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Bivalve-Enhanced Nitrogen Removal From Coastal Estuaries

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Abstract: Interest in use of bivalves to remediate estuarine eutrophication has increased in recent years. High variation among data sets, substantial focus on particle removal, and insufficient links to anthropogenic nitrogen (N) sources encouraged this empirical examination of N removal by bivalves from estuaries receiving different N loads. We determined the capacity of the oyster *Crassostrea virginica* to remove N by comparing N assimilated into tissues with anthropogenic N from land or available in phytoplankton. Oyster growth yielded 0.2–0.4 g N in tissues and depended on estuary-specific conditions. $\delta^{15}N$ values confirmed that N in oyster tissues derived from local anthropogenic sources. At representative restoration and aquaculture conditions ($\leq$400 oysters·m^{-2} at 0.5%–1.0% of estuary area), estimated N removal was $\leq$15% of land-derived loads and <1% of phytoplankton N. N removal via biogeochemical processes was negligible during grow-out, but became important after oysters attained harvestable size. This study explicitly demonstrates that oysters assimilated land-derived N, but suggests that bivalve bioremediation consider trade-offs between intensity of planting, ecological effects, and available space.

Résumé : Le recours aux bivalves pour contrer l'eutrophisation estuarienne suscite un intérêt croissant ces dernières années. La variabilité élevée d'un ensemble de données à l'autre, un accent important sur le retrait de particules et l'insuffisance de liens établis vers des sources anthropiques d'azote (N) ont motivé l'examen empirique du retrait de l'azote par des bivalves d'estuaires qui reçoivent différentes charges d'azote. Nous avons déterminé la capacité de l'huître, *Crassostrea virginica*, de retirer l'azote en comparant le N assimilé dans leurs tissus au N anthropique de source terrestre ou disponible dans le phytoplancton. La croissance d'une huître produit de 0,2 g à 0,4 g de N dans les tissus, selon les conditions propres à l'estuaire. Les valeurs de $\delta^{15}N$ ont confirmé que le N dans les tissus d'huîtres provient de sources anthropiques locales. Dans des conditions de restauration et d'aquaculture représentatives ($\leq$400 huîtres·m^{-2} sur de 0,5 % à 1,0 % de la superficie de l'estuaire), le taux de retrait estimé de l'azote était de $\leq$15 % des charges de sources terrestres et de <1 % du N provenant du phytoplancton. Le retrait du N par des processus biogéochimiques s'est avéré négligeable durant le grossissement, mais devenait important après que les huîtres aient atteint une taille commerciale. L'étude démontre explicitement que les huîtres ont assimilé du N de source terrestre, mais suggère que la biorestauration à l'aide de bivalves devrait tenir compte des compromis entre l'intensité de l'ensemencement, les effets écologiques et l'espace disponible.

[Traduit par la Rédaction]

Introduction

Increased awareness of the pervasive and often negative effects of eutrophication has prompted considerable interest in bivalves as a natural solution to remove particles from the water column or remediate nitrogen (N) loads to coastal waters (e.g., Gifford et al. 2007; Cerco and Noel 2007; Gren et al. 2009). At least 30 studies worldwide since 1980 have attempted to quantify some aspect of bioremediation by bivalves, with more than half of these studies in the last decade (Table 1). Nearly unanimously, these studies concluded that bivalves were potentially important bioremediators. Specific results, however, are not entirely consistent with this optimistic notion. Only ~40% of studies quantified some form of N removal, and results were typically reported in different units and using different spatial and time scales, which makes comparison among systems problematic. Overall, a careful review of the data indicates that while bivalves may remove 30%–45% of local particle concentrations, and in one case possibly as much as 90% of local chlorophyll *a* (chl *a*) concentration, N removal is lower, ranging from less than 1%–15% of total annual N loads to 25% of daily N load (Table 1). Although the available data are fragmentary and do not convey comprehensive results with respect to N removal, they do suggest that bivalves remediate some symptoms of eutrophication.

Emphasis on particle removal as a primary means to assess bioremediation among previous studies is a concern. In the past 30 years, shellfish bioremediation research has largely focused on reduction of particle loads, turbidity in the water

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Table 1. Comparison of bivalve bioremediation-related studies, including study locations, methods of remediation studied, density and shell height of bivalves, and primary conclusions.

		Method of remediation						
Species	Location	N stored in tissues	Particle removal	Biogeo-chemistry	Density (m^{-2})	Height (mm)	Conclusion	Source
Oysters								
Crassostrea virginica	Chesapeake Bay, USA	+	—	—	Up to 286	76	10^6 oysters removed 132 kg N; up to 10%–15% of annual N load	Higgins et al. 2011
Crassostrea gigas	Valdivia estuary, Chile	(+)	(+)	—	100	Seed	Net chlorophyll *a* and N reduction via filtration (modeled)	Silva et al. 2011
Crassostrea virginica	Bogue Sound, USA	—	—	+	—	—	Denitrification removed –20 to 35 μmol $N{\cdot}L^{-1}{\cdot}m^{-2}{\cdot}h^{-1}$	Piehler and Smyth 2011
Crassostrea virginica	South Carolina estuaries, USA	—	+	—	412–2931	23–51	Removed up to 28% of chlorophyll *a* in 0.3–1.3 h	Grizzle et al. 2008
Crassostrea virginica	Chesapeake Bay, USA	—	(+)	—	—	76	May remove 0.07%–1.4% of phytoplankton·day^{-1} (modeled)	Fulford et al. 2007
Crassostrea virginica	Chesapeake Bay, USA	—	(+)	(+)	—	—	Reduced total N concentration 10%–15% (modeled)	Cerco and Noel 2007
Pinctada imbricata	Port Stephens, Australia	+	—	—	—	—	Removed 7.5 kg N·$tonne^{-1}$ oyster; ~2% of wastewater N load·$year^{-1}$	Gifford et al. 2005
Crassostrea virginica	Chesapeake Bay, USA	—	(+)	(+)	—	—	Denitrification–burial removed 7.5 $\times 10^{-4}$ kg $N{\cdot}g^{-1}$ oyster; 0.6% of annual N load (modeled)	Newell et al. 2005
Crassostrea virginica	Chesapeake Bay, USA	—	—	+	—	—	Denitrification by simulated biodeposits removed 20% of local N load (lab)	Newell et al. 2002
Crassostrea gigas	Thau Lagoon, France	—	+	+	40	—	Reduced chlorophyll *a* but increased N in water column	Souchu et al. 2001
Pinctada imbricata	Port Stephens, Australia	(+)	(+)	(+)	—	—	May remove 19 kg N·$tonne^{-1}$ oysters	Gifford et al. 2004
Crassostrea virginica	North Carolina creek, USA	—	+	—	125	48	Some reduction of chlorophyll *a* and suspended solids	Nelson et al. 2004
Crassostrea gigas	Hiroshima Bay, Japan	+	(+)	(+)	Raft culture	—	Removed ~10% of N load·day^{-1}	Songsangjinda et al. 2000
Saccostrea commercialis	Moreton Bay, Australia	—	+	(+)	33–100	—	Removed particles (92% of chlorophyll *a*, 20% of N), increased sedimentation	Jones and Preston 1999
Mussels								
Mytilus edulis	Skagerrak Strait, Sweden	(+)	—	+	Long lines	—	Net N removal by harvest, burial, biogeochemical processes	Carlsson et al. 2012
Perna canaliculus	Firth of Thames, NZ	—	—	+	16 per chamber	—	34% of mineralized N was released as NH_4^+ (possible denitrification)	Giles and Pilditch 2006
Mytilus galloprovincialis	Goro lagoon, Italy	—	—	+	60 kg, long lines	—	Increased sedimentation with net input of N to sediments	Nizzoli et al. 2006
Mytilus galloprovincialis	Dokai Bay, Japan	+	+	—	Long lines	15–41	Removed ~25% of dissolved inorganic nitrogen (DIN) in 1 day (lab)	Kohama et al. 2002
Musculista senhousia	Lake Nakaumi, Japan	+	—	—	0–46 712	—	Shell burial removed 0.7%–4.9% of annual N load	Yamamuro et al. 2000
Mytilus edulis	Orust–Tjörn system, Sweden	+	—	+	100 kg, long lines	—	Removed 8.5–12 g $N{\cdot}kg^{-1}$ live mussel; removed 20% of DIN	Haamer 1996
Mytilus spp.	Upper South Cove, Canada	—	(+)	+	400, long lines	—	Increased sedimentation, released NH_4^+	Hatcher et al. 1994

Table 1 (*concluded*).

