this study, developing improved carbon budgets for systems with and without shellfish aquaculture is warranted to improve understanding of coastal carbon cycling (Doney 2010; Cai 2011; Bauer and others 2013; Laruelle and others 2014; Filgueira and others 2015; Gruber 2015).

Conclusions

Linking bivalve physiology, the physical environment, and ecosystem level processes to determine the overall effects of bivalves in a system is not a new concept (e.g. (Officer and others 1982; Prins and others 1998), however few recent studies that attempt to assess bivalves in terms of nutrient removal and carbon cycling fully consider the bivalves within the context of an ecosystem (as reviewed by (Filgueira and others 2015). This synthesis demonstrates the large influence clam cultivation has on C and N cycling, highlighting the importance of external food
sources in supporting high biomass of clams in a system with a fast water residence time.

Although a net sink for PN and PC from the aquatic system upon harvest, on a local ecosystem scale, bivalve aquaculture enhances benthic respiration and N mineralization. Depending on the physical characteristics of the ecosystem (e.g. residence time, depth, etc), food for clams may be derived from outside the immediate system (i.e. a subsidy). Thus the increased N mineralization associated with the bivalve cultivation is ‘new’ N as it would not be present in the system without bivalve facilitation. The primary production that is subsequently fueled by the regenerated N is a result of the cultivation operations. This synthesis demonstrates the importance of considering the ecological context of bivalve aquaculture when assessing effects on eutrophication, both removal of particulates but also influence on N cycling.
Literature Cited.


Emery, K. A. (2015). Coastal bivalve aquaculture carbon cycling, spatial distribution and resource use in Virginia, USA and Baja California, Mexico. MS Thesis, University of Virginia, Charlottesville, Virginia, USA.


Table 5-1. Seasonal average water column characteristics in 2012, Cherrystone Inlet.

<table>
<thead>
<tr>
<th>Season</th>
<th>Salinity</th>
<th>Temperature (°C)</th>
<th>POC (mg L⁻¹)</th>
<th>PN (mg L⁻¹)</th>
<th>TSS (mg L⁻¹)</th>
<th>Chl a (ug L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dec-feb</td>
<td>22.4</td>
<td>7.1</td>
<td>0.10</td>
<td>0.02</td>
<td>46.9</td>
<td>1.8</td>
</tr>
<tr>
<td>mar-may</td>
<td>19.5</td>
<td>16.2</td>
<td>0.87</td>
<td>0.13</td>
<td>77.5</td>
<td>15.2</td>
</tr>
<tr>
<td>june-aug</td>
<td>20.9</td>
<td>27.5</td>
<td>0.84</td>
<td>0.13</td>
<td>124.4</td>
<td>14.7</td>
</tr>
<tr>
<td>sept-nov</td>
<td>24.6</td>
<td>19.7</td>
<td>0.38</td>
<td>0.06</td>
<td>42.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Parameter</td>
<td>Seed</td>
<td>Button</td>
<td>Littlenecks</td>
<td>Middlenecks</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------</td>
<td>--------</td>
<td>-------------</td>
<td>-------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td><strong>Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg Length (mm)</td>
<td>12.0</td>
<td>38.5</td>
<td>45.7</td>
<td>56.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue Biomass (gDW ind⁻¹)</td>
<td>0.06</td>
<td>0.5</td>
<td>0.9</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shell Biomass (gDW ind⁻¹)</td>
<td>0.3</td>
<td>8.8</td>
<td>14.6</td>
<td>26.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Standing Stock</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (ind)</td>
<td>3.6x10⁷</td>
<td>6.4x10⁷</td>
<td>3.5 x10⁷</td>
<td>9.3 x10⁶</td>
<td>1.4 x10⁸</td>
<td></td>
</tr>
<tr>
<td>Tissue (Mg C)</td>
<td>0.8</td>
<td>12.6</td>
<td>11.5</td>
<td>5.3</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td>Tissue (Mg N)</td>
<td>0.3</td>
<td>4.4</td>
<td>4.0</td>
<td>1.6</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Shell (Mg C)</td>
<td>1.4</td>
<td>75.3</td>
<td>68.4</td>
<td>32.8</td>
<td>177.9</td>
<td></td>
</tr>
<tr>
<td><strong>Harvested (yr⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (ind)</td>
<td>0</td>
<td>1.5 x10⁶</td>
<td>1.3 x10⁷</td>
<td>1.6 x10⁷</td>
<td>3.0 x10⁷</td>
<td></td>
</tr>
<tr>
<td>Tissue (Mg C yr⁻¹)</td>
<td>0</td>
<td>0.3</td>
<td>4.2</td>
<td>9.0</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>Tissue (Mg N yr⁻¹)</td>
<td>0</td>
<td>0.1</td>
<td>1.5</td>
<td>3.1</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Shell (Mg C yr⁻¹)</td>
<td>0</td>
<td>1.7</td>
<td>25.2</td>
<td>55.3</td>
<td>82.2</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5-2.** Clam size and biomass in Cherrystone Inlet, 2012. Standing stock numbers, C and N content by size category; annual harvest information including total number and C and N removed by harvest.
Table 5-3. 2012 seasonal filtration rates of the standing stock clam population in Cherrystone Inlet including the magnitudes relative to the tidal exchange and volume of the creek.

