Enhanced nutrient regeneration at commercial hard clam (*Mercenaria mercenaria*) beds and the role of macroalgae

Anna E. Murphy*, Iris C. Anderson, Mark W. Luckenbach

Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, Virginia 23062, USA

ABSTRACT: 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 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 to 77%, while having no significant effect on phosphate. 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.

KEY WORDS: Aquaculture · Clam · Shellfish · Macroalgae · Eutrophication · Nitrogen cycling

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 eco-system, 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 et al. 2001, Valiela et al. 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. 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 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, Bricker et al. 2014, Petersen 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 bivalve 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. 1994, 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 and diffusion, changing sediment DO and nutrient profiles and subsequently biogeochemical process rates (Nizzoli et al. 2006). However, use of predator exclusion nets (i.e. plastic mesh placed flush on the sediment surface) by clam growers on the US east coast may reduce exchanges between overlying water and sediments. In addition, ephemeral macroalgae on the net surface affect DO, release particulate organic carbon (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 (e.g. self-shading) and/or environmental factors (e.g. increase in temperature), ephemeral macroalgae in the coastal bays of Virginia degrade rapidly, releasing nutrients and decreasing DO (Tyler et al. 2001, Hardison et al. 2010).

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* aquaculture on benthic respiration and nutrient regeneration, specifically with regard to modifying fluxes to the water column and contributing to seasonal macroalgal growth. 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. We expected macroalgae to decrease nutrient effluxes at clam beds and shift community metabolism to net autotrophy (see Fig. 4).
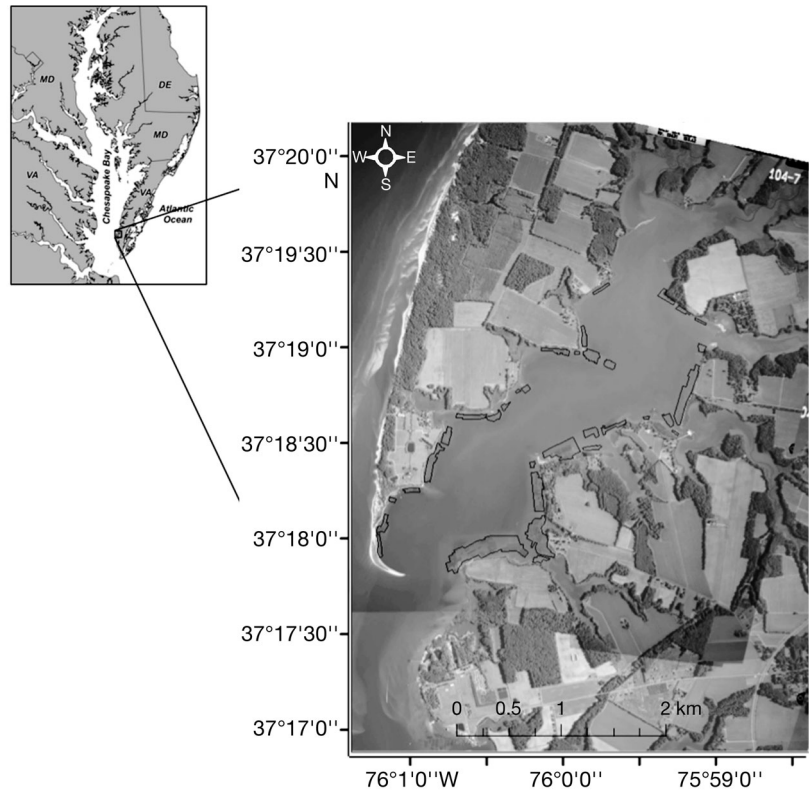

Fig. 1. Aerial photograph of Cherrystone Inlet, Chesapeake Bay, USA, taken in 2012. Black polygons delineate active clam aquaculture operations

MATERIALS AND METHODS

Site description

Field experiments were conducted in May, July, and October 2012 in Cherrystone Inlet, a small tributary (5.7 km^2) of Chesapeake Bay, located on the bayside of the eastern shore of Virginia, USA (Fig. 1). Average depth is <2 m and approx. one-third of its subtidal bottom is partitioned into private shellfish leases, most used to grow hard clams *Mercenaria mercenaria*. 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 yr. The clams in Cherrystone Inlet are planted at 700–800 ind. 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 into Chesapeake Bay. The sites experience little salin-

ity variation, which is driven largely by rain events. Macroalgae, including *Ulva lactuca*, *Gracilaria* spp., *Agardhiella tenera*, and *Cladophora* sp., are present on the commercial clam bed nets throughout the year.

