2010

Alternative substrates as a native oyster (Crassostrea virginica) reef restoration strategy in Chesapeake Bay

Russell Paul Burke
College of William and Mary - Virginia Institute of Marine Science

Alternative Substrates as a Native Oyster (*Crassostrea virginica*) Reef Restoration Strategy in Chesapeake Bay

---

A Dissertation
Submitted 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

---

by
Russell Paul Burke
2010

APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

_____

Russell P. Burke

Approved by the Committee, April 2010

_____

Romuald N. Lipcius, Ph.D.
Chair of Advisory Committee

_____

Mark W. Luckenbach, Ph.D.

_____

Rochelle D. Seitz, Ph.D.

_____

Harry V. Wang, Ph.D.

_____

Jian Shen, Ph.D.

_____

Sebastian J. Schreiber, Ph.D.
University of California, Davis

To my parents, Linda and Russ

"Taken together, paleontological, archaeological, historical, geological and ecological evidence shows that oysters set, survive and grow better on elevated reefs with substantial 'cores' of oyster shells and 'cinders', and other suitable substrate, and healthy 'veneers' of living oysters than on beds near or on the bottom. Spatfall is better, growth is faster, predation effects are lower and disease-related effects reduced. Oysters lying flat on the bottom or partially submerged in the bottom do not fare nearly as well. Relative successes of 'off-bottom culture' efforts employing man-made structures to maintain the living oysters off of the bottom in disease- and predation-prone areas confirm this."

> Hargis WJ, Haven DS (1995) Chesapeake Bay oyster reefs, their importance, destruction and guidelines for restoring them. p. 329-358 *In*: M. Luckenbach, R. Mann, and J. Wesson, eds. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

"Success can come only with realistic goals couched within comprehensive and quantitative analysis delineating planned actions in concert within the complex interplay between population dynamics and habitat maintenance."

> Mann R, Powell EN (2007) Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. *Journal of Shellfish Research*, 26(4), 1-13

"Well-intentioned yet poorly 'designed' reefs, when monitored and appraised against original expectations, may lead the assessors to conclude that 'reefs don't work' when, with the correct habitat requirement information for the target species, the end result would have been successful."

> Jensen AC, Collins KJ, Lockwood P (2000) Current issues relating to artificial reefs in European seas. p. 489–499 *In:* A.C. Jensen, K.J. Collins and A.P.M. Lockwood, eds. Artificial reefs in European seas. Kluwer Academics Publishers, London

TABLE OF CONTENTS

Chapter 5:
Living Oyster Reef Shorelines Using Shell and Alternative Structures in the Lynnhaven River, Chesapeake Bay

ACKNOWLEDGMENTS

I owe my utmost thanks to my parents, Linda and Russ Burke, and my wife, Candy Burke Huayhualla. Truly, there is no way I would have survived the many trials of my young adult life without their unconditional love and support. The best relationships I have seen, and been a part of, have had a healthy mix of independence and interdependence. I witnessed that balance between my parents and have found a similar dynamic relationship with my soul mate, Candy. I was seriously considering leaving graduate school in the fall of 2003. Meeting Candy changed me forever. . .she filled a hole in my heart and helped me discover real happiness. She has brought out the best in me; this degree is hers as much as it is mine. We'll share it together.

I owe a special thanks to my advisor, Rom Lipcius. The reason I decided to come work with Rom in 2002 is the same reason I remained here for the duration of my graduate career – his heartfelt compassion, positive outlook on life and science, and his ability to recognize a real opportunity even if it was simply the result of serendipity. When my father passed away in June 2003, he gave me a wide berth as I grieved and searched my soul for direction and meaning. The flexibility afforded me was crucial to my recovery and integral in my growing as a scientist, as I also wrestled with finding a project that fit me. Fortunately, Rom reached out to Dave Schulte and Captain Bob Jensen, men who ended up shaping my academic path in ways none of us could have predicted. I am grateful for Rom's willingness to trust my judgment and his support of my passion and ethics. I have learned much about science under Rom's tutelage, but even more about how politics can shape the way that science is implemented at the local, state, and federal levels of government. Finally, I wish to thank Rom for keeping me funded through my degree extension and for supporting my travel to professional conferences in Connecticut, Canada, France, North Carolina, California, and South Carolina. He has made a commitment to the professional development of his students and staff, and we are all grateful.

I am especially thankful to my committee members for their support and understanding. My dissertation research came together in an unconventional way and did not follow the milestone schedule well. Sebastian Schreiber helped me to understand mathematical modeling in fall 2003 when I was really struggling. His enthusiasm for math and science was infectious and, I'm happy to admit, I got the bug. And, despite my inability to incorporate modeling into my dissertation due to time constraints, Harry Wang and Jian Shen helped educate me on how physical oceanography affects oyster larval dispersal and metapopulation connectivity, and how hydrodynamic modeling can integrate biological and physical data to produce practical tools.

When it came to field work, Rochelle Seitz guided me through my earliest field days, instructing me and the many students, interns, and summer aides "what lies beneath" in the benthos. Rochelle's domain is secondary production and, during the years of my research, I have come to understand the value of benthic-pelagic coupling and importance of monitoring the benthic community before, during, and after implementing an experiment or survey.

Harry and Mark Luckenbach both gave me something I needed but have always had a problem requesting – constructive criticism. A scientist is no good without an honest critique of his/her work. Harry reached out to me and, though we both were outside of our comfort zone, he challenged me and my track record. It was what I needed at the time and I am grateful for his courage and his candor. Mark's critiques came in the form of detailed edits and comments in my prospectus and dissertation drafts. His thorough reviews of both documents contributed significantly (sorry, no *p*-value) to my progress through this degree program.

In summary, my committee and moderators (Steve Kaattari and Jim Perry) made a positive difference for me. Looking back on my dissertation research and the other projects I found myself working on, or even running, I am proud of my commitment and accomplishments; I believe that I earned the respect of those with whom I worked.

The cast of characters of the five-act play that has been my graduate experience is long and incredibly diverse. Captain Bob Jensen (Reeftek-McLean), our own King Lear, inspired us with his vision and passion for the Chesapeake Bay and the eastern oyster. David Bushey (Commonwealth Pro Dive), the jack of all marine science trades (diver, reef builder, side scan sonar specialist, etc.), helped make Captain Bob's vision of quantifying his reefs at Steamer Rock a reality and jumpstarted my research. Darryl Nixon (Getting It Done, Inc.), the self-taught scientist with an insatiable appetite for knowledge, conditioned and delivered reefballs for my final project, and stuck around to become a "shadow advisor" to these and potential future projects. The last character, but perhaps the most influential one, has been Dave Schulte (Army Corps/VIMS), our own Sisyphus, who carried the burden of designing and implementing a federal oyster restoration program that would solidify the paradigm shift from oyster harvest grounds to large-scale permanent oyster reef sanctuaries. Dave's commitment to do the right thing by the environment and the taxpayer, despite the political and peer pressure to do otherwise, inspired me. Our working relationship and friendship have deep meaning for me. We have humbly dubbed ourselves the next Bill Hargis and Dexter Haven, partly because of how we have fought together to save this oyster restoration program in Virginia, and partly due to our great respect for what they achieved in their decades of service to the institute, the state, and science, in general; in no small way, I feel like the torch needed to be passed and we were just naïve and stubborn enough to continue the epic journey started by Hargis and Haven long ago. As they could not have achieved what they did at VIMS without considerable assistance from others, there are many people who deserve credit for their participation and support.

The Marine Conservation Biology and Marine Community Ecology lab groups were the backbone of my field research. Mike Seebo, Jacques van Montfrans, Katie Knick, Alison Smith and Danielle McCulloch always brought their "A" game for long, busy days on the Rappahannock and Lynnhaven Rivers. Jill Dowdy, Paul Gerdes, and Ryan Gill, along with Gina Ralph, Gabby Saluta, Cassie Bradley, Allison Colden, Liza Hernandez, Justin Falls, Diane Tulipani, James Douglass, Bryce Brylawski, Chris Long, Dave Hewitt, Deb Lambert, Amanda Lawless, Justine Woodward, Caitlin Bovery, Rachel Ward, Ethan Theuerkauf, Seth Theuerkauf, Jessica Showalter, Emily Kimminau, Katelynn Jenkins, and Elena Tenore all logged in hours on the Lynnhaven River projects, which speaks to the required manpower for some of this research.

On top of an incredible team of staff and students, other individuals and groups at VIMS have played an integral part in the successful completion of this research. David Evans mentored me as his Statistics Teaching Assistant, an experience that prepared me for teaching a graduate course of my own design at Christopher Newport University in fall 2008. Ryan Carnegie and Shellfish Pathology Lab are responsible for all we discovered about Dermo and MSX disease in oysters from 2005-2009. Stan Allen and the Aquaculture Genetics and Breeding Technology Center contributed oyster larval production, hatchery setting on various substrates, and oyster ploidy testing through flow cytometry. PG Ross and the Eastern Shore Lab spent two grueling days in the summer sun transporting and deploying the alternative substrate experiment in Lynnhaven. Sharon Miller, Susan Rollins, and the rest of the Vessel Operations crew worked closely with us over many years meeting our needs during high vessel demand, inclement weather, ceased engines, blown trailer tires, and unpredictable incidents (i.e. a broken leg on the Rappahannock River). Cindy Forrester, Gail Reardon, and Grace Walser of the Fisheries Sciences Department saved the day when freezers died, impromptu travel authorizations had to be processed, when we all struggled our way through the eVA procurement program for the first time, international travel plans needed to be made, and other fun quandaries. Kevin Kiley, Gary Anderson, Bob Polley and the rest of the Information Technology and Networking Services team helped me resolve computer issues, set up classes during my teaching assistantship, and prepare for teleconferences and seminars. The Customer Service Center and Communications and Publications departments contributed to the success of this work, often providing small miracles at a moment's notice. Special thanks to Diane Perry, Fonda Powell, Sue Presson, and Louise Lawson for administrative support. Lastly, I wish to thank Tom Grose, Paul Nichols, and Gloria Rowe of the Safety Office and Howard Kator who all stuck by me through an incredible ordeal as I recovered from a field-related bacterial infection (MRSA) and fought insurance companies and prepared for Workmen's Compensation appeal hearings over an 18-month period.

External agency and individual support rounded out the whole graduate experience. Special thanks to the US Army Corps of Engineers (Norfolk District) and the National Oceanic and Atmospheric Administration Chesapeake Bay Office who provided funding for some of this research. The Rappahannock Preservation Society, Lynnhaven River Now (Laurie Sorabella-Carroll, Richard Serpe, Cliff Love; Mr. and Mrs. Chalmers, Handeland, Clifton, Yopp, and Guion), and the Chesapeake Bay Foundation (Tommy Leggett, Claire Hudson, and Christy Everett) are non-profit organizations that contributed planning and field support on two major projects. Justin Worrell's (Virginia Marine Resources Commission) patience and flexibility as I worked to submit two permit applications helped me tremendously.

I consider myself blessed to have had these years at the College of William and Mary and the Virginia Institute of Marine Science, and want everyone who has contributed to my life personally and professionally to know that I have carried you in my heart. When I was running on empty, the knowledge of your investment in me is what I called upon as my personal reserve. Thank you.

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF APPENDICES

ABSTRACT

Oyster shell for native oyster reef restoration is scarce in Chesapeake Bay and other estuaries (Chapter 1). Consequently, alternative substrates merit consideration in oyster restoration. This dissertation examines the suitability of shell alternatives, including granite, concrete, limestone marl, concrete modules and reefballs with reef surveys and experiments in the Rappahannock and Lynnhaven Rivers of Chesapeake Bay. Oyster recruitment, growth, survival, density, biomass, condition, and disease stress, as well as reef accretion and persistence, were measured. In the Lynnhaven River, intertidal riprap had a mean density of 978 oysters m^{-2} (165 g AFDM m^{-2}) and peak densities > 2000 oysters m^{-2} (Chapter 2), which are among the highest abundances on alternative reefs, shell or otherwise. Riprap reefs supported a robust population size structure, signifying consistent annual recruitment and reef sustainability. Riprap age (older > younger) and location influenced reef performance; granite and concrete both supported dense oyster-mussel assemblages. In 2005 and 2007, oyster and mussel population structure, density and biomass were quantified on a novel, subtidal concrete modular reef deployed in 2000 in the Rappahannock River (Chapter 3). The reef was not seeded or harvested. Densities (m^{-2} river bottom) were very high for oysters (2005: 991 m^{-2}; 2007: 2191 m^{-2}) and mussels (2005: 8433 m^{-2}; 2007: 6984 m^{-2}) and comparable to the highest densities on shell reefs. An adjoining 0.44 ha array of concrete reefs (Steamer Rock) was deployed in 1994 and sampled in 2006. These reefs contained > 4 million oysters and > 30 million mussels. Oysters from both reef systems had low disease prevalence and intensity. In a field experiment (Chapter 4), treatments simulating oyster habitat were placed at three intertidal sites in Long Creek of the Lynnhaven River. Granite had highest oyster recruitment and abundance (density > 1500 m^{-2} and biomass > 200 g AFDM m^{-2}). Many reefs reached a mature state after two years. By Year 3, some reefs had accreted 15-20 L of shell m^{-2} river bottom, and contained three year classes; some treatments had > 30 % of live oysters growing on other oysters. Large oysters (> 95 mm shell height) had lower intensities of Dermo infection than smaller (60-90 mm) oysters. These patterns indicate that oyster disease tolerance has developed in these high-salinity waters, and highlight the importance of substrate type and reef location in ecological oyster reef restoration. In summer 2006, nine reefs were constructed at two shoreline sites in the Lynnhaven River (Chapter 5), three each of oyster shell (OS), riprap (RR), and concrete modules (CM). Six reefballs were placed at each site, half pre-seeded with hatchery-reared oysters. Finally, *in situ* setting of triploid oyster larvae on OS, RR and CM reefs was attempted. After 2.5 yrs, all reefs had high oyster density and biomass (unseeded: 150-1200 m^{-2}, 150-600 g AFDM m^{-2}; seeded: 30-1800 oysters m^{-2}), and sustainable accretion rates (8-15 L m^{-2} yr^{-1}); diploid and triploid oysters had light Dermo infections. Consequently, alternative substrates can serve as effective oyster reefs under diverse conditions in subtidal and intertidal environments of Chesapeake Bay.

Alternative Substrates as a Native Oyster (*Crassostrea virginica*) Reef Restoration Strategy in Chesapeake Bay

Chapter 1

An Introduction to Native Oyster Reef Restoration and the Use of Alternative Substrates

ABSTRACT: Restoration efforts with native Eastern oyster (*Crassostrea virginica*) in Chesapeake Bay have been extensive, yet impeded by substrate and recruitment limitations along with numerous other environmental and political factors. Nearly 150 years of exhaustive and destructive oyster harvest techniques, combined with increased agricultural runoff, sedimentation, nutrient input and environmental pollution, have relegated the bay's population to < 1 % its historic standing stock. Increasingly intensive and mechanized fishing contributed to leveling the profile of oyster reefs, forcing most of the remaining oysters to struggle for survival in and around the sediment-water interface where sedimentation, poor food supply, and low dissolved oxygen impeded the immune system of susceptible oysters. Under such conditions, parasites (Dermo – *Perkinsus marinus* and MSX – *Haplosploridium nelsoni*) have thrived and suppressed the immune systems of a weakened oyster population for nearly half a century. The demand for a disease-resistant oyster intensified as the public oyster fishery and its economic infrastructure struggled. Political pressure from the bay's seafood industry led to efforts to selective breeding and consider the introduction of a nonnative oyster species with inherent resistance to Dermo and MSX. A critical, yet often overlooked, effect of the oyster fishery was the intense and systematic negative selection for slower-growing oysters. Those oysters best genetically-equipped to sustain the debilitating effects of disease were systematically removed from populations year after year, removing one of the native oyster's last natural mitigating defenses to disease – the worst possible scenario given the persistent habitat destruction preceding these disease outbreaks. More recently, however, natural native oyster disease resistance has been detected among sanctuary oyster populations in the Lynnhaven, Elizabeth, Rappahannock and the Great Wicomico Rivers. If oyster condition (including the debilitating effects of disease) is influenced by its ambient living conditions, then reef architecture and substrate quality play an important role in oyster population recovery. With oyster shell in limited supply, alternative substrates must be considered part of adaptive management in oyster restoration. This dissertation addresses the suitability of shell alternatives, including granite, recycled concrete, limestone marl, concrete modules and reefballs, for the large-scale ecological restoration of native oyster reefs in the Chesapeake Bay and its tributaries.

Introduction. Temperate estuaries such as Chesapeake Bay have undergone profound changes worldwide due to human exploitation and pollution, rendering them the most degraded of marine ecosystems (Jackson et al. 2001). One of the most critical alterations has been the near-complete eradication of oyster populations and their reefs (Rothschild et al. 1994). Their destruction can be linked primarily to the surge of humans around the bay and beyond and their demand for oysters and shells (Hargis and Haven 1999). Benthic invertebrates such as the oyster are extremely important in nutrient recycling and benthic pelagic coupling (Rhoads 1974, Boynton et al. 1980, Dame et al. 1980) and molluscan suspension feeders may act as a natural control of the adverse effects of eutrophication in estuaries (Cloern 1981, Cohen et al. 1984, Officer et al. 1982, Newell 1988). Thus, overfishing of oysters to the point of ecological extinction has dramatically changed the health of the bay (Jackson et al. 2001).

An adaptive, ecosystem-based management program for the Chesapeake Bay and the recovery of its oyster populations will require the application of diverse methods and a variety of substrates to be successful. It is the goal of this dissertation research to demonstrate the importance of alternative oyster reef substrates to the ongoing, large-scale restoration.

Ecological and fishery restoration, though separate in many ways, share one common thread – they both rely on the native eastern oyster (*Crassostrea virginica*) to be successful. Furthermore, successful large-scale restoration of native oyster metapopulations in Chesapeake Bay's tributaries will provide secondary benefits to the Bay oyster fishery as well as to general water quality. If, in fact, the Chesapeake Bay cannot be restored without also restoring the native oyster, construction of extensive

permanent oyster sanctuary reef networks will be required. With shell availability at a record low, other 'alternative' substrate options must be considered. This dissertation addresses that problem directly through extensive field surveys and experiments in two tributaries of Chesapeake Bay.

Chesapeake Bay oyster history. An expanded version of this historical depiction integrating the political element is provided in Appendix 1.1. The following introductory material emphasizes the scientific basis for the utility of alternative substrates in native oyster reef restoration.

Oyster reefs in most ecoregions where they historically occurred are in poor condition and at risk of extirpation as functional ecosystems (Kirby 2004, Lotze et al. 2006, Airoldi and Beck 2007, Coen and Grizzle 2007, Beck et al. 2009). The Chesapeake Bay's oyster population decline is amongst the most dramatic globally at less than 1 % of its historic abundance. One of the earliest documented acknowledgments that the Chesapeake Bay oyster fishery was in danger of damaging the plentiful reefs occurred in 1858 when numerous Virginia residents, led by James G. Paxton, Esq., testified in front of the House of Delegates of Virginia that the 'Oyster Fundum of Virginia' needed to be regulated and taxed (Paxton 1858). However, the committee voted unanimously to leave the Commonwealth's oyster fundum unregulated citing the doctrine of *Laissez-Faire* capitalism. A few decades later (1880s), the foretold oyster decline began due to extreme levels of harvesting and substrate removal (Ingersoll 1881, Winslow 1881, Brooks 1891, Stevenson 1894, Kennedy and Breisch 1981, Rothschild et al. 1994); despite long-term instability, the Chesapeake oyster fishery became the largest

in the world during the 1880s (MacKenzie 1981, NRC 2004). There was minimal support to regulate such a bourgeoning fishery, especially with the oyster becoming widely recognized as an important cultural symbol of the Chesapeake Bay region. And, only a few decades later, the oyster fishery crashed (Haven et al. 1978, Andrews 1996), ushering in the era of shell and oyster subsidies (state and federal) that perpetuated through the 1980s. These subsidies protracted the period of intense fishing pressure, accelerating the rate of oyster population decline and habitat destruction. Consequently, the current state of shell availability in Chesapeake Bay is one of severe limitation, such that alternative substrates must be considered for restoration efforts.

Oyster diseases. Another major contributing factor to this decline over the last half century was the action of MSX and Dermo, diseases caused by pathogens *Haplosporidium nelsoni* and *Perkinsus marinus*, respectively (Andrews 1988). The oyster population and its habitat were in very poor condition by the time disease mortality began taking its toll (Andrews 1996). The combined effect of both oyster diseases and overharvesting has been the recent elimination of commercial oyster production from essentially all waters in the Virginia portion of the bay with the exception of three oyster bars in the upper James River and very limited areas of the upper Rappahannock River (Mann et al. 1991).

Disease truly was 'the last straw' for the native oyster in Chesapeake Bay. The increasingly intensive and mechanized fishing contributed to leveling the profile of oyster reefs which, in turn, altered the flow regime over the reefs (Lenihan et al. 2001). In one experiment, oysters with the highest proportion of individuals infected with Dermo,

highest intensity of infection, and highest mortality were located at the base of reefs, where flow speeds and food quality were lowest and sedimentation rates highest (Lenihan et al. 1996). The restoration of oyster reefs, whether made of shell or of alternative substrates, with adequate reef height can improve flow, reduce sedimentation, and help alleviate the negative effects of disease on resident oysters.

Natural disease resistance is, however, developing in many sub-populations of native oysters in the lower Chesapeake Bay (Carnegie and Burreson 2009). Long-term monitoring of Dermo and MSX in the lower portion of most of Virginia's major Bay tributaries (classified as Zone 3, high salinity, high disease-intense waters), has uncovered significant populations of wild native oysters (Carnegie and Burreson 2009). Natural disease resistance has apparently evolved in the Lynnhaven River (Dissertation Chapters 3 and 4), Great Wicomico River (Carnegie and Burreson 2008, 2009), Rappahannock River (Dissertation Chapter 2, Lipcius and Burke 2006), and Tangier Sound (Encomio et al. 2005), where it was first documented. In most cases, these oysters occur in sanctuaries (intentional or *de facto*) and in high-salinity, high-disease zones where oysters are not expected to have persistent populations (Oyster Management Plan 2009).

Natural disease resistance in oysters benefits both ecological and fishery restoration, as well as aquaculture. Disease-resistant strains have been used in restocking programs for ecological restoration (Lynnhaven River, 2007-2009) and for hatchery-based aquaculture, including private leasehold-based aquaculture. What appears to be limiting is that these oysters do not exist in numbers sufficient to support a wild oyster fishery. This will continue to be the case, given ongoing harvest damage and the poor

condition of the remnant oyster habitat, which suppresses recruitment and survival of young oysters. Low recruitment due to low stock levels only compounds these problems, and inhibits recovery to commercially acceptable stock size (MacKenzie 1981, Southworth and Mann 2004).

Sanctuaries vs. harvest grounds. Leaving oysters undisturbed on constructed or natural reefs in sanctuaries may be the only way to restore high-quality oyster bottom in mesohaline Chesapeake Bay. Jordan and Coakley's (2004) oyster population model led them to conclude that harvest pressure must be curtailed before oyster stocks can recover. Such a recovery will help restore the crucial ecological role oyster reefs play in benthic-pelagic coupling (Newell 1988, Newell et al. 2005) and will provide hard substrate used by many other species (Coen et al. 1999). Posey et al. (1999) suggested that the vertical complexity of oyster reefs influences the degree to which reefs are utilized by benthic organisms, particularly decapod crustaceans, because reefs with higher vertical complexity contained higher abundances of epifaunal organisms. Soniat et al. (2004) determined that horizontal surface was preferable to vertical surface for oyster larval settlement under optimal conditions (low sedimentation, low predator pressure) but when conditions degraded, vertical surfaces with refuge led to higher oyster survival than horizontal and vertical surfaces without refuge. Thus, the restoration strategy of harvest or managed grounds/reserves, which protects reefs for a period of 1-3 years before exploitation, appears to be unsustainable. These reefs are eventually degraded by reduction of their height, which reduces oyster growth rates and exposes them to catastrophic mortality during hypoxic events (Lenihan and Peterson 1998). Alternative

substrate reefs can be built with the benefits of vertical complexity in mind (Nestlerode et al. 2007), as well as protection from illegal poaching and cownose ray predation. Vertical complexity of alternative substrate reefs also allows for greater flow and lower overall sedimentation (Soniat et al. 2004, Dissertation Chapter 3).

Alternative substrates vs. shell. Traditionally, low-relief shell reefs (5-10 cm thick; Smith et al. 2005) and oyster shell mounds (~1 m tall) have been created in an attempt to mimic natural reef conditions and accelerate recruitment (Southworth et al. 2008b). To date these efforts have met with limited success (Mann and Powell 2007).

Availability of good quality shell for oyster reef restoration projects has been a growing problem. Equally serious are the documented limitations of using dredged, fossil shell for such projects. Given the severe shortage of oyster shell for restoration efforts and recognition that greater reef height or relief is an important characteristic of successfully restored oyster reefs, the use of alternative substrates for restoration reefs has received considerable attention. For example, the state of Maryland has teamed up with the USACE (Baltimore District) to utilize substrates such as granite, concrete, and steel slag as reef alternatives in the recent construction of a 5.4-ha reef in the lower Severn River (Wood 2009). In addition, ecological oyster restoration efforts (construction and monitoring) in Virginia in the Great Wicomico River (Schulte et al. 2009), Lynnhaven River (Lipcius et al. 2008; Dissertation Chapters 2, 4 and 5) and Rappahannock River (Dissertation Chapter 3) provide evidence for the use of alternative substrates, which has been an established oyster reef restoration technique in the southeastern United States, including the Gulf of Mexico.

A restoration strategy only works if reefs are built at a biologically meaningful scale, in optimized locations, with a durable substrate, and protected from physical degradation (e.g. harvesting) and have sufficient recruitment. Well-intentioned yet poorly 'designed' reefs, when monitored and appraised against original expectations, may lead assessors to conclude that 'reefs don't work' when, with the correct habitat requirement information for the target species, the end result would have been successful (Jensen et al. 2000).

Artificial reefs around the world. Since World War II national artificial reef programs have been developed in Japan, the United States of America (US), Thailand, India, Taiwan, Malaysia, Australia, and the South Pacific Islands. Countries of the European Union (EU), including Italy, Spain, France, Portugal, the United Kingdom, and Monaco also have artificial reef programs.

By far, the largest financial obligation of a federal government is in Japan, with funding in recent years of billions of yen annually (Yamane 1989). Here, significant government support for construction has led to establishment of an industrial infrastructure, while a large research program has also evolved. Geographically, roughly 10 % of Japan's ocean shelf has received what Yamane refers to as "improvements." No other federal government is as heavily involved as Japan (Stone et al. 1991). The principal materials or structures used to enhance fishery species in Japan include: (1) rocks (in layers, piles, or in cages), (2) substrate blocks (concrete), (3) breakwater blocks (concrete), (4) chamber structures (concrete cubes and cylinders), (5) large chamber structures (concrete, plastic, fiberglass, and steel frameworks), (6) longline, (7) plastic

seaweeds, (8) bamboo rafts, and (9) floating devices (Mottet 1981, Stone et al. 1991). Though fish remain the principal focus of many of these reef projects, the rock (sea urchins and abalone), substrate block (larval fishes and invertebrates), and breakwater block (seagrass and clam culture grounds) reefs were deployed, in part, with shellfish recovery in mind.

In many other nations, efforts have been made with a more limited geographic range or on a feasibility basis. European countries have been experimenting with various types of artificial reefs for over 30 years (Jensen 2002). Often, such reefs serve a dual purpose, as habitat and as an outlet for excess materials produced by regional industry (e.g. pelletized coal ash). Some of the oldest and best document reefs have been deployed by Italy and other Mediterranean countries. At least 11 artificial reefs exist along the Italian Adriatic coast (Bombace et al. 2000). Seven of these serve as the best European examples to date of reefs that have provided successful commercial harvests, especially of bivalves, and which are used both by fishers and in aquaculture (Jensen 2002). The first Italian reef to be planned scientifically was deployed in 1974 (Bombace et al. 1989). The aims of the scheme were protection from illegal trawling, repopulation of biota through the provision of habitat, and enhancement of harvestable sessile biomass, especially mussels and oysters, through the introduction of suitable surfaces. The initial costs were recovered three times over in about four years through small-scale fisheries and collection of the mussels settled on the artificial substrata (Bombace et al. 1994). One reef was used for experimental work on suspended shellfish culture (mussels and oysters; Fabi and Fiorentini 1997, Fabi et al. 1986). On this oyster reef, species richness, species diversity, and fish abundance increased after reef deployment (Fabi and Fiorentini

1994), particularly for reef-dwelling nekto-benthic species. Three years after deployment, the increase in average catch weight for these species was 10–42 times the initial values. In eutrophic waters, annual settlement of bivalves on these structures provides mariculture opportunities for coastal communities; annual production was measured as 8 kg of mussels per m of rope (Fabi and Fiorentini 1990).

Recently there has been a shift to deploy reef modules following baseline assessment of fish diversity and biomass. In Portugal, reefs were deployed off the island of Madeira and near the mainland (Neves dos Santos and Costa Monteiro 1997). On the mainland, there were two reefs off the Ria Formosa, an estuarine system on the Algarve coast. There were two reef types, a "production" reef and an "exploitation" reef. The production reef (735 concrete lattice units each 2.7 m^3) was deployed to provide shelter for juveniles migrating from the lagoon to open coastal water. The exploitation reef (20 concrete structures in two sizes, 130 m^3 and 174 m^3) was placed farther from the lagoon mouth to aggregate fish. The structures were physically stable, developed an epibiotic community within months, and concentrated fish (Neves dos Santos and Costa Monteiro 1998, Costa Monteiro and Neves dos Santos 2000). The success of these reefs led to the development of a much larger reef system for commercial exploitation, involving a 35-km^2 area of seabed off the Algarve coast, using more than 19,000 modules with a combined weight of 66,690 t, which represented one of the largest artificial reef systems in Europe.

Artificial habitats have been used for over 100 years in the US but have only recently been recognized by fishery managers as a viable resource enhancement technique (McGurrin et al. 1989, Stone et al. 1991). Artificial habitats have been

deployed throughout the US in a variety of temperature zones and in fresh, estuarine, and saltwater environments. Their use is perhaps more ubiquitous than in Japan. They have been deployed for many purposes such as recreational and commercial fishing, sport diving, waste disposal, pollution control, and environmental mitigation.

Prior to 1984, only a few coastal states, including Virginia, had well-developed programs directed at enhancing fisheries and fish habitat with artificial reef structures. In 1985, the USA developed its first National Artificial Reef Plan (NARP) under direction of the National Fishing Enhancement Act of 1984 and with the participation of coastal state artificial reef program managers (US Dept. of Commerce 1985). In 1986, the Atlantic States Marine Fisheries Commission formed an Interstate Artificial Reef Program to promote effective artificial reef fishery development and provide information to satisfy present and upcoming reef management needs (McGurrin et al. 1989). A similar program was soon developed by the Gulf States Marine Fisheries Commission (Stone et al. 1991). Approximately half of the coastal state natural resources agencies in the US have approved plans for construction of artificial fish habitats based on the national plan (US Dept. of Commerce 2007). From 1986-1990, the states of North Carolina (NCDMF 1988), Louisiana (Wilson 1986), New York, New Jersey, California, and Texas (Stone et al. 1991) developed artificial reef management plans with guidance from the NARP. The US NARP was amended in 2007 to reflect the progress and state of knowledge surrounding guidelines for site selection, construction, development, and assessment of artificial reefs (US Dept. of Commerce 2007).

Virginia's current Artificial Reef Program, which is managed by the Marine Resources Commission, traces its roots back over 40 years. In the 1950s recreational

fishermen spearheaded efforts, resulting in the sinking of automobiles, tires and over 100 surplus U.S. Navy landing craft and pontoon barge sections off Virginia Beach. The Marine Resources Commission became formally involved in reef building as the authorized recipient of six World War II Liberty ships in the early 1970s. The vessels were sunk in offshore waters to form the popular Triangle Reef off Virginia Beach and the Parramore Reef off Wachapreague. During the 1970s and early 1980s, the Artificial Reef Program primarily used "materials of opportunity" to create artificial reefs. Concrete pipe, ships, and automobile tires were used most often. In addition to simple deployments, attempts were made to use these materials to develop structures that provided stability, durability and a maximum amount of surface area and interior space. For example, tires were split and sunk vertically into concrete bases and concrete pipe was bundled into pyramids (VMRC 2009).

Currently, the Virginia Artificial Reef Program is manufacturing all concrete structures to augment the materials of opportunity which are still in use. High profile structure is created with concrete "igloos." These structures are 1.3 m in height with a base spanning over 4 m and weigh approximately 5440 kg. Low profile habitat is created with concrete tetrahedrons, which have a profile of approximately 1 m and a weight of 227 kg. The tetrahedron shape was chosen for its low center of gravity, which makes the unit very stable (VMRC 2009).

In the arena of oyster restoration, one of the earliest and largest artificial reef deployments took place in 1994 in the Rappahannock River, Virginia. The Steamer Rock oyster sanctuary reef (~0.4-ha footprint) was made of a “material of opportunity” (reinforced concrete bridge pieces), was of no cost to the state of Virginia, and is the

largest, most prolific artificial (alternative substrate) oyster reef ever built in the lower Chesapeake Bay. Its popularity as a perennial fishing hotspot for recreational fishers speaks to the resident and visiting fish populations it sustains. The strategy of stacking the bridge material and its careful placement in concentric rings maximized its potential as an oyster reef, and sets it apart from most other artificial reefs in the region. Dumping haphazardly from the surface, though potentially adequate for recreational fish attraction, appears to be much less effective for the construction of oyster reefs. A central goal of this dissertation was to quantify features of alternative substrates that optimize the effectiveness of restored native oyster reefs.

Summary. The preceding examples demonstrate that alternative reef structures can provide the stability and complexity of natural reefs, and lead to higher abundance, biomass and diversity of species under restoration. Alternative substrate reefs can serve as the foundation from which natural oyster reefs can grow and reclaim their dominant role as ecosystem engineers. The focus of this dissertation is to assess the performance of alternative oyster reef substrates relative to unconsolidated oyster shell both subtidally and intertidally within two Chesapeake Bay tributaries. In addition, the results of this research will inform oyster restoration efforts by assisting federal, state, and local agencies in Chesapeake Bay and beyond.

Chapter 2

Granite and concrete riprap as intertidal native oyster (*Crassostrea virginica*) reefs

ABSTRACT: In recent years, oyster shell for native oyster restoration projects has been in short supply, requiring examination of alternative substrates useful in reef construction. In addition, "living shoreline" buffers that protect salt marsh and other coastal habitats from erosion may also be constructed from man-made materials. Consequently, we investigated the efficacy of alternative substrates to serve as oyster reefs along the intertidal zone of a subestuary in Chesapeake Bay. Intertidal shorelines comprised of granite and concrete riprap, built as revetments for erosion control, were sampled for oyster density, biomass (ash-free dry mass – AFDM), population structure, and condition at 17 locations throughout the Lynnhaven River System (LRS). For comparative purposes, restored oyster shell reefs were also sampled, including two high-density samples that represent peak reef productivity. The two high-density samples, 956 oysters m^{-2} for Hume's Marsh and 776 oysters m^{-2} for Keeling's Drain, were 8-10 times higher than the remainder of restored oyster shell reefs (97 +/- 16 (SEM) oysters m^{-2}). With a mean density of 978 +/- 177 oysters m^{-2}, and peak densities > 2000 oysters m^{-2}, the intertidal riprap shorelines supported some of the highest abundances of oysters ever recorded for Chesapeake Bay manmade oyster reefs, shell or otherwise. Mean riprap oyster biomass was 165.02 +/- 24.63 g AFDM m^{-2}. Five sites maintained > 295 g AFDM m^{-2}, which far exceeds a stated restoration goal of 177 g AFDM m^{-2}. Many of the riprap reefs supported a robust oyster population size structure which denotes consistent annual recruitment, a quality important for reef persistence. Riprap age (older > younger) and location (downriver > upriver) influenced oyster reef performance more so than composition (granite or concrete); both substrates supported dense oyster-mussel assemblages. Finally, despite being immersed in a high-salinity zone noted for high oyster disease pressure, many of these oysters had grown quite large indicating some level of disease tolerance. These results indicate that riprap reefs are effective in enhancing abundance of oysters and mussels, and serve as viable alternative substrates in oyster reef restoration and living shoreline construction to mitigate habitat degradation.

INTRODUCTION

Historically, natural marsh dominated Atlantic coastal shorelines and estuarine tributaries of North America. As coastal communities became more developed, shallow-graded marshes were filled in and steeper gradients were created at the water's edge (Komar and Holman 1986, Berman et al. 2000). Shoreline erosion became exacerbated by forest and marsh development to the extent that considerable shorefront marshes and forested buffers were lost. To control shoreline erosion along residential, commercial and government-owned waterfront properties, numerous strategies were employed, including creation of bulkheads (wooden, metal or concrete) and riprap revetments (granite or concrete). More recently, conversion of natural marsh to riprap or bulkhead has caused concerns about the effects of shoreline hardening and marsh loss on flora, fauna, and estuarine water quality (Dauer et al. 2000). In response to these concerns, the effects of shoreline development and oyster reefs on benthic (Lawless 2008) and riprap (this study) communities were investigated in the Lynnhaven River System (LRS) in Virginia Beach, Virginia.

Oysters are ecosystem engineers that provide habitat, influence population and food web dynamics, maintain biodiversity, and affect nutrient cycling and other ecosystem processes (Ruesink et al. 2005). The eastern oyster, *Crassostrea virginica*, and its reefs can enhance several critical ecosystem functions by (1) reduction of water turbidity through active filtration (Newell 1988, Nelson et al. 2004), (2) stabilization of substrate, (3) erosion reduction (Meyer et al. 1997), (4) provision of habitat for many marine organisms (Coen et al. 1999), and (5) alteration of current flow over the reefs which, in turn, results in a reduction of susceptibility to disease (Lenihan et al. 1996,

Lenihan et al. 1999) and enhancement of larval recruitment (Abelson and Denny 1997). Thus, the loss of oyster reefs through over-harvesting, disease, and pollution can cause complex changes in coastal ecosystems.

The prospect that intertidal oyster communities on granite and concrete riprap revetments can stabilize substrate and reduce erosion has only recently been considered by proponents of 'living shoreline' projects and native oyster restoration (Erdle et al. 2006). From New England (Hardwick-Witman and Mathieson 1983, Capone et al. 2008) to the Mid-Atlantic Bight (Baylor 1894, Taylor and Bushek 2008, Ross and Luckenbach 2009) to the South Atlantic (Harris 1980, Bahr and Lanier 1981, Burrell 1986, O'Beirn et al. 1996, Coen et al. 1999, Manley et al. 2008) and Gulf Coasts (Ritter 1896, May 1971), intertidal eastern oyster populations have persisted despite the species becoming ecologically extinct throughout much of its range (Kirby 2004). Consequently, the potential exists to combine shoreline protection and oyster reef construction to achieve mutually beneficial goals.

The concept of using 'alternative substrates,' such as granite, concrete, gypsum, clam shells, and limestone for the construction of fringing intertidal reefs for native oyster restoration has been tested in the mid-Atlantic (Luckenbach and Ross 2006, Nestlerode et al. 2007), South Atlantic (Powers et al. 2009) and Gulf states (Soniat et al. 1991, Haywood and Soniat 1992, LADWF 2004). In 2004, we observed that oysters appeared to be thriving on intertidal riprap along the shorelines of the LRS, similar to the recent discovery of thriving New England rocky intertidal oyster communities (Capone et al. 2008). Consequently, we set out to quantify abundance of bivalves on granite and concrete riprap to determine the viability of riprap as a restoration tool. In summer 2005,

we quantified oyster and mussel biomass on granite and concrete riprap shoreline in the LRS to determine if: (1) alternative (non-shell) substrate (concrete and granite riprap) was suitable for native oyster restoration, (2) oyster populations on riprap were persistent, (3) performance of riprap oyster reef populations were dependent on location, and (4) age of riprap reefs influenced oyster and mussel abundance.

MATERIALS AND METHODS

Riprap field survey. Granite and concrete riprap make up a significant portion (Luckenbach and Ross 2006) of mitigated shoreline in the LRS (Fig. 2.1a-c). In Broad Bay, Long Creek and Lynnhaven Bay, in particular, riprap is a common shoreline type; we thus focused our sampling in these three areas of the LRS. A combination of 19 samples (10 concrete, 9 granite) was collected at 17 locations from 30 June through 25 July 2005. Along a single granite riprap shoreline, one site (6a) was fully exposed to sunlight while another site (6b) was shaded beneath a wooden dock; along one property containing concrete, one site (12a) was fully exposed to sunlight and another site (12b) was shaded (Table 2.1a).

Site selection was nominally random through haphazard sampling. Shoreline maps were used for navigation to all available riprap shoreline in these three areas (Luckenbach and Ross 2006). A site was selected on the map. A specific point on the property, distinguishable from a distance far enough as not to bias our sample location toward those with higher oyster density, was selected as the vessel neared the shoreline.

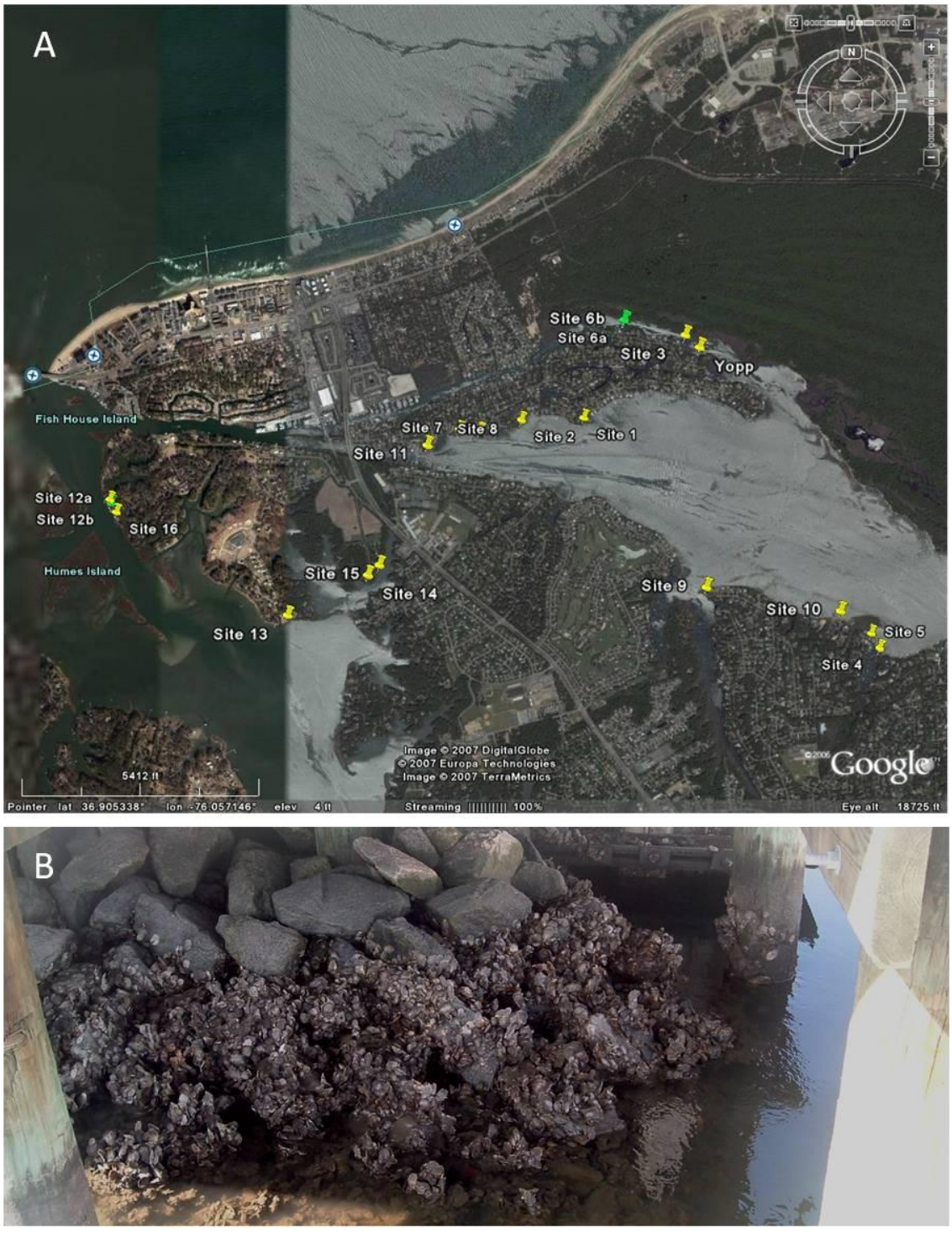

A
Site 6b
Site 6a
Site 3
Yopp
Fish House Island
Site 7
Site 8
Site 2
Site 1
Site 11
Site 12a
Site 12b
Site 16
Humes Island
Site 15
Site 14
Site 9
Site 10
Site 5
Site 4
Site 13
Image © 2007 DigitalGlobe
© 2007 Europa Technologies
Image © 2007 TerraMetrics
Google
5412 ft
Pointer lat 36.905338° lon -76.057146° elev 4 ft
Streaming 100%
Eye alt 18725 ft
B

Figure 2.1 A-B.

Figure 2.1: (A) Aerial map of the lower Lynnhaven River System containing markers for riprap shoreline sample sites; yellow = exposed, green = shaded (Map generated using Google Earth™). (B) Riprap oyster reefs shaded beneath a wooden dock along Long Creek. (C) Collage of riprap oyster photographs taken in and around Broad Bay, 2005.

Table 2.1: (A) Riprap sample site and (B) oyster and mussel data for Broad Bay, Long Creek and Lynnhaven Bay (SH – shell height, SA – surface area, E – exposed, S – shaded)

Region	Site #	Sample Date	Latitude (°N)	Longitude (°W)	Riprap Type	Sediment Type
N. Broad Bay	1	6/30/2005	36.90428	76.04892	Granite	Muddy Sand
N. Broad Bay	2	6/30/2005	36.90419	76.05370	Granite	Muddy Sand
Long Creek	3	7/6/2005	36.90957	76.04092	Concrete	Muddy Sand
S. Broad Bay	4	7/8/2005	36.88950	76.02570	Concrete	Muddy Sand
S. Broad Bay	5	7/8/2005	36.89040	76.02620	Granite	Coarse Sand
Long Creek	6a – E	7/11/2005	36.91048	76.04602	Granite	Mud
Long Creek	6b – S	7/11/2005	36.91048	76.04602	Granite	Mud
Long Creek	Yopp	7/11/2005	36.90873	76.03965	Concrete	Mud
N. Broad Bay	7	7/19/2005	36.90371	76.05869	Concrete	Muddy Sand
N. Broad Bay	8	7/19/2005	36.90332	76.05711	Granite	Coarse Sand
S. Broad Bay	9	7/19/2005	36.89414	76.03976	Concrete	Muddy Sand
S. Broad Bay	10	7/19/2005	36.89214	76.02883	Granite	Coarse Sand
N. Broad Bay	11	7/21/2005	36.90231	76.06105	Concrete	Coarse Sand
Lynnhaven Bay	12a – E	7/21/2005	36.89911	76.08601	Concrete	Coarse Sand
Lynnhaven Bay	12b – S	7/25/2005	36.89911	76.08601	Concrete	Coarse Sand
Lynnhaven Bay	13	7/25/2005	36.89164	76.07219	Concrete	
Lynnhaven Bay	14	7/25/2005	36.89423	76.06588	Granite	
Lynnhaven Bay	15	7/25/2005	36.89487	76.06493	Granite	
Lynnhaven Bay	16	7/25/2005	36.89841	76.08545	Concrete	

A.

Table 2.1 B.

Site #	Oyster Density (m^{-2})	Oyster Biomass (g AFDM m^{-2})	Mean Oyster SH (mm)	Oyster Volume (mL m^{-2})	Mussel Density (m^{-2})	Mussel Volume (mL m^{-2})	Riprap SA: Bottom Area Ratio	Oyster Condition Index 1	Oyster Condition Index 2	Oyster Condition Index 3
1	260	58.03	43.2	2840	256	520	4.68	10.84	6.56	2.80
2	756	253.59	48.8	9932	1608	2200	6.23	10.38	7.66	3.71
3	1628	294.02	44.3	16500	308	360	7.40	7.17	5.03	2.21
4	32	0.17	24.2	28	264	100	2.78	--	--	--
5	112	12.95	37.1	340	216	80	2.46	12.54	9.70	2.36
6a – E	356	58.06	41.7	2600	140	320	4.81	8.66	6.42	2.49
6b – S	568	119.19	41.5	4800	152	520	3.56	9.23	6.61	2.71
Yopp	1328	285.02	46.7	15000	2048	4000	5.17	9.68	6.71	2.26
7	2028	216.81	40.7	12720	196	160	3.09	10.83	6.60	2.21
8	940	174.38	45.2	8000	480	600	2.17	11.02	7.31	2.25
9	224	48.65	46.0	984	428	100	1.32	9.64	8.10	4.24
10	928	143.12	41.0	6344	196	40	5.80	9.47	6.25	2.16
11	1228	175.46	38.9	7000	328	360	5.15	9.29	8.24	2.21
12a – E	2540	294.94	39.4	19980	1624	3820	4.16	10.05	8.04	2.31
12b – S	2000	236.39	33.7	8880	1088	2560	3.58	10.90	9.16	3.73
13	1092	249.44	44.2	10440	760	1188	1.24	8.98	7.40	2.97
14	432	105.82	47.4	6560	328	508	2.60	9.25	7.02	2.61
15	116	62.06	56.9	2560	44	60	1.86	13.63	10.75	3.74
16	2012	347.27	43.6	1640	1196	2000	2.05	8.20	6.40	2.82
Mean	978	165.02	42.3	7220	612	1028	3.69	9.99	7.44	2.76
SE Mean	177	24.63	1.5	1324	136	292	0.40	0.35	0.32	0.15

B.

Upon arriving at the sample location, a 0.5-m x 0.5-m polyvinylchloride (PVC) quadrat was tossed on the riprap – wherever it landed in the intertidal/upper-subtidal zone (where we expected to find high oyster biomass) was where we sampled. Each rock that was > 50 % within the quadrat was scraped clean of all organisms, including dead shells.

One plastic freezer bag was used for each rock sampled; this bag contained a sample identification number, the sampling date, and the estimated surface area (SA) of the rock sampled. The SA measurement was made with measuring tape on each discernible face of the rock. The sum of individual face SA measurements produced the total SA estimate for each rock. Each bag was placed in a cooler with ice and kept in freezer storage at VIMS until it was processed. Oyster counts and shell heights (SH), and SA of each rock were recorded (Appendix 2.1).

Lab processing included counts of oysters, mussels, crabs, and fish. SH was measured for all oysters, live or dead. Dry mass (DM), ash free dry mass (AFDM), and condition index (CI) were calculated for selected oysters. Simple linear regressions of log AFDM versus log SH were run to predict oyster biomass from SH (Fig. 2.2). Oyster condition (Mercado-Silva 2005) was calculated for each sample site (see equations below). A subsample of oysters collected throughout the range of oyster shell heights was processed by removing fouling organisms and rinsing. After cleaning, oysters were blotted dry before being measured. Measurements made on each oyster included total mass (nearest 0.001 g), SH (nearest 0.1 mm), and wet shell mass (nearest 0.001 g). After shucking, shells and tissue were dried at 60°C for at least 48 h and weighed, followed by 6 h at 550°C in a muffle furnace to account for the ash in DM and produce AFDM estimates. The following CIs were calculated:

CI1 = [dry tissue weight (g) / shell cavity volume] x 100 (Abbe and Albright 2003)

CI2 = [dry tissue weight (g) / dry shell cavity volume] x 100 (Abbe and Sanders, 1988)

CI3 = [dry tissue weight (g) / dry shell weight (g)] x 100 (Rainer and Mann 1992)

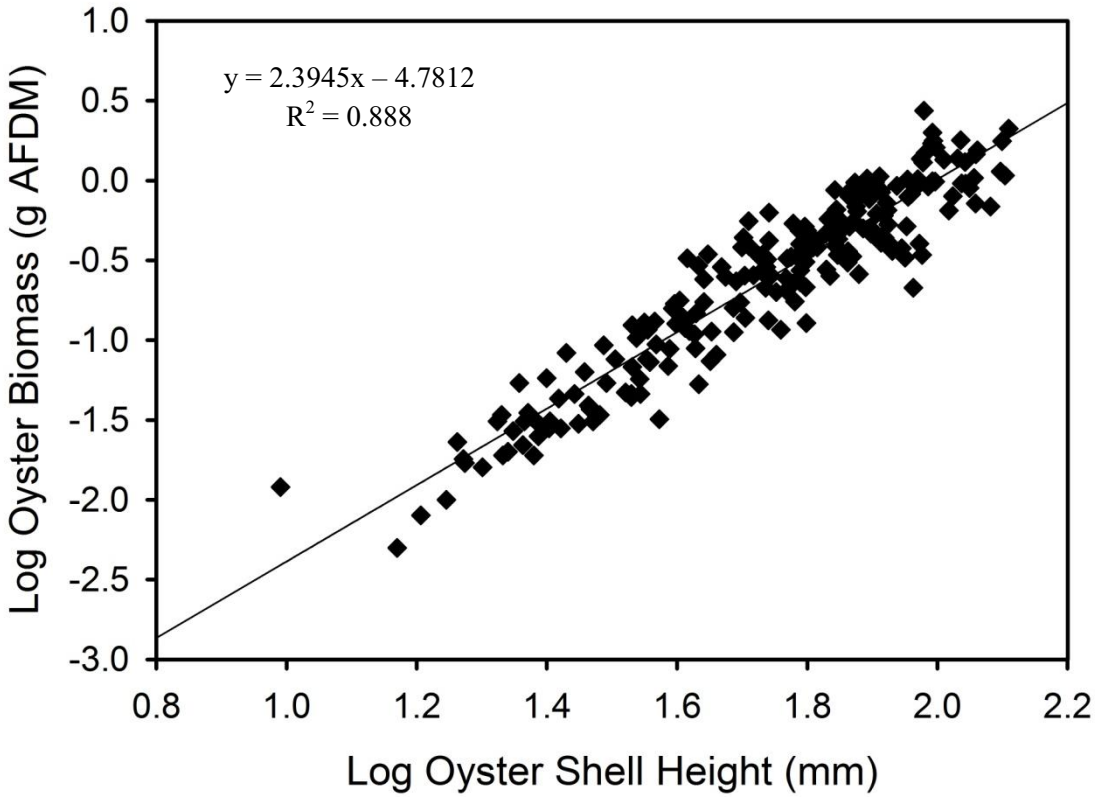


Figure 2.2: Regression model of log oyster AFDM (g) versus log oyster shell height (mm) used for oyster biomass estimation for riprap oysters (pooled across all sites).

These indices are considered to be the most accurate indicators of condition (Hickman and Illingworth 1980, Davenport and Chen 1987). For CI1 and CI2, shell cavity volume is equal to the difference between the mass of the whole oyster (g) and the mass of the empty valves (g) (Abbe and Sanders 1988, Crosby and Gale 1990). CI1 considered the mass of the empty shells immediately after shucking whereas CI2 used the mass of the shells after a period of drying (Abbe and Albright 2003). For all analyses, condition

indices were used where shell volume was calculated by a gravimetric method. These measures are linearly related to those where CI3 is calculated by a volumetric method (i.e. by water displacement of the shells, Schumacker et al. 1998).

Restored oyster shell reef survey. Restored oyster shell reef samples were taken to compare with metrics of oysters sampled on riprap. The site selection method was identical to that used for riprap samples. For the restored oyster shell reefs, sets of samples were taken from a reef in Long Creek and in Hume's Marsh (Lynnhaven Bay), as they appeared to represent the range from low to high oyster density. The oyster reef samples were selected in a stratified random sampling design with location in the intertidal zone (lower, mid, upper) as the stratum. The Hume's Marsh Reef was sampled completely with five samples from each intertidal zone. The Long Creek reef was not fully sampled (insufficient number of samples: only 5 of 15 retrieved). A single high-density sample was taken from the Keeling's Drain reef (lower Eastern Branch of Lynnhaven River) and another part of Hume's Marsh Reef; both represent peak reef productivity on restored oyster shell reefs.

The method for collection on a restored oyster shell reef was the excavation of a selected sample down to a depth of 15 cm using a 0.5-m x 0.5-m quadrat. Only live oysters, or dead shells with live oysters attached to them, were brought back and placed in freezer storage. SH and shell volume were measured for all oysters and the number of oysters per base shell was noted. A subset of 5-14 oysters was selected across the range of SH to determine AFDM, and CI. Simple linear regressions of log AFDM versus log SH were run to produce a model that could predict oyster biomass from SH alone.

In the analysis of size structure for oysters, peaks were analyzed with FISAT II (Gayanilo et al. 2002) to delineate individual year classes. The peaks were separated using Bhattacharya's Method (Bhattacharya 1967). The program uses a set of equations that yields mean lengths, population sizes, standard deviations and separation indices (SI) for each year class, where SI is the difference between two successive means divided by the difference between their estimated standard deviations. However, measurement of peak height and width were secondary to the primary goal of distinguishing individual peaks (size classes as proxies for age classes).

RESULTS

Riprap oyster population structure. In total, 4551 oysters were collected and used in the size structure analysis (Fig. 2.3a). Oyster SH ranged from 1.4-133.4 mm, with some variability in size structure across sites (Fig. 2.3b).

Riprap oyster density. Oyster counts ranged from 8-635 with a mean of 244 +/- 44 oysters (SE) sample^{-1}. Mean oyster density was 978 +/- 177 oysters m^{-2} with a mean SH of 42.3 +/- 1.5 mm (Table 2.1b). On average, concrete samples contained 219 more oysters than granite samples (Table 2.2). Along a single granite riprap shoreline in Long Creek, site 6a was fully exposed to sunlight while site 6b was shaded beneath a wooden dock. At this site, the shaded sample contained 37 % more oysters and the oyster band extended 6-7 cm higher in the intertidal zone than that of the exposed sample. In a Lynnhaven Bay shoreline containing concrete riprap, site 12a was exposed and site 12b

was shaded. At this site, both samples had 2000 or more oysters m^{-2}, and although the shaded oyster band extended higher in the intertidal, the exposed sample contained 27 % more oysters than the shaded sample.

Table 2.2: Comparison of mean oyster count, shell height (SH), dry tissue mass (DM), ash-free dry tissue mass (AFDM), and oysters per unit surface area (SA) between concrete and granite riprap (+/- 1 SEM).

Riprap Type	Oyster Density (m^{-2})	Oyster Biomass (g AFDM m^{-2})	Mean Oyster SH (mm)	Oyster Volume (mL m^{-2})	Mussel Density (m^{-2})	Mussel Volume (mL m^{-2})	Riprap SA: Bottom Area Ratio	Oyster Condition Index 1	Oyster Condition Index 2	Oyster Condition Index 3
Concrete	1411	214.82	40.2	9316	824	1464	3.592	9.42	7.30	2.77
	(255)	(35.29)	(2.2)	(2188)	(204)	(488)	(0.612)	(0.40)	(0.41)	(0.25)
Granite	496	109.69	44.8	4888	380	540	3.796	10.56	7.59	2.76
	(107)	(24.46)	(1.9)	(1024)	(160)	(220)	(0.544)	(0.55)	(0.53)	(0.20)

Oyster density clearly increased closer to the mouth of the LRS. In many cases, there was an order of magnitude difference in oyster density between the upper bay sites (1, 4, 5, 9) and the lower bay sites (7, 11, 12a, 12b, 13, 16). Overlaying the riprap sites with the hydrodynamic and source-sink modeling results (Lipcius et al. 2008) allowed for classification of each site as a: (1) source, (2) sink, (3) putative source, (4) self replenishing, or (5) exporting sink (Table 2.1a). Sites 4, 5 and 9 (upper bay sites) were designated sources while all six of the aforementioned lower bay sites were designated sinks.

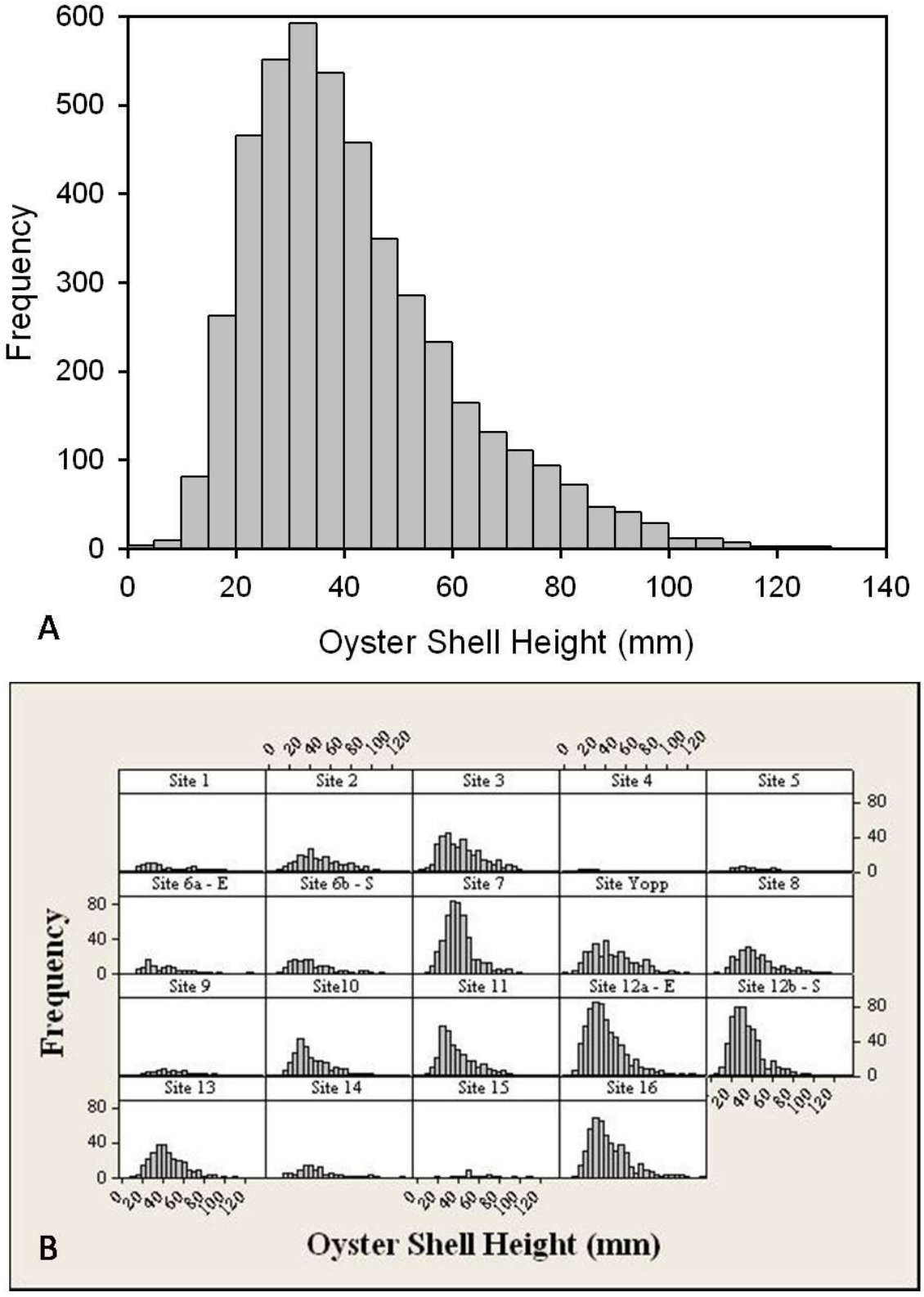


Figure 2.3: (A) Population size structure of oysters on riprap in Lynnhaven River System (LRS). (B) LRS riprap oyster population size structure, by site.

Riprap oyster biomass. Comparison of biomass estimates (total g AFDM m^{-2} bottom +/- 1 SEM) generated from regression equations using pooled and site-specific data (Fig. 2.4) led to site-specific equations. Regressions from pooled data were robust ($R^2 > 0.83$ for AFDM), but regressions from the site-specific data were better (R^2: 0.83 – 0.96). Thus, site-specific regression equations were used (Table 2.3).

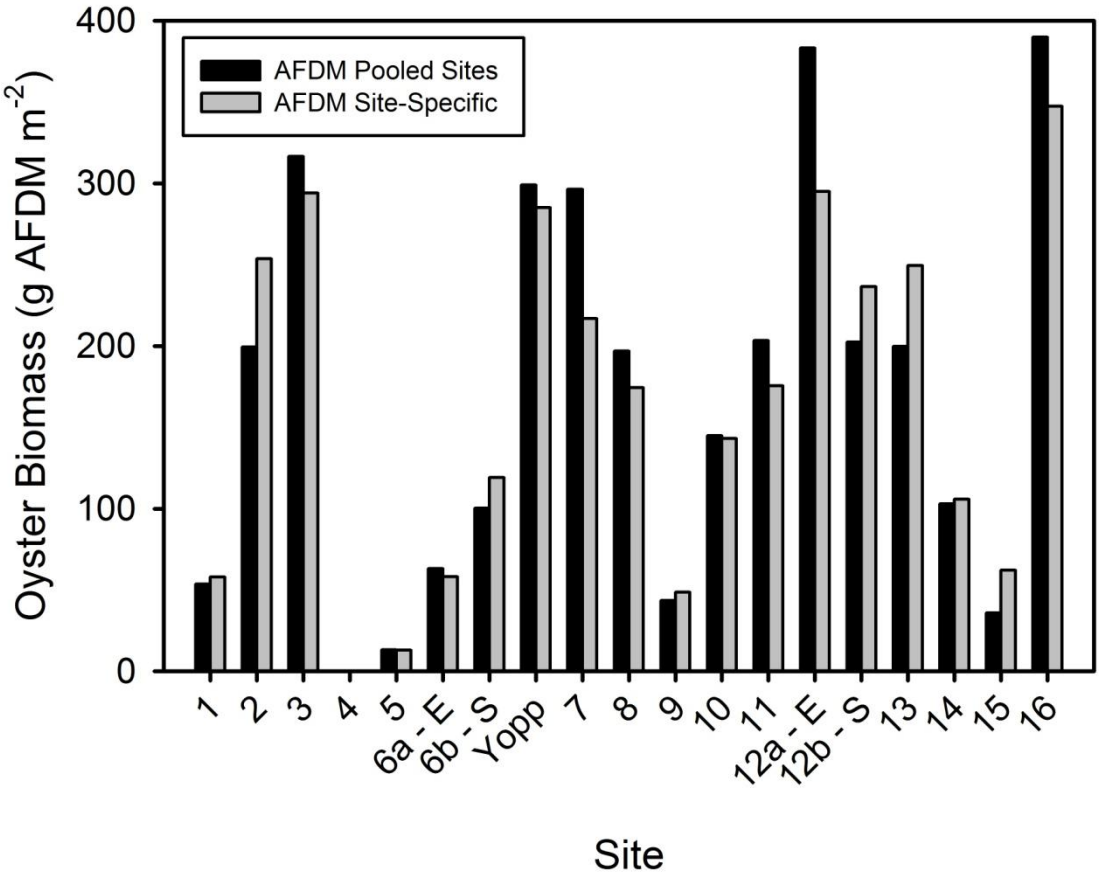


Figure 2.4: Riprap oyster biomass estimates (g AFDM m^{-2} river bottom), by site, generated from regression models using pooled (gray bars) and site-specific data (black bars).

Table 2.3: Linear regression models of oyster dry mass (DM) and ash-free dry mass (AFDM) derived for each site in Broad Bay, Long Creek and Lynnhaven Bay.

Site #	Linear Regression Model Log DM (g) v. Log SH (mm)	R^2	Linear Regression Model Log AFDM (g) v. Log SH (mm)	R^2
1	y = 2.7195x - 5.2963	0.9437	y = 2.6913x - 5.2896	0.9443
2	y = 2.1542x - 4.1889	0.8787	y = 2.3667x - 4.629	0.9211
3	y = 2.9185x - 5.7011	0.9062	y = 2.8588x - 5.6484	0.9023
4	Used Pooled Model	--	Used Pooled Model	--
5	y = 2.5548x - 4.9925	0.9183	y = 2.5029x - 4.9646	0.9196
6a – E	y = 2.7465x - 5.3957	0.9578	y = 2.7378x - 5.4344	0.9561
6b – S	y = 2.5372x - 4.905	0.9396	y = 2.5157x - 4.9229	0.9408
Yopp	y = 2.3532x - 4.6608	0.9176	y = 2.276x - 4.5876	0.9121
7	y = 2.4237x - 4.9242	0.8524	y = 2.3351x - 4.8152	0.8341
8	y = 2.1328x - 4.3117	0.8810	y = 2.1163x - 4.3336	0.8833
9	y = 2.2899x - 4.4348	0.8703	y = 2.2186x - 4.4228	0.8625
10	y = 2.2822x - 4.5432	0.9556	y = 2.2231x - 4.4894	0.9566
11	y = 2.3766x - 4.7392	0.9356	y = 2.4556x - 4.9494	0.9421
12a – E	y = 2.2607x - 4.5951	0.9224	y = 2.2991x - 4.7284	0.9247
12b – S	y = 2.4562x - 4.7326	0.9240	y = 2.5183x - 4.9208	0.9233
13	y = 2.5461x - 4.9518	0.8726	y = 2.5789x - 5.1085	0.8744
14	y = 2.1531x - 4.2479	0.8633	y = 2.1614x - 4.3434	0.8536
15	y = 2.4052x - 4.5013	0.8744	y = 2.3727x - 4.5026	0.8712
16	y = 2.3708x - 4.6887	0.9325	y = 2.2385x - 4.5517	0.8920
Pooled Linear Regression Model	y = 2.3984x - 4.7198	0.8849	y = 2.3945x - 4.7812	0.8880

Oyster biomass ranged from 0.17-347.27 g AFDM m^{-2} with a mean (+/- 1 SEM) of 165.02 +/- 24.63 g AFDM m^{-2}. In many cases, the age of the riprap shoreline determined how developed the oyster reef had become. For example, riprap at sites #4 and #5 had been replaced within two years of sampling due to damage from Hurricane Isabel (18 September 2003); granite and concrete take many months to obtain the appropriate surface pH to support oyster settlement (Weiner et al. 1989, Bonar et al. 1990) and, in this case, we would not expect to find multiple year classes of oysters. In contrast, some of the riprap sites were five or more years old and had thriving oyster populations on and within them. We were informed by homeowners along Lynnhaven Bay that concrete riprap at sites 12a-b and 16 were close to 30 years old; as might be expected, riprap at these sites supported higher densities of small and large oysters, with more than 1.5-2.0 times the oyster AFDM the Lynnhaven Decision Document expects for unseeded restored oyster reefs five years or older.

Riprap oyster volume and reef accretion. Oyster volume (live and dead shell) ranged from 0.03-19.98 L m^{-2}, with a mean volume of 7.2 +/- 1.3 L m^{-2}. The oldest riprap oyster reefs accreted more shell than those deployed only a few years prior to this survey. On rock reefs, such as these, shell accretion is an important oyster reef restoration metric.

Riprap oyster condition index. Mean CIs were calculated from all sites except site #4 (Table 2.2) because that site had low sample size. All three CIs are documented in the literature (Rainer and Mann 1992) and assume that the oysters processed have both valves. However, the process of removing oysters from riprap with hand scrapers left

some of the oysters with only a single valve. Thus, a correction factor was used to avoid inflation of condition index for any oyster missing significant shell mass. For example, an oyster with only one valve has its CI divided by two and an oyster with half of one valve missing (1.5 valves remaining) had its CI divided by 1.5. Since in no case were all oysters in a sample missing whole, or portions of their, valves, the mean 'corrected CI' is not simply one half of the original CI. CI did vary amongst sites, but the means (+/- 1 SEM) of CI1 (9.99 +/- 0.35), CI2 (7.44 +/- 0.32) and CI3 (2.76 +/- 0.15) indicate that the riprap oysters were healthy. There was no trend in the CIs among sites by region or riprap type ($p > 0.16$ for all three CIs). Also, there was no distinct effect of concrete versus granite on oyster CI (CI 1: $R^2 = 0.15$; CI2: $R^2 = 0.01$; CI3: $R^2 = 0.00$).

Restored shell reef oyster population structure. In total, 362 oysters were collected over 15 samples in the intertidal zone on the Hume's Marsh restored oyster shell reef. Oyster SH ranged from 8.9-116.3 mm, with three peaks representing two to three year classes (Fig. 2.5).

