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Effects of age class on N removal capacity of oysters and implications for bioremediation

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ABSTRACT: Previous estimates of nitrogen (N) removal from coastal systems by either assimilation into tissues and shells of oysters or stimulated through biogeochemical processes have not accounted for ontogenetic changes in N sequestration. To understand how N removal may change with ontogeny, we compared N in tissue, shell, and biodeposits between juvenile and adult oysters. Juvenile oysters assimilated 165 ± 8 mg (±SE) N oyster^{-1} into soft tissues and shell, while adult oysters lost mass and returned N to the estuary (–48 ± 21 mg N oyster^{-1}). The percentage of N in soft tissues (11.80 ± 0.01) did not differ between age classes but was significantly higher than measured elsewhere. The percentage of N in shell averaged 0.46 ± 0.01 and 0.26 ± 0.01 in juveniles and adults, respectively, accounting for ≥50% of total N in oysters. N released in biodeposits was estimated at 0.21 ± 0.00 to 0.23 ± 0.00 mg N oyster^{-1} d^{-1} and did not differ between age classes. Accurate quantification of N removal by oysters, therefore, should account for age and location-specific variation by including direct measurements of N assimilated into tissues and shell, released in biodeposits, and removed by biogeochemical processes. Continuous N sequestration and removal from local waters for bioremediation may require balancing recruitment or planting of young actively growing oysters with harvest of larger animals to ensure net N removal by assimilation before growth declines. This strategy may be particularly useful where environmental conditions do not support significant N removal through biogeochemical processes.

KEY WORDS: Aquaculture · Eutrophication · Shellfish · Assimilation · Restoration

INTRODUCTION

Oysters benefit coastal ecosystems by providing ecologically and economically valuable ecosystem services (Coen et al. 2007, Grabowski et al. 2012). Oyster reefs provide habitat for other commercially important species (Peterson et al. 2003) and help reduce shoreline erosion by attenuating wave action along coastlines (Meyer et al. 1997). As filter feeders, oysters have the potential to improve water quality by remediating the effects of anthropogenically-driven eutrophication by sequestering land-derived nitrogen (N), a limiting nutrient for primary production in many coastal systems (Dame & Libes 1993, Cerco & Noel 2007, Carmichael et al. 2012b). N contained in phytoplankton, which are consumed by oysters, is assimilated into oyster tissues and shell and released in biodeposits to the benthos. Upon harvest, the N assimilated into oyster tissues and shell is removed from the system. The portion of N that is excreted (feces) or ejected (pseudofeces) as biodeposits may further stimulate benthic microbial processes, indirectly removing additional N through coupled nitrification-denitrification (Newell 2004, Porter et al. 2004, Grabowski & Peterson 2007, Smyth et al. 2013). Nitrogen remediation is often a goal of oyster restoration and promoted as a secondary benefit of oyster aquaculture (Rice 2001, Gifford et al. 2004, Dumbauld et al. 2009, Bricker et al. 2014, Kellogg et al. 2014).

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Despite increasing demand for estimates of N removal rates for oysters and other filter feeders to guide bioremediation activities (Higgins et al. 2011, Carmichael et al. 2012b, Grabowski et al. 2012), few studies have directly and comprehensively quantified oyster-mediated N removal under different biological or environmental conditions (reviewed in Carmichael et al. 2012b, Kellogg et al. 2013, Kellogg et al. 2014). Because total N assimilated in oyster tissues and shell is a function of N content (as a percentage) and total tissue or shell weight, N removal capacity by assimilation into tissues and shell is dependent upon the growth rate of oysters (Songsangjinda et al. 2000, Higgins et al. 2011, Carmichael et al. 2012b). In addition to affects on age-at-length relationships due to variation in environmental attributes among grow-out locations or time periods (Harding et al. 2008, Liddel 2008, Carmichael et al. 2012b), oyster growth, as in many organisms, does not occur at a constant rate throughout their life cycle. Growth occurs at the highest rate in juvenile oysters and gradually decreases as oysters approach maximum size (Raillard & Ménesguen 1994, Kennedy 1996, Wang et al. 2008). Biodeposit production is also known to change relative to growth rate and metabolism associated with age (Haven & Morales-Alamo 1966, Songsangjinda et al. 2000, Mitchell 2006). Therefore, ontogeny along with environmental attributes is likely to affect both the rate of direct N assimilation into oyster tissue and shell as well as indirect N removal via stimulation of benthic processes, mediating the N-removal capacity of oysters throughout life.

By combining data for direct N assimilation into tissues and shell with data on N released in biodeposits for different age classes of oysters, we can substantially improve understanding of N sequestration and removal as a potential ecosystem service and better define possible management applications. In this study, we compared N assimilated into tissues and shell as well as released in biodeposits of juvenile and adult oysters at sites in the Gulf of Mexico, USA. To understand the environmental factors that mediated N assimilation and biodeposition rates at study sites, we compared oyster growth and N content in tissues, shell, and biodeposits to environmental attributes including water temperature, salinity, dissolved oxygen concentration, current speed, food resources (suspended and benthic chlorophyll *a* as well as total and organic suspended particles), and nutrient concentrations. Sources of N assimilated and potentially removed by oysters were identified by comparing N and C stable isotope ratios in oyster tissues and biodeposits to values in suspended particles available as food. The resulting age class-based estimate of N removal by oysters provides a framework for designing and evaluating the bioremediation capacity of oyster restoration and aquaculture projects and highlights the potential importance of oysters, at different life stages, to estuarine nutrient cycling and associated ecosystem services.

MATERIALS AND METHODS

Transplanted oysters

To investigate the effects of ontogeny on N removal by oysters, juvenile and adult hatchery-reared oysters were deployed in June 2011 and allowed to grow under natural field conditions during the typical growing season (early June through mid-October). Oysters obtained from the Auburn University Shellfish Laboratory (AUSL) on Dauphin Island, Alabama were grown at 2 sites, approximately 2 km apart, located along the northern shore of the Fort Morgan peninsula in Mobile Bay, Alabama, USA (Fig. 1).

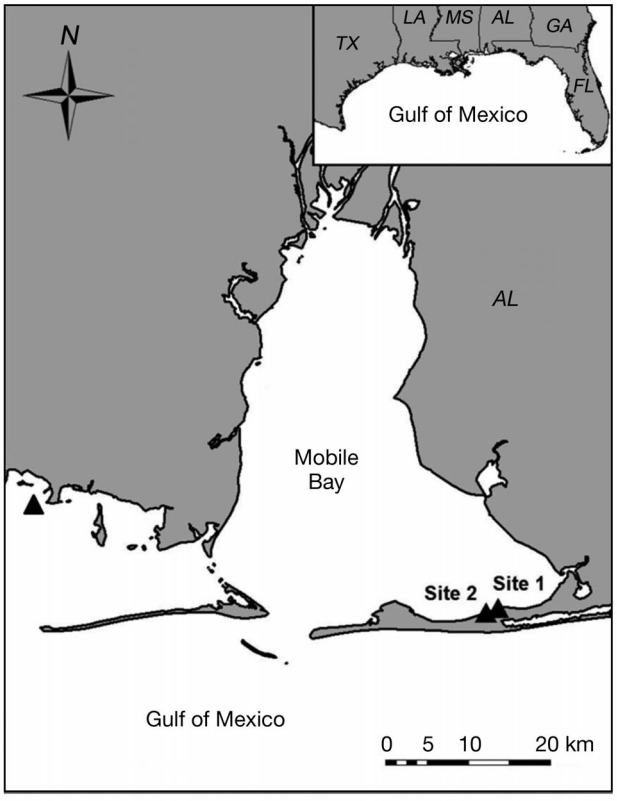

Fig. 1. Study sites in Mobile Bay, AL and pre-transplant hatchery grow-out site at Point aux Pins, AL (Site 1: 30.2825° N, 87.7514° W; Site 2: 30.2539° N, 87.8228° W; Point aux Pins: 30.3705° N, 88.3158° W)

Sites were chosen based on similar physical and chemical conditions and due to the presence of wooden piers (measuring 2 m wide × 30 m long), suitable for hanging oyster cages in relatively mild wave action.

