

2014

## **Biophysical Control of Oyster Reef Performance in Chesapeake Bay.**

Allison M. Colden  
*College of William and Mary*

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**Biophysical Control of Oyster Reef Performance in Chesapeake Bay**

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**A Dissertation**

**Presented to**

**The Faculty of the School of Marine Science**

**The College of William and Mary in Virginia**

**In Partial Fulfillment**

**of the Requirements for the Degree of**

**Doctor of Philosophy**

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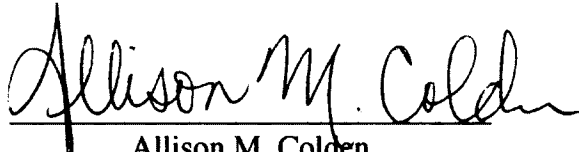
**by**

**Allison M. Colden**


**December 16, 2014**

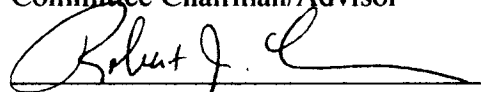
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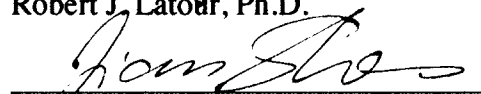
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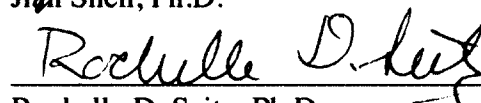
  
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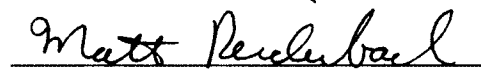
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## ACKNOWLEDGEMENTS

*“Nothing can stop the man with the right mental attitude from achieving his goal; nothing on earth can help the man with the wrong mental attitude.”*

– Thomas Jefferson

There are times while completing a doctoral dissertation in which maintaining the right mental attitude can be challenging. As such, I am deeply grateful to all those who have assisted me throughout my doctoral program through their advice, encouragement, and willingness to lend a helping hand. Foremost, I would like to thank my advisor, Dr. Rom Lipcius, and the members of my committee, Dr. Rob Latour, Dr. Rochelle Seitz, Dr. Jian Shen, and Dr. Matt Reidenbach, for their suggestions, advice, and encouragement. Rom’s mentorship, support, and sense of humor were incredibly helpful throughout my program. Rom allowed me the independence to work through issues on my own but provided guidance at critical moments. His willingness to play ‘devil’s advocate’ in our collegial, sometimes spirited discussions helped immensely in the final stages of my doctoral program.

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fauna. Enhanced deposition in the vicinity of reefs, along with the probable drawdown of resources by filtration, provides evidence for spatial pattern formation of reefs. Overall, these studies suggest that biophysical interactions drive the persistence and performance of oyster reefs at multiple spatial scales. This comprehensive view of the mechanistic drivers of restoration reef performance should be considered in an integrated, estuary-wide approach to oyster restoration in Chesapeake Bay and beyond.

## ABSTRACT

Biogenic reefs of the eastern oyster (*Crassostrea virginica*) are prominent features of the estuarine landscape in Chesapeake Bay and throughout its range. Oyster reefs provide complex structured habitat in otherwise unstructured systems. Reefs enhance substrate not only for the settlement of oysters, but also for a diverse suite of fauna. The reefs and their communities further provide valuable ecosystem services. Unfortunately, oyster reefs in Chesapeake Bay have been decimated by overfishing, disease, and habitat loss that has led to a widespread decline. Efforts to restore oysters by construction of shell and artificial reefs has had limited success due to degradation of constructed habitat by sedimentation. The presence of reefs within the landscape inherently changes flow and sediment flux, but these biophysical interactions are not well defined; thus, I used mesocosm studies, large-scale field experiments, and detailed hydrodynamics observations to quantify interactions between oysters, reef geometry, and sediment dynamics at the individual and reef scales.

Catastrophic burial of reefs by episodic events, such as storms, causes mass mortality of oysters, but the effects of less severe, chronic sedimentation are unknown. I conducted a mesocosm study in which oysters were partially or completely buried with sediment and measured their survival, growth, condition, and biodeposition rate over time. Only oysters that were buried at greater than 90% of their shell height experienced significant mortality. Biodeposition and condition were negatively impacted by burial depth; however, growth increased with burial depth. This demonstrates a mechanism for oyster survival under chronic sedimentation conditions – oysters increased shell growth rate by depleting metabolic reserves to reach the sediment surface to feed and ventilate. These sublethal impacts of partial burial on oysters affect reef condition, as growth and biodeposition are major contributors to reef accretion.

Historical oyster reefs in Chesapeake Bay were highly productive and self-sustaining prior to heavy exploitation in the late 19<sup>th</sup> century. Distinct reef morphologies were present throughout the oyster's range, suggesting aspects of reef structure were contributing to reef productivity. I conducted two large-scale field experiments to determine how reef height and orientation affected sediment dynamics and reef productivity. Reefs in the Great Wicomico and Lynnhaven Rivers showed distinct changes in sediment deposition and oyster abundance – deposition was lowest and abundance highest on high-relief reefs with a threshold in oyster density at 0.3 m. This indicates a non-linear response of oyster populations to sediment dynamics driven by reef height, which can be exploited for effective and efficient restoration of oyster reefs.

Reef orientation significantly impacted hydrodynamics and sediment dynamics by altering the spatial distribution of sediment deposition. Significant differences in reef area loss impacted productivity by reducing available habitat area for settlement of reef fauna. Secondary productivity of reefs was enhanced on perpendicular reefs whose orientation maintained high flow conditions over the reef crest, which promotes faster growth of reef

## AUTHOR'S NOTE

The chapters that comprise this dissertation were written in manuscript format for scientific publication. Thus, the formatting of each chapter follows the guidelines of the publication to which the manuscript was or will be submitted. At the time of writing, citations for individual chapters are as follows:

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### Chapter 4:

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## **Biophysical Control of Oyster Reef Performance in Chesapeake Bay**



## INTRODUCTION

The eastern or American oyster (*Crassostrea virginica*) was once a dominant ecosystem engineer in many estuaries along the east coast of the United States, particularly in Chesapeake Bay (Jones et al. 1994). This bivalve influences estuarine processes and structures benthic habitats and their associated communities. Individual oysters contribute to ecosystem services and functioning by filtering water, removing particulate matter and bacteria, and depositing the processed material on the benthos (Haven and Morales-Alamo 1972). The filtration and biodeposition processes link the benthic and pelagic environments and facilitate biogeochemical cycling (Kellogg et al. 2013). Large populations of oysters form aggregations, called reefs, which provide structured habitat in largely unstructured soft-sediment systems. In addition to playing a critical role in the ecological functioning of estuarine systems, oysters are harvested commercially throughout their range for human consumption. The biogenic reefs formed by oysters also serve as habitat and foraging grounds for commercial fish and shellfish species, contributing further to their economic importance (Zimmerman 1989, Coen and Grizzle 2007).

Unfortunately, oyster populations in the mid-Atlantic region have experienced sequential collapses throughout the 19<sup>th</sup> and 20<sup>th</sup> centuries due to overharvesting, habitat loss, water quality degradation, and disease (Rothschild et al. 1994, Kirby 2004, Beck et al. 2011). The progression of population collapse demonstrates a pattern of destructive harvesting that leads to habitat degradation and later to conditions that cannot support viable populations (Kirby 2004). This pattern has been repeated many times over worldwide, and current estimates are that 70% or more of the world's oyster populations

are at less than 10% of their prior abundances (Beck et al. 2011). This represents a global crisis in which ecosystems will be or have already been dramatically altered by the loss of oyster populations and their attendant ecosystem services.

Study Species: *Crassostrea virginica*

The eastern oyster is a monomyarian lamellibranch bivalve of the family Ostreidae. Its range spans most of the Atlantic and Gulf coasts, from the Gulf of St. Lawrence to Brazil and Argentina, in areas where salinity ranges between 5-28 psu (Carriker and Gaffney 1996). It is a eurytopic species with wide temperature and salinity tolerances and an efficient filter feeder capable of clearing up to 98% of particles > 3  $\mu\text{m}$  (Haven and Morales-Alamo 1970) at a rate of 7.0 L of water per hour (Gedan et al. 2014). Filtration rates are enhanced by increasing flow speed at low velocities (< 2  $\text{cm s}^{-1}$ ), but may be inhibited at high flow speeds (Grizzle et al. 1992). Temperature and seston concentration also affect filtration rates. Oysters maintain high filtration rates under high seston conditions (up to 25  $\text{mg L}^{-1}$ ; Newell and Langdon 1996), but the production of pseudofeces increases as more material is rejected without ingestion (Newell and Langdon 1996).

The eastern oyster is a protandric broadcast spawner with prolific reproductive capacity. Female oysters can produce as many as 500 million eggs per season over several spawning events (Galtsoff 1930 in Kennedy et al. 1996), and fecundity increases with age. After spawning, eggs are fertilized externally in the water column. Larvae remain in the plankton for 2-3 wks before becoming competent to settle. Larvae have vertical swimming capabilities that allow them to exploit local currents to promote

retention in the ecosystem. Competent larvae are induced to settle by several cues, including chemical signals from living oysters and bacterial biofilms on oyster reefs (Tamburri et al. 1996), acoustic signals from oyster reef communities (Lillis et al. 2013), and turbulence over rough substrates (Fuchs et al. 2013). Pediveliger larvae use their muscular foot to probe potential settlement areas. If the area is suitable, larvae will cement to the substrate and undergo metamorphosis to become a juvenile oyster or “spat.” If the substrate is unsuitable, larvae will not settle and can be swept away to a new habitat on the next tidal cycle. Juvenile oysters grow rapidly and usually become sexually mature within a year from settlement. The timing of the protandric shift from male to female varies geographically but usually occurs at approximately 1.6 years of age, and the majority of individuals are female by the time they reach market size (>76 mm) (Harding et al. 2012).

The induction of larval settlement by chemical and physical cues results in gregarious setting that forms biogenic reefs over the course of many generations. Larvae are induced to settle on the surface of live and dead oysters, which causes accretion of the shell-oyster matrix and the formation of complex three-dimensional structures over time.

#### Oyster Reef Development in Chesapeake Bay

Historical literature on the structure of oyster reefs suggests that Chesapeake Bay is a geographical transition area for *C. virginica* reef forms (Woods et al. 2004). As such, several different reef types were found within the Bay, but different reef forms dominated in different areas (Kennedy and Sanford 1999). In lower Chesapeake Bay, the dominant form was the highly productive southern-style reef. These reefs were large, 3-

dimensional structures that often protruded from the water during low tide (Woods et al. 2004). They were oriented perpendicular to tidal flow and the shoreline, but were not usually connected to the shore. In mesohaline portions of the Bay, northern-style reefs were dominant. These were flatter, bed-like reefs that formed on the shoulders of and were oriented parallel to paleochannels. They were more ephemeral on geologic time scales and less productive than southern-style reefs (Smith et al. 2003, Woods et al. 2004).

Southern-style reefs were thought to form as small oyster clumps attached to protrusions from the shore grew vertically and channelward (Grave 1905). Increased water flow at the crest and tip of the reef allowed oysters there to grow faster. Decreased flow velocities shoreward and upstream of the reefs enhanced sedimentation causing reefs to become detached from shore (Grave 1905). This decrease in flow also explains the even spacing of reefs along the estuary; sufficient time was needed for flow speed to increase before encountering the next reef (Woods et al. 2004). Northern-style reefs formed along the sides of channels where fast-flowing water kept oysters free from sediment and enhanced food and oxygen fluxes, enhancing oyster growth. As paleochannels filled with sediment, many of these reefs were lost (Smith et al. 2005, Woods et al. 2004).

Historically, the majority of reefs in the Virginia portion of the Bay were typical southern-style reefs. They were described as very large, up to 3 km in length, with a wide, shoal-like form (Haven and Whitcomb 1983, Kennedy and Sanford 1999). They dominated the environment and caused significant changes in circulation in areas where large intertidal reefs caused a diversion of flow. For example, at Point of Shoals in the James River, Virginia, over 6 km of intertidal reefs oriented perpendicular to the channel

axis presented major obstacles to tidal flow moving up the river (DeAlteris 1988). Historical reefs were “platform-like” with steeply sloping sides and a wide crest where large live oysters were found (Smith et al. 2003). These reef types presumably formed as the result of interactions of the reef with local hydrodynamics that produced favorable conditions for oyster growth and survival (Grave 1905). This interaction allowed for the persistence of reefs for several centuries prior to European colonization and the commercialization of oyster harvesting that reduced these reefs to their present degraded state.

#### Declines in Chesapeake Bay Oyster Populations

*Crassostrea virginica* is economically and culturally important to the Chesapeake Bay region. The cultural significance of the oyster can be traced back to the Native Americans in the area who named the bay “Chesepiooc,” meaning “Great Shellfish Bay” (Woods et al. 2004). At the peak of the oyster industry, Chesapeake Bay produced more than 50 million pounds of oysters, and dominated the total oyster harvest for the United States (Hargis and Haven 1988). Following peak harvests in the 1880s, oyster populations began to decline steadily. By 2000, bay-wide oyster harvests were less than 2% of the harvests in 1880, and the current population size is estimated at less than 1% of the historical population of the 1800s (Newell 1988, Hargis and Haven 1988, Kirby 2004). In mesohaline portions of the bay, oyster populations have declined by 99.7%, and if trends in habitat loss and harvest continue, extirpation of oysters is expected by 2027 (Wilberg et al. 2011).

The dramatic decline in oyster populations is often attributed to overharvesting, habitat loss and water quality degradation (DeAlteris 1988, Hargis and Haven 1988, Rothschild et al. 1994, Smith et al. 2005, Kemp et al. 2005). Harvesting of oyster populations on a commercial scale began in the mid-19<sup>th</sup> century, with the legalization of oyster dredges. Unlike hand-tonging and other harvest methods, oyster dredging is highly damaging to reef structures. Hand tongs and patent tongs are limited in their efficiency and area, meaning that only small areas could be worked and much of the shell material (cultch) remains on the oyster reef. The legalization of oyster dredges led to widespread exploitation of oyster reefs, since dredges could cover an area more quickly and efficiently than tonging (Rothschild et al. 1994). The efficacy of the dredge at exploiting oyster populations and their shell resources was evident from sequential oyster fishery collapses along the coast of North America (Kirby 2004).

The effects of dredging were dramatic, as large three-dimensional structures were essentially flattened within a century (Rothschild et al. 1994, DeAlteris 1988, Woods et al. 2005). Comparison of bathymetric charts of oyster bottom in the James River, Virginia indicates that from the 1870s to the 1940s, approximately 18,000 m<sup>3</sup> of oyster shell had been removed from the system. Of the 6 km<sup>2</sup> of intertidal reef that were present in the 1870s survey, only remnant footprints of the reefs remained in the 1940s (DeAlteris 1988, Woods et al. 2005). In mesohaline portions of the Bay, reef area decreased by 3.5% annually since 1911 (Smith et al. 2005). Reefs that remain have been degraded by destructive fishing methods that reduce reef elevation. In 100 years of fishing on Wreck Shoal in the James River, 200 years or more of natural reef accretion was eliminated by a 1 m reduction in reef elevation (DeAlteris 1988). The reduction in vertical relief makes

remaining reefs more susceptible to sedimentation, rendering the reef unsuitable for recruitment, and possible burial, resulting in a complete loss of habitat (Smith et al. 2003). Contemporaneous with reductions in reef elevation, anthropogenic changes in land use and changes in sea level caused a 2-3 fold increase in sediment flux to the Bay, which has continued to increase (Colman and Bratton 2003). It is likely that a significant amount of reef habitat was lost as a result of reduced elevation coupled with increased sediment loading (DeAlteris 1988).

In addition to the loss of substrate from reefs, harvesting caused a decline in reproductive yield-per-recruit. Because oysters are protandric, fishing that removes the largest individuals consequently removes a large proportion of females from the population. Thus, the reproductive potential of the population is diminished and rebuilding of the stock is more difficult (Rothschild et al. 1994, Kennedy 1996). Estimates of current yield-per-recruit suggest that present levels are about 8.4% of those of an unexploited stock (Rothschild et al. 1994).

Chesapeake Bay also continued to experience anthropogenically-induced changes in nutrient loading during the early 20<sup>th</sup> century. Nutrient loading tends to support smaller, less nutritious phytoplankton species and promotes blooms of toxic species. Sediment cores from the mid-20<sup>th</sup> century indicate a shift in the ratio of pennate to centric diatoms, an indicator of decreased water quality and a shift from benthic to pelagic primary production (Cooper and Brush 1993, Kemp et al. 2005). These indicators of eutrophication became evident more than a century after large-scale land-use changes began. This suggests that before overharvesting, oysters were able to mitigate the influx

of nutrients and sediment through filtration and biodeposition (Kirby and Miller 2005). After overexploitation, these factors precluded oysters' recovery.

After initial declines in the early 1900s, oyster populations stabilized at relatively low levels until a further decline occurred in the early 1960s associated with the proliferation of two oyster diseases, Dermo (*Perkinsus marinus*) and MSX (*Haplosporidium nelsoni*). Dermo is caused by an apicomplexan parasite that was first identified following large-scale mortalities in Gulf of Mexico in the 1940s. Dermo is transmitted between oysters through their feces and also through the water column; infective cells of *P. marinus* are encountered as oysters feed. Oysters infected with Dermo experience reduced growth rates and eventually die of tissue lysis. Once infection takes hold in a particular location it is exacerbated by the release of infective cells when oysters die (Ford and Tripp 1996). The introduction of MSX to the United States likely accompanied the import of *Crassostrea gigas* from Japan (Burreson et al. 2000). The disease is caused by a haplosporidian parasite whose infective stage and method of transmission are unknown (Ford and Tripp 1996). Both diseases can cause significant mortality, particularly in polyhaline portions of the Bay. Prevalence and intensity of both diseases tend to be higher in warm, high-salinity waters, like those of lower Chesapeake Bay. To date, disease prevalence remains high, although there is some recent evidence for the development of resistance to MSX by oysters in lower Chesapeake Bay (Carnegie and Burreson 2011).



## Dissertation Rationale and Objectives

The recognition of the ecological and economic importance of the eastern oyster has led to the development of habitat management and restoration programs aimed at improving oyster stocks and reestablishing ecosystem services. In Chesapeake Bay, with few exceptions (Schulte et al. 2009), these programs have been unsuccessful at producing significant improvements in oyster populations, despite several decades of directed effort (Southworth et al. 1998, Wilberg et al. 2011). Examination of the literature and previous restoration projects suggests that a mechanistic understanding of the processes controlling reef success is necessary to comprehend why some projects succeed when others fail.

The objectives of this study are to investigate the influence of aspects of restoration reef structure on oyster population dynamics and identify mechanisms controlling restoration success. A mesocosm experiment quantifying the effect of sediment burial on oysters was conducted to determine if sediment affects reef-building processes, such as growth and biodeposition, to which oysters contribute (Chapter 1). Two large-scale field experiments were used to determine the effects of reef elevation above the bed and orientation to tidal currents on oyster production and the provision of habitat for benthic macrofauna (Chapters 2-3). Observational studies of the impacts of various reef structures on local hydrodynamics elucidated the feedbacks between reef structure, oyster dynamics, and sediment transport that are thought to control restoration success at the reef and landscape scale (Chapter 4). The reef design that resulted from this study will help improve the probability of restoration success and prevent wasteful use of monetary and substrate resources in projects that are unlikely to succeed.

## Literature Cited

- Beck, M.W. et al. 2011. Oyster reefs at risk and recommendations for conservation, restoration and management. *Bioscience* 61: 107-116.
- Burreston, E.M., Stokes, N.A., and Friedman, C.S. 2000. Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. *Journal of Aquatic Animal Health* 12: 1-8.
- Carnegie, R.B., and Burreston, E.M. 2011. Declining impact of an introduced pathogen: *Haplosporidium nelsoni* in the oyster *Crassostrea virginica* in Chesapeake Bay. *Marine Ecology Progress Series* 432:1-15.
- Carriker, M.R. and Gaffney, P.M. 1996. A Catalog of Selected Species of Living Oysters (*Ostreacea*) of the World. In Kennedy, V.S., Newell, R.I.E., and Eble, A.F. *The Eastern Oyster: Crassostrea virginica*. Maryland Sea Grant Publications: College Park, MD.
- Coen, L., and Grizzle, R. 2007. The importance of habitat created by molluscan shellfish to managed species along the Atlantic coast of the United States. *Atlantic States Marine Fisheries Commission. Habitat Management Series*, Washington, DC, 1–108.
- Colman, S. M., and Bratton, J. F. 2003. Anthropogenically induced changes in sediment and biogenic silica fluxes in Chesapeake Bay. *Geology* 31: 71–74.
- DeAlteris, J. 1988. The geomorphic development of Wreck Shoal, a subtidal oyster reef of the James River, Virginia. *Estuaries and Coasts* 11: 240-249.
- Ford, S.E. and Tripp, M.R. 1996. Diseases and Defense Mechanisms. In Kennedy, V.S., Newell, R.I.E., and Eble, A.F. *The Eastern Oyster: Crassostrea virginica*. Maryland Sea Grant Publications: College Park, MD.

- Fuchs H.L., Hunter E.J., Schmitt E.L., Guazzo R.A. 2013. Active downward propulsion by oyster larvae in turbulence. *Journal of Experimental Biology* 216:1458-1469.
- Galtsoff, P.S. 1930. The fecundity of the oyster. *Science*: 97-98.
- Gedan, K.B., L, Kellogg, and D.L. Breitburg. 2014. Accounting for multiple foundation species in oyster reef restoration benefits. *Restoration Ecology* 22: 1-8.
- Harding, J.M., E.N. Powell, R. Mann, and M.J. Southworth. 2012. Variations in eastern oyster (*Crassostrea virginica*) sex-ratios from three Virginia estuaries: protandry, growth, and demographics. *Journal of the Marine Biological Association of the United Kingdom* 93: 519-531.
- Hargis, W.H. and Haven, D.S. 1988. Rehabilitation of the troubled oyster industry of the lower Chesapeake Bay. *Journal of Shellfish Research* 7: 271-279.
- Haven, D.S. and R. Morales-Alamo. 1972. Biodeposition as a factor in sedimentation of fine suspended solids in estuaries. In *Geological Society of America Memoir No. 133*, 121-130. New York: Geological Society of America.
- Haven, D.S. and R. Morales-Alamo. 1970. Filtration of particles from suspension by the American oyster *Crassostrea virginica*. *Biological Bulletin* 139: 248-264.
- Haven, D.S., and J.P. Whitcomb. 1983. The origin and extent of oyster reefs in the James River, Virginia. *Journal of Shellfish Research* 32: 141-151.
- Jones, C.G., J.H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. *Oikos* 69: 373-386.
- Kellogg, M.L., J.C. Cornell, M.S. Owens, and K.T. Paynter. 2013. Denitrification and nutrient assimilation on a restored oyster reef. *Marine Ecology Progress Series* 480: 1-19.

- Kemp, W., Boynton, W., and Adolf, J. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology Progress Series* 303: 1-29.
- Kennedy, V. 1996. The ecological role of the eastern oyster, *Crassostrea virginica*, with remarks on disease. *Journal of Shellfish Research* 15: 177-183.
- Kennedy, V.S. and L.P. Sanford. 1999. The morphology and physical oceanography of unexploited oyster reefs in North America. In *Oyster reef habitat restoration: a synopsis and synthesis of approaches*, eds. Luckenbach, M.W., R. Mann, and J.A. Wesson, 25-46. Gloucester Point: VIMS Press.
- Kirby, M. 2004. Fishing down the coast: Historical expansion and collapse of oyster fisheries along continental margins. *Proceedings of the National Academy of Sciences* 101:13096-13099.
- Kirby, M., and Miller, H. 2005. Response of a benthic suspension feeder (*Crassostrea virginica* Gmelin) to three centuries of anthropogenic eutrophication in Chesapeake Bay. *Estuarine, Coastal and Shelf Science* 62: 679-689.
- Lillis, A., D.B. Eggleston, and D.R. Bohnenstiehl. 2013. Oyster larvae settle in response to habitat-associated underwater sounds. *PLoS ONE* 8: 1-10.
- Newell R.I.E. 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American oyster, *Crassostrea virginica*? In *Understanding the Estuary: Advances in Chesapeake Bay Research*, eds. Lynch, MP and Krome E.C., 536-546. Solomons: Chesapeake Research Consortium.

- Newell, R.I.E. and C.J. Langdon. 1996. Mechanisms and physiology of larval and adult feeding. In *The eastern oyster: Crassostrea virginica*, eds. Kennedy, V.S., R.I.E. Newell, and A.F. Eble, 185-230. College Park: Maryland Sea Grant College.
- Rothschild, B., Ault, J., Gouletquer, P., and Heral, M. 1994. Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. *Marine Ecology Progress Series* 111: 29–39.
- Schulte, D.M., Burke, R.P., and Lipcius, R.N. 2009. Unprecedented restoration of a native oyster metapopulation. *Science* 325:1124-1128.
- Smith, G. F., Bruce, D. G., Roach, E. B., Hansen, A., Newell, R. I. E., and McManus, A. M. 2005. Assessment of Recent Habitat Conditions of Eastern Oyster *Crassostrea virginica* Bars in Mesohaline Chesapeake Bay. *North American Journal of Fisheries Management* 25: 1569–1590.
- Southworth, M. and Mann, R. 1998. Oyster reef broodstock enhancement in the Great Wicomico River, Virginia. *Journal of Shellfish Research* 17: 1101–1114.
- Tamburri, M.N., C.M. Finelli, D.S. Wethey, and R.K. Zimmer-Faust. 1996. Chemical induction of larval behavior in flow. *Biological Bulletin* 191: 367-373.
- Wilberg, M.J., Livings, M.E., Barkman, J.S., Morris, B.T., and Robinson, J.M. 2011. Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. *Marine Ecology Progress Series* 436: 131-144.
- Woods, H., Hargis, W., Jr, Hershner, C., and Mason, P. 2004. Improving Siting and Construction Criteria for Oyster Reef Restoration. *VIMS Special Report in Applied Marine Science and Ocean Engineering*, 387, 1–16.
- Woods, H., Hargis, W., Jr, Hershner, C., and Mason, P. 2005. Disappearance of the

natural emergent 3-dimensional oyster reef system of the James River, Virginia,  
1871–1948. *Journal of Shellfish Research* 24:139–142.

Zimmerman, R.J., T. Minello, T. Baumer, and M. Castiglione. 1989. Oyster reef as  
habitat for estuarine macrofauna. NOAA Technical Memorandum, NMFS-SEFC-249,  
16 p.

## CHAPTER 1

### **Lethal and sublethal effects of sediment burial on the eastern oyster, *Crassostrea virginica***

#### Abstract

Eastern oysters (*Crassostrea virginica*) are important ecosystem engineers in estuarine systems, building biogenic reef structures through reproduction, mortality, and biodeposition. Persistence of reefs relies on reef growth outpacing degradation processes, including sediment deposition, which can kill resident oysters and inhibit oyster growth and larval settlement. Sediment deposition may result from episodic events, such as storms and dredging, or by more chronic inputs due to run-off. Despite its importance, the quantitative impacts of burial depth on oyster survival and sublethal effects of sediment deposition on function are undefined. In this mesocosm study, we quantified the effects of partial and complete burial (0, 50, 70, 90, and 110 % of oyster shell height) on survival, biodeposition, condition index and growth of eastern oysters (shell height: 25-75 mm). The estimated burial-survival function indicated survival only declined significantly when 90 % or more of an oyster was buried; the critical burial depth that induced 50 % mortality ( $LD_{50}$ ) was 108 %. This finding will allow scaling of mortality rates of oysters as a function of sediment load and oyster size. Biodeposition and condition index were negatively related to burial depth, whereas shell growth increased with burial depth. Decreases in biodeposition and condition index were likely related to the disruption of feeding by burial and reallocation of energy resources to shell growth. The increase in shell growth was analogous to vertical migration in benthic infaunal

species such that buried oysters increased growth in the vertical axis to breach the sediment surface to feed and respire, at the expense of tissue growth. While oysters are tolerant to partial burial in terms of survival, burial has negative impacts on metabolic processes that contribute to oyster population demographics and reef habitat quality. Future management efforts should consider the effects of partial burial, which often occur more frequently than catastrophic burial events causing mass mortality.



## INTRODUCTION

With more than one-third of the world's population living within 100 km of the coastline (Cohen et al. 1997), the potential for human impacts on coastal and estuarine environments is profound. One such impact is the dramatic increase in terrestrial sediment flux to estuaries as a result of deforestation, land use change, and agriculture (Syvitski et al. 2005). Generally, estuaries act as sediment traps, steadily infilling until an erosional event, such as a strong storm, removes sediment from the system (Dellapenna et al. 1998). Massive land clearance by European settlers of the U.S. mid-Atlantic region in the late 18<sup>th</sup> century resulted in a 10-fold increase in sediment inputs to coastal habitats (Meade 1982). Additionally, sediments resulting from this initial land clearance are stored in watershed reservoirs, which are expected to continue to augment sediment inputs for several centuries (Meade 1982). Predicted changes in the frequency and intensity of storm activity, precipitation and streamflow associated with climate change are likely to further increase sediment inputs (Pyke et al. 2008, Najjar et al. 2010) and mobilize watershed sediment stores (Meade 1982). As such, estuaries will continue to experience a constant influx of sediment from exogenous sources.

Sediment inputs influence the structure and function of estuarine habitats through modification of sediment characteristics, nutrient availability, and water clarity. Sediment grain size is an important factor structuring benthic communities (Gray 1981), and inputs that significantly alter grain size or bury epifaunal or infaunal organisms can diminish benthic diversity and productivity (Maurer et al. 1981). Suspended sediments reduce light availability for estuarine autotrophs; sediment cores from Chesapeake Bay indicate a reduction in microphytobenthos due to reduced water clarity and a shift to pelagic

phytoplankton species due to nutrient inputs from run-off after watershed deforestation (Cooper & Brush 1993). Sediment and nutrient inputs have also been implicated in the widespread reduction in seagrass cover (Orth et al. 2006), with serious consequences for estuarine biodiversity (Waycott et al. 2009).

Like seagrasses, biogenic reefs constructed by the eastern oyster, *Crassostrea virginica*, are important features structuring the estuarine landscape. Oyster populations and reefs provide a suite of ecosystem services (Coen et al. 2007, Grabowski & Peterson 2007), such as habitat for benthic macrofauna (Zimmerman et al. 1989), enhanced benthic-pelagic coupling and nutrient cycling (Lenihan 1999, Kellogg et al. 2013), and shoreline stabilization (Meyer et al. 1997, Piazza et al. 2005). Unfortunately, oyster population biomass and associated reef habitat along the Gulf of Mexico and Atlantic coasts in the United States have declined by 88 % and 64 %, respectively (Zu Ermgassen et al. 2012). Declines in reef quality have been attributed to overharvesting, habitat degradation, and disease (Rothschild et al. 1994). Moreover, the removal of shell material and disassociation of the reef structure by harvesting reduces reef elevation, rendering reefs more susceptible to siltation and eventual burial (Rothschild et al. 1994, Smith et al. 2001).

Reef burial can be caused by episodic events such as storms (Livingston et al. 1999), run-off (Twichell et al. 2010), or dredging (Wilber & Clark 2001) whose associated deposition can rival or exceed annual rates (Norris 1953, Miller et al. 2002, Suedel et al. 2014). Winds, waves, and storm surge causes the erosion and mass transport of sediments (Livingston et al. 1999), the deposition of which can kill oysters and bury entire reefs (Norris 1953, Miller et al. 2002, Twichell et al. 2010). Run-off events



associated with changes in freshwater inputs to the watershed by precipitation, seasonal inputs, or controlled releases from man-made reservoirs can deposit up to 31 cm of sediment in a single event (Kniskern & Kuehl 2003). These events can also bury reefs, particularly those adjacent to river mouths due to their proximity to sediment plumes (Twichell et al. 2010). Additionally, freshwater pulses can cause oyster mortality and exacerbate burial effects. Each year, approximately 400 million m<sup>3</sup> of sediment are dredged throughout the United States to maintain channels (Jones & Lee 1981). Dredged sediments produce a plume that increases suspended sediment concentrations and affects areas up to 500 m from the dredge site (Wilber & Clark 2001, Suedel et al. 2014). Suspended sediments can abrade gill tissues, inflicting metabolic stress (Suedel et al. 2014), and associated deposition may increase oyster mortality by 40 % (Rose 1973).

Despite the presumed importance of sediment deposition on oyster reef persistence (Jordan-Cooley et al. 2011), few studies have addressed burial effects quantitatively and results of those studies have been variable. At the reef scale, sediment deposition has been cited as the main cause of intertidal and subtidal reef failure (Bahr & Lanier 1981, Taylor & Bushek 2008, Powers et al. 2009). In contrast, Fodrie et al. (2014) found no correlation between sedimentation and various oyster metrics at a small sub-reef scale (0.25 m<sup>2</sup> patches). To elucidate the effects of burial, we experimentally examined the lethal and sublethal effects of sediment deposition on oysters. Specifically, in mesocosm experiments, we (i) quantified survival in various burial depth treatments; (ii) determined a critical burial depth at which 50 % of oysters experience mortality (LD<sub>50</sub>); and, (iii) measured sublethal responses to partial burial by quantifying biodeposition, growth, and condition index. Biodeposition and growth are the key metabolic processes

contributing to reef accretion (DeAlteris 1988), which is critical for sustaining reef habitat. Condition index, a ratio of somatic tissue weight to shell cavity volume, is an indicator of oyster health, identifying whether oysters are negatively impacted by burial even if they do not experience mortality. The results of this study can be incorporated into mathematical models (Jordan-Cooley et al. 2011, Wilberg et al. 2013) to determine reef-scale impacts to inform management actions for imperiled eastern oyster populations throughout their range.

## **METHODS**

### **Mesocosm experiment I**

We measured survival, growth, condition index, and biodeposition of 278 hatchery-reared triploid oysters over a 28-d period in July 2013. Episodic wind events and spring tidal currents control sediment resuspension and transport in shallow estuarine habitats, indicating that partial or complete burial conditions dominate on time scales of days to weeks (Sanford et al. 1991, Kniskern & Kuehl 2003). Consequently, we limited our experiments to the most relevant time frame for deposition in shallow areas, about 1 month, which would encompass spring-neap cycles and episodic events. Triploid oysters were used to minimize individual variation due to reproductive effort and to provide a wide range of sizes of available oysters, given the time of year. Using hatchery oysters from a single source also minimized variation due to differences in acclimation and handling stress, as all oysters were reared in the same area and under the same methods of cultivation.

Oysters of 25-75 mm shell height (SH) were obtained from a local oyster grower on the York River, Virginia, and transported to the Virginia Institute of Marine Science in

Gloucester Point, Virginia. Shell height (mm) and wet weight (g) were measured for each oyster, and oysters were randomly assigned to one of the following burial treatments: 0 %, 50 %, 70 %, 90 %, and 110 %. We hypothesized that burial depth relative to the size of the oyster was important; therefore, treatments represented the percentage of an individual oyster's shell height that was buried by sediment (Fig. 1). Oysters in the 0 % burial treatment (control) were placed on top of the sediment.

To ensure proper burial depth and to prevent subsidence of the oyster during the experiment, an individual experimental container (473 mL) with an elevated bottom was prepared for each oyster. Portland cement poured into each container was used to elevate the oyster off the bottom of the experimental container such that the appropriate percentage of the oyster's height given the assigned treatment would be exposed when the container was filled flush with sediment. Each oyster was placed in the prepared container upright with the umbo of the oyster resting on the cement bottom (Fig. 1). The containers were filled to the rim with sand (mean  $\pm$  SE;  $96.6 \pm 0.2$  % sand) obtained approximately 5 m offshore of Gloucester Point, Virginia. This sediment grain size typifies sediments in shallow estuarine areas throughout the eastern oysters' range, including the Gulf of Mexico (Lisle & Comer 2011) and other mid-Atlantic estuaries (Kiddon & Buffum 2000). Prior to use, sand was sieved with a 63- $\mu$ m sieve to remove debris and defaunated by air-drying.

Experimental containers were distributed evenly into 6 large outdoor mesocosm tanks (2.43 m x 1.11 m x 0.91 m) at the Virginia Institute of Marine Science. Tanks were first divided into 50 even quadrants and containers with oysters were randomly distributed into the quadrants, excluding those directly below the tank inflow and near the

tank drain pipe. This distribution ensured even spacing of experimental units throughout the tanks. A downspout was added to the inflow to direct the flow of water towards the bottom of the tank to minimize resuspension of biodeposits collected during the experiment. Tanks were supplied with a continuous flow of unfiltered York River water at a rate of approximately  $5.33 \text{ L min}^{-1}$ , resulting in full replacement at least three times daily. Aquarium bubblers were added to each tank to ensure adequate oxygenation.

Environmental conditions in the tanks were monitored using data loggers (Tidbit v2 Water Temperature Data Logger, Onset, Inc.) and a hand-held data sonde (Yellow Springs Instruments, Inc.). Temperature data to the nearest  $0.01^{\circ}\text{C}$  was collected every 30 min for the duration of the experiment by data loggers. Additional measurements of temperature, salinity, and dissolved oxygen (DO) were taken in the water column at the approximate height of experimental containers periodically with the data sonde to validate the readings from data loggers and to monitor additional environmental variables expected to influence oyster metabolism.

Random samples of oysters in each burial treatment were removed from the tanks at weekly intervals, for a total of 4 sampling events over the 28-d period. This resulted in 14 replicates per withdrawal-treatment combination, with the exception of the 50 %-week 1 and 90 %-week 4 treatments. In these 2 treatment-week combinations, 1 oyster each was found to be dead prior to the start of the experiment, resulting in 13 replicates. The subsampled oysters were assessed for survival, growth, and condition index. Biodeposit collection trays were placed on those oysters selected for sampling 6 d prior to the sampling event. Biodeposits were then collected and processed along with the oysters each week. After removing the oysters, the experimental containers were filled with

additional sediment and returned to the mesocosms to avoid changes in flow within the tanks.

### **Lethal Effects**

Oysters were removed from their containers, scrubbed, and visually assessed for survival as follows: live oysters were those whose valves were either tightly closed or closed after handling. Gaping or empty oysters were considered dead. Two oysters, one each in the 50 %-week 1 and 90 %-week 4 treatment combinations, were thought to have been dead at the start of the experiment (valves filled with mud); therefore, these 2 data points were excluded from all analyses. Shell height (mm) and wet weight (g) were measured for all oysters and used to determine growth rates. Live oysters were retained for biodeposition and condition index analyses.

### **Sublethal Effects**

Biodeposits were collected in aluminum trays placed on top of each experimental container. A small slit was cut in the bottom of each collection tray to allow the oyster to protrude through the tray and gape sufficiently, with the exception of collection trays for the 0 % and 110 % treatments for which the tray was left intact. Oysters in the 0 % burial treatment were placed on top of the intact collection tray, which was situated on the sediment surface. Oysters in the 110 % burial treatment did not protrude from the sediment, and the collection tray was placed on top of the oyster on the sediment surface.

After 6 d, collection trays were capped and removed and biodeposits rinsed into pre-weighed dishes. Any biodeposits remaining on the sediment surface in partial burial treatments after the removal of the collection tray were collected by pipette. Biodeposits were easily distinguished from underlying sediments due to their size and pelletized

nature. Biodeposits were dried to constant weight at 60°C and weighed to the nearest 0.001 g ( $\pm 0.0001$  g). Biodeposition rate was calculated as the dry weight of biodeposits divided by the number of days of biodeposit collection (6 d), expressed as g dw d<sup>-1</sup>.

Condition index was determined for all live oysters following each sampling event. Oyster condition index was assessed using the index of Abbe & Albright (2003), the ratio of dry tissue weight (g) to shell cavity volume, which is approximated by subtracting the wet weight of the shells immediately after removing oyster tissue from the total wet weight. This condition index accounts for the partitioning of resources into tissue vs. shell growth. Higher condition indices suggest that growth effort is directed toward tissue growth, whereas lower condition indices suggest the dominance of shell growth over tissue growth or the deterioration of tissue quality (weight) over time.

Prior to the start of the experiment and after withdrawal, each oyster's shell height, width, depth, and total wet mass were recorded. Shell height difference was chosen to represent growth rate, as it was the least variable of all growth responses. The difference in shell height before and after the experiment was divided by the number of days in the trial to determine the growth rate in mm d<sup>-1</sup>. Oysters with calculated growth rates < 0 were considered to be 0, since it is likely that the negative change in shell height was due to measurement error rather than loss of shell at the margin.

### **Statistical analyses**

Oyster survival was analyzed by logistic regression with burial treatment and time as predictor variables and tank as a blocking factor. Combinations of these variables were used to define the *a priori* candidate model set (Table 1). Initial analyses of the effect of oyster size on survival indicated that size was not a significant factor ( $p = 0.56$ );



therefore, oyster size was not included as a factor. Akaike's information criterion (AIC) was used to compare candidate models (Anderson 2008). Effect sizes and likelihood ratio tests were used to assess model fit. The 50 % mortality level ( $LD_{50}$ ) for burial treatments was determined by solving for the inflection point of the best-fitting logistic model. The 95 % confidence interval for  $LD_{50}$  was determined by bootstrapping ( $n = 1000$ ) with replacement.

Oyster growth, condition index, and biodeposition rate were analyzed using multiple linear regression, with burial treatment and week as predictor variables and tank as a blocking factor. To account for oyster size, an initial regression of size against each response variable was conducted. If oyster size was significant, then the residuals of that regression were analyzed with the candidate model set (Table 1). If size was not significant, then the original data were used in regressions with the candidate model set. We used AIC to select the best model for each response variable. If AIC values indicated more than one plausible model ( $w > 0.1$ ), effect sizes and model fit ( $r^2$ ) were examined to determine the most parsimonious model (Anderson 2008). Shapiro-Wilk and Levene's tests and visual inspection of model residuals were used to assess normality and homogeneity of variance assumptions. Biodeposition rate data were log transformed to meet the assumptions of normality and homogeneity of variance; all other variables satisfied linear regression assumptions without transformation. Differences in environmental variables among tanks were assessed with a one-way analysis of variance (ANOVA) model. All statistical analyses were conducted using R statistical software, version 3.1.0 (R Core Development Team 2013).

## Mesocosm experiment II

A second burial trial using wild, diploid oysters collected from the Great Wicomico River, Virginia was conducted to determine if ploidy would affect the burial-survival function. Experimental containers for 56 adult oysters (60-90 mm SH) were prepared as described previously. Oysters were randomly assigned to 0, 25, 50, 70, 90, 100, and 110 % burial treatments ( $n = 8$ ) and randomly distributed into 3 outdoor mesocosm tanks used in the triploid trial. After 12 d, all oysters were removed and assessed for survival. Oysters that were tightly closed or closed upon handling were considered live; gaping oysters that did not respond to handling or empty valves were considered dead. Diploid oysters were expected to experience greater mortality and metabolic stress than triploid oysters due to gametogenesis and reproduction; therefore, diploid trials were shorter than triploid trials. The trial duration (12 d) represents the approximate interval of partial burial due to spring-neap tidal cycles in the absence of other episodic events. Diploid survival was analyzed using logistic regression and AIC model selection with burial treatment and oyster size as factors (Table 2). Tank and week were not included as factors because no significant tank effects were found in previous trials and all oysters were sampled at the conclusion of the 12 d trial. The diploid burial-survival function was estimated from the best-fitting logistic model, and the point of 50 % mortality ( $LD_{50}$ ) was estimated from the inflection point of the function. The 95 % confidence interval about the inflection point was determined by bootstrapping with replacement ( $n=1000$ ).

## **RESULTS**

### **Mesocosm experiment I**

#### **Mesocosm Conditions**

Temperatures ranged from 23.2 to 36.6°C, which is well below the maximum thermal tolerance of eastern oysters (48.5°C; Shumway 1996). The mean temperature observed in our mesocosms (27.2°C; Table 3) is within the 15-y mean temperature range for Virginia (23-29°C; Southworth & Mann 2014) and for other mid-Atlantic estuaries (19-31°C; Kiddon & Buffum 2000). Elevated temperatures (>30°C) were observed for <10 % of the experiment's duration, indicating that any effects of thermal stress are likely minimal relative to stress due to burial. The data logger for one tank failed to deploy, and did not collect data for the duration of the experiment; therefore, the temperature data collected by the YSI data sonde was used for comparison. Salinity varied from 17.5 to 22.4, which was also within the tolerances of this euryhaline species (Table 3). Dissolved oxygen (DO) levels within experimental tanks remained within normoxic limits throughout the experiment (Table 3), indicating that water column DO levels were sufficient to maintain normal metabolic functions. There were no significant differences between mesocosm tanks in any of the environmental variables (Table 3).

#### **Lethal Effects**

The estimated burial-survival function indicated that survival was not significantly impacted by burial up to 70 %; the LD<sub>50</sub> was 108 % burial (Fig. 2a). In total, 40 oysters died over the course of the experiment, all of which occurred at burial depths 70 % and greater (Fig. 2a). The highest mortality occurred in the first week and in the highest burial treatment. Burial treatment was the only significant factor affecting

survival (Table 1), and this model provided a significantly better fit than the null (intercept only) model (Wald test,  $df = 276$ ,  $p < 0.01$ ). The remaining models did not improve the fit significantly over the treatment-only model (Table 1).

