Oyster Reef Restoration in Virginia: Broodstock Addition & Nutrient Exchanges

Laurie Ann Sorabella
College of William and Mary - Virginia Institute of Marine Science

Follow this and additional works at: https://scholarworks.wm.edu/etd
Part of the Fresh Water Studies Commons, Oceanography Commons, and the Water Resource Management Commons

Recommended Citation
https://dx.doi.org/doi:10.25773/v5-xvg3-ja55

This Thesis is brought to you for free and open access by the Theses, Dissertations, & Master Projects at W&M ScholarWorks. It has been accepted for inclusion in Dissertations, Theses, and Masters Projects by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.
OYSTER REEF RESTORATION IN VIRGINIA:
BROODSTOCK ADDITION & NUTRIENT EXCHANGES

A Thesis
Presented to
The Faculty of the School of Marine Science
The College of William & Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Science

by
Laurie Ann Sorabella
2002
APPROVAL SHEET

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

Master of Science

Laurie A. Sorabella

Approved, September 2002

Kenneth A. Moore, Ph.D.
Committee Chairman/Co-Advisor

Mark W. Luckenbach, Ph.D.
Co-advisor

Iris C. Anderson, Ph.D.

John M. Brubaker, Ph.D.

Roger L. Mann, Ph.D.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS .............................................................................................................. v
LIST OF TABLES ........................................................................................................................ vi
LIST OF FIGURES ...................................................................................................................... vii
THESIS ABSTRACT ................................................................................................................... ix

THESIS INTRODUCTION ........................................................................................................ 2
LITERATURE CITED .................................................................................................................. 7

CHAPTER 1
OYSTER REEF RESTORATION STRATEGY: BROODSTOCK ADDITION ..................................... 11

ABSTRACT ................................................................................................................................. 12
INTRODUCTION ......................................................................................................................... 13
   Reef Restoration Strategy ..................................................................................................... 15
   Oyster Stock Potential as Broodstock .................................................................................. 18
METHODS .................................................................................................................................. 21
   Site Description .................................................................................................................... 21
   Growth & Survival ................................................................................................................. 24
   Female Fecundity .................................................................................................................. 26
   Disease Prevalence & Intensity ............................................................................................ 28
   Cumulative Egg Production ................................................................................................. 30
RESULTS .................................................................................................................................... 31
   Growth & Survival ............................................................................................................... 31
   Disease Prevalence & Intensity ............................................................................................ 35
   Female Fecundity .................................................................................................................. 43
   Cumulative Egg Production ................................................................................................. 49
DISCUSSION ............................................................................................................................... 49
   Disease Pressure 2000 & 2001 ............................................................................................ 51
   High MSX Pressure - Tanner Point Reef ............................................................................. 54
   High Dermo Pressure - Larchmont Reef ............................................................................. 59
   Broodstock Strategies ........................................................................................................... 63
CONCLUSION ............................................................................................................................. 68
LITERATURE CITED .................................................................................................................. 70

CHAPTER 2
OYSTER REEF RESTORATION IN VIRGINIA: NUTRIENT EXCHANGES WITH THE WATER COLUMN ................................................................................................................... 76

ABSTRACT ................................................................................................................................. 77
INTRODUCTION ........................................................................................................................ 78
   Water Quality Changes Associated with Oyster Restoration ............................................. 78
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential Relationships Between Oyster Reefs and Seagrasses</td>
<td>84</td>
</tr>
<tr>
<td>Objectives</td>
<td>87</td>
</tr>
<tr>
<td>METHODS</td>
<td>87</td>
</tr>
<tr>
<td>Site Description</td>
<td>87</td>
</tr>
<tr>
<td>Sample Collection</td>
<td>89</td>
</tr>
<tr>
<td>Flux Calculations for Nutrients</td>
<td>96</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>98</td>
</tr>
<tr>
<td>RESULTS</td>
<td>98</td>
</tr>
<tr>
<td>Dissolved Nutrient Fluxes</td>
<td>98</td>
</tr>
<tr>
<td>Chlorophyll-a Fluxes</td>
<td>106</td>
</tr>
<tr>
<td>Sediment Biogeochemistry</td>
<td>109</td>
</tr>
<tr>
<td>Net Exchange of Nitrogen and Phosphorus</td>
<td>109</td>
</tr>
<tr>
<td>Net Impact of Reef Restoration on Nitrogen Exchange in the System</td>
<td>115</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>118</td>
</tr>
<tr>
<td>Nitrogen Processes</td>
<td>118</td>
</tr>
<tr>
<td>Phosphorus Processes</td>
<td>125</td>
</tr>
<tr>
<td>Sediment Biogeochemistry</td>
<td>129</td>
</tr>
<tr>
<td>Rate of Exchange Processes</td>
<td>132</td>
</tr>
<tr>
<td>Link to Seagrass Habitat &amp; Potential Recolonization</td>
<td>135</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>138</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>139</td>
</tr>
<tr>
<td>THESIS SUMMARY</td>
<td>147</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>151</td>
</tr>
<tr>
<td>VITA</td>
<td>152</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

My graduate experience at VIMS has surpassed my expectations. I owe tremendous thanks to my major advisers, Dr. Mark Luckenbach and Dr. Ken Moore, for their constant support, guidance, and insight into the design and implementation of this research project. Both are excellent scientists and have been superb role models for me. In addition, I would like to thank the members of my committee: Dr. Iris Anderson for her continual confidence in me, Dr. Roger Mann for sharing his extensive oyster experience, and Dr. John Brubaker for his curiosity about oyster restoration. I also thank Dr. Bob Diaz, who made my statistical stumbling blocks manageable.

I have tremendous gratitude for all those who have helped me to complete this study. Three people, in particular, gave very generously of their time in order to assist me with this research. Betty Berry Neikirk spent many hours collecting samples with me in the field and teaching me nutrient analysis techniques in the lab. Betty’s company and good humor throughout my study made me truly look forward to field and lab days. PG Ross managed all boat logistics and helped me with every Lafayette River field day, despite my gift for scheduling the rainiest possible field days. Rita Crockett offered me her patience, knowledge and microtome-talent as she taught me to process, prepare and read histology slides. There were also many, many students and friends who deserve thanks for joining me on my field days.

I greatly appreciate the funding that I have received for my education and my research, provided by: Sea Grant’s Oyster Disease Research Program through Virginia Sea Grant Graduate Marine Science Consortium, the Kelley Watson Memorial Fellowship through VIMS’ Dean’s Office, the Nancy Foster Scholarship through the National Oceanic and Atmospheric Administration, and the Chesapeake Bay Foundation for oysters for my broodstock study.

As always, I wholeheartedly thank my family for their perpetual support of my endeavors. I thank my father for whisking me away to far away marine locations that make me dream of my next step in life; my mother for her unending belief in my ability; Jean for her understanding of the life of a graduate student; Bill for his family spirit which has encouraged me to leave school to come home on holidays; and Robert for sharing my curiosity for science. I also wish to thank my grandfather, Philip Tobia Sorabella, who has inspired my interest in marine systems with his life-long fascination with the sea and marine life. Finally, I thank my awesome new husband, Bob Carroll, who bought me my first waterproof paper, who encouraged my progress in graduate school and who will continue to be my fellow adventurer on many marine science explorations in the future. May I continue to make all of you proud and to excite you about oyster restoration.
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHAPTER 1: BROODSTOCK ADDITION</strong></td>
<td></td>
</tr>
<tr>
<td>1.1 ANOVA table for size</td>
<td>34</td>
</tr>
<tr>
<td>1.2 ANOVA table for survival</td>
<td>37</td>
</tr>
<tr>
<td>1.3 ANOVA table for survival – year 1</td>
<td>37</td>
</tr>
<tr>
<td>1.4 ANOVA table for survival – year 2</td>
<td>37</td>
</tr>
<tr>
<td><strong>CHAPTER 2: NUTRIENT EXCHANGES</strong></td>
<td></td>
</tr>
<tr>
<td>2.1 Sediment Organic Content</td>
<td>110</td>
</tr>
<tr>
<td>2.2 Sediment Nutrients</td>
<td>111</td>
</tr>
<tr>
<td>2.3 Sediment Chlorophyll-a &amp; Sediment Phaeophytin</td>
<td>112</td>
</tr>
<tr>
<td>2.4 Sediment Bulk Density and Percent Water</td>
<td>113</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Study site - Hampton Roads, Virginia</td>
<td>17</td>
</tr>
<tr>
<td>1.2</td>
<td>Locations of student nursery sites</td>
<td>23</td>
</tr>
<tr>
<td>1.3</td>
<td>Diagram of the experimental set up in the Lafayette River</td>
<td>25</td>
</tr>
<tr>
<td>1.4</td>
<td>Oyster growth as mean shell height (± SE) verses time</td>
<td>32</td>
</tr>
<tr>
<td>1.5</td>
<td>Water quality at the Lafayette River reefs</td>
<td>33</td>
</tr>
<tr>
<td>1.6</td>
<td>Oyster mortality plotted as mean % survival (± SE) verses time</td>
<td>36</td>
</tr>
<tr>
<td>1.7</td>
<td><em>Haplosporidium nelsoni</em> prevalence (± SE)</td>
<td>38</td>
</tr>
<tr>
<td>1.8</td>
<td>Weighted prevalence of <em>Haplosporidium nelsoni</em> (± SE)</td>
<td>40</td>
</tr>
<tr>
<td>1.9</td>
<td><em>Perkinsus marinus</em> prevalence (± SE)</td>
<td>41</td>
</tr>
<tr>
<td>1.10</td>
<td>Weighted prevalence of <em>Perkinsus marinus</em> (± SE)</td>
<td>42</td>
</tr>
<tr>
<td>1.11</td>
<td>Mean female egg production (± SE)</td>
<td>44</td>
</tr>
<tr>
<td>1.12</td>
<td>Individual female egg production verses oyster shell height</td>
<td>46</td>
</tr>
<tr>
<td>1.13</td>
<td>Proportion of reproductive females in populations</td>
<td>48</td>
</tr>
<tr>
<td>1.14</td>
<td>Cumulative egg production (± SE) over the entire experiment</td>
<td>50</td>
</tr>
<tr>
<td>1.15</td>
<td>Cumulative egg production (± SE) for year 1 (Cumulative$_1$) and year 2 (Cumulative$_2$) of deployment</td>
<td>50</td>
</tr>
<tr>
<td>1.16</td>
<td>Distribution of <em>H. nelsoni</em> in Virginia in the fall of 2000</td>
<td>65</td>
</tr>
<tr>
<td>1.17</td>
<td>Distribution of <em>P. marinus</em> in Virginia in the fall of 2000</td>
<td>66</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES (cont.)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Chapter 2: Nutrient Exchanges</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>General oyster population nutrient budget</td>
</tr>
<tr>
<td>2.2</td>
<td>Study site – Fishermans Island</td>
</tr>
<tr>
<td>2.3</td>
<td>Oyster biomass and density in the three chambers (± SE)</td>
</tr>
<tr>
<td>2.4</td>
<td>Flux study chamber</td>
</tr>
<tr>
<td>2.5</td>
<td>Seasonal fluxes of NO$_2^-$ and NO$_3^-$</td>
</tr>
<tr>
<td>2.6</td>
<td>Seasonal fluxes of NH$_4^+$ and DON</td>
</tr>
<tr>
<td>2.7</td>
<td>Seasonal fluxes of PO$_4^{3-}$</td>
</tr>
<tr>
<td>2.8</td>
<td>Seasonal fluxes of chlorophyll-a</td>
</tr>
<tr>
<td>2.9</td>
<td>Net nitrogen exchange calculated by summing DIN, DON and particulate nitrogen (calculated from chlorophyll-a values)</td>
</tr>
<tr>
<td>2.10</td>
<td>Net phosphorus exchange calculated by summing DIP and particulate phosphorus (calculated from chlorophyll-a values)</td>
</tr>
<tr>
<td>2.11</td>
<td>Net impact of reef rehabilitation on nitrogen cycling</td>
</tr>
<tr>
<td>2.12</td>
<td>Nutrient cycling as a function of grazing rate</td>
</tr>
</tbody>
</table>
Oyster (*Crassostrea virginica*) reef restoration is being undertaken in the Chesapeake Bay with the dual purpose of re-establishing viable native oyster populations on sanctuary reefs and restoring reef ecosystem functions, such as water filtration. To enhance reproduction and larval settlement on the sanctuary reefs, shell plants are often stocked with hatchery-produced brood stock oysters. An important unresolved issue in such stocking efforts is the suitability of specific oyster stocks for achieving the desired goal. In particular, oysters stocks selected for aquaculture are currently available and there is uncertainty about the efficacy of using these stocks versus wild stocks for restoration efforts. Brood stock addition strategy was explored by comparing the performance of two hatchery-reared oyster stocks, the selectively-bred CROSBreed stock and a local wild-caught stock, after transplant onto a sanctuary reef in the Lafayette River (Chesapeake Bay, Virginia) to determine which should be used as brood stock in reef restoration efforts. Performance was evaluated based on growth, survival, female fecundity, sex of the population and disease level of the two stocks. These data were used to calculate cumulative egg production, which served as a measure of reproductive potential. Results indicated that reproductive performance of the two stocks was dependent on disease pressure. CROSBreed stock oysters provided greater cumulative egg production when MSX pressure was high. Wild-caught stock oysters provided greater cumulative egg production when dermo pressure was high. The relationship between disease pressure and cumulative egg production from these oyster stocks offers a potential criterion for the selection of stock for use as brood stock to maximize the rate of sanctuary reef population development.

Once these created reefs develop viable oyster populations, their function in water filtration has not previously been determined in the Chesapeake Bay. Flux study measurements were performed to examine the seasonal impact of reef rehabilitation on phytoplankton grazing and nutrient cycling from created oyster reefs at Fishermans Island, VA. In situ mesocosms were used to measure dissolved inorganic nitrogen, dissolved organic nitrogen, dissolved inorganic phosphorus and chlorophyll exchanges over the reefs, and fluxes were compared to those over an adjacent intertidal flat similar to the section that had been replaced by reef construction. In most seasons, the oyster reef community showed greater dissolved nutrient release and greater chlorophyll uptake than the adjacent intertidal flat. Net seasonal nitrogen exchange for both communities was determined by combining dissolved and particulate (calculated from chlorophyll flux) fluxes. The oyster reef was determined to be a sink for nitrogen in the spring and fall, while the intertidal flat was determined to be a source for nitrogen throughout the study. These data suggest that these revitalized oyster reefs at Fisherman’s Island may increase the potential for nitrogen storage and decrease phytoplankton standing stocks in spring and fall, as phytoplankton are nitrogen-limited in the region. This potential seasonal impact on phytoplankton abundance in spring and fall coincides with the critical growth period for eelgrass (*Zostera marina*) in this region, and suggests the potential for created oyster reefs to locally improve habitat for eelgrass.
OYSTER REEF RESTORATION IN VIRGINIA:
BROODSTOCK ADDITION & NUTRIENT EXCHANGES
THESIS INTRODUCTION

In the twentieth century, the Chesapeake Bay's once prominent oyster population and oyster reef habitat have experienced tremendous declines (Brumbaugh et al. 2000a, Hargis & Haven 1999, Luckenbach et al. 2000, Newell 1988, Rothschild et al. 1994). Near collapse of the oyster population, *Crassostrea virginica* (Gmelin 1791), was the result of a combination of factors, most notably over harvesting, habitat destruction, and disease (Brumbaugh et al. 2000a, Hargis & Haven 1999, Kennedy 1996, Rothschild et al. 1994). Population rebound has been difficult for *C. virginica* because of continued disease pressure, low broodstock density with associated decreased fertilization efficiency, and insufficient larval settling substrate (Hargis & Haven 1999).

Oysters and oyster reefs have historically been valuable in the Chesapeake Bay ecosystem because they impact water quality through phytoplankton grazing, sediment removal from the water column, and nutrient-cycling (Coen et al. 1999a, Dame et al. 1989, Haven & Morales-Alamo 1970, Newell 1988, Newell et al. 1999, Ulanowicz & Tuttle 1992). From March through December, oysters in the bay filter water at a rate of 5 L • h\(^{-1}\) • g\(^{-1}\) (dry tissue weight) (Newell 1988) removing phytoplankton and suspended sediments from the water column, and they stabilize nutrient cycling by retaining nitrogen and phosphorus in long-lived tissue (Dame et al. 1989). Additionally, oysters and oyster reefs provide food and habitat for myriad
commercial and non-commercial species (Coen et al. 1999b) and oysters serve as a valuable link in benthic-pelagic coupling (Haven & Morales-Alamo 1966).

Many field and modeling studies have concluded that the filter-feeding of bivalves can improve water clarity and reduce the potential for eutrophication in estuaries (Cloern 1982, Cohen et al. 1984, Dame 1999, Newell 1988, Officer et al. 1982, Tuttle et al. 1987, Ulanowicz & Tuttle 1992). Newell (1988) concluded that the Chesapeake Bay's historic oyster population was likely the dominant species filtering phytoplankton and suspended solids from the water in pre-colonial times. He hypothesized that the water was substantially clearer historically because oysters had the potential to filter all of the water in the bay in less than a week. He also theorized that the decline in the oyster population had resulted in the oysters' inability to graze the spring bloom, which has left high levels of phytoplankton available for microbial decomposition and led to summer anoxia problems.

Provided that oyster reef filtration can improve water clarity, oyster reef restoration may be linked with improved conditions for the growth and revitalization of polyhaline seagrass beds (Dame et al. 1984, Dennison et al. 1993, Hargis & Haven 1999, Newell 1988, Newell et al. 1999, Ulanowicz & Tuttle 1992). Seagrass beds are a second critical shallow water habitat that has experienced tremendous declines in the twentieth century (Orth & Moore 1983). Destruction of polyhaline seagrasses beds, formed by species such as *Zostera marina*, has resulted mainly from periodic natural and human disturbances, wasting disease in the 1930's, and the decline of
water quality (Kemp 1989, Short & Wylie-Echeverria 1996, Stevenson et al. 1993). Currently, the Chesapeake Bay Z. marina population is limited primarily by light availability (Batiuk et al. 1992, Moore et al. 1996, Moore & Wetzel 2000, Wetzel & Penhale 1983). Restoration of seagrass beds is being undertaken in the Chesapeake Bay to reestablish the myriad ecological benefits that this habitat provides to the ecosystem (Batiuk et al. 1992). Seagrass beds impact water quality by stabilizing nutrient cycling as they remove inorganic nutrients from the water column and substrate and store nitrogen and phosphorus in long-lived tissue, and by initiating deposition of inorganic suspended matter from the water column and minimizing sediment resuspension (Kemp 1989, Ward et al. 1984). Like oyster reefs, seagrass beds provide habitat and nursery for many bay species (Gerloff & Krumbholz 1966, Kemp 1989).

Since the late 1980's, there has been increasing emphasis on restoring oyster reefs in the bay because their presence and ecosystem functions contribute to the overall ecological rehabilitation of the bay (Gottlieb & Schweighofer 1996, Hargis & Haven 1999, Luckenbach et al. 1999, Mann 2000). Restoration of sanctuary oyster reefs in the Chesapeake Bay is now considered to be integral to the revitalization of the devastated oyster population, to the re-establishment of a viable oyster fishery, and locally, to improved water quality and habitat availability (Brumbaugh et al. 2000b, Coen et al. 1999a, Gottlieb & Schweighofer 1996, Hargis & Haven 1999, Luckenbach et al. 2000, Rothschild et al. 1994, Ulanowicz & Tuttle 1992). Since 1994, eastern oyster reef restoration has been undertaken in the Chesapeake Bay with
the primary purpose of developing self-sustaining oyster populations on constructed reef habitat, and a secondary purpose of reviving oyster ecosystem filter and habitat function (Hargis & Haven 1999). Oyster population revitalization and reef restoration are being undertaken by (1) placing oyster shell in the bay to serve as settling substrate for planktonic larval oysters; (2) restricting harvest; and (3) in areas where recruitment is limited, adding broodstock to increase reproduction and potential settlement to the reef structure (Luckenbach et al. 2000).

This thesis research has two objectives that relate directly to the primary and secondary objectives of oyster reef restoration. The first explores the strategy behind stocking reefs with broodstock oysters and compares the performance of two hatchery-reared oyster stocks to determine which stock should be used as broodstock to initiate the development self-sustaining oyster populations on sanctuary oyster reefs in large-scale reef restoration efforts. The second objective deals with restored oyster reef filtration function and uses flux study measurements to identify the nutrient exchanges associated with a developed oyster reef in comparison to the adjacent intertidal flat that was replaced by the constructed reef.