Species	Location	Method of remediation: N stored in tissues	Method of remediation: Particle removal	Method of remediation: Biogeochemistry	Density (m^{-2})	Height (mm)	Conclusion	Source
Mytilus edulis	North Sea, Netherlands	—	+	(+)	Field flume	—	Removed chlorophyll *a* and seston, released NH_4^+ (possible denitrification)	Dame et al. 1991
Mytilus edulis	Northern Baltic Sea, Sweden	—	—	+	535–1693 g chambers	—	Increased annual N, C, P sedimentation by 10%	Kautsky and Evans 1987
Perna canaliculus	Kenepuru Sound, NZ	+	—	+	Long lines	—	Harvest and denitrification removed 68% more N than reference sites	Kaspar et al. 1985
Geukensia demissa	Cape Cod, USA	+	+	(+)	34–365	10–100	Mussels retained and recycled N within the marsh system	Jordan and Valiela 1982
Clams								
Tapes philippinarum	Goro lagoon, Italy	—	—	+	100–3000	—	Increased sedimentation with net removal of N from sediments	Nizzoli et al. 2006
Corbicula japonica	Lake Shinji, Japan	—	+	(+)	0–1000	—	Removed chlorophyll *a*, released NH_4^+	Nakamura and Kerciku 2000
Mya arenaria	Laholm Bay, Sweden	—	+	+	0–2000	1–25	Removed up to 27% of new local production	Loo and Rosenberg 1989
Mercenaria mercenaria	Narragansett Bay, USA	—	+	+	16 mesocosm	32–107	Increased C sedimentation; models may overestimate particle removal	Doering et al. 1986, 1987
Corbicula fluminea	Potomac River, USA	—	+	—	1.2–1467	1 – >25	Removed 30% of chlorophyll *a* in 2 h	Cohen et al. 1984
Scallops								
Chlamys farreri	Sishili Bay, China	—	+	(+)	0–40	32±4	Removed up to 45% of particles·day^{-1}	Zhou et al. 2006
Cockles								
Cardium edule	Laholm Bay, Sweden	—	+	+	0–8000	4–21	Removed up to 27% of new local production	Loo and Rosenberg 1989
Various								
	Various	(+)	(+)	—	25–500	—	Bioremediaiton was location- and condition-specific (modeled)	Ferreira et al. 2007
	San Francisco Bay, USA	(+)	(+)	(+)	200	—	Defined conditions for remediation (model)	Officer et al. 1982

Note: Methods of remediation include nitrogen removal by assimilation into shell or soft tissues, particle removal (measured in terms of suspended particulates, chlorophyll *a* concentration, or filtration rate), and stimulation of biogeochemical processes via biodeposits. Parentheses indicate studies for which results were calculated from literature values, estimated, or modeled and not directly measured. A long dash (—) indicates not reported.

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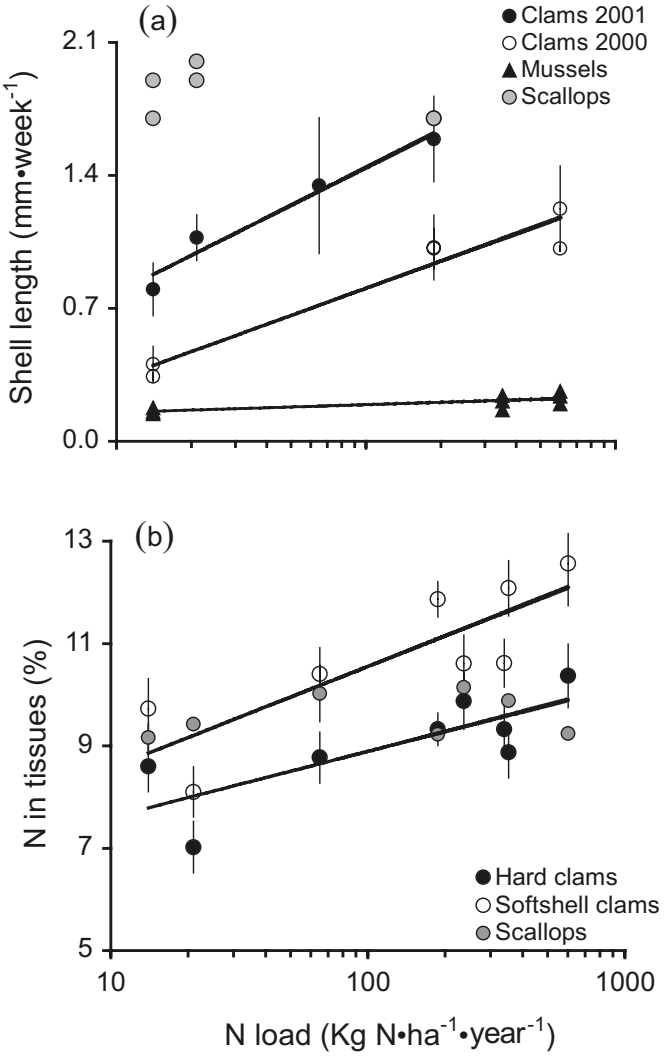
column, and biogeochemical processes, with fewer studies (~20%) directly quantifying N removal by assimilation into tissues (Table 1). Focus on particle removal and associated processes, however, may not be adequate to quantify N removal. Phytoplankton particles are subject to hydrodynamic forces, and it is difficult to assess consumption by bivalves relative to export down-estuary or consumption by other suspension feeders (Nelson et al. 2004; Grizzle et al. 2008). Similarly, measurement of particle or N removal using filtration rates may be inaccurate (Pomeroy et al. 2006; Grizzle et al. 2008), requiring careful consideration of environmental conditions that affect shellfish metabolism and likely overestimating N removal when bivalve condition or growth declines (Jørgensen 1966; Bayne and Newell 1983; Rice 1999). Consideration of N removal by tissue assimilation, however, reflects net potential N removal regardless of variation in environmental factors that alter filtration rates or physiological condition. Since many species used for bioremediation are commercially valuable, these species directly remove N in tissues when they are harvested as well as producing biodeposits that potentially alter downstream biogeochemical processes (Rice 1999; Newell 2004). Bivalve biodeposition has been credited with stimulating N removal as well as N addition to estuaries worldwide (Table 1), and the effects appear to be location-specific and inconclusive (Souchu et al. 2001; Zhou et al. 2006; Coen et al. 2007). These findings encourage a more comprehensive empirical examination of N removal by tissue assimilation along with the potential for biogeochemical processes to complement this mechanism of N removal.

Estimates of bioremediation also need to be made at relevant spatial scales, preferably considering entire estuaries. Previous studies reported largely local effects often based on generalized data or literature values, with the assumption that local effects and general conditions will broadly scale up (Pomeroy et al. 2006; Grizzle et al. 2008; Dumbauld et al. 2009). The large variation in findings reported in Table 1 suggests that local controls on N removal may also vary greatly. Location-specific factors such as salinity, temperature, dissolved oxygen (DO), hydrology, bivalve density and species composition, and total N load can affect estimates of bioremediation by affecting the quantity and quality of particle loads or bivalve responses (e.g., Officer et al. 1982; Carmichael et al. 2004*a*; Grizzle et al. 2008), rendering generalized data unreliable. Studies across a range of N-enriched estuaries show that the influence of N loads on bivalve growth and N content depended on species and location-specific attributes (Fig. 1: data from Evgenidou and Valiela 2002; Weiss et al. 2002; Shriver et al. 2002; and Carmichael et al. 2004*a*). Space available for cultivating shellfish may also strictly limit bioremediation relative to estuary size (Ferreira et al. 2007) but has rarely been considered in bioremediation assessments (Table 1). Calculations of N removal by tissue assimilation can avoid some of these potential hurdles by using location-specific measures of growth and N content in tissues relative to local planting areas, N loads, and particle supply. These caveats highlight the need for location-specific data and the potential utility of tissue assimilation to define N removal capacity of bivalves across locations and through time.

To collect data that are meaningful for assessment of bioremediation, restoration, or management efforts, it is also important to demonstrate that bivalves remove N from land-derived sources. Although most studies claim to test bivalve capacity to remediate cultural eutrophication or anthropogenic N loads (e.g., Officer et al. 1982; Zhou et al. 2006; Cerco and Noel 2007), they have not demonstrated that bivalves actually removed land-derived or autochthonous N, rather than N in particles conveyed from adjacent waters.

Fig. 1. Mean (± standard error) bivalve growth rates (*a*) and %N content in tissues (*b*) compared with N loading rates to four estuaries in Cape Cod, Massachusetts. Clams include combined data for softshell (*Mya arenaria*) and northern hard clams (*Mercenaria mercenaria*), which differed between seasons (2000, 2001), but not between species. Mussels refer to *Geukensia demissa*, and scallops refer to *Argopectin irradians*. Data are from Evgenidou and Valiela (2002), Weiss et al. (2002), Shriver et al. (2002), and Carmichael et al. (2004*a*). Nitrogen (%) in scallops was determined by Shriver et al. (2002), but not previously published. Shell length: $y = 0.29 \ln(x) + 0.12$, $R^2 = 0.96$, $F_{\text{regression } 3} = 48.62$, $P = 0.02$ (clams 2001); $y = 0.21 \ln(x) - 0.15$, $R^2 = 0.93$, $F_{\text{regression } 5} = 55.80$, $P = 0.002$ (clams 2000); $y = 0.02 \ln(x) + 0.11$, $R^2 = 0.56$, $F_{\text{regression } 8} = 9.02$, $P = 0.02$ (mussels). Nitrogen (%): $y = 0.56 \ln(x) + 6.31$, $R^2 = 0.61$, $F_{\text{regression } 7} = 9.31$, $P = 0.02$ (hard clams); $y = 0.86 \ln(x) + 6.58$, $R^2 = 0.69$, $F_{\text{regression } 7} = 13.13$, $P = 0.01$ (softshell clams).



Fortunately, the specific combination of N sources on a watershed confers unique N stable isotope signatures to nutrients delivered to a water body (McClelland et al. 1997; Carmichael et al. 2004*b*; Fertig et al. 2009). Producers may assimilate these nutrients and become food for consumers such as bivalves, which subsequently acquire the location-specific N stable isotope ratio (Evgenidou and Valiela 2002; Weiss et al. 2002; Shriver et al. 2002). Hence, N stable isotopes provide a tool to link estuary-specific N sources to bivalves and determine the efficacy of bioremediation.

Although the full complement of bivalves in a system may contribute to N removal at some level (if harvested and possibly via biogeochemistry), oysters or other commercially harvested species with similar feeding behavior and physiological capacity will likely have the greatest potential for use as a management or N bioremediation tool. Oysters are well documented to maintain high feeding rates at high food concentrations by coupling high particle capture rates with an efficient pre-ingestion and sorting mechanism (Newell and Langdon 1996). Higher growth rates combined with resulting greater dry mass to height ratios compared with other species (Shumway 1996; Carmichael et al. 2004*a*) and higher volume of ejecta production (Tenore and Dunstan 1973; Newell and Langdon 1996) render oysters potentially more effective at assimilating N into tissues or removing N via biogeochemical processing than some other species. Oysters are also abundantly harvested throughout the world and frequently targeted for aquaculture, restoration, and ecosystem assessment activities, making them of high interest for bioremediation study (Table 1; Fulford et al. 2010; Beck et al. 2011).

