ABSTRACT

Title of Thesis: EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) GROWTH AND EPIFAUNAL COMMUNITY DEVELOPMENT ON BARS OF VARYING OYSTER DENSITY IN CHESAPEAKE BAY

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The eastern oyster, *Crassostrea virginica,* is a gregarious, reef-forming organism. Oyster populations that once dominated the Chesapeake estuary have declined significantly and interest has recently arisen to restore the economic and ecological benefits of native oyster populations. Understanding the ecological importance of oysters and oyster reefs is critical to the restoration of the estuary's ecosystem as a whole. Oyster densities on most Maryland reefs are very low, however, natural reefs formed in other areas are comprised of high densities of oysters. In order to maximize the effectiveness of oyster restoration, it is important to determine how oyster density may affect oyster growth, parasite prevalence and the formation of reef habitat utilized by the benthic community.

In the fall of 1999, twelve 0.2-acre experimental plots were constructed in the Patuxent River, a tributary of the Chesapeake Bay, by placing fossil oyster shell on a barren natural oyster bar. The plots were assigned one of four treatments, zero, 124, 247, 494 oysters/m^2, in a completely randomized design. Oyster growth was 0.117 (± 0.0037 SEM) mm/day for the 2000 season and slowed to 0.067 (± 0.0061) mm/day in 2001. Throughout the study, oyster growth was independent of the density of oysters observed. Colonization of the oyster reefs with fouling organisms was correlated to the density of oysters.

EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) GROWTH AND EPIFAUNAL COMMUNITY DEVELOPMENT ON BARS OF VARYING OYSTER DENSITY IN CHESAPEAKE BAY

by

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Introduction

Decline of the Chesapeake Estuary

Estuaries such as the Chesapeake Bay are among the most degraded marine systems in the world (Jackson et al 2001). The watershed of the Chesapeake Bay has changed dramatically since 1607, when Captain John Smith ventured into its waters. At that time, dense beds of underwater grasses reached from the estuarine shoreline to expansive oyster reefs, which could be found along the main channel of the estuary and throughout many of its tributaries. Historically, oyster reefs followed the edges of the deep channels of the estuary, at times posing a hazard to ships that ventured from the safety of deep water, yet oyster reefs were more than an obstacle in John Smith's Chesapeake Bay. The oysters that colonized these reefs were a keystone species in the estuary. The filter feeding activity of oysters dominated the ecology of the Chesapeake by removing massive amounts of phytoplankton from the water column. Oyster reefs also provided habitat for numerous species of fish and invertebrates. In these ways, oysters linked benthic and pelagic food webs (Newell 1988, Kennedy 1989). The intensive harvest of oysters that would come with the colonization of the watershed would forever change the balance of the estuarine ecosystem. The productivity of the estuary would no longer be controlled by dense populations of filter feeders, but rather would be dominated by overabundant phytoplankton. This transformation would ultimately lead to expensive financial commitments from federal, state and private groups with an interest in restoring not only the oyster, but also the health of the Chesapeake estuary.

Upon arrival, English settlers started to clear the landscape of the watershed to produce lumber and grow crops. By the 19th century, the watershed had changed significantly, with 50% of the land cleared for agriculture (Cooper 1995). The amount of cleared land

increased to 80% of the watershed by the end of the 19th century. This was accompanied by increased surface runoff of water, which carried with it an increased sediment load (Cooper and Brush 1993, Cooper 1995). The suspended sediment from runoff reduced water clarity and the ability of sunlight to penetrate into the water column. This increased light attenuation and reduced the abundance of underwater grasses in deeper water. During the rapid land conversion and high sedimentation of the mid 18th and early 19th centuries, underwater grasses in the Bay declined rapidly (Davis 1985). Although sedimentation rates declined during the 20th century, they were still four times greater than pre-settlement rates (Davis 1985, Cooper 1995, Jackson 2001). In addition to increasing sedimentation, the runoff that flowed from the degraded watershed carried with it commercial fertilizers, which were becoming more widely used in the 20th century (Cooper 1995). The increase in nutrients led to an increase in primary productivity, especially the growth of planktonic algae. This further contributed to the turbidity of the water, reducing the penetration of light into the water column, increasing growth of periphyton on the leaf surfaces (Orth and Moore 1983), and exacerbating the decline of underwater grasses. The cumulative effect was continued declines in underwater grass habitat (Valentine and Stevenson 1981).

The transformation of the watershed from forested to agricultural land use accelerated the nutrient enrichment (eutrophication) of the estuary. In addition to enhancing primary production and turbidity, phytoplanktonic species composition changed, and the coupling of benthic and pelagic systems was diminished (Kemp and Boynton 1992). Sediment cores have documented a shift in diatom composition since European settlement indicating a shift from a benthic pelagic system to a pelagic dominated system (Brush and

Davis 1984, Davis 1985, Cooper 1995). In a balanced system, grazers such as oysters consume this additional phytoplankton growth. Without adequate grazing pressure, bacteria degrade unconsumed phytoplankton. Bacterial decomposition of phytoplankton consumes oxygen and may lead to hypoxia, anoxia or both. In a well-oxygenated system, nitrogen inputs are transformed to nitrogen gas through the nitrification-denitrification process; but anoxic conditions inhibit this microbial activity, resulting in less nitrogen gas released to the atmosphere and more nitrogen recycled and available for primary producers (Kemp and Boynton 1992, Newell and Cornwell 2000). Without adequate grazing of phytoplankton, an estuarine ecosystem becomes prone to anoxia, leading to increased nutrient recycling and increased phytoplankton production (Officer et al 1984, Nixon 1995).

In the Chesapeake estuary, over-harvesting of oysters led to a reduced capacity of the system to remove the byproducts of nutrient enrichment, over abundant phytoplankton (Newell 1988). Increased nutrient enrichment led to a system in which productivity was no longer controlled by climatic factors, but rather was largely dominated by anthropogenic nutrient inputs (Cooper 1995). Nutrients fueled water column primary production unimpeded by the moderating influence of benthic filter feeding. Restoration of dense populations of oysters to the Chesapeake estuary would aid in reducing the eutrophication of the system, as oysters would be able to consume much of this excess phytoplankton.

Oyster Reef Structure

Oysters naturally form dense aggregations and were found in high density throughout the Chesapeake prior to European settlement (Alford 1973, Bahr 1976, Kennedy 1989,

Hargis 1999). Maps from surveys completed by Winslow and Yates in the late 19th and early 20th century show multiple large oyster bars following the edges of the estuary's deep channels and dominating much of its shallow portions (US Coast and Geodetic survey 1913). These reefs were referred to as 'fringing', and generally formed on the shoulders of channels (Stevenson 1894, Hargis, 1999). Similar to fringing coral reefs, the oyster reefs of the Chesapeake estuary were narrow and dropped off sharply to the channel, as fringing coral reefs tend to drop sharply on the seaward side (Davis 1928).