Researchers have begun to investigate the potential for multi-habitat restoration and synergistic relationships between oyster reef restoration and seagrass bed restoration (Hargis & Haven 1999, Moore et al. 1999, Newell et al. 1999, Peterson & Heck 1999 & 2001, Ulanowicz & Tuttle 1992). Nutrient exchange values associated with the oyster reef were used to speculate on the potential for oyster reefs
to impact water clarity and thus improve conditions for seagrasses on a local scale.

Results may be useful in further defining restoration strategies for these habitats.
LITERATURE CITED


aquatic vegetation: Habitat requirements as barometers of Chesapeake Bay health. *Bioscience* 43(2): 86-94.


CHAPTER 1

OYSTER REEF RESTORATION STRATEGY:

BROODSTOCK ADDITION
ABSTRACT

Over the past several years, eastern oyster (*Crassostrea virginica*) restoration efforts in Virginia have focused on constructing reef structures to act as sanctuaries. Increasingly, shell plants are stocked with hatchery-produced broodstock oysters that spawn and increase recruitment to the reefs. An important unresolved issue in such stocking efforts is the suitability of specific oyster stocks for achieving the desired goal. In particular, oysters stocks selected for aquaculture are currently available and there is uncertainty about the efficacy of using these stocks verses wild stocks for restoration efforts. To date, few studies have concentrated on determining which oyster stock provides the most suitable and productive broodstock for these large-scale restoration efforts. For comparison, this study tracks the performance of two hatchery-reared oyster stocks, the CROSBreed oyster stock (generation 3 line) and a Chesapeake Bay wild-caught oyster stock (Lynnhaven), after deployment onto reefs in the Lafayette River (Chesapeake Bay, VA) to establish which stock has the potential to produce the greatest number of offspring. Performance is evaluated based on growth, survival, female fecundity, sex of the population and disease affliction of the two stocks. These data were used to calculate cumulative egg production of the stocks. Results from the Lafayette River indicate that reproductive performance of the two stocks is dependent on disease pressure. Where MSX disease pressure is high, the CROSBreed stock oysters experienced lower infection levels and outperformed the Lynnhaven stock for cumulative egg production because of their superior survival and female fecundity. Where dermo disease pressure was high, the Lynnhaven stock was more robust in resisting the disease and outperformed the CROSBreed stock for cumulative egg production due to the stock’s greater size and female fecundity, and despite lower survival. Ultimately, to maximize potential reproduction and settlement to the reef, the choice of broodstock for use in reef restoration efforts should be based on the disease history of the region. However, due to the episodic nature of disease pressure, transplant of both stocks to a reef would ensure reproduction regardless of disease pressure. Results from this study suggest that MSX-resistance is more easily bred into oysters than dermo-resistance. Therefore, selective-breeding for dual disease resistance may find success by breeding MSX-resistance into the Lynnhaven stock oyster, which is heartier with regard to dermo.
INTRODUCTION

The eastern oyster, *Crassostrea virginica*, was once a prominent and extremely valuable organism in the Chesapeake Bay (Luckenbach et al. 1999), both ecologically and economically. Ecologically, oysters served a critical role in the Chesapeake Bay ecosystem by functioning as bioengineers in structuring habitat for settlement, survival and growth of oysters and reef-associated fauna (Coen et al. 1999, Jones et al. 1994) and in creating food for deposit-feeding organisms (Haven & Morales-Alamo 1966). It has been suggested that oysters once controlled water quality, phytoplankton abundance, and influenced productivity in the Bay (Dame 1996, Newell 1988) because of the magnitude of the historic population’s capacity to filter phytoplankton from the water column (Newell 1988). Economically, the eastern oyster once supported the most valuable fishery in the Chesapeake Bay (Newell 1988).

In 1988, Newell used catch statistics to estimate that the eastern oyster standing stock in the Chesapeake Bay had declined by 99%, from $188 \times 10^6$ Kg dry tissue to $1.9 \times 10^6$ Kg dry tissue, over the last 125 years. Heavy fishing pressure over the previous 125 years had negatively impacted the eastern oyster population in five ways: (1) by destroying three-dimensional reef structure and habitat with use of the power dredge (Rothschild et al. 1994); (2) by removing large, highly reproductive female broodstock from the spawning population; (3) by removing large oysters that had survived disease pressure that would have been important broodstock in natural selection (Hargis & Haven 1999, Kennedy 1996); (4) by permanently removing
oyster shells that provided necessary settling substrate for planktonic oyster larvae (Rothschild et al. 1994, Crisp 1967); and (5) by decreasing oyster density, thereby reducing fertilization efficiency of broadcast spawning (Levitan 1991, Mann & Evans 1998).

Adding to the devastation of Virginia’s oyster population, two parasite-induced diseases (MSX caused by *Haplosporidium nelsoni* and dermo caused by *Perkinsus marinus*) have thrived in the lower Chesapeake Bay since the 1950’s causing extensive oyster mortality (Burreson & Calvo 1996) – up to 90% mortality in some river systems from MSX (Burreson et al. 2000) and between 25-67% mortality in oysters ≥ 2 years from dermo (Andrews 1988). *Haplosporidium nelsoni* was introduced to the Delaware Bay and the Chesapeake Bay in the 1950’s and is now present along the Atlantic coast from Maine to Florida (Burreson et al. 2000). This parasite is confined to the high salinity (Andrews 1977) regions of the estuary and is most virulent over the late summer and early fall (Calvo & Burreson 2001) when water temperature is just below 20°C (Ford & Figueras 1988, Kennedy et al. 1996). *Perkinsus marinus* is native to Atlantic coast embayments south of the Delaware Bay and to the Gulf of Mexico and has increased in virulence along with increased oyster stress (Burreson & Calvo 1996). *P. marinus* thrives in waters characterized by high temperature (> 20°C) and salinity (>15 ppt) levels, but has a wide tolerance (8-50 ppt) for salinity (Mackin 1956). Dermo disease has historically been widespread in the southern Chesapeake Bay (Calvo & Burreson 2001 & 2002) but was only first recorded in the Delaware Bay in 1990 (Ford 1992).
Reef Restoration Strategy

Restoration of sanctuary oyster reefs in the Chesapeake Bay is now considered to be integral to the revitalization of the devastated oyster population, to the re-establishment of a viable oyster fishery, and locally, to improved water quality and habitat availability (Brumbaugh et al. 2000b, Hargis & Haven 1999, Rothschild et al. 1994, Ulanowicz & Tuttle 1992). Through a partnership between federal and state agencies and a private, non-governmental organization (NGO), large-scale oyster reef restoration was begun in Virginia in 1994 (Wesson et al. 1999). To date, a series of 50 high-relief oyster shell bases have been constructed in Virginia’s tributaries to act as oyster sanctuaries. Beginning in 1997, the NGO began stocking these shell bases in recruitment-limited waterways with hatchery-produced broodstock oysters raised through a community-based restoration effort (Brumbaugh et al. 2000 a & b, Luckenbach et al. 2000). This program, called the Student Oyster Corps (SOC), is composed of teachers and students who raise juvenile oysters in off-bottom trays (called “Taylor Floats”) in local tributaries throughout the school year. In the spring, oysters are transplanted at high density (200 oysters - m²) onto shell bases. Transplanted oysters spawn throughout the summer and increase potential recruitment to the reefs and to the river at large (Brumbaugh et al. 2000a).

Early results from this oyster restoration effort indicate that the addition of broodstock provided an effective means to initiate increased settlement to the reefs. Results from the recruitment-limited Lynnhaven River (Chesapeake Bay, VA)
showed that settlement onto the sanctuary reef increased by an order of magnitude, from 8 spat \( \text{m}^2 \) in 1997 to 181 spat \( \text{m}^2 \) in 1998, during the spawning season following the addition of 40,000 student-raised oysters (Brumbaugh et al. 2000a). These promising results were encouraging to scientists and resource managers and similar reef stocking projects were planned for successive years.

In addition to enhancing settlement to the sanctuary reefs, the reef restoration initiative aims to stock reefs with hearty broodstock oysters that will survive and reproduce in spite of disease, and that will pass their disease tolerance traits to their offspring and accelerate natural selection in the restored population. In the aforementioned 1997-1998 project, the SOC raised wild-caught, hatchery-produced Lynnhaven stock oysters. The Lynnhaven River is located in the polyhaline portion of the lower Chesapeake Bay (Figure 1.1) where disease pressure from both \( P. \) marinus and \( H. \) nelsoni were presumed to be high based on disease distribution maps (Burreson & Calvo 1996, Burreson 1991) coupled with recent records of extensive oyster mortality (Brumbaugh et al. 2000a). Oysters growing in regions where \( P. \) marinus is enzootic have shown heightened resistance to the parasite, demonstrating that resistance to \( P. \) marinus is heritable and can be built through natural selection (Andrews 1954, Gaffney and Bushek 1996). Similarly, selective breeding programs have demonstrated that resistance to \( H. \) nelsoni is also heritable and can be rapidly developed by breeding MSX survivors (Ford & Haskin 1987). Provided that large adult oysters collected from the Lynnhaven River had indeed built up a resistance after years of exposure to dermo and MSX, their hatchery-produced offspring used to
Figure 1.1 Study Site - Hampton Roads, Virginia
Figure marks location of two oyster reefs (marked with black circles) and source for wild-caught oyster stock. Tanner Point reef is at the mouth of the Lafayette River (36° 54.2' N, 76° 19.15'W) and the Larchmont reef is located about 1.5 km up river (36° 54.25’N, 76° 18.05’W).
stock the Lynnhaven reef in 1998 should have increased broodstock survival on the reef and enhanced disease resistance of offspring.

During the second year of this restocking project (1998-1999), schools raised the CROSBreed oyster stock (Brumbaugh et al. 2000b) that had originally been produced for aquaculture purposes (Haskin & Ford 1987). This CROSBreed stock is the result of a regional cooperative breeding effort (the Cooperative Regional Oyster Selective Breeding program) that has built upon a selection program initiated in 1962 for resistance to the parasite *H. nelsoni* (Haskin & Ford 1987). The CROSBreed stock, originally known as DB HSRL (Delaware Bay high-survival-resistant lines), was bred through a repetitive process of spawning Delaware Bay oysters who had survived the original MSX epizootic, out-planting the offspring to MSX-infected waters for 33 months, and collecting and crossing the survivors (Haskin & Ford 1987, Ford & Haskin 1987). After 1990, when *P. marinus* was discovered in Delaware Bay, selection for resistance to the parasite *P. marinus* and for rapid growth were incorporated into the selection regime for the CROSBreed oyster (Allen, pers. com).

**Oyster Stock Potential as Broodstock**

The efficacy of using the CROSBreed stock for population revitalization is not well established. The CROSBreed stock was originally developed for aquaculture and not with the intention that it would serve as broodstock for restoration. While efforts were made to out-cross the offspring to avoid inbreeding, the effective
population size ($N_e$) of the stock is quite low. $N_e$ should be between 30 and 50 parents per generation (Kennedy et al. 1996), and CROSBreed $N_e$ estimates range from 4.1–16.2 (Vrijenhoek et al. 1990). Inbreeding has the potential to decrease fecundity (Rodhouse et al. 1986), increase mortality (Zouros et al. 1983), and increase homozygosity, leaving the population less able to respond to and survive environmental challenges or disease in the future (Kennedy et al. 1996).

Additionally, there are questions as to the usefulness of stocking the Chesapeake Bay with Delaware Bay-native oysters for disease reasons. While CROSBreed stock oysters are highly selected for resistance to $H. nelsoni$, they have not experienced the level or duration of $P. marinus$ challenge experienced by Chesapeake Bay oysters (Burreson & Calvo 1996, Ford & Haskin 1987) and dermo is the biggest disease challenge in the Chesapeake Bay region (Burreson 1991, Burreson & Calvo 1996, Calvo & Burreson 2001).

While some of the methods for oyster reef restoration have been established (Hargis & Haven 1999), few studies have concentrated on determining which oyster stock provides the most suitable and productive broodstock for large-scale restoration efforts in Chesapeake Bay. The primary objective of oyster restoration is the re-establishment of self-sustaining reefs. Therefore, the oyster stock with the highest cumulative reproductive potential is considered to be the most desirable stock to use as broodstock since it will maximize the potential for new recruits to the reefs.
An individual oyster’s fecundity is affected by the oyster’s size (Cox & Mann 1992) and disease infection level (Dittman et al. 1998, Ford et al. 1990, Ford & Figueras 1988, Kennedy et al. 1995). Female fecundity ranges from 10,000 to 66 million eggs per spawn (Davis & Chanley 1956). Oyster fecundity is directly related to oyster size and increases exponentially with oyster shell height (Cox & Mann 1992), thus the faster growing stock should produce more gametes. However, while older oysters have the potential to produce more eggs per spawning season, they also have higher incidence of disease (Mann & Evans 1998, Burreson & Calvo 1996). Dermo and MSX have both been linked with dramatic decreases in oyster egg production and spawning potential (Dittman et al. 1988, Ford & Figueras 1988, Kennedy et al. 1995).

Cumulative community fecundity is affected by survival of the stock, number of females in the assemblage, and duration of the spawning season. At the community level, cumulative egg production for an oyster stock will continue to increase the longer the oysters survive. A stock is favored for survival if it has a fast growth rate allowing oysters to reach predator size-refuge more quickly (Dittman et al. 1998), if it is able to resist mortality associated with P. marinus and H. nelsoni parasites, and if inbreeding doesn’t affect it’s survival (Kennedy et al. 1996) or egg production (Rodhouse et al. 1986).

The objective of this study was to compare the performance two hatchery-reared stocks of Crassostrea virginica, the CROSBreed stock and the Lynnhaven stock,
after deployment onto sanctuary reefs in the Lafayette River (Chesapeake Bay, VA) to establish which stock had the highest cumulative reproductive potential. Due to the limitations of tracking larvae, reproductive performance was evaluated by counting eggs. The two stocks were compared based on their growth, survival, female fecundity, percent of the population that was reproductively female, and prevalence and intensity of *Haplosporidium nelsoni* and *Perkinsus marinus* infections. From these data, cumulative egg production for each oyster stock was calculated. Cumulative egg production provides one basis for evaluating the suitability of an oyster stock to serve as broodstock for sanctuary reef restoration in Virginia.

**METHODS**

**Site Description**

In June 1999, the Virginia Marine Resources Commission (VMRC) constructed two intertidal shell bases in the polyhaline region of the Lafayette River, 36° 54’ N, 76° 19’ W (Figure 1.1). Reef locations were established collaboratively by VMRC, the Virginia Institute of Marine Science (VIMS) and Old Dominion University (Atkinson et. al. 1998) and were designed specifically as replicates for this research project. The two reefs, Tanner Point and Larchmont, were within 1.5 km of one another, were expected to experience similar water quality conditions, and were constructed in approximately the same water depth, 4 ft at MLW and 5 ft at MLW, respectively.
In order to evaluate the suitability of the two oyster stocks for use in large-scale restoration projects, the research was coordinated with the existing SOC oyster restoration grow-out program. On July 10, 1999, a private aquaculture facility spawned oysters from both the selectively-bred CROSBreed stock (supplied by VIMS) and the wild-caught Lynnhaven stock (supplied from a field collection in 1998). Juvenile oysters were treated identically in the hatchery, both placed in downwellers on July 27 and in a flow-through nursery on August 12, 1999. In October 1999, 46,000 juveniles of the selectively-bred CROSBreed stock (generation 3 line) and 46,000 juveniles of the Lynnhaven stock were distributed to 28 SOC classes. Each class received oysters of only one stock to eliminate the potential for confusing the stocks during the student nursery phase. Classes raised their oysters in local tributaries (Figure 1.2) from October 1999 through May 2000. Salinity range experienced by the two oyster stocks during the student nursery phase of the project was not significantly different (average = 20 ppt, t=-0.011, p = 0.495) and oysters distributed throughout this same vicinity during the 1998-1999 growing season did not show different growth rates (Brumbaugh et al. 2000b). Concurrently, 4,000 oysters of each stock were raised at a control site (average of 18 ppt, range 13-22 ppt) on the York River to ensure that differences in growth rate recorded during the student grow out phase were due to innate differences in the stocks and not due to different water quality or handling techniques experienced by the two stocks. In May 2000, approximately 24,000 oysters of each stock were transplanted by the SOC onto each of the two replicate sanctuary reefs in the Lafayette River (Chesapeake Bay, VA). At the time oysters were deployed, a random sub-sample of 1,200 oysters...
Figure 1.2 Locations of student nursery sites

\( \Delta \) = Locations of Lynnhaven Strain floats
\( \blacklozenge \) = Locations of CROSBreed Strain floats
was collected from each treatment (reef and stock), divided into two bags, and stored in bottom cages at the base of the reefs for sampling throughout the following 17 months (Figure 1.3). Samples were taken from the bottom cages to establish growth, survival, female fecundity, % female, and disease.

**Growth & Survival**

Growth and survival were measured monthly from May through October 2000, once in February 2001, and monthly from May through September 2001. Mean shell height was determined (to the nearest 0.1 mm) for each treatment by measuring a sub-sample of 100 oysters, 50 per bag, with calipers. The sub-sample was gathered by placing the oysters into a tub, mixing them, and pulling all oysters from the lower-left-quadrant for measurement. From each bag, dead oysters were counted, measured to the nearest 0.1 millimeter, and removed. Monthly mortality was calculated by dividing the number of dead oysters per bag by the number of oysters alive at the beginning of the interval. Cumulative survival was established at each sample period with the equation:

\[
\text{cum } \% \text{ survival}_{i} = \text{cum } \% \text{ survival}_{i-1} - (\text{cum } \% \text{ survival}_{i-1} \times \text{monthly mortality})
\]

where \( i \) is the sample number.

Growth and survival data were analyzed with 3-way ANOVA (response: growth or % survival; factors: stock, reef, year). For all statistical analyses conducted in this study, significance level was set at 0.05 and data was checked for homogeneity.
Figure 1.3 Diagram of the experimental set up in the Lafayette River (Norfolk)

Oysters were deployed in bottom cages to facilitate sampling and to negate the effects of large predators. At each reef, replicate bags, each containing 600 oysters, were deployed in May 2000. Each bottom cage containing a bag of CROSBreed strain oysters and a bag of Lynnhaven strain oysters
of variance and normal distribution prior to statistical analysis with ANOVA, as stipulated by Zar (1999) and Underwood (1997).

Water quality data from the Lafayette River were collected at the same time as the growth and survival samples. At each reef, salinity (ppt) and water temperature (°C) were determined in the field by use of a hand refractometer and a mercury thermometer. Water samples were collected for chlorophyll a analysis and were chilled immediately in the field. Upon return to the lab, 5ml water samples were filtered through Whatman GF/F filters, filters were extracted for 24 hours in a DMSO/acetone mixture (45% acetone : 45% DMSO : 10% DI water with 0.01% diethylamine) and fluorescence was determined using a Turner Designs Fluorometer (model 10-AU) after Shoaf and Lium (1976).

**Female Fecundity**

Female fecundity is defined as the total number of ripe eggs present per female at any given time. Fecundity was measured using similar methods to those used by Cox (1988) and was determined from bi-weekly samples collected during the normal spawning season for Chesapeake Bay oysters (Andrews 1979, Haven & Fritz 1985). In 2000, fecundity sampling was conducted from late-June through late September. In 2001, sampling was begun 3 weeks earlier, to account for possibly earlier spawning from Delaware Bay natives (CROSBreed stock), as has been found for more northern stocks (Barber et al. 1991b, Loosenoff & Nomejko 1951). On each sampling date, 13 oysters from each bag (2 bags per reef) were collected and brought
back to the lab where shell heights were measured to the nearest 0.1 millimeter, oysters were shucked, their condition evaluated (as “R” ripe, “N” normal, or “W” watery), and their sex determined with a gonad smear under light microscopy. Eggs were counted from 5 females for each reef-stock treatment on each sampling date during the peak of the spawning season. Toward the end of their spawning season (after late August), it was not uncommon for the CROSBreed stock to have fewer than 5 females in the 26-oyster sample.