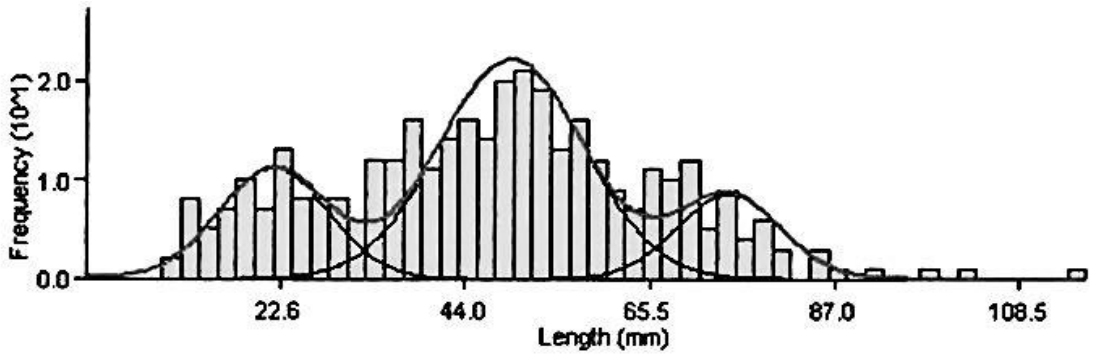


Figure 2.5: Separation of oyster shell length-frequency data from Hume's Marsh restored oyster shell reef into individual oyster classes.

The population size structure was similar for all three intertidal zones (Appendix 2.2), thus allowing us to collapse across intertidal zone and provide for a robust analysis of the entire reef. Although the Long Creek restored oyster shell reef (Appendix 2.3) was incomplete, similar size structure patterns emerged. The two high-density oyster reef samples (Fig. 2.6a-b) supported the full complement of oysters throughout the entire size range observed in the LRS.

Restored shell reef oyster density. Mean oyster density on the Hume's Marsh restored oyster shell reef was 97 +/- 16 oysters m^{-2} with a mean SH of 46.0 +/- 2.6 mm and a mean mussel density of 8 +/- 4 mussels m^{-2} (Table 2.4a). No trends were detected amongst the individual samples or intertidal zones (Appendix 2.4).

The Hume's Marsh high-density oyster shell reef sample had an oyster density of 956 oysters m^{-2}, with a mean SH of 53.2 +/- 1.7 mm, and a mussel density of 300 mussels m^{-2}. The Keeling's Drain high-density oyster reef sample had an oyster density of 776 oysters m^{-2}, with a mean SH of 50.5 +/- 1.9 mm, and a mussel density of 68 mussels m^{-2}. Both samples were collected within the low- to mid-intertidal zone.

Restored shell reef oyster biomass. Oyster biomass was estimated for the Hume's Marsh restored oyster shell reef using separate regressions for each sample and a single regression including all samples to determine the relative error associated with regressions containing a small percentage of the total sample size. All regressions fit the data well ($r^2 > 0.77$). The Hume's Marsh restored oyster shell reef mean oyster biomass was 26.48 +/- 5.45 g AFDM m^{-2}.

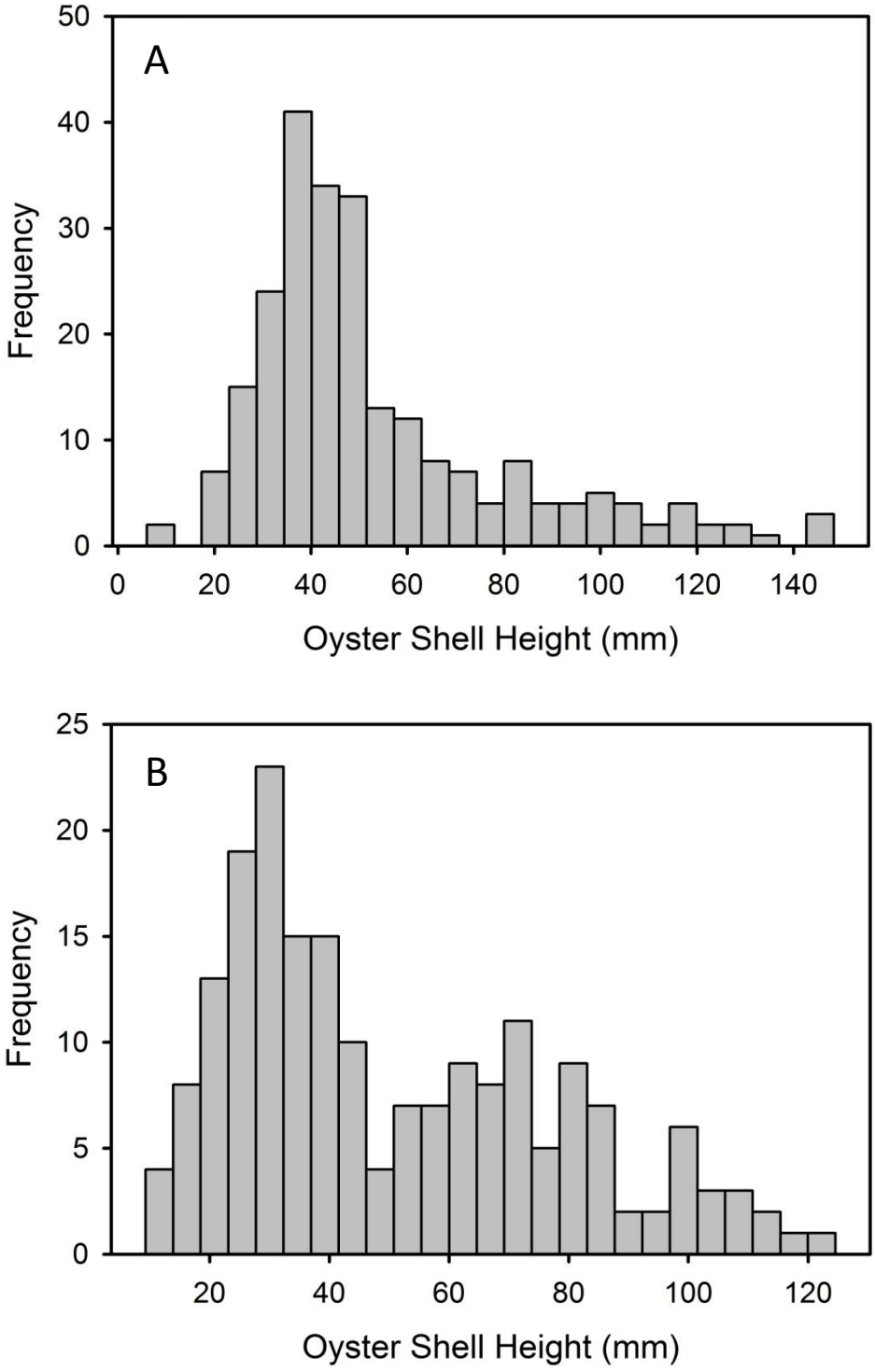


Figure 2.6: (A) Population size structure of oysters in Hume's Marsh high-density oyster sample. (B) Population size structure of oysters in Keeling's Drain high-density oyster sample.

A single oyster SH-biomass regression model for the whole reef (y = 2.8838x - 5.5426) produced different mean oyster biomass estimates than three separate models for each intertidal zone (Table 2.4a). Regardless of the method chosen, the mid intertidal zone had the highest biomass.

The Hume's Marsh high-density oyster reef sample (y = 2.1677x – 4.4791) had an oyster biomass of 232.22 g AFDM m^{-2}. The oyster biomass was equal to 58% of the sum of all 15 samples taken from Hume's Marsh restored oyster shell reef, which highlights the variable productivity of previously deployed restored oyster shell reefs. The Keeling's Drain high-density oyster reef sample (y = 2.7219x - 5.3600) also had a high oyster biomass of 251.54 g AFDM m^{-2}.

Restored shell reef oyster volume and reef accretion. Oyster volume for the Hume's Marsh restored oyster shell reef, ranged from 0.0-6.0 L m^{-2}, with a mean volume of 2.9 +/- 0.5 L m^{-2}. This reef was built very high with large shucked oyster shells, which have allowed it to persist for a number of years.

Restored shell reef oyster condition index. CIs were calculated for all samples on the Hume's Marsh restored oyster shell reef. Over the entire reef, the mean CI values for the three indices (CIs 1-3) were 12.6 +/- 0.6, 10.1 +/- 0.6, and 3.4 +/- 0.3 while the mean corrected CI values were 10.8 +/- 0.4, 8.6 +/- 1.7, and 2.7 +/- 0.2 (Table 2.4b), indicating oysters in similar health to the average riprap oyster. Lower intertidal oysters had a greater mean CI than those sampled from the upper intertidal zone. Note that no CIs were calculated for the Long Creek reef because AFDM estimates were not available.

Table 2.4: (A) Mean oyster density, shell height (SH), biomass (dry mass (DM), and ash-free dry mass (AFDM)) of Hume's Marsh restored oyster shell reef and (B) condition indices (CI) across intertidal zones

Intertidal Zone	Depth (cm)	Oyster Density (m^{-2})	Oyster Biomass (g AFDM m^{-2})	Oyster Biomass (g DM m^{-2})	Mean Oyster SH (mm)	Mussel Density (m^{-2})
Upper	0.8 (0.8)	88 (31)	23.25 (10.07)	26.71 (11.46)	44.8 (3.4)	8 (4)
Mid	9.6 (2.3)	107 (25)	32.52 (11.56)	37.11 (13.01)	47.1 (3.8)	8 (4)
Lower	34.4 (5.3)	94 (31)	23.67 (7.95)	27.14 (9.05)	46.0 (6.5)	16 (12)

A.

Intertidal Zone	Oyster Condition Index 1	Oyster Condition Index 2	Oyster Condition Index 3	Oyster CI 1 Corrected	Oyster CI 2 Corrected	Oyster CI 3 Corrected
Upper	10.9 (1.0)	8.6 (1.2)	2.9 (0.7)	9.1 (0.5)	7.0 (0.4)	2.3 (0.4)
Mid	12.4 (0.7)	10.0 (0.8)	3.1 (0.2)	10.8 (0.6)	8.5 (0.6)	2.5 (0.1)
Lower	14.1 (0.8)	11.6 (0.8)	4.1 (0.6)	12.3 (0.5)	9.9 (0.6)	3.2 (0.3)

B.

The Hume's Marsh high-density oyster reef sample had CIs (1-3) of 6.7 +/- 0.3, 5.1 +/- 0.2, and 2.1 +/- 0.1. The Keeling's Drain high-density oyster reef sample had CIs of 8.8 +/- 0.3, 7.3 +/- 0.3 and 2.3 +/- 0.1. The CIs were similar for both high-density samples (Table 2.5). However, the mean oyster CI for the Hume's Marsh high-density sample was much less than the adjusted CIs for the Hume's Marsh restored oyster shell reef.

Table 2.5: Mean oyster density, shell height (SH), biomass (dry mass (DM), and ash-free dry mass (AFDM)), and condition indices (CI) of Hume's Marsh and Keeling's Drain high-density restored oyster shell reef sample, and mussel density across intertidal zones.

Sample ID	Oyster Density (m^{-2})	Oyster Biomass (g DM m^{-2})	Oyster Biomass (g AFDM m^{-2})	Mean Oyster SH (mm)	Mussel Density (m^{-2})	Oyster Condition Index 1	Oyster Condition Index 2	Oyster Condition Index 3
Hume's Marsh	956	289.20	232.22	53.2 (1.7)	300	6.7 (0.3)	5.1 (0.2)	2.1 (0.1)
Keeling's Drain	776	287.12	251.54	50.5 (1.9)	68	8.8 (0.3)	7.3 (0.3)	2.3 (0.1)

DISCUSSION

The riprap oyster populations of Broad Bay, Long Creek and Lynnhaven Bay had high condition index, biomass often exceeding 177 g AFDM m^{-2}, and very high densities averaging nearly 1000 oyster m^{-2} (978 +/- 177 SEM), which is among the top estimates for intertidal artificial reef substrates in Chesapeake Bay (Nestlerode et al. 2007). Both concrete and granite riprap supported robust oyster populations with a size structure indicative of consistent annual recruitment, an important attribute for reef persistence. The smooth curve of the single peak in size structure made it difficult to distinguish year classes, and is usually characteristic of consistent recruitment from year to year. High and variable growth rates and multiple spawning events could explain also the lack of distinction between size classes.

We also compared the performance of the riprap reefs with the nearby Hume's marsh restored oyster shell reef. The Hume's Marsh oyster shell reef harbored oysters with a healthy condition index at a mean density of 97 +/- 16 (SEM) oysters m^{-2}. Moreover, oyster in the lower intertidal had a greater mean CI than those in the upper intertidal zone. Bartol and Mann (1999) reported that oysters in the lower intertidal on a

constructed oyster shell reef had higher survival than those in the high intertidal. Lower intertidal oysters experience less thermal and desiccation stress, and can continue to feed during periods in the tidal cycle when upper intertidal oysters are exposed and unable to feed. Apparently, indirect benefits of aerial exposure in the mid to upper intertidal zone, such as reduced parasite load (Encomio and Chu 2007), fewer fouling organisms, and a partial refuge from predation, do not overwhelm the disadvantage of reduced duration of inundation in the high intertidal zone (Bishop and Peterson 2006).

Oyster reefs constructed of loose oyster shells offer a buffered, protected habitat for oysters that settle and grow within the reef interstices, but only to a shallow depth of 10 cm (Bartol and Mann 1999). Riprap reefs provide much larger pore spaces and supported oysters three to four rocks deep, greater than 50 cm deep, in many cases. The considerable surface area, diversity of exposed and shaded surfaces, and good flow within the reef interstices of concrete and granite riprap likely promote high oyster and mussel densities.

The two high-density restored oyster shell reef samples represent peak values for oyster density and biomass on such man-made reefs. These values were 8-10 times higher than the Hume's Marsh and Long Creek restored oyster shell reefs, and validate claims that portions of constructed shell oyster reefs can perform well. The two high-density samples each supported a robust oyster population size structure (Mann et al. 2009a); however, they had lower mean oyster condition than the rest of the Hume's Marsh restored oyster shell reef. This substantial difference in oyster condition suggests that food competition became compounded with increased oyster and mussel density, a phenomenon often exacerbated by low water flow over the reef (Wildish et al. 1987,

Eckman et al. 1989, Lenihan et al. 1996). This was likely the case as this sample was collected on the lee side of the Hume's Marsh restored oyster shell reef where buffering of wind and wave action occurs due to the presence of Hume's Marsh on one side and the restored oyster shell reef on the other. The Keeling's Drain high-density sample may have fared better in this respect since it has marsh behind it, but open, unabated water flow in front of it (mouth and channel of the Eastern Branch of the Lynnhaven River) (Artabane 2006).

Finally, despite residing in a high salinity zone noted for high oyster disease pressure (Carnegie and Burreson 2009), many of the oysters grew much larger than the assumed size at which disease causes high mortality. Some possible explanations for the high survival and growth are (1) evolved disease tolerance, (2) reduced pathogen virulence or abundance, (3) high, but not complete disease mortality, and (4) oysters grow fast in the LRS and give the impression of oyster disease resistance without really expressing it. Unfortunately, this study did not test disease prevalence or intensity. Beyond a lack of disease data, oysters in the LRS do often grow to 75 mm SH (market size) faster than in many other parts of Chesapeake Bay, so assuming that a 75-mm oyster is three years old would not be appropriate for the LRS. Thus, use of oyster SH alone as a proxy for oyster disease resistance was not justified in this study. Disease-tolerant or not, riprap oyster densities eclipsing 1000 m^{-2} with oyster biomass > 200 g AFDM m^{-2} indicate that concrete and granite riprap do serve as effective oyster reef habitat in the LRS and should be considered for use in future native oyster restoration reef projects. Furthermore, these alternative reefs can serve as "living shorelines" to mitigate erosion in place of detrimental bulkheads (Meyer et al. 1997, Seitz et al. 2009).

In summary, alternative substrate reefs of granite and concrete supported some of the highest abundances of healthy oysters ever recorded for Chesapeake Bay artificial oyster reefs, shell or otherwise. Furthermore, the oyster population size structure was indicative of consistent annual oyster recruitment, a quality critical for reef persistence. Consequently, alternative oyster reefs constructed of concrete or granite riprap can enhance ecological native oyster restoration efforts in the LRS and other high-salinity Chesapeake Bay tributaries.

Acknowledgments. We express appreciation to students and staff of the Marine Conservation Biology and Marine Community Ecology Programs at VIMS, particularly, Katie Knick, Amanda Lawless, Alison Smith, and Justine Woodward for their assistance in the lab and field. Special thanks to Dave Schulte for his field help and expertise. Funding was provided by grants to R.N. Lipcius and R.D. Seitz from the Norfolk Office of the USACE (D. Schulte, program manager). We wish to thank all waterfront property owners who allowed us to sample their riprap and often provided us critical site-specific background information. Finally, we have dedicated the name of one riprap sample site as "Yopp" for the late Mr. Luther Yopp, who graciously welcomed our sampling of his riprap and placement of an alternative substrate experiment outside his property along Long Creek.

Chapter 3

Population structure, density and biomass of the Eastern oyster (*Crassostrea virginica*) and hooked mussel (*Ischadium recurvum*) on artificial oyster reefs in the Rappahannock River, Chesapeake Bay

ABSTRACT: Restored Eastern oyster (*Crassostrea virginica*) shell reefs have generally experienced marginal success and require reseeding to sustain populations in Chesapeake Bay. Many shell reefs vary significantly from natural reefs in having: (1) limited vertical complexity, (2) low reef stability, (3) reduced substrate area for larval settlement, and (4) diminished reef community structure. Some alternative reef structures may overcome these deficiencies and serve as effective oyster reefs. In 2005 and 2007, oyster and mussel population structure, density and biomass were quantified on a novel concrete modular reef deployed subtidally in the Rappahannock River, Chesapeake Bay. The modular reef was neither seeded artificially nor harvested. The reef was colonized heavily by oysters and mussels, which recruited and survived at high densities for the 5-7 years since reef deployment in 2000. Oyster and mussel biomass and density were among the highest recorded for natural and restored oyster reefs. Additionally, a large array of artificial concrete reefs known as 'Steamer Rock' (deployed in 1994, and located adjacent to the concrete modular reef), was sampled in 2006. We estimated that, within a subtidal footprint of < 0.4 ha, these reefs contained > 4 million oysters and > 30 million mussels. In disease prevalence and intensity tests, oysters from both reef systems were healthy. These artificial reefs support mature oyster communities, as they provide vertical structure and stability required to buffer environmental stress and predation pressure. We posit that the Steamer Rock reef system: (1) supports a significant fraction of the Rappahannock River oyster and mussel breeding stock, (2) is the largest, most successful artificial (alternative substrate) oyster reef in Chesapeake Bay, and (3) sustains a diverse assortment of fish and invertebrates. Thus, large-scale, subtidal, alternative substrate reefs are a viable native oyster restoration strategy.

INTRODUCTION

Restoration of the eastern oyster, *Crassostrea virginica*, in Chesapeake Bay has traditionally relied on low-relief shell reefs (2-4 inches thick; Smith et al. 2005) and oyster shell mounds (~1 m tall, personal observation) created in an attempt to mimic natural reef conditions and accelerate recruitment (Mann 2001, Southworth et al. 2008b). To date these efforts have met with limited success (Mann and Powell 2007), though some recent restored oyster reefs in the Great Wicomico River (Schulte et al. 2009), and Lynnhaven River (Lipcius et al. 2008) have been thriving.

Due to limitations in natural hard substrate, artificial habitats have been used for various species over 100 years and recently recognized by fishery managers as a viable enhancement technique (McGurrin et al. 1989, Stone et al. 1991). The utility of artificial reefs led the United States to develop its a National Artificial Reef Plan (NARP) in 1985 (amended in 2007) under direction of the National Fishing Enhancement Act of 1984 and with the participation of coastal state artificial reef program managers (US Dept. of Commerce 1985, 2007). For instance, the Commonwealth of Virginia's Artificial Reef Program, which is managed by the Marine Resources Commission, has a rich history, using World War II Liberty ships in the early 1970s and “materials of opportunity” such as demolished concrete bridges in the 1970s and as structures that provide stability, durability and a maximum amount of surface area and interior space.

Traditional oyster repletion programs (those that condition harvest bottom to receive natural spatset) and restoration projects (those that rehabilitate formerly productive oyster bottom) have relied almost exclusively on supplies of oyster shell from either processing houses or deposits of 3,000-4,000-year-old buried fossil shell.

However, oyster shell has become a limiting resource. Thus, substrates other than oyster shell (shucked or dredged), including surf clam shells, gypsum (gypment), crushed concrete, limestone, porcelain toilets, and pelletized coal ash, have been used experimentally as alternative oyster reef substrate (Soniat et al. 1991, Haywood et al. 1999, LADWF 2004, Nestlerode et al. 2007).

More recent oyster restoration efforts have integrated construction of artificial (alternative substrate) reefs such as reefballs (Brumbaugh et al. 2006), limestone (Lukens 1997, Davis et al. 2006) and concrete structures (Cowan 2003). Out of 11 oyster reef sanctuaries comprised of constructed shell and limestone reefs, and one natural reef, seven were performing well over 10 years after their creation (Powers et al. 2009). Disease prevalence and severity were low in sanctuary reefs despite high densities and older oysters, which in the past have been highly susceptible to disease (Powers et al. 2009). We extend these results by providing an in-depth analysis of a massive artificial reef system to discern the key features of artificial oyster reefs that drive reef success. Our sampling design addressed factors such as reef layer and face, and edge vs. interior reef locations, enabling us to determine the role of substrate orientation and position for oyster and mussel abundance. Vertical orientation and predation refuge are critical to the early development of the oyster reef community and must be included in restoration designs (Soniat et al. 2004).

Forecasting benthic community development on artificial substrata is difficult and controversial (Sara' 1987). According to the classical view, development of the epifaunal benthic community was seen as a successional sequence leading to a climax community through deterministic and predictable stages (Clements 1916, Scheer 1945). Conversely,

the community of a newly immersed artificial substratum is linked to stochastic larval recruitment that is also characterized by seasonal and annual variability and by the relative dominance of pioneer species, so that a classic succession cannot be determined (Sutherland and Karlson 1977). In either case, five years is sufficient for a relatively stable community to develop in waters other than the most oligotrophic waters (Jensen et al. 2000). In our study, eastern oyster (*C. virginica*) and hooked mussel (*Ischadium recurvum*) were quantitatively sampled on a concrete modular reef in 2005 and 2007 approximately 5 and 7 years after deployment, respectively. In 2006, the Steamer Rock reef complex was similarly sampled, 11 years after deployment. Hence, both sets of reefs had ample time for a mature oyster reef community to develop.

MATERIALS AND METHODS

Concrete Module Reef

Construction. In 1994, a non-profit organization (Rappahannock Preservation Society - RPS) deployed 176 stacks (7 layers per stack) of recycled bridge pieces (materials of opportunity) subtidally at 7 m depth on sand bottom at Steamer Rock (SR), near the mouth of the Rappahannock River, a western-shore tributary of Chesapeake Bay. Steamer Rock remains the largest (0.4-ha footprint) alternative oyster reef in lower Chesapeake Bay. In October 2000, RPS deployed an experimental rebar-reinforced concrete modular reef (CM) at the same depth and location. The designer of both reef complexes, a retired engineer for the United States Navy (Captain Robert Jensen), intended to provide suitable substrate for oysters in a high-flow, low-siltation habitat (Abelson and Denny 1997) while testing his own design aimed at maximizing flow,

surface area and vertical structure (Baynes and Szmant 1989). The site selected met Virginia state and federal artificial reef requirements (US Dept. of Commerce 1985, 2007) and benefited from strong tidal currents, ample depth assuring the tall reefs would not pose a navigational hazard, and conservative distance from depths frequently associated with hypoxia.

Sampling procedure and design. The CM reef (5.02-m^2 footprint) consisted of five Module Layers (ML, see one ML in Fig. 3.1) stacked on each other (Fig. 3.2a), with four faces (Top, Side, Hole, Bottom) per ML (dimensions: 2.24 m x 2.24 m x 0.66 m). Due to logistical constraints, we were only able to sample the top three layers (MLs 5, 4, and 3) on 27 May 2005 (Fig. 3.2b-c). During reef retrieval, a commercial diver indicated that the lowest two layers appeared similar in oyster and mussel abundance to the upper three layers, when MLs 3, 2, and 1 were sampled (Fig. 3.2d). The three MLs were secured simultaneously with straps by a commercial diver and brought to the surface by a crane aboard a commercial barge for sampling (ML 4 was sampled in May 2007; technical problems limited sampling to only one ML). To access all faces on each ML, the crane on the commercial barge lifted one ML off the lower ML until all samples were collected. Upon completion, the layers were stacked in order aboard the barge and returned to the river bottom. Photographic and videographic documentation of each reef recovery and sampling procedures were compiled the day of removal.

The CM reef was sampled using a stratified random sampling design (Appendix 3.1) following Cochran (1977) and Williams et al. (2002). Two types of strata were defined, ML and face. The SA for each face was calculated using a schematic (Fig. 3.1)

provided by Reeftek-McLean (McLean constructed and deployed the CM reef). All potential sample plots for each ML-face treatment (Appendix 3.1) were calculated with Microsoft Excel®; sample plots were selected using random numbers generated by Excel. On site, surface area of each sample was defined using a 25.4-cm x 25.4-cm quadrat (2.54-cm x 2.54-cm Riverdale mesh). In 2005, a total of 120 samples were collected over 7.250 m^2 of concrete SA; 10 samples were taken from each of the 12 ML-face treatments. In 2007, a total of 150 samples, over 8.048 m^2 of concrete SA, were collected from MLs 4, 3, 2 and 1 (ML 1 – Bottom was barren; no samples were taken). Half of all samples collected from MLs 4 and 3 in 2007 were ML-face plots previously sampled/cleared in 2005 to quantify oyster reef recovery, and are henceforth referred to as 'resamples' in the 'resampling' sections. Careful attention was paid to the total SA sampled since oyster and mussel density were ultimately calculated using recorded sample plot areas.

Upon removal of the MLs, it became apparent the lifting straps had removed the epifauna present at each strap-reef interface. Sample plots that were impacted by the straps were discarded and the next random plot selected. Epifauna were removed from each sample plot with hand scrapers, placed in large trays, stored in freezer bags, placed in ice-filled coolers, and transported to freezers at the Virginia Institute of Marine Science (VIMS).

Laboratory processing. Samples were processed in the laboratory in increments of 24 samples (3 MLs x 4 faces x 2 replicates). The first 24 samples were haphazardly selected from freezer storage. Each sample was thawed and rinsed over a 1-mm mesh sieve.

Bivalve (oyster and mussel, live and dead) and sponge volume were measured using volumetric displacement. Shell height (SH), width, and depth were measured for all bivalves, living and dead. For oysters, SH was considered as the distance from the umbo to the farthest posterior end of the shell. Additionally, all internal tissues were collected for selected oyster in pre-weighed aluminum 'weigh boats' for dry mass (DM) and ash-free dry mass (AFDM) measurements. Of the 924 mussels collected, 138 mussels representing the full range of SH values were processed for DM and AFDM.

Condition Indices (CIs; Mercado-Silva 2005) were calculated for 66 of the 108 oysters from among the first 24 samples processed during the summer 2005. The oysters were selected from all faces on all three MLs. For the remaining 96 samples, bivalve volume was quantified and SH was measured for all oysters. Live and dead mussels were counted but no SHs were measured. Details regarding laboratory biomass procedures and condition index calculations can be found in the 'Materials and Methods' section of Dissertation Chapter 2.

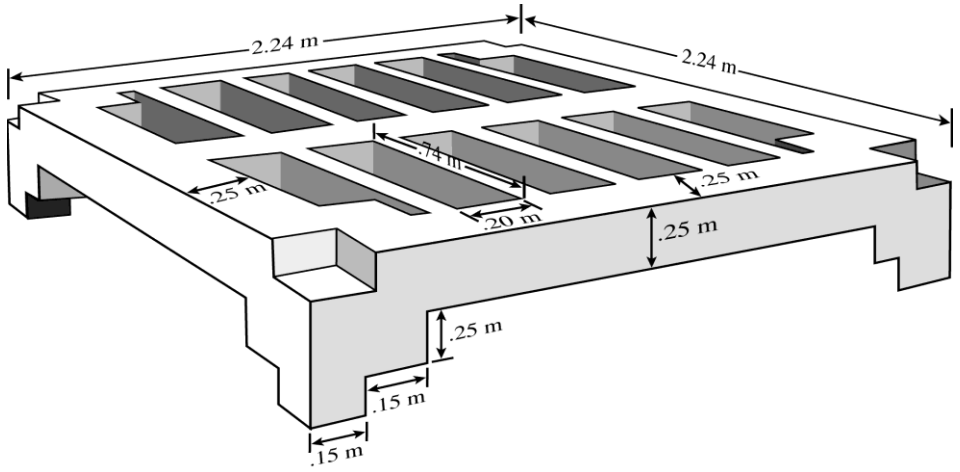


Figure 3.1: Schematic design of a single concrete module (image credit: Harold Burrell, VIMS).

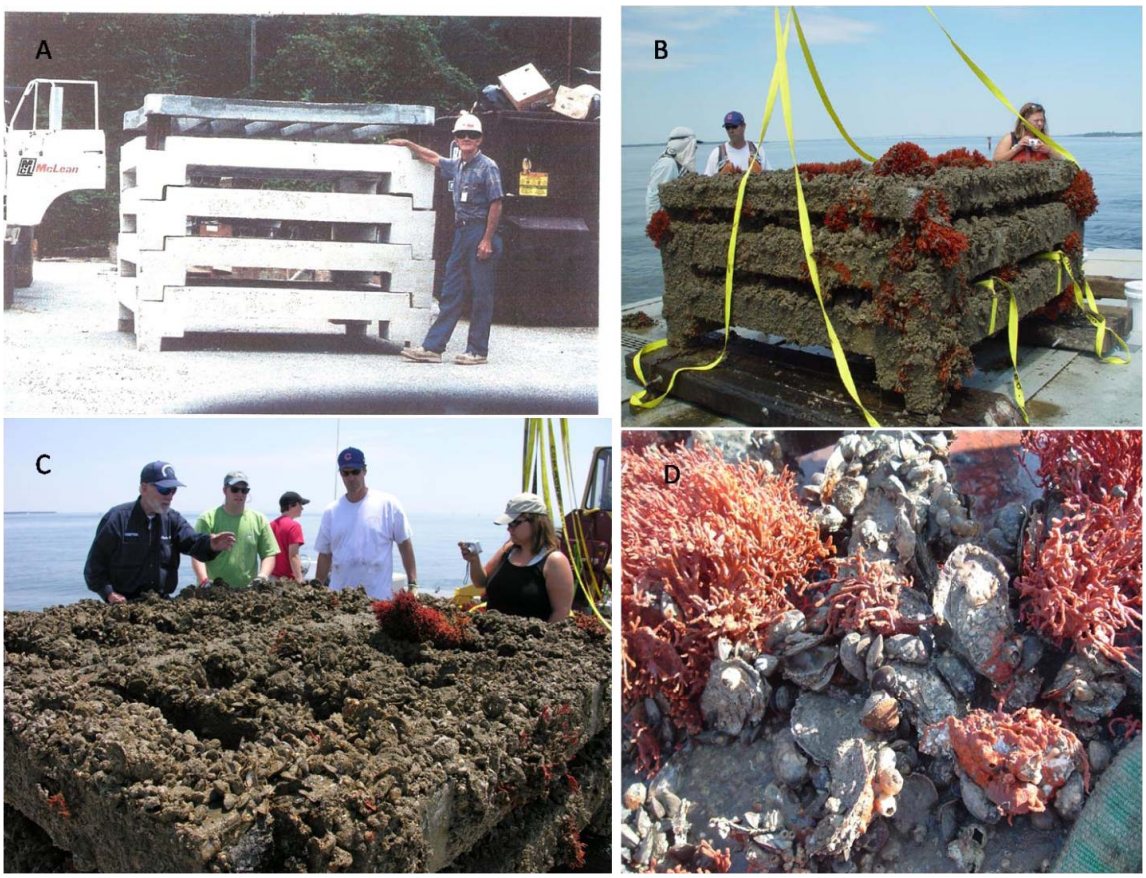


Figure 3.2: (A) The five concrete module layers (MLs) stacked pre-deployment, fall 2000 (image credits: Capt. Robert Jensen), (B) The top three MLs on a barge in the Rappahannock River, May 2005, (C) ML 4 with the reef designer, Capt. Jensen (pictured far left), May 2005, (D) Close-up of the Top face of ML 2, May 2007.

Population structure. In the analysis of population size structure for oysters we used all 120 samples (523 oysters), whereas for mussels we used only the first 24 randomly-selected (stratified by ML and face) samples (924 mussels). Peaks were analyzed with FISAT II (Gayanilo et al. 2002) to delineate individual year classes (YCs). The peaks were separated using Bhattacharya's Method (Bhattacharya 1967). The program uses a set of equations that yields mean lengths, population sizes (in numbers), standard deviations and separation indices for each YC, where a 'separation index' is the

difference between two successive means divided by the difference between their estimated standard deviations. Note, peak height and width were secondary to the primary goal of effectively distinguishing individual peaks (size classes as a proxy for age classes).

Density and abundance. Density of individuals m^{-2} of river bottom is a common metric for assessing oyster and mussel density of reefs. Mean oyster and mussel density for each ML and face was calculated by dividing live oyster and mussel counts by the sample area (not constant) for each sample. This density metric is referred to as 'surface area density' since it measures the number of individuals on a given concrete surface.

Oyster and mussel abundance were defined as the total unit measure for one ML, face, or the whole reef. Abundance was calculated by multiplying surface area (SA) density times the total available concrete SA for a given stratum (ML or face). The MLs were assumed to be uniform in shape from MLs 1-5 despite ML 5 being the slightly smaller prototype. For a single ML, the Top and Bottom faces each contained 3.1 m^2 of concrete SA, the Side face had 2.9 m^2, and the Hole face (eight holes) had 5.7 m^2.

The CM reef (5 MLs) contained nearly 15 times more surface area than the 5 m^2 of river bottom that it covered (Table 3.1). Confidence bounds were calculated using an estimator based on a stratified random sampling design with unequal sample areas (Williams et al. 2002). Oyster density and mussel density were analyzed using a two-way analysis of variance (ANOVA) examining the effects of ML, face, and the interactions. Student-Neuman-Keuls (SNK) *post hoc* comparison tests (Zar 1996) were conducted when significant interaction effects were detected ($p < 0.05$).

Biomass. The AFDM data for oysters and mussels were used in a length-weight regression to estimate biomass over the entire CM reef, given that the size structure produced from all 120 samples was consistent with the size structure produced from the first 24 samples.

Pathology. A total of 30 (May 2005: 75.6-125.2 mm SH) and 25 (Nov. 2007: 51.4-153.8 mm SH) oysters were haphazardly sampled from the different ML faces for pathology tests performed within two weeks of sampling. Samples were brought back live and on ice. The VIMS Shellfish Pathology group processed the oysters and determined presence and intensity of Dermo (*Perkinsus marinus*) and MSX (*Haplosporidium nelsoni*) parasites. Two methods of Dermo analysis were conducted in 2007, histology and RFTM (Ray fluid thioglycollate medium). The RFTM method has a lower detection limit.

Table 3.1: Surface area (SA) and SA-to-River Bottom (RB) ratios of the Steamer Rock (SR) and Concrete Module (CM) reefs.

	SR SA (m^2)	CM SA (m^2)
Top	16	3.1
Side	4	2.9
Hole	---	5.7
Bottom	16	3.1
Layer^{-1}	36	14.8
Stack^{-1}	236	74.2
SA:RB Ratio	14.75	14.78

Steamer Rock Reef

Sampling procedure and design. Adjacent to the CMs, the much larger SR concrete reef was systematically sampled in April and August 2006. The layout of the reef (Fig. 3.3a-b) is a set of seven concentric rings consisting of 176 seven-layer stacks of bridge sections (from the Rte. 3 Norris Bridge) covering 2816 m^2 of river bottom within a 0.40-ha footprint. The stacks (Fig. 3.4a) are about 4 m tall and were deployed in 1994 at depths of 6-8 m in the Lower Rappahannock River off Mosquito Point (Lancaster County, VA) to create sanctuary oyster and recreational fish reefs. Unlike the CM survey, these stacks could not be lifted to the surface. Sample collection required SCUBA diving over two sampling days. We expected that the intermediate concentric rings (interior habitat) would be more similar to each other than the outer- and inner-most rings (edge habitat).

The outer- and inner-most rings were sampled in April 2006; the intermediate rings were sampled in August 2006. Diver safety prompted the decision to sample the least obstructed (outer/innermost) rings first. Additionally, intense Dermo oyster infections were more likely late in the summer (August) than in spring (April), especially in the intermediate rings where bivalve densities were projected to be highest.

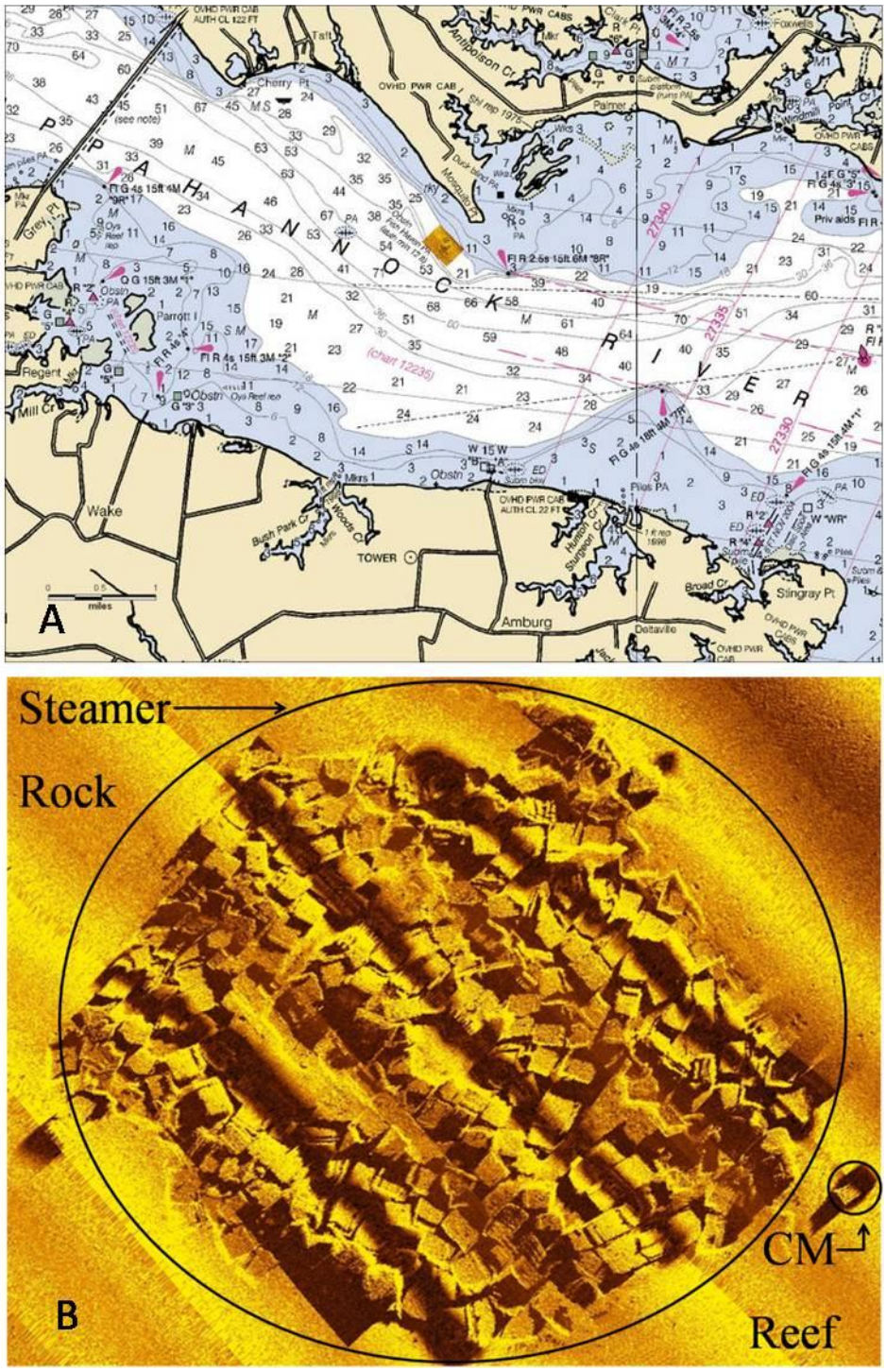


Figure 3.3: (A) Bathymetic map of the lower Rappahannock River including (B) a mosaic of side scan sonar of the Concrete Module (CM) and Steamer Rock reefs (images credit: Gary Smith, Random Motion, LLC).

The method of sample collection included the diver haphazardly selecting a sample location visually from about 1 m away from the structure to avoid bias, proceeding towards the location, setting a 0.3-m x 0.3-m sampling device flush against the site, scraping all encrusted growth into a mesh bag until the concrete was fully exposed, cinching the bag shut, and sending the sample to the surface using a dive bag filled with air. For each sample, depth, GPS coordinates of the stack, concentric ring identity, and the face of the structure (top, side, inner/between slabs) were recorded. When moving to the next ring, GPS coordinates were once again recorded because turbidity limited underwater visibility in August within the intermediate rings, increasing the probability of diver disorientation. A color video camera mounted to the diver's helmet was used for quality control (Fig. 3.4b); we could confirm that the area sampled was 0.3 m x 0.3 m. This video footage was also important because three different divers conducted the sampling in April. In August, a single diver did the sampling, minimizing individual-based sampling variance. Sample contents were removed from the mesh bags into freezer bags, placed in ice-filled coolers, and transported to freezers at VIMS.

Laboratory processing. The same laboratory procedures used for the CM samples were used for the SR survey, except that disease testing was only completed for the August 2006 samples. Measurements included oyster SH, mussel and mud crab counts, and bivalve and sponge volume. Population size structure, density, biomass and CI estimates were calculated as well. General linear models (GLMs) were conducted to test for differences in oyster SH, abundance, biomass, and CI amongst concentric ring classes; Kruskal-Wallis rank tests were conducted if tests of normality failed.

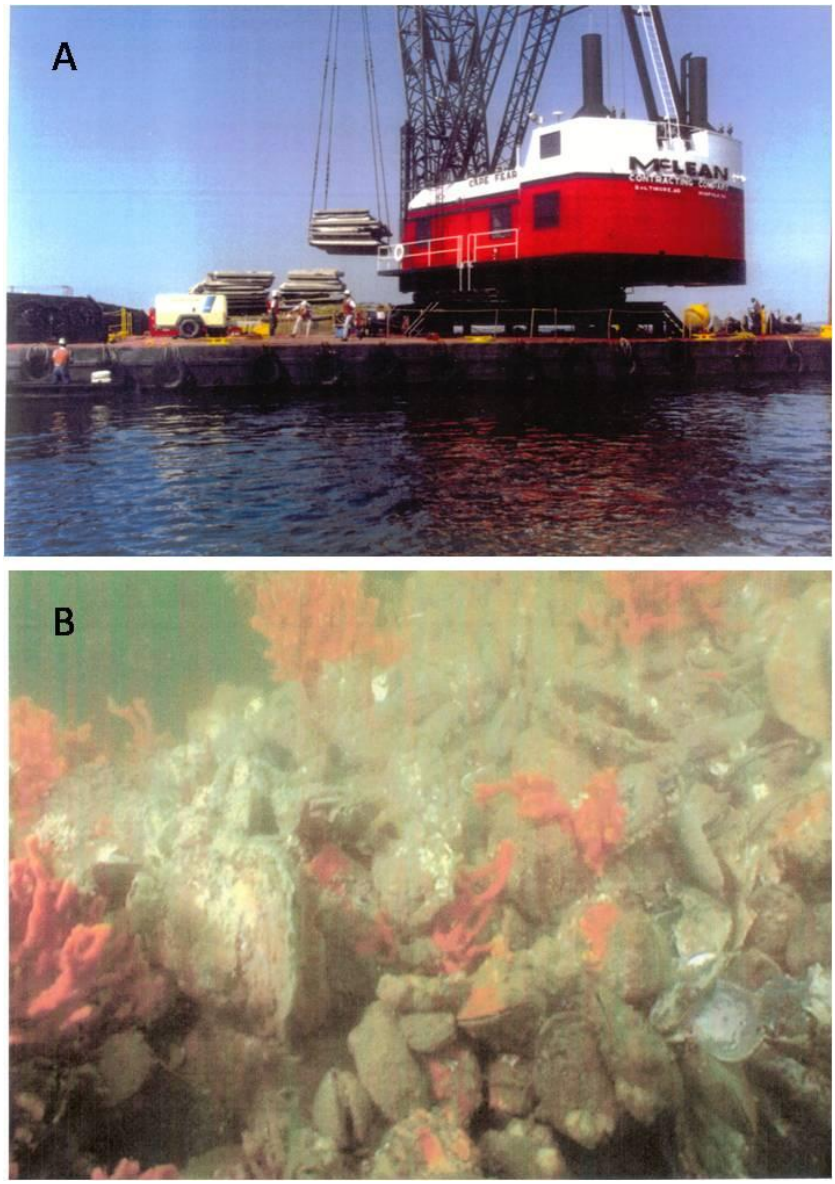


Figure 3.4: (A) Deployment of the first Steamer Rock reef stacks by McLean Contracting Co., September 1994, (B) Underwater image of an SR reef surface ~10 years post-deployment (image credits: Capt. Robert Jensen).

Pathology. A SCUBA diver haphazardly sampled 25 oysters (Aug. 2006: 73.3 – 141.3 mm SH) from the SR stacks for pathology tests. Samples were brought back to the VIMS Pathology group live and on ice, and were performed within two weeks of sampling. Presence and intensity of Dermo (*Perkinsus marinus*) and MSX (*Haplosporidium nelsoni*) parasites were determined in the same manner as for the 2007 CM oysters – histology and RFTM (Dermo only).

RESULTS

Concrete Module Reef

Population structure – 2005. Oyster size structure was constructed using a length-frequency histogram (Fig. 3.5a) that included SHs (mm) for 520 of the 523 live oysters sampled. Oyster SHs ranged from 7.1-139.0 mm. Peaks were analyzed with FISAT II (Gayanilo et al. 2002) to delineate individual year classes. Four distinct peaks were distinguished from the composite distributions (Fig. 3.5a). The Top face of MLs 5 and 4 had the most pronounced oyster spat peak, while MLs 4 and 3 had more large oysters (Fig. 3.5b). This trend of increasing oyster size with depth was confirmed by diver observation for MLs 2 and 1. The mussel size structure (Fig. 3.6a) had 4-5 peaks with a robust size distribution of mussel SHs (9.2-61.0 mm). ML 4 contained the most mussels, many of which were > 30.0 mm (Fig. 3.6b). The Top face had more large mussels than the other three faces combined (Fig. 3.6b).

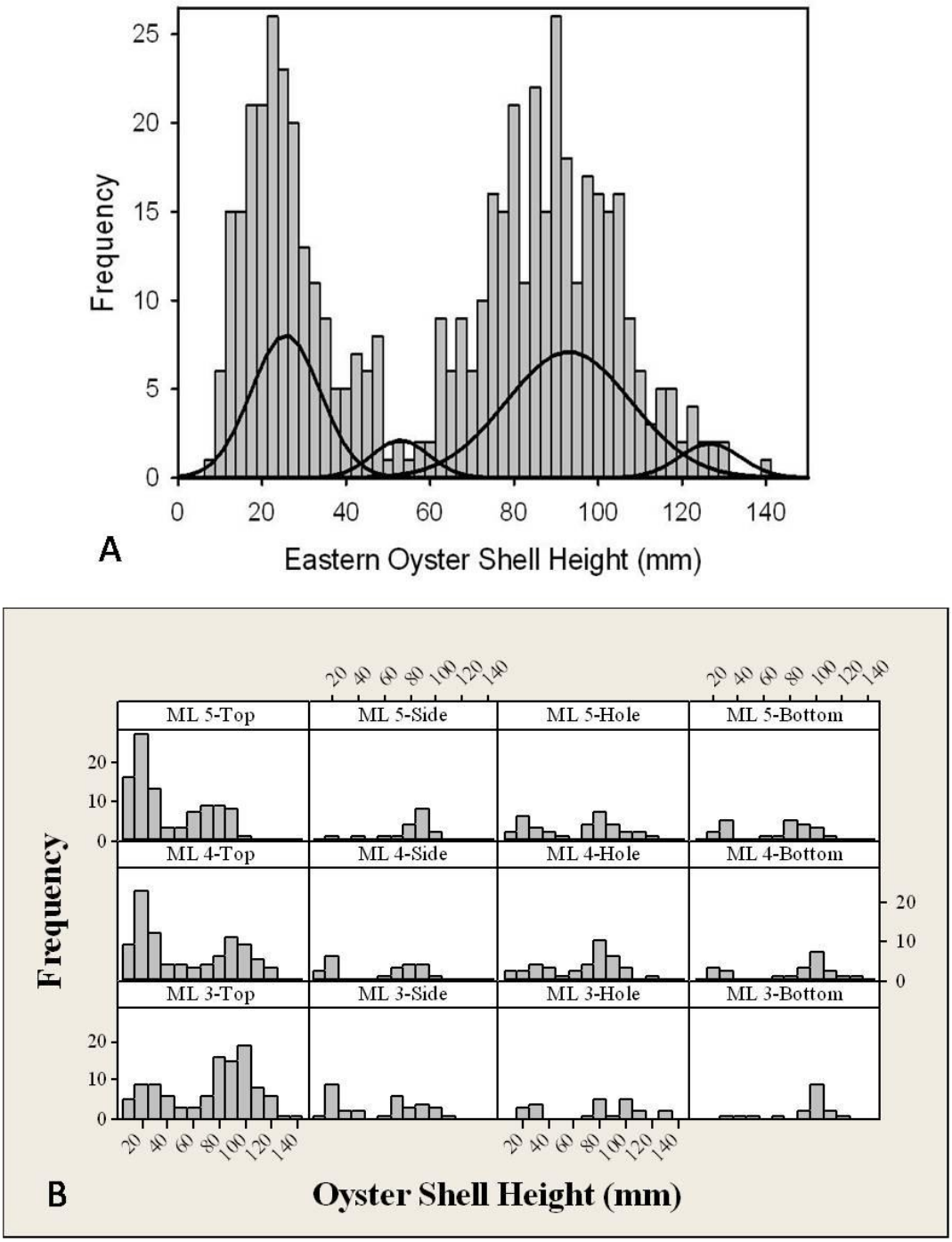


Figure 3.5: (A) Population size structure (PSS) of oysters on the concrete modular reef (May 2005) with separation of normal distributions using Bhattacharya's method of decomposing composite distributions (FiSAT 2: FAO-ICLARM Stock Assessment Tools), (B) Oyster PSS by Module Layer (ML)-face (May 2005).

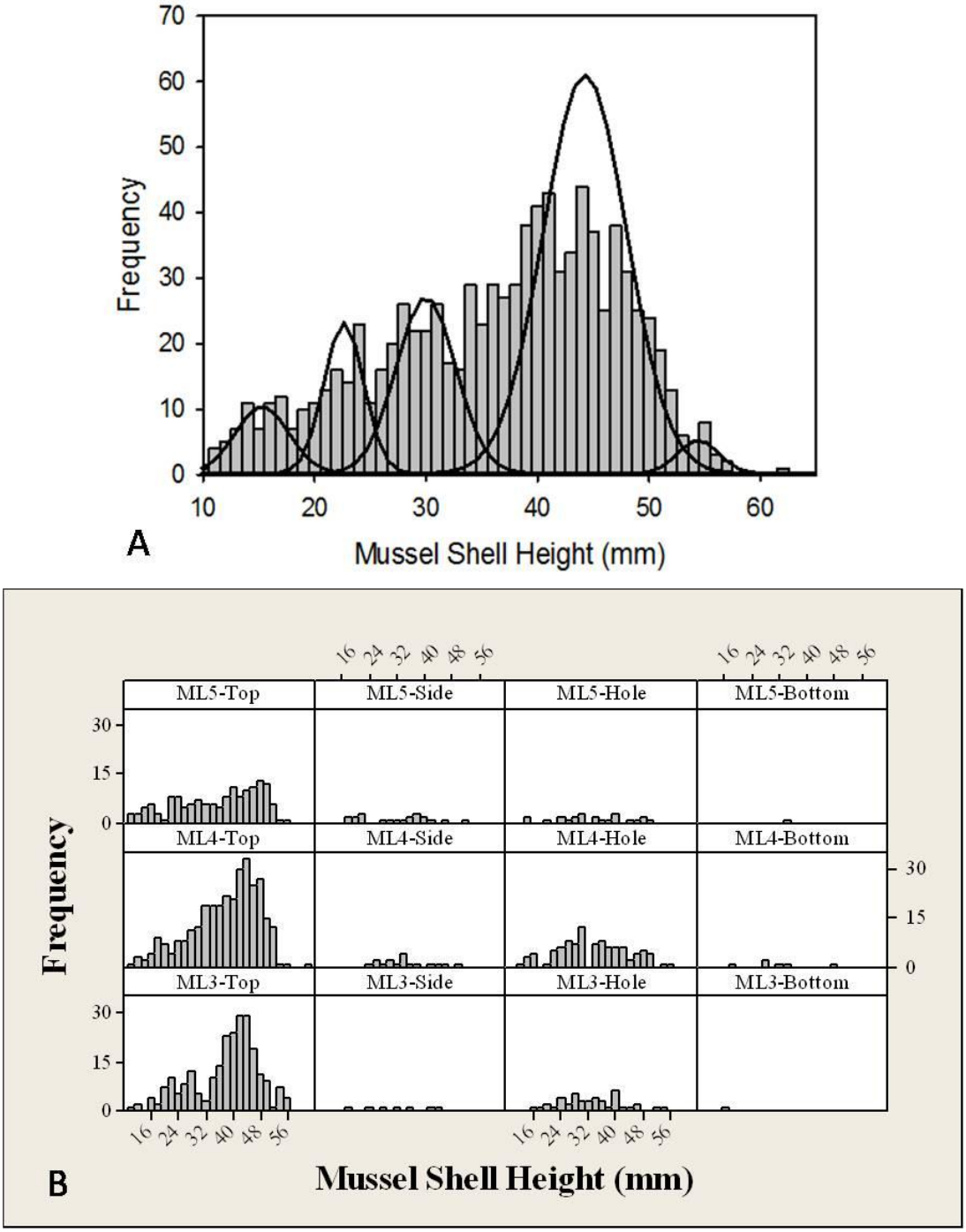


Figure 3.6: (A) Population size structure (PSS) of mussels on the concrete modular reef (May 2005) with separation of normal distributions using Bhattacharya's method (B) Mussel PSS by Module Layer (ML)-face (May 2005).

Population Structure – 2007, 1st Sampling. Oyster size structure (1230 oysters, SH: 3.0-148.4 mm) of undisturbed ML concrete surfaces (Fig. 3.7a) was dominated by the 2006 and 2007 year classes, a trend most pronounced on the Top and Side faces of MLs 3, 2, and 1. To resolve the older year classes, spat (SH < 40.0 mm) were removed, revealing a size structure trend similar to that seen in 2005 with 3+ YCs present (Fig. 3.7b). Mussel SHs were not recorded in 2007, but ranged from small recruits to full-sized adults (> 40 mm).

Population Structure – 2007, Resampling. Oyster size structure on concrete surfaces previously sampled in 2005 (Fig. 3.8a-b) was predictably truncated (oyster SH < 50.0 mm). The Top and Side faces of ML 4 (24 oysters, SH: 15.0-47.3 mm) contained oysters from the 2005 and 2006 year classes (Fig. 3.8a); ML 3 (430 oysters, SH: 4.0 to 43.0 mm), although dominated by the Top face, contained oysters from YCs 2005-2007 on all faces (Fig. 3.8b). Mussels SHs were not recorded in 2007, but were dominated by smaller size classes.

Density – 2005. Oyster abundance and density differed significantly across face (ANOVA, $F = 30.91$, $p < 0.0005$) with the highest densities on the Top face (Fig. 3.9a). There was no effect of ML and no ML-face interaction effect. By SA density (Fig. 3.9b), the Top face contained significantly more oyster than the other faces. For large adult oysters (SH > 76 mm) there were significant ML ($F = 3.56$, $p = 0.032$), face ($F = 13.59$, $p < 0.0005$), and ML-face interaction ($F = 4.89$, $p < 0.0005$) effects. Spat abundance varied significantly only by face ($F = 7.59$, $p < 0.0005$).

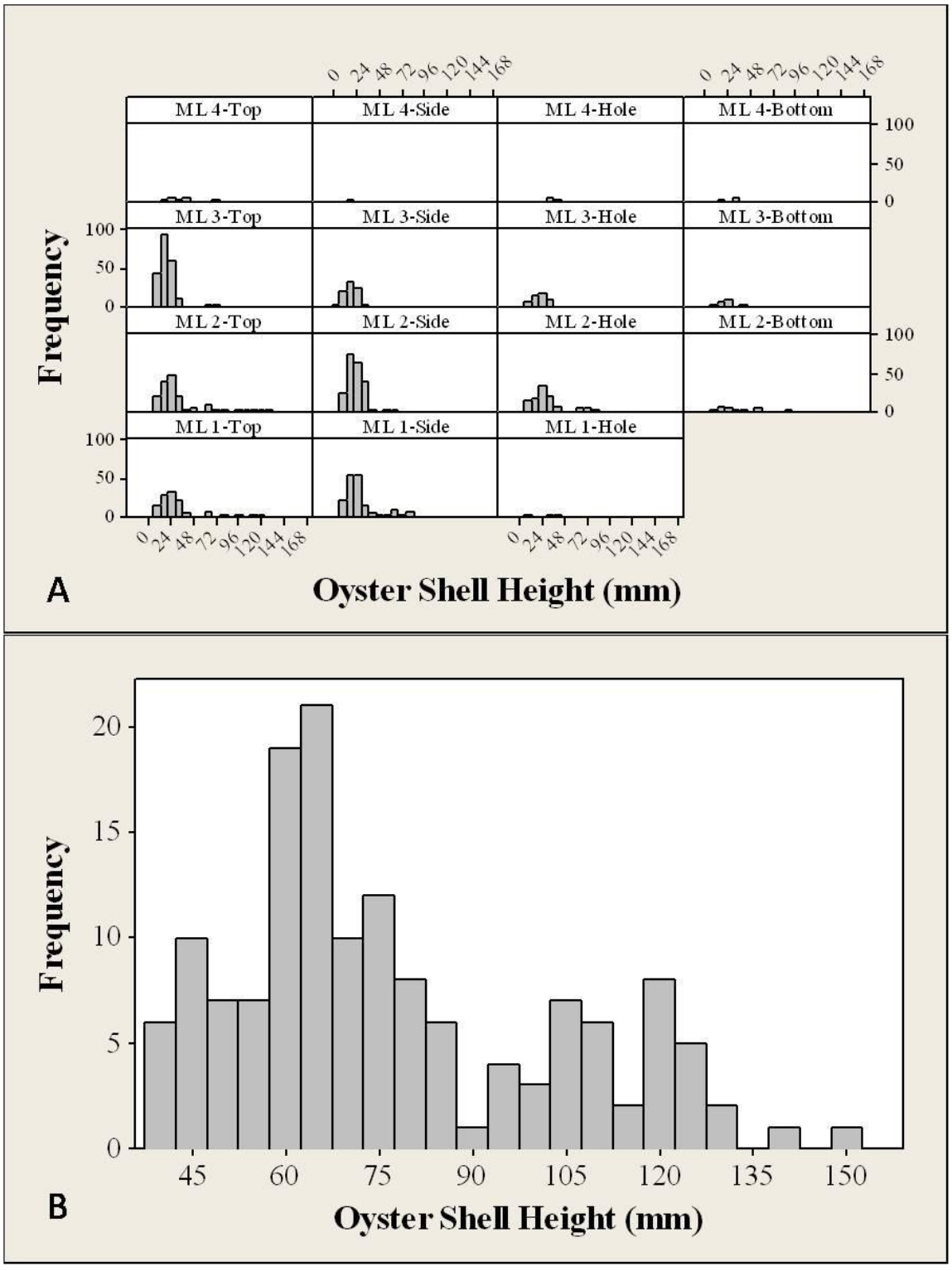


Figure 3.7: (A) Population size structure (PSS) of oysters from undisturbed plots (1st Sampling) on the concrete modular reef (Module Layer (ML) 4 – May 2007; MLs 1, 2, 3 – Nov. 2007), (B) PSS of adult oysters (Shell Height > 40.0 mm), otherwise obscured by strong 2007 oyster recruitment.

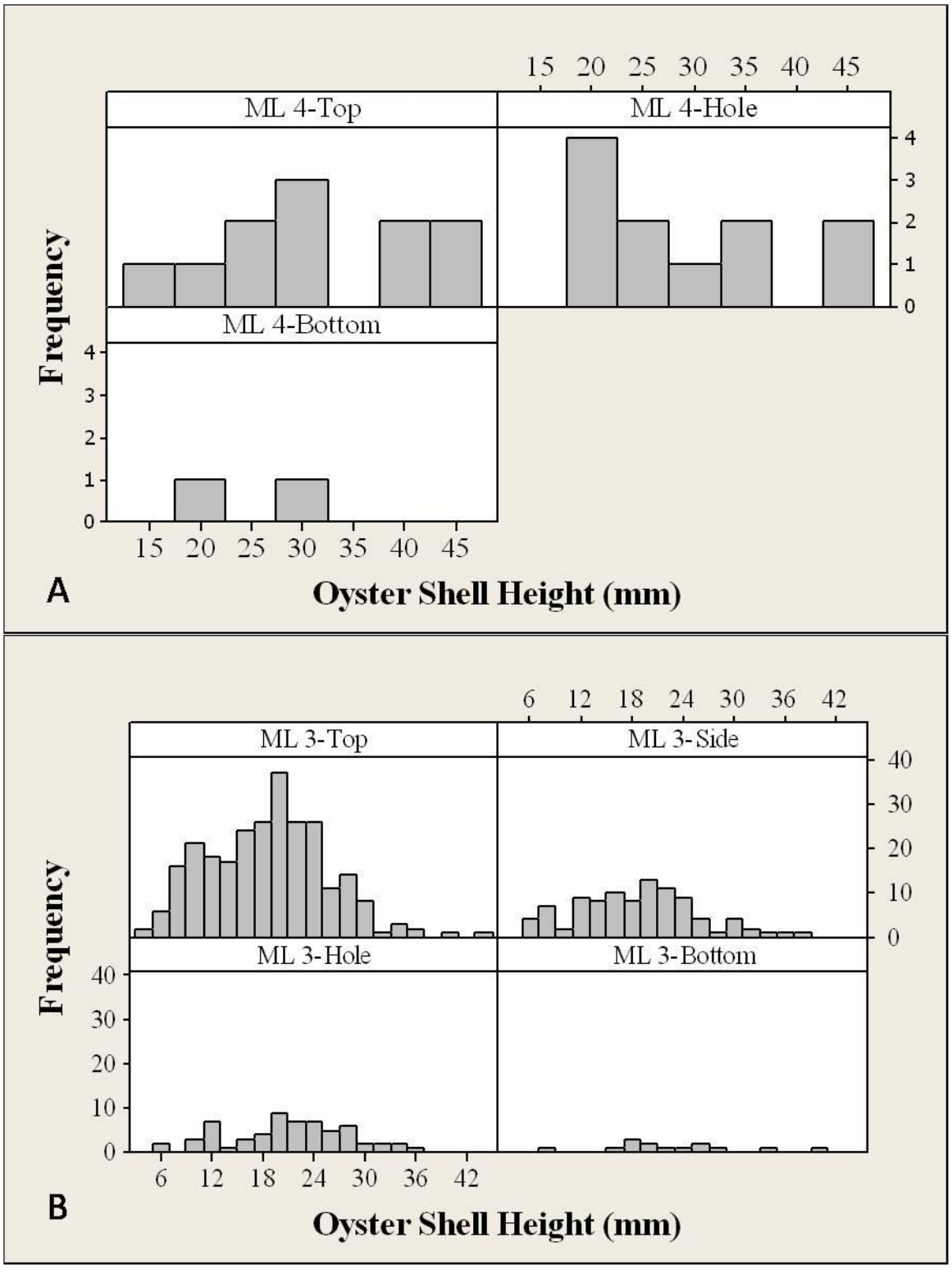


Figure 3.8: Population size structure of oysters from previously-denuded plots (Resampling) on the concrete modular reef in (A) May 2007 (Module Layer – ML 4) and (B) Nov. 2007 (ML 3).

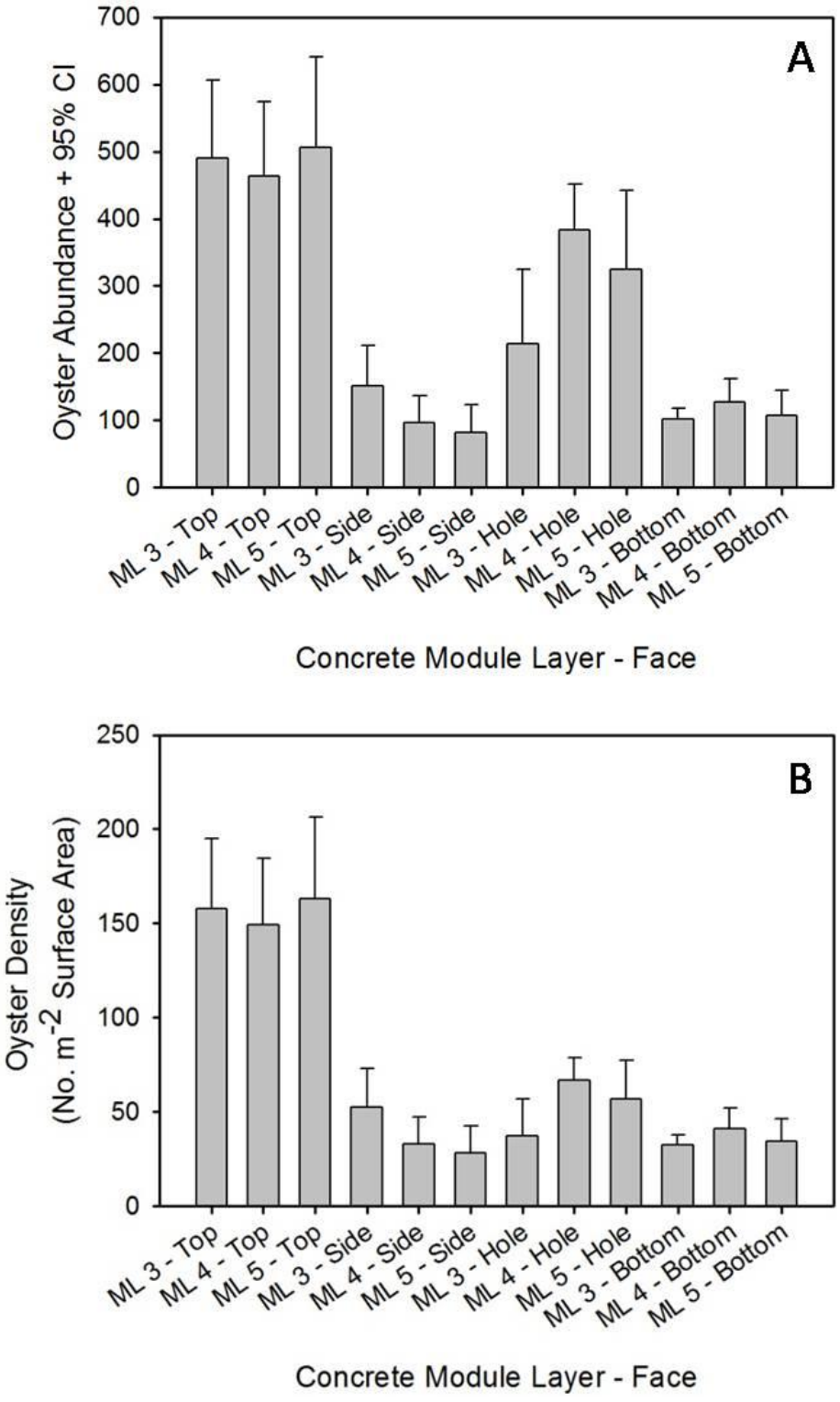


Figure 3.9: Concrete module reef (A) oyster abundance, and (B) surface density, estimates (+ 95% confidence interval) by Module Layer (ML)-face (May 2005).

Mussel abundance and density differed significantly across ML (ANOVA, $F = 15.33$, $p < 0.0005$), face ($F = 233.59$, $p < 0.0005$), and the ML-face ($F = 12.07$, $p < 0.0005$) interaction (Fig. 3.10a-b). ML 4 was mussel-dominated with the Top and Hole faces maintaining much higher surface densities than the Side and Bottom faces ($F = 96.63$, $p < 0.0005$); ML 3 had the same relationship between faces ($F = 45.86$, $p < 0.0005$). On ML 5 ($F = 100.50$, $p < 0.0005$), however, the Hole face was statistically similar to the Side and Bottom faces. Oyster density was strongly correlated ($R^2 = 0.54$, $p < 0.0001$) with mussel density (Fig. 3.11).

Bivalve volume (oyster and mussels) differed significantly across ML (ANOVA, $F = 6.12$, $p = 0.003$), face ($F = 102.04$, $p < 0.0005$), and the ML-face ($F = 4.32$, $p = 0.001$) interaction. Bivalve volume was similar on ML 4 ($F = 55.14$, $p < 0.0005$) and ML 3 ($F = 27.37$, $p < 0.0005$); ML 4 > ML 5 ($F = 25.83$, $p < 0.0005$) (Fig. 3.12a). By face, Top > Hole > Side = Bottom (Fig. 3.12a). By SA, the Side, Hole and Bottom faces were similar; the Top face contained significantly higher bivalve volume (Fig. 3.12b).

Red beard sponge (*Microciona prolifera*) volume did not substantially vary between MLs but was more abundant on the Hole and Bottom faces (Fig. 3.13a). By SA, the Side, Hole and Bottom faces were similar; the Top face contained little to no sponge (Fig. 3.13b). The sponge and oyster community contained mud crabs, juvenile clams (*Mya arenaria, Mercenaria mercenaria*, *Macoma balthica*, *Macoma mitchelli*), mussels (*I. recurvum*, *Mytilus edilus*), barnacles, amphipods, isopods, Nereid polychaete worms, tunicates (*Molgula manhattensis*), and resident fish including oyster toadfish (*Opsanus tau*), gobies (G*obiosoma* sp.), blennies, and skilletfish (*Gobiesox strumosus*). Large spadefish (*Chaetodipterus faber*) were also observed near the CM reef.

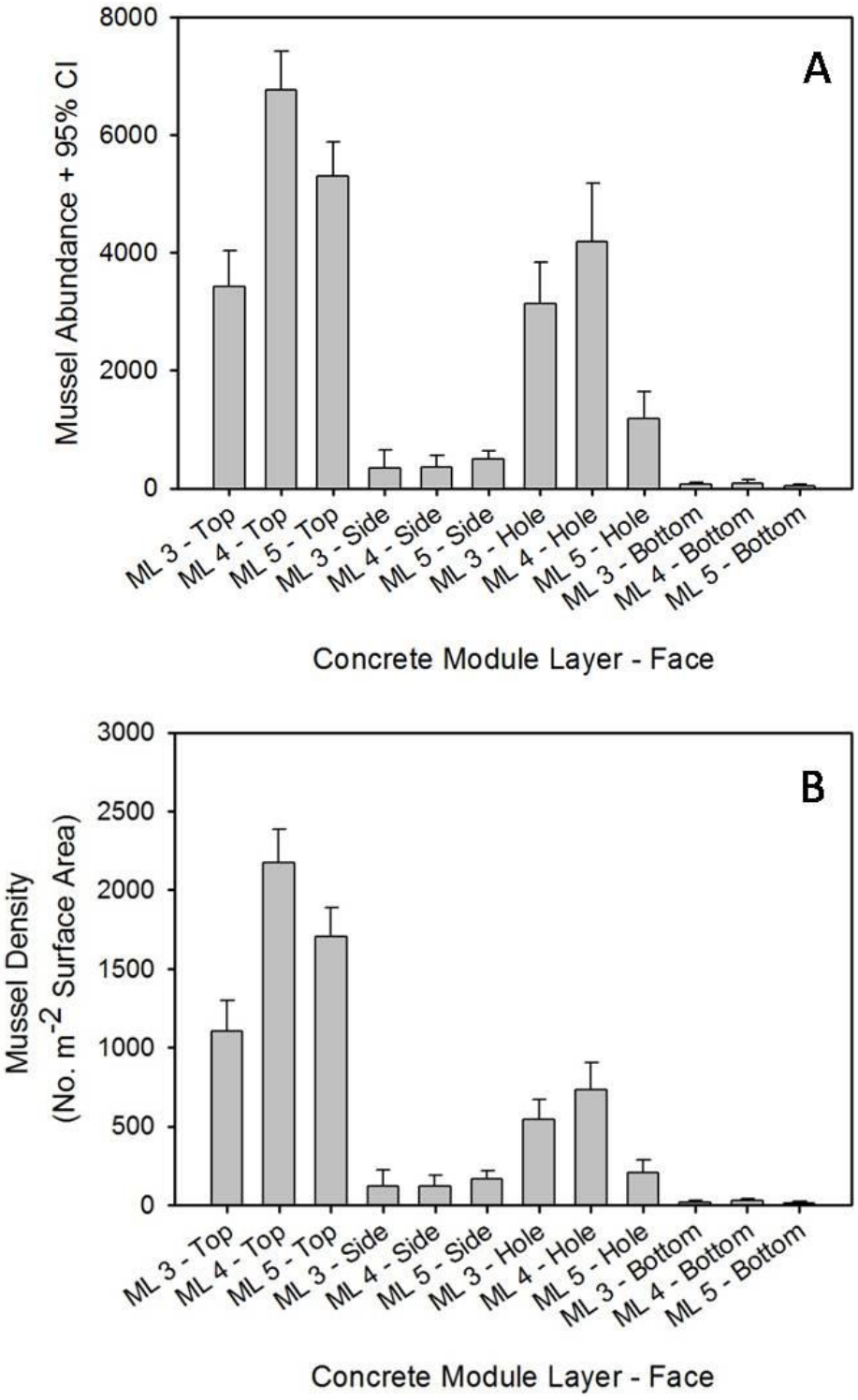


Figure 3.10: Concrete module reef (A) mussel abundance, and (B) surface density, estimates (+ 95% confidence interval) by Module Layer (ML)-face (May 2005).