Juvenile oysters were spawned in spring 2011 and had mean shell height (longest shell dimension) of 42.4 ± 0.3 mm at the initiation of the study. Adult oysters were 3 to 4 yr old with mean shell height of 98.0 ± 1.3 mm. Adult oysters were maintained in the field at Point Aux Pins, Alabama, prior to transplanting (Fig. 1). Oysters were placed in plastic-coated wire mesh aquaculture cages measuring 53 cm × 122 cm × 15 cm deep. Cages had wire mesh dividers, which crossed in the center to create 4 identical compartments to maintain cage balance and avoid oyster crowding. Three replicate cages per treatment (juvenile and adult) were deployed at each site, and the position of cages relative to shore was randomized between the sites. Cages were suspended from piers using nylon rope such that the bottom of each cage hung 10 to 20 cm above the sediment surface. Juvenile (n = 600 per cage; 928 oysters m^{-2}) and adult (n = 200 per cage; 309 oysters m^{-2}) oysters were stocked in a single layer along the bottom of each cage. These densities are representative of those used in (USA) aquaculture activities and found on some restored and natural oyster reefs (Luckenbach et al. 2005, Carmichael et al. 2012b). Transplanted oysters were divided evenly among the 4 compartments of each cage for a total of 150 or 50 oysters per compartment for juvenile and adult cages, respectively.

Sampling scheme

To determine N assimilation and biodeposition rates relative to environmental attributes, water, sediment, and oysters were sampled biweekly throughout the study period. Initial environmental conditions were sampled 28 d prior to the start of the study (Day −28) to establish baseline conditions for oyster acclimation from the hatchery to the field. Oysters were obtained from the hatchery (Day 0) and held at ambient conditions in flow-through tanks, while initial size and weight data were collected before deployment (on Day 8), 2 wk prior to the first biweekly field sampling (Day 22). Ambient water for flow-through tanks at AUSL is drawn from the Gulf of Mexico just seaward of Dauphin Island, Alabama, where oysters were held prior to the study, before processing at the hatchery. Oysters were then placed at field sites (Fig. 1) for up to 139 d (Day 147).

On each biweekly sampling date after water and sediments were sampled, cages were removed from the water, and 3 adult oysters were randomly collected from each cage compartment for a total of 12 oysters per cage at each site, plus 6 juvenile oysters per cage compartment for a total of 24 oysters per cage at each site. These oysters were used to measure growth, N content, and stable isotope ratios in tissues as well as biodeposit production. Sampled oysters were placed in plastic bags and kept alive on ice until processing. Oyster cages were cleaned on shore to remove fouling prior to replacement at grow-out sites.

Environmental attributes

Temperature, salinity, and dissolved oxygen (DO) concentration were measured using a hand-held YSI 85 meter. A Marsh McBirney Flo-Mate model 2000 rod suspended flow meter was used to collect flow data. All measurements were taken at the depth of transplant cages at each end of the experimental setup at both sites. Salinity was determined using the Practical Salinity Scale.

Food resources

Food resources for oysters were measured in terms of benthic and water column chlorophyll *a* (chl *a*), suspended particulate matter (SPM), and particulate organic matter (POM) concentrations. Whole water samples were collected using a LaMotte model JT-1 horizontal water sampler. Samples were pre-filtered through a 200 µm mesh sieve to remove large particles and macrofauna before pouring into 1 l dark Nalgene bottles. Samples were stored on ice until processing (within 10 h). Samples were vacuum filtered through pre-ashed 0.7 µm Whatman glass fiber filters (GF/F) to separate suspended particulates from filtrate.

Benthic and water column chl *a* concentrations were determined by 2:3 DMSO:90% acetone extraction according to the methods of MacIntyre & Cullen (2005) and analyzed using a Turner Designs TD700 fluorometer. For sediments, three 1 cm diameter × 3 cm deep sediment cores were collected beneath each set of oyster cages at both sites. Cores were combined into a 50 ml dark plastic bottle and stored on ice for transport. Sample bottles were then frozen at −20°C until processing. SPM and POM concentrations in water were determined from the mass of fil-

ters dried to a constant mass at 60°C before and after ashing at 500°C for 4 h. Three subsamples from each water or sediment sample collected in the field were processed to obtain 1 mean chl *a* value per sample and to account for variation within individual samples. Additionally, 2 filters were processed to obtain 1 SPM and POM value per sample, to yield 2 true replicate samples for each parameter.

The concentrations of nutrients available to support phytoplankton production were measured in water sample filtrates. Filtrate from each water sample was frozen at −20°C prior to N and P analysis using a Skalar Autoanalyzer following the methods of Strickland & Parsons (1972). Measured nutrient concentrations included ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), and total dissolved nitrogen (TDN).

Oyster growth and survival

Mean oyster growth was measured throughout the experiment by measuring shell height and soft tissue dry mass of oysters collected on each sample date. Shell height was measured to the nearest 0.1 mm using vernier calipers. Soft tissue dry mass was measured after separating soft tissue from shell and drying to a constant mass at 60°C. Slopes of the best fit regression line comparing mean shell height or tissue dry mass to day of collection were used to determine oyster growth rates. Survival was determined by counting oyster mortality in each cage during collection, and dead oysters were removed from cages.

Oyster N content and assimilation

N content in oyster soft tissues was determined by combustion during stable isotope analysis (SIA). The percentage of N in tissues was calculated by dividing the amount of N in each sample by the total weight of the sample and multiplying by 100. Total tissue N content per oyster was calculated by multiplying the percentage of N by the whole tissue dry weights of individual oysters for each treatment on each sample date. Whole tissue samples were not available prior to Day 79, and N content was calculated for adductor muscle only. To calculate total N content in oysters for this time period, we applied a correction factor (87.8 ± 1.1%) derived from the relationship between adductor muscle and whole tissue N content for samples collected on and after Day 79, which did not change through time or differ among treatments.

Shell N content was determined by direct combustion during SIA, and results were confirmed by analyzing duplicate samples using a Costech 4010 ECS elemental analyzer. To prepare shells for elemental analysis, after removing soft tissue, shells were thoroughly cleaned and dried. Three complete shells (both left and right valve) were randomly selected from each cage on the initial and final sampling dates. Within treatments, material from the 3 shells was combined to form 1 aggregate shell sample for each cage and each date (n = 6 replicates per age class for each date). To sample shell deposited throughout the study, a 3 mm wide lengthwise strip was cut from umbo to outer margin along the center line of each shell using a Buehler IsoMet 1000 precision saw. Shell slices were subsequently ground to a powder using a Ryobi hand-held rotary tool. Samples were further homogenized using a mortar and pestle and packed in tin capsules for elemental analyses. Because results did not differ between the 2 combustion analyses, values for each sample were averaged and treated as a single sample for further analyses and calculations. Shell N percentage was calculated in the same way as tissue N percentage, and the resulting mean value was multiplied by dry shell weights of individual oysters to determine the total shell N content per oyster.

Total N assimilated into oyster tissues and shell was determined by subtracting the mean N content at time of deployment from the mean N content at harvest.

Oyster biodeposition

To determine the effects of age class on potential indirect N removal by stimulation of benthic microbial processes, we measured the quantity and N content of oyster biodeposits. To collect biodeposits, immediately following field collection, live oysters (n = 4 adult and n = 6 juvenile oysters per cage) were placed in aquaria containing ultra-pure artificial seawater and allowed to clear their guts. For this study, 18 h was determined to be the optimal time period for gut clearance based on preliminary lab trials with oysters of the same cohort. Aquaria were separated by site, treatment (juvenile or adult), and cage replicate. Salinity of artificial seawater was matched to field conditions ± 1 psu on the day of collection, and temperature was maintained at ambient lab conditions of approximately 23°C. Upon gut clearance, biodeposits were collected and sorted into their 2 components, feces and

pseudofeces, according to the methods of Fila et al. (2001), which were developed by a member of our lab group and are regularly used to successfully separate the components of ejecta by visual examination (e.g. Arakawa 1965). A Zeiss Stereodiscovery V12 microscope with cold light source was used to aid visualization during biodeposit sorting. Once separated, each form of ejecta was vacuum filtered onto a pre-weighed and ashed 0.7 µm Whatman GF/F, dried to a constant mass at 60°C, and reweighed to determine biodeposit quantity. Dry mass of biodeposits collected on filters was compared to dry weight and standardized by number of oysters for each replicate.

The amount of N in each sample was determined by combustion during SIA. The percentage of N in oyster biodeposits was calculated by dividing the amount of N by the total sample weight for each sample. The percentage of N was multiplied by the mean quantity of biodeposits produced to determine the biodeposit N content in terms of mg N released per oyster.