To determine female fecundity, the gonad of female oysters was manually stripped with a scalpel and was copiously flushed with 1 μm-filtered York River water into a small beaker. The contents of the beaker were then poured through stacked 103μm and 20μm mesh Nitex nylon sieves. Large debris was caught on the 103μm sieve and ripe eggs 40 - 62μm in size (Loosanoff & Davis 1963) were caught on the 20μm sieve. Eggs were then placed into a 2000 ml beaker along with 1000 – 2000 ml of York River water (filtered to 1μm), depending on egg concentration. The mixture was plunged for 30 seconds to keep negatively buoyant eggs in suspension while three 1 ml aliquots were taken with a pipette and placed into three Sedgwick-Rafter cells. Ripe eggs were counted from each Sedgwick-Rafter cell under light microscopy and values were averaged. The average number of eggs from the 3 slides was then multiplied by the amount of water in the 2000 ml beaker to determine the total number of eggs per female. Fecundity was analyzed in two ways: (1) with 3-way ANOVA (response: mean female fecundity; factors: stock, reef, year) to determine whether the two stocks produced significantly different numbers of eggs;
and (2) with regression analysis run on fecundity vs. shell height to determine if the two stocks had different fecundity-shell height relationships.

The proportion of the population that was reproductively female, or % female, was determined from histology slides produced in disease processing (see below) and from sex as determined in fecundity sampling. Percent female was calculated by dividing the number of oysters with a developed female gonad by the total number of oysters sampled. In June, July and August of 2000 and 2001, % female was calculated from at least 26 animals per reef-stock treatment. In May and September 2000 and 2001, % female was determined from at least 50 animals. Oysters were scored as 1 or 0 based on the presence or absence of a developed female gonad and data were analyzed with logistic regression (Zar 1999) accounting for stock, reef and date (N = 1687).

**Disease Prevalence & Intensity**

Disease prevalence and intensity were sampled during the summers of 2000 and 2001 in May, July and September for *P. marinus* and in May and September for *H. nelsoni*. Sampling dates were selected based on the timing of disease onset and development of infection (Burreson & Calvo 1996, Ford & Figueras 1988). *P. marinus* infection was determined by collecting a sample of gill, mantle and rectum from 20 oysters per bag and incubating the tissue in separate tubes containing fluid thioglycollate for 5-7 days while *P. marinus* hypnospores enlarged but did not replicate, after Ray (1952 & 1966). After the incubation, the tissue was removed
from the tube, stained with a lugol iodine stain that turned the hypnospores blue-black and cells were counted under a light microscope (40x). Each oyster's infection was rated according to the seven-point scale defined by Ray (1954), where 0 is negative and 6 is heavy infection. In total, 960 oysters were sampled for dermo throughout this study.

*H. nelsoni* samples were processed in May and September using standard tissue histology (Howard & Smith 1983). Twenty oyster bodies per bag were fixed in Davidson’s Alcohol, Formalin, and Acedic acid solution (AFA) for at least 24 hours, after which, a section of visceral mass was taken from between the gills and labial palps. The tissue was then dehydrated, cleared and embedded in a paraffin cube. The cube was sliced into 6 μm-thick sections, mounted on a slide, and stained with Harris’ Hematoxylin & Eosin Y for examination under light microscopy. Disease severity was scored in Little Ford Units (Ford et al. 1999) where 0 was negative and 6 was a heavy, systemic infection. In total, 640 oysters were sampled for MSX throughout this study.

Disease was scored in two ways: as percent prevalence and as weighted prevalence. “Percent prevalence” measures the proportion of the oysters sampled that were infected with parasites and was calculated by dividing the number of infected oysters by the total number sampled. For statistical analysis, each oyster was scored 1 or 0 based on presence or absence of parasites and percent prevalence was analyzed with logistic regression (Zar 1999) accounting for the effects of stock,
reef, season and year on disease prevalence. “Weighted prevalence” (or intensity) is a measure that combines the prevalence and the intensity of the infections. This index was calculated by rating the infection level for each oyster and dividing the sum of the infection intensities by the number of oysters sampled. Weighted prevalence was analyzed with a Cochran-Mantel-Haenszel test (Agresti 1990) and also accounted for the effects of stock, reef, season and year on disease intensity.

**Cumulative Egg Production**

Cumulative egg production (CEP) is a measure of the spawning potential for the oyster stocks and was calculated separately for each oyster stock on each reef using the following equation:

\[
\text{CEP} = \# \text{ stocked} \times \sum \left[ \text{survival} \times \text{mean fecundity} \times \% \text{ female} \right]_{\text{bi-weekly samples}}
\]  

This equation is similar to that used by Cox (1988) to calculate cumulative egg production on four James River reefs. Standard error \( \delta \text{CEP}_{bi} \) was calculated for each bi-weekly interval (indicated with \( bi \) subscript) by taking the standard error for each measurement as a fraction of the mean (i.e. \( \delta \text{fecundity}_{bi}/\text{fecundity}_{bi} \)), and performing the following calculation:

\[
(\delta \text{survival}_{bi}/\text{survival}_{bi})^2 + (\delta \text{fecundity}_{bi}/\text{fecundity}_{bi})^2 = (\delta \text{CEP}_{bi}/\text{CEP}_{bi})^2
\]

then multiplying the sum \( (\delta \text{CEP}_{bi}/\text{CEP}_{bi}) \) by the value for cumulative egg production for that bi-weekly period. Standard errors for each of the 13 bi-weekly intervals were
then summed with the following equation to calculate the standard error for cumulative egg production:

\[ \delta CEP = \sqrt{(\delta CEP_{bil})^2 + (\delta CEP_{bil2})^2 + \ldots (\delta CEP_{bil3})^2} \]  

(4)

Cumulative egg production was also calculated separately for year one (cumulative\(_1\)) and year two (cumulative\(_2\)) using the same calculations as above.

**RESULTS**

**Growth & Survival**

Oyster growth followed a typical seasonal pattern (Figure 1.4) with shell height increasing during the spring, summer and fall and growth plateaus over the winter when oysters ceased filtration (Haven & Morales-Alamo 1970). The Lynnhaven stock had significantly faster growth during the student-run nursery phase than the CROSBreed stock (ANOVA, \(p=0.0005\)) despite similar water quality conditions at the various grow out sites (Figure 1.5). Results were consistent with growth rate trends recorded at the York River control site, where the Lynnhaven stock grew significantly faster than the CROSBreed stock from July 1999 through May 2000 (ANOVA, \(p=0.0001\)). Once oysters were deployed onto the two reefs in May 2000, oyster growth data were analyzed with a 3-way ANOVA (response: shell height, factors: stock, reef, year). Results (Table 1.1) indicated that stock (\(p<0.0001\)), reef (\(p=0.0035\)) and year (\(p<0.0001\)) were all significant factors, with the Lynnhaven stock growing faster than the CROSBreed stock and oysters on the Larchmont reef growing faster than those on the Tanner Point reef.
Figure 1.4 Oyster growth as mean shell height (±SE) verses time

Oyster growth on the reefs was analyzed with 3-way ANOVA. Lynnhaven strain oysters were larger than CROSBreed strain oysters (p<0.0001) and oysters on the Larchmont reef were larger than oysters on the Tanner Point reef (p=0.0035). Larger shell height translates to faster growth rates since all oysters were spawned on the same day.
Figure 1.5 Water quality at the Lafayette River reefs throughout sampling period. (a) Salinity and temperature experienced by the two reefs, (b) chlorophyll-a concentration. Salinity did differ significantly with date based on results of 2-way ANOVA (p<0.0001) but did not differ between reefs (p=0.0527). Temperature did differ by date (p<0.0001) and by reef (p=0.0155) however temperature differences were less than 1°C different, thus the difference is considered to be negligible. Chlorophyll-a was significantly lower at TP (p=0.0357) but did not differ based on date (p=0.0588).

(a) Salinity & Temperature

(b) Chlorophyll-a concentration
Table 1.1 ANOVA table for size

Asterices (**) indicate significance at the 0.05 $\alpha$-level.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>stock</td>
<td>1</td>
<td>34.63</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>reef</td>
<td>1</td>
<td>9.03</td>
<td>$0.0035^{**}$</td>
</tr>
<tr>
<td>year</td>
<td>1</td>
<td>119.78</td>
<td>$&lt;0.0001^{**}$</td>
</tr>
<tr>
<td>stock*reef</td>
<td>1</td>
<td>0.64</td>
<td>0.4270</td>
</tr>
<tr>
<td>stock*year</td>
<td>1</td>
<td>0.81</td>
<td>0.3715</td>
</tr>
<tr>
<td>reef*year</td>
<td>1</td>
<td>2.19</td>
<td>0.1421</td>
</tr>
<tr>
<td>stock<em>reef</em>year</td>
<td>1</td>
<td>0.007</td>
<td>0.9347</td>
</tr>
</tbody>
</table>
Oyster survival data showed that mortality was similar between the two stocks during the first summer that the oysters were deployed on the reefs (Figure 1.6). Survival from May 2000 through September 2001 was initially analyzed with a 3-way ANOVA (response: % survival; factors: stock, reef, year) and a significant interaction for stock * year was observed (Table 1.2). Therefore, separate 2-way ANOVA tests were run (response: % survival; factors: stock, reef) for spawning season 1 (May – October 2000, Table 1.3) and spawning season 2 (May – September 2001, Table 1.4). Results from year 1 indicated that there was no significant difference in survival between stocks (p=0.9061) or reefs (p=0.6667), nor was there significant interaction. Results from year 2 indicated a significant reef * stock interaction, with the CROSBreed stock oysters on the Tanner Point reef consistently having the highest survival throughout year 2, and by the end of the study survival was significantly greater (p<0.05) than for the other reef by stock treatments, based on Scheffe’s post-hoc test (Zar 1999).

**Disease Prevalence & Intensity**

*H. nelsoni* percent prevalence (Figure 1.7) was found to differ based on stock (p<0.0001), reef (p=0.0426), season (p=0.0029) and year (p=0.0225). None of the oysters were infected with *Haplosporidium nelsoni* when they were transplanted onto the reef in May 2000. Over the course of this study, an oyster’s likelihood of contracting MSX was higher for the Lynnhaven stock than for the CROSBreed stock and was higher for oysters on the Tanner Point Reef than on the Larchmont reef. MSX infections followed a predictable seasonal cycle in which onset occurred from...
Figure 1.6 Oyster mortality plotted as mean % survival (± SE) verses time

Oysters mortality was analyzed separately for year 1 and year 2 using 2-way ANOVA. During the first spawning season (May-October 2000), there was no significant difference in survival for either reef or strain. Analysis of survival in year 2 has significant reef * strain interactions. Mortality patterns in year 2 are discussed in detail in the text.
Table 1.2 ANOVA table for survival

Asterices (**) indicate significance at the 0.05 α-level.

<table>
<thead>
<tr>
<th>DF</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>stock</td>
<td>1</td>
<td>13.14</td>
</tr>
<tr>
<td>reef</td>
<td>1</td>
<td>2.10</td>
</tr>
<tr>
<td>year</td>
<td>1</td>
<td>176.35</td>
</tr>
<tr>
<td>stock*reef</td>
<td>1</td>
<td>2.86</td>
</tr>
<tr>
<td>stock*year</td>
<td>1</td>
<td>5.08</td>
</tr>
<tr>
<td>reef*year</td>
<td>1</td>
<td>0.74</td>
</tr>
<tr>
<td>stock<em>reef</em>year</td>
<td>1</td>
<td>1.82</td>
</tr>
</tbody>
</table>

Table 1.3 ANOVA table for survival – year 1

Asterices (**) indicates significance at the 0.05 α-level.

<table>
<thead>
<tr>
<th>DF</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>stock</td>
<td>1</td>
<td>0.014</td>
</tr>
<tr>
<td>reef</td>
<td>1</td>
<td>0.176</td>
</tr>
<tr>
<td>stock*reef</td>
<td>1</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Table 1.4 ANOVA table for survival – year 2

Asterices (**) indicates significance at the 0.05 α-level.

<table>
<thead>
<tr>
<th>DF</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>stock</td>
<td>1</td>
<td>28.88</td>
</tr>
<tr>
<td>reef</td>
<td>1</td>
<td>4.55</td>
</tr>
<tr>
<td>stock*reef</td>
<td>1</td>
<td>6.00</td>
</tr>
</tbody>
</table>
Figure 1.7 *Haplosporidium nelsoni* % Prevalence (± SE) (a) Larchmont reef (b) Tanner Point reef. The CROSBreed strain experienced lower prevalence of *H. nelsoni* than the Lynnhaven strain (p<0.0001) and oysters on the Larchmont reef experienced lower prevalence of *H. nelsoni* then on the Tanner Point reef (p=0.0426). As was expected based on the seasonal cycle of *H. nelsoni*, MSX prevalence increases with season (p=0.0029) and year (p=0.0225). Black bars mark the distinction between years.
the late-summer to early-fall (Calvo & Burreson 2002, Ford & Figueras 1988) and
developed through the winter (Ford & Figueras 1988). MSX prevalence was higher
in 2001 than in 2000, as is consistent with parasite lifecycle and survey results (Calvo
& Burreson 2001 & 2002, Ford & Figueras 1988). MSX weighted prevalence (Figure
1.8) also differed with stock (p<0.0001), season (p<0.0001) and year (p=0.0388), but
was not significantly different with regard to reef (p=0.1133).

*P. marinus* percent prevalence (Figure 1.9) differed significantly with stock
(p<0.0001), reef (p<0.0001), season (p<0.0001) and year (p<0.0001). Dermo
infection also followed a predictable seasonal pattern with onset in the early summer
as water temperatures increased and infections intensifying over the summer
(Burreson & Calvo 1996). Dermo infections were infrequent in May 2000 when the
oysters were deployed to the reefs and incidence of infection increased with season
and year. The CROSBreed stock was more likely to become infected with *P. marinus*
than the Lynnhaven stock and oysters on the Larchmont reef were more likely to be
infected than oysters on the Tanner Point reef. This is opposite from the results for
MSX % prevalence. By the end of the second summer, both stocks had 100%
prevalence of Dermo infection, however; weighted prevalence data from September
2001 (Figure 1.10) showed that the Lynnhaven stock had lower intensity infections.
Results from the C-M-H statistic indicated that weighted prevalence for *P. marinus*
was significantly higher for the CROSBreed stock than for the Lynnhaven stock
(p=0.0004), for the Larchmont reef than for the Tanner Point reef (p<0.0001), and
increased by season (p<0.0001) and year (p<0.0001).
Figure 1.8 Weighted Prevalence of *Haplosporidium nelsoni* (± SE) (a) Larchmont reef (b) Tanner Point reef. Disease severity is scored in Little Ford Units (Ford et al. 2000) in which 0 is negative and 6 is a heavy, systemic infection. Note that the scale on the y-axis only ranges from 0-2. Weighted prevalence was lower for the CROSBreed than for the Lynnhaven strain (p<0.0001), and increased with season (p<0.0001) and year (p<0.0001). Black bars mark the distinction between years.
Figure 1.9 *Perkinsus marinus* Prevalence (± SE) (a) Larchmont Reef (b) Tanner Point reef. The Lynnhaven strain experienced lower prevalence of *P. marinus* than the CROSBreed strain (*p*<0.0001) and that oysters on the Tanner Point reef experienced lower prevalence of *P. marinus* than those on the Larchmont reef (*p*<0.0001). As was expected based on the seasonal cycle of *P. marinus*, dermo prevalence increased with season (*p*<0.0001) and year (*p*<0.0001). Black bars mark the distinction between years.

(a) Larchmont

(b) Tanner Point
Figure 1.10 Weighted Prevalence of *Perkinsus marinus* (± SE) (a) Larchmont reef (b) Tanner Point reef. Dermo severity was scored using the methods established by Ray (1954) in which 0 is negative and 6 is heavy infection. Weighted prevalence was lower for the Lynnhaven than for the CROSBreed strain (p=0.0004), was lower for the Tanner Point reef than for the Larchmont reef (p<0.0001) and increased with season (p<0.0001) and year (p<0.0001). Black bars mark the distinction between years.
Female Fecundity

In the Chesapeake Bay, oyster spawning occurs periodically throughout the summer, with approximately a 4 week period of reconditioning between spawns (Mann & Evans 1998), as is consistent with shell string settlement data (Southworth et al. 2001 & 2002). This caused great variation in mean egg number per female for each sampling date since some females were ripe while others had recently spawned (Figure 1.11). The Lynnhaven stock, native to the Chesapeake Bay, appeared to have a longer spawning season on both reefs than the CROSBreed, native to Delaware Bay. This is consistent with “physiological race” data from Barber et al. (1991b) and Loosanoff & Nomejko (1951). There was a spawning peak in early June of 2001 for the CROSBreed and Lynnhaven stocks on the Tanner Point reef. Oyster fecundity was not sampled in early June 2000 thus, it is possible that the first spawning period was missed. However, both stocks had the same temperature spawning cue (Barber et al. 1991b, Loosanoff & Nomejko 1951) and thus the spawn would have been similarly missed for both stocks. Average female fecundity was analyzed with 3-way ANOVA (response: mean egg number; factors: stock, reef, year). Results indicated that significant variation in mean egg number per female occurred between years (p=0.0293), but that variation due to reef (p=0.8043) and stock (p=0.6239) were not significant.

Oyster fecundity did appear to be related to shell height (Figure 1.12) but regression values could not be used since the data were not normally distributed, as
Figure 1.11 Mean Female Egg Production (± SE)

(a) Larchmont reef (b) Tanner Point reef. There is great variation in mean egg number per female due to a variety of factors (differences in female shell height, variation in disease infection level between females, incomplete spawning synchrony such that females may be extremely fecund while others have recently spawned out). There was no significant difference in female egg production between reefs or stocks. There was a significant difference between egg production in year 1 & 2 (p=0.0293). The Lynnhaven stock appeared to have a longer spawning season (extending into late August and September) than the CROSBreed (ending by mid-August) on both reefs and both years. Black bars mark the distinction between years.
(a) Larchmont

- CROSBreed
- Lynnhaven

(b) Tanner Point

- CROSBreed
- Lynnhaven
Figure 1.12 Individual female egg production verses oyster shell height

Lines indicate trends between shell height and egg production for the four strain-reef combinations. Trend lines are conservative. CROSBreed strain oysters on Tanner Point have the highest egg production relative to size, followed by the Lynnhaven strain oysters on Larchmont, CROSBreed on Larchmont and Lynnhaven strain oysters on Tanner Point, that have essentially no relationship between egg production and size.
was found by Cox (1988). Trend lines (Figure 1.12) estimated the relationships between shell height and egg production for the different treatments. Trend lines were conservative, since trends were biased by low egg counts that were generally the result of recently spawned gonads. Female fecundity was predicted to vary based on oyster shell height (Cox & Mann 1992, Kennedy et al. 1996); however, female fecundity has also been found to be strongly affected by both disease infection (Dittman et al. 1988, Ford & Figueras 1988, Kennedy et al. 1995) and by period of the spawning cycle (Cox 1988). Trend lines indicated that egg production for the CROSBreed stock on the Tanner Point reef had the strongest positive relationship with shell height and that the Lynnhaven stock on the Tanner Point reef had no relationship with shell height. On the Larchmont reef, the Lynnhaven stock egg production was more positively related to shell height than the CROSBreed stock.

Data are presented for the ratio of reproductive females to total oysters sampled (Figure 1.13). These data were analyzed with logistic regression (Zar 1999), for which individual oysters were scored as female (1) or not-female (0) accounting for factors of reef, stock, year and date. The statistic indicated that the likelihood of being reproductively female increased from mid-June (p=0.0026) through mid-July (p=0.0076) and decreased from late August (p<0.0001) through late September (p=0.0013). These results are consistent with those of Mann et al. (1994) who found that the proportion of reproductive females was highest during peak spawning season and was lower at the beginning and end of the season. Analysis also indicates that the likelihood of being reproductively female was higher for the Lynnhaven stock.
Figure 1.13 Proportion of reproductive females in populations (a) CROSBreed on Larchmont (b) Lynnhaven on Larchmont (c) CROSBreed on Tanner Point and (d) Lynnhaven on Tanner Point. The proportion of reproductive females is typically highest at the peak of the spawning season and declines toward the beginning and end of the season. Logistic regression results indicate that % female increases from mid-June through mid-July and decreases from late August through late September. Since the CROSBreed strain has a shorter spawning season than the Lynnhaven strain, logistic regression results indicate that the Lynnhaven strain has higher proportion of females. Black bars mark the distinction between years.
than for the CROSBreed stock (p<0.0001), possibly resulting from the shorter spawning season of the CROSBreed stock. Year (p=0.9992) and reef (p=0.8434) were not significant factors controlling the proportion of reproductive females.

**Cumulative Egg Production**

Results from the cumulative egg production calculation (Figure 1.14) indicate that the Lynnhaven stock had greater spawning potential than the CROSBreed stock on the Larchmont reef. This trend was reversed on the Tanner Point reef, where the CROSBreed stock had higher spawning potential than the Lynnhaven stock. Cumulative egg production values were also calculated as cumulative egg production in year 1 (cumulative₁) and in year 2 (cumulative₂) (Figure 1.15). These data show that in year 1, the Lynnhaven stock had higher reproductive potential than the CROSBreed stock on both reefs. In year 2, the Lynnhaven stock continued to have higher reproductive potential than the CROSBreed on the Larchmont reef, however the CROSBreed stock outperformed the Lynnhaven stock on the Tanner Point reef.