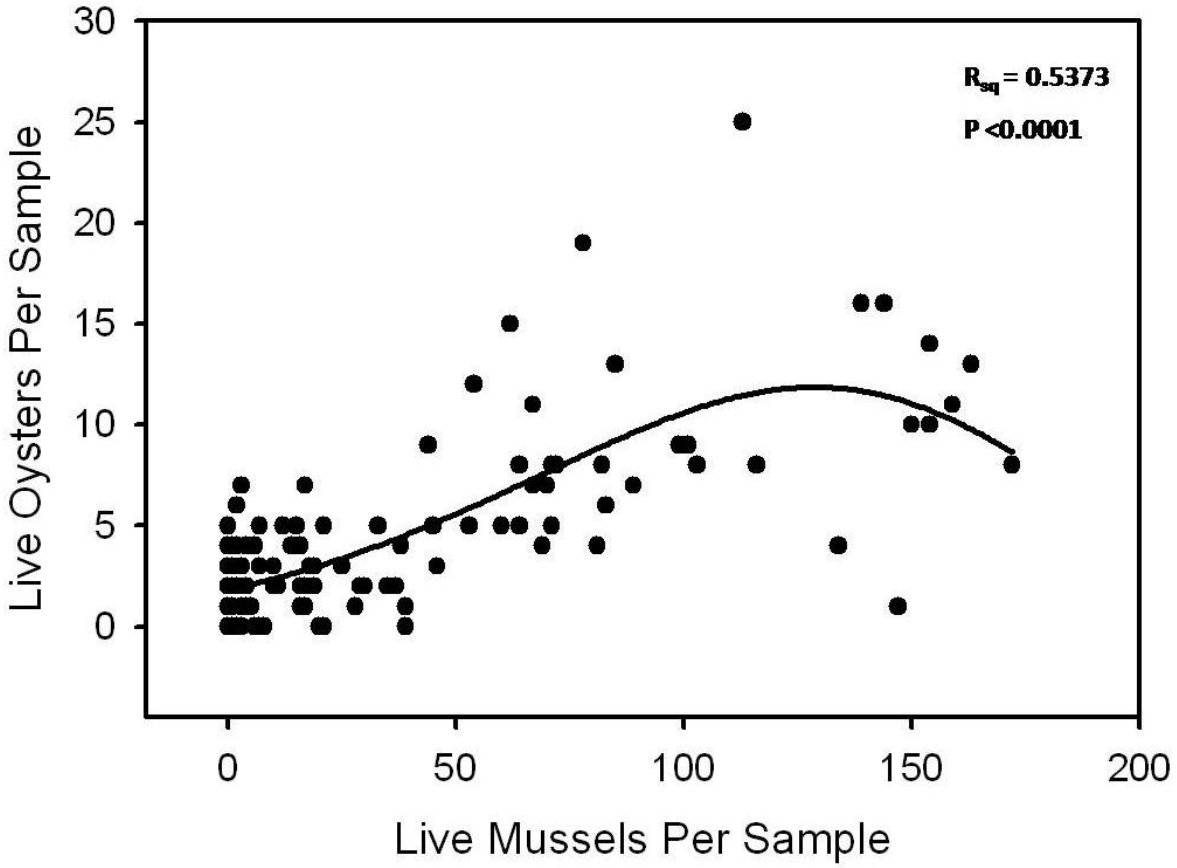


Figure 3.11: Regression of live oysters per sample versus live mussels per sample on the concrete module reef (May 2005).

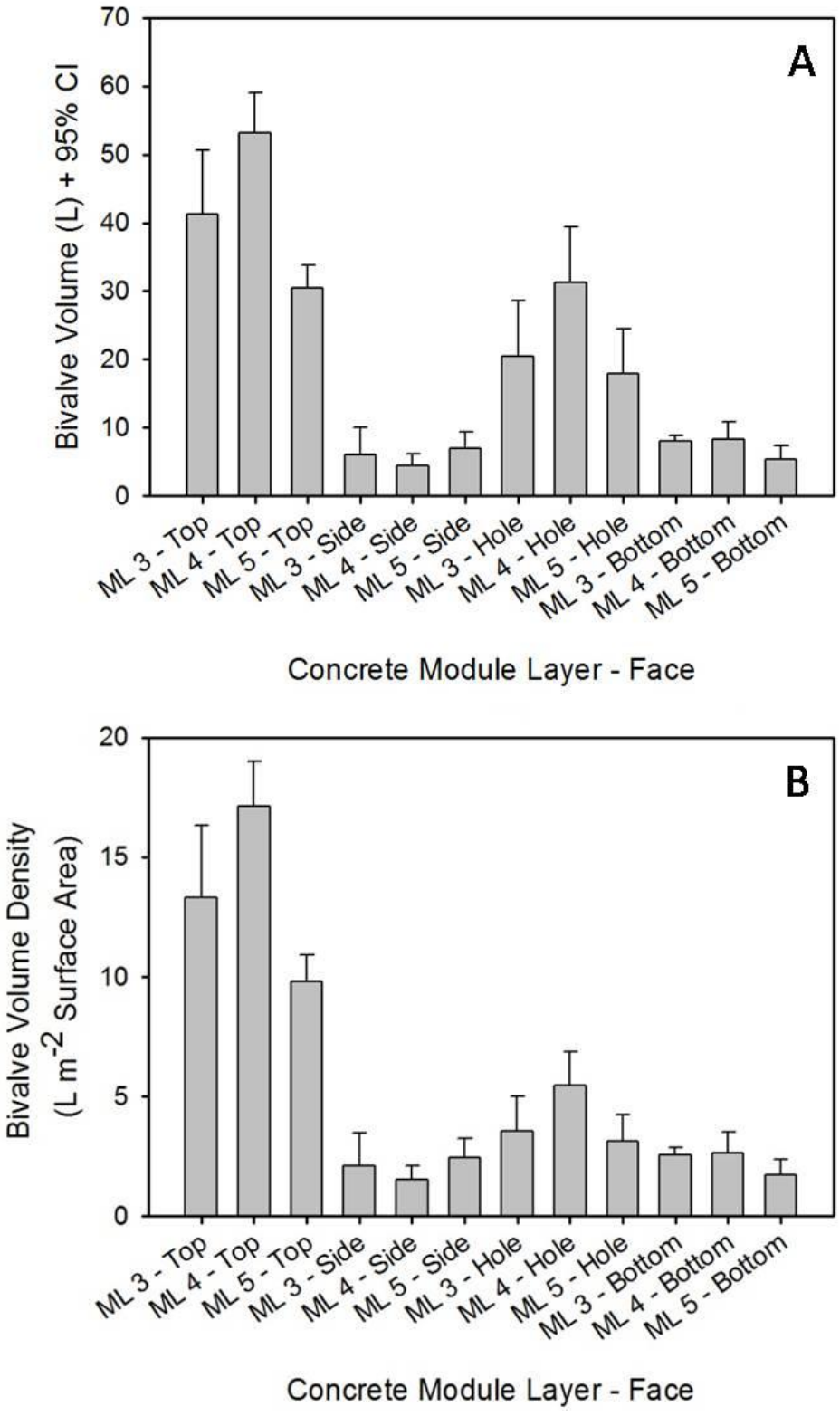


Figure 3.12: Concrete module reef (A) bivalve (oyster and mussel) volume, and (B) surface density, estimates (+ 95% confidence interval) by Module Layer (ML)-face (May 2005).

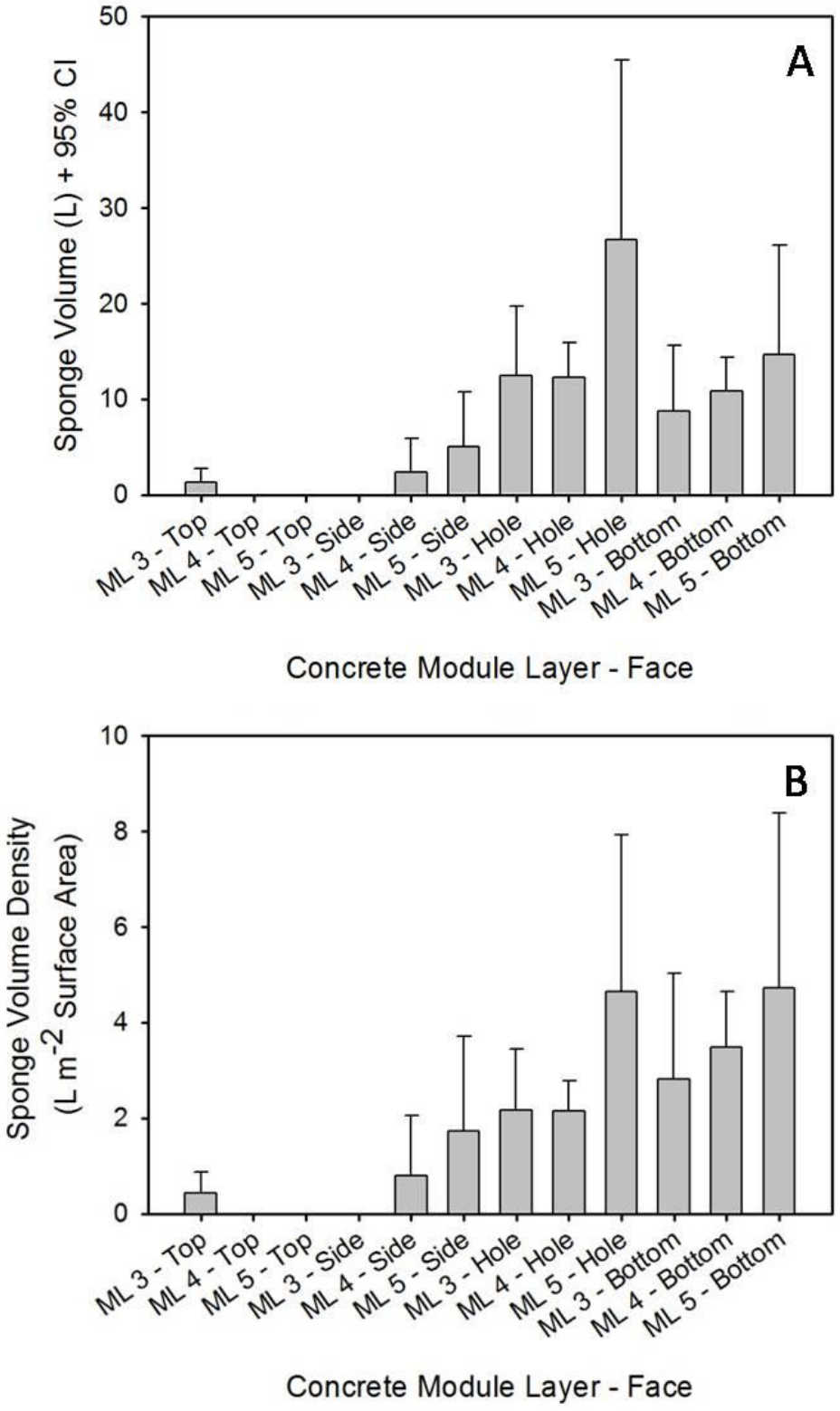


Figure 3.13: Concrete module reef (A) sponge volume, and (B) surface density, estimates (+ 95% confidence interval) by Module Layer (ML)-face (May 2005).

Density – 2007, 1st Sampling. ML 4 was sampled before the 2007 recruitment event; MLs 3, 2, and 1 were sampled after that event and should be considered separate from ML 4. Oyster density differed significantly across ML (ANOVA, F = 7.21, $p = 0.001$), face (F = 17.54, $p < 0.0005$), and ML-face (F = 4.05, $p = 0.001$) interaction even with ML 4 excluded. The Top face of ML 3 was oyster-dominated with a density more than twice any other ML-Top (Fig. 3.14a). Across all MLs, the other Top faces as well as the Side and Hole faces maintained similar oyster densities; the Bottom faces contained the fewest oysters. By SA, the trends were similar. Thirty-three percent of all oysters measured were located on other oysters or mussels (Table 3.2a).

Mussel density (Fig. 3.14b) differed significantly across ML (ANOVA, F = 25.58, $p < 0.0005$), face (F = 64.77, $p < 0.0005$), and ML-face (F = 24.91, $p < 0.0005$) interaction, and followed a similar trend to oyster density (ML 3-Top was more than three times higher than other Top faces) except that the Top > Hole faces, and both Side and Bottom faces maintained low densities. Also, mussel density of ML 3 was greater than MLs 4, 2, and 1. By SA, the trends were similar.

Bivalve volume (oyster and mussels) differed by face only: Hole and Top faces were greater than the Bottom and Side faces (Fig. 3.14c). By SA, the trends were similar. Oyster volume (live oysters only) did not significantly differ by ML or face (Fig. 3.14d). Sponge volume differed by face only with the Hole greater than the Top face (Fig. 3.14e). By SA, there was no statistical difference by ML or face. Mud Crab density was higher on MLs 2 and 3 than MLs 4 and 1; and higher in the Hole than the Top face (Fig. 3.14f). By SA, there was no statistical difference by ML or face.

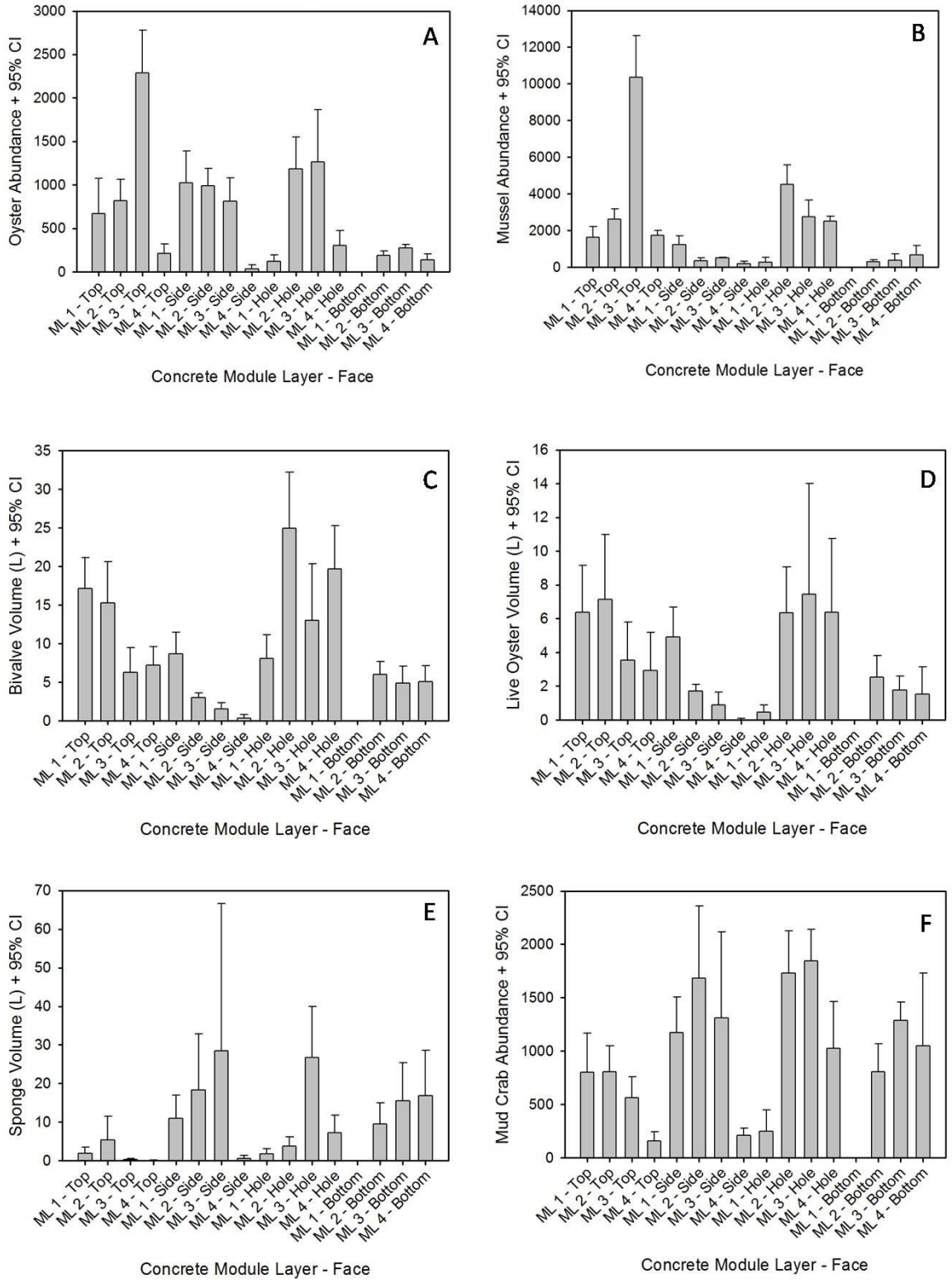


Figure 3.14: Concrete module reef (1st sampling) (A) oyster and (B) mussel abundance, (C) bivalve, (D) live oyster, and (E) sponge volume (L), and (F) mud crab abundance estimates (+ 95% confidence interval) by Module Layer (ML)-face (May and November 2007).

Table 3.2: (A) Percentage of oysters cohered to other oysters or mussels by Module Layer-face for undisturbed (1st Sampling) and (B) previously-denuded (Resampling) plots (May and November 2007).

2007 CM 1st Sampling	Top	Side	Hole	Bottom	Total
ML 4	2/20	3/3	4/14	3/10	12/47; 25.5%
ML 3	38/219	3/81	19/54	6/22	66/376; 17.6%
ML 2	87/159	31/210	70/108	10/30	198/507; 39.1%
ML 1	69/123	61/173	4/11	0/0	134/307; 43.6%
Total	196/521; 37.6%	98/467; 21.0%	97/187; 51.9%	19/62; 30.6%	410/1237; 33.1%

A.

2007 CM 1st Sampling	Top	Side	Hole	Bottom	Total
ML 4	8/11	0/0	1/11	1/2	10/24; 41.7%
ML 3	41/260	4/95	29/50	2/14	76/419; 18.1%
Total	49/271; 18.1%	4/95; 4.2%	30/61; 49.2%	3/16; 18.8%	86/443; 19.4%

B.

Density – 2007, Resampling. As ML 4 and 3 were resampled in May and Nov. 2007, respectively, they are considered separately. For ML 3, the Top face recruited oyster spat at a higher density than the Side and Bottom faces. The Top and Hole faces maintained the bulk of ML 4's oyster abundance, but at an order of magnitude lower than ML 3, which emphasizes the strength of the 2007 oyster recruitment class (Fig. 3.15a). By SA, the trends were similar. Of all oysters measured, 19 % were on other oysters or mussels (Table 3.2b).

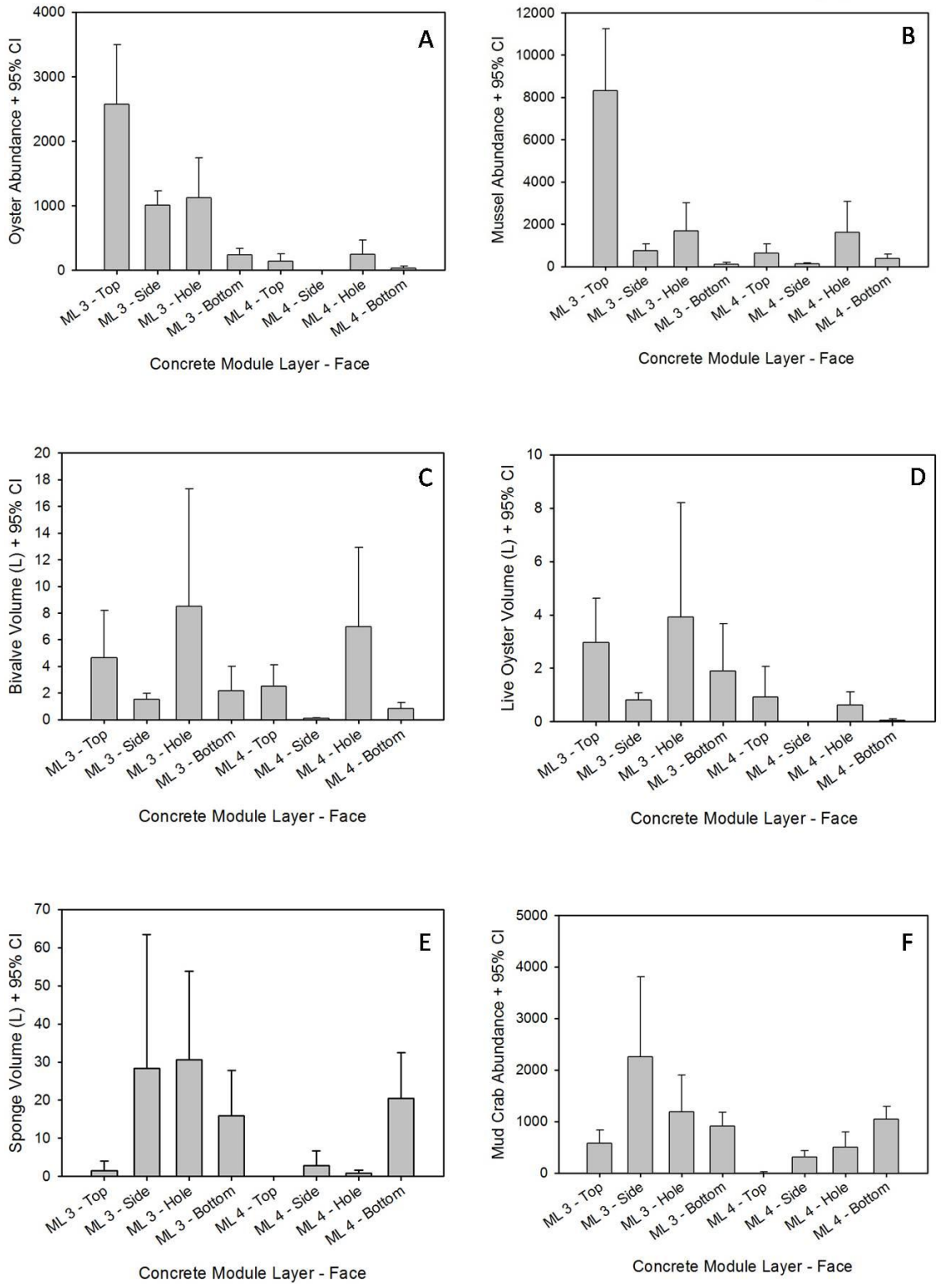


Figure 3.15: Concrete module reef (Resampling) (A) oyster and (B) mussel abundance, (C) bivalve, (D) live oyster, and (E) sponge volume (L), and (F) mud crab abundance estimates (+ 95% confidence interval) by Module Layer (ML)-face (May and November 2007).

Mussel recruitment/density (Fig. 3.15b) followed the same trend as oyster density, similar to the trend observed in 2005. One notable similarity between MLs 4 and 3 was the high mussel density on the Hole and Bottom faces, but not on the Top and Side faces. By SA, the trends were similar.

Bivalve volume (Fig. 3.15c) of ML 3 was greater on the Top face; ML 4's Hole and Top faces were greater than the Side and Bottom faces (Side > Bottom). By SA, no statistical difference was detected for ML 3; the Side face of ML 4 maintained lower bivalve volume than the other faces. Oyster volume (live oysters only) followed the same trends (Fig. 3.15d).

Sponge volume (Fig. 3.15e) was greater on the Hole face of ML 3 and Bottom face of ML 4. By SA, no statistical difference was detected for ML 3; the Bottom face of ML 4 maintained greater sponge volume than the other faces.

No statistical difference was detected for ML 3's mud crab density (Fig. 3.15f). For ML 4, the Bottom > Side > Top face; by SA, Bottom > Side = Hole > Top face.

Biomass – 2005. Sixty-two oysters and 138 mussels throughout the full size range (SH) were processed to yield reliable estimates of oyster (Fig. 3.16a) and mussel (Fig. 3.16b) biomass with the regression model of log AFDM versus log SH. Oyster biomass differed significantly across ML (ANOVA, $F = 4.06$, $p = 0.020$), face ($F = 10.51$, $p < 0.0005$), and ML-face ($F = 2.81$, $p = 0.014$) interaction with the highest densities on the Top and Hole faces (Fig. 3.17a). ML 4 ($F = 3.98$, $p = 0.015$) and ML 3 ($F = 7.00$, $p = 0.001$) varied significantly by face.

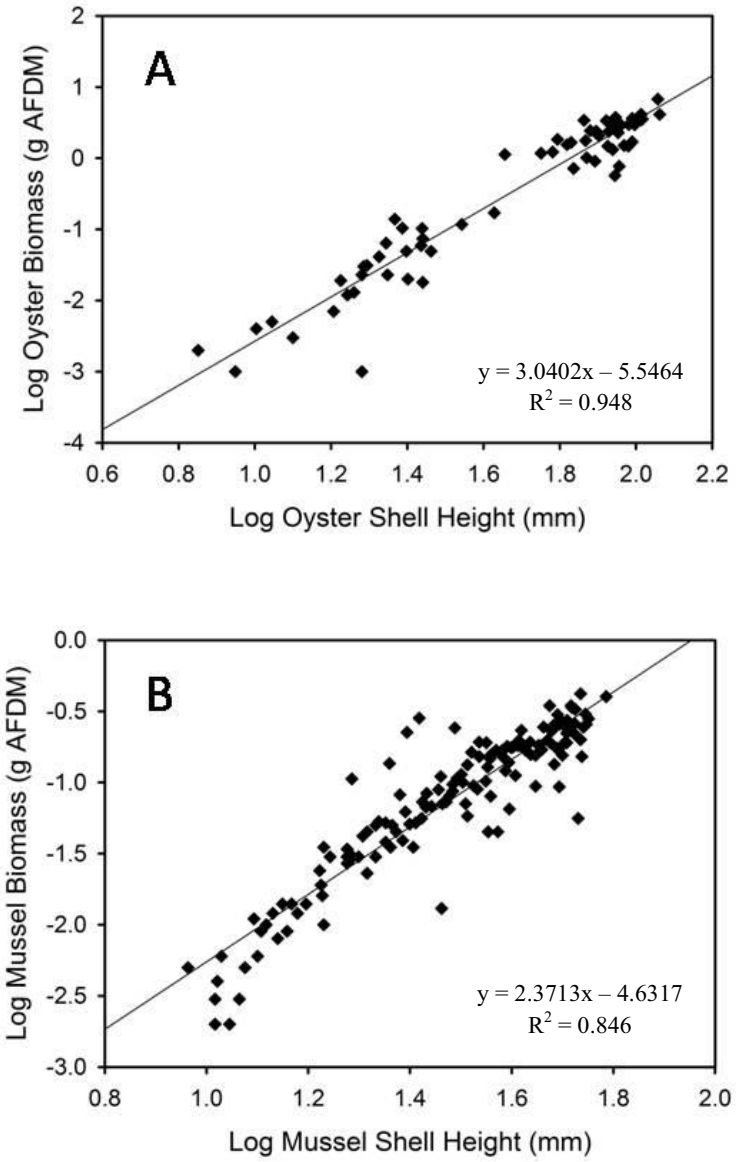


Figure 3.16: Regression models of log AFDM (g) versus log SH (mm) for (A) oyster and (B) mussel biomass estimation on the concrete module reef (May 2005).

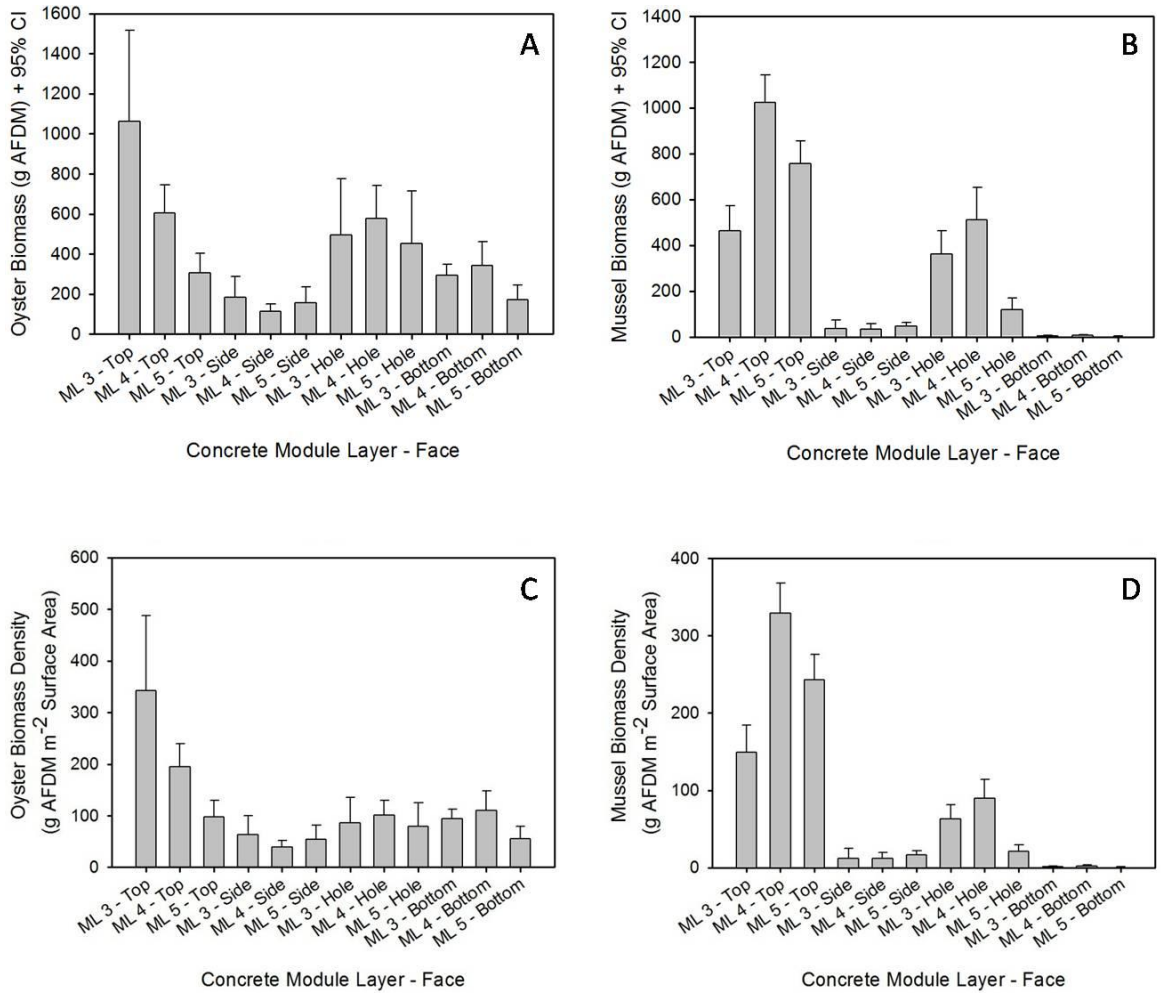


Figure 3.17: Concrete module reef (A) oyster and (B) mussel biomass, and (C) oyster and (D) mussel biomass surface area density estimates (+ 95% confidence interval) by Module Layer (ML)-face (May 2005).

Mussel biomass (Fig. 3.17b) differed significantly across ML (ANOVA, F = 13.34, p = 0.001), face (F = 92.77, $p < 0.0005$), and ML-face (F = 6.07, p = 0.004) interaction. ML 5 (F = 18.31, p = 0.008), ML 4 (F = 103.31, $p < 0.0005$) and ML 3 (F = 13.20, p = 0.015) varied significantly by face. Across all MLs, mussel biomass ranked the same: Top > Hole > Side > Bottom. By SA, the Top face maintained much higher oyster and mussel biomass than the other faces (Fig. 3.17c-d).

Biomass – 2007, 1st Sampling. Sample variance was much higher for oyster biomass estimates than oyster density. The face (GLM, F = 4.27, p = 0.007), but not ML (F = 1.82, p = 0.167) or ML-face (F = 2.05, p = 0.067) interaction effects, significantly influenced oyster biomass. Oyster biomass increased with depth, and biomass was higher on Top and Hole faces (Fig. 3.18a-b). Higher oyster density on the Top face of ML 3 (> 2 times MLs 2 and 1) did not equate to higher oyster biomass; oyster biomass on ML 3's Top face was roughly half that of the Top faces of ML 2 and ML 1. Oyster size structure for the three Top faces revealed a greater number of spat on ML 3 with fewer adults; ML 2 and ML 1 had half as many spat, but many more adults.

Biomass – 2007, Resampling. Oyster biomass on resampled plots of ML 3 varied by face (Top > Side > Bottom; Top = Hole); undisturbed plots did not differ. Similarly, ML 4 maintained higher oyster biomass on the Hole and Top faces than on the Bottom and Side faces (Fig. 3.19a). By SA, these trends shift slightly, but reflect variable oyster recruitment patterns to recently denuded surfaces (Fig. 3.19b).

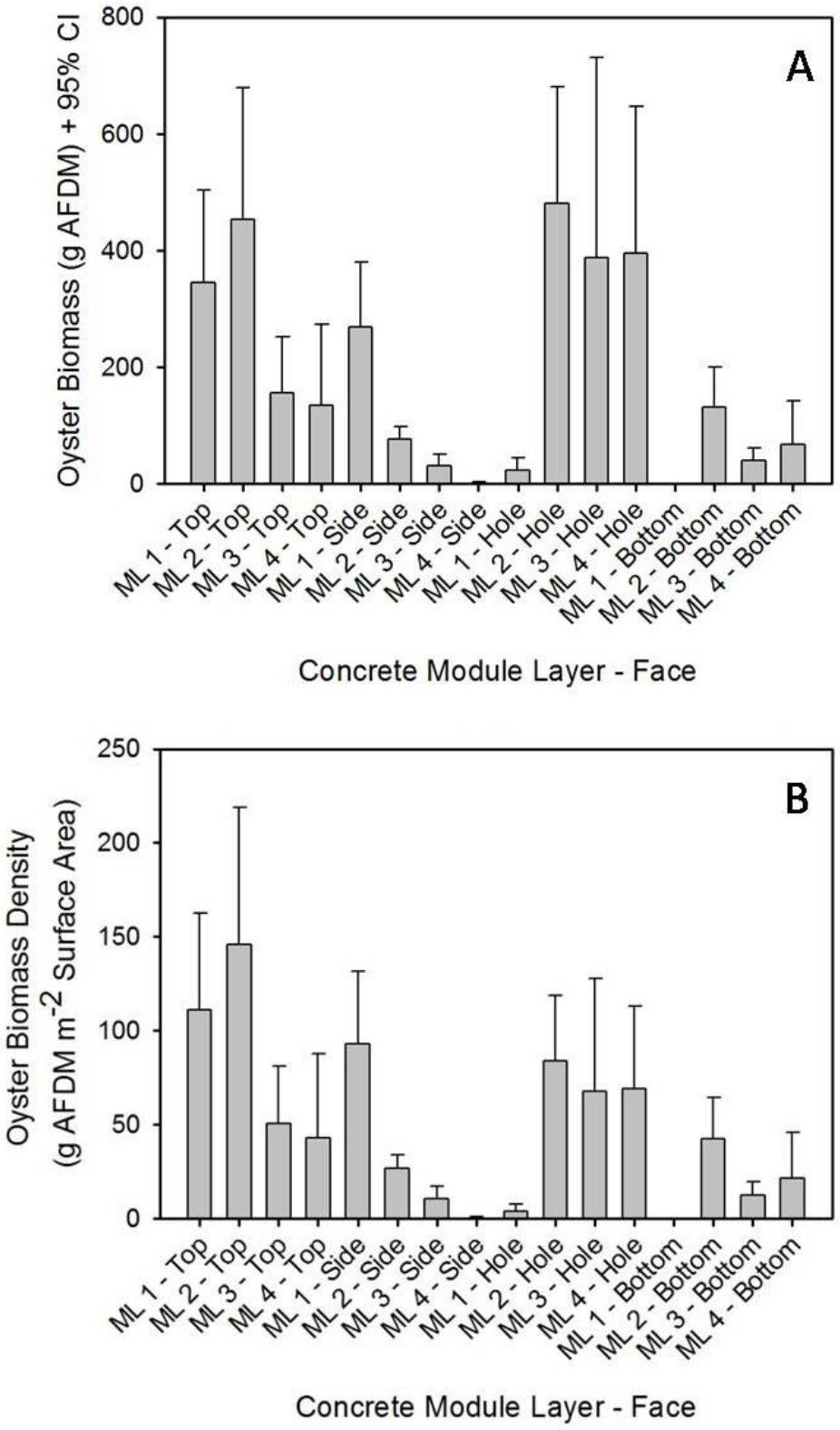


Figure 3.18: Concrete module reef (1st Sampling) (A) oyster abundance, and (B) surface density, estimates (+ 95% confidence interval) by Module Layer (ML)-face (May and November 2007).

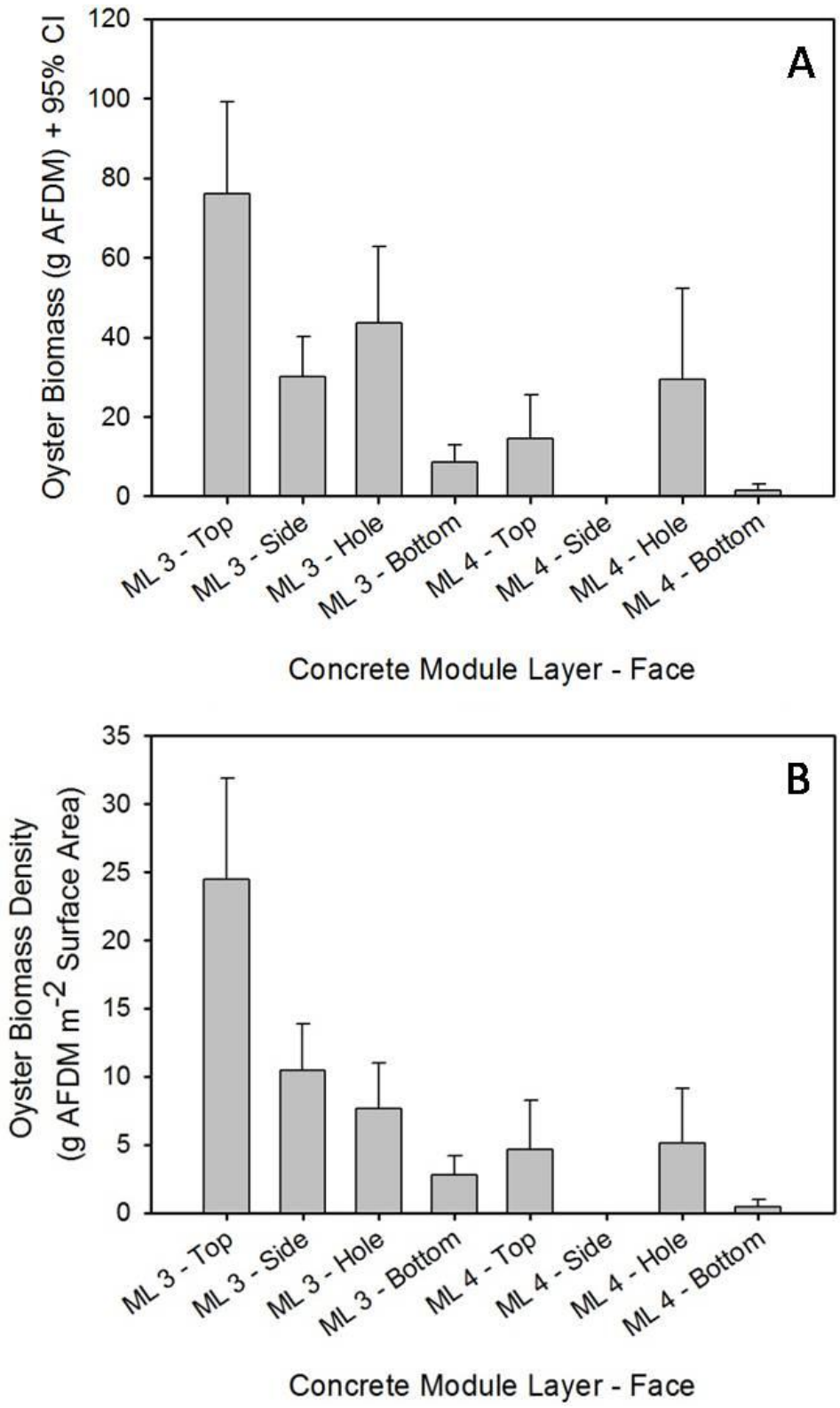


Figure 3.19: Concrete module reef (Resampling) (A) oyster abundance, and (B) surface density, estimates (+ 95% confidence interval) by Module Layer (ML)-face (May and November 2007).

Pathology – 2005. Of the 30 large oysters processed for disease assessment, none were infected with MSX and 30% were infected with Dermo. Of the infected with Dermo, none had serious infections (4 infections were *light*, 5 were *rare*, 21 *negative*). There was positive correlation (Regression, $R^2 = 0.14$, $p = 0.043$) between individual oyster size and Dermo intensity rank (Fig. 3.20a). The following pathogens were found in one or more oysters: *Nematopsis* (1), *Rickettsia*-like organisms (1), *Sphenophyra*-like ciliates (11), *Stegotricha* spp. ciliates (1), and viral gametocytic hypertrophy (1).

Pathology – 2007. Of the 25 oysters processed for disease assessment, six were infected with MSX and 84% were infected with Dermo.

P. marinus, RFTM: 84% prevalence (21/25 positive), intensities: 0-16-3-2 (Heavy-Moderate-Light-Rare)
P. marinus, histology: 56% prevalence (14/25 positive), intensities: 0-2-8-4
H. nelsoni, histology: 24% prevalence (6/25 positive), intensities: 1-1-2-2

The weighted prevalence of *P. marinus*, calculated from RFTM data, would be 2.08, indicating that "serious mortality" should be occurring. Note however that no infections were "heavy" by RFTM, and just 4 were of intensity greater than "light-moderate" – a category collapsed together with the "moderates" in generating the 0-16-3-2 intensity count, and the weighted prevalence value. By RFTM metrics, therefore, 21 of 25 oysters were no more than "light-to-moderately" infected and so were probably generally healthy. The histology data suggest the proportion of healthy (with respect to *P. marinus*) oysters was probably even higher. Just one or two additional oysters, at most, were detrimentally affected by *H. nelsoni*. There was no correlation (Regression, $R^2 = 0.07$, $p = 0.220$) between individual oyster size and Dermo intensity rank (Fig. 3.20b) across a very wide size range.

Condition index. Sixty-six oysters throughout the full range of SHs were processed to yield three CIs that (Rainer and Mann 1992). CI results for the 2005 CM oysters were CI1: 11.9 +/- 0.4; CI2: 8.4 +/- 0.3; CI3: 5.3 +/- 0.3 (+/- 1 SE_{Mean})), indicating that these oysters were healthy. Oyster condition was not tested by ML, but was significantly influenced by face for CI3 ($F = 4.81$, $p = 0.005$) and not CI1 ($F = 0.02$, $p = 0.997$) or CI2 ($F = 0.07$, $p < 0.975$). In addition, there was no correlation between oyster condition and SH.

Steamer Rock Reef

Population structure. Steamer Rock oyster size structure (Fig. 3.21) was robust throughout the full size range, containing a minimum of five year classes (2001-2005). Oyster SHs ranged from 5.0-122.0 mm for the Outer Ring (132 oysters), 12.6-128.7 mm for the Inner Ring (54 oysters), and 17.4-142.5 mm for the Intermediate Rings (171 oysters). Mussel SHs were not recorded in 2007, but ranged from small recruits to full-sized adults (> 40 mm).

Density. Oyster density (ANOVA, $F = 1.13$, $p = 0.336$) (Fig. 3.22a) and live mussel volume ($F = 1.93$, $p = 0.162$) (Fig. 3.22b) did not significantly differ between the Intermediate, Outer or Inner rings of concrete stacks. Mussel ($F = 6.69$, $p = 0.004$) and mud crab density ($F = 8.71$, $p = 0.001$) (Fig. 3.22c), as well as oyster volume ($F = 7.68$, $p = 0.002$) (live oysters only; Fig. 3.22b), were greater on the Intermediate rings than the Outer ring (Inner = Outer ring).

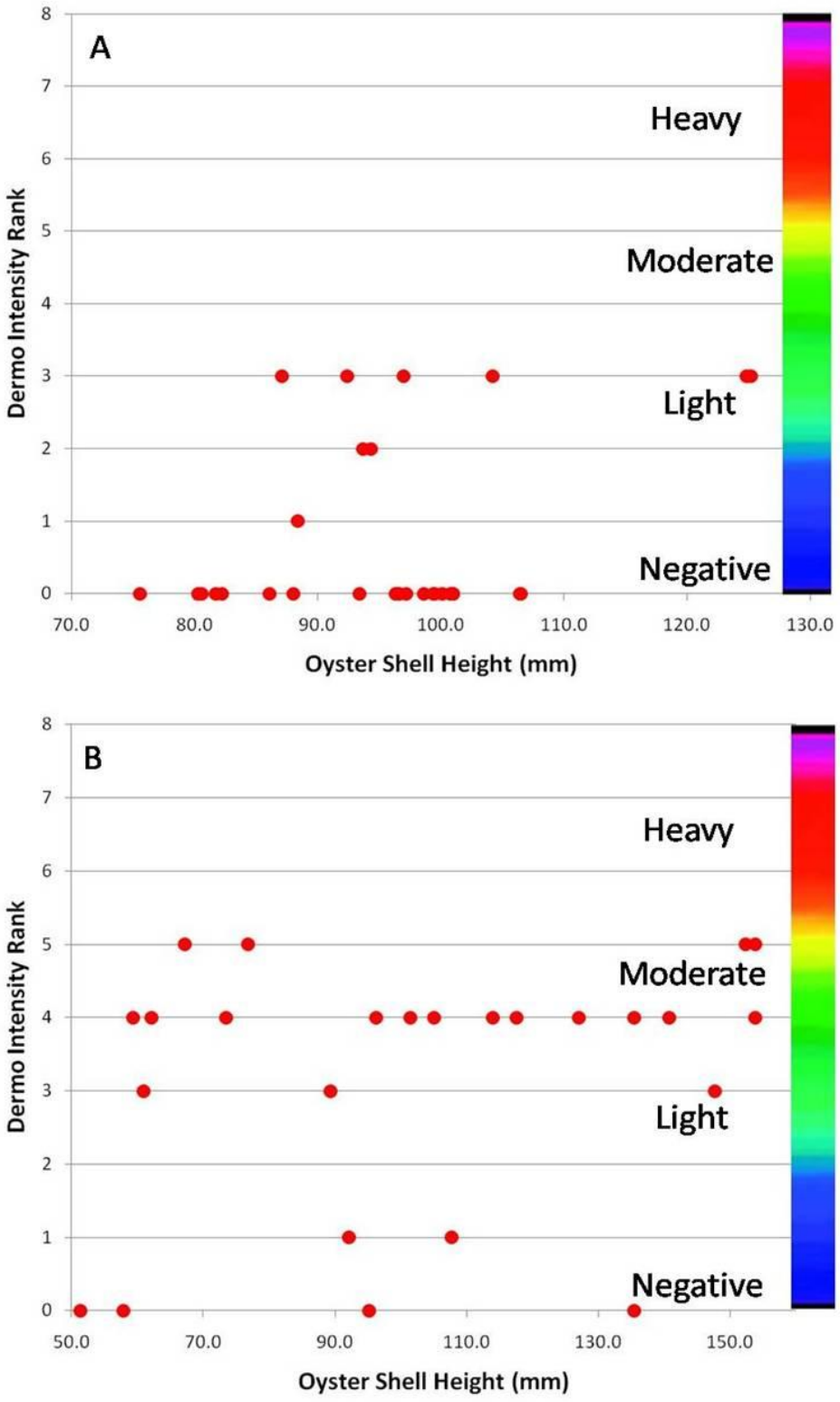


Figure 3.20: Dermo intensity rank versus oyster shell height from the concrete module reef in (A) May 2005 and (B) November 2007.

Biomass. Oyster biomass (Fig. 3.22b) did not significantly differ between the Intermediate, Outer and Inner Ring strata (ANOVA, F = 0.11, p = 0.894). Two hundred thirty nine oysters (Outer – 85; Inner – 51; Intermediate – 103 oysters, respectively) throughout the full range of SHs were processed to yield reliable estimate of oyster biomass (Fig. 3.23a-c) across all three strata.

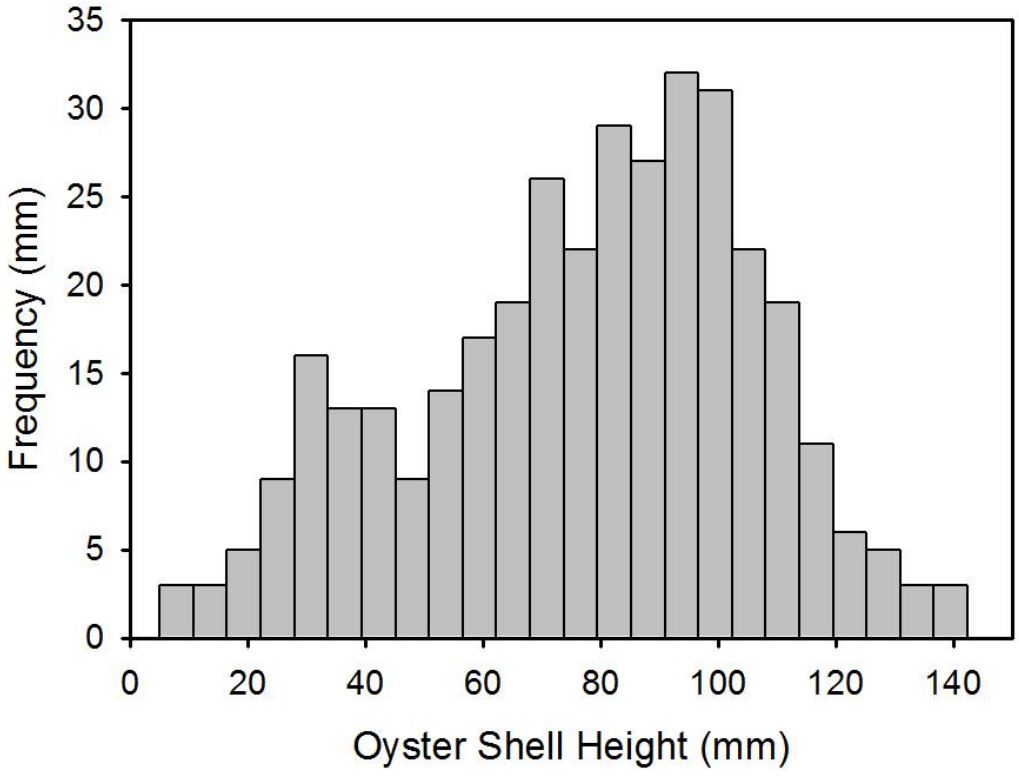


Figure 3.21: Population size structure of oysters on the Outer, Inner, and Intermediate Rings of the Steamer Rock reef complex (2006).

Pathology. Of the 25 oysters processed for disease assessment, none were infected with MSX and 96% were infected with Dermo.

P. marinus, RFTM: 96% prevalence (24/25 positive), intensities: 1-16-4-3
P. marinus, histology: 56% prevalence (14/25 positive), intensities: 0-1-4-9
H. nelsoni, histology: 0/25 positive

The weighted prevalence of *P. marinus*, calculated from RFTM data, would be 2.34, indicating that "serious mortality" should be occurring. Note, however, that just one infection was "heavy" by RFTM, and just 8 were of intensity greater than "light-moderate." By RFTM metrics, therefore, 17 of 25 oysters were no more than "light-moderately" infected and so were probably generally healthy. The histology data suggest the proportion of healthy oysters was probably even higher. There was no correlation (Regression, F = 0.03, p = 0.873) between individual oyster size and Dermo intensity rank (Fig. 3.24).

Condition index. Two hundred forty six oysters throughout the full range of SHs were processed to yield three CIs across all three strata (Outer – 82; Inner – 62; Intermediate – 102 oysters). Oyster condition for the Outer (CI1: 11.7 +/- 0.3; CI2: 8.3 +/- 0.3; CI3: 3.5 +/- 0.1) and Inner (CI1: 11.1 +/- 0.4; CI2: 7.7 +/- 0.3; CI3: 3.6 +/- 0.2) stacks sampled in April 2006 were similar to each other and to the 2005 CM reefs. The Intermediate stacks sampled in August 2006 contained oyster with lower average condition (CI1: 9.0 +/- 0.3; CI2: 6.6 +/- 0.2; CI3: 2.5 +/- 0.1).

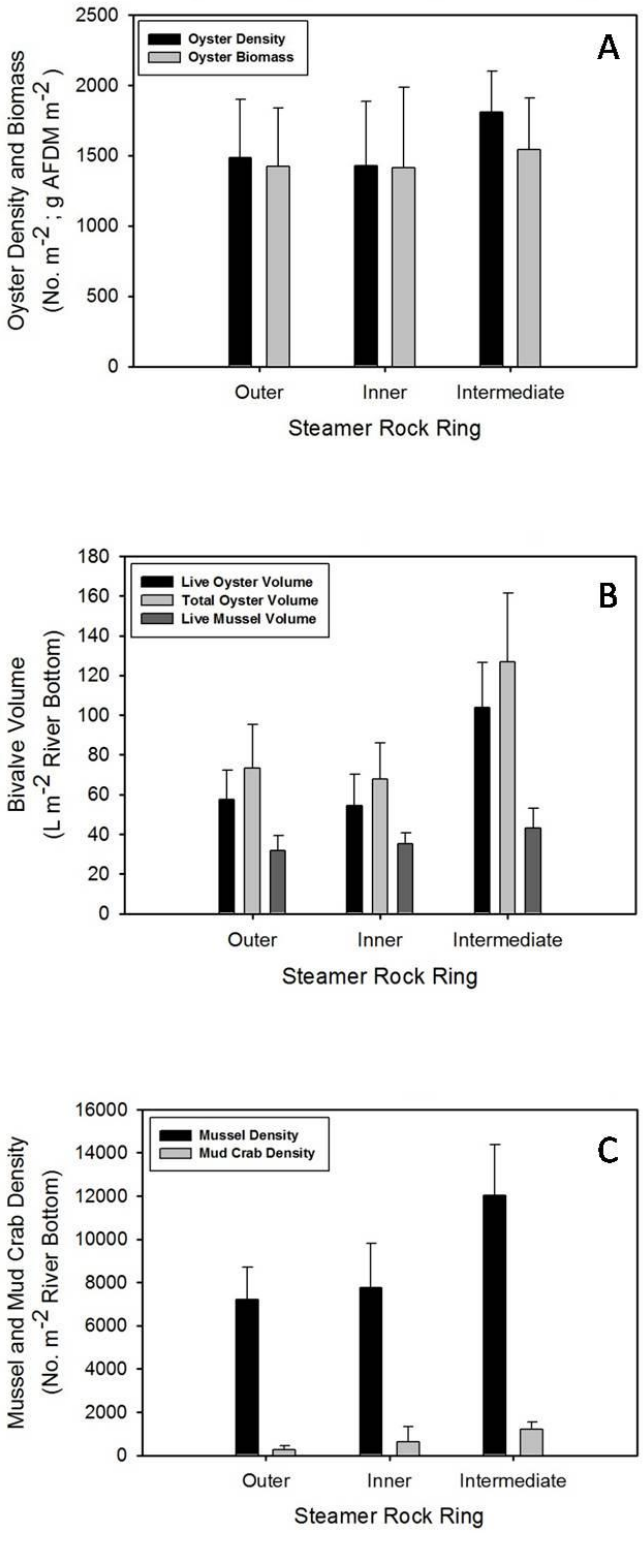


Figure 3.22: Oyster (A) density (m^{-2} river bottom - RB) and biomass (g AFDM m^{-2} RB), (B) live oyster, total oyster, and live mussel volume (L m^{-2} RB), and (C) mussel and mud crab density (m^{-2} RB) on the Outer, Inner, and Intermediate Rings of the Steamer Rock reef complex (2006).

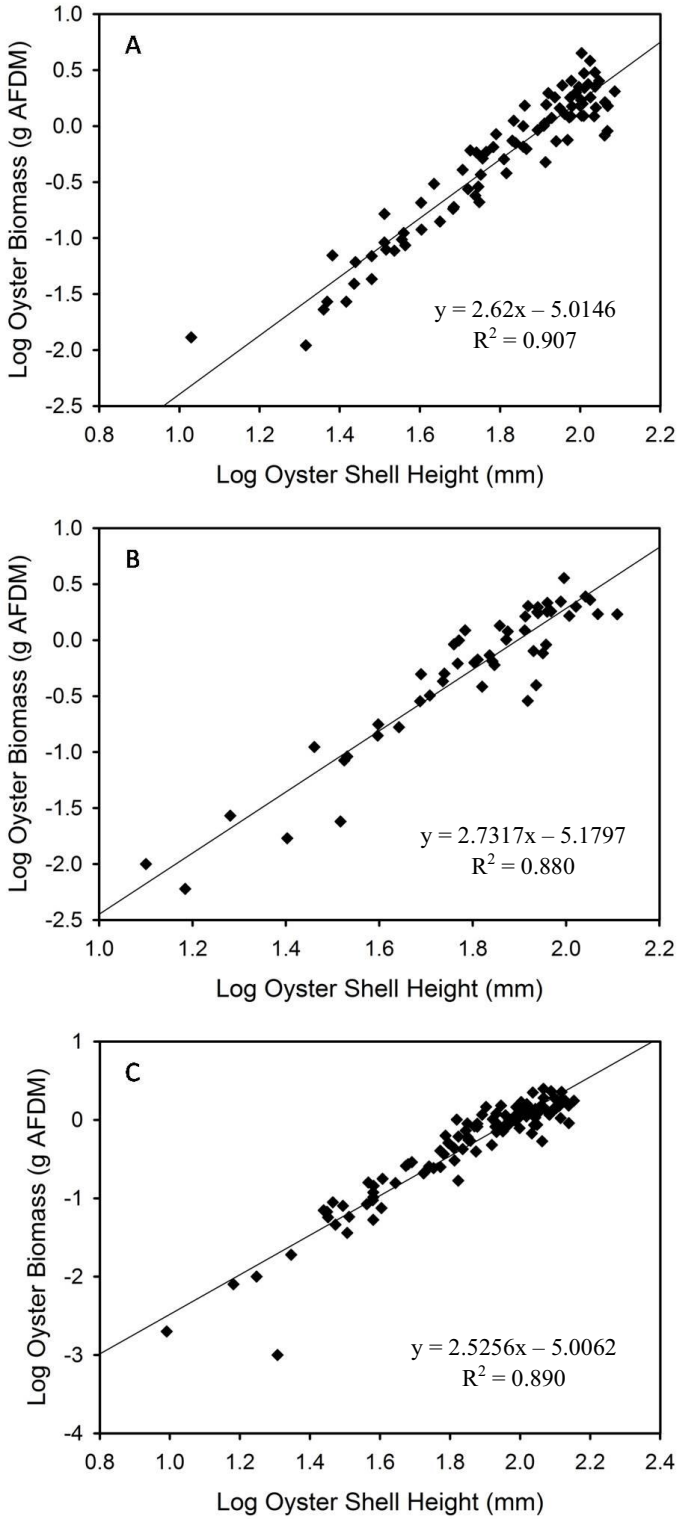


Figure 3.23: Regression models of AFDM (g) versus log SH (mm) for oyster biomass estimation on the (A) Outer, (B) Inner, and (C) Intermediate Rings of the Steamer Rock reef complex (2006).

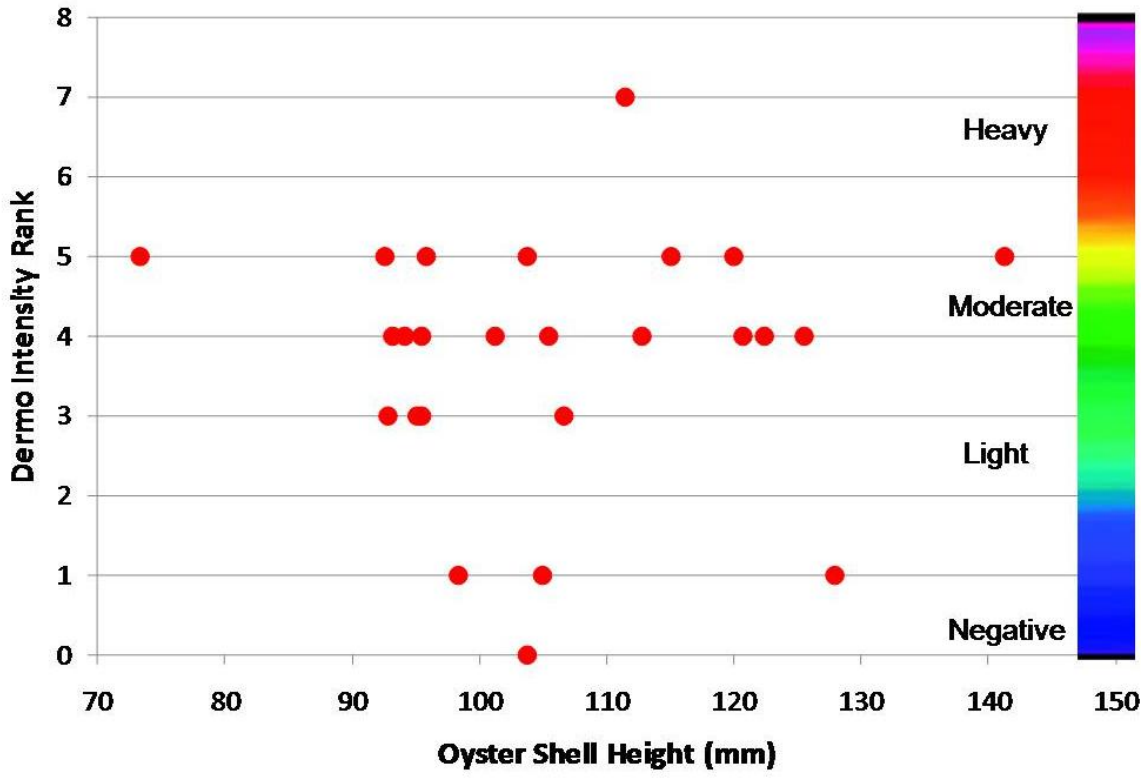


Figure 3.24: Steamer Rock oyster Dermo intensity rank versus oyster shell height (mm), August 2006.

DISCUSSION

Concrete Module Reef

Population structure. The 2005 CM oyster size structure could contain a maximum of four year classes since the modular reef system had been deployed 4.5 years prior to sampling. Upon plotting the size-frequency data, visual estimates suggested that there were at least three, if not four, year classes present. To help interpret possible factors affecting the patterns we observed, Rappahannock River discharge data, oyster density estimates from the Commonwealth of Virginia's Marine Resources Commission Dive Survey, and data from the Virginia Dredge Survey (Southworth et al. 2003, Southworth

et al. 2002) were examined (Fig. 3.25). The discharge data showed low-flow, drought (high salinity) conditions in 2001 and 2002 – good for oyster recruitment (larval retention is protracted) but bad for disease-associated mortality (Albright et al. 2007). 2003 and 2004 were high flow (wet), low salinity years – conditions conducive to adult oyster survival and lower oyster recruitment. In theory, two years of good recruitment with high disease pressure would not be an issue for young oysters with Dermo; *P. marinus* does not usually attack young oyster in Chesapeake Bay, in contrast to MSX, and natural spatfall can be grown 1 or 2 years before the animals acquire the disease (Andrews and Ray 1988). In order to thrive through adulthood, though, these growing oysters would need a lower salinity environment or some level of disease resistance. Fortunately, 2003 and 2004 brought increased precipitation and, thus, diminished disease pressure. The two peaks representing 2003 and 2004 are pronounced with the high peak for 2004 and smaller peak for 2003, with oysters that had undergone an extra year of natural mortality. Despite two years of recruitment failure in the rest of the Rappahannock River Estuary (Wesson Dive Survey 2005 update; Fig. 3.25), the CMs experienced moderate recruitment.

Although settlement throughout the Rappahannock River system was low from 2003-2005, 2006 and 2007 were good recruitment years, only slightly less than 2001 and 2002 (Southworth et al. 2008a). The CMs received these recruits on undisturbed and denuded surfaces in very high numbers. Recovery of previously-cleared concrete surfaces, as well as a persistent population of larger adults, was encouraging.

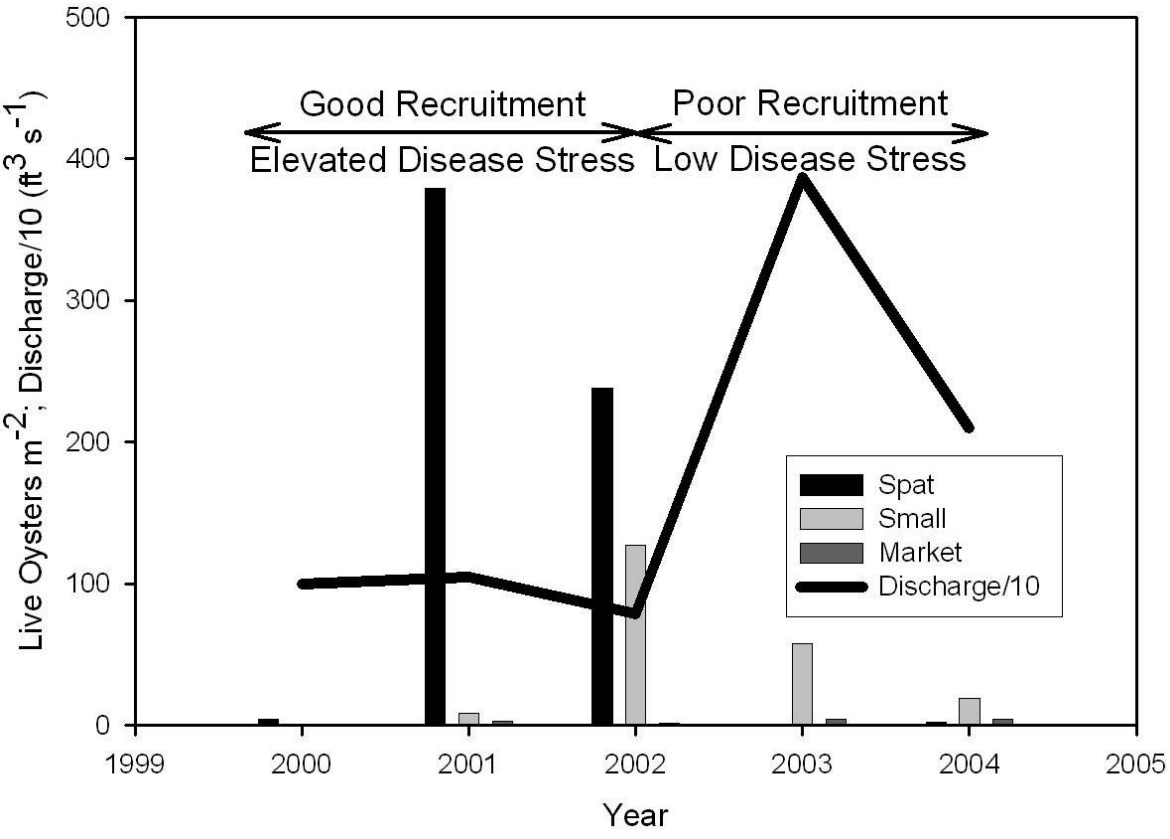


Figure 3.25: Rappahannock River discharge ($ft^3\ s^{-1}$) at Fredericksburg, VA and oyster density (m^{-2}) on Parrott's Rock, Lower Rappahannock River, 2000-2004 (VMRC Annual Dive Survey).

At first glance, mussel size structure (2005) appeared to be one continuous distribution possibly indicating successive year classes of similar levels of recruitment and survival. However, closer scrutiny and the application of FISAT II revealed 4-5 separate size classes. The largest peak, however, was the 40-50 mm mussel size class. Assuming that each peak represents one year class, this large peak coincides with the 2001 recruitment event noted within the CM oyster population as well as other surveyed Rappahannock River populations (Southworth et al. 2008a). We did not see a large spike of small mussels in spring of 2005 or 2007, but the Nov. 2007 sampling captured the strong recruitment from preceding months.

Oyster and mussel size structure varied by ML and face. The protected Top faces of MLs 4 and 3 in 2005, and the MLs 3, 2, and 1 in 2007, contained more oysters and mussels throughout the full size range than the uppermost MLs. The Bottom faces supported fewer mussels amongst a thriving sponge community. Hole and Side faces contrasted more for mussels than oysters. The primary benefit of having a diversity of concrete surface area (vertical/horizontal and protected/exposed) was that the oyster-mussel-sponge community could develop under heterogeneous conditions, making the reef community more diverse, stable, and resilient.

Density and biomass. The CM reef estimates of density were high for oysters (991 +/- 284 m^{-2} river bottom (+/- 95% confidence interval); 2191 +/- 777) and mussels (8433 +/- 1581; 6984 +/- 1822). These densities are comparable to the highest on restored oyster shell reefs in Chesapeake Bay (Nestlerode et al. 2007, Schulte et al. 2009). The estimates of biomass (g AFDM m^{-2} RB) were also high for oysters (1584 +/- 621; 715 +/- 443) and mussels (1117 +/- 235; not available for 2007). Bivalve volume (L m^{-2} RB), an important measure of reef growth and persistence, was very high for an oyster restoration reef (77.7 +/- 18.2; 34.5 +/- 11.8). Live oyster volume (12.9 +/- 8.0 L m^{-2} RB) and mud crab density (3414 +/- 1292 m^{-2} RB) was also recorded in 2007. The apparent decline in the oyster reef from ~78 L m^{-2} RB in 2005 to 34.5 L m^{-2} RB in 2007 has multiple possible explanations: (1) Oyster density was actually higher in fall 2007 than in May 2005, but increased adult oyster mortality and reduced oyster condition after two dry, disease-intense years (relative to pre-spawned oysters sampled in May 2005 after two wet years) exacerbated the difference in bivalve volume (Austin et al. 1993), (2) bivalve (oyster and

mussel) populations experience cycles related to salinity and temperature with a lag in response time (Austin et al. 1996), and (3) the oyster-mussel reef has, in fact, entered a regressive period from which it may not recover (Ardizzone et al. 1989).

As the oyster community structure developed on the CMs, a higher proportion of the oyster population was found on live or dead oysters or mussels. The percentage of oysters living on other oysters or mussels (Table 3.2a-b) increased with depth, likely due to oyster larval tendency to settle towards the bottom where luminosity is lower and less direct (Kennedy et al. 1996). By face, the Hole face had the highest proportion (undisturbed: 52 %; denuded: 49 %) of oysters on other oyster or mussels. The Top faces (38 %) and Bottom faces (31 %) of undisturbed plots, maintained a much higher proportion of oysters on other oyster or mussels than the Top (18 %) and Bottom (19 %) faces of denuded plots.