Oyster reefs were the primary three-dimensional hard substrate for benthic organisms in the soft-sediment dominated environment of the Chesapeake (Wells 1961, Newell 1988, Kennedy 1989, McCormick-Ray 1998). Oysters themselves are the ecosystem engineers that develop reefs slowly over time (Bahr 1976, Lenihan and Peterson 1998, Hargis 1999). It took thousands of years for Chesapeake oyster reefs to develop and shape the ecosystem (Mann 2000). These reefs provide habitat for numerous species of fish and invertebrates. Complexity and species diversity of organisms on reefs increases as reef size increases, with numerous species on reefs that are absent from the surrounding barren bottom (Lenihan and Peterson 1998, Coen and Luckenbach 2000, Breitburg et al 2000, Harding and Mann 2001). Fish species associated with oyster reefs have been characterized into reef resident, facultative resident and transient (Breitburg 1999). Resident fish rely on the structure of the reef itself for nesting and feeding (Breitburg 1999, Coen and Luckenbach 2000), whereas transient fish exploit a much broader geographic range (Coen et al 1999).

In order for reefs to support diverse communities, they must provide refuge from predation (Korringa 1952). This is done through the creation and maintenance of interstitial space by the growth and settlement of high densities of oysters (O'Beirn et al 2000, Lenihan and Peterson 1998). In a study of a restored reef in Virginia, spat and juvenile oysters were protected from predation within the interstitial space of the reef (Bartol and Mann 1997). Prey species of decapods and fish have also been shown to move into the structure of oyster reefs in the presence of potential predators (Posey et al 1999, Coen and Luckenbach 2000).

The massive size of reefs in the Chesapeake estuary altered the flow of water (McCormick-Ray 1998, Kennedy and Sanford 1999). This in turn increased the delivery of food to the reef by increasing turbulence and reducing the benthic boundary layer (Dame 1996, Lenihan 1999). Settlement of larvae has also been shown to increase with reef size (Breitburg et al 1995), and as reefs continue to grow, they become more stable due to the recruitment of oysters and epifaunal organisms (Kennedy and Sanford 1999). These large structures produce more larvae as oysters in high density and close proximity typically have increased fertilization success (Levitan 1991, Pavlos & Paynter 2001). This enhances the ability of the reef to alter water flow, allowing the reef to support greater growth, higher abundances of oysters or both (Butman et al 1994).

In tropical systems, coral reefs provide complex habitat, with varying microhabitats allowing for the coexistence of diverse species of fish (Sale 1977, Hixon and Beets 1993). Reef holes, a larger and more complex version of interstitial space within an oyster reef, provide the necessary protection for prey species of fish (Hixon and Beets

1993). On a smaller scale, oyster reefs may have performed a similar function to coral reefs, providing refuge for resident species of fish. Species of coral reef fish respond quickly to removal of competitors by predation or other causes, by occupying vacant habitat that remains intact (Sale 1977, Stone 1995). Complications arise when removal of fish is accompanied by destruction of the reef structure. If fish species are not able to re-colonize, due to limited habitat, species richness and diversity begin to decline. This decline continues if only a few protected areas remain, with the local extinction of species following soon after (Stone 1995). A similar phenomenon may have occurred in the Chesapeake estuary, with diversity of oyster reef fish species declining due to the destruction of oyster reef habitat.

Given the historical abundance of oyster reefs in the Chesapeake estuary combined with the habitat and filtering contributions of reefs and oysters to estuarine ecosystems, the significance of oyster reefs to the estuary is apparent. The balance achieved through thousands of years of reef development was evident in that oysters did not overwhelm the entire estuary, but instead formed distinctive reefs, which charted its deep channels. As with the destruction of coral habitats, the destruction of oyster reef structures reduced species diversity and removed a vital link from the estuary, altering the balance of the ecosystem.

Oyster Fishery

Indigenous people of the Chesapeake estuary likely exploited intertidal and other easily accessible oysters for thousands of years, yet this sustainable harvest expanded significantly within two hundred years of European colonization (Brooks 1891, Stevenson 1894, Hargis 1999). Historically, the oyster harvest from the Chesapeake was

the most valuable fishery of the mid-Atlantic region (Alford 1973, Kennedy 1989, Mann 2000) with peak harvests occurring in the late 19th century, and subsequent harvests declining throughout the 20th century (Brooks 1891, Stevenson 1894, Alford 1973). Destructive harvesting gear, intense fishing pressure and inadequate management of the Bay's oyster fishery combined with the effects of disease and reduced water quality ensured the decline of the oyster fishery (Gross and Smyth 1946, Kennedy and Breisch 1983, Kennedy 1989, Rothschild et al 1994, Lenihan and Peterson 1998, Mann 2000).

Historical oyster harvesting has been described as destructive, in that clumps of the reef structure are broken and all sizes of oysters are harvested. The use of dredges in the Chesapeake estuary started in the early part of the 19th century (Brooks, 1891, Stevenson 1894). Vessels dredged for oysters primarily in the lower portion of the estuary and transported their catch to New England markets. Due to the perception that the dredging of oysters would decimate the oyster population of the estuary, a law was enacted in 1820 prohibiting the use of dredges to catch oysters (Stevenson 1894, Coen and Luckenbach 2000). The law was short lived, as by 1865, it was repealed and a licensing system was put into place (Stevenson 1894).

The licensing system in Maryland allowed residents to use dredges, scrapes and hand tongs to catch oysters. Dredges and scrapes are similar, in that both are metal implements towed behind a vessel while underway. Dredges are typically larger, weighing up to 100 lbs., and are usually towed in pairs; while scrapes are smaller and are typically towed behind smaller vessels. The use of dredges and scrapes allowed the fishery to utilize more efficient gear in the catching of oysters. Due to the increased

efficiency of dredges and scrapes, the law permitted the use of this gear exclusively in the Chesapeake proper and only under sail power. In spite of this attempt to manage the harvest of oysters, overharvesting of the estuaries most valuable resource continued.

The low demand for oysters in the Chesapeake region combined with the prohibition on dredging from 1820 to 1865 allowed for the expansion of the local hand tong fishery. Hand tongs, which had been used for over half a century in the Chesapeake, were made from two rake-like metal heads attached to long wooden shafts. A waterman would articulate the shafts to gather oysters from shallower (<24') areas of the estuary. Late in the 19th century, deep water tongs, a precursor to today's patent tongs, were developed to catch oysters in water too deep for hand tongs.