Experimental design

In each sampling month, fluxes were measured at 3 randomly selected clam beds and 3 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 (approx. 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 sampling month 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, 3 sets of randomly-positioned blocks of 4 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, 3 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).

Ambient macroalgal biomass was obtained by randomly tossing a ring sampler (0.014 m^2) 5 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

During each sampling month, *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 i.d. by 40 cm tall) were inserted into the sediment to a depth resulting in a 20 cm water column, allowed to equilibrate for 1 h, 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 h incubation period. DO was measured by pulling the sample with a syringe into an airtight chamber containing a Hach LDO101 Luminescent DO sensor. Dissolved inorganic carbon (DIC) samples, stored in 8 ml hungate tubes (Bellco Glass), were preserved with 15 µl saturated mercuric chloride and kept cold under water until analyzed within 1 mo 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 analysis for DIN, dissolved inorganic phosphate (DIP), DOC, DON, and chlorophyll *a* (chl *a*) as described below.

Flux calculations

Hourly fluxes for each analyte were calculated as:

$$\text{Flux} = (m \times V)/A \tag{1}$$

where m is equal to the slope of the linear regression of concentration (µM or mM) versus time (h); 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 in mmol C m^{-2} day^{-1}) and daily nutrient fluxes were calculated as follows:

$$R = F_d \times 24 \text{ h} \tag{2}$$
$$\text{GPP} = h_l \times (F_l - F_d) \tag{3}$$
$$\text{NCP} = \text{GPP} + R \tag{4}$$
$$\text{Daily nutrient flux} = (F_l \times h_l) + (F_d \times h_d) \tag{5}$$

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} h^{-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 macroalgal 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 h. All macroalgae were removed from the CML and CMD chambers, and the DW was determined for each species present. A subset of dried macroalgal tissue samples from the dominant species during each sampling month were stored in the freezer until analyzed on a Carlo Erba (Thermo Electron 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):

$$\log_{10}(E) = 0.94 \times \log_{10}(DW) + 1.33 \qquad (6)$$

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 clam plus macroalgae treatment from the clam only 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 sampling month as:

$$\text{N or C content} = \Sigma\, W_i X_i \qquad (7)$$

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:

$$MP = NCP_C - NCP_{CM} \qquad (8)$$

$$MG = MP/C \text{ content} \qquad (9)$$

$$MN = MG \times N \text{ content} \times (1\ \text{mol}/14\ \text{g}) \times (1000\ \text{mmol}/1\ \text{mol}) \qquad (10)$$

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 Eq. 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 h). Dried subsamples were acidified and analyzed on a Carlo Erba (Thermo Electron 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 (Knepel & Bogren 2001), and DON (Koroleff 1983) on a Lachat QuikChem 8000 automated ion analyzer (Lachat Instruments; 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 to 7 cm below the sediment surface using a stainless steel push-point sampler (MHE Products), and also analyzed for hydrogen sulfide (Cline 1969). Water column samples were filtered (0.7 μm GF/F) and extracted for chl *a* and phaeophytin analysis as described by Anderson et al. (2014). Salinity, temperature, DO, chl *a*, and turbidity were monitored continuously throughout each 3 d experiment using a YSI model 6600 datasonde 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 d experiment using a Li-Cor quantum deck sensor (LI-190SA) and underwater quantum sensor (LI-192SA).

Statistical analysis

Daily metabolic rates and descriptive measurements (e.g. 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 with 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 (v. 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 in the spring to 25.3 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 (1–2 µM) than the bare sites (0.3–1 µM) despite their close proximity. Porewater DIN, dominated by NH_4^+, and SRP were signifi-

Table 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). Data are means and SE. nd: no data were collected. *Significant difference between treatments within each month (post hoc results, implies significant interactions were observed between month and season). ‡Significant difference between treatments across all months (implies no significant interactions were observed)