**DISCUSSION**

Results of this study indicated that reproductive performance of these two oyster stocks on the reefs in the Lafayette River was largely dependent on disease pressure. *H. nelsoni* and *P. marinus*, the parasites that cause MSX and dermo respectively, have different life cycles and both influence survival, growth, female fecundity and gonadal development of the oyster hosts. The CROSBreed stock that
Cumulative egg production is calculated by multiplying bi-weekly measured values for % survival, % female and mean egg production per female and summing the products over the two year period. Standard errors from each measurement are calculated accordingly. The data show that the Lynnhaven strain provides higher cumulative egg production than the CROSbreed strain on the Larchmont reef, whereas the CROSbreed strain may have higher cumulative egg production than the Lynnhaven strain on the Tanner Point reef.

Cumulative egg production (same as bars in Figure 1.14) are divided into cumulative values for year 1 and year 2. In year 1, the Lynnhaven strain had higher cumulative egg production than the CROSbreed on both reefs. In year 2, the Lynnhaven strain continued to outperform the CROSbreed strain on the Larchmont reef, whereas the CROSbreed strain on Tanner Point outperformed the Lynnhaven strain.
had originally been bred for resistance to MSX disease did demonstrate higher resistance to MSX than the Lynnhaven stock. Where MSX pressure was high, the CROSBreed stock had higher cumulative egg production because this stock had higher survival rates and higher female fecundity. The Lynnhaven stock that had been spawned from parents collected in the Lynnhaven River where dermo disease was enzootic, demonstrated higher resistance to dermo than the CROSBreed stock. Where dermo pressure was high, the Lynnhaven stock had higher cumulative egg production due to higher female fecundity (secondary to larger shell heights) and despite lower survival.

**Disease pressure 2000 & 2001**

Throughout the Chesapeake Bay, salinities were anomalously high in 2000 and 2001 as a result of a series of dry years beginning in 1999 (Calvo & Burreson 2001 & 2002). This high salinity created an environment that was conducive to the survival and propagation of both *H. nelsoni* and *P. marinus* (Calvo & Burreson 2001 & 2002) resulting in anomalously high disease levels in 2000 and 2001. In 2001, both diseases were measured at record high levels relative to the last 15 years of research that tracks disease status in Virginia (Calvo & Burreson 2002). High salinity also allowed for the spread of both parasites into areas that were previously uninfested (Calvo & Burreson 2002).

In this study conducted in the Lafayette River, disease pressure appeared to be the major factor governing survival and fecundity dynamics of the two oyster
stocks. The CROSBreed stock had been bred since 1962 for resistance specifically to MSX disease (Ford & Haskin 1987) and this selection program has been successful at producing oysters with increased resistance to *H. nelsoni* (Barber et al. 1991a, Burreson 1991, Ford & Haskin 1987). As expected based on the results of similar studies (Barber et al. 1991a, Burreson 1991, Ford & Haskin 1987), the CROSBreed stock oysters transplanted onto reefs in the Lafayette River did exhibit lower infection rates and lower MSX-related mortality than the Lynnhaven stock oysters. Also, as is consistent with previous research (Burreson 1991, Andrews 1954), the CROSBreed stock appeared to have lower levels of dermo tolerance and resistance than the Lynnhaven stock, which was spawned from oysters that lived in regions with dermo enzootic waters. These results suggest that the Lynnhaven stock oysters have likely developed some level of natural resistance to dermo disease from extended exposure in the Lynnhaven River, as has been found for oysters living in areas with a long history of disease exposure (Gaffney & Bushek 1996).

Both *H. nelsoni* and *P. marinus* severely impact the growth, survival and gonad development of their oyster hosts. *Haplosporidium nelsoni* has been found to slow oyster growth by reducing oyster clearance rates (Barber et al. 1991a & b) and by reducing the oysters’ production of glycogen, the major storage compound that nourishes the oyster over the winter and fuels gametogenesis in the spring (Barber et al. 1988, Kennedy et al. 1995). Once infected with MSX, oyster condition declines rapidly and the animal tends to die quickly, in a matter of months, after contracting the disease (Barber et al. 1991a). In lightly infected oysters, MSX severely impairs
gonad production because of the relationship between the parasites and glycogen production (Barber 1996, Ford & Figueras 1988) and *H. nelsoni* completely inhibits gonad production in systemically infected oysters (Ford et al. 1990, Ford & Figueras 1988). *Perkinsus marinus* has similar impacts on the oyster host but the effects are less dramatic because the trajectory of infection is slower (Burreson 1991, Kennedy et al. 1996). Dermo has been found to cause different growth responses in Chesapeake Bay native oysters and in the Delaware Bay native CROSBreed stock. Chesapeake Bay native oysters have been found to increase in shell height despite dermo infection (Barber & Mann 1994, Burreson 1991), whereas growth rate of dermo-infected DB HSRL stock oysters (forerunner to CROSBreed stock) tends to be reduced due to the disease (Burreson 1991). Dermo causes oyster mortality after two consecutive years of exposure, once disease levels intensify to moderate/heavy levels in the oysters’ tissue (Burreson 1991). Given appropriate water quality over the winter, disease levels may decrease over the winter (Barber & Mann 1994, Burreson & Calvo 1996, Kennedy et al. 1996). Reproduction of dermo-infected oysters is only impaired once disease has intensified during the second summer of exposure to *P. marinus* (Dittman 1993), when egg quantity is compromised but egg quality is not reduced (Kennedy et al. 1995).

The reefs in the Lafayette River had originally been designed as replicates with the assumption that the two reefs would experience similar water quality and disease conditions throughout the experiment. This was not the case in this study. The Tanner Point reef was found to experience greater pressure from *Haplosporidium*...
nelsoni and the Larchmont Reef was found to experience greater pressure from Perkinsus marinus. Water quality also differed slightly between the reefs (Figure 1.5). These disease results provided an interesting opportunity to compare the response of these two oyster stocks given different levels of pressure from the two parasites.

**High MSX Pressure – Tanner Point Reef**

**Disease Prevalence and Intensity – Tanner Point**

MSX tends to infect oysters from the late summer to early fall. Consistent with the seasonal pattern of MSX onset, *H. nelsoni* parasitized oysters of both stocks in the Lafayette River during the late summer 2000, the first year that oysters were deployed on the reef. Late summer infections can cause oyster mortality by late-fall or the parasites may overwinter in the oyster body and kill the animal in the early spring (Calvo & Burreson 2002, Ford & Figueras 1988, Kennedy et al. 1996). CROSBreed stock oysters on both reefs were minimally affected by MSX disease throughout the sampling period. Beginning during summer 2000 and continuing throughout the study (Figure 1.6), the Lynnhaven stock oysters deployed on the Tanner Point reef (LY-TP) experienced very high levels of MSX, relative to findings from ongoing disease monitoring research in Virginia’s portion of the Chesapeake Bay (Calvo & Burreson 2000 & 2001). MSX % prevalence on Tanner Point reef was significantly higher than on the Larchmont Reef throughout the study. MSX weighted prevalence for the Lynnhaven stock oysters was not different between the reefs at the point of initial infection (September 2000) but was significantly higher at the Tanner Point reef in 2001.
At Tanner Point, oysters of both stocks were also infected with *P. marinus* (Figure 1.9b) although dermo pressure was lower than on the Larchmont reef (Figure 1.9a). Onset of dermo began in early summer 2000 and percent prevalence remained relatively low through the first summer, as is consistent with the cycle for the parasite. Dermo infections decreased slightly over the winter, but the second consecutive year of high disease pressure caused prevalence and intensity to increase through the end of summer 2001. The CROSBreed stock oysters on both reefs were more susceptible to *Perkinsus marinus* than the Lynnhaven stock oysters, but both stocks did reach 100% prevalence by the end of the second summer. The Tanner Point reef is discussed primarily as a heavy MSX pressure treatment, but results are confounded by the presence of dermo disease.

**Growth & Survival – Tanner Point**

MSX has been found to slow growth by reducing oyster clearance rate (Barber et al. 1991a). Oyster growth rates on the Tanner Point reef were similar between the CROSBreed stock and Lynnhaven stock oysters from May 2000 through September 2000, prior to MSX infection of the LY-TP oysters. From October 2000 through August 2001, growth rate for LY-TP oysters was slower than their pre-infection rate (Figure 1.4), which may have resulted from MSX infection. Growth rate for the CROSBreed stock (XB-TP) slowed as well, despite low levels of MSX infection. The reduced growth rate for the XB-TP oysters may have resulted from dermo infections
(Burreson 1991) and oysters at Tanner Point may simply have grown more slowly than oysters at Larchmont due to lower phytoplankton availability (Figure 1.5).

Oyster mortality on the Tanner Point reef was low in summer 2000 prior to the onset of MSX. After September 2000, the mortality pattern experienced by LY-TP oysters (Figure 1.6) was consistent with the MSX mortality trajectory, with approximately 35% mortality of LY-TP oysters occurring between September 2000 and May 2001. This % mortality was similar to the MSX % prevalence for LY-TP oysters in September 2000. Morality continued for LY-TP oysters through July 2001, when mortality rates declined. This decline in LY-TP oyster mortality may have occurred because many of the MSX-susceptible LY-TP oysters had already died or because MSX virulence declines in the hottest months of the summer (Ford & Figueras 1988). XB-TP oysters experienced low levels of mortality in this study (Figure 1.6), likely due to their MSX resistance and the lower dermo pressure at Tanner Point. Approximately twenty percent of the mortality experienced by XB-TP oysters occurred between May 2001 and September 2001, which was consistent with the timing for dermo-related mortality (Calvo & Burreson 1996).

**Female Fecundity & Proportion of Females – Tanner Point**

In 2000, prior to the onset of MSX, the two oyster stocks had similar mean egg numbers per female (Figure 1.11b). The LY-TP oysters appeared to have a longer spawning season in 2000, with a main spawning peak on Jul-12-00, and two smaller peaks on Jul-25-00 and Aug-12-00, whereas XB-TP oysters only appeared to
have one peak on Jul-12-00. The magnitude of the spawning peak on Jul 12-00 was similar between the two stocks. Spawning peaks recorded for both reefs in the Lafayette River in 2000 roughly matched the timing of spawning in other similar salinity areas in the Chesapeake Bay, based on comparison with annual spat settlement data (Southworth et al. 2001).

In 2001, XB-TP oyster egg production increased two to three-fold over year 1 values at the main spawning peaks on Jun-1-01 and Jul-2-01 (Figure 1.11b). This increase in egg production of XB-TP oysters was likely the result of increased size (Figure 1.12) (Cox 1988, Cox & Mann 1992). Conversely, mean egg number per LY-TP female at both spawning peaks (Jun-1-01 and Jul-2-01) was the same magnitude in 2001 as it was 2000 (Figure 1.11b) despite larger average size (Figure 1.4). This indicated that gonad development in the LY-TP oysters was impaired because oysters should have produced more eggs in 2001 given larger average shell height (Cox & Mann 1992). The lack of a positive relationship between shell height and egg production per individual LY-TP oyster (Figure 1.12) suggested that larger oysters were infected with disease (Mann & Evans 1998) and therefore, had severely impaired gonad development.

The proportion of reproductive females per total for LY-TP oysters fluctuated in 2000 (Figure 1.13d) but remained more consistent than the proportion of females for XB-TP oysters in 2000 (Figure 1.13c). In 2001, MSX-related gonad inhibition in LY-TP oysters was indicated by a decreased proportion of reproductive females
(Figure 1.13d) from 2000 to 2001. These data were consistent with other studies of MSX-infected oysters that have found abnormal and inhibited gonad development in infected animals (Ford & Figueras 1988). All other treatments had increased proportions of reproductive females from 2000 to 2001 (Figure 1.13a, b & c).

Cumulative Egg Production – Tanner Point

During year 1 (Figure 1.15), before MSX infections were present, LY-TP oysters had higher cumulative egg production than the CROSBreed stock due to a longer spawning period and higher female : total ratios, along with similar survival between the stocks. Once *H. nelsoni* infected the Lynnhaven stock oysters by year 2, their cumulative reproductive output quickly declined due to high mortality, decreased egg production per individual, and decreased proportion of reproductive females. Conversely, XB-TP oysters served as weaker broodstock than LY-TP oysters in year 1 with only one spawning peak (Figure 1.11), a short spawning season, and low female : total ratios. In year 2, the XB-TP oysters showed increased egg production, had two spawning peaks, and had a higher proportion of reproductive females than in year one (Figure 1.13c). Together with superior survival relative to LY-TP oysters, the XB-TP oysters had higher cumulative reproductive output. Over the entire two-year study period, the CROSBreed stock oysters served as better broodstock with higher cumulative female fecundity than the Lynnhaven stock oysters on the Tanner Point reef (Figure 1.14). This difference was attributed to extreme MSX pressure on this reef.
High Dermo Pressure – Larchmont Reef

Disease Prevalence & Intensity – Larchmont Reef

Dermo prevalence and weighted prevalence were both significantly higher for the CROSBreed stock than for the Lynnhaven stock on both reefs. Dermo infection began early in the summer of 2000 (Figure 1.9), but infections were of low intensity (Figure 1.10) until September 2000. Dermo infections decreased over the winter for all four reef-stock treatments. *Perkinsus marinus* tends to infect oysters over the summer in year one, reach maximum prevalence and intensity in September of the second year (Burreson 1991, Burreson & Calvo 1996, Calvo & Burreson 2001 & 2002), and cause oyster mortality from July to September during the second summer of infection. As is consistent with this trajectory, dermo infections increased and intensified through the summer and reached 100% prevalence by September 2001.

Weighted prevalence was a useful index to identify the relative rate of dermo disease onset between the stocks. Unlike the rapid progression trajectory for MSX, dermo proliferation and dermo-related mortality tend to occur more slowly and oysters are capable of performing normal functions given low levels of dermo disease (Dittman 1993, Burreson 1991). Although both stocks reached 100% prevalence by September 2001, the Lynnhaven stock oysters had lower weighted prevalence, indicating that the intensity of their infections was lower. Therefore, they may have had a longer period of normal functioning than the CROSBreed stock oysters.
Lynnhaven stock oysters on the Larchmont reef (LY-LR) did also become infected with *Haplosporidium nelsoni*, although MSX % prevalence was lower at Larchmont than at Tanner Point (Figure 1.7 a & b). MSX % prevalence was highest for LY-LR in September 2000 (approximately 30%) and decreased in 2001. This reef was considered to as a high dermo pressure condition, however presence of MSX may confound results.

**Growth & Survival – Larchmont Reef**

Dermo has been found to cause different responses in Chesapeake Bay native oysters and in the Delaware Bay native CROSBreed stock. Chesapeake Bay native oysters have been found to increase in shell height despite dermo infection (Barber & Mann 1994, Burreson 1991), whereas growth rate of dermo-infected CROSBreed oysters tends to slow down (Burreson 1991). A similar pattern was found for oysters on the Larchmont reef during the second summer of dermo infection (Figure 1.4), after dermo infections had intensified. Growth rates for LY-LR oysters were clearly faster in 2001 than for the CROSBreed stock on the Larchmont reef (XB-LR), since the stocks were approximately 7 mm different in mean shell height in May 2001 and were approximately 13 mm different in shell height by August 2001. This growth rate reduction was consistent with the timing of high dermo prevalence and intensity for XB-LR oysters.

Oyster mortality on the Larchmont reef appeared to be closely related to disease affliction. LY-LR oysters experienced roughly 33% mortality from
September 2000 – May 2001. This mortality was likely MSX-related, since there was approximately 29% MSX prevalence in the LY-LR population in September 2000, and timing was consistent with the trajectory for the disease. Throughout summer 2001, LY-LR oyster mortality did continue at a steady rate, which was likely both dermo and MSX-related mortality. XB-LR oysters experienced lower levels of mortality than LY-LR oyster from deployment through May 2001, which probably was due to their relatively lower MSX prevalence. Mortality of XB-LR oysters accelerated during summer 2001, as was consistent with the expected dermo-related mortality (Burreson 1991, Calvo & Burreson 2001 & 2002), and occurred at a more rapid rate during summer 2001 than mortality for LY-LR oysters.

Female Fecundity & % Female – Larchmont Reef

During the first year of dermo exposure, oyster gonad development was unlikely to be impaired by *P. marinus* (Dittman 1993). In 2000, LY-LR oysters appeared to have three spawning peaks on Jun-28-00, Jul-12-00 and Aug-10-00, but spawning activity did continue for the stock through Sep-26-00. XB-LR oysters appeared to have only two spawning peaks on Jul-12-00 and Aug-10-00 and both were either similar to or lower in magnitude than LY-LR oysters. This difference may have been related to average shell height, which was smaller for the CROSBreed stock.

Heavier dermo intensities found in the second summer of infection were expected to reduce the quantity of eggs produced for both stocks (Kennedy et al.
Infection intensities for *P. marinus* remained fairly low through July 2001 despite high prevalence (Figures 1.9a and 1.10a) thus gonad development may not have been impacted by the disease since XB-LR egg production appeared to be completed by mid-July. Gonad development has been found to be most sensitive to parasites in the beginning stages of gamete development in the spring, more so than once gametes are mature in the summer (Ford et al. 1990). XB-LR oysters appeared to have two spawning peaks in 2001, on Jul-2-01 and Jul-18-01, after which their spawning season was essentially completed (Figure 1.11). All of their spawning peaks were of larger magnitude in 2001 than in 2000, which was expected given their increased average shell height (Cox 1988, Cox & Mann 1992). LY-TP oysters appeared to have three spawning peaks on Jul-2-01, Jul-18-01 and Aug-1-01 that were also higher in magnitude than their egg production in 2000 (Figure 1.11). Their Aug-1-01 peak was extremely high despite dermo infection intensities of approximately 4 (on 0-6 scale). Given the findings of Kennedy et al. (1995), it was possible that this Aug-1-01 spawn would have been even higher in the absence of dermo infections.

LY-LR oysters had higher egg production relative to shell height than did XB-LR oysters (Figure 1.12), despite their higher MSX infections. This indicated that egg production in larger XB-LR oysters was more impacted by dermo disease than egg production in larger LY-LR oysters and demonstrated that these Lynnhaven stock oysters were heartier with respect to dermo resistance.
There was not an obvious trend between the ratio of female : total oysters (Figure 1.13a & b) between the stocks on the Larchmont reef. This suggested that dermo disease did not cause complete inhibition of gonad production and was consistent with results from Kennedy et al. (1995). There is one study that found that joint affliction of MSX and dermo did inhibit gonad production (Barber 1996), but this may have been more of a result of MSX infection than dermo infection.

Cumulative Fecundity - Larchmont Reef

During year one, cumulative reproductive output (Figure 1.15) was slightly higher for LY-LR oysters than for XB-LR oysters due to similar survival, longer spawning season and larger shell heights. In year two, LY-LR oysters continued to have higher cumulative egg production than XB-LR oysters due to greater dermo resistance, their physiological relationship between shell height and egg production (Cox 1988, Cox & Mann 1992) and despite lower cumulative survival. XB-LR oysters’ cumulative egg production also increased from year one to year two, but the magnitude of the increase was much lower than for LY-LR oysters. Over the course of the two-year study, the Lynnhaven stock had higher cumulative reproductive output than the CROSBreed stock on the Larchmont reef (Figure 1.14). This difference was primarily attributed to the increased ability of the Lynnhaven stock oysters to resist or tolerate Perkinsus marinus.

Broodstock strategies

Results from this study indicated that the CROSBreed stock had a higher level of resistance to the parasite Haplosporidium nelsoni and therefore, outperformed the
Lynnhaven stock in terms of cumulative egg production in an area with high pressure from *H. nelsoni*. However, the Lynnhaven stock was more resistant than the CROSBreed stock with respect to resistance to *Perkinsus marinus*, and therefore outperformed the CROSBreed stock for cumulative egg production in an area experiencing high pressure from *P. marinus*. Presumably, the Lynnhaven stock would have had improved survival, thus even higher cumulative egg production, on the Larchmont reef (high dermo condition) in the absence of MSX pressure. In Virginia’s portion of the Chesapeake Bay, dermo has historically been more widespread than MSX (Burreson 1991, Burreson & Calvo 1996, Calvo & Burreson 2001). In 2000, dermo prevalence was ≥ 61% (Figure 1.16) at 31 out of 35 stations sampled by the ongoing monitoring program (Calvo & Burreson 2001) and MSX prevalence was ≤ 10% (Figure 1.17) at 22 of the 33 stations sampled (Calvo & Burreson 2002). At all but one of the stations (Nansemond Ridge), dermo was present wherever MSX was present.