Sponge volume (L m^{-2} RB) was more variable (31.3 +/- 20.6; 34.2 +/- 26.7) across the CM reef strata, with the majority present on the Hole and Bottom faces. Sponge grew densest in close proximity to a crevice or crack and may gain a spatial refuge when small and grow out from a strong foundation where even occasional high current and abrasion by large fish would not cause them to be dislodged. Sponges compete for space with oysters and mussels, though, in a defensive space utilization strategy (Karlson 1978). Oysters can sustain some level of sponge overgrowth, but some 'boxes' (whole dead oyster shells) were found with a film of sponge on them. At the very least, sponge inhibits settlement of competent oyster larvae where they have overgrown suitable substrate (Gunter 1955). Only one oyster (spat, 2007) was found growing on sponge. Alternative oyster settlement sites included live mussels, or their

byssal threads (only very small oysters). Mussels appear to be more easily overgrown than oysters, but can, although in much lower density, adhere to a living sponge (byssal thread attachment) and survive. A sponge has the highest surface area per individual of all biota on the reef, but appears limited in its capacity to support many mussels.

One strong year class of oyster or mussel can dominate a population for years. Broadcast spawning is most efficient when adults are at high densities (Mann and Evans 1998), populations are hydrodynamically linked (Lipcius et al. 2008), quality substrate is available (Schulte et al. 2009), and ambient conditions, such as salinity (Kennedy et al. 1996), are optimized. Ctenophores can inhibit oyster larval abundance (Breitburg and Fulford 2006). In 2006 and 2007, river conditions were the best they had been since 2001-2002 and the Rappahannock River adult oyster population (as well as populations in many other subestuaries) responded (Southworth et al. 2002, 2003). In 2007, settlement was relatively high and among the highest over the previous 15 years of monitoring (Southworth et al. 2008a). In sampling the CM reefs in May and November 2007, distinctions between the 2006 and 2007 recruitment events were possible. Oyster and mussel abundance were seven- and ~three-fold greater, respectively, on ML 3 than ML 4. However, oyster and mussel biomass were equal between MLs 3 and 4, indicating evidence that the abundance discrepancy was due, in large part, to the strong 2007 bivalve year classes.

The resampling (2007) of previously denuded surfaces (2005) provided additional support for this hypothesis – oyster and mussel abundance were ten- and four-fold greater on ML 3 than ML 4. Furthermore, the ratios of oyster (ML 3 – 1:1; ML 4 – 1.5:1) and mussel (ML 3 – 1.3:1; ML 4 – 2:1) abundance on undisturbed versus previously-denuded

plots were positive indicators of recovery of cleared surfaces in fewer than three years. The 3:1 ratio of oyster biomass (ML 3:ML 4) on previously-denuded surfaces highlights the strong influence of the 2007 year class.

Large adult (> 75.0 mm SH) oyster abundance (2005) varied by face and ML, with highest abundance on the middle layer (ML 3). Spat were significantly impacted by face and covaried with juvenile abundance possibly indicating preferential oyster larval settlement on surfaces occupied by conspecifics, and higher subsequent spat survival into the young adult oyster (juvenile) size class (Bartol and Mann 1999, Bartol et al. 1999). The resident oysters may have served as substrate, provided refuge, or both. However, there was no covariance of large oysters with spat. Crowding as the reef matures in that region of the reef, due to growth of oysters, mussels, sponges, bryozoans, and tunicates, may limit settlement substrate, increase the probability of larval loss due to the high density of filter feeders (Thorson 1966, Woodin 1976, Peterson and Black 1987, Osman et al. 1992, Tamburri et al. 2007), or increase post-set mortality due to elevated predator densities (Newell et al. 2000). Nevertheless, recruitment potential on these reefs may decrease while still maintaining a high density of large adult oysters. Schulte et al. (2009) showed a similar relationship between oyster adults and spat on the restored shell reefs of the Great Wicomico River, whereby at high adult densities (> 800 oysters m^{-2} RB) the trend of increasing spat shifted downward.

A similar relationship exists between mussels and oysters. They share a facilitative relationship at low to medium density (early stage of reef development) and greater space and resource competition at higher densities (as the reef stabilizes and matures) (Dittman 1990, Bruno and Bertness 2000, Witman and Dayton 2000). This

relationship was most pronounced on the Top faces. On some oyster grounds in Chesapeake Bay, *I. recurvum* forms dense colonies attached to live oysters. Engle and Chapman (1952) found that oysters with attached mussels were characteristically more elongate than were mussel-free controls. More meat, relative to shell, was produced in mussel-free oysters, with these oysters having a condition factor about 28 % better than the oysters with attached mussels. Spatial competition occurs at high bivalve densities, but the outward and upward growth of oysters and mussels can reduce this effect. These findings support the assertion that the CMs had experienced the 'pioneer stage' of succession and persisted through the stage of invertebrate (oyster and mussel) dominance (Badalamenti et al. 2002).

The oyster and mussel community on the Top face of the uppermost ML (ML 5 in 2005; ML 4 in 2007) was the least productive. Conversely, the base ML's Bottom face is lost due to burial in the sediment, and the Top and Hole faces become buried in pseudofeces over many years, eventually killing most of the organisms attached to them. Overall, the base ML is not fully lost as an oyster-mussel community, but its lower productivity should be factored into calculations of expected benefits from production of such structures as restoration or mitigation reefs. Continued monitoring of the CMs will reveal whether the bivalve assemblage has reached a stable state, will start to degrade, or will continue to a more diverse, climax community.

Pathology and condition. MSX and Dermo were the two main pathogens of interest. By 2005, MSX had become relatively rare in the Chesapeake Bay after two years (2003-2004) of heavy streamflows and depressed salinities (Carnegie and Burreson 2005)

indicating that neither parasite (*P. marinus* and *H. nelsoni*) was seriously impacting oyster populations in the survey area (included the Rappahannock River), and that mortality caused by these parasites in 2004 was probably low. In addition, disease pressure and mortality should have been low until at least through summer 2005, and perhaps longer if normal rainfall and streamflow conditions were slow to return. From a disease-perspective, these oysters were healthy.

Condition indices of the 2005 CM oysters were high. Elevated oyster condition following two years (2003-2004) of increased precipitation, with the resultant increase in river discharge, had been also occurred in the Rappahannock River in the 1970s and 1980s, not far from where the SR reefs reside (Austin et al. 1993). These patterns were further explained by a significant relation between spat count and the Palmer Drought Index (PDI), which is published monthly by the Office of the Virginia State Climatologist at University of Virginia (Austin et al. 1996). The drought index is a combination of rainfall, soil type, and evapotranspiration. The responses of the spatfall to changes in the PDI were reflected both in the 1960s, as conditions evolved from "damp" to "drought," and in the more prolonged "drying" period of the mid-1970s to mid-1980s, as the spatfall reflected a short and a longer period of increased set. The cyclic nature of the PDI results in rapid and cyclic changes in the spatfall (Austin et al. 1996).

Studies in the upper part of Chesapeake Bay (Engle 1951), in Canada (Medcoff and Needler 1941), and in Louisiana (Hopkins et al. 1954) showed CI cycles similar to those in the Rappahannock River (Austin et al. 1993). The finding of no difference between face and ML may indicate that, although face can affect oyster density, the influence of river discharge may overwhelm the anticipated effects of bivalve density on

oyster condition. Another possible explanation may be that water flow around, and circulation through, the reef provides ample food delivery and sediment relief irrespective of oyster location.

Disease assessment was conducted for 25 additional oysters from the 4 November 2007 sampling. Unlike the oysters tested from May 2005, these oysters had experienced two seasons of relative drought, were in a post-spawning recovery state, and were sampled at peak Dermo/MSX prevalence and intensity. By convention, these oysters should have been in poor condition. However, the RFTM metrics and histology revealed that 21 of 25 oysters were no more than "light-to-moderately" infected and so were probably generally healthy. The histology data suggest the proportion of healthy (with respect to *P. marinus*) oysters was probably even higher. Just one or two additional oysters, at most, were detrimentally affected by *H. nelsoni*. Thus, the prognosis for the CM oyster population's sustainability is good.

Steamer Rock Reef

Population structure. Oyster size structure was robust throughout the full size range, containing a minimum of five year classes (2001 – 2005). More than half of all oysters sampled were large adult oysters (> 75 mm SH) which indicates: (1) a hardy broodstock with some level of disease resistance, (2) adequate shell accretion (volume is the proxy for reef persistence), and (3) longevity. The largest peak was between 80-100 mm SH and represented the strong year classes of 2001 and 2002. The smaller peaks (< 80 mm) represent the 2003-2005 year classes both the SR and CM reefs recruited oysters during

these years. Lighter recruitment during wet years was apparently important for oyster reef stability.

An important observation for SR was that the 2006 oyster survey detected five year classes (presumably 2001-2005). Since the reefs were deployed in 1994, an entire generation of oyster likely recruited, had grown and died through the late 1990s. The reefs persisted and grew. This finding of reef longevity and durability is critical. The population quantified here was the second generation, indicating that alternative substrate reefs can persist through years of poor recruitment and subsequent coverage by fouling species and still flourish when more favorable conditions emerge.

Density and biomass. Oyster density, biomass, and bivalve volume did not significantly differ between the Intermediate, Outer or Inner rings of concrete stacks. The Intermediate stacks had higher densities and volumes. These differences were exacerbated for estimates of total reef abundance since the Intermediate stacks made up 71 % of the SR reef. Across 176 stacks covering approximately 0.4 ha of river bottom, we estimate a population of 4.818 +/- 0.927 million oysters (SEM), 30.055 +/- 6.116 million mussels, and 2.713 +/- 0.965 million mud crabs, with a total oyster biomass of 4242 +/- 1112 kg AFDM, a total oyster volume of 312,260 +/- 86,210 L, and a total mussel volume of 113,360 +/- 24,890 L. The CM reef design is the more efficient use of space (SA); however, the SR reef complex also supports a vibrant oyster reef community comprised of high bivalve and sponge densities and volumes in an otherwise substrate-limited system; these reef structures provide stable and heterogeneous settlement surfaces.

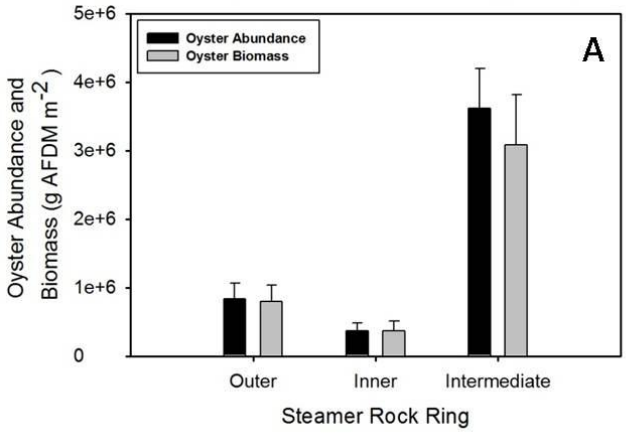
By SA, the SR and CM reefs are both productive (Table 3.3), especially when compared to other restored shell reef sites in the Rappahannock River (Drumming Ground Reef, Parrot Rock Reef). These restored shell reefs experienced low (2005) to marginal (2006/2007) oyster densities at least one order of magnitude lower than the SR/CM reefs (Southworth et al. 2006, 2007, 2008a). Poaching of oysters is still a problem on many Chesapeake Bay restored oyster shell reefs and may affect the recorded densities for reefs such as Drumming Ground or Parrot Rock reefs. The benefit of large, alternative concrete oyster reefs is the impracticality of poaching oysters off of them. Thus, our population estimates are not confounded by a potential negative (poaching) bias (a genuine concern for restored shell reefs) and the reefs are left to function and progress naturally.

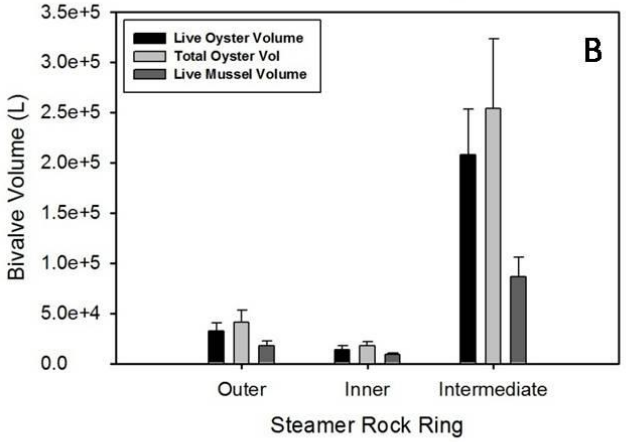
Pathology and condition. The effect of Dermo and MSX on SR oysters collected in August 2006 was similar to oysters tested from the CM reefs in November 2007. By RFTM metrics and histology, 17 of 25 oysters were no more than "light-to-moderately" infected and so were probably generally healthy. Once again, the histology data suggest the proportion of healthy (with respect to *P. marinus*) oysters was probably even higher. No MSX infections were detected. The SR reef pathology data suggest that the population of oysters living on these lower Rappahannock River reefs tolerate the effects of disease, with greater than half of the oysters growing into the large oyster size class (> 75 mm SH). To date, this study is the first to document disease resistance on subtidal alternative substrate oyster reefs. This emergence of disease resistance has been documented in the Great Wicomico River (Carnegie et al. 2008, Schulte et al. 2009),

upper and lower Lynnhaven River (Dissertation Chapters 3 and 4), and Elizabeth River (Burke and Schulte, *unpublished data*); each of these examples (including the SR and CM reefs) have been fully-protected sanctuary oyster populations where harvest activities cannot remove the largest, fastest-growing oysters. Selective premature harvest of oysters on unprotected reefs may result in the suppression of disease resistance development (Munch et al. 2005, Edeline et al. 2007).

Table 3.3: Density and surface area density metrics for the Concrete Module (2005, 2007) and Steamer Rock (2006) reef complexes.

Reef, Year (+/- 95% CI)	Oyster Density (m^{-2} River Bottom)	Oyster Biomass (g AFDM m^{-2} River Bottom)	Bivalve Volume (L m^{-2} River Bottom)	Oyster SA Density (m^{-2})	Oyster Biomass SA Density (g AFDM m^{-2})	Bivalve Volume SA Density (L m^{-2})
Concrete Modules, 2005	991 (284)	1584 (621)	77.7 (18.2)	284 (82)	439 (168)	21.8 (4.8)
Steamer Rock, 2006	1575 (386)	1459 (451)	126.1 (32.5)	107 (26)	99 (31)	8.6 (2.2)
Concrete Modules, 2007	2191 (777)	715 (443)	34.5 (11.8)	626 (214)	183 (112)	8.7 (3.0)





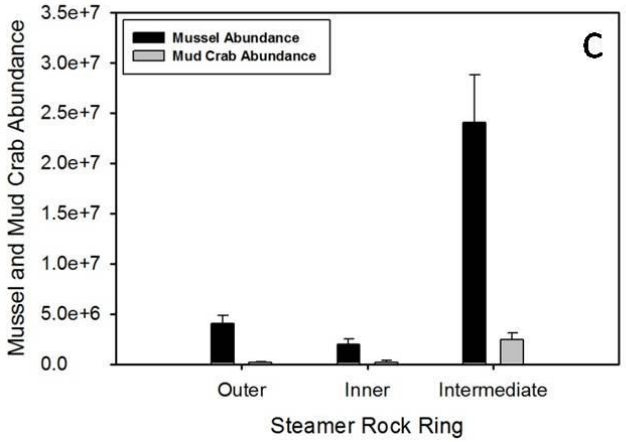


Figure 3.26: Total oyster (A) abundance and biomass (g AFDM m^{-2} RB), (B) live oyster, total oyster, and live mussel volume (L), and (C) mussel and mud crab abundance on the Outer, Inner, and Intermediate Rings of the Steamer Rock reef complex (2006).

CONCLUSIONS

Throughout the Chesapeake Bay, substrate limitation has been a considerable issue. Many areas have also contended with significant recruitment limitation. Much of the Rappahannock River is heavily silted, experiences annual summer hypoxia, and has low recruitment. The SR and CM reef systems were deployed to a subtidal depth of 7 m upon solid, sandy bottom at a location that experiences strong semidiurnal tidal currents. These site-specific characteristics likely reduce siltation, reef subsidence, and hypoxic stress, but increase flow of plankton-rich water, as well as increase exposure of oyster and mussel larvae to the reef during periods of recruitment. In 1994, 176 seven-layer stacks of concrete bridge 'material of opportunity' were deployed in concentric rings as a new oyster reef restoration strategy. In October 2000, an experimental five-layer CM reef was deployed adjacent to the larger reef complex. The experimental CM reef increased settlement surface area by nearly 15 times that of the sediment bottom below it. Its design makes it ideal for use as a sanctuary reef because it: (1) is difficult to harvest on or around with patent tongs or dredges, (2) provides a plethora of niche spaces as evidenced by its diverse community, (3) increases reef stability and, thus, the capacity for increased vertical complexity, and (4) can act as a source reef in a metapopulation-based restoration reef strategy. Other reefs were restored in the lower Rappahannock River and have shown marginal success at the same time this reef has proven quite productive and healthy.

A five-ML reef performed well. A taller reef may not be stable unless the MLs are made wider; a shorter, three-ML reef may only contain one fully-functioning ML and not be worth the investment. Given a fixed budget for CM reef construction (i.e.

maximum of 60 MLs) for a single project, the optimal allocation of MLs may be 12 five-ML or 15 four-ML reefs rather than 20 three-ML or 30 two-ML reefs. Although single-ML reefs would cover the most area, the strategy would result in the greatest loss of concrete surface area for oyster and mussel settlement and the highest likelihood of individual reef failure due to siltation or fouling. The internal spaces would quickly fill with sediment and sponges; water flow along the estuary floor would likely bury the whole structure in a few years, resulting in a considerable loss of a restoration program's investment.

We continue to see high survival ratios of multiple year classes on intertidal reefs constructed of alternative substrates such as concrete and granite (Dissertation Chapters 2, 4). The results presented here make a convincing argument for the consideration of subtidal deployment of alternative substrate reefs to supplement the current native oyster restoration efforts in the Chesapeake Bay. Based on high oyster and mussel density (and the resultant elevated fertilization efficiency), robust population structure, good oyster condition and the detection of tolerance to Dermo and MSX diseases, we posit that Steamer Rock is a significant contributor to the lower Rappahannock River oyster and mussel larval pool each year and that this reef complex is the largest, most successful artificial (alternative substrate) oyster reef built in the Chesapeake Bay. Its popularity as a perennial fishing hotspot for recreational fishers speaks to the resident and visiting fish populations it sustains. The CM reef design uses space efficiently and might be economically viable considering the long-term production. The Steamer Rock reef is made of a 'material of opportunity' and little, but requires careful placement.

Nonetheless it is clear that effectively-designed, alternative substrate reefs are a viable restoration method for the native eastern oyster in subtidal habitats of Chesapeake Bay.

Acknowledgments. We express appreciation to Captain Robert W. Jensen, the Rappahannock Preservation Society, Reeftek-McLean, Commonwealth Pro Dive, and the VIMS Shellfish Pathology Group. Special thanks to the entire Marine Conservation Biology and Marine Community Ecology groups at VIMS for their support of this research in both the lab and field.

Chapter 4

Eastern oyster (*Crassostrea virginica*) recruitment, growth, and survival on alternative reef substrates

ABSTRACT: Reef restoration efforts with native Eastern oyster (*Crassostrea virginica*) in Chesapeake Bay have been extensive, yet impeded by predation and by substrate limitation due to the diminishing availability of oyster shell. Consequently, we experimentally tested the effects of large predators (e.g. cownose ray and blue crab) and the performance of various substrates as alternative intertidal oyster reefs in the Lynnhaven River System (LRS), a southern subestuary of Chesapeake Bay. In a three-year field experiment, treatments simulating intertidal oyster habitat were placed at three sites (marsh, riprap and restored oyster shell reef) within a tidal creek of the LRS. The two factors included (1) substrate type, involving granite, concrete, limestone marl, and oyster shell of various sizes and (2) predation, involving experimental plots either open or caged to exclude large predators. The response variables included native oyster recruitment, density, biomass, growth, survivorship, condition, and reef accretion. Across all sites, granite of two size classes had the highest oyster recruitment and long-term abundance (density > 1500 m^{-2} and biomass > 200 g ash-free dry mass m^{-2}). Survival was high in most treatments. No significant caging or long-term handling effects were detected. Oyster weight-at-age and condition index decreased with increasing oyster density, indicating density-dependence. Many reefs reached a mature state after two years. By Year 3, some treatments accreted 15-20 L of shell m^{-2} of river bottom, and contained three strong oyster year classes; a few treatments had > 30 % of live oysters growing on other oysters. Exterior portions of the treatments maintained > 70 % of oyster density, biomass, and oyster shell volume indicating that exterior oyster density was a major control of oyster biomass. Disease (i.e. Dermo) intensity was lower in large oysters (> 95 mm shell length) than in smaller oysters (60-90 mm shell length). This trend was most pronounced in treatments with high oyster densities, suggesting that oyster disease tolerance had developed in these high salinity waters. These findings confirm that, in high-salinity intertidal habitats, (1) large predators such as blue crab and cownose ray do not control oyster population dynamics, (2) disease is not an absolute impediment to oyster recovery, and (3) alternative substrates can be extremely effective as native oyster reefs.

INTRODUCTION

Oyster reefs in most ecoregions where they historically occurred are in poor condition and at risk of extirpation as functional ecosystems (Lotze et al. 2006, Airoldi and Beck 2007, Beck et al. 2009). The Chesapeake Bay's eastern oyster (*Crassostrea virginica*) population decline is amongst the most dramatic globally, placing it at less than 1 % of its historic abundance. The decline was due to extreme levels of harvesting and substrate removal since the 1800s (Stevenson 1894, Kennedy and Breisch 1981, Rothschild et al. 1994), and in the last half century due to the action of MSX or Dermo, diseases caused by the pathogens *Haplosploridium nelsoni* and *Perkinsus marinus*, respectively (Andrews 1988). The combined effect of disease and overharvesting has been the elimination of the commercial oyster fishery from essentially all waters in the lower bay with the exception of three oyster bars in the upper James River and very limited areas of the upper Rappahannock River (Mann et al. 1991). Other areas that appear to have self-sustaining populations are the lower Rappahannock River and the Lynnhaven River, and, more recently, the Great Wicomico River (Schulte et al. 2009). Oyster populations in these and other bay subestuaries have shown signs of recovery and disease resistance (Encomio et al. 2005, Lipcius and Burke 2006, Carnegie and Burreson 2008, 2009, Dissertation Chapter 3).

In recent years, a dramatic shift away from unsustainable harvest ground (public fishery subsidy) practices (Santopietro et al. 2009, Herberich 2006) towards the reestablishment of large sanctuary reef networks has occurred. These sanctuary networks are aimed at restoring oyster metapopulation structure that would provide the larvae for ample recruitment to sanctuary reefs and public oyster grounds. Persistent harvesting of

most large oysters likely slowed the natural genetic rebound one would expect according to Darwinian selection (Munch et al. 2005), but this sanctuary network strategy should serve to dampen such effects. Ecological oyster reef restoration is now focused on a tributary-by-tributary strategy to achieve sustainable oyster populations (Schulte et al. 2006).

The oyster shell necessary to build reefs has, unfortunately, diminished greatly, requiring examination of substrates, either natural or artificial, to construct oyster reefs. Alternative reefs may incorporate shells but are not made solely of dredged or shucked oyster shells. Use of alternative reef materials for eastern oyster, restoration has been an established technique in the southeastern United States, including many areas along the Gulf of Mexico (Cowan 2003, LADWF 2004, Schulte and Ray 2009), and throughout Europe for native and introduced oyster species (Jensen 2002). In Chesapeake Bay, restored oyster reefs built of alternative substrates have shown promise in the Rappahannock River, Lynnhaven River, and Fisherman's Island (Lipcius and Burke 2006, Luckenbach and Ross 2006, Nestlerode et al. 2007, Dissertation Chapters 2, 3).

Disease, declines in water quality, and frequent shellfish closures shut down the fishery in the Lynnhaven River System (LRS) in the early 1970s (Schulte et al. 2006). More recently, the LRS has been the focus of oyster restoration because it was a historical source of coveted oysters ("Lynnhaven Fancies"), it has supported natural oyster populations in recent years (Brumbaugh et al. 2000), it had historical oyster grounds delineated by the Baylor Survey in the late 1890s (Baylor 1894, Chipman 1948), and it had a history of regular spat settlement and significant private oyster production before the oyster disease MSX became established in the 1960s (Chipman 1948).

Given the impediments of predation by large predators and of substrate limitation due to the lack of oyster shell, we experimentally tested the performance of various substrates as alternative intertidal oyster reefs in the LRS. Specifically, in a three-year field experiment, we examined the effects of substrate type (granite, concrete, limestone marl and oyster shell of various sizes) and predation (caged and uncaged reefs) at three sites (marsh, riprap and restored oyster shell reef) within a tidal creek of the LRS. Reef performance was measured as native oyster recruitment, density, biomass, growth, survivorship, condition, and reef accretion. We relied solely on natural settlement to the substrates due to consistent recruitment in the LRS. The experiment took place in Long Creek, a narrow creek connecting Broad Bay and Lynnhaven Bay in the LRS (Fig. 4.1). Long Creek has a distinct intertidal oyster band, and there were several shoreline property owners along its shores willing to allow us to deploy these oyster treatments for three years. The area is also not prone to interference or tampering, nor does it experience large boat wakes that might disturb experimental plots.

MATERIALS AND METHODS

Sampling procedure and design. Experimental oyster trays (n = 108; L x W x H - 0.50 m x 0.50 m x 0.23 m; mesh size = 2.54 cm x 2.54 cm) were deployed in Long Creek in late August 2005 (Fig. 4.1) in a replicated randomized block design (Fig. 4.2). The mesh size of caged plots was selected to exclude large predators, but still allow water and small predators to pass easily through the trays. Hypotheses (Table 4.1) were tested at three sites: (1) a marsh fringe (36 trays), (2) the Long Creek restored oyster shell reef (36 trays), and (3) two stands of riprap revetments (12 trays on granite; 24 trays on concrete).

The fixed factors were substrate type and caging (caged/uncaged); site was a random factor. There were six substrate classes (Table 4.2, Fig. 4.3a-h) including small pieces of recycled concrete (CVS), large and small granite (GL, GS), large and small limestone marl (LML, LMS), and unconsolidated, loose oyster shell (OSU) reclaimed from the Long Creek restored oyster shell reef.

The trays were filled with each substrate class and deployed in replicate sets of 12 (6 caged trays and 6 uncaged trays) with 3 replicate sets per site. The uncaged trays did not contain a mesh cover and were open to blue crabs, large fish (e.g. cownose ray and oyster toadfish), birds (e.g. oystercatcher and seagulls), and mammals (e.g. raccoons and muskrats). The caged trays were closed with the same material and mesh size using 8 cable ties (2 per side). Placement of the trays was adjacent to the marsh, on the Long Creek restored oyster shell reef, and on the riprap. The riprap was the most variable in terms of tray placement. The goal was uniform placement in the intertidal zone; sometimes that meant placing them on the sediment (mud usually) or on the base of the riprap. In extreme cases, pieces of riprap from the revetment were placed below the tray to keep it from sinking into the mud; a few of these trays were half sunk in mud early in the experiment which accounted for anoxic conditions in some lower tray portions.

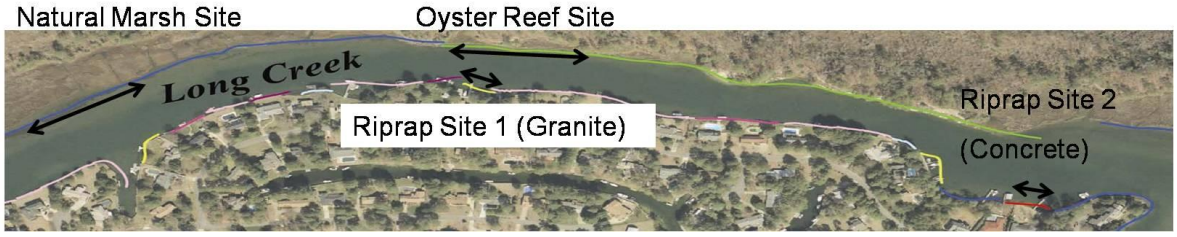


Figure 4.1: The Lynnhaven River System (Chesapeake Bay, Virginia) contains the deployment sites for the Alternative Substrate Experiment trays in Long Creek.

Table 4.1: *A priori* hypotheses for the Alternative Substrate Experiment (ASE).

Hypo-thesis	*a priori* Hypothesis Description followed by observation(s) and associated logic behind hypotheses
ASE #1	Oyster Recruitment differs across 1) substrate type and 2) caging (large predator exclusion) 1) Substrate Type: General hypothesis is that certain substrates perform better than others; more specifically, large rocks will fare better than loose shells/small rocks. 2) Caging (Predator Exclusion): Mud crab predators will have a more detrimental impact on spat survival in closed rather than open trays; blue crabs and cownose rays can eat oysters in open trays.
ASE #2	Oyster Recruitment and survival increases on substrates with greater fractal dimension and interstitial volume 1) Increasing fractal dimension equates to increasing surface area, which equates to more potential settlement substrate and, thus, higher recruitment and lower post-set mortality 2) Interstitial Volume (Pore space): Increasing interstitial volume correlates to increased flushing or water flow (lower sedimentation & more available food) which results in increased settlement and survival.
ASE #3	Oyster Survival (%) differs across 1) substrate type and 2) caging (large predator exclusion) Observation(s)/Logic outlined by ASE #1 and ASE #2.
ASE #4	Oyster Growth does not differ across site, substrate and/or caging (large predator exclusion) Water flow in Long Creek is similar amongst sample sites. H_A: Increased Interstitial Volume (pore space) equates to increased growth due to lower siltation stress and more available food.
ASE #5	Exterior > Interior for: Oyster 1) Recruitment, 2) Density, 3) Survival (%), and 4) Growth Exterior substrate surfaces are more readily available (higher larval encounter rate) than interior surfaces deeper within treatments; food is more limiting on substrate types with low interstitial volume.
ASE #6	Good recruitment one year on a substrate facilitates relatively higher recruitment in subsequent years on that substrate Studies have shown that oysters selectively settle on clean substrate, seemingly cued chemically by presence of calcium carbonate ($CaCO_3$) and live oysters (conspecifics). Thus, the presence of live oysters or their recently dead shells on a given substrate increases the probability of a higher oyster larval settlement rate compared to substrate unoccupied by oysters/shells.
ASE #7	Disease tolerance does exist and is detected in Dermo/MSX intensity data with the largest oysters having lower intensity parasitic infections. The null hypothesis is that disease prevalence and intensity do not differ between site, substrate, or caging. An alternative hypothesis is oyster populations that experience the fewest metabolic stresses are expected to have the lowest intensity infections. Thus, site and caging are not expected to have a significant effect; larger substrates with high interstitial volume and good flow should perform best and favor oysters recruiting to them.

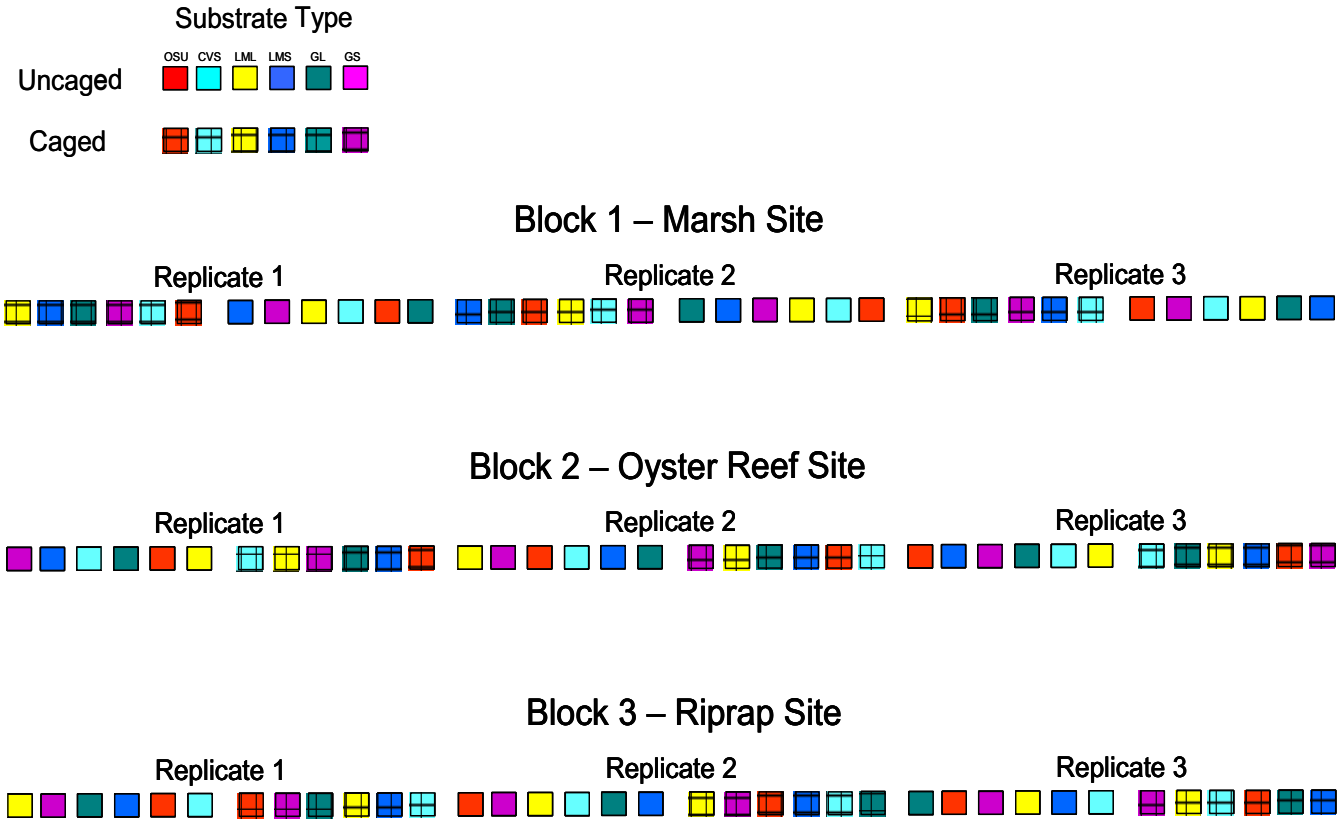


Figure 4.2: Long Creek Alternative Substrate Experiment design layout.

Sampling occurred in the spring and the fall of each year (fall 2005 - spring 2008) and was non-destructive, except for the final sampling. All measurements were made on-site (except for spring 2008). Trays were lifted out of the water onto adjacent rocks, hauled into the boat with a davit winch, or sampled *in situ* when the tide was low enough. Each tray was divided into 4 quadrants; each quadrant was systematically split into 4 categories – Top, Edge, Middle, and Bottom, with one quadrant sampled each time. Quadrants were marked systematically with cable ties, where "Exterior" = Top + Edge, and "Interior" = Middle + Bottom. The sampling regime (Table 4.3) was established to address each hypothesis (Table 4.1) quantitatively throughout the experiment.

Table 4.2: The six substrate classes tested in the Alternative Substrate Experiment.

Substrate	Acronym	Size Dimensions
Demolished/Recycled Concrete Very Small	CVS	2-3" diameter
Large Granite	GL	10-14" L, 4-6" W, 4-6" H
Small Granite	GS	4-6" diameter
Large Limestone Marl	LML	12-16" L, 10-14" W, 6-8" H
Small Limestone Marl	LMS	4-6" diameter
Unconsolidated Oyster Shell	OSU	2-4" Shell Height

Figure 4.3: Images of experimental treatments (marsh site, fall 2006): (A) CVS, (B) CVS with spat (SC1/YC2), (C) OSU with small adult (SC2/YC1) oysters, (D) LML, (E) GL with large adult (SC3/YC1) oysters, (F) GL with spat, (G) GS (side view), (H) GS (bottom view).

Table 4.3: Field sampling protocols delineated by sampling period and tray quadrant.

Sampling Period	Quad 1	Additional Quad
fall 2005	Oyster Count (live/dead); mud crab count; 1/3 trays from each site had oyster SH measured	--
spring 2006	Resampled as before but measured SH for all oysters in all 108 trays	Quad 2: Selected randomly and sampled same as Quad 1
fall 2006	Resampled as in spring 2006	Quad 3: Selected randomly and sampled same as Quad 1 except that oysters < ~10 mm were counted (not measured) as YOY
spring 2007	Resampled as in spring 2006	--
fall 2007	Resampled as in spring 2006	--
spring 2008	Two thirds (72 Trays) resampled as in spring 2006; a subset of oysters from the remaining 36 trays (12 from each site) were processed in the laboratory for disease (Dermo, MSX)	Quad 4 (only Quad remaining): sampled same as Quad 1

YOY = Young-of-the-Year, also known as spat

Density, biomass and condition index. Oyster density and biomass are the primary metrics of success in Chesapeake Bay native oyster restoration. A thorough description of annual oyster recruitment can aid in determining the success of an oyster reef, but density and biomass through time are important criteria for determining oyster reef sustainability. Density – the number of live oysters per m^2 of river bottom – is calculated by multiplying the sum of all oysters in a tray quadrant (0.0625 m^2) by 16.

Measures of biomass – grams of ash free dry mass (AFDM) of oyster tissue per m^2 of river bottom – were calculated from the pooled linear regression model of log AFDM vs. log SH from the LRS Riprap Survey (Dissertation Chapter 2) for fall 2005

through fall 2007 (Fig. 4.4). The regression model (Log AFDM = 2.3945 * (Log SH) - 4.7812) predicts oyster biomass from oyster SH. In spring 2008, thirty separate regressions (~1400 oysters) were generated for exterior and interior segments of all treatments (Table 4.4). Individual oyster AFDM (g) estimates are summed for all live oysters in a given category (i.e. one tray quadrant of CVS) and multiplied times 16 to estimate oyster biomass per m^2 of river bottom.

Shell height (SH), width, and depth were measured for all oysters, living and dead. SH was considered as the distance from the umbo to the farthest posterior end of the shell. Additionally, all internal tissues were collected for selected oyster in pre-weighed aluminum 'weigh boats' for dry mass (DM) and AFDM measurements. More than 1400 oysters representing the full range of SH values were processed to yield reliable estimates of oyster biomass via regression of log AFDM (g) versus log SH (mm). The oysters were selected across all factors, including site, substrate, caged/uncaged, exterior/interior, and handled/undisturbed quadrants (Table 4.4). Details regarding laboratory biomass procedures and condition index calculations can be found in the 'Materials and Methods' section of Dissertation Chapter 2.

Repeated Measures ANOVA tests were appropriate for this experimental design but produced significant interaction effects for Sampling Period (fall 2005, spring 2006, etc.) by caging or substrate type and, thus, required the use of Two-Way ANOVAs for each site during each sampling period (Underwood 1997). Visual differences evident in the associated tables and graphs were confirmed statistically using the Information-Theoretic (I-T) approach (Burnham and Anderson 1998, Anderson 2008), which allowed us to compare multiple candidate linear models (one- and two-way ANOVAs) and linear-

mixed models (general linear models with fixed and random factors) (Appendix 4.2). Interaction effects between factors caused us to abandon the use of a Linear Mixed Model (two fixed factors: Substrate and Caging; one random factor: Site) and turn to separate Two-Way Analysis of Variance (ANOVA) tests for each site. Further interaction effects were analyzed with Student-Newman-Keuls (SNK) *post-hoc* comparison tests (Underwood 1997).

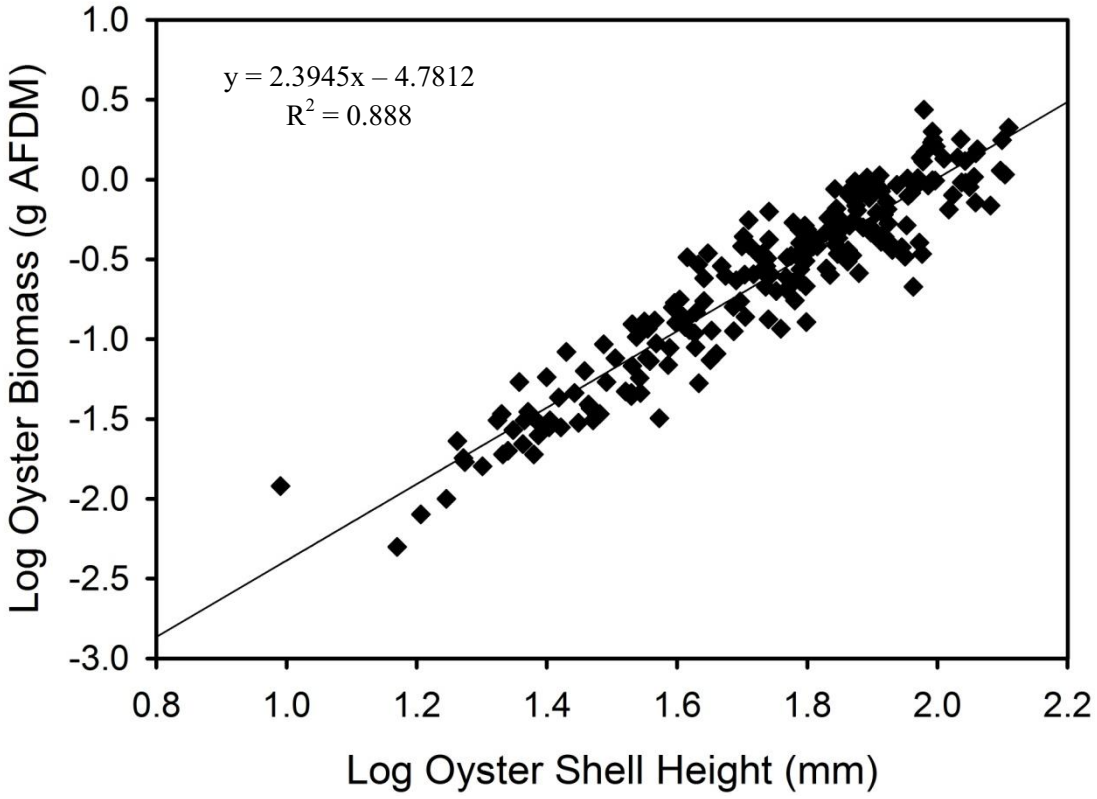


Figure 4.4: Regression model of log AFDM (g) versus log oyster shell height (mm) for biomass estimation of oysters measured in the Lynnhaven Riprap Survey (Dissertation Chapter 2) and used for oyster biomass estimation for this Alternative Substrate Experiment (ASE) from fall 2005 to fall 2007.

Table 4.4: Regression models of log AFDM (g) versus log oyster shell height (mm) used for oyster biomass estimation of exterior and interior segments of experimental treatments.

Site	Substrate	Exterior Sample Size (n)	Exterior Biomass Regression Equation	Interior Sample Size (n)	Interior Biomass Regression Equation
Marsh	CVS	95	10^((2.1422*LOG10(SH))-4.2327)	21	10^((2.4203*LOG10(SH))-4.7906)
Marsh	GL	69	10^((2.4141*LOG10(SH))-4.776)	36	10^((2.2811*LOG10(SH))-4.5101)
Marsh	GS	65	10^((2.5047*LOG10(SH))-4.9667)	21	10^((1.9855*LOG10(SH))-4.1671)
Marsh	LML	60	10^((2.3006*LOG10(SH))-4.5332)	33	10^((2.3213*LOG10(SH))-4.5216)
Marsh	LMS	84	10^((2.3926*LOG10(SH))-4.7431)	17	10^((1.8781*LOG10(SH))-3.7813)
Marsh	OSU	109	10^((2.2284*LOG10(SH))-4.4474)	35	10^((2.2449*LOG10(SH))-4.5365)
Oyster Reef	CVS	57	10^((2.2091*LOG10(SH))-4.2808)	9	10^((2.1983*LOG10(SH))-4.2574)
Oyster Reef	GL	44	10^((2.2695*LOG10(SH))-4.3963)	14	10^((2.6191*LOG10(SH))-4.9958)
Oyster Reef	GS	71	10^((2.7227*LOG10(SH))-5.2603)	36	10^((2.7547*LOG10(SH))-5.3581)
Oyster Reef	LML	--	Use Riprap LMS Exterior	--	Use Riprap LMS Exterior
Oyster Reef	LMS	--	Use Riprap LMS Exterior	--	Use Riprap LMS Exterior
Oyster Reef	OSU	54	10^((2.6867*LOG10(SH))-5.2135)	24	10^((2.247*LOG10(SH))-4.4636)
Riprap	CVS	52	10^((2.4865*LOG10(SH))-4.8774)	--	Use Riprap CVS Exterior
Riprap	GL	104	10^((2.305*LOG10(SH))-4.4947)	13	10^((2.0862*LOG10(SH))-4.1763)
Riprap	GS	106	10^((2.4224*LOG10(SH))-4.7282)	18	10^((1.8744*LOG10(SH))-3.9027)
Riprap	LML	22	10^((2.539*LOG10(SH))-4.8159)	11	10^((3.0689*LOG10(SH))-5.7337)
Riprap	LMS	13	10^((2.5017*LOG10(SH))-4.8094)	--	Use Riprap LMS Exterior
Riprap	OSU	70	10^((2.1952*LOG10(SH))-4.3076)	31	10^((2.3103*LOG10(SH))-4.6348)

Oyster and substrate volume. Oyster volume, a direct assay of oyster reef accretion, was measured for live oysters and dead shells separately through volumetric displacement. Oyster and substrate volume measurements were made for 72 of the 108 experimental trays in spring 2008 across all factors, including site, substrate, caged/uncaged, exterior (Top, Edge)/interior (Middle, Bottom), and tray quadrant (Quads 1 and 4). Oysters (live and dead) and substrates were scraped clean of oysters, mussels, large barnacle clusters and other fouling organisms to avoid overestimation.

Pathology and condition. Dermo (*Perkinsus marinus*) and MSX (*Haplosporidium nelsoni*) prevalence and intensity were tested in 110 oysters. Oysters were collected in

early September since peak infection intensity occurs in September and October. Oysters ranging in SH from 46.3-121.4 mm were haphazardly sampled from granite, limestone marl, oyster shell, and recycled concrete at each site (n = 6-10 from each treatment). No distinction was made between caged or uncaged treatments during oyster selection for disease testing; nor were the large and small categories for granite and limestone marl distinguished. Samples were brought back to the VIMS Shellfish Pathology group live and on ice. Two methods of Dermo and MSX testing were conducted, histology and RFTM (Ray fluid thioglycollate medium). The RFTM has a lower detection limit and, thus, those data were selected for analysis. The oysters were assigned one of nine disease ratings (Ray 1954, Table 4.5). SH and other condition metrics (i.e. emaciated tissue, thin shell, presence of boring sponge, etc.) were noted for each oyster measured. A linear mixed model (fixed factor = substrate type; random factor = site) with oyster SH as a covariate and Dermo intensity rank as the response variable was used to determine if oyster disease intensity varied by site or substrate type.

Table 4.5: Dermo disease intensity ranking system for oysters.

Dermo Disease Intensity Rank	Nominal Rank
Negative (N; no cells detected)	0
Rare (R)	1
Very Light (VL)	2
Light (L)	3
Light to Moderate (LM)	4
Moderate (M)	5
Moderate to Heavy (MH)	6
Heavy (H)	7
Very Heavy (VH)	8

RESULTS

Recruitment. At least one recruitment spike was detected during each fall sampling, which produced three year classes (YC1 = 2005, YC2 = 2006, YC3 = 2007). We classified three size classes by shell height: Spat (SC1) were < 30.0 mm SH, small adults (SC2) were 30.1 - 70.0 mm, and large adults (SC3) were > 70.0 mm. Most new oyster recruits were small (SC1), with only a small number of oysters – often those that settled earliest in the season and almost exclusively on treatments at the marsh site – growing large enough to be placed in SC2. For example, in fall 2007 (Fig. 4.5), YC1 was almost entirely within SC3, YC2 in SC2, and YC3 in SC1, with minimal overlap. These classifications were selected to assist in analyses and descriptions. To further simplify comparisons of three years of recruitment across site, substrate type, and caging factors, we adopted a ranking system from 0 to 8 (Table 4.6). Note that qualitative descriptors of recruitment such as 'Low' and 'Extremely High' refer only to the Chesapeake Bay and its subestuaries; locations outside of Chesapeake Bay could have different rankings.

The first recruitment event (YC1 - 2005) was deemed 'average' with a mean rank value of 3.0 across all sites (Table 4.7, Appendix 4.1). Treatments at the oyster reef site received the fewest recruits; treatments at the riprap and marsh sites received 2 and 3 times as many, respectively (Fig. 4.6b). Across the sites, limestone marl (LML and LMS) treatments received the fewest recruits; the recycled concrete (CVS) and loose oyster shell (OSU) treatments received 1.5 times as many recruits, while the granite (GL and GS) treatments received nearly 2.5 times as many recruits as the limestone marl treatments. Across all sites and substrates, there was no detectable caging effect (General Linear Model – GLM, F = 5.77, p = 0.138). Significant effects of site (F = 26.33, p =

0.008) and substrate type (F = 7.82, p = 0.003) were detected; however, the trends were fairly consistent between and within substrate types. For example, GL treatments had a mean rank of 6.0 at the marsh site, 4.5 at the riprap site and 2.5 at the oyster reef site; OSU treatments had a mean rank of 4.5 at the marsh site, 2.5 at the riprap site and 1.0 at the oyster reef site. The between-substrate (i.e. GL vs. OSU) and within-substrate (e.g. OSU, among sites) oyster recruitment trends were consistent across all factors. Further analysis among the treatments is described in the 'Density and biomass' section.

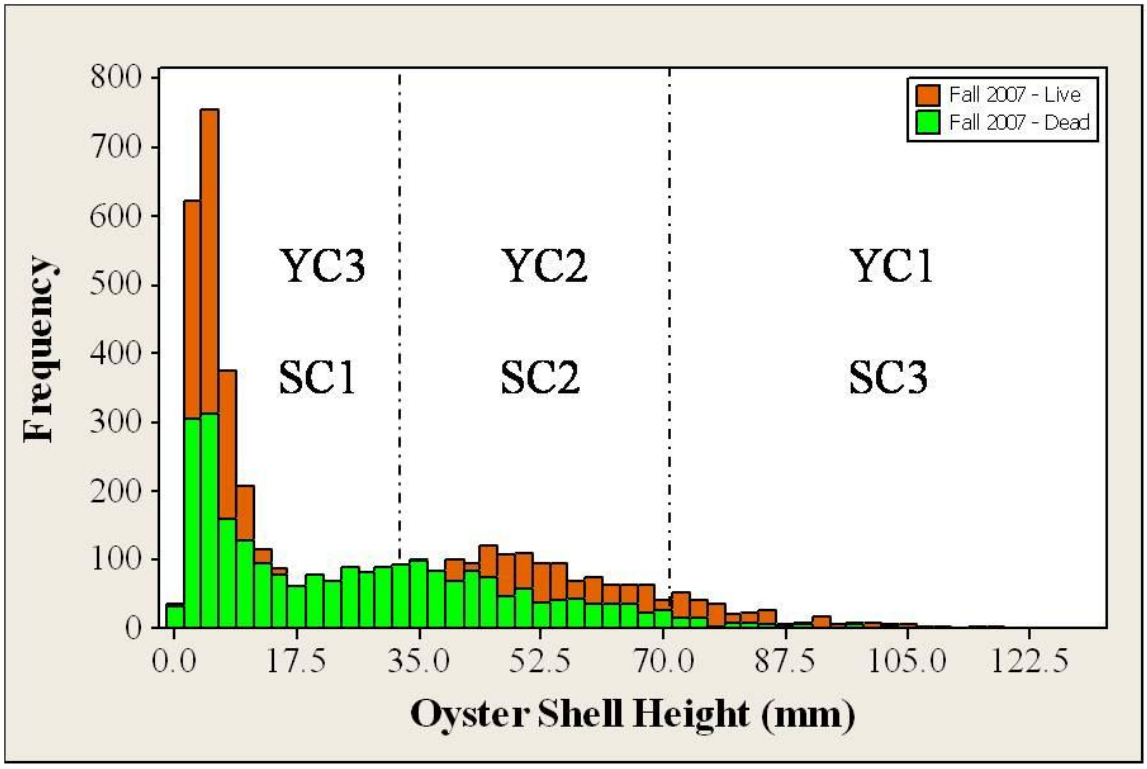


Figure 4.5: Population size structure of live/dead oysters in fall 2007, where YC1 = 2005, YC2 = 2006, YC3 = 2007; spat (SC1) < 30.0 mm, small adults (SC2) = 30.1 to 70.0 mm, and large adults (SC3) > 70.0 mm.

Table 4.6: Nominal oyster recruitment ranking scale for lower Chesapeake Bay waters.

Recruitment Description	Recruitment (Spat m^{-2})	Nominal Rank
None	0	0
Very Low	1 – 50	1
Low	51 – 200	2
Average	201 – 400	3
Good	401 – 700	4
Very Good	701 – 1000	5
High	1001 – 1500	6
Very High	1501 – 2000	7
Extremely High	> 2000	8

The second recruitment event (YC2 - 2006) ranked as 'good' with a mean value of 3.7 across all sites. Treatments at the oyster reef and riprap sites received roughly the same number of spat which, for treatments at the oyster reef site, was 2.3 times greater than YC1 oyster recruitment. Treatments at the marsh site received 1.5 times as many spat as the other two sites which was less than a 10 % increase relative to YC1. These two successive years of high recruitment fostered considerable clustering of oysters into a solid reef matrix on all treatments at the marsh site, and the granite treatments at the riprap site.

Across sites, LML and LMS treatments attracted the fewest recruits. OSU and CVS treatments received 2.2 and 2.5 times as many recruits, respectively. For GL and GS treatments, YC2 was three times that of LML and LMS treatments, and was only slightly higher (~1.2x) than YC1 recruitment on granite treatments. Although statistically non-significant ($p > 0.05$), uncaged treatments often attracted > 25 % more recruits than the caged treatments. Wherever this disparity between uncaged and caged

treatments emerged during this recruitment event (most pronounced in the granite treatments), it tended to remain throughout the rest of the experiment.

Density and biomass. By site, mean oyster densities (+/- 1 SEM) and mean oyster biomass estimates were: Marsh (759 +/- 92 m^{-2}, 11.4 +/- 2.6 g m^{-2}), Riprap (365 +/- 64 m^{-2}, 5.3 +/- 1.8 g m^{-2}), and Oyster Reef (84 +/- 17 m^{-2}, 0.4 +/- 0.2 g m^{-2}), yielding ranks of 'Very Good', 'Average' and 'Low', respectively (Table 4.7). Here we compare Exterior (reef surface) and Interior (substrate beneath the reef surface) treatment segments. By substrate volume, the Exterior:Interior ratio was roughly 1:1; thus, we posited an expected Exterior:Interior ratio of ~1:1 for oyster density and biomass as the null hypothesis of no difference. However, mean Exterior and Interior oyster density and biomass estimates, respectively, for the three sites were:

Site	Exterior Density (m^{-2})	Interior Density (m^{-2})	Exterior Biomass (g AFDM m^{-2})	Interior Biomass (g AFDM m^{-2})
Marsh	541 (73)	228 (40)	7.5 (2.1)	4.0 (0.9)
Riprap	262 (47)	104 (22)	4.3 (1.5)	1.0 (0.4)
Oyster Reef	64 (14)	19 (5)	0.3 (0.2)	0.1 (0.1)

Table 4.7: Mean recruitment rankings (as defined in Table 4.6) for YC1, YC2, and YC3 by substrate-site, site, substrate, and caging effects (n = 108).

Substrate-Site	YC1	YC2	YC3	Mean
CVS-Marsh	4.0	5.5	4.0	4.5
CVS-Oyster Reef	2.0	4.5	8.0	4.8
CVS-Riprap	2.5	3.5	2.5	2.8
GL-Marsh	6.0	6.5	4.0	5.5
GL-Oyster Reef	2.5	4.0	7.5	4.7
GL-Riprap	4.5	4.5	1.0	3.3
GS-Marsh	6.0	6.5	4.5	5.7
GS-Oyster Reef	2.5	5.0	8.0	5.2
GS-Riprap	5.0	4.5	2.0	3.8
LML-Marsh	3.0	2.5	3.0	2.8
LML-Oyster Reef	0.5	1.0	1.0	0.8
LML-Riprap	2.0	1.5	2.5	2.0
LMS-Marsh	3.5	3.0	2.5	3.0
LMS-Oyster Reef	0.0	1.0	2.0	1.0
LMS-Riprap	2.0	1.5	2.0	1.8
OSU-Marsh	4.5	4.5	6.0	5.0
OSU-Oyster Reef	1.0	3.5	6.0	3.5
OSU-Riprap	2.5	4.0	4.5	3.7

Site	YC1	YC2	YC3	Mean
Marsh	4.5	4.8	4.0	4.4
Oyster Reef	1.4	3.2	5.4	3.3
Riprap	3.1	3.3	2.4	2.9

Substrate	YC1	YC2	YC3	Mean
CVS	2.8	4.5	4.8	4.1
GL	4.3	5.0	4.2	4.5
GS	4.5	5.3	4.8	4.9
LML	1.8	1.7	2.2	1.9
LMS	1.8	1.8	2.2	1.9
OSU	2.7	4.0	5.5	4.1

Caging	YC1	YC2	YC3	Mean
Caged	2.9	3.3	3.8	3.3
Uncaged	3.1	4.2	4.1	3.8

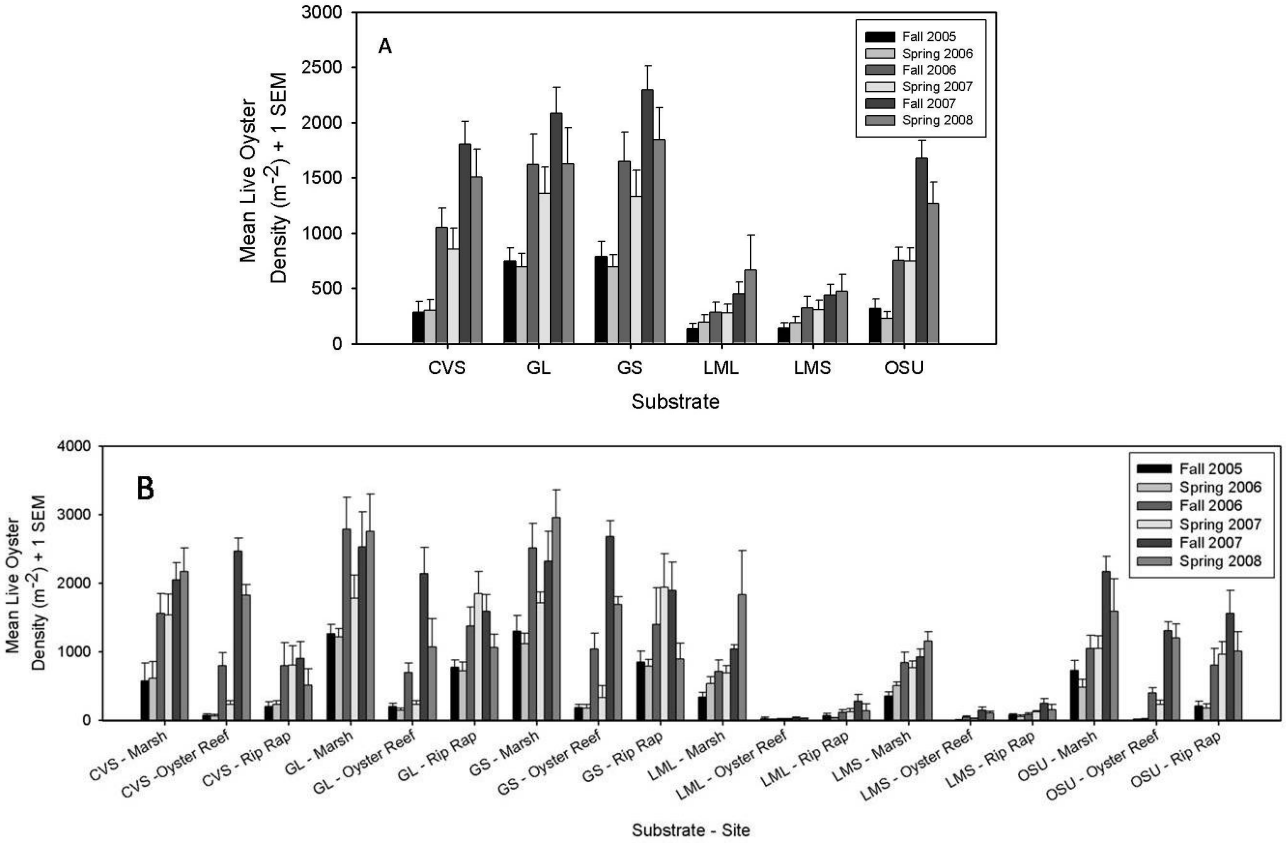


Figure 4.6: Mean live oyster density per unit area of river bottom (No. m^{-2}) + 1 SEM by (A) substrate and (B) substrate-site from fall 2005 to spring 2008.

When comparing the different substrate types, site alone did not account for differences in mean oyster density (Fig. 4.6a-b) and biomass (Fig. 4.7a-b):

Substrate Type	Oyster Density (m^{-2})	Exterior Biomass (g AFDM m^{-2})
CVS	284 (99)	4.9 (2.2)
GL	747 (119)	41.3 (13.9)
GS	786 (140)	29.4 (10.5)
LML	137 (43)	6.2 (4.8)
LMS	143 (42)	7.4 (4.3)
OSU	318 (88)	13.8 (6.8)

The differences by substrate between mean Exterior and Interior oyster density (Fig. 4.8a-b) and between Exterior and Interior biomass (Fig. 4.9a-b) were:

Substrate Type	Exterior Density (m^{-2})	Interior Density (m^{-2})	Exterior Biomass (g AFDM m^{-2})	Interior Biomass (g AFDM m^{-2})
CVS	227 (86)	58 (15)	1.3 (0.6)	0.4 (0.1)
GL	506 (96)	251 (69)	11.3 (3.9)	2.5 (0.9)
GS	567 (111)	219 (44)	6.0 (2.2)	3.8 (1.5)
LML	88 (30)	49 (19)	0.7 (0.5)	1.4 (1.1)
LMS	111 (33)	35 (11)	1.7 (1.1)	0.8 (0.4)
OSU	236 (62)	92 (38)	3.2 (1.4)	1.4 (1.4)

For the caging factor, mean oyster density and biomass estimates were:

Caging	Oyster Density (m^{-2})	Oyster Biomass (g AFDM m^{-2})
Caged	343 (51)	5.1 (1.7)
Uncaged	463 (76)	6.3 (2.0)

The mean Exterior and Interior oyster density and biomass for caging were:

Caging	Exterior Density (m^{-2})	Interior Density (m^{-2})	Exterior Biomass (g AFDM m^{-2})	Interior Biomass (g AFDM m^{-2})
Caged	220 (33)	129 (29)	4.0 (1.4)	1.7 (0.6)
Uncaged	358 (61)	106 (20)	4.0 (1.4)	1.7 (0.6)

Exterior treatment segments received many more recruits than the interior. Oyster density (presented in 'Recruitment' section) and biomass both differed significantly by site (GLM, F = 12.07, $p = 0.008$) and substrate type (F = 3.93, $p = 0.031$); oyster biomass had a significant site x substrate interaction (F = 4.07, $p = 0.018$), which required separate two-way ANOVAs for each site. Lower larval encounter rates with, or survival within, interior treatment segments may have occurred due to lower water flow, less food, effects of pore size, or sedimentation. Treatments at the marsh site had the highest oyster density and biomass, but oyster density and biomass in the interior treatment segments did not differ between substrate types or caging through spring 2008; the treatments at the riprap site showed this same trend by spring 2008. Most of the exterior segment treatments at the marsh site became so heavily encrusted with oysters that substrate type (and caging, to an even lesser extent) within the treatment interior were not significant factors.

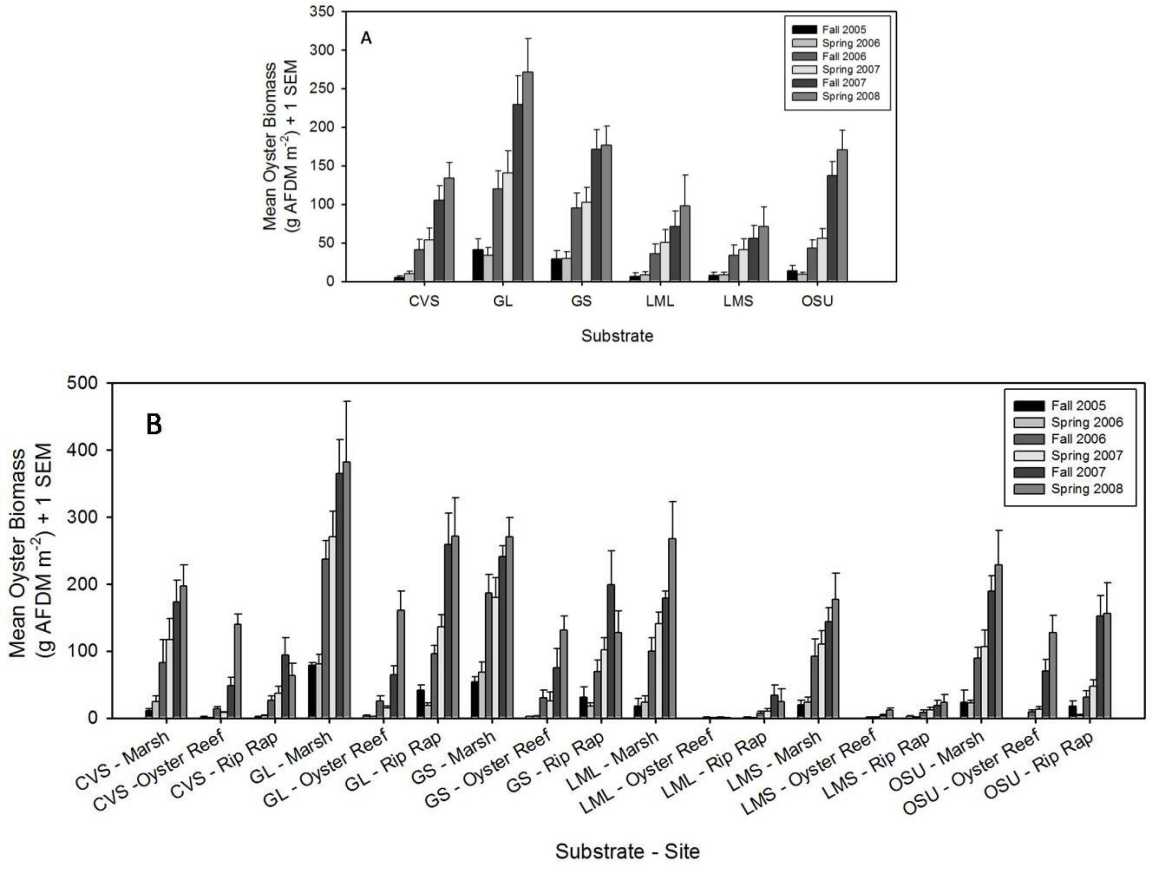


Figure 4.7: Mean oyster biomass per unit area of river bottom (g AFDM m^{-2}) + 1 SEM by (A) substrate and (B) substrate-site from fall 2005 to spring 2008.

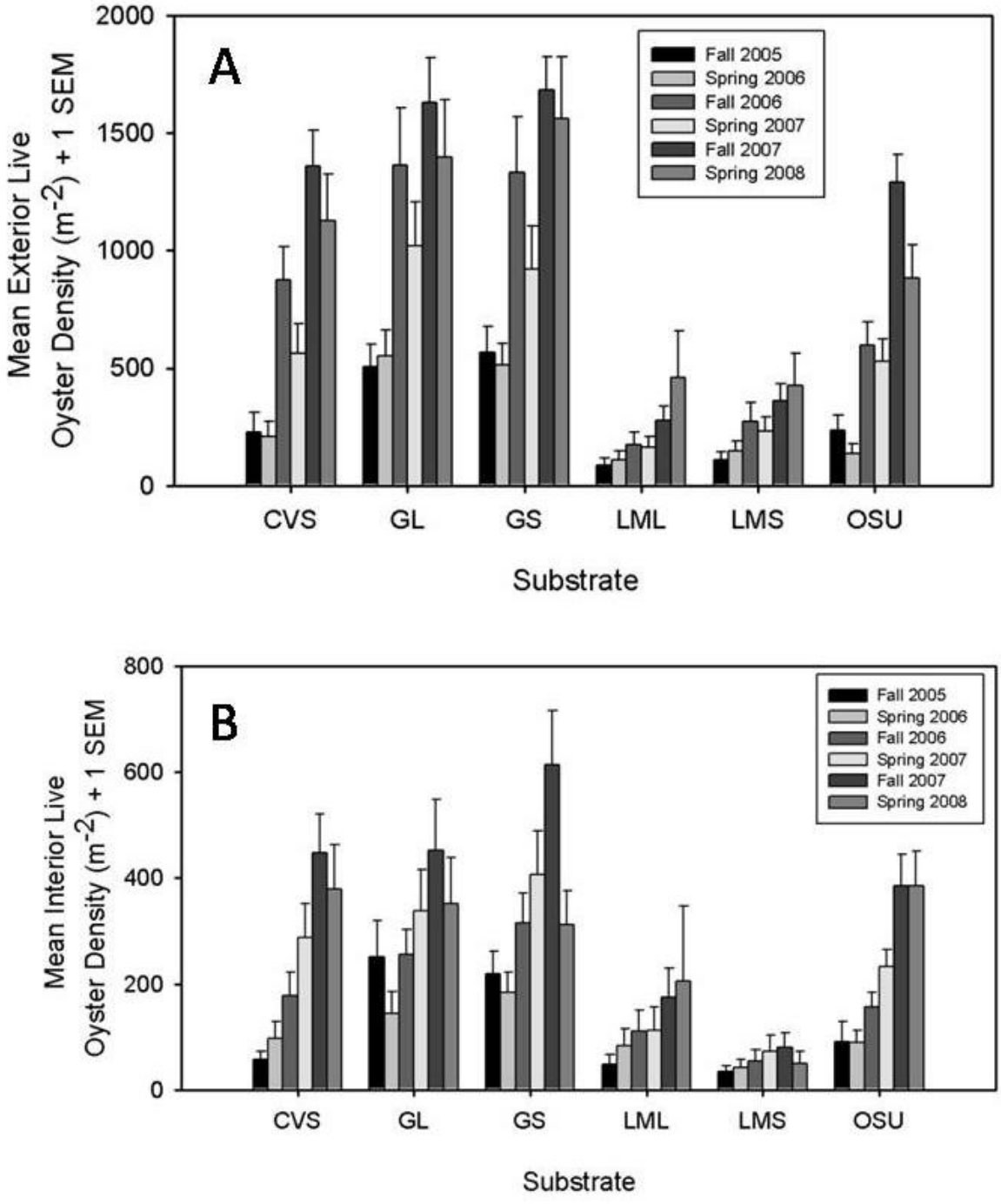


Figure 4.8: Mean (A) exterior and (B) interior live oyster density per unit area of river bottom (No. m^{-2}) + 1 SEM by substrate from fall 2005 to spring 2008.

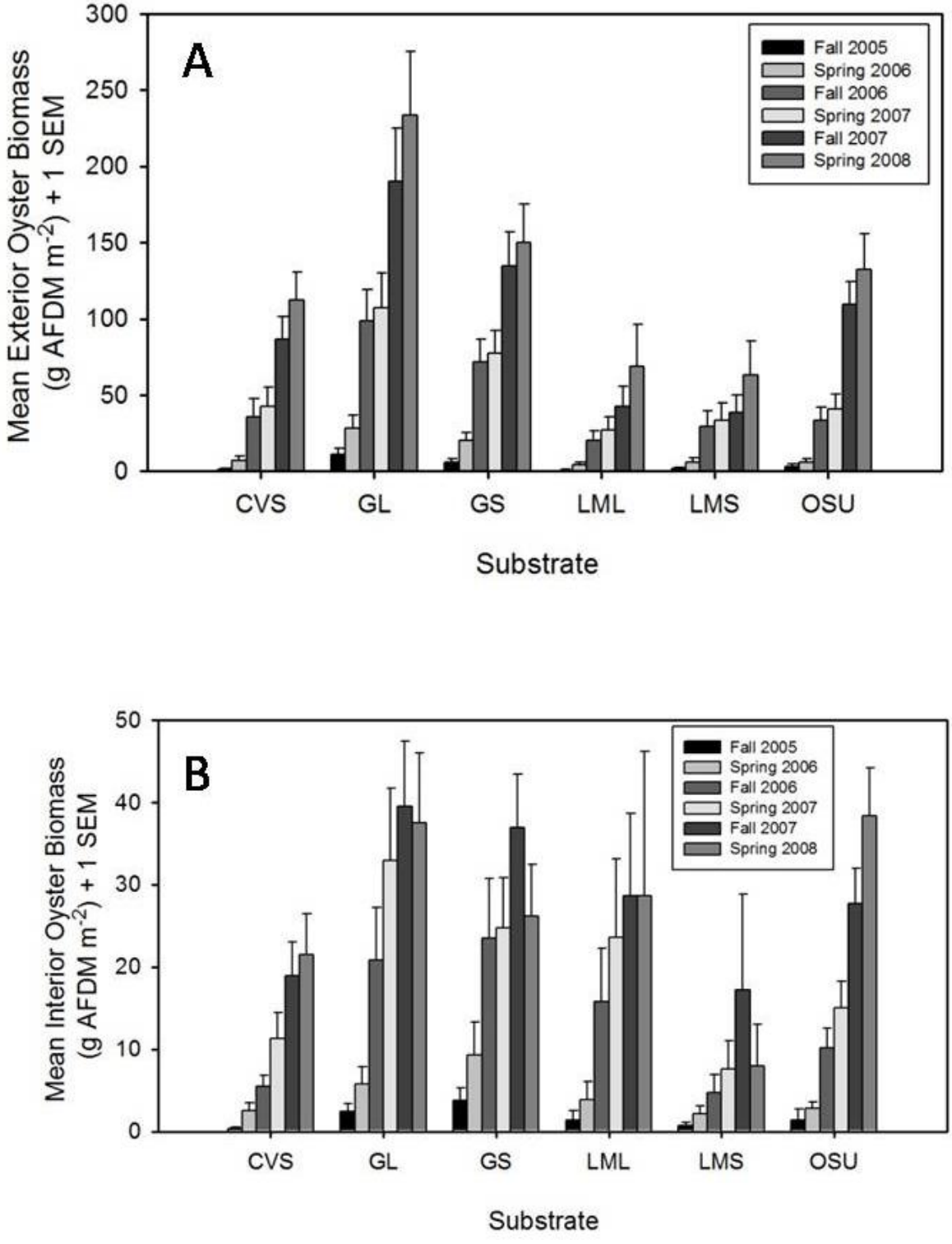


Figure 4.9: Mean (A) exterior and (B) interior oyster biomass per unit area of river bottom (g AFDM m^{-2}) + 1 SEM by substrate from fall 2005 to spring 2008.