		May	July	October
PW NH_4^+	Bare	20.95 (5.4)*	56.3 (6.5)	46.15 (4.5)*
	Clam	118.7 (25.2)	66.1 (14.3)	113.2 (14.2)
WC NH_4^+	Bare‡	0.31 (0.17)	0.56 (0.10)	0.94 (0.20)
	Clam	0.99 (0.15)	1.98 (0.36)	1.12 (0.05)
PW NO_x	Bare	0.75 (0.11)	0.34 (0.09)	1.16 (0.56)
	Clam	0.37 (0.04)	0.39 (0.09)	0.39 (0.07)
WC NO_x	Bare‡	0.24 (0.04)	0.12 (0.05)	0.11 (0.03)
	Clam	0.11 (0.02)	1.38 (1.27)	0.20 (0.04)
PW PO_4^{3-}	Bare	1.32 (0.39)*	4.12 (0.40)	3.02 (0.44)*
	Clam	8.5 (1.25)	5.9 (1.53)	7.8 (1.23)
WC PO_4^{3-}	Bare	0.06 (0.004)	0.08 (0.01)*	0.11 (0.01)
	Clam	0.04 (0.004)*	0.15 (0.02)	0.11 (0.01)
PW sulfide	Bare	nd	91.9 (24.0)	116.9 (23.2)
	Clam	nd	127.6 (13.0)	206.8 (63.5)
Benthic chl a	Bare	3.50 (0.34)	5.62 (0.42)	4.78 (0.38)
	Clam	1.31 (0.28)	3.09 (0.18)*	3.40 (0.13)*
Benthic phaeophytin	Bare	1.31 (0.11)*	3.09 (0.25)*	3.40 (0.50)*
	Clam	5.53 (0.38)	6.98 (0.40)	6.75 (0.38)
Sediment OM	Bare	0.99 (0.13)	0.84 (0.09)*	0.90 (0.11)
	Clam	1.09 (0.19)	1.87 (0.28)	1.02 (0.13)
Sediment C:N	Bare	7.55 (0.37)	7.40 (0.17)	7.34 (0.16)
	Clam	6.63 (0.46)	7.39 (0.13)	7.03 (0.09)

Table 1. Seasonal site characteristics, clam biomass, density, and shell length observed at the clam beds where flux incubations were conducted. k_d: light attenuation; PAR: photosynthetically active radiation. Data are means and SE

Month	Salinity	Temperature (°C)	Chlorophyll a (µg l^{-1})	Dissolved oxygen (mg l^{-1})	k_d (PAR m^{-1})	Clam biomass (g ash-free DW m^{-2})	Clam density (ind. m^{-2})	Clam size (shell length, mm)
May	20.3 (0.03)	20.8 (0.08)	5.19 (0.09)	8.42 (0.11)	1.48 (0.03)	242.9 (19.0)	821 (82.5)	40.3 (0.3)
July	22.2 (0.05)	29.3 (0.13)	4.41 (0.08)	7.18 (0.16)	2.16 (0.09)	228.9 (16.6)	790 (47.7)	39.8 (0.4)
October	25.3 (0.01)	18.5 (0.06)	2.05 (0.07)	8.19 (0.05)	1.43 (0.03)	319.27 (18.8)	999 (70.5)	40.4 (0.3)

cantly 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. Average (±SD) shell length was 39.1 ± 6.2 mm; clam biomass averaged 263.7 ± 103.1 g ash-free DW m^{-2}, 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 Fig. 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 (Cl) 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 Cl. 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 Cl and CM. Within treatments, no significant seasonal effect was observed in either the Cl 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 dif-

Table 3. Mean biomass (g DW m^{-2}) and SE of macroalgae by species retrieved from the clams plus macroalgae in the light (CML) and dark (CMD) cores after the incubations during each season. The species within the experimental cores reflects the ambient species composition found *in situ* each month. Also shown is the mean percent total N and organic C content of each species per month. nd: no data; (−) not applicable. 'Other' species in May were predominately *Cladophora* sp.