Given the paucity of comprehensive empirical assessments of bivalve bioremediation relative to estuary-specific N loads and the likelihood that oysters are a most effective bioremediator species, in this paper we directly measured N removal capacity of the oyster *Crassostrea virginica*. To test N removal under different N loading regimes, we quantified N assimilated into tissues and removed at harvest by oysters transplanted into five Cape Cod estuaries that receive different land-derived N loads. Estuary-specific growth rates and N content in oyster tissues were measured to determine total N stored in oyster tissues. To determine whether oysters assimilated (and therefore had potential to remove) anthropogenic N, we measured estuary-specific N stable isotope ratios in suspended particles and oyster tissues and compared them with the percent wastewater contribution to each estuary. To roughly estimate the potential N removal stimulated by oyster biodeposition associated with our empirical growth measurements, we applied literature estimates of N removal by denitrification and burial. Data on N removal by tissue assimilation were further compared with estuary-specific, land-derived N loads and N in phytoplankton to calculate the number of oysters required to completely remediate N loads and the approximate percent N (%N) removed by oysters at typical restoration and aquaculture densities if planted over different percentages of embayment area. We used both land-derived and phytoplankton N to account for dissolved inorganic N loaded to the estuary from external sources as well as to roughly account for additional N regenerated within or otherwise conveyed to the estuary, including from biodeposits by oysters, which may contribute to algal production and symptoms of eutrophication (Kemp and Boynton 1984; Mayer et al. 1998; York et al. 2007).

Materials and methods

Study sites

This study was conducted in five estuaries on Cape Cod, Massachusetts, USA (Fig. 2), characterized by different land uses on their watersheds (Table 2). N loads to these shallow, well-mixed estuaries (Sage Lot Pond, Wild Harbor, Green Pond, Snug Harbor, Childs River) do not vary substantially among seasons because land-derived loads are delivered primarily through groundwater (Valiela et al. 1992; Jay et al. 1997). The N loads have been estimated and span most of the range of land-derived N loads to coastal estuaries in the United States (e.g., Valiela et al. 1992, 2000; Kroeger et al. 1999). Several studies on the effects of eutrophication on habitat and food supply for a variety of bivalve species have been conducted in these estuaries; site conditions are well documented, and this previous work (e.g., Evgenidou and Valiela 2002; Shriver et al. 2002; Carmichael et al. 2004*a*) provided context for the current study.

Field measurements

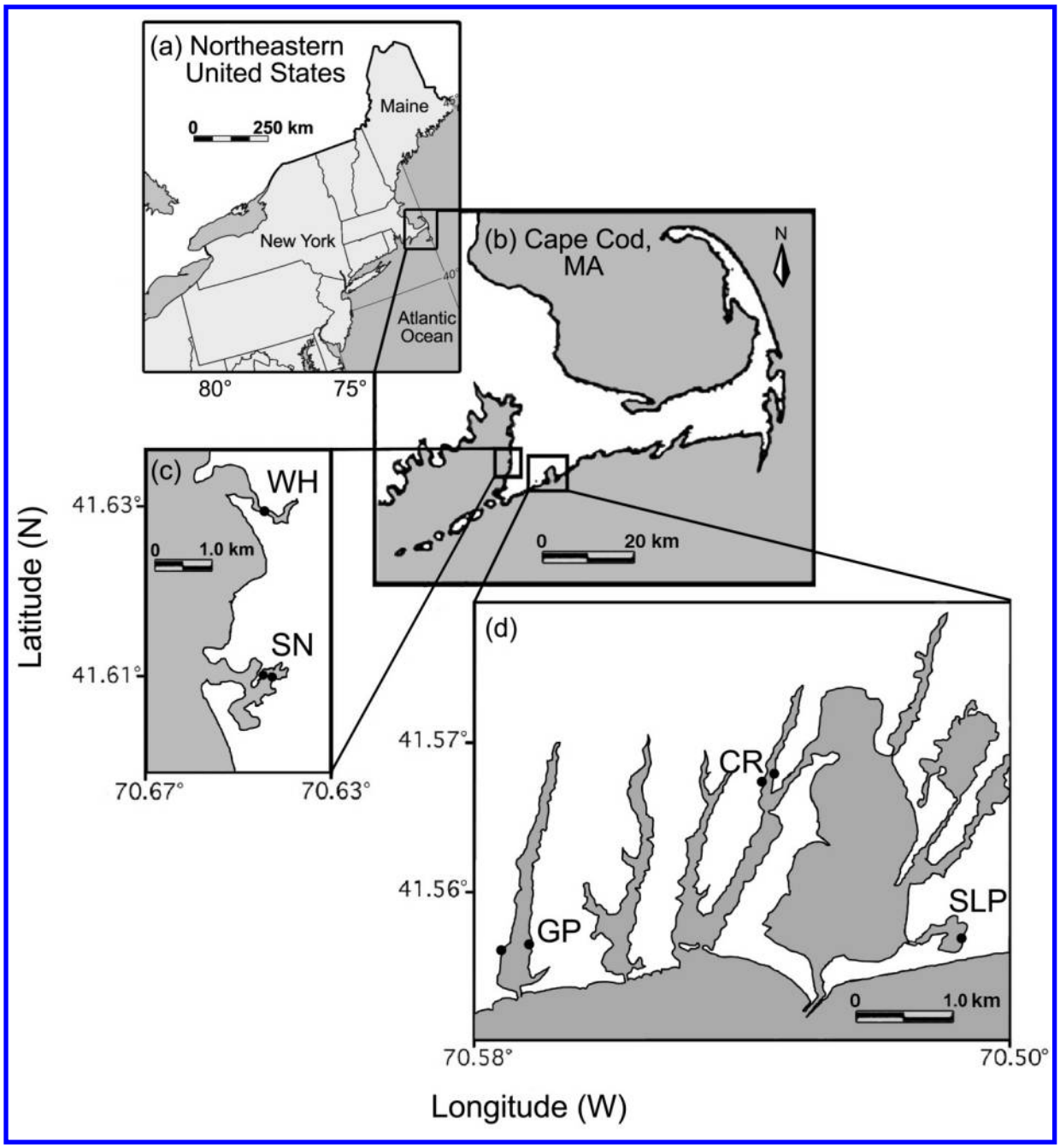
Oyster transplants

To quantify estuary-specific growth and N content in oysters, juvenile hatchery-reared oysters (8.2 ± 0.2 mm longest dimension) were transplanted at two sites in five Cape Cod estuaries (Fig. 2) during the primary growing season (June–October, starting on 29 June 2003), when bivalves in the estuaries are most actively assimilating particle and N loads (Shriver et al. 2002; Carmichael et al. 2004*a*). We used hatchery-reared oysters to ensure common stock was transplanted into each estuary and to most accurately reflect bivalves planted for culture or management purposes. Oysters were obtained from the Aquaculture Research Corporation in Dennis, Massachusetts. Oysters ($n = 67 \pm 2$) were placed in plastic-coated wire mesh aquaculture cages measuring 30 cm wide × 52 cm long × 8 cm deep. Cages were lined on the inside with 3 mm plastic mesh and elevated 6 cm above the sediment surface in approximately 1 m of water (at low tide). Cages were placed at this height to mimic typical natural settlement elevations for oysters in the area and allow access for sampling and cleaning with minimal disturbance while maintaining access to natural food sources. Four replicate cages were transplanted at each site (eight per estuary), and one randomly selected cage was removed from each site on days 28, 56, 84, and 112. This sampling scheme was chosen to capture spatial and temporal variation in growth, survival, and N content during the growing season.

Oyster growth and survival

To measure growth during the experimental period, we recorded shell dimensions of 50 randomly selected oysters from initial hatchery stock and from each cage at each removal date. Of the 50 oysters, 10 were further processed to determine mean soft tissue dry mass. Soft tissues from the remaining 40 oysters were reserved for N content and stable isotope analyses. In two cases (Snug Harbor and Green Pond), mortality limited the number of oysters measured to 33 and 49, respectively. Shell dimensions were recorded as the longest height (umbo to margin), width, and length of each oyster to the nearest 0.1 mm using vernier calipers. To measure soft tissue dry mass, whole tissues were separated

Fig. 2. Study sites in five northeastern USA (*a*) estuaries on Cape Cod, Massachusetts (*b*). WH, Wild Harbor; and SN, Snug Harbor (*c*); GP, Green Pond; CR, Childs River; and SLP, Sage Lot Pond (*d*).



from shell and dried to a constant mass at 60 °C. Whole tissues, except gut, were collected to reflect total N assimilated without including unassimilated foods. Growth rates were determined from the slope of the regression line comparing mean shell height and dry mass of oysters in each estuary to date of collection. To determine percent survival, we counted the number of living oysters in each transplant cage at collection, divided by the total number planted, and multiplied by 100.

Suspended particles

To determine the quantity and quality of suspended particles available as food for oysters during the growing season, we collected whole water at 10 cm from the sediment surface at each site (Fig. 2) every 2 weeks. Two 1 L samples (200 µm prefiltered) were passed through pre-ashed 0.7 µm Whatman GF/F filters. We identified components of available foods by measuringchl *a* concentration and total suspended and organic particulate matter. Chl *a* was determined by 90% acetone extraction and analyzed by spectrophotometry (Lorenzen 1967). Total suspended and organic particulate matter were determined from the mass of dried filters (at 60 °C to a constant mass) before and after ashing at 490 °C for 4 h.