<table>
<thead>
<tr>
<th>Season</th>
<th>Total Filtration Rate (m$^3$ day$^{-1}$)</th>
<th>% Tidal Exchange</th>
<th>% Inlet filtered daily</th>
<th>Time to filter Inlet volume (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>$0.4 \times 10^6$</td>
<td>10%</td>
<td>7%</td>
<td>14.5</td>
</tr>
<tr>
<td>Spring</td>
<td>$1.6 \times 10^6$</td>
<td>37%</td>
<td>26%</td>
<td>3.9</td>
</tr>
<tr>
<td>Summer</td>
<td>$2.3 \times 10^6$</td>
<td>52%</td>
<td>37%</td>
<td>2.7</td>
</tr>
<tr>
<td>Fall</td>
<td>$2.8 \times 10^6$</td>
<td>62%</td>
<td>44%</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Table 5-4. 2012 seasonal average clam bioenergetics (kg C or N day\(^{-1}\)) of the total standing stock in Cherrystone Inlet including ingestion, egestion, respiration and excretion, and assimilation. Total annual rates (Mg C or N yr\(^{-1}\)) of these physiological processes for the clam population in Cherrystone Inlet are provided.

<table>
<thead>
<tr>
<th>Season</th>
<th>Ingested</th>
<th>Egested</th>
<th>Respiration/Excretion</th>
<th>Net Assimilation (+ reproduction)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>N</td>
<td>C</td>
<td>N</td>
</tr>
<tr>
<td>Winter</td>
<td>43.9</td>
<td>6.6</td>
<td>11.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Spring</td>
<td>1380.1</td>
<td>208.3</td>
<td>345.0</td>
<td>52.1</td>
</tr>
<tr>
<td>Summer</td>
<td>1905.5</td>
<td>287.6</td>
<td>476.4</td>
<td>71.9</td>
</tr>
<tr>
<td>Fall</td>
<td>1051.2</td>
<td>158.7</td>
<td>262.8</td>
<td>39.7</td>
</tr>
<tr>
<td>Annual (Mg C or N/yr)</td>
<td>401.9</td>
<td>60.7</td>
<td>100.5</td>
<td>15.2</td>
</tr>
</tbody>
</table>

\(^1\)respiration and excretion has been subtracted from total assimilation rates
Table 5-5. Seasonal net primary pelagic production and turnover time in Cherrystone Inlet, derived from the output of an ecosystem model provided by Kuschner (2015), compared to the time it takes the standing clam population to filter the entire system ("Bivalve Clearance Time") and the water residence time of the embayment (Herman et al. 2007).

<table>
<thead>
<tr>
<th>Season</th>
<th>Phytoplankton Turnover time (d)</th>
<th>Bivalve Clearance Time (d)</th>
<th>Water Residence time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>3.2</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>1.4</td>
<td>3.9</td>
<td>~2-3</td>
</tr>
<tr>
<td>Summer</td>
<td>1.1</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>3.0</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>
Table 5-6. Annual primary production (Mg C yr\textsuperscript{-1}) and N demand (Mg N yr\textsuperscript{-1}) for phytoplankton (Kuschner 2015), benthic microalgae (BMA; Reay et al. 1995, Murphy et al. 2015), and macroalgae (Murphy et al. 2015). Also shown are clam ingestion rates and the percent of the phytoplankton production that the clams consume. Seasonal rates are provided in Figure 4.