#### Sublethal Effects

Biodeposition rate ( $g\ dw\ d^{-1}$ ) was highest in the 0 % burial treatment and decreased monotonically and significantly across partial burial treatments (Fig. 3, Table 4). The lowest deposition rate occurred in the 110 % burial treatment (Table 4); however, this value represents passive deposition in the tank rather than biodeposition, as indicated by the absence of fecal pellets. Oysters in the 110 % treatment were entirely buried for the duration of the experiment, and the biodeposit collection trays were situated on the sediment surface within the experimental container. Biodeposition rates in all other treatments (Table 4) may have included some passive deposition, but it was likely minimal because most of the sediment was bound in pseudofeces due to active filtration by oysters. Week was also a significant factor controlling biodeposition rates ( $p < 0.01$ ). Mean biodeposition decreased over time in all burial treatments, indicating increasing metabolic stress over time or temporal changes in seston inputs over the duration of the experiment.

Condition index varied additively with burial treatment and week (Table 1). AIC comparisons indicated that the model including tank effects was the best of the candidate set; however, parameter estimates for tank effects were non-significant ( $p = 0.07$ ), so the treatment-week model was chosen as most parsimonious (Table 1, Anderson 2008). Burial treatment and time had opposing effects on oyster condition index. Condition index generally declined with increasing burial (Fig. 4), but increased with time. The

highest condition index was in the 50 % burial treatment (Table 4). The 0, 50, and 70 % burial treatments tended to have similar condition indices, whereas the 90 and 110 % treatments tended to have lower condition indices (Table 4). Over all burial treatments, the highest condition index was in the 21-d sampling interval (mean  $\pm$  SE:  $11.5 \pm 0.2$ ), followed by the 28-d interval ( $11.0 \pm 0.3$ ).

Oyster size had a significant effect on growth rates ( $r^2 = 0.05$ ;  $p < 0.01$ ). To account for oyster size, we analyzed the residuals of the oyster size-growth rate linear regression with the candidate model set (Fig. 5, Table 1). Burial treatment was the only significant factor controlling growth rates (Table 1), which increased with burial depth (Fig. 5). The highest growth rate was in the 110 % burial treatment, which was nearly 3 times the mean growth rate of the 0 % burial treatment (Table 4). Mean growth rates in 70 and 90 % burial treatments were double the growth rate of the 0 % treatment (Table 4).

### **Mesocosm experiment II**

After 12 d, 8 of 56 oysters in the diploid trial died, which occurred in the 50, 70, 100 and 110 % burial treatments. The highest mortality was in the 110 % burial treatment ( $n=5$ ), and was equivalent in the 50, 70, and 100 % treatments in which a single oyster died. No mortality was observed in 0, 25, or 90 % treatments. As in the triploid trials, burial treatment was the only significant factor affecting survival (Table 2,  $p = 0.023$ ); oyster size and treatment-size interactions were not significant ( $p = 0.44$  and  $p = 0.41$ , respectively). The treatment only model provided a significantly better fit than the null model (Wald test;  $df = 2$ ;  $p = 0.001$ ) and the additive and interaction models were not significantly different from the treatment model (Fig. 2b, Table 2). The diploid burial-

survival function (Fig. 2b) had an estimated inflection point of 118 % burial (95 % CI: 96-154 %), which is higher than the LD<sub>50</sub> for triploids of 108 %.

## **DISCUSSION**

The key findings of our study were that (i) oysters buried in sediment did not exhibit significant mortality or sublethal effects until at least 70 % of the shell was buried, (ii) the survival response differed little between diploid and triploid oysters or by oyster size, (iii) biodeposition and condition index were inversely related to burial depth, and (iv) shell growth increased with burial depth.

### **Lethal Effects**

With the exception of 1 oyster in the 70 % burial treatment, all mortality occurred in the 90 and 110 % burial treatments over the 28-d experiment. The maximum mortality was 62 % during the first week in the 110 % treatment group, although oyster survival did not vary significantly with time. This mortality was much lower than those observed previously (Dunnington 1968, Lund 1957a), in which 100 % mortality of completely buried adult oysters occurred within 7 d. However, the burial depths in previous experiments were higher, up to 76 mm of sediment (Dunnington 1968). In experiments with comparable burial depths, lethal burial depths for adult oysters were 10-20 mm below the sediment surface (Kranz 1974, Essink 1999), though these values were not calculated relative to shell height. The maximum burial depth in the present study was 7.4 mm for a 73.5 mm oyster. Lund (1957a) suggested that shallowly buried oysters (<12.7 mm) may be able to clear sediment from their bill by repeatedly opening and closing their valves, a behavioral adaptation to burial. Exposed valves were not observed in any of the 110 % burial treatments; however, small depressions in the sediment surface

were evident, which may indicate the movement of buried oysters in an attempt to remove sediment (Lund 1957a, Hinchey et al. 2006).

Diploid and triploid oysters responded similarly to burial, with the highest mortality observed in the complete (100 %) burial treatments (Fig. 2). Diploid oysters exhibited mortality at lower partial burial (50 %) than triploids (70 %), but mortality in the 50 % diploid treatment was limited to a single oyster. The  $LD_{50}$  for diploids was 118 % burial, which is higher than the 108 %  $LD_{50}$  for triploids (Fig. 2); however, these estimates were not significantly different from one other, suggesting that both triploids and diploids are tolerant to partial burial in terms of survival. The higher  $LD_{50}$  for diploids may have resulted from a shorter experimental duration or the larger size of oysters used in diploid trials. Diploid trials were limited to 12 d, whereas triploid trials lasted up to 28 d. A longer trial duration for triploids may have led to exhaustion of metabolic reserves under non-feeding, anaerobic conditions leading to higher mortality rates and an associated decrease in the estimated  $LD_{50}$ . Additionally, diploid oysters were collected from the wild and were larger (60-90 mm SH) than triploids (25-75 mm SH). Although physiological studies suggest that larger oysters are better suited to survive anoxic conditions due to larger capacity for carbohydrate storage, which is catabolized in anaerobic pathways (de Zwaan et al. 1976), we detected no effects of oyster size on survival in either triploid or diploid trials. Overall, both diploid and triploid oysters were tolerant to partial burial and responded similarly to burial regardless of ploidy, which reaffirms the findings of a previous study on the lack of an effect of ploidy on oyster survival (Walton et al. 2013).

Oyster size did not have a significant effect on survival, which was unexpected. Under the anaerobic conditions of burial, oysters revert to catabolism of carbohydrates to maintain metabolic function (de Zwaan & Wijsman 1976). The ability of oysters to carry out anaerobic metabolism increases with developmental stage, as body size and the capacity for carbohydrate storage increases (Widdows et al. 1989), thus, we expected that juvenile oysters would experience higher mortality due to burial than adult oysters. Kranz (1974) also observed no size effects on survival for buried *C. virginica*, purple-hinge rock scallops (*Hinnites multirugosus*), which similarly adhere to hard surfaces, and bay scallops (*Aequipecten Irradians*), whereas larger individuals of two mussel species (*Modiolus demissus* and *Mytilus edulis*) did survive better than smaller individuals. Others have documented effects of burial and anaerobic metabolism on juvenile *C. virginica* (16 mm SH, Widdows et al. 1989; 9-12 mm SH, Hinchey et al. 2006), but did not examine size effects. The fact that oysters in this study did not differ in survival suggests that neither the capacity for glycogen storage facilitated by larger body size nor the ability to physically ventilate the sediment to relieve sediment overburden were driving factors in survival.

Conversely, if we assume that oyster mortality increases significantly at burial depths >110 % SH, then smaller oysters would experience significantly higher mortality at absolute sediment loads that would not affect larger oysters appreciably. For instance, a 6-mm sediment load should cause relatively low mortality (about 40 %) in adult oysters of 100 mm SH (106 % burial), whereas the same 6-mm load should cause 100 % mortality in juvenile oysters  $\leq 20$  mm SH ( $\geq 130$  % burial). A major benefit of our



findings is therefore that it will allow scaling of mortality rates of oysters as a function of sediment load and oyster size.

Epifaunal and infaunal species demonstrate varying degrees of tolerance to burial, based on their response to metabolic stress and life history strategy (Kranz 1974, Maurer et al. 1981, Hinchey et al. 2006). Motile infaunal bivalves are generally better suited than epifaunal bivalves to escape burial by vertical migration and siphon extension. Mortality of the infaunal bivalves *Mercenaria mercenaria* and *Nucula proxima* increased with burial depth and burial time (Maurer et al. 1981); however, *M. mercenaria* were able to overcome more than 16 cm of burial by vertical migration (Maurer et al. 1981). Epifaunal suspension feeders, such as oysters and mussels, are more susceptible to burial due to their sessile life history and lack of a digging foot (Kranz 1974, Hinchey et al. 2006). Overall, low mortality rates in the present study indicate that oysters are highly tolerant to partial and shallow total burial on weekly time scales, more so than other epifaunal suspension feeders (Maurer et al. 1981, Hinchey et al. 2006).

### **Sublethal Effects**

Biodeposition was inversely related to partial burial depth. The sediment collected in the 110 % treatment represented background deposition within the mesocosms, since oysters neither penetrated the sediment surface nor produced fecal pellets in that treatment. Similarly, no biodeposition was observed for juvenile oysters (9-12 mm SH) buried in 2-5 mm of silty sand (Hinchey et al. 2006). Biodeposition rates in 0-90 % burial treatments were 1.4-3.4 times greater than background sediment deposition (110 %). Mean biodeposition in the unburied (0 %) treatment ( $0.27 \text{ g dw d}^{-1}$ ) was comparable to values in previous studies ( $0.1-0.2 \text{ g dw d}^{-1}$ ; Haven & Morales-Alamo

1966, 1972). Although biodeposition was reduced in the 50 and 70 % treatments (0.15-0.18 g dw d<sup>-1</sup>), it also fell within normal ranges of biodeposition for oysters not subjected to burial (Haven & Morales-Alamo 1966).

We did not observe significant effects of oyster size on biodeposition. In contrast, Haven & Morales-Alamo (1972) found that biodeposition rate increased asymptotically with oyster size, though the rate per unit weight of oyster decreased with increasing oyster size. The difference in response may be due to the different sizes of oysters used in the two studies, or due to our use of triploid oysters. Diploid oysters experience additional metabolic stress relative to triploids during spawning periods (Dégremont et al. 2012), and reproductive effort in oysters scales with oyster size (Thompson et al. 1996); therefore, diploid oysters are more likely to exhibit a size-specific response to external stressors than are triploids.

Biodeposition is a critically important process contributing to reef sustainability (DeAlteris 1988) and modulating sediment supply on and around reefs (Widdows et al. 1998). Oyster biodeposits are composed primarily of small inorganic particles with very slow settling velocities. The repackaging of these suspended sediments by oysters into feces and pseudofeces increases the settling velocity and cohesiveness of the sediment, leading to rapid deposition (Haven & Morales-Alamo 1966, 1972, DeAlteris 1988). In areas where bivalves occur, biodeposition accounts for as much as 25 % of all deposition, and the rate of sediment delivery via biodeposition can be 8-fold higher than gravitational settling (Lund 1957a).

Long-term subtidal oyster reef persistence is determined by a balance between sediment accumulation, reef accretion, and sea level rise (DeAlteris 1988). Biodeposits

contribute to reef accretion by filling reef interstitial space, effectively elevating the reef surface. Biogenic sediments (biodeposits and shell) on subtidal reefs accumulate as quickly as 50 cm per 100 y (DeAlteris 1988). Maximum sea level rise estimates for the mid-Atlantic region are 20-29 cm by 2100 (Sallenger et al. 2012), indicating that subtidal oyster reefs should outpace sea level rise given ideal conditions for accretion, similarly to intertidal reefs (Rodriguez et al. 2014). In the absence of biodeposits, caused either by reduced biodeposit production or erosion, reef accretion depends solely on the addition of shell through oyster recruitment and mortality, the rate of which may be less than the current rate of sea level rise (DeAlteris 1988). Intertidal reefs experience similar depositional events due to shifting sediments and storms (Taylor & Bushek 2008), but the effects of these events may be exacerbated by reduced erosion due to lack of inundation, leading to further reductions in accretion on intertidal reefs relative to subtidal reefs. Thus, the reduction in biodeposit production associated with partial burial observed in this study suggests that non-catastrophic burial events can have lasting impacts on long-term oyster reef persistence even in the absence of mass mortality.

Condition index decreased significantly with increased burial depth. Oysters in the control treatment (0 % burial) had the highest condition index, indicating a higher tissue-to-shell ratio in these oysters than those in other treatments. The decline in condition index with burial depth could be indicative of either the deterioration of tissue due to metabolic stress and sustained anaerobic conditions or to the investment of energy into shell growth when access to food and oxygen are limited by burial. In contrast, high suspended-sediment loads had little effect on oyster condition index after 7 d (Suedel et

al. 2014), indicating that sediment deposition and burial are more detrimental to oyster condition index than are high suspended-sediment loads.

Shell growth was influenced by oyster size and burial treatment. Maximum growth rates were in the 110 % burial treatment ( $0.32 \pm 0.05 \text{ mm d}^{-1}$ ), which is higher than the growth rate for triploid *C. virginica* under normal conditions ( $0.2 \text{ mm d}^{-1}$ ; Harding 2007). In addition, mean growth rates of the 70, 90, and 110 % treatments ( $0.22\text{-}0.32 \text{ mm d}^{-1}$ ) exceeded previously published growth rates ( $0.1\text{-}0.2 \text{ mm d}^{-1}$ ) for both diploid and triploid *C. virginica* (Harding 2007, Kraueter et al. 2007, Dégremont et al. 2012, Walton et al. 2013). Growth rates for the 0 and 50 % burial treatments ( $0.12\text{-}0.16 \text{ mm d}^{-1}$ ) agreed well with published estimates.

The effect of partial burial on oyster growth has not previously been addressed, but sediment deposition has been shown to negatively affect oyster growth (Grant et al. 1990, Lenihan 1999). Accelerated growth in shell height of oysters has been recorded in response to high-density conditions, which produces long and narrow oysters with shells up to 36 cm long (McCormick-Ray 2005). Under resource competition, oysters grow in the vertical axis to reach phytoplankton higher in the water column. Additionally, oysters in muddy habitats, which are often found almost completely buried, also exhibit an elongated shape, a presumed adaptation to rapidly accumulating soft sediments (Galtsoff & Luce 1930, Chinzei 1986). This life history may represent a trade-off by which oysters are subjected to enhanced sedimentation but avoid shell-boring polychaetes and sponges, which cannot survive anoxic sediments (Carver et al. 2010). We suspect that oysters experiencing stress caused by partial or complete burial exhibit a similar vertical growth response to reach the sediment surface to feed and respire.

That the highest growth rate and lowest condition index were in the 110 % treatment suggests that decreases in condition index were more likely due to oyster responses to burial than deterioration of tissues under anoxic conditions. We surmise that oysters responded to sediment burial by allocating energy reserves to shell growth in an attempt to remain above the sediment surface. This response would be comparable to vertical migration behavior by clams buried by sediment (Maurer et al. 1981). Seagrasses also exhibit a similar response, in which buried shoots increase vertical growth in response to moderate burial (Marba & Duarte 1994, Cabaço et al. 2008). In oysters, this may represent a physiological response to accumulating biodeposits, in which oysters allocate resources to vertical growth. Oysters usually live in constant contact with biodeposits, which can accumulate rapidly and produce anoxic, reducing conditions similar to those below the sediment surface (Lund 1957b). The monotonic increase of growth rate with increasing burial suggests that this growth response was triggered before total burial and may serve as an important mechanism for oysters to outpace sediment accumulation. Growth rates were highest under complete burial, indicating that the likely limit to this increased growth is burial that induces mass mortality. Extrapolations from the estimated burial-survival function suggest that mass mortality occurs at approximately 130 % burial.

The use of hatchery-reared triploid oysters in this experiment allowed for control of individual variation, but it precludes precise application of our observations of sublethal burial effects to wild, diploid oysters. Our results indicated no significant difference between triploid and diploid oysters with respect to survival. This may suggest that diploid oysters would respond similarly in sublethal effects, but previous

studies indicate triploid oysters grow faster and have higher condition indices than diploid oysters under field conditions (Walton et al. 2013). Thus, our shell growth rates and condition indices are likely to overestimate those of diploid oysters (Walton et al. 2013), but we expect the observed trends to hold.

### **Conclusions**

The impacts of sediment deposition on estuarine habitats, particularly biogenic habitats, are expected to increase with climate change. Amplification in storm frequency and intensity will increase the likelihood of mass transport of sediment that can cause rapid deposition events (Najjar et al. 2010). Increases in precipitation intensity are likely to mobilize stored sediments and magnify sediment inputs to estuaries (Meade 1982, Najjar et al. 2010), while sea-level rise will increase available accommodation space and enhance coastal erosion due to changes in estuary volume and tidal currents (Short & Neckles 1999). Sediment inputs affect water clarity, nutrient availability, and sediment characteristics, all of which impact species' distributions and estuarine productivity. Overall, the impacts of sediment deposition and burial on biogenic habitats are likely to increase over time, indicating that a clear understanding of the response of oyster reefs to short- and long-term burial events is needed.

The findings of our study can improve population model predictions by accounting for sublethal impacts of burial and episodic events that may impact reef persistence. These predictions may be used to inform reef placement or construction criteria to minimize adverse effects of partial burial on reef-building processes, particularly biodeposition. This study indicated that oysters can survive burial up to 70 % of their shell height, but that sublethal effects of burial on biodeposition, growth,

and condition index may occur at lower levels of partial burial. Oyster metabolic processes contribute to the growth of oyster reefs over time through reproduction, growth, and the production of biodeposits (DeAlteris 1988). These reef-building processes help maintain ideal reef conditions by elevating the reef off the bottom to heights where oysters experience reduced sedimentation, higher survival, and faster growth (Lenihan 1999). Understanding the impacts of sediment burial on oyster survival and function is critical to the success of continued restoration efforts, the recovery of natural oyster populations, and the productivity of aquaculture operations. This study demonstrates that burial of oysters by sediment, even partially, can impact the sustainability of natural and man-made oyster reefs by impairing reef-building processes.

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## LITERATURE CITED

- Abbe GR, Albright BW (2003) An improvement to the determination of meat condition index for the Eastern Oyster *Crassostrea virginica* (Gmelin 1791). *J Shellfish Res* 22:3747-3752
- Anderson DR (2008) Model based inference in the life sciences. Springer Science Press, New York, NY
- Bahr LM, Lanier WP (1981) The ecology of intertidal oyster reefs of the south Atlantic coast: A community profile. US Fish and Wildlife Service, Washington, DC, FWS/OBS-81/15
- Cabaço S, Santos R, Duarte CM (2008) The impact of sediment burial and erosion on seagrass: a review. *Est Coast Shelf Sci* 79:354-366
- Carver CE, Thériault I, Mallet AL (2010) Infection of cultured eastern oysters *Crassostrea virginica* by the boring sponge *Cliona celata*, with emphasis on sponge life history and mitigation strategies. *J Shellfish Res* 29:905-915
- Chinzei K (1986) Shell structure, growth, and functional morphology of an elongate cretaceous oyster. *Palaeontology* 29:139-154
- Coen LD, Brumbaugh RD, Bushek D, Grizzle R, Luckenbach MW, Posey MH, Powers SP, Tolley SG (2007) AS WE SEE IT: Ecosystem services related to oyster restoration. *Mar Ecol Prog Ser* 341:304-307
- Cohen JE, Small C, Mellinger A, Gallup J, Sachs J, Vitousek PM, Mooney HA (1997) Estimates of coastal populations. *Science* 278:1211-1212
- Cooper SR, Brush GS (1993) A 2500-year history of anoxia and eutrophication in Chesapeake Bay. *Estuaries* 16:617-626



- De Alteris JT (1988) The geomorphic development of wreck shoal, a subtidal oyster reef of the James River, Virginia. *Estuaries* 11:240-249
- De Zwaan A, Wijsman TCM (1976) Anaerobic metabolism in bivalvia (Mollusca): Characteristics of anaerobic metabolism. *Comp Biochem Phys B* 54:313-323
- Dégremont L, Garcia C, Frank Lawale A, Allen SK (2012) Triploid oysters in the Chesapeake Bay: Comparison of diploid and triploid *Crassostrea virginica*. *J Shellfish Res* 31:21-31
- Dellapenna TM, Kuehl SA, Schaffner LC (1998) Seabed mixing and particle residence times in biologically and physically dominated estuarine systems: a comparison of lower Chesapeake Bay and the York River subestuary. *Est Coast Shelf Sci* 46:777-795
- Dunnington EA (1968) Survival time of oysters after burial at various temperatures. *Proc Natl Shellfish Assoc* 58:101-103
- Essink K (1999) Ecological effects of dumping of dredged sediments; options for management. *J Coastal Cons* 5:69-80
- Fodrie FJ, Rodriguez AB, Baillie CJ, Brodeur MC, Coleman SE, Gittman RK, Keller DA, Kenworthy MD, Poray AK, Ridge JT, Theuerkauf EJ, Lindquist NL (2014) Classic paradigms in a novel environment: inserting food web and productivity lessons from rocky shores and saltmarshes into biogenic reef restoration. *J Appl Ecol* 51:1314-1325
- Galtsoff PS, Luce RH (1930) Oyster investigations in Georgia. Bureau of Fisheries Document 1077 Appendix V, Rep. U.S. Comm Fisheries for 1930:61-100

- Grabowski JH, Peterson CH (2007) Restoring oyster reefs to recover ecosystem services. In: Cuddington K, Byers JE, Wilson WG, Hastings A (eds.) Ecosystem Engineers, Plants to Protists. Elsevier Academic Press, Burlington, MA, p 281-298
- Grant J, Enright CT, Griswold A (1990) Resuspension and growth of *Ostrea edulis*: a field experiment. Mar Biol 104:51-59
- Gray JS (1981) The ecology of marine sediments: An introduction to the structure and function of benthic communities. Cambridge University Press, New York, NY
- Harding JA (2007) Comparison of growth rates between diploid DEBY eastern oysters (*Crassostrea virginica*, Gmelin 1791), triploid eastern oysters, and triploid Suminoe oysters (*C. ariakensis*, Fugita 1913). J Shellfish Res 26:961-972
- Haven DS, Morales Alamo R (1966) Aspects of biodeposition by oysters and other invertebrate filter feeders. Limnol Oceanogr 11:487-498
- Haven DS, Morales Alamo R (1972) Biodeposition as a factor in sedimentation of fine suspended solids in estuaries. Geol Soc Am Bull 133:121-130
- Hinchey EK, Schaffner LC, Hoar CC, Vogt BW, Batte LP (2006) Responses of estuarine benthic invertebrates to sediment burial: the importance of mobility and adaptation. Hydrobiologia 556:85-98
- Jones RA, Lee GF (1981) The significance of dredging and dredged material disposal as a source of nitrogen and phosphorus for estuarine waters. In: Neilson BJ, Cronin LE (eds.) Estuaries and Nutrients. Humana Press, Clifton, NJ, p 517-530
- Jordan-Cooley WC, Lipcius RN, Shaw LB, Shen J, Shi J (2011) Bistability in a differential equation model of oyster reef height and sediment accumulation. J Theor Biol 289:1-11

- Kellogg ML, Cornwell JC, Owens MS, Paynter KT (2013) Denitrification and nutrient assimilation on a restored oyster reef. *Mar Ecol Prog Ser* 480:1-19
- Kiddon J, Buffum H (2000) Sediment characteristics data: Mid-Atlantic Integrated Assessment. U.S. Environmental Protection Agency, <http://www.epa.gov/emap/maia/html/data/estuary/9798/sedgrain.txt/>
- Kniskern TA, Kuehl SA (2003) Spatial and temporal variability of seabed disturbance in the York River subestuary. *Est Coast Shelf Sci* 58:37-55
- Kraeuter JN, Ford S, Cummings M (2007) Oyster growth analysis: A comparison of methods. *J Shellfish Res* 26:479-491
- Kranz PM (1974) The anastrophic burial of bivalves and its paleoecological significance. *J Geol* 82:237-265
- Lenihan HS (1999) Physical-biological coupling on oyster reefs: How habitat structure influences individual performance. *Ecol Monogr* 69:251-275
- Lisle JT, Comer NN (2011) Characterization of sediments from the Gulf of Mexico and Atlantic shorelines, Texas to Florida. U.S. Geological Survey Open-File Report 2011-1199
- Livingston RJ, Howell RL, Niu X, Lewis FG, Woodsum GC (1999) Recovery of oyster reefs (*Crassostrea virginica*) in a Gulf estuary following disturbance by two hurricanes. *Bull Mar Sci* 64:465-483
- Lund EJ (1957a) Self-silting by the oyster and its significance for sedimentation geology. Institute of Marine Science, University of Texas, Port Aransas, TX

- Lund EJ (1957b) Self-silting, survival of the oyster as a closed system, and reducing tendencies of the environment of the oyster. Institute of Marine Science, University of Texas, Port Aransas, TX
- Marbà N, Duarte CM (1994) Growth response of the seagrass *Cymodocea nodosa* to experimental burial and erosion. *Mar Ecol Prog Ser* 107:307-311
- Maurer D, Keck RT, Tinsman JC, Leathem WA (1981) Vertical migration and mortality of benthos in dredged material – Part I: Mollusca. *Mar Environ Res* 4:299-319
- McCormick-Ray J (2005) Historical oyster reef connections to Chesapeake Bay – a framework for consideration. *Est Coast Shelf Sci* 64:119-134
- Meade RH (1982) Sources, sinks, and storage of river sediment in the Atlantic drainage of the United States. *J Geol* 90:235-252
- Meyer DL, Townsend EC, Thayer GW (1997) Stabilization and erosion control value of oyster cultch for intertidal marsh. *Rest Ecol* 5:93-99
- Miller DC, Muir CL, Hauser OA (2002) Detrimental effects of sedimentation on marine benthos: what can be learned from natural processes and rates? *J Mar Res* 50:489-520
- Najjar R, Pyke CR, Adams MB, Breitburg D, Hershner C, Kemp M, Howarth R, Mulholland M, Paolisso M, Secor D, Sellner K, Wardrop D, Wood R (2010) Potential climate change impacts on the Chesapeake Bay. *Est Coast Shelf Sci* 86:1-20
- Norris RM (1953) Buried oyster reefs in some Texas bays. *J Paleontol* 27:569-576

- Orth RJ, Carruthers JB, Dennison WC, Duarte CM, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL (2006) A global crisis for seagrass ecosystems. *BioScience* 56:987-996
- Piazza BP, Banks PD, LaPeyre MK (2005) The potential for created oyster shell reefs as a sustainable shoreline protection strategy in Louisiana. *Restor Ecol* 13:499-506
- Powers SP, Peterson CH, Grabowski JH, Lenihan HS (2009) Success of constructed oyster reefs in no-harvest sanctuaries: implications for restoration. *Mar Ecol Prog Ser* 389:159-170
- Pyke CR, Najjar R, Adams MB, Breitburg D, Hershner C, Kemp M, Howarth R, Mulholland M, Paolisso M, Secor D, Sellner K, Wardrop D, Wood R (2008) Climate change and the Chesapeake Bay: State of the science review and recommendations. A Report from the Chesapeake Bay Program Science and Technical Advisory Committee 85 pp
- R Core Development Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>
- Rodriguez AB, Fodrie FJ, Ridge JT, Lindquist NL, Theuerkauf EJ, Coleman SE, Grabowski JH, Brodeur MC, Gittman RK, Keller DA, Kenworthy MD (2014) Oyster reefs can outpace sea-level rise. *Nat Clim Change* 4:493-497
- Rose CD (1973) Mortality of market-sized oysters (*Crassostrea virginica*) in the vicinity of a dredging operation. *Chesapeake Sci* 14:135-138

- Rothschild BJ, Ault JS, Gouletquer P, Heral M (1994) Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. *Mar Ecol Prog Ser* 111:29-39
- Sallenger AH, Doran KS, Howd PA (2012) Hotspot of accelerated sea-level rise on the Atlantic coast of North America. *Nat Clim Change* 2:884-888
- Sanford LP, Panageotou W, Halka JP (1991) Tidal resuspension of sediments in northern Chesapeake Bay. *Mar Geol* 97:87-103
- Short FT, Neckles HA (1999) The effects of global climate change on seagrasses. *Aquat Bot* 63:169-196
- Shumway SE (1996) Natural environmental factors. In: Kennedy VS, Newell RIE, Eble AF (eds.) *The eastern oyster: Crassostrea virginica*. Maryland Sea Grant Press, College Park, MD
- Smith GF, Bruce DG, Roach EB (2001) Remote acoustic habitat assessment techniques used to characterize the quality and extent of oyster bottom in the Chesapeake Bay. *Mar Geod* 24:171-189
- Southworth M, Mann R (2014) The status of Virginia's public oyster resource, 2013. Molluscan Ecology Program, Virginia Institute of Marine Science, Gloucester Point, Virginia
- Suedel BC, Clarke JU, Wilkens J, Lutz CH, Clarke DG (2014) The effects of a simulated suspended sediment plume on eastern oyster (*Crassostrea virginica*) survival, growth and condition. *Est Coasts in press* (DOI 10.1007/s12237-014-9835-0)
- Syvitski JPM, Vörösmarty CJ, Kettner AJ, Green P (2005) Impact of humans on the flux of terrestrial sediment to the global coastal ocean. *Science* 308:376-380

- Taylor J, Bushek D (2008) Intertidal oyster reefs can persist and function in a temperate North American Atlantic estuary. *Mar Ecol Prog Ser* 361:301-306
- Thompson RJ, Newell RIE, Kennedy VS, Mann R (1996) Reproductive processes and early development. In: Kennedy VS, Newell RIE, Eble AF (eds.) *The eastern oyster: Crassostrea virginica*, Maryland Sea Grant Press, College Park, MD
- Twichell D, Edmiston I, Andrews B, Stevenson W, Donoghue J, Poore R, Osterman L (2010) Geologic controls on the recent evolution of oyster reefs in Apalachicola Bay and St. George Sound, Florida. *Est Coast Shelf Sci* 88:385-394
- Walton WC, Rikard FS, Chaplin GI, Davis JE, Arias CR, Supan JE (2013) Effects of ploidy and gear on the performance of cultured oysters, *Crassostrea virginica*: Survival, growth, shape, condition index and *Vibrio* abundances. *Aquaculture* 414-415:260-266
- Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladine A, Fourqurean JW, Heck KL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT, Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *P Natl Acad Sci* 106:12377-12381
- Widdows J, Brinsley MD, Bowley N, Barrett C (1998) A benthic annular flume for *in situ* measurement of suspension feeding/biodeposition rates and erosion potential of intertidal cohesive sediments. *Est Coast Shelf Sci* 46:27-38
- Widdows J, Newell RIE, Mann R (1989) Effects of hypoxia and anoxia on survival, energy, metabolism, and feeding of oyster larvae (*Crassostrea virginica*, Gmelin). *Biol Bull* 177:154-166

Wilber DH, Clarke DG (2001) Biological effects of suspended sediments: A review of suspended sediment impacts on fish and shellfish with relation to dredging activities in estuaries. *North Am J Fish Manage* 21:855-873

Wilberg MJ, Wiedenmann JR, Robinson JM (2013) Sustainable exploitation and management of autogenic ecosystem engineers: application to oysters in Chesapeake Bay. *Ecol App* 23:766-776

Zimmerman RJ, Minello T, Baumer T, Castiglione M (1989) Oyster reef as habitat for estuarine macrofauna. *National Marine Fisheries Service Southeast Fisheries Science Center, Galveston, TX* 55 pp.

Zu Ermgassen PSE, Spalding MD, Blake B, Coen LD, Dumbauld B, Geiger S, Grabowski JH, Grizzle R, Luckenbach M, McGraw K, Rodney W, Ruesink JL, Powers SP, Brumbaugh R (2012) Historical ecology with real numbers: past and present extent and biomass of an imperiled estuarine habitat. *Proc R Soc B* 282:18



Table 1: AIC model comparison results for oyster response variables in triploid trials. The best model is indicated in bold. P-values reported for survival models are the result of likelihood ratio tests with the treatment-only model (model 1) as the null model.

<b>Survival</b>				
Model	Parameters	AICc	w	p ( $\chi^2$ )
<b>1</b>	<b>Burial</b>	<b>134.4</b>	<b>0.58</b>	---
2	Week	232.9	<0.01	---
3	Burial + Week	136.2	0.24	0.62
4	Burial + Week + Tank	138.1	0.09	0.78
5	Burial * Week	138.3	0.08	0.87
<b>Biodeposition</b>				
Model	Parameters	AICc	w	r <sup>2</sup>
1	Burial	630.1	<0.01	0.14
2	Week	558.1	<0.01	0.38
<b>3</b>	<b>Burial + Week</b>	<b>503.0</b>	<b>0.52</b>	<b>0.52</b>
4	Burial + Week + Tank	504.3	0.28	0.51
5	Burial * Week	505.0	0.20	0.51
<b>Condition index</b>				
Model	Parameters	AICc	W	r <sup>2</sup>
1	Burial	1102.5	<0.01	0.01
2	Week	1007.8	0.03	0.34
<b>3</b>	<b>Burial + Week</b>	<b>1003.8</b>	<b>0.24</b>	<b>0.35</b>
4	Burial + Week + Tank	1002.4	0.48	0.36
5	Burial * Week	1003.8	0.24	0.35
<b>Growth</b>				
Model	Parameters	AICc	W	r <sup>2</sup>
<b>1</b>	<b>Burial</b>	<b>-190.9</b>	<b>0.21</b>	<b>0.11</b>
2	Week	-164.4	0.38	0.01
3	Burial + Week	-191.7	0.33	0.12
4	Burial + Week + Tank	-190.7	0.20	0.12
5	Burial * Week	-191.3	0.26	0.12

Table 2: AIC model comparison results for oyster survival in diploid trials. The best model is indicated in bold. P-values reported for are the result of likelihood ratio tests with the treatment-only model (model 1) as the null model.

<b>Survival</b>				
Model	Parameters	AICc	$w$	$p(\chi^2)$
<b>1</b>	<b>Burial</b>	<b>40.2</b>	<b>0.69</b>	---
2	Size	50.3	<0.01	---
3	Burial + Size	42.5	0.22	0.97
5	Burial * Size	44.3	0.09	0.72

Table 3: Summary of environmental conditions in mesocosms for experiment I. Values given in parentheses are standard error (SE). P values are reported for one-way ANOVAs for differences in environmental variables between mesocosm tanks.

<b>Variable</b>	<b>Range</b>	<b>Mean (SE)</b>	<b>p-value</b>
Temperature (°C)	23.2 – 36.6	27.2 (0.1)	0.98
Salinity (ppt)	17.5 – 22.4	19.9 (0.2)	0.99
Dissolved oxygen (mg L <sup>-1</sup> )	3.2 – 9.3	4.8 (0.2)	0.62

Table 4: Summary of sublethal effects of burial treatments on mean biodeposition, condition index and growth. Values given in parentheses are standard error.

<b>Burial Treatment</b>	<b>Biodeposition (g dw d<sup>-1</sup>)</b>	<b>Condition Index</b>	<b>Growth (mm d<sup>-1</sup>)</b>
0 %	0.27 (0.03)	9.8 (0.3)	0.12 (0.01)
50 %	0.18 (0.03)	9.9 (0.5)	0.16 (0.02)
70 %	0.15 (0.02)	9.8 (0.3)	0.22 (0.02)
90 %	0.11 (0.01)	8.9 (0.4)	0.24 (0.03)
110 %	0.08 (0.01) *	8.9 (0.5)	0.32 (0.05)

\* Mean deposition reported for 110 % burial treatment represents background sedimentation in the tanks rather than oyster biodeposition.

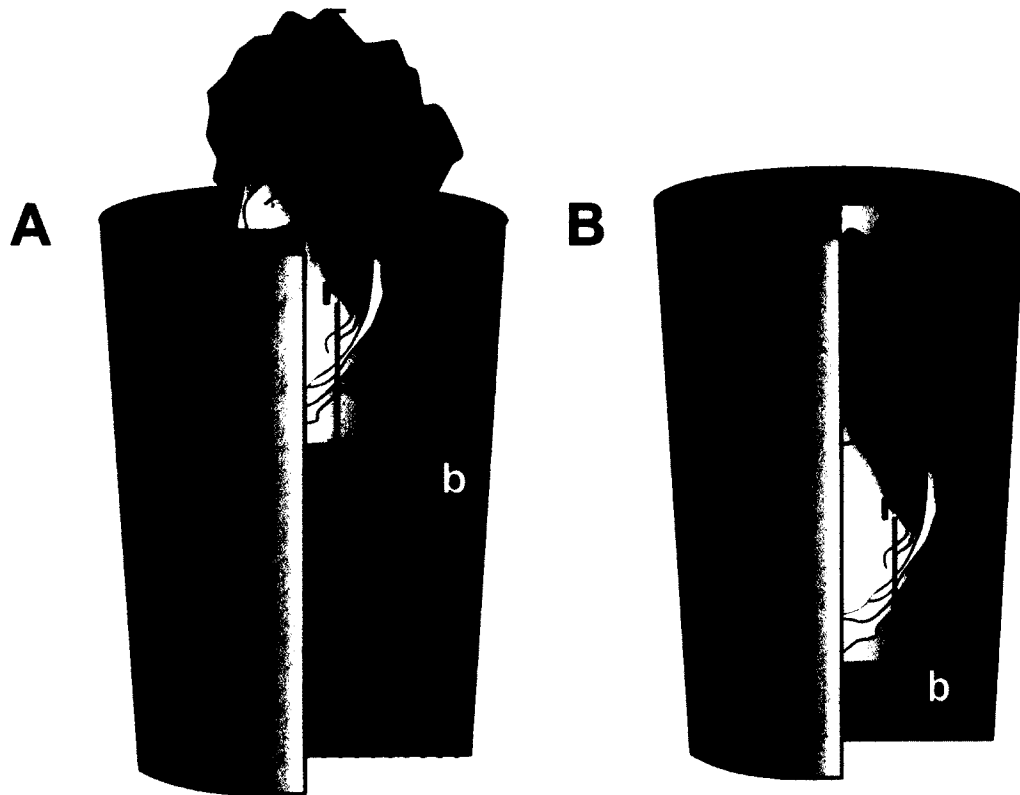


Fig. 1: Set-up of experimental containers. Panel A depicts an oyster in the 50 % burial treatment. Sediment is labeled "a" and the elevated bottom of Portland cement is labeled "b." Panel B depicts an oyster in the 110 % burial treatment; sediment and elevated bottom are labeled as in Panel A.

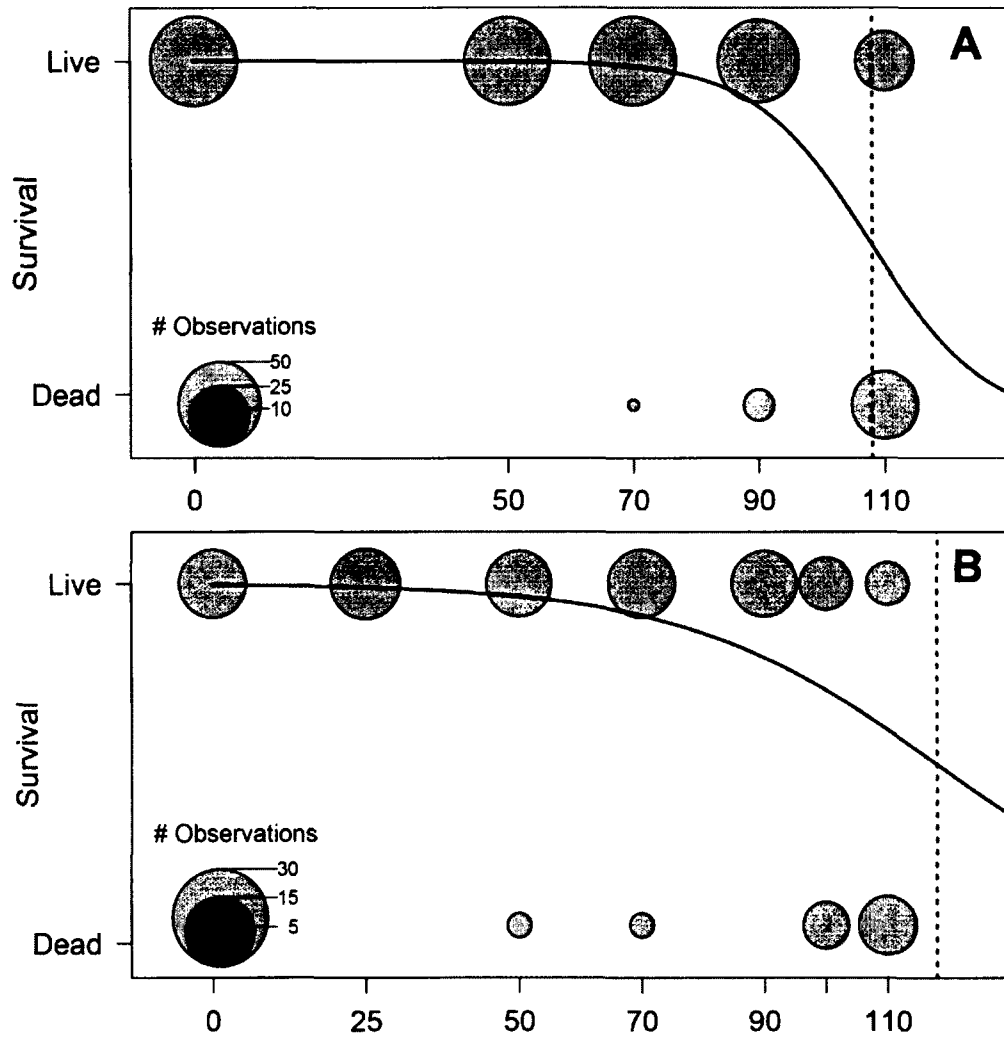


Fig. 2: Oyster survival across % burial treatments for triploid (A) and diploid (B) trials. The size of the circle is proportional to the number of observations at each survival status-treatment combination (smallest circle = 1 (triploid) or 2 (diploid) observations). Dashed lines indicate the % burial at which 50 % mortality occurred as estimated from the logistic regression. Burial treatment was the only significant factor (triploid:  $p < 0.01$ ; diploid:  $p = 0.02$ ) for survival.

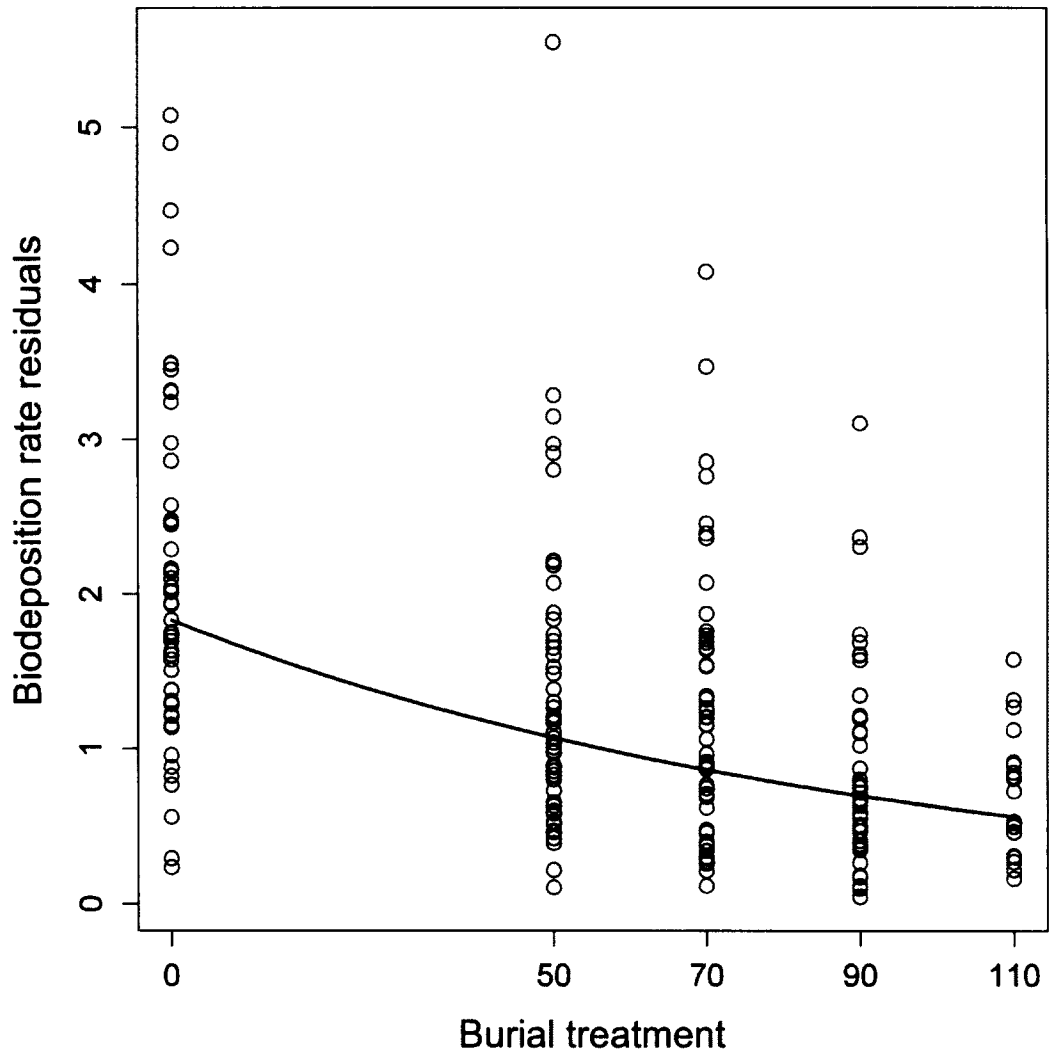


Fig. 3: Biodeposition rate residuals across % burial treatments in triploid trials. Regression shown is the back-transformed log-linear regression of biodeposition-week regression residuals ( $r^2 = 0.22$ ). Both treatment ( $p < 0.01$ ) and week ( $p < 0.01$ ) were significant factors controlling biodeposition. Oyster size ( $p = 0.08$ ) and tank ( $p = 0.40$ ) were not significant.