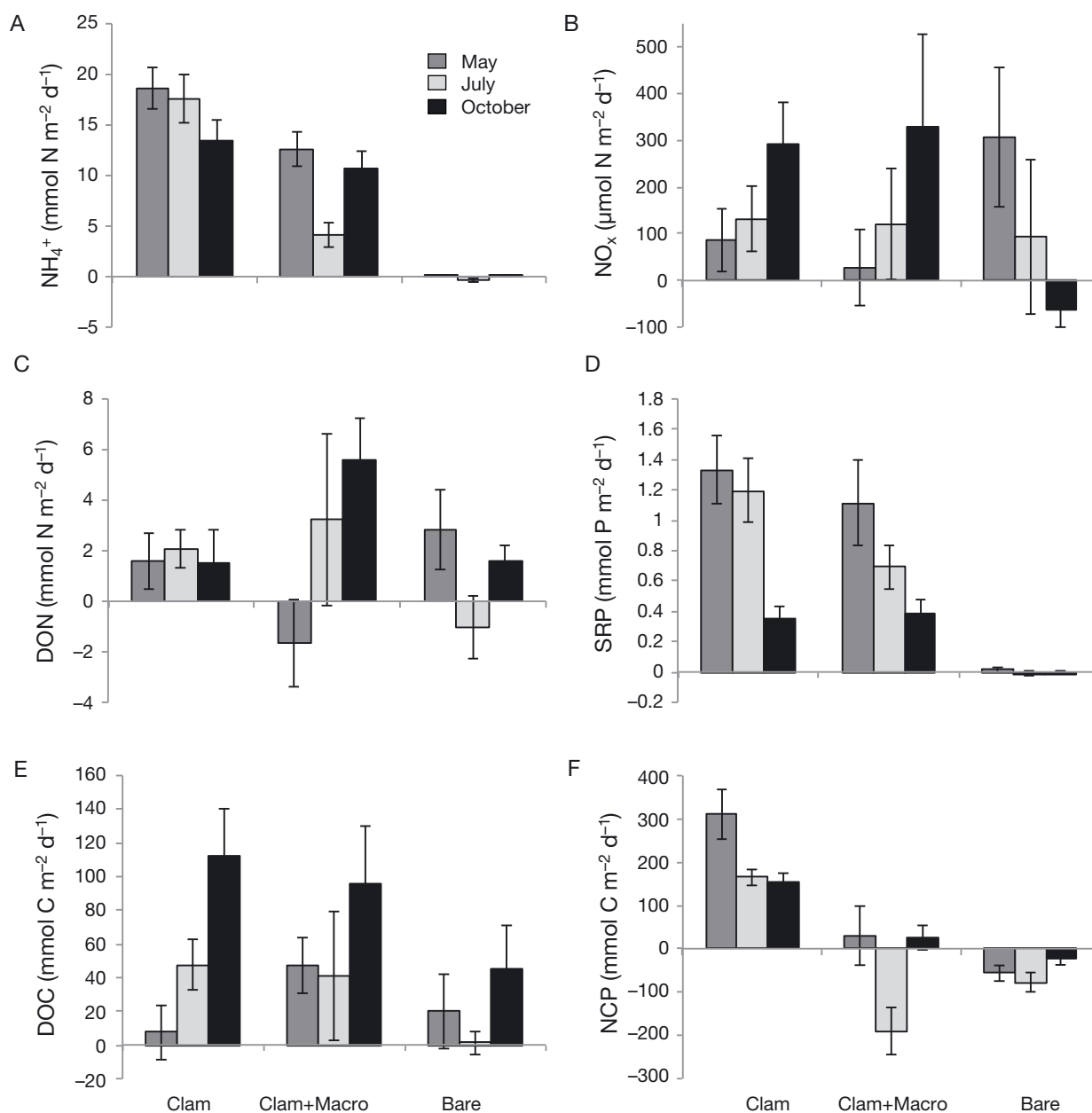| | May | | | July | | | October | | |
	Biomass	% N	% C	Biomass	% N	% C	Biomass	% N	% C
Ulva lactuca	0.52 (0.2)	2.44 (0.43)	27.08 (2.5)	1.54 (0.5)	nd	nd	0	–	–
Gracilaria spp.	10.35 (2.0)	1.51 (0.04)	27.84 (0.9)	84.1 (6.8)	3.40 (0.3)	33.96 (2.3)	41.1 (4.9)	3.53 (0.3)	34.61 (1.5)
Agardhiella	0	–	–	38.17 (6.7)	2.80 (0.2)	28.28 (0.8)	8.88 (2.9)	2.76 (0.4)	29.42 (1.1)
Other	0.57 (0.1)	nd	nd	0	–	–	0	–	–
Total biomass	24.13 (2.3)	–	–	123.8 (3.5)	–	–	52.8 (2.8)	–	–
Species-weighted mean of macro- algal community	–	2.02	27.32	–	3.22	32.19	–	3.39	33.69

Fig. 2. (A) Net daily flux rates of ammonium (NH_4^+), (B) nitrate + nitrite (NO_x), (C) dissolved organic nitrogen (DON), (D) soluble reactive phosphorous (SRP), (E) dissolved organic carbon (DOC), and (F) net benthic community production (NCP), calculated using DIC fluxes, for all treatments including clam only (Clam), clam plus macroalgae (Clam+Macro), and control sediment (Bare), in May, July, and October 2012. Data are mean ± SE

ference 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 Cl treatments, there was no effect of season. Within the CM treatment, May had significantly lower values than October, while July values were not different from those in May or October.

