Estuaries and Coasts (2021) 44:1363–1379
https://doi.org/10.1007/s12237-020-00874-2

Juvenile Oyster (*Crassostrea virginica*) Biodeposits Contribute to a Rapid Rise in Sediment Nutrients on Restored Intertidal Oyster Reefs (Mosquito Lagoon, FL, USA)

Bryan Locher[1] · Nia R. Hurst[1,2] · Linda J. Walters[3] · Lisa G. Chambers[1]

Received: 11 June 2020 / Revised: 6 October 2020 / Accepted: 16 November 2020 / Published online: 26 November 2020

Abstract

By filtering large volumes of water and releasing nutrient-rich biodeposits (feces and pseudofeces), oysters can locally enhance sediment biogeochemical cycling. An active *Crassostrea virginica* restoration program in Mosquito Lagoon, FL (USA), was leveraged to assess the immediate (first-year) effects of restoration on sediment nutrients. Measurements included extractable and total carbon, nitrogen, and phosphorus on dead, natural, and restored reefs using a before-after-control-impact design. To investigate an observed "age-nutrient paradox" in sediment nutrient concentrations, a laboratory experiment compared feeding rates and biodeposit nutrient content between juvenile and older oysters. The field study documented a 136% increase in ammonium, 78% increase in total nitrogen, 46% increase in total phosphorus, and 75% increase in organic matter concentrations 12 months post-restoration, with extractable nutrients responding more rapidly to restoration than total nutrients. Sediment nutrient increases were positively correlated with oyster density, shell length, and reef height. Moreover, the laboratory study indicated juvenile oysters had higher rates of chlorophyll-*a* removal and ammonium efflux and produced biodeposits with higher concentrations of dissolved organic carbon, nitrate, and ammonium than older oysters. Overall, this study documented increases in sediment nutrients on intertidal reefs within the first year of restoration, which may be explained by a greater filtration rate and more nutrient-enriched biodeposits contributed by young oysters as compared to older oysters. Sediment total nitrogen and ammonium content may be the most robust monitoring metrics for documenting the ecosystem service of enhanced biogeochemical cycling on restored oyster reefs.

Keywords *Crassostrea virginica* · Oyster reef restoration · Biogeochemistry · Biodeposits · Carbon · Nitrogen

Introduction

Oyster populations have precipitously declined during the past two centuries due to overharvesting, disease, and habitat degradation (Jackson et al. 2001; Kirby 2004; Beck et al. 2011). From 1900 to 1995, annual harvest yields of the eastern oyster, *Crassostrea virginica*, in most Atlantic coastal states decreased by more than 90%, prompting a wide-scale interest in oyster reef restoration projects (Mackenzie 1996). More recently, the goals of oyster restoration have begun to shift away from the creation of harvestable populations and towards enhancing ecosystem services, such as top-down control of phytoplankton populations, water clarity improvement, and habitat provisioning (Coen and Luckenbach 2000; Grabowski et al. 2005; Coen et al. 2007; Newell et al. 2007; Grabowski et al. 2012). Research has documented restoration can rapidly affect certain ecosystem services, such as water filtration (within weeks after recruitment) and the abundance and diversity of reef-dependent faunal communities (within 1 year of restoration) (Humphries et al. 2011; La Peyre et al. 2014; Pierson and Eggleston 2014; Dillon et al. 2015; Humphries and La Peyre 2015; Rezek et al. 2017).

From a biogeochemical perspective, oysters filter large volumes of water containing suspended organic matter and deposit it on the underlying sediment. This creates

Communicated by Lijun Hou

✉ Lisa G. Chambers
lisa.chambers@ucf.edu

[1] Aquatic Biogeochemistry Lab, Department of Biology, University of Central Florida, 4000 Central Florida Blvd., Bldg. 20, BIO 301, Orlando, FL 32816, USA

[2] Engineer Research and Development Center, US Army Corps of Engineers, Vicksburg, MS, USA

[3] Coastal and Estuarine Ecology Lab, Department of Biology, University of Central Florida, 4000 Central Florida Blvd., Bldg. 20, BIO 301, Orlando, FL 32816, USA

biogeochemical hot spots, areas with enhanced rates of biogeochemical cycling relative to the surrounding sediments (Dame et al. 1989; Mcclain et al. 2003; Chambers et al. 2018). During feeding, oysters assimilate organic matter to fulfill metabolic requirements and then release both digested and undigested materials in the form of feces and pseudofeces (herein referred to collectively as biodeposits). Once released, these mucus-bound biodeposits can be consumed by grazers and microbes in the benthos, resuspend back into the water column, or can be buried in the sediment (Dame 1999; Haven and Morales-Alamo 1966; Newell 2004; Testa et al. 2015). Biodeposition supplies labile nutrients for microbially mediated transformations of carbon (C), nitrogen (N), and phosphorus (P) (Newell et al. 2005; Smyth et al. 2016; Chambers et al. 2018). After several years, a restored reef can accumulate significant stores of C and N in the underlying sediment (Newell et al. 2005; Kellogg et al. 2013; Pollack et al. 2013; Fodrie et al. 2017; Chambers et al. 2018) as well as increase benthic primary productivity (Newell et al. 2002; Blomberg et al. 2017; Volaric et al. 2018). Due to the importance of N availability to coastal eutrophication, multiple studies have investigated the promotion of coupled nitrification/denitrification in sediments both on and adjacent to oyster reefs due to biodeposition (Pollack et al. 2013; Smyth et al. 2013b; Hoellein et al. 2015; Humphries et al. 2016). However, findings indicated denitrification rates can differ by orders of magnitude based on site, season, and habitat context (Kellogg et al. 2013; Mortazavi et al. 2015; Smyth et al. 2015, 2018; Westbrook et al. 2019), highlighting the need for additional research on oyster-mediated sediment nutrient cycling.

The magnitude and geographic extent to which biodeposition can impact biogeochemical cycling remains relatively understudied (Haven and Morales-Alamo 1968; Newell et al. 2005; Dalrymple and Carmichael 2015; Porter et al. 2018). Studies usually describe oyster reefs as providing biodeposits that are "high quality organic matter" (Smyth et al. 2016) or "C- and N-rich" (Hoellein et al. 2015), but most lack quantification or speciation of C, N, and P in biodeposits, with a particular lack of data on the availability of P or comparisons among age classes.

Recent research on the surface sediments of restored intertidal *C. virginica* reefs in Central Florida found total C content was 236% higher and total N was 260% higher on 1-year-old restored reefs, relative to dead/unrestored reefs (Chambers et al. 2018). Furthermore, sediment dissolved organic C (DOC), ammonium (NH_4^+), and total C concentrations peaked in 1-year-old restored reefs, even when compared to 4-year- and 7-year-old restored reefs and natural/reference reefs, despite 1-year-old restored reefs having significantly fewer live oysters (Chambers et al. 2018). We suggest this data demonstrates an age-nutrient paradox, whereby fewer, young oysters are able to produce equivalent, or even greater, sediment nutrient pools than higher-density, older oysters on restored or natural reefs. Enhancing the knowledge about the role of oyster reef restoration on coastal sediment cycling and how this may change as a reef ages will provide important data to restoration practitioners wanting to quantify the rate of return for ecosystem services following a restoration project.

The goals of this study were (1) to improve the understanding of the short-term (first year post-restoration) response of sediment biogeochemistry to oyster restoration, and how these nutrient pools relate to common management metrics for oyster reef monitoring (e.g., live oyster density, shell length, and reef height), and (2) to unravel the age-nutrient paradox using a controlled laboratory experiment that compares the filtration efficiency and biodeposit chemistry of young (< 14 months since recruitment) and older live oyster clusters. This was accomplished by pairing a before-after-control-impact (BACI) field study of recently restored intertidal *C. virginica* reefs in Mosquito Lagoon, FL, with replicate tank biodeposit studies containing young and older oysters. We predicted inorganic sediment nutrient pools (NH_4^+, soluble reactive phosphorus (SRP), and DOC), commonly considered to be the more "bioavailable" nutrient pools (Reddy and DeLaune 2008), would reach concentrations comparable to reference/natural reefs within the first year post-restoration. Also, we expected the controlled laboratory study would reveal that young oysters can contribute to this rapid increase in bioavailable nutrients through the production of more nutrient-rich biodeposits than older oysters.

Methods

Site Description

The Indian River Lagoon (IRL) is a shallow lagoon that stretches for 251 km along the Atlantic coast of Central Florida (Dybas 2002). The low-profile reefs of *C. virginica* for this study are located in the northernmost portion of the IRL known as Mosquito Lagoon, which is microtidal and low in energy and averages 1.7 m deep (Smith 1993). The subtropical climate allows water temperatures to generally exceed 20 °C from March to November and fall between 10 and 15 °C in winter months; long water residence times result in salinities that range from 22.6 to 45.2 ppt (Phlips et al. 2015).

Within Mosquito Lagoon, there is an abundance of intertidal reefs of *C. virginica* bordered by mangrove-dominated islands. Many of these reefs have experienced degradation within the past century, primarily due to the action of boat wakes (Grizzle et al. 2002; Wall et al. 2005). Reefs located along popular boating channels degrade into disarticulated shell piles that accumulate as oyster clusters become dislodged by the force of boat wakes (Garvis et al. 2015). Within the boundaries of Canaveral National Seashore where

the reefs for this study are located, 40% of oyster reef coverage has been lost since 1943 (Garvis et al. 2015). Since 2007, there has been a community-based restoration effort to restore the dead margins and degraded reefs. The restoration process involves leveling piles of shell in the selected area to an intertidal height equal to the elevation of adjacent live oyster clusters. Mats made of Vexar™-extruded polyethylene mesh commonly used in the aquaculture industry had oyster shells attached and were deployed over the leveled area and held in place with cement weights. The mesh openings in mats were 1 in. × 1 in. (22.2 mm × 22.2 mm) wide, with the mesh parts themselves being < 2 mm wide. The mats allowed for negligible amounts of sediment and oyster biodeposits getting trapped on top of the mats. This layer of stabilized shells provides the substrate for natural oyster larvae to recruit and forms a restored section of reef. As of Summer 2018, 3.25 acres of reef area has been restored on 89 individual reefs (L.J. Walters, pers. comm.).

Field Study Design

The field-based study utilized a BACI design where sediment samples were collected from oyster reefs at 7 timepoints: before the restoration, post-leveling of restored reefs, and 1 week, 1 month, 6 months, 9 months, and 12 months after restoration. Three treatments were utilized with a total of four reefs in each treatment: dead, restored, and natural reefs (Fig. 1). Restored sites were selected based on obtaining the largest latitudinal spread of sites within the Mosquito Lagoon's restoration area and working within the constraints of reef sites permitted for restoration. This study chose to leverage an active restoration program, which limited the ability to achieve an optimal spatial arrangement of sites but did improve the application of the findings to other restoration projects. Natural and dead sites near the restored reefs were selected to serve as positive and negative controls, while also allowing for assessment of confounding environmental variables (e.g., seasonal shifts in water chemistry and temperature) at each restored reef.