Stable isotope analysis

To identify the quantity and source of N assimilated into oyster tissues, shell, and biodeposits, N and C stable isotope ratios were determined in each sample type as well as in water column SPM (for comparison to available food sources). On each sampling date, 2 SPM filters from each water sample and 3 juvenile and adult oysters from each cage at each site were randomly selected for SIA. For tissues, 3 whole tissue samples plus 2 individual and 1 aggregate of 3 adductor muscle samples were analyzed for each age class on each sampling date at each site. Tissues and shell were processed as described above and homogenized using a mortar and pestle. Homogenized dry tissues and shell, biodeposit filters, and SPM filters were packed in tin capsules and analyzed on a PDZ Europa 20-20 mass spectrometer using continuous flow isotope ratio mass spectrometry (IRMS) following combustion in a PDZ Europa Automatic Nitrogen and Carbon Analyzer-Gas Solid Liquid at the University of California, Davis Stable Isotope Facility. Gases were separated on a Carbosieve G column prior to IRMS.

Table 1. Mean environmental attributes (±1 SE) measured at 2 experimental oyster grow-out sites in Mobile Bay, AL, and associated ANOVA statistics for comparison between sites

Attribute	Site 1	Site 2	*F*	df	p value
Temperature (°C)	28.2 ± 0.7	29.0 ± 0.7	409.61	45	<0.001
Salinity	16.2 ± 0.9	16.4 ± 1.0	40.21	45	<0.001
DO (mg l^{-1})	5.89 ± 0.27	6.91 ± 0.36	66.70	45	<0.001
Current speed (m s^{-1})	0.02 ± 0.01	0.02 ± 0.00	2.68	25	0.13
SPM (mg l^{-1})	53.20 ± 2.61	60.95 ± 0.52	25.74	78	<0.001
POM (mg l^{-1})	12.85 ± 3.31	14.86 ± 0.69	22.24	78	<0.001
Water chl *a* (µg l^{-1})	11.03 ± 0.73	11.98 ± 0.85	8.02	43	0.01
Benthic chl *a* (µg m^{-2})	445.35 ± 58.54	416.75 ± 44.22	1.37	59	0.25
TDN (µM)	34.59 ± 1.88	33.13 ± 0.84	0.64	43	0.43
NH_4^+ (µM)	1.19 ± 0.17	1.25 ± 0.20	0.11	43	0.75
NO_3^- (µM)	1.00 ± 0.18	0.90 ± 0.28	1.14	43	0.30
NO_2^- (µM)	0.02 ± 0.00	0.02 ± 0.01	0.29	43	0.60
PO_4^{-3} (µM)	0.83 ± 0.17	0.96 ± 0.21	0.82	43	0.37

Statistical analysis

All analyses were performed with Minitab 16® software, using a general linear model for analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons and linear regression, where appropriate. ANOVAs were performed using site and day as fixed factors for environmental variables and also included age class as a fixed factor for biological comparisons. We ran a full factorial linear model for differences among factors and examined all first-order interactions. Data were log-transformed to meet normality assumptions when needed. In cases where no differences were found between sites, data were combined for subsequent analyses. Results with a p-value < 0.05 were considered significant. All R^2 values are reported as adjusted R^2. Variability is reported as standard error of the mean, and error was propagated according to the methods summarized by Valiela (2009).

RESULTS

Environmental attributes

Site-specific and temporal differences in environmental attributes resulted in small differences in habitat conditions and available food supply at each site during the study period. Temperature, salinity, and DO concentration differed significantly between sites, with Site 2 values being slightly higher (Table 1). Current speed did not differ between sites (Table 1). Both sites showed a drop in temperature of ~8°C on Day 107 immediately following landfall of Tropical Storm Lee on 8 September 2011.

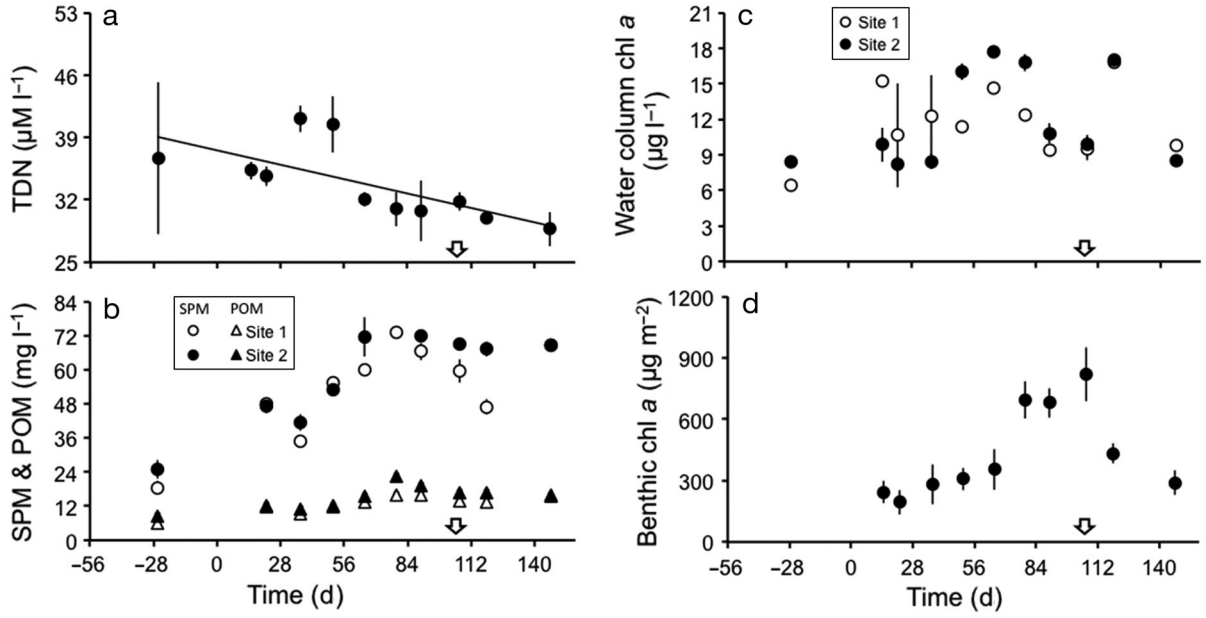

Fig. 2. Metrics of mean food resources (±1 SE) available to oysters at transplant sites in Mobile Bay measured 28 d prior to deployment (Day −28) and on each sample date (Days 0 to 147), including (a) total dissolved nitrogen (TDN), (b) suspended particulate matter (SPM) and particulate organic matter (POM), (c) water column chlorophyll *a* (chl *a*) and (d) benthic chl *a*. Regression line indicates a decrease in TDN through time: $y = -0.058x + 37.57$, $R^2 = 0.44$, $F_{reg\ 1,9} = 8.86$, $p < 0.05$. Arrows indicate landfall of Tropical Storm Lee in Mobile Bay, AL, on Day 107 of the study

Water column SPM, POM, and chl *a* concentrations differed significantly between study sites, with 10 to 15% higher values at Site 2, but benthic chl *a* and nutrient concentrations (TDN, NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-}) did not differ (Table 1). TDN decreased through time during the study, while SPM, POM, and water column chl *a* increased until Day 65, then leveled off or declined such that there was no consistent pattern through time (Fig. 2). Benthic chl *a* increased steadily through Day 107 (landfall of Tropical Storm Lee), then declined during the remainder of the study period (Fig. 2). C and N content and C:N in SPM did not differ between sites. Values ranged from 0.9 to 5.4 µg ml^{-1} for C and 0.3 to 1.1 µg ml^{-1} for N and increased through time (data not shown; C: $y = 0.22x + 175$, $R^2 = 0.27$, $F_{reg\ 1,20} = 9.03$, $p < 0.01$; N: $y = 0.041x + 0.44$, $R^2 = 0.34$, $F_{reg\ 1,20} = 11.97$, $p < 0.01$), resulting in a consistent mean C:N of 5.1 ± 0.2 in SPM through time.

