

# Changes in Sediment Characteristics upon Oyster Reef Restoration, NE Florida, USA

## Abstract

As ecosystem engineers, oysters alter a host of physical and chemical characteristics of their environment. Oyster reef construction (or restoration) can therefore be expected to have wide ranging effects, from increased refuge for organisms, to lower turbidity. We measured changes in sediment characteristics and benthic nutrient fluxes near a constructed intertidal oyster reef in the Guana Tolomato Matanzas National Estuarine Research Reserve in NE Florida, USA. Within one year of reef construction, particle size distribution shifted towards finer sediment, and organic matter content increased to  $7.3\% \pm 2.1$  (standard error) compared to  $2.9\% \pm 0.8$  (standard error) at control sites, where oysters were absent. After three years, up to 15 cm of this fine, organic-rich sediment had accreted immediately shoreward of the reef. In benthic flux experiments performed under dark conditions, reef sediments released  $167 \mu\text{M}/\text{m}^2/\text{hr}$   $\text{NH}_4^+$  compared to  $-4 \mu\text{M}/\text{m}^2/\text{hr}$  from control site sediments. Under light conditions both reef and control fluxes were insignificant. This was presumably due to uptake by benthic microalgae; chlorophyll *a* concentrations were 3 times higher in reef sediments compared to controls. Deposition of organic rich sediment near oyster reefs might render important ecosystem services from carbon burial and perhaps increased denitrification. However, burial of live oysters with excessive sediment accretion might threaten reef success in some cases. Our results highlight the complexity of physical-biological interactions and the need for complete assessment of ecosystem services in order to optimize design and thoroughly evaluate restoration outcomes.

**Keywords:** Oyster restoration; Benthic nutrient flux; Carbon sequestration; Sediment biogeochemistry; Shoreline protection; Sedimentary organic matter

## Introduction

Oysters, dominated by *Crassostrea virginica* on the U.S. East Coast, are widely recognized for their economic value and ecosystem services. In addition to direct revenue from oyster harvesting, oysters provide hard substrate for sessile organisms, habitat complexity and refuge for small mobile organisms, and attenuation of wave energy for shoreline protection [1,2]. Furthermore, the filtering capacity of oysters is thought to improve water clarity and influence the structure of estuarine ecosystems with respect to recycling of organic matter and nutrients [3-5], benthic-pelagic coupling, and energy flow to the microbial loop [6,7]. At the creek-level scale, oyster communities have been shown to significantly affect concentrations of particulate and dissolved nutrients and organic matter, though effects often depended on season and tidal state [5,8]. Due to a variety of factors such as overharvesting, destructive fishing practices, and disease, worldwide oyster abundance has declined by an estimated 85% [4,9,10]. In addition to the economic loss of harvestable oysters, the loss of ecosystem services is of great concern, and may exacerbate water quality declines from nutrient loading, land development, dredging, and other anthropogenic activities [4,2].

Therefore, restoration of oyster reefs has received increasing attention and investment from coastal municipalities. However, the wisdom of pursuing restoration is controversial [11-16]. Part of this disagreement may stem from differences in how the goals of each restoration project are defined, the complexity of ecosystem services rendered by oyster reefs, and whether benefits from all of these services are included in the assessment of restoration success [1,2]. The effect of oyster filtration on water quality has been extensively studied and

debated [11,12,14,15], but less research has been devoted to the effect of oyster reefs on the surrounding sediment. Reefs act to attenuate wave energy, possibly facilitating deposition of fine sediment [8]; this process may work in concert with oyster filtration to increase light penetration that may then shift ecosystems towards more benthic primary producers [6]. Finer particles and much higher organic matter (OM) content in oyster-associated sediments suggests a substantial role for carbon and nutrient removal by burial [8,17] and benthic algal uptake where light penetration is sufficient [6]. However, mesocosm experiments show that physical factors such as bottom shear can influence sediment resuspension and benthic micro-algal biomass, making the system more complex and the likelihood of OM burial versus remineralization more difficult to predict [7]. Several recent studies suggest that sediments associated with natural and restored oyster reefs have high rates of denitrification and may thus represent important sites for long term nitrogen removal [17-20]. Whole-creek studies and some mesocosm studies do not parse the contributions of the oysters themselves versus the associated sediments but rather consider the reef-sediment system as a whole [3,5,17]. Indeed, it is difficult to separate these effects because the presence of the reef will likely alter the depositional environment and ultimately the biogeochemistry of the surrounding sediment.