Sample collection from all reefs began in May 2017, 3 weeks before restoration activities occurred. Within 1 week post-leveling, samples were collected only from the four restored reefs to observe any effects of the perturbation to surface layer sediments caused by the leveling process. Otherwise, each timepoint included the sampling of all four dead, restored, and natural reefs (12 reefs in total). Restoration occurred in June 2017. For each field sampling, four replicate sediment samples were collected from each of the 12 reefs over two consecutive days at haphazardly selected points within the intertidal zone during low tide (± 3 h). Samples were collected during low tide so that the sediment and mats were exposed; therefore, sediment could not resuspend in the water column. Each core was collected using the push core

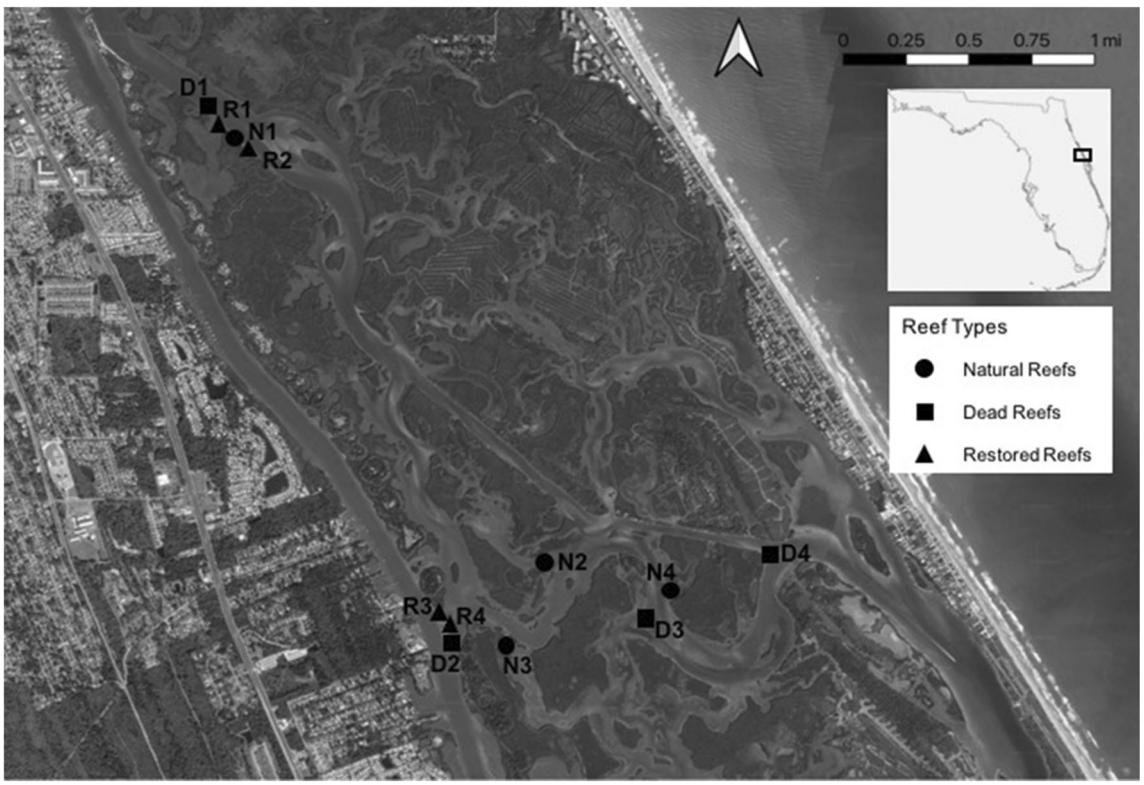

Fig. 1 Field experiment sites in Mosquito Lagoon, FL

method with a beveled 7-cm-diameter polycarbonate tube. Large pieces of shell (> 2 cm diameter) that interfered with penetration of the coring tube were carefully removed from the surface before coring. On restored reefs, oyster mats were pulled back by hand in order to access the sediment. Each sample consisted of two 0–5-cm-deep cores collected within 0.5 m from each other and composited into one sample in order to ensure a large enough mass of sediment was collected for lab analyses. Although the sediment accretion rate at these reefs is unknown, the 0–5 cm depth was chosen to represent the most recently deposited material, while minimizing older/deeper sediments that accumulated prior to the restoration activity. Each sediment core was field extruded, placed in sterile Whirl-Pak bags, and transported on ice back to the laboratory. At each reef, one surface water grab sample was collected from the top 10 cm of the water column 2–4 m adjacent to the reef in a 500-mL acid-washed Nalgene bottle and immediately placed on ice. Additionally, a handheld sonde (ProDSS; YSI Inc., Yellow Springs, OH, USA) was used to record surface water temperature, pH, dissolved oxygen (DO), salinity, and conductivity at a 10 cm depth, 2–4 m adjacent to each reef during sampling.

Once countable oysters appeared on the restored reefs (starting at 6 months post-restoration), oyster reef biophysical data were recorded at the same locations where sediment samples were collected. In order to accomplish this, marking flags were placed at the exact location of each sediment core during the 6-month, 9-month, and 12-month post-restoration samplings. Within 4 days of sediment collection, these sites were revisited and 0.25-m^2 quadrats were placed directly on top of the flagged locations. The number of live oysters, shell lengths of the first fifty oysters, reef height of the tallest point from the reef sediment, and five additional random points were recorded in each quadrat.

Surface Water Properties

Upon return to the laboratory, surface water samples were vacuum filtered through a 0.45-μm membrane filter and acidified with distilled, deionized H_2SO_4 to a pH value < 2. Samples were stored at 4 °C until analysis for nitrate + nitrite (herein referred to as NO_3^-), NH_4^+, SRP, and DOC. Concentrations of NO_3^-, NH_4^+, and SRP were determined colorimetrically on a Seal AQ2 Automated Discrete Analyzer (Seal Analytical, Mequon, WI, USA) using EPA methods 353.2 Rev. 2.0, 350.1 Rev. 2.0, and 365.1 Rev. 2.0, respectively (USEPA 1993). A Shimadzu TOC-L Analyzer (Shimadzu Scientific Instruments, Kyoto, Japan) was used to measure the concentration of non-purgeable DOC.

Sediment and Biodeposit Nutrient Pools

Upon return from the field, sediment samples were weighed and homogenized by hand in the laboratory. All samples contained some amount of shell; any shell fragments > 2 cm in diameter were excluded in sample processing. Extractable pools of bioavailable nutrients comprise nutrients in the porewater and are adsorbed to the surface of sediment particles that are removed by the addition of salts. Extractable nutrient pools were determined within 72 h of collection for NO_3^-, NH_4^+, SRP, and DOC by placing 3–4 g of sediment into a 40-mL centrifuge tube. A solution of 2 M KCl was added for NO_3^-, NH_4^+, and SRP extraction and 0.5 M K_2SO_4 for DOC extraction; the samples were agitated on an orbital shaker at 100 rpm for 1 h. Samples were then centrifuged at 4000 rpm at 10 °C for 10 min. The supernatant was filtered through a Supor 0.45-μm filter (Pall Corporation, Port Washington, NY, USA); acidified with distilled, deionized H_2SO_4 to a pH value < 2; and stored at 4 °C. Subsequent analysis for NO_3^-, NH_4^+, and SRP was performed as described above for surface water nutrients.

Total nutrient pools include nutrients both adsorbed and occluded with sediment particles and were measured on dried reef sediment only. Samples were dried at 70 °C until constant weight was achieved, then ground in a stainless steel ball mill container with a SPEX Sample Prep 8000M Mixer/Mill (SPEX, Metuchen, NJ, USA). Ground subsamples were used to determine total C and N on a Vario Micro Cube CN Analyzer (Elementar Americas Inc., Mount Laurel, NJ, USA). A subsample of dried, ground reef sediment was also combusted at 550 °C for 5 h to determine organic matter (OM) content via loss on ignition. Following loss-on-ignition analysis, solid-phase total P was determined by boiling the resulting ash in 1 M HCl on a hot plate for 1 h and filtering through Whatman #41 filter papers (Andersen 1976). Samples were then analyzed for total P on the Seal AQ2 Automated Discrete Analyzer via method 365.1 Rev. 2.0 (USEPA 1993).

Laboratory Experiment

The controlled laboratory experiment utilized *C. virginica* collected in Mosquito Lagoon from a single fringing reef composed of a recently restored dead margin on one side and an intact live, natural portion on the other side. Restoration of the 65.5-m^2 dead margin occurred in June 2017. Live oyster clusters were collected from the restored and natural portions of the reef during Summer 2018 to create two treatment groups based on time elapsed for oyster recruitment to occur: (1) less than or equal to 14 months old (herein referred to as "juveniles") on the restored portion of the reef and (2) a mix of ages but dominated by larger oysters (herein referred to as "older") from the intact portion of the reef. Juveniles are defined by others as those with a mean shell length < 43 mm (Dalrymple and Carmichael 2015). Oyster length measurements were conducted at the end of each of the four feeding experiments (herein referred to as "rounds") to confirm that the juvenile treatment group consisted of significantly smaller mean shell

sizes than the older treatment. During four separate rounds of experiments (carried out on June 1, July 5, July 14, and August 7, 2018), seven clusters of 30–40 oysters were collected from each portion of reef (14 total clusters) during low tide and were placed on ice for transport back to the laboratory. At the time of each collection, a handheld sonde (ProDSS; YSI Inc., Yellow Springs, OH, USA) was placed at a 10 cm depth, 2–4 m off the reef edge to measure water temperature, dissolved oxygen, pH, salinity, and turbidity. A chlorophyll-*a* probe (Manta Plus; Eureka Water Probes, Austin, TX, USA) was also deployed at a 10 cm depth to measure chlorophyll concentrations as a proxy for phytoplankton concentration. Directly after oyster collection, approximately 1300 L of surface water was collected 4–5 m off shore at the Canaveral National Seashore kayak ramp (450 m from the site of oyster collection) and transferred into a PVC-coated Kolaps-A-Tank (Burch Inc., Fort Dodge, IA, USA) for transport to the lab.

Upon return to the laboratory, oysters were immediately scrubbed clean of epibionts and sediment and oyster clusters were placed in individual 75.7-L (20-gallon) glass aquarium tanks filled with site water for a total of 24 h. The 24-h time period was chosen based on a pilot study conducted with three tanks containing 30–40 oysters each sampled at 0 h, 4 h, 8 h, 12 h, 24 h, and 36 h after the introduction of oysters to determine the time needed for the oysters to remove all viable suspended organic matter, observed as chlorophyll-*a* concentrations approaching zero, while limiting the exposure time to the artificial tank conditions (see Supplementary Fig. 1 for example graphs of parameter change over time). Throughout the feeding period, a 120-gallon per hour (gph) recirculation pump was used to ensure even suspension of particulate matter in the water column and was placed below the surface of the funnel to prevent disturbance to biodeposits within the funnel (Fig. 2). The surface area of a 25-cm-wide plastic funnel was evenly covered with 30–40 oysters and placed in each of 14 total tanks. This allowed for the collection of biodeposits from all individuals within the cluster. Biodeposits fell with gravity into a glass mason jar attached to the bottom of the funnel (Fig. 2). No adjustment period was given for the oysters as water inside the tanks was identical to field conditions, and filtering began immediately upon tank setup. Three additional tanks with no oysters were used as controls in each round of the experiment.

During the 24-h feeding period, water quality parameters, chlorophyll-*a* concentration, and surface water were sampled five times in each tank at time 0 h, 2 h, 6 h, 12 h, and 24 h after placement into tanks, as described above. Surface water samples were collected in 20-mL scintillation vials at the tank's mid-depth beside the recirculation pump for later analysis of dissolved nutrient content. Water samples were immediately filtered through a 0.45-μm syringe filter, preserved, and quantified for NO_3^-, NH_4^+, and SRP, as described above for the field study.

After the 24-h feeding period, water was carefully siphoned out of the tanks while ensuring no disturbance to biodeposits, then the glass jars containing the biodeposits were removed. Biodeposits were separated from water in 40-mL centrifuge tubes by centrifuging at 4000 rpm and 10 °C for 5 min. The saltwater supernatant was discarded using a 5-mL pipette. Separation of feces and pseudofeces was not possible as both

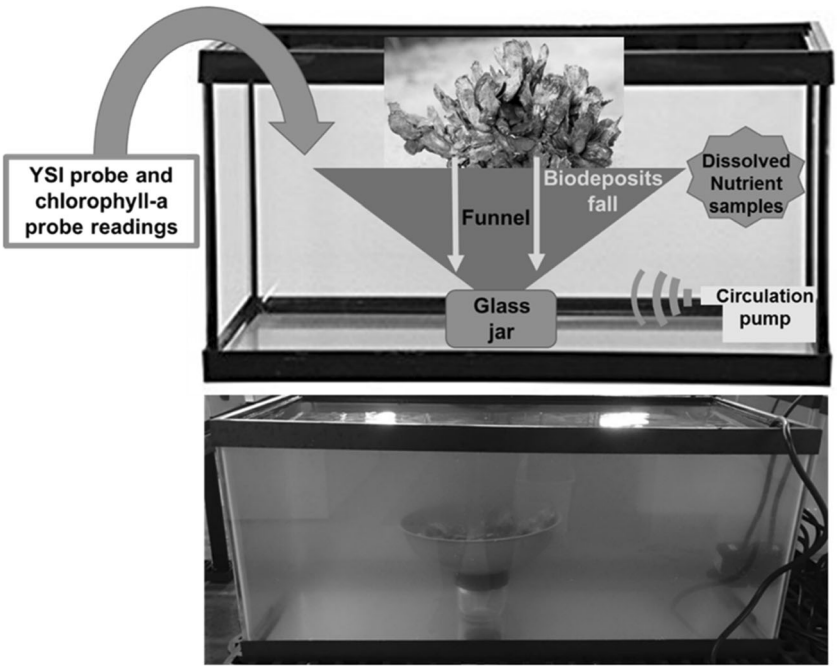

Fig. 2 Arrangement of tanks in the laboratory experiment. The placement of oysters and locations for taking water quality measurements and surface water samples in each tank are indicated

components mixed well to form an aggregate of biodeposits at the bottom of the jars. For the extraction of DOC, NO_3^-, NH_4^+, and SRP, 0.5 g of biodeposits was placed in 40-mL centrifuge tubes and mixed with 2 M KCl. The samples were agitated on an orbital shaker at 100 rpm for 1 h. Samples were then centrifuged at 4000 rpm and 10 °C for 10 min. The supernatant was filtered through a Supor 0.45-μm filter; acidified to a pH value < 2 with distilled, deionized H_2SO_4; stored at 4 °C; and analyzed for DOC, NO_3^-, NH_4^+, and SRP. The remainder of the biodeposits was placed in aluminum tins, weighed for total wet weight, and dried at 70 °C in a gravimetric drying oven for at least 3 days until a constant weight was achieved. The sum of the mass for extraction and gravimetric water content was used to estimate the total mass of biodeposits produced in each experimental tank.