Given the history and distribution of disease in the Chesapeake Bay, restoration initiatives have two options. Option 1: Broodstock can be selected based on the salinity regime and disease history of an area, such that the CROSBreed stock would be transplanted to sanctuary reefs in areas with a history of high *H. nelsoni* pressure (♦ symbol (≥ 25% prevalence) in Fig. 1.16 from Calvo & Burreson 2001) and the Lynnhaven stock would be transplanted to sanctuary reefs in areas with a history of high *P. marinus* pressure (♦ symbol (≥ 61% prevalence) in Fig 1.17 from Calvo & Burreson 2001). Provided that disease pressure can accurately be predicted for an area, this option is more practical and economical because purchasing, raising and
Figure 1.16 Distribution of *Haplosporidium nelsoni* in Virginia in the fall of 2000. Copied from Calvo & Burreson 2001.
Figure 1.17 Distribution of *Perkinsus marinus* in Virginia in the fall of 2000. Copied from Calvo & Burreson 2001.
transplanting oysters to sanctuary reefs is a costly and labor intensive process. With resources enough to raise and transplant a finite number oysters to a sanctuary reef, reef rehabilitation will be more likely if all oysters stocked to the reef are of the stock with the higher potential reproductive output. Option 2: Both oyster stocks can be transplanted to sanctuary reefs in order to ensure egg production in case of high pressure from either or both diseases. With resources enough to raise and transplant 100,000 oysters to a sanctuary reef, 50,000 oysters of each stock would be transplanted. With this option, if dermo pressure was strong and MSX pressure was not, reproductive output from the CROSBreed stock oysters would have been low and the stocking of those 50,000 oysters would not have been worth the money and effort. However, in light of recent proliferation of the diseases into previously uninfected areas (Calvo & Burreson 2002), the second option may be most effective given the episodic nature of disease patterns.

Resource managers must consider the genetic consequences of stock enhancement, in addition to selecting oyster broodstock based on cumulative egg production. Although the Lynnhaven stock has not developed as thorough a resistance to dermo as the CROSBreed stock has developed against MSX, this study provides strong evidence that the Lynnhaven stock is heartier with regard to \textit{P. marinus} than is the CROSBreed. Dermo resistance in Lynnhaven stock likely developed through natural selection after centuries of exposure to the disease (Gaffney & Bushek 1996). Natural selection is inevitably a slower process than laboratory selective breeding because the most resistant animals are not repeatedly crossed with one another. However, the natural selection process is also less likely to
cause genetic bottleneck from “sweepstake” spawning success, therefore; a naturally selected stock should demonstrate greater genetic variability than a laboratory selected stock, keeping the oyster population more able to handle stresses in the future (Kennedy et al. 1996). MSX resistance is rapidly bred into oysters (Ford & Haskin 1987) and dermo resistance is not (demonstrated by the third generation CROSBrreed stock used in this study). Therefore, scientists should consider using the more genetically diverse and dermo-tolerant Lynnhaven stock to perform selective-breeding techniques (Ford & Haskin 1987) for resistance to MSX over two generations. This would create a robust stock of oysters relative to disease, as well as a genetically diverse stock of oysters to serve as broodstock for future Chesapeake Bay oyster populations.

CONCLUSION

Results from this study indicate that the CROSBrreed stock has superior resistance to MSX, which makes the stock more highly reproductive in regions with high MSX pressure. In regions with high dermo pressure, which is more widespread in the Chesapeake Bay than MSX (Burreson 1991, Burreson & Calvo 1996), the Lynnhaven stock is more robust in resisting the disease and therefore offers higher potential for reproduction. While it is possible to select broodstock based on the disease history of an area to maximize potential reproduction and recruitment, it may also be valuable to stock reefs with both stocks in order to favor likely reproduction regardless of disease pressure.
Results from this study are encouraging for the oyster reef restoration initiative because 20-50% of the oysters stocked to reefs in the Lafayette River continued to survive after two spawning seasons despite two consecutive years of anomalously high disease pressure. Consistent with the idea behind stocking the reefs with broodstock oysters, these survivors likely have genetically heightened resistance to the diseases and therefore may contribute to a heartier revitalized oyster population through their reproduction. This study measured reproductive potential by counting eggs. Future research will be necessary to establish the viability of the offspring produced by these oyster stocks and also to track development of disease resistance in the revitalized population. Molecular tools are being employed in research that is currently underway on oyster stocks in the Great Wicomico River (Chesapeake Bay, VA), where CROS Breed stock oysters have been transplanted to the reefs, to determine if disease resistance is maintained when CROS Breed stock oysters mate with the local population. Future research may also include modeling the potential oyster population development with the creation of a Leslie Matrix. This would allow researchers to establish an expected trajectory for oyster population rehabilitation using survival and fecundity data for these two stocks and would allow the manipulation of numbers of oysters stocked to quantify transplant targets.


73


CHAPTER 2

OYSTER REEF RESTORATION IN VIRGINIA:

NUTRIENT EXCHANGES WITH THE WATER COLUMN
ABSTRACT

One of the goals of oyster (*Crassostrea virginica*) reef restoration in the Chesapeake Bay (Virginia) is recovery of reef ecosystem functions such as water filtration. In this study, flux measurements were performed to examine the seasonal effects of reef rehabilitation on phytoplankton uptake and nutrient cycling from created oyster reefs at Fisherman’s Island, VA. In situ mesocosms were used to measure dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), dissolved inorganic phosphorus (DIP) and chlorophyll exchanges over the reef and fluxes were compared to those over an adjacent intertidal flat similar to the section that had been replaced by reef construction. The oyster reef community consistently showed greater chlorophyll uptake than the adjacent intertidal flat and generally displayed greater release of DIN, DON and DIP than the intertidal flat. Faster rates of nutrient cycling indicated that the reef had greater potential for benthic-pelagic coupling and secondary production than the intertidal flats. Net seasonal nitrogen exchange for both communities was determined by combining particulate (calculated from chlorophyll-a flux) and dissolved organic and inorganic nitrogen fluxes. The intertidal flat was determined to serve as a source for nitrogen throughout the study while the oyster reef was a sink for nitrogen in spring and fall and a source only during summer. Net nitrogen uptake by the reef was significantly different than release from intertidal flat in spring and fall. These data suggest that revitalized oyster reefs at Fisherman’s Island may increase potential nitrogen storage and decrease phytoplankton standing stocks in the spring and fall as phytoplankton are nitrogen-limited at Fisherman’s Island. This potential seasonal impact on phytoplankton abundance in spring and fall coincides with the critical growth periods for eelgrass (*Zostera marina*) in this region, and suggests the potential for created oyster reefs to locally improve habitat for eelgrass.
INTRODUCTION

Water Quality Changes Associated with Oyster Reef Restoration

The eastern oyster, *Crassostrea virginica*, was once a prominent and ecologically valuable organism in the Chesapeake Bay (Luckenbach et al. 1999). Oysters served a critical role in the Chesapeake Bay ecosystem by functioning as bioengineers in structuring reef habitat for a diverse assemblage of organisms (Coen et al. 1999 a & b, Harding & Mann 1998, Meyer & Townsend 2000), in creating food for other organisms (Haven & Morales-Alamo 1966), and in influencing water quality, eutrophication, and productivity in the water column (Dame 1996, Newell 1988, Ulanowicz & Tuttle 1992). By 1988, the eastern oyster population was estimated to have declined by 99% over the previous 125 years (Newell 1988). Since 1994, oyster reef restoration has been undertaken in the Chesapeake Bay with a joint purpose of revitalizing a devastated oyster population and reviving oyster ecosystem filter and habitat function (Hargis & Haven 1999).

*C. virginica* is a highly effective filter feeder (Dame 1999, Dame et al. 1984, Galtsoff 1964, Haven & Morales-Alamo 1970, Hily 1991). Oysters filter phytoplankton and suspended sediments (Haven & Morales-Alamo 1970, Jones & Preston 1999), the two major components of light attenuation in the water column (Batiuk et al. 1992). They sort particulate-rich estuarine water with their gills and labial palps (Newell & Jordan 1983), consuming phytoplankton and rejecting inorganic sediments into undigested pellets called pseudofeces (Haven & Morales-
Ingested organic matter is metabolized by the oyster and nutrients are either (1) assimilated and stored in long-lived tissue (Dame et al. 1989, Dame & Libes 1993); (2) excreted to the water column in dissolved inorganic and organic forms (Dame 1996, Boucher & Boucher-Rodoni 1988); or (3) egested from the oyster into the underlying sediment as feces (Clark & Wikfors 1998, Dame et al. 1984 & 1989, Haven & Morales-Alamo 1966 & 1970, Kim 1983) (Figure 2.1). Through this process, oysters release nutrients that were biologically bound by phytoplankton allowing further primary production in the water column (Dame et al. 1991, Dame 1996 & 1999, Herman & Scholten 1990). They also convert particulate organic and inorganic matter from suspended form to pelletized form, rendering it accessible to deposit-feeding organisms (Dame et al. 1980, Dame et al. 2001, Haven & Morales-Alamo 1966, Kautsky & Evans 1987, Kemp & Boynton 1992). Oyster reefs have been regarded as “filters” or “cleaners” of estuarine water because of the magnitude of their role in phytoplankton consumption, nutrient cycling, nutrient retention and benthic-pelagic coupling (Dame et al. 2001).

Bivalves are considered to be ideal organisms to stabilize the effects of nutrient loading in estuaries because (1) they have relatively slow biomass turnover rates, (2) their population is present year-round in the water column and is opportunistically ready to consume phytoplankton; thus there is no lag time between primary production and consumption, and (3) bivalve grazing doesn’t level off with increased phytoplankton concentrations (Herman & Scholten 1990). By coupling increased phytoplankton production to production at higher trophic levels, oysters
Figure 2.1. General oyster population nitrogen budget


Many published studies measure the potential for bivalves to affect water clarity in estuarine systems through particle consumption (suspended sediment and phytoplankton), deposition, and nutrient excretion. There is generally agreement in the literature that bivalves mediate sedimentation of inorganic particles from the water column to the bottom (Kautsky & Evans 1987, Peterson & Heck 1999). However, two dominant paradigms emerge from researchers quantifying bivalve impact on water clarity and phytoplankton standing stocks. Studies from several regions conclude that bivalve filtration provides a negative feedback loop where bivalve grazing rates are sufficient to stabilize and reduce phytoplankton biomass in entire embayments regardless of nutrient loading (Cloern 1982, Cohen et al. 1984, Dame 1996, Dame et al. 1991, Herman & Scholten 1990, Hily 1991, Newell 1988, Newell et al. 1999, Riemann et al. 1988, Officer et al. 1982, Smaal & Zurburg 1997). Other studies, mostly those conducted in South Carolina estuaries, conclude that bivalve filtration provides a positive feedback loop through which bivalve grazing and nutrient regeneration, primarily ammonium release, leads to enhanced and faster phytoplankton production that does not reduce phytoplankton biomass or impact water clarity (Dame 1999, Dame et al. 1984, Dame & Libes 1993). High
productivity would be a benefit to the system provided that the production is transferred up the food chain but not if the result is high phytoplankton biomass.

The impact of oyster reefs on water clarity can be measured in three ways: (1) by tracking rates of phytoplankton consumption, (2) by quantifying the reef's role in nutrient cycling and storage, or (3) by directly tracking changes in water column light attenuation. In 1988, Newell modeled potential phytoplankton uptake of the Chesapeake Bay's historic oyster population. He found that oysters had the ability to filter 52% of the daily phytoplankton production in Chesapeake Bay in the short term, and from this he concluded that they contributed to clearer water in the Bay (Newell 1988). However, Newell's calculations were based on phytoplankton consumption and overlooked the fate of excreted and biodeposited nutrients and hence, he may have overestimated the impact of bivalves on phytoplankton biomass and water clarity. A more thorough approach for studying the longer-term impact of oyster reefs on water clarity may be through tracking nutrient pools and flows. This method accounts for determinations of particulate nutrient uptake (measured by phytoplankton consumption), nutrient storage, inorganic nutrient release from excretion and remineralization of biodeposits, and the fate of the released inorganic nutrients. In polyhaline areas, nitrogen levels generally limit phytoplankton production (Caraco et al. 1987) and thus, evaluation of nitrogen cycling serves as a particularly useful measure of the oyster reef's impact on phytoplankton biomass and, consequently, on water clarity as phytoplankton can comprise up to 45% of water column light attenuation in the Chesapeake Bay (Batiuk et al. 1992).
Ultimately, the best measure of the oyster reef’s impact on water clarity is the direct tracking of changes in light attenuation, however, in the Chesapeake Bay, oyster biomass is very low relative to water volume and changes in attenuation which may be attributed to specific reefs may be difficult to detect. Therefore, tracking nitrogen and phosphorus cycling on the reef seems to be the best current technique to measure the function and value of reef restoration.

Bivalves facilitate the removal of nitrogen from the water column directly by sequestering nitrogen in their tissue and shells (Dame et al. 1989, Dame & Libes 1993, Kim 1983, Rice et al. 2000), and indirectly by influencing the further removal of nitrogen from the water column, both through deposition and subsequent consumption by deposit feeders (Dame et al. 1991, Hily 1991, Peterson & Heck 1999), and by creating an environment that is conducive to release of nitrogen via coupled nitrification–denitrification (Newell et al. 1999, Dame et al. 1991). Laboratory mesocosm work has shown that bivalve biodeposits, when mixed into sediments under oxic water conditions, initiate coupled nitrification-denitrification causing release of nitrogen from the system as N₂ gas (Newell et al. 1999). Studies have also demonstrated that ammonium diffusing out of the sediment is intercepted and utilized by benthic microalgae causing only low levels of inorganic nitrogen release to the water column from biodeposit remineralization (Baudinet et al. 1990, Newell et al. 1999).
Since water column nitrogen levels tend to limit the growth of phytoplankton in coastal areas (Caraco et al. 1987, Ryther & Dunstan 1971), one can conclude that oyster reefs have the potential to reduce the phytoplankton standing stocks and thereby increase water clarity, if it can be demonstrated that these reefs retain nitrogen or otherwise render nitrogen inaccessible to pelagic phytoplankton via nitrogen storage or release from the system. The model created by Ulanowicz & Tuttle (1992) supports this concept. This model, which tracks nutrient cycling in the Chesapeake Bay, predicts that a 150% increase in the oyster population will lead to a 12% decrease in water column primary production and a 29% increase in benthic primary production (Ulanowicz & Tuttle 1992).

**Potential Relationships Between Oyster Reefs and Seagrasses**

Recently, researchers have begun to speculate on the potential for bivalves to create a more habitable growth environment for seagrasses through two mechanisms. First, by improving water clarity (Dennison et al. 1993, Hargis & Haven 1999, Moore et al. 1999, Newell et al. 1999, Ulanowicz & Tuttle 1992, Reusch et al. 1994), and second, by elevating sediment-nutrient levels and creating a source of nutrients that is available to seagrasses via root and rhizome uptake but unavailable to pelagic phytoplankton (Peterson & Heck 1999 & 2001, Reusch et al. 1994). The latter is more a benefit from benthic infauna, such as clams and mussels, rather than of oyster reefs (Peterson & Heck 1999 & 2001, Reusch et al. 1994).
Seagrass populations in the Chesapeake Bay and in shallow water estuarine and coastal environments throughout the world are in many cases limited in distribution by water clarity (Moore & Wetzel 2000, Dennison et al. 1993, Kemp 1989) and low sediment-nutrient levels (Peterson & Heck 1999, Short 1987, Orth 1977), but may also be limited by exposure, substrate, wave activity and sediment anoxia (Fonseca et al. 1983, Smith et al. 1988). Elevated water column nutrient levels from watershed sources can cause phytoplankton blooms that increase the turbidity of the water, thereby shading seagrasses and reducing their photosynthesis (Short & Wyllie-Echeverria 1996). Phytoplankton further decrease the light available to seagrasses by absorbing photons in the same spectral wavelengths as seagrasses require for photosynthesis, and thus alter the light quality that reaches the plants (Dennison et al. 1993, Pierce et al. 1986).

Eelgrass, *Z. marina*, has a high light requirement, needing 20% of surface irradiance in order to grow (Duarte 1991). High light requirements result from the challenging habitat in which these seagrasses exist, in anoxic sediments and in physically dynamic systems that require the plant to have large below-ground, non-photosynthetic root structures (Smith et al. 1988). Light attenuates exponentially in the water column according to the Beer-Lambert equation:

\[
\ln \left( \frac{I_z}{I_0} \right) = -K_d \cdot z
\]

where \( I_z/I_0 \) is the percent of surface irradiance required by seagrasses, which is a function of the water column light attenuation \( (K_d) \) and the water depth \( (z) \). In the Chesapeake Bay, phytoplankton and suspended sediments are the major components
of light attenuation in the water column (Batiuk et al. 1992). The Beer-Lambert equation shows that a change in $K_d$, from altered phytoplankton or suspended sediment concentrations in the water column, will directly affect the depth at which seagrasses can grow.

Water column nutrients are not usually beneficial for seagrasses because they tend to promote the growth of light-competitive phytoplankton and epiphytic algae that negatively impact seagrasses by blocking their access to light (Valiela 1995). Although seagrasses can take up nitrogen from the water column through their leaves (McRoy & Alexander 1975), they absorb much of their nitrogen and all of their phosphorus through their roots and rhizomes from sediment pore water (Short & McRoy 1984). Due to these physiological light and nutrient requirements, seagrasses generally thrive in nutrient-rich sediments under high light, low water-column nutrient conditions (Valiela 1995).

Conversely, phytoplankton and epiphytes (microalgae that grow on seagrass blades) are generally considered to be nutrient-limited. Like seagrasses, phytoplankton preferentially exhibit uptake of ammonium over nitrite and nitrate, but they can also use dissolved organic nitrogen (DON) (McCarthy 1980). They thrive in lower light, high water-column nutrient conditions because (1) they have low light requirements, needing only 0.5-1% of surface irradiance for survival, growth and reproduction (Wetzel 1975); and (2) their nutrient uptake occurs only from the water column.
Objectives

One of the goals of oyster reef restoration in the Chesapeake Bay is the return of reef ecosystem functions such as water filtration (Hargis & Haven 1999). In addition to the filtering capacity of the oysters themselves, the developed reef serves as a habitat for a diverse assemblage of other filter-feeding organisms (Meyer & Townsend 2000). To estimate the impact of oyster reef rehabilitation on water quality, this study measured the seasonal impact of a rehabilitated reef on phytoplankton grazing and nutrient cycling by quantifying nutrient exchanges associated with the oyster reef community in comparison to those associated with the intertidal flat community that the reef had replaced. Flux study results were also used to estimate the potential for oyster reefs to create a more habitable environment for *Z. marina* on a local scale.

METHODS

Site Description

The study was conducted on the constructed oyster reef and adjacent intertidal flat at Fisherman’s Island on the southern tip of Virginia’s Eastern Shore, 37° 06’ N, 75° 58’ W (Figure 2.2). In July 1996, the Virginia Marine Resources Commission constructed eleven intertidal reefs of various substrates to increase settlement habitat for larval oysters (Coen & Luckenbach 2000). These reefs were built upon the shallow intertidal sand flat (<0.5m) that extends from a *Spartina alterniflora* marsh to the edge of a shallow channel (2m MLW). The two reefs created
Fishermans Island is located at the base of Virginia’s Eastern Shore. Eleven reef bases were constructed out of various substrates in 1996 (Coen & Luckenbach 2000). For this research, only the reefs constructed with oyster shell bases were sampled. (Figure created by Janet Nestlerode)
with oyster shells were used for this research. Jointly, they cover 364 m² of area that was previously intertidal flat. Since their construction in 1996, a viable oyster community has developed on the created oyster shell bases through natural recruitment. From 1996-1999, oyster density on the oyster shell reefs varied seasonally from 523 to 1650 oysters • m⁻², with oysters ranging from 5 mm to over 100 mm in shell height (O’Beirn, unpub. data). During this study, oyster density ranged from 745 to 882 oysters • m⁻² with an average biomass range of 207 to 302 gdw • m⁻² (Figure 2.3). Fisherman’s Island is located in the polyhaline region of the Bay where salinity (= 25 ppt) is appropriate for both *C. virginica* and *Z. marina*. Phytoplankton biomass in this region has previously been determined to be limited by nitrogen availability in the water column (Reay et al. 1993).