Consequently, the dominant mode of reef expansion (i.e. reef accretion) occurred at the reef surface, as seen in comparisons of Exterior and Interior mean oyster density (Fig. 4.10a-b) and biomass (Fig. 4.11a-b) at the marsh site in fall 2007 (n = 36):

Substrate Type	Exterior Density (m^{-2})	Interior Density (m^{-2})	Exterior Biomass (g AFDM m^{-2})	Interior Biomass (g AFDM m^{-2})
CVS	1429 (183)	619 (108)	137.5 (25.5)	35.6 (8.2)
GL	2059 (470)	467 (79)	292.5 (56.0)	72.1 (14.1)
GS	1803 (274)	517 (202)	195.3 (26.8)	45.3 (13.9)
LML	563 (53)	477 (65)	101.7 (21.4)	77.1 (17.1)
LMS	725 (83)	197 (60)	95.5 (19.8)	48.1 (33.0)
OSU	1688 (187)	480 (75)	153.9 (16.9)	35.9 (8.0)

and Spring 2008 (n = 24) using substrate-site-specific biomass regression models:

Substrate Type	Exterior Density (m^{-2})	Interior Density (m^{-2})	Exterior Biomass (g AFDM m^{-2})	Interior Biomass (g AFDM m^{-2})
CVS	1728 (248)	444 (146)	178.2 (26.7)	19.0 (4.7)
GL	2280 (434)	584 (118)	321.0 (90.7)	60.7 (6.7)
GS	2672 (253)	360 (98)	244.6 (32.9)	26.0 (7.6)
LML	1284 (286)	548 (399)	196.2 (13.7)	71.6 (48.1)
LMS	1036 (113)	120 (52)	158.4 (28.9)	18.6 (14.4)
OSU	1144 (333)	444 (169)	184.3 (45.0)	44.0 (14.8)

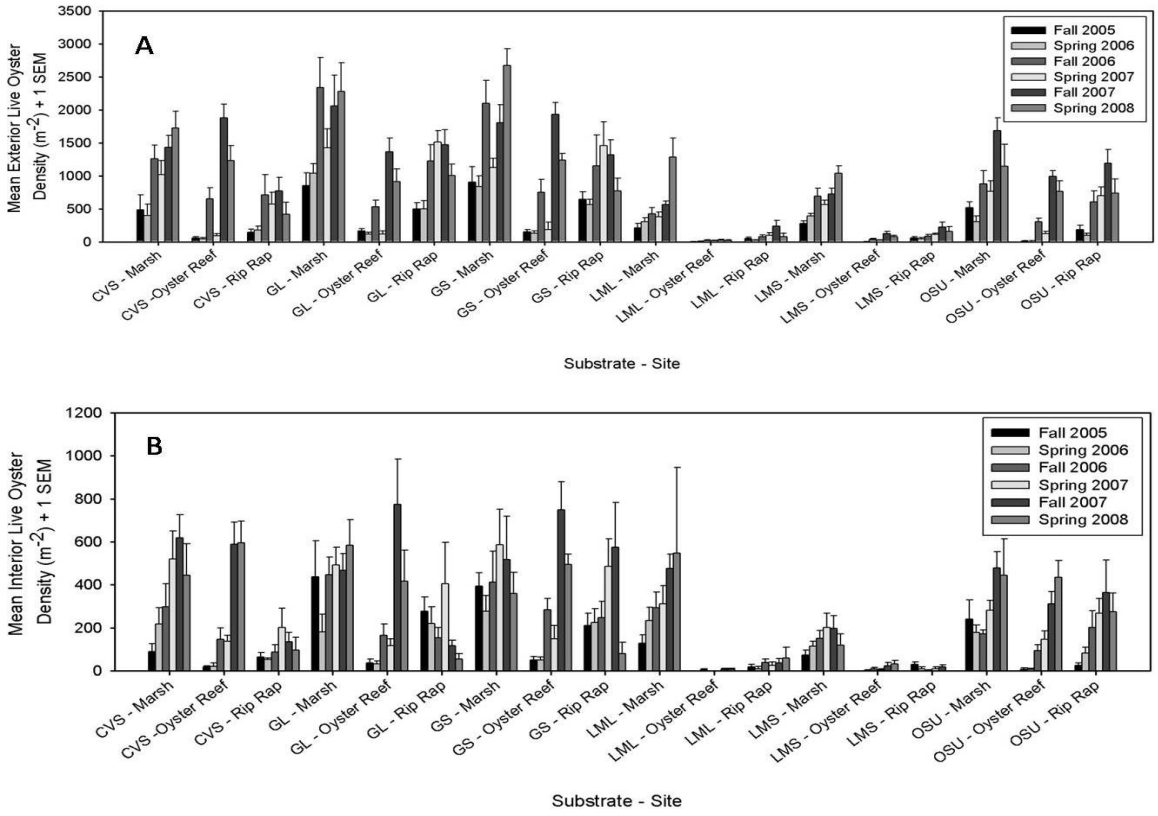


Figure 4.10: Mean (A) exterior and (B) interior live oyster density per unit area of river bottom (No. m^{-2}) + 1 SEM by substrate-site from fall 2005 to spring 2008.

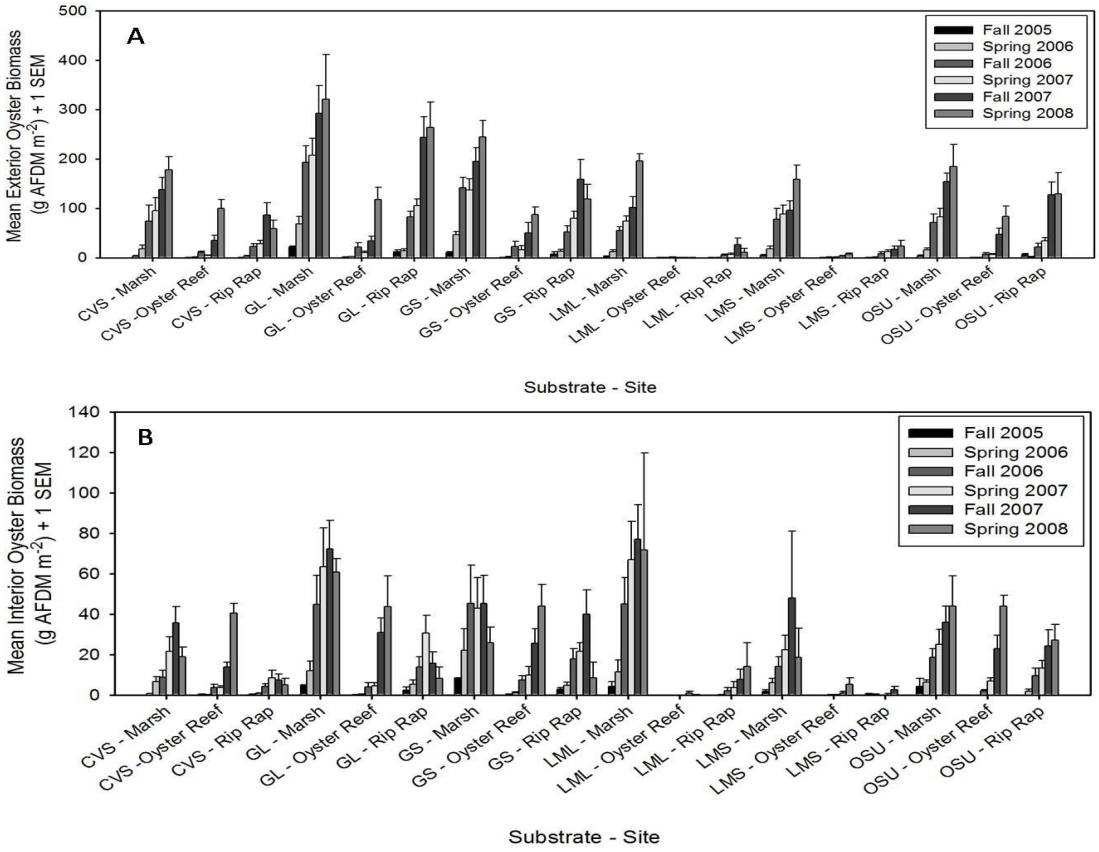


Figure 4.11: Mean (A) exterior and (B) interior oyster biomass per unit area of river bottom (g AFDM m^{-2}) + 1 SEM by substrate-site from fall 2005 to spring 2008.

By spring 2008, the interior oyster density and biomass estimates did not differ significantly between treatments (GLM, $p > 0.05$), while in the within-substrate comparisons, exterior surfaces had much higher oyster density (Paired t-tests, $p < 0.0005$) and biomass ($p < 0.0005$) than interior treatment segments. Substrate-site (Fig. 4.12a-b) and substrate only (Fig. 4.13a-b) oyster density and biomass comparisons between previously-handled (Quad 1) and undisturbed (Quad 4) treatment quadrants (Paired t-tests, $p > 0.05$) revealed no significant deviations. The first tests of this kind were conducted in spring and fall 2006 where Quads 2 and 3, respectively, were sampled alongside Quad 1. No apparent positive cleansing effects or destructive, handling effects were realized by oysters in the handled quadrant in spring 2006 (Quad 1 vs. 2, n = 108, $p = 0.798$). However, a handling effect was detected in fall 2006 (Quad 1 vs. 3, n = 108, $p = 0.016$). Further analysis of exterior ($p = 0.147$) and interior ($p = 0.002$) portions of the quadrants revealed that some benefits of cleansing may have been experienced by substrates in the interior of the previously-handled treatment. But by spring 2008, no differences between previously-handled (Quad 1) and undisturbed (Quad 4) treatment quadrants were apparent for oyster density (n = 72, $p = 0.739$) and biomass ($p = 0.146$). Linear regressions were conducted to determine the effect of oyster density on oyster tissue mass at a given SH in exterior and interior treatment segments. Biomass data from Quads 1 and 4 were combined to increase sample size, based on the absence of handling effects in the spring 2008 sampling. There was a relationship between oyster density and oyster AFDM at a given size ($R^2 = 0.39$, $p = 0.009$); oysters of a given size decreased in tissue mass with increasing oyster density (Fig. 4.14a). The trend was the same for the effect of exterior oyster density on exterior oyster biomass ($R^2 = 0.42$, $p = 0.006$; Fig.

4.14b), but no such trend existed in the interior locations (R^2 = 0.008, p = 0.763; Fig. 4.14c). In fact, it was the exterior oyster density that drove the biomass of oysters present in the interior of the treatments tested in this experiment (R^2 = 0.23, p = 0.080; Fig. 4.14d). Effectively, the more developed, or mature, the face of an oyster reef (treatment) becomes, the greater the influence of the exterior oyster density on the interior oyster growth.

When exterior and interior treatment segments were combined, mean oyster density and biomass estimates at the marsh site after 2+ years were:

Substrate Type	Oyster Density (m^{-2})	Exterior Biomass (g AFDM m^{-2})
CVS	2048 (252)	173.1 (32.4)
GL	2525 (516)	364.6 (50.2)
GS	2320 (437)	240.7 (16.5)
LML	1040 (59)	178.8 (10.6)
LMS	923 (130)	143.6 (21.4)
OSU	2168 (219)	189.8 (22.4)

And, after 3 years, mean oyster density and biomass estimates in treatments at the productive marsh site were:

Substrate Type	Oyster Density (m^{-2})	Exterior Biomass (g AFDM m^{-2})
CVS	2172 (343)	197.2 (30.9)
GL	2760 (540)	381.7 (90.7)
GS	2952 (406)	270.6 (28.7)
LML	1832 (644)	267.7 (54.9)
LMS	1156 (138)	176.9 (39.0)
OSU	1588 (477)	228.4 (51.4)

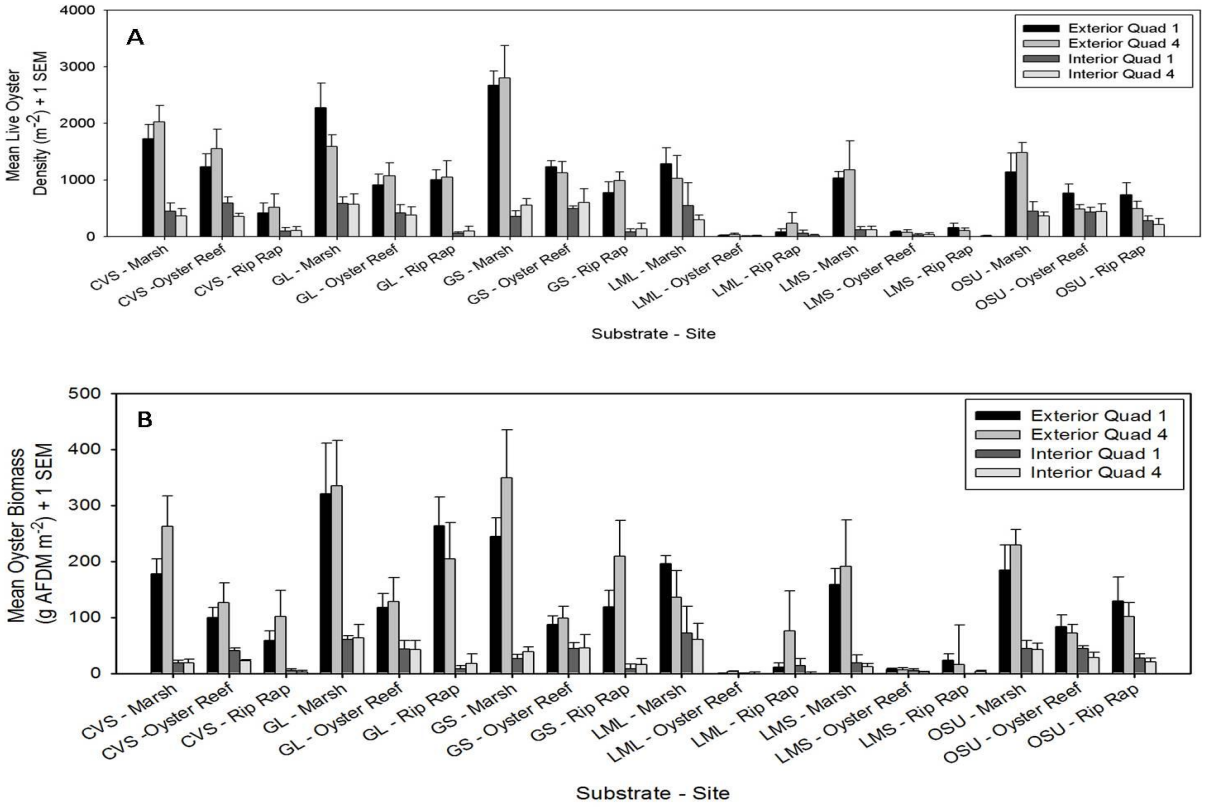


Figure 4.12: Mean live oyster (A) density and (B) biomass per unit area of river bottom (No. m^{-2}; g AFDM m^{-2}) + 1 SEM by substrate-site for the exterior and interior segments of previously-handled (Quad 1) and undisturbed (Quad 4) quadrants of experimental trays (n = 72) sampled in spring 2008.

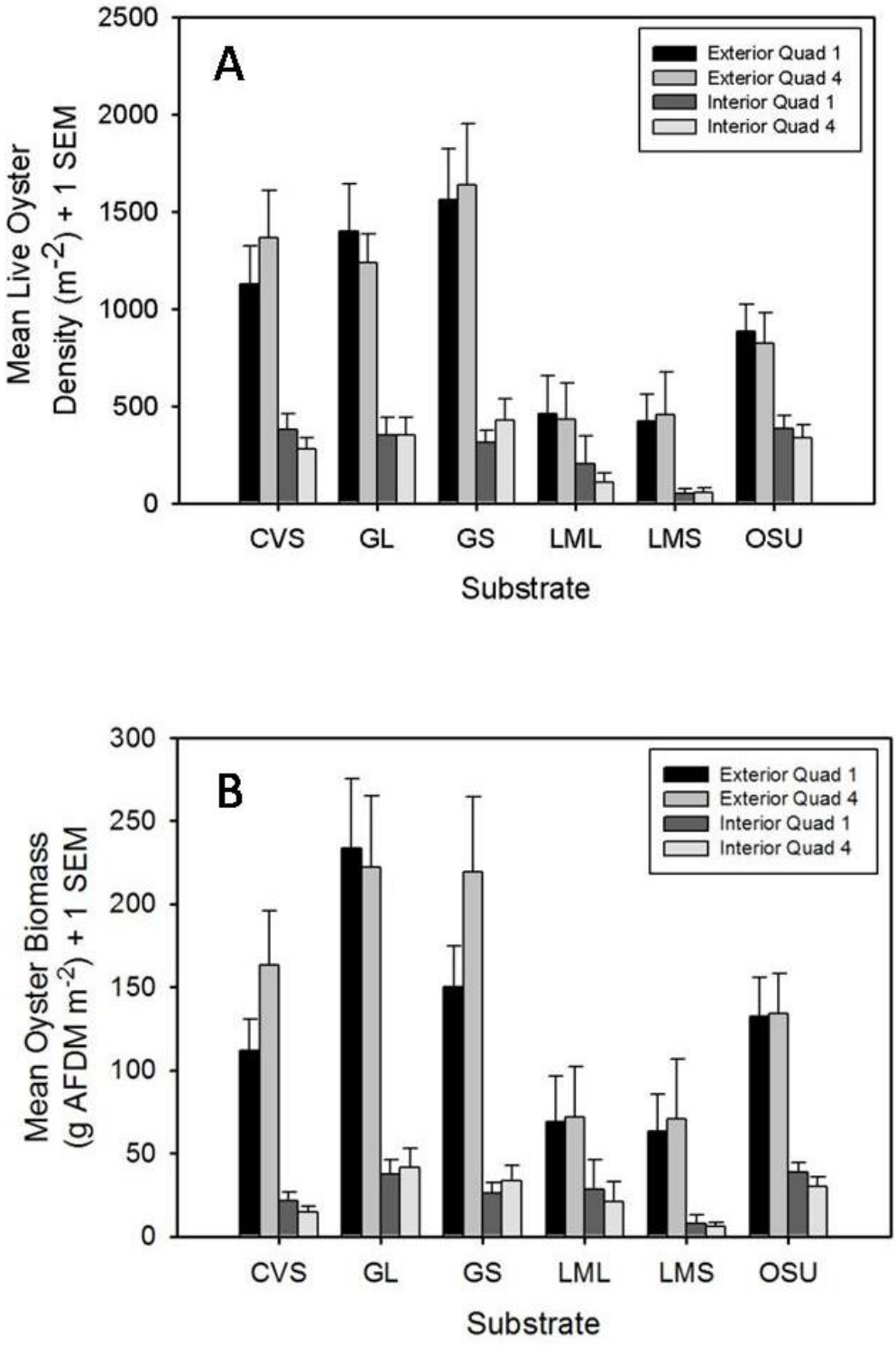


Figure 4.13: Mean live oyster (A) density and (B) biomass per unit area of river bottom (No. m^{-2}; g AFDM m^{-2}) + 1 SEM by substrate for the exterior and interior segments of previously-handled (Quad 1) and undisturbed (Quad 4) quadrants of experimental trays (n = 72) sampled in spring 2008.

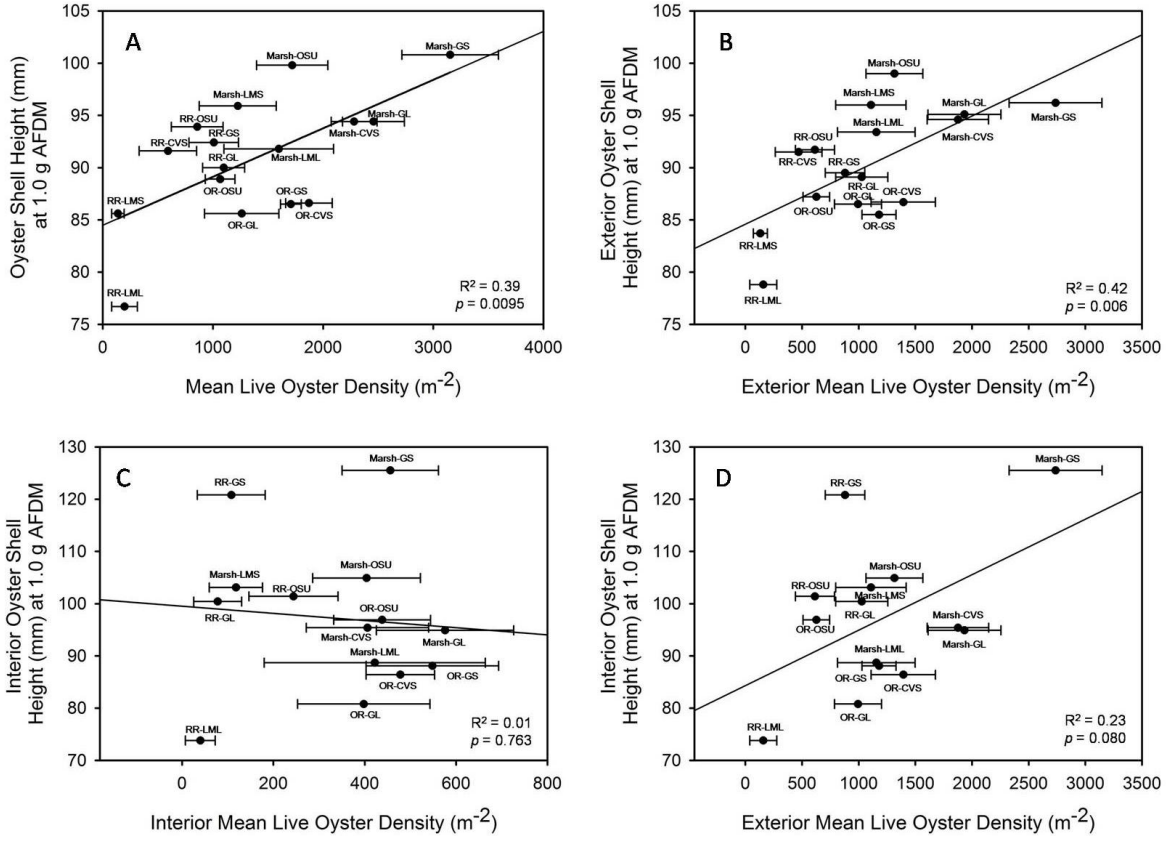


Figure 4.14: Regression models of (A) oyster shell height – SH (mm) at 1.0 g AFDM versus mean live oyster density, (B) exterior oyster SH at 1.0 g AFDM versus exterior live oyster density, (C) interior oyster SH at 1.0 g AFDM versus interior live oyster density, and (D) interior oyster SH at 1.0 g AFDM versus exterior live oyster density, three years after deployment.

The USACE's Lynnhaven Decision Document (Schulte et al. 2006) set the 5-yr mean oyster biomass benchmark at 177 g AFDM m^{-2} river bottom based on the best reef information available at the time – some of the most productive, sustainable oyster reefs located on Virginia's eastern shore. Treatments at the marsh site (fall 2007: 215.1 +/- 16.4 g m^{-2}; spring 2008: 253.8 +/- 23.8 g m^{-2}) reached that oyster biomass benchmark in just over 2 years; GL (fall 2007: 259.3 +/- 46.0 g m^{-2}; spring 2008: 271.5 +/- 57.5 g m^{-2}), GS (fall 2007: 198.6 +/- 50.4 g m^{-2}; spring 2008: 127.4 +/- 32.8 g m^{-2}), and OSU (fall 2007: 151.8 +/- 31.1 g m^{-2}; spring 2008: 156.2 +/- 46.0 g m^{-2}) treatments also reached similar oyster biomass levels at the riprap site.

Population structure. Population size structure (PSS) varied between site (Fig. 4.15a), substrate (Fig. 4.15 b-d) and, to a lesser extent, caging (Fig. 4.15 e-f). PSS was most "developed" on treatments at the marsh site, slightly less at the riprap site, and was considerably less developed at the oyster reef site, where a "highly developed" PSS means a mature oyster population with multiple size classes (proxy for year classes) present and abundant. Notable, however, was the robust PSS across all sites (Fig. 4.16a) after less than two years (spring 2007) and its similarity to the PSS for the entire LRS Riprap Survey (Fig. 4.16b). This indicates that many of these experimental intertidal treatments, particularly the granite treatments – across all sites, and all treatments at the marsh site – reached a mature reef state in three years or less (Fig. 4.15a-b, e-f).

By fall 2007, we recorded three year classes (YC1 = 2005, YC2 = 2006, YC3 = 2007), as demarcated by three size classes in shell height (SH): Spat (SC1) were < 30.0 mm, small adults (SC2) were 30.1 - 70.0 mm, and large adults (SC3) were > 70.0 mm; only a few oysters grew > 100.0 mm. We elected not to refer to oysters as 'markets'

(market oysters are > 75 mm SH) because the break at 75 mm SH is economically, not ecologically, defined. Oyster PSS confirmed trends observed in oyster density and biomass analysis. Caging and handling effects were minimal, exterior treatment segments contained > 70% of the oyster population, consistently attracting more recruits than the interior of the treatments, and most importantly, alternative oyster reef substrates performed as well or better than loose oyster shell in treatments at all three sites.

Growth and survival. In fall 2005, the proportion of live oysters was 0.92, which is assumed to be the proportion of settled spat that survived the post-settlement mortality period (~ 6 wks) and the time subsequent to that period. By spring 2006, the proportion surviving dropped to 0.87, which provided an estimate of overwintering survivorship.

Fall 2006 was the first measure of mortality over the period of greatest predation and disease stress. Since these oysters started as spat and very small adults in the spring, significant disease-driven mortality was not anticipated. A quantitative estimate of predation- vs. disease-related mortality was not realized; instead, we made qualitative observations on mud crab, blue crab, and fish predation, or simply gaping, undamaged valves. Some substrates were partially or totally covered by sediment; many oysters that recruited to those substrates died due to siltation or burial, and their shells (and the surrounding substrate) were a distinct black color typically associated with anoxia.

Spat recruitment and densities were high in fall 2006. These oysters grew quickly such that some of the oysters reached shell heights > 70 mm in the first year, with most of these occurring on exterior portions of granite treatments at the marsh site. The rapid growth was likely due to the warm spring and fall of 2006, and may represent near-

maximum growth rate for oysters on intertidal reefs in the LRS. Those oysters that grew into SC3 (YC1) had done so recently, so the proportion of live oysters was high (0.95). For newly-recruited spat (YC2, SC1), the proportion live (0.77) was slightly higher than that of the small adults (YC1, SC2) – 0.71. The proportion of live recruits may have been slightly underestimated because all dead oysters were measured, not just those that had died recently. Thus, some of the dead oysters may have died in the previous year.

Over the following winter a major mortality event occurred, presumably due to the severe cold, which reduced the proportion live to 0.61. YC2 spat and small adults suffered the highest relative mortality, especially at the oyster reef site, where many treatments lost 50-70 % of their oysters. Mortality was generally restricted to the substrates highest in the intertidal zone, so that treatments containing significant biomass in the lower intertidal appeared to be less affected. Despite this major mortality event, most oysters on oyster shell survived, possibly due to thermal buffering of the shell-oyster matrix by reducing the substrate surface area exposed to the cold.

In fall 2007, most of YC1 had entered SC3 with a proportion live = 0.80. YC2 dominated SC2 with a proportion live = 0.77; YC3 was another good recruitment class and had a proportion live = 0.74. These are underestimates of survival because of the repetitive measurement of dead oysters that had died months to years earlier.

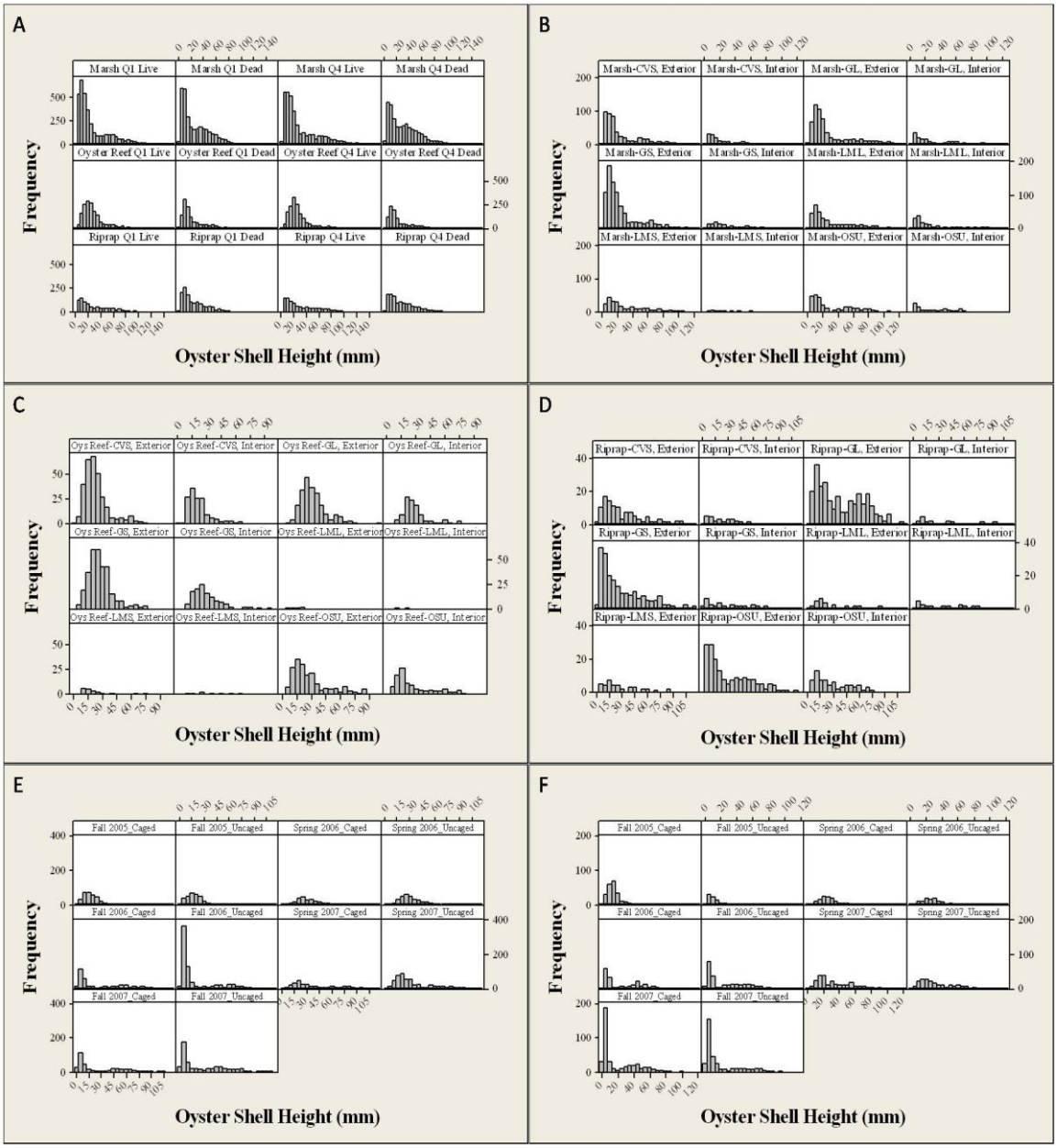


Figure 4.15: Population size structure (PSS) of oysters on (A) all substrates at each site separated by handled/undisturbed quadrants (Quads 1, 4) and live/dead in spring 2008. PSS at the (B) marsh, (C) oyster reef, and (D) riprap sites are separated by substrate and exterior/interior segments of each treatment in spring 2008. PSS on (E) large granite (GL), and (F) unconsolidated oyster shell (OSU) at the marsh site separated into caged/uncaged treatments from fall 2005 to fall 2007, which are representative of many of the treatments over time.

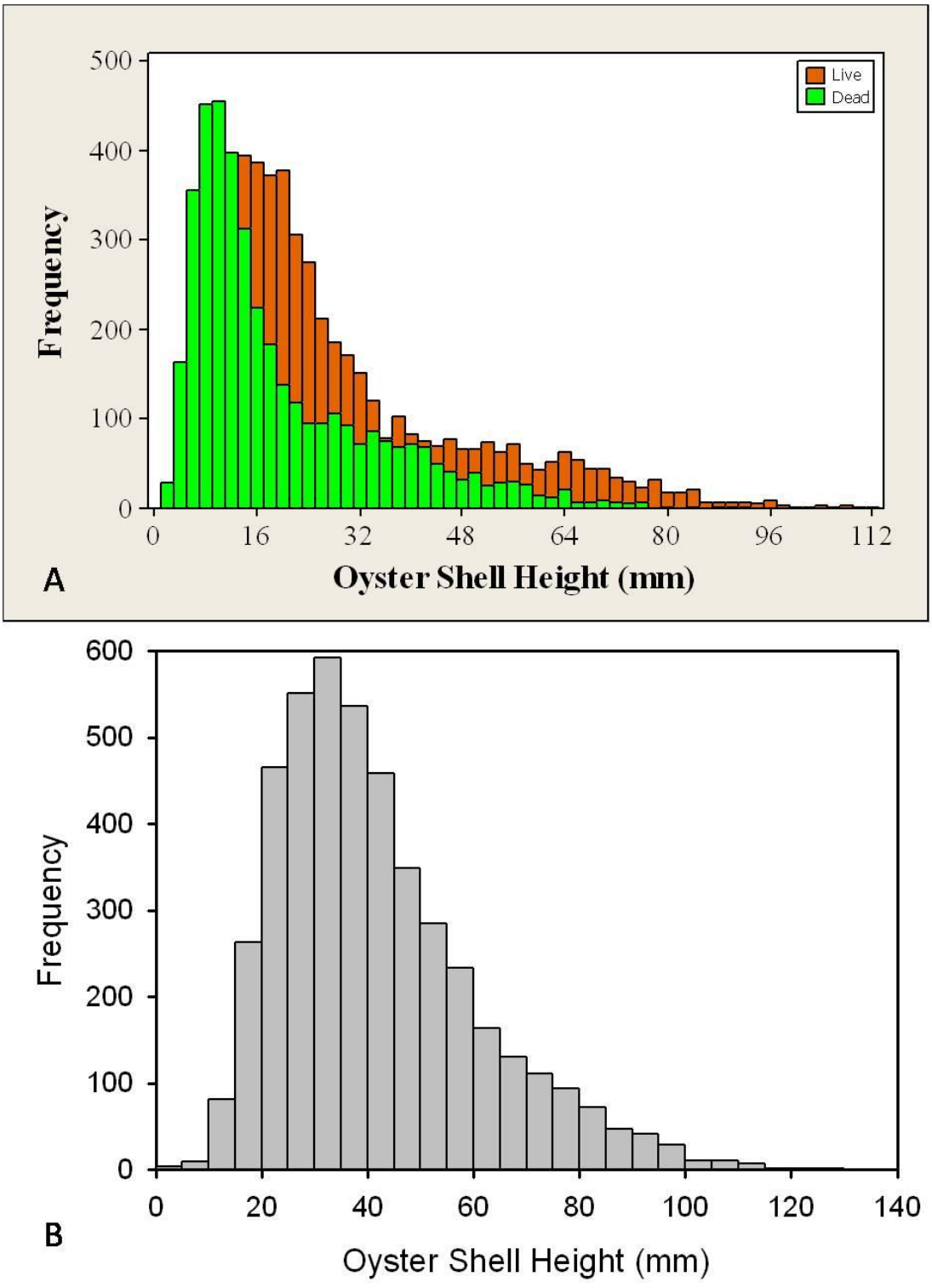


Figure 4.16: Population size structure (PSS) of oysters (A) across the entire Alternative Substrate Experiment (ASE) in spring 2007 and (B) on riprap in Lynnhaven Bay, Broad Bay and Long Creek (Lynnhaven Riprap Survey – Dissertation Chapter 2).

In spring 2008, the proportion of live oysters across all treatments was 0.52 and 0.54 for Quads 1 and 4, respectively. By site, experimental treatments at the oyster reef (Q1: 0.59, Q4: 0.64) > marsh (Q1: 0.55, Q4: 0.56) > riprap (Q1: 0.46, Q4: 0.48); by substrate, loose oyster shell (OSU – Q1: 0.61, Q4: 0.64) and limestone marl (LML – Q1: 0.58, Q4: 0.53; LMS – Q1: 0.53, Q4: 0.58) treatments had a higher proportion of live oysters than the granite (GL – Q1: 0.49, Q4: 0.50; GS – Q1: 0.48, Q4: 0.52) and crushed concrete (CVS – Q1: 0.49, Q4: 0.54) treatments. However, a site x substrate type interaction effect (GLM, $F = 2.57$, $p = 0.014$) required separate one-way ANOVAs to be conducted for each site. Although, no differences were detected among substrates at the marsh and riprap sites, most substrates had higher mean oyster survival than the LML treatment ($F = 4.98$, $p = 0.005$).

By site-substrate-exterior/interior for Quads 1 and 4 (Table 4.8), the proportion of live oysters was much lower on the interior than the exterior treatment segments at the riprap site (Paired t-test, $p < 0.005$) and the marsh site ($p < 0.005$). In contrast, the proportion of live oysters on oyster reef site's exterior and interior treatment segments was similar ($p = 0.612$):

Site	SC1	SC2	SC3
Marsh	0.52	0.56	0.69
Riprap	0.59	0.56	0.73
Oyster Reef	0.40	0.37	0.70

SC3 contained the fewest oysters but had the best survival; SC1 and SC2 (spat and juveniles) remained the more vulnerable life stages, incurring a higher relative mortality.

After a second year of recruitment in fall 2006, younger oysters began to settle and grow on older oysters. It became apparent that a test of substrate viability for oysters

would require the distinction between oysters recruiting to the base substrate or to other oysters – live or dead – already present on the substrates. In fact, oysters recruited to other oysters on the treatment exterior at higher proportions than on the treatment interior (Fig. 4.17a). In spring 2008, the proportion of live oysters on other oysters on the treatment exterior was equivalent or greater than that on the treatment interior (Fig. 4.17b). Thus, more oysters recruited to the treatment exterior AND a greater proportion of those oysters survived. This trend was most pronounced in treatments at the riprap site, where experimental tray subsidence was most dramatic. The vertical structure provided by live and recently dead oysters represented some of the only viable settlement surfaces as much of the available substrate in many treatments was covered in sediment and algae.

Overall, considering this subestuary's high salinity, high predator concentrations during the warmer months, and the winter 2007 mortality event, survival and growth rates were high. Oysters in treatments at the marsh and riprap sites consistently grew faster with lower mortality than oysters in treatments at the oyster reef site.

Oyster volume and reef accretion. Oyster volume varied significantly ($p < 0.020$) by substrate and site (Fig. 4.18a) with the highest accretion on treatments at the marsh site (GL treatments exceeded 20 L m^{-2}), reflecting trends of oyster density and biomass. Total oyster volume was dominated by live oysters and, thus, the treatment exteriors (Fig. 4.18b), where > 70 % oysters were located. In three years, < 2 L m^{-2} yr^{-1} accreted on treatments at the oyster reef site, treatments ranged from < 2 to 5 L m^{-2} yr^{-1} at the riprap site, and 3-8 L m^{-2} yr^{-1} at the marsh site.

Pathology and condition. Mean oyster condition (+ 1 SEM) was calculated for CIs 1-3 by site-substrate-exterior/interior (Fig. 4.19). No handling effects on oyster condition were detected ($p > 0.05$) between Quad 1 (n = 712) and Quad 4 (n = 695) and, thus, samples were pooled. Treatments at the oyster reef site had the highest CI values. The marsh and riprap treatments were similar to each other but maintained significantly lower oyster condition.

Site	CI1	CI2	CI3
Marsh	9.9 (0.2)	7.5 (0.1)	2.9 (0.1)
Riprap	9.6 (0.2)	7.4 (0.1)	2.7 (0.1)
Oyster Reef	13.5 (0.3)	10.5 (0.2)	3.1 (0.1)

Regarding disease (Dermo and MSX) condition, 93 % of the 110 tested oysters contained *Perkinsus* cells, with weighted prevalence (WP) of 2.35 +/- 0.17 (Table 4.9a). Only one oyster had *Haplosploridium* cells, and it was a rare infection (a few cells), so no MSX analyses were conducted. Dermo WP varied by site (Table 4.9b) and substrate (Table 4.9c). Dermo intensity varied significantly by site (ANOVA, F = 16.27, p = 0.003; Fig. 4.20a) and substrate (F = 4.97, p = 0.042; Fig. 4.20b) with no interaction (F = 0.41, p = 0.868). The marsh site had a mean Dermo intensity of 'Moderate' (4.68 +/- 0.25), the oyster reef site 'Light-to-Moderate' (3.91 +/- 0.28), and the riprap site 'Light' (3.29 +/- 0.30). The two smallest substrates – recycled concrete (mean rank = 4.31 +/- 0.32) and loose oyster shell (4.41 +/- 0.33) – maintained higher Dermo intensities than the larger substrates – granite (3.83 +/- 0.28) and limestone marl (3.28 +/- 0.41).

Table 4.8: Proportion of live oysters for the handled (Quad 1) and undisturbed (Quad 4) tray quadrants by site-substrate-exterior/interior for spring 2008.

Site-Substrate-Exterior/Interior	Quad 1	Quad 4
Marsh-CVS-Exterior	0.58	0.57
Marsh-CVS-Interior	0.28	0.33
Marsh-GL-Exterior	0.52	0.48
Marsh-GL-Interior	0.46	0.39
Marsh-GS-Exterior	0.53	0.53
Marsh-GS-Interior	0.37	0.41
Marsh-LML-Exterior	0.57	0.53
Marsh-LML-Interior	0.67	0.53
Marsh-LMS-Exterior	0.60	0.62
Marsh-LMS-Interior	0.31	0.41
Marsh-OSU-Exterior	0.60	0.71
Marsh-OSU-Interior	0.49	0.56
Oyster Reef-CVS-Exterior	0.60	0.66
Oyster Reef-CVS-Interior	0.58	0.65
Oyster Reef-GL-Exterior	0.52	0.61
Oyster Reef-GL-Interior	0.61	0.61
Oyster Reef-GS-Exterior	0.58	0.64
Oyster Reef-GS-Interior	0.55	0.56
Oyster Reef-LML-Exterior	0.29	0.59
Oyster Reef-LML-Interior	0.40	0.60
Oyster Reef-LMS-Exterior	0.54	0.63
Oyster Reef-LMS-Interior	0.73	0.83
Oyster Reef-OSU-Exterior	0.71	0.65
Oyster Reef-OSU-Interior	0.73	0.69
Riprap-CVS-Exterior	0.39	0.41
Riprap-CVS-Interior	0.15	0.27
Riprap-GL-Exterior	0.45	0.52
Riprap-GL-Interior	0.18	0.24
Riprap-GS-Exterior	0.41	0.46
Riprap-GS-Interior	0.12	0.28
Riprap-LML-Exterior	0.61	0.52
Riprap-LML-Interior	0.43	0.33
Riprap-LMS-Exterior	0.42	0.42
Riprap-LMS-Interior	0.00	0.23
Riprap-OSU-Exterior	0.63	0.57
Riprap-OSU-Interior	0.44	0.44

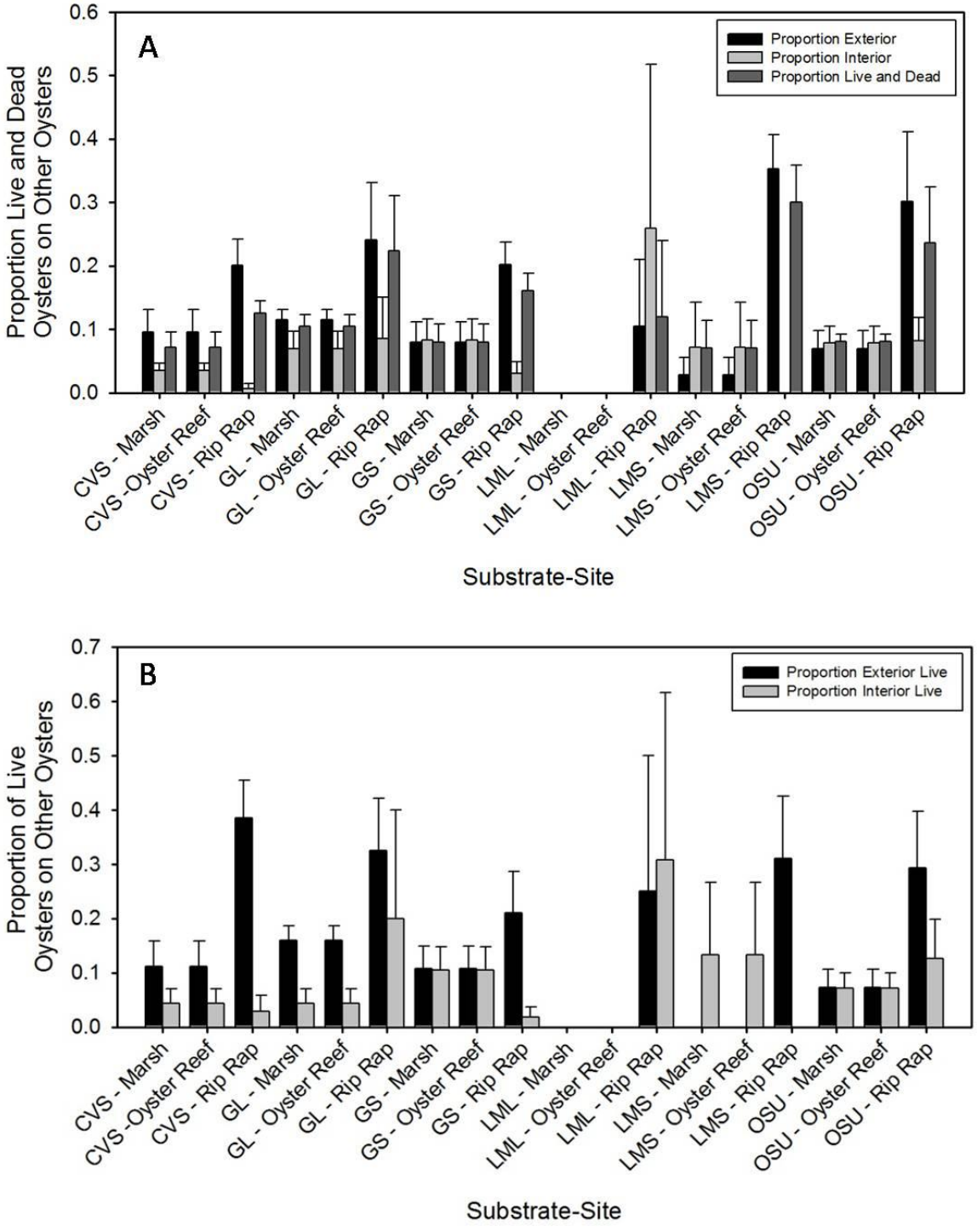


Figure 4.17: (A) Proportion of live and dead oysters present on other oysters in the exterior and interior reef segments, and exterior plus interior, by substrate-site. (B) Proportion of live oysters only on other oysters in the exterior and interior reef segments.

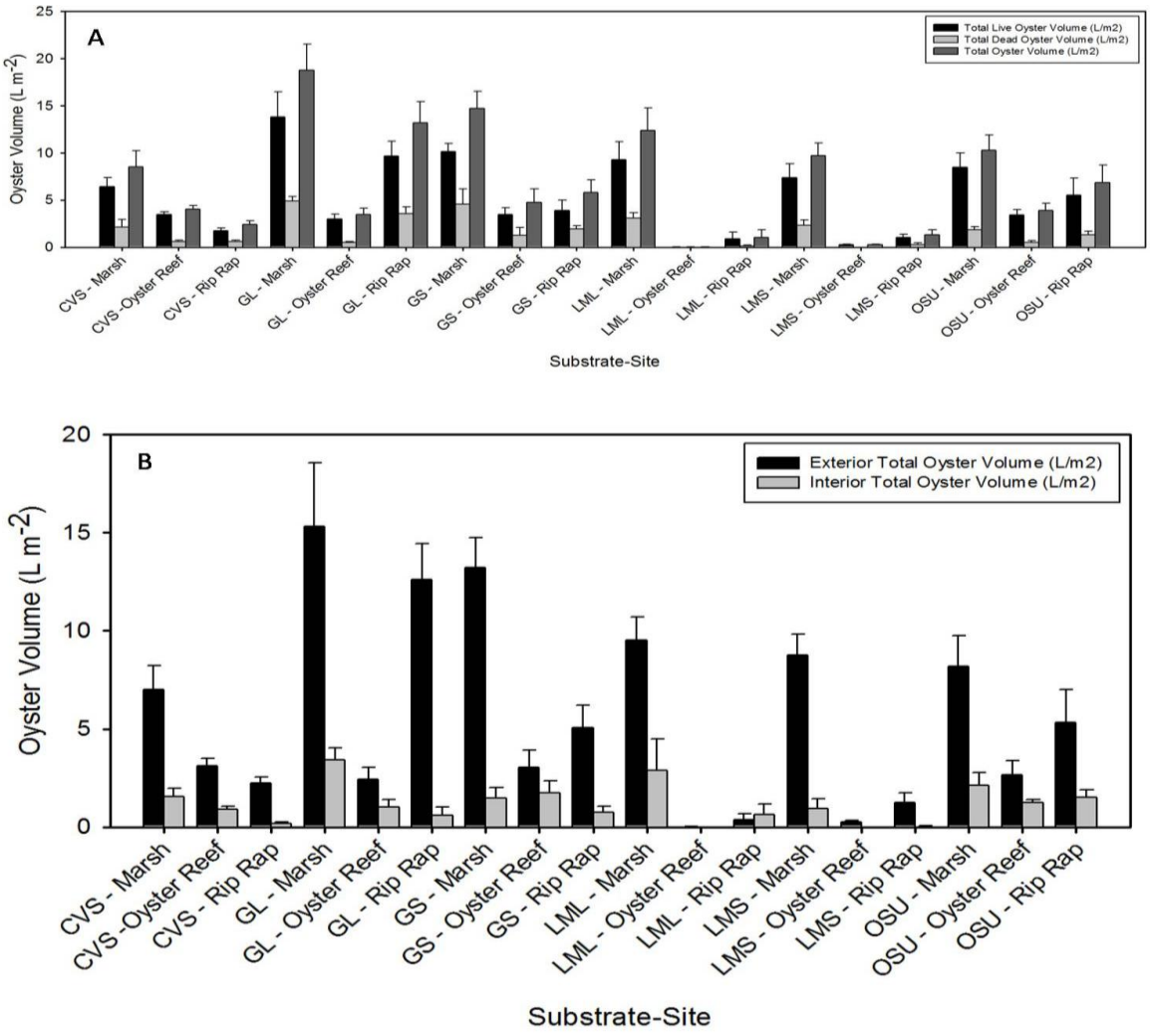


Figure 4.18: (A) Live, dead, and total (live + dead) oyster volume (L m^{-2}), and (B) exterior and interior oyster volume, by substrate-site.

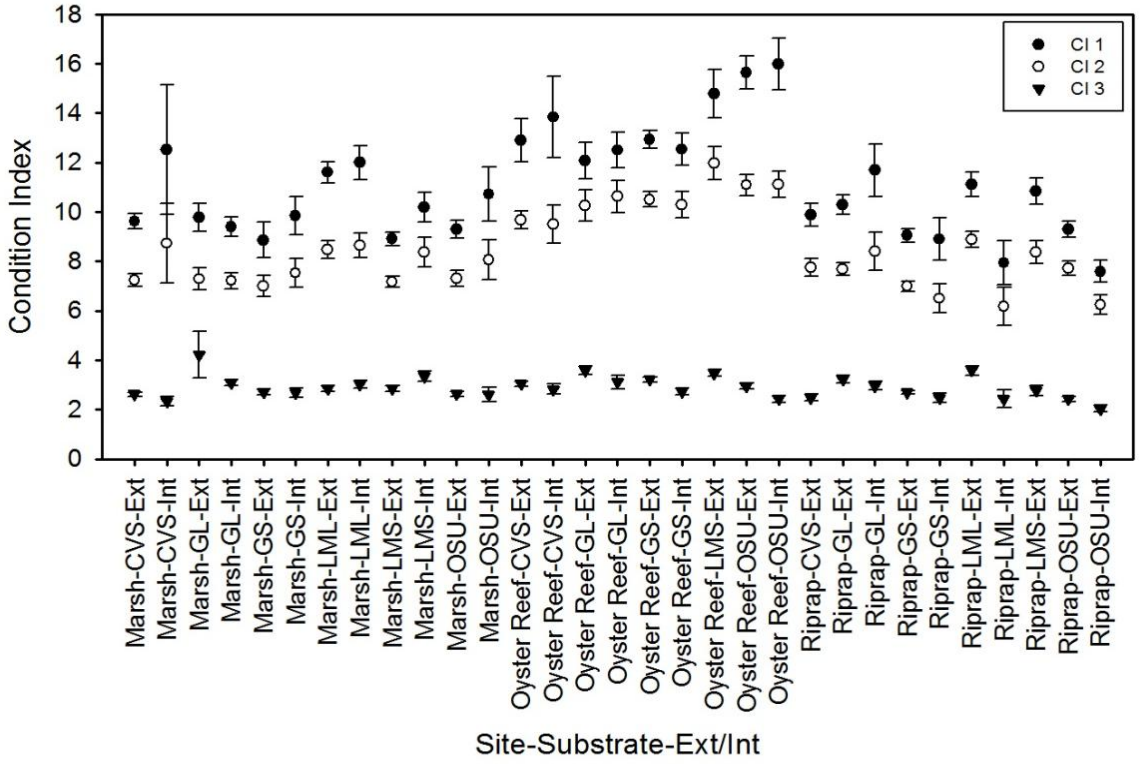


Figure 4.19: Mean oyster condition index – CI (+ 1 SEM) for CIs 1-3 by site-substrate-exterior/interior.

Table 4.9: Dermo disease intensity data by (A) site-substrate, (B) site and (C) substrate, where Dermo disease intensity ranks as follows: Heavy-Moderate-Light-Rare-Negative.

Site-Substrate	Oysters Tested	Proportion Infected	Weighted Prevalence	Oyster Disease Intensity	Nominal Disease Intensity Rank	Mean Oyster Density (No. m^{-2} +/- 1 SEM)	Mean Oyster Biomass (g AFDM m^{-2} +/- 1 SEM)
Marsh-CVS	10	1.00	3.20	3-5-2-0-0	5.2	2048 (252)	173.1 (32.4)
Marsh-Granite	10	1.00	2.60	2-4-4-0-0	4.4	2423 (477)	302.6 (33.3)
Marsh-LM	10	0.90	2.45	1-6-1-1-1	3.7	981 (89)	161.2 (16.0)
Marsh-OSU	10	1.00	3.60	5-3-2-0-0	5.4	2168 (219)	189.8 (22.4)
Oyster Reef-CVS	6	1.00	2.58	2-1-2-1-0	4	2467 (191)	48.8 (12.4)
Oyster Reef-Granite	10	0.90	1.90	1-3-5-0-1	3.6	2408 (306)	69.9 (20.6)
Oyster Reef-LM	6	1.00	2.00	0-3-3-0-0	3.8	93 (28)	2.5 (1.1)
Oyster Reef-OSU	10	1.00	2.40	1-5-4-0-0	4.2	1307 (130)	70.3 (17.5)
Riprap-CVS	10	1.00	1.95	0-5-4-1-0	3.6	901 (244)	48.8 (12.4)
Riprap-Granite	10	0.90	1.90	0-5-4-0-1	3.5	1741 (329)	229.0 (48.2)
Riprap-LM	9	0.56	1.67	2-1-2-0-4	2.4	260 (86)	26.5 (11.5)
Riprap-OSU	9	0.89	2.00	1-3-4-0-1	3.6	1560 (338)	151.8 (31.1)

A.

Substrate	Oysters Tested	Proportion Infected	Weighted Prevalence	Disease Intensity	Nominal Disease Intensity Rank	Mean Oyster Density Fall 2007 (m^{-2})	Mean Biomass Fall 2007 (g AFDM m^{-2})
Recycled Concrete	26	1.00	2.58	5-11-8-2-0	4.3	1805 (203)	105.3 (18.4)
Granite	30	0.93	2.13	3-12-13-0-2	3.8	2191 (225)	200.5 (31.3)
Limestone Marl	25	0.80	2.04	3-10-6-1-5	3.3	445 (103)	63.4 (18.2)
Oyster Shell	29	0.97	2.67	7-11-10-0-1	4.4	1678 (159)	137.3 (17.9)

B.

Substrate	Oysters Tested	Proportion Infected	Weighted Prevalence	Disease Intensity	Nominal Disease Intensity Rank	Mean Oyster Density Fall 2007 (m^{-2})	Mean Biomass Fall 2007 (g AFDM m^{-2})
Marsh	40	0.98	2.96	11-18-9-1-1	4.7	1837 (158)	215.1 (16.4)
Oyster Reef	32	0.97	2.22	4-12-14-1-1	3.9	1463 (195)	44.0 (7.8)
Riprap	38	0.84	1.88	3-14-14-1-6	3.3	1077 (148)	126.1 (19.2)

C.

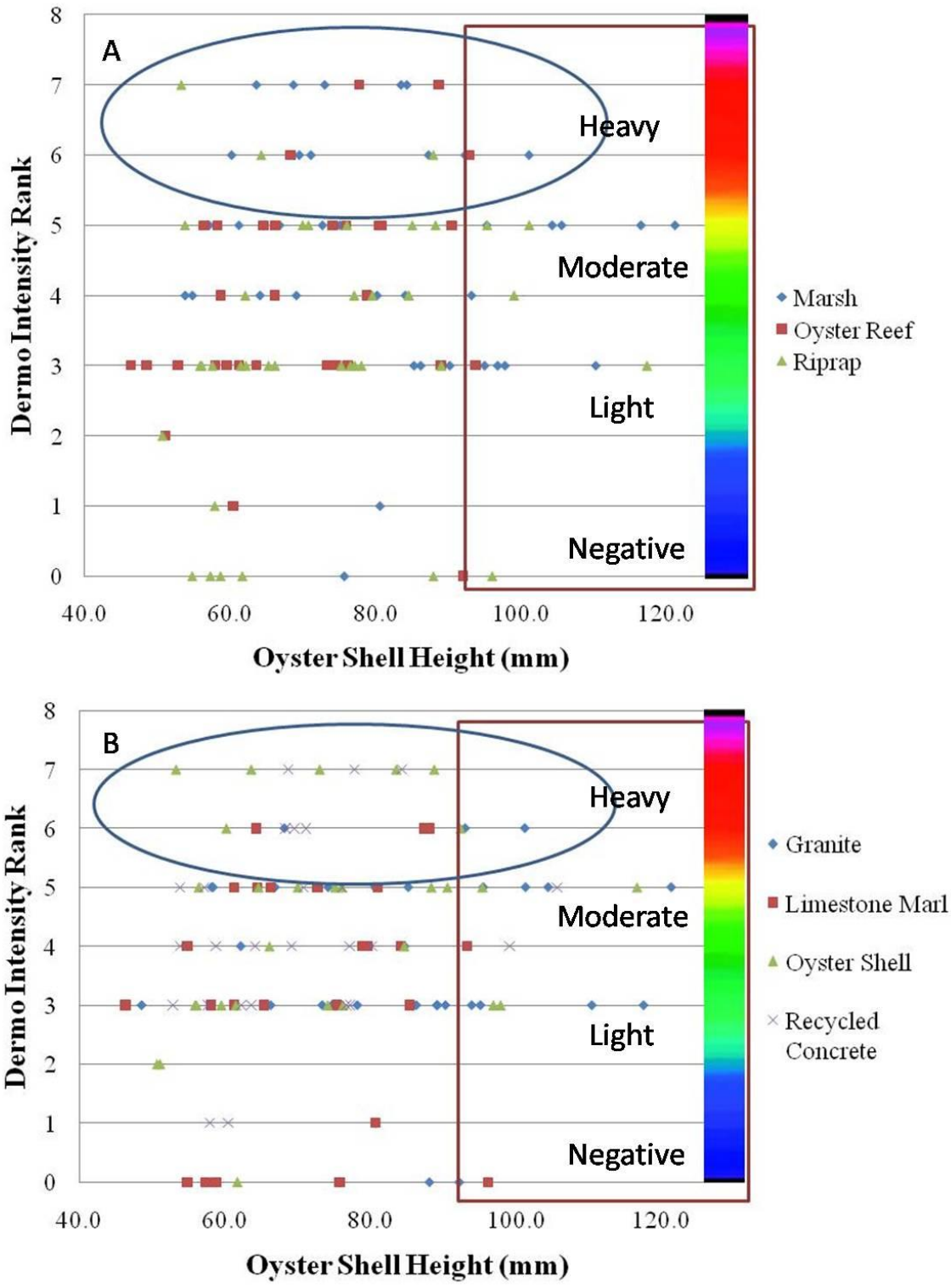


Figure 4.20: Dermo intensity rank versus oyster shell height by (A) site and (B) substrate.

DISCUSSION

Recruitment. Restoration efforts with native eastern oyster (*Crassostrea virginica*) in Chesapeake Bay have been extensive, yet impeded by substrate and recruitment limitations along with other environmental factors. This experiment focused on effectiveness of a number of substrates as oyster reef habitat and, over a 3-yr span, yielded excellent results. Recruitment was not limiting during the lifetime of this experiment; in fact, recruitment was good in all three years of the study (2005, 2006 and 2007), with the latter two years being exceptionally high. The YC1 recruitment was important in determining substrate suitability for oyster settlement because all treatments were vacant when they were deployed, devoid of oysters, mussels, sponges and any other fouling organisms (e.g. barnacles and tunicates) that may have competed with oysters for space.

Baywide, in summer and fall of 2006, oyster recruitment throughout lower Chesapeake Bay was above the 14-yr average (Southworth et al. 2007). For example, in the Great Wicomico River recruitment levels were the highest in decades (Southworth and Mann, 2007). Recruitment in summer and fall of 2007 was not as high as in 2006 (Southworth et al. 2008a). However, YC3 in Long Creek was even larger than YC2, and there were more recruits on the treatments at the oyster reef site than at the other two sites – 2.3 times as many spat as at the riprap site and 1.4 times as many spat as at the marsh site. Furthermore, YC3 on treatments at the oyster reef site reflected a 70 % increase from YC2 recruitment and almost 300 % more recruits than in YC1, due in large part to high recruitment to OSU and very high recruitment on GL, GS, and CVS. Across all

sites, OSU treatments had the most recruits, 2.5 times more than LML and LMS treatments, 30 % more than GL treatments, and 15 % more than GS and CVS treatments.

The results at the oyster reef site were puzzling. How could treatments at the oyster reef site do so much better after low recruitment during the first two years? Moreover, cold temperatures in the previous winter had killed most of the spat that had settled at the oyster reef site as YC2 in 2006, indicating that oyster densities at the oyster reef site were unlikely to be high enough to attract the record number of settlers. Lastly, why would the mature, high-oyster density treatments at the marsh site not continue to attract the greatest number of recruits?

To explain these results, we adopted the following conceptual model. Reefs reach a carrying capacity, whereby they cannot continue to support much higher densities of filtering oysters; intraspecific competition becomes too great for new spat to survive and thrive as they might in conditions of lower competitor or predator density. On Virginia's Eastern Shore, intertidal oyster reefs maintain this state and tend to be dominated by high densities of small oysters that cover a smaller number of large oysters (B. Truitt, pers. comm.). In this system, substrate is at a premium and is generally limiting. In Long Creek, quality substrate is also limiting. However, the die-off only months earlier should have opened up surface area for the oyster larvae that would eventually make up YC3. Furthermore, the remaining shells of those recently dead spat and small adults, as well as a number of surviving small and large adults, provided the physical and chemical cues – calcium carbonate (Zimmer-Faust and Tamburri 1994) and oyster pseudofeces (Tamburri et al. 1996, Turner et al. 1994), respectively – that can attract oyster larvae as they prepare to settle out of the water column. These cues were undoubtedly stronger on

treatments at the riprap and marsh sites, but the major difference was the high percentage of vacant substrate area within the treatments at the oyster reef site. The resulting density of oyster recruits on treatments at the oyster reef site in 2007 was higher than any other recorded throughout the entire experiment. We also dismiss the hypothesis that the water flow favored the treatments at the oyster reef site over those at the marsh and riprap sites, since (1) tidal flow in the narrow Long Creek is strong during each tidal cycle, and (2) the LRS Riprap Survey (Dissertation Chapter 2) provided oyster size frequency data for both stands of concrete and granite riprap as well as the Long Creek restored oyster shell reef, which confirmed the presence of multiple year classes.

Much of the LRS oyster population exists in the intertidal and upper subtidal zones (Luckenbach and Ross 2006, this study). This zonation may be partially due to intense predation (Menge and Branch 2000, Witman and Dayton 2000), though reef architecture, scale, and substrate quality may be equally or more critical to the success of subtidal restored oyster reefs.

Density, biomass and condition. Density often exceeded 1000 oysters m^{-2} of river bottom after one year and 2000 m^{-2} after three years. Biomass was similarly high on most substrates, except limestone marl, and generally ranged from 150-300 g AFDM m^{-2} after 3 years. In particular, granite treatments at the marsh site generated very high biomass (270-382 g AFDM m^{-2}). As a comparison, a 75-mm SH oyster, which is considered commercial (market) size, will weigh approximately 1 g AFDM. More than 70 % of oyster density and biomass was on the exterior segments, with that percentage increasing over time as the reefs matured.

These oyster density and biomass estimates highlight the effectiveness of alternative substrates for oyster reef restoration and the ability of oysters to survive and persist at high density and biomass in high salinity waters of Chesapeake Bay. Previously it was thought that oyster biomass would be too low at high salinity habitats, particularly when compared to biomass in low-salinity sanctuaries (Paynter 1999). These results refute that hypothesis. Moreover, the magnitude and frequency of recruitment improve the chances that established reefs will be sustained by new recruits and persist indefinitely. Comparably high density and biomass on oyster reefs has been achieved in the high-salinity waters of coastal lagoons bordering Chesapeake Bay (Ross and Luckenbach 2009).

Population structure. Oyster PSS varied between sites, substrates, location in the reef, and, to a lesser extent, whether or not a plot was caged to exclude large predators. PSS was most developed at the marsh site, slightly less at the riprap site, and considerably less at the oyster reef site. Caging (large predator exclusion) was not necessary for development of PSS on shell or non-shell substrates.

Growth and survival. Oyster growth was high at the marsh and riprap sites, and less so at the oyster reef site. Growth was not significantly affected by location within the treatment (exterior vs. interior), though we often observed the largest oysters on the exterior segments. Oyster survival was high in all treatments except for limited mortality due to anoxia, siltation and burial in some treatments, and due to the severe winter cold in 2007. The proportion of oysters settling and surviving on other previously-settled oysters

(live or dead) increased over time, with a greater number recruiting to the treatment exterior. This trend indicates that underlying substrate becomes less important as the reef matures above and around it; the developing shell reef becomes the main settlement substrate. In areas of consistent, annual recruitment, substrate type may be less important. However, where recruitment is infrequent or marginal, oyster reef substrate quality may become critical. Additionally, if quality shell is limiting for large-scale restoration and reef architecture is also deemed important, managers may consider using less desirable materials of opportunity for the reef base with a veneer of higher-quality substrate (Clarke et al. 1999, Priest et al. 1999, Nestlerode et al. 2007).

Oyster volume and reef accretion. A key feature of a thriving oyster reef is the rate of shell accretion. Shell reefs must maintain a positive shell balance (Mann and Powell 2007). The durability structure of a substrate such as large granite likely lowers the accretion threshold since the rocks themselves persist, even if the reef were denuded of oysters by ice, storms, predation or poaching.

Historical records indicate that oyster reefs required about 5 L m^{-2} yr^{-1} of shell accretion (Mann et al. 2009b) to balance natural sources of shell loss, including burial, dissolution, and fouling (Smith et al. 2005). In a post-industrial Chesapeake Bay, the necessary shell accretion to balance loss is likely higher. In three years, treatments at the oyster reef site accreted < 2 L m^{-2} yr^{-1}; treatments at the riprap site ranged from < 2 to 5 L m^{-2} yr^{-1}. Treatments at the marsh site (3-8 L m^{-2} yr^{-1}) were all in a state of positive shell balance with no apparent threat of being buried or lost. Intertidal oyster reefs do not experience the same type of sedimentation, fouling and predation that many subtidal reefs

experience, but they do contend with more variable, often extreme, physical conditions (Menge and Branch 2000).

Pathology and condition. Oyster condition at all three sites and all six substrate classes decreased with increasing oyster density, similarly to that observed in oyster aquaculture (Rheault and Rice 1996). The oyster reef site maintained the lowest oyster densities and the highest mean CI values. Oysters recruited, grew, and survived well despite the heavy disease challenge in these high-salinity waters, suggesting that oysters can express disease resistance when afforded protection on reefs of high quality, whether shell or alternative materials.

Previously it was hypothesized that disease would kill older oysters in high-salinity areas, such that Dermo intensity would correlate positively with oyster shell height, because the largest and oldest oysters contain the heaviest infections. Under the contrasting hypothesis that disease tolerance exists in native oyster populations, some large and old oysters could have significantly lower intensity infections than they should have according to the former hypothesis. In this study, the largest oysters had light to moderate infections, some with no infection at all, whereas many of the intermediate-sized oysters had moderate to heavy infections. It is possible that some large oysters died of Dermo infections and were thus not sampled, but we would still expect to sample numerous large oysters with heavy infections. The densest populations of oysters with the greatest Dermo prevalence (marsh site) also harbored some of the largest, most Dermo-tolerant oysters. Consequently, we reject the hypothesis that disease tolerance cannot be expressed in native oyster populations, and conclude instead that a percentage

of oysters can indeed survive, reproduce and persist under high disease challenge in high-salinity areas. Along with the recent findings of disease tolerance in disease-challenged (Dermo and MSX) oyster populations (Carnegie and Burreson 2009), these results indicate that restoration efforts of native oyster populations in high-salinity, disease-challenged areas can indeed succeed.

CONCLUSIONS

This experiment represents the most comprehensive quantitative test of alternative, non-shell materials as intertidal oyster reef substrate in the Chesapeake Bay, with emphasis on oyster recruitment success, growth, survival, and reef development. The experimental treatments recruited ample oysters to distinguish subtle differences between substrates in all three years of the study. The findings of this study were: (1) density often exceeded 1000 oysters m^{-2} of river bottom after one year and 2000 m^{-2} after 2 ½ years, in many cases, (2) exterior treatment segments maintained > 70 % of oyster density and biomass, (3) most treatments exceeded a biomass of 150 g AFDM m^{-2} after three years, (4) caging (large predator exclusion) was not necessary for development of oyster reefs on shell or non-shell substrates, (5) reef accretion rates were high, (6) survival was high in all treatments except for limited mortality due to anoxia, siltation and burial in some treatments, and due to the severe winter cold in 2007, and (7) oysters recruited, grew and survived well despite heavy disease challenge. Throughout the entire experiment, granite treatments performed as well as, or better than, oyster shell. Moreover, rock reefs are easier to establish and more persistent than shells which can

degrade quickly and are more easily perturbed by storms and poaching. We conclude that construction of intertidal oyster reefs with shell and alternative materials, such as granite and concrete, is a viable restoration strategy in the high-salinity waters of Chesapeake Bay.