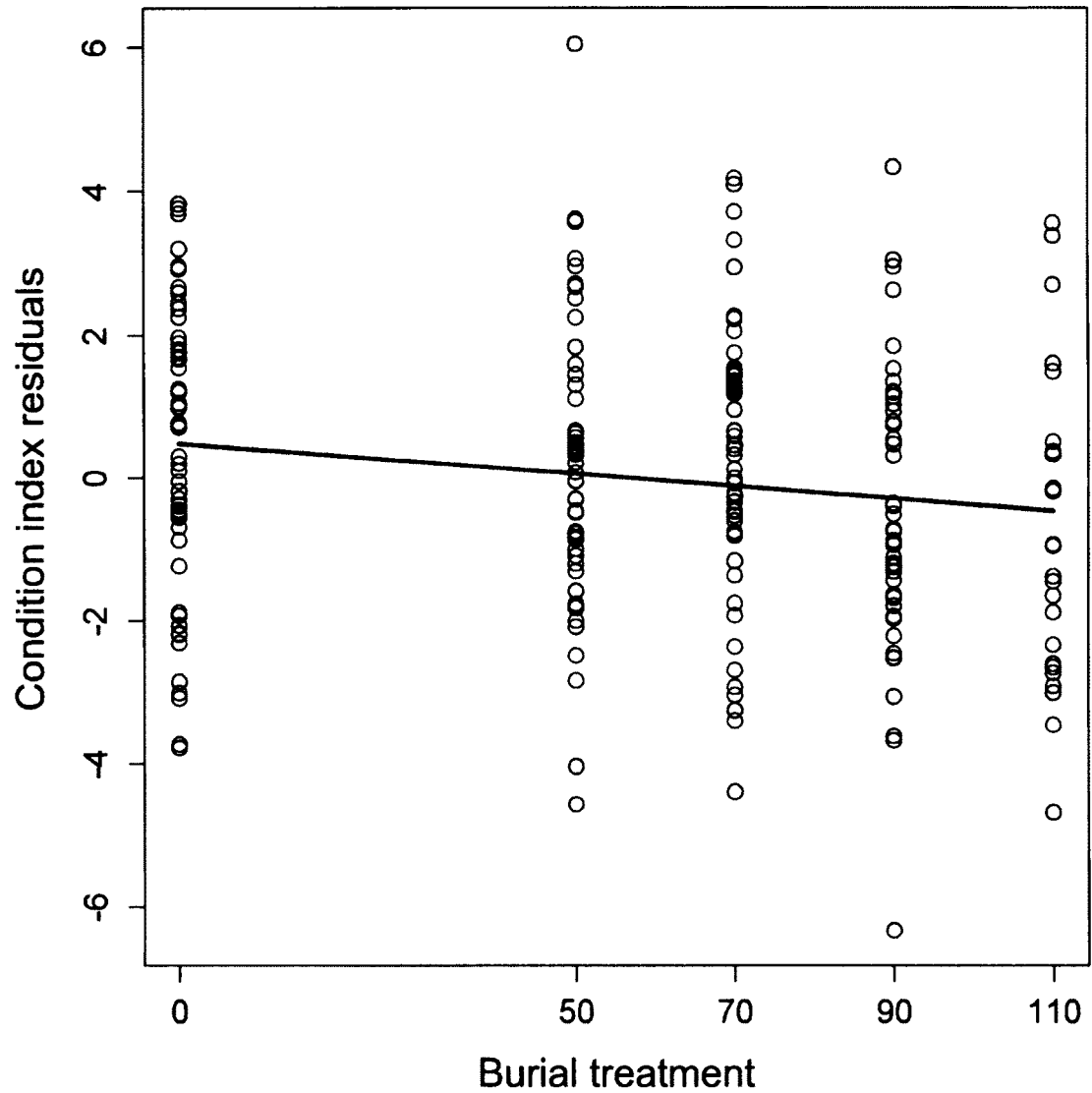


Fig. 4: Oyster condition index across % burial treatments in triploid trials. Model estimates indicated a significant effect of week on oyster condition index ( $p < 0.01$ ); therefore, data shown are the residuals from the condition index-week regression as a function of burial treatment ( $p = 0.01$ ;  $r^2 = 0.35$ ). Oyster size ( $p = 0.15$ ) and tank ( $p = 0.07$ ) were not significant.



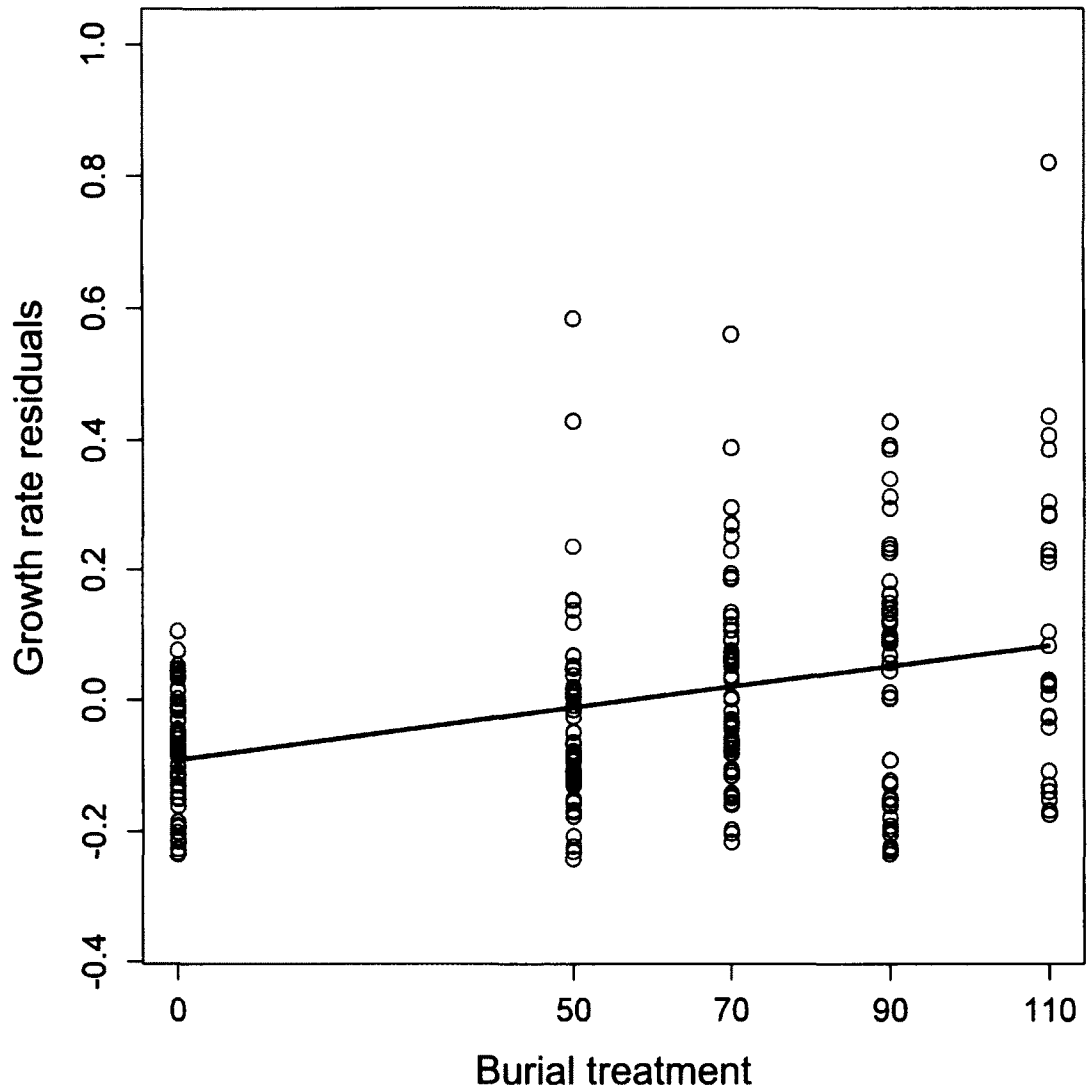


Fig. 5: Oyster growth-rate residuals as a function of % burial treatment in triploid trials. Initial regression indicated a significant effect of oyster size on growth rate ( $p < 0.01$ ); therefore, data shown are the residuals from the size-growth rate regression of growth rate as a function of burial treatment ( $p < 0.01$ ;  $r^2 = 0.11$ ). Tank and week effects were not significant ( $p = 0.32$  and  $p = 0.10$ , respectively).

## CHAPTER 2

### **Initial conditions drive threshold dynamics of oyster reefs**

#### Abstract

Globally, ecosystem engineers have been devastated, resulting in altered ecosystems with degraded ecological function and triggering widespread, costly restoration efforts that have typically faltered. Threshold responses to biotic or abiotic forcing may produce multiple trajectories due to different initial conditions, which can determine population recovery or local extinction, yet experimental tests of this phenomenon are lacking in marine ecosystems. We experimentally demonstrate a threshold response of native oyster reef populations to initial conditions of reef height and define the underlying mechanisms. After two years, oyster reefs exhibited diverging trajectories toward either degradation or persistence, depending upon initial reef height. Reefs higher than 0.3 m supported greater oyster density, survival, and reef complexity, whereas sediment deposition was reduced. Reefs lower than 0.3 m were eventually buried. These observations (i) provide experimental evidence for threshold dynamics in marine species, (ii) suggest that the collapse of oyster populations was to a large extent due to anthropogenic habitat degradation that eliminated positive feedbacks and which may have created alternative, stable reef trajectories towards local extinction, and (iii) indicate an avenue by which oyster restoration success is achievable.

The world's structure-forming ecosystem engineers have been devastated over the past two centuries, followed by minimal success in their restoration despite extensive efforts (1-3). These alterations generally involve phase shifts from a productive state to one that is severely degraded (4-13). Such phase shifts and nonlinear responses to perturbations have been reported in marine, freshwater, and terrestrial ecosystems (4), including savannas (5), forests (6), lakes (7), kelp forests (8), rocky intertidal zones (9,10), and coral reefs (11-13). It has been hypothesized that a major obstacle preventing recovery stems from the elimination of positive feedbacks that maintain productive ecosystem states (14), and which are dependent on initial conditions of the system. Although the concepts of thresholds, critical points, phase shifts, and alternative states are pervasive in the ecological literature, clear experimental demonstration of these phenomena with empirical studies is limited and often equivocal (15). Large-scale dramatic shifts in ecosystem state can occur suddenly and unpredictably, and mechanisms driving ecosystem change are not well understood and difficult to observe directly (16). There is also ongoing debate about what constitutes evidence for alternative stable states and regime shifts, which complicates the design of experiments needed to discern these nonlinear responses in nature (15, 17).

In mollusks, alternative states resulting from nonlinear dynamics have been suggested for beds of the horse mussel *Atrina zelandica* in New Zealand (18), patches of the blue mussel *Mytilus edulis* along the northeast Atlantic coast of North America (10), and eastern oyster *Crassostrea virginica* reefs in Chesapeake Bay (19). For eastern oyster reefs, restoration success, as measured by oyster abundance and persistence, was associated with the initial condition of reef height at construction (20,21). In these

studies, high-relief reefs (0.25-2 m) performed better than low-relief (0.08-0.12 m) reefs in oyster recruitment, growth, and survival. Population responses to initial reef height resulted in high-relief reefs supporting oyster populations twice as abundant as those on low-relief reefs and which persisted over time through continued reef accretion, whereas low-relief reefs degraded over time leading to burial (20) and a return to the unrestored state (21). This dichotomy in responses between high-relief and low-relief reefs indicates that there may be a threshold of initial reef height at which the reef trajectory switches from degradation to persistence. We propose that these responses represent alternative trajectories of the ecosystem state and are driven by hydrodynamic responses to the initial condition of reef height. We present an experimental field study designed to capture the proposed threshold at which reef trajectories switch from degradation to persistence and to quantify the mechanisms controlling nonlinear dynamics.

Elevation of the reef surface above the seafloor changes the position of the bed relative to the water surface. In doing so, the effective height of the water column is restricted as it passes over the reef, resulting in faster flow rates relative to non-constricted flow (20). If faster flow rates result in reduced sediment deposition and increased erosion at the reef surface, increasing reef height would result in higher-quality habitat for oyster settlement. Also, faster flow supports faster oyster growth due to the breakdown of feeding-induced gradients in phytoplankton concentration and the direct increase in filtration due to physical flow (22). Where predation or disease pressure is substantial, a survival advantage could be conferred from increased flow rates if faster growth shortens the time to reach a size refuge from predation (23) or if improved flow

enhances resistance to disease (24). As such, we predict that the trajectory of the oyster population and the reef habitat depends upon feedbacks controlling sediment dynamics.

We constructed 24 experimental oyster reefs across 4 sites in the Great Wicomico (GWR) and Lynnhaven (LR) Rivers, two sub-estuaries of Chesapeake Bay in Virginia. Sites were selected to represent a range of environmental conditions, with one site each of high and low energy in each river. The subtidal reefs were constructed of oyster shell with a 0.03 m base layer of crushed surf clam shell at 6 initial reef heights ranging from 0.05-0.5 m above the bed (Fig. 1). We quantified oyster density, size, and survival at 8 and 24 mos post-construction and measured sediment deposition rates and reef surface rugosity to characterize sediment dynamics. Nonlinear threshold models (logistic and Gompertz) were compared to linear models to confirm nonlinearity of the response and to characterize the form of the functional relationship between reef height and oyster density, size, and survival.

After 24 mos, two reef trajectories were evident--one of persistence of high-relief reefs and the other of degradation of low-relief reefs (Fig. 2). At two sites with intermediate sediment deposition (GWR1, GWR2, mean:  $0.07 \pm 0.02$  and  $0.02 \pm 0.002$  g dw d<sup>-1</sup>, respectively), the 2 lowest-relief reefs (0.05, 0.1 m) were nearly buried (> 90%) by the conclusion of the experiment. The remaining 2 sites (LR1, LR2) represented the extremes in sediment deposition. LR2 experienced the highest deposition rates (mean:  $3.42 \pm 1.31$  g dw d<sup>-1</sup>), two orders of magnitude greater than all other sites, and all reefs, regardless of initial height, were buried after 24 mos. At LR1, sediment deposition was extremely low ( $0.01 \pm 0.001$  g dw d<sup>-1</sup>); all of these reefs persisted through the study, although oyster population responses varied across reef heights. These differing

responses as a function of sediment deposition are characteristic of a threshold response and divergent trajectories as a function of a controlling environmental parameter (16).

At all sites in both sampling periods, we observed nonlinear threshold responses of oyster density with respect to reef height (Fig. 3, Table S2). Estimated inflection points of  $0.27 \pm 0.03$  and  $0.33 \pm 0.07$  m in the 8 and 24 mos sampling periods, respectively, corresponded with previous studies that found reefs constructed at  $< 0.2$  m degraded over time in the field (20,21) and in theoretical models (19). Oyster densities on reefs at or above the estimated thresholds (0.3-0.5 m) were 3.5 times higher ( $499.3 \pm 56.2$  m<sup>-2</sup>) than on reefs below the estimated threshold (0.05-0.2 m,  $140.6 \pm 18.2$  m<sup>-2</sup>) with a significant difference in density between reef trajectories (ANOVA,  $p < 0.001$ ).

Threshold dynamics in oyster density were likely driven by sediment transport. The two most productive sites at 24 mos (GWR2, LR1) experienced sediment deposition rates that were two orders of magnitude lower than the other sites (Fig. 4). These sites were located in low-energy environments with muddy-sand sediments. The high-energy sites (GWR1, LR2) were characterized by sandy sediments, most likely due to the erosion of mud from the seabed. Sand provides hard substrate to support the weight of the reef structure, but it is also less cohesive than muddy sediments and may become mobile under high-energy conditions. The burial of all reefs at LR2, the sandiest site with highest exposure, suggests that there is a critical point in sediment deposition beyond which high oyster density and reef accretion cannot sustain the reef over time, irrespective of initial reef height.

Patterns in oyster density may also have arisen from the effect of reef height on reef complexity. Rugosity, a measure of surface complexity, increased monotonically

with reef height (Fig. 4). Recent studies indicate that eastern oyster larvae respond to turbulence by altering their swimming behavior (25), which increases the probability of settlement and is presumably beneficial in increasing the likelihood that an oyster will contact an oyster reef before being advected away by the current (26). Observed patterns in oyster density likely resulted from reduced sediment deposition helping maintain clean substrate for larval settlement and strong settlement cues derived from increased turbulence associated with higher rugosity derived from reduced sediment accumulation.

Survival increased with reef height at the LR2 site in the first sampling period with a threshold in survival occurring at approximately 0.4 m (Fig. 3). The other sites in the first sampling period did not have significant effects of reef height on survival ( $p > 0.05$ ; Fig. 3). At 24 mos, survival increased with reef height at all sites except LR2 (Fig. 3), which was the location that experienced complete burial and total mortality. This result demonstrates the dichotomy of responses along the sediment deposition gradient. At 8 mos, only the site with high sediment loads demonstrated a response of survival to reef height (LR2, Fig. 3). Over time, the remaining sites (GWR1, GWR2, LR1) approached an equilibrium and responded similarly to physical forcing, even at relatively low sediment loading; however, reefs experiencing sediment deposition beyond a certain threshold, like those at LR2, were more likely to be lost due to burial. The loss of low-relief reefs ( $< 0.1\text{m}$ ) within 24 mos of construction is comparable to that observed in other studies in which reefs below our estimated threshold (e.g., those at 0.1 m) experienced heavy siltation and were 90% buried within 16 mos (20). Oyster mortality may have resulted directly from sediment burial (27) or by increasing oysters' susceptibility to disease by increasing metabolic stress (24).

In contrast to oyster density and survival, oyster size responded linearly (Fig. S1). This suggests that the interactions between reef height and sediment dynamics leading to differences in reef trajectories are acting at the time of settlement and early post-settlement. Although variable, size was linearly related to reef height, and decreased with increasing reef height (Fig. S1). If the relationship had been related to sediment, we would expect to see a positive response of oyster size to reef height, since high-relief reefs experience lower sedimentation rates. Inclusion of oyster density as a covariate revealed an effect of recruitment on the slope of the reef height relationship (Table S9), suggesting that the negative relationship between reef height and oyster size was due to intraspecific competition (Fig. S1). This negative feedback is in contrast to previous studies in which the largest oysters occurred on high-relief reefs (21). The densities observed on our reefs were 4-fold higher than those observed on restoration reefs in Chesapeake Bay and 30 times higher than densities typical of harvested reefs (21), so density-dependent effects may have been enhanced due to the extremely high oyster densities.

The threshold response in oyster density and survival is best explained by the physical responses to initial reef height over time. Sediment deposition rates decreased with reef height with the exception of one site (LR1) where deposition rates were extremely low ( $0.017 \pm 0.001 \text{ g m}^{-2} \text{ d}^{-1}$ ). An examination of experimental reefs above and below the predicted reef height threshold demonstrates that high-relief reefs were on an increasing trajectory while low-relief reefs experienced decreasing oyster density over time (Fig. 6). There was convergence in the response over time within groups and divergence between groups, where the density of the two groups was clearly separated in



the second year. This observation indicates that differences in oyster density across reef heights were sufficient to produce diverging trajectories in reef condition and persistence over time.

Native oyster species have been decimated globally due to overfishing, eutrophication, and habitat degradation (24, 1-2). Concern over these declines and losses of attendant oyster ecosystem services, such as nutrient cycling, water filtration and habitat structure, have spurred efforts to restore oyster populations globally (1), but these efforts have seen limited success (29).

The threshold dynamics observed in this study are a likely explanation for both the initial loss of reef habitats and for the failure of many restoration efforts. Destructive harvesting techniques like dredging and hydraulic tonging reduce the height of reefs by removing accreted oyster shells along with live oysters. The reduction of reef height below a critical threshold would cause the reef to move from a persistent to a degrading trajectory, even without the effects of continued harvest. Metabolic stress from sedimentation exacerbates the effects of oyster disease, further contributing to reef decline (24).

Restoration projects utilizing low-relief reef designs (0.05-0.1 m) in Chesapeake Bay have required continual shell and oyster additions since their construction (29), indicating that the initial reef heights used were too low to sustain high oyster density and maintain low sedimentation rates required for persistence. Similar resilience of degraded ecosystems to restoration has been observed in wetlands and grasslands (30). Repeated field observations of divergent oyster reef trajectories in multiple systems suggest that the nonlinear responses to gradients in sediment deposition observed here are the likely

mechanism controlling oyster reef success in this and other systems (20, 31).

Understanding the nonlinear response of restored systems to different initial conditions is critical for the success of ecological restoration. More importantly, understanding the mechanistic drivers can provide insights into the dramatic shifts observed in natural systems, which may lead to better restoration and management.

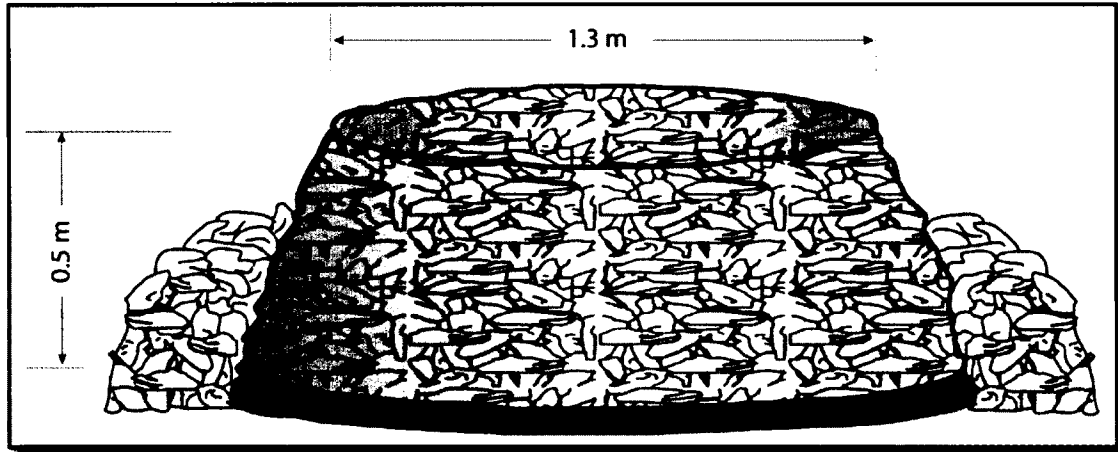
### References and Notes:

1. M.W. Beck *et al.*, Oyster reefs at risk and recommendations for conservation, restoration, and management. *Bioscience*, **61** 107-116 (2011).
2. P.S.E. Zu Ermgassen *et al.*, Historical ecology with real numbers: past and present extent and biomass of an imperiled estuarine habitat. *Proc. R. Soc. Biol.*, 1-8 (2012).
3. Airolidi, L., Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe. In: Gibson, R.N., Atkinson, R.J.A., Gordon, J.D.M. (Eds.), *Oceanography and Marine Biology: An Annual Review* vol. 45. CRC Press, pp. 345–405.
4. C. Folke *et al.*, Regime shifts, resilience and biodiversity in ecosystem management. *Annu. Rev. Ecol. Evol. Syst.* **33**, 557-581 (2004).
5. J.M. Anderies, M.A. Janssen, B.H. Walker, Grazing management, resilience and the dynamics of a fire-driven rangeland system. *Ecosystems*, **5** 23-44 (2002).
6. G.D. Peterson, Forest dynamics in the southeastern United States: managing multiple stable states. In: *Resilience and the Behavior of Large-scale Ecosystems*. L.H. Gunderson, L. Pritchard (eds.) (Island Press, Washington DC, 2002).
7. R. Carpenter, D. Ludwig, W.A. Brock, Management of eutrophication for lakes subject to potentially irreversible change. *Ecol. Appl.*, **9** 751-771 (1999).
8. B. Konar, J.A. Estes, The stability of boundary regions between kelp beds and deforested areas. *Ecology*, **84**, 174-185 (2003).
9. R.T. Paine, A.C. Trimble, Abrupt community change on a rocky shore – biological mechanisms contributing to the potential formation of an alternative state. *Ecol. Lett.* **7**, 441-445 (2004).
10. P. Petraitis, E.T. Methratta, E.C. Rhile, N.A. Vidargas, S.R. Dudgeon, Experimental confirmation of multiple community states in a marine ecosystem, *Oecologia* **161**, 139-148 (2009).
11. T.P. Hughes *et al.*, Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Curr. Biol.*, **17** 360-365 (2007).
12. P.J. Mumby, A. Hastings, H.J. Edwards, Thresholds and the resilience of Caribbean coral reefs. *Nature*, **450** 98-101 (2007).
13. T. Elmhirst, S.R. Connolly, T.P. Hughes, Connectivity, regime shifts and the resilience of coral reefs. *Coral Reefs*, **28** 949-957 (2009).
14. Donadi, Serena, et al. "The bivalve loop: intra-specific facilitation in burrowing cockles through habitat modification." *J. Exp. Mar. Biol. Ecol.*, **461** 44-52 (2014).
15. P.S. Petraitis, S.R. Dudgeon, Detection of alternative stable states in marine communities. *J. Exp. Mar. Biol. Ecol.* **300**, 343-371 (2004).
16. M. Scheffer, S. Carpenter, J.A. Foley, C. Folk, B. Walker, Catastrophic shifts in ecosystems. *Nature* **413**, 591-596 (2001).

17. C. Hsieh, S.M. Glaser, A.J. Lucas, G. Sugihara, Distinguishing random environmental fluctuations from ecological catastrophes for the North Pacific Ocean, *Nature Lett.* **435**, 336-340 (2005).
18. G. Coco, S.F. Thrush, M.O.Green, J.E. Hewitt, Feedbacks between bivalve density, flow and suspended sediment concentration on patch stable states. *Ecology* **87**, 2862-2870 (2006).
19. W.C. Jordan-Cooley, R.N. Lipcius, L.B. Shaw, J. Shen, J. Shi, Bistability in a differential equation model of oyster reef height and sediment accumulation. *J. Theor. Biol.* **289**, 1-11 (2011).
20. H.S. Lenihan, Physical-biological coupling on oyster reefs: how habitat structure influences individual performance. *Ecol. Monogr.* **69**, 251-275 (1999).
21. D.M. Schulte, R.P. Burke, R.N. Lipcius, Unprecedented restoration of a native oyster metapopulation. *Science*, **325**, 1124-1128 (2009).
22. H.S. Lenihan, C.H. Peterson, J.M. Allen, Does flow speed also have a direct effect on growth of active suspension-feeders: an experimental test on oysters. *Limnol. Oceanogr.* **41**, 1359-1366 (1996).
23. D.B. Eggleston, Foraging behavior of the blue crab, *Callinectes sapidus*, on juvenile oysters, *Crassostrea virginica*: effects of prey density and size. *Bull. Mar. Sci.*, **46** 62-82 (1990).
24. H.S. Lenihan, F. Micheli, S.W. Shelton, C.H. Peterson, The influence of multiple environmental stressors on susceptibility to parasites: an experimental determinations with oysters. *Limnol. Oceanogr.* **44** 910-924 (1999).
25. H.L. Fuchs, E.J. Hunter, E.L. Schmitt, R.A. Guazzo, Active downward propulsion by oyster larvae in turbulence. *J. Exp. Biol.*, **216**, 1458-1469 (2013).
26. H.L. Fuchs, M.A. Reidenbach, Biophysical constraints on optimal patch lengths for settlement of a reef-building bivalve. *PLoS ONE*, **8**, 1-11.
27. E.A. Dunnington, Survival time of oysters after burial at various temperatures. *Proc. Natl. Shellfisheries Assoc.* **58** 101-103 (1968).
28. H.K. Lotze *et al.*, Depletion, degradation and recovery potential of estuaries and coastal seas. *Science*, **312** 1806-1809 (2006).
29. V.S. Kennedy *et al.*, Lessons learned from efforts to restored oyster populations in Maryland and Virginia, 1990 to 2007. *J. Shellfish Res.*, **30** 719-731 (2011).
30. K.N. Suding, K.L. Gross, G.R. Houseman, Alternative states and positive feedback in restoration ecology. *Trends Ecol. Evol.* **19**, 46-53 (2004).
31. S.P. Powers, C.H. Peterson, J.H. Grabowski, H.S. Lenihan, Success of constructed oyster reefs in no-harvest sanctuaries: implications for restoration. *Mar. Ecol. Prog. Ser.*, **389** 159-170 (2009).
32. H. Woods, W.J. Hargis, C.H. Hershner, P. Mason, "Improving siting and construction criteria for oyster reef restoration" (Spec. Rep. App. Mar. Sci. Oc. Eng. No. 387, Virginia Institute of Marine Science, 2004).
33. I.K. Bartol, R. Mann, M. Luckenbach, Growth and mortality of oysters (*Crassostrea virginica*) on constructed intertidal reefs: effects of tidal height and substrate level. *J. Exp. Mar. Biol. Ecol.*, **237** 157-184 (1999).
34. J. White, The use of sediment traps in high-energy environments. *Mar. Geophys. Res.*, **12** 145-152 (1990).
35. N.J. Frost, M.T. Burrows, M.P. Johnson, M.E. Hanley, S.J. Hawkins, Measuring surface complexity in ecological studies. *Limnol. Oceanogr. Methods*, **3** 203-210 (2005).

36. H. Akaike, A new look at the statistical model identification, *IEEE T. Automat. Contr.* **19** 716-723 (1974).
37. D.R. Anderson, *Model Based Inferences in the Life Sciences* (Springer Science, New York, 2008).
38. D.K. Kimura, Extending the von Bertalanffy growth model using explanatory variables. *Can. J. Fish. Aquat. Sci.*, **65** 1879-1891 (2008).
39. D.I. Warton, F.K.C. Hui, The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, **92** 3-10 (2011).

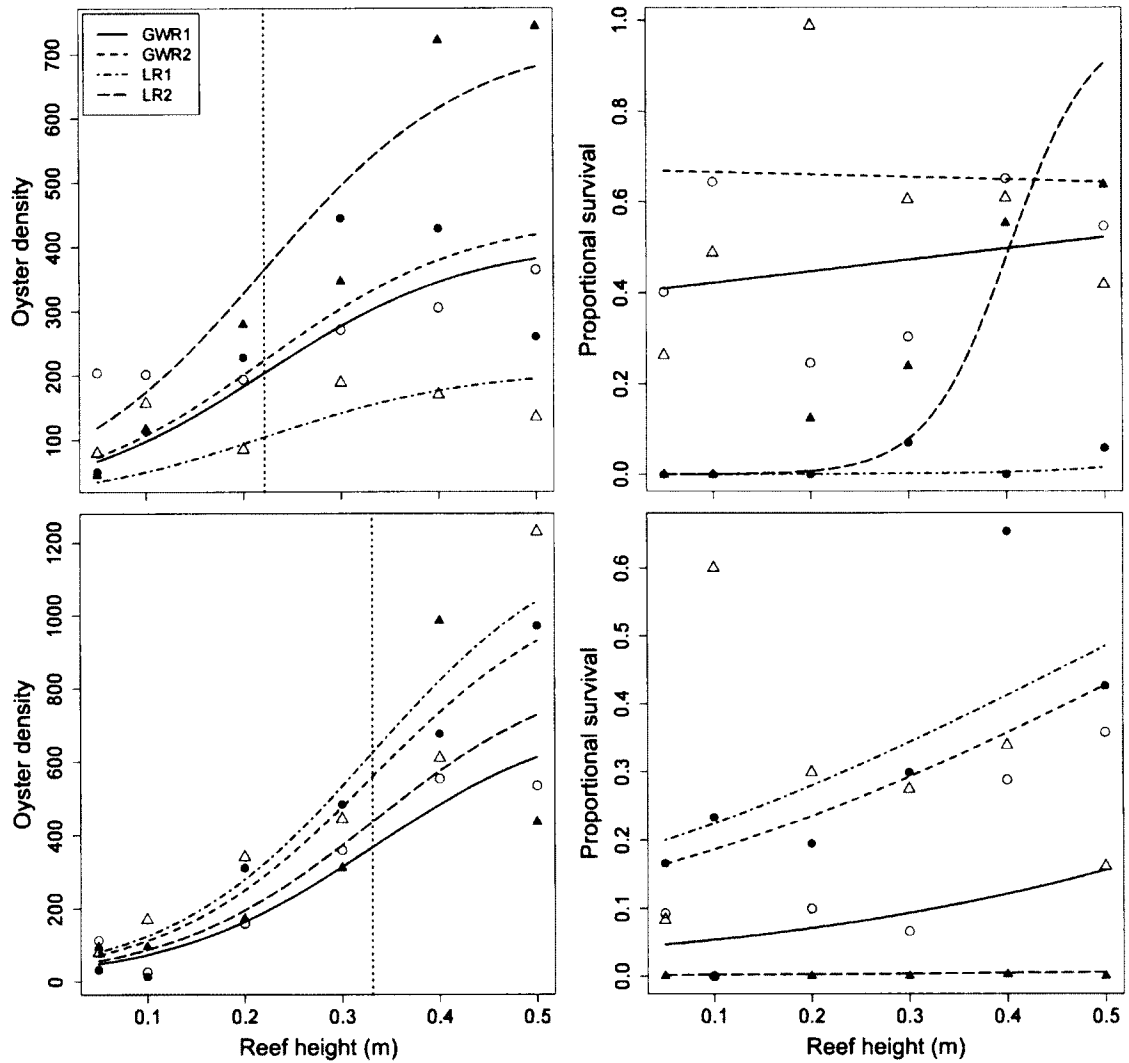
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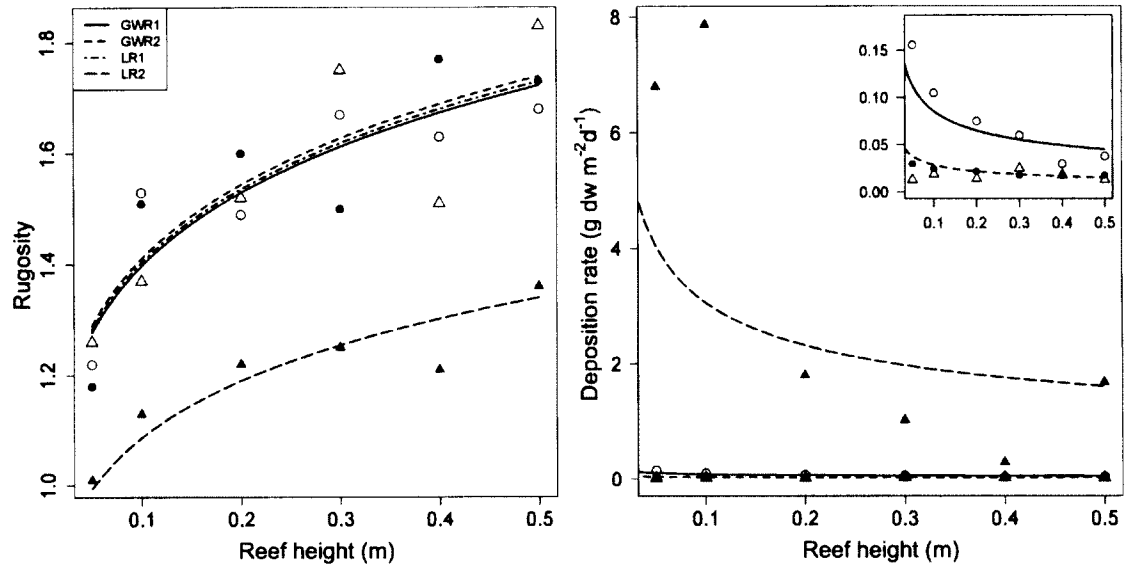
**Fig. 1: Experimental reef design.** Darker shading indicates a 0.03 m base layer of crushed surf clam (*Spisula solidissima*) shell. The remainder of the reef was constructed using oyster shell. The highest reef height treatment (0.5 m) is depicted; other treatments were 0.05, 0.1, 0.2, 0.3, and 0.4 m.



**Fig. 2: Reef condition.** Remotely operated vehicle (ROV) images of low- (A) and high-relief (B) reefs 2 mos after construction. Panel A shows a 0.1 m reef at LR2, and Panel B shows a 0.5 m reef at GWR1. The ROV's sampling arm is visible in the lower left of each photograph.

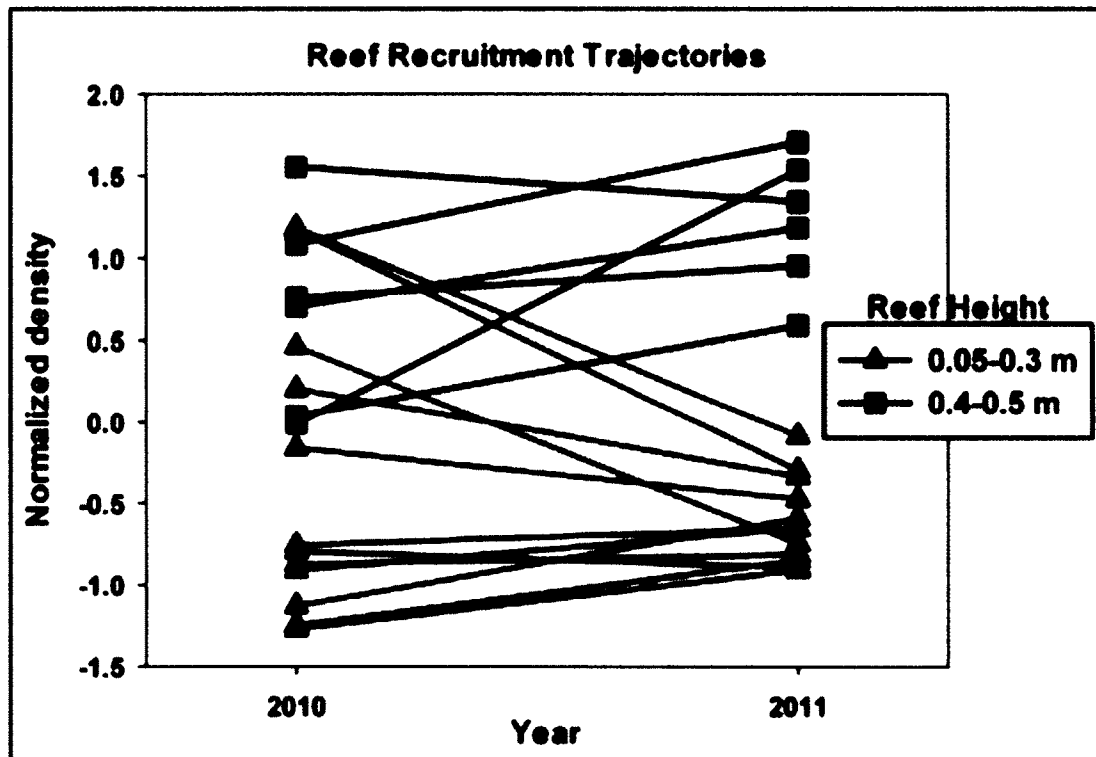


**Fig. 3: Oyster density and survival.** Nonlinear response of oyster density to initial reef height at 8 (top left panel) and 24 mos (bottom left panel) post-construction. Lines are the site-specific logistic model fit. The threshold in initial reef height estimated by the inflection point of the curve is shown by the vertical dotted line. Nonlinear response of survival to initial reef height is shown for 8 (top right panel) and 24 mos (bottom right panel) post-construction. Regressions are the best model fit of the site-specific logistic and power functions for 8 and 24 mos, respectively. Symbols indicate different experimental sites: GWR1 (○), GWR2 (●), LR1 (△), LR2 (▲).



**Fig. 4: Physical responses to reef height.** Rugosity (left panel) and sediment deposition rate (right panel) with respect to reef height. Sediment deposition rates at LR2 were two orders of magnitude larger than other sites (shown in inset). No regression is shown for LR1 for sediment deposition because regression parameters were not significantly different from zero, due to extremely low levels of deposition. Symbols indicate different experimental sites: GWR1 (○), GWR2 (●), LR1 (△), LR2 (▲).





**Fig. 5. Reef trajectories.** Trajectories for oyster density over 2 years are shown for reefs above (red) and below (blue) the average estimated reef height threshold (0.3 m).

### **Supplementary Materials:**

**Materials and Methods:** The field experiment was carried out from July 2009 to August 2011 in two sub-estuaries of lower Chesapeake Bay, the Great Wicomico River (GWR) and Lynnhaven River (LR), Virginia, USA. Experimental reefs of various heights (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 m) were constructed at two sites within each river. The range of reef heights used encompasses those typically used in oyster restoration or repletion activities in Chesapeake Bay (0.05-0.1 m) and the minimum reef height previously observed to produce “successful” reefs (0.4-0.5 m, 21). Sites were chosen based on standard restoration site criteria: water depth less than 3 m, sand to muddy-sand bottom and proximity to available oyster broodstock (32). Reefs were circular (1.3 m diameter) and plateau-shaped to maximize the reef area of the intended height (Fig. 1). Oyster shell was spread evenly over a 0.03 m base layer of crushed surf clam (*Spisula solidissima*) shell until the designated reef height was achieved.

Oyster density, size, and survival were measured 8 and 24 mos post-construction. At each sampling interval, the reef was divided into 9 equal areas (0.048 m<sup>2</sup>), and 2 areas were chosen at random for sampling. Within selected areas, all oyster shell and associated organisms were excavated down to 0.15 m (33). For reefs with less than 0.15 m relief, shell was excavated to the depth at which no shell material was present. All material from the 2 subsamples was combined into a single sample for analysis. Quadrats that were sampled at 8 mos were excluded from sampling at 24 mos. For reefs that were buried at the time of sampling, sediment was removed and the reef was excavated down to 15 cm or until no shell material was present. Oysters were counted, classified as live or dead, and measured to the nearest 0.1 mm shell height.

To evaluate the proposed mechanisms controlling divergent reef trajectories, we measured sediment deposition rate and surface complexity, two important components of the proposed sediment-reef interactions. Sediment deposition rates were measured using sediment traps deployed on the reefs for 7 d in Fall 2009. Seasonal deposition rates observed previously were highest in fall (20); therefore, deployments were carried out in the fall to capture maximum deposition rates. Cylindrical sediment traps were constructed from PVC pipe (25 cm x 2.1 cm; aspect ratio: 11.9; 34). Traps were embedded within the reef matrix such that the opening of the sediment trap was flush with the reef surface. Traps remained capped during placement and were later uncapped and left for 7 d. At retrieval, traps were capped under water, transported to the lab on ice, and processed immediately. Because of the aspect ratio of the traps used and the energetic conditions of our study sites, we suspect very little resuspension and subsequent erosion of sediment from the traps (34).

Sediment volume within each trap was determined by vacuum filtering the contents on a glass fiber filter (Whatman GF/F, 47 mm diameter) to remove excess water. The sediment was dried for 24 h at 70°C and weighed to the nearest 0.001 g. Sediment volumes were converted to deposition rates by scaling the trap opening area to 1 m<sup>2</sup> and dividing the total sediment dry volume by the deployment time (7 d).

Surface rugosity, an index of surface complexity, of the reefs was measured using the chain and tape method (35). This method involves laying a fine link chain over the surface of the reef, allowing the chain to conform to the crevices in the reef surface. The length of the chain needed to conform to the reef is divided by the linear dimension of the reef to give an index of rugosity. Four replicate measurements were made parallel to the

adjacent shoreline along the diameter of the reef.

## **Analysis**

For oyster density, size, sediment deposition, and rugosity, nonlinear least squares regression was used to fit a suite of candidate base models to the response variables to determine the form of the relationship between reef height and the response variables (Table S1). The candidate models included a linear function to rule out a lack of nonlinearity in the response (17), a symmetrical threshold function (logistic), an asymmetrical threshold function (Gompertz), and a nonlinear monotonic function (power function). Akaike's Information Criterion (AIC) was used to compare base models to determine the function that best described the form of the relationship between reef height and the response variables. Models with AIC weights greater than 0.1 were examined to determine the most parsimonious model, which was retained for covariate analysis. (36-37; Tables S2-S5). Covariates were added to the chosen base model to account for site-specific variation in responses and to quantify the influence of covariates of the proposed mechanism on the form of the base model (38; Table S6). Significance of the covariates was determined by comparing the AIC value of the covariate model with that of the base model (Tables S7-S11). Model validity was confirmed by likelihood ratio tests comparing each candidate model to the null (intercept-only) model (Tables S7-S11).

Oyster survival was measured as the proportion of oysters alive at the time of sampling. Instead of comparing the suite of base models, proportional survival data were analyzed by logistic regression (39). A suite of covariate models was fit to the data with AIC model comparisons as previously described (Table S8).

**Supplementary Tables and Figures:**

Table S1. Candidate base models for nonlinear regression analysis. X marks the models that were under consideration for each response variable. The best model identified by AIC model comparison is indicated in bold.

<b>Model</b>	<b>Function</b>	<b>Density</b>	<b>Size</b>	<b>Sediment Deposition</b>	<b>Rugosity</b>
Linear	$y = ax + b$	X	<b>X</b>	X	X
Power	$y = ax^b$		X	<b>X</b>	<b>X</b>
Logistic	$c + \frac{d - c}{1 + e^{-a(x-b)}}$	<b>X</b>	X	X	X
Gompertz	$a + (b - a)e^{-e^{-c(x-d)}}$	X	X	X	X

Table S2. AIC model comparison results for oyster density base models. The model chosen for covariate analysis is indicated in bold.

<b>8 mos post-construction</b>			
<b>Model</b>	<b>AIC</b>	<b><math>\Delta</math>AIC</b>	<b>Weights</b>
<b>Logistic</b>	<b>312.8</b>	<b>1.284</b>	<b>0.224</b>
Gompertz	312.9	1.428	0.209
Linear	311.5	0.000	0.426
<b>24 mos post-construction</b>			
<b>Logistic</b>	<b>315.0</b>	<b>0.000</b>	<b>0.323</b>
Gompertz	315.2	0.203	0.290
Linear	319.1	4.087	0.042

Table S3. AIC model comparison results for oyster size base models. The model chosen for covariate analysis is indicated in bold.