**Sample Collection**

The flux study undertaken here quantified in situ changes in dissolved ammonium (NH₄⁺), nitrate (NO₃⁻) and nitrite (NO₂⁻), dissolved organic nitrogen (DON), dissolved inorganic phosphorus (DIP), chlorophyll a (chl a) and dissolved oxygen (DO) concentrations over time in sealed, stirred, clear mesocosms chambers placed directly on the developed *C. virginica* reef and the adjacent intertidal flat (see Moore et al. 1999). Chambers were chosen to constrain a water mass over the substrate surfaces relative to normal flow conditions so that measurable rates of change of water column constituents could be determined. Although the water was in contact with the substrate for a long period of time in the chambers, uptake and
Figure 2.3. Oyster biomass (a) and density (b) in the three chambers (± S.E.)

(a) Mean oyster biomass shows a seasonal pattern that is consistent with data collected from 1996-1999 (O’Beirn, unpublished data) with oyster growth through the summer. From late summer through early spring, disease causes mortality in larger oysters (2-3” shell height) (Burreson 1991), leading to greatly reduced biomass in the spring. (b) Mean oyster density is consistent from June to August, drops in October with some disease induced mortality, and increases by spring due to early fall recruitment of larvae which are plentiful but small (O’Beirn, unpublished data).
release rates for the two substrates could be relatively compared and results could be extrapolated to the system to identify the potential effects of oyster reef filtration.

The chambers were deployed and sampled on June 8, August 10 and October 20, 2000 and on April 5, 2001. Bi-monthly sampling dates were selected between the months of March and November because oysters and *Z. marina* are both active during this period (Dennison et al. 1993, Haven & Morales-Alamo 1970) and this seasonal schedule matches that of Moore et al. (1999) and thus facilitates relative comparisons with other habitats. In May 2000, six round collar bases (31 cm x 14.5 cm, height x radius) were permanently placed into either the oyster reef or adjacent intertidal flat to a depth of 30 cm to isolate identical areas of substrate (662.9 cm²). Bases were all deployed on the crest of the oyster reef and 20 meters to the east of the reef on the intertidal flat. Specific locations were selected by dividing the reef and adjacent flat into one-meter segments and randomly identifying 3 locations for collar deployment in each substrate type. Oyster biomass and density per collar base were determined prior to each sampling event (Figure 2.3). Biomass was determined non-destructively by measuring oyster shell height to the nearest millimeter with calipers and converting height to biomass using the following equation from Mann and Evans (1998):

\[
\text{Dry weight} = 0.000423 \times \text{Shell height}^{1.7475}
\]

On each of the four sampling days, 6 round clear Plexiglas chambers (14.5 cm x 61 cm, radius x height) were snugly fitted onto the bases and gaps were sealed with
neoprene tape (Figure 2.4). The chambers enclosed the entire reef community including oysters, associated reef fauna, biodeposits and underlying sediments. Three additional chambers of identical dimensions but with a sealed bottom served as water blanks, allowing for determination of water quality changes that were associated with the water column alone. Inside each chamber, there was a small stirrer that kept the water slowly circulating to avoid stratification. The chambers were held in place with plastic coated steel reinforcing bars, filled with ambient water, and sealed at the top with a stopper, enclosing approximately 40 liters of water for the duration of the experiment. Water volume was recorded in each chamber at the beginning and end of the study and flux rates were corrected for water volume differences between chambers.

A TYGON R-3603 silicone tube with a watertight seal was suspended into the center of the chamber for sample collection. Water samples were removed from the chamber using a 30cc plastic syringe that attached to a one-way valve on the end of the silicone tube. Two replicate water samples were taken from each of the 9 chambers at half-hour intervals (T0, T1, T2, T3, T4) for the first 2 hours of the experiment. After sample T4, the chambers were darkened with black plastic bags and were allowed to acclimate for one hour. After acclimation, half-hour sampling (T5, T6, T7) resumed for another three cycles. Samples were filtered in the field through 0.45μm sterile Acrodisc syringe filters. Water samples were immediately chilled before they were taken back to the lab and frozen for NH₄⁺, NO₃⁻, NO₂⁻, DON and orthophosphate (PO₄³⁻) analysis. NH₄⁺ and PO₄³⁻ were analyzed.
Figure 2.4. Flux Study Chamber

(a) Chamber deployed on reef substrate at low tide during flux study data collection  (b) Diagram indicating dimensions of the chamber, placement of the collar within the substrate and location of the stirrer, sampling hose and stopper.
spectrophotometrically using the techniques defined by Solorzano (1969) and Parsons et al. (1984), respectively. NO$_3$ was reduced to NO$_2$ using a cadmium reduction column and determined by diazotization using a Alpkem “Flow Solution” autoanalyzer (Perstorp 1992). DON was analyzed using the alkaline persulfate method in sealed glass ampoules following the method of Parsons et al. (1984).

Replicate chlorophyll $a$ samples were collected from each chamber at the beginning, middle and end of the experiment (T0, T4, T7) and were immediately chilled before they were brought back to the lab. Upon return, 5ml water samples were filtered through Whatman GF/F filters, extracted for 24 hours in a DMSO/acetone mixture (45% acetone : 45% DMSO : 10% DI water with 0.01% diethylamine) and fluorescence was determined using a Turner Designs Fluorometer (model 10-AU) after Shoaf and Lium (1976). Replicate ambient chlorophyll $a$ samples were also taken from the water column at T0 and T7.

Since the chlorophyll $a$ concentrations in the oyster chambers were reduced from ambient concentrations to very low levels sometime during the interval between T0 to T4 in the June and August samplings, it was decided that chl $a$ should be sampled from the oyster chambers at shorter intervals to more precisely measure uptake rates. Therefore, during the October 2000 and April 2001 sampling dates all 9 chambers were sampled for chl $a$ at T0, T4 and T7 as before, but intensive chlorophyll $a$ sampling at 10-minute intervals was also added between T0 and T4 for the oyster chambers.
Dissolved oxygen samples were taken at T0, T4 and T7, were fixed in the field and were brought back to the lab for analysis using the Winkler Titration method (Standard Methods for the Examination of Water and Wastewater 1992). DO samples were collected to ensure that water in chambers did not become anoxic ($O_2 = 0.0$ ml/l, Tyson & Pearson 1991), which would have drastically altered nutrient fluxes from the sediments (Cowan & Boynton 1996, Kemp & Boynton 1992).

Replicate sediment cores (73 cm$^2$ x 2 cm deep) were taken from the reef and from the adjacent intertidal flat in April, June, August and October 2001. Half of each core was extracted for sediment-nutrient analysis in KCl and analyzed for extractable NO$_2^-$, NO$_3^-$, NH$_4^+$, and PO$_4^{3-}$ as described above. The other half was placed in pre-weighed foil envelopes, weighed, dried at 50°C to a constant weight, and reweighed to determine dry bulk density and percent water content. Samples from the dried sediment were ground and processed for % total organic carbon (TOC) and % total nitrogen (TN) using an Exeter Analytical CE-24000 CHN elemental analyzer (Exeter Analytical CE-2400 CHN Elemental Autoanalyzer Instruction Manual 1998, Menzel & Vaccaro 1964) and the remainder of the sediment was combusted at 500°C for 5 hours and reweighed to determine organic matter content. Replicate surface sediment samples (1 cm$^2$) were collected from the oyster reef and the adjacent intertidal flat in June, August and October 2001 and
were processed for sediment-chlorophyll and sediment-phaeophytin using the methods defined by Lorenzen (1967).

**Flux Calculations for Nutrients**

Fluxes from the two substrates were determined as the linear rate of change in concentrations of dissolved nutrients and chlorophyll-a in each chamber over the course of 4.5 hours. While chambers were darkened 2 hours into the experiment in an attempt to simulate nighttime conditions, the chambers did not appear to have adequate acclimation time to adjust and no significant differences in light/dark rates could be determined. Therefore, fluxes were calculated using nutrient concentration data over the entire 4.5 hours rather than separately as light and dark fluxes. Fluxes between both substrates and the overlying water were calculated as follows: (1) for all nine chambers and for each sampling period, mean nutrient concentrations were obtained from duplicate samples; (2) mean concentrations were regressed against time; (3) mean water column slope for the three water blank chambers was calculated and subtracted from individual slopes for each reef or intertidal flat chamber to correct for water column processes; (4) corrected slopes were multiplied by the measured water volume in the chamber and divided by substrate area enclosed by the collar (0.0729 m²); (5) exchange rates for substrates were reported in μM · m⁻² · hr⁻¹ for all DIN, DON and DIP fluxes and in μg-chl a · m⁻² · hr⁻¹ for chlorophyll fluxes and per m³ for water blank chambers (multiplying per m² fluxes by 1m water depth); (6) exchange rates from the three reef and three intertidal flat chambers were averaged to obtain NH₄⁺, NO₃⁻, NO₂⁻, DON, PO₄³⁻ and chl-a exchange rates between
each substrate and the overlying water for each sampling date. For all flux values, negative values indicate uptake from the water column and positive values indicate release to the system.

Flux values for the oyster reef community were reported as flux per square meter of reef rather than as flux per oyster because (1) normalizing to oyster biomass did not reduce the error variance of the data and (2) flux data fairly reflected the reef processes associated with the entire reef community, which may be only partially a function of oyster biomass.

Chlorophyll-a exchange rates were converted to rates of particulate nitrogen (PN) and particulate phosphorus (PP) exchange and seasonal rates of net exchange of total nitrogen (TN) and net phosphorus (NP) (µg · m⁻² · hr⁻¹) were calculated by combining rates of particulate and dissolved nutrient exchanges for each sampling period. In calculating the net exchange of total nitrogen, fluxes for particulate nitrogen, dissolved inorganic nitrogen and dissolved organic nitrogen were used. In calculating NP, only dissolved inorganic and particulate phosphorus were summed since dissolved organic phosphorus (DOP) was not measured. Chlorophyll flux was converted to PN and PP fluxes using a N:Chl-a weight ratio of 8.8 and a P:Chl-a weight ratio of 1.22, calculated with a C:Chl-a weight ratio of 50 and Redfield (1958) weight ratio values for phytoplankton (C:N = 5.68 and C:P = 1.22). Phytoplankton C:Chl-a can range from 25 in nitrate-rich water to 60 in nitrate-depleted water (Parsons & Takahashi 1973). The C:Chl-a weight ratio of 50 was used for this system.
since phytoplankton are known to be nitrogen limited (Reay et al. 1993) and the ambient dissolved nitrogen concentration in the water was between 1-2 μM-N in April, June, August and October.

**Statistical Analysis**

All flux data were square root transformed in order to correct for non-consistency of error variance (Zar 1999). For clarity, non-transformed data are presented in graphs. Oyster reef and intertidal flat fluxes were statistically compared on each sampling date using one-way ANOVA (response: flux, factor: substrate). Water column fluxes are presented in graphs for reference although fluxes from these chambers were not statistically compared to fluxes from the substrates. Sediment nutrients, sediment chlorophyll, bulk density, % water, % TOC, %TN, TOC : TN results were analyzed using two-way ANOVA (factors: substrate & date). When interactions were significant, Scheffe’s post-hoc test was used to determine significance (Zar 1999). Significance level for all statistics was set at 0.05.

**RESULTS**

**Dissolved Nutrient Fluxes**

Inorganic nitrogen fluxes were predominantly related to NH₄⁺ flux for both oyster reef and intertidal flat substrates. NO₂⁻ and NO₃⁻ fluxes showed low levels of exchange during all months sampled (Figure 2.5). The oyster reef treatment did show significantly greater release of NO₂⁻ than the intertidal flat in April (p=0.0130), June (p=0.0031), August (p<0.0001) and October (p=0.0019) and significantly
Figure 2.5. Seasonal fluxes of \( \text{NO}_2^- \) and \( \text{NO}_3^- \)

Means (± SE) of fluxes per sampling season. Fluxes from the intertidal flat and oyster reef substrates were based on substrate area and reported in \( \mu\text{M-N} \cdot \text{m}^2 \cdot \text{hr}^{-1} \). Fluxes from the water blanks were multiplied by the depth of water (1m) and reported in \( \mu\text{M-N} \cdot \text{m}^3 \cdot \text{hr}^{-1} \). All were plotted on the same axis for comparison. Samples were collected from June 2000 through April 2001. Asterices (*) indicate significant differences in fluxes between the oyster reef and the intertidal flat substrates on each date at the 0.05 \( \alpha \)-level as determined by ANOVA. Positive fluxes indicate release to the water column and negative fluxes indicate uptake from the water column.
greater release of NO3⁻ than the intertidal flat in April (p=0.0281), June (p=0.0127), August (p=0.0040) and October (p=0.0002).

Both oyster reefs and intertidal flats released NH4⁺ to the overlying water column throughout the study period with the reef showing highest release rates in August and the intertidal flat showing a consistent rate of release from June through October (Figure 2.6). The oyster reef community showed significantly higher release rates than the intertidal flat for NH4⁺ in June (p=0.0010), August (p=0.0065) and October (p=0.0325), but not in April (p=0.7716). Fluxes of DON were generally about half the magnitude of NH4⁺ fluxes and showed similar seasonal patterns. The oyster reef community release rates for DON were significantly higher than release rates for the intertidal flat in June (p=0.0020) and October (p=0.0354), but not in April (p=0.2091) or August (p=0.071). In most seasons, NH4⁺ and DON were taken up from the water column chambers.

PO4³⁻ fluxes were consistent in all seasons for the intertidal flat, but fluxes varied seasonally for the reef with highest PO4³⁻ release in August and October (Figure 2.7). Release of PO4³⁻ was significantly higher for the oyster reef than for the adjacent flat in August (p=0.0167), significantly higher for the flat in June (p=0.0005) and not significantly different between the substrates in April (p=0.2936) or October (p=0.0637). PO4³⁻ was removed from the water column in all seasons.
Figure 2.6. Seasonal fluxes of NH$_4^+$ and DON

Means (± SE) of fluxes per sampling season. Fluxes from the intertidal flat and oyster reef substrates were based on substrate area and reported in μM-N · m$^2$ · hr$^{-1}$. Fluxes from the water blanks were multiplied by the depth of water (1m) and reported in μM-N · m$^3$ · hr$^{-1}$. All were plotted on the same axis for comparison. Samples were collected from June 2000 through April 2001. Asterices (*) indicate significant differences in fluxes between the oyster reef and the intertidal flat substrates on each date at the 0.05 α-level as determined by ANOVA. Positive fluxes indicate release to the water column and negative fluxes indicate uptake from the water column.
Means (± SE) of fluxes per sampling season. Fluxes from the intertidal flat and oyster reef substrates were based on substrate area and reported in \( \mu M \cdot m^2 \cdot hr^{-1} \). Fluxes from the water blanks were multiplied by the depth of water (1m) and reported in \( \mu M \cdot m^3 \cdot hr^{-1} \). All were plotted on the same axis for comparison. Samples were collected from June 2000 through April 2001. Asterices (*) indicate significant differences in fluxes between the oyster reef and the intertidal flat substrates on each date at the 0.05 \( \alpha \)-level as determined by ANOVA. Positive fluxes indicate release to the water column and negative fluxes indicate uptake from the water column.
Chlorophyll-a Fluxes

For the water blank and intertidal flat treatments, chlorophyll-a fluxes were calculated as described above for nutrient fluxes. However, chlorophyll-a concentrations decreased exponentially in the oyster reef chambers, as was similarly found by Coughlan (1969), with the oyster reef virtually depleting the phytoplankton in the chamber by the end of the first two hours. A linear fit of chlorophyll-a concentration to the first hour of each experiment was used to determine uptake rates because of the relatively short duration of exposure a water mass would be expected to have over an individual reef’s surface. This method provided a conservative estimate of chlorophyll-a depletion relative to time. The most representative rate occurs immediately after the chamber was filled with ambient phytoplankton concentrations because oyster consumption rates necessarily decline once they have depleted the phytoplankton supply by grazing.

The oyster reef and intertidal flat communities consistently removed phytoplankton from the water column (Figure 2.8). The oyster reef removed chlorophyll-a at significantly faster rates than the intertidal flat in April (p<0.0001), August (p=0.0080) and October (p=0.0027), but not in June (p=0.0686). Chlorophyll-a was also removed from the water column chambers in April, June and October, except in August when production exceeded consumption.
Means (± SE) of fluxes per sampling season. Fluxes from the intertidal flat and oyster reef substrates were based on substrate area and reported in μg-chl-a · m\(^2\) · hr\(^{-1}\). Fluxes from the water blanks were multiplied by the depth of water (1m) and reported in μg-chl-a · m\(^3\) · hr\(^{-1}\). All were plotted on the same axis for comparison. Samples were collected from June 2000 through April 2001. Asterices (*) indicate significant differences in fluxes between the oyster reef and the intertidal flat substrates on each date at the 0.05 α-level as determined by ANOVA. Positive fluxes indicated release to the water column and negative fluxes indicate uptake from the water column.
Sediment Biogeochemistry

The sediment inside the oyster reef contained higher % organic matter, higher % TOC and higher % TN throughout the sampling period than the adjacent intertidal flat (Table 2.1). Despite higher total organic carbon and total nitrogen content in reef sediments, the carbon to nitrogen ratio was not significantly different (p>0.05) between the reef and intertidal flat sediments. Oyster reef sediments had higher levels (p<0.05) of extractable NH₄⁺ and PO₄³⁻ throughout the study period compared to the flat (Table 2.2), but sediments from the two substrates had inconsistent trends for sediment NO₂⁻ and no difference (p>0.05) in sediment NO₃⁻ concentrations. Sediment chlorophyll and phaeophytin levels were both significantly (p<0.05) higher in the oyster reef sediments than in the intertidal flat sediments (Table 2.3). Bulk density was significantly lower and % water was significantly higher in oyster reef sediments (Table 2.4).

Net Exchange of Nitrogen and Phosphorus

Net nitrogen exchange data demonstrated that the intertidal flat served as a source of nitrogen in all periods sampled (Figure 2.9) while the oyster reef community served as a storage mechanism for nitrogen in April and October. Although oyster reefs had higher dissolved nitrogen release rates than the adjacent flat in June, August and October, the nitrogen release was counterbalanced by high particulate nitrogen (calculated from chlorophyll-a fluxes) consumption in April and October. Nitrogen uptake by the reef was significantly different from nitrogen release by the intertidal flat in April (p=0.0001) and October (p=0.0490). Summer
Table 2.1. Sediment Organic Content

Sediment percent organic matter is a weight measure. Percent total organic carbon and percent total nitrogen are atomic weight measures. Different subscripts indicate significant differences (2-way ANOVA (factors: date, substrate, $\alpha = 0.05$)) between substrate types on each date. Where interactions are significant, for %TOC, Scheffe's post-hoc test was used to determine significance. SE = standard error.