After biodeposit processing, all oysters were measured for shell length. The wet tissue was removed and dried at 70 °C until constant weight was achieved to obtain total dry tissue weight in order to standardize all nutrient measurements to grams of dry weight (herein referred to as g dry wt) in each tank. The total process of oyster collection, 24-h feeding, and biodeposit analysis was repeated four times (rounds 1–4) to reach an *n* value of 28 for each treatment group.

Statistical Analyses

Data analysis was performed in R, version 3.5.1 (R Core Team 2018). For the field experiment, linear mixed-effects models were used to analyze nutrient pools with the command "lmer" in the lme4 package (Pinheiro et al. 2016; Bates et al. 2015). The interaction of treatment (dead, natural, or restored reef) and sampling time were applied as fixed effects in the models, and individual reef was a random effect. Homogeneity of variance was assessed using Levene's tests, and normality of the residuals was assessed by visually inspecting Q-Q plots. *p* values were obtained from the models by using the lmerTest package and running ANOVA on the models (Kuznetsova et al. 2017). A least squares means post hoc test was used to identify significant differences among the pairwise comparisons between the treatments and between the timepoints. All results were considered statistically significant at $\alpha = 0.05$. Additionally, principal component analysis was utilized to assess the variability between individual reefs with the package FactoMineR (Le et al. 2008). In order to assess the effect of geography on sediment properties and the random effect of reef, Google Earth (Google, 2019) was utilized to calculate distance to the nearest inlet and channel width for each reef. Distance to the nearest inlet was determined using lines drawn through major boating channels to the inlet entrance, and channel width was determined using the nearest shoreline perpendicular to the reef. Correlation tables were used to assess the strength of correlations between sediment properties and geographical parameters (latitude, channel width). A separate correlation table analyzed the relationship between sediment properties and oyster reef biophysical data at the 6-month, 9-month, and 12-month samplings. All correlation tables were computed with Microsoft Excel, and the critical value was calculated using the *r* statistic to assess the significance of the coefficients at $\alpha = 0.05$ and $\alpha = 0.01$.

For the lab experiment, measurements were standardized to grams of dry tissue weight in each tank. For water quality parameters and dissolved nutrients over the 24-h feeding period, end (24-h) measurements were subtracted from start (0-h) measurements for every water quality and nutrient parameter. Data was tested for the assumptions of a normal distribution with the Shapiro-Wilk test and for homogeneity of variance with Levene's test. Water quality parameters and surface water nutrients met both of these assumptions, and a logarithmic transformation was applied to the biodeposit nutrient data in order to meet assumptions. A two-way ANOVA was used to assess differences between the treatment groups and the interaction between treatment and rounds 1–4 of the experiment. Where the interactive effect was significant, Tukey's HSD post hoc test was used to assess the differences between individual rounds. All results were considered statistically significant at $\alpha = 0.05$.

Results

Field Study

Surface Water Properties

Time was a significant predictor for all water quality parameters and dissolved nutrients measured ($p < 0.001$), and treatment was not significant for any. Surface water DOC concentrations ranged from a low concentration of 1.6 mg L^{-1} during the March sampling to a high concentration of 13.1 mg L^{-1} during the first June sampling. Surface water NO_3^- concentrations were consistently below detection (BD) or near the detection limit (0.003 mg NO_3^- L^{-1}). Surface water NH_4^+ was below detection at both 6 months and 12 months post-restoration (detection limit 0.07 mg NH_4^+ L^{-1}). The average temperature during the summer samplings was 29.0 ± 0.3 °C (mean ± standard error), and that during the winter samplings was 16.5 ± 0.3 °C. The average % dissolved oxygen in the surface waters was 94.1 ± 1.9% over 12 months and did not differ between treatments. Salinity changed between every sampling, with a high level of 44.6 ± 0.1 ppt before restoration (June 2017) and a low level of 29.7 ± 0.2 ppt at 12 months post-restoration (June 2018). Surface water pH averaged 7.96 ± 0.03 for all reefs over 12 months.

Sediment Nutrients

The random effect of reef and the interaction of treatment with time were significant predictors for extractable nutrient pools (Table 1). Extractable DOC concentrations on dead and natural reefs did not differ throughout the study and averaged 0.083 ± 0.004 g kg^{-1} and 0.095 ± 0.004 g kg^{-1}, respectively. Restored reef DOC concentrations decreased after the leveling of loose shells and measured higher than natural and dead reefs by 9 months post-restoration (Fig. 3a). Extractable NO_3^- concentrations were BD at 1 month and 6 months and only differed by treatment during before restoration (Fig. 3b). After the oyster mats were deployed, extractable NH_4^+ concentrations generally increased on restored reefs, from 6.83 ± 1.20 g kg^{-1} at 1 week to 10.78 ± 1.61 g kg^{-1} at 1 month post-restoration (Fig. 3c). From 1 month onwards, restored reef NH_4^+ concentrations did not change over time and averaged 9.98 ± 0.81 g kg^{-1}, which was consistently higher than that of dead reefs (3.63 ± 0.38 g kg^{-1}). Extractable SRP was generally higher in dead reef sediments (1.29 ± 0.08 g kg^{-1}) throughout the study, compared to natural reefs (0.51 ± 0.05 g PO_4^{3-} kg^{-1}), while restored reefs remained intermediate in value (Fig. 3d).

The random effect of reef and the interaction of treatment with time were significant for soil OM and all total nutrient pools (Table 1, Fig. 4). Organic matter content was generally greater on natural reefs (0.95 ± 0.03 g kg^{-1}) compared to dead reefs (0.55 ± 0.03 g kg^{-1}), while restored reef concentrations were between natural and dead and generally increased over time (Fig. 4a). Total C maintained an average of 54.6 ± 2.1 g C kg^{-1} over the entire study on dead reefs, which was similar to natural reef concentrations (53.9 ± 1.6 g C kg^{-1}). Total C on restored reefs peaked 1 month post-restoration but remained similar in concentration to natural and dead reefs for the rest of the study (Fig. 4b). Total N concentrations were generally higher on natural reefs (1.40 ± 0.04 g N kg^{-1}) compared to dead reefs (0.58 ± 0.05 g N kg^{-1}) throughout the entire study (Fig. 4c). Restored reef sediments increased a total of 79% from the 1- to 6-month samplings; from 6 months onwards, restored reef sediments measured an average of 0.98 ± 0.07 g N kg^{-1}, compared to 0.57 ± 0.07 in dead reef sediments and 1.52 ± 0.07 g N kg^{-1} in natural reef sediments (Fig. 4c). Natural reefs contained higher total P concentrations than both dead and restored reefs at every timepoint. Total P on restored reef sediment increased by 48% from 1 to 6 months post-restoration and began to approach natural reef levels at 9 months and 12 months (Fig. 4d).

Reef Geographical and Biophysical Variables

Extractable NH_4^+, SRP, DOC, and total C were all negatively correlated with distance to the Lagoon inlet (north of the study region), indicating higher concentrations in these parameters on reefs closer to the point of tidal exchange. The width of the channel on which the reef was located was not significantly correlated with sediment nutrients. Principal component analysis of all sediment samples (n = 48) taken at every timepoint showed clustering of replicates on each individual reef but significant separation between many of the reefs within the same treatment (Fig. 5). The four natural reefs showed the most similarity within treatment, with three out of four reefs clustered together in the PCA.

Correlations of sediment nutrients with oyster reef biophysical parameters measured at 6 months, 9 months, and 12 months (once recruitment began on restored reefs) showed positive correlations between oyster density, reef height, and shell length at all three timepoints (Table 2). Extractable DOC and NH_4^+ also demonstrated positive correlations with reef biophysical parameters that generally strengthened over the study period, while extractable SRP was negatively correlated with biophysical properties, particularly at 6 months and 9 months post-restoration. Extractable NO_3^- and total C were not correlated with oyster reef parameters. Reef height had the greatest number of significant correlations with sediment nutrients (n = 24), but all three biophysical parameters were similarly strong correlates (Table 2).

Lab Experiment

Over the four repetitions of the experiment (rounds), the oysters collected from the 12–14-month-old portion of reef (juveniles) averaged 34.7 ± 0.4 mm in shell length, while the

Table 1 p values from the results of linear mixed-effects models of all reef sediment properties

	DOC	NO_3^-	NH_4^+	SRP	OM	TC	TN	TP
Treatment	0.194	0.806	0.082	**0.0497**	0.079	0.124	**0.049**	***0.004***
Time	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	0.070	***< 0.001***	***< 0.001***
Treatment:time	***< 0.001***	***< 0.001***	***< 0.001***	***0.004***	**0.045**	***< 0.001***	**0.032**	***< 0.001***
Random reef	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***

Values in bold denote significance at the critical value of $p \leq 0.05$, and bold and italics denote values where $p \leq 0.01$

DOC dissolved organic carbon, *NO_3^-* nitrate, *NH_4^+* ammonium, *SRP* soluble reactive phosphorus, *OM* organic matter, *TC* total carbon, *TN* total nitrogen, *TP* total phosphorus

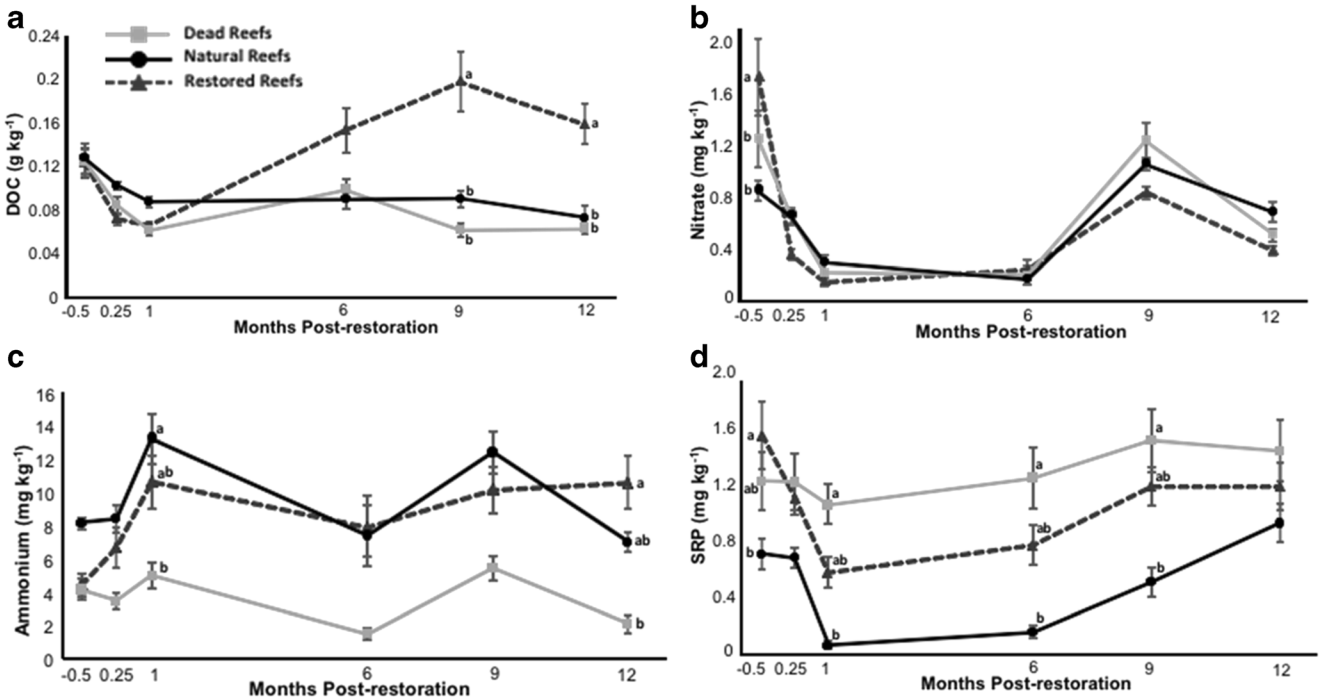

Fig. 3 Sediment DOC (**a**), NO_3^- (**b**), NH_4^+ (**c**), and SRP (**d**) pools over time in dead, natural, and restored reefs. Error bars indicate standard error, and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means pairwise comparison

oysters collected from the natural portion of the reef (older) averaged 57.1 ± 1.6 mm, resulting in a significant difference in size distribution between treatments ($p < 0.001$). Over all four rounds, juvenile oyster tanks contained 36 ± 8 individual oysters (5.38 ± 1.84 g dry tissue wt per tank), compared to 25 ± 5 individuals for the older tanks (10.69 ± 3.77 g dry tissue wt per tank). Due to the variability in the number of individuals and total biomass in each tank, data were standardized according to g dry wt^{-1} of oyster biomass for each experimental unit (i.e., tank).