<b>8 mos post-construction</b>			
<b>Model</b>	<b>AIC</b>	<b><math>\Delta</math>AIC</b>	<b>Weights</b>
<b>Linear</b>	<b>3108.0</b>	<b>0.000</b>	<b>0.407</b>
Logistic	3108.6	0.602	0.301
Gompertz	3108.7	0.661	0.292
<b>24 mos post-construction</b>			
<b>Linear</b>	<b>7304.2</b>	<b>0.000</b>	<b>0.684</b>
Gompertz	7301.1	2.791	0.169
Logistic	7303.9	3.075	0.147

Table S4. AIC model comparison results for rugosity base models. The model chosen for covariate analysis is indicated in bold.

<b>Model</b>	<b>AIC</b>	<b><math>\Delta</math>AIC</b>	<b>Weights</b>
<b>Power</b>	<b>-10.81</b>	<b>0.000</b>	<b>0.492</b>
Linear	-8.867	1.942	0.186
Gompertz	-8.616	2.193	0.164
Logistic	-8.541	2.268	0.158



Table S5. AIC model comparison results for sediment deposition rate base models. The model chosen for covariate analysis is indicated in bold.

<b>Model</b>	<b>AIC</b>	<b><math>\Delta</math>AIC</b>	<b>Weights</b>
<b>Power</b>	<b>68.88</b>	<b>0.000</b>	<b>0.960</b>
Linear	75.23	6.352	0.040
Logistic	101.4	32.51	<0.001
Gompertz		Did not converge	

Table S6. Candidate covariate models for nonlinear regression analysis. X marks the covariates that were under consideration for each response variable.

<b>Response</b>	<b>Base Model</b>	<b>Site</b>	<b>Density</b>	<b>Sediment Deposition</b>
Oyster Density	Logistic	X		
Survival	Logistic	X	X	X
Oyster Size	Linear	X	X	
Sediment Deposition	Power	X		
Rugosity	Power	X		

Table S7. AIC model comparison results for site covariate models of oyster density. Site covariate column lists the parameters in which the site covariate was included. P-values are for likelihood ratio tests against the null (intercept-only) model. The best model is indicated in bold.

<b>8 mos post-construction</b>					
<b>Model</b>	<b>Site Covariate</b>	<b>AIC</b>	<b>ΔAIC</b>	<b>Weights</b>	<b>P-value</b>
<b>3</b>	<b>Asymptote</b>	<b>315.0</b>	<b>1.767</b>	<b>0.237</b>	<b>0.005</b>
1	None	312.8	0.000	0.574	0.001
2	Slope	314.5	2.228	0.188	0.006
4	Slope + Asymptote		Did not converge		
<b>24 mos post-construction</b>					
<b>3</b>	<b>Asymptote</b>	<b>315.0</b>	<b>0.000</b>	<b>0.827</b>	<b>0.006</b>
2	Slope	319.4	4.453	0.089	0.038
1	None	319.6	4.569	0.084	0.058
4	Slope + Asymptote		Did not converge		

Table S8. AIC model comparison results for site covariate models of proportional survival. Site covariate column lists the parameters in which the site covariate was included. P-values are for likelihood ratio tests against the null (intercept-only) model. The best model is indicated in bold.

<b>Model</b>	<b>Site Covariate</b>	<b>AIC</b>	<b>ΔAIC</b>	<b>Weights</b>	<b>P-value</b>
<b>4</b>	<b>Slope+Asymptote</b>	<b>113.12</b>	<b>0.000</b>	<b>0.980</b>	<b>&lt;0.001</b>
3	Asymptote	120.86	7.740	0.020	<0.001
2	Slope	135.48	22.35	<0.001	0.015
1	None	138.58	25.48	<0.001	0.072
<b>24 mos post-construction</b>					
<b>4</b>	<b>Slope+Asymptote</b>	<b>75.16</b>	<b>0.000</b>	<b>0.715</b>	<b>&lt;0.001</b>
3	Asymptote	77.01	1.844	0.284	<0.001
2	Slope	93.56	18.39	<0.001	<0.001
1	None	112.27	37.10	<0.001	0.305

Table S9. AIC model comparison results for oyster density covariate models of oyster size. Density covariate column lists the parameters in which the density covariate was included. P-values are for likelihood ratio tests against the null (intercept-only) model. The best model is indicated in bold.

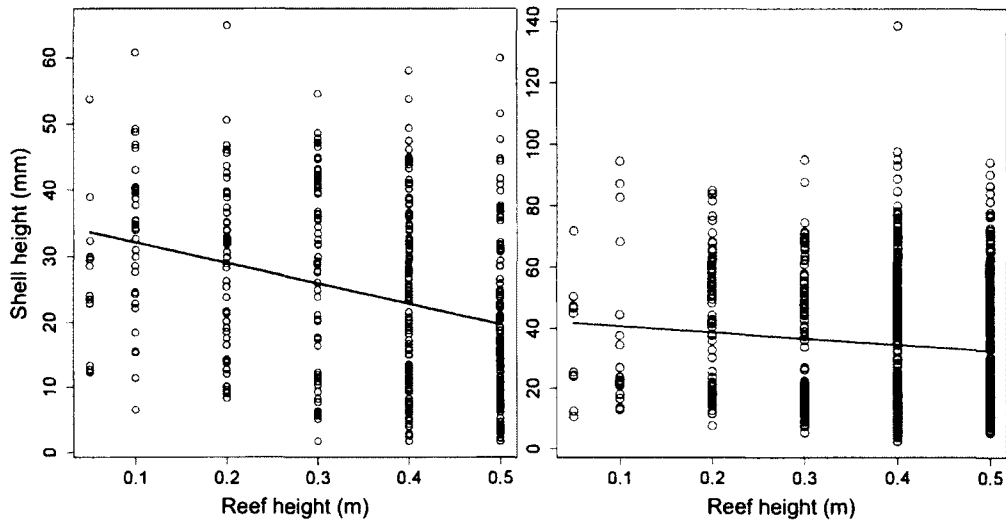
<b>8 mos post-construction</b>					
<b>Model</b>	<b>Density Covariate</b>	<b>AIC</b>	<b>ΔAIC</b>	<b>Weights</b>	<b>P-value</b>
<b>3</b>	<b>Intercept</b>	<b>3074.8</b>	<b>0.000</b>	<b>0.424</b>	<b>&lt;0.001</b>
2	Slope	3075.1	0.271	0.370	<0.001
4	Slope+Intercept	3076.3	1.452	0.205	<0.001
1	None	3108.0	33.21	<0.001	<0.001
<b>24 mos post-construction</b>					
<b>4</b>	<b>Slope+Intercept</b>	<b>7166.5</b>	<b>0.000</b>	<b>0.991</b>	<b>&lt;0.001</b>
3	Intercept	7176.1	9.510	0.009	<0.001
2	Slope	7196.7	34.16	<0.001	<0.001
1	None	7301.1	134.6	<0.001	0.002

Table S10. AIC model comparison results for site covariate models of rugosity. Site covariate column lists the parameters in which the site covariate was included. P-values are for likelihood ratio tests against the null (intercept-only) model. The best model is indicated in bold.

<b>Model</b>	<b>Site Covariate</b>	<b>AIC</b>	<b><math>\Delta</math>AIC</b>	<b>Weights</b>	<b>P-value</b>
<b>2</b>	<b>Slope</b>	<b>-42.15</b>	<b>0.000</b>	<b>0.918</b>	<b>&lt;0.001</b>
4	Slope+Intercept	-37.31	4.840	0.081	<0.001
3	Intercept	-26.54	15.61	<0.001	<0.001
1	None	-10.81	31.34	<0.001	<0.001

Table S11. AIC model comparison results for site covariate models of sediment deposition rate. Site covariate column lists the parameters in which the site covariate was included. P-values are for likelihood ratio tests against the null (intercept-only) model. The best model is indicated in bold.

<b>Model</b>	<b>Site Covariate</b>	<b>AIC</b>	<b><math>\Delta</math>AIC</b>	<b>Weights</b>	<b>P-value</b>
<b>2</b>	<b>Slope</b>	<b>-49.37</b>	<b>0.000</b>	<b>1.000</b>	<b>&lt;0.001</b>
1	None	68.88	118.2	<0.001	<0.001
3	Intercept	9.116	58.49	<0.001	<0.001
4	Slope+Intercept		Did not converge		



**Fig. S1: Oyster size.** Oyster size across reef heights at 8 (left panel) and 24 mos (right panel). Regressions depict the best-fit linear model.



## CHAPTER 3

### **Orientation affects oyster reef productivity in Chesapeake Bay**

#### Abstract

The functional extinction of a majority of the world's oyster reef habitats has led to a resurgence of restoration efforts aimed at increasing oyster population size and enhancing attendant ecosystem services of oyster reef habitat. Reef design is a critical part of the restoration process, which is often limited to considerations of vertical relief and restoration location. Historically, large, highly productive reefs of the eastern oyster, *Crassostrea virginica*, in Chesapeake Bay were oriented perpendicular to tidal current at evenly-spaced intervals, which were thought to arise from interactions with local flow conditions that created optimal conditions for oyster growth and survival. To investigate if historical reef geometries could improve oyster reef performance, we conducted a long-term, large-scale field experiment in multiple locations in two sub-estuaries of Chesapeake Bay, the Piankatank and Great Wicomico Rivers. We monitored oyster population demographics and macrofaunal community density, biomass, and diversity on parallel, perpendicular, and circular reefs seasonally over 2 consecutive years to capture seasonal and interannual variability and resolve differences between study locations. After 2 years, an abundant oyster and macrofaunal community was established on all experimental reefs. Although reef orientation did not have a significant effect on oyster population metrics (density and biomass), macrofaunal community metrics were positively correlated with oyster biomass. In particular, macrofaunal biomass increased proportionally with oyster biomass. Perpendicular reefs supported higher macrofaunal

biomass than either parallel or perpendicular reefs, at the community level and for most taxonomic groups individually. That this trend was evident across both rivers indicates that the mechanism driving macrofaunal biomass enhancement is likely due to the reef orientation itself, rather than site-specific conditions, lending support for the proposed hydrodynamic mechanism. Circular reefs lost a significant amount of reef area, which resulted in significantly lower abundance and total reef biomass for both oysters and macrofauna relative to parallel and perpendicular reefs. Secondary production of benthic macrofauna is an important link in the estuarine food web and also enhances ecosystem functions such as denitrification. Positive associations of reef orientation and oyster biomass with macrofaunal secondary production indicates that efforts aiming to maximize oyster biomass will also enhance community biomass and attendant reef ecosystem benefits.

## INTRODUCTION

The eastern oyster, *Crassostrea virginica*, is an ecosystem engineer whose biogenic reef structures were once prominent features of the estuarine landscape throughout its geographic range along the east coast of North America. Eastern oyster reefs are constructed by the gregarious settlement of multiple generations of oyster larvae onto hard substrates that can evolve over time into large reef complexes up to several km long (Price 1954, Haven and Whitcomb 1983). Oyster reefs are critical not only for the oysters that rely on them for settlement substrate, but also for a diverse suite of estuarine species that utilize them as foraging grounds, nurseries, predation refuge, or habitat (Zimmerman 1989, Coen and Grizzle 2007). Oyster reef communities carry out a variety of ecosystem services including nutrient cycling (Kellogg et al. 2013), benthic-pelagic coupling (Grizzle et al. 2008), shoreline stabilization (Meyer et al. 1997), and improvement of water clarity (Porter et al. 2004). Unfortunately, overexploitation, disease pressure, and worsening water quality (Rothschild et al. 1994, Mackenzie 2007) has led to the functional extinction of more than 85% of the world's oyster reefs (Beck et al. 2011), and the eastern oyster is no exception (Lotze et al. 2006). The systematic depletion and degradation of oyster populations and habitat along the North American coast demonstrates the long-term pressures that these populations have faced (Kirby 2004). Consequently, oyster populations in Chesapeake Bay, which once supported the largest oyster fishery in the United States, are at less than 1% of peak populations in the late 1800s (Hargis and Haven 1988, Beck et al. 2011, Wilberg et al. 2011).

Chesapeake Bay represents a geographic transition zone for eastern oyster reef morphology. Along the Atlantic coast, areas south of Chesapeake Bay exhibit

predominantly intertidal reefs, while most reefs north of the bay are subtidal (Kennedy and Sanford 1999, Woods et al. 2004). Both intertidal and subtidal reefs occur within the bay, though extant reefs are largely subtidal.

Historically, oyster reefs exhibited 3 distinct morphologies: 1) string reefs: long, shoal-like reefs oriented at right angles to tidal currents, 2) fringe reefs: reefs oriented parallel to tidal currents along the channel in areas of sharp changes in relief, and 3) patch reefs: small, amorphous reefs with dimensions approaching unity (Haven and Whitcomb 1983, Kennedy and Sanford 1999, Smith et al. 2003). String reefs were generally largest and most productive of the three types. They have been described as “upthrusting” since many were intertidal prior to heavy exploitation in the 19<sup>th</sup> century (Woods et al. 2005). Fringe reefs were more ephemeral on geological time scales and had lower relief than string reefs (Smith et al. 2003). Patch reefs were common near the mouths of rivers and in deeper water than string or fringe reefs (Haven and Whitcomb 1983, Kennedy and Sanford 1999). Examples of string and fringe reefs have been described from areas throughout the eastern oysters’ geographic range, including Texas (Norris 1953, Price 1954) and North Carolina (Grave 1905). Perpendicularly-oriented reefs of the clams *Anomalocardia subrugosa* (= *Globivenus fordii*) and *Protothaca grata* in Panama (Lewis and Macdonald 1972) and parallel beds of horse mussels in the Bay of Fundy (Wildish and Fader 1998) are also thought to develop by similar processes as those for oysters. That these reef morphologies are pervasive throughout the oysters’ range and for other bivalve species suggests that the underlying mechanism for reef formation is similar across diverse ecosystems.

The interactions of nascent reef structures with local flow dynamics has long been cited as the mechanism for the formation of these distinct reef types. Grave (1905) was one of the first to describe the formation of long, shoal-like reefs in North Carolina. He proposed that these evenly spaced, perpendicularly-oriented reefs formed when small oyster clusters along the shore experienced high flow rates at their leading edge, improving conditions for oyster growth and settlement. Higher growth rates at the edge led to elongation of the emerging reef away from the shoreline. High flow rates over the reef crest further enhanced oyster growth leading to accretion in the vertical axis and lateral growth by colonization of cultch material that had fallen from the crest. Eventually, the reef formed such an obstruction to flow that water pressure caused a break in the reef, usually near shore, forming the familiar string reefs found throughout the eastern oyster's range. Similarly, fringe reefs are thought formed along channel edges as fast flowing water maintained optimal conditions for oyster growth and settlement (Powell et al. 1995, Kennedy and Sanford 1999, Smith et al. 2003). Fringe reefs were the closest available hard substrates for mature oyster larvae being transported upstream in the channel, and thus reefs were formed. Patch reefs were usually formed in areas that lack strong bidirectional currents (Haven and Whitcomb 1983, Kennedy and Sanford 1999).

Although the mechanisms described for the formation of each reef type are intuitively sound, the ecological consequences of these reef geometries has not been explored empirically. Since the early 1990s, extensive oyster reef restoration has been carried out in Chesapeake Bay, first in an effort to restore fishery capital and later to reestablish ecological functioning present on pre-exploitation reefs (Wesson et al. 1999).

The design of restoration reefs was guided primarily by the restoration goal. Initially, reefs were constructed as thin veneers of shell spread over a large area intended to provide maximum harvest return after several years. The shift in focus to ecological restoration in recent years was accompanied by a shift in reef design, focused more on creating 3-dimensional habitat, a main feature of which was vertical relief (Wesson et al. 1999). Vertical relief can affect oyster survival and growth and exposure to hypoxia (Lenihan 1999), and reefs in several Chesapeake Bay tributaries have benefitted from a shift to high relief reefs (Schulte et al. 2009). Despite the presumed importance of reef orientation in shaping the growth of natural oyster reefs historically and a shift toward reef design to maximize ecosystem services, reef geometry has yet to be addressed in a restoration framework. If the three historical reef geometries did alter hydrodynamics to optimize habitat for oysters, then the application of these geometries to reef restoration may improve oyster population recovery. Additionally, recent studies have indicated that the ecosystem services provided by oyster reefs (nutrient cycling, benthic-pelagic coupling, and denitrification) are enhanced by macrofaunal communities that colonize reef habitat (Hadley et al. 2010, Kellogg et al. 2013). Several species of reef macrofauna are positively associated with oyster abundance (Luckenbach et al. 2005); therefore, if reef geometry improves oyster settlement, growth, or survival, it follows that it may also impact reef communities and the level of ecological services provided by restoration reefs.

We conducted a long-term field experiment to test the hypothesis that reef orientation impacts the performance of restoration reefs by altering local hydrodynamics. We followed oyster reef populations and reef communities from the time of construction through several years of development. We hypothesized that in shallow, subtidal areas

currently targeted for restoration, reefs oriented perpendicular to tidal currents would support larger oyster populations because of higher flow rates associated with having the short axis of the reef in the direction of flow. Positive associations between macrofaunal species and oyster abundance would then drive increases in abundance of macrofaunal communities capable of enhancing the ecosystem functioning capacity of the reef overall.

Studies of state or federally-managed restoration reefs are often complicated by restoration activities, such as seeding with oysters or shell addition, during the course of the study that interrupt the natural progression of reef development (Kennedy et al. 2011). This study utilized shell reefs with no addition of oysters, simply natural recruitment, to determine if the hydrodynamic interactions described for historical reefs support the development of reef communities on restoration reefs. This study was carried out in multiple locations in 2 rivers over 2 years to demonstrate that the proposed reef orientation-flow mechanism is a fundamental driver of reef community development, rather than a fleeting local phenomenon. Oyster reef restoration is a costly venture; improvements to reef design that would enhance the performance of these reefs and increase returns on extensive investments are therefore highly desirable. This study provides insights into the ecological significance of oyster reef morphology for oyster population recovery and reef community development to improve restoration outcomes and enhance secondary productivity and ecosystem services.

## **MATERIALS AND METHODS**

### **Study Sites**

Two tributaries on the western shore of Chesapeake Bay were selected--the Piankatank River (PR) and the Great Wicomico River (GWR; Fig. 1). The Piankatank

River has a drainage area of approximately 466 km<sup>2</sup> and a tidal range of 0.4 m (Chen et al. 1977). Small tidal amplitude in PR results in maximum tidal currents of approximately 0.12 m s<sup>-1</sup> though the system is generally well mixed. The Great Wicomico River has a drainage area of 182.8 km<sup>2</sup>, and is also microtidal. Both rivers are trap-type estuaries that flow directly into the bay (Chen et al. 1977, Andrews 1979). Trap-type estuaries are characterized by low flushing rates, limited freshwater inputs, small tidal amplitude, and restricted entrances leading to high retention of planktonic larvae within the system (Andrews 1979). Both the PR and GWR were historically highly productive seed areas, where larval availability and settlement is high, and where moderate seed harvest continues today (Harding et al. 2010, Southworth et al. 2010).

In each river, one site was chosen on the north and south shores, for a total of 4 sites (Fig. 1). Sites were chosen based on several criteria including depth, bottom type, and proximity to oyster broodstock. Standard restoration siting criteria in Chesapeake Bay targets areas less than approximately 3 m in depth to limit navigational interference and exposure to hypoxia (Wesson et al. 1999). The average depth in PR and GWR ranges from 3.2 to 3.4 m, and sites chosen for the experiment ranged in depth from 1.25 to 2 m. Hard substrates, such as shelly sand or relict oyster bars, are preferred over muddy substrates for restoration, to support the weight of the reef without loss of substrate. Sediments at the sites in this study were predominantly sand (95-99%), with 1-5% muds and occasional oyster shell or shell hash. Proximity to broodstock ensures an adequate larval supply to sustain reef populations. Both PR and GWR have undergone restoration carried out by state and federal agencies (Wesson et al. 1999, Schulte et al. 2009). A large-scale restoration project in GWR in 2004 (35 ha) resulted in



unprecedented oyster recovery in that system (Schulte et al. 2009); therefore, broodstock limitation was not a concern. Smaller-scale efforts in PR have included the construction of several small sanctuary reefs (< 1 ha) by the Virginia Marine Resources Commission (Wesson et al. 1999). To ensure adequate larval supply to experimental reefs, sites were chosen that were in close proximity, within 1 km, to established restoration oyster reefs.

### Reef Construction

Six experimental reefs of three different reef types were constructed in each of the four study sites to mimic the shape and orientation of historic reefs in Chesapeake Bay. The reef types were parallel or perpendicular to tidal currents and circular (control). Parallel and perpendicular reefs were rectangular (1 m x 5 m x 0.4 m) with the long axis oriented parallel or perpendicular to tidal currents. Circular reefs were 2.5 m in diameter (0.4 m height), which maintained approximately equal surface area and volume across reef types (Fig. 2).

Reefs were constructed using oyster shell obtained from local seafood processors that had been air-dried for a minimum of 6 months. Experimental reefs were built to an initial height of 0.4 m above the bottom, which supports long-term reef persistence of restored oyster reefs (Schulte et al. 2009). Two reefs of each orientation were built at each location for a total of 6 reefs per site and 8 replicates of each reef type across all locations. Reefs were built in a 3 x 2 matrix configuration, with reef types randomly assigned within a site (Fig. 2). Distance between reefs was 20 m in the alongshore direction and 15 m in the across shore direction. This arrangement maximized the available space at each study site to minimize interactions between reefs.

During construction, 5 sampling trays were placed within the reef matrix such that the edge of the tray was flush with the surface of the reef. Trays were constructed of vinyl-coated steel mesh lined with 6 mm plastic mesh (25 cm x 25 cm x 15 cm, sample volume: 9.4 L). These trays were used to sample oyster and macrofaunal populations twice yearly in October and April between 2011 and 2013, resulting in 4 sampling events. One reef each at the GWR and PR south shore sites had the fifth sampling tray intact in October 2013, so these trays were sampled and included in the analysis. Density values reflect the number of individuals per tray (area: 0.06 m<sup>2</sup>; volume: 9.4 L).

#### Environmental Monitoring

Environmental conditions were measured at each site weekly from June-September in 2011 and 2012. Temperature, salinity, and dissolved oxygen measurements were taken with a hand-held YSI data sonde (YSI Professional Plus, Yellow Springs Instruments, Inc., Yellow Springs, OH) approximately 10 cm from the seabed at 10 locations within each site. Additionally, shellstrings were deployed weekly at each site to determine oyster larval supply. Shellstrings were constructed of oyster shell obtained from a local processor that had been air-dried for a minimum of 6 months. Seven single valves (> 76 mm shell height) were strung on heavy gauge wire, which was suspended from an anchored buoy at 10 locations at each site. Each week, shellstrings were collected, replaced with new shellstrings, and transported back to the Virginia Institute of Marine Science in Gloucester Point, Virginia for processing. Shellstring design and deployment protocol was modeled after the state-sponsored annual spatfall monitoring program conducted by the Virginia Institute of Marine Science (Southworth and Mann

2014). Power analysis of 2011 shellstring data indicated that 8 shellstrings provided sufficient replication, so the number of replicates was reduced to 8 in 2012.

Shellstrings were air-dried overnight, and 3 shells were randomly selected for examination under magnification (40 x). The number of spat on the concave surface of each valve was recorded. Mean spat per shell was determined weekly for each site.

#### Reef Characteristics

Reef area was measured in August 2011, two months following construction, and in May 2013, after the last reef sampling event. In 2011, 3 transects were measured in the across-shore and alongshore directions, and the mean of each dimension was used to determine reef area. In 2013, measurements were taken every 1 m, and the means of dimensions used to determine area. Surface roughness or rugosity of the reefs was determined using the chain-to-tape ratio, in which the length of a fine-link chain laid along the crevices of the reef surface is divided by the linear length of the same transect (Frost et al. 2005). The resulting ratio has a lower bound at 1, which would indicate a completely featureless surface. Nine rugosity transects were taken in the alongshore and across-shore directions on each reefs with chains of 3 different link lengths (14.8, 19.6, and 24.7 mm) to investigate the fractal geometry of the reef surface. Because link length measurements did not differ significantly (ANOVA,  $p = 0.91$ ), all chain measurements were pooled to determine rugosity. Distance from a known height to the reef surface was measured every 10 cm along the long axis or along shore axis (circular reefs) to calculate mean reef height. One-way analysis of variance (ANOVA) was used to identify differences in reef area, height, rugosity, and area change by reef type. Spring 2013 reef area was used to calculate oyster and macrofaunal total abundance and total reef biomass

from Spring 2013 oyster and macrofaunal density and biomass, which was analyzed by one-way ANOVA with respect to reef type.

### Oyster Sampling

Twice yearly, oyster reef trays were sampled to quantify oyster population density and biomass as well as macrofaunal community density, biomass, and diversity. During sampling, one randomly selected tray from each reef was covered with 1 mm mesh to prevent loss of organisms or shell material while it was removed from the reef. Trays were placed intact into a 1 mm mesh bag for transport back to the lab. Samples were rinsed thoroughly with fresh water in the mesh bags then transferred to plastic bags and frozen until further processing.

During processing, all live and dead oysters were counted and measured (shell height) to the nearest 0.1 mm. Macrofauna on or within oysters were removed and processed separately. Live oysters were retained from each sample for biomass. Biomass was calculated as the g ash-free dry mass of oyster tissue following drying for 48 hr at 65 °C and combustion for 4 hr at 550 °C measured to the nearest 0.001 g. For the first two sampling periods (October 2011 and April 2012), all live oysters were processed for biomass. High oyster density in later sampling periods necessitated subsampling in which 30 oysters were randomly selected from 10-mm shell height size bins. For size classes with fewer than 30 oysters, all live oysters were processed. The subsampled biomass data was used to construct site-specific shell height-biomass regressions for each sampling period, which were used to determine biomass for all oysters in the sample. Biomass (g AFDM tray<sup>-1</sup>) was summed over all oysters on each reef for biomass analyses.

## Macrofaunal Sampling

Macrofauna visible to the naked eye were removed from oyster shells during oyster processing. After sorting, all oysters and shell were rinsed thoroughly with fresh water, which was passed through a 1 mm sieve to ensure all organisms had been removed. All fish, crabs, shrimp, worms, and bivalves were counted and identified to the lowest possible taxonomic level. For the first sampling period (October 2011), all individuals were counted and biomassed. In all subsequent sampling periods, macrofaunal samples were sieved over 3 successively smaller sieves (12.7 mm, 3 mm, 1 mm) to divide the sample into 3 distinct size classes. All organisms retained on the 12.7 mm mesh were counted and biomassed. Organisms in the 3 mm and 1 mm size classes were subsampled by randomly selecting one-quarter of the sample for counts, biomass, and species identification. Biomass (g AFDM tray<sup>-1</sup>) was determined by drying and combusting organisms and taking the difference of dry weight (g) and ash weight (g).

## Statistical Analyses

### *Oyster Population Response*

The repeated measures arising from successive sampling of reefs over time are advantageous in that they reduce the number of individual reefs required; however, within-reef samples cannot be assumed to be independent (Underwood 2002). As such, we must account for temporal autocorrelation within reef samples to avoid biased estimates of the variance of fixed effects (Littell et al. 2006). To do so, we fit a suite of candidate correlation structures to oyster density and biomass data separately using a global ANOVA model with river, season, and reef orientation as fixed effects (Littell et al. 2006). This model is a reduced version of the *a priori* models that included two- and

three-way interaction effects. No interaction terms were significant; therefore, interaction models were removed from consideration, as overfitting models can lead to spurious effects (Anderson 2008).

Candidate correlation structures examined were unstructured, compound symmetry, autoregressive order 1 (AR (1)), and autoregressive moving average (ARMA (p,q)). In unstructured correlation, a unique correlation is calculated between each pair of observations, allowing the data to define the correlation structure, whereas compound symmetry assumes a constant correlation between all observations. Both AR (1) and ARMA (p,q) assume that correlation is a function of the distance between two observations in space or time (Littell et al. 2006). That is, observations taken closely together are more highly correlated than those taken farther apart. In ARMA (p,q) correlation, the relationship between subsequent observations depends on a polynomial function that includes both autoregression of order  $p$  and a moving average of order  $q$ . AR (1) correlation is a special case of ARMA (p,q) in which the subsequent observation depends linearly on one observation prior with no moving average. Preliminary analysis indicated ARMA (1,1) to be the most appropriate ARMA structure for this data set, although  $p$  and  $q$  can take on any positive integer value. Because there were only 4-5 observations per reef, the auto-regressive order ( $p$ ) was chosen to be 1. The order for the moving average ( $q$ ) was chosen by comparing models fit with  $p = 1$  and  $q = 1, 2, \text{ or } 3$ . ARMA (1,1) was found to be the most parsimonious ARMA model and was thus included in the correlation candidate model set.

Candidate correlation structures were fit to the data with the global model using R v.3.1.0 (R Core Development Team 2013). Akaike's Information Criterion (AIC) was

used to determine the best correlation structure for each oyster response variable (Littell et al. 2006). When  $\Delta AICc$  values were  $< 2$ , the model with fewer parameters was chosen for parsimony (Anderson 2008). The unstructured correlation models did not converge for any density or biomass response due to the large number of parameter estimates relative to sample size ( $N = 98$ ).

### *Reef Community Response*

Macrofaunal community responses examined included density, biomass, and Shannon diversity, a measure ranging from 0 to approximately 4.5 with higher values indicating higher diversity. In addition to the fixed factors of interest in the previous global model (river, season, reef orientation), we surmised that oyster population structure might also have an impact on macrofaunal community response. Oysters may increase the habitat complexity or surface area of the reef, facilitating greater abundance of reef organisms, or may compete for resources. To elucidate these interactions, we included oyster biomass in the analyses of macrofaunal density, biomass, and diversity. Oyster biomass was chosen over oyster density because the size and surface area of oysters reflected in biomass is likely to be more important in provisioning of habitat and resource consumption than is the actual number of oysters. However, oyster density and biomass was strongly collinear in all sampling periods, indicating that the use of density in place of biomass would likely produce similar results (Fig. 3, Table 1).

Macrofauna were collected concurrently with oyster samples; therefore, the issue of temporal autocorrelation is also relevant for macrofauna. As for oyster analyses, a global model was fit to each response variable with a number of correlation structures, including unstructured, compound symmetry, AR (1), and ARMA (1,1), and AIC was

used to select the best correlation structure (Littell et al. 2006). The global model was a generalized additive model (GAM) that included all the fixed effects of the previous model (river, season, reef type) with the addition of a non-parametric term for oyster biomass, fit by thin plate regression splines (Wood 2003, 2006). The generalized additive model framework allows the data to define the shape of the oyster-macrofauna relationship while taking into consideration other factors that contribute to the response (Wood 2006), which was advantageous as the variation due to location and season was often quite large. Significance of oyster biomass as a factor was determined by examining the p-value of the spline parameter and examination of model fit by residual analysis. Because the density data are counts of organisms and the biomass data were positive, real numbers, the GAM model was fit with the negative binomial and Gamma distributions for density and biomass, respectively.

To determine which species were most characteristic of reef communities, we used the similarity percentages (SIMPER) procedure in Primer (Clarke 1993, Clark and Gorley 2006, v.6, PRIMER-E Ltd., Plymouth, UK). SIMPER identifies the species that contribute the most to the similarity between samples within a group, such as within a river, using the Bray-Curtis similarity matrix. The result is a rank order list of species in order of importance in structuring the macrofaunal community within groups (Clarke 1993). Prior to analysis, the data were square-root transformed to down weight the significance of abundant species (Clarke and Gorley 2006).

#### *Taxonomic Group Response*

Additional analyses were carried out on four taxonomic groups within the reef community: mussels, mud crabs, worms, and resident fish. These groups not only



represent the most abundant species on oyster reefs but also have important ecological links to oysters and oyster reef habitat. Species included in each group are as follows: (1) mussels: *Ischadium recurvum*, *Mytilus edulis*, *Mytilopsis leucophaeta*, (2) mud crabs: *Eurypanopeus depressus*, *Dyspanopeus sayi*, *Panopeus herbstii*, *Rhithropanopeus harrisi*, (3) polychaetes: *Alitta succinea*, and (4) resident fish: *Gobisoma bosc*, *Gobiesox strumosus*, *Chasmodes bosquianus*, *Opsanus tao*. Density and biomass of each species group was analyzed using the GAM global model as described for total macrofaunal density and biomass. Polychaete and fish biomass data contained a small number of zeros; therefore, 1 was added to all data points prior to analysis, as the Gamma distribution is only defined for positive, real numbers.

## RESULTS

### Environmental Conditions

Environmental conditions were similar in GWR and PR. Water temperatures were higher earlier in the season in 2011 than in 2012, and differed by a maximum of 5 °C between years (Fig. 4). Salinity also varied between years, with salinity in 2012 approximately 4 units higher than in 2011 (Fig. 4). Dissolved oxygen was the most variable environmental response, but with no clear trend across rivers or between years. Dissolved oxygen levels at experimental sites remained within normoxic limits (> 4 mg L<sup>-1</sup>) throughout both summers (Fig. 4).

### Spatfall Monitoring

Shellstring deployments indicated a marked difference in larval supply between experimental sites in PR and GWR, with spatfall in GWR 5-6 times greater than in the PR (Fig. 5). Spatfall in GWR peaked approximately 1 week earlier than in PR (Fig. 5).

In 2011, a primary summer recruitment event was evident in late June with a secondary, smaller recruitment event in late August (Fig. 5). This secondary recruitment event was observed only in PR; a hurricane precluded the deployment of shellstrings in GWR during this time period. In 2012, a single recruitment event was observed in both rivers in early July, with the peak in GWR again 1 week prior to peak recruitment in PR (Fig. 5). Although only a single recruitment event was observed, oyster spatfall on shellstrings in 2012 was 10-fold greater than in 2011 (Fig. 5). Oysters in PR were larger (maximum SH) than those in GWR in all time periods, despite recruitment peaking 1 week earlier in GWR (Fig. 6-7). The structure of the oyster population in both rivers remained consistent over time, with populations dominated by small oysters (< 60 mm SH); large oysters only made up approximately 10% of the population (> 70 mm SH; Fig. 6-7).

#### Reef Characteristics

After 2 years, all reefs were emergent with moderate vertical relief (12.9-28.7 cm). Reef area in 2011 and 2013 were significantly different by reef type (ANOVA;  $p = 0.01$  and  $p \ll 0.01$ , respectively). Tukey's honest significant difference (HSD) tests indicated that in 2011 perpendicular reefs had significantly larger area than circular reefs ( $p = 0.01$ ), but were not different from parallel reefs ( $p = 0.66$ ). Parallel reefs were not significantly different from circular reefs ( $p = 0.06$ ). In 2013, perpendicular reefs again had the greatest area, followed by parallel then circular reefs; however, in 2013, both perpendicular and parallel reefs had significantly larger area than circular reefs ( $p \ll 0.01$  for both comparisons). Perpendicular and parallel reef areas were not significantly different in 2013 ( $p = 0.99$ ). Mean reef area difference was similar to trends in area in both years. All circular reefs experienced a loss in reef area over the 2 year study.

Percent area change was positive on average for perpendicular and parallel reefs (mean  $\pm$  SE; perpendicular:  $4.30 \pm 4.43$ , parallel:  $6.71 \pm 6.82$ ). Mean reef height and rugosity did not differ by reef type (ANOVA;  $p = 0.42$  and  $p = 0.67$ , respectively).

Differences in reef area led to significant differences in oyster abundance and biomass and macrofaunal abundance and biomass between reef types (Fig. 8). For all abundance and total biomass variables, circular reefs had the lowest mean followed by perpendicular then parallel reefs. Parallel and perpendicular reefs supported similar oyster biomass, which was significantly greater than that on circular reefs (Fig. 8B). For oyster abundance, macrofaunal abundance, and macrofaunal biomass, parallel reefs had significantly higher abundance and biomass than circular reefs and perpendicular reefs were intermediate (Fig. 8 A,C,D).

#### Temporal Autocorrelation

The best correlation structure identified by AIC differed between oyster density and biomass (Table 2). Correlation structures for oyster density were functionally equivalent, as evidenced by similar AICc values ( $\Delta AICc < 2$ ) and weights (Table 2). Compound symmetry was selected as the correlation structure for oyster density as it had the highest AIC weight. For oyster biomass, ARMA (1,1) was identified as the best correlation structure with AICc values and weights supporting the use of this model over the other two candidate structures (Table 2).

Correlation structures for macrofaunal density and biomass did not differ significantly and were functionally equivalent according to AIC comparisons (Table 3). ARMA (1,1) requires estimation of one additional parameter over compound symmetry and AR (1), and so was rejected as the most parsimonious model. The AR (1) correlation

structure was chosen for both density and biomass. Compound symmetry and AR (1) had equivalent AIC weights (Table 3), but AR (1) was more likely to capture interannual variation than compound symmetry as it assumes samples taken closer in time are more tightly correlated than samples farther separated in time. Because all taxonomic groups were included in total macrofaunal density and biomass, the AR (1) correlation structure was also applied to group-level density and biomass responses.

### Oyster Population Response

#### *River*

Oyster density varied significantly between rivers, with densities in GWR double those in PR (Table 2, Fig. 9). Higher oyster density in GWR reflected trends in spatfall on weekly shellstring deployments (Fig. 5). In contrast, oyster biomass did not differ significantly between rivers (Table 2, Fig. 9). GWR had a greater density of oysters, but an equivalent biomass, because oysters in PR were larger; mean shell height was 30.9 mm in PR and 25.8 mm in GWR (Fig. 6-7).

#### *Season*

Seasonal trends in oyster density and biomass reflected annual cycles of recruitment and mortality. Oyster density was highest in fall, following recruitment, which was also evident in the shift of size frequencies toward smaller individuals (Table 2, Fig. 6-9). Biomass was significantly lower in the fall when the population was composed primarily of small juvenile oysters (Table 2, Fig. 9).

#### *Reef Orientation*

Neither oyster density nor biomass was affected by reef orientation (Table 2). In PR, there was a trend toward higher density and biomass on parallel reefs relative to

circular and perpendicular reefs, which was only true for oyster biomass in GWR. Oyster density in GWR was highest on circular reefs and lowest on perpendicular reefs (Table 2).

### Macrofaunal Community Response

#### *River*

In total, 21 species representing 20 genera in 5 taxonomic classes were observed on experimental reefs (Table 4). Both the density and biomass of macrofauna were dominated by a few abundant species. Hooked mussel (*I. recurvum*), the flatback mud crab (*E. depressus*), a polychaete worm (*A. succinea*), and the naked goby (*G. bosc*) contributed most (> 90%) to community similarity across both rivers (SIMPER, Table 5, Appendix A). Overall, diversity was higher in PR than in GWR, with an average of 1 additional species per sample relative to GWR (Fig. 10). Species evenness was also greater in PR (Fig. 10), due to the overwhelming dominance of a single species of mussel in GWR (Table 6).

Macrofaunal density was significantly higher in GWR than PR; biomass was marginally higher in GWR (Table 3, Fig. 11). The difference in macrofaunal density between rivers was driven primarily by high mussel (*I. recurvum*) density in GWR (Table 5, Fig. 12A). Xanthid crab and resident fish densities were higher in PR, whereas polychaete densities did not differ between rivers (Fig. 12, Appendix B). Macrofaunal biomass across taxonomic groups followed similar trends, with higher mussel biomass in GWR, higher crab and fish biomass in PR, and similar polychaete biomass in both rivers (Appendix B).

### *Season*

Macrofaunal diversity varied significantly across seasons, with higher diversity in spring (Table 6). These trends were driven by changes in species evenness rather than richness, which remained at moderate levels (Fig. 10). Macrofaunal density did not change seasonally (Table 3), which mirrored the lack of a seasonal trend in the most abundant group, mussels (Appendix B). Fish density and biomass were also not significantly different across seasons, though there was a trend toward higher biomass in spring relative to fall due to the dominance of small individuals in fall following mid-summer recruitment (Breitburg 1999). Mud crab density and biomass were significantly higher in fall, whereas polychaete density and biomass were significantly higher in spring (Appendix B). In fall, the species with > 90% species contribution were *I. recurvum*, *E. depressus*, and *G. bosc*; in spring, *G. bosc* was replaced by polychaetes in dominance (Table 5, Appendix A).

### *Reef Orientation*

Effects of reef orientation were not evident for macrofaunal diversity or density at the community or taxonomic group level (Table 3, Fig. 14-15). However, macrofaunal biomass varied significantly by reef orientation (Fig. 14), both at the community level and at the taxonomic group level. Across both rivers, biomass was significantly higher on perpendicular reefs than on parallel and circular reefs (Fig. 14). Mussel, mud crab, and fish biomass followed the pattern of total macrofaunal biomass with significantly higher biomass on perpendicular reefs, whereas polychaete biomass did not vary with reef orientation (Fig. 16, Appendix B).

## *Oyster Biomass*

Oyster biomass was fit to macrofaunal response variables by a thin plate regression spline smoother within the generalized additive model. Both macrofaunal density and biomass had significant positive relationships with oyster biomass (Fig. 17). Macrofaunal biomass approximately doubled for every doubling of oyster biomass, and the relationship was nearly linear (Fig. 17B), whereas macrofaunal density increased asymptotically with oyster biomass (Fig. 17A).

Taxonomic-level relationships were more complex. Both mussel and mud crab densities were positively related to oyster biomass (Fig. 18 A-B, Appendix B), whereas polychaete density was strongly nonlinear and dome-shaped with maximum density at intermediate oyster biomass (Fig. 18-C). Fish density was not significantly related to oyster biomass (Fig. 18-D, Appendix B).

Macrofaunal biomass across taxonomic groups had similar patterns as density. Both mussel and mud crab biomass increased monotonically with increasing oyster biomass (Fig. 19 A-B, Appendix B). Polychaete biomass was maximal at intermediate oyster biomass, and fish biomass had a weak positive relationship with oyster biomass (Fig. 19 C-D, Appendix B).

## **DISCUSSION**

This long-term field experiment revealed important processes shaping oyster reef communities in Chesapeake Bay, and provides empirical evidence to evaluate hypotheses of reef-building mechanisms. The key findings of this study are as follows: (1) restoration location is an important determinant of reef community development as environmental conditions and local dispersal patterns will influence the larval pool

available to settle newly-restored habitat, (2) seasonal variation in oyster reef community structure necessitates repeated sampling of reef communities over time, (3) reef orientation does not affect oyster population structure or settlement of reef species, but does significantly impact the secondary production of reef communities, (4) reef orientation produces significant changes in reef area over time that determine the productivity and persistence of reefs, and (5) macrofaunal production is significantly related to oyster production.

#### Location (River) Effects

Restoration location has a significant influence on the establishment of oyster populations (Grabowski et al. 2005, Gregalis et al. 2009), and much emphasis has been placed on restoration siting criteria (Powell et al. 1995, Smith et al. 2003, Woods et al. 2004). River-specific differences were the dominant driver of the density and diversity of reef fauna, but was only marginally significant in its effect on biomass. Reef orientation and oyster biomass were the significant drivers of trends in macrofaunal biomass. Generally, external factors, such as local circulation patterns, broodstock availability, and environmental conditions, influence the larval pool available to newly established reef habitat. The density and diversity of organisms will depend on the delivery of those larvae to the reef as well as post-settlement mortality and interspecific interactions. Once the reef community is established, however, reef-specific characteristics like orientation and oyster biomass seem to exert a stronger influence.

River-specific and interannual differences in larval supply were the main drivers of oyster population variation in this study. Shellstring surveys indicated that larval supply in GWR was 5-6 fold higher than PR in both years. Spatfall in 2012 was



markedly higher than in 2011, and the temporal patterns in recruitment varied. In 2011, two recruitment peak signals were evident in both GWR and PR with low levels of recruitment present in intervening periods. In 2012, a single recruitment event was present with little to no recruitment evident in following the peak, though the spatfall from this single event exceeded the cumulative spatfall for the 2011 season. A sharp increase in temperature (2.8 °C), the largest weekly increase over the spawning season, two weeks prior to the 2012 recruitment peak may have induced a synchronous spawning event leading to high recruitment peaks two weeks later (Thompson et al. 1996). Patterns in oyster recruitment reflected long-term observations for GWR, that populations are maintained by episodic high recruitment events that support population persistence under levels of high mortality (0.62-0.88%; Southworth et al. 2010). Oyster densities on experiments reefs over 4 sampling periods reflected both interannual and system-specific differences in larval supply observed on shellstrings.

#### Reef Orientation

Macrofaunal density and diversity did not differ between reef types likely because all reefs were exposed to the same larval pool due to their close proximity within rivers. Differences in diversity were minimal, but significant. On average, PR supported 1 additional species than GWR; however, species found only in PR were rare, and lack of strong environmental gradients in salinity across rivers suggests this may be a sampling effect. Macrofaunal density was significantly different across rivers, but was driven primarily by the overwhelming abundance of hooked mussels in the GWR.

Across both river systems, higher biomass was associated with perpendicular reefs and increasing oyster biomass for all macrofaunal responses examined, with the

exception of polychaetes. Perpendicular reefs have their shortest axis in the direction of tidal flow; therefore, velocity over these reefs will increase due to constriction of the water column as it passes over the reef crest. Turbulent eddies that slow flow will be minimized relative to circular or parallel reefs because of the short area over which currents interact with the reef. Higher flow rates lead to higher survival and growth of oysters (Kennedy and Sanford 1999, Lenihan 1999), but not necessarily settlement as larvae may pass over the reef too quickly to settle successfully (Fuchs and Reidenbach 2013). This is reflected in our observations of macrofaunal density and biomass; density was not enhanced by any particular reef orientation, but biomass was significantly higher on perpendicular reefs. This suggests that the processes controlling macrofaunal community establishment and production are somewhat decoupled. Large-scale processes such as larval dispersal control to the density of reef populations, but reef-scale processes control survival and growth once organisms have settled. In contrast, oyster density and biomass showed no reef-specific effect. For oysters, the placement of reefs in an area of good larval retention with adequate vertical reef seems to be more important than the shape or orientation of the reef itself.