This paper documents the changes in sedimentary characteristics that occurred upon construction of an intertidal oyster reef in the Guana Tolomato Matanzas National Estuarine Research Reserve (GTM NERR) in NE Florida, USA. Specifically, we compared the particle size distribution and organic matter (OM) content of sediments near the reef to those in adjacent area without reef structures. We measured changes in “reef” sediments compared to control sites and to “baseline” (pre- restoration) samples over a period of three years. We also measured chlorophyll *a* concentrations and dissolved inorganic nitrogen (DIN) fluxes, and estimated accretion of sediments in restored and control areas. We then discuss the implications of these changes in the context of shoreline protection, carbon sequestration, nitrogen cycling, and oyster restoration success. The results highlight the connection between oyster reefs and the surrounding coastal landscape, and the need to understand how their physical, biological, and chemical effects interact in order to better predict ecological outcomes, and ultimately to maximize benefits from restoration efforts.

## Materials and Methods

### Sampling site

The study site is the intertidal zone of the Tolomato River in the Guana Tolomato Matanzas National Estuarine Research Reserve in Ponte Vedra Beach, Florida, USA at N 30°00.2' and W 81° 20.2'. An oyster restoration project was performed by the reserve staff and volunteers and was constructed of bagged oysters stacked to form segments of artificial reef (Figures 1 and 2). The reef segments are oriented parallel to the shore along 328 m of the beach near the mean low tide line. The segments are on average 1.8 m wide and 5.3 m long and spaced 6 m apart. The installation began in June 2012 at the northern end of the site and 28 segments were added to the south through summer 2013. Surface sediment samples were first collected in May 2012, prior to the installation of the reef, and resampled in May 2013, January 2014, March 2014, and May 2015. Samples were obtained at low tide from 8 cross- shore transects, 4 intersecting reef segments, and 4 located at equivalent elevations at control sites 25-125 m north along the shoreline (Figure 1). Control transects were 25 m apart, and reef transects were centered on reef segments at least 25 m apart. For most sampling dates, three elevations were sampled along each transect: subtidal (2 m seaward of the reef midpoint), low (2 m shoreward of the reef midpoint) or high (5 m shoreward of the reef midpoint; Figure 2). On May 2012 high samples were not collected, and on March 2014 sub tidal samples were not collected.

### Particle size distribution and OM content

In 2012-2013, sediment samples for OM content and particle size analysis were collected by push core, followed by sectioning; only the top 2 cm were used in this study. For 2014 and 2015, samples were obtained by pressing a petri dish into the sediment and sliding a thin plastic sheet under the dish; this method recovers the top 1.5 cm of sediment. Sediment samples were placed in zip-lock bags and transported back to the lab at ambient temperature. The sediment was stored in the freezer until it was dried at 80°C for 1-3 days or until mass was constant. The sediment was then gently ground with a mortar and pestle to break up aggregates and passed through a 2 mm (fine gravel) sieve to remove oyster shell hash. Particle size was determined for

the baseline sample taken in May 2012 and the sample taken in May 2013 using the rapid method for particle size determination as outlined [21]. OM content was determined using the loss-on-ignition method; combustion was performed at 550°C for 12 hours [22]. Observationally, the area behind the reef had noticeably finer sediment after 1 year, and after 2 years the sediment surface was visibly elevated behind the reef segments (Figure 2B). This finer sediment accumulated in a distinct layer over a deeper layer of sandy sediments that were visually apparent in sediment push cores (Figure 4, inset photo). In order to map the accretion of fine sediment and estimate the total deposition after three years, we took sediment cores in a 2 m by 2 m grid pattern and measured the thickness of the distinctly finer layer of sediment at the surface. The cores were taken in 2014, shoreward of Reef #3, one of the earliest established oyster reefs in the restoration project (locations indicated in Figure 1). The grid covered an area 8 m by 16 m and a control grid measuring 6 m by 16 m was also sampled in the same manner. Using Surfer 13 (Golden Software, 2015), the thickness values were gridded using a Kriging interpolation, in order to create a topographic map of the surface fines, and to estimate a total volume of fine sediment in the sampled area.