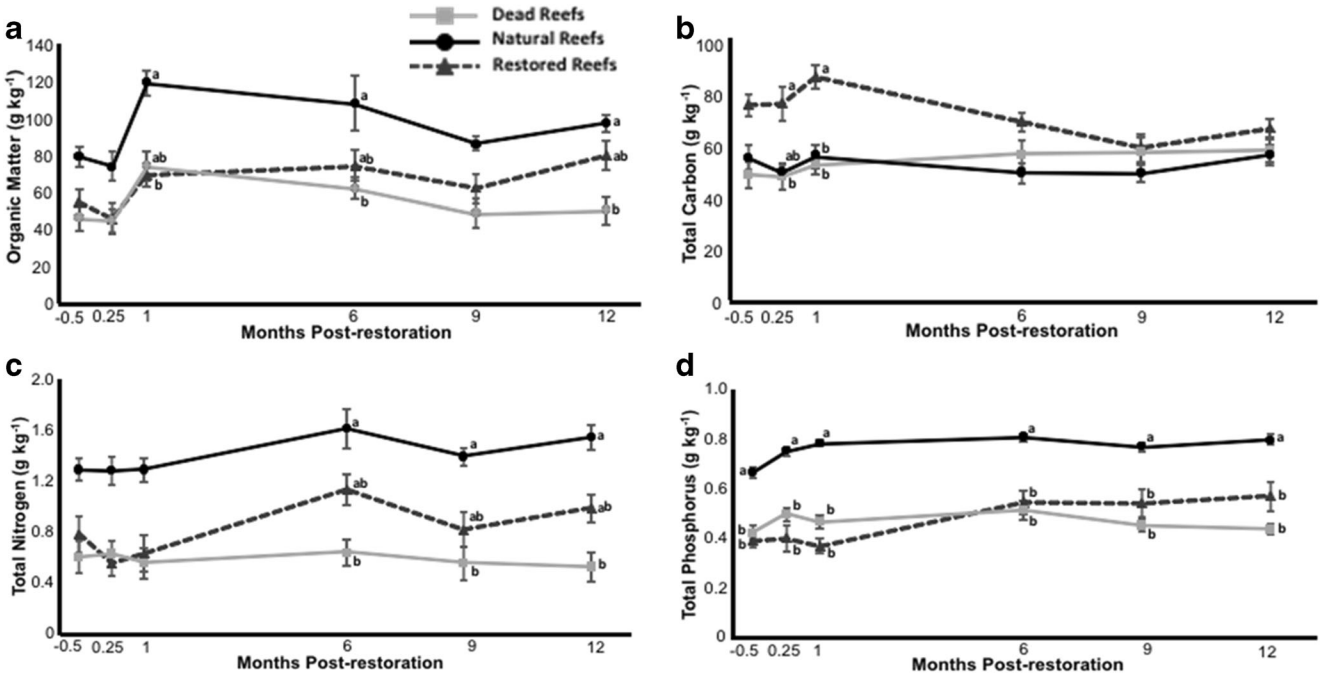

Fig. 4 OM (**a**), total C (**b**), total N (**c**), and total P (**d**) pools over time in dead, natural, and restored reefs. Error bars indicate standard error, and letters denote significantly different means ($p \leq 0.05$) between treatments according to a post hoc least squares means pairwise comparison

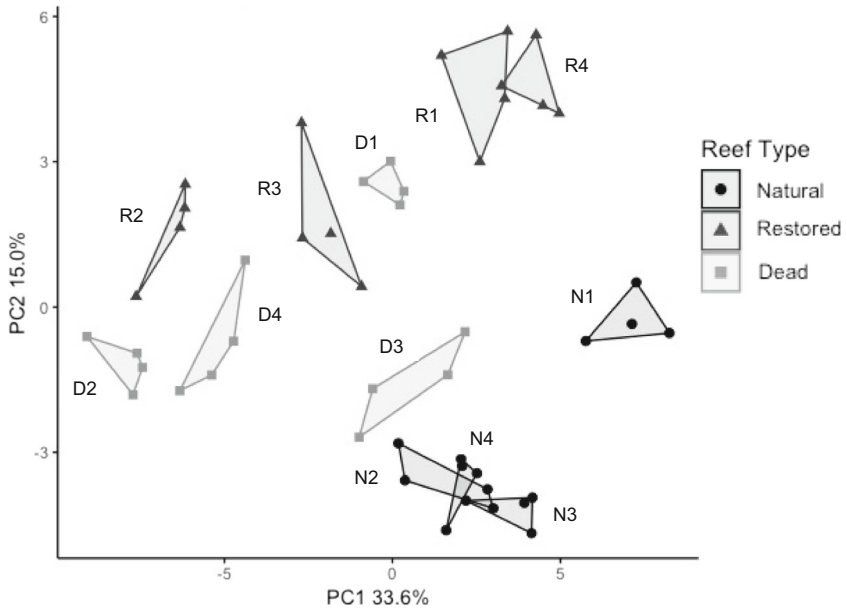

Fig. 5 Results of principal component analysis of all 48 samples taken from oyster reefs (4 samples per reef, 4 reefs per treatment) for all sediment properties

Surface Water

Surface water salinity averaged 33.8 ± 0.1 ppt and did not differ between treatments (control, juvenile, and older) during any round of the experiment (Supplementary Table 1). Water temperature stabilized at room temperature (25.0 ± 0.8 °C) in all tanks during each round of the experiment. The control treatment (without oysters) generally differed from both of the oyster treatments for dissolved oxygen, pH, and turbidity. Dissolved oxygen content in the water column decreased by 40.4 ± 7.8% over 24 h in the oyster tanks but only by 1.6 ± 1.2% in the control tanks ($p < 0.001$; Fig. 6a). Starting pH was 8.04 ± 0.01 in all tanks and generally remained constant in the control treatments. In oyster treatments, pH declined over 24 h by an average of 0.5 and differed between treatments in every round (Supplementary Table 1). Turbidity declined in all treatments and differed between juvenile and older oysters ($p < 0.001$), with an overall decrease of 4.1 ± 0.64 nephelometric turbidity unit (NTU) for controls, 1.43 ± 0.12 NTU g dry wt^{-1} for juvenile oysters, and 0.71 ± 0.06 NTU g dry wt^{-1} for older oysters (Fig. 6b).

Both juvenile and older oysters reduced chlorophyll-*a* concentrations to levels below the control treatments ($p < 0.001$). On average, chlorophyll-*a* concentrations decreased by 36 ± 3% in controls due to the natural deposition of suspended particulate matter, 81 ± 2% in the juvenile oysters, and 76 ± 2% in older oysters (Fig. 6c). When concentrations were standardized to gram of dry body weight in each tank, juvenile oysters decreased chlorophyll-*a* by 2.9 ± 0.2 μg L^{-1} g dry wt^{-1} and older oysters by 1.5 ± 0.1 μg L^{-1} g dry wt^{-1} during the 24-h experiments.

Table 2 Pearson's correlation coefficients between sediment properties and reef biophysical properties

	Months	DOC	NO_3^-	NH_4^+	SRP	OM	TC	TN	TP
Oyster density	6	0.043	−0.067	0.203	***−0.480***	***0.390***	0.014	***0.643***	***0.622***
	9	***0.519***	−0.195	0.229	−0.273	***0.548***	0.179	***0.488***	***0.465***
	12	***0.596***	0.132	***0.657***	−0.158	***0.531***	0.181	***0.540***	***0.512***
Reef height	6	0.072	−0.057	**0.352**	***−0.599***	***0.368***	0.033	***0.715***	***0.650***
	9	***0.384***	−0.227	***0.462***	***−0.452***	***0.485***	−0.035	***0.538***	***0.548***
	12	***0.416***	0.186	***0.584***	−0.138	***0.593***	0.150	***0.577***	***0.575***
Shell length	6	−0.091	−0.105	0.100	***−0.596***	***0.374***	−0.124	***0.634***	***0.559***
	9	0.119	0.033	**0.357**	***−0.614***	***0.645***	−0.066	***0.634***	***0.595***
	12	**0.346**	0.182	***0.469***	−0.245	***0.607***	0.216	***0.586***	***0.495***

Values in bold denote significance at the critical value of $p \leq 0.05$, and bold and italics denote values where $p \leq 0.01$

DOC dissolved organic carbon, NO_3^- nitrate, NH_4^+ ammonium, *SRP* soluble reactive phosphorus, *OM* organic matter, *TC* total carbon, *TN* total nitrogen, *TP* total phosphorus

Surface water DOC concentrations in the tanks fluctuated between 1.6 and 16.3 mg L^{-1} (average 5.2 ± 0.2 mg L^{-1}) over the various tanks and rounds but showed no clear pattern or difference between treatments. Similarly, surface water NO_3^- concentrations showed no treatment effect but differed by round (ranging from BD in rounds 2 and 3 to 0.05 mg L^{-1} in round 4). Surface water NH_4^+ exhibited a significant treatment × round interaction ($p < 0.001$). Over all four rounds, juvenile oysters increased surface water NH_4^+ concentrations after 24 h by 0.15 ± 0.02 mg NH_4^+ L^{-1} g dry wt^{-1} and older oysters by 0.11 ± 0.01 mg NH_4^+ L^{-1} g dry wt^{-1}, but overall NH_4^+ concentrations were lowest during round 2 and highest during round 1 (Fig. 6d). Surface water SRP showed high between-round variability and no significant treatment effect. On average, SRP increased by 1.5 ± 0.6 μg L^{-1} g dry wt^{-1} in oyster treatments over 24 h, while remaining steady in control treatments. Example graphs of changes over time for the water chemistry of round 1 are included in the Supplementary Material.

Biodeposit Nutrient Content

Despite the differences in the number of individual oysters needed to evenly fill the funnel, dry tissue weight between tanks, and filtration rates between treatments, the total wet weight of biodeposits collected over all four rounds was comparable (mean of 4.16 ± 2.52 g for juvenile tanks and 5.32 ± 3.32 g for older oyster tanks). All four biodeposit extractable nutrients (DOC, NO_3^-, NH_4^+, and SRP) indicated a significant effect of round ($p < 0.001$, $p = 0.008$, $p < 0.001$, and $p < 0.001$, respectively), and three of the four nutrients (DOC, NO_3^-, and NH_4^+) showed higher concentrations in juvenile oyster biodeposits compared to older oyster biodeposits ($p < 0.001$, $p < 0.001$, and $p = 0.046$, respectively; Table 3).