Net effluxes of SRP in the Cl and CM treatments were significantly greater than in B during all sea-

sons (Fig. 2D). However, the presence of macroalgae had no significant effect on the clam bed SRP flux. Seasonal trends were detected in the Cl 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.

Table 4. Statistical parameters from the mixed-effect models with treatment and month as fixed factors and day sampled as the random factor on daily flux rates. n = 9 for each treatment per month; treatments were sampled over 3 consecutive days. Summaries of the post hoc test results are provided when a significant interaction was observed. NO_x: nitrate + nitrite; DON: dissolved organic nitrogen; SRP: soluble reactive phosphorous; DOC: dissolved organic carbon; NCP: net community production. Months — M: May; J: July; O: October. Treatments — B: bare, Cl: clam only; CM: clam plus macroalgae. ns: no significant difference among groups

| Response | Factors | F | df | p | Post hoc summary | |
					Month within treatment	Treatment within month
NH_4^+	Treatment	220.99	2	<0.001	Cl: ns	May: B < CM < Cl
	Month	5.47	2	0.044	CM: J < M = O	July: B < CM < Cl
	Treatment × Month	5.17	4	0.001	B: ns	Oct: B < CM = Cl
NO_x	Treatment	0.60	2	0.55	Cl: ns	May: B > CM
	Month	0.13	2	0.88	CM: J < M = O	July: ns
	Treatment × Month	4.07	4	0.005	B: ns	Oct: B < Cl = CM
DON	Treatment	0.45	2	0.64	Cl: ns	May: B > CM
	Month	0.76	2	0.51	CM: M < O	July: ns
	Treatment × Month	4.42	4	0.003	B: ns	Oct: ns
SRP	Treatment	122.40	2	<0.001	Cl: J = M > O	May: B < Cl = CM
	Month	4.17	2	0.07	CM: M > O	July: B < Cl = CM
	Treatment × Month	4.67	4	0.002	B: ns	Oct: B < Cl = CM
DOC	Treatment	2.77	2	0.07		
	Month	2.86	2	0.13		
	Treatment × Month	1.36	4	0.26		
NCP	Treatment	52.60	2	<0.001	Cl: J = O < M	May: B = CM < Cl
	Month	6.30	2	0.033	CM: J < M = O	July: B = CM < Cl
	Treatment × Month	4.50	4	0.003	B: ns	Oct: B = CM < Cl