Oyster growth and survival

Growth and survival patterns differed between juvenile and adult oysters, but not by site. Thus, data from both sites were combined for analyses. Juvenile oysters grew significantly throughout the study in terms of shell height and tissue dry weight (Fig. 3). Juvenile oysters grew at a rate of 0.19 ± 0.06 mm d^{-1}

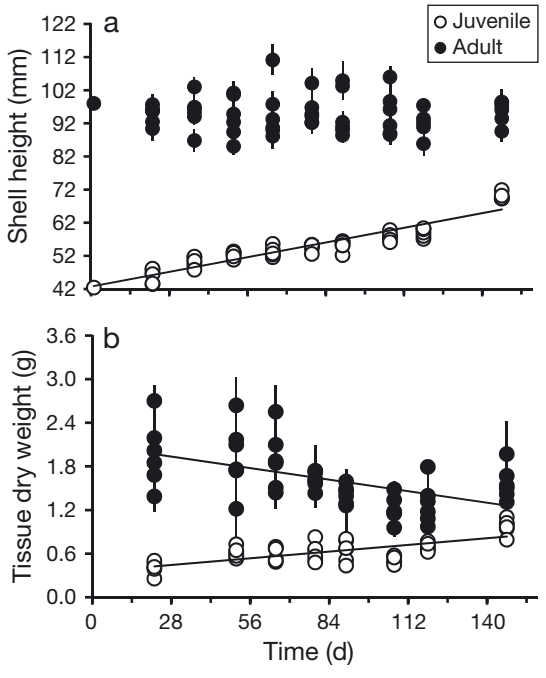

Fig. 3. Mean oyster size (±1 SE) in terms of (a) shell height (mm) and (b) soft tissue dry weight (g) relative to number of days in field. Significant growth rates are indicated by regression lines: juvenile shell height — $y = 0.16x + 42.70$, $R^2 = 0.88$, $F_{reg\ 1,53} = 392.81$, $p < 0.001$; juvenile tissue dry weight — $y = 0.003x + 0.035$, $R^2 = 0.47$, $F_{reg\ 1,46} = 43.00$, $p < 0.001$; adult tissue dry weight — $y = -0.006x + 2.093$, $R^2 = 0.26$, $F_{reg\ 1,546} = 17.57$, $p < 0.001$)

shell height and 4.5 ± 0.4 mg d^{-1} soft tissue dry weight, and these juveniles measured 46 ± 1 to 70 ± 1 mm shell height and 0.41 ± 0.03 to 0.97 ± 0.04 g dry weight at harvest. In contrast, adult oysters did not show measurable shell growth and lost soft tissue weight during the study at a rate of −3.2 ± 1.4 mg d^{-1} with tissue dry weight of 1.57 ± 0.09 to 1.97 ± 0.15 g at harvest (Fig. 3b). Juvenile oysters also had a slightly higher survival rate than adult oysters (juvenile: 99%; adult: 98%; ANOVA: $F_{1,32} = 16.04$, $p < 0.001$).

Table 2. Mean (±SE) %N, N content at harvest, assimilated N (calculated as N content at harvest − N content at deployment) in oyster tissue and shell, and total N assimilated by juvenile and adult oysters

	Juvenile	Adult
$\%N_{soft\ tissue}$	11.80 ± 0.01	11.80 ± 0.01
$\%N_{shell}$	0.46 ± 0.01	0.26 ± 0.01
N content at $harvest_{soft\ tissue}$ (mg $oyster^{-1}$)	116 ± 6	170 ± 16
N content at $harvest_{shell}$ (mg $oyster^{-1}$)	109 ± 6	217 ± 14
Assimilated $N_{soft\ tissue}$ (mg $oyster^{-1}$)	68 ± 6	−48 ± 21
Assimilated N_{shell} (mg $oyster^{-1}$)	97 ± 6	0
Total assimilated N (mg $oyster^{-1}$)	165 ± 8	−48 ± 21

N content in soft tissues and shell

Juvenile oysters assimilated N into both soft tissues and shell throughout the study, while adult oysters lost N due to lack of growth (Table 2). For soft tissues, the percentage of N did not differ by site or between juvenile and adult oysters (ANOVA site: $F_{1,79} = 0.49$, $p = 0.49$; age class: $F_{1,79} = 1.37$, $p = 0.25$). Hence, values were combined for subsequent analyses. The percentage of N in oyster tissues (11.8 ± 0.1%) remained constant through time such that soft tissue N content per oyster was directly related to tissue dry weight (Fig. 4a). The resulting total N content in oyster tissues also did not differ between sites (ANOVA site: $F_{1,71} = 3.59$, $p = 0.18$), and tissue N content per oyster was higher in adults than juveniles, due to the larger adult size (Fig. 4b, circles; ANOVA: $F_{1,71} = 380.76$, $p < 0.001$). Adult oysters, however, lost N from soft tissues due to the loss of tissue dry mass during the study (Table 2, Fig. 4b). This finding is demonstrated by the black triangles in Fig. 4b, which show net N content in adult oysters below zero after initial N content at planting was subtracted from final N content at harvest. In contrast, juveniles showed net N assimilation into soft tissues, demonstrated by the open triangles in Fig. 4b, which fell above zero.

For oyster shell, the percentage of N did not differ by site but was >40% higher on average in juvenile than in adult oysters at both sites (Table 2; ANOVA site: $F_{1,17} = 0.03$, $p = 0.85$; age class: $F_{1,17} = 23.96$, $p < 0.001$). Percentage of N in oyster shell was higher at harvest than at the time of transplant for both age classes (transplant: juvenile = 0.16 ± 0.02%, adult = 0.21 ± 0.01%; harvest: juvenile = 0.46 ± 0.01%, adult = 0.26 ± 0.01%; ANOVA: $F_{1,17} = 159.68$, $p < 0.001$). Percent N was 96 to 99% lower in shell than in soft tissues, but shell weight at harvest was higher (juvenile: 23.57 ± 1.04 g; adult: 82.62 ± 5.04 g), resulting in similar or greater total N content in shell compared to soft tissues (Table 2). As with soft tissues, adult oysters maintained higher total shell N content due to their larger size (ANOVA: $F_{1,19} = 162.24$, $p < 0.001$). While the shell material of adults did not lose N content like the soft tissues, no additional N was assimilated into shell by adult oysters during the study period (Table 2). The total N assimilated into soft tissues

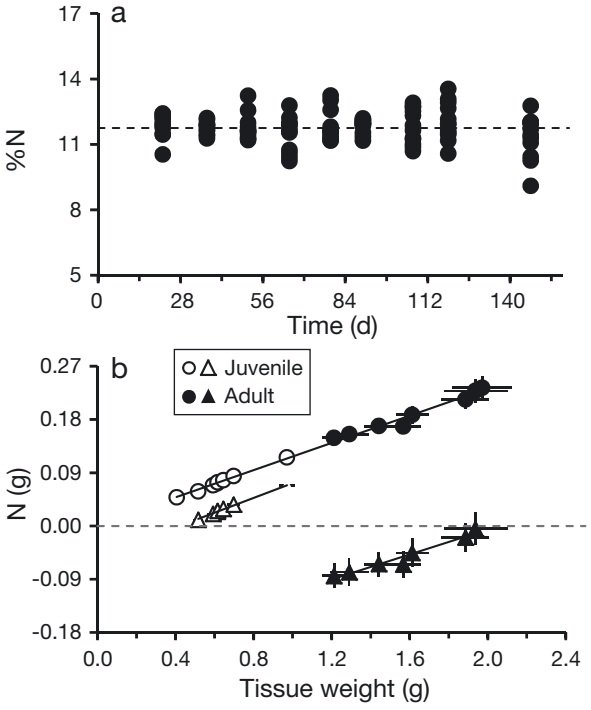

Fig. 4. (a) %N in oyster soft tissues through time and (b) mean (±1 SE) N content in oyster soft tissues relative to dry weight at harvest (circles) and net N assimilated during transplant period (N content at harvest − N content at deployment; triangles). Dashed lines indicate mean %N (a) and point of no net assimilation (b). Regression lines: N content at harvest — $y = 0.12x + 0.002$, $R^2 = 0.99$, $F_{reg\ 1,14} = 2338.09$, $p < 0.001$; net N assimilated — $y = 0.13x - 0.05$, $R^2 = 0.99$, $F_{reg\ 1,5} = 1089.18$, $p < 0.001$ juveniles and $y = 0.11x - 0.22$, $R^2 = 0.94$, $F_{reg\ 1,5} = 90.84$, $p < 0.001$ adults