Yet possibly more destructive to oysters than the harvesting gear used in the Chesapeake oyster fishery was the ineffective management of the resource. The Chesapeake's oyster resource has long been considered a common property (Alford 1973). The lack of conservation that accompanies common property resources led to the over exploitation of the oyster fishery (Brooks 1891, Hardin 1968, Alford 1973).

Oyster harvesting in the 19th century may have increased the productivity of bars, by dispersing oysters and allowing them to grow more quickly without the intense competition for food and space (Stevenson 1894); however, continued destruction of habitat and the by-catch of undersized oysters led to irreparable damage to the reef structure (Korringa 1952, Kennedy 1989). Oyster reefs were flattened, altering not only the shape of reefs but also their productivity (Mann 2000, Coen and Luckenbach 2000).

Winslow and Brooks (1891) described harvested reefs as containing muddy patches within the structure of the reef itself. Due to the apparent destruction of oyster reefs by harvesting and the accompanying drop in the oyster fishery, several surveys were undertaken (Brooks 1891). A survey by Brooks in 1892-1893 showed a relative drop of ~50% in the abundance of oysters at some locations compared to a similar survey by Winslow five years earlier (Brooks 1891).

In the late 19th century, watermen harvested 12-15 million bushels per year from Maryland's portion of the Bay (Brooks 1891, Alford 1973, Kennedy 1989). Prior to the turn of the century, conservation measures for the oyster population were recommended (Stevenson 1894, Brooks 1891, Hargis 1999). Kennedy and Breisch (1983) summarize the various management strategies recommended throughout the past 160 years, many of which were ignored.

As Maryland's oyster fishery entered the 20th century, a decline in the oyster harvest and a history of mismanagement of the oyster fishery set the tone for the future. By 1905, Maryland's annual harvest of oysters dropped to approximately five million bushels. In the 1920's shells from harvested oysters were returned to the estuary in an attempt to increase the production of oysters. From the 1930s to the mid 1980s, the harvest of oysters was relatively stable at two to three million bushels per year (Kennedy and Breisch 1983, Alford 1973). In the 1960s, the repletion program of the Maryland Department of Natural Resources (MDNR) was initiated to increase the harvest of oysters from the estuary.

In the eight years from 1987 to 1994, Maryland's oyster industry once again declined dramatically. This decline was most likely due to disease, (Kennedy 1989, MacKensie 1996), but more than a century of habitat destruction and over harvesting could not be ignored. The harvest of oysters from the Maryland portion of the Bay was a mere eighty thousand bushels for the 1993 – 1994 season, (MDNR 2001) a shadow of the fifteen million harvested over a century before.

Disease

Although the decline of oysters in Chesapeake Bay is known to be largely the result of overharvesting (Brooks 1891, Alford 1973, MacKenzie 1996, Hargis and Haven 1999), the recent drop in harvest has been attributed to disease (Kennedy 1989, Ulanowicz and Tuttle 1992). Drought conditions in the mid 1980s accelerated the intensity of the protozoan parasite *Perkinsus marinus* throughout the Chesapeake (Burreson and Andrews 1988). It is likely that the combination of disease and poor water exacerbated the recent decline of the oyster population (Rothschild et al 1994).

P. marinus, first described as *Dermocystidium marinum* (Mackin, Owen, Collier 1950), favors salinities of 20 to 30ppt (Ray and Chandler 1955) although it is found in oyster beds throughout the Chesapeake estuary (Andrews and Ray 1988). Studies in the 1950s by Ray and Chandler (1955) and in the 1960s by Andrews (1965) demonstrated that *P. marinus* is spread to other oysters by proximity. The intensity and resultant mortality of oysters from *P. marinus* infection was related to the initial number of infected animals for proximity studies. The distance between infected and uninfected animals was also found to be important (Ray and Chandler 1955, Andrews 1988). Other studies have shown that disease free spat grown in low salinity areas will not acquire infection, or will acquire

low levels of infection throughout their growth to market size (Paynter and Burreson 1991, Paynter 1999).

P. marinus infections typically increased during the warm summer months, especially in high salinity waters (Ray and Chandler 1955, Andrews and Hewatt 1957). Management practices to reduce the transmission of *P. marinus* to other stocks have been developed (Andrews and Ray 1988, Ford 1992, Paynter 1999). The following recommendations for the management of *P. marinus* in oyster populations were made: plant only disease free seed, maintain 0.4 km between bars, allow bars to go fallow between crops, monitor disease levels in summer and fall, and harvest diseased oysters early, before large mortalities occur (Andrews and Ray 1988).

In addition to the management advice noted above, scientists found that low salinity areas, those less than 10ppt, often reduce the virulence of disease in oysters, leading to greater survival (Ragone and Burreson 1990, 1993). Areas with salinities from 6 to 9 ppt had more negative and light infections, while higher salinity areas, 12 and 20 ppt, had more medium and heavy infections. Mortality was greater in high salinity areas and oysters transferred from low to high salinity suffered mortality more quickly than oysters that remained in low salinity.

Failure of managers to follow the previously mentioned recommendations combined with periods of drought has allowed *P. marinus* to spread throughout the estuary to all areas where oysters are found (Andrews 1988, Ragone and Burreson 1993). Although oysters could be grown to market size by transplanting seed from high salinity, high disease areas

to low salinity grow out areas; this annual importation of parasites established a system for the maintenance of low levels of *P. marinus* in low salinity grow-out areas (Paynter 1999).

Restoration of Oysters in the Ecosystem

Oysters did more than provide a commercial fishery for the Chesapeake estuary. Bivalve filter feeders link the benthic and pelagic food webs through the feeding process (Newell 1988, Kennedy 1989, Dame 1999, Mann 2000). As filter feeders, oysters remove suspended particles from the water column and consume phytoplankton. Oysters aggregate and excrete the remaining particles, which are used by other reef inhabitants. Through this filtering process, oysters have the potential to control phytoplankton populations.