DOC was typically released from sediments at all treatments and during all sampling months (Fig. 2E). There was no significant effect of season or treatment on DOC fluxes, and 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 Cl treatment was net heterotrophic during all sampling months 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 sampling months, 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 Cl 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 mean ± SD clam NH_4^+ excretion rates (using the equation from Sma & Baggaley 1976) ranged from 233.6 ± 66.6 µmol N m^{-2} h^{-1} in July to 542.8 ± 225.0 µmol N m^{-2} h^{-1} in May and an intermediate of 410.5 ± 136.2 µmol N m^{-2} h^{-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 3 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 = 0.15; 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^+,
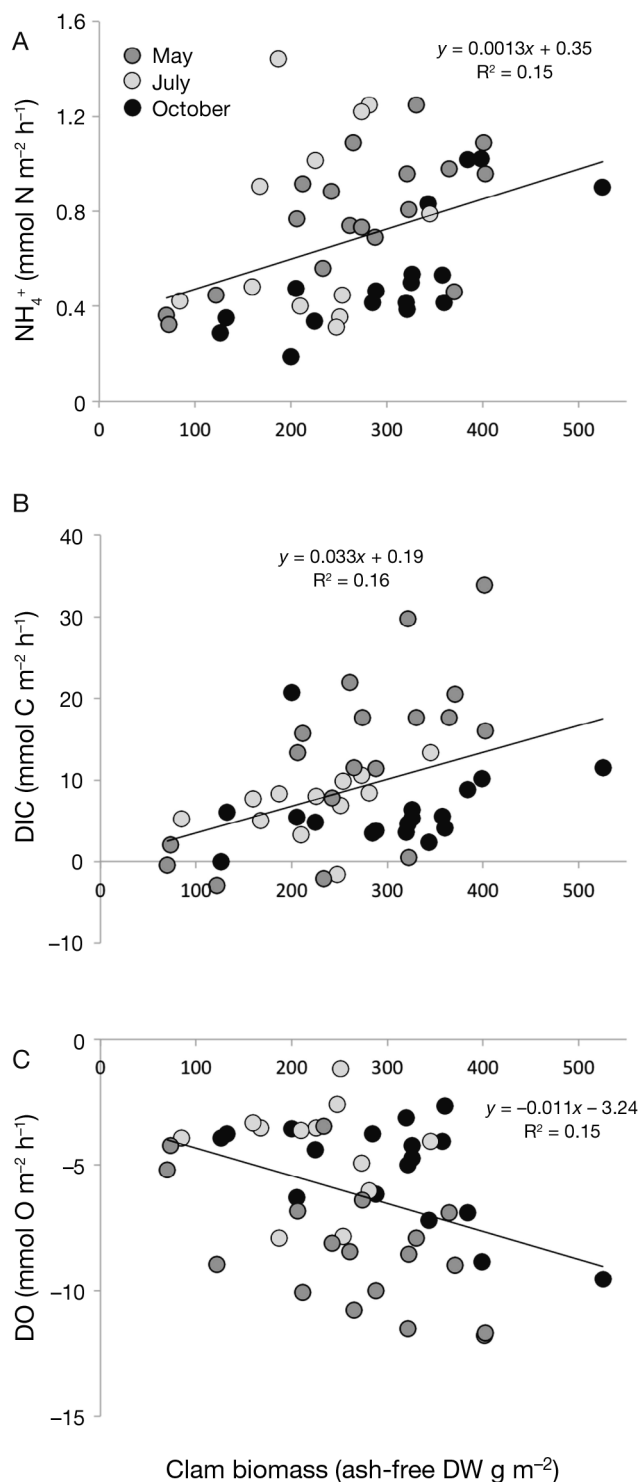
Fig. 3. (A) Hourly (light and dark) ammonium (NH_4^+), (B) dissolved inorganic carbon (DIC), and (C) dissolved oxygen (DO) fluxes as a function of clam biomass (ash-free DW g m^{-2}), in May, July, and October 2012. Analyses included clam dark and clam light treatments only. The solid line is the regression including all months. Slopes and regression statistics are provided in Table 5

Table 5. Regression statistics of hourly fluxes of ammonium (NH_4^+), 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. Fig. 3 provides graphical representation of the data. Significant results ($p < 0.05$) in **bold**

Parameter	Month	Slope	R^2	p
NH_4^+	May	0.002	0.42	**0.002**
	July	0.001	0.04	0.533
	October	0.002	0.52	**<0.001**
	All	0.001	0.15	**0.008**
DIC	May	0.071	0.44	**0.002**
	July	0.024	0.10	0.16
	October	0.008	0.03	0.52
	All	0.033	0.16	**0.005**
DO	May	−0.014	0.31	**0.010**
	July	−0.002	0.01	0.822
	October	−0.011	0.29	**0.015**
	All	−0.011	0.15	**0.007**

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 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. 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 C:N of the fluxes for each treatment (i.e. C:N = the slope). 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, C:N increased in clam treatments with and without macroalgae. However, C:N was higher in the dark than in the 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,

Table 6. Linear regression estimates of the relative proportion (slope) of dissolved inorganic carbon to ammonium fluxes on a net daily basis, as well as in the dark and in the light for all treatments: clam only (Cl), clam plus macroalgae (CM), and control sediment (B). Significant results (p < 0.05) in **bold**

	Treatment	Slope	R^2	p-value
Daily	Cl	9.9	0.23	**0.007**
	CM	23.7	0.56	**0.000**
	B	66.1	0.16	**0.022**
Dark	Cl	8.3	0.07	0.098
	CM	10.3	0.29	**0.002**
	B	39.9	0.10	0.062
Light	Cl	13.1	0.27	**0.003**
	CM	30.1	0.47	**0.000**
	B	26.7	−0.02	0.466