Environmental attributes

Salinity was measured using a handheld salinity refractom-

Table 2. Land-derived annual N loading rate, area-weighted N loading rate, the percent contribution of wastewater to total annual N load, volume and surface area of five Cape Cod estuaries in which oysters were transplanted, and seasonal mean (± standard error) suspended particulate matter (SPM), particulate organic matter (POM), temperature, salinity (practical salinity scale), and percent survival of oysters at two sites in each estuary.

	Estuary characteristics						Water column				
Estuary	N loading rate (kg N·year^{-1})	Area-weighted N load (kg N·m^{-2}·year^{-1})	Wastewater (%)	Volume (m^3)	Surface area (m^2)	Site	SPM (mg·L^{-1})	POM (mg·L^{-1})	Temperature (°C)	Salinity	Oyster survival (%)
Sage Lot Pond[a]	990	14×10^{-4}	4	0.59×10^6	0.70×10^6	1	59±16	16±4	22±2	28±1	93±3
Wild Harbor[b]	3 185	65×10^{-4}	21	0.49×10^6	0.49×10^6	1	49±5	14±1	21±1	26±1	83±5
Green Pond[c]	10 716	178×10^{-4}	54	1.28×10^{6d}	0.60×10^6	1	67±7	15±3	22±2	28±0	96±2
						2	65±12	12±2	22±2	28±0	98±1
Snug Harbor[b]	4 263	236×10^{-4}	89	0.21×10^6	0.18×10^6	1	42±6	17±9	22±1	25±1	87±10
						2	42±5	16±3	22±1	25±1	96±2
Childs River[a]	8 114	601×10^{-4}	63	0.18×10^6	0.14×10^6	1	55±5	14±3	23±2	27±1	96±0
						2	38±6	16±2	23±2	26±1	97±1

Note: Data were available for only one site in Sage Lot Pond and Wild Harbor.
[a]Data from Valiela et al. (2000).
[b]Data from Costa 1994.
[c]Data from Kroeger et al. (1999).
[d]Data from Ramsey 2000.

eter with automatic temperature correction (Fisher Scientific). Temperature was measured using a Carolina armored water thermometer (NIST certified, 0.5 °C accuracy). Parameters were measured adjacent to cages at each transplant site prior to collecting water samples.

Stable isotope analysis and N content in oysters

To determine whether N assimilated into oysters was derived from anthropogenic sources, we measured the N stable isotope ratios in suspended particulate matter (available foods) and oyster tissues. Soft tissues were aggregated from 10 randomly selected oysters on each removal date to yield four replicate aggregate samples for each site (because of mortality, two replicates were processed for Snug Harbor on day 84). Samples were dried to a constant mass at 60 °C and ground to a powder with a mortar and pestle. Tissues and dried filters containing suspended particulate matter were analyzed by continuous flow isotope ratio mass spectrometry (IRMS) at the University of California Davis Stable Isotope Facility (Davis, California). All samples were analyzed on a PDZ Europa 20-20 mass spectrometer after combustion in a PDZ Europa Automatic Nitrogen and Carbon Analyzer-Gas Solid Liquid. Gases were separated on a Supelco Carbosieve G column before IRMS. Machine reproducibility was ≤0.02‰ and was determined by analyzing randomly selected subsamples for 10% of the samples.

N content in oyster soft tissues was determined by combustion during stable isotope analysis. To ensure N content reflected ambient rather than residual hatchery conditions, N content data were used only from oysters sampled on day 112.

N removal and bioremediation calculations

To calculate the capacity of oysters to remove N and remediate eutrophication, we used a two-step process. First, we applied empirical estuary-specific growth and N content data from transplanted oysters to estimate estuary-specific times to reach harvestable size (T_h) and used regression analysis to extrapolate the corresponding soft tissue N content when oysters did not reach harvestable size within the study season. We opted to define harvestable size as 76.2 mm shell height because it is representative of practices of the USA oyster fisheries (T. Getchis, East Coast Shellfish Growers Association, 1623 Whitesville Road, Toms River, NJ 08755, USA, personal communication, 2010; MacKenzie 1996; Higgins et al. 2011).

Second, we estimated the number of oysters required to assimilate and store the N in each estuary during the time period between planting and harvest (T_h) based on (*i*) land-derived N load to each estuary and (*ii*) N in phytoplankton available in each estuary. We used these approaches to account for dissolved inorganic N loaded to the estuary from external sources (land-derived N loads) as well as to roughly account for additional N regenerated within or otherwise conveyed to the estuary, including from biodeposits by oysters, which may contribute to algal production (phytoplankton N) and symptoms of eutrophication (Kemp and Boynton 1984; Mayer et al. 1998; Souchu et al. 2001). We used phytoplankton N rather than N content in suspended particulate matter for this calculation because it is likely most reflective of consumed diet and is consistent with estuary-specific phyto-

plankton dynamics and bivalve growth responses measured during this and other studies (Riera et al. 1999; Carmichael et al. 2004*a*; York et al. 2007).

Determining T_h

The number of growing seasons required to reach harvestable size (T_h) was defined as the time period between planting and when oysters reach a length of 76.2 mm. T_h was calculated from estuary-specific growth rates, assuming growth occurred only during the growing season (mid-May – mid-October) in Cape Cod. T_h was estimated by first determining the number of days to reach harvest size by extrapolating from the equation for the lines best fit to the regression of shell height compared with sampling day for each estuary. We divided the number of days to reach 76.2 mm by the number of days estimated for a typical growing season (~153 days) to determine the number of seasons (T_h) to reach 76.2 mm. This estimate of growing season length was consistent with conditions during this study and previous observations for the mid to northern Atlantic region (Loosanoff and Nomejko 1949; Rheault and Rice 1996; Soniat et al. 1998). This estimate is also reasonable given the relatively short period of growth to harvestable size for these estuaries, and because the smaller amount of growth that may occur during the remainder of the year (colder periods) is likely balanced by a reduction in growth with age as bivalves approach legal size and not accounted for in a linear model (Askew 1972).

Land-derived and phytoplankton N loads

To calculate land-derived N loads and phytoplankton N in each estuary during oyster growth to harvestable size, we multiplied T_h by total annual land-derived N load (Table 2) and by estimated seasonal phytoplankton N in the estuary. Seasonal phytoplankton N load was calculated by multiplying the empirical mean chl *a* concentrations measured in each estuary by estuary volume at mean tide height (Table 2) and assuming a N:chl *a* ratio of 12.8 ± 1.5 (MacIntyre et al. 2002). Although the N:chl *a* ratio will necessarily vary with the composition of phytoplankton in a given area, we opted to use this value because it is comparable to values previously applied to make similar calculations (Newell et al. 2005) but was refined based on raw data from a long-term data set (MacIntyre et al. 2002). To roughly capture interannual and spatial variation in production, we used mean chl *a* concentrations measured during this study and in two previous studies, including measurements throughout the estuary and along a salinity gradient (Weiss et al. 2002; Shriver et al. 2002; Carmichael et al. 2004*a*). Because the concentration of chl *a* will also vary with tidal flow and season, we opted to use a mean seasonal value based on samples collected across different tidal cycles during the period of greatest bivalve growth in our region. The resulting values were also within the range of median seasonal chl *a* concentrations in estuaries worldwide (Cebrian and Valiela 1999). Previous work indicates this approach is appropriate to the physical and biological dynamics of these estuaries (Jay et al. 1997; Shriver et al. 2002; York et al. 2007).

Because of the spatial and temporal heterogeneity inherent in making estimates of primary production and biomass in estuaries (Malone et al. 1988; Underwood and Kromkamp 1999), we emphasize that this effort to scale-up estimates of phytoplankton N to the whole estuary volume provides only a rough estimate of the possible N in phytoplankton that may be available to oysters in these estuaries (Shaffer and Onuf 1985). Our approach composites data from a variety of spatial and temporal scales, including values generally representative of similar shallow water bodies, to generate an estimate based on the suite of best available data (e.g., Shaffer and Onuf 1985). These estimates, however, may not capture short-term horizontal or vertical patchiness in biomass that may occur in response to environmental variation or grazing, despite the shallow, well-mixed attributes of the estuaries (Cloern et al. 1985; Monbet 1992). These values also do not account for the potentially greater compensatory responses of phytoplankton to the presence of oysters as estimates are scaled up to higher densities. While approximate, this scaling-up effort in terms of phytoplankton N is grounded in empirical site-specific data and important given that both land-derived and regenerated N sources may contribute to eutrophic conditions and available food supply for bivalves in the estuary (Malone et al. 1988; Underwood and Kromkamp 1999; York et al. 2007). Hence, phytoplankton N load is considered a rough estimate of maximum N available in foods for oysters in our study estuaries.

N removal by tissue assimilation

To determine the amount of N assimilated into oyster soft tissues and potentially removed at harvest, we used estuary-specific dry mass to shell height relationships (Table 3) to extrapolate dry mass at legal size. We multiplied the resulting value by the mean %N content measured in soft tissues at the end of the study (day 112). For simplicity and because oysters were planted at a small size (~8 mm), we assumed N content in oysters was negligible at the start of this study and did not subtract initial N content from our N removal estimates. N assimilated into shell (estimated at 0.08%–0.8%; derived from Carriker 1996; Lee et al. 2011; R.H. Carmichael, unpublished data) was not included in these calculations because of the questionable ability to accurately estimate organic N content in shell at the time of study (Carriker 1996).

N removal by denitrification and burial

To include rough estimates of potential biogeochemical N removal (N_d) stimulated by bivalve biodeposits, we combined our field data with estimates of N removal by denitrification and burial (0.75 g N per gram of oyster dry mass per year) predicted by Newell et al. (2005). Since oysters were actively growing and changing size during the study, we extrapolated this additional N removed during T_h by plotting estuary-specific mean dry mass at each sampling day compared with calculated N_d for 1 day at that mass (assuming 0.002 g N removed per gram of oyster dry mass per day). We used the slope of the resulting best-fit regression line for each estuary as a rate of estuary-specific N removal (mg N·day^{-1}) via denitrification and burial for oysters in this study. To determine total N_d during time to harvest, we then multiplied each rate by the estuary-specific number of days required for oysters to reach harvest size. We determined the percent enhancement of N removal provided by denitrification and burial by dividing N_d at harvest by N in tissues at harvest and multiplying by 100. To roughly estimate N removal by oysters if not har-

Table 3. Equations and regression statistics describing the change in shell height through time and corresponding soft tissue dry mass and shell height relationships shown in Fig. 3.