Numerous examples exist of dense populations of filter feeders controlling phytoplankton production through grazing (Dame 1996, Rheault and Rice 1996, Coen and Luckenbach 2000). Oysters and other bivalve filter feeders that form dense assemblages are important to the nutrient cycling in estuarine and coastal systems (Dame 1999, Newell et. al. 2002). Specifically, oysters have been shown to utilize up to 70% of organic filtrate (Newell 1988, Coen and Luckenbach 2000). Oysters control phytoplankton biomass through predation, or grazing. Sediment and inorganic particles removed from the water column, but not digested, are expelled as pseudofeces (Korringa 1952). Under oxidized conditions, the nitrification-denitrification process is enhanced leading to reduced recycling of nutrients to fuel primary productivity (Kemp and Boynton 1992, Newell and Cornwell 2000, Newell et. al. 2002). As a result, restoration of dense populations of oysters in estuarine ecosystems will reduce phytoplankton productivity (Ulanowicz and

Tuttle 1992, Newell and Cornwell 2000). The Chesapeake estuary often experiences periods of anoxia, especially during the summer months (Officer et al 1984, Malone 1992). Under these anoxic conditions, nutrient rich material is quickly remineralized and used to fuel primary production (Kemp and Boynton 1992, Newell and Cornwell 2000). This leads to further eutrophication of the estuary. Through the filtering process and due to the potential for rapid recycling of nutrients, oysters are able to support both negative and positive feedback loops, affecting nutrient cycling and primary production (Kemp and Boynton 1992, Dame 1999). In the historic, well-oxygenated Chesapeake estuary, dense populations of oysters controlled primary productivity through this process.

The shallow-water, phytoplankton rich system of the estuary provided an ideal situation for the development of an oyster dominated ecosystem (Alford 1975). The combined effects of grazing and rapid recycling of nutrients in the system provided stability and high productivity (Newell 1988, Dame 1996). Oysters utilize tidal energy for the delivery of food and the removal of waste products. The loss of oyster populations in the Chesapeake estuary eliminated this coupling and accelerated the eutrophication of the system, further reducing the trophic complexity of the estuary (Newell 1988).

Oyster Life History

As gregarious, reef-forming organisms, oysters create habitat for other organisms, provide a fishery, and form a link between benthic and pelagic food webs. Historically, densities of oysters were much higher in the Chesapeake estuary than they are today. During the time of Winslow's survey, a 12-tooth dredge, (hand dredge by today's terms), would be filled in 30-45 seconds, (Brooks 1891, McCormick-Ray 1998). This is quite impressive, considering the inefficiency of a dredge (Chai et al 1992). Presently, surveys

of Natural Oyster Bars in the Chesapeake estuary reveal densities of oysters varying from zero to two hundred fifty oysters/m^2 (Vanisko et al 2002).

Given the historical abundance of oysters in estuarine systems and the ecological role oysters and oyster reefs played in the ecosystem, the importance of dense populations of oysters to the benthic community is evident. The role of oyster density in determining the development of this community, however, is not as apparent. Several studies have discussed the effect of oyster stocking density on the fouling community, especially as related to the aquaculture of oysters. For example, Adams et al (1994) found that bags with 500 oysters, (~1000 oysters/m^2), had fewer spat than bags with lower densities of oysters. Moroney and Walker (1999) also found that higher densities of oysters grown in bags, (250 to 500 oysters/bag, ~500 to 1,000 oysters/m^2), resulted in lower fouling. This decrease in fouling may be due to the tendency for high numbers of oysters to migrate towards the center of the bags, thereby reducing the available surface area (Adams et al 1994). A more recent study performed on intertidal oyster reefs constructed in Virginia showed that the presence of oysters increased the settlement of spat onto reefs (O'Beirn et al 2000). This may be due to the maintenance of interstitial space by the growth of oysters, to the presence of oysters or both.

Food limited growth of suspension feeders has been considered in aquaculture settings, where it is possible for high densities of animals to deplete local food supplies (Grizzle and Morin 1989, Rheault and Rice 1996, Moroney and Walker 1999). This type of food-limited growth is generally not considered in a natural setting. Studies of oysters grown on intertidal reefs in Georgia revealed that oyster growth would cease or progress very

slowly once oysters reached 140 to 150 mm due to the high energy costs of intertidal reef existence (Bahr 1976). Sub-tidal populations of oysters, however, increase the roughness of the bottom, increasing turbulence and therefore reducing the benthic boundary layer (Kemp and Boynton 1992). This increased turbulence increases the flux of particles and in turn controls growth (Grizzle and Morin 1989).

Laboratory flume experiments have demonstrated that oysters, with a mean shell height of 43 mm, are able to deplete the food supply. This results in reduced growth of oysters downstream (Rheault and Rice 1996). Rheault and Rice (1996) also stocked oysters into mesh bags to determine if stocking density had an effect on growth. Bags with lower stocking density showed greater oyster growth than oysters stocked at higher density, indicating an effect of density on growth. Alternatively, oysters with an initial mean shell height of 47 to 50 mm were grown in mesh bags at densities of 100, 250 or 500 oysters/bag, approximately 200 to 1000 oysters/m^2, and density had no effect on growth or survival of oysters (Adams et al 1994). Intertidal reefs were constructed in Virginia and were seeded by a natural spat settlement event. Densities of oysters on these constructed reefs were as high as 834 oysters/m^2, yet no reduction in growth was noted at this density (O'Beirn et al 2000).

In general, oysters grow from March to December along the mid-Atlantic, with the limits of growth typically controlled by local conditions. In Georgia, oyster growth varied from 0.9 to 5.1 mm/month throughout the growing season for oysters with an initial mean shell height of 40mm (Moroney and Walker 1999). *P. marinus* was detected in this study and likely influenced the growth rate of oysters.

In the Chesapeake estuary, the growing season is confined from late May to November (Paynter and Mallonee 1990, Paynter and DiMichele 1990). Tray studies have yielded oyster growth rates ranging from 8.3 to 16.7 mm/month depending on genetic strain and salinity, with higher salinities supporting faster growth (Paynter and Mallonee 1990, Paynter and DiMichele 1990). In all of these studies, density of oysters was considered to be non-limiting. Growth rates were lower in the second year, as an increase in shell height of larger oysters resulted in a greater increase in biomass (Paynter and DiMichele 1990, Rheault and Rice 1996). Growth of oysters was determined to be constant at each site during the growing season and had high R^2 values, yet varied from site to site (Paynter and Mallonee 1990).

Beaven (1952) studied oyster growth in the Chesapeake estuary and found that oysters on various oyster bars typically reached 76mm by the end of the third growing season. Another study in the Chesapeake, growing oysters in trays, reported maximum growth rates of 10 and 15 mm/month (Brown et al 1998). Initial mean shell height of oysters was 15 mm and final mean shell height was 74 mm for sites with low *P. marinus* prevalence.

Genetics as well as physiological mechanisms are known to influence the growth of *C. virginica* (Paynter and DiMichele 1990). In the late 19th century, oysters achieve market size, or 75 mm within two to three growing seasons (Brooks 1891). It is generally accepted today that oysters found on natural oyster bars in Chesapeake Bay reach market size in three to four growing seasons (Alford 1975, Kennedy 1989).