Discussion

Previous research has demonstrated the rapid recovery of several sediment nutrient pools and biogeochemical properties beneath recently restored intertidal oyster reefs as indicated by a convergence of concentrations between restored and natural (live, control) reefs during a space-for-time substitution study (Chambers et al. 2018). In some cases, concentrations of key nutrients were highest 1 year post-restoration, compared to 4-year- and 7-year-old restored reefs. The current study focuses on the short-term (first year) response of biogeochemistry to restoration and disentangles a key cause of the observed age-nutrient paradox (i.e., how can some nutrient concentrations be *higher* in young reefs that have fewer, smaller

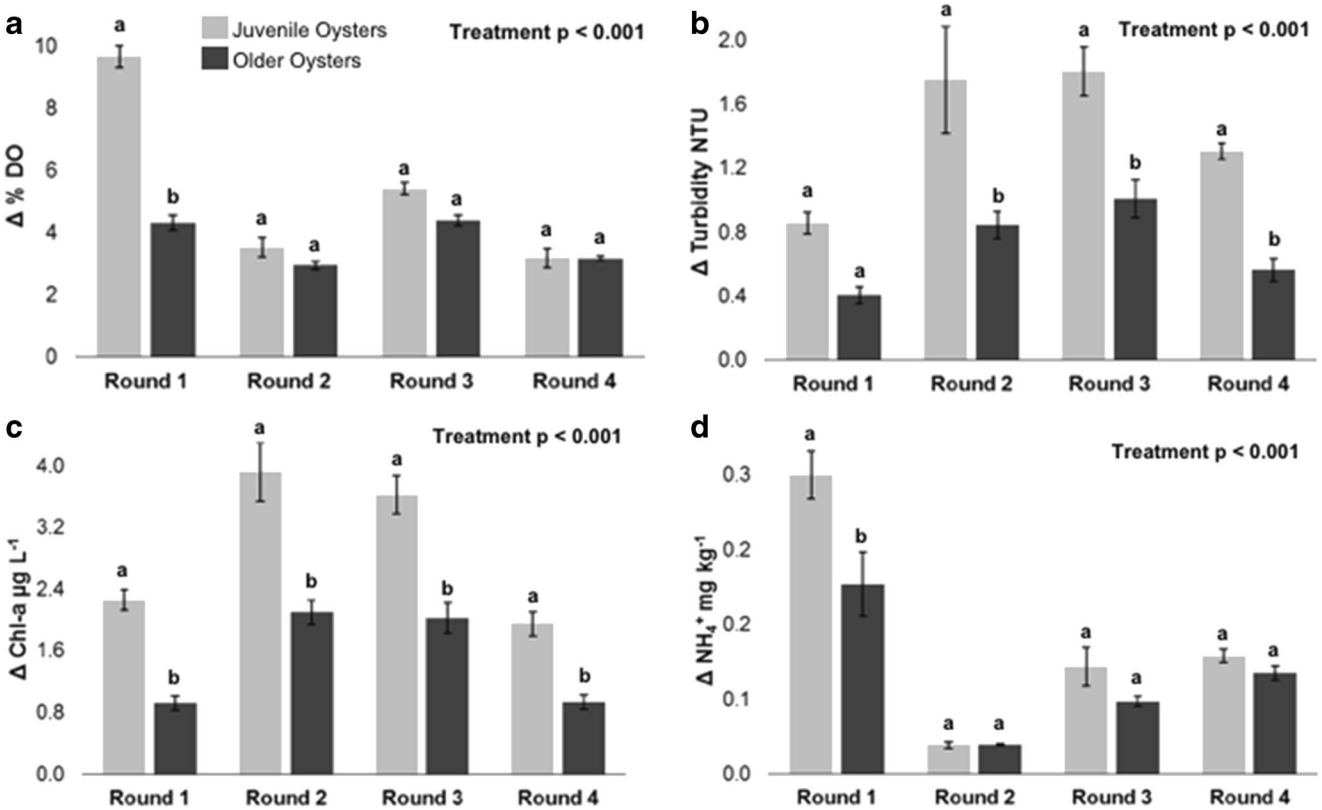

Fig. 6 Mean of the change (24–0 h values) in % DO (**a**), turbidity (**b**), Chl-*a* (**c**), and NH_4^+ (**d**) in tank waters for all rounds of the laboratory experiment. Letters indicate significant differences between the treatment means in each round

Table 3 Mean concentrations of extractable nutrients for juvenile and older oyster biodeposits, all oysters, and natural reef sediments from the field experiment

	DOC	SRP	NO_3^-	NH_4^+
Juvenile biodeposits	**183 ± 26.7**	9.77 ± 1.33	**2.04 ± 0.23**	**36.7 ± 5.30**
Older biodeposits	**108 ± 11.7**	7.74 ± 0.75	**1.06 ± 0.13**	**31.4 ± 4.97**
All oyster biodeposits	146 ± 15.3	8.76 ± 0.77	1.55 ± 0.15	34.1 ± 3.62
Natural reef sediment (0–5 cm)	95.2 ± 3.94	0.51 ± 0.05	0.62 ± 0.04	9.58 ± 0.55

Values in bold denote significance between the juvenile and older treatments at $p \leq 0.05$ according to Tukey's HSD post hoc test. Units are mg kg^{-1}

DOC dissolved organic carbon, *SRP* soluble reactive phosphorus, NO_3^- nitrate, NH_4^+ ammonium

oysters than older, more densely colonized reefs?) with a controlled laboratory experiment. Specifically, results indicate one of the key contributors to nutrient increases in surface sediments during the first year of restoration is the ability of younger oysters to produce, on a gram-for-gram basis, more nutrient-rich biodeposits than older oysters.

Sediment Extractable Nutrients Respond Rapidly to Restoration

Extractable nutrient pools, which are comprised of both the nutrients dissolved within the sediment porewater and those loosely adsorbed to the cation exchange complex, are considered the most bioavailable, or labile, forms of sediment nutrients (Reddy and DeLaune 2008). In this study, extractable DOC and NH_4^+ both demonstrated a significant increase during the first year of restoration, with DOC concentrations of 60% greater at 9 months post-restoration than at pre-restoration, and NH_4^+ concentrations of 136% higher at 12 months post-restoration than at pre-restoration (Fig. 3). Using natural reef concentrations as an index for success, DOC concentrations exceeded natural reefs by 9 months post-restoration, whereas NH_4^+ concentrations were statistically equivalent to natural reefs and different from dead reefs by 12 months post-restoration. This rapid rise of extractable nutrients so early in the reef development process is interesting, given that the biophysical parameters of reef height and shell length (and inferred oyster body mass) are significantly less than those of natural reefs for the duration of the field experiment. Prior work on Mosquito Lagoon's oyster reefs also showed that despite 1-year-old restored reefs having both a lower reef thickness and density of live oysters than natural reefs, the restored reef NH_4^+ concentrations were statistically equivalent to and DOC concentrations exceeded that of the natural reef sediments (Chambers et al. 2018).

These findings concur with prior research indicating the highest sediment extractable DOC concentrations occurred 1 year post-restoration and were strongly correlated with several indicators of C and N cycling (including extracellular enzyme activity and total C and N), suggesting DOC may be a strong indicator of organic matter accumulation and general biogeochemical activity in reef sediments (Chambers et al. 2018). The factors that influence a net accumulation or release of organic C on restored reefs are currently not well understood. Although this study shows rapid accumulation of DOC and organic matter in surface sediments, reef type and habitat context can create differences in C deposition rates (Fodrie et al. 2017; Westbrook et al. 2019). Dissolved organic C may accumulate in reef sediments due to the physical structure of reefs, which influences water velocities, promoting deposition of suspended organic matter (Lenihan 1999; Reidenbach et al. 2013; Southwell et al. 2017; Kitsikoudis et al. 2020). On oyster reefs, biodeposits are likely the dominant autochthonous source of DOC. In our laboratory, biodeposit DOC concentrations were a full order of magnitude greater than concentrations found in the top 0–5 cm of sediment in the field and demonstrate the ability of biodeposition to serve as a DOC source (Table 3). It also suggests that a large portion of C and other nutrients in biodeposits is distributed into multiple sinks. The large difference in nutrient concentrations between biodeposits and surface sediments may be attributed to the action of heterotrophic bacteria that consume DOC as oyster density and nutrient availability increase rapidly on restored reefs (Kellogg et al. 2014; Volaric et al. 2018). Although mucus-bound feces and pseudofeces settle to the reef surface several times faster than natural particle deposition (Widdows et al. 1998; Dame 1999), the biodeposits only reach the sediment when bottom water velocities are low and they may be resuspended in the water column prior to deposition (Newell et al. 2005; Porter et al. 2018).

Significantly more research has been published regarding N cycling on natural and restored oyster reefs due to the interest in quantifying denitrification (Newell et al. 2002; Kellogg et al. 2013; Smyth et al. 2013b, 2016; Humphries et al. 2016; Westbrook et al. 2019). In this study, as in others, the dominant form of inorganic N on oyster reefs was found to be NH_4^+ (NO_3^- concentrations were low or BD for most sediment samples). The majority of N assimilated by oysters is used for tissue growth, and the rest is excreted as urine, 70% of which is NH_4^+ (Bayne and Hawkins 1992). The dominance of NH_4^+-N can also be attributed to oyster biodeposition; the increased availability of organic C and N from biodeposits stimulates microbial respiration, driving sediments to become anaerobic and promoting N mineralization and dissimilatory nitrate

reduction to ammonium (Smyth et al. 2013a) over nitrification (Dame et al. 1985, 1989; Christensen et al. 2000; Carlsson et al. 2012; Lunstrum et al. 2018). Evidence for the connection between field sediment NH_4^+ and biodeposits is also found in this laboratory experiment, where biodeposits contained on average 34 ± 3.6 mg NH_4^+ kg^{-1} g dry wt^{-1}, 1 order of magnitude greater than concentrations of 9.6 ± 0.6 mg NH_4^+ kg^{-1} found in natural reef sediments (Table 3). Other studies have documented NH_4^+ fluxing from the sediments of *C. virginica* reefs (Plutchak et al. 2010; Kellogg et al. 2013; Smyth et al. 2013b; Southwell et al. 2017). In addition to this autochthonous source, the field study revealed sediment NH_4^+ is also influenced by seasonal trends and periods of oyster recruitment. For example, an early increase (58% between 1 week and 1 month) in NH_4^+ in restored reef sediments was mirrored by a similar increase in natural reef NH_4^+. This is assumed to be related to estuary-wide allochthonous sources of N, particularly since measurable oyster physical parameters did not occur until month 6 on restored reefs. Seasonal influences in NH_4^+ dynamics were also measured in sediment on intertidal oyster reefs in South Carolina (Dame et al. 1989) and North Carolina (Smyth et al. 2013b); caged oysters in Jamaica Bay, New York (Hoellein and Zarnoch 2014); and subtidal oyster reefs in the Chesapeake Bay (Kellogg et al. 2013).

Extractable SRP concentrations declined immediately following restoration activity then tracked an intermediate concentration between dead reefs (highest) and reference reefs (lowest). Past studies have demonstrated that bivalves excrete low concentrations of SRP (Dame et al. 1989; Magni et al. 2000) and bivalve reef sediments can be a net source of SRP (Asmus et al. 1995; Newell et al. 2005; Kellogg et al. 2013). One of the few studies of sediment SRP flux in eastern oyster reefs found that very little (8%) of the annual total P flux is deposited as SRP into sediments (Dame et al. 1989). This study, along with that of Chambers et al. (2018), found a generally negative relationship between sediment extractable SRP and both oyster density and reef age, which seems to contradict the idea of oyster reefs serving as a new source of SRP. This may be explained by an observed decrease in sediment pH as reefs age, which can promote P adsorption to iron (Krom and Berner 1981), as well as greater Ca^{2+} from the dissolution of $CaCO_3$ shell causing occlusion of P.

Total Sediment Nutrient Pools Respond More Slowly to Restoration

Organic matter and total nutrient pools can accumulate on oyster reefs in response to both allochthonous and autochthonous sources. The physical structure of reefs is known to influence water velocities, which can enhance deposition of suspended organic matter (Lenihan 1999; Reidenbach et al. 2013; Southwell et al. 2017; Kitsikoudis et al. 2020). Since suspended particulate matter, such as phytoplankton, varies seasonally (Ubertini et al. 2012), this may account for the observed fluctuations in surface sediment organic matter content throughout the year. Despite seasonality, natural reefs maintained relatively higher organic matter than dead reefs, while concentrations increased in restored reefs (Fig. 4). This trend indicates organic matter content was an effective measure in discerning between the three reef types. In contrast, total C content did not show treatment differences within the first year. This may be because total C includes both organic and inorganic forms of C. While natural reef sediments have a higher abundance of organic C (as indicated by organic matter content), dead reefs are assumed to have greater inorganic C due to the dissolution of accumulated dead shells. This idea, in combination with the loss of CO_2 that occurs during shell calcification, has led to an ongoing debate regarding whether oyster reefs are a net C source or sink (Fodrie et al. 2017). Both total N and P sediment pools slowly increased during the first year of restoration but did not achieve concentrations similar to natural reefs within the 12-month timeframe. In the study by Chambers et al. (2018), it took 7 years for total N concentrations to mimic those of natural reefs, while total P did not differ significantly with reef age.