	Shell height					Dry mass				
Estuary	y	R^2	df	F	P	y	R^2	df	F	P
Sage Lot Pond	$0.25x + 6.85$	0.94	4	50.13	0.01	—	—	—	—	—
Wild Harbor	$0.30x + 10.77$	0.97	4	109.75	0.002	$0.005^{0.089x}$	0.85	38	202.40	<0.0001
Green Pond	$0.39x + 11.06$	0.95	4	61.37	0.004	$0.008^{0.082x}$	0.88	38	259.16	<0.0001
Snug Harbor	$0.26x + 11.07$	0.93	4	38.37	0.01	$0.007^{0.085x}$	0.91	39	390.20	<0.0001
Childs River	$0.31x + 12.26$	0.91	4	31.53	0.01	$0.010^{0.072x}$	0.84	34	171.54	<0.0001

Table 4. Mean (± standard error) oyster shell and soft tissue growth rates, number of growing seasons to reach typical harvestable size of 76.2 mm (T_h), and soft tissue dry mass (DW) and N content at harvestable size in Cape Cod estuaries.

	Oyster growth rates				
Estuary	Shell height ($mm \cdot day^{-1}$)	Soft tissue ($mg \cdot day^{-1}$)	T_h	DW at harvest ($g \cdot oyster^{-1}$)	Tissue N at harvest ($g \cdot oyster^{-1}$)
Sage Lot Pond	0.25±0.04	1.7±0.5	1.8±0.3	—	—
Wild Harbor	0.30±0.03	1.8±0.1	1.4±0.2	4.1±1.3	0.35±0.11
Green Pond	0.39±0.05	6.8±2.0	1.1±0.2	4.2±1.2	0.36±0.10
Snug Harbor	0.26±0.04	2.3±0.4	1.7±0.3	4.4±1.1	0.38±0.09
Childs River	0.31±0.06	2.1±0.5	1.4±0.3	2.4±1.2	0.20±0.11

Note: Oysters in Sage Lot Pond did not grow sufficiently to reliably determine dry mass at harvest size. Tissue N at harvest was based on a mean of 8.6% ± 0.2% N in oyster tissues on day 112.

vested, we calculated N_d after 1 year at legal size by assuming no further growth, multiplying dry mass at harvest (Table 4) by 0.75 g N (Newell et al. 2005), and adding this number to N_d during T_h.

Quantifying capacity for bioremediation

Given the estuary-specific N removal per oyster at harvest size, the actual capacity for bioremediation depends on the density and area on which bivalves are planted. Because density and area planted can vary, we opted to first determine the number of oysters needed to remove 100% of N loads in each estuary. We then determined the density of oysters required to support this N removal if the entire bottom area of each embayment were available and suitable for oyster growth. The number of oysters required to remediate 100% of land-derived and phytoplankton N loads was determined by dividing each N load during T_h (the cumulative load during growth to harvest size) by the quantity of N in each oyster at harvest size.

To estimate more realistic N removal capacities at lower coverage areas, we calculated N removal by oysters if planted at typical restoration and aquaculture grow-out densities of varying intensity (which also may account for different gear types) at 0.5%–5.0% bottom area coverage. We multiplied the N in each oyster at harvest size by 75 and $150 \cdot m^{-2}$ (restoration) and 400, 550, and $1650 \cdot m^{-2}$ (aquaculture) densities and by the appropriate surface area of each estuary (calculated from values in Table 2). The resulting N removal estimates were compared to land-derived and phytoplankton N loads during the time to reach harvest size (T_h).

Statistical analysis

All growth and environmental data are reported as the mean of data from two replicate cages at two sites in each estuary, except for Sage Lot Pond (SLP) and Wild Harbor (WH), in which cages were lost after day 28 and data were collected from only one site. To compare the rate and magnitude of growth among estuaries, regression analyses of shell height and dry mass through time were followed by a test for homogeneity of slopes (Sokal and Rohlf 1981) and analysis of covariance (ANCOVA). Data were log-transformed, as needed, before testing for significance of regression and higher-order statistics. Type II regression was used when error was present in the independent variable (comparison between growth rate and chl *a* concentration, $\delta^{15}N$ in tissue, and suspended particulate matter). A one-way ANCOVA was used to compare survival, suspended and organic particulate matter concentrations, and environmental attributes among estuaries. Since sample sizes were not equivalent among estuaries (owing to cage loss from two sites), we averaged site values for each sampling date to obtain estuary means for each variable on each sampling day. Regression analyses, including F tests, were performed in Microsoft Excel 11.3.7. All other analyses were performed in Stat View 5.0.1 (SAS Institute Inc., Cary, North Carolina). A significance value of $P < 0.05$ was used for all tests.

Each N stable isotope ratio data point represents the mean of data from two sites in each estuary from which aggregates of 10 individuals were replicated two to four times per site, depending on the number of individuals available from each estuary (indicated above). Error reported for calculated values were propagated from empirical field measurements (Valiela 2001). All error is reported as standard error unless otherwise noted. For higher-order extrapolations (cumulative N loads, numbers of oysters, and area required for remediation), error is reported as coefficient of variation (CV) to most accurately reflect the scale of relative variation in these data among estuaries. Where error bars are not visible in figures, error was smaller than the symbol.

Fig. 3. Mean (± standard error) shell height (*a*) of oysters transplanted into five Cape Cod estuaries on day 0 and removed on days 28, 56, 84, and 112 and the corresponding soft tissue dry mass (*b*). Regression statistics are shown in Table 3.

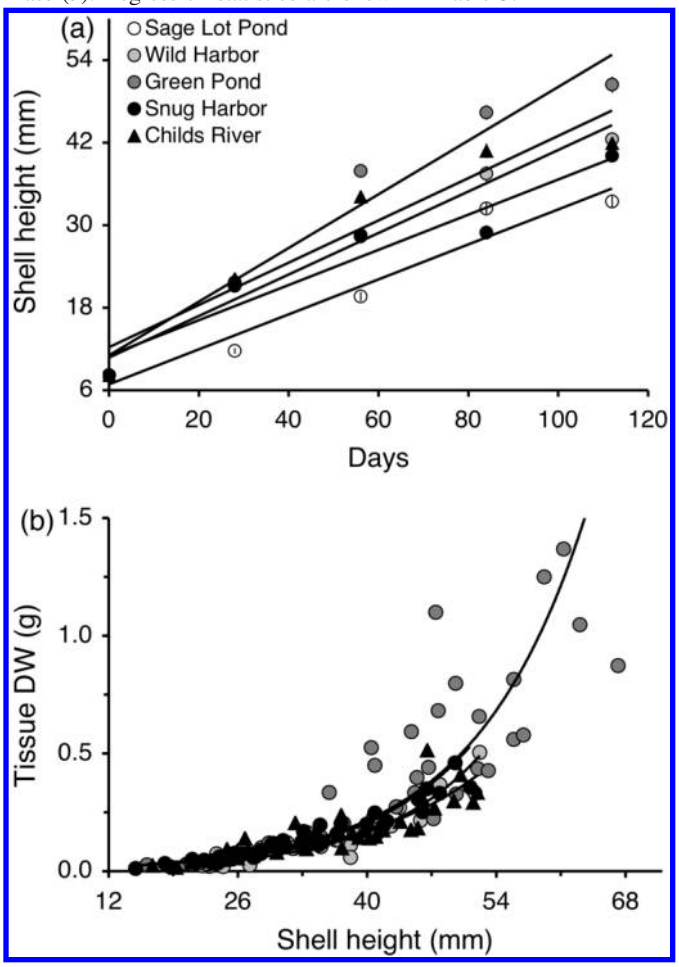
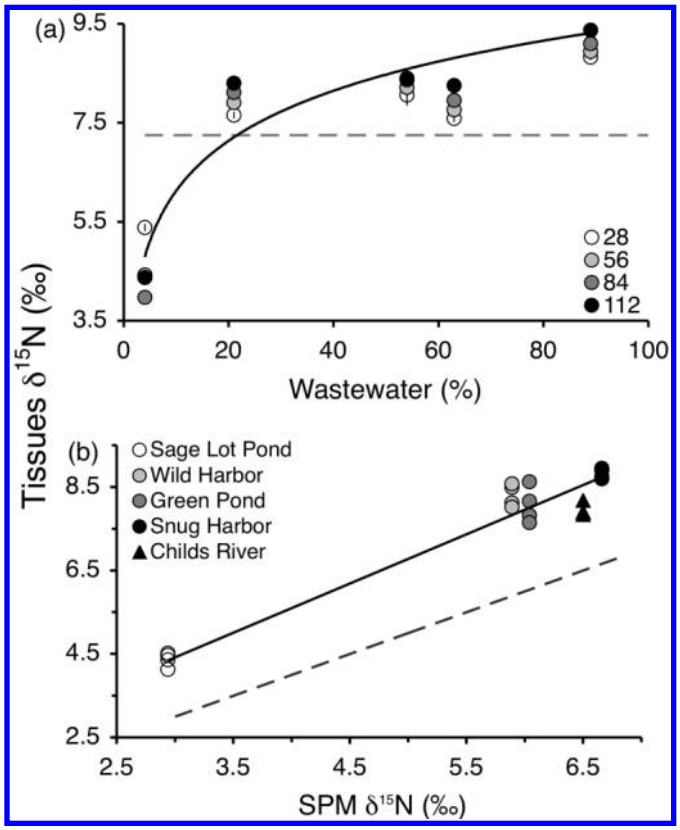


Fig. 4. $\delta^{15}N$ in oyster tissues on days 28, 56, 84, and 112 compared with percent contribution of wastewater to N load (from Table 2) received by five Cape Cod estuaries (*a*) and on day 112 compared with $\delta^{15}N$ in suspended particulate matter (SPM; *b*). Dashed grey lines show mean $\delta^{15}N$ in oyster tissues at day 0 (*a*) and the 1:1 line where points would fall if there were no isotope fractionation from food sources (SPM) to oyster tissues (*b*). Data points in panel (*a*) show the mean (± standard error) of four samples for each date. Where no error bars are present, error is smaller than the symbol. Panel (*b*) shows all four data points for day 112. Wastewater: $y = 1.46 \ln(x) + 2.77$, $R^2 = 0.88$, $F_{\text{regression } 4} = 23.34$, $P = 0.02$; SPM (type II regression): $y = 1.18x + 0.88$, $R^2 = 0.94$, $F_{\text{regression } 19} = 267.85$, $P < 0.001$.