Summary and Challenges

The ecology of the Chesapeake estuary has changed dramatically since the time of colonial settlement. The watershed was altered initially for agriculture and later for the development of urban and industrial areas. As this transformation took place, the natural resources of the estuary were heavily exploited. Fisheries developed and expanded at the expense of native fish populations. Many of these fisheries, especially the oyster fishery, were not sustainable. Water quality steadily declined as sediment and nutrient inputs to the estuary accelerated. The overharvesting of oysters and reduction in underwater grass habitat accelerated this decline in water quality, increasing the eutrophication of the estuary.

With a compromised oyster population and poor water quality, the health of the modern estuary is less than before European colonization. There have been periods of prosperity for the Chesapeake oyster fishery, yet over harvesting and disease have resulted in a fishery that is a shadow of what it once was and a population of oysters that is at or near an all time low (MacKensie 1996). Newell (1988) estimated that the population of oysters in the Chesapeake estuary in the late 1980's was approximately 1% of historic levels. A survey of the landings of oysters for Maryland quickly shows the rapid decline of both the population and the fishery. The combined pressure of over fishing and habitat destruction likely overwhelmed the compensation mechanisms of oysters (McCormick-Ray 1998). Efforts to restore oysters and the oyster fishery to the estuary are compromised due to continued sedimentation, anoxia and the persistence of disease (Lenihan and Peterson 1998, Jackson et al 2001).

In 1983, the Chesapeake Bay Program released the first Chesapeake Bay Agreement, marking an effort by Maryland, Pennsylvania, Virginia and the District of Columbia to take action to restore the health of the Chesapeake estuary. The 1987 signing of the Bay Agreement included specific goals for restoring the living resources of the estuary. In 1993, the Maryland Oyster Roundtable released an action plan to restore oysters in Maryland. The recent Chesapeake 2000 Bay Agreement sets specific habitat and water quality goals for restoring and protecting the living resources of the Chesapeake estuary. In addition, goals to encourage stewardship of public resources through community involvement and education are included. This message is not new, as Brooks (1891) and Stevenson (1894) called for the protection of the oyster resource of the Chesapeake estuary over a century ago. Only after dense beds of oysters are restored to the estuary, can significant progress be made to improve the health of the Chesapeake estuary. While it is unrealistic to think that we will return the estuary to its condition in the 17th century, it is not unrealistic to imagine a stable resource that can support a sustainable harvest of oysters and exert a moderating influence on the nutrient inputs from anthropogenic sources. One essential component of a restored estuary is the restoration of dense populations of oysters and the associated benthic reef community.

Although there is a great deal of knowledge on the biology of oysters and their ecological and economic importance, efforts to rebuild the oyster population of the Chesapeake estuary have not made much progress in the 20th century (Kennedy and Breisch 1983). Answers to questions such as, (1) how many oysters need to be planted per acre to restore the ecological function of oysters, (2) at what density of oysters is growth inhibited, (3) do high density plots of oysters show greater incidence of *P. marinus* than low density

plots, are not fully understood. Given the vast sums of money being spent on oyster restoration in the Chesapeake estuary, it is important to answer some of these questions in order to maximize the potential for success as well as to use funds as efficiently as possible.

In an attempt to answer some of these questions, an experiment was undertaken at a former, but now barren, natural oyster bar in the Patuxent River. The goal of the project was to determine how the density of oysters planted on the bottom affected oyster growth and mortality as well as how density affected the acquisition of *P. marinus* in oysters grown on the bottom. In addition to monitoring these factors, the development of the epifaunal community associated with oysters was followed for two growing seasons. In this study, I hypothesized that oysters planted at densities greater than $250/m^2$ to compete for food and space and thus grow at a slower rate than oysters planted at a lower density. The same high-density plots of oysters are also expected to have greater prevalence and weighted intensity of *P. marinus*, as the close proximity of oysters should facilitate the transmission of disease. The combination of higher disease and competition for food and space is expected to result in higher mortality of oysters on high-density plots.

High-density of oysters will also result in some positive effects for the oyster reef community. The close proximity of oysters on high-density plots should increase the fertilization success of spawning oysters. I expect high-density plots to equalize at some moderate density that will likely increase fertilization success, without causing massive disease related mortality.

Due to the difficulty associated with the measuring of some of these parameters, growth, disease and the development of the epifaunal community will be the focus of this project. Surprisingly, the high-densities of oysters planted in this study did not result in reduced growth rates as would be expected.

Methods

The Kitts Marsh project was designed to test the effect of varying densities of oysters on the growth, survival and development of *P. marinus* in a population of oysters. Twelve individual 0.2-acre experimental plots were constructed in the Patuxent River by placing fossil oyster shell on a former, but now barren, natural oyster bar (Figure 1).

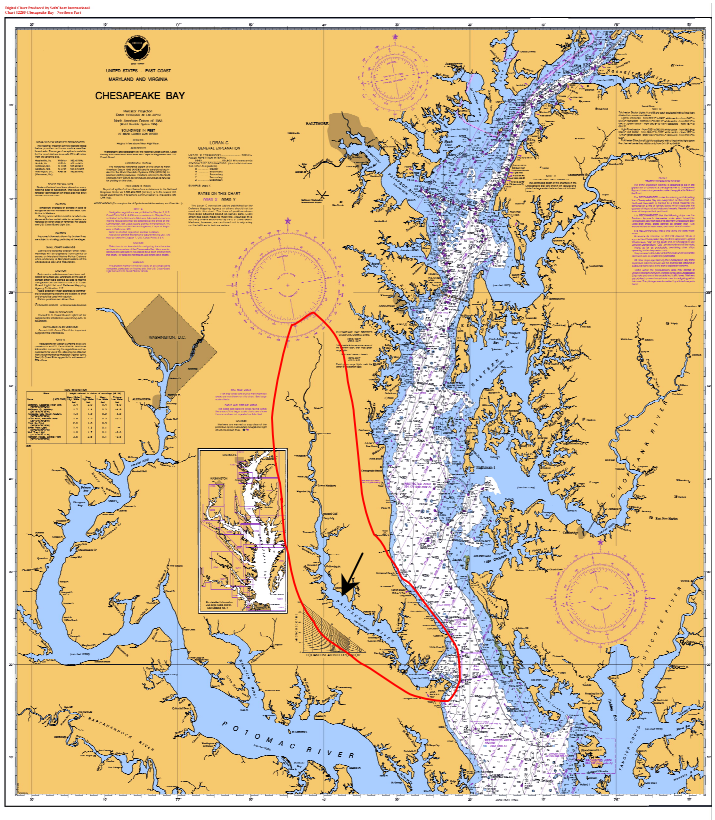

Figure 1. NOAA chart of the Maryland portion of the Chesapeake Estuary (purchased by SoftChart International). The Kitts Marsh project was located in the Patuxent River, (circled), just offshore of Kitt Point (arrow).