Higher density in GWR reflected trends in larval supply, whereas biomass was more heavily influenced by season. That GWR had higher density but equivalent biomass to PR suggests that density-dependent competition for food or space is limiting growth in GWR.

Interactions of nascent oyster reefs with flow as the mechanism for lateral reef growth and accretion has been cited for many years (Grave 1905). Our results indicate that oyster density and biomass do not exhibit a strong response to reef orientation, but

that orientation effects on physical reef characteristics can modulate reef productivity by changing the reef footprint over time. Circular reefs were significantly smaller in area than their original size by the end of the study, whereas parallel and perpendicular reefs remained nearly the same or increased slightly across all study sites. That the mean height between the reefs was not significantly different suggests that differences in reef area were not due to differences in spreading rates or subsidence between reef types. Rather, the mechanism was likely the burial of circular reefs at the margin, which would result in smaller reef footprint without a concomitant loss of height. Because of the larger diameter of circular reefs relative to rectangular reefs, the slope from the reef plateau to the reef edge was inherently less steep. More gently sloping sides may make these reefs more susceptible to sediment deposition causing burial and reef loss at the perimeter. Although oyster density and biomass and macrofaunal density did not differ by reef type, the changes in reef area did affect overall reef productivity. Reefs used in this study were small (5 m<sup>2</sup>), but changes in reef area and macrofaunal biomass suggest that reefs altered flow in a manner that led to similar area and biomass across multiple locations within a river system and across study systems. Thus, the mechanisms described by Grave (1905) are qualitatively supported by our results, but were not consistent across all variables examined.

#### Season

Strong seasonal signals for both oysters and macrofauna indicate that a single snapshot of the reef community may not be representative, and that multiple samples should be taken to understand the structure and function of the community. Oyster density and biomass reflected seasonal changes in the population due to recruitment,

growth and mortality. High densities of oysters in fall from seasonal recruitment were not evident in spring, likely due to predation and overwintering mortality. Higher biomass in spring reflected growth throughout late fall and early spring and the proportionally greater loss of smaller individuals due to mortality. Similarly, macrofaunal diversity reflected seasonal changes in the dominance of certain species, particularly naked gobies and clam worms. The clam worm, *A. succinea*, was the dominant polychaete in our samples, and has a semelparous life history. Adult worms die shortly after spawning in summer; therefore, the strong seasonal effect was reflective of the life cycle (Pardo and Dauer 2003). Community composition and the ecosystem services carried out by various reef species is likely to vary seasonally, and should be taken into consideration in restoration monitoring.

#### Macrofaunal Community Response

The enhancement of nekton and benthic macrofaunal abundance by oyster reefs is well documented (Peterson et al. 2003, Rodney and Paynter 2006, Kellogg et al. 2013), but the role of oysters in this enhancement is unclear (Tolley and Volety 2005). While some studies have shown positive correlations between oyster abundance and size structure and community metrics (Luckenbach et al. 2005, Boudreaux et al. 2006, Hadley et al. 2010), others indicate that there is no functional difference between live oysters and clustered, dead shell (Tolley and Volety 2005).

Our results indicate positive relationships between total macrofaunal density and biomass with oyster biomass. These relationships were reflected in group-level responses for mussels and crabs. The positive association of mussels and oysters has been documented previously (Hadley et al. 2010), and likely represents a facilitative relationship in which

surface complexity created by living oysters produces low flow areas conducive to settlement (Soniati et al. 2005). Mud crabs rely on oyster habitat both for refuge from predation as well as on oyster spat as a prey resource (MacDonald 1982); therefore, their positive correlation is not unexpected. Fish density and biomass were not strongly correlated with oyster biomass, which is likely a reflection of the oyster metric used. Resident fish species, such as gobies, blennies, and skillettfish, rely on oyster reefs as refuge from predation by larger transient fish, such as striped bass, and as foraging grounds where they feed on a variety of benthic invertebrates and oyster larvae (Breitburg 1999, D'Aguillo et al. 2014). These species also use disarticulated shells to lay their eggs (D'Aguillo et al. 2014). Oyster biomass does not account for refuges or nest sites provided by dead shell, which may explain the lack of a relationship between fish and oysters in this analysis.

The relationship between *Alitta succinea* density and biomass and oyster biomass was dome-shaped, with maximum worm density or biomass at intermediate oyster biomass. *Alitta succinea* density is enhanced on oyster reefs relative to soft-bottom habitat (Pardo and Dauer 2003), but our study indicated that the role of oysters in this enhancement is not straightforward. *Alitta succinea* is predominantly a deposit feeder (Pardo and Dauer 2003), but it can also feed heavily on oyster larvae (Barnes et al. 2010). Oysters may also provide structural refuge for worms that are often fed on by reef fishes (D'Aguillo et al. 2014). The increase in polychaete density and biomass at low oyster biomass may have resulted from the enrichment of reef sediments by oyster biodeposition, which enhances the worms' microbial food source, or via increasing complexity, which provides refuge from predation. The decline in polychaete density and biomass at high

oyster biomass may have resulted from hypoxia due to high oxygen demand within the reef matrix at high levels of biodeposition.

## CONCLUSIONS

In recent years, focus in the restoration community has shifted from restoring single species to reestablishing ecosystem services and functioning. For oyster reefs, one of the most important functions is the provision of habitat for benthic species. In turn, resident species can further enhance ecosystem functioning; for example, mussels increase benthic-pelagic coupling and denitrification through filtration and biodeposition (Kellogg et al. 2013, Gedan et al. 2014). Secondary production provides the trophic linkage from the benthos to the pelagic zone, supporting commercial fisheries. This study shows that projects aiming to maximize oyster biomass will enhance reef secondary production through positive relationships between macrofaunal biomass with oyster biomass. Secondary production is also improved by perpendicular reef orientation, which promotes high flow rates over the reef. Hence, reef structure is a critical factor for ecological functioning even in the absence of oyster population enhancement. Reef persistence is of utmost importance for long-term viability of restoration efforts; therefore, reef designs should avoid patch or circular reefs with gently sloping sides that are prone to area loss. The existence of similar reef geometries across several estuarine systems suggests that restoration efforts outside Chesapeake Bay may be improved by similar reef geometries, but further investigation is necessary to confirm this conclusion. Ecological restoration of oyster reefs is a complex process, but may be improved with careful consideration of restoration location and reef design.

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## LITERATURE CITED

- Anderson, D.R. 2008. Model based inference in the life sciences: a primer on evidence. Springer Science and Business Media, New York, NY, USA.
- Andrews, J. D. 1979. Pelecypoda: Ostreidae. Pages 293-341 in Giese, A. C. and J. S. Pearse, editors. Reproduction of marine invertebrates. Academic Press, New York, NY, USA.
- Barnes, B.B., M.W. Luckenbach, and P.R. Kingsley-Smith. 2010. Oyster reef community interaction: the effect of resident fauna on oyster (*Crassostrea spp.*) larval recruitment. *Journal of Experimental Marine Biology and Ecology* 391: 169-177.
- Beck, M.W. et al. 2011. Oyster reefs at risk and recommendations for conservation, restoration and management. *Bioscience* 61: 107-116.
- Boudreaux, M.L., J.L. Stiner, and L.J. Walters. 2006. Biodiversity of sessile and motile macrofauna on intertidal oyster reefs in Mosquito Lagoon, Florida. *Journal of Shellfish Research* 25: 1079-1089.
- Breitburg, D.L. 1999. Are three-dimensional structure and healthy oyster populations the keys to an ecologically interesting and important fish community? in Luckenbach, M.W., R. Mann, and J.A. Wesson, editors. Oyster reef habitat restoration: a synopsis and synthesis of approaches. VIMS Press, Gloucester Point, VA, USA.
- Chen, H.S., P.V. Hyer, A.Y. Kuo, and C.S. Fang. 1977. Hydrography and hydrodynamics of Virginia estuaries XI: Mathematical model studies of water quality of the Piankatank estuary. Special Report No. 124 in Applied Marine Science and Ocean Engineering, Virginia Institute of Marine Science, Gloucester Point, VA, USA.
- Clarke, K.R. 1993. Non-parametric multivariate analyses of changes in community



- structure. *Australian Journal of Ecology* 18: 117-143.
- Clarke, K.R., and R.N. Gorley, RN. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, UK.
- Coen, L.D., and R.E. Grizzle. 2007. The importance of habitat created by molluscan shellfish to managed species along the Atlantic coasts of the United States. ASMFC Habitat Management Series #8, Atlantic States Marine Fisheries Commission, Washington, DC. 115 pp.
- D'Aguillo, M.C., A.S. Harold, and T.L. Darden. 2014. Diet composition and feeding ecology of the naked goby *Gobiosoma bosc* (Gobiidae) from four western Atlantic estuaries. *Journal of Fish Biology* 85: 355-373.
- Fuchs, H.L., and M.A. Reidenbach. 2013. Biophysical constraints on optimal patch lengths for settlement of a reef-building bivalve. *PLoS ONE* 8: 1-11.
- Gedan, K.B., L. Kellogg, and D.L. Breitburg. 2014. Accounting for multiple foundation species in oyster reef restoration benefits. *Restoration Ecology* 22: 1-8.
- Grabowski, J.H., A.R. Hughes, D.L. Kimbro, and M.A. Dolan. 2005. How habitat setting influences restored oyster reef communities. *Ecology* 86: 1926-1935.
- Grave, C. 1905. Investigation for the promotion of the oyster industry of North Carolina. Pages 247-341 in U.S. Fish Commission Report for 1903, U.S. Commission for Fish and Fisheries.
- Gregalis, K.C., M.W. Johnson, and S.P. Powers. 2009. Restored oyster reef location and design affect responses of resident and transient fish, crab, and shellfish species in Mobile Bay, Alabama. *Transactions of the American Fisheries Society* 138: 314-327.

- Grizzle, R.E., J.K. Greene, and L.D. Coen. 2008. Seston removal by natural and constructed intertidal oyster (*Crassostrea virginica*) reefs: a comparison with previous laboratory studies, and the value of *in situ* methods. *Estuaries and Coasts* 31: 1208-1220.
- Hadley, N.H., M. Hodges, D.H. Wilber, and L.D. Coen. 2010. Evaluating intertidal oyster reef development in South Carolina using associated faunal indicators. *Restoration Ecology* 18: 691-701.
- Harding, J.M., R. Mann, M.J. Southworth, and J.A. Wesson. 2010. Management of the Piankatank River, Virginia, in support of oyster (*Crassostrea virginica* Gmelin 1791) fishery repletion. *Journal of Shellfish Research* 29: 867-888.
- Hargis, W.H., and D.S. Haven. 1988. Rehabilitation of the troubled oyster industry of the lower Chesapeake Bay. *Journal of Shellfish Research* 7: 271-279.
- Haven, D.S., and J.P. Whitcomb. 1983. The origin and extent of oyster reefs in the James River, Virginia. *Journal of Shellfish Research* 32: 141-151.
- Kellogg, M.L., J.C. Cornell, M.S. Owens, and K.T. Paynter. 2013. Denitrification and nutrient assimilation on a restored oyster reef. *Marine Ecology Progress Series* 480: 1-19.
- 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. Lesson learned from efforts to restore oyster populations in Maryland and Virginia, 1990 to 2007. *Journal of Shellfish Research* 30: 719-731.
- Kennedy, V.S. and L.P. Sanford. 1999. The morphology and physical oceanography of unexploited oyster reefs in North America. Pages 25-46 *in* Luckenbach, M.W., R.

- Mann, and J.A. Wesson, editors. Oyster reef habitat restoration: A synopsis and synthesis of approaches. Virginia Institute of Marine Science Press, Gloucester Point, VA, USA.
- 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* 101:13096-13099.
- Lenihan, H.S. 1999. Physical-biological coupling on oyster reefs: how habitat structure influences individual performance. *Ecological Monographs* 69: 251-275.
- Lewis, A.J., and H.C. Macdonald. 1972. Mapping of mangrove and perpendicular-oriented shell reefs in southeastern Panama with side-looking radar. *Photogrammetria* 28:187-199.
- Littell, R.C., G.A. Milliken, W.W. Stroup, R.D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models, Second edition. SAS Institute, Cary, NC, USA.
- Lotze, H.K. et al. 2006 Depletion, degradation and recovery potential of estuaries and coastal seas. *Science* 312: 1806-1809.
- 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* 40: 64-78.
- Mackenzie, C.L. 2007. Causes underlying the historical decline in eastern oyster (*Crassostrea virginica* Gmelin, 1791) landings. *Journal of Shellfish Research* 26: 927-938.

- McDonald, J. 1982. Divergent life history patterns in the co-occurring intertidal crabs *Panopeus herbstii* and *Eurypanopeus depressus* (Crustacea: Brachyura: Xanthidae). *Marine Ecology Progress Series* 8: 173-180.
- Meyer, D.L, E.C. Townsend, and G.W. Thayer. 1997. Stabilization and erosion control value of oyster cultch for intertidal marsh. *Restoration Ecology* 5: 93-99.
- Norris, R.M. 1953. Buried oyster reefs in some Texas bays. *Journal of Paleontology* 274: 569-576.
- Pardo, E.V. and D.M. Dauer. 2003. Particle size selection in individual from epifaunal versus infaunal populations of the nereidid polychaete *Neanthes succinea* (Polychaeta: Nereididae). *Hydrobiologia* 496: 355-360.
- Peterson, C.H., J.H. Grabowski, and S.P. Powers. 2003. Estimated enhancement of fish production resulting from restoring oyster reef habitats: quantitative valuation. *Marine Ecology Progress Series* 264: 249-264.
- Porter, E.T., J.C. Cornwell, and L.P. Sanford. 2004. Effect of oysters *Crassostrea virginica* and bottom shear velocity on benthic-pelagic coupling and estuarine water quality. *Marine Ecology Progress Series* 271: 61-75.
- Price, W.A., 1954. Oyster reefs of the Gulf of Mexico. *in Gulf of Mexico: Its Origin, Waters, and Marine Life*. Pages 39-65 *in* Galtsoff, P. editor. U.S. Fish and Wildlife Service Fishery Bulletin No. 89.
- Powell, E.N., J. Song, M.S. Ellis, and E.A. Wilson-Ormond. 1995. The status and long-term trends of oyster reefs in Galveston Bay, Texas. *Journal of Shellfish Research* 142: 439-457.
- R Core Team. 2014. R: A language and environment for statistical computing. R

Foundation for Statistical Computing, Vienna, Austria.

- 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: 39-51.
- Rothschild, B.J., J.S. Ault, P. Gouilletquer, and M. Héral. 1994. Declines of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. *Marine Ecology Progress Series* 111: 29-39.
- Schulte, D.M., R.P. Burke, and R.N. Lipcius. 2009. Unprecedented restoration of a native oyster metapopulation. *Science* 325: 1124-1128.
- Smith, G.F., E.B. Roach, and D.G. Bruce. 2003. The location, composition, and origin of oyster bars in mesohaline Chesapeake Bay. *Estuarine, Coastal and Shelf Science* 56: 391-409.
- Soniat, T.M., C.M. Finelli, and J.T. Ruiz. 2004. Vertical structure and predator refuge mediate oyster reef development and community dynamics. *Journal of Experimental Marine Biology and Ecology* 310:163-182.
- Southworth, M. and R. Mann. 2014. The status of Virginia's public oyster resource, 2013. Molluscan Ecology Program, Virginia Institute of Marine Science, Gloucester Point, Virginia. 48 pp.
- Southworth, M., J.M. Harding, J.A. Wesson, and R. Mann. 2010. Oyster *Crassostrea virginica*, Gmelin 1791 population dynamics on public reefs in the Great Wicomico River, Virginia, USA. *Journal of Shellfish Research* 292: 271-290.
- Thompson, R.J., R.I.E. Newell, V.S. Kennedy, and R. Mann. 1996. Reproductive processes and early development. Pages 335-370 in Kennedy, V.S., R.I.E. Newell,

- and A.F. Eble, editors. The eastern oyster: *Crassostrea virginica*. Maryland Sea Grant College, College Park, MD, USA.
- Tolley, S.G., and Volety, A.K. 2005. The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. *Journal of Shellfish Research* 24: 1007-1012.
- Underwood, A. J. 2002. *Experiments in Ecology: Their logical design and interpretation using analysis of variance*. Cambridge University Press, New York, NY, USA.
- Wesson, J.A., R. Mann, and M.W. Luckenbach. 1999. Oyster restoration efforts in Virginia. Pages 117-129 *in* Luckenbach, M.W., R. Mann, and J.A. Wesson, editors. *Oyster reef habitat restoration: A synopsis and synthesis of approaches*. Virginia Institute of Marine Science Press, Gloucester Point, VA, USA.
- Wilberg, M.J., M.E. Livings, J.S. Barkman, B.T. Morris, and J.M. Robinson. 2011. Overfishing, disease, habitat loss and potential extirpation of oysters in upper Chesapeake Bay. *Marine Ecology Progress Series* 436: 131-144.
- Wildish, D.J., and G.B.J. Fader. 1998. Pelagic-benthic coupling in the Bay of Fundy. *Hydrobiologia* 375/376: 369-380.
- Wood, S.N. 2006. *Generalized Additive Models: An Introduction with R*. Chapman and Hall/CRC, Boca Raton, FL, USA.
- Wood, S.N. 2003. Thin-plate regression splines. *Journal of the Royal Statistical Society* 65:95-114.
- Woods, H., W.J. Hargis, Jr., C.H. Hershner, and P. Mason. 2004. Improving siting and construction criteria for oyster reef restoration. *Special Report in Applied Marine Science and Ocean Engineering #387*, Virginia Institute of Marine Science,

Gloucester Point, VA, USA.

Woods, H., W.J. Hargis, C.H. Hershner, and P. Mason. 2005. Disappearance of the natural emergent 3-dimensional oyster reef systems of the James River, Virginia, 1871-1948. *Journal of Shellfish Research* 24: 139-142.

Zimmerman, R.J., T. Minello, T. Baumer, and M. Castiglione. 1989. Oyster reef as habitat for estuarine macrofauna. NOAA Technical Memorandum, NMFS-SEFC-249, 16 p.

Table 1. Linear regressions of oyster density as a function of oyster biomass; data were  $\log_{10}$ -transformed to meet statistical assumptions.

Season	Intercept		Slope		$r^2$	$P$
	<i>Estimate</i>	<i>SE</i>	<i>Estimate</i>	<i>SE</i>		
Fall 2011	-2.09	0.55	0.99	0.17	0.60	< 0.001
Spring 2012	0.52	0.41	0.32	0.15	0.15	< 0.05
Fall 2012	-0.26	0.37	0.74	0.16	0.48	< 0.001
Spring 2013	0.29	0.44	0.51	0.16	0.33	< 0.005



Table 2. Oyster response AIC model comparisons for the following candidate autocorrelation structures: CS=compound symmetry, AR (1) = autoregressive, ARMA (1,1) = autoregressive moving average order. Parameter estimates are relative to the reference condition of GWR circular reefs in fall. Both density and biomass were log-transformed prior to analysis.

<b>Oyster Density</b>			
<i>Correlation</i>	<i>AICc</i>	<i>ΔAICc</i>	<i>w</i>
CS	232.5	0.00	0.37
AR (1)	233.1	0.66	0.27
ARMA (1,1)	232.5	0.03	0.36
<i>Parameter</i>	<i>Estimate</i>	<i>SE</i>	<i>P-value</i>
Intercept	7.02	0.13	< 0.001
PR	-0.59	0.11	< 0.001
Spring	-1.00	0.15	< 0.001
Parallel	0.22	0.14	0.11
Perpendicular	0.05	0.14	0.72
<b>Oyster Biomass</b>			
<i>Correlation</i>	<i>AICc</i>	<i>ΔAICc</i>	<i>w</i>
CS	820.8	11.4	0.00
AR (1)	812.4	2.98	0.18
ARMA (1,1)	809.4	0.00	0.81
<i>Parameter</i>	<i>Estimate</i>	<i>SE</i>	<i>P-value</i>
Intercept	23.7	4.32	< 0.001
PR	-3.69	4.30	0.39
Spring	19.3	1.94	< 0.001
Parallel	4.25	5.25	0.42
Perpendicular	-1.56	5.25	0.77

Table 3. Macrofaunal density and biomass GAM model results. The global GAM model was fit with the following candidate autocorrelation structures: CS=compound symmetry, AR (1) = autoregressive, ARMA (1,1) = autoregressive moving average order 1. The selected correlation structure is indicated in italics. Parameter estimates are relative to the reference condition of GWR circular reefs in fall.

<b>Macrofaunal Density</b>			
<i>Correlation</i>	<i>AICc</i>	<i>ΔAIC</i>	<i>w</i>
CS	1473.2	0.00	0.36
<i>AR (1)</i>	1473.2	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	1473.7	0.48	0.28
<i>Parameter</i>	<i>Estimate</i>	<i>SE</i>	<i>P-value</i>
Parametric variables:			
Intercept	7.08	0.10	< 0.001
PR	-0.43	0.09	< 0.001
Spring	-0.02	0.10	0.88
Parallel	0.14	0.10	0.19
Perpendicular	0.15	0.10	0.16
Smoothed variable:			
Oyster biomass			< 0.001
<b>Macrofaunal Biomass</b>			
<i>Correlation</i>	<i>AICc</i>	<i>ΔAIC</i>	<i>w</i>
CS	863.6	0.00	0.36
<i>AR (1)</i>	<i>863.6</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	864.0	0.43	0.29
<i>Parameter</i>	<i>Estimate</i>	<i>SE</i>	<i>P-value</i>
Parametric variables:			
Intercept	3.58	0.19	< 0.001
PR	-0.36	0.18	0.05
Spring	-0.41	0.20	0.05
Parallel	0.01	0.21	0.97
Perpendicular	0.50	0.21	0.02
Smoothed variable:			
Oyster biomass			< 0.01

Table 4. Species encountered in macrofaunal community samples. \* Indicates species included in group-level macrofaunal analyses.

Scientific Name	Common Name	Locations Present	Seasons Present
<b>Bivalvia</b>			
<i>Ischadium recurvum</i> *	Hooked mussel	GWR, PR	Fall, Spring
<i>Macoma balthica</i>	Baltic clam	GWR, PR	Fall, Spring
<i>Macoma mitchelli</i>	Matagorda macoma	PR	Spring
<i>Mya arenaria</i>	Soft shell clam	GWR, PR	Fall, Spring
<i>Mytilus edulis</i> *	Blue mussel	GWR, PR	Fall, Spring
<i>Mytilopsis leucophaeta</i> *	Dark false mussel	PR	Fall
<b>Crustacea</b>			
<i>Alpheus heterochelitus</i>	Big-claw snapping shrimp	GWR	Fall
<i>Callinectes sapidus</i>	Blue crab	GWR, PR	Fall
<i>Dyspanopeus sayi</i> *	Even-clawed mud crab	GWR, PR	Fall, Spring
<i>Eurypanopeus depressus</i> *	Flatback mud crab	GWR, PR	Fall, Spring
<i>Palaemonetes spp.</i>	Grass shrimp	GWR, PR	Fall, Spring
<i>Panopeus herbstii</i> *	Black-fingered mud crab	GWR, PR	Fall, Spring
<i>Pinnotheres ostreum</i>	Pea crab	PR	Fall, Spring
<i>Rhithropanopeus harrisi</i> *	White-clawed mud crab	GWR, PR	Fall, Spring
<b>Gastropoda</b>			
<i>Boonea impressa</i>	Impressed odostome	PR	Spring
<b>Osteichthyes</b>			
<i>Anguilla rostrata</i>	American eel	GWR, PR	Fall, Spring
<i>Chasmodes bosquianus</i> *	Striped blenny	GWR, PR	Fall, Spring
<i>Gobisoma bosc</i> *	Naked goby	GWR, PR	Fall, Spring
<i>Gobiesox strumosus</i> *	Skilletfish	GWR, PR	Fall, Spring
<i>Opsanus tao</i> *	Oyster toadfish	GWR, PR	Spring
<b>Polychaeta</b>			
<i>Alitta succinea</i> *	Clam worm	GWR, PR	Fall, Spring

Table 5. SIMPER analysis results for macrofaunal density. The species contributing > 90% to community similarity within rivers and seasons are shown with respect to river and season.

<b>River</b>			
<i>GWR</i>		<i>PR</i>	
Species	Cumulative %	Species	Cumulative %
<i>I. recurvum</i>	57.6	<i>I. recurvum</i>	33.5
<i>E. depressus</i>	74.4	<i>E. depressus</i>	66.8
<i>A. succinea</i>	85.6	<i>A. succinea</i>	78.1
Unidentified xanthid crabs	93.8	Unidentified xanthid crabs	89.0
		<i>G. bosc</i>	94.3
<b>Season</b>			
<i>Fall</i>		<i>Spring</i>	
Species	Cumulative %	Species	Cumulative %
<i>I. recurvum</i>	49.1	<i>I. recurvum</i>	41.7
<i>E. depressus</i>	75.5	<i>E. depressus</i>	65.4
Unidentified xanthid crabs	85.6	<i>A. succinea</i>	85.2
<i>G. bosc</i>	90.4	Unidentified xanthid crabs	94.0

Table 6. Generalized additive model results for Shannon diversity. The AR (1) correlation structure was applied to diversity because it was the most appropriate structure for both macrofaunal density and biomass.

<b>Shannon Diversity</b>			
<i>Parameter</i>	<i>Estimate</i>	<i>SE</i>	<i>P-value</i>
Parametric variables:			
Intercept	0.57	0.03	< 0.001
PR	0.52	0.03	< 0.001
Spring	0.16	0.03	< 0.001
Parallel	-0.02	0.04	0.61
Perpendicular	0.03	0.04	0.38
Smoothed variable:			
Oyster biomass			<0.64

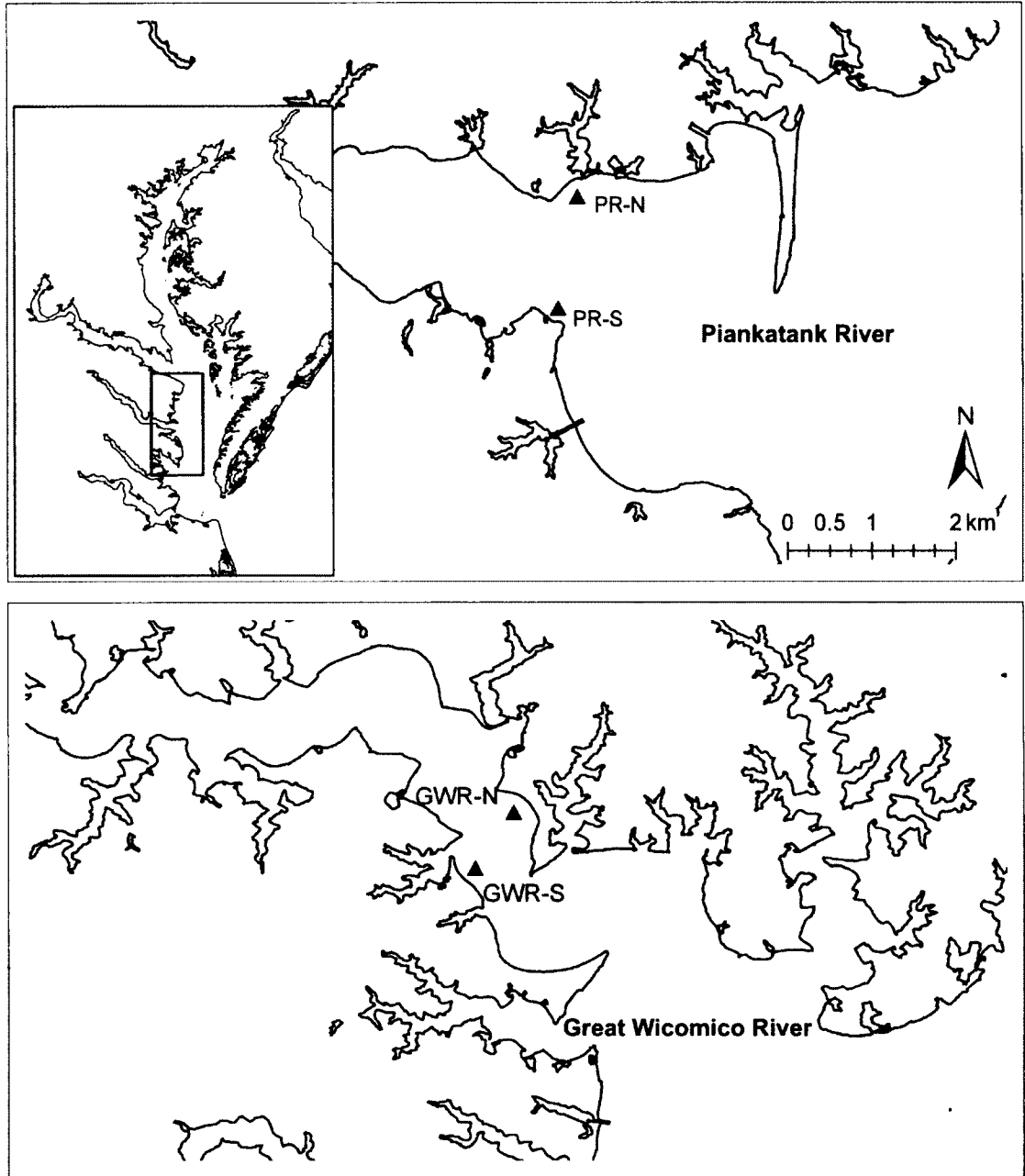


Fig. 1: Locations of the Piankatank and Great Wicomico Rivers on the western shore of Chesapeake Bay (inset). Individual study locations within each river are indicated by triangles. PR = Piankatank River, GWR = Great Wicomico River, N = North shore, S = South shore.

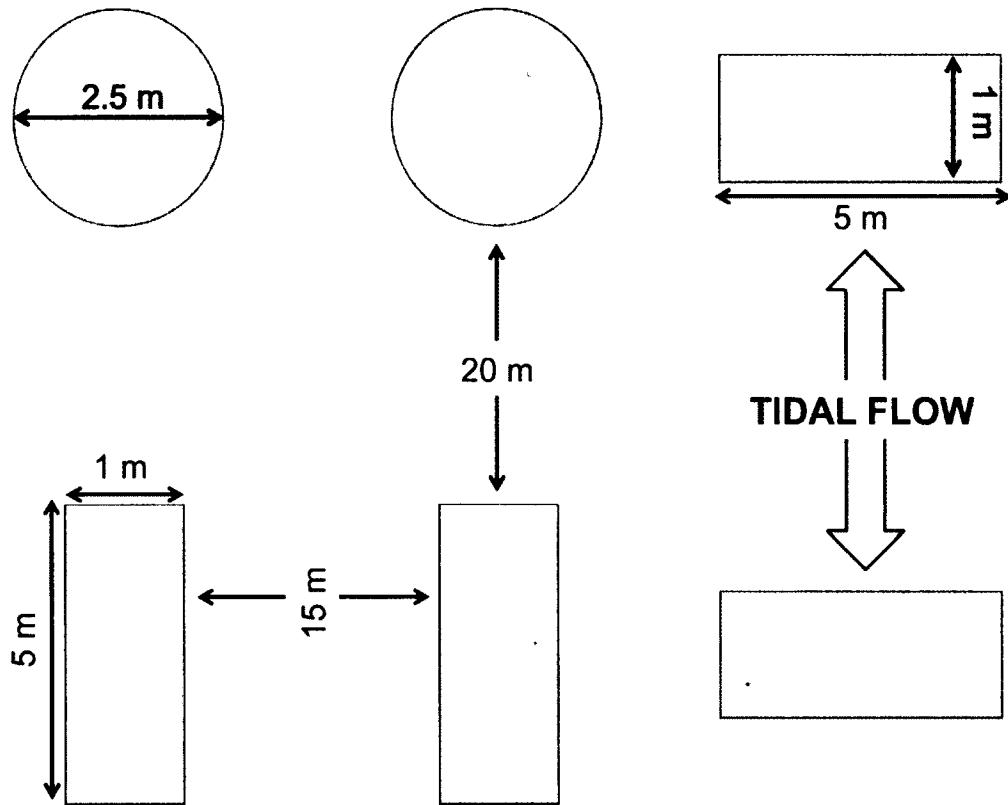


Fig. 2: Plan-view schematic of reef dimensions and placement at the Piankatank River-North shore site. Each site had 2 replicate reefs of each type. Assignment of reef types was chosen randomly for each site, so the arrangement varied across sites. Tidal flow arrow indicates the predominant tidal current direction along the channel to which the orientation treatments are relative.

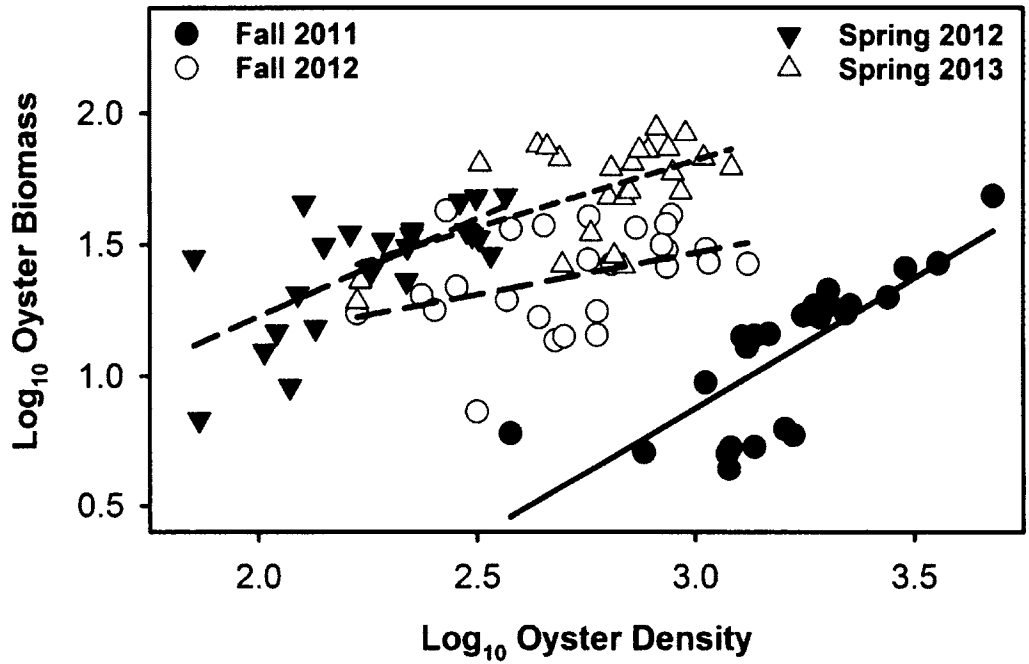


Fig. 3: Oyster density as a function of oyster biomass; data were  $\text{log}_{10}$ -transformed to meet statistical assumptions. Parameter estimates and statistics are in Table 1.



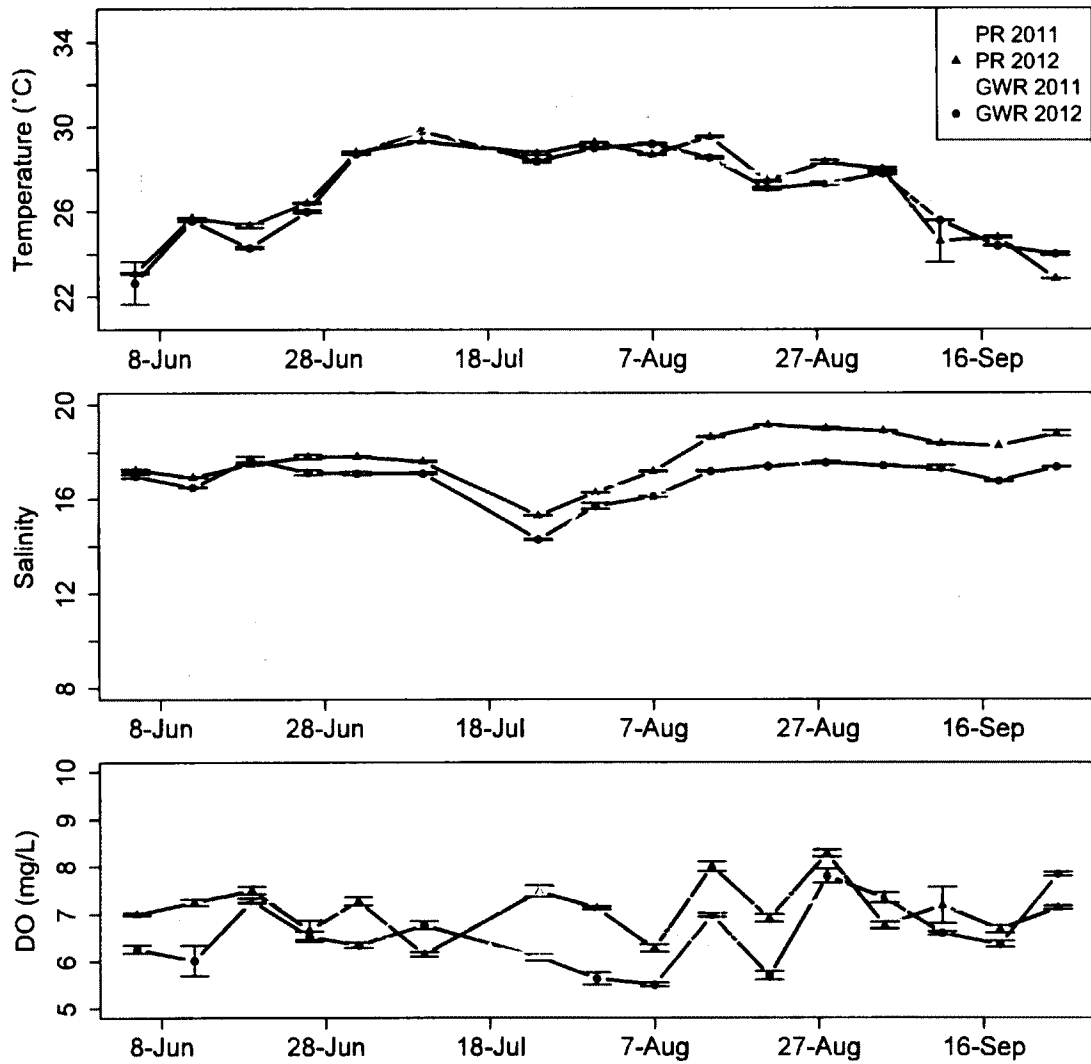


Fig. 4: Environmental conditions at study sites during 2011 and 2012. Points represent weekly means ( $\pm 1$  SE) in each river. Data are pooled over north and south shore sites within each river (n=20).

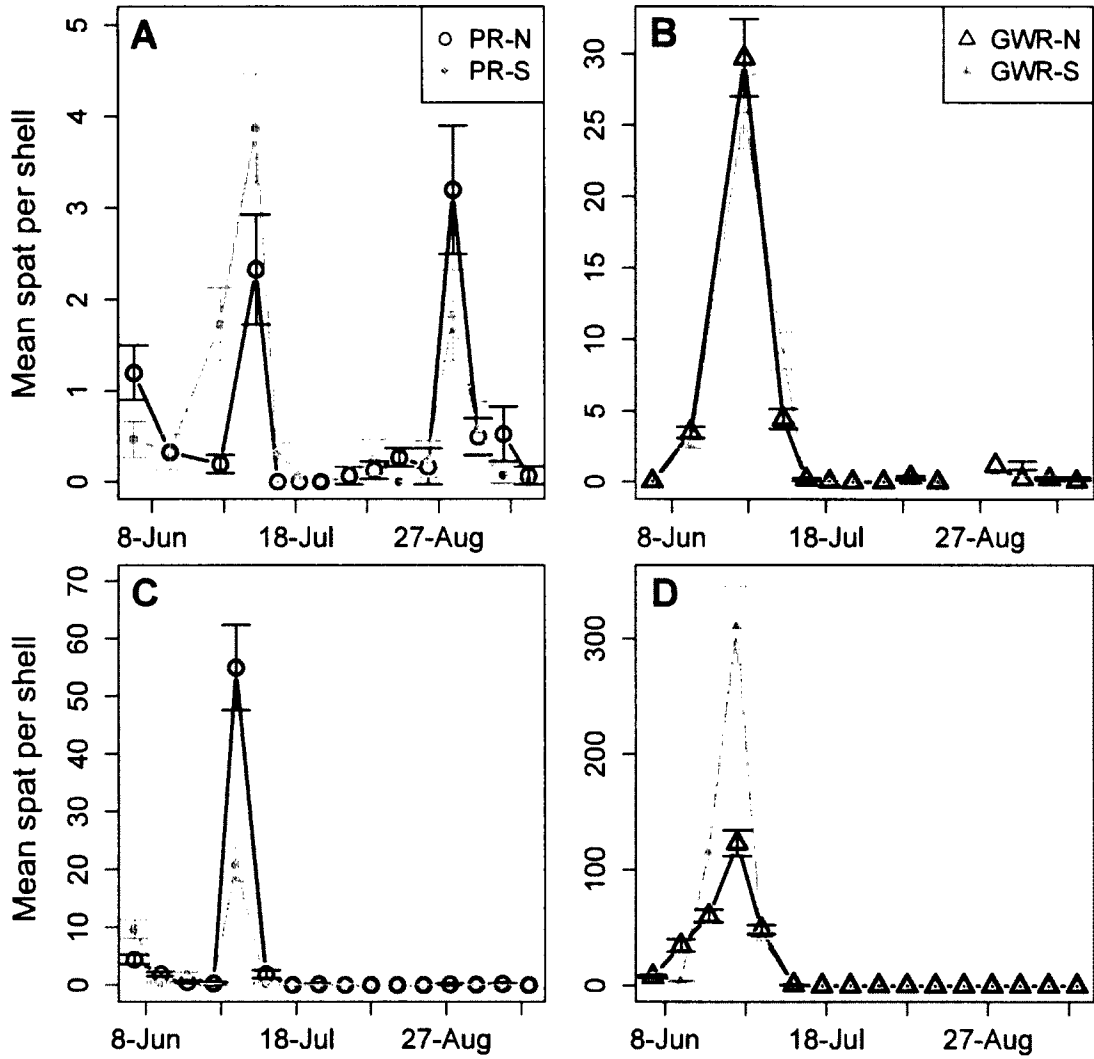


Fig. 5: Weekly spatfall from shellstring deployments for 2011 (A-B) and 2012 (C-D) in each river (PR: A, C; GWR: B, D). Points represent mean spat per shell  $\pm$  1 SE. North and south shore sites within each river are denoted by open and closed symbols. Note the difference in scale for the y-axis between the two years.

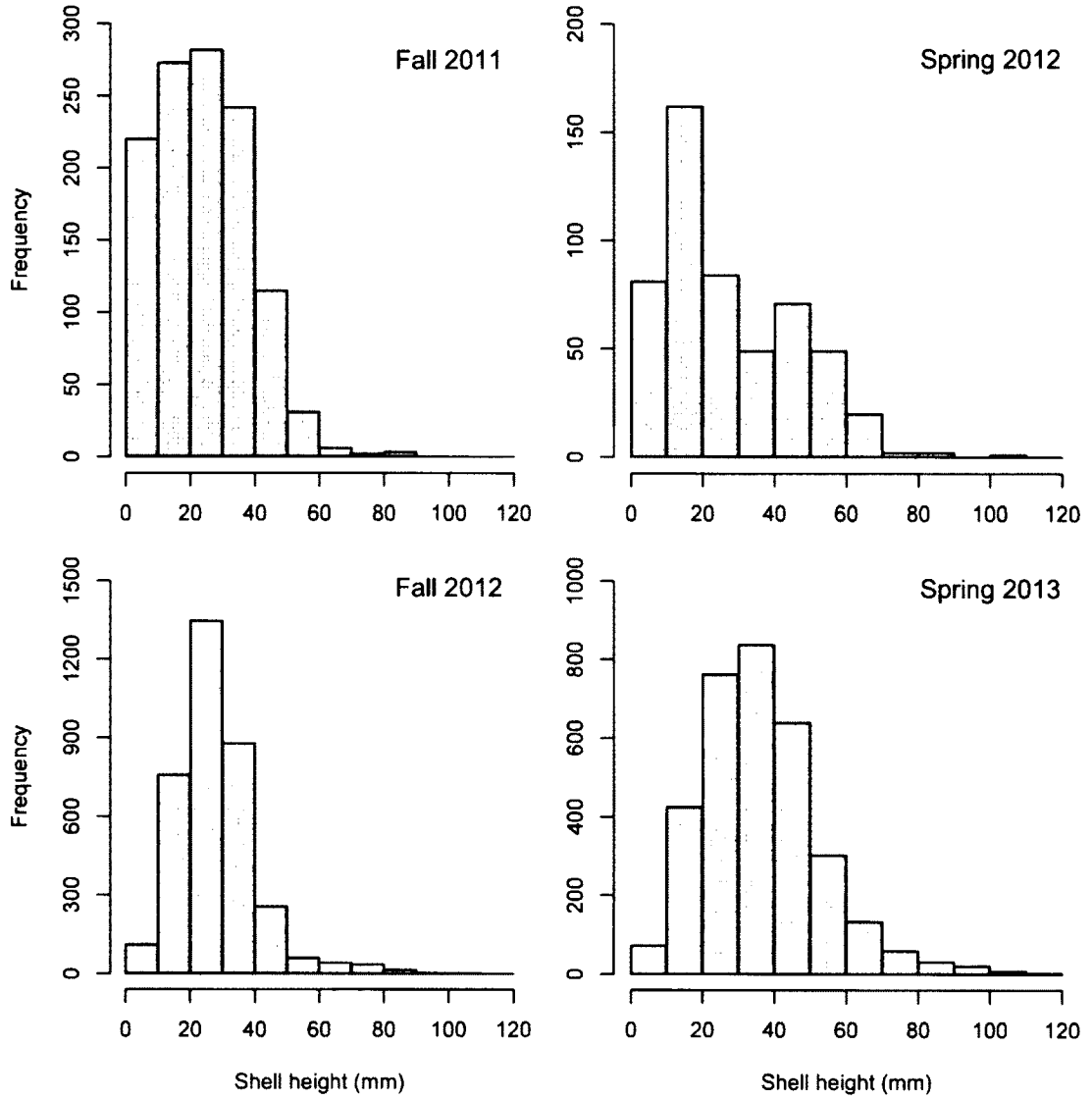


Fig. 6: Size frequencies of live oysters in the Piankatank River (PR) across sampling periods.