Acknowledgments. We express appreciation to students and staff of the Marine Conservation Biology and Marine Community Ecology Programs at VIMS, Mrs. Candy Burke Huayhualla, and Mr. Darryl Nixon, for their assistance in the lab and field. Special thanks to the VIMS Shellfish Pathology Lab for testing > 100 oysters for Dermo and MSX. Funding was provided by grants to R.N. Lipcius and R.D. Seitz from the Norfolk Office of the USACE (Norfolk District) and from the NOAA Chesapeake Bay Office through the Blue Crab Advanced Research Consortium. Finally, we wish to thank First Landing State Park, Mr. and Mrs. Robert Clifton, Mr. Jonathan and Laura Guion, and recognize the late Mr. Luther Yopp, who welcomed our placement and sampling of the Alternative Substrate Experiment alongside their properties on Long Creek.

Chapter 5

Living oyster reef shorelines using shell and alternative structures in the Lynnhaven River, Chesapeake Bay

ABSTRACT: Some physical barriers that protect shorelines from erosion are constructed of mollusk shell or alternative materials, which may also serve as reefs (i.e. "living shorelines") for ecological restoration of native bivalve species such as oysters and mussels. Despite their increasingly popular use, the efficacy of "living shoreline" reefs in enhancing bivalve abundance has rarely been tested. We experimentally examined the effectiveness of several types of living shoreline reef in augmenting abundance of Eastern oyster, *Crassostrea virginica*, at two locations in the Lynnhaven River System, a subestuary of lower Chesapeake Bay. In summer 2006, nine reef structures were constructed at two sites: three each of oyster shell (OS), riprap (RR), and concrete modular (CM) reefs. We also placed six reefballs (RBs) at each site, half of which had been seeded with oysters in culture tanks. In addition, we attempted *in situ* setting of triploid oyster larvae on OS, RR, and CM reefs. The primary objective of this construction was the development of vital oyster reef habitat as a living shoreline in the upper subtidal zone adjacent to natural marshes. The performance of unseeded and seeded reefs was assessed for oyster recruitment, density, biomass (ash-free dry mass – AFDM), condition, growth, survival, reef structural integrity, and disease. After 2.5 yrs: (1) mean oyster density and biomass were high on most unseeded (150-1200 m^{-2}, 150-600 g AFDM m^{-2}) and seeded (LB: 30 – 1800 oysters m^{-2}) reefs, (2) most reefs reached a mature state, as indicated by population structure and high accretion rates (8-15 L m^{-2} yr^{-1}), (3) the proportion of live oysters was high (unseeded: 0.53- 0.77; seeded: 0.63-0.75), (4) reefs were covered with oysters, often obscuring the base substrate and causing subsequent oysters to recruit to the living portion of the reef (up to 44 % of oysters grew on other oysters), and (5) both diploid and triploid oysters tested for disease had below average Dermo (i.e. disease) infections, and were therefore healthy. Moreover, *in situ* setting of triploid oyster larvae was successful. These findings indicate that living shoreline reefs can serve both as physical barriers and as restoration reefs for native bivalve species, such as eastern oyster.

INTRODUCTION

Estuaries such as Chesapeake Bay have extensive shorelines lined with marshes, beaches, and tidal mudflats that provide a rich habitat for plants and animals (VA CZM 2009). In many estuaries, however, shorelines are eroding at rapid rates (VA CZM 2009). For instance, in Chesapeake Bay, as much as one third of all shorelines have eroded, with some areas losing as much as 20-40 cm of shoreline per year (Chesapeake Bay Program 2005); an estimated 57 % of the sediment in the Bay comes from these eroding shorelines (Langland and Cronin 2003). With the heavy industrial, agricultural, and residential development of watersheds, including shorelines of rivers and estuaries, the percentage of forested land has decreased. The increasing proportion of surfaces that are impervious to drainage has exacerbated erosion problems and associated delivery of pollutants to our coastal waters (NOAA 1998). The resultant increased sediment and nutrient loads have numerous negative effects on estuarine flora and fauna by: (1) blocking light required for submerged aquatic vegetation, (2) burying low-lying sessile invertebrate reefs (e.g. oyster reefs) or clogging the filtration system of filter feeders, and (3) increasing the frequency and intensity of harmful algal blooms that subsequently lead to zones of low dissolved oxygen (NOAA 1998, Rabalais et al. 2001).

As coastal populations continue to grow, and as sea level continues to rise, the need for shoreline stabilization has intensified. There is growing concern that erosion control efforts that use "hardened" shoreline (e.g. rock revetments, wood or vinyl bulkheads) are damaging natural, shoreline habitats (VA CZM 2009). Effective shoreline protection may be achieved, however, with a technique called "living shorelines." Living shorelines incorporate materials such as marsh plantings, shrubs and trees, low profile

sills and breakwaters, and strategically placed organic material, which can recreate the ecological functions of a natural shoreline (VA CZM 2009). Living shorelines also promote local participation in “best management practices” with structures that do not diminish environmental conditions while concurrently suiting the needs of the shoreline property owner (Dept. Conservation and Recreation 2009). These benefits include: (1) reduction of erosion and property loss, (2) lower erosion control construction costs, (3) natural and aesthetically pleasing views, (4) restored marine habitat and spawning areas for fish and invertebrates, and (5) improved water quality (VA CZM 2009).

Ecological restoration of the eastern oyster, *Crassostrea virginica*, in Chesapeake Bay is one means of mitigating the effects of increased turbidity and phytoplankton production, given suitable environmental conditions (Hargis and Haven 1999). Reefs (i.e. "living shorelines") used for ecological restoration of native oyster may also serve as physical barriers that protect shorelines from erosion. However, the efficacy of "living shoreline" reefs in enhancing oyster abundance has not been tested. We experimentally examined the effectiveness of several types of living shoreline reef in augmenting abundance of eastern oyster, *Crassostrea virginica*, at two locations in the Lynnhaven River System, a subestuary of lower Chesapeake Bay.

In early 2006, two homeowners in Virginia Beach’s LRS shoreline community agreed to allow the construction of a living shoreline experiment adjacent to each of their properties. In summer 2006, three oyster shell (OS), riprap (RR) and concrete module (CM) reefs were constructed at these two subtidal sites in the upper reaches of the LRS, one in Linkhorn Bay (LB) and one in the Upper Eastern Branch (EB) of the Lynnhaven River (Fig. 5.1). In addition, we placed six reefballs (RB) at each site, half of which had

been seeded with oysters in controlled culture tanks two months prior to deployment. The primary goal of this construction (Fig. 5.2a-c) was the development of healthy oyster reef habitat as a living shoreline in the subtidal zone adjoining natural marshes. We assessed the comparative success of the reef types (unseeded and seeded) with respect to oyster recruitment, density, biomass, condition, growth, survival, disease intensity, and reef structural integrity (Fig. 5.3a-j). The *a priori* hypotheses (Table 5.1) outline expectations for this study.

Figure 5.1: Chesapeake Bay (inset) contains the deployment sites for the Living Shoreline Experiment reefs in Linkhorn Bay and the Eastern Branch of the Lynnhaven River (Virginia Beach, Virginia).

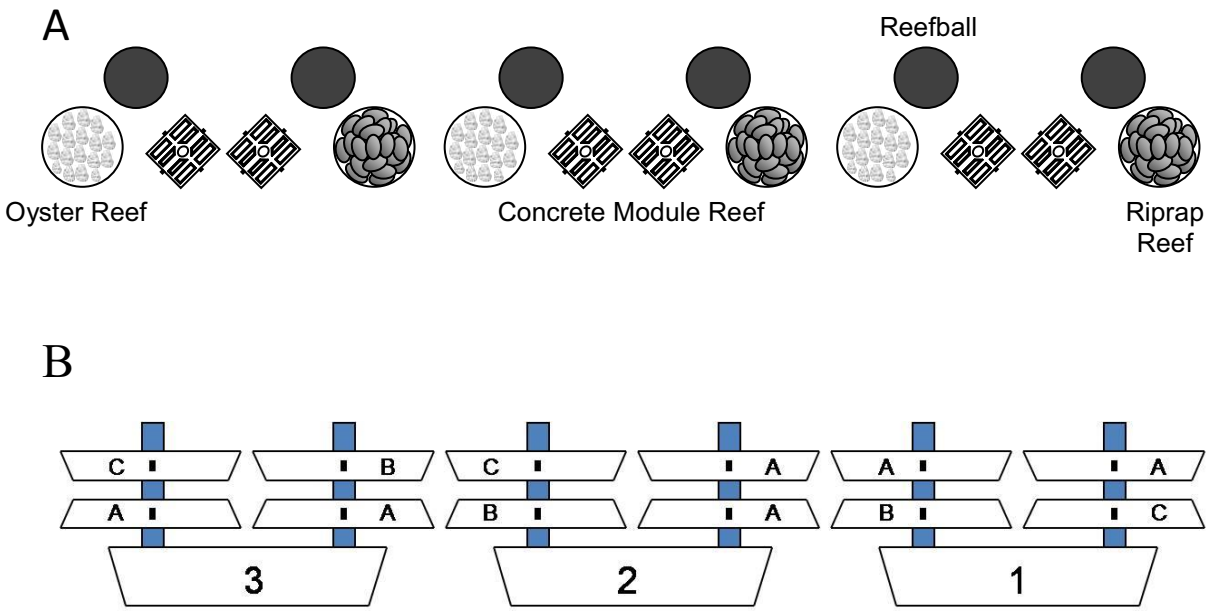


Figure 5.2: (A) General schematic of the Living Shoreline Experiment located at two properties in the Lynnhaven River System. (B) Schematic of concrete module reef replicates (Proprietary Design: ReefTek Model 1105) in the Eastern Branch (EB) site of the Lynnhaven River. Note, modules labeled "B" were inoculated with triploid (3N) larvae via a remote field larval setting experiment; modules labeled "A" and "C" were both deployed barren ("A": July 2006; "C": August 2006). (C) The EB site, post-deployment.

Figure 5.3 A-F.

Figure 5.3: (A) Captain Robert Jensen (Rappahannock Preservation Society; ReefTek) and his concrete module prototype. (B) A seeded reefball suspended by a crane prior to deployment. (C) An oyster cluster from an oyster shell reef. (D) Oysters (2.5 yrs old) from a seeded reefball with shell heights > 177 mm (7 inches). (E) Granite covered in oysters from a riprap reef. (F) Submerged concrete modules removed from a reef, with the seeded module at the top left. (G) Large oysters from an oyster shell reef at the EB site. (H) Oysters covering > 90 % of a seeded concrete module at the LB Site. (I) Seeded reefball (1.5 yrs post-deployment) with oysters thriving in every nook and cranny. (J) Oyster reef restoration ecologists on a mission.

Table 5.1: *A priori* hypotheses for the Living Shoreline Experiment (LSE).

Hypothesis	*a priori* Hypothesis Description followed by the observation(s) and associated logic behind hypotheses
LSE #1	Year 1: Oyster Recruitment/Density is higher on restored oyster shell reefs than on riprap and concrete module reefs
	Riprap and concrete modules were not conditioned prior to deployment; oyster shells, by definition, are conditioned to receive oyster larvae and had a distinct advantage in Year 1 of this experiment.
LSE #2	Year 2: Oyster Recruitment on riprap and concrete module reefs is equal to Recruitment on restored oyster shell reef from Year 1
	The 1st sampling of the Alternative Substrate Experiment (Fall 2005) revealed alternative substrates recruited oysters as well or better than loose oyster shell.
LSE #3	Year 1: Oyster Survival (%) is higher on riprap and concrete module reefs than on restored oyster shell reef
	Lower oyster density and higher interstitial volume on riprap and concrete module reefs equate to higher survival (%).
LSE #4	Oyster Growth does not differ across 1) substrate/reef type, and 2) site
	There were no data available to suggest that one reef type or site would have oysters growing faster than any other.
LSE #5	Oyster Recruitment and Density are higher at the Eastern Branch (Lynnhaven River) site than at the Linkhorn Bay site.
	The extant population at the Eastern Branch (Lynnhaven River) site was considerably larger than the Linkhorn Bay site.

Additionally, oyster larvae were set *in situ* on some modules, rocks and shell bags to detect differences between seeded and barren substrates. This work is an expansion of a study conducted by Coon and Fitt (1999) on unconsolidated (loose) OS reefs. To date, this was the first attempt at deploying eyed-larvae on alternative substrates *in situ*. We sought to determine the physical and economic feasibility of deploying larvae *in situ* (as opposed to setting oyster larvae in tanks on land) on alternative oyster reef substrates. The *a priori* hypotheses (Table 5.2) outline expectations for this corollary study.

Table 5.2: *A priori* hypotheses for the Remote Field Larval Setting Experiment (RFLSE), the corollary to the Living Shoreline Experiment.

Hypothesis	*a priori* Hypothesis Description followed by the observation(s) and associated logic behind hypotheses
RFLSE #1	Oyster recruitment to oyster shells (in mesh bags) and conditioned granite rip rap is greater than on unconditioned concrete modules
	Unconditioned rock and concrete surfaces are acidic and lack a biofilm that oyster larvae detect when landing on a substrate to test its viability for permanent settlement
RFLSE #2	Oyster growth and survival is high on all substrates since these larvae were triploid
	Oyster growth and survival is higher on riprap and concrete modules than the oyster shells that are crowded in, and restricted by, mesh bags.
RFLSE #3	Triploid oysters set on riprap and concrete modules have higher survival and growth than diploid oysters that recruited naturally.
	Triploid oysters do not have to expend energy for gamete production which frees up energy for shell and somatic growth, as well as energy to combat parasites and associate disease
RFLSE #4	Substrates set with triploid oyster larvae have higher recruitment in Year 2 than substrates with only diploid oysters present (excluding seeded reefballs).
	A greater number of larger oysters equates to more fresh calcium carbonate shell surface available and stronger chemical cues for oyster larval attraction.

MATERIALS AND METHODS

Sampling procedure and design. After obtaining each homeowner's consent to construct the general design (Fig. 5.2a) of three replicates of the granite RR (0.91 m diameter, 0.51 m height), loose OS (0.91 m diameter, 0.51 m height), and CM reefs (bases: 1.22 m x 1.22 m x 0.13 m; mini-modules: 0.61 m x 0.61 m x 0.09 m), scale drawings were constructed and submitted to the Commonwealth of Virginia as two joint permit applications with the Chesapeake Bay Foundation. The official permits were issued on 24 July 2006; we deployed the granite RR and OS reefs that same day. The next day, 18 of 24 CM structures were deployed. Due to construction delays, the last six modules were deployed a few weeks later. A permit addendum was sought and granted for the addition of six RBs to each living shoreline. Half of the RBs were conditioned and seeded with diploid oysters; the other RBs were devoid of oysters and unconditioned. The RBs are called Mini-Bay Balls (dimensions: Base diameter – 0.71 m, Top diameter – 0.51 m, Height – 0.51 m, and estimated surface area of 2.74 m^2). The RBs were deployed on 26 September 2006 and likely missed the 2006 oyster larval recruitment window.

Sampling of the reefs was conducted in July 2007, fall 2007, and September-October 2008 (henceforth referenced as spring 2007, fall 2007, and fall 2008, respectively) and was non-destructive, except for the final sampling. Oyster shell height (SH) of live and dead oysters, as well as mud crab counts, were recorded from one of four quadrants of each reef. One quarter of the granite RR and OS reefs were non-destructively sampled *in situ* (except for fall 2008), recording the percent of substrate present below the sediment line. Care was taken to return reef material to its original

orientation (i.e. rocks at the reef base were returned to the base position of the reef; oyster clusters from shell reefs were placed on top of the empty, anoxic shell). This process was important to the maintenance of reef integrity and ensuring that the shells and rocks that were at the top, middle, or bottom were returned in that order to the reef. These precautions were especially critical at the EB site where reef bases were buried in mud. Any oysters that were accidentally placed at the bottom in the mud would have likely died. One quarter of each CM layer was also non-destructively sampled noting its position (upper/lower), condition, and locations of oysters measured (top, sides, holes or bottom). Since one out of every four CMs on a reef was used in the RFLSE, the mean (density, biomass, etc.) of the other three units was used to estimate the fourth. RBs were photographed and notes taken regarding oyster reef progression (estimated growth, density, and presence/absence of oyster recruits). At the close of the experiment (fall 2008), one quarter of each RB was destructively sampled. For all reef types, a fixed number of oysters throughout the range of oyster SHs were retained for disease (Dermo/MSX), biomass, and condition index analysis.

Approximately one million eyed-larvae were obtained from the VIMS Aquaculture Genetics and Breeding Technology Center (ABC) and were deployed at the two LSE sites on 7 August 2006. The objective was to set these larvae on three CMs directly from the LSE, six pieces of conditioned granite RR from the adjacent shoreline, and six mesh bags of OS. The modules were set on cinder blocks to maximize the surface area for settlement and a silt fence enclosure built around them with the granite and shell bags placed around the inside perimeter of the fence, to secure the bottom of the fence to the sediment and avoid larval loss. Once larvae had warmed to room

temperature (~20 min), they were mixed in a pitcher filled will local river water, and dispersed within the enclosure using a small cup. The silt fence was removed two days later. Six weeks post-deployment, recruitment and post-set mortality were recorded. The CMs were then returned to their respective reefs. The six granite pieces and three shell bags were set near the LSE reefs of similar substrate, but not on them. Three shell bags were recovered for sampling. The bags were broken down into four categories (top exterior, top interior, bottom exterior and bottom interior) to detect patterns in recruitment and post-set mortality. Oyster counts (live/dead) were recorded, noting the face of the shell (inner/outer). Twelve weeks post-set, a single shell bag and three pieces of granite from each site were recovered. The shell bags were broken down as before and all oyster SHs were recorded. Oyster SHs were also recorded for the granite. Subsequently, we determined the ploidy of a subset of the oysters. Since the larvae from the VIMS ABC were triploid, we could distinguish them by their ploidy using flow cytometry. A fixed number of oysters from both sites (½ from granite; ½ from OS, with the shell half divided by the four sub-categories: top exterior, top interior, bottom exterior, and bottom interior) were provided to the VIMS flow cytometry lab who provided analytical results (Appendix 5.1).

Two shell bags, three pieces of granite, and three CMs remained at each site, so progress of the triploid oysters and reef succession could be monitored with each sampling of the LSE. One oyster bag was opened and the contents spread out in a 0.29-m^2 tray at each site during the spring 2007 LSE sampling. The second oyster bag was not opened or sampled until the final sampling (fall 2008) to determine if growth and survival were affected by the mesh bag.

We used a linear-mixed model design (General Linear Model: fixed factor: Reef Type; random factor: Site) in the statistical analysis. Due to a significant interaction effect, we tested the Reef Type effect with separate one-way ANOVA models for each site (Underwood 1997).

Recruitment. To simplify comparisons of multiple years of oyster recruitment across site, reef type, and seeding factors, a ranking system from 0 to 8 was adopted (Table 4.6). Note that qualitative descriptors of oyster recruitment such as 'Low' and 'Extremely High' refer only to the Chesapeake Bay and its subestuaries; locations outside of Chesapeake Bay could have different rankings. Full-scale sampling of unseeded reefs was first conducted in spring 2007. Though the live oysters were considerably larger than they would have been in November 2006, it is appropriate to consider any oyster – live or dead – greater than 5.0 mm (a SH that indicates survival past post-set mortality – 6 wks) as the 2006 YC (YC1 = 2006, YC2 = 2007). Distinct differences in size classes between sites resulted in classification by YC only. The seeded substrates were measured six weeks post-deployment (RFLSE). The cinder blocks were not measured during each sampling; RBs were only measured during the last sampling. A different method, using population size structure and notation of 'newly-dead' and 'old dead' oysters, was employed to estimate YC1 and YC2; this method was validated using other data collected in this study.

Biomass and condition index. SH, width, and depth were measured for all oysters, living and dead. SH was considered as the distance from the umbo to the posterior end of

the shell. Additionally, all internal tissues were collected for selected oysters in pre-weighed aluminum 'weigh boats' for dry mass (DM) and AFDM measurements. More than 1200 oysters representing the full range of SH values were processed to yield reliable estimates of oyster biomass via regression of log AFDM (g) versus log SH (mm). The oysters were selected for unseeded RR, OS, CM reefs, RBs, cinder blocks, as well as seeded (diploid) RBs and triploid (188) oysters from RR, OS, CM reefs and cinder blocks (Table 5.3). Details regarding laboratory biomass procedures and calculation of oyster condition indices can be found in the 'Materials and Methods' section of Dissertation Chapter 2.

Oyster and substrate volume. Oyster volume, a direct assay of oyster reef accretion, was measured for live oysters and dead shells separately through volumetric displacement. Oyster volume measurements were made for all unseeded and most seeded reefs/substrates in fall 2008. Oysters (live and dead) and substrates were scraped clean of oysters, mussels, large barnacle clusters, etc., to avoid overestimation.

Pathology and condition. Dermo (*Perkinsus marinus*) and MSX (*Haplosporidium nelsoni*) prevalence and intensity were tested in 59 oysters. Oysters were collected in early November since peak infection intensity occurs in the fall. Oysters ranging in SH from 59.0-176.8 mm were selected haphazardly from unseeded and seeded reefs at each site; n = 10-15 from each treatment. Samples were brought back to the VIMS Pathology group live and on ice. Dermo testing was conducted using RFTM (Ray fluid thioglycollate medium). The RFTM has a lower detection limit than histology and, thus, was selected for analysis. The oysters were assigned one of nine disease ratings (Ray

1954, Table 4.5). SH and other condition metrics (i.e. emaciated tissue, thin shell, presence of boring sponge, etc.) were noted for each oyster tested.

Table 5.3: Regression models of log AFDM (g) versus log oyster shell height (mm) used for oyster biomass estimation for unseeded oyster shell, riprap and concrete module reefs, reefballs (unseeded and seeded), and cinder blocks (LB site only) at both sites in fall 2008.

Site	Reef Type	Sample Size (n)	Biomass Regression Equations
LB	Oyster Shell	131	10^((2.5868*LOG10(SH))-4.9846)
EB	Oyster Shell	129	10^((2.0948*LOG10(SH))-3.9713)
LB	Riprap	128	10^((2.5556*LOG10(SH))-4.8866)
EB	Riprap	93	10^((2.0037*LOG10(SH))-4.0046)
LB	Concrete Modules	121	10^((2.5612*LOG10(SH))-4.9126)
EB	Concrete Modules	38	10^((2.9195*LOG10(SH))-5.4654)
LB	Unseeded Reefballs	87	10^((2.3107*LOG10(SH))-4.5075)
EB	Unseeded Reefballs	61	10^((1.8240*LOG10(SH))-3.6156)
LB	Seeded Reefballs	86	10^((1.8242*LOG10(SH))-3.7435)
EB	Seeded Reefballs	59	10^((1.6811*LOG10(SH))-3.4102)
LB	Cinder Blocks	100	10^((2.0924*LOG10(SH))-4.0555)

RESULTS

Recruitment. In 2006, the unseeded granite RR reefs and CM reefs experienced low to moderate recruitment; recruitment at the OS reefs was moderate at the LB site, and high at the EB site (YC1). Across all reef types, the EB site outrecruited the LB site by an order of magnitude (Table 5.4a). The unseeded and seeded RBs were deployed too late in the season (late September) to receive more than a few oyster larvae, but the seeded RBs (Fig. 5.3b) were uniformly packed with ~35-mm oysters, mimicking high natural recruitment.

The release of triploid larvae within enclosures containing OS, RR, and CMs resulted in oyster recruitment to those substrates. A site x substrate type interaction (General Linear Model – GLM, F = 3.78, p = 0.032) required a separate one-way ANOVA for each site. Triploid oyster recruitment was significantly higher (LB: F = 8.16, p = 0.008; EB: F = 20.76, $p < 0.0005$) on OS high) than on RR (low to moderate) and CMs (low). Though not part of the initial experimental design, cinder blocks were included due to low recruitment to seeded and unseeded blocks.

By fall 2007, unseeded reefs at both sites had experienced recruitment (YC2), but the LB received more recruits than the EB site (Table 5.4b), a reversal from the previous year. Unseeded reefs at both sites were developing well: OS reefs at the EB site were beginning to cohere (cluster), a critical indicator of reef succession. Although OS reefs received more recruits for the second year in a row (moderate to high), the other reef types experienced much higher recruitment levels than in 2006 (moderate). Seeded substrates at the LB site recruited well in 2007 (moderate to high); seeded OS at the EB site also recruited well, but the RR and CMs experienced very low recruitment. The cinder blocks had sunken in the mud and were no longer measured. The seeded RBs recruited well (high) at both sites.

Table 5.4: Oyster recruitment rankings for YC1 (fall 2006) and YC2 (fall 2007) by (A) site and (B) site-reef type, for seeded and unseeded oyster shell, riprap, concrete modules, reefballs, and cinder blocks.

Site	Seeded/Unseeded	YC1	YC2	Mean
LB	Unseeded	2.0	4.4	3.2
EB	Unseeded	2.6	3.5	2.8
LB	Seeded	4.6	5.0	4.8
EB	Seeded	3.6	3.5	3.3

A.

Site	Reef Type	Seeded/Unseeded	YC1	YC2	Mean
LB	Oyster Shell	Unseeded	3	6	4.5
EB	Oyster Shell	Unseeded	7	5	6.0
LB	Riprap	Unseeded	2	4	3.0
EB	Riprap	Unseeded	3	4	3.5
LB	Concrete Module	Unseeded	2	4	3.0
EB	Concrete Module	Unseeded	2	2	2.0
LB	Reefball	Unseeded	0	4	2.0
EB	Reefball	Unseeded	0	3	1.5
LB	Cinder Block	Unseeded	3	4	3.5
EB	Cinder Block	Unseeded	1	--	1.0
LB	Oyster Shell	Seeded	8	5	6.5
EB	Oyster Shell	Seeded	6	5	5.5
LB	Riprap	Seeded	3	5	4.0
EB	Riprap	Seeded	2	1	1.5
LB	Concrete Module	Seeded	2	3	2.5
EB	Concrete Module	Seeded	1	1	1.0
LB	Reefball	Seeded	7	7	7.0
EB	Reefball	Seeded	8	7	7.5
LB	Cinder Block	Seeded	3	5	4.0
EB	Cinder Block	Seeded	1	--	1.0

B.

Density. Unseeded oyster reefs were first sampled in spring 2007. Recruitment on these reefs in 2006 – YC1 (Table 5.4a-b) was roughly equivalent to oyster density (Fig. 5.4a) recorded the following spring, where the EB site maintained higher densities of live oysters than the LB site. An interaction effect between site and reef type ($F = 24.77$, $p < 0.0005$) dictated the need to conduct separate one-way ANOVAs of reef type for each site. At the EB site ($F = 32.37$, $p = 0.001$), oyster densities ranked: OS >> RR = CM, with mean OS oyster density near 1000 m^{-2} and RR-CM densities of ~200 m^{-2} (Fig. 5.4a). At the LB site ($F = 11.62$, $p = 0.009$), oyster densities ranked: OS > RR = CM, with mean oyster densities less than 150 m^{-2} (Fig. 5.4a). Oyster densities on seeded substrates commenced in fall 2006. As mentioned earlier, oyster recruitment to OS at both sites was extremely high, exceeding 9000 spat m^{-2} at the LB site, and 2800 spat m^{-2} at the EB site (Fig. 5.4b). RR recruited between 260 (EB site) and 530 spat m^{-2} (LB site); CMs recruited between 40 (EB site) and 150 spat m^{-2} (LB site).

Across most seeded substrates in spring 2007, oyster densities were similar as in fall 2006. RR oyster densities were lower, but this decrease was artificial; the rocks with densest triploid settlement were recovered 12 wks post-deployment in 2006 for ploidy testing to determine performance of the RFLSE (Appendix 5.1). Thus, the rocks left behind for monitoring in spring 2007 and beyond had lower relative oyster density.

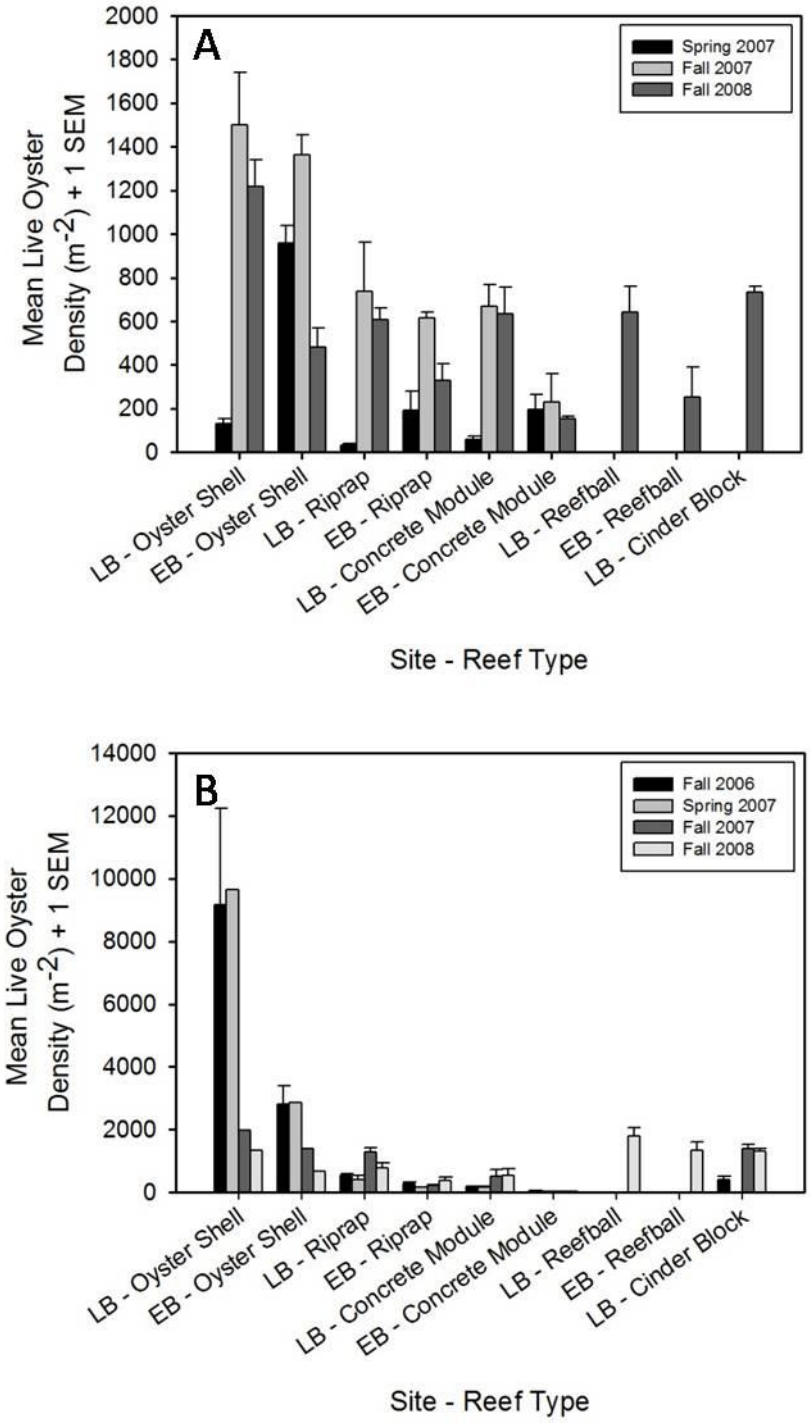


Figure 5.4: Mean live oyster density per unit area of river bottom (No. m^{-2}) + 1 SEM site-reef type from spring 2007 to fall 2008 for (A) unseeded reefs, and from fall 2006 to fall 2008 for (B) seeded reefs.

By fall 2007, a second oyster recruitment event and a year of mortality (winter and summer) reshaped oyster density on the unseeded and seeded reefs. The 2007 recruits (YC2) settled more heavily on unseeded reefs at the sandy, LB site – oyster densities for OS, RR, and CM reefs were statistically equivalent ($F = 5.13$, $p = 0.152$) between the sites (LB: 968 +/- 166; EB: 735 +/- 172 m^{-2} (+/- SE)) but varied significantly between reef types ($F = 33.45$, $p = 0.029$). Oyster density ranks were: OS > RR = CM at the LB site ($F = 5.34$, $p = 0.009$); OS > RR > CM at the EB site ($F = 39.83$, $p < 0.0005$) (Fig. 5.4a). The most dramatic increases, in many cases > 10-fold, occurred on the OS and CM reefs at the LB site, and RR at both sites.

Seeded OS densities declined from spring to fall 2007 (Fig. 5.4b) because oysters removed from their mesh bags in spring 2007 were spread out, thus artificially decreasing their densities. In an attempt to detect the benefits or disadvantages of long-term enclosure in a mesh bag, one OS bag from each site was deconstructed and the remaining one was left intact for the final sampling (fall 2008). For all other substrates, oyster density increased in fall 2007 (GLM, site x substrate interaction effect, $F = 5.57$, $p = 0.046$), particularly at the LB site (one-way ANOVA, $F = 9.48$, $p = 0.014$): RR – 1295 +/- 140 m^{-2}, CM – 514 +/- 200 m^{-2}, and CB – 1398 +/- 120 m^{-2} (Fig. 5.4b). These oyster assemblages were accreting at an impressive rate (1.5 yrs), especially the RBs (Fig. 5.3i) where oysters were beginning to accrete laterally (reef expansion).

All unseeded and seeded reefs were destructively sampled and frozen in fall 2008, to determine reef performance. This sampling occurred before most 2008 recruits (YC3) could grow large enough to be measured and, thus, excluded estimates for YC3. By fall 2008, no significant differences were detected between sites (GLM, $F = 14.47$, $p =$

0.063), reef types (F = 4.64, p = 0.177), or due to the site x reef interaction (F = 3.33, p = 0.071). However, oyster density on unseeded reefs differed by reef type at each site (ANOVA, LB: F = 10.83, p = 0.010; EB: F = 5.93, p = 0.038). When we included RB reefs in the analysis, oyster density ranks for unseeded reef types at the EB site (ANOVA, F = 11.74, p = 0.001) were: OS = RR > CM = RB, with no live oyster density > 500 m^{-2}; at the LB site (F = 11.55, p = 0.001), they were: OS (1216 +/- 123 m^{-2}) > RR = CM = RB, with all densities > 600 m^{-2} (Fig. 5.4a).

Excluding RBs (LB = EB site), seeded substrate oyster densities at the LB site were twice those at the EB site (10:1 for CM), by fall 2008 (Fig. 5.4b). Note that the near doubling of RR density at the EB site was artificial because one of the seeded rocks at this site was not recovered and the resulting density was based on two samples instead of three (Fig 5.4b). Oyster density ranks for seeded substrates at the LB site were: RB = OS > RR = CM, with most densities > 750 m^{-2} (Fig. 5.4b). At the EB site, they were: RB > OS > RR > CM, with most densities < 700 m^{-2} (Fig. 5.4b).

The OS bags opened during the final sampling had oyster densities of 3008 m^{-2} (LB site) and 2171 m^{-2} (EB site), given the small footprint (0.0645 m^2); if these oysters were spread across a 0.292-m^2 tray in the manner the other OS samples had been, the densities drop to 664 m^{-2} and 480 m^{-2}, respectively. Compared to the previously spread and seeded OS samples, densities were lower, with a smaller mean oyster SH at the LB site (71.7 +/- 1.4 vs. 66.2 +/- 2.3 mm).

Seeded RB oyster densities were 1795 +/- 268 m^{-2} at the LB site and 1347 +/- 257 m^{-2} at the EB site (Fig. 5.4b). Oysters that had fallen off the RBs created a ring or 'halo'

that was included in the RB density estimate. Nearly half of all RB oysters, including some of the largest ones, were present in these halos.

Biomass. In spring 2007, unseeded oyster biomass followed the same trend as for density (GLM, F = 26.91, $p < 0.0005$). The EB site (OS >> RR = CM; ANOVA, F = 44.07, $p < 0.0005$) contained > 10-fold more oyster biomass than the LB site (OS > RR = CM, F = 3.55, $p = 0.096$), 245.8 +/- 78.4 vs. 17.2 +/- 3.1 g AFDM m^{-2} (Fig. 5.5a).

In fall 2007, the difference in oyster biomass between the two sites was still considerable but the GLM analysis was confounded by an interaction effect between site and unseeded reef type (F = 13.17, $p = 0.001$); separate one-way ANOVAs of reef type were conducted for each site. Oyster biomass ranks were: OS > RR = CM at the LB site (F = 13.17, $p = 0.017$) and OS > RR > CM at the EB site (F = 13.17, $p < 0.0005$).

The interaction effect was not significant in fall 2008 (GLM, F = 2.22, $p = 0.151$), but oyster biomass trends between sites on OS significantly differed from trends between RR and CM among sites, leading to significant effects of both site (F = 16.75, $p = 0.001$) and reef type (F= 14.86, $p = 0.001$). By fall 2008, most reef types contained high oyster biomass (Fig 5.5a):

Site	Oyster Shell (OS)	Riprap (RR)	Concrete Modules (CM)	Reefballs (RB)	Cinder Blocks (CB)
Linkhorn Bay (LB)	616 (99)	413 (64)	443 (53)	380 (64)	603 (30)
Eastern Branch (EB)	554 (77)	156 (39)	122 (18)	156 (91)	--

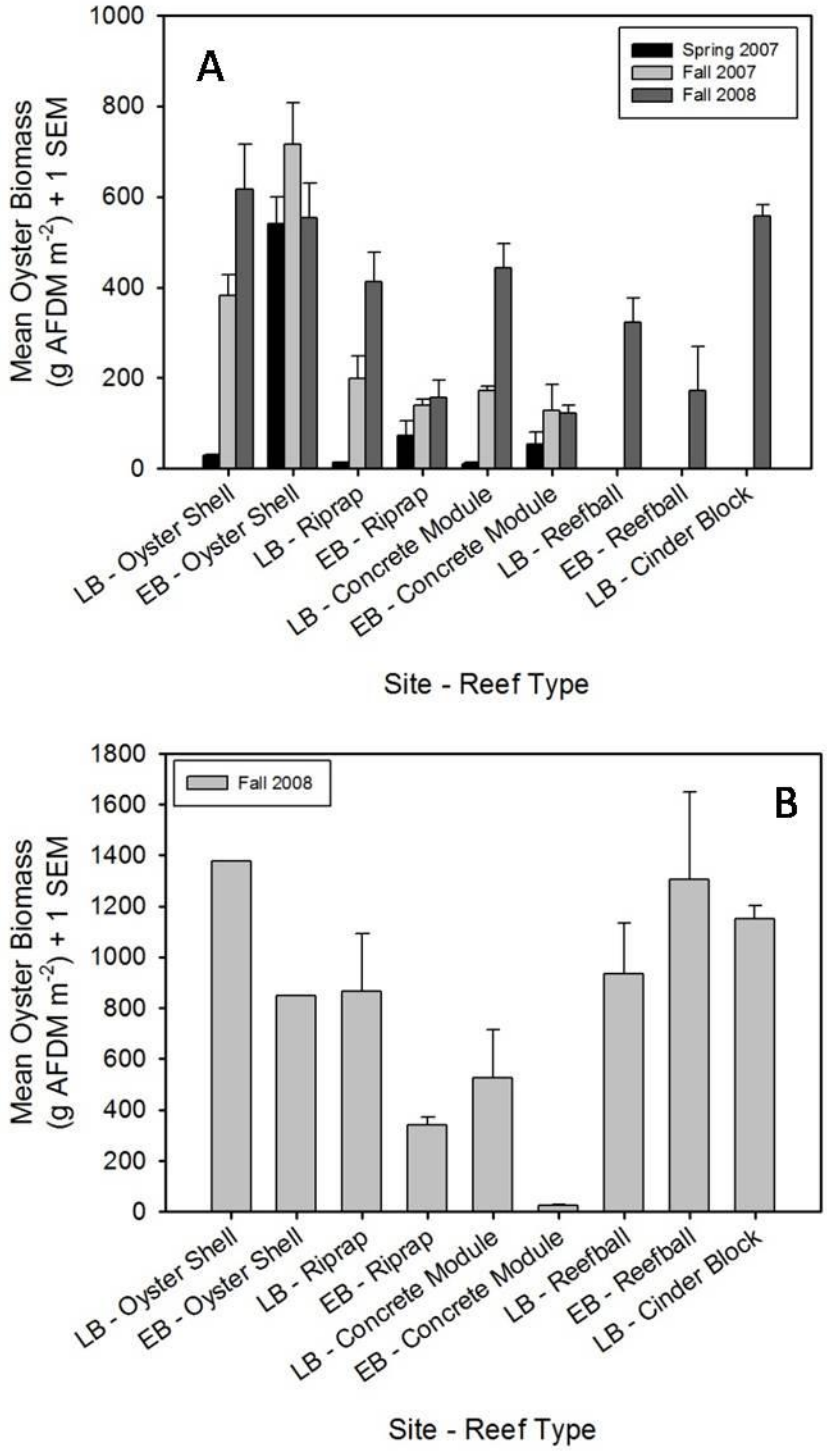


Figure 5.5: Mean oyster biomass per unit area of river bottom (g AFDM m^{-2}) + 1 SEM by site-reef type for (A) unseeded reefs from spring 2007 to fall 2008, and (B) seeded reefs in fall 2008.

The unseeded oyster biomass regression models (Fig. 5.6a-k) were also used to estimate the biomass of diploid oysters on seeded substrates. Additionally, 188 triploid oysters were processed to estimate their contribution to the biomass of the seeded reefs at both sites. The biomass regression models were not reliable (R^2 values < 20%, with many approaching zero) compared to those produced for diploid oysters (Table 5.3), so individual biomass estimates were matched to each triploid oyster within each site-reef type. For triploid oysters that were not processed, the site-reef type mean biomass was used.

Population structure (PSS). PSS was similar across unseeded OS, RR and CM reefs in spring 2007 (Fig. 5.7a). In fall 2007, a strong recruitment event (YC2) dwarfed YC1 across site and reef type (Fig. 5.7b). Oysters on reefs at the LB site must have settled earlier in the season or grew faster after settlement; oyster SH was 25-30 mm larger on reefs at the LB site. This trend was maintained through fall 2008 (Fig. 5.7c-d); RBs and cinder blocks showed similar trends (Fig. 5.7e-f).

PSS of oysters was recorded for seeded substrates from fall 2006 through fall 2008 (Fig. 5.8a-g). Some oysters measured on OS and RR were diploid spat that had settled after the deployment of the triploid larvae in August 2006. The triploid oysters grew quickly (some > 60 mm) on both the OS and RR (Fig. 5.8a). By spring 2007, many oysters on RR and CMs achieved SHs > 80 mm, with a mean SH near 70 mm (Fig. 5.8b). The trend of larger oyster recruits at the LB site on unseeded reefs also occurred on seeded substrates in fall 2007 (Fig. 5.8c-d). By fall 2008, oysters from YC1 were > 120 mm; oysters from YC2 were 50-75 mm at the LB site and 30-60 mm at the EB site (Fig.

5.8e-f). Oysters on the seeded RBs were < 150 mm at the LB site with peaks at 50 and 100 mm. Oyster SH exceeded 170 mm at the EB site with no obvious dominant size class (Fig. 5.8g).

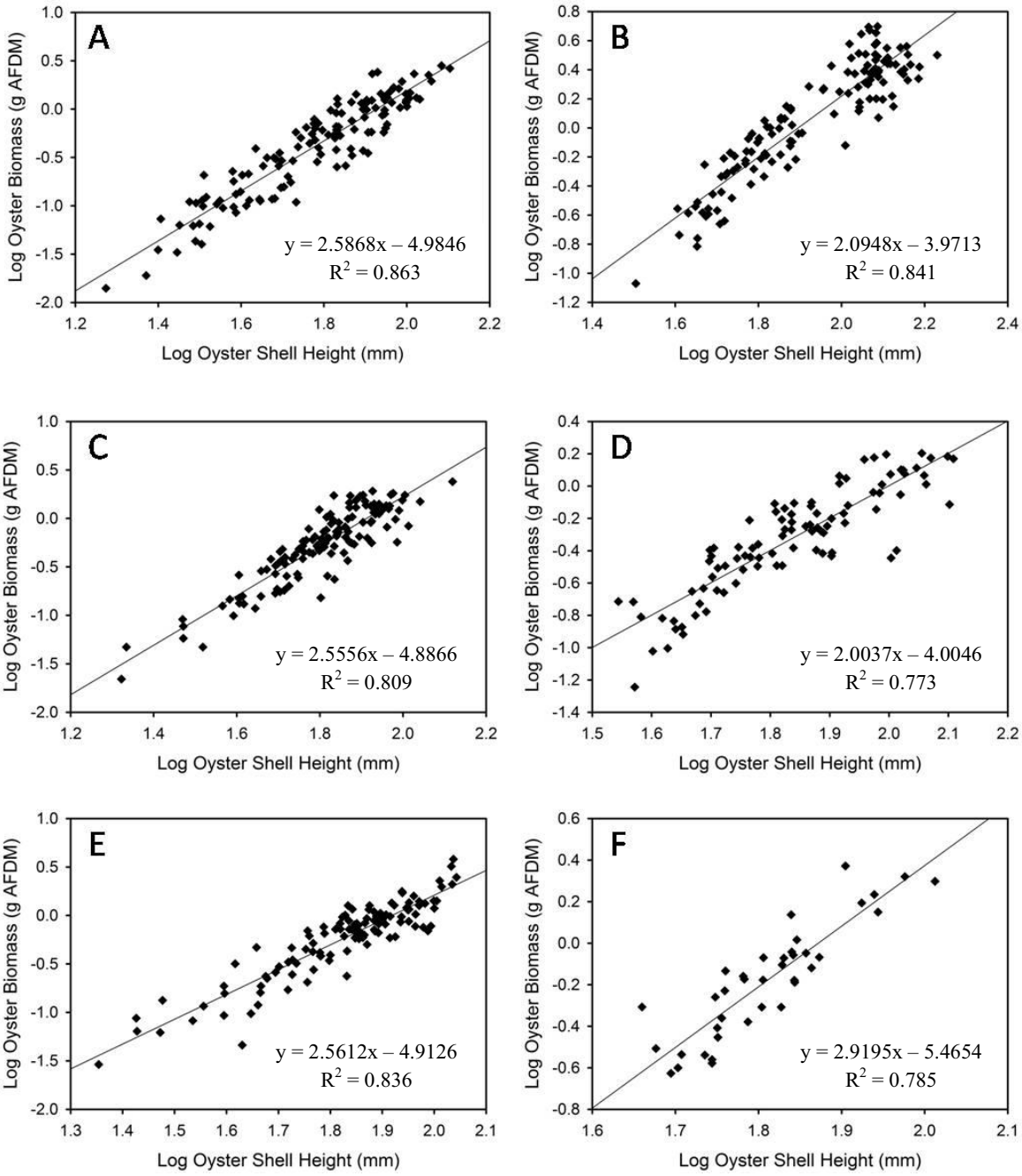


Figure 5.6 A-F.

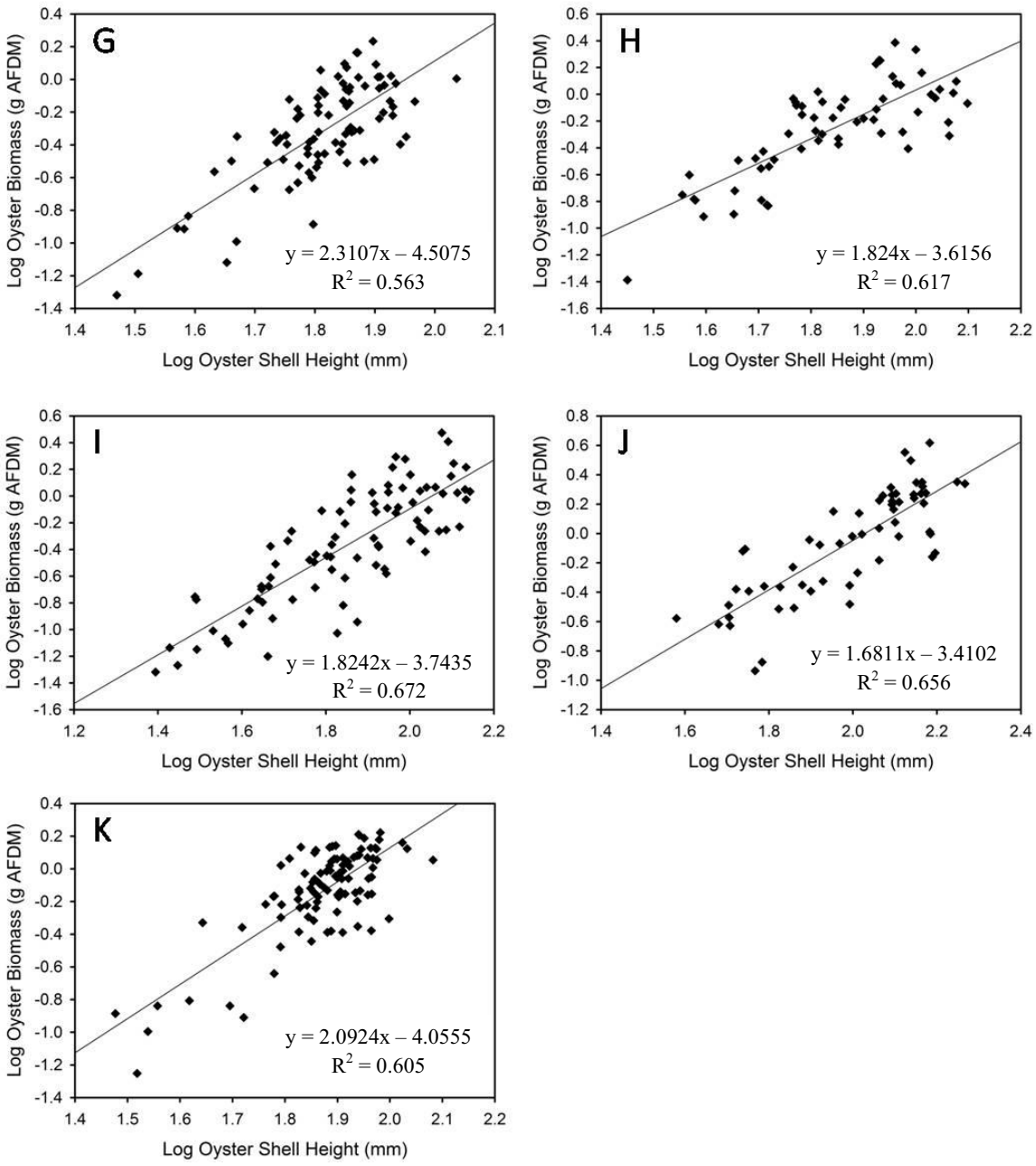


Figure 5.6: Regression models of log AFDM (g) versus log oyster shell height (mm) for biomass estimation of oysters on the unseeded oyster shell(A, B), riprap (C, D), and concrete module reefs (E, F), unseeded (G, H) and seeded reefballs (I, J), and unseeded cinder blocks (K, at LB site only) at the LB and EB sites, respectively.

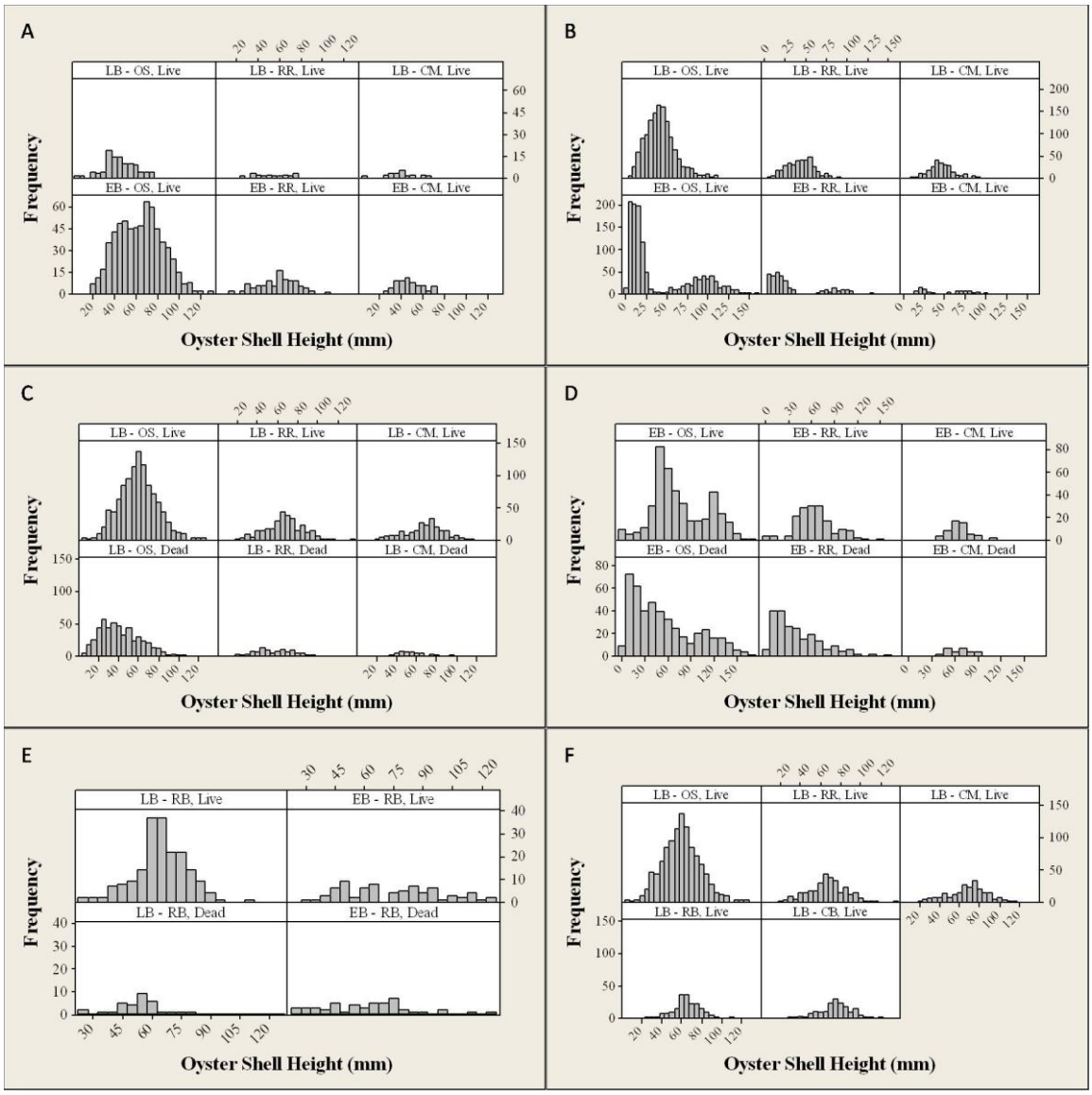


Figure 5.7: Population size structure (PSS) on unseeded oyster shell, riprap, and concrete module reefs in (A) spring 2007, (B) fall 2007, and (C-D) fall 2008, where (C) LB and (D) EB sites include PSS of dead oysters. (E) PSS of live and dead oysters on unseeded reefballs in fall 2008. (F) PSS of live oysters on unseeded reefs, including cinder blocks, in fall 2008.

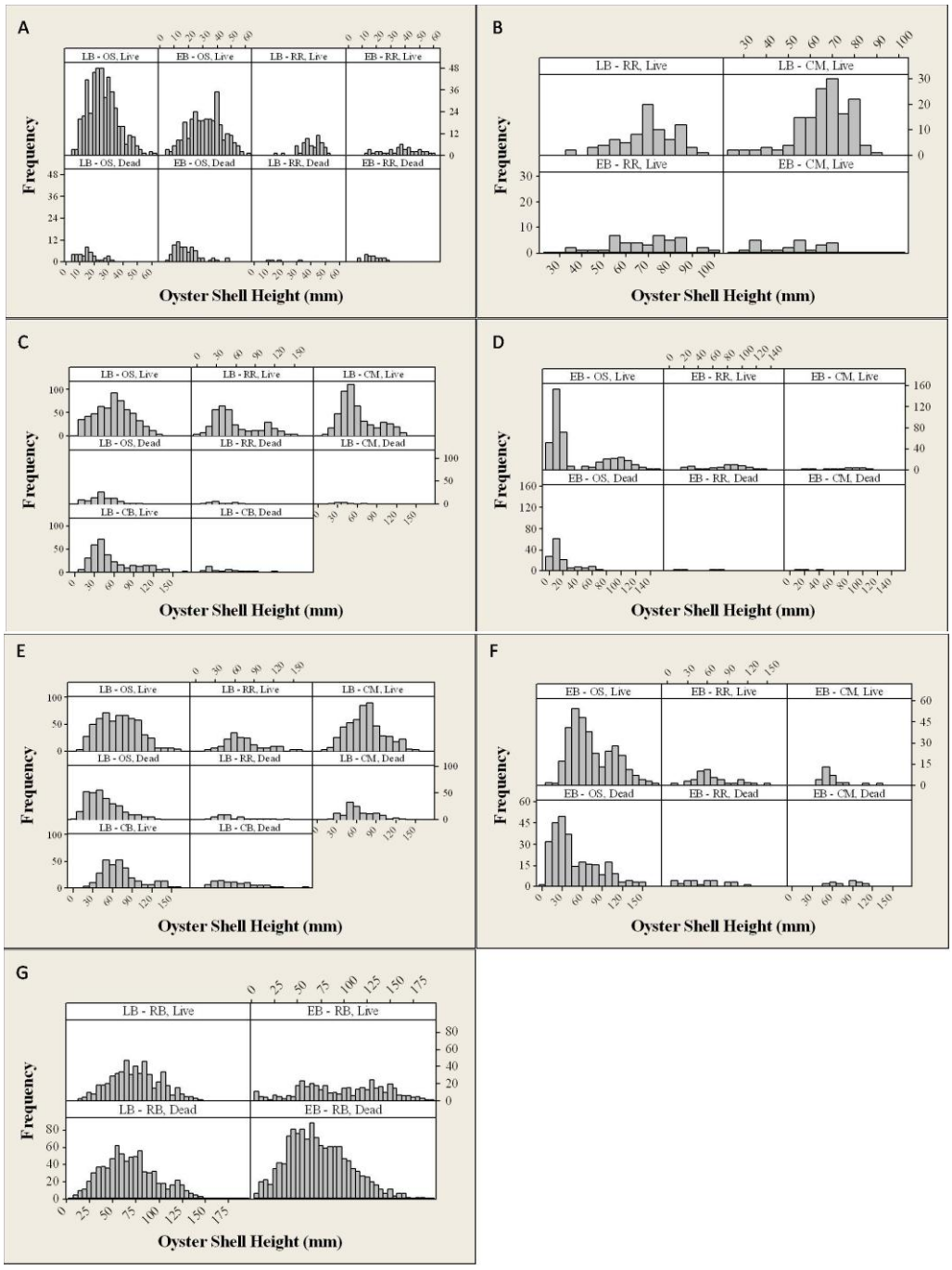


Figure 5.8: (A) Population size structure (PSS) of live and dead oysters on seeded oyster shell (OS) and granite riprap (RR) at the LB and EB sites in fall 2006. (B) PSS of live oysters on seeded RR and concrete modules (CM) at both sites in spring 2007. PSS of live and dead oysters on (C) seeded OS, RR, CMs, and cinder blocks (CB) at the LB site and (D) seeded OS, RR, and CMs at the EB site in fall 2007. PSS of live and dead oysters on seeded OS, RR, and CMs at the (E) LB (including CBs) and (F) EB site. (G) PSS of live and dead oysters on seeded reefballs at both sites in fall 2008.

Survival. In spring 2007, the mean proportion of live oysters across site and unseeded reef type was 0.80 +/- 0.04. The ranked order at the LB site was: CM (0.96 +/- 0.04) = OS > RR (0.61 +/- 0.13); at the EB site, CM (0.83 +/- 0.12) = OS = RR (Fig. 5.9a). By fall 2007, the only change in the proportion of live oysters was at the LB site where CM (0.94 +/- 0.02) > OS = RR (0.82 +/- 0.03). And by fall 2008 (GLM, site x reef type effect, F = 4.53, p = 0.034), RB (0.86 +/- 0.03), CM, CB and RR were all > OS (0.68 +/- 0.02) at the LB site (ANOVA, F = 15.37, p = 0.004). The muddy, EB site (F = 10.24, p = 0.012) experienced the greatest mortalities with proportions of live oysters of 0.68 +/- 0.01 for CM reefs, 0.48 +/- 0.05 for OS reefs, and 0.42 +/- 0.05 for RR reefs (CM > OS = RR); RB was 0.57 +/- 0.06 (Fig 5.9a). Overall, survivorship was high on the unseeded reefs at the EB site and very high at the LB site.

On seeded substrates, oyster survival was similar at both site, but some reef types differed from others. At the LB site in fall 2006, seeded CMs (0.99 +/- 0.01) and cinder blocks (1.00 +/- 0.00) had near-zero mortality. RR (0.96 +/- 0.04) and OS (0.90 +/- 0.02) had high survival as well (Fig. 5.9b). At the EB site, CM (0.98 +/- 0.02) > OS (0.84 +/- 0.00), and the proportion of live oysters on RR reefs was 0.89 +/- 0.03. In spring 2007, the proportion live was similar to fall 2006 (CM > OS = RR, at both sites). By fall 2007, survival was still high, but by fall 2008 (ANOVA, LB: F = 14.67, p = 0.001; EB: F = 32.73, $p < 0.0005$), survivorship dropped by 20 % or more on most seeded substrates (Fig 5.9b):

Site	Oyster Shell (OS)	Riprap (RR)	Concrete Modules (CM)	Reefballs (RB)	Cinder Blocks
Linkhorn Bay (LB)	0.63 (0.09)	0.80 (0.04)	0.79 (0.02)	0.41 (0.05)	0.77 (0.03)
Eastern Branch (EB)	0.56 (0.03)	0.63 (0.03)	0.67 (0.04)	0.23 (0.03)	--

The proportions of oysters settling on other oysters (live or dead) were recorded in fall 2007-2008. By fall 2007, OS (0.04 +/- 0.01) = RR (0.04 +/- 0.02) = CM (0.01 +/- 0.01) at the LB site (Fig. 5.10a) on unseeded substrates. The proportions were much higher at the EB site: OS (0.44 +/- 0.01) > RR (0.28 +/- 0.02) > CM (0.04 +/- 0.04). By fall 2008 (GLM, site x reef type, F = 37.29, $p < 0.0005$), these trends were maintained except for RR (0.15 +/- 0.04) at the EB site (F = 48.82, $p < 0.0005$) where more oysters recruited to the substrates, and CMs (0.09 +/- 0.02) at the LB site (F = 0.96, $p = 0.433$) where more oysters recruited to live and dead oysters. Unseeded RBs had similar proportions at each site (LB: 0.03 +/- 0.02; EB: 0.11 +/- 0.06), as did cinder blocks (0.02 +/- 0.00) at the LB site (Fig. 5.10a).

In fall 2007, proportions of oysters on other oysters on seeded substrates varied between sites and substrates, but were similar by fall 2008 (Fig. 5.10b). In 2007, RR (0.24 +/- 0.03) > CM (0.11 +/- 0.02) = OS (0.07) at the LB site. OS (0.46) > RR (0.08 +/- 0.06) = CM (0.00 +/- 0.00) at the EB site. By fall 2008, the proportions of oysters on other oysters was (Fig. 5.10b):

Site	Oyster Shell (OS)	Riprap (RR)	Concrete Modules (CM)	Reefballs (RB)	Cinder Blocks
Linkhorn Bay (LB)	0.16 (0.03)	0.29 (0.05)	0.17 (0.02)	0.16 (0.02)	0.27 (0.08)
Eastern Branch (EB)	0.31 (0.17)	0.38 (0.18)	0.00 (0.00)	0.16 (0.03)	--

Oyster volume and reef accretion. Oyster volume (L m^{-2} of live and dead oyster shell) is an important reef feature because reefs must accrete at a rate high enough to combat the loss of shell due to physical and chemical weathering, and outright removal (Mann and Powell 2007, Powell and Klinck 2007). By fall 2008, many of the unseeded reefs

(GLM, F = 30.55, p = 0.009) at both sites (F = 12.79, p = 0.037) had accreted substantial new shell (Fig. 5.11):

Site	Oyster Shell (OS)	Riprap (RR)	Concrete Modules (CM)	Reefballs (RB)	Cinder Blocks
Linkhorn Bay (LB)	38.4 (4.1)	13.4 (1.8)	15.7 (0.8)	14.0 (2.3)	19.5 (1.2)
Eastern Branch (EB)	28.9 (3.6)	9.9 (1.8)	3.7 (0.2)	9.7 (4.4)	--

Note the similarities and extreme differences between some of the seeded substrates and the unseeded reef types (ANOVA, LB: F = 12.30, p = 0.002; EB: F = 36.42, p = 0.002) in the following seeded substrate oyster volumes (Fig. 5.11):

Site	Oyster Shell (OS)	Riprap (RR)	Concrete Modules (CM)	Reefballs (RB)	Cinder Blocks
Linkhorn Bay (LB)	39.8	30.9 (8.6)	20.2 (7.2)	77.2 (4.5)	45.6 (2.0)
Eastern Branch (EB)	36.9	1.2 (0.4)	1.2 (0.4)	102.5 (10.2)	--

Pathology and condition. After 2.5 yrs, oysters from the LB and EB sites were tested for Dermo disease. Of 59 oysters tested, 80 % contained *Perkinsus* cells, equating to a weighted prevalence of 1.66 +/- 0.21 (Table 5.5a). No MSX analyses were conducted because *Haplosporidium* was rare in the LRS (Dissertation Chapter 4). Oysters were only tested from unseeded and seeded OS at both sites, and seeded RBs at the EB site (Fig. 5.12, Table 5.5b). Dermo intensity differed somewhat by site (GLM: F = 5.35, p = 0.062) and oyster ploidy (F = 558.64, p = 0.141) with oyster SH as a covariate (F = 0.03, p = 0.0853). Without the SH covariate, ploidy explained most of the variance (F = 164.08, p = 0.050). Without site as a factor, triploid DEBY oysters had significantly lower Dermo intensity than diploid oysters (F = 6.35, p = 0.015).

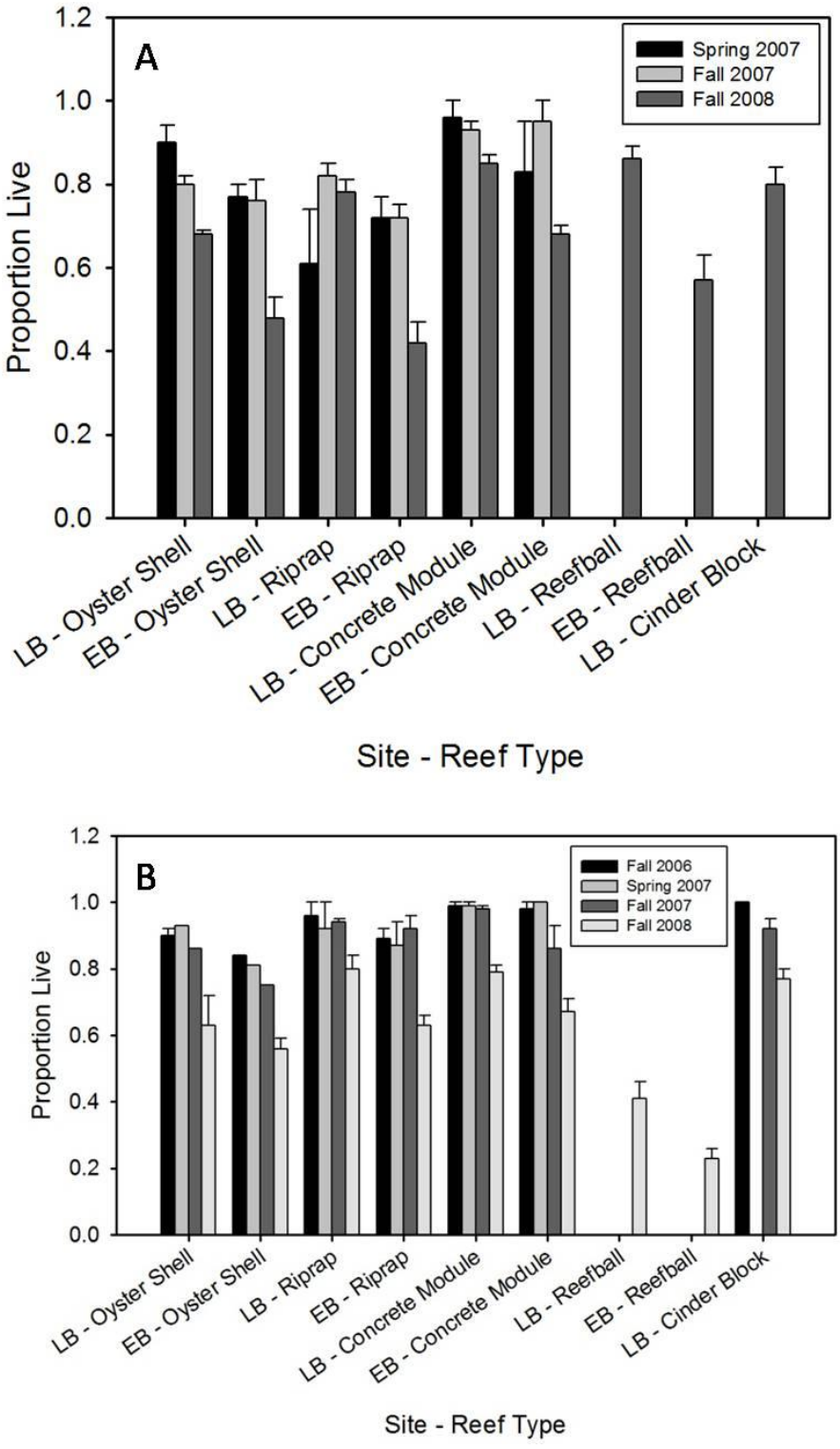


Figure 5.9: Proportion of live oysters by site-reef type on (A) unseeded (spring 2007 – fall 2008) and (B) seeded (fall 2006 – fall 2008) oyster shell, riprap, concrete modules, reefballs, and cinder blocks.

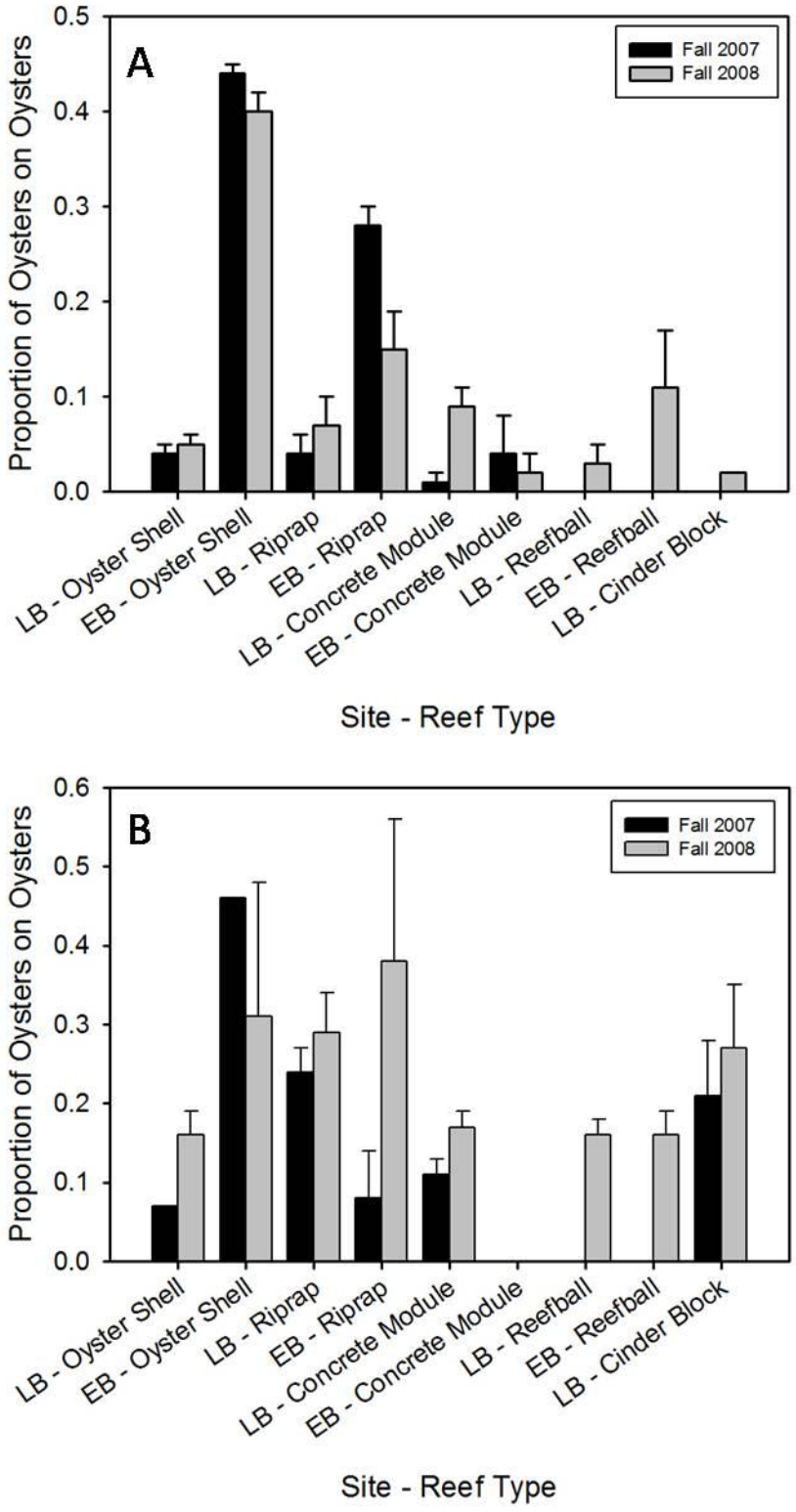


Figure 5.10: Proportion of live oysters on other oysters by site-reef type on (A) unseeded and (B) seeded oyster shell, riprap, concrete modules, reefballs, and cinder blocks in fall 2007 and fall 2008.