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, and 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 (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 (Fig. 4). 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.
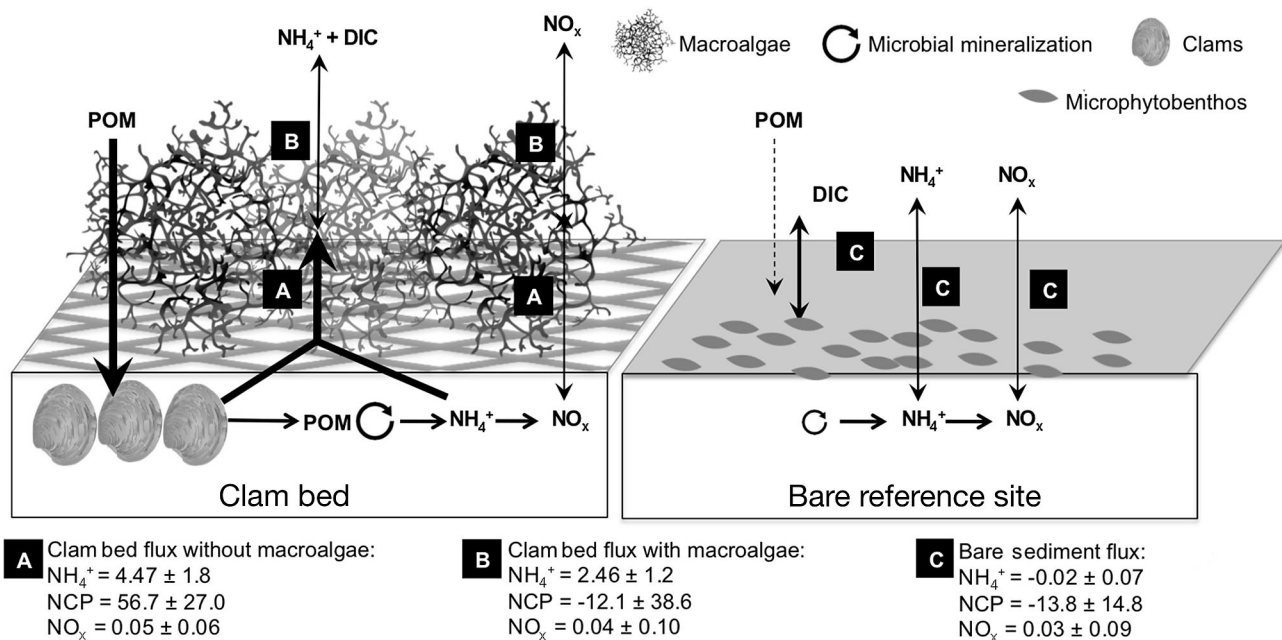

Fig. 4. A conceptual model illustrating the net annual fluxes of NH_4^+, NO_x (mol N m^{-2} yr^{-1}) and net community production (NCP; mol C m^{-2} yr^{-1}); a positive flux represents net heterotrophy; a negative flux net autotrophy. POM: particulate organic matter; NH_4^+: ammonium; NO_x: nitrate + nitrite; DIC: dissolved inorganic carbon

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 and 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 was significantly correlated with DO and DIC fluxes when data from all sampling months 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 the 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 Inlet is relatively low (6.6–7.6) compared to nearby systems such as Hog Island Bay, Virginia, which averages 13.3 (Anderson et al. 2003). In Cherrystone Inlet 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, high clam densities and the predator exclusion net may constrict clam movement and decrease bioturbation.

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 is included 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 increased grazing by clams under the nets (Sauriau & 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 macroalgae 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 by up to 43%. Other studies have similarly reported that macroalgae effectively assimilate nutrients fluxing from sediments, temporarily sequestering them in their tissue (i.e. McGlathery et al. 1997, Sundbäck 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 Inlet 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 Inlet, we found that the clam aquaculture sediments provided 58 to 122% of the macroalgal N demand. The percent of macroalgal N demand supplied by the benthos exceeds estimates reported in nearby systems (27 to 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 29 838 kg N yr^{-1} (Kuschner 2015). According to the Virginia Marine Resources Commission, while the entire embayment is leased for shellfish aquaculture, only some of the leases are active. Based on actual coverage of clam aquaculture in Cherrystone Inlet of 181 008 to 476 048 m^2 (estimated using aerial photographs taken in 2012 and GIS delineation; Emery 2015), we found that the total NH_4^+ released from clam operations (without macroalgal uptake) is 38 to 99.8% 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, and depth. For example, nutrient regeneration facilitated by bivalve aquaculture is likely more consequential in pristine systems with low external nutrient loading such as Cherrystone Inlet than in 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 (approx. 700, each 72 m^2) within the studied farm, and assuming negligible N uptake in the winter months, macroalgae have the capacity to assimilate approx. 3652 kg N yr^{-1} on this single farm, an amount equivalent to 116% of the annual NH_4^+ released from the clam operation if no macroalgae were on the nets (3158 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 effi-

cient 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 Inlet would require economic sustainability. In the United States, 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 and allowing them to drift away. The fate of these 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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Appendix