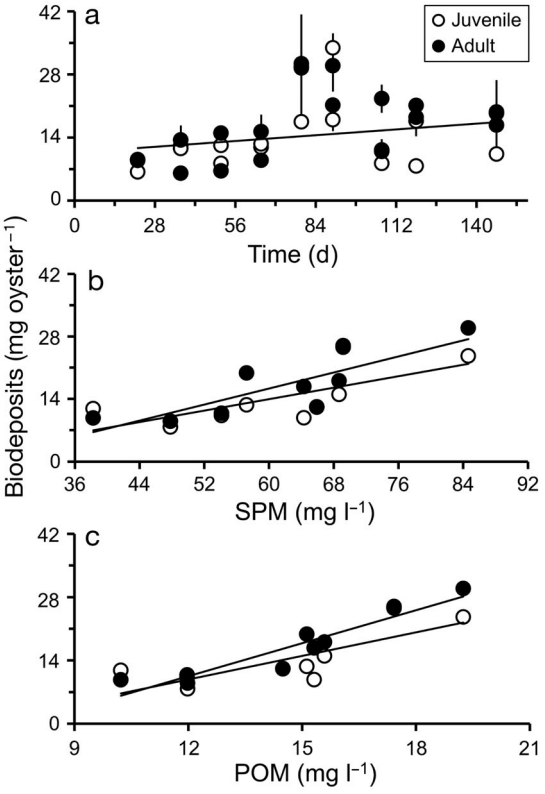

Fig. 5. Total biodeposits produced by juvenile and adult oysters (a) on each sample date (error bars show SE) and compared to bulk water column (b) SPM and (c) POM concentration. Biodeposits were collected after an 18 h period when no additional food resources were available to oysters. Regression lines: Time, adult oysters — $y = 1.53x + 9.19$, $R^2 = 0.22$, $F_{reg\ 1,16} = 5.84$, p = 0.03; SPM, juvenile oysters — $y = 0.32x - 5.33$, $R^2 = 0.41$, $F_{reg\ 1,8} = 6.47$, p = 0.04 and adult oysters — $y = 0.45x - 10.66$, $R^2 = 0.65$, $F_{reg\ 1,8} = 15.98$, $p < 0.01$; POM, juvenile oysters — $y = 1.74x - 11.06$, $R^2 = 0.56$, $F_{reg\ 1,8} = 11.13$, p = 0.01 and adult oysters — $y = 2.43x - 18.64$, $R^2 = 0.87$, $F_{reg\ 1,8} = 54.71$, $p < 0.001$

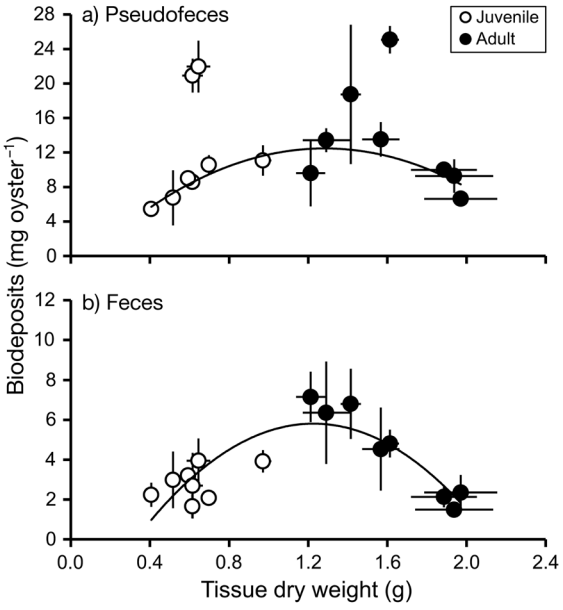

Fig. 6. Mean biodeposit production (±1 SE) by juvenile and adult oysters, separated as (a) pseudofeces and (b) feces, relative to mean soft tissue dry weight (±1 SE) on each sample date. Regression lines indicate relationships between biodeposit production and soft tissue dry weight, with juvenile and adult data pooled: pseudofeces — $y = -8.00x^2 + 22.58x - 2.02$, $R^2 = 0.68$, $F_{reg\ 2,9} = 12.62$, $p < 0.01$; feces — $y = -7.20x^2 + 17.65x - 5.00$, $R^2 = 0.66$, $F_{reg\ 2,13} = 15.55$, $p < 0.001$. Days 79 and 90 were outliers to the regression for pseudofeces and were thus excluded from analysis

and shell during the study period was 165 ± 8 mg N oyster^{-1} for juveniles and –48 ± 21 mg N oyster^{-1} for adults (Table 2), with N in shell accounting for nearly 50% of the total N content in juvenile oysters and >50% of total N content in adult oysters at harvest.

N released in biodeposits

There were no differences in feces, pseudofeces, or total biodeposit production between sites (ANOVA feces: $F_{1,77} = 2.20$, p = 0.14; pseudofeces: $F_{1,77} = 0.61$, p = 0.44; total: $F_{1,77} = 0.73$, p = 0.40), and data from each site were combined for subsequent analyses. Adult oysters produced more total biodeposits than juvenile oysters (ANOVA: $F_{1,77} = 6.28$, p = 0.01), with total biodeposit production by adult oysters increasing through time during the study (Fig. 5a). This pattern was driven by somewhat greater production of feces by adult compared to juvenile oysters (ANOVA: $F_{1,77} = 3.97$, p = 0.049), while there was no difference in pseudofeces production between age classes (Fig. 6). Total biodeposit production for both juvenile and adult oysters was also related to bulk water column SPM and POM concentrations on each sample day (Fig. 5), largely due to the effect of suspended particle loads on pseudofeces production (Table 3). Overall, pseudofeces accounted for the majority of

Table 3. Regression statistics for oyster pseudofeces production compared to bulk water column SPM and POM concentrations on each sample day

	Regression equation	R^2	F_{reg}	df	p
SPM					
Juvenile	$y = 0.30x - 6.57$	0.38	5.97	1.8	0.04
Adult	$y = 0.35x - 8.79$	0.62	14.13	1.8	<0.01
POM					
Juvenile	$y = 1.61x - 11.96$	0.54	10.27	1.8	0.01
Adult	$y = 1.82x - 13.74$	0.74	24.11	1.8	<0.01

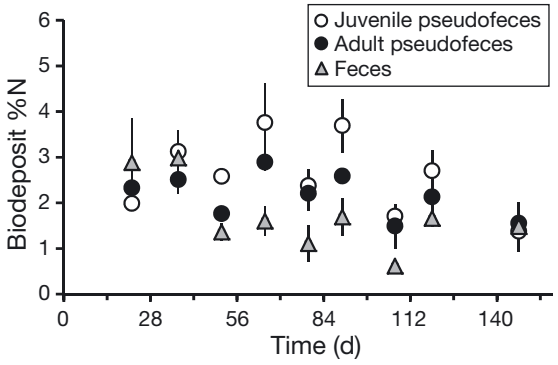

Fig. 7. Mean percentage of N (±1 SE) in oyster biodeposits collected on each sample date

biodeposits produced by both juvenile and adult oysters (ANOVA: $F_{1,206}$ = 213.01, p < 0.001), and both forms of ejecta increased with oyster dry weight to about 1.2 to 1.5 g, then declined for larger oysters (Fig. 6).

Percentage of N content in feces and pseudofeces did not differ between sites and showed no significant pattern through time. However, the N content (as a percentage) was higher in oyster pseudofeces (2.6 ± 0.2% juveniles; 2.2 ± 0.1% adults) than in feces (1.9 ± 0.2%) (ANOVA: $F_{1,123}$ = 10.16, p < 0.01), and juvenile pseudofeces had higher N content than pseudofeces produced by adults (ANOVA: $F_{1,8}$ = 6.79, p = 0.03) (Fig. 7). The percentage of N in oyster feces did not differ between age classes. The greater production of pseudofeces with larger percentages of N resulted in greater total mean N content released in pseudofeces (0.11 ± 0.01 to 0.81 ± 0.17 mg N oyster^{-1}) compared to feces (0.02 ± 0.01 to 0.12 ± 0.04 mg N oyster^{-1}; Fig. 8) for both age classes.

Overall, oysters released N in biodeposits at a mean rate of 19.6 ± 1.8 µg N oyster^{-1} h^{-1} (for an 18 h period where no additional food resources were available), and this rate did not differ between sites or age classes (ANOVA site: $F_{1,103}$ = 1.14, p = 0.29; age class: $F_{1,103}$ = 0.06, p = 0.81). Given the specific number of oysters in the field during each biweekly sampling period (total ranging from 2245 to 3600 for juveniles and 432 to 1200 for adults) and the known rates of N released in biodeposits per oyster on each sampling date (Fig. 8), we extrapolated a total quantity of N potentially released back to the estuary in oyster biodeposits during this study (94.4 ± 12.5 g N by juveniles and 24.0 ± 10.1 g N by adults). Normalizing these values relative to the average number of oysters (2932 ± 144 juveniles and 805 ± 81 adults) during the study and days in the field (n = 139) resulted in estimated N-release rates of 0.23 ± 0.00 mg

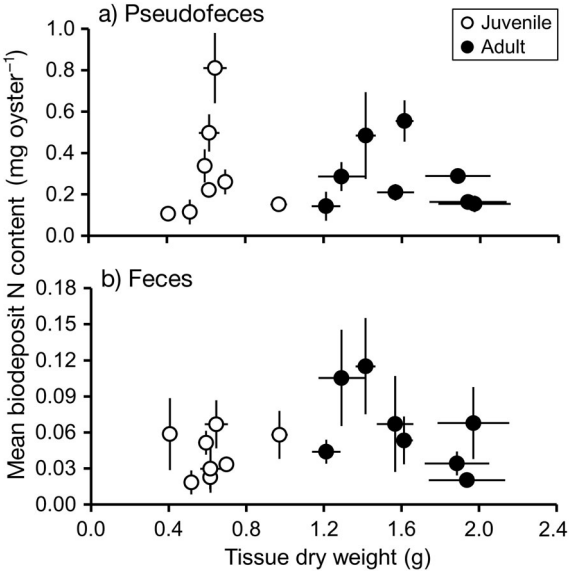

Fig. 8. Mean N content (±1 SE) in (a) pseudofeces and (b) feces produced by juvenile and adult oysters compared to mean soft tissue dry weight (±1 SE) on each sample date

N oyster^{-1} d^{-1} and 0.21 ± 0.00 mg N oyster^{-1} d^{-1} (or 0.02 ± 0.00 mmol N oyster^{-1} d^{-1}) by juvenile and adult oysters, respectively.