Individual Reefs Have Unique Biogeochemical Properties

Regardless of reef type (i.e., treatment), both extractable and total sediment nutrient pools were strongly influenced by which individual reef the sample was collected on (i.e., a significant random "reef" effect in the statistical model; Fig. 5). These reef-to-reef differences in sediment biogeochemistry are not surprising, given natural variations in reef size, shape, history, landscape context, etc., which could not be fully controlled for in a study that leverages real, on-the-ground, community-driven restoration activities (although all efforts were made to choose study reefs with similar geophysical properties). This field study approach was chosen over a heavily controlled experimental approach to increase the applicability and transferability of the data to other restoration projects by including field variation. In Mosquito Lagoon, the north-to-south gradient of study reefs is thought to be a significant contributor to individual reef differences (Chambers et al. 2018). Northern sites are closer to the ocean inlet and experience greater tidal amplitude (Smith 1993), which may contribute to greater reef heights and oyster densities on these reefs (K. Kibler and L.J. Walters, personal communication). In general, individual natural reefs were more similar to each other than individual dead or restored reefs (Fig. 5). This may suggest the environmental conditions that allow a reef to persist over time are similar for all natural reefs, whereas the conditions that lead to degradation are more diverse.

Juvenile Oysters Produce Nutrient-Rich Biodeposits

Juvenile oysters are known to have a generally linear shell growth rate in the first year of life (Munroe et al. 2017). This quantifiable daily growth of shell and the net assimilation of N into soft tissues differs from adult oysters, which do not always have measurable shell growth and can even lose soft tissue biomass and N content over time (Dalrymple and Carmichael 2015). These life history traits can help explain the results of both the field study and laboratory tank experiment, which documented an overall significant effect of age class on the change (end–start concentration) in surface water chlorophyll-*a*, % DO, pH, and turbidity over 24 h, whereby juvenile oysters decreased concentrations though filtration/feeding by a greater quantity than older oysters, when corrected for the mass of dry body tissue in each tank (Supplementary Fig. 2). The 93% greater average decrease in chlorophyll-*a* concentrations per g dry wt over a 24-h feeding period by juvenile oysters suggests more rapid feeding rates on a g dry wt basis when compared to older oysters. Similarly, calculated filtration rates under ideal conditions at 23 °C using densities and shell lengths of oysters collected from intertidal restored reefs in LA found that oysters < 75 mm accounted for 70% of filtration capacity on a reef while oysters > 75 mm accounted for the remaining 30% (La Peyre et al. 2014). Other studies investigating chlorophyll-*a* reductions above oyster reefs in the field show varying results and generally did not account for oyster size or density or temporal scale (Dame et al. 1992; Dame and Libes 1993; Wilson-Ormond et al. 1997; Cressman et al. 2003; Grizzle et al. 2008, 2018). Significant reductions in chlorophyll-*a* have been documented just downstream of a reef (Dame et al. 1992; Nelson et al. 2004; Grizzle et al. 2008) and 10–20 m away from a reef (Grizzle et al. 2018), while no reductions were observed in other studies (Plutchak et al. 2010).

Concurrently with the decrease in chlorophyll-*a*, the average decline in surface water turbidity in the juvenile treatment was double that of the older treatment, while the reduction in DO was 50% greater in the juvenile treatment (all corrected per g dry wt). The greater decrease in % DO in the juvenile oyster tanks matches the response in water pH, and as respiration (CO_2 production) begins to dominate over photosynthesis, the excess CO_2 reacts with H_2O to drive down the pH (Supplementary Fig. 1). As oysters consume phytoplankton, DO will naturally decrease. However, this decrease is not expected to have negatively impacted *C. virginica*; prior studies have shown *C. virginica* is able to tolerate oxygen concentrations as low as 80% below saturation, as well as a wide range of temperature and salinity combinations (Shumway and Koehn 1982). Overall, this study provides corroborating evidence that restoring oyster reefs can provide measurable improvements to water clarity within the first year (Dame et al. 1984; Grizzle et al. 2008; Kellogg et al. 2013; zu Ermgassen et al. 2013; La Peyre et al. 2014).

This study also quantified nutrient concentrations in oyster excretions. Juvenile oysters produced biodeposits that had, on average, 69% greater DOC, 82% greater NO_3^-, and 19% greater NH_4^+ concentrations (per g dry wt) than older oysters (Table 3). These elevated extractable nutrient concentrations in juvenile biodeposits could be a result of more particles being rejected as pseudofeces, thus enriching total biodeposits in inorganic particles deemed not nutritious enough by the labial palps (Newell and Jordan 1983). Additionally, the higher chlorophyll-*a* reduction rates could indicate that juvenile oysters have higher success in ingesting organic-rich particles and then convert them into a higher concentration of inorganic particles present in feces (Jordan 1987). A prior study by Dalrymple and Carmichael (2015) also showed that juvenile pseudofeces had significantly higher N content than adult pseudofeces. Haven and Morales-Alamo (1966) found that the largest group of oysters in their study (mean weight 73.3 g) deposited less material per unit weight than the three smaller groups, again supporting the notion that juvenile oysters capture more particles and produce more pseudofeces. These results were repeated later by Dalrymple and Carmichael (2015), where production of both feces and pseudofeces increased with oyster size up to 1.2–1.5 g dry wt oyster^{-1} and then decreased for adult oysters larger than this. A true mass balance of nutrients exchanged between water column and oysters could not be determined with our study design but could be a promising future research direction. Overall, the results from this laboratory experiment begin to elucidate the age-nutrient paradox—why sediment nutrient concentrations are so high on recently restored (≤ 1-year-old) reefs in the field despite the lower density and body mass of the juvenile oysters colonizing them.

Biogeochemical Properties Reflect Common Monitoring Metrics

Researchers and restoration practitioners have been promoting the universal inclusion of monitoring into restoration activities as a way to evaluate success and improve methodologies and approaches (e.g., Kennedy et al. 2011; Baggett et al. 2015). However, metrics for success have generally focused on an organismal approach to monitoring, such as reef area, reef height, density of live oysters, and oyster size-frequency distributions (Coen and Luckenbach 2000; Baggett et al. 2015). As the recognized benefits of oyster reef restoration expand to include ecosystem services beyond the oysters themselves (Peterson et al. 2003; Coen et al. 2007; Grabowski et al. 2012; La Peyre et al. 2014) and these benefits change depending upon landscape context (Grabowski et al. 2005; Smyth et al. 2015; Westbrook et al. 2019), it follows that additional metrics for success may need to be added to the repertoire of

restoration monitoring to evaluate the development of functional ecosystem services, such as biogeochemical cycling and nutrient storage. Measuring the availability of sediment nutrients in conjunction with reef biophysical parameters can help give context to and indicate the progression of the downstream effects of restoration in a complex reef-dependent food web (Peterson et al. 2003; Rodney and Paynter 2006; Humphries et al. 2011; Rezek et al. 2017). The increase in sediment nutrient pools within months after the restoration of Mosquito Lagoon's intertidal reefs matches the recovery timeframe of other ecosystem services such as water filtration capacity and habitat provision for fish and invertebrates (La Peyre et al. 2014; Pierson and Eggleston 2014; Dillon et al. 2015; Rezek et al. 2017). Based on this study and previous work, we proposed that N pools are the sediment biogeochemical parameters most useful in (1) showing a rapid and quantifiable response to oyster reef restoration, (2) correlating strongly with traditional biophysical monitoring metrics, and (3) having ecological significance as a biogeochemical property. Specifically, sediment extractable NH_4^+ began to increase and differentiate from dead reef sediments within 1 month post-restoration, reaching concentrations equivalent to natural reefs (and statistically different from dead reefs) by 12 months post-restoration. Extractable NH_4^+ was positively correlated with oyster density, reef height, and shell length during the first year post-restoration, with a particularly strong correlation with reef height at every timeframe. The metric of reef height is thought to be the most consistent of our biophysical metrics; shell length and oyster density are known to vary greatly between sites during the first year of growth (Dillon et al. 2015; Munroe et al. 2017) and within an individual reef (Lenihan 1999; Luckenbach et al. 2005; Hanke et al. 2017). Ammonium has also been identified by this and other studies as having a strong link to oyster metabolism (as the primary nitrogen form found in biodeposition; Hammen et al. 1966; Dame et al. 1984) and is an ecologically significant nutrient readily available for assimilation by bacteria, phytoplankton, and higher plants (Reddy and DeLaune 2008). Total N pools represent all forms of N (organic and inorganic N, including N occluded to mineral matter). Total N accumulates more slowly than extractable NH_4^+ but appears to do so in a generally linear pattern (Chambers et al. 2018). Total N was also the only parameter to show a strong ($p \leq 0.01$) correlation with all three traditional monitoring metrics measured at every timepoint quantified (6 months, 9 months, and 12-months post-restoration) and had the greatest number of significant correlations to the other biogeochemical parameters measured in this study (seven correlations at $p \leq 0.01$). This suggests total N accumulation is a very robust indicator of long-term biogeochemical development of restored oyster reef sediments.

Conclusion

This study found that young (1-year-old or less) restored oyster reefs can significantly change C, N, and P pools in both the sediment and water column on a reef within months of restoration. With the recognition of oyster reefs as areas of enhanced sediment biogeochemical cycling, measuring changes in sediment properties can be considered for restoration monitoring plans where ecosystem-level impacts are a primary objective. The timeframe for the development of ecosystem services related to nutrient cycling is also important to restoration managers aiming to improve water quality and reduce nutrient loading with the enhancement of eastern oyster populations (Kellogg et al. 2014 and references therein).

The laboratory study presented helps to unravel the age-nutrient paradox where juvenile oysters, on a g dry tissue wt basis, reduced water column chlorophyll-*a* concentrations at a significantly greater rate and produced biodeposits with higher extractable nutrient concentrations than older oysters. This showed that juvenile oysters on restored reefs are able to provide a greater contribution to DOC and inorganic sediment nutrient pools than older oysters on natural reefs. Dalrymple and Carmichael (2015) compared N content in biodeposits from juvenile and adult oysters, but to our knowledge, no studies have measured extractable nutrient concentrations in biodeposits. Further characterization of the fate and transport of oyster biodeposits is necessary to fully understand the magnitude and extent of their role in biogeochemical cycling within the reef. Furthermore, this BACI study is the first to describe the impacts of restoration on reef sediment properties in the time scale of months. Despite the influence of estuary-wide seasonal trends, comparisons between dead, natural, and restored intertidal reefs revealed clear differences in nutrient pools that correlated highly with the physical condition of oysters. The lack of significant differences of certain variables between treatments or times can be attributed to the high within-treatment variability due to the irregularities in individual reefs and to non-biogenic factors which affect the surface of reefs. Filling knowledge gaps on C and P dynamics in oyster reef sediments as well as understanding the biogeochemical impacts beyond 1 year post-restoration will help to further define oyster reefs as valuable biogeochemical hot spots in coastal ecosystems.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12237-020-00874-2.

Acknowledgments The authors would like to thank Jessica Chevallier, Chelsea Nitsch, Evan Duga, Hayden Denton, Joshua Breithaupt, Havalend Steinmuller, Sarah Harttung, Paul Sacks, Christian Pilato, Iris Fang, and members of the Coastal & Estuarine Ecology Lab and Kibler Ecohydraulics Lab at the University of Central Florida for help with field sampling and lab processing. A special thanks to Jennifer Bennett and Angela Ferebee for their help with the field sampling and lab processing for the oyster feeding experiment.

Funding This research was funded by the National Science Foundation under the Coupled Human-Natural Systems Program, Award # 1617374 to LGC and LJW.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Andersen, J.M. 1976. An ignition method for determination of total phosphorus in lake sediments. *Water Research* 10 (4): 329–331.