May

July

October

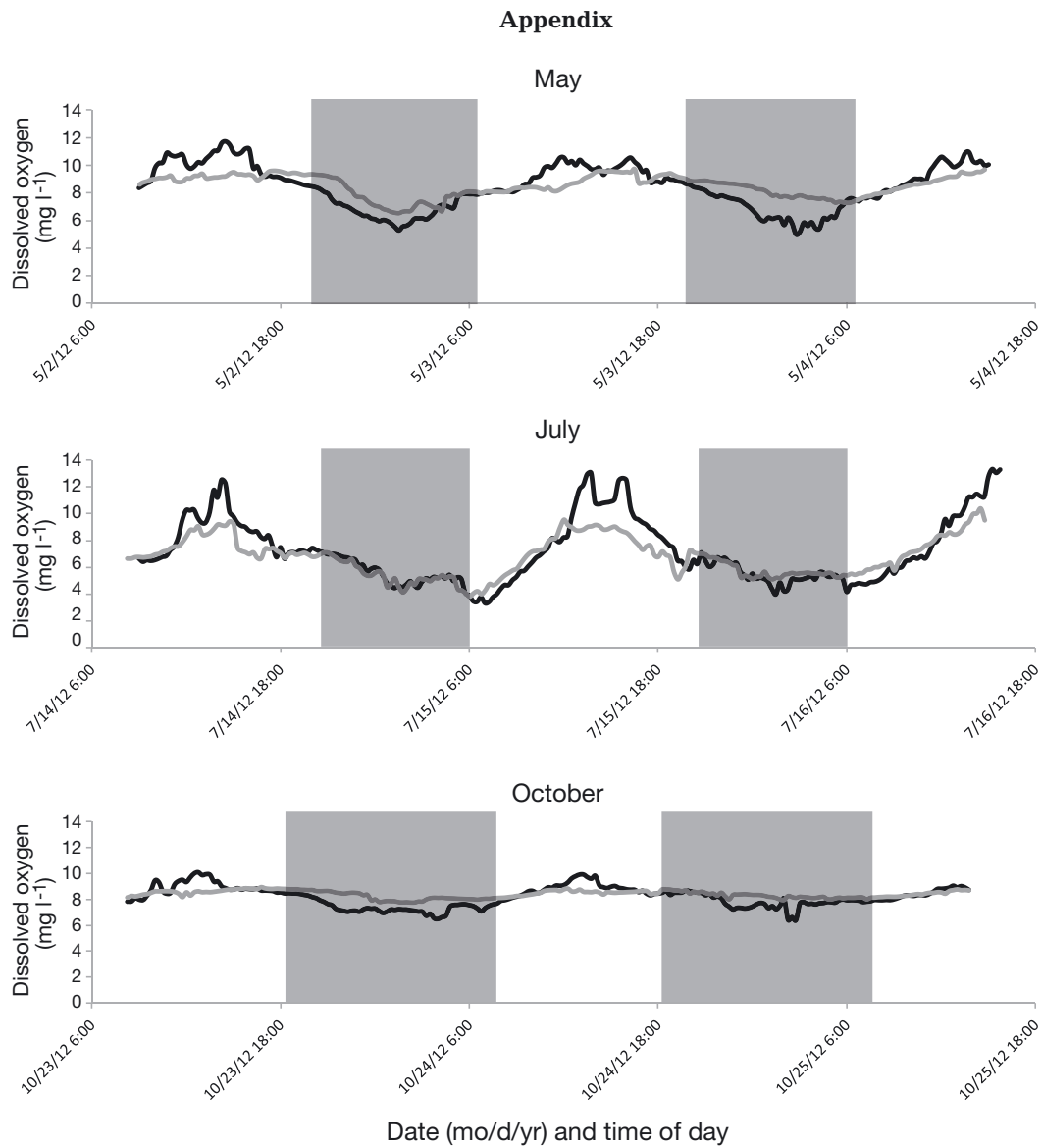Date (mo/d/yr) and time of day

Fig. A1. Continuous dissolved oxygen in the bottom water at the clam bed (black) and bare, uncultivated (gray) sites during each 3 d experiment. Shaded boxes represent nighttime hours. Dissolved oxygen probes were placed approx. 5 cm from the sediment–water interface and continuously monitored over the 3 d period