### DIN and chlorophyll *a*

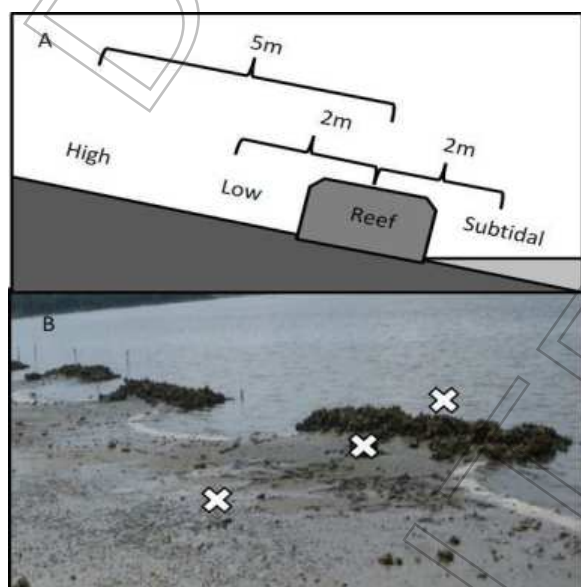
DIN fluxes were measured using a core incubation experiment in July 2014. Six sediment push cores were obtained at the “low” elevation as described above, 3 from control sites and 3 from reef sites. Visible fauna were avoided when taking the cores but infaunal organisms were not excavated. The cores were approximately 12 cm deep and 10 cm in diameter. The cores were placed in a cooler and transported to Flagler College at ambient temperature with no overlying water in order to avoid resuspension during transport (~1.5 hr). The core tops remained open throughout the entire experiment. Upon arrival at the lab, 1L artificial seawater was siphoned slowly onto each core. The artificial seawater was made from Instant Ocean salts and deionized,



**Figure 1:** Map of sampling locations along the shore of the Tolomato river at the Guana Tolomato Matanzas National Estuarine research reserve.

sterilized water. The flux experiments were conducted over the next 2 days, with the light experiment on day 1 and the dark experiment on day 2. Before the incubation began, the overlying water was siphoned off and replaced with new artificial seawater. The cores were stirred at 60 rpm using a small motor and chain drive system linked to stir bars in each core. The stirring speed was sufficient to mix the water within about 1 min but not fast enough to resuspend the surface sediments. Ten-mL aliquots of overlying water were removed periodically for measurement of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  plus  $\text{NO}_2^-$  (hereafter  $\text{NO}_x^-$ ). The initial sample was taken after 5 minutes of stirring to ensure a homogeneous water column.  $\text{NH}_4^+$  samples were measured immediately, and  $\text{NO}_x^-$  samples were frozen and analyzed within 1 week. Ammonium was measured using the OPA method [23], modified for smaller sample sizes [24].  $\text{NO}_x^-$  was measured by a modified Cd reduction method, using a Turner Designs Trilogy fluorometer/spectrophotometer to measure absorbance [25]. After the flux experiments were completed, surface sediments were subsampled by scraping the surface sediment layer (~ top 2mm) of the cores. The surface sediment was stored in centrifuge tubes at  $-20^\circ\text{C}$  until analyzed for chlorophyll *a* content, which was within 2 weeks. Chlorophyll *a* was analyzed using the non-acidification method (EPA method 445.0). The method was modified to use 100% acetone to account for the water content of the sediment. The chlorophyll *a* fluorescence was then compared to a chlorophyll *a* standard purchased from Turner Designs.

Statistical analysis of organic matter content was performed using IBM SPSS Statistics 21 using a generalized linear model with sample type (baseline, control, and reef) and elevation (high, low, and subtidal) as fixed factors. Particle size distribution was analyzed similarly, with % sand, silt, and clay each tested separately. For comparisons of chlorophyll *a* concentration in surface sediments, 2-sided *t*-tests were used. For the  $\text{NH}_4^+$  fluxes a one-way ANOVA with time as the independent variable was used to determine significance. *P*-values of less than 0.05 were considered significant in all tests.



**Figure 2:** Sample elevations A) diagram of 3 sampling elevations (high, low, and sub-tidal) and B) photo of oyster reef site, taken June 2015 (3 years after construction).