<table>
<thead>
<tr>
<th>Month</th>
<th>Substrate</th>
<th>% organic mean</th>
<th>% organic SE</th>
<th>% TOC mean</th>
<th>% TOC SE</th>
<th>% TN mean</th>
<th>% TN SE</th>
<th>TOC:TN mean</th>
<th>TOC:TN SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>Reef</td>
<td>4.63</td>
<td>0.18</td>
<td>1.15</td>
<td>0.04</td>
<td>0.14</td>
<td>0.01</td>
<td>8.01</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>1.50</td>
<td>0.06</td>
<td>0.26</td>
<td>0.04</td>
<td>0.04</td>
<td>0.01</td>
<td>6.53</td>
<td>0.16</td>
</tr>
<tr>
<td>June</td>
<td>Reef</td>
<td>3.58</td>
<td>0.16</td>
<td>0.91</td>
<td>0.08</td>
<td>0.12</td>
<td>0.01</td>
<td>7.87</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>0.87</td>
<td>0.19</td>
<td>0.22</td>
<td>0.00</td>
<td>0.02</td>
<td>0.00</td>
<td>9.25</td>
<td>1.00</td>
</tr>
<tr>
<td>August</td>
<td>Reef</td>
<td>2.64</td>
<td>0.35</td>
<td>0.74</td>
<td>0.08</td>
<td>0.10</td>
<td>0.01</td>
<td>7.48</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>0.78</td>
<td>0.10</td>
<td>0.15</td>
<td>0.04</td>
<td>0.02</td>
<td>0.00</td>
<td>6.16</td>
<td>0.67</td>
</tr>
<tr>
<td>October</td>
<td>Reef</td>
<td>3.49</td>
<td>0.47</td>
<td>0.83</td>
<td>0.05</td>
<td>0.11</td>
<td>0.01</td>
<td>7.48</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>1.21</td>
<td>0.19</td>
<td>0.29</td>
<td>0.08</td>
<td>0.04</td>
<td>0.01</td>
<td>7.34</td>
<td>0.41</td>
</tr>
</tbody>
</table>
Table 2.2. Sediment Nutrients

Different subscripts indicate significant differences (2-way ANOVA (factors: date, substrate, $\alpha = 0.05$)) between substrate types on each date. Where interactions are significant, for sediment $\text{NO}_2$, Scheffe's post hoc test was used to determine significance. SE = standard error

<table>
<thead>
<tr>
<th>Month</th>
<th>Substrate</th>
<th>Sediment $\text{NO}_2$ (uM-N $\cdot$ m$^2$)</th>
<th>Sediment $\text{NO}_3$ (uM-N $\cdot$ m$^2$)</th>
<th>Sediment $\text{NH}_4$ (uM-N $\cdot$ m$^2$)</th>
<th>Sediment $\text{PO}_4$ (uM-P $\cdot$ m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>SE</td>
<td>mean</td>
<td>SE</td>
</tr>
<tr>
<td>April</td>
<td>Reef</td>
<td>0.1</td>
<td>0.1</td>
<td>24.4</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>0.1</td>
<td>0.1</td>
<td>35.2</td>
<td>7.3</td>
</tr>
<tr>
<td>June</td>
<td>Reef</td>
<td>2.9$^a$</td>
<td>1.5</td>
<td>24.6</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>0.1$^b$</td>
<td>0.1</td>
<td>16.6</td>
<td>2.5</td>
</tr>
<tr>
<td>August</td>
<td>Reef</td>
<td>0.4$^a$</td>
<td>0.4</td>
<td>17.1</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>1.3$^b$</td>
<td>0.2</td>
<td>24.9</td>
<td>4.2</td>
</tr>
<tr>
<td>October</td>
<td>Reef</td>
<td>5.6$^a$</td>
<td>0.8</td>
<td>32.8</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>3.0$^b$</td>
<td>0.1</td>
<td>17.3</td>
<td>3.0</td>
</tr>
</tbody>
</table>
Table 2.3. Sediment Chlorophyll-a and Sediment Phaeophytin

Different subscripts indicate significant differences (2-way ANOVA (factors: date, substrate, $\alpha = 0.05$)) between substrate types on each date. Sediment chlorophyll measures healthy benthic diatoms whereas phaeophytin is an indicator of degraded chlorophyll, perhaps resulting from degradation or incomplete digestion (Dame & Dankers 1988). SE = standard error

<table>
<thead>
<tr>
<th>Month</th>
<th>Substrate</th>
<th>Sediment Chlorophyll-a (ug-chl-a $\cdot$ m$^{-2}$)</th>
<th>Sediment Phaeophytin (ug-phaeo $\cdot$ m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>SE</td>
</tr>
<tr>
<td>June</td>
<td>Reef</td>
<td>15,079$^a$</td>
<td>1,160</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>12,654$^b$</td>
<td>2,113</td>
</tr>
<tr>
<td>August</td>
<td>Reef</td>
<td>15,325$^a$</td>
<td>3,576</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>10,194$^b$</td>
<td>3,832</td>
</tr>
<tr>
<td>October</td>
<td>Reef</td>
<td>14,552$^a$</td>
<td>798</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>8,858$^b$</td>
<td>531</td>
</tr>
</tbody>
</table>
Table 2.4. Sediment Bulk Density and Percent Water

Different subscripts indicate significant differences (2-way ANOVA (factors: date, substrate, $\alpha = 0.05$)) between substrate types on each date. SE = standard error

<table>
<thead>
<tr>
<th>Month</th>
<th>Substrate</th>
<th>Sediment Bulk Density (g cm$^{-3}$)</th>
<th>Sediment % H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>SE</td>
</tr>
<tr>
<td>April</td>
<td>Reef</td>
<td>1.02$^a$</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>1.79$^b$</td>
<td>0.05</td>
</tr>
<tr>
<td>June</td>
<td>Reef</td>
<td>1.38$^a$</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>1.99$^b$</td>
<td>0.07</td>
</tr>
<tr>
<td>August</td>
<td>Reef</td>
<td>1.55$^a$</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>2.06$^b$</td>
<td>0.08</td>
</tr>
<tr>
<td>October</td>
<td>Reef</td>
<td>1.34$^a$</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Flat</td>
<td>1.74$^b$</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Figure 2.9 Net nitrogen exchange calculated by summing DIN, DON and particulate nitrogen (calculated from chlorophyll-a values) fluxes. Means (± SE) of flux per month shown. Samples were collected from June 2000 through April 2001. * indicates significant difference in fluxes between the oyster reef and intertidal flat substrates on each date at the 0.05 α-level as determined by ANOVA. Positive values indicate release to the overlying water column and negative values indicate uptake from the water column.
nitrogen release was not significantly different between the substrates in June (p=0.6477) or August (p=0.0889).

Although this study did not measure dissolved organic phosphorus, net phosphorus exchange calculated by summing DIP and PP fluxes demonstrated that the intertidal flat was a source of phosphorus to the water column throughout the sampling period (Figure 2.10), whereas the oyster reef treatment demonstrated net phosphorus uptake in April and June and release in August and October. The net phosphorus uptake by the reef was significantly different from phosphorus release by the intertidal flat in April (p=0.0002) and June (p=0.0005). In August, reef release of DIP was significantly higher than intertidal flat release (p=0.0167) and in October, phosphorus exchange from the two substrates was not significantly different (p=0.7598).

*Net Impact of Reef Restoration on Nitrogen Exchange in the System*

Intertidal flat flux values were subtracted from the oyster reef flux values in order to measure the effect of replacing a section of intertidal flat with oyster reef. Results were similar to those for reef net nitrogen exchange however; the magnitudes of uptake are higher and the magnitudes of release are lower, since the flat had released nitrogen in all seasons (Figure 2.11).
Figure 2.10 Net phosphorus exchange calculated by summing DIP and particulate phosphorus (calculated from chlorophyll-a values) fluxes. Means (± SE) of flux per month shown. Samples were collected from June 2000 through April 2001. * indicates significant difference in fluxes between the oyster reef and intertidal flat substrates on each date at the 0.05 α-level as determined by ANOVA. Positive values indicate release to the overlying water column and negative values indicate uptake from the water column.
Figure 2.11 Net impact of reef rehabilitation on nitrogen cycling. Means (± SE) of flux per month were calculated by summing TN fluxes of oyster reef and intertidal flat to gauge the impact of replacing a square meter of intertidal flat with reef. Positive values indicate release to the overlying water column and negative values indicate uptake from the overlying water column.
DISCUSSION

Nitrogen Processes

During all seasons both the oyster reef and the intertidal flat were sources of dissolved nitrogen to the overlying water column; however dissolved nitrogen fluxes from the oyster reef were significantly higher than fluxes from the intertidal flat in June, August and October. In April, August and October, the oyster reef showed greater particulate nitrogen (PN) uptake (calculated from chlorophyll-a fluxes) than the intertidal flat. When dissolved and particulate nitrogen fluxes were summed, the oyster reef was determined to be a sink for total nitrogen in spring and fall, while the intertidal flat was determined to be a consistent and low-level nitrogen source to the water.

Dissolved nitrogen exchanges

Both substrates showed low levels of NO$_3^-$ and NO$_2^-$ exchange during all seasons, however; the intertidal flat generally took up NO$_2^-$ and NO$_3^-$ while the oyster reef consistently released these oxidized forms. Fluxes of NO$_3^-$ and NO$_2^-$ from the intertidal flat followed the seasonal pattern found by others in similar habitats (Cowan & Boynton 1996, Reay et al. 1995) with uptake during all seasons, except for NO$_3^-$ release in August and October. Release of these oxidized forms from the sediment in August and October may have been from infaunal excretion or they may have been the result of nitrification processes within the sediment that were not directly coupled with denitrification (Cowan & Boynton 1996). NO$_3^-$ and NO$_2^-$ fluxes were significantly higher from the oyster reef community than fluxes from the
intertidal flat during all seasons. Consistent release of NO$_2^-$ and NO$_3^-$ from the reef may have been evidence of nitrification processes, or may have been the product of NH$_4^+$ that underwent nitrification within the oysters’ shells, as was observed by Boucher and Boucher-Rodoni (1988).

NH$_4^+$ was the major component of dissolved nitrogen exchange and was consistently released from both reef and flat communities throughout the study period. Release from the intertidal flat was of similar order of magnitude and followed similar seasonal patterns to other studies (Cowan & Boynton 1996, Reay et al. 1995), but was considerably higher (up to 7x for NH$_4^+$) at Fisherman’s Island than in nearby Cherrystone Inlet (Reay et al. 1995). This flux difference may have been due to higher levels of remineralization of organic matter (Cowan & Boynton 1996, Sloth et al. 1995) since the organic content of the sediment at Fisherman’s Island ranged from 11% to 114% higher than that reported in Reay et al. (1995).

NH$_4^+$ release from the reef was significantly higher than NH$_4^+$ flux from the intertidal flat over the summer and early fall and was 2 – 3 times higher than would have been expected from excretion alone in June and August, based on particulate nitrogen consumption rates and oyster energetics (Kim 1983, Hammen 1969). Previous oyster flux studies have calculated that only 40% of summer NH$_4^+$ release comes from oyster excretion and that the rest is due to sediment processes (Boucher & Boucher-Rodoni 1988). Therefore, NH$_4^+$ releases found in this study likely resulted from a combination of high summer excretion rates due to increased
metabolic demands of reef organisms and from high biodeposit remineralization due to increased bacterial metabolic demands (Dame 1999, Smaal & Zurburg 1997). In August, NH$_4^+$ releases from the reef were at their highest seasonal levels. This timing coincides with the timing of the typically highest seasonal remineralization rates (Hayakawa et al. 1999, Valiela 1995) in coastal areas.

NH$_4^+$ fluxes from the reef at Fisherman’s Island were of a similar magnitude to those for oyster reefs in South Carolina (Dame et al. 1985, 1989). However, reefs in South Carolina had NH$_4^+$ releases of approximately 2,600 μM · m$^{-2}$ · h$^{-1}$ in June and 6,200 μM · m$^{-2}$ · h$^{-1}$ in August (from Figure 2 in Dame et al. 1985), which were roughly double the release rates at Fisherman’s Island. These higher NH$_4^+$ fluxes in South Carolina were not explained by increased phytoplankton consumption, which was double that of Fisherman’s Island in June, but was 3 - 4 times less than at Fisherman’s Island in August. The difference may be due to the structure of the reef habitats. In Virginia, reefs are mounds composed mostly of shell and organisms within the sediment 2-3 inches below the surface of the mound, whereas in South Carolina, oysters are completely surrounded by and growing out of thick detrital material (Dame 1999, Dame et al. 1985). Reefs in South Carolina are therefore likely to have higher mineralization rates in the associated sediments that would cause increased efflux of NH$_4^+$ to the water column (Dame 1999). The differences in flux values may also result from the different sampling techniques used in the two studies, chambers used in this study verses flow-through tunnels used in South Carolina (Dame et al. 1984, 1985, 1989)
Nitrogen fluxes for the oyster reefs on Fisherman’s Island study were compared to summer flux rates from mussel beds, *Mytilus edulis*, in the Wadden Sea (Dame & Dankers 1988) because much of the published bivalve flux study data has been collected on mussel beds. In the Wadden Sea, where mussel beds had densities of 4919 individuals \( \cdot \) m\(^2\) and 818 g (ash free dry weight) \( \cdot \) m\(^2\), chlorophyll uptake rates of 15,370 – 55,100 \( \mu \text{g-chl-a} \cdot \text{m}^2 \cdot \text{hr}^{-1} \) were 7-10 times higher than at Fisherman’s Island and NO\(_3^-\) + NO\(_2^-\) fluxes of 1,428 – 5,714 \( \mu \text{M-N} \cdot \text{m}^2 \cdot \text{hr}^{-1} \) were 10-26 times higher (Dame & Dankers 1988). However, NH\(_4^+\) flux rates in the Wadden Sea, 2,857 – 7,857 \( \mu \text{M-N} \cdot \text{m}^2 \cdot \text{hr}^{-1} \), were only double the rates at Fisherman’s Island. The authors attribute these relatively low levels NH\(_4^+\) in the water column to in situ phytoplankton uptake (Dame & Dankers 1988), as they preferentially take up NH\(_4^+\) over oxidized forms of nitrogen. Phytoplankton productivity is presumably high in this system, given the magnitude of NH\(_4^+\) uptake that the pelagic primary producers would have to be responsible for. In the Wadden Sea, these mussel beds appear to be exerting top down control (Valiela 1995) on the benthic primary producer biomass (Dame & Dankers 1988) since phytoplankton biomass is low despite high phytoplankton productivity.

Exchange of DON from both substrates was approximately one-half to one-third the magnitude of NH\(_4^+\) fluxes and followed similar seasonal patterns. DON was generally released to the water column from the intertidal flat. This release was likely from infaunal excretion and organic matter decomposition. In June, the flat
removed DON from the water column, as is consistent with fluxes recorded in June for intertidal flats at nearby Hungar’s Creek (Moore et al., unpublished data). This uptake may have been due to low sediment organic content in June, which would have caused benthic microbes to respire pelagic-derived DON or may have been due to benthic microalgal utilization of DON.

DON release rates from the oyster reef community were significantly higher than releases from the intertidal flat in June and October. Released DON was likely generated by both biodeposit decomposition and organism excretion. DON release into the water column should be proportionally 2.3 times lower than NH$_4^+$ release to the water column, based on the oyster nitrogen budget (Hammen 1969). This was consistent with the ratio of DON to NH$_4^+$ release recorded for the Fisherman’s Island reefs, however, both NH$_4^+$ and DON were higher than would be expected based on particle consumption and oyster energetics alone, further suggesting that remineralization of biodeposits was contributing to these fluxes.

NH$_4^+$ and DON uptake from the water blank chambers during most seasons was likely the result of phytoplankton growth within the chambers. In August, chlorophyll-a concentrations increased in the water blank chambers, but chl-a was removed from the water column in all other seasons. These decreases in chl-a may have been due to passive settlement or zooplankton grazing of the phytoplankton in the water column.
Particulate Nitrogen Exchanges

Chlorophyll-a was consistently removed from the water column by both oyster reef and intertidal flat communities throughout the sampling period, however; uptake from the oyster reef community was significantly higher than uptake by the intertidal flat community in April, August and October. This difference in chlorophyll-a consumption between the two communities was most likely due to the greater biomass of suspension feeding organisms (both oysters and other reef-associated fauna) present in the oyster reef relative to suspension feeding biomass in the intertidal flat.

There was some passive settlement or zooplankton grazing of phytoplankton in the water column, as was demonstrated by declines in chlorophyll-a concentrations in water blank chambers in April, June and October. All chlorophyll-a fluxes from the reef and intertidal flat have been corrected for water column processes and therefore, reported chl-a fluxes represent only benthic community (reef or intertidal flat) chl-a consumption and were not due to passive particle settlement or zooplankton grazing.

Chlorophyll-a consumption rates for the reefs, when converted into nitrogen units, were similar to the nitrogen requirement estimates calculated by Luckenbach et al. (2000), who modeled the minimum nitrogen consumption requirements per m² of the reef using oyster growth and population development data collected seasonally on the Fisherman’s Island reefs from 1996-1999. Their modeling study estimated
nitrogen consumption rates to range between approximately 10 – 62 mg-N • m$^2$ • hr$^{-1}$ (Luckenbach et al. 2000) with great seasonal variation. These values are the same as the range measured in this flux study of 24 – 62 mg-N • m$^2$ • hr$^{-1}$, which excluded winter flux rates when feeding is greatly reduced (Haven & Morales-Alamo 1970).

**Total Nitrogen (TN) Exchange**

When the DIN, DON and PN fluxes from each substrate were combined, the intertidal flat was found to serve as a consistent source of nitrogen, while the oyster reef was found to store nitrogen in April and October and to release nitrogen over the summer. Low levels of TN release from the intertidal flat throughout the study period were consistent with results of other sediment-water column flux studies conducted at similar locations in the polyhaline portion of the Chesapeake Bay (Cowan & Boynton 1996, Reay et al. 1995). Constant release from the intertidal flat throughout the study period indicates that there must be an external source of nitrogen to this intertidal flat, likely from deposition of organic matter from the water column.

Although the oyster reef substrate had significantly higher NO$_2^-$, NO$_3^-$, NH$_4^+$ and DON releases in most sampled seasons than the adjacent intertidal flat, this high dissolved nitrogen release was counterbalanced by the oyster reef’s significantly higher particulate nitrogen consumption rates. Therefore, in April and October, when the reef’s chlorophyll-a uptake exceeded dissolved nitrogen release, the oyster reefs served as a net nitrogen sink. In June and August, particulate nitrogen uptake
did not exceed dissolved nitrogen release, thus during that period the reef served as a source of total nitrogen, however, TN flux was not significantly different from that of the intertidal flat. These results imply that net impact on nitrogen cycling from replacing intertidal flat with oyster reef at Fisherman’s Island is the creation of a potential reservoir for nitrogen storage in the spring and fall. The net seasonal impact of reef rehabilitation of nitrogen cycling, measured by subtracting the intertidal flat TN flux from the oyster reef TN flux, revealed that the construction of the Fisherman’s Island reef provided nitrogen storage potential of 2.25 and 1.75 mg-N · m² · hr⁻¹ in April and October, respectively, relative to the flats that they replaced.

**Phosphorus Processes**

During all seasons, DIP was released from both oyster reef and intertidal flat substrates and particulate phosphorus (calculated from chlorophyll-a flux) was taken up by both substrates. When dissolved inorganic and particulate phosphorus fluxes were combined, the intertidal flat was determined to be a net source for phosphorus throughout the study, whereas the reef was a sink in April and June and a source only in August and October. Unlike nitrogen, where 33% of ingested nitrogen has been found to be excreted in dissolved form to the water column by oysters (Hammen 1969, Kim 1983), only 8% of ingested phosphorus has been found to be excreted to the water column (Dame et al. 1989). Therefore, phosphorus release from both substrates in this study was either driven by organic matter remineralization or by changing redox potential within the sediments that caused release of PO₄³⁻ to the water column.
Dissolved and Particulate Phosphorus Exchanges

PO$_4^{3-}$ release from the intertidal flat was consistent throughout the study and followed similar patterns as those measured for other nearby flats (Cowan & Boynton 1996, Reay et al. 1995). However, fluxes measured from the intertidal flats at Fisherman’s Island were at least 10 times higher than those reported by Reay et al. (1995). As was the case with DIN release from the flats, this flux difference is likely driven by the high organic content of the intertidal flat sediments at Fisherman’s Island relative to those reported in Reay et al. (1995), as organic matter supply rates regulate the magnitude of remineralization (Cowan & Boynton 1996, Sloth et al. 1995).

PO$_4^{3-}$ fluxes from the oyster reef showed great seasonal variation with low levels of release in April and June and higher levels in August and October. In June, the oyster reef showed significantly lower release of PO$_4^{3-}$ than the adjacent flat although the organic content in reef sediments was much higher. In August, PO$_4^{3-}$ release was approximately 150 $\mu$M $\cdot$ m$^{-2}$ $\cdot$ hr$^{-1}$ higher than would have been expected from particle consumption and oyster energetics alone (Dame et al. 1989). This excess PO$_4^{3-}$ release may provide evidence of remineralization occurring in reef sediments during late-summer and is consistent with very high NH$_4^+$ release from the reef, which was also attributed to remineralization of biodeposits. October PO$_4^{3-}$ fluxes were consistent with levels that would be expected based on chlorophyll-a
consumption and indicate that remineralization rates had substantially slowed in reef sediments during the early fall.

PO$_4^{3-}$ was consistently removed from the water column in all seasons that, like results from NH$_4^+$ and DON uptake, likely indicated phytoplankton utilization of dissolved nutrients.

Results for particulate phosphorus exchange were in direct proportion to results for particulate nitrogen exchange since both were calculated using the Redfield (1958) ratio to convert chlorophyll-a flux. Particulate phosphorus uptake by the reef was significantly higher than uptake by the intertidal flat in April, August and October.

**Total Phosphorus Exchange**

Although DOP was not measured in this study, results from Dame et al. (1989) indicate that total phosphorus exchange for oyster reefs is closely related to particulate phosphorus uptake. Therefore, chlorophyll-a fluxes (converted to PP) measured by this study may serve as a suitable estimate of total phosphorus flux from the reef. However, I also calculated phosphorus exchange by combining dissolved inorganic phosphorus and particulate phosphorus fluxes (DIP+PP). DIP+PP indicated that the intertidal flat was determined to be a net source of phosphorus to the water column throughout the study. This is consistent with sediment-water column flux studies conducted at similar locations in the polyhaline portion of the
Chesapeake Bay (Cowan & Boynton 1996, Reay et al. 1995). Consistent phosphorus release from the intertidal flat indicates that there must have been an external source of phosphorus to this intertidal flat, again likely due to sedimentation of particles from the water column.