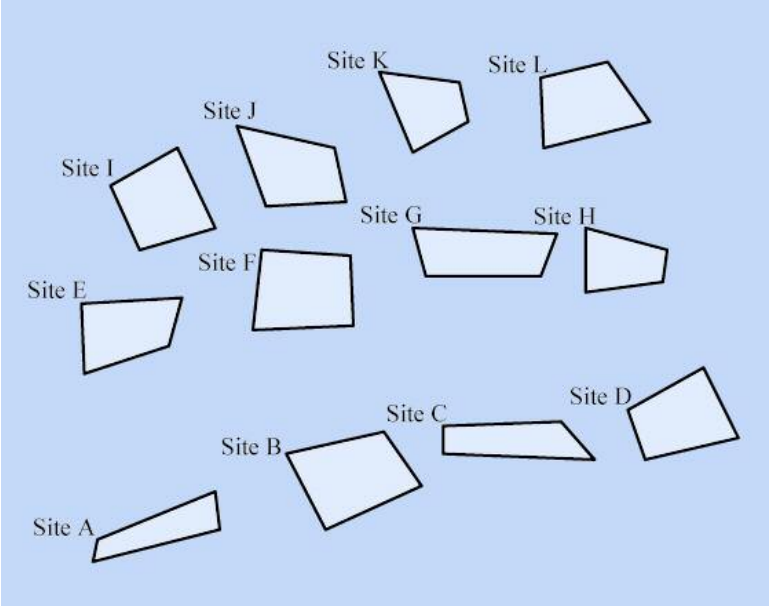

Figure 2. Layout of the Kitts Marsh site. Twelve distinct 0.2-acre plots were constructed and seeded with spat-on-shell. Plots were randomly assigned treatments of 0, 124, 247 or 494 oysters/m^2.

The plots, (Figure 2), were assigned one of four treatments, zero, 124, 247, and 494 oysters/m^2, in a completely randomized design. Each plot was seeded with oysters that were attached to individual pieces of oyster shell, spat-on-shell. The oysters were produced at the Maryland Department of Natural Resources (MDNR) Piney Point hatchery. In September of 1999, when the plots were seeded, the mean size of oysters planted was 25.0 (±0.51 SEM) mm. After the initial seeding, the plots were sampled in spring, summer and fall of 2000 and 2001. Oyster size, mortality, and condition index were measured with each sampling. Epifaunal colonization of the oysters and shells was also sampled. This was done by counting the number of organisms or noting their presence per quadrat grab.

The depth of the study site was approximately 3.5 meters. The corners of each of the twelve plots were identified by divers and marked with buoys. A Northstar 952XD

DGPS was used to obtain latitude and longitude coordinates for each corner of the plots. The coordinates were saved on the DGPS as well as recorded for future use. To determine the size of the plots, the DGPS coordinates were downloaded into a Nobletek chart plotting software program. Lines were drawn between points and the resulting boxes were printed on a laser printer. Each of the plots were cut out of the paper and weighed to the nearest 0.001g. The area of a single reference plot (plot L) was calculated using the formula for a trapezoid, $\frac{h(a_1 + a_2)}{2}$, where h = height, and a_1 and a_2 = length of the two parallel sides. Due to the irregular shape of the remaining plots, their areas were determined by solving the ratio,

$$\frac{Area\_of\_plot\_X}{Weight\_of\_plot\_X} = \frac{Area\_of\_plot\_L}{Weight\_of\_plot\_L}$$

for the area of the plot in question.

The marked plots were assigned treatments so that spat-on-shell oysters could be planted by boat. The boat was maneuvered into the area of the plot to allow time for volunteers to drop the spat covered shells overboard. The boat position was maintained until the proper number of oysters had been planted on the site, per the assigned treatment. The number of oysters needed was determined by sampling several mesh bags of spat-on-shell to determine the average number of oysters per bag. This number was then used to determine the number of oysters needed per plot, assuming a plot size of 0.2-acre and treatments of zero, 124, 247, and 494 oysters/m^2.

During each sampling trip, dissolved oxygen, temperature and salinity measurements were taken at the surface and bottom. Water quality was initially not different among

sites and therefore only one set of water quality data was collected for the whole site. A Yellow Springs Instruments, (YSI), model 37 salinity conductivity and temperature meter, was used to determine salinity and temperature. A YSI model 58 dissolved oxygen meter was used to measure dissolved oxygen levels.

In August of 2000, a YSI Model 6000 UPG3 continuously recording water quality monitor was deployed to measure water quality on the bottom every fifteen minutes. Cable ties were used to anchor the monitor to a cement block. The block was secured to a screw anchor, previously installed by divers, and located in the center of the experimental plots. A ¼ inch plastic-coated steel aircraft cable was then used to attach the block and monitor to the screw anchor. The block was used to ensure that the monitor was not buried by sediment and took bottom water quality measurements representative of the water approximately six inches above the bottom surface. Continuous monitoring occurred from August to September 2000, and from May through December 2001.

The plots were sampled initially in 1999 to ensure proper planting of the sites, and then on a seasonal basis during the spring, summer and fall of 2000 and 2001. During each sampling trip, a buoy was haphazardly dropped on a location inside the plot not near the edge as determined by the pre-recorded DGPS coordinates. The same vessel and DGPS unit were used each time to locate the plots. Divers entered the water at the location of the buoy, descended to the bottom and located the plot. Once on the bottom, divers moved away from the buoy and collected samples by haphazardly placing a $0.11m^2$ quadrat within the site and collecting the oyster and shell material within the quadrat to a

depth that included the first layer of planted fossil shell. This was done three times on each site for a total of thirty-six samples per sampling trip. The samples were placed into mesh dive bags and transported to the surface. Once at the surface the contents of the bags were emptied into plastic bags, labeled and transported to the lab for analysis.

In the lab, samples were stored in a refrigerator at 4°C and processed in a haphazard order. For each sample, shell height in mm of both live and dead oysters, number of oysters per quad, *P. marinus* prevalence and intensity and condition of oysters was tabulated. The following formula was used to calculate the condition index of oysters (Paynter and Burreson 1991),

$$\frac{oyster\ tissue\ dry\ weight}{(oyster\ total\ weight - oyster\ shell\ weight)} * 100$$

Clumps of oysters were cleaned of mud and fouling organisms, measured and weighed to the nearest 0.1g. If possible, individual oysters were weighed, but clumps were used if separation of oysters was not possible. A specific oyster was then shucked and the empty shell, or clump of oysters containing the empty shell was reweighed. The oyster meat was placed into a weigh boat, weighed wet, and then dried at 60° for three days. Dry weight was recorded to the nearest 0.01g.