Results

Field measurements

Oyster growth and survival

Oyster shell and soft tissue growth rates were in the range of 0.25–0.39 and 1.7–6.8 mg·day^{-1}, respectively (Fig. 3; Tables 3 and 4). Morphometric ratios between shell height, length, and width were similar among estuaries (one-way analysis of variance (ANOVA) for each ratio L:W, L:H, W:H compared among estuaries, $F_{[4,15]} < 2.04$, $P > 0.14$; data not shown). Hence, shell height (shown in Fig. 3*a*) was a suitable measure of relative shell growth for this study. Shell growth was generally highest among oysters in Green Pond and lowest in Sage Lot Pond (Fig. 3; test for homogeneity of slopes: $F_{[1,4,4]} = 1.65$, $P = 0.21$; ANCOVA: $F_{[1,4]} = 5.93$, $P = 0.003$). On average, oysters achieved 61% ± 4% of harvest size (76.2 mm height) within the study period, with the fastest growing oysters exceeding 80% of harvest size (Fig. 3*a*). Data for Sage Lot Pond were excluded from subsequent calculations because oysters in this estuary did not grow sufficiently to achieve at least 50% of harvest size (Fig. 3*a*). The dry mass of tissues increased exponentially with shell height in all estuaries (Fig. 3*b*). Relative dry mass content during the time to reach harvest size, however, differed by estuary (Table 4; test for homogeneity of slopes: $F_{[1,3,3]} = 1.37$, $P = 0.26$; ANCOVA: $F_{[1,3]} = 5.80$, $P < 0.001$), with the larger oysters in Green Pond having greater corresponding dry mass (Fisher's partial least-squares difference for dry mass, $P < 0.0001$ for all comparisons with Green Pond). Survival ranged from 82% to 97% and did not differ among estuaries (Table 2; one-way ANOVA: $F_{[1,4]} = 2.40$, $P = 0.12$).

Environmental attributes

Chl *a* concentrations at oyster transplant sites increased with increasing land-derived N load to estuaries (Supplemental Fig. S1*a*[1]). Oyster growth rates, in turn, increased with increasing chl *a* concentration among estuaries, but were stratified by salinity (Supplemental Fig. S1*b*[1]). In higher N-loaded estuaries in which salinity measured at or below 23

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/f2012-057.

Table 5. Mean (± standard error) chlorophyll *a* (chl *a*) concentration measured in four Cape Cod estuaries during bivalve growing seasons in 2000, 2001, and 2003 (this study), estimated seasonal phytoplankton N (Ph N) in each estuary, and total cumulative Ph N and land-derived N loaded to each estuary during the time oysters grew to harvestable size (T_h).

	Chl *a* (mg·m^{-3})								
Estuary	2000[a]	2001[a]	2003	Mean	Ph N (kg N·season^{-1})	CV (%)	Cumulative Ph N (kg N·T_h^{-1})	CV (%)	Cumulative land-derived N (kg N·T_h^{-1})	CV (%)
Wild Harbor	—	13.6±1.4	10.8±1.9	12.2±2.5	7.7×10^4	16	10.9×10^4	27	0.5×10^4	25
Green Pond	11.3±3.1	22.8±2.9	13.0±1.9	15.7±3.6	25.7×10^4	39	28.1×10^4	49	1.2×10^4	34
Snug Harbor	—	27.3±3.3	15.2±2.4	21.3±3.5	5.7×10^4	40	9.6×10^4	54	0.7×10^4	42
Childs River	14.0±2.4	26.4±2.3	16.0±3.2	18.8±2.7	4.4×10^4	35	6.0×10^4	53	1.1×10^4	48

Note: Ph N was determined from mean chl *a* concentration and volume of each embayment (Table 1), assuming a N:chl *a* ratio of 12.84 (MacIntyre et al. 2002). Ph N (kg N·T_h^{-1}) = Ph N (kg N·season^{-1}) multiplied by T_h (Table 4). N load (kg N·T_h^{-1}) = total N load from Table 2 multiplied by T_h. CV, coefficient of variation.

[a]Data from Shriver et al. (2002) and Carmichael et al. (2004*a*).

on 10%–30% of sampling dates (Snug Harbor and Childs River), oyster growth was depressed despite higher chl *a* concentrations (>14 mg·m^{-3}; Supplemental Fig. S1*b*[1]). Suspended and organic particulate matter and water temperature did not vary significantly among estuaries and showed no relationship to oyster growth (Table 2).

N stable isotope ratios

δ^{15}N values in tissues confirmed that transplanted oysters assimilated N from local anthropogenic sources. Through time, δ^{15}N values in oysters moved away from hatchery values (Fig. 4*a*, grey dashed line) toward estuary-specific N stable isotope ratios that increased with increasing percent wastewater inputs to the adjacent watersheds. Oyster tissues also showed an approximately 2‰–4‰ enrichment compared with suspended particulate matter in each estuary, typical of a single trophic step from the estuary-specific food source to consumer (Fig. 4*b*).

Land-derived and phytoplankton N loads

Total land-derived N loads to study estuaries during T_h (roughly one to two seasons) ranged from 4 500 to 12 000 kg N (Table 5). The estimated N available in phytoplankton standing stock during the same period was up to 24 times higher than land-derived N loads and ranged from 60 000 to 281 000 kg N·T_h^{-1} (Table 5). The greatest cumulative N loads were estimated in Green Pond, owing to the high annual land-derived N load and larger embayment area and volume of this estuary (Tables 2 and 5).

N removal and estimated bioremediation

In this section, we quantified oyster capacity for N removal via harvest and estimated theoretical N removal by denitrification and burial. We then compared the empirical estimates of N removal by tissue assimilation with land-derived N loads and N in phytoplankton to evaluate embayment-scale remediation of eutrophication.

N removal by assimilation into tissues

N content in oyster tissues averaged 8.6% ± 0.2% and did not differ among estuaries. Hence, the mean value was used for subsequent N removal calculations in all estuaries. Based on estuary-specific oyster growth rates (Tables 3 and 4) and %N content, N assimilation and potential removal was estimated at 0.3–0.5 g N per oyster, if harvested at 76.2 mm (Table 4). The estimated time for oysters to reach harvest size was less than two growing seasons (T_h = 1.1–1.8 years) and resulted in mean dry mass of 2–4 g per oyster (Table 4). Although the %N content in tissues was similar among estuaries, the significant differences in relative dry mass at harvest size (Fig. 3 and Table 4) resulted in different N content per oyster and therefore different N removal capacities via tissue assimilation (Table 4).

Estimated N removal by denitrification and burial

Assuming the potential for increased denitrification due to biodeposit production by oysters was equivalent to 0.75 g N removed per gram of oyster dry mass annually (Newell et al. 2005), we estimated that denitrification and burial could theoretically enhance N removal by 1%–2% during growth to harvestable size (Table 6). Hence, N removal by biogeochem-

Table 6. Equations and regression statistics for estimated N removal (mg) through burial or denitrification per oyster compared with sampling day (0, 28, 56, 84, 112), based on estuary-specific dry mass measured in this study and N removal rates reported by Newell et al. (2005).

Estuary	y	R^2	df	F	P	T_h (days)	N_d during T_h (g N·oyster^{-1})	Additional N removed (%)	N_d 1 year after T_h (g N·oyster^{-1})
Wild Harbor	$0.03x - 1.49$	0.84	5	20.79	0.01	217	0.007±0.002	2.1±1.0	3.11±0.99
Green Pond	$0.04x - 1.19$	0.83	5	19.98	0.01	167	0.006±0.001	1.7±0.7	3.19±0.92
Snug Harbor	$0.03x - 1.52$	0.89	5	33.24	<0.01	255	0.009±0.002	2.3±0.8	3.34±0.86
Childs River	$0.02x - 0.73$	0.86	5	25.11	<0.01	208	0.004±0.001	2.1±1.5	1.78±0.93

Note: These relationships and T_h (reported in days) were used to estimate N removal due to burial or denitrification (N_d) during growth to harvest size, the %N removed by these processes in addition to N assimilation into tissues, and potential N removal by denitrification and burial if oysters are retained in the estuary for 1 year after reaching harvest size (assuming no further growth).

Table 7. The number and density of harvest size (76.2 mm) oysters needed to assimilate 100% of land-derived and phytoplankton N (Ph N), assuming the entire bottom area was available for rearing oysters in four Cape Cod estuaries.