## Results

### Sediment particle size distribution and accretion

Large changes were observed in the sedimentary environment after only one year of the oyster restoration. Sediment particle size was markedly finer, with more silt and less sand compared to the controls and baseline samples in the low elevation position behind the reef (Figure 3). In the generalized linear model for % sand and % silt, sample type (baseline, control, or reef), sample elevation (low, high, or subtidal), and type\*elevation were all significant (SI Tables 2 and 3). For % clay, sample type was not significant, but elevation and type\*elevation were significant (SI Table 4). The spatial survey of fine sediment distribution showed up to 16 cm of fine sediment directly shoreward of the reef (Figure 4), and the total volume of fine sediment behind the reef segment was  $2.9 \text{ m}^3$ . This corresponds to an average of  $0.36 \text{ m}^3$  of fine sediment per linear meter of shoreline, including both reef segments and gaps. A thinner, much less distinct surface layer was detected in the control area. The volume of this layer was  $0.50 \text{ m}^3$  or  $0.12 \text{ m}^3$  per m of shoreline.

### Sediment OM

OM content of sediment samples ranged from 0.7% to 9.9%. Statistical analysis showed that effects for sample type, elevation, and type\*elevation were all significant (SI Table 1). The largest change was at the low position, where OM content was more than 3 times that of controls (Figure 5A). Repeated sampling over time showed substantial variation in the % OM content of reef sediments at this low position, yet the values were always significantly higher than comparable controls (Figure 5B).

### $\text{NH}_4^+$ flux and chlorophyll *a* concentrations

In the core flux experiments, differences between reef and control cores were apparent in the dark but not in the light as shown in (Figure 6.) In the light core incubations,  $\text{NH}_4^+$  fluxes were not significantly different from zero for both control and reef cores ( $p=0.93$  and  $0.20$ , respectively.) For the dark incubation, control cores showed a small but significant decrease of  $-0.03 \mu\text{mol/hr}$ , whereas reef cores released  $1.35 \mu\text{mol/hr}$  ( $p=0.03$  and  $0.0002$  respectively.) Using the rate of accumulation in the 1L of overlying water in the cores, this translates to a surface efflux of  $167 \mu\text{mol/m}^2/\text{hr}$  for reef sediments and an influx of  $4 \mu\text{mol/m}^2/\text{hr}$  for control sediments.  $\text{NO}_x^-$  fluxes were not significant for any cores (data not shown.) Chlorophyll *a* content ranged from 23-75 ng/g dry sed (Figure 6C) and was approximately 3 times higher in reef sediments;  $p<0.05$ .

## Discussion

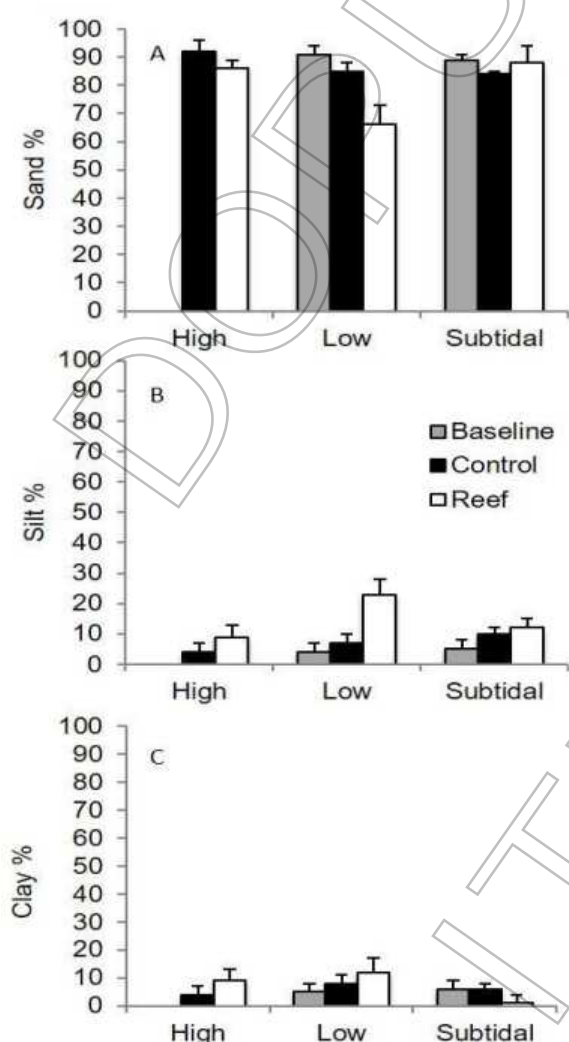
The sedimentary environment surrounding the restored oyster reef changed dramatically over the course of the study, with effects concentrated in the area immediately shoreward of the reef (Figure 2). Particle size distribution (Figure 3) and OM content (Figure 5) confirm that finer, more organic-rich sediments were deposited in the protected area behind the newly created reef. Statistical analysis of the dataset indicated that this change was a function of reef presence and sample elevation. Decreased particle size is likely caused by a combination of reduction of physical energy from wave attenuation by the reef structure [26] plus accretion of bio-deposits from live oysters [27]. Our site is located along the Intracoastal Waterway, and receives