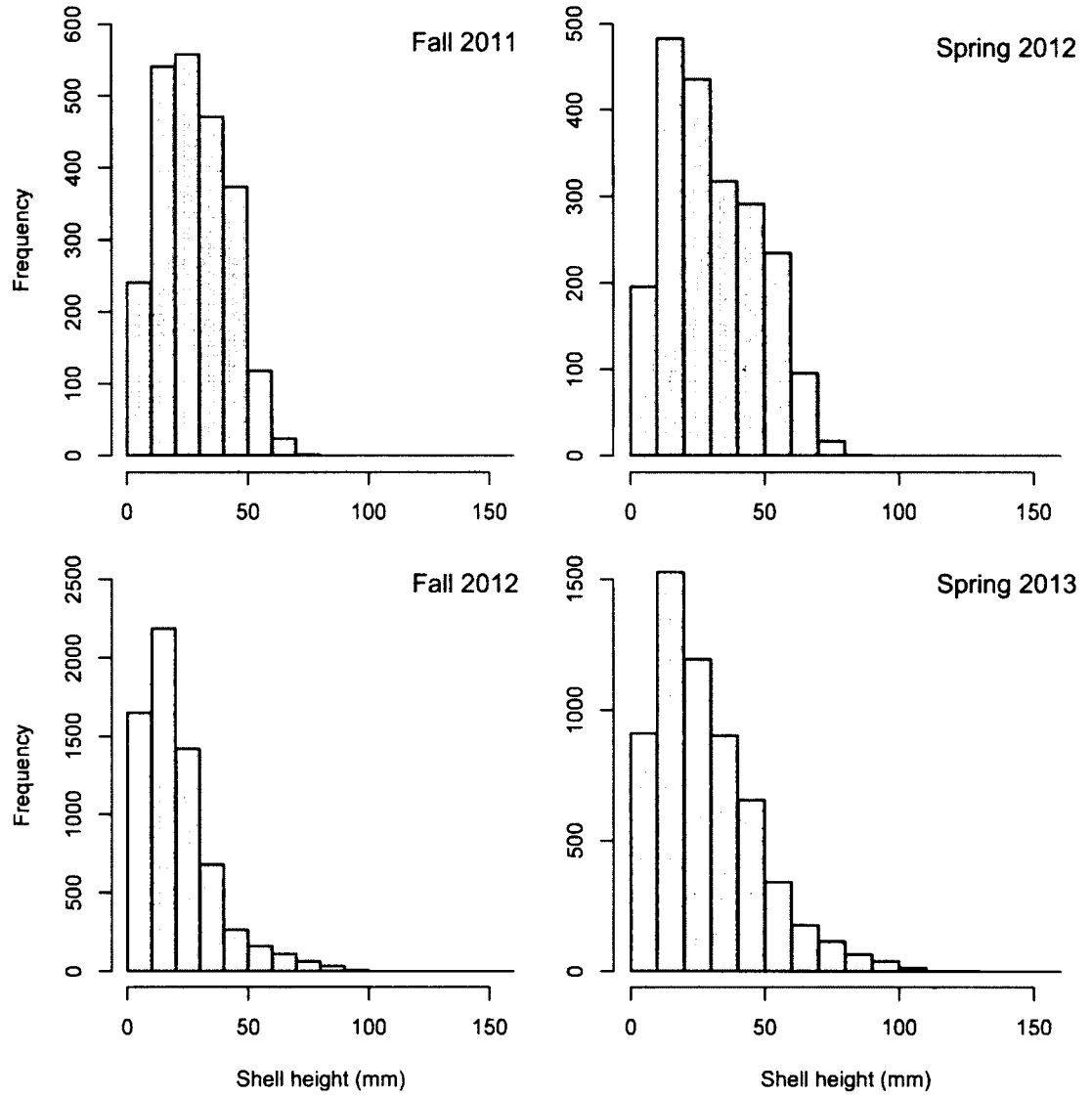


Fig. 7: Size frequencies of live oysters in the Great Wicomico River (GWR) across sampling periods.

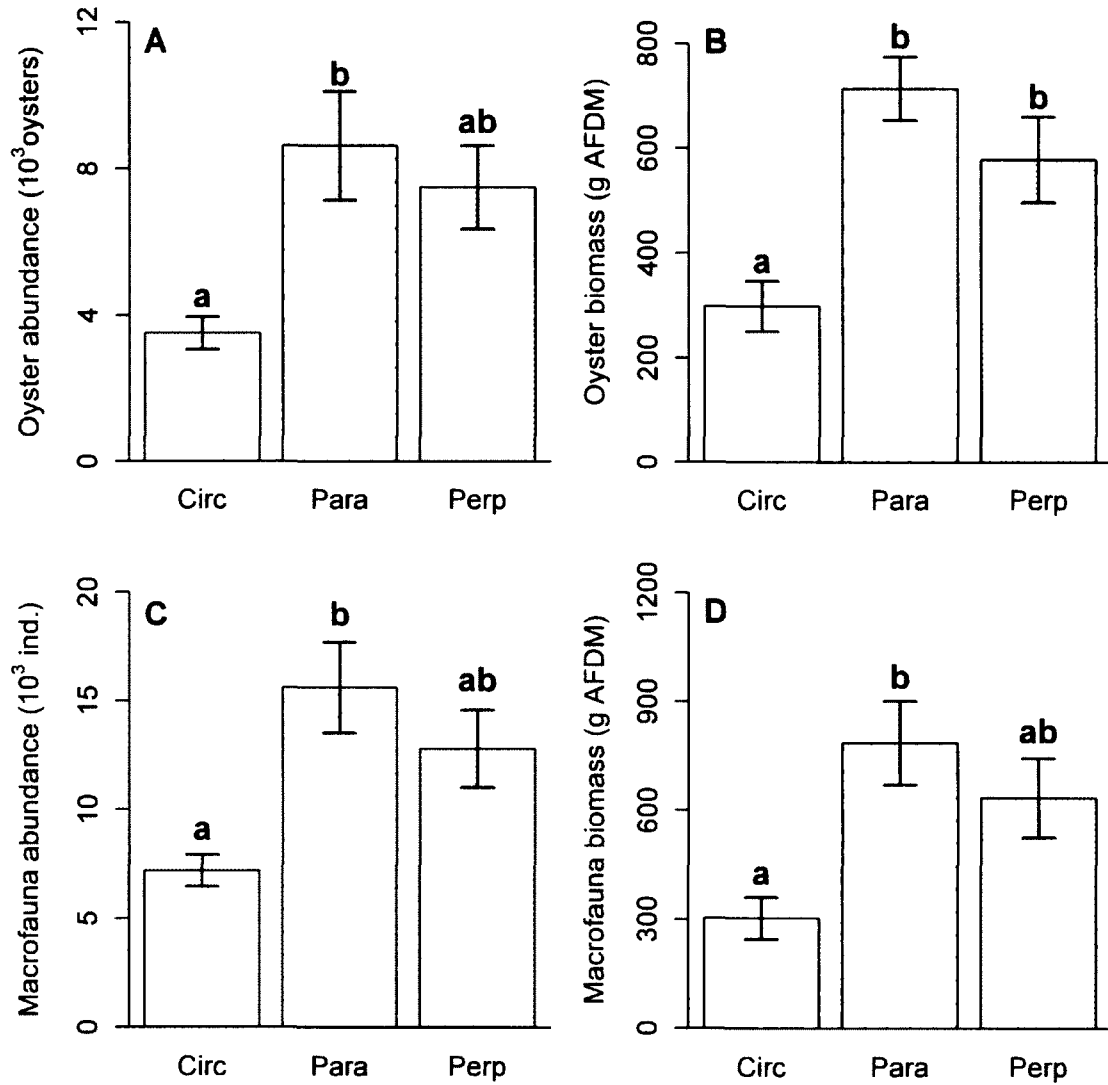


Fig. 8: Mean ( $\pm$  1 SE) oyster abundance (A), biomass (B), macrofaunal abundance (C), and biomass by reef type. Letters indicate significant differences (Tukey HSD;  $p < 0.05$ ) between reef types. Reef types: Circ = circular, Para = parallel, Perp = perpendicular.

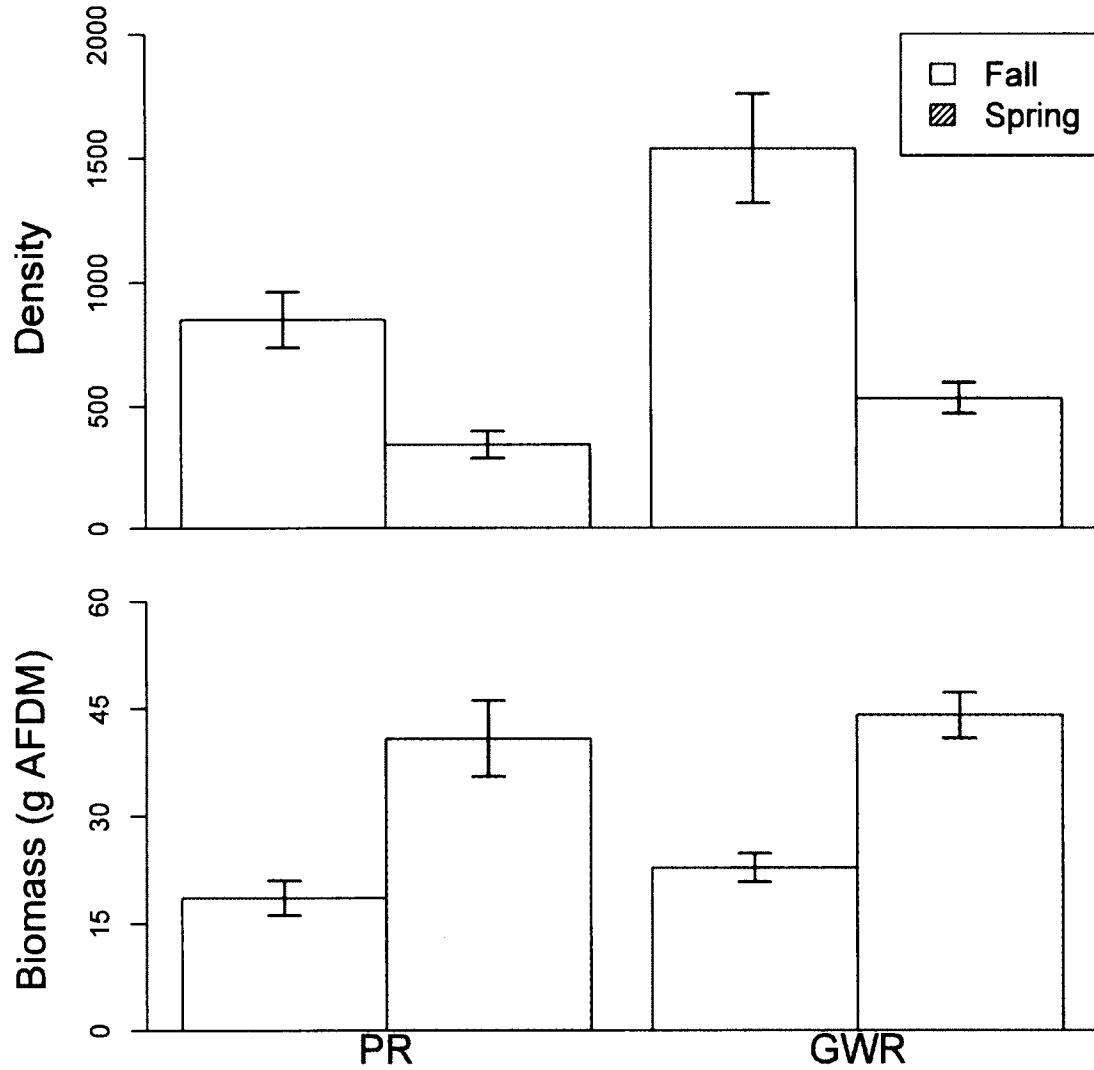


Fig. 9: Mean ( $\pm 1$  SE) oyster density and biomass in each river across seasons. GWR had higher oyster density, but biomass did not differ between rivers. Density was significantly higher in fall and biomass higher in spring across both study systems. Density and biomass are reported for the tray sample volume ( $0.06 \text{ m}^2$ ; 9.4 L shell volume).

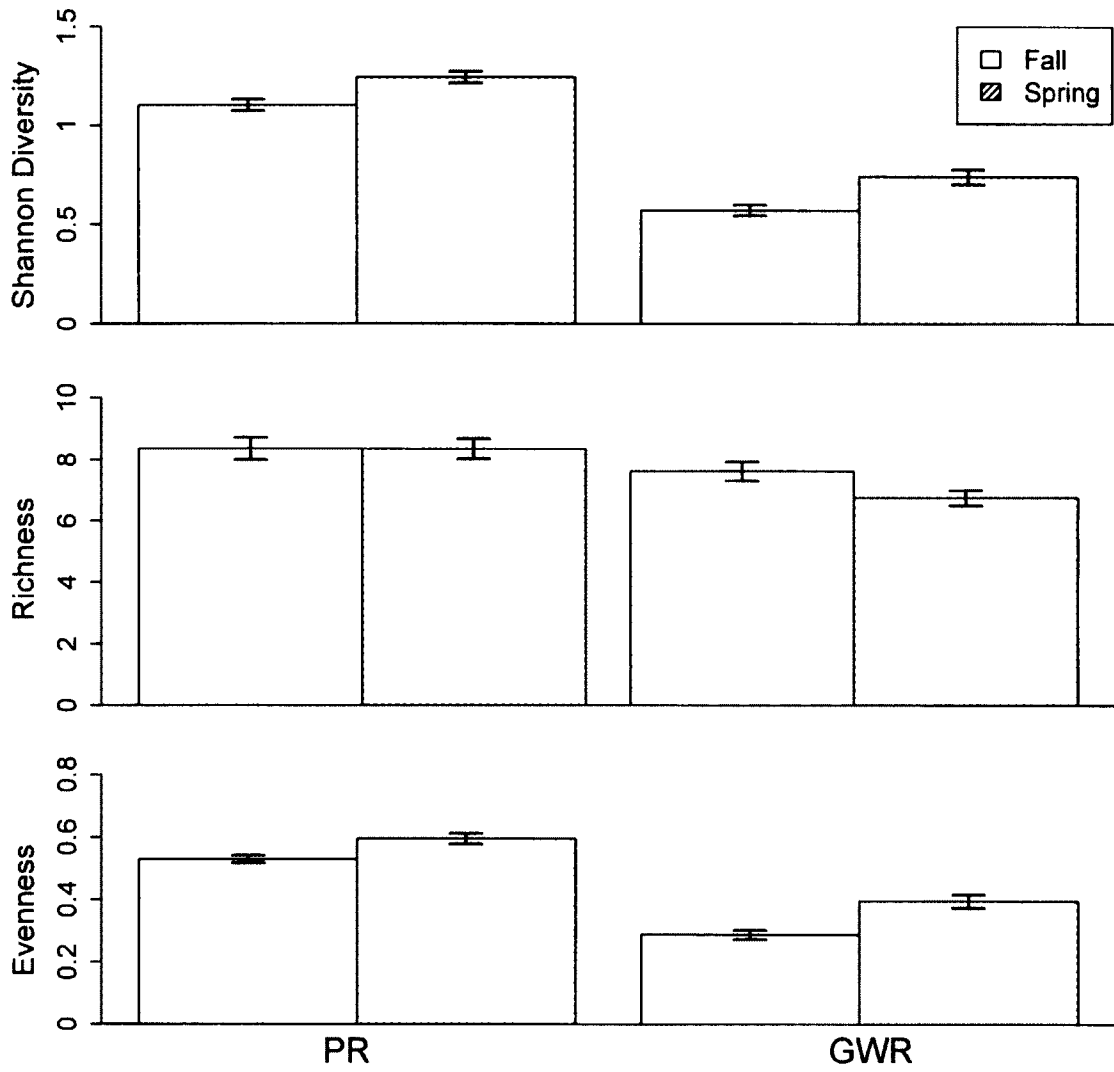


Fig. 10: Macrofaunal diversity responses across season in each study system. Data are means pooled across reef type; error bars = 1 SE.

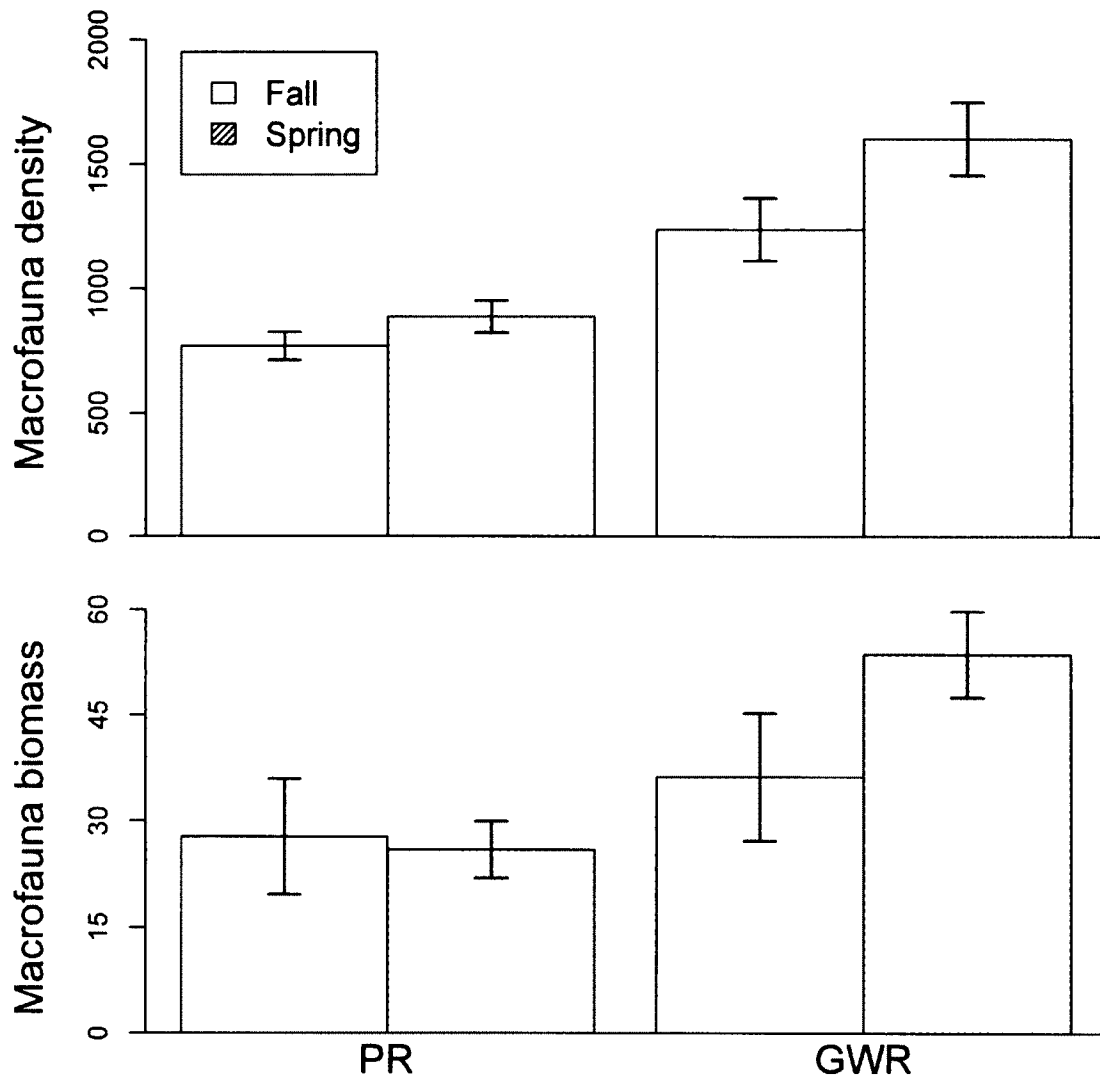


Fig. 11: Mean ( $\pm 1$  SE) macrofaunal density and biomass across seasons in each river system. Total macrofaunal density and biomass was summed across all species for each sample and pooled across reef types such that means shown represent 24 replicate samples of each season-river combination. Density and biomass were significantly higher in GWR than PR but did not differ significantly across seasons within rivers. Density and biomass are reported for the tray sample volume ( $0.06 \text{ m}^2$ ; 9.4 L shell volume).



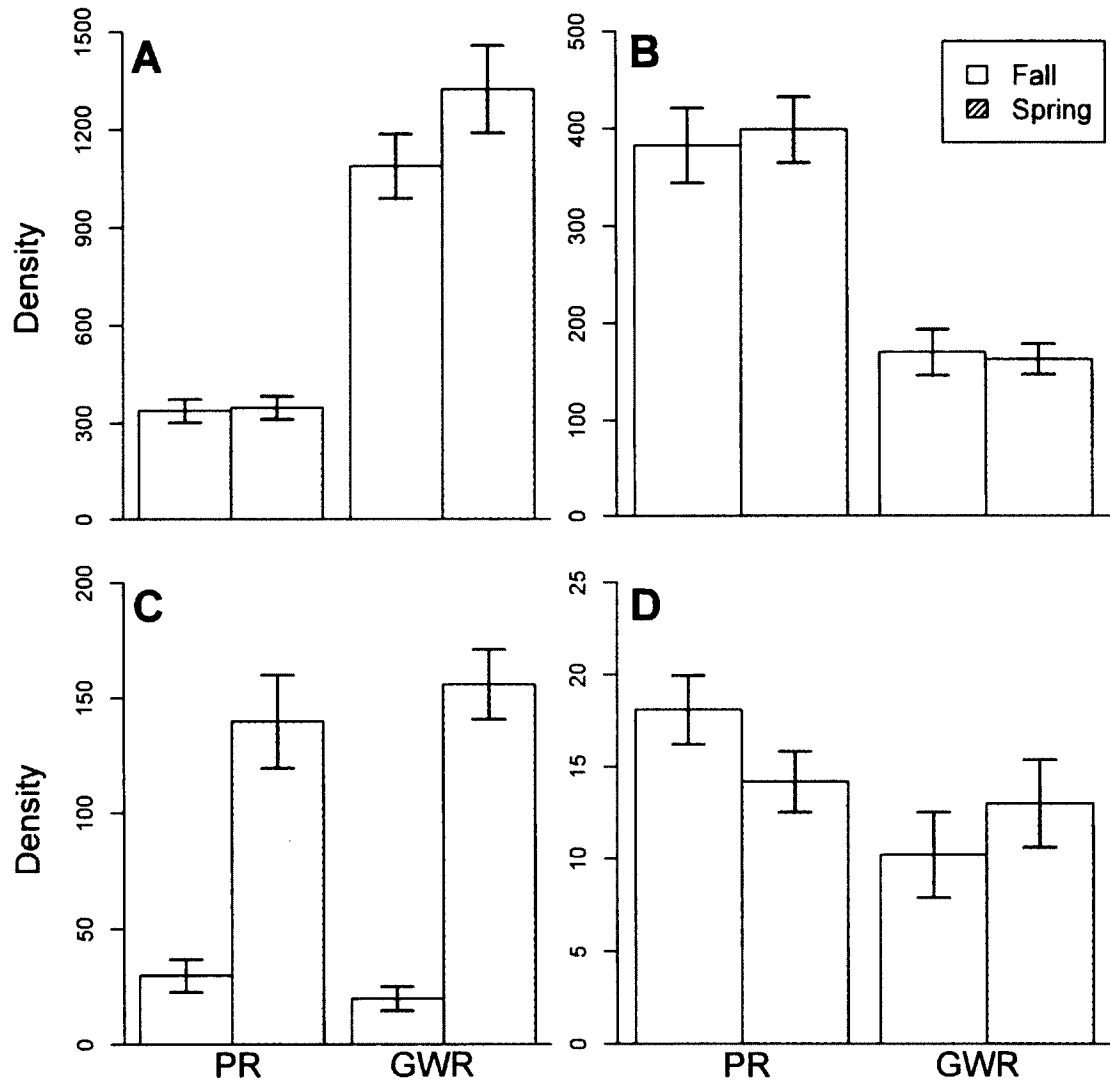


Fig. 12: Taxonomic group level mean density ( $\pm 1$  SE) for mussels (A), mud crabs (B), polychaetes (C), and fish (D) across seasons. Data were pooled across reef type for a total of 24 replicate samples. Mussel, mud crab, and fish density differed significantly between rivers; polychaete density did not. Mud crab and polychaete density varied with season, with higher mud crab density in fall and higher polychaete density in spring. Seasonal effects on mussel and fish density were not significant. Density represents the number of individuals per tray sample volume ( $0.06 \text{ m}^2$ ; 9.4 L shell volume).

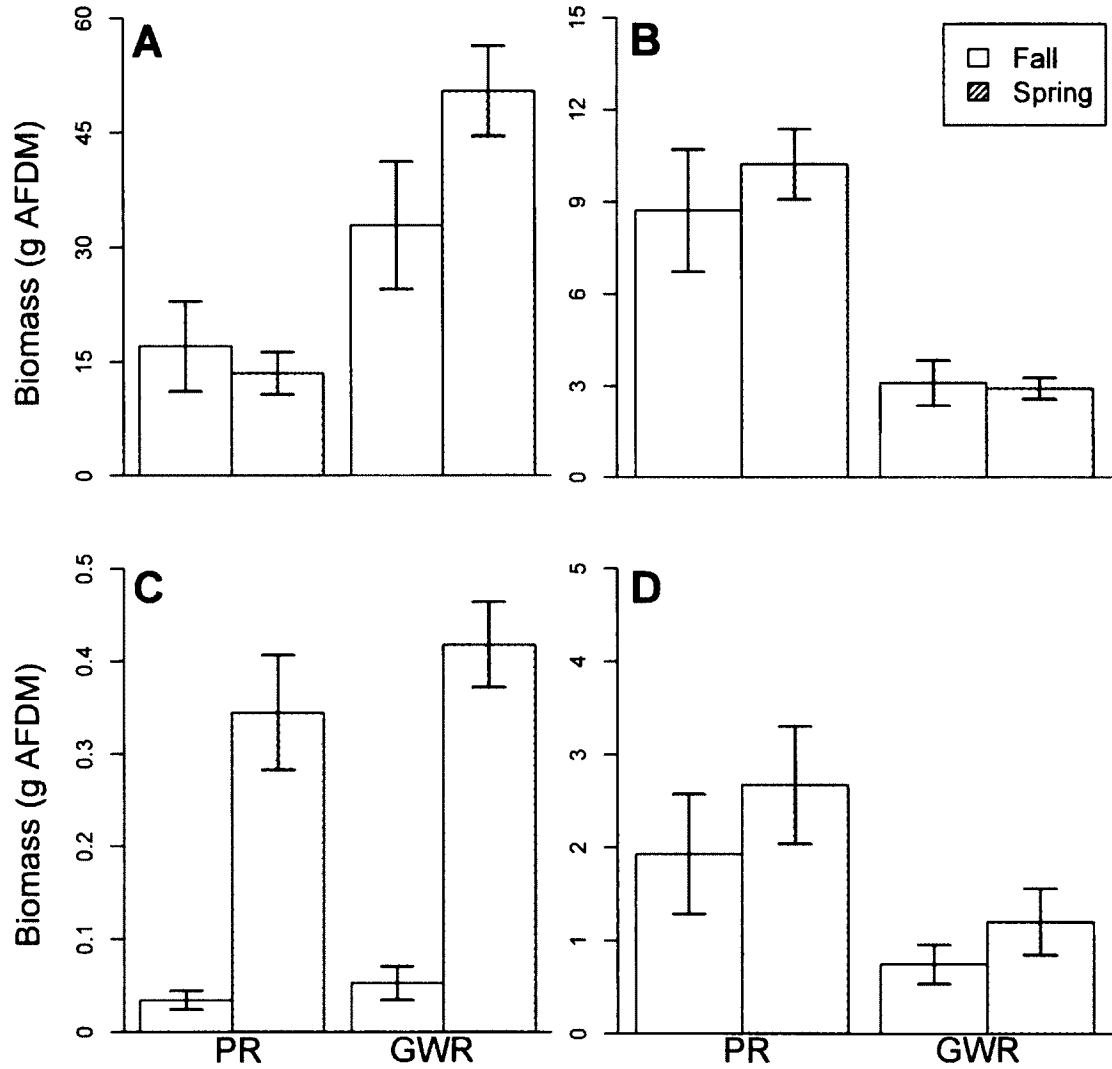


Fig. 13: Taxonomic group level mean biomass ( $\pm 1$  SE) for mussels (A), mud crabs (B), polychaetes (C), and fish (D) across seasons. Data were pooled across reef type for a total of 24 replicate samples. Mussel biomass was significantly higher in GWR while mud crab and fish biomass were higher in PR. Polychaete biomass varied only with season and was significantly higher in spring. Biomass represents the g AFDM per tray sample volume ( $0.06 \text{ m}^2$ ; 9.4 L shell volume).

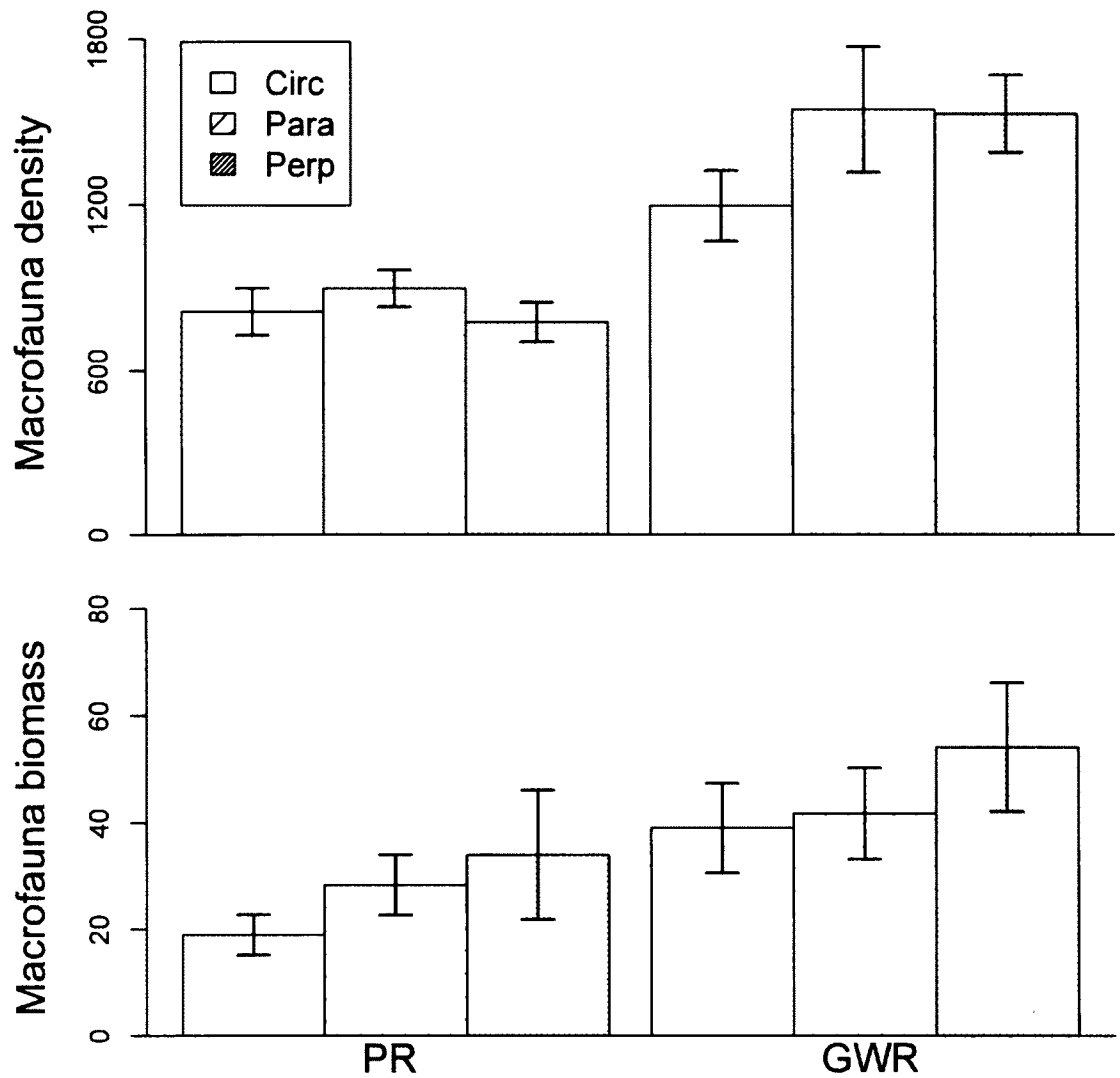


Fig. 14: Mean ( $\pm 1$  SE) total macrofaunal density and biomass for reef type treatments. Macrofaunal density was significantly higher in GWR but did not vary with reef type. Biomass was also higher in GWR, and perpendicular reefs had significantly higher biomass than circular or parallel reefs (perpendicular > parallel = circular). Density and biomass are reported for the tray sample volume ( $0.06 \text{ m}^2$ ; 9.4 L shell volume).

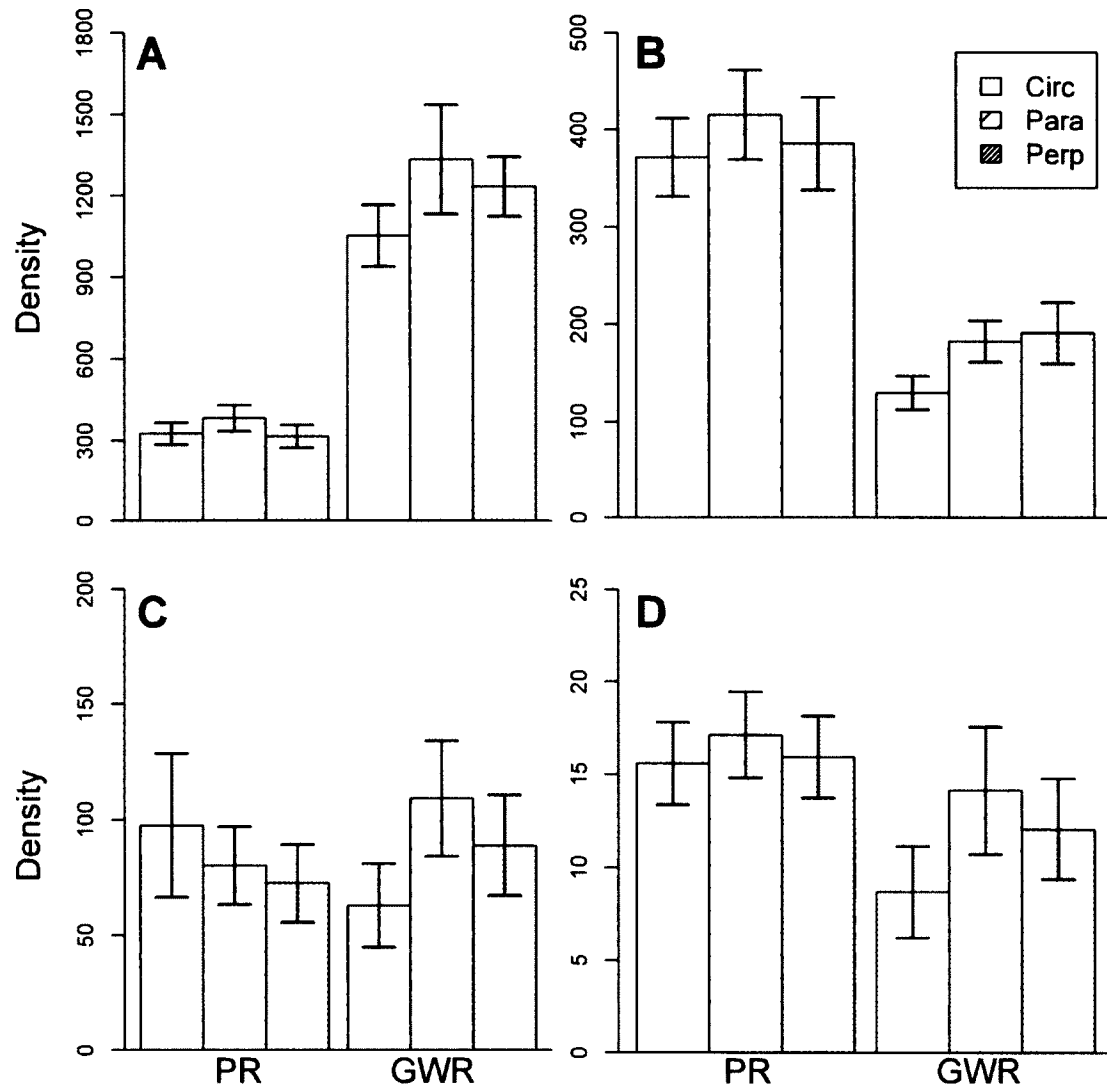


Fig. 15: Taxonomic group level mean densities ( $\pm 1$  SE) for mussels (A), mud crabs (B), polychaetes (C), and fish (D). Densities differed significantly between river systems for all groups except polychaetes. Mussel density showed a marginal effect of reef type in which parallel reefs had greater density (parallel > perpendicular = circular), but this effect was not significant at  $\alpha = 0.05$ . There were no other significant reef type effects for any of the other macrofaunal groups. Density is reported for the tray sample volume ( $0.06 \text{ m}^2$ ; 9.4 L shell volume).

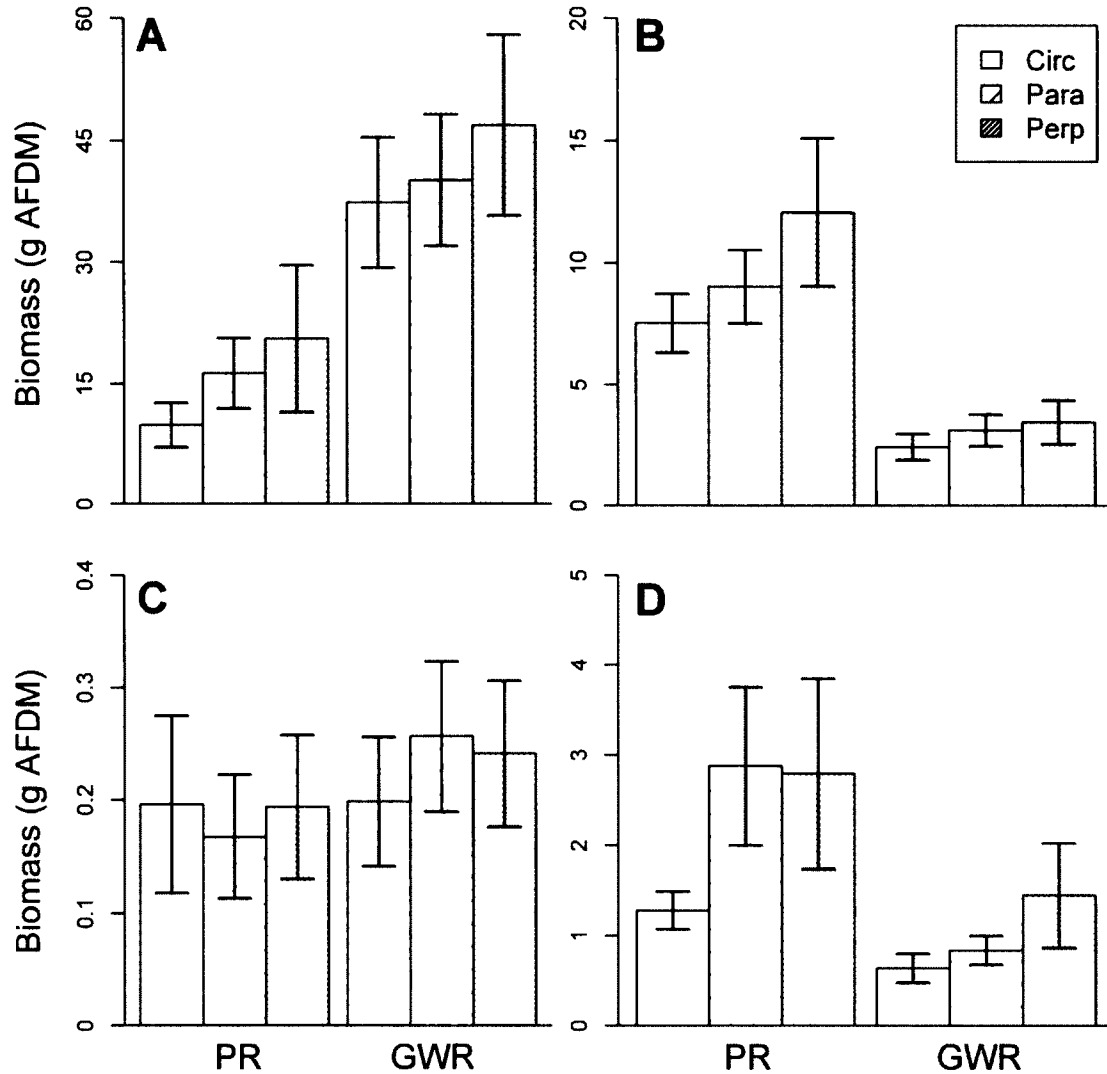


Fig. 16: Mean ( $\pm 1$  SE) macrofaunal biomass for individual taxonomic groups: (A) mussels, (B), mud crabs, (C) polychaetes, and (D) fish. Mussel biomass was significantly higher in GWR than PR. Mud crab and fish biomass were higher in PR and polychaete biomass did not differ between rivers. Mussel and mud crab biomass on perpendicular reefs was significantly higher than on parallel and circular reefs (perpendicular > parallel = circular). Fish biomass indicated a similar trend, but this effect was only marginally significant ( $p = 0.06$ ). Biomass represents the g AFDM per tray sample volume ( $0.06 \text{ m}^2$ ; 9.4 L shell volume).

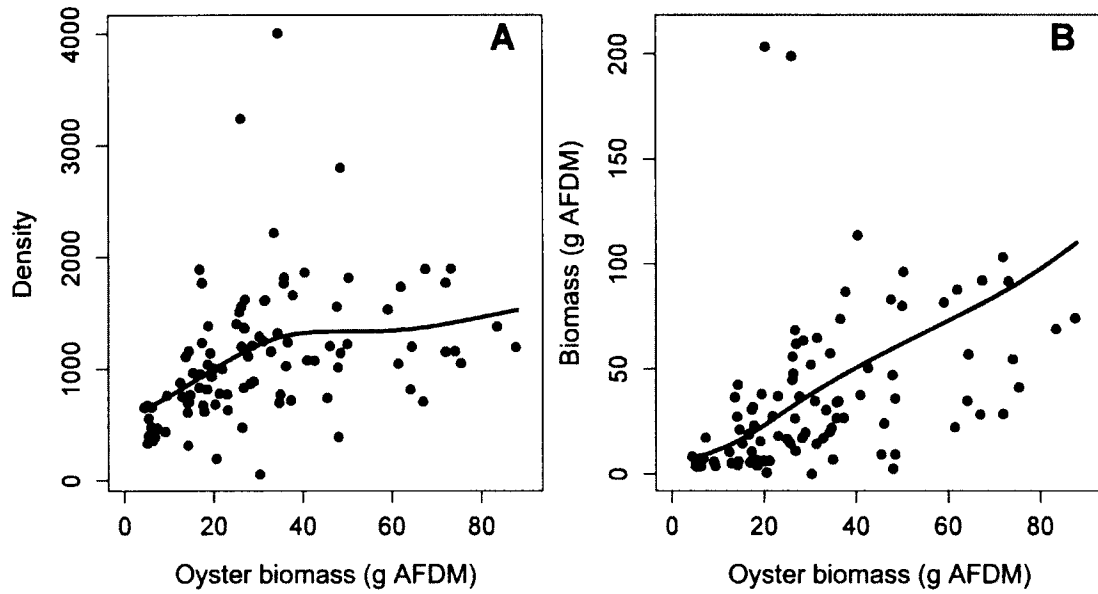


Fig. 17: Total macrofaunal density (A) and biomass (B) as a function of oyster biomass. Curves represent the smoothed spline for each response with respect to oyster biomass. Significant smoothing parameters indicated a significant effect of oyster biomass on macrofaunal density and biomass after accounting for fixed effects of all other factors (river, season, reef type). Density and biomass are reported for the tray sample volume (0.06 m<sup>2</sup>; 9.4 L shell volume).