<table>
<thead>
<tr>
<th></th>
<th>Annual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>437.6</td>
</tr>
<tr>
<td>BMA (Reay et al. 1995)</td>
<td>298.2</td>
</tr>
<tr>
<td>BMA (Murphy et al. 2015)</td>
<td>1295.6</td>
</tr>
<tr>
<td>Macroalgae</td>
<td>149.4</td>
</tr>
<tr>
<td>Clam Ingestion</td>
<td>401.9</td>
</tr>
<tr>
<td>% of phytoplankton clams ingest</td>
<td>92%</td>
</tr>
</tbody>
</table>

200
**Figure 5-1.** Cherrystone Inlet, VA. Clam aquaculture operations are delineated by black polygons.
Figure 5-2. Seasonal dataflow extrapolations of chl a in Cherrystone Inlet.
Figure 5-4. Seasonal net primary production rates (Mg C d⁻¹) including phytoplankton (gray), benthic microalgae (hatched), and macroalgae (striped). Seasonal clam ingestion rates are shown in black and correspond to 9%, 110%, 85%, and 131% of the phytoplankton production for winter, spring, summer, and fall, respectively.
Figure 5-5. Benthic respiration rates (A) and net NH$_4^+$ fluxes (B) scaled to the ecosystem at the uncultivated sediments (gray) and clam beds, including contribution from the clams (i.e. respiration and excretion) (white) and microbial respiration and mineralization (black).
Figure 5-6. Annual C and N budget showing fluxes in Mg C or N yr⁻¹ associated with the clam beds and water column. Net DIC and DIN fluxes (FLUX), macroalgal primary production (P.P.) and denitrification (DNF) were directly measured (Murphy et al. 2015, Murphy et al. in review) and subsequently scaled here. Clam physiological rates including clam ingestion (ING), respiration (RESP), excretion (EXC), egestion (EGE), and growth (GROWTH) were modeled and scaled to the standing stock of clams in the system (current study); phytoplankton primary production (P.P.) and the input of DIN from the watershed and Chesapeake Bay were modeled (Kuschner 2015); export of PN and PC via harvest (HARVEST) were provided by the clam growers. *GROWTH here does not
account for reproduction or mortality; \( GROWTH = \text{ING} - \text{RESP/EXC} - \text{EGE} \).
## APPENDIX B.

<table>
<thead>
<tr>
<th>Clam Physiological Rate</th>
<th>Equation(s)</th>
<th>Measured Variables</th>
<th>Reference(s)</th>
</tr>
</thead>
</table>
| Filtration Rate (FR) (ml ind⁻¹ d⁻¹) | FR = FRₘₐₓ ⊕ f(T) ⊗ f(Sal) ⊗ f(TSS) | T: temperature (°C)  
L: shell length (mm)  
S: salinity (ppt) | Doering and Oviatt 1986; Hofmann et al. 2006; Wiseman 2010 |
|  | FRₘₐₓ = ((1.0.96 ⊗ T^{0.95})/2.95) ⊗ 60 ⊗ 24 |  |  |
|  | f(T) = 0.277 ⊗ \left( 1 + \frac{e^{(T-7.5)/3}-1}{1 + e^{(T-7.5)/3}} \right) ⊗ \left( 1 - \frac{e^{(T-20)/3}-1}{e^{(T-20)/3}+1} \right) |  |  |
|  | f(Sal) = -8.1027 \times 10^{-3} S^2 + 0.4144 S-4.302 |  |  |
| Ingestion Rate (Iₑ, Iₙ) (g PC or PN ind⁻¹ d⁻¹) | Iₑ = FR \times chl \alpha \times 57.214  
Iₙ = FR \times chl \alpha \times 7.025 | chl \alpha: mean seasonal chl \alpha (mg l⁻¹) | Hofmann et al. 2006; Wiseman 2010 |
| Respiration (R) (gDIC ind⁻¹ d⁻¹) | R = 24^*(a^*W^{0.8484})^* e^{0.1012*(T-20)*\left(\frac{10^{-6}}{22.414}\right)}^*12 | a: base respiration rate (200 μl O₂ hr⁻¹ gDW⁻¹)  
W: clam biomass (g DW)  
T: temperature (°C) |  |
| Excretion (U) (gN ind⁻¹ d⁻¹) | Log U = 0.94 Log W + 1.33 | W: clam tissue biomass (g DW) | Srna and Baggaley 1978 |
| Assimilation (Aₑ, Aₙ) (gC or gN ind⁻¹ d⁻¹) | Aₑ = Cₑ \times 0.75  
Aₙ = Cₙ \times 0.75 |  | Tenore and Dunstan 1973; Kuschner 2015 |
| Egestion (E) (gC or gN ind⁻¹ d⁻¹) | Eₑ = Cₑ \times 0.25  
Eₙ = Cₙ \times 0.25 |  | Tenore and Dunstan 1973; Kuschner 2015 |