P. marinus prevalence was determined by Ray's fluid Thioglycollate method, (RFTM) (Ray 1952). Up to ten oysters from each sample were shucked and the rectum removed if the oyster was sufficiently large, or whole or half oysters were used if the oyster was not large enough to remove only the rectum. The meat was placed into a 1.5 mL centrifuge tube containing 1.2 mL fluid thioglycollate, penicillin streptomycin solution. Tubes were

placed into a dark drawer for five to ten days, at which time the tissue was removed and macerated on a slide. The tissue was stained with 100% Lugol's iodine solution and examined for the presence of *P. marinus* cells. The severity of infection was characterized using weighted prevalence, with the following weights assigned, no infection = 0, light = 1, moderate = 3, heavy = 5 (Paynter and Burreson 1991).

To examine the relationship between oyster density and the oyster reef community, the abundance of epifaunal organisms was recorded. Clumps of oysters were examined and the number of fouling organisms was noted. Additionally, organisms dislodged while processing oysters were enumerated and recorded. Therefore, data on fouling community organisms was documented as abundance per quadrat.

The data collected throughout the Kitts Marsh study was analyzed using JMPIN 4® software (SAS Institute Inc.). It was determined that the density of the oysters changed through time (see results). As a consequence, for some analyses, the observed density at time t rather than the initial assigned density group was used. Multiple general linear model (ANOVA and regression) analyses were performed to determine effects of time and initial density on several variables including the number of oysters per quad, oyster growth and oyster condition. Assumptions of all models used were checked to ensure approximate normality and constant variance. Where needed, remedial measures such as weighted regression analyses were used. Generally, heteroscedasticity[1] of variances was the most common problem. Mussel, barnacle and anemone data was weighted to allow for multiple variance groups. Additional analyses were performed on the community

[1] Heteroscedasticity – inequality of variances among samples (Sokal and Rohlf, 1996)

data to determine the effect of oyster density group and time on the abundance of fouling organisms. For significant model results, Tukey HSD contrasts were made to determine the nature of the significance. Additional contrasts were performed based on *a priori* assumptions to determine the specific effect of time, presence of oysters or oyster density group on macro-invertebrate abundance.

Results

Temperature, Salinity and Dissolved Oxygen

To assess the suitability of the Kitts Marsh project site for oyster growth, water quality data were collected during the summer of 2000 and again during the growing season in 2001. Regional drought conditions were noted throughout the study, potentially altering the dynamics of oyster growth and disease acquisition at the project site. Daily mean temperature, salinity, and dissolved oxygen were plotted over time from August to September 2000 and from May to December 2001 (Figure 3). As expected, mean water temperature declined from August to September 2000. In 2001, daily mean water temperatures followed a seasonal pattern, increasing from May through July, leveling off through mid September, and then dropping to a low in December 2001, (Figure 3).

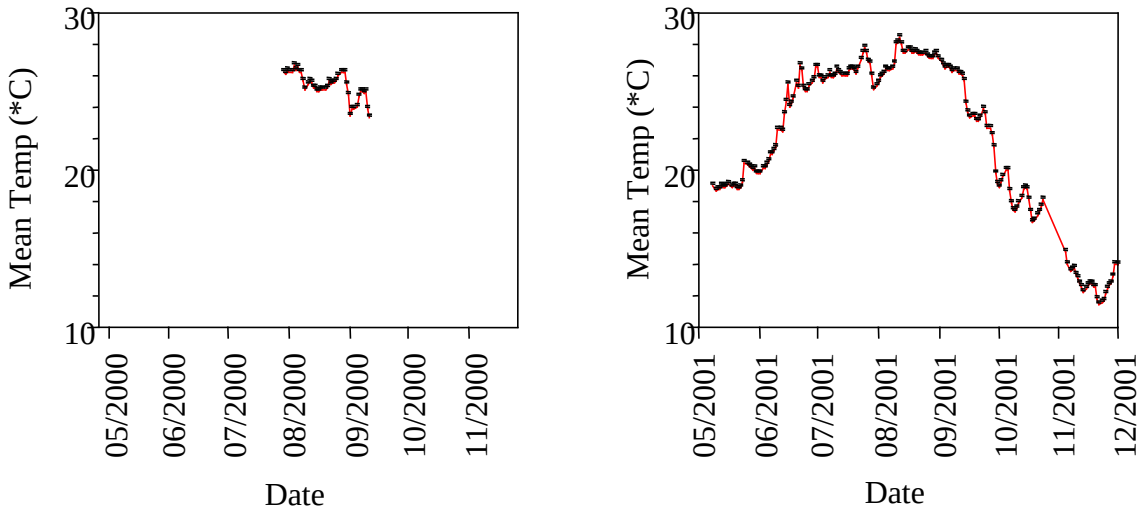

Figure 3. Bottom water temperature measured from early August to late September 2000 and from May to December 2001. Water temperature declined from August to September 2000. In 2001, water temperature increased from 19°C May to 25°C in July, then fluctuated between 25°C and 29°C until mid September, when they declined to 12°C in November.

In August of 2000, daily mean salinity increased initially but remained constant through September. Due to ongoing drought conditions in 2001, salinities were higher than

would normally have been expected and generally increased throughout the year, with the exception of a slight drop in salinity during August in both years (Figure 4).

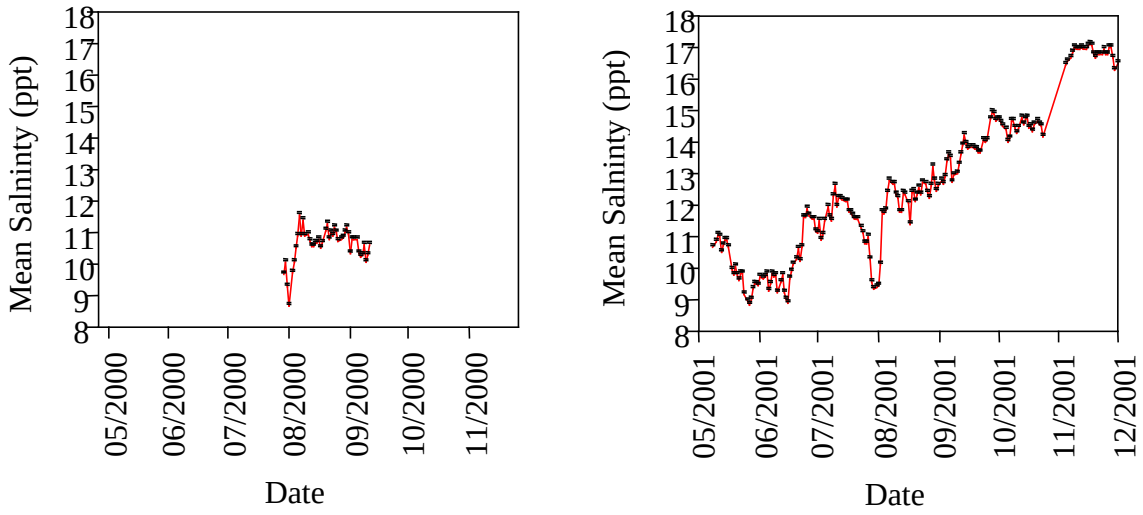

Figure 4. Salinity was recorded from early August to late September 2000 and again from May to December 2001. Salinity varied from 8.7 to 11.6 for August and September 2000. In 2001, salinity varied from 8.9 to 17.2, with salinities fluctuating from 9.5 to 15.0 in August and September 2001 and increasing to 17 in December.