Asmus, H., R.M. Asmus, and G.F. Zubillaga. 1995. Do mussel beds intensify the phosphorus exchange between sediment and tidal waters? *Ophelia* 41 (1): 37–55.

Baggett, L.P., S.P. Powers, R.D. Brumbaugh, L.D. Coen, B.M. Deangelis, J.K. Greene, B.T. Hancock, et al. 2015. Guidelines for evaluating performance of oyster habitat restoration. *Restoration Ecology* 23 (6): 737–745. https://doi.org/10.1111/rec.12262.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.

Bayne, B. L. and A. J. S. Hawkins. 1992. Ecological and physiological aspects of herbivory in benthic suspension-feeding molluscs. In *Plant-animal interactions in the marine benthos*, ed. D. M. John, S. J. Hawkins, and J. H. Prince, 46th ed., 265–388. Oxford: Clarendon.

Beck, M.W., R.D. Brumbaugh, L. Airoldi, A. Carranza, L.D. Coen, C. Crawford, O. Defeo, G.J. Edgar, B. Hancock, M.C. Kay, H.S. Lenihan, M.W. Luckenbach, C.L. Toropova, G. Zhang, and X. Guo. 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. *BioScience* 61 (2): 107–116.

Blomberg, B.N., B. Lebreton, T.A. Palmer, G. Guillou, J.B. Pollack, and P.A. Montagna. 2017. Does reef structure affect oyster food resources? A stable isotope assessment. *Marine Environmental Research* 127: 32–40. https://doi.org/10.1016/j.marenvres.2017.03.003.

Carlsson, M.S., P. Engström, O. Lindahl, L. Ljungqvist, J.K. Petersen, L. Svanberg, and M. Holmer. 2012. Effects of mussel farms on the benthic nitrogen cycle on the swedish west coast. *Aquaculture Environment Interactions* 2 (2): 177–191. https://doi.org/10.3354/aei00039.

Chambers, L.G., S.A. Gaspar, C.J. Pilato, H.E. Steinmuller, K.J. McCarthy, P.E. Sacks, and L.J. Walters. 2018. How well do restored intertidal oyster reefs support key biogeochemical properties in a coastal lagoon? *Estuaries and Coasts* 41 (3): 784–799. https://doi.org/10.1007/s12237-017-0311-5.

Christensen, P.B., S. Rysgaard, N.P. Sloth, T. Dalsgaard, and S. Schwærter. 2000. Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. *Aquatic Microbial Ecology* 21: 73–84. https://doi.org/10.3354/ame021073.

Coen, L.D., and M.W. Luckenbach. 2000. Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? *Ecological Engineering* 15 (3-4): 323–343. https://doi.org/10.1016/S0925-8574(00)00084-7.

Coen, L.D., R.D. Brumbaugh, D. Bushek, R.E. Grizzle, M.W. Luckenbach, M.H. Posey, S.P. Powers, and S.G. Tolley. 2007. Ecosystem services related to oyster restoration. *Marine Ecology Progress Series* 341: 303–307. https://doi.org/10.3354/meps341299.

Core Team, R. 2018. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Cressman, K., M.H. Posey, M.A. Mallin, L.A. Leonard, and T.D. Alphin. 2003. Effects of oyster reefs on water quality in a tidal creek estuary. *Journal of Shellfish Research* 22: 753–762.

Dalrymple, D.J., and R.H. Carmichael. 2015. Effects of age class on N removal capacity of oysters and implications for bioremediation. *Marine Ecology Progress Series* 528: 205–220. https://doi.org/10.3354/meps11252.

Dame, R.F. 1999. Oyster reefs as components of estuarine nutrient cycling: Incidental or controlling? In *Oyster reef habitat restoration: A synopsis and synthesis of approaches*, ed. M.W. Luckenbach, R. Mann, and J.A. Wesson, 267–280. Williamsburg: Virginia Institute of Marine Science.

Dame, R.F., and S. Libes. 1993. Oyster reefs and nutrient retention in tidal creeks. *Journal of Experimental Marine Biology and Ecology* 171 (2): 251–258. https://doi.org/10.1016/0022-0981(93)90007-B.

Dame, R.F., R.G. Zingmark, and E. Haskin. 1984. Oyster reefs as processors of estuarine materials. *Journal of Experimental Marine Biology and Ecology* 83 (3): 239–247.

Dame, R.F., T.G. Wolaver, and S.M. Libes. 1985. The summer uptake and release of nitrogen by an intertidal oyster reef. *Netherlands Journal of Sea Research* 19 (3-4): 265–268.

Dame, R.F., J.D. Spurrier, and T.G. Wolaver. 1989. Carbon, nitrogen and phosphorus processing by an oyster reef. *Marine Ecology Progress Series* 54: 249–256. https://doi.org/10.3354/meps054249.

Dame, R.F., J.D. Spurrier, and R.G. Zingmark. 1992. In situ metabolism of an oyster reef. *Journal of Experimental Marine Biology and Ecology* 164 (2): 147–159. https://doi.org/10.1016/0022-0981(92)90171-6.

Dillon, K.S., M.S. Peterson, and C.A. May. 2015. Functional equivalence of constructed and natural intertidal eastern oyster reef habitats in a northern Gulf of Mexico estuary. *Marine Ecology Progress Series* 528: 187–203. https://doi.org/10.3354/meps11269.

Dybas, C.L. 2002. Florida's Indian River Lagoon: An estuary in transition. *BioScience* 52 (7): 555–559.

Fodrie, F.J., A.B. Rodriguez, R.K. Gittman, J.H. Grabowski, N.L. Lindquist, C.H. Peterson, M.F. Piehler, and J.T. Ridge. 2017. Oyster reefs as carbon sources and sinks. *Proceedings of the Royal Society B: Biological Sciences* 284 (1859): 20170891. https://doi.org/10.1098/rspb.2017.0891.

Garvis, S.K., P.E. Sacks, and L.J. Walters. 2015. Formation, movement, and restoration of dead intertidal oyster reefs in Canaveral National Seashore and Mosquito Lagoon, Florida. *Journal of Shellfish Research* 34 (2): 251–258. https://doi.org/10.2983/035.034.0206.

Grabowski, J.H., R.A. Hughes, D.L. Kimbro, and M.A. Dolan. 2005. How habitat setting influences restored oyster reef communities. *Ecology* 86 (7): 1926–1935.

Grabowski, J.H., R.D. Brumbaugh, R.F. Conrad, A.G. Keeler, J.J. Opaluch, C.H. Peterson, M.F. Piehler, S.P. Powers, and A.R. Smyth. 2012. Economic valuation of ecosystem services provided by oyster reefs. *BioScience* 62 (10): 900–909. https://doi.org/10.1525/bio.2012.62.10.10.

Grizzle, R.E., J.R. Adams, and L.J. Walters. 2002. Historical changes in intertidal oyster (*Crassostrea virginica*) reefs in a Florida lagoon potentially related to boating activities. *Journal of Shellfish Research* 21: 749–756.

Grizzle, R.E., J.K. Greene, and L.D. Coen. 2008. Seston removal by natural and constructed intertidal eastern oyster (*Crassostrea virginica*) reefs: A comparison with previous laboratory studies, and the value of in situ methods. *Estuaries and Coasts* 31 (6): 1208–1220. https://doi.org/10.1007/s12237-008-9098-8.

Grizzle, R.E., A. Rasmussen, A.J. Martignette, K. Ward, and L.D. Coen. 2018. Mapping seston depletion over an intertidal eastern oyster (*Crassostrea virginica*) reef: Implications for restoration of multiple habitats. *Estuarine, Coastal and Shelf Science* 212: 265–272. https://doi.org/10.1016/j.ecss.2018.07.013.

Hammen, C.S., H.F. Miller Jr., and W.H. Geer. 1966. Nitrogen excretion of *Crassostrea virginica*. *Comparative Biochemistry and Physiology* 17 (4): 1199–1200.

Hanke, M.H., M.H. Posey, and T.D. Alphin. 2017. The influence of habitat characteristics on intertidal oyster (*Crassostrea virginica*) populations. *Marine Ecology Progress Series* 571: 121–138.

Haven, D.S., and R. Morales-Alamo. 1966. Aspects of biodeposition by oysters and other invertebrate filter feeders. *Limnology and Oceanography* 11 (4): 487–498. https://doi.org/10.4319/lo.1966.11.4.0487.

Haven, D.S., and R. Morales-Alamo. 1968. Occurrence and transport of faecal pellets in suspension in a tidal estuary. *Sedimentary Geology* 2 (2): 141–151.

Hoellein, T.J., and C.B. Zarnoch. 2014. Effect of eastern oysters (*Crassostrea virginica*) on sediment carbon and nitrogen dynamics in an urban estuary. *Ecological Applications* 24 (2): 271–286. https://doi.org/10.1890/12-1798.1.

Hoellein, T.J., C.B. Zarnoch, and R.E. Grizzle. 2015. Eastern oyster (*Crassostrea virginica*) filtration, biodeposition, and sediment nitrogen cycling at two oyster reefs with contrasting water quality in Great Bay Estuary (New Hampshire, USA). *Biogeochemistry* 122 (1): 113–129. https://doi.org/10.1007/s10533-014-0034-7.

Humphries, A.T., and M.K. La Peyre. 2015. Oyster reef restoration supports increased nekton biomass and potential commercial fishery value. *PeerJ* 3: e1111. https://doi.org/10.7717/peerj.1111.

Humphries, A.T., M.K. La Peyre, M.E. Kimball, and L.P. Rozas. 2011. Testing the effect of habitat structure and complexity on nekton assemblages using experimental oyster reefs. *Journal of Experimental Marine Biology and Ecology* 409 (1-2): 172–179. https://doi.org/10.1016/j.jembe.2011.08.017.

Humphries, A.T., S.G. Ayvazian, J.C. Carey, B.T. Hancock, S. Grabbert, D. Cobb, C.J. Strobel, and R.W. Fulweiler. 2016. Directly measured denitrification reveals oyster aquaculture and restored oyster reefs remove nitrogen at comparable high rates. *Frontiers in Marine Science* 3: 1–10. https://doi.org/10.3389/fmars.2016.00074.

Jackson, J.B., M.X. Kirby, W.H. Berger, K.A. Bjorndal, L.W. Botsford, B.J. Bourque, R.H. Bradbury, R. Cooke, J. Erlandson, J.A. Estes, T.P. Hughes, S. Kidwell, C.B. Lange, H.S. Lenihan, J.M. Pandolfi, C.H. Peterson, R.S. Steneck, M.J. Tegner, and R.R. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293 (5530): 629–639.

Jordan, S.J. 1987. *Sedimentation and remineralization associated with biodeposition by the American oyster Crassostrea virginica (Gmelin)*. Doctoral Dissertation: University of Maryland, College Park, MD, USA.

Kellogg, M.L., J.C. Cornwell, M.S. Owens, and K.T. Paynter. 2013. Denitrification and nutrient assimilation on a restored oyster reef. *Marine Ecology Progress Series* 480: 1–19. https://doi.org/10.3354/meps10331.

Kellogg, M.L., A.R. Smyth, M.W. Luckenbach, R.H. Carmichael, B.L. Brown, J.C. Cornwell, M.F. Piehler, M.S. Owens, D.J. Dalrymple, and C.B. Higgins. 2014. Use of oysters to mitigate eutrophication in coastal waters. *Estuarine, Coastal and Shelf Science* 151: 156–168. https://doi.org/10.1016/j.ecss.2014.09.025.

Kennedy, V.S., D.L. Breitburg, M.C. Christman, M.W. Luckenbach, K. Paynter, J. Kramer, K.G. Sellner, J. Dew-Baxter, C. Keller, and R. Mann. 2011. Lessons learned from efforts to restore oyster populations in Maryland and Virginia, 1990 to 2007. *Journal of Shellfish Research* 30 (3): 719–731.

Kirby, M.X. 2004. Fishing down the coast: Historical expansion and collapse of oyster fisheries along continental margins. *Proceedings of the National Academy of Sciences of the United States of America* 101 (35): 13096–13099. https://doi.org/10.1073/pnas.0405150101.

Kitsikoudis, V., K.M. Kibler, and L.J. Walters. 2020. In-situ measurements of turbulent flow over intertidal natural and degraded oyster reefs in an estuarine lagoon. *Ecological Engineering* 143: 105688. https://doi.org/10.1016/j.ecoleng.2019.105688.