Stable isotope analysis

δ^{15}N values in water column SPM, available as food for oysters, did not differ between study sites and averaged ~5‰ throughout the study period (Fig. 9a). δ^{13}C values in SPM were heavier between Days 51 and 90, coincidental with a period of low rainfall during July and August 2011, but became lighter after Day 107 with the landfall of Tropical Storm Lee (Fig. 9b). These events were also coincidental with a spike in benthic chl *a* (Fig. 2), a drop in temperature, and an increase in DO concentration.

δ^{15}N values in tissues of the initial hatchery stock oysters differed between juveniles and adults (ANOVA: $F_{1,10}$ = 359.74, p < 0.001), with juvenile tissues averaging 8.77 ± 0.03‰ and adults averaging 10.81 ± 0.10‰ on Day 0 (Fig. 9a). Stable isotope ratios in juveniles and adults converged during the study period, so that by Day 147, isotope ratios remained different but averaged 9.55 ± 0.09‰ in tissues of juveniles and 10.19 ± 0.06‰ in tissues of adults (ANOVA: $F_{1,10}$ = 37.19, p < 0.001). On Day 147, the mean difference between δ^{15}N in tissues and bulk available SPM was 5.80 ± 0.17‰ for juveniles and 6.44 ± 0.16‰ for adults.

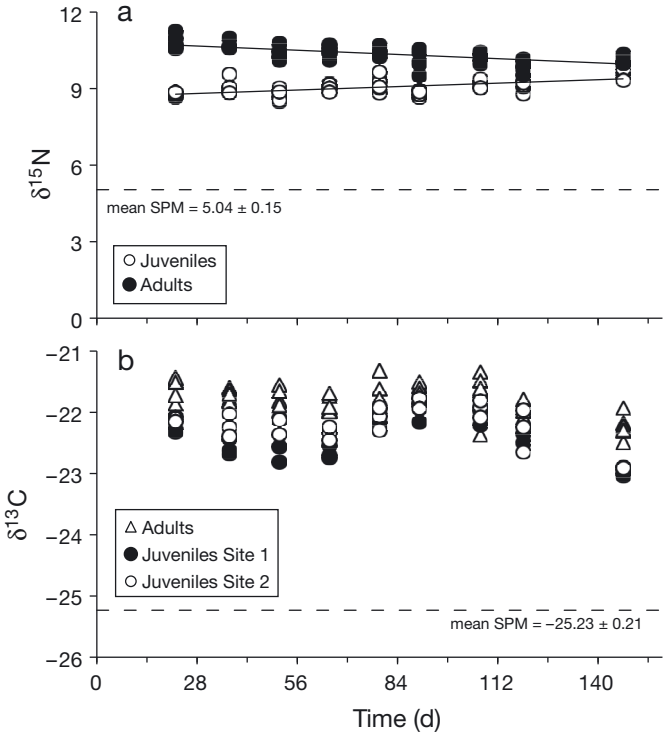

Fig. 9. (a) $\delta^{15}N$ and (b) $\delta^{13}C$ in oyster tissues and water column suspended particulate matter (SPM) through time. Dashed lines indicate mean (±SE) values in SPM throughout the study. Regression lines indicate tissue $\delta^{15}N$: juvenile — $y = 0.005x + 8.67$, $R^2 = 0.37$, $F_{reg\ 1,52} = 0.06$, $p < 0.001$; adult — $y = -0.006x + 10.80$, $R^2 = 0.41$, $F_{reg\ 1,52} = 36.53$, $p < 0.001$

$\delta^{13}C$ values in SPM did not differ significantly between sites and had a mean value of −25‰ during the study period (Fig. 9b). Like $\delta^{15}N$, $\delta^{13}C$ values in tissues of the initial hatchery stock oysters differed between juveniles and adults (ANOVA: $F_{1,10} = 86.22$, $p < 0.001$), with juvenile tissues averaging −22.18 ± 0.04‰ and adults averaging −21.58 ± 0.07‰, respectively, on Day 0 (Fig. 9b). During the study, $\delta^{13}C$ values in adult oyster tissues remained heavier than those of juveniles. While $\delta^{13}C$ in adult oysters did not differ between sites (ANOVA: $F_{1,80} = 200.55$, $p < 0.001$), values in juvenile oyster tissues were heavier at Site 2 than at Site 1 (ANOVA: $F_{1,80} = 4.57$, $p < 0.001$). $\delta^{13}C$ values in oyster tissues followed changes in SPM through time, showing heavier values between Days 51 and 90, reflecting drought-like conditions which persisted during most of July and August 2011, but became lighter after Day 107 (Fig. 9b) such that $\delta^{13}C$ values in oyster tissues were significantly lighter in both age classes on Day 147 (ANOVA: $F_{8,80} = 21.69$, $p < 0.001$). On Day 147, the mean difference between $\delta^{13}C$ in tissues and SPM was 2.28 ± 0.19‰ for juveniles and 3.01 ± 0.19‰ for adults.

DISCUSSION

Results of this study demonstrate that age class may be an important factor in determining the capacity for N removal from coastal waters by oysters. Direct N removal via assimilation into tissues and shell as well as biodeposit production that may mediate indirect N removal through biogeochemical processes differed between juvenile and adult age classes.

N assimilation into soft tissues and shell

Juvenile oysters were more important than adults for active N removal due to direct N assimilation among oysters in this study. Because the percentage of N in soft tissues did not differ between age classes and remained constant through time, the rate of N assimilation into soft tissues was driven by tissue growth rate. As a result, although adult oysters contained a greater amount of N per oyster, they did not grow and actually lost biomass, acting as a net source of N back to the estuary. Juvenile oysters, in contrast, actively grew and assimilated N into soft tissues during the study. Similarly, oysters showed a significant ontogenetic difference in N assimilation into shell, driven by differences in growth as with soft tissues but also due to a difference in percentage of N content in shell. Juveniles showed significant shell growth and associated assimilation of N. Among adults, however, while shell did not grow, shell mass was maintained during the period of inactive growth, and N stored in shell of adult oysters was not readily released back into the system. While we do not know the specific mechanism for lack of growth in adult oysters (some possibilities are discussed below), these data demonstrate that different size or age classes of oysters can assimilate N differently under the same environmental conditions.

These findings demonstrate that N may be sequestered in oyster shell and retained despite loss of soft tissue in living animals. Because shell material is not readily metabolized (Liu et al. 2010) and dissolution typically occurs on time scales of years to decades (Powell et al. 2006), shell is less responsive to short-term environmental or physiological variation that could result in N loss from soft tissues. N stored in shell, therefore, has potential to be retained for much longer periods of time compared to soft tissues (E. S. Darrow unpubl. data). The specific timescale on which N may be released from shell is likely location-specific, depending on site-specific environmental conditions, including pH (Waldbusser et al. 2011), sediment burial rate (Davies et al. 1989), and abun-

dance of shell-boring organisms (Carver et al. 2010). Some previous studies have ignored the potential contribution of N stored in shell to N-removal estimates, based on the assumption that the amount of N stored in shell is small (Carmichael et al. 2012b). While the percentage of N stored in shell is small compared to soft tissues (shell has been reported to comprise 0.02 to 0.46% N, depending on the species), because of the relatively higher weight of shell compared to soft tissues, our data and others show that N assimilated into oyster shell can be a significant portion of N stored and potentially removed from estuaries at harvest (Kellogg et al. 2014). It is important to note, however, that shell returned to the estuary for disposal or as part of restoration efforts may provide N storage for some period of time but should not be considered a net source of N removal.