	To assimilate land-derived N			To assimilate Ph N		
	No.	Density (m^{-2})	CV (%)	No.	Density (m^{-2})	CV (%)
Wild Harbor	1.3×10^7	26	26	3.1×10^8	632	28
Green Pond	3.2×10^7	54	34	7.7×10^8	1287	49
Snug Harbor	1.9×10^7	104	42	2.5×10^8	1396	54
Childs River	5.5×10^7	405	48	3.0×10^8	2206	54

Note: Estimates do not include potential N removal by denitrification or burial. CV, coefficient of variation.

ical processes appeared to be small compared with N assimilated into tissues during the first one to two seasons of growth (compare Tables 4 and 6). This same approach suggests that retaining harvest-sized oysters (76.2 mm) in the estuary for up to 1 year after reaching harvest size could produce sufficient biodeposits to increase N removal by nearly an order of magnitude beyond N assimilation in tissues (when immediately harvested) (Table 6).

Determining the relevance and scale of N removal estimates

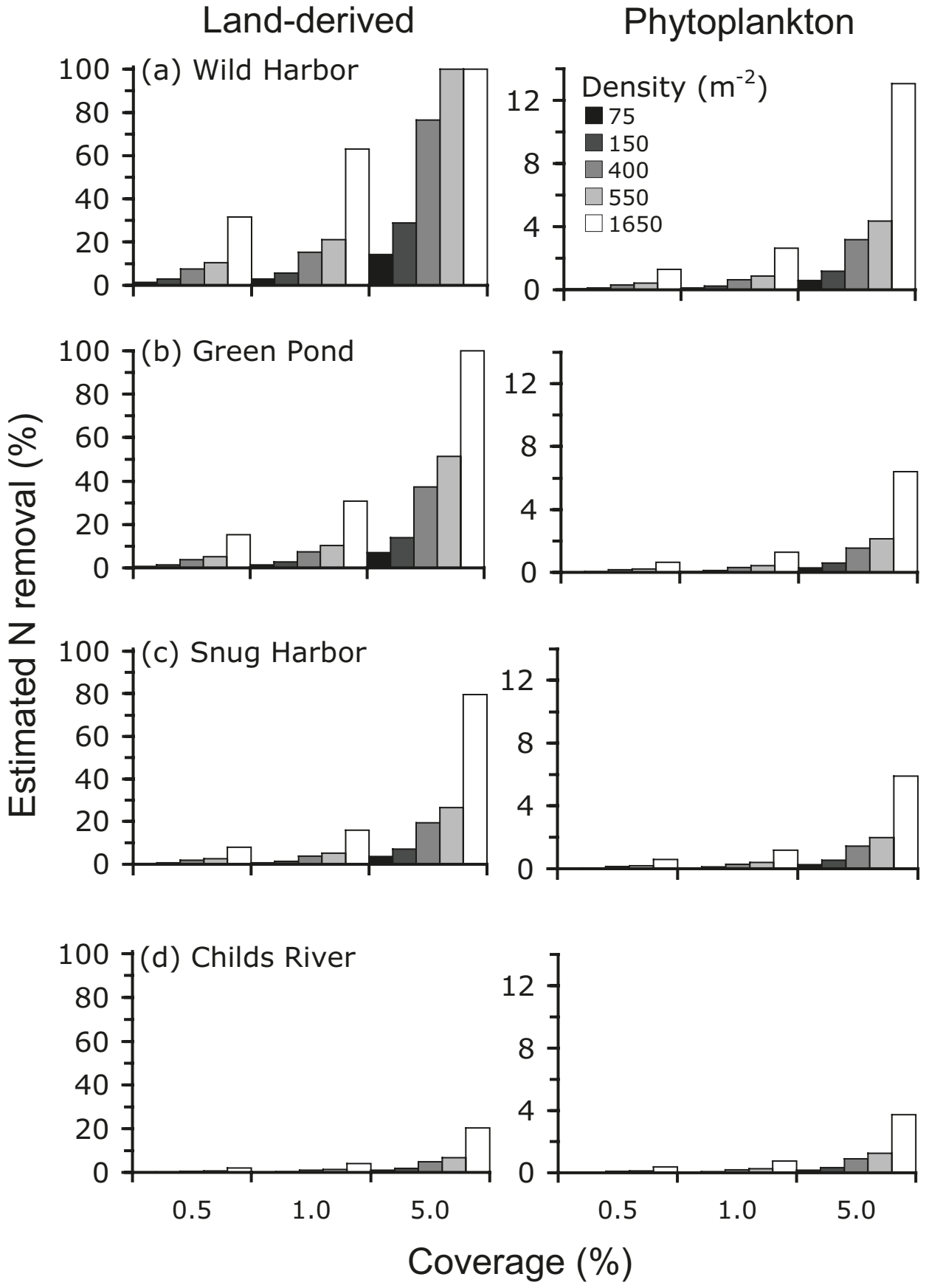
N removal by assimilation into tissues or by stimulation of biogeochemical processes depends on N removal per oyster as well as the density and area on which bivalves are grown. As a point of departure, we calculated how many oysters were needed to remove 100% of the land-derived or phytoplankton N loads to each estuary and determined the density of planting that this level of remediation would require. We do not suggest 100% N removal as a remediation goal, but rather use this value as an endpoint to further evaluate the scale of potential N removal. We estimated that 13–55 million oysters would be required to assimilate and remove all embayment-wide land-derived N loads and at least 250 million to assimilate all of the estimated phytoplankton N load (Table 7). Assuming the entire estuary were available and suitable for planting, these numbers of oysters correspond to densities of roughly 30–400 m^{-2} to remediate 100% of the land-derived N load and 600–2200 m^{-2} to remediate 100% of phytoplankton N (Table 7).

To give our findings a more biologically relevant context, we also determined %N removal capacity under different planting densities and at more realistic bottom coverage areas of 0.5%–5% of the estuary (corresponding to approximately 0.1–3.0 ha coverage in these estuaries; Fig. 5). Under the various N removal scenarios we tested, maximum N removal capacity was estimated at 20%–100% of land-derived N loads and 4%–13% of phytoplankton N and occurred at 5% bottom coverage in lower N loaded estuaries or where oyster growth was highest (Wild Harbor and Green Pond; Figs. 5*a* and 5*b*). Under restoration and aquaculture conditions that seem most common in the USA at present (oyster densities ≤ 400 m^{-2} and when planting area is relatively small compared with total estuary area), estimated N removal capacity decreased to ≤15% of land-derived loads and <1% of phytoplankton N (Fig. 5). We considered only N removal via soft tissue assimilation for these comparisons because we empirically quantified this metric, and tissue assimilation was estimated to be the dominant N removal process during growth to harvest size.

Discussion

In this study, as in many others, land-derived N loads fed local production and contributed to high local phytoplankton biomass, in turn providing useful endpoints for the range of potential N loads to be remediated in receiving estuaries. While land-derived N loads to our estuaries are typical of estuarine systems in the USA, our estimates of phytoplankton N were high relative to N contributed by phytoplankton reported elsewhere (Malone et al. 1988). These high estimates are likely due to heterogeneity in phytoplankton biomass throughout the estuary, which cannot be systematically accounted for with the available data. These values, therefore, are considered a maximum value for N potentially available in foods for oysters within the estuary during the time to reach harvest size (roughly two growing seasons), and attention should be given to the error associated with these values. These values are important, however, because phytoplankton biomass directly relates to water turbidity and low dissolved oxygen concentrations that are important management concerns (Cloern 2001; Valiela 2006). Hence, phytoplankton N may be the form most important to assessing remediation of the symptoms or negative effects of eutrophication. We assessed N removal capacity by oysters in these estuaries rela-

Fig. 5. Estimated removal of land-derived or phytoplankton N (%) by assimilation into oyster tissues if planted at typical restoration and aquaculture densities (75, 150, 400, 550, 1650·m^{-2}) over different areas of percent bottom coverage (0.5, 1.0, 5.0) in four Cape Cod estuaries.



tive to both the land-derived N load (external N sources alone, reflecting the minimum N load to be remediated) and the phytoplankton N load (total N theoretically available in food for bivalves, representing a possible maximum N load to be remediated).

N removal via tissue assimilation

Oysters assimilated anthropogenic N but showed the greatest potential to remediate N loads in estuaries that were not highly eutrophied. Our estimates indicated that lower N-loaded estuaries or those in which oyster growth rates were particularly high could support enough oysters to assimilate land-derived N when planting area and density were relatively high. Even at high densities, however, phytoplankton N removal of more than a few percent required planting over large areas of bottom that may make remediation impractical relative to estuary size. It is important to consider that a variety of factors limit the area available for planting shellfish in suburban estuaries like those we studied, including variability in habitat quality, structures (e.g., docks, revetments, piers, jetties, bottom debris), zoning and regulations protecting public rights for commercial and recreational capture fisheries and swimming, national shellfish sanitation program classifications, and aesthetic preferences. When you consider that only 1.0% of bottom area in our study sites represented as much as 0.5 ha (an area larger than a standard football or soccer field), it is easy to perceive why N removal may be spatially impractical in some water bodies. Application of bivalve remediation measures should consider the potential for enhanced N removal at high oyster densities to be mitigated by overcrowding or changes in habitat quality such as reduced dissolved oxygen concentrations that can impair the growth and sustainability of shellfish stocks and ultimately diminish the capacity for bioremediation (Rheault and Rice 1996; Ferreira et al. 2007). Overall, our data suggest N removal capacity of 1%–15% is most realistic given the conditions typical to restoration and aquaculture activities in USA estuaries and depending on available space. This finding is consistent with previous estimates across a range of different estuaries (e.g., Haamer 1996; Songsangjinda et al. 2000; Higgins et al. 2011). We conclude that the capacity for N removal by oyster harvest is likely to be modest relative to total N load and limited by the area of available habitat in many estuaries where remediation efforts are most needed: those with active, urbanized watersheds and high particle loads.