Krom, M.D., and R.A. Berner. 1981. The diagenesis of phosphorus in a nearshore marine sediment. *Geochimica et Cosmochimica Acta* 45 (2): 207–216.

Kuznetsova, A., P.B. Brockhoff, and R.H. Christensen. 2017. lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software* 82: 1–26.

La Peyre, M.K., A.T. Humphries, S.M. Casas, and J.F. La Peyre. 2014. Temporal variation in development of ecosystem services from oyster reef restoration. *Ecological Engineering* 63: 34–44. https://doi.org/10.1016/j.ecoleng.2013.12.001.

Lê, S., J. Josse, and F. Husson. 2008. FactoMineR: A package for multivariate analysis. *Journal of Statistical Software* 25: 1–18.

Lenihan, H.S. 1999. Physical-biological coupling on oyster reefs: How habitat structure influences individual performance. *Ecological Monographs* 69 (3): 251–275. https://doi.org/10.1890/0012-9615(1999)069[0251:PBCOOR]2.0.CO;2.

Luckenbach, M.W., L.D. Coen, P.G. Ross, and J.A. Stephen. 2005. Oyster reef habitat restoration: Relationships between oyster abundance and community development based on two studies in Virginia and South Carolina. *Journal of Coastal Research* 21: 64–78.

Lunstrum, A., K. McGlathery, and A.R. Smyth. 2018. Oyster (*Crassostrea virginica*) aquaculture shifts sediment nitrogen processes toward mineralization over denitrification. *Estuaries and Coasts* 41 (4): 1130–1146. https://doi.org/10.1007/s12237-017-0327-x.

Mackenzie, C.L. 1996. History of oystering in the United States and Canada, featuring the eight greatest oyster estuaries. *Marine Fisheries Review* 58: 1–78.

Magni, P., S. Montani, C. Takada, and H. Tsutsumi. 2000. Temporal scaling and relevance of bivalve nutrient excretion on a tidal flat of the Seto Inland Sea, Japan. *Marine Ecology Progress Series* 198: 139–155. https://doi.org/10.3354/meps198139.

Mcclain, M.E., E.W. Boyer, C.L. Dent, S.E. Gergel, N.B. Grimm, P.M. Groffman, S.C. Hart, et al. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems* 6 (4): 301–312. https://doi.org/10.1007/s10021-003-0161-9.

Mortazavi, B., A.C. Ortmann, L. Wang, R.J. Bernard, C.L. Staudhammer, J.D. Dalrymple, R.H. Carmichael, and A.A. Kleinhuizen. 2015. Evaluating the impact of oyster (*Crassostrea virginica*) gardening on sediment nitrogen cycling in a subtropical estuary. *Bulletin of Marine Science* 91 (3): 323–341. https://doi.org/10.5343/bms.2014.1060.

Munroe, D., S. Borsetti, K. Ashton-Alcox, and D. Bushek. 2017. Early post-settlement growth in wild eastern oyster (*Crassostrea virginica* Gemlin 1791) populations. *Estuaries and Coasts* 40 (3): 880–888. https://doi.org/10.1007/s12237-016-0185-y.

Nelson, K.A., L.A. Leonard, M.H. Posey, T.D. Alphin, and M.A. Mallin. 2004. Using transplanted oyster (*Crassostrea virginica*) beds to improve water quality in small tidal creeks: A pilot study. *Journal of Experimental Marine Biology and Ecology* 298 (2): 347–368. https://doi.org/10.1016/S0022-0981(03)00367-8.

Newell, R.I.E. 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review. *Journal of Shellfish Research* 23: 51–61.

Newell, R.I.E., and S.J. Jordan. 1983. Preferential ingestion of organic material by the American oyster *Crassostrea virginica*. *Marine Ecology Progress Series* 13: 47–53.

Newell, R.I.E., J.C. Cornwell, and M.S. Owens. 2002. Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: A laboratory study. *Limnology and Oceanography* 47 (5): 1367–1379. https://doi.org/10.4319/lo.2002.47.5.1367.

Newell, R.I.E., R.R. Holyoke, and J.C. Cornwell. 2005. Influence of eastern oysters on nitrogen and phosphorus regeneration in Chesapeake Bay, USA. In *The comparative roles of suspension-feeders in ecosystems*, ed. R. Dame and S. Olenin, 93–120. Dordrecht: Springer.

Newell, R.I.E., W.M. Kemp, J.D. Hagy III, C.F. Cerco, J.M. Testa, and W.R. Boynton. 2007. Top-down control of phytoplankton by oysters in Chesapeake Bay, USA: Reply to Newell et al. (2007). *Marine Ecology Progress Series* 341: 299–301. https://doi.org/10.3354/meps341299.

Peterson, C.H., J.H. Grabowski, and S.P. Powers. 2003. Estimated enhancement of fish production resulting from restoring oyster reef habitat: Quantitative valuation. *Marine Ecology Progress Series* 264: 249–264. https://doi.org/10.3354/meps264249.

Phlips, E.J., S. Badylak, M.A. Lasi, R. Chamberlain, W.C. Green, L.M. Hall, J.A. Hart, J.C. Lockwood, J.D. Miller, L.J. Morris, and J.S. Steward. 2015. From red tides to green and brown tides: Bloom dynamics in a restricted subtropical lagoon under shifting climatic conditions. *Estuaries and Coasts* 38 (3): 886–904. https://doi.org/10.1007/s12237-014-9874-6.

Pierson, K.J., and D.B. Eggleston. 2014. Response of estuarine fish to large-scale oyster reef restoration. *Transactions of the American Fisheries Society* 143 (1): 273–288. https://doi.org/10.1080/00028487.2013.847863.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. nlme: Linear and Nonlinear Mixed E ects Models. R package version 3.1-127.

Plutchak, R., K. Major, J. Cebrian, C.D. Foster, M.E. Miller, A. Anton, K.L. Sheehan, K.L. Heck, and S.P. Powers. 2010. Impacts of oyster reef restoration on primary productivity and nutrient dynamics in tidal creeks of the north central Gulf of Mexico. *Estuaries and Coasts* 33 (6): 1355–1364. https://doi.org/10.1007/s12237-010-9327-9.

Pollack, J.B., D. Yoskowitz, H. Kim, and P.A. Montagna. 2013. Role and value of nitrogen regulation provided by oysters (*Crassostrea virginica*) in the Mission-Aransas. *PLoS One* 8 (6): e65314. https://doi.org/10.1371/journal.pone.0065314.

Porter, E.T., H. Franz, and R. Lacouture. 2018. Impact of eastern oyster *Crassostrea virginica* biodeposit resuspension on the seston, nutrient, phytoplankton, and zooplankton dynamics: A mesocosm experiment. *Marine Ecology Progress Series* 586: 21–40. https://doi.org/10.3354/meps12417.

Reddy, K.R., and R.D. DeLaune. 2008. *Biogeochemistry of wetlands: Science and applications*. New York: CRC.

Reidenbach, M.A., P. Berg, A. Hume, J.C. Hansen, and E.R. Whitman. 2013. Hydrodynamics of intertidal oyster reefs: The influence of boundary layer flow processes on sediment and oxygen exchange. *Limnology and Oceanography: Fluids and Environments* 3 (1): 225–239. https://doi.org/10.1215/21573689-2395266.

Rezek, R. J., B. Lebreton, E. B. Roark, T. A. Palmer, and J. B. Pollack. 2017. How does a restored oyster reef develop? An assessment based on stable isotopes and community metrics. *Marine Biology* 164. Springer Berlin Heidelberg: 1–17. https://doi.org/10.1007/s00227-017-3084-2.

Rodney, W.S., and K.T. Paynter. 2006. Comparisons of macrofaunal assemblages on restored and non-restored oyster reefs in mesohaline regions of Chesapeake Bay in Maryland. *Journal of Experimental Marine Biology and Ecology* 335 (1): 39–51. https://doi.org/10.1016/j.jembe.2006.02.017.

Shumway, S., and R.K. Koehn. 1982. Oxygen consumption in the American oyster *Crassostrea virginica*. *Marine Ecology Progress Series* 9: 59–68.

Smith, N.P. 1993. Tidal and nontidal flushing of Florida's Indian River Lagoon. *Estuaries* 16 (4): 739–746.

Smyth, A.R., S.P. Thompson, K.N. Siporin, W.S. Gardner, M.J. McCarthy, and M.F. Piehler. 2013a. Assessing nitrogen dynamics throughout the estuarine landscape. *Estuaries and Coasts* 36 (1): 44–55. https://doi.org/10.1007/s12237-012-9554-3.

Smyth, A.R., N.R. Geraldi, and M.F. Piehler. 2013b. Oyster-mediated benthic – pelagic coupling modifies nitrogen pools and processes. *Marine Ecology Progress Series* 493: 23–30. https://doi.org/10.3354/meps10516.

Smyth, A.R., M.F. Piehler, and J.H. Grabowski. 2015. Habitat context influences nitrogen removal by restored oyster reefs. *Journal of Applied Ecology* 52 (3): 716–725. https://doi.org/10.1111/1365-2664.12435.

Smyth, A.R., N.R. Geraldi, S.P. Thompson, and M.F. Piehler. 2016. Biological activity exceeds biogenic structure in influencing sediment nitrogen cycling in experimental oyster reefs. *Marine Ecology Progress Series* 560: 173–183. https://doi.org/10.3354/meps11922.

Smyth, A.R., A.E. Murphy, I.C. Anderson, and B. Song. 2018. Differential effects of bivalves on sediment nitrogen cycling in a shallow coastal bay. *Estuaries and Coasts* 41 (4): 1147–1163. https://doi.org/10.1007/s12237-017-0344-9.

Southwell, M.W., J.J. Veenstra, C.D. Adams, E.V. Scarlett, and K.B. Payne. 2017. Changes in sediment characteristics upon oyster reef restoration, NE Florida, USA. *Journal of Coastal Zone Management* 20: 442.

Testa, J.M., D.C. Brady, J.C. Cornwell, M.S. Owens, L.P. Sanford, C.R. Newell, S.E. Suttles, and R.I.E. Newell. 2015. Modeling the impact of floating oyster (*Crassostrea virginica*) aquaculture on sediment-water nutrient and oxygen fluxes. *Aquaculture Environment Interactions* 7 (3): 205–222. https://doi.org/10.3354/aei00151.

Ubertini, M., S. Lefebvre, A. Gangnery, K. Grangeré, R. Le Gendre, and F. Orvain. 2012. Spatial variability of benthic-pelagic coupling in an estuary ecosystem: Consequences for microphytobenthos resuspension phenomenon. *PLoS One* 7 (8): e44155. https://doi.org/10.1371/journal.pone.0044155.

USEPA 1993. Methods for the Determination of Inorganic Substances in Environmental Samples (EPA/600/R-93/100).

Volaric, M.P., P. Berg, and M.A. Reidenbach. 2018. Oxygen metabolism of intertidal oyster reefs measured by aquatic eddy covariance. *Marine Ecology Progress Series* 599: 75–91. https://doi.org/10.3354/meps12627.

Wall, L.M., L.J. Walters, R.E. Grizzle, and P.E. Sacks. 2005. Recreational boating activity and its impact on the recruitment and survival of the oyster *Crassostrea virginica* on intertidal reefs in Mosquito Lagoon, Florida. *Journal of Shellfish Research* 24: 965–973.

Westbrook, P., L. Heffner, and M.K. La Peyre. 2019. Measuring carbon and nitrogen bioassimilation, burial, and denitrification contributions of oyster reefs in Gulf coast estuaries. *Marine Biology* 166 (1): 1–14. https://doi.org/10.1007/s00227-018-3449-1.

Widdows, J., M.D. Brinsley, P.N. Salkeld, and M. Elliott. 1998. Use of annular flumes to determine the influence of current velocity and bivalves on material flux at the sediment-water interface. *Estuaries* 21 (4): 552–559.

Wilson-Ormond, E.A., E.N. Powell, and S.M. Ray. 1997. Short-term and small-scale variation in food availability to natural oyster populations: Food, flow and flux. *Marine Ecology* 18 (1): 1–34. https://doi.org/10.1111/j.1439-0485.1997.tb00424.x.

zu Ermgassen, P.S., M.D. Spalding, R.E. Grizzle, and R.D. Brumbaugh. 2013. Quantifying the loss of a marine ecosystem service: Filtration by the eastern oyster in US estuaries. *Estuaries and Coasts* 36 (1): 36–43. https://doi.org/10.1007/s12237-012-9559-y.