Our results further highlight that oyster tissue dry weight is crucial to defining N assimilation into tissues and potential N removal at harvest, particularly for larger (older) age classes in which growth may be slower. Oyster shell dimensions typically have a positive allometric relationship with soft tissue weight, and therefore, it has been assumed that measurements such as shell length can be used as a proxy for tissue weight (Dame 1972, Higgins et al. 2011, Pollack et al. 2011) and thus N content (Rose et al. 2015). Our data demonstrate that shell length to tissue dry weight (and N content) relationships can be decoupled (Fig. 3). Basing assimilatory N-removal estimates on shell dimensions alone (using shell length as a proxy), without making direct measurements of tissue weight, would have resulted in an overestimate of tissue N content in adult oysters. Results of this study demonstrate the importance of basing oyster N storage or removal estimates on direct measurements of tissue biomass and N content.

Potential for indirect N removal via biodeposition

On a per oyster basis, juvenile and adult age classes released similar quantities of N in biodeposits, despite differences in production of and N content in feces and pseudofeces. Hence, both age classes would be expected to show similar potential for indirect N removal via biodeposit-stimulated biogeochemical processes on a per oyster basis, assuming biodeposits would be retained in nearby sediments. Due to their smaller size, however, juvenile oysters may be stocked at a higher density and could represent a larger potential source of N deposited to the benthos per unit surface area. Stocking density, therefore, may be an important factor in determining potential for indirect N removal by oysters through biodeposition (Mitchell 2006, Higgins et al. 2013, Hoellein & Zarnoch 2014) in locations where conditions promote nitrification-denitrification coupled processes that result in N removal. As with N assimilation into tissues, data from this study demonstrate that the N released in biodeposits is not necessarily positively correlated with shell size or tissue dry weight. Hence, biogeochemistry-based N removal may be overestimated by making this assumption.

Estimates of N removal by burial and denitrification relative to biodeposition rate made by Newell et al. (2002) suggest that ~20% of N released in oyster biodeposits may be removed from a system under the appropriate environmental conditions. Applying this estimate to data collected during this study suggests that N released in oyster biodeposits could result in removal of an additional ~0.07 mg N oyster^{-1} at a mean rate of ~3.9 µg N oyster^{-1} h^{-1} via benthic denitrification when conditions are such that coupled nitrification-denitrification processes can occur, which can be highly variable among seasons and locations (Piehler & Smyth 2011, B. Mortazavi & A. Ortmann unpubl. data). This estimate of indirect N removal via biodeposition may be conservative if biodeposition rates in the field, where food may be continuously available, are higher than we predicted from gut clearance alone. Accordingly, our estimates of N released in biodeposits are comparable to previous reports (Higgins et al. 2013), but our estimated total N removal is lower than previously reported (Haven & Morales-Alamo 1966: ~0.14 to 0.22 g N oyster^{-1} d^{-1}; Carmichael et al. 2012b: ~1.78 to 3.34 g N oyster^{-1} yr^{-1}). Estimates made by Carmichael et al. (2012b) were most similar to our estimates but were based on an assumed positive allometric relationship between oyster weight and biodeposition; a pattern not observed in the present study because biodeposition decreased for larger oysters, which lost biomass during this study. Haven & Morales-Alamo (1966) measured biodeposition by oysters in isolated laboratory chambers, where rates exceeded estimates from field studies due to experimental design that promoted deposition. That study made direct measurements of biodeposition by individual oysters, held and continuously fed in the laboratory, as opposed to measuring oysters immediately upon collection from the field and in the absence of continuous particle inputs as in this study. These differences highlight potential sources of variation in estimating N removal via biodeposit-stimulated biogeochemical processes and indicate the value of direct measurements of biodeposit

production and biogeochemical processes to accurately quantify N removal that may occur *in situ* due to biodeposition.

Factors affecting N-removal capacity

N removal via assimilation into oyster tissues and shell is dependent on oysters actively feeding and growing. Aside from age class, therefore, any factors that affect oyster feeding, growth, and condition could affect the N-removal capacity of oysters. Although some of the environmental attributes measured during this study differed significantly between the 2 study sites, this variation was not significant enough to cause differences in oyster growth between sites. Values for water temperature, salinity, and DO were typical of summer conditions in the study area (Stumpf et al. 1993, Cowan et al. 1996, Park et al. 2007) and consistent with suitable oyster growth conditions in Mobile Bay (Biancani et al. 2012). Water-column chl *a* values were within the range reported in previous studies (Cowan et al. 1996, Biancani et al. 2012), and SPM and POM concentrations were similar to or higher than those reported for suitable oyster growth elsewhere (Grizzle et al. 1992, Ferreira et al. 1997, Carmichael et al. 2012b). As a result, although adult oysters did not show significant growth during the study, growth rates of juvenile oysters were similar to those reported in Mobile Bay and other urbanized estuaries during periods of peak growth (Biancani et al. 2012: 0.18 ± 0.01 mm d^{-1}, shell height; Carmichael et al. 2012b). Overall food availability may also have been influenced by high flow rates in the study area and high SPM concentrations that are known to affect oyster feeding (Grizzle et al. 1992, Ferreira et al. 1997, Carmichael et al. 2012b). While food supply did not appear to be limiting for juvenile oysters, it could have been limiting for the larger adults, despite stocking at lower density.

It is also possible that larger adult oysters were experiencing some other size or ontogenetic-related stressor not captured during this study. A shift to a reproductive state (and associated gamete production) or environmental stressors can contribute to lower tissue mass and reduced N content in some cases (Thompson et al. 1996). Seasonal changes in glycogen, lipid, and protein content of tissues have been reported due to reproductive condition in oysters and in response to general stressors such as low temperature or disease in many bivalves (Thompson et al. 1996). Since oysters used in this study were larger than (adults) or grew to (juveniles) typical spawning sizes during the study period and were planted at the same locations, we might expect effects of spawning or environmental variation to similarly affect biomass and N content in both of the age classes during this study. While lowest tissue mass among adults was found during Days 79 to 119 (mid-August to mid-September), consistent with a post-spawning period in the Gulf of Mexico (Hayes & Menzel 1981, Kennedy 1996), the percentage of N did not show a corresponding change, and juveniles showed no pattern in tissue composition that could be related to reproduction. The ontogenetic difference we found in biomass, therefore, suggests a more complex explanation. Regardless of the reason, we documented a loss of soft tissue mass and associated N from adult oysters during the study period. Even if this effect was due to seasonal reproduction or a temporary environmental stressor and oysters were to subsequently regain weight, N in tissues was lost and the oysters were (even temporarily) a source of N to the system. If oysters are harvested as part of N remediation efforts, then this loss could significantly affect N-removal estimates that are based on shell length rather than direct measures of biomass. Overall, poor growth only among adult oysters demonstrates the potential for ontogenetic-based differences in N-removal capacity of oysters that may further vary by location or season with environmental conditions.

Biodeposition is also closely linked to environmental variables and food resource quality and availability (Haven & Morales-Alamo 1966, Tenore & Dunstan 1973, Mitchell 2006), particularly bulk water column SPM and POM. In this study, the quantity of biodeposits and their N content showed variability among sample days that was not reflected in oyster growth and tissue N content, indicating that biodeposition was more sensitive to small-scale environmental perturbations, such as storm events, compared to oyster growth. In areas with high flow rates, oyster biodeposits may be exported and diluted into nearby waters prior to reaching the sediment surface (Haven & Morales-Alamo 1968, Widdows et al. 1998, Newell et al. 2005). Like many estuaries, Mobile Bay is a dynamic system with variable flow patterns affected by wind, riverine, and tidal influences (Stumpf et al. 1993). A large velocity shear has been reported in Mobile Bay near the sites used for this study (Park et al. 2007), suggesting that biodeposit export was likely in our area. Accordingly, benthic N and C content in sediments did not increase during the transplant period (data not shown), and denitrification was not clearly related to oyster biodeposition (B. Mortazavi & A. Ortmann unpubl. data). These findings confirm

that, despite significant biodeposition, N removal cannot be assumed to occur in association with oyster culture or restoration. Because indirect N removal via biogeochemical processes in sediments below oyster cages or reefs can only occur in areas where the benthic conditions can support denitrification (Piehler & Smyth 2011, Higgins et al. 2013, Hoellein & Zarnoch 2014), physical and chemical environmental conditions should be considered relative to rates of biodeposit production, and biogeochemical N removal should be directly measured.