N removal by biogeochemical processes

Inclusion of rough literature estimates for N removal stimulated by biodeposition suggests that biogeochemical processes have potential to enhance capacity for N removal by oysters. Our findings indicate that during the first one to two seasons of growth, tissue assimilation was the dominant form of N removal for individual oysters, but after reaching harvestable size, denitrification could become the dominant process. These estimates should be taken with caution because the rates of biogeochemical N removal applied in this study were based on a lab study (using the N_2:Ar method applied to simulated biodeposits in cores) and a subsequent modeling effort (Newell et al. 2005). The differences we estimated in N removal by denitrification among oyster age classes may vary if younger, smaller oysters are planted at higher densities than larger counterparts and if juvenile oysters produce more biodeposits than we predicted based on literature estimates from larger-sized oysters (Newell et al. 2005). It is also important to consider the practical limitations to grow-out practices that may be required to encourage bivalve-stimulated denitrification. The N removal benefits of retaining oysters in the estuary after harvest size, particularly for commercial growers, may not outweigh the increased production costs, heightened risk of loss, and greater exposure to diseases (Ewart and Ford 1993; Lafferty et al. 2004). N removal by biogeochemical processes through time, therefore, may be more important to restoration projects focused on ecosystem services as opposed to fishery yields.

The extent to which these estimates are relevant to the natural environment and can be scaled up to an estuary is not clear. In particular, the effect of estuary-specific variation in sediment type and composition, hydrology, microbial community composition, benthic consumers, and other factors may affect rates of N removal via burial or denitrification and other biogeochemical processes (Newell et al. 2005; Seitzinger et al. 2006; Ferguson and Eyre 2007). For example, variation in local tidal or current flow may dilute and redistribute biodeposits to remote locations so that effects may be diminished or difficult to detect and relate to grow-out efforts (Tenore et al. 1985; Chamberlain et al. 2001; Forrest et al. 2007). In some cases, high intensity aquaculture activities may change local environmental conditions in ways that mediate capacity for denitrification (Newell 2004), such as by reducing dissolved oxygen and enhancing sulfide concentrations (Chamberlain et al. 2001; Christensen et al. 2003; Forrest and Creese 2006). Measurements in Cape Cod estuaries (i.e., Sage Lot Pond and Childs River) indicate naturally occurring denitrification could account for 32%–37% dissolved inorganic N loss in the estuaries we studied (Lamontagne and Valiela 1995; Lamontagne et al. 2002). Denitrification rates in other estuaries reportedly range from 7% to nearly 60% of total dissolved inorganic nitrogen load, depending on location (Nowicki et al. 1997). If N removal by denitrification stimulated by oyster biodeposits equals or dominates N assimilated into soft tissues by the time oysters reach harvestable size, our data suggest total N removal by oysters would be comparable to these previously reported naturally occurring rates. A recent field study that measured denitrification in sediments adjacent to oyster reefs found somewhat lower denitrification rates than predicted by the literature values we applied, but corroborated that rates were highest in summer during peak feeding and growth and strongly correlated with sediment oxygen demand (Piehler and Smyth 2011). Denitrification capacity, therefore, is likely to be location-specific, and variation with intensity and type of bivalve aquaculture or restoration activity needs to be determined.

Further empirical and estuary-specific study is needed to refine and corroborate N removal estimates via stimulation of biogeochemical processes. These data, in turn, will help quantify ecosystem services provided by bivalves and guide how oysters may best function as a N management or restoration tool (Pomeroy et al. 2007; Coen et al. 2007; Grizzle et al. 2008). A number of shellfish-related denitrification studies have been performed on large scale, deepwater mussel farms in Europe and Japan (e.g., Haamer 1996; Kohama et al.

2002; Carlsson et al. 2012). The growing data set from these farms will be useful to evaluate nutrient removal due to intensive aquaculture operations, but the applications may not translate well to smaller urbanized USA waters (Dumbauld et al. 2009). New molecular methods offer alternatives to define the magnitude and scale of denitrification processes in estuaries (e.g., Smith et al. 2007; Cao et al. 2008). If coupled with existing rate-defining methods and empirical measurements, these tools may more accurately define N removal via denitrification processes in the natural environment (Groffman et al. 2006).

Comparison and application to other systems

The range of N loads and estuary conditions we tested are generally representative of temperate estuaries in which studies on N enrichment and bivalves have been conducted (Newell et al. 2005; Carmichael et al. 2012). Although the estuaries we studied are relatively shallow and have short residence times, the high chl *a* concentrations we measured are consistent with the productive conditions associated with eutrophication in estuaries worldwide (Cebrian and Valiela 1999; Cloern 2001; Valiela 2006). The relatively high estimates of phytoplankton N content in our study estuaries were accompanied by relatively high rates of oyster growth, typical among bivalves in N enriched estuaries (Carmichael et al. 2004*a*; Valiela 2006). In turn, oyster shell and soft tissue growth rates were comparable to or higher than growth rates reported elsewhere (Ortega and Sutherland 1992; Shumway 1996; Dame et al. 2000). When extrapolated to harvest size, estimated dry mass and resulting N content in tissues were also high, consistent with the higher available food supply (Shumway 1996; Hyun et al. 2001; Carmichael et al. 2004*a*). Accordingly, time to reach harvest size and %N content in oysters were consistent with values measured in previous studies (e.g., Rheault and Rice 1996; Shumway 1996; Higgins et al. 2011). These comparisons suggest food resources were not limited in the estuaries, and the key N input and removal variables we measured were representative of conditions in many estuaries where bioremediation by bivalves is likely to be of interest.

Since N inputs increase food supply for bivalves, and in turn increase bivalve growth, we could expect N removal by oysters or other bivalves to be relative to N load across estuaries and occur at a given rate up to the physiological capacity of the oyster (Newell and Langdon 1996; Newell et al. 2005). Estuary-specific environmental factors, however, can disrupt this pattern. For example, in this study salinity limited oyster growth in higher N-loaded estuaries, despite high food supply, so that maximum shell and soft tissue growth were found in a moderately N-loaded estuary. The combined effects of salinity and food supply provided a serendipitous opportunity to test both lower and higher growth scenarios that helped capture some effect of environmental variation. Similar variation in bivalve growth due to salinity has been observed in many locations, including in response to episodic exposure (Calvo et al. 1999; Dekshenieks et al. 2000; Weiss et al. 2002). While the general approach and findings of this study are likely to be applicable to many estuaries (for example, the quantitative data presented in Fig. 5 and Table 7 can be applied to calculate parameters for any desired %N removal), application will require location-specific data collection, particularly for environmental attributes that affect oyster growth and denitrification.

Comparison to N removal by other species

Comparison of our data with similar data collected for other bivalves transplanted in Cape Cod waters suggests oysters had the highest capacity for N removal. We limited this comparison to Cape Cod waters to avoid intrasystem or regional variation and highlight species-specific responses across a common set of estuaries. As found for oysters in this study, other species, including *Mercenaria mercenaria*, *Mya arenaria*, *Argopectin irradians*, and *Geukensia demissa*, acquired $\delta^{15}N‰$ in tissues that reflect land-derived N sources, indicating they assimilated local N from anthropogenic sources and have potential to remove land-derived N from a system if harvested (Valiela 2006). Most of these species showed a significant increase in growth as land-derived N loads increased available food supply, consistent with the notion that the initial and perhaps a primary effect of eutrophication on many species is increased secondary production (Nixon and Buckley 2002; Carmichael et al. 2004*a*). Our results suggest that compared with some other commercially harvested species, oysters have a higher capacity for growth under N-enriched and subsequently higher food supply conditions. Blue mussels (*Mytilus edulis*), which also have high feeding and assimilation rates (Tenore and Dunstan 1973), have been extensively used to manage nutrient loads to European waters (Gren et al. 2009; Lindahl and Kollberg 2009). Blue mussel farms are operated on a large scale (e.g., Haamer 1996; Kohama et al. 2002; Carlsson et al. 2012) and in relatively open, deep waters, where the suite of spatial constraints we presented for Cape Cod estuaries is less of an impediment (Inglis et al. 2000). Even in the European systems where nutrient and pollutant removal has been reportedly successful, the need to balance stocking density and planting area with local environmental conditions and consequences is recognized (Inglis et al. 2000; Gren et al. 2009; Rosland et al. 2011). These data corroborate our initial assumption that oysters or species with similar feeding behavior and physiological capacity have the greatest potential for use as a management or N bioremediation tool. These comparisons also highlight important regional differences in shellfish farming techniques and locations that may affect the capacity for bioremediation by different species.

We provide the first data to quantify N removal by assimilation into oyster tissues across a range of N loads and estuary-specific conditions, including explicit demonstration that oysters assimilated N from anthropogenic sources. Our data suggest bioremediation by bivalves will be most effective when N loads are lower, high-quality bivalve habitat is not limited, and oysters or physiologically similar bivalves are readily available. In many systems, particularly in the USA, these conditions may not be inherent to highly altered and eutrophic waters where remediation is most in demand. Critical review of bioremediation studies during the past 30 years reveals similar findings, typically reporting removal of less than 20% of local N loads. Like other authors, we agree that bivalves can be a useful N removal device and could function as part of a larger N removal strategy (Fulford et al. 2007; Cerco and Noel 2007; Dumbauld et al. 2009; and others), but our results also confirm that bivalve bioremedia-

tion should be considered in the context of trade-offs between intensity of shellfish culture, ecological consequences, and available space (Kemp et al. 2005; Ferreira et al. 2007; Fulford et al. 2010). How meaningful bivalves may be to bioremediation will depend on community-specific bioremediation goals and estuary-specific conditions (Ferreira et al. 2007; Burkholder and Shumway 2011). Further empirical and estuary-specific study is needed to refine N removal estimates for biogeochemical processes in comparison with N removal by assimilation into tissues. Empirical data like those presented here in combination with newly developed modeling techniques (e.g., Fulford et al. 2010; Rosland et al. 2011; Silva et al. 2011) have potential to greatly improve site selection for shellfish culture and restoration activities and guide productive N remediation efforts.

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