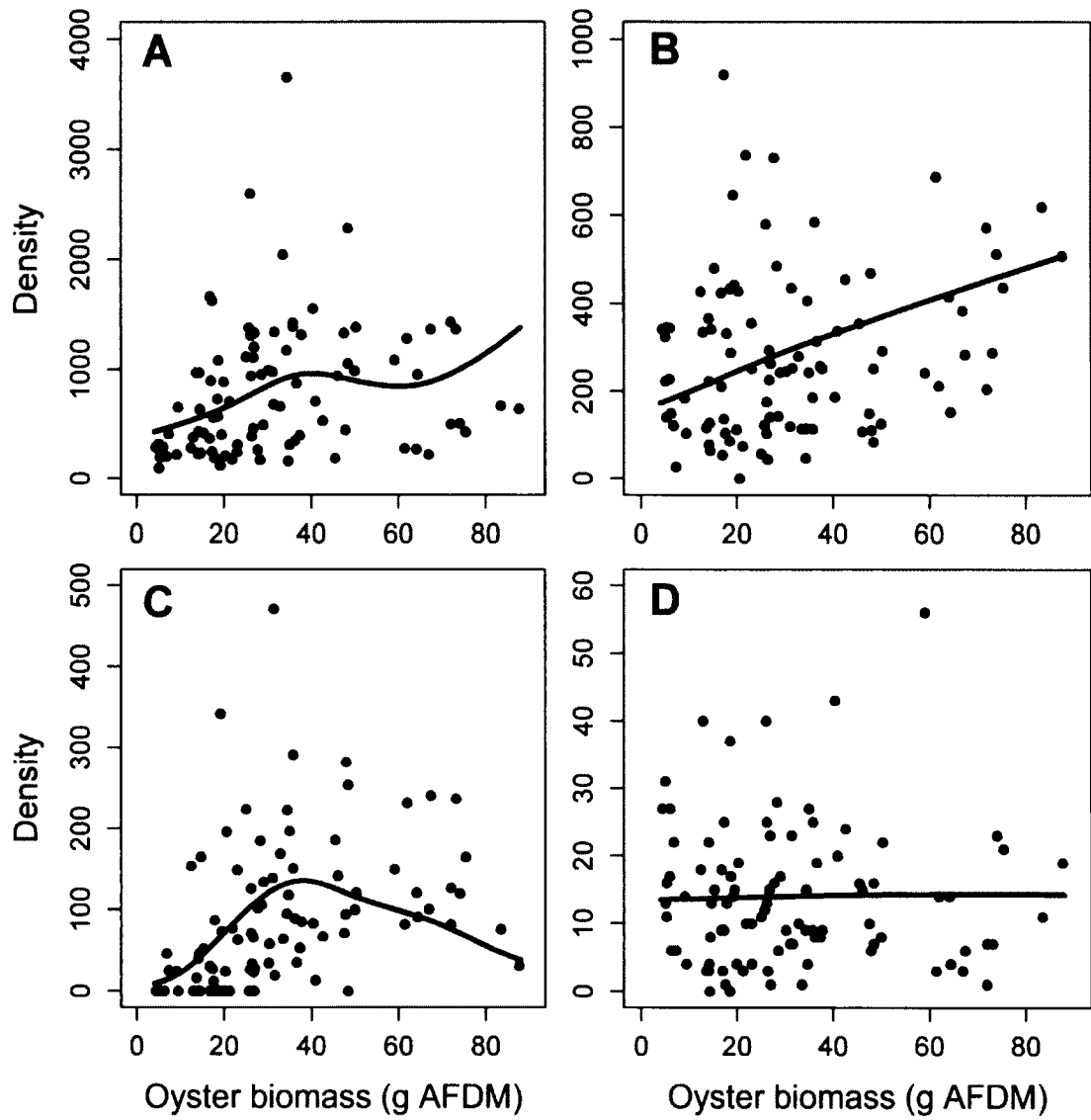


Fig. 18: Macrofaunal group density as a function of oyster biomass for mussels (A), mud crabs (B), worms (C), and fish (D). Curves are the smoothed spline for each response variable with respect to oyster biomass. Mussels, mud crabs, and worms had significant spline parameters indicating a significant relationship between oyster biomass and density. The relationship between oyster biomass and fish density was not significant. Density represents the number of individuals per tray sample volume (0.06 m<sup>2</sup>; 9.4 L shell volume).

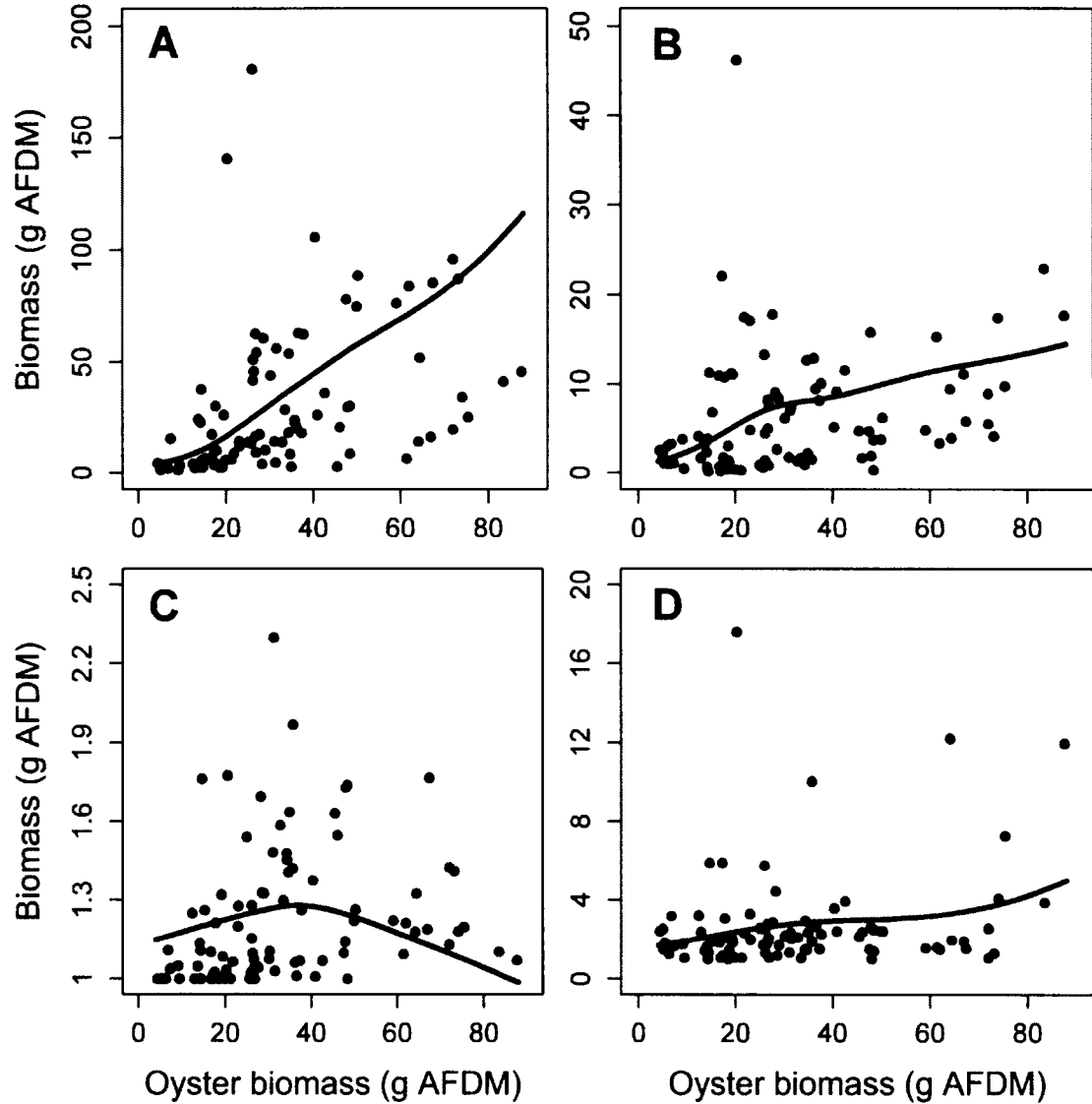


Fig. 19: Macrofaunal group biomass as a function of oyster biomass for mussels (A), mud crabs (B), worms (C), and fish (D). Curves are the smoothed spline for each response variable with respect to oyster biomass. Mussels, mud crabs, and worms had significant spline parameters indicating a significant relationship between biomass and density. The relationship between oyster biomass and fish density was positive, but not significant ( $p = 0.09$ ). Biomass represents the g AFDM per tray sample volume ( $0.06 \text{ m}^2$ ; 9.4 L shell volume).



APPENDIX A.

Community composition results of similarity percentage (SIMPER) analysis of macrofaunal biomass.

Table A1. Similarity percentage (SIMPER) analysis results for macrofaunal biomass. The rank order contribution of species accounting for > 90% of community dissimilarity is shown with respect to river and season.

<b>River</b>			
<b>Great Wicomico River</b>		<b>Piankatank River</b>	
<b>Species</b>	<b>Cumulative %</b>	<b>Species</b>	<b>Cumulative %</b>
I. recurvum	63.8	I. recurvum	34.8
E. depressus	78.2	E. depressus	64.3
Xanthid crabs	86.4	Xanthid crabs	76.0
A. succinea	90.7	G. bosc	86.7
		A. succinea	90.5

<b>Season</b>			
<b>Fall</b>		<b>Spring</b>	
<b>Species</b>	<b>Cumulative %</b>	<b>Species</b>	<b>Cumulative %</b>
I. recurvum	50.9	I. recurvum	48.0
E. depressus	73.0	E. depressus	69.7
G. bosc	82.6	Xanthid crabs	81.0
Xanthid crabs	91.2	A. succinea	88.2
		G. bosc	93.2

APPENDIX B.

Generalized additive model (GAM) results for macrofaunal taxonomic group level density and biomass.

Table B1. Mussel density and biomass AIC model comparisons for the GAM global model with the following candidate autocorrelation structures: CS=compound symmetry, AR (1) = autoregressive, ARMA (1,1) = autoregressive moving average order. The correlation model selected is shown in italics. GAM parameter estimates presented are relative to the reference condition of Great Wicomico River (GWR) circular reefs in fall.

<b>Mussel Density</b>			
Correlation	AICc	$\Delta$ AICc	w
CS	1323.6	0.00	0.36
<i>AR (1)</i>	<i>1323.6</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	1324.1	0.50	0.28
Parameter	Estimate	SE	P-value
Parametric variables:			
Intercept	6.90	0.09	< 0.001
PR	-1.11	0.09	< 0.001
Spring	0.05	0.08	0.51
Parallel	0.14	0.11	0.19
Perpendicular	0.05	0.10	0.61
Smoothed variable:			
Oyster biomass			< 0.001
<b>Mussel Biomass</b>			
Correlation	AICc	$\Delta$ AICc	w
CS	757.1	0.00	0.36
<i>AR (1)</i>	<i>757.1</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	757.6	0.44	0.29
Parameter	Estimate	SE	P-value
Parametric variables:			
Intercept	3.55	0.23	< 0.001
PR	-0.94	0.32	< 0.001
Spring	-0.69	0.25	< 0.001
Parallel	0.09	0.25	0.73
Perpendicular	0.58	0.25	0.02
Smoothed variable:			
Oyster biomass			< 0.001

Table B2. Mud crab density and biomass AIC model comparisons for the GAM global model with three candidate correlation structures. The correlation model selected is shown in italics. Parameter estimates presented for best autocorrelation model are relative to the reference condition of GWR circular reefs in fall.

<b>Mud Crab Density</b>			
Correlation	AICc	$\Delta$ AICc	w
CS	1211.0	0.00	0.36
<i>AR (1)</i>	<i>1211.0</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	1211.5	0.50	0.28
Parameter	Estimate	SE	P-value
Parametric variables:			
Intercept	5.04	0.12	< 0.001
PR	0.96	0.11	< 0.001
Spring	-0.29	0.13	0.03
Parallel	0.17	0.13	0.19
Perpendicular	0.24	0.13	0.07
Smoothed variable:			
Oyster biomass			< 0.001
<b>Mud Crab Biomass</b>			
Correlation	AICc	$\Delta$ AICc	w
CS	491.9	0.00	0.36
<i>AR (1)</i>	<i>491.9</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	492.3	0.44	0.29
Parameter	Estimate	SE	P-value
Parametric variables:			
Intercept	0.91	0.17	< 0.001
PR	1.35	0.16	< 0.001
Spring	-0.37	0.19	0.05
Parallel	0.03	0.19	0.86
Perpendicular	0.36	0.19	0.06
Smoothed variable:			
Oyster biomass			< 0.001

Table B3. Polychaete density and biomass AIC model comparisons for the GAM global model with three candidate correlation structures. The correlation model selected is shown in italics. Parameter estimates presented for best autocorrelation model are relative to the reference condition of GWR circular reefs in fall.

<b>Polychaete Density</b>			
Correlation	AICc	$\Delta$ AICc	w
CS	979.2	0.00	0.36
<i>AR (1)</i>	<i>979.2</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	989.7	0.43	0.29
Parameter	Estimate	SE	P-value
Parametric variables:			
Intercept	2.33	0.30	< 0.001
PR	0.59	0.28	0.03
Spring	2.84	0.27	< 0.001
Parallel	-0.19	0.34	0.58
Perpendicular	-0.18	0.34	0.59
Smoothed variable:			
Oyster biomass			< 0.001
<b>Polychaete Biomass</b>			
Correlation	AICc	$\Delta$ AICc	w
CS	-78.5	0.00	0.36
<i>AR (1)</i>	<i>-78.5</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	-78.1	0.43	0.29
Parameter	Estimate	SE	P-value
Parametric variables:			
Intercept	0.04	0.03	0.22
PR	-0.01	0.03	0.68
Spring	0.29	0.03	< 0.001
Parallel	0.003	0.03	0.93
Perpendicular	0.01	0.03	0.82
Smoothed variable:			
Oyster biomass			< 0.001

Table B4. Fish density and biomass AIC model comparisons for the GAM global model with three candidate correlation structures. The correlation model selected is shown in italics. Parameter estimates presented for best autocorrelation model are relative to the reference condition of GWR circular reefs in fall.

<b>Fish Density</b>			
Correlation	AICc	$\Delta$ AICc	<i>w</i>
CS	689.8	0.00	0.36
<i>AR (1)</i>	<i>689.8</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	690.3	0.44	0.29
Parameter	Estimate	SE	P-value
Parametric variables:			
Intercept	2.33	0.17	< 0.001
PR	0.32	0.16	0.05
Spring	-0.05	0.16	0.76
Parallel	0.29	0.19	0.13
Perpendicular	0.16	0.19	0.41
Smoothed variable:			
Oyster biomass			0.82
<b>Fish Biomass</b>			
Correlation	AICc	$\Delta$ AICc	<i>w</i>
CS	317.6	0.00	0.36
<i>AR (1)</i>	<i>317.6</i>	<i>0.00</i>	<i>0.36</i>
ARMA (1,1)	318.1	0.44	0.29
Parameter	Estimate	SE	P-value
Parametric variables:			
Intercept	0.41	0.16	0.01
PR	0.54	0.15	< 0.001
Spring	0.01	0.17	0.98
Parallel	0.20	0.17	0.25
Perpendicular	0.45	0.17	< 0.001
Smoothed variable:			
Oyster biomass			0.09

## CHAPTER 4

### **Sediment suspension and deposition across subtidal oyster reefs: implications for restoration**

#### Abstract

The eastern oyster, *Crassostrea virginica*, is a prominent ecosystem engineer, whose reefs exhibit strikingly consistent morphologies at multiple spatial scales throughout its North American range. These distinct morphologies are thought to form by interactions of nascent reef structures with hydrodynamics, but this mechanism has yet to be quantified. We collected seabed and water column observations across constructed reefs of 3 orientations representative of those found throughout the oyster's range: parallel or perpendicular to tidal currents or circular. Areas adjacent to reefs were sites of fine sediment trapping, with lower flow velocities, evidence of particle settling, and more fine sediments on the seabed relative to off-reef reference sites. The water column above the reef crest exhibited higher acoustic backscatter, higher flow velocities, and larger particles in suspension, consistent with local dispersal of flocculated fine sediment from the reef crest and/or active filtration by oysters. Particle trapping in areas between reefs has the potential to inhibit reef growth between existing reef structures. This is the first study to provide empirical evidence of hydrodynamic mechanisms contributing to historical oyster reef morphology and landscape-scale spatial self-organization of oyster metapopulations. Oyster reef restoration efforts can benefit from this improved understanding of biophysical interactions that govern sediment dynamics on constructed oyster reefs.

## INTRODUCTION

Distinct spatial patterns arising from self-organizing processes are prolific in coastal and marine habitats as varied as mudflats, salt marshes, mussel beds (van de Koppel et al. 2012), and coral reefs (Mistr and Bercovici 2003). These patterns arise from small-scale feedbacks that produce larger patterns (Reitkirk and van de Koppel 2008), which may be evident on multiple spatial scales due to scaling of underlying processes (Mister and Vercovici 2003; Rietkirk and van de Koppel 2008). For sessile benthic species, such as mussels, corals, and oysters, which rely on water currents to supply planktonic food and oxygen and remove waste products, these spatial patterns function to maximize material fluxes, resource utilization, and niche partitioning (Gili and Coma 1998). In temperate estuaries of the east coast of North America, the eastern oyster (*Crassostrea virginica*) is a dominant ecosystem engineer whose biogenic reef structures provide complex 3-dimensional habitat in the estuarine landscape. Eastern oysters build reefs through the gregarious settlement of multiple generations onto existing oyster substrate. These reefs provide habitat for other estuarine organisms (Coen and Grizzle 2007; Zimmerman 1989) and modify the environment through biophysical interactions that affect local current flow and sediment dynamics (Reidenbach et al. 2013).

Throughout the eastern oyster's range from the Gulf of St. Lawrence, Canada to the Gulf of Mexico (Carriker and Gaffney 1996), distinct reef morphologies and spatial patterns, thought to arise from feedback mechanisms between oyster reefs and local hydrodynamics, are evident (Grave 1905). Three reef types or morphologies are described: 1) string reefs: long, shoal-like reefs oriented perpendicular to prevailing tidal

currents, 2) fringe reefs: narrow reefs located along tidal channels in areas of sharp change in relief, and 3) patch reefs: small, irregularly shaped reefs with length-to-width ratios approaching one (Grave 1905; Kennedy and Sanford 1999; Price 1954; Smith et al. 2003). String reefs are generally the most productive and prominent reef form, and are characterized by high vertical relief. Fringe reefs have much lower vertical profiles than string reefs and are considered more ephemeral on geologic time scales (Smith et al. 2003). Patch reefs are generally found in deeper waters than string and fringe reefs and near the mouths of rivers or creeks (Haven and Whitcomb 1983). The similarity of morphologies found throughout the oyster's range and for other species that aggregate or form reefs suggests that there is a similar underlying mechanism driving reef morphology across diverse ecosystems (Mistr and Bercovici 2003).

Grave (1905) was the first to propose a mechanism for the formation of oyster reefs in North Carolina. He surmised that interactions of nascent reef structures with hydrodynamic flow led to the formation of perpendicularly oriented reefs in areas with strong bidirectional currents. Oysters at the outer edge of small clusters along the shoreline experienced the fastest growth rate because of enhanced food delivery due to faster currents away from the shoreline. Faster growth at the periphery led to an elongation of the protrusion into the flow. Reef accretion in the lateral and vertical directions was then enhanced by faster flows over and around the end of the reef. Eventually, poor growth and sediment deposition near the shoreline caused a break, forming the recognizable string reef morphology. Fringe reefs formed along tidal channels where fast flows maintained clean substrate for oyster settlement (Kennedy and Sanford 1999; Powell et al. 1995; Smith et al. 2003). The development of these reefs was



enhanced by the transport of mature oyster larvae by tidal currents in the channel, as fringe reefs provided the closest available hard substrate for the settlement of competent larvae. Patch reefs were usually formed in areas lacking strong bidirectional currents (Haven and Whitcomb 1983; Kennedy and Sanford 1999).

At a larger scale, reefs are not randomly distributed within the estuarine landscape. String reefs in Copano and Matagorda Bays, Texas, are evenly spaced along the axis of the estuary (Hedgpeth 1953). In Tangier and Pocomoke Sounds in Chesapeake Bay, reefs are distributed along the meanders of the channel (McCormick-Ray 1998; 2005). Similarly, coral reef patches off the coast of Queensland, Australia exhibit even spacing of perpendicularly oriented growth forms along a parallel axis that follows the East Australia Current (Mistr and Vercovici 2003), as do mussel beds in the Netherlands (van de Koppel et al. 2012). Spatial pattern formation in corals and mussels is attributed to local positive feedbacks that facilitate reef-scale growth and large-scale negative feedbacks between reef patches (Rietkirk and van de Koppel 2008; van de Koppel et al. 2012). For mussels, beds oriented perpendicular to currents allow for maximum uptake of phytoplankton, leading to rapid growth and persistence of the bed; however, food-depleted waters carried downstream of the mussel bed and sediment deposition behind the bed limit the formation of additional beds in close proximity due to sedimentation and resource limitation (van Leeuwen et al. 2010; van de Koppel et al. 2012). Thus, the regular spacing between beds represents the distance over which currents must travel to accumulate enough particulate matter to support additional mussel beds (Liu et al. 2014). Similarly, the depositional influence of intertidal *Crassostrea gigas* oyster reefs in the Netherlands scales with reef size and in the direction of prevailing winds (Wallis et al.

2014). Thus, the regular spacing of oyster reefs, as observed in Chesapeake Bay and the Gulf of Mexico, could result from similar feedbacks of sediment dynamics between oyster reefs on a landscape scale.

Despite the presumed importance of oyster reef orientation in the formation of historical reef structures, this process has not been studied empirically. The few studies that have quantified the effects of oyster reefs on local hydrodynamics and sediment transport have focused solely on intertidal reefs (Reidenbach et al. 2013; Walles et al. 2014; Whitman and Reidenbach 2012). Subtidal reefs are likely to respond differently than intertidal reefs, due to differences in reef metabolism and flow conditions. Oysters on intertidal reefs are exposed for several hours at a time and may experience temperature and desiccation stress. Stress may influence metabolic functions, such as filtration and biodeposition, which contribute to sediment dynamics and reef accretion (DeAlteris 1988; Haven and Morales-Alamo 1972). Oyster filtration is a large contributor to sediment uptake by oyster reefs (Dame et al. 1984; Nelson et al. 2004; Reidenbach et al. 2013); therefore, conditions that preclude filtration are likely to affect the sediment balance. Additionally, subtidal reefs are subject to sediment transport at all stages of the tide, whereas intertidal reefs are not subject to sediment deposition when exposed.

The purpose of this study was to quantify the effect of historical reef geometries on hydrodynamics and sediment dynamics in shallow subtidal systems currently targeted for oyster restoration (Wesson et al. 1999). Unfortunately, overfishing, disease, and habitat degradation (Rothschild et al. 1994; Wilberg et al. 2011) have led to the loss of 64% of oyster habitat and 88% of oyster biomass in the U.S. since the 19<sup>th</sup> century (Zu Ermgassen et al. 2012). Enhanced understanding of the hydrodynamic mechanisms that

support oyster reef growth could be a boon to oyster restoration efforts. Existing natural and constructed oyster reefs often succumb to siltation and burial that limit their productivity and can lead to habitat loss (Smith et al. 2005). To determine if the proposed mechanisms of reef-flow interactions on historical reef forms are present on small-scale restored oyster reefs, we conducted two field studies to examine how sediment dynamics responded to these interactions to better understand reef- and landscape-scale processes. A closer examination of the interactions of historical reef forms with flow and their consequences for sediment dynamics should provide valuable insight for future restoration efforts.

## **METHODS**

### **Study Locations**

Oyster reef studies were carried out in the Piankatank (PR) and Great Wicomico (GWR) Rivers in Virginia, two sub-estuaries on the western shore of Chesapeake Bay (Fig. 1). Both rivers are small, trap-type estuaries characterized by small tidal amplitude, weak tidal currents, and high residence times relative to their size (Andrews 1979; Chen et al. 1977). These conditions are conducive to the retention of planktonic larvae, and these systems once supported highly productive natural oyster beds from which seed oysters were harvested. Both rivers have undergone restoration by state and federal agencies, and each has been designated a “Tier 1” priority restoration area in the U.S. Army Corps of Engineers Native Oyster Restoration Master Plan (USACE 2012).

Within each river, two sites were selected for reef construction, one each on the north and south shores, for a total of 4 sites (Fig. 1). Sites were selected based on standard restoration criteria for subtidal reefs, including depths < 3 m, hard bottom

substrate, and proximity to oyster broodstock (Gregalis et al. 2009; Wesson et al. 1999). The average depth of PR and GWR ranges from 3.2 - 3.4 m (Andrews 1979; Chen et al. 1977) and chosen study locations had mean depths ranging from 1.3 – 2.0 m. Sediments at the sites were predominantly sand (mean  $\pm$  SE: 97%  $\pm$  1% sand) with some oyster shell or shell hash present. Each site was located within 1 km of an established restoration reef to ensure oyster broodstock were present nearby.

### Reef Construction

Six experimental reefs of varying orientation were constructed at each of the study sites in June 2011. Reef orientations, perpendicular or parallel to tidal currents or circular, were designed to mimic string, fringe, and patch reef configurations historically found throughout the oyster's range. Two reefs of each type were constructed in each location for a total of 24 reefs (Fig. 1). Oyster shell obtained from local seafood processors was used to construct the reefs. Perpendicular and parallel reefs were 1 m x 5 m x 0.4 m, with their orientation determined relative to the long axis. Circular reefs were 2.5 m in diameter and 0.4 m tall, which assured approximately equal surface area and volume for each reef type. Reefs were placed in a 2 x 3 grid configuration, with reef orientation randomly assigned to each reef; therefore, the spatial distribution of reef types varied across study sites (Fig. 2).

### Reef Dimensions

Physical characteristics of the experimental reefs were measured in early summer 2013, two years following reef construction. Cross-sectional reef height profiles on parallel and perpendicular reefs were taken every meter along the long axis. Heights were recorded every 10 cm along the profile, using a probe to measure from a known

height to the reef surface. For circular reefs, profiles were taken every meter in both the alongshore and cross-shore directions. Reef area was determined by probing the perimeter of the reef to establish the reef boundary, which was often covered by a thin layer of sediment, and measuring the linear dimension. For parallel and perpendicular reefs, a single measurement was made of the length of the long axis, the mean width was determined from replicate measurements every meter along the reef, and the length and mean width were used to calculate reef area. For circular reefs, the mean diameter from replicate measurements in the alongshore and cross-shore directions was used to determine area. Area measured in summer 2013 was compared to similar measurements from summer 2011, two months following reef construction, to determine the change in area of each reef.

Rugosity, a measure of surface roughness or heterogeneity, was measured on each reef using chain profiles. The length of small link chain (14.8-27.2 mm) laid along the reef surface and allowed to conform to the contours of the reef is divided by the linear length of the same axis to determine the rugosity index (Frost et al. 2005). Twenty-seven replicate measurements were made on each reef, 3 transects each in the alongshore and cross-shore directions with 3 replicate measurements using 3 different chain link sizes (14.8, 19.6, 24.7 mm link length). Rugosity estimates did not differ significantly between transects or chain link sizes (ANOVA,  $p > 0.05$ ); therefore, all measurements were pooled to determine the mean rugosity on each reef. Area in 2011 and 2013, percent change in area, and rugosity across reef types was examined by one-way analysis of variance (ANOVA;  $\alpha = 0.05$ ) with Tukey's honest significant difference (Tukey HSD) post-hoc comparisons.

## Sediment Grain Size

We hypothesized that tidal currents interacting with reef structures would create a spatial gradient in sediment grain size due to settlement of fine particles in areas of low flow immediately adjacent to reefs in accordance with theory and observation on intertidal reefs (Grave 1905; Walles et al. 2014). To investigate this hypothesis, grain size transects were taken in summer 2013 on each reef at the northern shore sites in PR and GWR (PR-N, GWR-N; Fig. 1) at distances of 0, 0.5, 1, 2, and 4 m from the reef. Sample transects were taken in the upstream, downstream, onshore, and offshore directions. Percent sand, silt, and clay were determined by sieve and pipette methods (Plumb 1981), and transects were averaged across study sites for each reef type. Percent sand was analyzed by 3-way ANOVA model with reef orientation, transect direction (*e.g.* upstream), and distance from the reef and their interactions as factors. Additionally, grain size samples from summer 2013 were compared to grain size samples taken in 2011 prior to reef construction with Student's t-test to determine the overall effect of reef addition to the larger study area. Assumptions of normality and homogeneity of variance were examined visually and with Shapiro-Wilks and Levene's tests, respectively.

### Pilot Acoustic Doppler Current Profiler (ADCP) Surveys

Pilot ADCP surveys were conducted in June and July 2013 to investigate the effects of reef orientation on hydrodynamics and sediment suspension and to determine the optimal deployment and sampling protocol for additional instruments. Logistical considerations precluded surveying all four locations; therefore, two study sites, (PR-N) and (GWR-N; Fig. 1), were selected for the pilot study. Reefs at each of these sites supported high oyster densities (mean  $\pm$  SE; PR-N:  $484 \pm 83$ , GWR-N:  $767 \pm 187$  0.06 m

<sup>2</sup>), and had similar predicted tidal current velocities (0.1 - 0.3 m s<sup>-1</sup>) over the study period. The use of northern shore sites in both rivers also minimized differences in wind-driven currents between shores due to dissimilarities in fetch. The placement of reef types within each study location varied due to random assignment of reef orientation treatments during construction.

A downward-looking 1200 kHz Acoustic Doppler Current Profiler (ADCP, RDI Inc.) was mounted on a floating platform (Fig. 3a) used for transects across each study site at 2 m intervals with a 0.1 m vertical resolution. The floating platform was pulled by hand across the study area via towlines attached to the platform frame. Patterns inferred from the ADCP backscatter (see Results section) suggested conditions favorable for sediment trapping were present between the perpendicular reefs present at PR-N. To further investigate the trends observed at PR-N, a second, in-depth hydrodynamics study was carried out at that site.

#### Piankatank River (PR-N) Main Hydrodynamics Study

A second deployment designed to better characterize tidal velocity and particle suspension at the PR-N site was conducted in March 2014. A stationary platform (Fig. 3b) was deployed on 5 consecutive days (March 14-18, 2014) at 4 locations at the PR-N site: an offshore soft-bottom reference site (reference), the downriver perpendicular reef crest (crest), and on the upriver side of the downriver and upriver perpendicular reefs (Fig. 2). The platform was deployed at each location for at least 24 hours to capture changes in flow conditions across tidal phases. Again, deployment dates were chosen during periods of consistent predicted tidal currents. Current velocities measured at a nearby monitoring

station were used to determine relative differences between currents during the study period (Stingray Point, CBIBS 44508).

The instrumentation platform (Fig. 3b) in the main hydrodynamics study included 2 Nortek Acoustic Doppler Velocimeters (ADV), a Sequoia Laser *in situ* Scattering and Transmissometry instrument (LISST 100X), which measures suspended particle size, and a Sontek Pulse-Coherent Acoustic Doppler Profiler (PC-ADP). Unfortunately, the signal-to-noise ratio in the ADV velocity data was too low to allow resolution of turbulent velocities, but piezoelectric pressure sensors within the ADVs were used to determine water depth and characterize the wave climate at each location (Jones and Monismith 2007). Pressure spectra from the sensor were transformed to wave spectra using the methods described by Jones and Monismith (2007) for shallow-water systems to obtain characteristic wave parameters such as significant wave height ( $H_s$ ), mean wave period ( $T$ ), and wave frequency ( $f$ ) during each tidal phase.

Suspended particle size was measured by the LISST at a single point 0.4 m above the bed. The LISST measures the size distribution and volume concentrations of particles in 32 distinct log-spaced size classes from 2.5-500  $\mu\text{m}$  (Fugate and Friedrichs 2002). The LISST sampled every 15 min in 5 min bursts with 900 records per burst. Particle size distributions were averaged over tidal phase to produce mean size distributions for each sampling location. The downward-looking PC-ADP was mounted on a bar approximately 0.55 m above the bed and extending away from the platform to prevent interference between instruments. The PC-ADP sampled at 1 Hz for 2 min every 15 min, with a vertical resolution of 0.01 m. Profiles were averaged over flood and ebb tide separately to obtain mean velocity profiles for each tidal phase. Burst-averaged current



velocity for each tidal phase was examined by one-way ANOVA with deployment location as a factor with Tukey's HSD post-hoc comparisons. Shapiro-Wilks and Levene's tests were used to assess the assumptions of normality and homogeneity of variance.

## **RESULTS**

### **Reef Dimensions**

Two months following construction, reef area was significantly different between reef types at all study locations (ANOVA,  $p = 0.01$ ). Perpendicular reefs had significantly greater area than circular reefs (Tukey HSD;  $p = 0.01$ ), and parallel reefs were intermediate. In 2013, reef area was again significantly different between reef types (ANOVA,  $p < 0.01$ ); parallel and perpendicular reefs were similar (Tukey HSD;  $p = 0.99$ ) and larger than circular reefs ( $p < 0.01$  for both comparisons). Circular reefs exhibited the largest change in area over time, which was negative on average, whereas perpendicular and parallel reefs increased in area over time (Fig. 4a; ANOVA,  $p < 0.05$ ). Rugosity also varied significantly over reef types with perpendicular and parallel reefs having similar and higher surface roughness than circular reefs (Fig. 4b; ANOVA,  $p < 0.05$ ). Perpendicular reefs displayed the highest mean reef height after 2 years cm (mean  $\pm$  SE;  $19.1 \pm 1.3$  cm) followed by parallel ( $17.9 \pm 0.8$  cm) then circular reefs ( $16.9 \pm 1.2$  cm), but these differences were not significant (ANOVA,  $p = 0.42$ ).

### **Sediment Grain Size**

Overall, grain size at the study locations was significantly finer following reef construction than before reef construction (t-test,  $p < 0.01$ ). At the PR-N site, % sand decreased from  $99.3 \pm 0.1\%$  (mean  $\pm$  SE) to  $98.5 \pm 0.3\%$ ; at GWR-N it decreased from

98.1 ± 0.6% to 96.2 ± 0.3%. Grain size within sites varied significantly by reef type (3-way ANOVA;  $p < 0.01$ ) and transect direction ( $p = 0.04$ ), with a significant interaction of type and transect ( $p = 0.04$ ). Sediments surrounding perpendicular reefs were significantly finer than those associated with parallel and circular reefs (Fig. 5). Perpendicular offshore transects had the lowest mean % sand overall, and were the only type-transect combination that was significantly different from any other (Tukey HSD, Fig. 6a). Although distance from the reef was not a significant factor in determining grain size ( $p = 0.38$ ), there was a trend toward lower grain size immediately adjacent to perpendicular reefs in downstream, upstream, and onshore transects (Fig. 6a). Parallel and circular reefs maintained relatively consistent grain size along transects (Fig. 6b-c).

#### Pilot ADCP Surveys

Results from the pilot ADCP surveys indicated lower acoustic backscatter (ABS) between reefs than above them (Fig. 7a, c), consistent with lower water column turbulence between the reefs and the removal of sediment from the water by settling. The observed decrease in ABS in the overlying water column between the two perpendicular reefs at PR-N (Fig. 7c) is indicative of lower sediment concentration (Holdaway et al. 1999) and/or lower intensity of turbulence (Seim et al. 1995). Although the highest velocities from individual ADCP bins tended to occur over the reef crests (e.g., at  $x = 5$  m in Fig. 7b and at  $x = 6$  m in Fig 7d), the overall weak current speeds and short sampling times at any one location resulted in velocity data that were insufficient to determine whether current speeds between the reefs were also lower in magnitude than those over the reefs. Because trends in ABS were most pronounced between the perpendicular reefs at PR-N, the second hydrodynamics study was carried out at that site. The PR-N site was

also the only study location in which two perpendicular reefs were located adjacent to one another.

#### PR-N Main Hydrodynamics Study

The results of the main hydrodynamics study suggest that the presence of reefs reduced flow intensity at PR-N. Depth-averaged current speed at the upriver and downriver reef sites was reduced relative to the reference site where reefs were absent, with an average overall reduction (pooling ebb and flood) of 77.1% and 72.1%, respectively (Fig. 8). Depth-averaged current speed over the reef crest was decreased 2.6% relative to the reference site. All differences in current speed were significant at  $p < 0.05$  (ANOVA; Tukey HSD). Tidal current profiles from the PC-ADP (Fig. 8) at downriver and upriver reef sites displayed kinks in current speed at approximately 0.2 m above the bed. The kink in velocity profiles was coincident with the mean height of the reef at each location and suggests eddies shed by the reef were affecting the structure of the flow.

The presence of perpendicular reefs enhanced resuspension of flocculated muds from the reef crest and deposition of these muddy flocs adjacent to the reefs. Because variation in particle size over the tidal cycle was not clearly related to tidal phase and was temporally more complex than velocity, particle size distributions are presented as a single time-average over the entire tidal cycle at each location (Fig. 9). For particles greater than 15  $\mu\text{m}$  in size, the two sites adjacent to the perpendicular reefs (Fig. 9c,d) each had a lower concentration of particles by volume than that observed over the reef crest (Fig. 9b). For sizes  $> 15 \mu\text{m}$ , the downriver site (Fig. 9d) also exhibited concentrations below that observed at the offshore reference site. For 15 to 150  $\mu\text{m}$

particles, the upriver site was likewise lower in concentration than the reference, but for those larger than 150  $\mu\text{m}$ , the upriver and reference sites were not significantly different. Together, these results are consistent with preferential suspension of flocs off of the reef crest and settling adjacent to the crest. At all four sites, a peak in particle volume concentration was observed in the range of 200 to 400  $\mu\text{m}$ , which is consistent with the incorporation of much of the total suspended mud into macroflocs. For the smallest observed particles (between 2.5 and 15  $\mu\text{m}$  in size), all four sites exhibited a similar concentration distribution, suggesting that the smallest suspended particles were composed of uniformly dispersed, non-settling washload sediments.

Wave activity at the study site was minimal during the study period. Significant wave height ranged from 0.04 - 0.06 m, and did not differ significantly across days (ANOVA,  $p = 0.66$ ); therefore, the influence of waves on our observations was negligible (Reidenbach et al. 2013). Current velocities measured at a downstream monitoring station (Stingray Point, CBIBS 44508) ranged from 0.41 - 0.60  $\text{m s}^{-1}$  and did not differ significantly between days (ANOVA;  $p = 0.12$ ), suggesting that temporal changes in tidal current velocities over the study period were not responsible for observed differences in velocity at the study site.

## **DISCUSSION**

### **Reef Characteristics**

Reef orientation produced considerable differences in reef physical characteristics over the course of 2 y. Circular reefs lost significantly greater area than either perpendicular or parallel reefs; which was most likely due to burial of circular reefs at the reef margin. Mean reef height did not differ between reef types and the minimum width

of rectangular reefs was less than that of circular reefs, indicating that the slopes of circular reefs were less steep. The lower steepness near the edges of circular reefs may have allowed sediment to more easily encroach on its borders, leading to a loss in reef area over time by burial. This explanation is further supported by differences in rugosity. Rugosity was lower on circular reefs, which may have been caused by infilling of reef interstitial space by sediment leading to lower overall surface heterogeneity.

These biophysical interactions have important implications for oyster reef restoration. Changes in reef area have both direct and indirect impacts on reef productivity. Loss of reef habitat to burial limits the reef surface area available for settlement and decreases the effectiveness of restoration. Indirectly, sediment deposition can reduce the quality of remaining habitat. Reduced bed roughness due to infilling of interstitial space decreases turbulent mixing, which supplies food and oxygen to the bed (Reidenbach et al. 2010). Additionally, sediment deposition negatively impacts oysters survival (Lenihan 1999; Colden and Lipcius, *in press*), leading to poor habitat quality even in the absence of burial. Thus, circular reefs are likely to be less productive in the long term than parallel and perpendicular reefs due to sediment transport dynamics causing burial and loss of reef area. Rather than a stable reef morphology, earlier studies suggested that patch reefs may be the end product of the evolution of fringe or patch reefs (Grave 1905), which accounts for their small, ephemeral nature relative to string and fringe reefs (Smith et al. 2003). That parallel and perpendicular reef area remained stable over time while circular reefs lost area supports this hypothesis and suggests that sediment transport dynamics on historical reefs are mimicked on restored reefs of similar configurations.

## Reef-Scale Hydrodynamics

Overall, sites adjacent to perpendicular reefs displayed reduced velocity relative to the reference or reef crest sites, indicating that the presence of reefs had a dampening effect on tidal currents. ADCP, PC-ADP and LISST data indicated that areas behind perpendicular reefs tended to trap flocculated fine sediments. Behind the reefs, there was a consistent kink in the velocity profile coincident with the height of the reef, suggesting that flow changes direction at the top of the reef (Reidenbach et al. 2010). Data were generally consistent across ebb and flood tidal phases, suggesting that deposition is occurring adjacent to perpendicular reefs throughout most of the tidal cycle due to alteration of flow by the reef structure. In contrast, velocities over the reef crest were as strong as or stronger than conditions observed at the reference location. Depth-averaged mean velocity on the crest was  $0.04 \text{ m s}^{-1}$ , which was double the mean velocity observed at the downriver reef location. Higher flow velocities prevented deposition on the reef surface, which was evident in the large peak in volume concentration of large particles ( $> 200 \text{ }\mu\text{m}$ ) measured by the LISST at 0.4 m above the bed. Grain size of suspended sediments measured at the reef crest is similar to the mean grain size of sediments on oyster reefs in tidal creeks in North Carolina ( $104 \text{ }\mu\text{m}$ ; Nelson et al. 2004), suggesting the reef is the net source of suspended material.

Particle size distributions from the LISST indicated a bimodal distribution with peaks in volume concentration at approximately  $10$  and  $250 \text{ }\mu\text{m}$  in diameter, which are likely sediment micro- and macroflocs as opposed to individual inorganic grains eroded from the bed. Mean velocities at the upriver and downriver reef sites ranged from  $0.01$ - $0.02 \text{ m s}^{-1}$ , and inorganic particles in the  $100$ - $200 \text{ }\mu\text{m}$  range (i.e., sand) would not be

found in suspension at these low velocities. In contrast, macroflocs in low energy estuarine environments are loose aggregations of fine inorganic particles and organic material that settle much more slowly than similarly-sized inorganic particles. Active bivalve beds tend to resuspend larger particles at lower bed stress because large particles are primarily composed of relatively low density, organic-rich flocs (Fugate and Friedrichs 2003). The micro- and macro-floc particles observed here are of similar size to those near clam beds (30-300  $\mu\text{m}$ ; Fugate and Friedrichs 2003), and on oyster reefs (104  $\mu\text{m}$ ; Nelson et al. 2004), suggesting that the macroflocs observed in this study were likely composed, at least in part, of biodeposits resuspended from the reefs.

At the reef scale, our findings support Grave's proposed hypothesis that perpendicular reef forms enhance resuspension of sediments from the reef crest, including reef waste products (biodeposits), to help to maintain a suitable substrate for oyster settlement. Enhanced sediment organic matter and the presence of aggregated particles downstream of restored reefs in a small tidal creek in North Carolina (Nelson et al. 2004) also supports the inference that sediments produced on the reef are resuspended from the crest and deposited adjacent to the reef. Higher organic matter in sediments immediately adjacent to our reefs suggests a similar pattern of erosion of biodeposits from the reef and deposition of biogenic sediments adjacent to the reef, resulting in a fining of sediments observed in grain size transects. In this way, oyster reefs are similar to other structured estuarine habitats, such as seagrass beds, that have long been recognized for their capacity to slow currents and promote sediment deposition (Chen et al. 2007; Hansen and Reidenbach 2012; Widdows et al. 2008).

## Mechanisms of Spatial Pattern Formation

Interactions of oyster reefs with hydrodynamics and sediment dynamics indicated the potential for feedbacks capable of large-scale pattern formation. At the GWR-N and PR-N sites, the addition of reefs to the study area resulted in finer sediments over time, suggesting that the presence of reef structures influences the spatial distribution of sediment deposition within the system. In grain size transects, areas of fine sediment deposition extended 0.5-1 m away from reefs, indicating an influence of the reef structure outside the immediate reef boundary. For intertidal *C. gigas* reefs, silt fraction and organic matter were enriched up to 100s of meters from the reef (van der Zee et al 2012). The sphere of influence of our reefs was much smaller, which corresponds to reef size (Walles et al. 2014). For *Crassostrea gigas* reefs on tidal mudflats, the size of the depositional area behind oyster reefs was best explained by the length of the reef in the direction perpendicular to flow (Walles et al. 2014). In our study, perpendicular reefs were much longer (5 m) in that direction relative to parallel (1 m) and circular (2.5 m) reefs, suggesting that the reef length of parallel and circular reefs may have been too small to exert a significant effect on flow. Additionally, clear spatial patterning requires physical forcings strong enough to produce the small-scale feedbacks that drive self-organization (van de Koppel et al. 2012). When water flow is slow, mussel beds do not exhibit pattern formation at scales  $> 1$  m (Ysebaert et al. 2009); therefore, the low velocities observed in this experiment may have also contributed to the lack of strong spatial patterns where slow flow was combined with small reef “length” on parallel and circular reefs.



Self-organization or pattern formation is present in many ecosystems, and is characterized by small-scale positive feedbacks and large-scale negative feedbacks (Rietkirk and van de Koppel 2008). If we consider the adjacent, perpendicular reefs in this study, the observed spatial distribution of sediment trapping and deposition provides a plausible mechanism for long-distance negative feedback on the formation of adjacent reefs. At the reef scale, reef growth is maintained by hydrodynamic interactions that promote erosion from the reef crest, maintaining suitable substrate for oyster settlement and maximizing filtration potential by breaking down concentration gradients. Sediment deposition behind the reef inhibits the growth of reefs in the surrounding area, until the area of depositional influence is surpassed. Both upriver and downriver reefs experienced sediment deposition upstream and downstream on the flood and ebb tides, respectively. This indicates an area of particle trapping between the two reefs, which is consistent with our hypothesis. Gradients in food availability are also implicated in pattern formation in bivalve species, and likely work in concert with sediment dynamics to produce spatial patterns. This was not the focus of this study, but similar hydrodynamic processes contribute to filtration and sediment deposition; therefore, the anticipated self-organizing effects are likely compounded when filtration is considered. Although additional studies are necessary to confirm this mechanism, this study provides a starting point for understanding mesoscale spatial pattern formation in oyster reefs (Grave 1905; McCormick-Ray 1998; 2005).