Direct measurements of oyster tissue and biodeposit N content are scarce in literature, making it difficult to fully assess the significance of grow-out location for the N-removal capacity of oysters. The N percentages measured in soft tissues during this study were higher than those previously reported (Newell et al. 2005, Higgins et al. 2011, Carmichael et al. 2012b). Comparison to literature values and unpublished data suggests that this difference may be regional, with oysters in the northern Gulf of Mexico having higher tissue %N content than those grown on the Atlantic coast of the USA by >25% on average (ANOVA: $F_{1,18} = 66.47$, $p < 0.001$; Fig. 10). Our measurements of the fraction of N stored in oyster shell are consistent with previous reports for adult oysters (0.2 to 0.3% N) but slightly higher than previously reported for juveniles (Newell et al. 2005, Higgins et al. 2011, Kellogg et al. 2014). These differences may reflect specific ontogenetic differences in resource partitioning among tissues that have not been studied in detail among different age classes. Overall, regional differences in N content in tissues, shell, and biodeposits may be due to differences in composition and N content of food resources, which may be in part attributed to differences in nutrient inputs among estuaries (Carmichael et al. 2004, Carmichael et al. 2012a). A general lack of published data for these values along with regional and interannual variation in environmental attributes among studies make it impossible to define a clear relationship between food quality and N content in oyster soft tissues at this time (Fig. 10). Food quality, however, has been shown to affect N content in tissues of other bivalves (Carmichael et al. 2004) and may be an important factor in determining oyster tissue N content and, in turn, the N-removal capacity of oysters.

Our findings highlight the importance of considering environmental and oyster condition, in this case related to ontogeny, when planning any grow-out activities designed to enhance N removal. The distinct age classes of oysters used for this study represent sizes typical during aquaculture and restoration grow-out but do not specifically mimic either scenario from planting to harvest or maturity. Juvenile oysters used in this study are similar in size to those harvested from aquaculture operations in USA waters (MacKenzie 1996, Higgins et al. 2011). Adult oysters exceeded the typical harvest size for aquaculture applications but represent older oysters that can occur on natural or restored reefs (Luckenbach et al. 2005). Grow-out conditions (and the resulting size range of oysters planted, harvested, or allowed to mature) vary considerably among aquaculture and restoration activities and locations, making knowledge of variation in these conditions critical to guide N remediation efforts. The total N removal from an oyster aquaculture operation, for example, would be equivalent to the difference in N content between planting and harvest. This time period would be somewhat predictable and would likely be repeated at regular intervals. In contrast, on a restored reef, N removal from harvest is not necessarily appli-

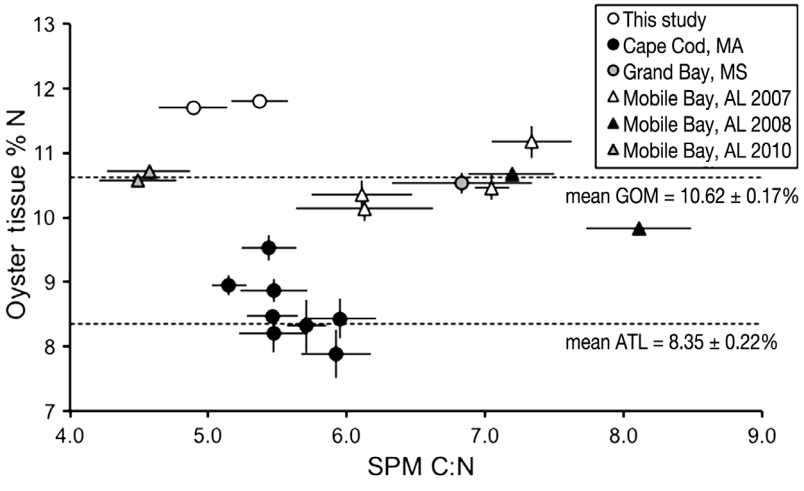

Fig. 10. Percentage of N in oyster soft tissues compared to C:N in SPM from this study and others for which similar data were available. All error bars show SE. Dashed lines indicate mean (±1 SE) percentage of N in oyster tissues reported in the northern Gulf of Mexico (GOM) and at sites on the Atlantic coast (ATL) of the USA. Data are from Carmichael et al. (2012b) (Cape Cod, MA), E. S. Darrow unpubl. data (Grand Bay, MS), unpublished data from Daskin et al. (2008) (Mobile Bay, AL, 2007), and H. Patterson unpubl. data (Mobile Bay, AL, 2008, 2010). Mean % N for ATL was calculated from 8 Cape Cod estuaries (Carmichael et al. 2012b) and single published values for Chesapeake Bay (Newell et al. 2005, Higgins et al. 2011). Chesapeake Bay data are not shown because data for C:N in SPM were not available

cable if reefs are protected and harvest is limited or prohibited. Results of this study, therefore, have important implications for both aquaculture and restoration activities by capturing and reporting differences in N assimilation capacity for age classes of oysters that are relevant to each type of activity.

Stable isotope ratios

Comparison of $\delta^{15}N$ and $\delta^{13}C$ values in oyster tissues and SPM confirmed that juvenile and adult oysters assimilated locally derived N and were exposed to both marine and land-derived food sources at various times throughout the study. Local N assimilation was evidenced by changes in tissue $\delta^{15}N$ values in juveniles and adults from the initial hatchery stock to reflect local food resources (SPM). The shifts from slightly heavier to lighter $\delta^{13}C$ values in oyster tissues were not surprising, given the typical patterns of freshwater discharge and corresponding salinity variation in Mobile Bay (Schroeder et al. 1990, Park et al. 2007), including a period of low freshwater discharge associated with landfall of Tropical Storm Lee during the study period. Differences in tissue $\delta^{15}N$ and $\delta^{13}C$ between juvenile and adult oysters may be due to selection and assimilation of N from different specific food sources by the different size classes of oysters (Shumway et al. 1985). Despite the overall deviation from the expected trophic fractionation between oysters and bulk SPM, stable isotope ratios confirmed that oysters grown during this study were assimilating locally derived particles and effectively sequestering local N and C sources.

Conclusions and implications

Natural oyster reefs in many coastal waters have been in decline during the last century due to unsustainable harvesting practices, decreased water quality, and coastal urbanization (Rothschild et al. 1994, Seavey et al. 2011, Zu Ermgassen et al. 2012). This decline represents not only a loss of commercial species and valuable habitat but also the loss of the natural filtering function that oysters provide to the system (Cloern 2001, Coen et al. 2007, Zu Ermgassen et al. 2013). Because oysters sequester N and contribute to the coupling of pelagic and benthic processes through biodeposition, large losses of oysters may change the dynamics of nutrient cycling and biogeochemistry within coastal ecosystems and contribute to trophic shifts through the loss of top-down control of phytoplankton (Newell 1988, Ulanowicz & Tuttle 1992). Oyster restoration is a viable option for recovery and prevention of further loss of these same ecosystem services, particularly in the northern Gulf of Mexico, which supports the only remaining viable native oyster capture fishery in the USA (Beck et al. 2011). Additionally, oyster aquaculture can provide a sustainable alternative to wild harvest by meeting seafood market demands, relieving pressure on native stocks, and potentially yielding environmental benefits through N sequestration and removal. Accurately quantifying the magnitude of these benefits will require direct measurement of N removal (via assimilation and biogeochemical processes) in the context of location-specific environmental conditions and oyster growth patterns.

Our data demonstrate that different size or age classes of oysters can assimilate N differently under the same environmental conditions. Specifically, actively growing juvenile oysters assimilated N at higher rates than adult oysters due to faster growth rates, but adult oysters represented a potentially important source of N storage in shell. Regardless of differences in growth and quantity or quality of biodeposits produced, juvenile and adult oysters had equivalent estimated potential to stimulate biogeochemical N removal via biodeposition per individual. These findings demonstrate an important disconnect between shell length and N assimilation, storage, and release rates between age classes. Given their smaller size and potential for higher stocking density, juvenile oysters may also yield higher quantities of biodeposits per unit area to fuel biogeochemically driven N removal from sediments. Furthermore, N removal by tissue and shell assimilation may be uncoupled from biodeposition and resulting biogeochemical N removal (which did not occur during this study). These results demonstrate the need for direct measurements of oyster growth, tissue and shell N content, biodeposit production, and biogeochemical processes to make accurate comprehensive estimates of N-removal. For continuous N sequestration and removal, oyster aquaculture or restoration projects may benefit from balancing recruitment of young, actively growing oysters with harvest of larger animals to support net N removal by assimilation. Consideration should be given to environmental stressors or intrinsic factors, such as reproduction, that may affect oyster condition and subsequent N removal at the time and location of grow-out. Strategies to maximize tissue-based N removal will be particularly important in areas where environmental conditions do not support substantial N removal through biogeochemical processes.

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