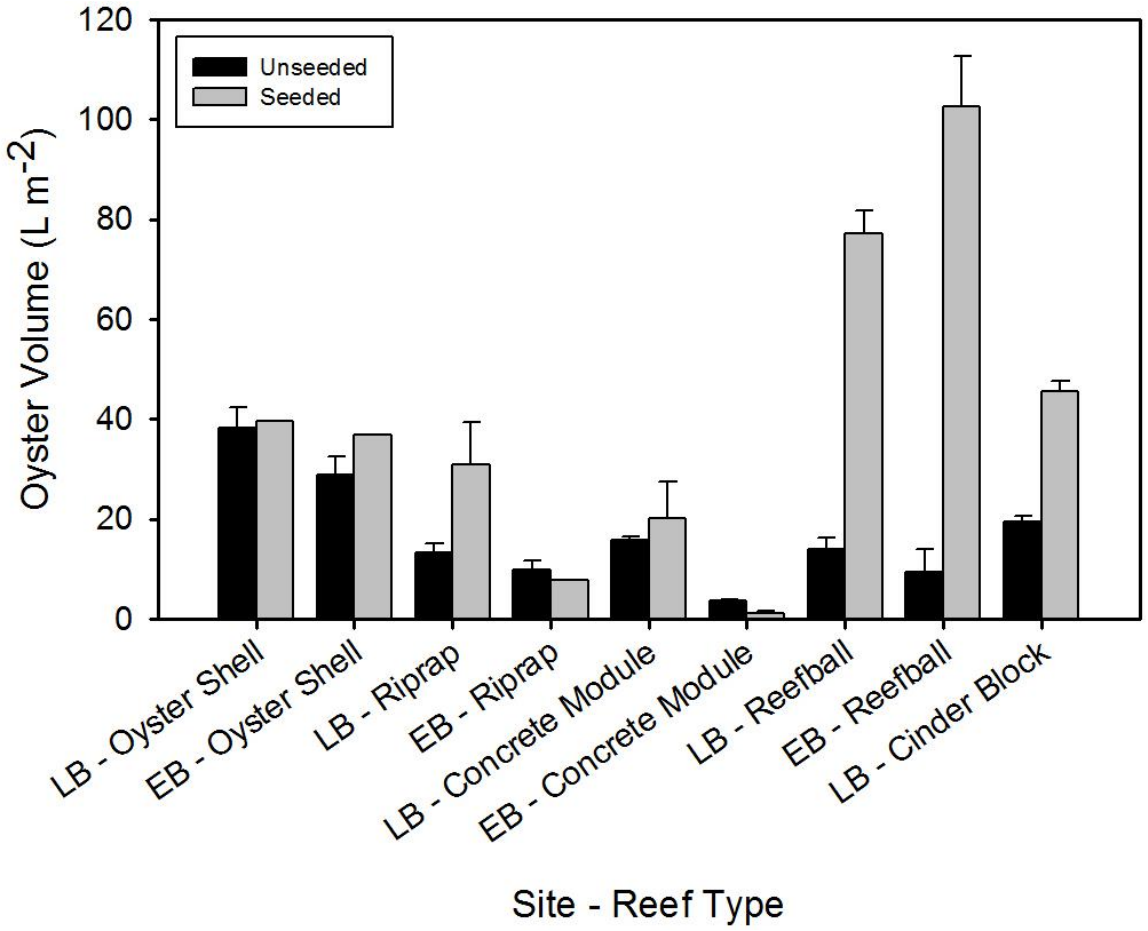


Figure 5.11: Oyster volume (L m^{-2}) by site-reef type for unseeded and seeded oyster shell, riprap, concrete modules, reefballs, and cinder blocks in fall 2008.

Table 5.5: Dermo disease intensity by (A) site-substrate-ploidy and (B) ploidy only (OS = oyster shell, RB = reefball), where Dermo disease intensity ranks are as follows: Heavy-Moderate-Light-Rare-Negative.

Site-Substrate-Ploidy	Oysters Tested	Proportion Infected	Weighted Prevalence	Oyster Disease Intensity	Nominal Disease Intensity Rank	Mean Oyster Density (No. m^{-2} +/- 1 SEM)	Mean Oyster Biomass (g AFDM m^{-2} +/- 1 SEM)
LB-OS-Diploid	10	1.00	1.95	0-5-4-1-0	3.3	1216 (123)	616.0 (99.2)
EB-OS-Diploid	10	0.80	1.80	0-5-3-0-2	2.8	483 (87)	553.6 (76.5)
EB-OS-Triploid	10	0.60	1.00	0-2-4-0-4	2.0	1339	848.4
LB-OS-Triploid	14	0.67	1.40	1-4-3-2-5	2.3	671	1376.9
EB-RB-Diploid	15	0.93	2.18	0-9-3-1-1	3.5	1347 (257)	1306.5 (341.0)

A.

Ploidy	Sample Size (n)	Disease Intensity	Disease Intensity Description
Diploid	34	3.24 (0.24)	Light
Triploid	25	2.16 (0.38)	Very Light

B.

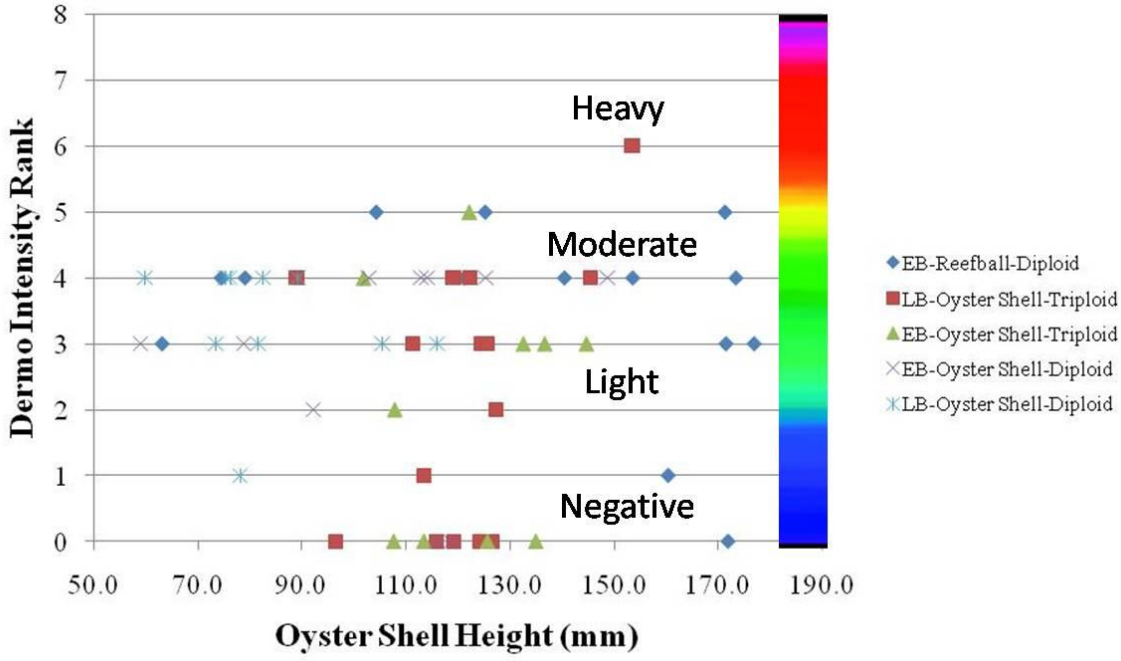


Figure 5.12: Dermo intensity rank versus oyster shell height by site-reef type-ploidy.

Mean oyster condition (CIs 1-3) was calculated for both diploid (Fig. 5.13a) and triploid (Fig. 5.13b) oysters by site-reef type. For diploid oysters, similar trends existed for CI1 (GLM, LB: $F = 318.55$, $p < 0.0005$; EB: $F = 36.71$, $p < 0.0005$) and CI2 (LB: $F = 71.92$, $p < 0.0005$; EB: $F = 8.50$, $p < 0.0005$), where CMs, and unseeded RBs at both sites had significantly higher oyster condition than the RR, OS, and seeded RB oysters (Fig. 5.13a). For triploid oysters, similar trends also existed for CI1 (GLM, LB: $F = 6.01$, $p = 0.001$; EB: $F = 26.07$, $p < 0.0005$) and CI2 (LB: $F = 4.84$, $p = 0.003$; EB: $F = 18.64$, $p < 0.0005$), where OS had significantly lower CI than RR and CMs (LB site only). Oyster density influenced the condition of both diploid (CI1: $F = 2.89$, $p = 0.123$; CI2: $F = 11.62$, $p = 0.008$) and triploid (CI1: $F = 14.12$, $p = 0.013$; CI2: $F = 10.70$, $p = 0.022$) oysters, with the lowest-density reefs containing oysters with the highest mean CI values (Fig. 5.14a-f).

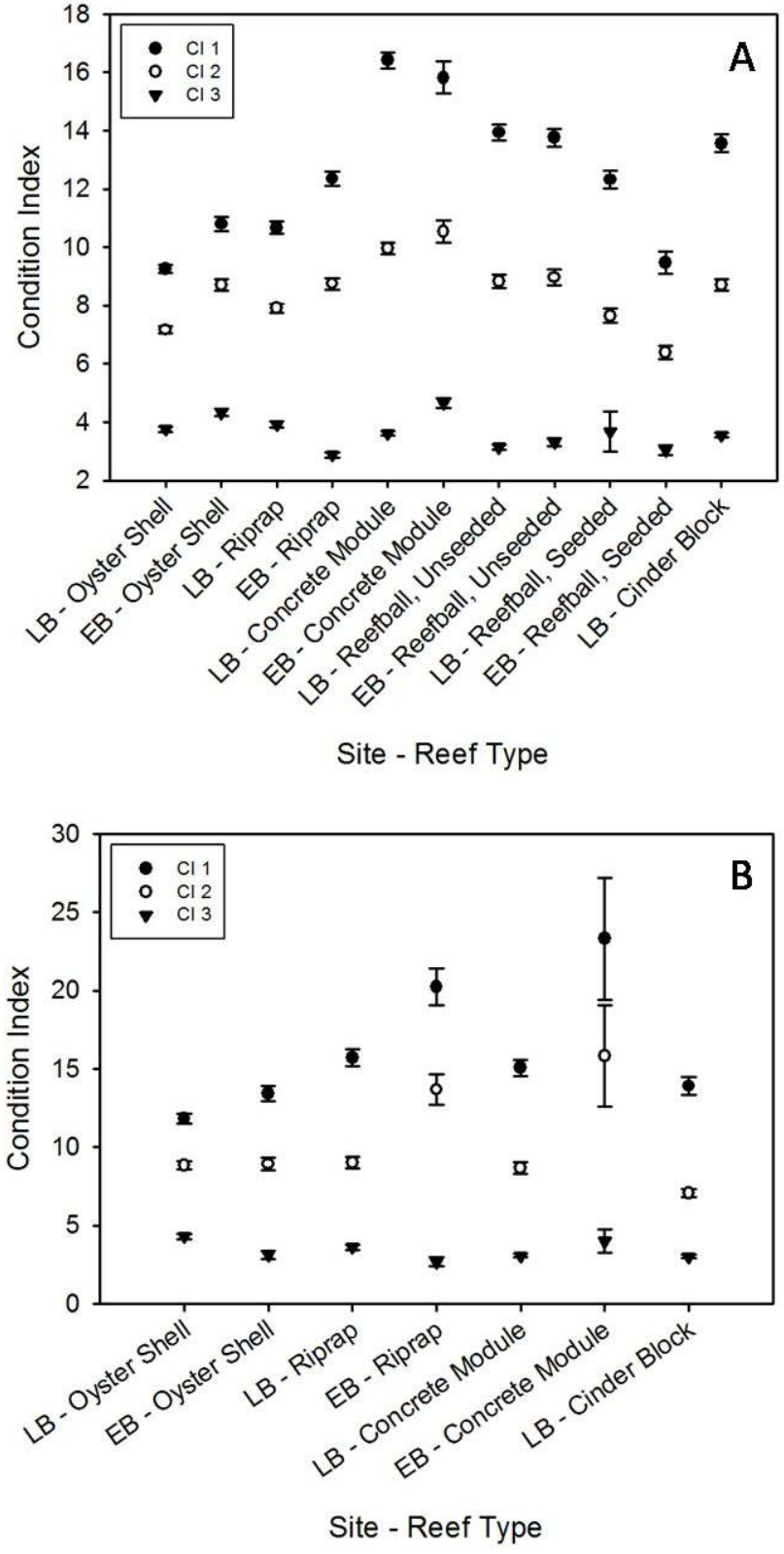


Figure 5.13: Mean oyster condition index – CI (+/- 1 SEM) by site-reef type for (A) diploid and (B) triploid oysters. CIs 1, 2, and 3 were calculated for all oysters processed for biomass.

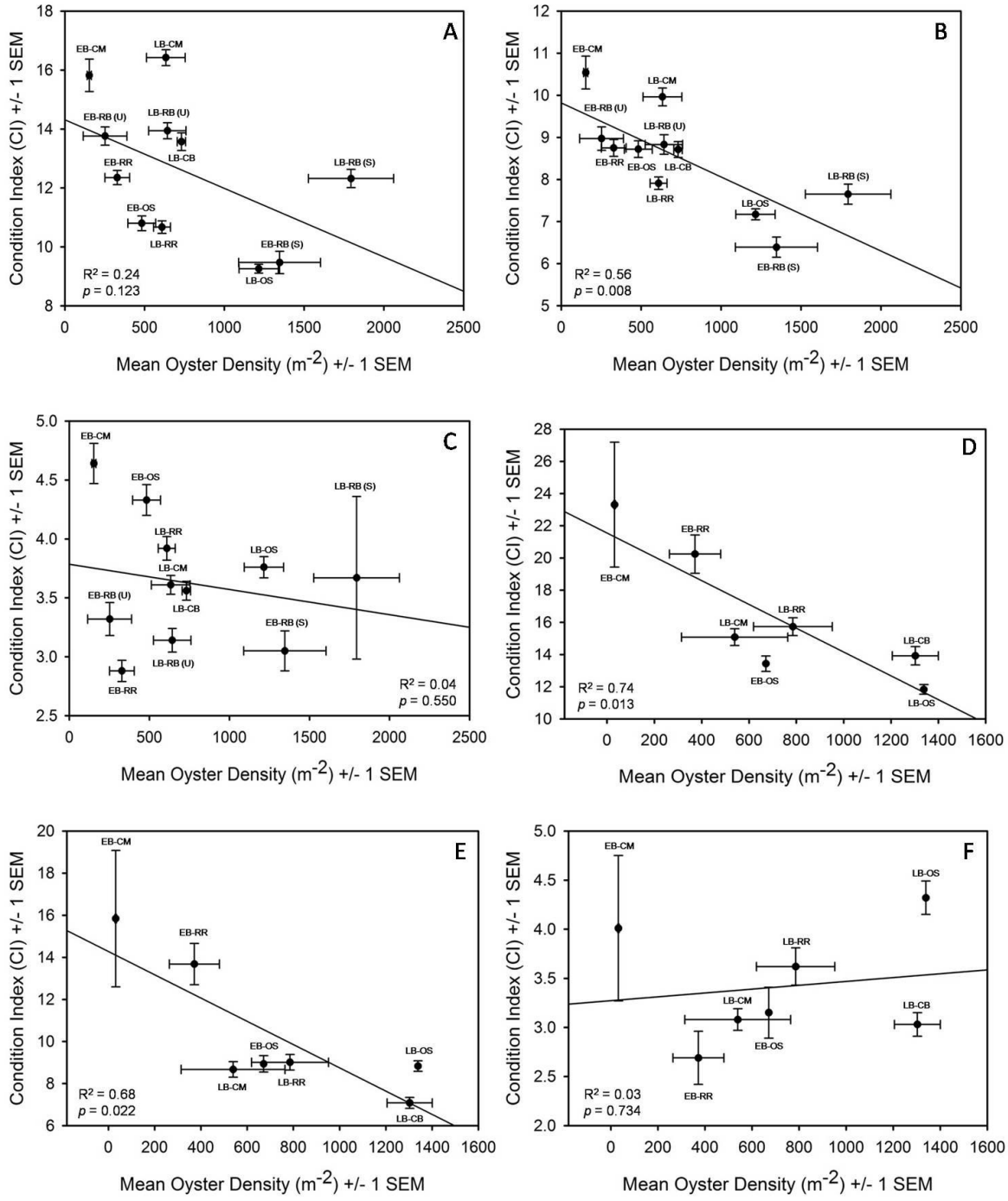


Figure 5.14: Regressions of mean oyster condition index (CI – for (A) CI 1, (B) CI 2, (C) CI 3) versus mean oyster density for unseeded oyster shell (OS), riprap (RR), concrete module (CM) reefs, reefballs (unseeded – U, seeded – S) at both sites (Linkhorn Bay – LB, Eastern Branch – EB) in fall 2008 (cinder blocks – CBs, at LB site only). Regression of mean oyster CI ((D) CI 1, (E) CI 2, (F) CI 3) versus mean oyster density for seeded (remotely-set) triploid oysters on OS, RR, CMs and CBs (at LB site only) in fall 2008.

DISCUSSION

Recruitment. In 2006, oyster recruitment was high in the Great Wicomico River, Lynnhaven River, and other Chesapeake Bay subestuaries (Southworth et al. 2007). In our study, recruitment was moderate to high in 2006 on OS reefs, but low on unseeded granite RR and CM reefs. The apparent delay of substantial recruitment on RR and CM reefs until 2007 was likely due to a need for protracted substrate conditioning. Recruitment on reefs at the muddy, EB site was generally higher than recruitment at the sandy, LB site by an order of magnitude. Similar differences were noted in oyster population densities on granite and concrete riprap lining the shores of the two sites before the experiment began. The unseeded and seeded RBs were deployed too late in the season (late September) to receive significant recruitment, except for seeded RBs, which had artificially high recruitment of hatchery-reared larvae, thereby mimicking high natural recruitment.

The release of hatchery-reared triploid larvae within enclosures containing OS, RR, and CMs produced different results at each site. Larvae recruited to substrates more readily at the LB than the EB site. The difference was likely due to better larval retention within the silt fence at the LB site than at the EB site, fence placement at LB was closer to the shoreline, minimizing larval loss above the top of the fence during high tide. OS had higher larval settlement than RR and CMs, probably because the RR and CMs were either insufficiently conditioned or because they lacked a calcium carbonate cue, which is liberated by the clean, shucked shell used in the OS treatments.

By the second year of oyster recruitment, unseeded reefs were recruiting at levels similar to seeded substrates; this apparent increase is attributable to the conditioned state of the substrates as well as the presence of live oysters from YC1. Only the seeded RBs were significantly outrecruiting their unseeded counterparts. The main goal of the RFLSE, to determine if setting oyster larvae on shells and alternative substrates *in situ* was achievable, was met and should be considered as a viable tool in oyster restoration. Our findings also demonstrate the value of reef seeding prior to deployment, as pre-seeded reefs reached an advanced stage of reef development in less than two years, which has previously been uncommon (Mann and Powell 2007).

Density and biomass. After one year post-deployment (July 2007), unseeded reefs at the EB site ranged from 200 (CM and RR) to nearly 1000 oysters m^{-2} of river bottom (OS); unseeded reefs at the LB site had mean oyster densities < 150 m^{-2}. Seeded substrates, such as RBs and OS, had mean oyster densities > 2000 m^{-2}, while the unconditioned RR and CMs had < 450 oysters m^{-2}. By the end of the experiment (fall 2008), oyster density and biomass were high on most unseeded (LB: 600-1200 oysters m^{-2}, 400-600 g AFDM m^{-2}; EB: 150-480 oysters m^{-2}, 120-550 g AFDM m^{-2}) and seeded (LB: 540-1800 oysters m^{-2}, 525-1375 g AFDM m^{-2}; EB: 30-1350 oysters m^{-2}, 20-1300 g AFDM m^{-2}) reef substrates. Reef performance was excellent relative to that of most restored oyster reefs in Chesapeake Bay (Nestlerode et al. 2007, Brumbaugh et al. 2009).

Reefs are suitable for oyster restoration and living shoreline production. Such reefs serve as a buffer for adjoining salt marshes buffeted by storms and waves, and promote marsh expansion towards the line of reefs, similarly to the function of historical fringing oyster reefs (Winslow 1881, Hargis and Haven 1999). This was hypothesized to

be the mechanism by which the Chesapeake Bay's early reefs first expanded, eventually becoming the oyster rocks catalogued by early Colonial explorers.

The alternative substrate reefs performed well, but did not outperform OS reefs, in contrast to our previous study (Dissertation Chapter 4), where granite RR had equal or higher oyster biomass than OS after three years. In that study, all substrates were conditioned at the outset of the experiment. In this study, conditioned (aged 1+ yrs) shucking-house shell was used for the OS reefs, compared to unconditioned granite and concrete reefs. This difference in conditioning likely explains the disparity between the two studies. More importantly, though, both studies demonstrated that most shell and non-shell substrates eventually reached high oyster biomass between 200 and 400 g AFDM m^{-2}, and were thus successful.

Population structure (PSS). By fall 2008, most of the unseeded and seeded reefs had obtained a PSS generally associated with mature intertidal and upper subtidal oyster reefs (Dissertation Chapters 2 and 4). Oyster PSS was more dependent on site than reef type or seeding factors. Substrate conditioning and seeding did, however, provide a catalyst for new reefs, which is particularly important in regions of low or inconsistent recruitment. Moreover, oysters on muddy bottoms (EB site) trended toward the long, thin growth form, whereas oysters on sandy, stable bottoms (LB site) tended to growth in a more round, robust form.

Growth and survival. Oyster growth of naturally-recruiting diploid oysters and seeded triploid oysters was high at both sites on nearly all substrates. Many oysters reached 70+

mm SH in one year. Triploid oysters grew to 50-60 mm in just three months after deployment. In 2+ yrs, diploid and triploid oysters grew well beyond 120 mm, with many > 150 mm. Oysters of this size are generally more fecund (diploid only) and attract many other oyster larvae during their lives and post-mortem (Galtsoff 1930). The proportion of live oysters on most unseeded (0.77-0.92 after 1 yr and 0.53-0.77 after 2 yrs) and seeded (0.92-0.95 after 1 yr and 0.63-0.75 after 2 yrs) reefs was above average for this high-salinity, disease-intense subestuary (CRC 1999). Unusually low survival on seeded RBs at the EB site was likely due to dislodgement of oysters which subsequently died of anoxia in the mud, or space and resource competition due to the high initial oyster densities on the RBs. In future oyster restoration efforts, a lower initial oyster density may allow RBs to develop optimally. The higher proportion of oysters on other living or dead oysters for seeded OS at the EB site was likely due to natural oyster recruitment in 2006 and 2007. In contrast, the RR and CMs at the EB site did not have high triploid larval recruitment and were at an initial disadvantage due to lower concentrations of chemical cues for recruitment (Turner et al. 1994, Tamburri et al. 1996).

Recruitment was often high on live and dead oysters that had previously recruited to the reefs, a phenomenon previously documented for limestone marl along the marsh in Long Creek, LRS (Dissertation Chapter 4). In that study, newly recruiting larvae generally set on the few oysters that had previously recruited to the marl, and not on the marl itself.

Oyster volume and reef accretion. Oyster volume (live and dead shell above the sediment) is critical for reef persistence because reefs need sufficiently high accretion to

offset calcium carbonate (shell) loss due to natural or fishing mortality, and physical or chemical weathering of shells (Mann and Powell 2007, Powell and Klinck 2007). Unseeded and seeded reefs with the highest oyster densities and biomasses also maintained the highest oyster volumes. The oyster shell accreted by all of the seeded substrates at the LB site and the OS and RBs at the EB site was exceedingly high, with unseeded reefs accreting 8-15 L m^{-2} yr^{-1}, and seeded RBs accreting 25-34 L m^{-2} yr^{-1}. These accretion rates are well above the minimum necessary to maintain a positive shell balance and assure reef persistence (Smith et al 2005, Mann et al. 2009b).

Pathology and condition. After nearly three years in a high-salinity subestuary of Chesapeake Bay diploid and triploid oysters had relatively light Dermo infections. Not only did oysters of the Lynnhaven River show signs of disease resistance, but many of the triploid oysters had no Dermo cells present in their tissues (a character trait selectively bred within the DEBY oyster strain). In addition, there was no relationship between Dermo intensity and oyster SH (and presumably, age). This is inconsistent with the hypothesis that oysters become more intensely infected with Dermo parasites as they grow and reach 2-3 years of age (Andrews and Ray 1988). Instead, it appears that these oysters have developed some level of disease resistance (Carnegie and Burreson 2009). Similarly, oysters from Tangier Sound (Encomio et al. 2005), Lynnhaven River (Carnegie and Burreson 2009, Dissertation Chapter 4), Rappahannock River (Dissertation Chapter 3), and Great Wicomico River (Carnegie and Burreson 2008, 2009) have all revealed the presence of disease (Dermo and MSX) resistance in the native eastern oyster, when adverse selection (e.g. fishing) is absent (Hargis and Haven 1999). Oyster

condition diminished with increasing oyster density across all reef types and for remotely-set triploid oysters on OS, RR and CM reefs, similar to that on shell and alternative substrates in the LRS (Dissertation Chapter 2) and on experimental reefs in Long Creek, LRS from 2005 to 2008 (Dissertation Chapter 4). In addition, oyster condition seemed to be better on substrates elevated above the sediment, despite oyster densities as high as 700 m^{-2}. At similar densities, RR oysters closer to the sediment had much lower oyster condition, possibly due to an effect of lower flow and sedimentation, and suggesting that reef architecture mediates the effects of high oyster density and sedimentation on oyster condition (Lenihan et al. 1999).

CONCLUSIONS

The major findings of this study were that: (1) oyster density, biomass, and reef accretion were high on unseeded and seeded reef substrates, (2) unseeded alternative substrates recruited fewer oysters and were less developed at a muddy site than at a sandy site, (3) oyster condition varied by oyster density and reef type, and (4) diploid and triploid oysters had light to moderate Dermo infections. Furthermore, *in situ* oyster larval recruitment or pre-setting of oysters on reefs gave them a relative advantage over unseeded reefs, especially when ambient physical conditions were stressful such as at the heavily silted muddy site). Therefore, living shoreline reefs, which can minimize shoreline erosion and loss of marsh, seagrass, and oyster habitat, can also serve as highly effective native oyster reefs whether constructed of shell or alternative substrates.

Additionally, the *in situ* deployment of competent oyster larvae to various reef types was successful, adding a tool for effective oyster restoration.

Acknowledgments. We express appreciation to students and staff of the Marine Conservation Biology and Marine Community Ecology Programs at VIMS, Lynnhaven River Now, Mr. Dave Schulte and the US Army Corps of Engineers, Captain Robert Jensen and Reeftek for use of a proprietary concrete module reef design and assistance in reef construction, the Chesapeake Bay Foundation for assistance in joint permitting and reef deployment, Mr. Darryl Nixon and Sea Search, Inc. for the construction, seeding and transportation of reefballs, Dr. Stan Allen and the VIMS Aquaculture Genetics and Breeding Technology Center (ABC) for assistance in reefball seeding, oyster growout, provision of larvae for remote field larval settlement, and oyster ploidy testing, Dr. Ryan Carnegie and the VIMS Shellfish Pathology Lab for Dermo testing, and Capp's Boat Works in Virginia Beach, VA for substrate storage and use of their pier. Funding was provided by grants to R.N. Lipcius and R.D. Seitz from the Norfolk Office of the USACE (Norfolk District) and from the NOAA Chesapeake Bay Office through the Blue Crab Advanced Research Consortium. Special thanks to Mr. Hap Chalmers and Dr. Wally Handeland for permission to construct the living shoreline reefs adjacent to their properties and moral support throughout the entire study.

Closing Thoughts

This dissertation explored the potential of alternative, non-oyster shell substrates to serve as restored oyster reefs through an array of surveys (Chapters 2 and 3) and experiments (Chapters 3, 4 and 5) in the Rappahannock and Lynnhaven Rivers, subestuaries of lower Chesapeake Bay. Oyster restoration in this system has proven challenging, with its fair share of scientific, logistical, fiscal, and political difficulties. It became apparent that various issues required consideration when implementing oyster restoration projects, including: (1) disease, (2) recruitment, (3) scale, and (4) reef design. Despite these potential difficulties, the results of this dissertation demonstrated conclusively that reefs constructed of shell and alternative materials were extremely effective in native oyster restoration in intertidal and subtidal habitats, even in the high-salinity waters of Chesapeake Bay where disease was thought to be insurmountable. Consequently, alternative reef structures, including living shoreline reefs, should be integrated into a comprehensive strategy to achieve successful native oyster restoration in Chesapeake Bay.

Appendices

Appendix 1.1: A Discourse on the History and Politics of Chesapeake Bay Oyster Restoration

Chesapeake Bay oyster history. Overfishing of oysters to the point of ecological extinction has dramatically changed the health of the Bay (Jackson et al. 2001), and led to the realization amongst scientists and managers that: "The Chesapeake Bay will not be restored without also restoring the native oyster." This quotation may be one of the few occasions where consensus is obtained amongst those involved in native oyster restoration in Chesapeake Bay, but emphasizes the underlying importance of oyster population recovery to the fate of the Bay in the 21st century.

The fate of the Chesapeake Bay ecosystem has been formally addressed by the President of the United States. The Administration of President Barack H. Obama (May 12, 2009) released Executive Order 13508 – Chesapeake Bay Protection and Restoration – declaring:

> "The Chesapeake Bay is a national treasure constituting the largest estuary in the United States and one of the largest and most biologically productive estuaries in the world. Restoration of the health of the Chesapeake Bay will require a renewed commitment to controlling pollution from all sources as well as

> protecting and restoring habitat and living resources, conserving lands, and improving management of natural resources, all of which contribute to improved water quality and ecosystem health. The Federal Government should lead this effort. Executive departments and agencies, working in collaboration, can use their expertise and resources to contribute significantly to improving the health of the Chesapeake Bay."

The Executive Order includes a section on 'Shared Federal Leadership, Planning and Accountability' which could set the modern precedent on how agencies interact on such extensive restoration missions. The assessment, and potential use, of alternative substrates for construction of native oyster restoration reefs is in direct alignment with the Executive Order, Part 8 – Monitoring and Decision Support for Ecosystem Management:

> Sec. 801(c): "using adaptive management to plan, monitor, evaluate, and adjust environmental management actions."

An adaptive, ecosystem-based management program for the Chesapeake Bay and the recovery of its oyster populations will require the application of diverse methods and a variety of substrates to be successful. It is the goal of this dissertation research to contribute to the ongoing, large-scale restoration (1000s of acres, > $500 million over the next 10 years) described in the Final Nonnative Oyster Programmatic Environmental Impact Statement – PEIS (USACE 2009) and outlined by President Obama and his administration.

Oyster reefs in most ecoregions where they historically occurred are in poor condition and at risk of extirpation as functional ecosystems (Kirby 2004, Lotze et al. 2006, Airoldi and Beck 2007, Coen and Grizzle 2007, Beck et al. 2009). Oyster reef

restoration, protection, and construction are important to meeting harvest, water quality, and fish habitat goals (Breitburg et al. 2000). By definition, a 'sustainable oyster harvest' is some level of oyster removal that can be sustained by oyster populations without causing further decline of the populations. If, in fact, the goal of a sustainable harvest is tenable, an unbiased quantification of such measures is necessary. Stock assessment experts are the best qualified to take on such a task. However, Chesapeake Bay oyster politics have been heavily skewed toward fishery interests for more than a century; thus, control of the definition of sustainable oyster harvest has fallen to industry-dominated, Blue- and Green-Ribbon oyster panels (BROP 2007). The conclusion reached by these panels influenced the goals set by the recent nonnative oyster PEIS which stated:

> "The purpose of this proposal is to establish an oyster population that reaches a level of abundance in Chesapeake Bay that would support **sustainable harvests** comparable to harvest levels during the period 1920-1970."

To aim for harvest levels comparable to those of the period 1920-1970 may be achievable. The greater concern is the claim that harvest levels from that period were 'sustainable.' The argument that has often been offered up by the oyster industry supporting this notion of sustainable harvest is that there was "no economic cost" to taxpayers/co-owners of the Commonwealth of Virginia's shared oyster resources.

More recently, at the Sept. 10th, 2008 Congressional Subcommittee meeting on Chesapeake Bay native oyster restoration, a representative from the Virginia Seafood Council (VSC), testified that "sustainable oyster harvests" were, in fact, maintained for the period 1920-1980 (Kellum 2008), but failed to mention that many of the spat obtained for private oyster production over those years were harvested from public

grounds. Shells had been planted in Virginia waters of the Chesapeake Bay to replenish depleted seed beds, as well as other beds, for many decades. The VSC representative's exact statement (Kellum 2008) was:

> "*tax revenues provided funding for replenishment along with* ***only small amounts of State General Funds***."

This statement is not really true, considering the multi-million dollar annual operating costs paid for by state general funds; amounts that far exceeded the tax revenue created by the oyster fishery itself (Haven et al. 1978).

Taxes were first levied on harvests in 1926. Taxes remained at token levels compared to the Commonwealth's incurred direct costs of maintaining the fishery up until 1962 (Haven et al. 1981) when a major change in the tax structure was initiated by a special "Seafood Study Commission" (Commission to Study and Revise Title 28 of the Code of Virginia: House Document No. 14, 1961) because, "prior to that time, the token taxes required were totally inadequate" (Haven et al. 1978).

The following example emphasizes the extent of the subsidy provided to the Virginia oyster fishery, public and private, during the period 1970-1975. All costs are adjusted from Haven et al. (1978) and represent 2008 dollars:

Shells planted by repletion program cost – **$ 10,076,772.00**
Tax revenue generated by "repletion tax" – $ 1,203,254.00
Tax revenue generated by public ground fishery – $ 120,325.00
Tax revenue generated by tax on seed taken from public grounds – $ 168,312.00

The revenue shortfall was > $8.5 million. It is important to note that most of the planted shells were planted to maintain "seed beds" that provided seed to the private leasehold fishery, though a significant portion was planted on public grounds of the wild oyster fishery to help maintain their integrity. What is clear here, though, is that the

public/private oyster fishery was not self-sustaining from a tax revenue standpoint and that significant outlays of state general funds were used to keep it operational. Seed removed from public grounds in the James, Great Wicomico, Piankatank and other Rivers in both states resulted in: (1) loss of the oyster reproductive base (markets and smalls), (2) habitat/reef damage (impacting oysters and most of the other species that rely on them as part of their life cycle), and (3) depletion of oyster recruits (the next generation of oysters, the spat).

Harvests were not 'sustainable;' they were 'sustained' as a "state-run farm" for oyster fishermen paid for with state and, more recently, federal funds with massive infusions of shell, seed, and money. Despite all this activity, it is important to note that the oyster populations and habitat quality steadily declined throughout this period. An honest accounting of the Chesapeake Bay oyster's history enables oyster restoration practitioners to set realistic goals with a clear understanding of what contributed to the oyster fishery and population collapse, and what, if any, practices were sustainable.

The Norfolk District of the USACE has led a successful, large-scale restoration effort for native oysters in Virginia's Great Wicomico River (GWR) – the first of its kind (Schulte et al. 2009). The distinct difference of the GWR and other native oyster restoration efforts was a move away from two-dimensional low-relief reefs (LRRs) – 2 to 4 inches – to three-dimensional high-relief reefs (HRRs) – 12 to 18 inches (Schulte et al. 2009). Since 1993, the Virginia Marine Resources Commission (VMRC), led by a state mandate to support the oyster fishery, maintained a repletion program that included LRRs and tall, mounded HRRs; however, limited funds and minimal industry support for building large sanctuary reefs caused VMRC to avoid constructing larger, more gently

sloping HRRs such as those created by the USACE in the GWR. Major concerns were raised regarding the direction of Chesapeake Bay native oyster restoration soon after the signing of the 2000 Chesapeake Agreement. The following were a few of the major issues revealed by a number of seminal ecological oyster restoration papers (VIMS 1996, Lenihan and Peterson 1998, Breitburg 1999, CRC 1999, Coen et al. 1999, Eggleston 1999, Eggleston et al. 1999, Lenihan 1999, Allen et al. 2000, Coen and Luckenbach 2000, Peterson et al. 2003, Grabowski 2004, Newell 2004, Newell et al. 2004, Luckenbach et al. 2005, Smith et al. 2005, Mann and Powell 2007, Powers et al. 2009, Beck et al. 2009): (1) LRRs are generally not sustainable due to heavy siltation and infrequent recruitment, (2) the scale of sanctuary reefs likely needs to be many tens of acres, up to a few hundred acres in some tributaries, to boost or create a self-sustaining oyster metapopulation, (3) both Maryland and Virginia have a severe shortage of available dredged and shucked house shell, and (4) to attempt a bay-wide restoration effort all at once too expensive (> $200 million). Oyster models (Smith et al. 2005, Mann and Powell 2007) show that the limited annual areal extent of a LRR-centric restoration program is ineffective in restoring oyster bottom at a rate commensurate with its rate of degradation. Predictions from the models indicate that even greatly expanding the scale of existing restoration activities will not be successful at restoring oyster habitat unless other reef configurations and higher quality substrates are utilized. Ecological oyster reef restoration is now focused on a tributary-by-tributary strategy, with a large-scale investment in HRRs to achieve sustainable oyster populations.

Oyster diseases. After years of destructive overfishing, massive cultch removal, and degrading water quality conditions (Rothschild et al. 1994), disease was the final insult for the native oyster in Chesapeake Bay. This leads to another major point of refutation with the VSC representative's Congressional testimony (Kellum 2008):

> "There is no scientific evidence that any significant disease resistance is occurring naturally in the [Chesapeake] Bay."

This quotation is wrong despite the frequency of similar statements in the media and marine resource commission meetings.

First, the increasingly intensive and mechanized fishing contributed to leveling the profile of oyster reefs which, in turn, altered the flow regime over the reefs (Lenihan et al. 2001). In one experiment, oysters with the highest proportion of individuals infected with Dermo, highest intensity of infection, and highest mortality were located at the base of reefs, where flow speeds and food quality were lowest and sedimentation rates highest (Lenihan et al. 1996). The restoration of oyster reefs of adequate reef height can improve flow, reduce sedimentation, and help alleviate the negative effects of disease on resident oysters.

Second, oyster pathology experts at the Virginia Institute of Marine Science (VIMS) have presented oyster disease resistance data at meetings of the latest Virginia Blue Ribbon Oyster Panel (BROP 2007); meetings that included the VSC representative who testified before Congress. Natural disease resistance is developing in many sub-populations of native oysters in the lower Chesapeake Bay. The long-term monitoring of Dermo and MSX in Virginia's native oysters have revealed that, in the lower portion of most of Virginia's major Bay tributaries (classified as Zone 3, high-salinity, high disease-

intense waters), there are significant populations of wild native oysters (Carnegie and Burreson 2009). The systems where natural disease resistance has been documented include the Lynnhaven River (Dissertation Chapters 4 and 5), Great Wicomico River (Carnegie and Burreson 2008, 2009), Elizabeth River (Burke and Schulte *unpublished data*), Rappahannock River (Dissertation Chapter 3, Lipcius and Burke 2006), and Tangier Sound (Encomio et al. 2005), where it was first documented. In most cases, these oysters have been found in sanctuaries (intentional or *de facto*) and in the high-salinity, high-disease zones that are "not supposed to have any large, old oysters"; many of these areas have not been fished in decades.

The Lynnhaven and Elizabeth Rivers have not had a public fishery in 30 and 50 years, respectively, due to fecal coliform levels, chemical/metal contamination, etc. Large (4" and longer), wild oysters found in Lynnhaven River have been used as hatchery broodstock for a 'spat-on-shell' program (paid with funds from the City of Virginia Beach, ~\$100,000 yr^{-1}) designed to support ecological restoration headed by the Norfolk District of the USACE in that system and serve as a model for future stocking efforts with wild strains of native oysters with significant disease resistance. One noteworthy find has been the monitoring of a group of oysters (2.5 years) from larval set to > 177 mm (7 inches) in shell height (Dissertation Chapter 5). The VIMS Aquaculture and Biotechnology Center and the Chesapeake Bay Foundation use large, wild, disease-resistant oysters from various Bay tributaries as broodstock for their respective spat-on-shell programs.

The lower Rappahannock River, below the Norris Bridge (Rte. 3), was maintained as a no-harvest area for 20 years. For 15 of those 20 years, an artificial concrete reef

strategically-placed at a depth of 7 m on hard sand by the Rappahannock Preservation Society (designed by Captain Robert Jensen, 1994) has been home to a natural oyster reef with oysters as large as 127-152 mm (5-6 inches) in shell height (Dissertation Chapter 3). Despite these and other examples of large healthy oysters in Virginia's lower Bay tributaries, claims that there is "no natural disease resistance in oysters" and that "sanctuaries don't work" have persisted.

At the 2008 Congressional Subcommittee hearing, the VSC representative expounded upon the participation of watermen to deliver oysters that are > 4 inches to sanctuary reefs in a buy-back program that has the intended outcome of enhancing recruitment in the nearby fished areas. Acknowledging the presence of these oysters recognizes that oysters do grow to that size; disease testing of such oysters as part of a unique, 50-year dataset at the Virginia Institute of Marine Science has revealed that oysters such as these have lower than average Dermo and MSX intensities, indicative of the development of some level of disease resistance (Carnegie and Burreson 2009). Additionally, the Commonwealth of Virginia spent $480,000 of general funds in 2007 and 2008 to purchase 40,000 bushels of spat-on-shell produced downriver of the USACE Great Wicomico River sanctuary oyster reefs, conservatively estimated at roughly 75 million oyster spat. Justification for selecting these oysters over the traditional seed-producing areas of the James River was the 'presence of greater disease resistance amongst the Great Wicomico River oysters.' The promoters of this shift from the status quo were representatives from the VSC and the Virginia Marine Resources Commission (VMRC) staff oyster specialist, two of the most vocal opponents of the notion that natural oyster disease resistance has emerged in native oyster populations (VMRC 2007, VMRC

2008). One important point of clarification is that this argument does not refer to naïve stocks of oysters such as those located in the low-salinity reaches of the upper Rappahannock River at Ross Rock versus downriver oyster populations consistently exposed to Dermo/MSX disease intensity associated with higher salinities; the argument here is that, within regions of perennial exposure to these disease parasites, Chesapeake Bay oysters have started to develop resistance to both Dermo and MSX (Carnegie and Burreson 2009).

Sanctuaries v. harvest grounds. A major component of an effective oyster restoration strategy is its inherent ability to be scaled up to a level appropriate to the prescribed estuary. Natural recovery of different Chesapeake Bay tributary oyster stocks (i.e. the Lynnhaven strain, the Elizabeth River strain, etc.), especially to the extent documented thus far, is particularly encouraging for those conducting ecological restoration at a large scale in a tributary-by-tributary fashion. What we are learning from focusing intensely in one tributary aids us when we begin a similar process of restoration in each subsequent tributary. With limited funds to deploy and effectively monitor ecological oyster restoration projects, this strategy has allowed the Norfolk District of the USACE to scale up to a size that is biologically meaningful for the oyster stock in that tributary. For example, prior to the Great Wicomico River Oyster Restoration Project, oyster sanctuaries in Virginia were only built in one-acre plots and make up < 1 % of the original oyster grounds of a given tributary and < 10 % of the total acreage restored in a project, often in sub-estuaries with many 1,000s of acres of formerly productive oyster reefs. Given the suggested scale of restoration in marine protected areas include up to 50% of the original population to hedge successfully against overfishing (Lauck et al.

1998), such small sanctuaries were likely ecologically trivial, and should not have been expected to significantly influence the oyster stock where they were present. However, the Great Wicomico River Project built sanctuary reefs on > 80 of the 480 acres of Baylor (public) grounds available for restoration. This sanctuary reef network was the first built large enough to significantly impact the local oyster population, and it has (Schulte et al. *manuscript in prep*). The opportunity to build 'sanctuaries exclusively' emerged only after an economic analysis revealed that the typical Maryland/Virginia strategy of building harvest grounds/reserves for the 'put-and-take' public fishery was not in the federal interest. Defining what the 'federal interest' means to oyster restoration is critical in discerning how this paradigm shift occurred.

In 2003, the Norfolk District of the USACE conducted an in-house economic analysis of harvest ground production in Virginia. To be in the 'public interest' and allow the USACE to support a 'put-and-take' fishery, the benefit-to-cost ratio needs to be a minimum of 1:1, with a preferred ratio of 3:1. The study revealed that harvest ground production yielded only 7 cents for each dollar spent, or a ratio of 0.07:1. The USACE at Norfolk District could not continue "put-and-take" fishery restoration and switched their program to its current focus of population enhancement and recovery, tributary by tributary.

More recent information has revealed that the Norfolk District economic analysis was indeed accurate. An analysis entitled, "Estimated Return to Harvest due to the Maryland Department of Natural Resources Repletion Activities 1990-2006" revealed that the benefit-to-cost ratio was 0.05:1, or 5 cents to the dollar (Herberich 2006). In 2008, Maryland's Oyster Advisory Commission (OAC) also stated that, despite their best

efforts, they could not show > 1:1 ratio of benefit-to-cost (OAC website) for their managed reserve system; however, they remain hopeful that one day they will.

Smith et al. (2005), from 1999 to 2001, used an acoustic seabed classification system and underwater videography to assess oyster habitat conditions throughout Maryland's portion of Chesapeake Bay relative to eastern oyster recruitment and habitat restoration activities. They concluded that the majority of oyster bottom in Maryland is extremely degraded and that no reasonable increase in the scale of present management practices (i.e., restoration and harvest) will reverse this habitat decline. Their results indicate that the ultimate fate of oyster shell spread by the Maryland Department of Natural Resources over the last 40 years has been to revert to barren, sedimented bottom covered by sand or mud. Thus, the program's contribution to the enhancement of eastern oyster populations and habitat has had minimal long-term benefit – a considerable negative endorsement for harvest or managed reserves.

Finally, a recent study concluded that harvest grounds are not in the Commonwealth of Virginia's public interest (Santopietro et al. 2009). This fishery restoration strategy, should it be continued, is a clear statement that the public fishery is still being subsidized at the cost of taxpayer dollars and degrades the credibility of those engaged in current and future Chesapeake Bay native oyster restoration activities. Ecological oyster reef restoration efforts applying the strategy of large-scale sanctuaries are preferable to the construction of more harvest grounds. It is clear that the past (and for the most part current) approaches have failed to produce desired results.

Alternative vs. shell substrate. Native oyster restoration in Chesapeake Bay has become a multi-agency effort with non-profit, local, state, and federal partners who have

committed > $50 million over the last 15 years and are poised to invest an estimated $500 million over the next ten years (USACE 2009). Ecological oyster reef restoration's new tributary-by-tributary strategy, focused on large-scale sanctuary reef production, is geared toward achieving sustainable oyster populations, with the Great Wicomico River (Schulte 2003) serving as the first full-scale attempt; the Lynnhaven River System – LRS (Schulte et al. 2006) is the second. The LRS was a well-known oyster-producing estuary and was the source of the historically-coveted oysters, "Lynnhaven Fancies" (Chipman 1948), until disease, declines in water quality, and frequent shellfish closures shut down the fishery in the early 1970s (Schulte et al. 2006). Led by the Norfolk District of the USACE, project partners have sought to revive the river's oyster stocks through a combination of reef construction and planting of disease-tolerant native oysters. The LRS was selected for large-scale ecological oyster restoration because it has supported natural oyster populations in recent years (Brumbaugh et al. 2000), had historical oyster grounds delineated by the Baylor Survey in the late 1890s (Baylor 1894, Chipman 1948), had engaged local non-profit organizations (i.e., Lynnhaven River NOW), and had a history of regular spat settlement and significant private oyster production before the oyster disease MSX became established in the 1960s (Chipman 1948). The USACE's projected overall investment in the LRS is $6.59 million to restore up to 111.3 acres of oyster habitat and by Year 5 (2012) with an associated oyster biomass (predicted) of approximately 130,000 kg on the restored habitat alone (Schulte et al. 2006). The proposition to construct oyster habitat out of shells and/or alternative materials represented an expansion of substrate options. Alternative materials/substrates are natural or artificial structures that may be used to construct oyster reefs. Such reefs can

incorporate shells but are not made solely of dredged or shucked oyster shells. Use of alternative materials as substrate for eastern oyster, *C. virginica*, recruitment and reef accretion is an established oyster reef restoration technique in the southeastern United States, including the Gulf of Mexico oyster-producing states. The USACE incorporated alternative materials into this adaptive management plan because available shell resources have been limited and recent research on alternative substrates for oyster reef restoration in Chesapeake Bay has shown promise in the Rappahannock and Lynnhaven River. Intertidal and subtidal oyster shell reefs were surveyed throughout the LRS since 2005 (Luckenbach and Ross 2006, Dissertation Chapter 2). Nestlerode et al. (2007) found that small-scale intertidal and subtidal restored oyster reefs succeeded in high salinity waters along Fisherman's Island. Another subtidal oyster reef population in a high salinity zone was quantified in 2005 at the mouth of the Rappahannock River in the form of a prominent sustainable constructed concrete modular reef (Lipcius and Burke 2006, Dissertation Chapter 3).

Finally, the most convincing evidence of large-scale subtidal oyster populations on restored oyster reefs has come from the Great Wicomico River with an oyster metapopulation more than 50 times the river's estimated oyster stock in 1994 (Schulte et al. 2009). Within the Great Wicomico River system, as in the LRS, there is a thriving intertidal band of oysters on the granite and concrete riprap revetments that line the shores of many homeowners. The USACE applied lessons of success learned in the Great Wicomico River native oyster restoration project, including reef scale (tens, instead of tenths, of acres) and reef height (25-45 cm, instead of 8-12 cm), when it constructed roughly 60 acres of subtidal high-relief oyster shell reefs (fall/winter of 2007 and 2008)

throughout the LRS. With a system-wide background oyster population of 10-20 million oysters (Luckenbach and Ross 2006), the USACE restored reefs should not be recruitment (spat) limited and, hopefully, will perform as well as the Great Wicomico River restored oyster reefs. Underwater video monitoring of these reefs (June 2009) confirmed that they are progressing in a trajectory similar to the Great Wicomico River sanctuary reefs.

Final remarks. Alternative substrate reefs can serve as the foundation from which natural oyster reefs can grow and reclaim their dominant role as ecosystem engineers. If we heed the lessons of history and don't become overwhelmed by the politics, Science and Nature may prevail in a mutual partnership. The renewed commitment made by President Obama's administration, Congress, and the scientific community at large presents us with hope for the future of the Chesapeake Bay ecosystem and the oysters within it.

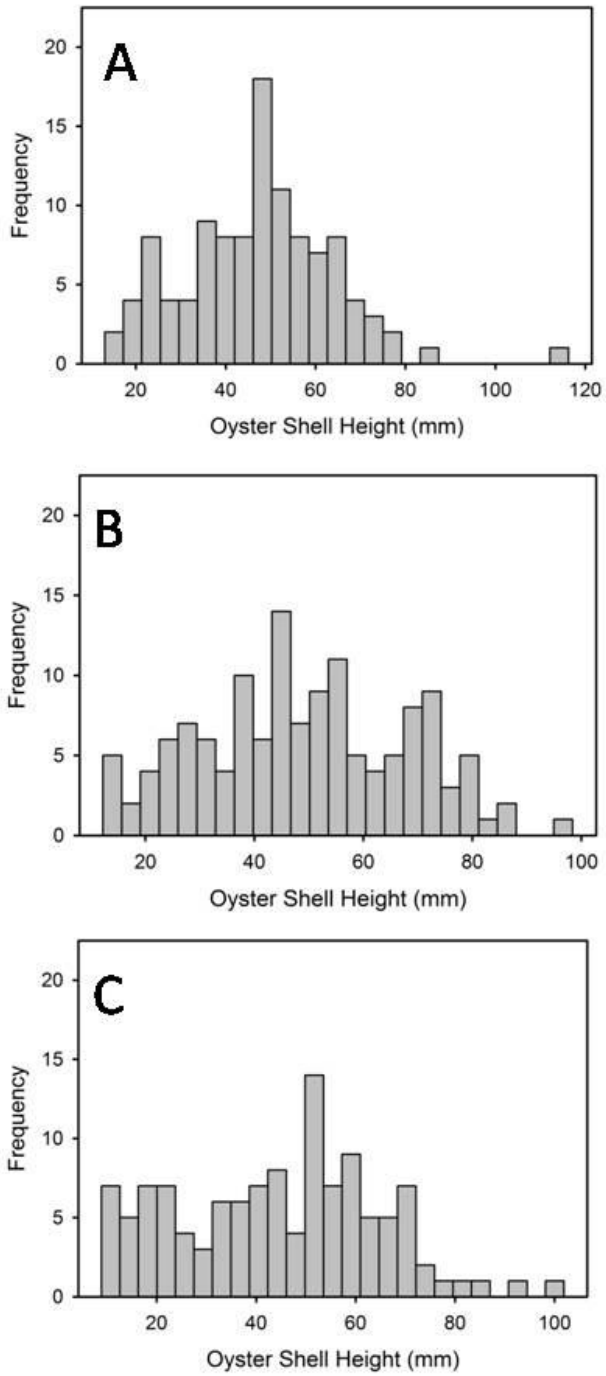
Appendix 2.1: Riprap oyster counts, oyster density, DM, AFDM, and surface area data.

Site	Rock	SA (m^2)	No. of Oysters	No. of Mussels	Mean Oyster SH (mm)	Oysters/SA (No./m^2)	DM (g) Pooled	AFDM (g) Pooled	DM (g) Ind. Site	AFDM (g) Ind. Site	DM/SA Pooled (g/m^2)	AFDM/SA Pooled (g/m^2)	DM/SA Ind. Site (g/m^2)	AFDM/SA Ind. Site (g/m^2)
1	1	0.145	7	64 Total	33.3	48	0.789	0.675	0.723	0.658	5.442	4.655	4.989	4.540
1	2	0.036	5	--	43.8	138	1.124	0.961	1.113	1.006	31.057	26.536	30.732	27.792
1	3	0.360	9	--	57.2	25	3.640	3.107	3.863	3.472	10.111	8.632	10.731	9.645
1	4	0.202	5	--	33.6	25	0.821	0.702	0.838	0.756	4.066	3.473	4.150	3.741
1	5	0.041	8	--	54.2	195	3.072	2.623	3.271	2.939	74.938	63.972	79.783	71.691
1	6	0.036	8	--	45.2	221	1.823	1.558	1.783	1.614	50.351	43.027	49.254	44.590
1	7	0.160	13	--	35.3	81	2.370	2.024	2.477	2.228	14.810	12.647	15.481	13.924
1	8	0.106	5	--	46.8	47	1.340	1.144	1.381	1.244	12.592	10.754	12.978	11.691
1	9	0.054	4	--	40.1	74	0.665	0.569	0.626	0.568	12.316	10.530	11.584	10.522
1	10	0.030	1	--	22.3	33	0.033	0.028	0.023	0.022	1.089	0.934	0.782	0.728
2	1	0.111	2	3	22.8	18	0.120	0.103	0.183	0.133	1.079	0.924	1.648	1.198
2	2	0.302	42	75	50.7	139	12.302	10.507	15.126	13.284	40.802	34.851	50.170	44.060
2	3	0.049	1	7	73.3	20	0.567	0.484	0.674	0.610	11.498	9.816	13.679	12.368
2	4	0.176	2	9	75.1	11	1.246	1.063	8.300	7.346	7.093	6.054	47.269	41.834
2	5	0.080	2	0	64.4	25	6.449	5.508	1.460	1.337	80.615	68.854	18.246	16.714
2	6	0.230	20	111	45.4	87	4.874	4.163	6.047	5.269	21.190	18.102	26.290	22.907
2	7	0.300	73	0	47.1	243	18.532	15.832	23.032	20.039	61.773	52.772	76.772	66.797
2	8	0.310	32	122	45.4	103	8.417	7.189	10.305	9.084	27.153	23.191	33.243	29.305
2	9	0.093	15	75	58.9	161	5.837	4.985	7.084	6.294	62.699	53.544	76.086	67.606
3		Dislodged	44	0	41.4	***	8.694	7.429	7.656	6.755	***	***	***	***
3	1	0.395	38	1	44.3	96	8.790	7.510	7.837	6.909	22.252	19.012	19.840	17.492
3	2	0.020	4	0	57.1	204	1.549	1.323	1.503	1.313	79.031	67.479	76.677	66.965
3	3	0.050	34	10	48.5	680	8.970	7.663	8.120	7.144	179.400	153.263	162.401	142.882
3	4	0.103	17	0	50.2	165	4.779	4.082	4.357	3.831	46.394	39.632	42.298	37.191
3	5	0.099	37	2	40.1	375	7.021	6.000	6.162	5.439	71.210	60.851	62.491	55.161
3	6	0.240	12	0	58.0	50	4.170	3.563	3.765	3.317	17.376	14.844	15.687	13.820
3	7	0.147	11	13	54.4	75	3.884	3.316	3.695	3.234	26.419	22.561	25.135	22.002
3	8	0.093	40	7	39.5	432	7.181	6.136	6.292	5.552	77.629	66.339	68.017	60.019
3	9	0.043	6	1	42.8	138	1.112	0.950	0.899	0.802	25.616	21.901	20.704	18.472
3	10	0.138	24	4	53.1	174	8.396	7.169	8.204	7.155	60.844	51.950	59.448	51.846
3	11	0.113	14	0	32.7	124	1.409	1.206	1.033	0.931	12.528	10.719	9.183	8.278
3	12	0.162	68	34	38.7	421	12.000	10.255	10.569	9.321	74.305	63.496	65.444	57.716
3	13	0.011	1	1	33.5	92	0.087	0.074	0.056	0.051	7.951	6.809	5.156	4.720
3	14	0.116	42	0	47.1	362	3.168	2.704	3.019	2.789	27.307	23.309	26.026	24.047
3	15	0.094	12	3	42.6	127	0.642	0.548	0.616	0.499	6.811	5.820	6.538	5.301
3	16	0.027	3	1	48.3	110	10.767	9.199	10.329	8.462	395.835	338.198	379.748	311.107
4	1	0.171	4	0	25.8	23	0.197	0.169	0.197	0.169	1.152	0.987	1.152	0.987
4	2	0.212	1	61	26.1	5	0.048	0.041	0.048	0.041	0.225	0.193	0.225	0.193
4	3	0.169	1	0	15.6	6	0.014	0.012	0.014	0.012	0.082	0.071	0.082	0.071
4	4	0.143	2	5	***	14	***	***	***	***	***	***	***	***
5	1	0.022	7	11	34.7	324	0.723	0.618	0.683	0.602	33.453	28.632	31.605	27.880
5	2	0.120	5	4	42.6	42	0.883	0.755	0.867	0.755	7.356	6.290	7.227	6.293
5	3	0.010	1	3	59.4	96	0.342	0.293	0.346	0.299	32.917	28.125	33.276	28.706
5	4	0.036	2	3	28.9	56	0.138	0.119	0.128	0.114	3.856	3.302	3.561	3.165
5	5	0.069	1	21	63.4	14	0.400	0.342	0.409	0.351	5.801	4.955	5.924	5.093
5	6	0.095	1	4	43.4	11	0.161	0.138	0.155	0.136	1.698	1.452	1.634	1.433
5	7	0.120	5	8	31.3	42	0.505	0.432	0.489	0.427	4.204	3.596	4.071	3.561
5	8	0.143	1	0	40.3	7	0.135	0.116	0.128	0.113	0.944	0.808	0.898	0.791
5		Dislodged	5	0	32.7	Unconsolidated	0.522	0.447	0.502	0.440	***	***	***	***
6a CE	1	0.270	15	4	38.2	56	2.681	2.291	2.364	2.086	9.930	8.485	8.755	7.725
6a CE	2	0.212	7	2	45.4	33	1.359	1.162	1.115	0.986	6.410	5.481	5.259	4.650
6a CE	3	0.226	3	2	31.2	13	0.254	0.218	0.189	0.167	1.124	0.962	0.835	0.740
6a CE	4	0.358	48	23	43.3	134	11.170	9.540	10.210	9.001	31.244	26.686	28.558	25.176
6a CE	5	0.040	1	0	39.3	25	0.198	0.170	0.160	0.142	4.956	4.238	4.002	3.540
6a CE	6	0.098	5	2	47.3	51	1.079	0.922	0.962	0.849	10.974	9.375	9.786	8.634
6a CE		Dislodged	10	2	41.4	Unconsolidated	1.723	1.473	1.454	1.285	***	***	***	***
6b CS	1	0.136	24	18	44.2	176	5.896	5.036	6.897	6.046	43.352	37.027	50.715	44.454
6b CS	2	0.293	24	9	40.4	82	4.591	3.923	5.269	4.632	15.696	13.413	18.012	15.836
6b CS	3	0.460	94	11	41.1	204	18.856	16.111	21.768	19.120	40.992	35.024	47.322	41.564
Yopp	1	0.138	20	9	52.6	145	6.405	5.471	6.071	5.202	46.585	39.787	44.155	37.832
Yopp	2	0.147	14	50	49.7	95	4.116	3.516	3.905	3.351	28.002	23.918	26.565	22.795
Yopp	3	0.115	7	27	45.2	61	1.431	1.223	1.371	1.196	12.497	10.684	11.972	10.443
Yopp	4	0.170	37	45	43.9	218	10.874	9.285	10.272	8.753	63.965	54.615	60.423	51.490
Yopp	5	0.114	37	74	47.4	325	11.650	9.949	11.030	9.431	102.197	87.276	96.752	82.724
Yopp	6	0.030	2	9	50.0	67	0.622	0.531	0.593	0.514	20.721	17.707	19.771	17.125
Yopp	7	0.157	30	97	57.1	191	7.337	6.268	6.978	6.015	46.734	39.927	44.448	38.310
Yopp	8	0.390	124	84	46.0	318	29.044	24.812	27.611	23.783	74.472	63.621	70.796	60.982
Yopp	9	0.032	2	1	44.7	63	0.220	0.188	0.213	0.188	6.884	5.889	6.647	5.878
Yopp	10	0.150	5	12	32.6	33	1.165	0.994	1.111	0.931	7.765	6.629	7.405	6.209
Yopp	11	0.155	5	40	73.8	32	1.427	1.220	1.371	1.181	9.207	7.868	8.847	7.616
Yopp	12	0.105	2	20	54.1	19	1.193	1.019	1.145	0.981	11.330	9.681	10.872	9.317

Appendix 2.1 continued

Site	Rock	SA (m^2)	No. of Oysters	No. of Mussels	Mean Oyster SH (mm)	Oysters/SA (No./m^2)	DM (g) Pooled	AFDM (g) Pooled	DM (g) Ind. Site	AFDM (g) Ind. Site	DM/SA Pooled (g/m^2)	AFDM/SA Pooled (g/m^2)	DM/SA Ind. Site (g/m^2)	AFDM/SA Ind. Site (g/m^2)
7	1	0.145	114	5	39.1	786	16.662	14.250	11.519	10.493	114.909	98.277	79.442	72.366
7	2	0.325	212	10	40.2	652	33.062	28.274	22.883	20.789	101.730	86.998	70.409	63.968
7	3	0.303	181	34	42.3	598	36.930	31.556	25.569	22.920	122.002	104.249	84.471	75.719
8	1	0.144	16	18	60.2	111	6.824	5.826	5.597	4.959	47.489	40.543	38.947	34.510
8	2	0.165	63	46	43.3	382	14.241	12.166	12.219	10.860	86.311	73.733	74.054	65.815
8	3	0.234	130	47	49.5	555	28.844	24.641	24.770	22.017	123.160	105.212	105.764	94.008
8		Dislodged	26	9	43.2	Unconsolidated	7.714	6.588	6.488	5.759	***	***	***	***
9	1	0.330	47	69	47.5	142	11.419	9.758	14.160	10.901	34.604	29.569	42.910	33.032
9	2	0.101	0	37	***	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9	3	0.029	9	1	38.2	313	1.266	1.083	1.224	1.263	43.946	37.588	42.483	43.847
10	1	0.380	51	11	37.2	134	3.865	3.305	3.725	3.367	10.170	8.699	9.802	8.860
10	2	0.460	95	4	46.5	207	11.687	9.988	10.992	9.810	25.406	21.712	23.895	21.327
10	3	0.460	51	4	42.5	111	19.897	17.000	18.598	16.554	43.253	36.957	40.431	35.987
10	4	0.065	29	15	33.1	446	5.133	4.393	5.022	4.573	78.976	67.583	77.254	70.347
10	5	0.085	6	15	53.3	70	1.779	1.520	1.707	1.476	20.883	17.843	20.036	17.327
11	1	0.340	125	55	34.7	368	16.014	13.694	14.074	11.786	47.101	40.277	41.393	34.664
11	2	0.068	8	2	27.5	118	0.485	0.416	0.431	0.348	7.136	6.113	6.331	5.121
11	3	0.020	1	0	40.8	278	4.131	3.532	3.628	3.043	45.897	39.245	40.317	33.813
11	4	0.069	8	0	36.4	117	0.248	0.212	0.217	0.183	3.610	3.086	3.168	2.667
11	5	0.090	25	3	51.9	50	1.150	13.233	1.010	11.379	12.781	147.029	11.221	126.432
11		Dislodged	21	2	51.1	Unconsolidated	15.595	0.983	13.710	0.849	***	***	***	***
11	6	0.234	49	9	46.3	209	11.719	10.013	10.251	8.731	50.082	42.791	43.806	37.312
11	7	0.040	2	0	35.2	50	0.207	0.177	0.183	0.150	5.189	4.442	4.585	3.763
11	8	0.426	68	11	38.2	160	10.042	8.587	8.823	7.396	23.573	20.157	20.710	17.361
12a BE		Dislodged	140	65	37.8	Unconsolidated	23.599	20.167	18.038	15.490	***	***	***	***
12a BE	1	0.099	125	87	38.2	1266	22.162	18.936	16.858	14.495	224.535	191.851	170.800	146.864
12a BE	2	0.195	173	155	38.9	887	28.121	24.041	21.782	18.638	144.212	123.286	111.704	95.582
12a BE	3	0.028	13	0	49.6	464	3.941	3.365	2.933	2.538	140.749	120.189	104.764	90.641
12a BE	4	0.054	2	5	17.7	37	0.045	0.039	0.040	0.033	0.842	0.723	0.736	0.609
12a BE	5	0.072	1	6	26.7	14	0.050	0.043	0.043	0.036	0.701	0.601	0.595	0.496
12a BE	6	0.102	48	9	46.2	471	11.601	9.912	8.790	7.568	113.739	97.174	86.175	74.192
12a BE	7	0.040	34	5	44.1	850	6.958	5.947	5.349	4.587	173.949	148.677	133.716	114.666
12a BE	8	0.069	33	5	42.1	478	6.402	5.472	4.914	4.215	92.788	79.304	71.212	61.092
12a BE	9	0.158	3	22	46.9	19	0.725	0.620	0.551	0.474	4.599	3.929	3.495	3.007
12a BE	10	0.051	4	4	63.4	78	1.868	1.595	1.374	1.193	36.625	31.265	26.950	23.395
12a BE	11	0.070	34	38	34.3	486	3.986	3.410	3.151	2.681	56.944	48.709	45.010	38.297
12a BE	12	0.058	13	3	36.4	224	1.534	1.313	1.226	1.040	26.450	22.632	21.132	17.929
12a BE	13	0.029	4	0	42.1	138	0.711	0.608	0.553	0.473	24.531	20.974	19.061	16.297
12a BE	14	0.017	8	2	25.1	476	0.391	0.335	0.329	0.275	23.299	19.965	19.574	16.376
12b BS	1	0.423	268	96	35.1	633	32.102	27.460	38.824	31.883	75.856	64.887	91.740	75.339
12b BS	2	0.160	118	101	31.0	738	11.890	10.170	14.386	11.823	74.310	63.563	89.915	73.892
12b BS	3	0.136	99	41	32.9	728	12.664	10.826	15.464	12.835	93.119	79.603	113.709	94.376
12b BS	4	0.176	15	34	36.5	85	2.489	2.127	3.058	2.556	14.149	12.090	17.388	14.530
13	1	0.110	66	65	40.2	600	10.796	9.231	11.283	11.283	98.149	83.921	102.574	102.574
13	2	0.078	78	60	43.8	1006	15.976	13.654	16.970	16.970	206.140	176.180	218.967	218.967
13	3	0.016	39	21	52.3	2500	12.014	10.262	13.065	13.065	770.144	657.807	837.468	837.468
13	4	0.097	44	33	45.5	455	10.153	8.675	10.921	10.921	104.891	89.619	112.822	112.822
13	5	0.010	8	5	32.1	800	0.731	0.625	0.734	0.734	73.058	62.532	73.383	73.383
13		Dislodged	38	6	44.9	Unconsolidated	8.727	7.456	9.389	9.389	***	***	***	***
14	1	0.230	15	10	53.4	65	5.118	4.370	5.361	4.457	22.252	19.001	23.310	19.376
14	2	0.260	20	16	62.1	77	10.777	9.194	10.680	8.895	41.449	35.361	41.078	34.210
14	3	0.111	58	53	41.0	523	10.403	8.892	11.665	9.675	93.890	80.257	105.284	87.316
14	4	0.051	15	3	46.8	296	3.832	3.273	4.130	3.430	75.728	64.692	81.615	67.777
15	1	0.090	6	5	56.3	67	1.920	1.640	3.264	2.849	21.328	18.223	36.270	31.654
15	2	0.086	5	0	68.4	58	2.848	2.430	4.853	4.192	32.998	28.158	56.238	48.572
15	3	0.059	10	3	56.8	169	3.384	2.891	5.757	5.016	57.360	48.999	97.579	85.025
15	4	0.100	5	0	41.9	50	0.844	0.722	1.433	1.260	8.441	7.218	14.331	12.602
15	5	0.130	3	3	64.3	23	1.491	1.273	2.540	2.198	11.470	9.790	19.542	16.907
16	1	0.075	22	10	42.0	293	3.886	3.323	3.746	3.060	51.816	44.302	49.953	40.796
16	2	0.113	70	81	40.7	619	12.741	10.890	12.255	9.906	112.751	96.369	108.450	87.661
16		Dislodged	76	13	40.3	Unconsolidated	14.215	12.147	13.653	10.963	***	***	***	***
16	3	0.152	169	144	39.9	1112	31.106	26.581	29.876	23.995	204.644	174.874	196.555	157.864
16	4	0.173	166	51	50.2	958	52.124	44.506	49.779	38.893	300.774	256.813	287.239	224.425

Appendix 2.2: Population structure of oysters in the (A) upper, (B) mid and (C) lower intertidal zone on the Hume's Marsh restored oyster shell reef.



Appendix 2.3: Long Creek restored oyster shell reef oyster and mussel counts, mean oyster SH and oyster density.

Intertidal	Coordinates				Depth	No. of	No. of	Mean Oyster	Density
Zone	Length	Width	Latitude	Longitude	(cm)	Mussels	Oysters	SH (mm)	(m^{-2})
Upper	14	4	36.91007°N	76.04079°W	0	65	3	28.9 (9.8)	12
Upper	2	5	36.91006°N	76.04066°W	0	61	0	--	0
Mid	20.5	5	36.91010°N	76.04084°W	19	23	2	57.6 (1.8)	8
Mid	4	3	36.91022°N	76.04064°W	4	8	11	44.3 (6.3)	44
Lower	25	1	36.91007°N	76.04086°W	30	0	84	57.9 (2.2)	336

Appendix 2.4: Hume's Marsh restored oyster shell reef oyster and mussel counts, oyster density, mean SH, DM_{pooled}, AFDM, $DM_{site\text{-}specific}$, and condition data.