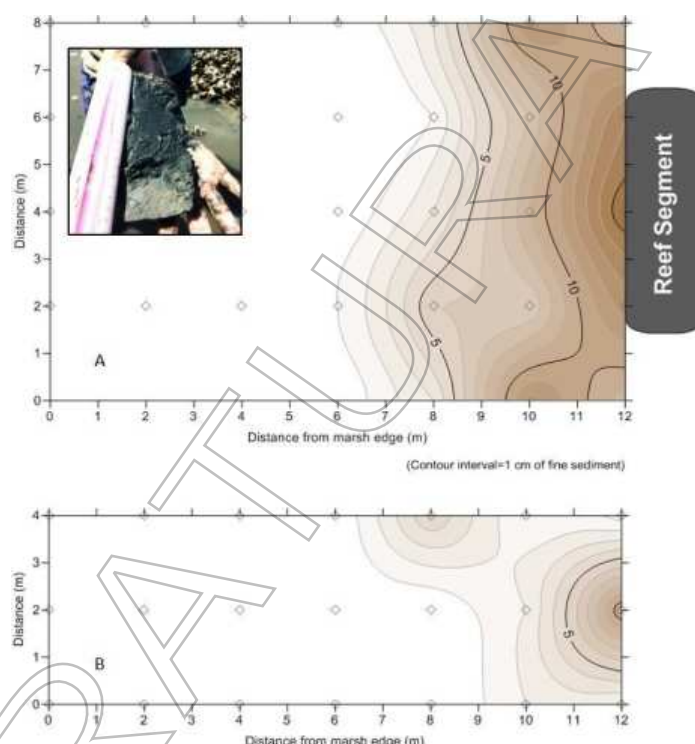
wave energy from boat wakes as well as wind driven waves. The placement of the bagged oyster mounds parallel to shore was designed, in part, for shoreline protection and was therefore meant to substantially alter the wave energy and depositional environment in the intertidal zone. The visible patterns in sediment accretion behind the oyster reefs (Figure 2) suggest that wave energy reduction is indeed a key component of OM enrichment at our site, either through the trapping of fine allocthonous sediment, or by facilitating sedimentation of OM-rich particles from oysters and commensal organisms [28].

Because of the change in particle size and texture of sediment a distinct sand-silt horizon was identifiable in reef sediments, often co-occurring with oyster shell hash. Using this horizon as an indicator, we estimate the average accretion rate over the last 3 years to be as much as 5 cm/yr for the area directly shoreward of the reef. We estimated the total volume of fine sediment in this upper layer behind one reef plus the adjacent area between it and the next reef, and found that it contained 2.9 m<sup>3</sup> of fine sediment, or 0.36 m<sup>3</sup> of fine sediment per linear meter of reef structure. A thinner, less distinct layer was also observed in the control area, and this layer

corresponds to 0.5 m<sup>3</sup> in the survey area or 0.12 m<sup>3</sup> per linear meter of shoreline. The presence of a fine layer in the control area may be due to the redistribution of fines from the adjacent reef site, but to be conservative we subtract it from the reef values to estimate a difference of 0.24 m<sup>3</sup> of fine sediment accreted per meter of constructed reef. One study [16] commented that burial by sediment appears to be an important factor in failed oyster restoration projects, and some oyster burial was evident at our site. A recent study indicates that oyster reefs can grow vertically up to 11 cm/yr when not limited by water column depth [29]. However, intertidal reefs, whose vertical growth rate is likely to be constrained by sea level, may experience significant stress from 5 cm/yr of sedimentation. Therefore, though sediment deposition confers ecosystem services with regard to erosion control and organic matter burial, predicting and managing sediment accretion may be an important consideration for long-term successful recruitment of oysters to similarly constructed reefs.