#### Implications for Oyster Restoration

This study indicates that subtidal oyster reefs alter hydrodynamics and sediment deposition at multiple scales, which has important implications for oyster reef restoration.

The differential loss in reef area between reef orientations has significant impacts on the potential productivity and persistence of oyster reefs. In shallow, subtidal areas targeted for restoration, the gentler slope of circular reefs relative to parallel and perpendicular reefs can result in loss of habitat at the margin; therefore, these reef configurations should be avoided to maximize productivity and ensure reef persistence. The sphere of influence of oyster reefs extends beyond the reef boundaries due to hydrodynamic changes affecting spatial patterns of erosion and deposition. The extent of this influence should determine the placement of oyster reefs relative to one another in an integrated, estuary-wide approach to restoration. Further inquiries about the scale and nature of pattern-formation mechanisms are necessary, but this study demonstrates that oyster reef restoration efforts can benefit from implementing physical aspects of historical reefs that contributed to their success.

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## LITERATURE CITED

- Andrews, J. D. 1979. Pelecypoda: Ostreidae. In *Reproduction of marine invertebrates*, eds. Giese, A. C. and J. S. Pearse, 293-341. New York: Academic Press.
- Carriker, M.R. and P.M. Gaffney. 1996. A catalog of selected species of living oysters (*Ostreacea*) of the world. In *The eastern oyster: Crassostrea virginica*, eds. Kennedy, V.S., R.I.E. Newell, and A.F. Eble, 1-18. College Park: Maryland Sea Grant College.
- Chen, H.S., P.V. Hyer, A.Y. Kuo, and C.S. Fang. 1977. Hydrography and hydrodynamics of Virginia estuaries XI: Mathematical model studies of water quality of the Piankatank estuary. Special Report No. 124 in *Applied Marine Science and Ocean Engineering*, Virginia Institute of Marine Science, Gloucester Point, VA, USA.
- Chen, S., L.P. Sanford, E.W. Koch, F. Shi, and E.W. North. 2007. A nearshore model to investigate the effects of seagrass bed geometry on wave attenuation and suspended sediment transport. *Estuaries and Coasts* 30: 296-310.
- Chesapeake Bay Interpretative Buoy System. 2014. <http://buoybay.noaa.gov>. Accessed 30 October 2014.
- Coen, L.D., and R.E. Grizzle. 2007. The importance of habitat created by molluscan shellfish to managed species along the Atlantic coasts of the United States. *ASMFC Habitat Management Series #8*, Atlantic States Marine Fisheries Commission, Washington, DC. 115 pp.
- Colden, A.M. and R.N. Lipcius. *in press*. Lethal and sublethal effects of sediment burial on oysters. *Marine Ecology Progress Series*.

- 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: 239-247.
- DeAlteris, J.T. 1988. The geomorphic development of Wreck Shoal, a subtidal oyster reef of the James River, Virginia. *Estuaries* 11: 240-249.
- Frost, N.J., M.T. Burrows, M.P. Johnson, M.E. Hanley, and S.J. Hawkins. 2005. Measuring surface complexity in ecological studies. *Limnology and Oceanography: Methods* 3: 203-210.
- Fugate, D.C. and C.T. Friedrichs. 2003. Controls on suspended aggregate size in partially mixed estuaries. *Estuarine, Coastal and Shelf Science* 58: 389-404.
- Fugate, D.C. and C.T. Friedrichs. 2002. Determining concentration and fall velocity of estuarine particle populations using ADV, OBS, and LISST. *Continental Shelf Research* 22: 1867-1886.
- Gedan, K.B., L. Kellogg, and D.L. Breitburg. 2014. Accounting for multiple foundation species in oyster reef restoration benefits. *Restoration Ecology* 22: 1-8.
- Gili, J. and R. Coma. 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends in Ecology and Evolution* 13: 316-321.
- Grave, C. 1905. Investigation for the promotion of the oyster industry of North Carolina. In U.S. Fish Commission Report for 1903, U.S. Commission for Fish and Fisheries, 247-341.
- Gregalis, K.C., Johnson, M.W., and S.P. Powers. 2009. Restored oyster reef location and design affect responses of resident and transient fish, crab, and shellfish species in Mobile Bay, Alabama. *Transactions of the American Fisheries Society* 138: 314-327.

- 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 on in situ methods. *Estuaries and Coasts* 31: 1208-1220.
- Harsh, D., and M.W. Luckenbach. 1999. Materials processing by oysters in patches: interactive roles of current speed and seston composition. In *Oyster reef habitat restoration: a synopsis and synthesis of approaches*, eds. Luckenbach, M.W., R. Mann, and J.A. Wesson, 251-266. Gloucester Point: VIMS Press.
- Hansen, J.C.R., and M.A. Reidenbach. 2013. Wave and tidally driven flows in eelgrass beds and their effect on sediment suspension. *Marine Ecology Progress Series* 448: 271-287.
- Haven, D.S., and J.P. Whitcomb. 1983. The origin and extent of oyster reefs in the James River, Virginia. *Journal of Shellfish Research* 32: 141-151.
- Haven, D.S., R. Morales-Alamo. 1972. Biodeposition as a factor in sedimentation of fine suspended solids in estuaries. In *Geological Society of America Memoir No. 133*, 121-130. New York: Geological Society of America.
- Hedgpeth, J.L. 1953. An introduction to zoogeography of the northwestern Gulf of Mexico with reference to the invertebrate fauna. *Publications of the Institute of Marine Science of the University of Texas* 3: 107-224.
- Holdaway, G. P., P.D. Thorne, D. Flatt, S.E. Jones, and D. Prandle. 1999. Comparison between ADCP and transmissometer measurements of suspended sediment concentration. *Continental Shelf Research* 19: 421-441.

- Jones, N.L., and S.G. Monismith. 2007. Measuring short-period waves in a tidally forced environment with a subsurface pressure gauge. *Limnology and Oceanography: Methods* 5: 317-327.
- Kennedy, V.S. and L.P. Sanford. 1999. The morphology and physical oceanography of unexploited oyster reefs in North America. In *Oyster reef habitat restoration: a synopsis and synthesis of approaches*, eds. Luckenbach, M.W., R. Mann, and J.A. Wesson, 25-46. Gloucester Point: VIMS Press.
- Lenihan, H.S. 1999. Physical-biological coupling on oyster reefs: how habitat structure influences individual performance. *Ecological Monographs* 69: 251-275.
- Lenihan, H.S., C.H. Peterson, and J.M. Allen. 1996. Does flow speed also have a direct effect on growth of active suspension feeders: an experimental test on oysters. *Limnology and Oceanography* 41: 1359-1366.
- Liu, Q., E.J. Weerman, R. Gupta, P.M.J. Herman, H. Olf, and J. van de Koppel. 2014. Biogenic gradients in algal density affect the emergent properties of spatially self-organized mussel beds. *Journal of the Royal Society: Interface* 11: 1-9.
- McCormick-Ray, J. 2005. Historical oyster reef connections to Chesapeake Bay – a framework for consideration. *Estuarine, Coastal and Shelf Science* 64: 119-134.
- McCormick-Ray, M.G. 1998. Oyster reefs in 1878 seascape pattern – Winslow revisited. *Estuaries* 4B: 784 – 800.
- Mistr, S. and D. Vercovici. 2003. A theoretical model of pattern formation in coral reefs. *Ecosystems* 6: 61-74.
- 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: 347-368.
- Plumb, R.H Jr. 1981. Procedures for Handling and Chemical Analysis of Sediment and Water Samples. Technical Report EPA/CE-81-1. Great Lakes Laboratory, State University College at Buffalo, Buffalo, NY. pp 403.
- Powell, E.N., J. Song, M.S. Ellis, and E.A. Wilson-Ormond. 1995. The status and long-term trends of oyster reefs in Galveston Bay, Texas. *Journal of Shellfish Research* 142: 439-457.
- Price, W.A., 1954. Oyster reefs of the Gulf of Mexico. In *Gulf of Mexico: Its Origin, Waters, and Marine Life*, ed. P. Galtsoff, 39-65. U.S. Fish and Wildlife Service Fishery Bulletin No. 89.
- Reidenbach, M.A., P. Berg, A. Hume, J.C.R. 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: 225-239.
- Reidenbach, M. A., M. Limm, M. Hondzo, and M. T. Stacey. 2010. Effects of bed roughness on boundary layer mixing and mass flux across the sediment-water interface. *Water Resources Research* 46: 1-15.
- Rietkirk, M., and van de Koppel, J. 2008. Regular pattern formation in real ecosystems. *Trends in Ecology and Evolution* 23: 169-175.
- Rothschild, B.J., J.S. Ault, P. Gouletquer, and M. Héral. 1994. Declines of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. *Marine Ecology Progress Series* 111: 29-39.

- Seim, H.E., J.C. Gregg, and R.T. Miyamoto. 1995. Acoustic backscatter from turbulent microstructure. *Journal of Atmospheric and Oceanic Technology* 12: 367-380.
- Smith, G.F., D.G. Bruce, E.B. Roach, A. Hansen, R.I.E. Newell, and A.M. McManus. 2005. Assessment of recent habitat conditions of eastern oyster *Crassostrea virginica* bars in mesohaline Chesapeake Bay. *North American Journal of Fisheries Management* 25: 1569-1590.
- Smith, G.F., E.B. Roach, and D.G. Bruce. 2003. The location, composition, and origin of oyster bars in mesohaline Chesapeake Bay. *Estuarine, Coastal and Shelf Science* 56: 391-409.
- U.S. Army Corps of Engineers. 2012. Chesapeake Bay oyster recovery: Native oyster restoration master plan for Maryland and Virginia.
- van de Koppel, J., T.J. Bouma, and P.M.J. Herman. 2012. The influence of local- and landscape-scale processes on spatial self-organization in estuarine ecosystems. *Journal of Experimental Biology* 215: 962-967.
- van der Zee, E.M., van der Heide, T., Donadi, S., Eklof, J.S., Eriksson, B.K., Olf, H., van der Veer, H.K. and Piersma, T. 2012. Spatially extended habitat modification by intertidal reef-building bivalve has implications for consumer-resource interactions. *Ecosystems* 15: 664-673.
- van Leeuwen, B., D.C.M. Augustijn, B.K. van Wesenbeeck, S.J.M.H. Hulscher, and M.B. de Vries. 2010. Modeling the influence of a young mussel bed on fine sediment dynamics on an intertidal flat in the Wadden Sea. *Ecological Engineering* 26: 145-153.

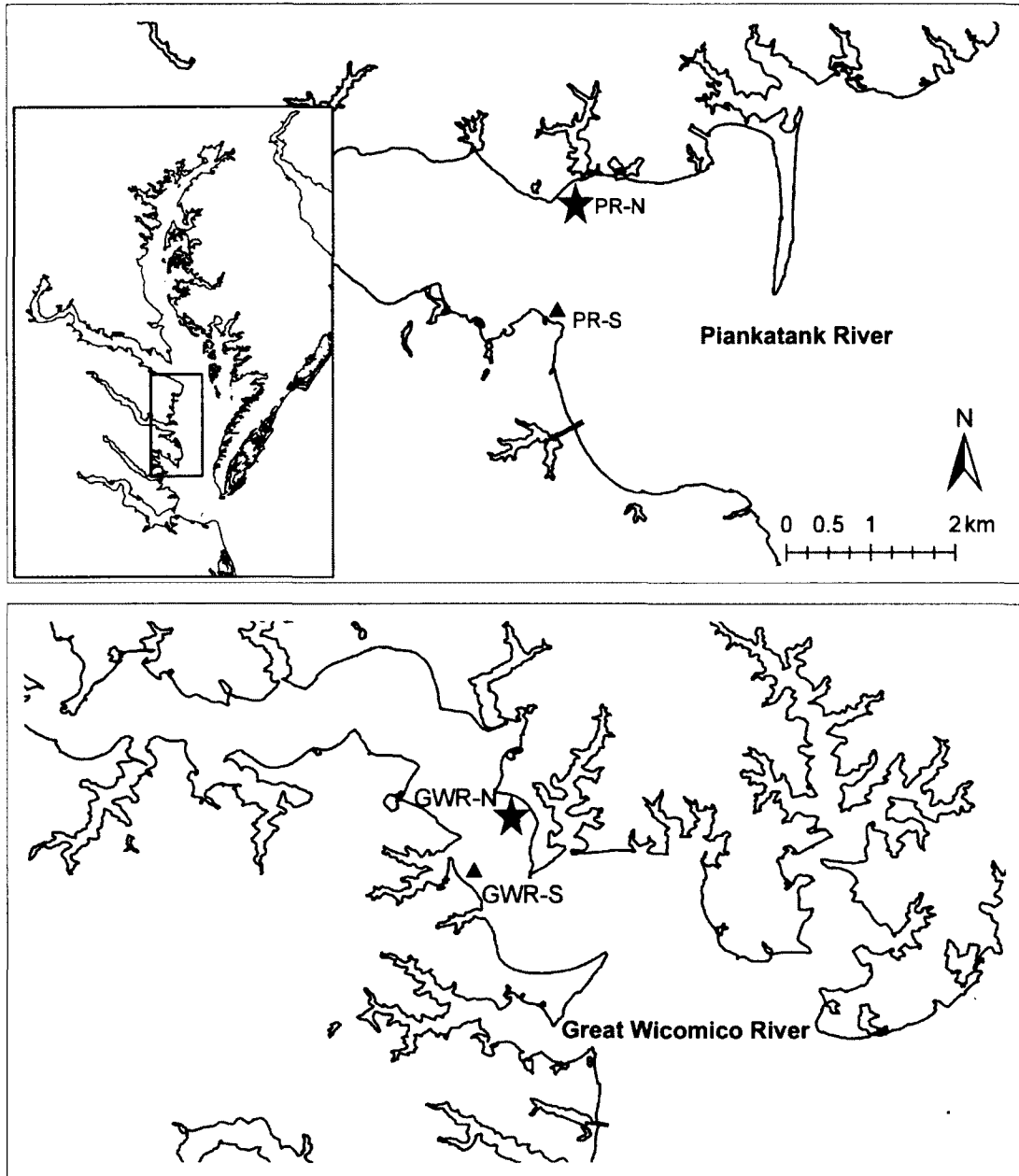


- Walles, B., J.S. de Paiva, B.C. van Prooijen, T. Ysebaert, and A.C. Smaal. 2014. The ecosystem engineer *Crassostrea gigas* affects tidal flat morphology beyond the boundary of their reef structures. *Estuaries and Coasts*. doi: 10.1007/s12237-014-9860-z.
- Wesson, J.A., R. Mann, and M.W. Luckenbach. 1999. Oyster restoration efforts in Virginia. In *Oyster reef habitat restoration: a synopsis and synthesis of approaches*, eds. Luckenbach, M.W., R. Mann, and J.A. Wesson, 117-129. Gloucester Point: VIMS Press.
- Whitman, E.R. and M.A. Reidenbach. 2012. Benthic flow environmental affect recruitment of *Crassostrea virginica* larvae to an intertidal oyster reef. *Marine Ecology Progress Series* 463: 177-191.
- Widdows, J., N.D. Pope, M.D. Brinsley, H. Asmus, and R.M. Asmus. 2008. Effects of seagrass beds (*Zostera noltii* and *Z. marina*) on near-bed hydrodynamics and sediment resuspension. *Marine Ecology Progress Series* 358: 125-136.
- Wilberg, M.J., M.E. Livings, J.S. Barkman, B.T. Morris, and J.M. Robinson. 2011. Overfishing, disease, habitat loss and potential extirpation of oysters in upper Chesapeake Bay. *Marine Ecology Progress Series* 436: 131-144.
- Woods, H., W.J. Hargis, C.H. Hershner, and P. Mason. 2005. Disappearance of the natural emergent 3-dimensional oyster reef systems of the James River, Virginia, 1871-1948. *Journal of Shellfish Research* 24: 139-142.
- Ysebaert, T., Hart, M., and P.M.J. Herman. 2009. Impacts of bottom and suspended cultures of mussels *Mytilus* spp. on the surrounding sedimentary environment and macrobenthic biodiversity. *Helgoland Marine Research* 63: 59-74.

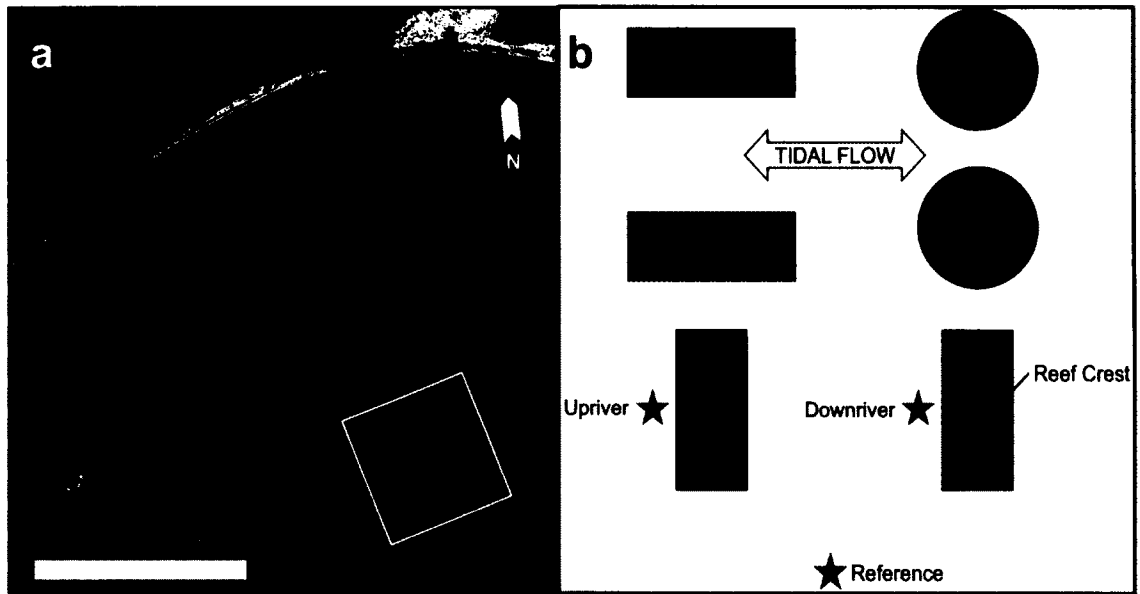
Zimmerman, R.J., T. Minello, T. Baumer, and M. Castiglione. 1989. Oyster reef as habitat for estuarine macrofauna. NOAA Technical Memorandum, NMFS-SEFC-249, 16 pp.

Zu Ermgassen, P.S.E., Spalding, M.D., Blake, B., Coen, L.D., Dumbauld, B., Geiger, S., Grabowshi, J.H., Grizzle, R., Luckenbach, M., McGraw, K., Rodney, W., Ruesink, J.L., Powers, S.P., and R. Brumbaugh. 2012. Historical ecology with real numbers: past and present extent and biomass of an imperiled estuarine habitat. Proceedings of the Royal Society B. doi:10.1098/rspb.2012.0313.

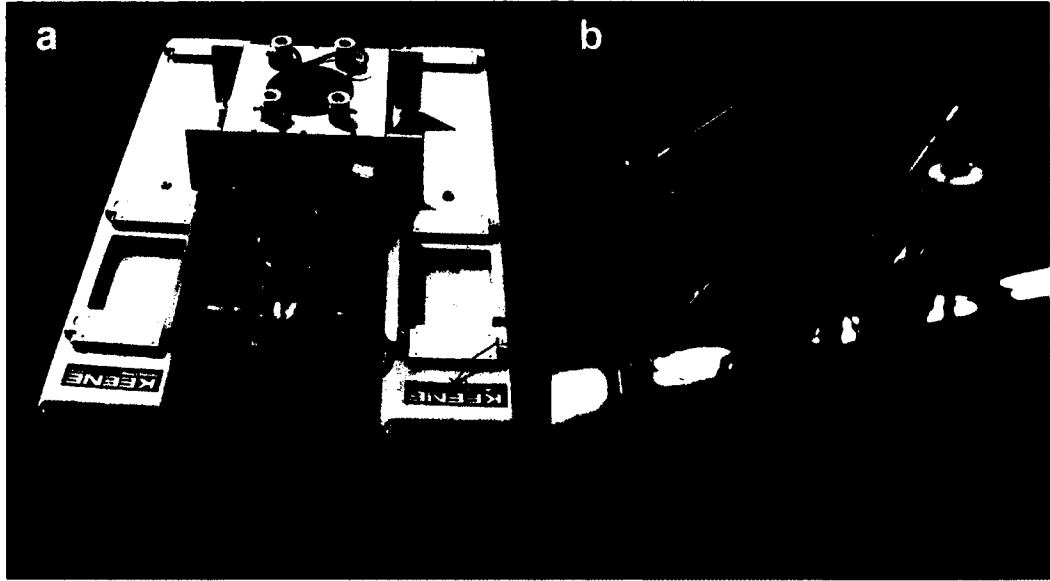
Zimmerman, R.J., T. Minello, T. Baumer, and M. Castiglione. 1989. Oyster reef as habitat for estuarine macrofauna. NOAA Technical Memorandum, NMFS-SEFC-249, 16p.



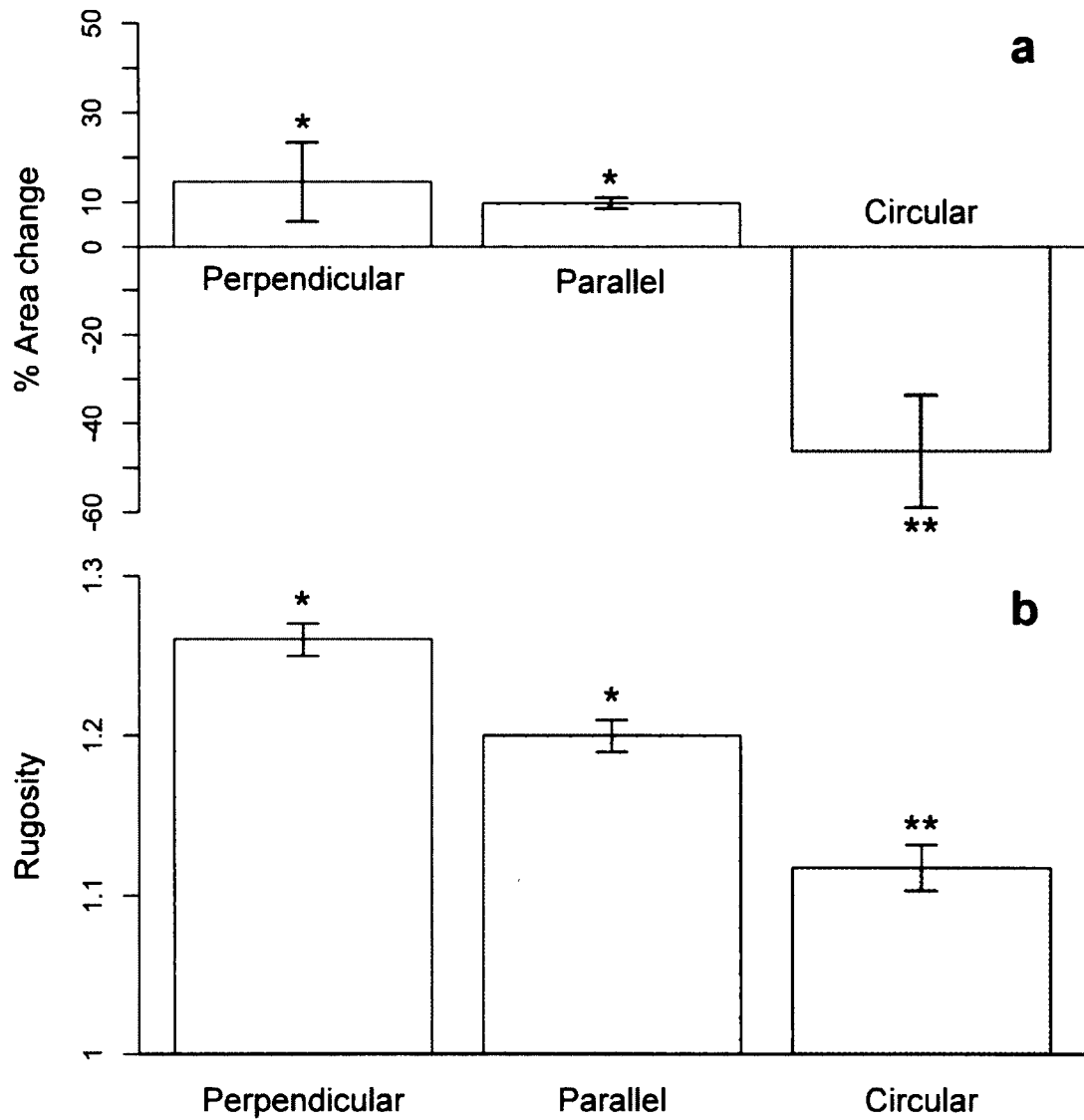
**Fig. 1** Location of reef orientation study locations within two sub-estuaries of Chesapeake Bay (inset), the Piankatank River (PR) and Great Wicomico River (GWR). Stars indicate pilot study locations. The Piankatank hydrodynamics study was conducted at PR-N



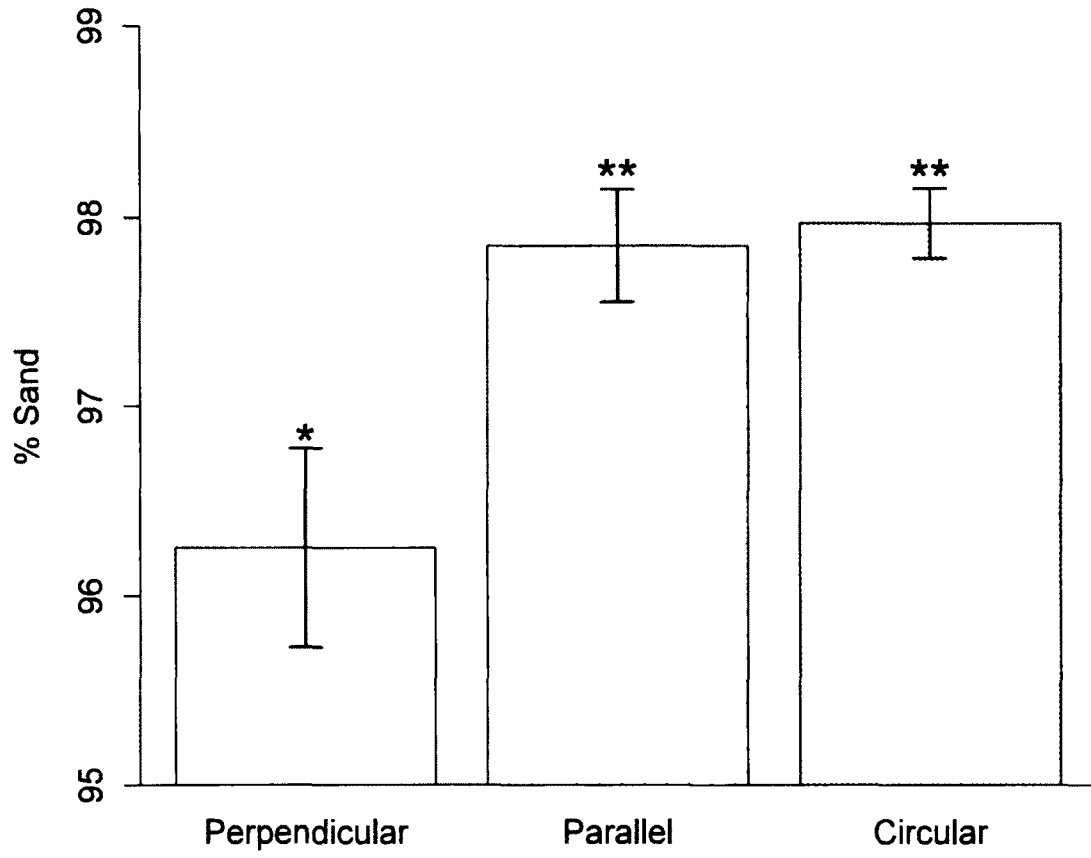
**Fig. 2** Satellite imagery of reefs at the PR-N site (a) highlighted by the white box and (b) reef layout and dimensions at PR-N. Platform deployment locations are indicated by stars. Satellite imagery obtained from Google Earth



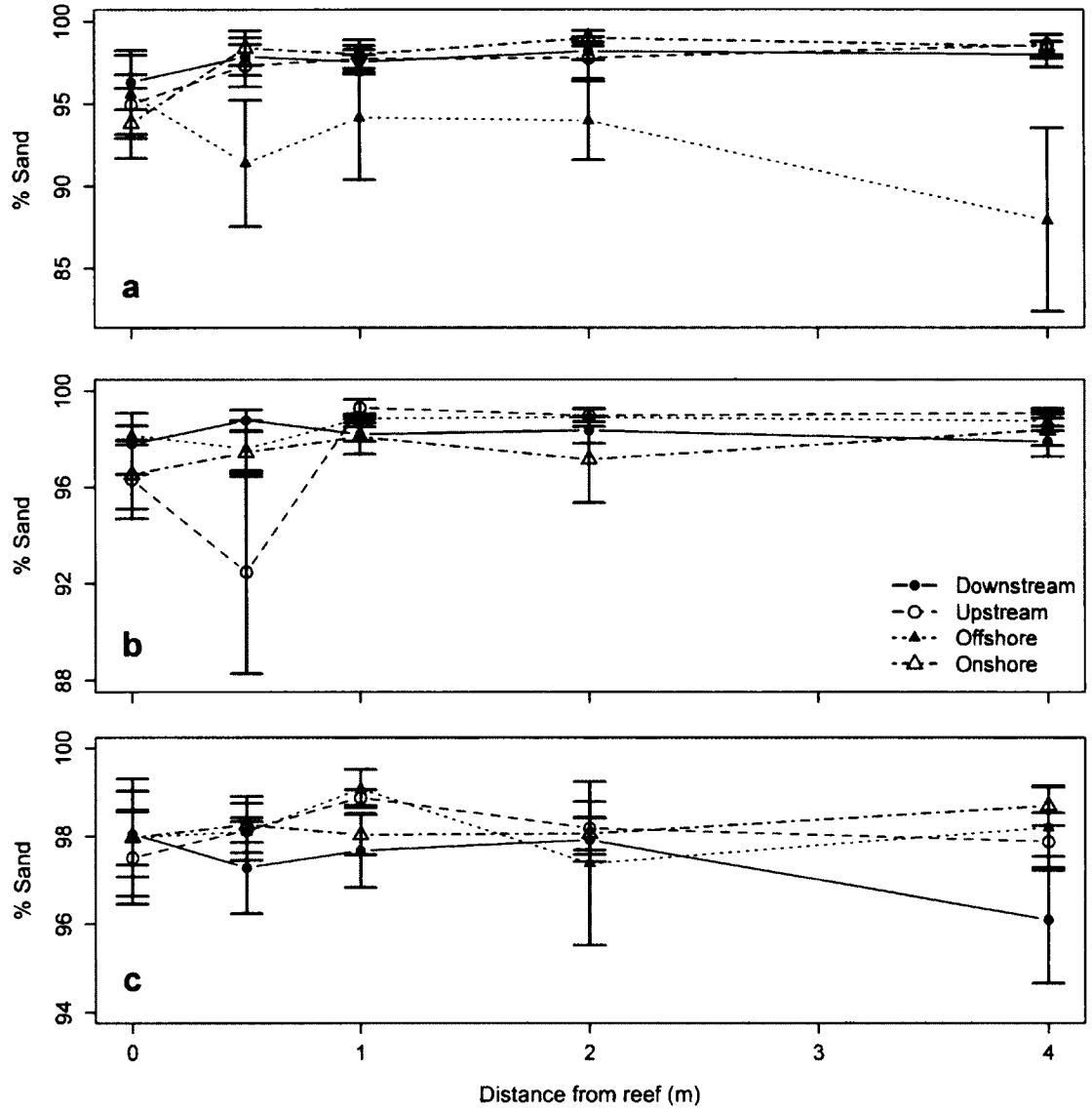
**Fig. 3** Field instrumentation for water column observations: (a) Floating ADCP platform used in pilot study; (b) Stationary instrument platform used in main hydrodynamics study



**Fig. 4** Mean ( $\pm 1$  SE) % area change (a) and rugosity (b) across reef types at PR-N. Asterisks indicate significant differences between reef orientations (ANOVA, Tukey HSD;  $p < 0.05$ )

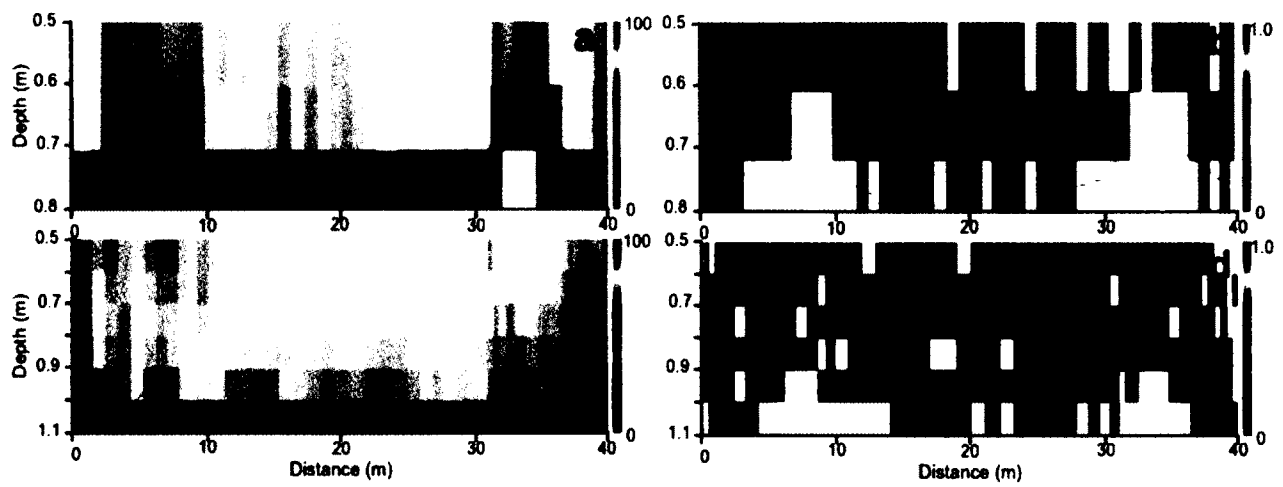


**Fig. 5** Mean ( $\pm 1$  SE) grain size as a function of reef type. Asterisks indicate significant differences in grain size across reef types (ANOVA, Tukey HSD;  $p < 0.05$ )

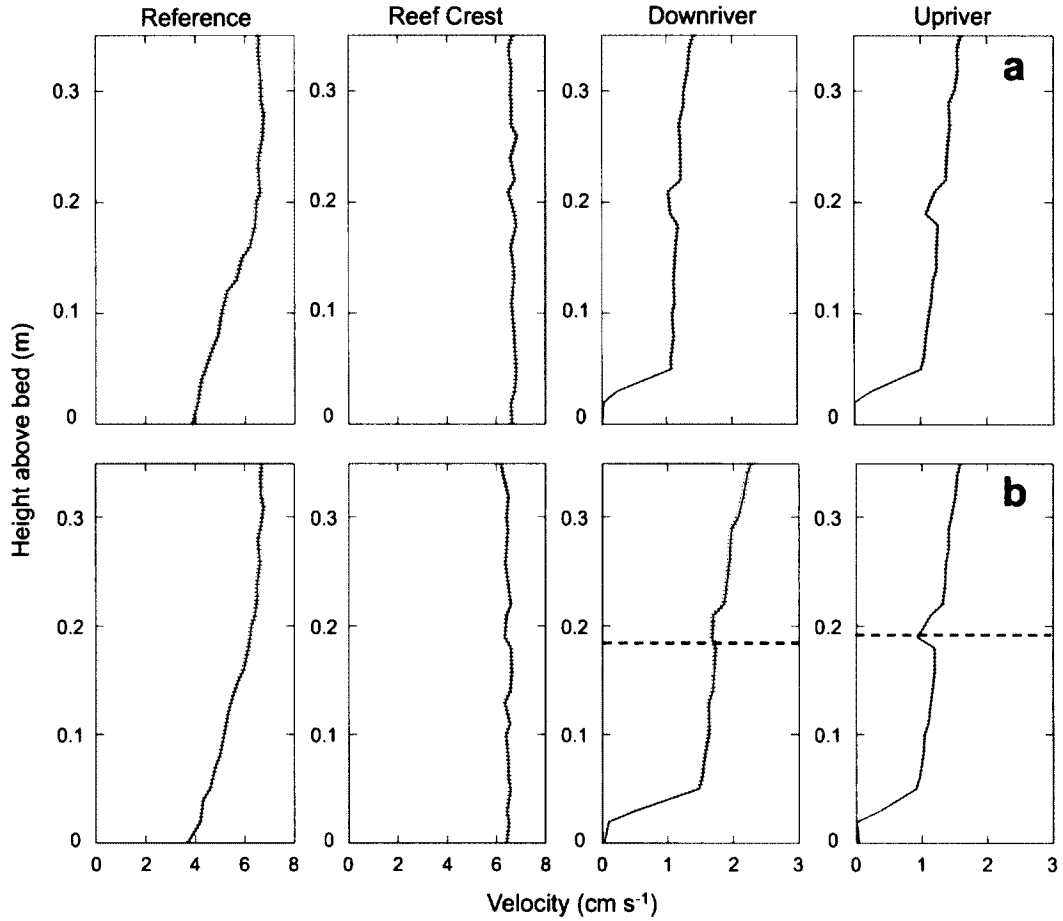


**Fig. 6** Mean grain size ( $\pm 1$  SE) as a function of distance from the reef for (a) perpendicular, (b), parallel, and (c) circular reefs across all 4 study sites ( $n = 8$ )

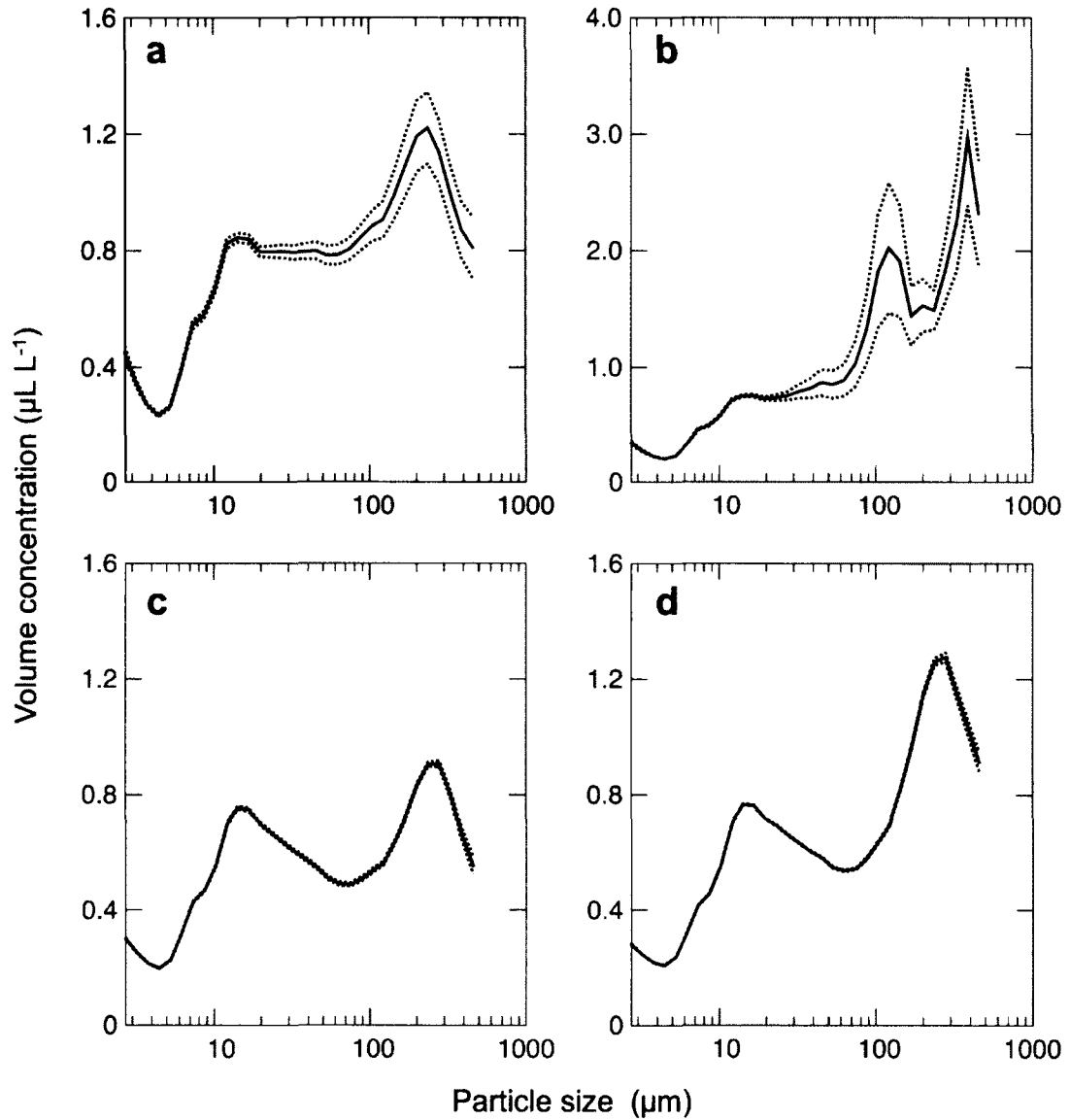




**Fig. 7** ADCP (a,c) acoustic backscatter (dB) and (b,d) velocity transects ( $m s^{-1}$ ) from the pilot hydrodynamics study at PR-N between (a,b) parallel and circular reefs and (c,d) between two perpendicular reefs. The solid black line indicates the height above which velocity data is not contaminated by side-lobe reflection off the bottom



**Fig. 8** Tidal phase-averaged current speed profiles for (a) flood and (b) ebb tides for PR-N main hydrodynamics study. Dashed lines on the upriver and downriver reef plots indicate the mean height of the reef. Height above the bed for the reef crest location is measured from the reef surface; all other locations are measured from the seabed. Note differences in velocity scale between sample locations



**Fig. 9** Mean particle size distributions (solid line) for PR-N main hydrodynamics study: (a) offshore reference site; (b) reef crest; (c) adjacent to upriver perpendicular reef; (d) adjacent to downriver perpendicular reef. Data are averaged over tidal phase. Dashed lines represent  $\pm 1$  SE. SE intervals for the downriver and upriver locations are present, but not visible due to scale

## CONCLUSIONS

The objectives of this research were to identify and quantify the biophysical interactions between oysters, reefs, and local hydrodynamic conditions controlling oyster reef performance in Chesapeake Bay. My results indicated that oyster-sediment interactions are complex and operate on various organizational and spatial scales. These findings provide insights into the role of biophysical interactions in previous oyster population collapses and provide valuable information for improving current oyster restoration efforts.

Oyster populations exhibited threshold dynamics in response to sedimentation modulated by reef height. Individual oysters partially buried by sediment demonstrated reduced metabolic activity, namely biodeposition, that contributes to reef accretion and persistence. From these observations, a new hypothesis of the sequential collapse of oyster populations emerged in which the reduction in reef height of exploited oyster reefs due to destructive harvesting led to increased sedimentation. Reef accretion was slowed due to reductions in metabolic output because of sedimentation stress. Increased vulnerability to siltation due to reductions in reef height and the inability of smaller, stressed populations to process additional material pushed the reef population to a tipping point at which habitat loss was inevitable. Strong positive feedbacks between these processes could explain how the once-dominant Chesapeake Bay went from its peak abundance to near extirpation in less than 150 y. Other factors such as disease and water

quality also contributed to the decimation of oyster populations, but that the largest decline in oyster stocks occurred before large-scale epizootics and eutrophication suggests that other mechanisms likely spurred the decline.

Using lessons from the past can help to improve restoration efforts today. The design of individual restoration reefs to maximize oyster population enhancement and ecosystem services would be improved by the application of reef structural characteristics that typified historical oyster reefs, namely high vertical relief and orientation to prevailing hydrodynamic forcings. The aim of this element was not to ask “how high?” but rather “how high is high enough?” Recommendations for reef design criteria must be balanced with realistic expectations of logistics and budget, which was achieved through the identification of reef trajectory thresholds at which degradation trajectories switch to persistence trajectories, maximizing the probability of long-term reef productivity while minimizing cost.

The tendency for oyster reefs to influence areas far afield of the reef suggests that current piecemeal approaches to restoration are inadequate. The construction and design (size, shape, orientation) of reefs must take into account the role of each reef in the larger estuarine reefscape. Depending on size and orientation, reefs have the potential to negatively influence other reefs in the vicinity and undermine costly restoration efforts that could have been avoided. This research indicates that biophysical interactions influence the performance of oysters and oyster reefs at multiple scales. The influence of hydrodynamics and sediment dynamics is a critical part of reef design and should be considered in a comprehensive, estuary-scale approach to restoration.

## VITA

Born in Norfolk, Virginia, 24 March 1987. Graduated from the International Baccalaureate Program at Princess Anne High School in Virginia Beach, Virginia in 2005. Earned B.S. in Biology with a concentration in Ecological and Biological Conservation from the University of Virginia in 2009. Entered the doctoral program at the College of William and Mary, School of Marine Science in 2009.