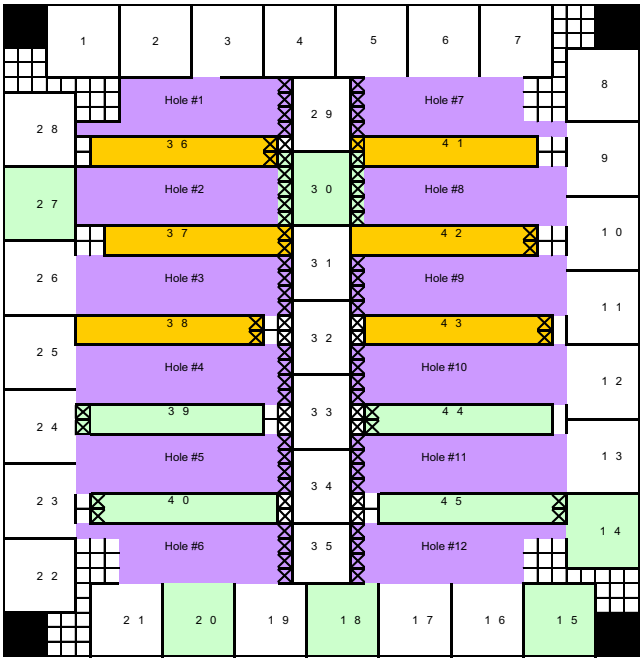
Hume's Marsh Oyster Reef

Intertidal	Coordinates				Depth	Total No.	Total No.	Mean Oyster	Density
Zone	Length	Width	Latitude (°N)	Longitude (°W)	(cm)	Mussels	Oysters	SH (mm)	(m^{-2})
Upper	34	3	36.89745	76.08886	4	0	0	--	0
Upper	37	7	36.89758	76.08876	0	6	37	49.2 (3.2)	148
Upper	21	3	36.89755	76.08874	0	0	9	35.8 (3.4)	36
Upper	14	4	36.89758	76.08868	0	1	24	43.7 (3.1)	96
Upper	7	3	36.89762	76.08860	0	3	40	50.6 (2.4)	160
Mid	21	4	36.89753	76.08875	17	3	8	46.4 (4.3)	32
Mid	12	2	36.89759	76.08868	4	3	20	32.9 (3.5)	80
Mid	20	4	36.89758	76.08823	10	2	42	53.9 (2.5)	168
Mid	32	7	36.89751	76.08885	6	0	25	49.7 (3.2)	100
Mid	15	7	36.89762	76.08869	11	0	39	52.6 (3.5)	156
Lower	24	11	36.89756	76.08882	33	0	10	59.8 (8.2)	40
Lower	34	6	36.89750	76.08886	23	3	37	53.5 (2.5)	148
Lower	33	7	36.89751	76.08885	28	1	20	56.0 (2.9)	80
Lower	35	8	36.89749	76.08889	54	0	5	30.5 (2.3)	20
Lower	11	2	36.89761	76.08868	34	15	46	30.0 (2.3)	184
Mean						2 (1)	24 (4)	46.0 (2.6)	97 (16)

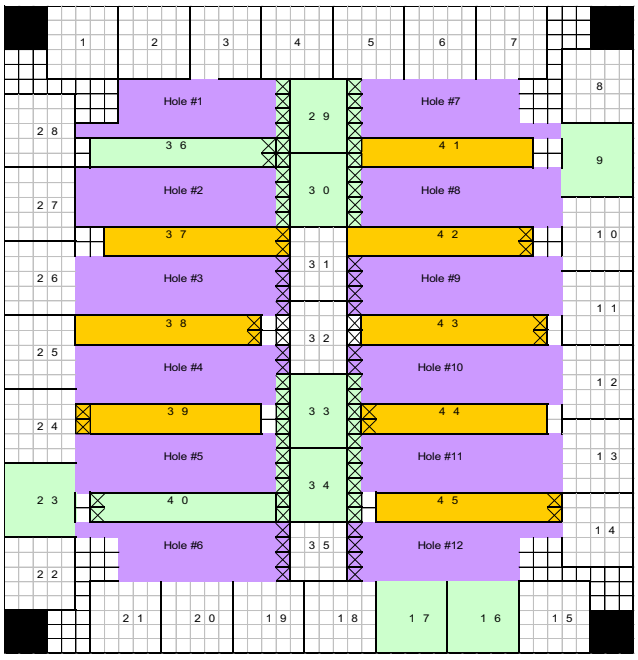
Intertidal			Total g	Total g	Total g	Condition	Condition	Condition	CI 1	CI 2	CI 3
Zone	L	W	DM_{pooled}	AFDM	DM_{site}	Index 1	Index 2	Index 3	Corrected	Corrected	Corrected
Upper	34	3	0.00	0.00	0.00	--	--	--	--	--	--
Upper	37	7	12.83	11.22	12.68	10.9 (0.9)	8.2 (0.8)	2.7 (0.2)	10.3 (0.9)	7.7 (0.7)	2.6 (0.2)
Upper	21	3	1.13	0.93	1.27	13.7 (1.5)	12.0 (1.7)	4.9 (1.4)	9.0 (1.0)	7.7 (0.7)	3.1 (0.7)
Upper	14	4	5.68	4.86	5.12	9.2 (0.6)	7.3 (0.6)	2.3 (0.3)	8.0 (0.8)	6.2 (0.5)	1.9 (0.2)
Upper	7	3	13.74	12.05	8.31	9.8 (0.7)	6.8 (0.6)	1.7 (0.2)	9.2 (0.5)	6.3 (0.4)	1.6 (0.1)
Mid	21	4	2.03	1.73	2.03	14.8 (1.3)	12.3 (1.1)	3.5 (0.3)	12.8 (1.2)	10.7 (1.1)	3.0 (0.2)
Mid	12	2	2.59	2.16	2.99	13.0 (0.9)	11.1 (0.9)	3.3 (0.8)	10.5 (0.9)	8.9 (0.7)	2.3 (0.3)
Mid	20	4	16.78	14.71	15.49	12.0 (0.8)	9.4 (0.8)	3.3 (0.4)	10.2 (0.7)	7.7 (0.5)	2.6 (0.2)
Mid	32	7	8.19	7.13	10.48	11.7 (0.9)	8.9 (0.9)	2.7 (0.4)	10.9 (0.9)	8.3 (0.8)	2.4 (0.2)
Mid	15	7	16.81	14.93	17.80	10.7 (0.6)	8.2 (0.4)	2.8 (0.4)	9.6 (0.9)	7.2 (0.5)	2.4 (0.1)
Lower	24	11	6.16	5.59	9.01	15.4 (1.3)	13.0 (0.5)	6.2 (1.7)	12.4 (0.9)	10.2 (0.6)	4.2 (0.8)
Lower	34	6	14.23	12.43	12.12	12.1 (0.6)	9.5 (0.6)	3.1 (0.4)	10.8 (0.7)	8.4 (0.5)	2.6 (0.2)
Lower	33	7	8.20	7.16	9.96	13.4 (0.6)	10.6 (0.5)	3.6 (0.5)	11.8 (1.3)	9.2 (0.9)	2.9 (0.2)
Lower	35	8	0.37	0.29	0.37	16.4 (1.0)	13.8 (0.8)	4.6 (1.0)	13.9 (1.8)	11.7 (1.5)	3.6 (0.4)
Lower	11	2	4.97	4.12	6.01	13.1 (0.9)	10.8 (0.7)	2.9 (0.3)	12.4 (1.0)	10.2 (0.8)	2.7 (0.2)
Mean			7.58 (1.54)	6.62 (1.36)	7.58 (1.45)	12.6 (0.6)	10.1 (0.6)	3.4 (0.3)	10.8 (0.4)	8.6 (1.7)	2.7 (0.2)

Appendix 3.1: Examples (Module Layer 3, all faces) of the stratified random sampling design used for the Concrete Module reef (Blue = plots sampled; yellow = unsampled interior Top/Bottom plots; purple = interstices).

Module 3 – Top face

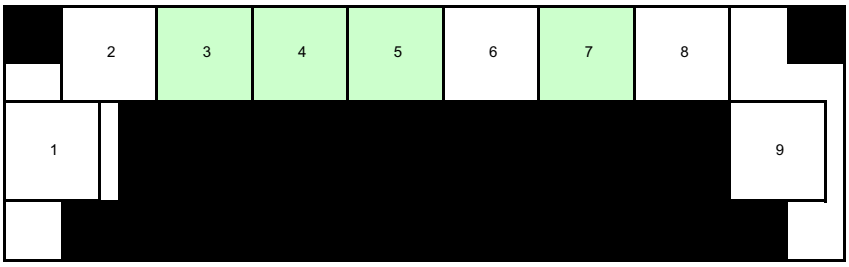


Module 3 – Bottom face

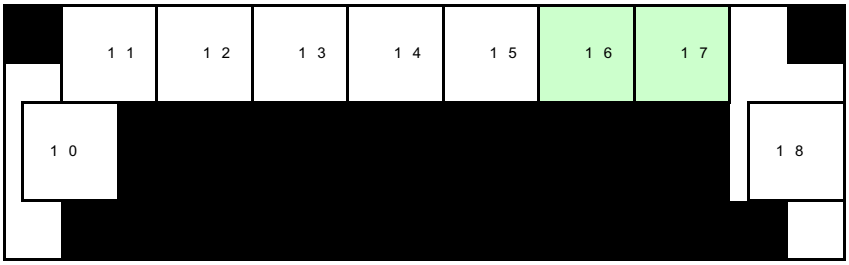


Appendix 3.1 continued

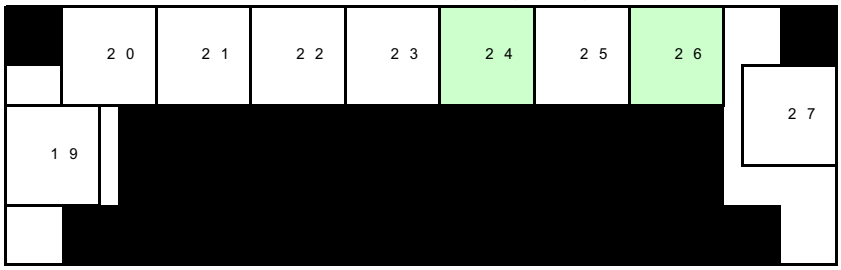
Module 3 – Side face (North)



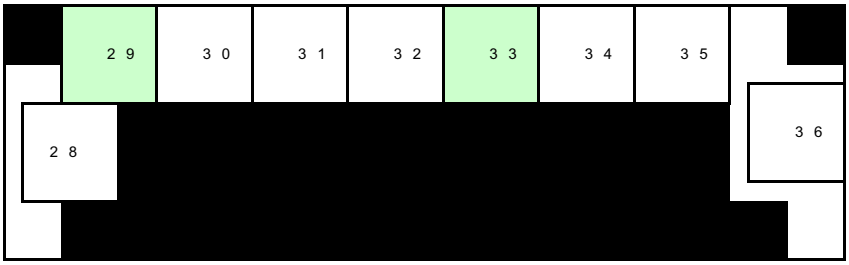
Module 3 – Side face (East)



Module 3 – Side face (South)

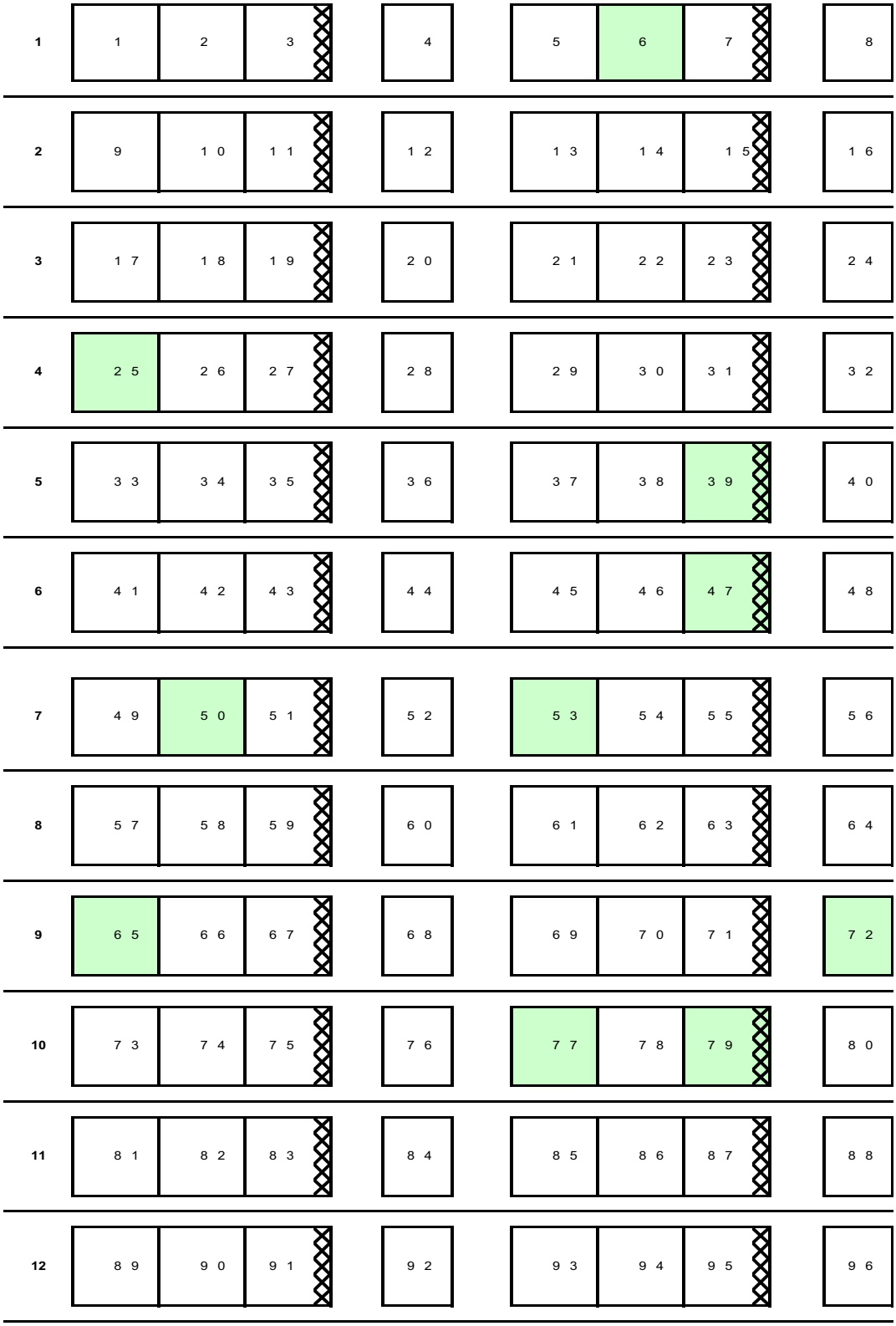


Module 3 – Side face (West)



Appendix 3.1 continued

Module 3 – Hole face



Appendix 4.1: Recruitment rankings for YC1, YC2, and YC3 by site-substrate-cage control.

Site	Substrate	Caging	YC1	YC2	YC3	Mean
Marsh	CVS	Caged	3	6	3	4.0
Marsh	CVS	Uncaged	5	5	5	5.0
Oyster Reef	CVS	Caged	2	4	8	4.7
Oyster Reef	CVS	Uncaged	2	5	8	5.0
Riprap	CVS	Caged	3	1	4	2.7
Riprap	CVS	Uncaged	2	6	1	3.0
Marsh	GL	Caged	6	5	4	5.0
Marsh	GL	Uncaged	6	8	4	6.0
Oyster Reef	GL	Caged	2	3	7	4.0
Oyster Reef	GL	Uncaged	3	5	8	5.3
Riprap	GL	Caged	4	5	1	3.3
Riprap	GL	Uncaged	5	4	1	3.3
Marsh	GS	Caged	5	6	4	5.0
Marsh	GS	Uncaged	7	7	5	6.3
Oyster Reef	GS	Caged	3	5	8	5.3
Oyster Reef	GS	Uncaged	2	5	8	5.0
Riprap	GS	Caged	4	5	1	3.3
Riprap	GS	Uncaged	6	4	3	4.3
Marsh	LML	Caged	3	3	3	3.0
Marsh	LML	Uncaged	3	2	3	2.7
Oyster Reef	LML	Caged	0	1	1	0.7
Oyster Reef	LML	Uncaged	1	1	1	1.0
Riprap	LML	Caged	2	1	2	1.7
Riprap	LML	Uncaged	2	2	3	2.3
Marsh	LMS	Caged	4	2	2	2.7
Marsh	LMS	Uncaged	3	4	3	3.3
Oyster Reef	LMS	Caged	0	1	2	1.0
Oyster Reef	LMS	Uncaged	0	1	2	1.0
Riprap	LMS	Caged	2	1	1	1.3
Riprap	LMS	Uncaged	2	2	3	2.3
Marsh	OSU	Caged	5	4	6	5.0
Marsh	OSU	Uncaged	4	5	6	5.0
Oyster Reef	OSU	Caged	1	3	6	3.3
Oyster Reef	OSU	Uncaged	1	4	6	3.7
Riprap	OSU	Caged	3	3	5	3.7
Riprap	OSU	Uncaged	2	5	4	3.7
		Mean	3.0	3.7	3.9	

Appendix 4.2: The Information-Theoretic (I-T) Approach – Multi-Model Inference to test "candidate" statistical models – was used to examine the importance of hypotheses (Table 4.1). Linear mixed models, analyses of variance (ANOVAs), and regressions were the primary statistical analyses applied in the Alternative Substrate Experiment. The following is an outline as to how these statistics were completed and is included here since the I-T Approach is a newer tool in the field of Applied Ecology.

One-Way ANOVAs (1 – 3)

Linear Mixed Models (4 – 8)

The weighted probabilities (w_i) assist in model selection, but interaction effects in these models can be problematic

Test Info	Model	Residual SS	n	RSS/n	K	n*log(RSS/n)	AIC_c	Delta	w_i	Pre-Weight
Fall 2007 MLD	1	107324864.0	108	993748.7	3	1491.40	1497.63	111.24	0.00	0.00
	2	116629509.0	108	1079902.9	3	1500.38	1506.61	120.22	0.00	0.00
	3	57981881.0	108	536869.3	3	1424.90	1431.13	44.74	0.00	0.00
	4	106176640.0	108	983117.0	5	1490.24	1500.82	114.44	0.00	0.00
	5	37805781.0	108	350053.5	5	1378.71	1389.30	2.91	0.11	0.23
	6	54814720.0	108	507543.7	5	1418.83	1429.42	43.04	0.00	0.00
	7	34583234.0	108	320215.1	8	1369.09	1386.54	0.16	0.43	0.92
	8	33779200.0	108	312770.4	9	1366.55	1386.38	0.00	0.46	1.00
										2.16

Test Info	Model	Residual SS	n	RSS/n	K	n*log(RSS/n)	AIC_c	Delta	w_i	Pre-Weight
Fall 2007 AFDM/m^2	1	880335.0	108	8151.3	3	972.64	978.87	110.82	0.00	0.00
	2	1360394.0	108	12596.2	3	1019.64	1025.88	157.83	0.00	0.00
	3	1024769.0	108	9488.6	3	989.05	995.28	127.23	0.00	0.00
	4	823571.4	108	7625.7	5	965.44	976.03	107.98	0.00	0.00
	5	388072.1	108	3593.3	5	884.18	894.76	26.72	0.00	0.00
	6	953459.0	108	8828.3	5	981.26	991.85	123.80	0.00	0.00
	7	307181.1	108	2844.3	8	858.93	876.39	8.34	0.02	0.02
	8	278155.0	108	2575.5	9	848.21	868.05	0.00	0.98	1.00
										1.02

Legend

Model #	Model Info (Factors)	K
1	Site (S)	3
2	Cage Control (CC)	3
3	Substrate (Sub)	3
4	S, CC, Sub, S x CC	5
5	S, CC, Sub, S x Sub	5
6	S, CC, Sub, CC x Sub	5
7	S, CC, Sub, S x CC, S x Sub, CC x Sub	8
8	S, CC, Sub, S x CC, S x Sub, CC x Sub, S x CC x Sub	9

Legend

Acronym	Definition
MLD	Mean Live Oyster Density (No.m^2)
AFDM/m^2	Mean Oyster Biomass - AFDM (g/m^2)
EXT	Exterior ("Top" + "Edge")
INT	Interior ("Middle" + "Bottom")
n	Sample Size
K	Number of Estimable Parameters
RSS	Residual Sum of Squares
AIC_c	Akaike's Information Criterion (corrected)

$$AIC_c = -2\log(\mathcal{L}(\hat{\theta}\,|\,\text{data})) + 2K + \frac{2K(K+1)}{(n-K-1)}$$

$$\Delta_i = AIC_i - \min AIC$$

$$w_i = \frac{\exp\left(-\frac{1}{2}\Delta_i\right)}{\sum_{r=1}^{R}\exp\left(-\frac{1}{2}\Delta_r\right)}$$

Delta	Level of Empirical Support
0 – 2	Substantial
4 – 7	Considerably less
>10	Essentially None

Appendix 4.2 Continued

Sampling Period	Response Variable	Effect of Site	Effect of Caging	Effect of Substrate	Interaction: CC x Sub	Interaction Effects w/ Site	Models Tested	Sample Size (n)	Candidate Models	Identified Model #'s	Weighted Probabilities
Fall 2005	MLD	Yes	No	Yes	No	No	8	108	1	8	1.00
Fall 2005	Ext. MLD	No	No	Yes	No	No	8	108	1	8	1.00
Fall 2005	Int. MLD	No	No	Yes	No	Yes	8	108	1	8	1.00
Fall 2005	AFDM/m^2	Yes	No	Yes	No	Yes	7	36	1	7	1.00
Fall 2005	Ext. AFDM/m^2	Yes	No	Yes	No	Yes	7	36	1	7	1.00
Fall 2005	Int. AFDM/m^2	No	No	No	No	No	7	36	2	5, 7	0.06, 0.94
Spring 2006	MLD	Yes	No	Yes	No	No	8	108	1	8	1.00
Spring 2006	Ext. MLD	Yes	No	Yes	No	Yes	8	108	2	7, 8	0.01, 0.99
Spring 2006	Int. MLD	No	Yes	Yes	No	No	8	108	1	8	1.00
Spring 2006	AFDM/m^2	Yes	No	No	No	Yes	8	108	2	7, 8	0.31, 0.69
Spring 2006	Ext. AFDM/m^2	Yes	No	No	No	Yes	8	108	2	7, 8	0.12, 0.88
Spring 2006	Int. AFDM/m^2	Yes	No	No	No	No	8	108	2	7, 8	0.02, 0.98
Fall 2006	MLD	No	No	Yes	No	No	8	108	1	8	1.00
Fall 2006	Ext. MLD	Yes	No	Yes	No	No	8	108	1	8	1.00
Fall 2006	Int. MLD	Yes	No	Yes	No	No	8	108	2	7, 8	0.02, 0.98
Fall 2006	AFDM/m^2	Yes	No	Yes	No	Yes	8	108	3	5, 7, 8	0.03, 0.30, 0.68
Fall 2006	Ext. AFDM/m^2	Yes	No	Yes	No	Yes	8	108	2	7, 8	0.25, 0.75
Fall 2006	Int. AFDM/m^2	Yes	No	No	No	No	8	108	2	7, 8	0.01, 0.99
Spring 2007	MLD	Yes	No	Yes	No	Yes	8	108	2	7, 8	0.01, 0.99
Spring 2007	Ext. MLD	Yes	No	Yes	No	Yes	8	108	1	8	1.00
Spring 2007	Int. MLD	Yes	No	Yes	No	No	8	108	2	7, 8	0.01, 0.99
Spring 2007	AFDM/m^2	Yes	No	Yes	No	Yes	8	108	2	7, 8	0.11, 0.89
Spring 2007	Ext. AFDM/m^2	Yes	No	Yes	No	Yes	8	108	2	7, 8	0.02, 0.98
Spring 2007	Int. AFDM/m^2	Yes	No	No	No	No	8	108	2	5, 8	0.01, 0.99
Fall 2007	MLD	Yes	Yes	Yes	No	Yes	8	108	3	5, 7, 8	0.11, 0.43, 0.46
Fall 2007	Ext. MLD	Yes	No	Yes	No	Yes	8	108	3	5, 7, 8	0.01, 0.09, 0.90
Fall 2007	Int. MLD	No	No	No	No	Yes	8	108	3	5, 7, 8	0.04, 0.11, 0.85
Fall 2007	AFDM/m^2	Yes	No	Yes	No	Yes	8	108	2	7, 8	0.02, 0.98
Fall 2007	Ext. AFDM/m^2	Yes	No	Yes	No	No	8	108	1	8	1.00
Fall 2007	Int. AFDM/m^2	No	No	No	No	No	8	108	1	8	1.00

*Note: The highlighted rows represent viable models that do NOT contain significant interaction effects between a fixed factor (substrate/caging) and the random factor (site). Where there was a significant interaction between the random factor and the fixed treatments (linear mixed model), further analysis was done as comparisons of treatments at each site (three separate two-way ANOVAs) as in the following example where, for 1) Fall 2007 MLD, Model #'s 2 and 4 equally weighted, so both must be considered, and for 2) Fall 2007 AFDM/m^2, Model #4 is the only viable statistical model.

One-Way ANOVAs
Two-Way ANOVA
Two-Way ANOVA w/ Interaction Term

Marsh

Test Info	Model Info	Model	Residual SS	n	RSS/n	K	n*log(RSS/n)	AIC_c	Delta	w_i	Pre-Weight
Fall 2007 MLD	CC	1	31035449.0	36	862095.8	3	492.02	498.77	20.52	0.00	0.00
	Sub	2	17590699.0	36	488630.5	3	471.58	478.33	0.08	0.39	0.96
	CC, Sub	3	17041124.0	36	473364.6	4	470.43	479.72	1.47	0.20	0.48
	CC, Sub, CC x Sub	4	15171584.0	36	421432.9	5	466.25	478.25	0.00	0.41	1.00
											2.44

Test Info	Model Info	Model	Residual SS	n	RSS/n	K	n*log(RSS/n)	AIC_c	Delta	w_i	Pre-Weight
Fall 2007 AFDM/m^2	CC	1	320695.0	36	8908.2	3	327.41	334.16	40.68	0.00	0.00
	Sub	2	147344.0	36	4092.9	3	299.41	306.16	12.68	0.00	0.00
	CC, Sub	3	129634.0	36	3600.9	4	294.80	304.09	10.61	0.00	0.00
	CC, Sub, CC x Sub	4	89538.0	36	2487.2	5	281.48	293.48	0.00	0.99	1.00
											1.01

Appendix 5.1: Flow cytometry performed by the VIMS Aquaculture Genetics and Breeding Technology Center to determine the ploidy of oysters removed from seeded oyster shell and granite riprap in fall 2006, 12 wks post-deployment of triploid (3N) larvae as part of the Remote Field Larval Settling Experiment. The greater the percentage of 3N oysters detected, the more successful the experiment.

Aquaculture Genetics and Breeding Technology Center
Virginia Institute of Marine Science
PO Box 1346
Rt 1208 Greate Road
Gloucester Point, VA 23062

Russ Burke
Fisheries Science
Virginia Institute of Marine Science

11-15-06

On October 30th the flow cytometry lab received samples from Russ Burke of putative triploid *C. virginica* spat that were set on shell and rip rap in the Lynnhaven River from a batch of triploid eyed larvae. Spat were measured and gill snippets taken for flow analysis to confirm the ploidy in the sample of spat.

The results of the analysis by flow cytometry performed on 11/06/06 indicate that 99% and 77% were triploid at Chalmers and Handeland, respectively. There were a total of 16 diploids out of 120 spat, 14 of which came from the Handeland site

Table 1. Breakdown of results from 10/30/06 sampling

Site (River)	Category	no. triploid	no. diploid	average size (mm)
Chalmers	Shell Top: Exterior	8	0	35
Chalmers	Shell Top: Interior	8	0	31
Chalmers	Shell Bottom: Exterior	8	0	34
Chalmers	Shell Bottom: Interior	8	0	28
Chalmers	Rock 1	17	1	39
Chalmers	Rock 2	9	1	42
Handeland	Shell Top: Exterior	7	1	40
Handeland	Shell Top: Interior	6	2	35
Handeland	Shell Bottom: Exterior	8	0	36
Handeland	Shell Bottom: Interior	5	3	38
Handeland	Rock 1	16	2	41
Handeland	Rock 2	4	6	38

Table 2. Combined results

Chalmers	
number sampled	60
number of triploids	58
number of diploids	2
percent triploidy	99
Handeland	
number sampled	60
number of triploids	46
number of diploids	14
percent triploidy	77

Comments: See attached documents for raw data.

Katherine Blackshear
Flow Cytometry Specialist

Appendix 5.1 continued

Sample type = Shells			Site 1				Site 2	
			Chalmers				Handeland	
		FlowSampleID	Length (mm)	ploidy		FlowSampleID	Length (mm)	ploidy
Top: Exterior	1	*1*	25	3N		*33*	45	3N
	2	*2*	42	3N		*34*	34	3N
	3	*3*	26	3N		*35*	37	3N
	4	*4*	30	3N		*36*	44	2N
	5	*5*	40	3N		*37*	48	3N
	6	*6*	44	3N		*38*	29	3N
	7	*7*	28	3N		*39*	39	3N
	8	*8*	47	3N		*40*	45	3N
		avg	35			*avg*	40	
Top: Interior	1	*9*	50	3N		*41*	34	3N
	2	*10*	36	3N		*42*	51	2N
	3	*11*	26	3N		*43*	26	2N
	4	*12*	32	3N		*44*	39	3N
	5	*13*	44	3N		*45*	44	3N
	6	*14*	21	3N		*46*	29	3N
	7	*15*	27	3N		*47*	32	3N
	8	*16*	11	3N		*48*	23	3N
		avg	31			*avg*	35	
Bottom: Exterior	1	*17*	32	3N		*49*	40	3N
	2	*18*	23	3N		*50*	46	3N
	3	*19*	24	3N		*51*	27	3N
	4	*20*	50	3N		*52*	39	3N
	5	*21*	31	3N		*53*	38	3N
	6	*22*	31	3N		*54*	34	3N
	7	*23*	41	3N		*55*	40.5	3N
	8	*24*	38	3N		*56*	23	3N
		avg	34			*avg*	36	
Bottom: Interior	1	*25*	27	3N		*57*	35	3N
	2	*26*	37	3N		*58*	40.5	3N
	3	*27*	37	3N		*59*	51.5	3N
	4	*28*	25	3N		*60*	35	2N
	5	*29*	26	3N		*61*	51	3N
	6	*30*	26.5	3N		*62*	34	3N
	7	*31*	11	3N		*63*	40	2N
	8	*32*	32	3N		*64*	14	2N
		avg	28			avg	38	

Appendix 5.1 continued

Sample type = RIP RAP			Site 1				Site 2	
			Chalmers				Handeland	
		FlowSampleID	Length (mm)	ploidy		FlowSampleID	Length (mm)	ploidy
Rock 1	1	*65*	45	3N		*93*	43	3N
	2	*66*	41	3N		*94*	46	3N
	3	*67*	46	3N		*95*	30	2N
	4	*68*	41	3N		*96*	56	3N
	5	*69*	48	3N		*97*	43	3N
	6	*70*	38	3N		*98*	47	3N
	7	*71*	28	3N		*99*	28	3N
	8	*72*	40	3N		*100*	37	3N
	9	*73*	38	3N		*101*	60	3N
	10	*74*	38	3N		*102*	24	2N
	11	*75*	34	3N		*103*	44	3N
	12	*76*	30	2N		*104*	38	3N
	13	*77*	50	3N		*105*	45	3N
	14	*78*	40	3N		*106*	24	3N
	15	*79*	45	3N		*107*	37	3N
	16	*80*	38	3N		*108*	44	3N
	17	*81*	36	3N		*109*	54	3N
	18	*82*	33	3N		*110*	46	3N
		avg	39			*avg*	41	
Rock 2	1	*83*	47	3N		*111*	39	3N
	2	*84*	48	3N		*112*	33	2N
	3	*85*	48	3N		*113*	28	2N
	4	*86*	41	3N		*114*	33	2N
	5	*87*	35	3N		*115*	43	3N
	6	*88*	38	3N		*116*	38	2N
	7	*89*	37	3N		*117*	53	3N
	8	*90*	43	3N		*118*	29	2N
	9	*91*	51	3N		*119*	41	2N
	10	*92*	28	2N		*120*	40	3N
		avg	42			avg	38	

Literature Cited

Abbe GR, Sanders JG (1988) Rapid decline in oyster condition in the Patuxent River, Maryland. J Shellfish Res 7:57-59

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 22:747-752

Abelson A, Denny M (1997) Settlement of marine organisms in flow. Ann Rev Ecol Syst 28:317-339

Administration of Barack H Obama (2009) Executive Order 13508—Chesapeake Bay protection and restoration, May 12, 2009. Office of the Federal Register, DCPD200900352

Airoldi L, Beck MW (2007) Loss, status and trends for coastal marine habitats of Europe. Oceanogr Mar Biol Ann Rev 45:345-405

Albright BW, Abbe GR, McCollough CB, Barker LS, Dungan CF (2007) Growth and mortality of Dermo-disease-free juvenile oysters (*Crassostrea virginica*) at three salinity regimes in an enzootic area of Chesapeake Bay. J Shellfish Res 26(2):451-463

Allen SK, Brumbaugh RD, Fox JC (2000) A consensus report from the 2000 Chesapeake Bay delegation to review French aquaculture: Oyster recovery strategy for the Chesapeake Bay – Proactive recommendations

Anderson DR (2008) Model based inference in the life sciences: A primer on evidence. Springer, New York, NY 10013

Andrews JD (1996) Epizootiology of the disease caused by the oyster pathogen, *Perkinsus marinus* and its effects on the oyster industry. P. 47-63. In: (eds.) WS Fisher. American Fisheries Society Special Publication No. 18, Bethesda, MD

Andrews JD, Ray SM (1988) Management strategies to control the disease caused by *Perkinsus marinus*. P. 257-264. In: (eds.) WS Fisher. American Fisheries Society Special Publication No. 18, Bethesda, MD

Ardizzone GD, Gravina MF, Belluscio A (1989) Temporal development of epibenthic communities on artificial reefs in the central Mediterranean Sea. Bull Mar Sci 44(2):592-608

Artabane SJ (2006) The effects of proximity to a subtidal channel on habitat utilization of intertidal oyster reefs. Master's Thesis. University of North Carolina, Wilmington, NC

Austin HM, Haven DS, Moustafa MS (1993) The relationship between trends in a condition index of the American oyster, *Crassostrea virginica*, and environmental parameters in three Virginia estuaries. Estuaries 16(2):362-374

Austin HM, Evans DA, Haven DS (1996) A retrospective time series analysis of oyster, *Crassostrea virginica*, recruitment (1946-1993) J Shellfish Res 15(3):565-582

Badalamenti F, Chemello R, D'Anna G, Henriquez Ramos P, Riggio S (2002) Are artificial reefs comparable to neighbouring natural rocky areas? A mollusc case study in the Gulf of Castellammare (NW Sicily). ICES J Mar Sci 59:S127-S131

Bahr LN and Lanier WP (1981) The ecology of intertidal oyster reefs of the south Atlantic coast: A community profile. US Fish and Wildlife Service, Office of Biological Services, Washington DC. FWS/OBS-81/15. 105 pp

Bartol IK, Mann R (1999) Small-scale patterns of recruitment on a constructed intertidal reef: the role of spatial refugia. p. 159-170. In: (eds.) MW Luckenbach, R Mann, J Wesson. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Bartol IK, Mann R, Luckenbach MW (1999) 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

Baylor JB (1894) Complete survey of the natural oyster beds, rocks, and shoals of Virginia. Report to the Governor of Virginia, 1895. Superintendent of Public Documents, Richmond

Baynes TW, Szmant AM (1989) Effect of current on the sessile benthic community structure of an artificial reef. Bull Mar Sci 44(2):545-566

Beck MB, Brumbaugh RD, Airoldi L, Carranza A, Coen LD, Crawford C, Defeo O, Edgar GJ, Hancock B, Kay M, Lenihan H, Luckenbach MW, Toropova CL, Zhang G (2009) Shellfish reefs at risk: A global analysis of problems and solutions. The Nature Conservancy, Arlington VA. 52 pp

Berman MR, Berquist H, Dewing S, Glover J, Hershner CH, Rudnicky T, Schatt DE, Skunda K (2000) Mathews County shoreline situation report. Special Report in Applied Marine and Ocean Engineering No. 364, Comprehensive Coastal Inventory Program, Virginia Institute of Marine Science, College of William and Mary, Virginia. 52 pp

Bhattacharya, CG (1967) A simple method of resolution of a distribution into Gaussian components. Biometrics 23(1):115-135

Bishop MJ, Peterson CH (2006) Direct effects of physical stress can be counteracted by indirect benefits: oyster growth on a tidal elevation gradient. Oecologia 147:426-433

Blue Ribbon Oyster Panel (2007) Report and recommendations of the Blue Ribbon Oyster Panel. Virginia Marine Resources Commission. Newport News, Virginia 23607

Bombace G (1989) Artificial reefs in the Mediterranean Sea. Bull Mar Sci 44:1023-1032

Bombace G, Fabi G, Fiorentini L, and Speranza S (1994) Analysis of the efficacy of artificial reefs located in five different areas of the Adriatic Sea. Bull Mar Sci 55:559-580

Bombace G, Fabi G, Fiorentini L (2000) Artificial reefs in the Adriatic Sea. In Artificial reefs in European Seas, 31-64. Ed. by AC Jensen, KJ Collins, and APM Lockwood. Kluwer. 508 pp

Bonar DB, Coon SL, Walch M, Weiner RM, Fitt W (1990) Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. Bull Mar Sci 46(2):484-498

Boynton WR, Kemp WM, Osbourne CG (1980) Nutrient fluxes across the sediment-water interface in the turbid zone of a coastal plain estuary. In: Estuarine perspectives, p. 93-109 (ed) VS Kennedy, Academic Press, New York

Breitburg DL (1999) Are three-dimensional structure and healthy oyster populations the keys to an ecologically interesting and important fish community? Pages 239-249. In: Oyster reef habitat restoration: a synopsis and synthesis of approaches, (eds) MW Luckenbach, R Mann, JA Wesson. Virginia Institute of Marine Science Press. Gloucester Point, Virginia. 336 pp

Breitburg DL, Coen LD, Luckenbach MW, Mann R, Posey M, Wesson JA (2000) Oyster reef restoration: Convergence of harvest and conservation strategies. J Shellfish Res 19(1):371-377

Breitburg DL, Fulford RS (2006) Oyster-sea nettle interdependence and altered control within the Chesapeake Bay ecosystem. Estuaries and Coasts 29(5):776-784

Brooks WK (1891) The oyster. The Johns Hopkins University Press, Baltimore, Maryland 21218

Brumbaugh RD, Sorabella LA, Garcia CO, Goldsborough WJ, Wesson JA (2000) Making a case for community-based oyster restoration: An example from Hampton Roads, Virginia, U.S.A. J Shellfish Res 19(1):467-472

Brumbaugh RD, Beck MW, Coen LD, Craig L, Hicks P (2006) Practitioners' guide to design and monitoring of shellfish restoration projects: An ecosystem services approach. The Nature Conservancy, Arlington, VA. 28 pp

Brumbaugh RD, Coen LD (2009) Contemporary approaches for small-scale oyster reef restoration to address substrate versus recruitment limitation: A review and comments relevant for the Olympia oyster, *Ostrea lurida* Carpenter 1864. J Shellfish Res 28(1):147-161

Bruno JF, Bertness MD (2000) Habitat modification and facilitation in benthic marine communities. In Marine Community Ecology. Sinauer Associates, Inc. Sunderland, MA 01375-0407

Burnham KP, Anderson DR (1998) Model selection and multimodel inference: A practical information-theoretic approach. 2nd Edition. Springer, New York, NY 10013

Burrell VG (1986) Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic): American oyster. US Fish and Wildlife Service Biological Report 82 (11.57). Coastal Ecology Group, US Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS. TR EL08204. 17 pp

Capone M, Grizzle R, Mathieson AC, Odell J (2008) Intertidal oysters in northern New England. Northeastern Naturalist 15(2):209-214

Carnegie RB, Burreson EM (2005) Status of the major oyster diseases in Virginia 2004: A summary of the annual monitoring program. Virginia Institute of Marine Science. Gloucester Point, Virginia 23062

Carnegie RB, Burreson EM (2008) Gametogenesis and spawning in *Crassostrea virginica* from disease-intense waters of Virginia, USA. 11th International Conference on Shellfish Restoration, Charleston, South Carolina 29401

Carnegie RB, Burreson EM (2009) Status of the major oyster diseases in Virginia 2006-2008: A summary of the annual oyster disease monitoring program. Virginia Institute of Marine. Gloucester Point, Virginia 23062

Chesapeake Research Consortium (1999) Chesapeake Bay oyster restoration: Consensus of a meeting of scientific experts. Virginia Institute of Marine Science. Wachapreague, Virginia

Chipman, Jr. WA (1948) Conditions affecting shellfish production in Lynnhaven Bay, Virginia, and the possibilities of improving them by increasing tidal flow. Special Scientific Report No. 61. United States Dept. of the Interior, Washington, D.C.

Clarke D, Meyer D, Veishlow A, LaCroix M (1999) Dredged material as a substrate for fisheries habitat establishment in coastal waters. pp 305-313. In: (eds) MW Luckenbach, R Mann, JA Wesson. Oyster reef habitat restoration: a synopsis and synthesis of approaches. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Clements FE (1916) Plant succession. Carnegie Inst. Wash. Publ. 242 pp

Cloern JE (1981) Does the benthos control phytoplankton biomass in South San Francisco Bay? Mar Ecol Prog Ser 9:191-202

Cochran WG (1977) Sampling techniques. Wiley, New York

Coen LD, Luckenbach MW, Breitburg DL (1999) The role of oyster reefs as essential fish habitat: a review of current knowledge and some new perspectives. American Fisheries Society Symposium 22:438-454

Coen LD, Luckenbach MW (2000) Developing success criteria and goals for evaluating oyster reef restoration: Ecological function or resource exploitation? Ecol Eng 15:323-343

Coen LD, Grizzle RE (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 #8

Cohen RRH, Dresler PV, Philips EJP, Cory RL (1984) The effect of the Asiatic clam, *Corbicula Fluminea* on phytoplankton of the Potomac River, Maryland. Limnol Oceanogr 29:170-180

Coon S, Fitt WK (1999) Perspectives on induced settlement and metamorphosis as a tool for oyster reef enhancement p. 171-178. In: (eds) MW Luckenbach, R Mann, JA Wesson. Oyster reef habitat restoration: a synopsis and synthesis of approaches. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Costa Monteiro C, Neves dos Santos M (2000) Portuguese artificial reefs. In Artificial reefs in European Seas, 249-262. Ed. by AC Jensen, KJ Collins, and APM Lockwood. Kluwer. 508 pp

Cowan JLW (2003) Terrebonne Bay Shoreline Protection and Oyster Reef (DEMO). Louisiana Department of Natural Resources

Crosby MP, Gale LD (1990) A review and evaluation of bivalve condition index methodologies with a suggested standard method. J Shellfish Res 9:233-237

Dame R, Zingmark R, Stevenson H, Nelson D (1980) Filter feeding coupling between the estuarine water column and benthic substrate, p. 521-526. In V.S. Kennedy (ed.), Estuarine perspectives. Academic Press, New York

Dauer DM, Weisberg SB, Ranasinghe JA (2000) Relationships between benthic community condition, water quality, sediment quality, nutrient loads, and land use patterns in Chesapeake Bay. Estuaries 23:80–96

Davenport J, Chen X (1987) A comparison of methods for the assessment of condition in the mussel (*Mytilus edilus* L.). J Moll Stud 53:293-297

Davis JLD, Takacs RL, Schnabel R (2006) Evaluating ecological impacts of living shorelines and shoreline habitat elements: An example from the upper western Chesapeake Bay. Proceedings of the 2006 Living Shoreline Summit. Gloucester Point, VA 23062

Dittman S (1990) Mussel beds – amensalism or amelioration for intertidal fauna? Helgolander Meeresunters 44:335-352

Eckman JE, Peterson CH, Cahalan JA (1989) Effects of flow speed, turbulence, and orientation on growth of juvenile bay scallops, *Argopecten irradians concentricus* (Say). J Exp Mar Biol Ecol 132(2):123-140

Edeline E, Carlson SM, Stige LC, Winfield IJ, Fletcher JM, James JB, Haugen TO, Vollestad LA, Stenseth NC (2007) Trait changes in a harvested population are driven by a dynamic tug-of-war between natural and harvest selection. Proc Nat Acad Sci 104(40):15799-15804

Eggleston DB (1999) Application of landscape ecological principles to oyster reef habitat restoration. p. 213-228. In: (eds.) MW Luckenbach, R Mann, and JA Wesson. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Eggleston DB, Elis WE, Etherington LL, Dahlgren CP, Posey MH (1999) Organism responses to habitat fragmentation and diversity: Habitat colonization by estuarine macrofauna. J Exp Mar Biol Ecol 236:107-132

Encomio VG, Stickler SM, Allen, Jr. SK, Chu F-L (2005) Performance of "natural Dermo-resistant" oyster stocks—survival , disease, growth, condition and energy reserves. J Shellfish Res 24(1):143-155

Encomio VG, Chu F-L (2007) Heat shock protein (hsp70) expression and thermal tolerance in sublethally heat-shocked eastern oysters *Crassostrea virginica* infected with the parasite *Perkinsus marinus*. Dis Aquat Org 76:251-260

Engle JB (1951) The condition of oysters as measured by the carbohydrate cycle, the condition factor and the percent dry weight. Proc Nat Shellfisheries Assoc 41:20-25

Engle JB, Chapman CR (1952) Oyster condition affected by attached mussels. Southern Fisherman (August):28-30

Erdle SY, Davis JLD, Sellner KG (2006) Management, policy, science, and engineering of nonstructural erosion control in the Chesapeake Bay. Proceedings of the 2006 Living Shoreline Summit. Chesapeake Research Consortium Publication No. 08-164

Fabi G, Fiorentini L, Giannmi S (1986) Growth of *Mytilus galloprovincalis* Lamarck, 1891 on a suspended and immersed culture in the Bay of Portonovo (central Adriatic Sea). FAO Fisheries Reports 357:144–154

Fabi G, Fiorentini L (1990) Shellfish culture associated with artificial reefs. FAO Fish Rep 428:99-107

Fabi G, Fiorentini L (1994) Comparison of an artificial reef and a control site in the Adriatic Sea. Bull Mar Sci 55:538-558

Fabi G, Fiorentini L (1997) Molluscan aquaculture on reefs. In European artificial reef research, 123-140. Ed. by AC Jensen. Southampton Oceanography Centre. ISBN 0-904175-28-6. 449 pp

Galtsoff P (1930) The fecundity of the oyster. Science. 72:97-98

Gayanilo Jr. FC, Sparre P, Pauly D (2002) FISAT II version 1.2.0. Food and agriculture Organization (FAO) of the United Nations, Rome

Grabowski JH (2004) Habitat complexity disrupts predator-prey interactions but not the trophic cascade on oyster reefs. Ecology 85(4):995-1004

Gunter G (1955) Mortality of oysters and abundance of certain associates as related to salinity. Ecology 36(4):601-605

Hardwick-Witman MN, Mathieson AC (1983) Intertidal macroalgae and macroinvertebrates: Seasonal and spatial abundance patterns along an estuarine gradient. Est Coast Shelf Sci 16:113-129

Hargis WJ, Haven DS (1999) Chesapeake oyster reefs, their importance, destruction and guidelines for restoring them. p. 329-358. *In*: (eds) MW Luckenbach, R Mann, and JA Wesson. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Harris DC (1980) Survey of the intertidal and subtidal oyster resources of the Georgia coast. Georgia Department of Natural Resources Coastal Resources Division (Project no. 2-234-R). Brunswick, Georgia, 44 pp

Haven DS, Whitcomb JP, Kendall PC (1981) The present and potential productivity of the Baylor Grounds in Virginia. Special Report in Applied Marine Science and Ocean Engineering No. 243. Virginia Institute of Marine Science, Gloucester Point, VA 23062

Haven DS, Hargis WJ, Kendall PC (1978) The oyster industry of Virginia: its status, problems and promise. VIMS special papers in marine science, Virginia Institute of Marine Science, Gloucester Point, VA 23062

Haywood EL, Soniat TS (1992) The use of cement-stabilized gypsum as cultch for the eastern oyster, *Crassostrea virginica* (Gmelin, 1791). J Shellfish Res 11(2):417-419

Haywood EL, Soniat TM, Broadhurst RC (1999) Alternatives to clam and oyster shell as cultch for eastern oysters. p. 295-304. In: (eds) M. Luckenbach, R. Mann, and J.A. Wesson. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Herberich D (2006) Estimated return to harvest due to the Maryland Department of Natural Resources repletion activities 1990-2006. College Park, MD 20742

Hickman RW, Illingworth J (1980) Condition cycle of the green-lipped mussel *Perna canaliculus* in New Zealand. Mar Biol 60:27-38

Hopkins SH, Mackin JG, Menzel RW (1954) The annual cycle of reproduction growth, and fattening. Proc Nat Shellfisheries Assoc 44:39-50

Ingersoll E (1881) 10th Census of the United States, the history and present condition of the fishery industries. The oyster-industry. 251 pp

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001). Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629-637

Jensen AC (2002) Artificial reefs of Europe: perspective and future. ICES J Mar Sci 59:S3-S13

Jensen AC, Collins KJ, Lockwood P (2000) Current issues relating to artificial reefs in European seas. Pages 489–499 in A.C. Jensen, K.J. Collins and A.P.M. Lockwood, eds. Artificial reefs in European seas. Kluwer Academics Publishers, London

Jordan SJ, Coakley JM (2004) Long-term projections of eastern oyster populations under various management scenarios. J Shellfish Res 23:63-72

Karlson R (1978) Predation and space utilization patterns in a marine epifaunal community. J Exp Mar Biol Ecol 31(3):225-239

Kellum, T (2008) Written Testimony to the U.S. House of Representatives Committee on Natural Resources Subcommittee on Fisheries, Wildlife, and Oceans: Oversight Hearings on Past, Current, and Future Efforts to Restore Oysters in the Chesapeake Bay. September 10, 2008

Kennedy VS, Breisch LL (1981) Maryland's oysters: Research and management. Maryland Sea Grant Publication UM-SG-TS-81-04, 286 pp

Kennedy VS, Newell RIE, Eble AF (1996) The Eastern Oyster, *Crassostrea virginica* University of Maryland Sea Grant Press, College Park, MD. 734 pp

Kirby MX (2004) Fishing down the coast: historical expansion and collapse of oyster fisheries along continental margins. Proc Nat Acad Sci 101(35):13096-13099

Komar PD, Holman RA (1986) Coastal processes and the development of shoreline erosion. Ann Rev Earth Planet Sci 14:237-265

Langland M, Cronin T (2003) A summary report of sediment processes in Chesapeake Bay and watershed. U.S. Geological Survey, WRI 03-4123, 109 pp

Lauck T, Clark CW, Mangel M, Munro GR (1998) Implementing the precautionary principle in fisheries management through marine reserves. Ecol Appl 8(1):S72-S78

Lawless AS (2008) Effects of shoreline development and oyster reefs on benthic communities in Lynnhaven, Virginia. Master's Thesis. The College of William and Mary. Gloucester Point, Virginia 23062

Lenihan HS, Peterson CH, Allen JM (1996) Does flow speed also have a direct effect on growth of active suspension feeders: An experimental test on oysters. Limnol Oceanogr 41(6):1359-1366

Lenihan HS, Peterson CH (1998) How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. Ecol Appl 11:128–40

Lenihan HS, Micheli F (1999) The influence of multiple environmental stressors on susceptibility to parasites: An experimental determination with oysters. Limnol Oceanog 44(3, part 2):910-924

Lenihan HS, Peterson CH, Byers JE, Grabowski JH, Thayer GW, Colby DR (2001) Cascading of habitat degradation: Oyster reefs invaded by refugee fishes escaping stress. Ecol Appl 11(3):764-782

Lipcius RN, Burke RP (2006) Abundance, biomass and size structure of eastern oyster and hooked mussel on a modular artificial reef in the Rappahannock River, Chesapeake Bay. Spec. Rept. Appl. Mar. Sci. Ocean Eng. No. 390, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062

Lipcius RN, Eggleston DB, Schreiber SJ, Seitz RD, Shen J, Sisson M, Stockhausen, WT, Wang HV (2008) Importance of metapopulation connectivity to restocking and restoration of marine species. Rev Fish Sci 16(1-3):101-110

Lotze HK, Lenihan HS, Bourque BJ, Bradbury, RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806-1809

Louisiana Department of Wildlife and Fisheries (2004) Louisiana's oyster shell recovery pilot project. Socioeconomics research and development section and marine fisheries division

Luckenbach MW, Coen LD, Ross PG, Stephen JA (2005) Oyster reef habitat restoration: Relationships between oyster abundance and community development based on two studies in Virginia and South Carolina. J Coastal Res Special Issue 40:64-78

Luckenbach MW, Ross PG (2006) Lynnhaven River: Pre-deployment basin survey (http://web.vims.edu/mollusc/NORM/NORMdatahub/NORMlysum.htm)

Lukens RR (1997) Guidelines for marine artificial reef materials. Artificial Reef Subcommittee of the Technical Coordinating Committee, Gulf States Marine Fisheries Commission

MacKenzie CL (1981) Biotic potential and environmental resistance in the American oyster (*Crassostrea virginica*) in Long Island Sound. Aquaculture 22:229-268

McGurrin JM, Stone RB, Sousa RJ (1989) Profiling United States artificial reef development. Bull Mar Sci 44:1004-1013

Manley J, Power A, Walker R (2008) Patterns of eastern oyster, *Crassostrea virginica* (Gmelin, 1791), recruitment in Sapelo Sound, Georgia: Implications for commercial oyster culture. Occasional papers of the University of Georgia Marine Extension Service Vol. 3

Mann R, Burreson EM, Baker PK (1991) The decline of the Virginia oyster fishery in Chesapeake Bay: considerations for introduction of a non-endemic species, *Crassostrea gigas* (Thunberg, 1793). J Shellfish Res 10(2):379-388

Mann R, Evans DA (1998) Estimation of oyster, *Crassostrea virginica*, standing stock, larval production and advective loss in relation to observed recruitment in the James River, Virginia. J Shellfish Res 17(1):239-254

Mann R (2001) Restoration of the oyster resource in Chesapeake Bay: The role of oyster Reefs in population enhancement, water quality improvement and support of diverse species-rich communities. Bull Aquacul Assoc Canada 101(1):38-42

Mann R, Powell EN (2007) Why oyster restoration goals in the Chesapeake Bay are not and probably cannot be achieved. J Shellfish Res 26(4):1-13

Mann R, Southworth M, Harding JM, Wesson JA (2009a) Populations studies of the native eastern oyster, *Crassostrea virginica*, (Gmelin, 1791) in the James River, Virginia, USA J Shellfish Res 28(2):193-220

Mann R, Harding JM, Southworth M (2009b) Reconstructing pre-colonial oyster demographics in the Chesapeake Bay, USA. Est Coast Shelf Sci 85:217-222

May EB (1971) A survey of the oyster and oyster shell resources of Alabama. Ala Mar Res Bull 3:1-14

Medcoff J, Needler AWH (1941) The influence of temperature and salinity on the condition of oysters *Ostrea virginica*. J Fish Res Board Can 5:253-257

Menge BA, Branch GM (2000) Rocky intertidal communities. In Marine Community Ecology. Sinauer Associates, Inc. Sunderland, MA 01375-0407

Mercado-Silva, N (2005) Condition index of the eastern oyster *Crassostrea virginica* (Gmelin 1791) in Sapelo Island, Georgia—effects of site, position on bed and pea crab parasitism. J Shellfish Res 24:121-126

Meyer DL, Townsend EC, Thayer GW (1997) Stabilization and erosion control value of oyster cultch for intertidal marsh. Rest Ecol 5(1):93-99

Mottet MC (1981) Enhancement of the marine environment for fisheries and aquaculture in Japan. Washington Dept. Fish., Tech. Rep. 7 pp

Munch SB, Walsh MR, Conover DO (2005) Harvest selection, genetic correlations, and evolutionary changes in recruitment: one less thing to worry about? Can J Fish Aquat Sci 62:802-810

National Oceanic and Atmospheric Administration (1998) Oxygen depletion in coastal waters by Nancy N Rabalais. NOAA's State of the coast report. Silver Spring, Maryland:http://state_of_coast.noaa.gov/bulletins/html/hyp_09/hyp.html

National Oceanic and Atmospheric Administration (2009) Alternative substrates. http://noaa.chesapeakebay.net/AlternativeSubstrates.aspx

National Research Council (2004) Non-native oysters in the Chesapeake Bay. National Academy Press, Washington D.C.

Nelson KA, Leonard LA, Posey MH, Alphin TD, Mallin MA (2004) Transplanted oyster (*Crassostrea virginica*) beds as self-sustaining mechanisms for water quality improvement in small tidal creeks. J Exp Mar Biol Ecol 298:347-368

Nestlerode JA, Luckenbach MW, O'Beirn (2007) Settlement and survival of the oyster *Crassostrea virginica* on created oyster reef habitats in Chesapeake Bay. Rest Ecol 15(2):273-282

Neves dos Santos M, Costa Monteiro C (1997) The Olha˜o artificial reef system (south Portugal): fish assemblages and fishing yield. Fish Res 30:33-41

Neves dos Santos M, Costa Monteiro C (1998) Comparison of the catch and fishing yield from an artificial reef system and neighbouring areas off Faro (south Portugal). Fish Res 39:55-65

Newell RIE (1988) Ecological changes in Chesapeake Bay: Are they the result of overharvesting the eastern oyster (*Crassostrea virginica*)? Pages 536-546 In: M.P. Lynch and E.C. Krome, (eds) Understanding the Estuary: Advances in Chesapeake Bay Research. Chesapeake Research Consortium Publication 129 (CBP/TRS 24/88), Gloucester Point, VA

Newell RIE, Alspach GS, Kennedy VS, Jacobs D (2000) Mortality of newly metamorphosed eastern oysters (*Crassostrea virginica*) in mesohaline Chesapeake Bay. Mar Biol 136:665-676

Newell RIE (2004) Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve mollusks: A review. J Shellfish Res 23(1):51-61

Newell RIE, Fisher TR, Holyoke RR, Cornwell JC (2004) Influence of eastern oysters on nitrogen and phosphorous regeneration in Chesapeake Bay, USA. In: The comparative roles of suspension feeders in ecosystems (eds) R Dame, S Olenin. NATO Science Series: IV – Earth and Environmental Sciences, Kluwer Academic Publishers, Dordrecht, The Netherlands

Newell RIE, Fisher TR, Holyoke RR, Cornwell JC (2005) Influence of eastern oysters on N and P regeneration in Chesapeake Bay, USA. Pages 93-120 in R. Dame and S. Olenin, editors. The comparative roles of suspension feeders in ecosystems. Springer, The Netherlands. Available: www.hpl.umces.edu/faculty/newellcv.html

North Carolina Division of Marine Fisheries (1988) North Carolina artificial reef master plan. North Carolina Dept. of Natural Resources and Community Development

O'Beirn FX, Heffernan PB, Walker RL (1996) Recruitment of the eastern oyster in coastal Georgia: Patterns and recommendations. N Am J Fish Man 16:413-426

Officer CB, Smayda TJ, Mann R (1982) Benthic filter feeding: A natural eutrophication control. Mar Ecol Prog Ser 9:203-210

Osman RW, Whitlatch RB, Malatesta RJ (1992) Potential role of micropredators in determining recruitment into a marine community. Mar Ecol Prog Ser 83:35-43

Oyster Advisory Commission (2008) http://www.dnr.state.md.us/fisheries/oysters/

Oyster Management Plan (2009) 2007 Oyster management plan workshop: Proceedings and agreement statements. Chesapeake Bay Program, Environmental Protection Agency

Paxton JG Esq (1858) Speech of James G. Paxton, Esq. of Rockbridge, relative to the oyster fundum of Virginia. Delivered in the House of Delegates of Virginia, March 26, 1858 Richmond, VA. Printed by Ritchie and Dunnavant

Paynter KT (1999) Managing around oyster diseases in Maryland and Maryland oyster roundtable strategies. p. 317-328. *In*: (eds) MW Luckenbach, R Mann, and JA Wesson. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Peterson CH, Black R (1987) Resource depletion by active suspension feeders on tidal flats: Influence of local density and tidal elevation. Limnol Oceanogr 32(1):143-166

Peterson CP, Grabowski JH, Powers SP (2003) Estimated enhancement of fish production resulting from restoring oyster reef habitat: Quantitative valuation. Mar Ecol Prog Ser 264:249-264

Piazza BP, Banks PD, La Peyre MK (2005) The potential for created oyster shell reefs as a sustainable shoreline protection strategy in Louisiana. Rest Ecol 13(3):499-506

Posey MH, Alphin TD, Powell CM, Townsend E (1999) Use of oyster reefs as habitat for epibenthic fish and decapods. pp 229-237. *In*: (eds) MW Luckenbach, R Mann, and JA Wesson. Oyster reef habitat restoration: a synopsis and synthesis of approaches. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Powell EN, Kraeuter JN, Ashton-Alcox KA (2006) How long does oyster shell last on an oyster reef? Est Coast Shelf Sci 69:531-542

Powell EN, Klinck JM (2007) Is oyster shell a sustainable resource? J Shellfish Res 26(1):181-194

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

Priest WI, Nestlerode J, Frye CW (1999) Use of dredged material for oyster habitat creation in coastal Virginia. pp 283-293. *In*: (eds) MW Luckenbach, R Mann, and JA Wesson. Oyster reef habitat restoration: a synopsis and synthesis of approaches. Virginia Institute of Marine Science Press, Gloucester Point, Virginia 23062

Rabalais NN, Turner RE, Wiseman WJ (2001) Hypoxia in the Gulf of Mexico. J Env Qual 30:320-329

Rainer JS, Mann R (1992) A comparison of methods for calculating condition index in eastern oysters *Crassostrea virginica* (Gmelin 1791). J Shellfish Res 11:55-58

Ray SM (1954) Studies on the occurrence of *Dermocystidium marinum* in young oysters. Texas A & M Research Foundation.

Rheault RB, Rice MA (1996) Food-limited growth and condition index in the eastern oyster, *Crassostrea virginica* (Gmelin 1791), and the bay scallop, *Argopecten irradians* (Lamarck 1819). J Shellfish Res 15(2):271-283

Rhoads D (1974) Organism-sediment relations on the muddy sea floor. Oceanogr Mar Biol Ann Rev 12:263-300

Ritter HP (1896) Report on a reconnaissance of the oyster beds of Mobile Bay and Mississippi Sound, Alabama. Bull US Fish Comm for 1895:325-339

Ross PG, Luckenbach MW (2009) Population assessment of eastern oysters (*Crassostrea virginica*) in the seaside coastal bays. Report to Coastal Zone Management Program, Virginia Department of Environmental Quality, Richmond, VA

Rothschild BJ, Ault JS, Goulletquer 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

Ruesink JL, Lenihan HS, Trimble AC, Heiman KW, Micheli F, Byers JE, Kay MC (2005) Biotic resistance and facilitation of a non-native oyster on rocky shores. Annual Review of Ecol Evol Syst 36:643-689

Santopietro GD, Stephenson K, Satyal VA, Wesson J (2009) A bioeconomic analysis of management plans for the public oyster grounds of the Rappahannock River. J Shellfish Res 28(2):235-241

Sara' M (1987) Persistence and changes in marine benthic communities. Nova Thalassia 3 suppl.:7-30

Scheer BT (1945) The development of marine fouling communities. Biol. Bull. 89:103-121

Schulte DM (2003) Final Decision Document Amendment, Section 704B as Amended, Chesapeake Bay Oyster Recovery Phase III, Great Wicomico River, Virginia. Norfolk District – United States Army Corps of Engineers. Norfolk, Virginia 23510-1096

Schulte DM, Cleland M, Groth K (2006) Final Decision Document Amendment, Section 704B as Amended, Chesapeake Bay Oyster Recovery Phase IV, Lynnhaven River, Virginia. Norfolk District – United States Army Corps of Engineers. Norfolk, Virginia 23510-1096

Schulte DM, Burke RP, Lipcius RN (2009) Unprecedented restoration of a native oyster metapopulation. Science 325:1124-1128

Schulte DM, Ray G (2009) Use of alternative materials for oyster reef construction. Ecosystem management and restoration research program (EMRRP) Technical Note

Schumacker EJ, Dumbauld BR, Kauffman BE (1998) Investigations using oyster condition index to monitor the aquatic environment of Wilapa Bay Washington. J Shellfish Res 17:338-339

Seitz RD, Lipcius RN, Olmstead NH, Seebo MS, Lambert DM (2006) Influence of shallow-water habitats and shoreline development on abundance, biomass, and diversity of benthic prey and predators in Chesapeake Bay. Mar Ecol Prog Ser 326:11-27

Seitz RD, Dauer DM, Llanso RJ, Long WC (2009) Broad-scale effects of hypoxia on benthic community structure in Chesapeake Bay, USA. J Exp Mar Biol Ecol 381:S4-S12

Smith GF, Bruce DG, Roach EB, Hansen A, Newell RIE, McManus AM (2005) Assessment of recent habitat conditions of eastern oyster *Crassostrea virginica* bars in mesohaline Chesapeake Bay. N Am J Fish Man 25:1569-1590

Soniat TS, Broadhurst RC, Haywood EL (1991) Alternatives to clamshell as cultch for oysters, and the use of gypsum for the production of cultchless oysters. J Shellfish Res 10:405-410

Soniat TM, Finelli CM, Ruiz JT (2004) Vertical structure and predator refuge mediate oyster reef development and community dynamics. J Exp Mar Biol Ecol 310:163-182

Southworth M, Harding JM, Mann R (2002) The status of Virginia's public oyster resource 2001. Molluscan ecology program, Virginia Institute of Marine Science, Gloucester Point, Virginia. 53 pp

Southworth M, Harding JM, Mann R (2003) The status of Virginia's public oyster resource 2002. Molluscan ecology program, Virginia Institute of Marine Science, Gloucester Point, Virginia. 51 pp

Southworth M, Mann R (2004) Decadal scale changes in seasonal patterns of oyster recruitment in the Virginia sub estuaries of the Chesapeake Bay. J Shellfish Res 23(2):391-402

Southworth M, Harding JM, Mann R (2006) The status of Virginia's public oyster resource 2005. Molluscan ecology program, Virginia Institute of Marine Science, Gloucester Point, Virginia. 49 pp

Southworth M, Harding JM, Mann R (2007) The status of Virginia's public oyster resource 2006. Molluscan ecology program, Virginia Institute of Marine Science, Gloucester Point, Virginia. 49 pp

Southworth M, Harding JM, Mann R (2008a) The status of Virginia's public oyster resource 2007. Molluscan ecology program, Virginia Institute of Marine Science, Gloucester Point, Virginia. 49 pp

Southworth M, Mann R, Harding JM, Wesson JA (2008b) Multiyear recruitment patterns of oysters (*Crassostrea virginica*) on constructed reefs in the Chesapeake Bay. J Shellfish Res. 27:1053

Stevenson CH (1894) The oyster industry of Maryland. U.S. Fish Comm Bull 12:203-298

Stone RB, McGurrin JM, Sprague LM, Seaman W (1991) Artificial habitats of the world: Synopsis and major trends. In Artificial habitats for marine and freshwater fisheries. Eds W Seaman, Jr, LM Sprague. Academic Press, Inc. San Diego, California 92101

Sutherland JP, Karlson RH (1977) Development and stability of the fouling community at Beaufort, North Carolina. Ecol Monogr 47:425-446

Tamburri MN, Finelli CM, Wethey DS, Zimmer-Faust RK (1996) Chemical induction of larval settlement behavior in flow. Biol Bull 191:367-373

Tamburri MN, Zimmer RK, Zimmer CA (2007) Mechanisms reconciling gregarious larval settlement with adult cannibalism. Ecol Monogr 77(2):255-268

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

Thorson G (1966) Some factors influencing the recruitment and establishment of marine benthic communities. Netherlands J Sea Res 3:267-293

Turner EJ, Zimmer-Faust RK, Palmer MA, Luckenbach MW, Pentcheff ND (1994) Settlement of oyster (*Crassostrea virginica*) larvae: Effects of water flow and a water soluble chemical cue. Limnol Oceanogr 39(7):1579-1593

Underwood AJ (1997) Experiments in ecology: Their logical design and interpretation using analysis of variance. Cambridge University Press, United Kingdom. 504 pp

United States Army Corps of Engineers (2009) Final programmatic environmental impact statement for oyster restoration in Chesapeake Bay including the use of a native and/or nonnative oyster. Norfolk, VA 23510

United States Department of Commerce (1985) National artificial reef plan: Guidelines for siting, construction, development, and assessment of artificial reefs

United States Department of Commerce (2007) National artificial reef plan (as amended): Guidelines for siting, construction, development, and assessment of artificial reefs

Virginia Coastal Zone Management Program (2009) Living shorelines. . .the natural approach to controlling shoreline erosion. www.deq.state.va.us/coastal/livingshore.html

Virginia Commission to study and revise Title 28 of the code of Virginia relating to fish, oysters and shellfish to the Governor and the General Assembly (1961) House Document No. 14. Richmond, Virginia, 102 pp

Virginia Department of Conservation and Recreation (2009) Shoreline erosion problems? Think Green. . .control shoreline erosion, help restore the Chesapeake Bay and save money all at the same time. 8 pp

Virginia Institute of Marine Science (1996) Strategic plan for molluscan shellfish research; including a rational plan for testing application of non-native oyster species. Report to the Governor and the General Assembly of Virginia, House Document No. 16, Richmond, Virginia

Virginia Marine Resources Commission (2007) January 23, 2007 Virginia Marine Resource Commission meeting minutes item 17, Virginia oyster restoration: approval of the 2007 program and approval of procurement procedures

Virginia Marine Resources Commission (2008) February 26, 2008 Virginia Marine Resource Commission meeting minutes item 16, oyster restoration: approval of the 2008 program and procurement procedures

Virginia Marine Resources Commission (2009) Virginia's artificial reef program. http://www.mrc.virginia.gov/vsrfdf/reef.shtm

Weiner RM, Walch M, Labare MP, Bonar DB, Colwell RR (1989) Effect of biofilms of the marine bacterium *Alteromonas colwelliana* (LST) on set of the oysters *Crassostrea gigas* (Thunberg) and *C. virginica* (Gmelin). J Shellfish Res 8:117-123

Wildish DJ, Kristmanson DD, Hoar RL, DeCoste AM, McCormick SD, White AW (1987) Giant scallop feeding and growth responses to flow. J Exp Mar Biol Ecol 113(3):207-220

Williams BK, Nichols JD, Conroy MJ (2002) Analysis and management of animal populations. Academic Press. San Diego, California 92101-4495, USA

Wilson CA (1986) Development of an artificial reef plan for the State of Louisiana: A statement of needs. CFI Publication No. 85-37. 23 pp

Winslow F (1881) Deterioration of American oyster beds. Popular Science Monthly 20:145-156

Witman JD, Dayton PK (2000) Rocky subtidal communities. In Marine Community Ecology. Sinauer Associates, Inc. Sunderland, MA 01375-0407

Wood P (2009) Finding the right home for oysters. The Capital. 14 Dec 2009. http://www.hometownannapolis.com/news/env/2009/12/14-26/Finding-the-right-home-for-oysters.html

Woodin SA (1976) Adult-larval interactions in dense infaunal assemblages: patterns of abundance. J Mar Res 34:25-41

Yamane T (1989) Status and future plans of artificial reef projects in Japan. Bull Mar Sci 44(2):1038-1040

Zar JH (1996) Biostatistical analyses. 3rd edition. Prentice Hall, Inc. Upper Saddle River, NJ 07458

Zimmer-Faust RK, Tamburri MN (1994) Chemical identity and ecological implications of a waterborne, larval settlement cue. Limnol Oceanogr 39(5):1075-1087

VITA

RUSSELL PAUL BURKE

Russ was born in Bay Shore, New York on 8 December 1979 to Russell and Linda Burke. He was raised in North Babylon and graduated from North Babylon High School in 1998. Russ studied marine and environmental science as an undergraduate at Rider University in Lawrenceville, New Jersey, and graduated with a B.S. in Marine Science and Environmental Science in the spring of 2002. Russ began his Ph.D. program at VIMS in the fall of 2002.