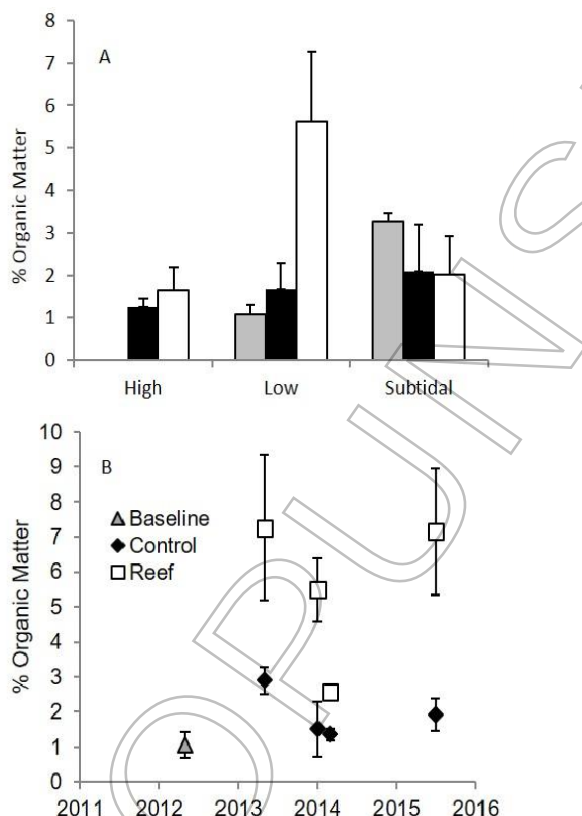
**Figure 3:** Particle size distributions; percentage sand (A), silt (B), and clay (C) of reef and control sediments from samples taken 1 year after restoration. N=4 for each elevation.



**Figure 4:** Topographic map of fine sediment layer thickness in reef sediments (A) and control areas (B) diamonds indicate grid sample locations and contour lines represent cm. Inset: photo of a reef sediment core showing upper layer of fine OM-rich sediment overlaying sandier sediments (foreground).

Our survey of sediment cores suggests that visible accretion of fines was limited to within approximately 7 m of the reef in the shoreward direction (Figure 4). Furthermore, differences in particle size and OM content between reef and control samples were only significant at the low position (Figures 3 and 5A). These results suggest that the effect on sediment was fairly localized. One goal of this restoration project was to conserve and expand the *Spartina* marsh, which is approximately 12 m from the reef. However, our results show no sedimentary differences on that portion of the upper beach, suggesting that to reduce marsh erosion additional structures might be required. One study [30]

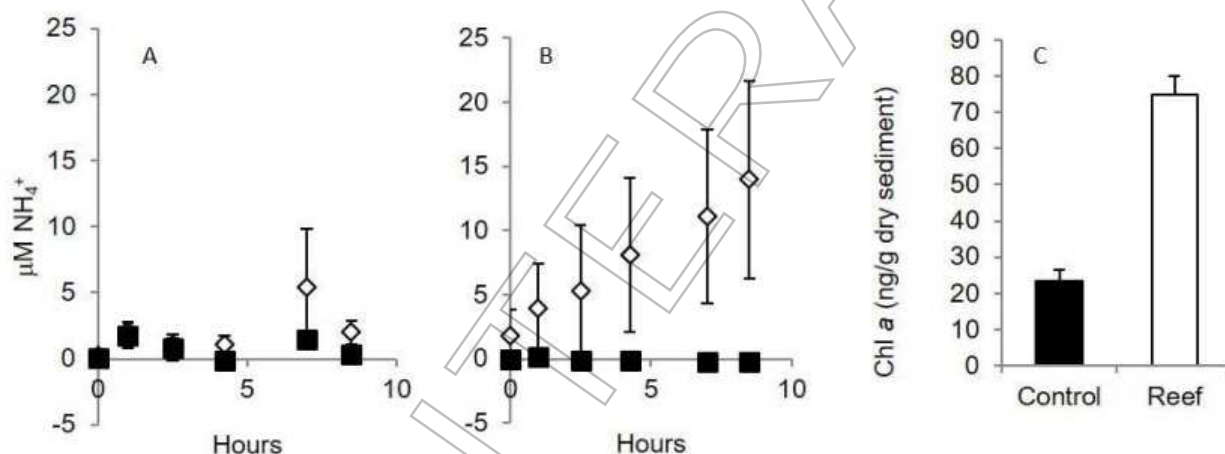
recently pointed out the value of vegetated coastal habitats such as *Spartina* marshes as carbon sinks; one reason for their efficacy is the ability to trap allochthonous carbon and to grow vertically in response to sea level rise. Our study suggests that the constructed oyster reef also exhibits these characteristics,