Supplementary Table S5-1. Summary of the equations, variables, and references used to model clam bioenergetics rates.
APPENDIX C.

Supplementary Table S5-2. A comparison of water quality parameters collected by dataflow surveys nearby the clam cultivation operations and outside of the operations. Averages and (standard deviations) are provided seasonally.

<table>
<thead>
<tr>
<th></th>
<th>May</th>
<th></th>
<th>July</th>
<th></th>
<th>October</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inlet</td>
<td>Clam beds</td>
<td>Inlet</td>
<td>Clam beds</td>
<td>Inlet</td>
<td>Clam beds</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>9.25 (0.47)</td>
<td>9.07 (0.44)</td>
<td>8.09 (0.16)</td>
<td>8.14 (0.17)</td>
<td>8.85 (0.37)</td>
<td>8.66 (0.25)</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>13.15 (6.7)</td>
<td>10.68 (4.1)</td>
<td>23.27 (15.6)</td>
<td>16.29 (6.8)</td>
<td>10.65 (5.3)</td>
<td>8.2 (2.5)</td>
</tr>
<tr>
<td>pH</td>
<td>8.19 (0.1)</td>
<td>8.15 (0.1)</td>
<td>7.98 (0.04)</td>
<td>7.99 (0.03)</td>
<td>7.99 (0.06)</td>
<td>7.97 (0.04)</td>
</tr>
<tr>
<td>Chl (ug/L)</td>
<td>15.2 (3.4)</td>
<td>13.99 (2.4)</td>
<td>15.5 (4.2)</td>
<td>13.7 (3.3)</td>
<td>7.73 (3.5)</td>
<td>5.85 (2.2)</td>
</tr>
</tbody>
</table>
APPENDIX D.

Supplementary Figure S5-1. Clam tissue dry weight (g) as a function of shell length (mm) measured in clams collected from Cherrystone Inlet in May, July, and November 2013.

\[
y = 0.0009x^2 - 0.028x + 0.266 \\
R^2 = 0.95 \\
n=159
\]
APPENDIX E.

Supplementary Figure S5-2. Clam oxygen demand (OD) and NH$_4^+$ flux from a closed-chamber flux experiment conducted in July 2013, in which clams were incubated without sediment. The average ratio of oxygen uptake and NH$_4^+$ release (respiration : excretion) was equal to 7.83, which was used to convert clam respiration rates to excretion rates.
APPENDIX F.

Supplementary Figure S3. Average seasonal light attenuation ($K_d$; m$^{-1}$) measured in 1990-91 (W. Reay, pers. comm.) (black) and 2012 (Murphy et al. 2015) (white).

Between the two time periods, light attenuation decreased by 25, 51, and 61% in Spring, Summer, and Fall, respectively.
VITA

Anna E. Murphy

Annie was born in Boston, MA on July 15, 1985. After graduating in 2003 from Scituate High School in Scituate, MA, she went on to earn a B.S. in Biology (minor in Marine Science) from Fairfield University, graduating cum laude in 2007. After college, Annie worked for Battelle Memorial Institute, an environmental consulting company in Duxbury, MA. In 2010 Annie entered the Masters program at the Virginia Institute of Marine Science (VIMS), College of William and Mary under graduate co-advisors Drs. Iris Anderson and Mark Luckenbach and bypassed into the Ph.D. program in 2012. During her tenure at VIMS she received numerous awards including the John M. and Marilyn Zeigler Achievement Award and the Kelly Watson Graduate Student Fellowship. She was a Virginia Sea Grant Research Fellow (2012-2013, 2014-2015) and an NSF GK-12 PERFECT Graduate Fellow (2013-2014). She will graduate in January 2016 with a Ph.D. in Marine Science, with a concentration in biological oceanography.