The oyster reef, however, was determined to be a net sink for phosphorus in April and June and became a source in August and October. This source of phosphorus in August was likely the result of remineralization of existing and newly produced biodeposits. In October, net phosphorus release from the reef was similar to DIP+PP release from the intertidal flat and may have come from similar sources, possibly deposition of water column particulates. Phosphorus exchange for the reef followed a similar seasonal pattern to that reported for oyster reefs in South Carolina (Dame et al. 1989) however, the magnitude of phosphorus storage in April and June at Fisherman’s Island was two times higher. This is consistent with the hypothesis that there was higher remineralization activity within reef sediments in South Carolina that caused higher release of dissolved inorganic nutrients.

This study does not attempt to quantify annual fluxes for Fisherman’s Island reefs because samples were not collected through the winter, when reefs are reported to store TN and TP (Dame et al. 1989). Therefore, annual fluxes based on this research would underestimate nitrogen and phosphorus storage on the Fisherman’s Island reefs. When averaged on an annual basis, South Carolina reefs had uptake rates for total nitrogen of 189 g N · m$^{-2}$ · yr$^{-1}$ and for total phosphorus of 98 g P · m$^{-2}$.
yr \(^{-1}\) (Dame et al. 1989). It is assumed that storage of nitrogen and phosphorus from a reef community at Fisherman’s Island would be higher based on seasonal comparisons of exchange rates and based on the structure of the reef and associated sediment biogeochemistry.

Net phosphorus data indicate that by replacing a section of intertidal flat with oyster reef, phosphorus storage is significantly increased in spring and early summer and phosphorus release is significantly higher in the late summer when remineralization of biodeposits occurs at a rapid rate. Phytoplankton at Fisherman’s Island have been shown to be limited primarily by nitrogen availability (Reay et al. 1993). In August, the reef increased the amount of DIP released into the system and would have contributed to increased phytoplankton production given adequate concentrations of nitrogen.

*Sediment Biogeochemistry*

Sediment characteristics, and presumably sediment biogeochemical processes, were significantly different between the two substrates. By enclosing the entire community (organisms and sediments) within the sampling chamber, nutrient fluxes reflected community fluxes that were a combination of biological and geochemical processes. The sediments within the oyster reef were significantly different from the sediments in the intertidal flat, as was expected based on the role of oysters in benthic-pelagic coupling. Oysters move substantial quantities of particulate organic and inorganic matter from the water column to the sediments both as feces and
pseudofeces (Haven & Morales-Alamo 1966 & 1970). These biodeposits are characteristically high in nutrients (Dame et al. 1984, Kautsky & Evans 1987). Reef sediments were predictably higher in organic content, %TN and %TOC than the adjacent intertidal flat. The ratio of carbon to nitrogen was not significantly different between the two substrates suggesting that the quality of organic matter is similar from reef to intertidal flat but that the quantity of organic matter available to deposit feeders is greater in the reef. This supports the concept that the reef plays a more important role in benthic-pelagic coupling than the intertidal flat. Bulk density was lower and % water was higher in oyster reef sediments than in intertidal flat sediments (Table 2.4), which is characteristic of sediments rich in biodeposit material (Kautsky & Evans 1987) and indicated that the sediments on the reef were finer than sediments on the intertidal flat.

Remineralization within the sediments is largely driven by organic matter content (Cowan & Boynton 1996, Sloth et al. 1995). The oyster reef had higher concentrations of extractable NH$_4^+$ and PO$_4^{3-}$, which are the main bi-products of remineralization, particularly in seasons when remineralization rates are typically high (Hayakawa et al. 1999, Valiela 1995). By August, for example, remineralization was indicated in reef sediments with a 26% decline in sediment organic content from June to August, despite high consumption of phytoplankton, concurrent with a 60% summer increase in sediment NH$_4^+$ levels. Due to high concentrations within the sediment, these extractable nutrients diffuse though the sediment-water interface, where they may be intercepted and utilized by benthic
primary producers (Newell et al. 1999). The higher sediment chlorophyll levels in reef sediments suggested that this process was enhanced on the reef relative to the intertidal flat. Higher sediment phaeophytin concentrations in the reef indicated chlorophyll degradation, possibly from incomplete digestion of both benthic diatoms (by deposit feeders) and pelagic phytoplankton (by suspension feeders), as well as bacterial remineralization.

Sediment characteristics provided information from which to speculate on chemical reactions within sediments in these two communities. Surface sediments (0-2 cm) from both habitats must have been oxygenated, based on the presence of \( \text{NO}_3^- \) and \( \text{NO}_2^- \) in the sediments of both, and this suggests that nitrification was occurring. In the absence of appropriate anoxic conditions for denitrification, there should have been a release of \( \text{NO}_2^- \) and \( \text{NO}_3^- \) from the sediments. Release of these oxidized forms was minimal from the intertidal flat and was high from the reef, however, oyster excretion and nitrification within the oyster body complicate this relationship. Release of \( \text{NH}_4^+ \) and nitrogen gas (the latter was not measured) from the sediments would have indicated that denitrification processes were occurring in the sediments as these are the end-products of coupled nitrification-denitrification. Both communities did release \( \text{NH}_4 \), however, this was also the primary component of organism excretion, thus results were confounded.

Denitrification may be estimated by calculating a ratio of the flux of \( \text{NH}_4^+ \) to the flux of \( \text{PO}_4^{3-} \) (LaMontagne et al. 2002). Low N:P values may indicate
denitrification because DIN is depleted relative to DIP through the denitrification process. Ratios from the intertidal flat for April, June, August and October were 2.9, 6.9, 9.9 and 7.4, and ratios from the oyster reef were 4.8, 46.1, 6.9 and 6.2 respectively. Comparison of these ratios between the two communities can be made only with the recognition that reef ratios are very conservative, since oyster excretion was responsible for a large proportion of the NH$_4^+$ flux. In August and October, this ratio comparison supports the conclusion by Newell et al. (1999) that states that the oyster reef enhances denitrification processes, more so than the intertidal flat.

**Rate of Exchange Processes**

While the magnitude and direction of net nutrient exchange is important to understanding the reef’s effect on water quality, so too is the rate of exchange (Cerco 2002, pers. com.). Overall, nutrient cycling was much faster for the reef than for the intertidal flat, with faster phytoplankton uptake rates, faster dissolved nutrients release rates and faster decomposition of organic matter. Benthic-pelagic coupling on the reef therefore provided higher quantities of organic matter to benthic deposit feeders than did the adjacent intertidal flat and had the potential to support a larger deposit-feeding community. Faster grazing rates on the reef also contributed more phaeopigment to the sediment and higher sediment inorganic nutrient levels which resulted from high rates of remineralization that supported increased concentration of benthic microalgae, both of which serve as food sources to deposit feeders (Bock & Miller 1995).
In 1987, the Chesapeake Bay Program defined a goal of reducing nutrient loads by 40% in order to decrease eutrophication-induced hypoxia in the Bay, increase water clarity and increase productivity at higher trophic levels (1987 Chesapeake Bay Agreement). Model simulations run with the Chesapeake Bay CH3D-WES Hydrodynamic model (Cerco 1995) showed that the reduction of nutrient levels by 40% (bottom-up control on primary production (Valiela 1995)) projected minimal decrease of hypoxia, did not result in significant changes in water clarity, and actually decreased productivity at higher trophic levels (Cerco, pers. com.). Instead, model simulations run with increased density of grazers indicated that top-down control (Valiela 1995) may be more tightly linked with decreased phytoplankton standing stock and increased productivity at higher trophic levels (Cerco, pers. com., Nixon 1988). When grazing levels are low, nutrients are biologically bound in high phytoplankton biomass, thus phytoplankton production is low (Figure 2.12). Intertidal flat nutrient cycling rates at Fisherman’s Island would fall close to the left-hand side in this diagram with low grazing levels, high phytoplankton biomass and low system primary productivity. With low community grazing rates, phytoplankton biomass is not transferred to production at higher trophic levels. As grazing levels increase, phytoplankton biomass is cropped and transferred up the food chain, dissolved nutrients are liberated from phytoplankton through consumer excretion, and phytoplankton productivity increases due to the increased dissolved nutrient reservoir. Oyster reef grazing rates at Fisherman’s Island were considerably higher than the intertidal flat grazing rates and would fall further to the right on the x-axis of the diagram. To increase productivity at higher
Figure 2.12 Nutrient cycling as a function of grazing rate. (a) At low levels of grazing, nutrients are biologically bound in phytoplankton biomass. As grazing increases, grazers excrete dissolved nutrients rendering them available to fuel phytoplankton production. (b) At some point along the continuum of grazing in diagram (a), phytoplankton productivity is at a maximum. Figure from Cerco (pers. com)
trophic levels, the goal should be to increase grazers in the bay to the point along the grazing continuum where phytoplankton productivity is at a maximum, while grazing pressure controls phytoplankton biomass. This concept is supported by research that found that decreased mussel grazing caused a decrease in phytoplankton productivity and a decrease in overall system productivity at higher trophic levels (Herman & Scholten 1990).

Figure 2.12 may help to explain the inconsistency between researchers who concluded that bivalve grazing supports a positive-feedback loop with greater productivity and little reduction in phytoplankton standing stock (Dame 1999, Dame et al. 1984, Dame & Libes 1993), and those who concluded that bivalve grazing is a negative-feedback loop with decreased phytoplankton standing stock resulting from increased grazing (Cloern 1982, Cohen et al. 1984, Dame 1996, Dame et al. 1991, Herman & Scholten 1990, Hily 1991, Riemann et al. 1988, Officer et al. 1982, Smaal & Zurburg 1997). The former group is likely conducting studies on systems with grazing pressure that is to the left of the peak in phytoplankton productivity, while the latter are conducting studies on systems with grazing pressure that is at or to the right of the peak in phytoplankton productivity.

**Link to Seagrass Habitat & Potential Recolonization**

As this study demonstrates, the oyster reef stored nitrogen in April and October, whereas the intertidal flat gradually released nitrogen throughout the sampling period. Therefore, by replacing intertidal flat with oyster reef, the net effect
would be to store nitrogen in the spring and fall. Since water column nitrogen levels have been shown to limit the growth of phytoplankton at Fisherman’s Island, (Reay et al. 1995), the nitrogen that is retained by the oyster reef is therefore rendered temporarily inaccessible to pelagic phytoplankton and should lead to a reduction in phytoplankton standing stock and increased water clarity. At Fisherman’s Island, phytoplankton comprise approximately 15% of $K_d$ (Moore, unpublished data from 1998-1999, converted with equation in Bannister 1974).

*Zostera marina* has two critical growth periods: Spring (March-May) and Fall (September-November) (Batiuk et al. 2000, Dennison et al. 1993, Moore et al. 1996). In spring, eelgrass primary production is channeled into generating seeds (Silberhorn et al. 1983) and building reserves to support the plant through the summer (Burke et al. 1996) when compensating light requirements are high in the Chesapeake Bay (Moore et al. 1997). In fall, eelgrass primary production contributes to vegetative reproduction that increases grass bed size the following spring (Orth & Moore 1983). Light availability is regarded as the most important determinant for seagrass survival and successful recolonization on shallow areas in the Chesapeake Bay during these periods (Moore et al. 1996, Moore & Wetzel 2000). By storing nitrogen in spring and fall, oyster reefs regulate phytoplankton abundance and have the potential to increase water clarity during these critical growth periods for *Z. marina*. A decrease in $K_d$ of 15% from oyster reef control of phytoplankton biomass will result in up to a 15% increase in the depth at which eelgrass can survive, photosynthesize, reproduce and potentially recolonize shoal areas (according to Beer-Lambert equation). Reefs
also increase water clarity by facilitating deposition of suspended inorganic material (Haven & Morales-Alamo 1966, Kautsky & Evans 1987) although this process was not measured in this study.

Over the summer, oyster reefs here released nitrogen to the water column, although not at a significantly higher rate than did the intertidal flat. Release of dissolved nitrogen over the summer has the potential to stimulate phytoplankton production as well as increase epiphytic loads on adjacent seagrass shoots (Moore & Wetzel 2000, Valiela 1995). This relationship is very complex, as summer mesograzer populations can potentially control high phytoplankton productivity in the water column and increased epiphytic growth on grass blades (Neckles et al. 1993, Moore & Wetzel 2000).

Conclusions from this study that extrapolate to the system level are speculative as changes in water clarity will depend heavily on water residence time and on the ratio of water volume to bivalve biomass and reef area (Officer et al. 1982). This study serves as a valuable first step is estimating the potential for rehabilitated oyster reefs to influence habitat conditions for seagrasses on a local scale. Inside the flux chambers, oyster reef area was adequate relative to water volume to exert top-down control on phytoplankton biomass in spring and fall, and possibly would have been adequate in June and August had the experiment been run for a longer period of time. Given adequate reef area, the oyster population would likely have been capable of exerting top-down control on phytoplankton biomass and
reducing $K_d$ in the creek at Fisherman’s Island, as has been found in other regions (Cloern 1982, Cohen et al. 1984, Dame 1996, Dame et al. 1991, Herman & Scholten 1990, Hily 1991, Riemann et al. 1988, Officer et al. 1982, Smaal & Zurburg 1997). A worthy goal for future research in this area should be the determination of the critical ratio of oyster reef area relative to creek volume that would allow reefs to exert top-down control on phytoplankton biomass.

**CONCLUSIONS**

This study demonstrates that the rehabilitation of oyster reefs at Fisherman’s Island does influence phytoplankton grazing and nutrient cycling in the water column. The oyster reefs have high grazing rates and high dissolved nutrient release rates relative to the intertidal flat habitat that their construction replaced. These faster cycling rates translate into increased benthic-pelagic coupling on reef substrate with increased secondary production for oysters and other reef dwelling organisms. This potentially contributes to top-down control of phytoplankton standing stocks in the water column. In spring and fall, oyster reefs serve as a temporary storage mechanism in the creek at Fisherman’s Island. This storage function renders nitrogen unavailable to phytoplankton production and, given sufficient reef area and oyster biomass, has the potential to increase water clarity seasonally.
LITERATURE CITED


THESIS SUMMARY

The undertaking and the science of oyster reef restoration in the Chesapeake Bay are in their infancy. Shell mound construction began in 1994 (Wesson et al. 1999) and oyster broodstock transplants began in recruitment-limited areas in 1996 (Brumbaugh et al. 2000, Southworth & Mann 1998). This research was conducted in the years 2000 and 2001 (4-5 years into the restocking effort) and therefore documents early progress in reaching the goals of (1) revitalizing the oyster population and (2) recovery of reef ecosystem function, specifically water filtration. The studies presented in this thesis provide necessary components in evaluating the intermediate progress toward attaining the goals of oyster reef restoration and results allow refinement of restoration techniques in process.

The decrease in bivalve suspension feeder populations in estuaries is a difficult trend to reverse because these systems tend to exhibit threshold effects (Dame et al. 2001). This suggests that revitalization of the oyster population in the Chesapeake Bay may be a slow process and that definitive progress may be difficult to detect in the beginning stages. Restocking efforts may need to be concentrated regionally in order to overcome threshold effects. In 2000, the state of Virginia, along with Maryland, Pennsylvania and the District of Columbia, made a ten-year commitment to oyster reef restoration (Chesapeake 2000 Agreement), with the recognition that the recovery of a Chesapeake Bay oyster population would require dedication on the order of decades.
Historically, overharvesting was the primary cause for the decline of the oyster population and disease-related mortality followed (Rothschild et al. 1994). In effort to revitalize the oyster population, harvest restrictions are in place throughout Virginia and reefs are designated as sanctuaries. Disease pressure currently seems to be the largest impediment to population rebound. Results from this study are encouraging because they document that oysters, *Crassostrea virginica*, transplanted onto reefs in the Lafayette River did indeed demonstrate survival and egg production through their second spawning season, despite two consecutive years of extreme disease pressure (Calvo & Burreson 2001 & 2002). In this study, 25-50% of the transplanted oysters survived and reproduced through two years, which demonstrates that disease resistant genes are present in the *C. virginica* broodstock (Mark Camera, pers. com.). Results indicate that reproductive performance of these two stocks differs based on pressure from dermo or MSX. This provides a simple criterion for the selection broodstock stock, which offers refinement to restoration techniques and increases the potential success of revitalizing the oyster population in the Chesapeake Bay.

This study measured potential reproduction by quantifying egg production and did not measure fertilization success, settlement to the reefs, or disease resistance in offspring. Each of these studies will be necessary in the future for thorough evaluation of these stocks as broodstock. Future research should also include similar studies conducted in various salinity regimes in the Chesapeake Bay to ensure that reproductive performance trends are consistent for euryhaline, mesohaline and
Oligohaline regions of the bay. Studies should also track reproductive potential from additional oyster stocks. Development of a Leslie Matrix model based on these fecundity and survival data would be useful in forecasting population development resulting from transplant of these two stocks, and could be used to manipulate numbers of oysters transplanted to define targets for overcoming threshold effects and initiating population development.

Subsequent to the development of oyster populations on reefs, the detection of water quality changes due to reef filtration may be difficult and may be gradually re-established. In the early stages of reef restoration, when the oyster population and reef-associated fauna are beginning to develop, increased grazing pressure should increase release of dissolved nutrients and increase primary productivity (Figure 2.12), but may not cause changes in water clarity (Dame & Libes 1993). As filter-feeding populations on the reef continue to develop and grazing pressure increases, at some point, there should be a shift toward top-down control on phytoplankton standing stocks that would favor improvement in water clarity.

In the short term, the potential for the reefs at Fisherman's Island to impact water quality can be estimated by measuring nitrogen cycling, since phytoplankton have been found to be limited by nitrogen availability in the region (Reay et al. 1993). Therefore, removal of nitrogen from the water column should lead to reduced phytoplankton standing stocks in the region. This study concludes that oyster reefs at Fisherman's Island serve as a nitrogen retention mechanism in spring and fall, and
may have the potential to improve water clarity during those seasons, but reefs release nitrogen over the summer. Some argue that oyster harvest during spring would provide a longer term removal mechanism for nitrogen. This concept is supported in the literature with nitrogen removal via the harvest of young, rapidly growing clams (Rice et al. 2000). However, by harvesting oysters from the sanctuary reefs at this point, the ecological benefits associated with oysters and oyster reef restoration would be lost along with the potential for a future oyster population. For long-term removal of nitrogen from the system via harvest, sanctuary reefs must have time to develop viable broodstock populations that can ultimately serve as a source of larvae to the waterway. Sanctuary reef populations will then consistently supply offspring to surrounding areas that will store nitrogen and export it upon harvest.

Nitrogen removal is a tool for estimating the potential for oyster reefs to improve water clarity, however; long-term monitoring of river-wide changes in light attenuation in areas with restored oyster reefs will ultimately be the best measure. Landscape-level experiments conducted in small waterways with stocked sanctuary reefs adjacent to seagrass restoration plots may help to determine the benefits of, and potential for multi-habitat restoration. Smaller scale mesocosm experiments may be useful in the short-term. Research that manipulates oyster density inside in situ chambers may allow determination of the necessary grazer to water volume ratio that causes the negative feedback loop. This would help to define restoration population targets based on river or sub-estuary water volume and would be a useful tool in identifying restoration success.
LITERATURE CITED


Chesapeake 2000 Agreement. 2000. Chesapeake Bay Program. www.chesapeakebay.net/c2k.htm


VITA

LAURIE ANN SORABELLA

Born in New York, New York on June 13, 1972 and raised in Ridgewood, New Jersey. In 1994, Laurie obtained a B.A. in Women’s Studies from Wesleyan University in Connecticut. After college, she was hired by the Chesapeake Bay Foundation to be the Education Manager on Great Fox Island and was later promoted to Virginia Habitat Restoration Coordinator where she organized and implemented oyster restoration, underwater bay grass restoration, and American Shad restoration projects with schools throughout Virginia. In 1999, Laurie began her graduate work at the Virginia Institute of Marine Science and she continued coordinating the student oyster restoration initiative in Virginia. Laurie is currently in the process of starting Oyster Reef Keepers of Virginia, Inc., a non-profit organization formed to restore and maintain oyster reefs in Virginia’s waterways through community-based restoration, public education, advocacy, monitoring and scientific research.