**Figure 5:** Average % OM in baseline (gray), reef (white), and control (black) sediments for three elevations (high, low, and subtidal; B) % OM content of low elevation samples versus sampling time. Error bars are 95% confidence intervals.

demonstrating that non-vegetated but structurally complex sites might also be important sites of carbon storage. However, in the case of oyster reefs, the carbonate structure itself also represents a long-term carbon storage mechanism, as well as potential buffering against future acidification [31]. Also, the protection afforded by the hard substrate may help stabilize the upland shoreline against suspension during storm events that threaten sequestered carbon [30,32]. Our data therefore support the idea that structurally complex benthic habitats, both natural and restored, are highly valuable for a variety of reasons, including sediment accretion and carbon storage. Furthermore, such secondary effects are often excluded from economic cost-benefit analysis [2].

Benthic nitrogen recycling was also affected by the construction of the oyster reef. Reef sediments became a source of  $\text{NH}_4^+$ , compared to a sink at control sites (Figure 6). The sediments surrounding this reef are therefore probably augmenting the considerable flux of recycled  $\text{NH}_4^+$  known to be produced from the oysters themselves [5]. Chlorophyll *a* concentrations show that these sediments also host more abundant benthic microalgae (Figure 6C), which were able to assimilate nearly all the ammonium released under light conditions (Figure 6A) and mitigate DIN transfer to the water column. Benthic microalgae may also stimulate denitrification by contributing labile organic carbon and increasing oxygenation of surface sediments [33]. Sediments from restored oyster reefs have been shown to be active sites of denitrification [18-20], with the highest sedimentary denitrification rate ever reported [17]. However, OM enrichment may also cause persistent anoxia within sediments, which may cause reduced  $\text{N}_2$  production if the supply of  $\text{NO}_3^-$  becomes limiting [33]. The areal  $\text{NH}_4^+$  fluxes we report here are from the low position only, and are likely to vary seasonally and spatially, and so more research is needed to characterize  $\text{NH}_4^+$  fluxes or predict effects on  $\text{N}_2$  production rates. Rather, these results highlight the potential for altered N-cycling upon reef construction, and the importance of benthic algal uptake in mediating benthic-pelagic coupling [6,7]. This is likely to be especially important for intertidal reefs such as the one in this study, which receive abundant sunlight.



**Figure 6:**  $\text{NH}_4^+$  concentrations during core flux experiments performed in the light (A) and dark (B); chlorophyll *a* concentrations in surface sediments of cores used in flux experiments (C) Black squares are control cores, white diamonds are reef cores. N = 3 and error bars are  $\pm 1$  S.D.

Higher sediment OM content could impart a variety of consequences for carbon and nitrogen storage, oysters and associated fauna, and for water quality. For example, addition of organic carbon may generate higher benthic oxygen demand, which creates more anoxic sediments and steepens gradients of redox-active compounds at the sediment-water interface. Although oysters are well adapted to withstand hypoxic and even anoxic conditions [34], hypoxic water negatively affects oyster recruitment [35]. Decreasing the depth of oxygen penetration in sediments may also limit the type and abundance of infaunal species.

## Conclusions

A recent assessment noted that intertidal constructed oyster reefs were more successful than subtidal reefs in terms of oyster survival and continued recruitment [16]. Given their proven efficacy and added potential benefits of shoreline stabilization [32], we expect to see continued construction of intertidal reefs. As such, a comprehensive understanding of physical, chemical, and biological effects of restoration is needed. At the GTM NERR oyster reef restoration site we observed significant changes in sediment characteristics within one year of reef installation. Many of these changes might be desirable or undesirable depending on the goals of a restoration project and the local environment. Sediment deposition was enhanced; this is a positive effect unless oyster burial occurs. Sediments became finer and more organic rich, which may enhance carbon storage and possibly denitrification.  $\text{NH}_4^+$  fluxes were significantly increased overall, though they were mitigated by an enhanced microalgal layer. Though often overlooked, these changes are important to document for assessing the full suite of ecosystem services provided by oyster restoration, and also for optimizing restoration design. Furthermore, such changes may affect the trajectory of restored oyster reefs over time and may have implications for the variety of commensal organisms that inhabit the sediment-reef system.