Saturated or 100% oxygen content of estuarine water is typically about 7.5 mg O_2/l. Hypoxic conditions are typically defined as water containing 1 to 3 mg O_2/l. Dissolved oxygen levels recorded at the site in the summer of 2000 indicated hypoxic conditions for much of the sampling period (Figure 5). Hypoxic conditions were also noted in 2001, when several nearly zero dissolved oxygen events were recorded (Figure 5). Dissolved oxygen levels increased with declining water temperatures from September to December. Although oysters are able to withstand periods of hypoxia, this response cannot be prolonged during the warmer summer months, when metabolic levels are higher. During the period in which water quality data was collected, anoxic conditions were noted twice in 2000, varying from one to two weeks in duration. Although these same anoxic

conditions were not noted for 2001, hypoxia was documented several times, with low dissolved oxygen conditions persisting for a week or more on three occasions.

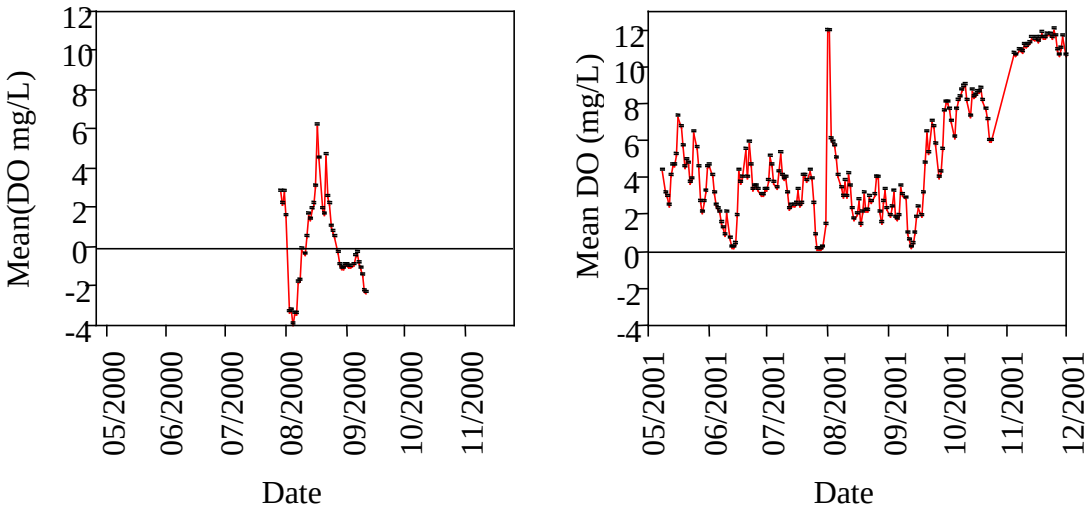

Figure 5. Dissolved oxygen, (DO), as measured from early August to late September 2000 and from May to December 2001, fluctuated. During 2000, DO levels indicated hypoxic (< 2 mg O_2/L) conditions for 35 of 45 days monitored. In 2001, hypoxic conditions were recorded during June, July August and September. The 2001 hypoxic events were not as long in duration as those recorded in August and September 2000.

Planted Densities of Oysters

In April 2000, samples collected from all sites indicated that the assigned treatments were not well represented on the bottom in the planned configuration (Table 1).

Site	Planned Density	Mean Density 2000 April	August	October	Mean Density 2001 May	August	November
A	0	9 (0)	9 (0)	9 (0)	30 (0)	0 (0)	0 (0)
B	494	690 (24)	860 (13)	960 (17)	300 (0.68)	150 (2.0)	156 (0.50)
C	124	160 (4.8)	640 (18)	440 (12)	237 (3.9)	230 (4.9)	158 (3.5)
D	247	730 (20)	660 (27)	780 (19)	320 (5.8)	130 (0)	208 (6.7)
E	124	820 (32)	340 (7.8)	330 (7.7)	20 (4.5)	197 (2.9)	176 (4.8)
F	247	1140 (11)	490 (4.1)	630 (13)	255 (2.9)	170 (2.7)	173 (2.8)
G	0	9 (0)	18 (0)	18 (0)	20 (3.0)	0 (0)	9 (0)
H	494	0 (0)	9 (0)	9 (0)	9 (0)	9 (0)	9 (0)
I	247	0 (0)	80 (4.9)	390 (13)	93 (4.3)	327 (12)	154 (6.0)
J	494	290 (8.4)	410 (7.8)	610 (17)	9 (0)	350 (9.0)	75 (9.1)
K	0	18 (0)	0 (0)	9 (0)	9 (0)	0 (0)	0 (0)
L	124	620 (19)	230 (4.5)	210 (11)	80 (5.7)	0 (0)	102 (7.9)

Table 1. Least Squares Means (± SE) densities of oysters for 2000 and 2001.

Based upon the observed densities of oysters on the sites, individual sites were characterized as high, medium and low and zero density. Due to the variation in the density of oysters across all sites from year one to year two, sites were assigned site densities for each year based on the mean density of oysters observed during that year. Mean oyster shell height was then plotted by sampling date for the high, medium and low density plots (Figure 6). With the exception of the April 2000 ($F_{2, 1676} = 15.5$, $p < 0.0001$) and the August 2001 samples ($F_{1, 610} = 21$, $p < 0.0001$), there was no difference in oyster shell height among site density groups for the study period. This difference may be due to the low numbers of live oysters found a the low density sites. In April 2000, only five live oysters overall were found on all of the low-density sites. These five oysters had a mean shell height of 29 (± 3.9) mm, as compared to the medium and high-density sites, which had oyster shell heights of 37.8 (± 0.39) mm and 40.0 (± 0.26) mm (Figure 6).

Following the April 2000 sampling, oyster shell height increases throughout the study period (Figure 6). In 2001, the high site density category was no longer distinguishable from the medium site density category due to a drop in oyster density across all sites. Medium and low-density sites continued to show no effect of oyster density on shell height.

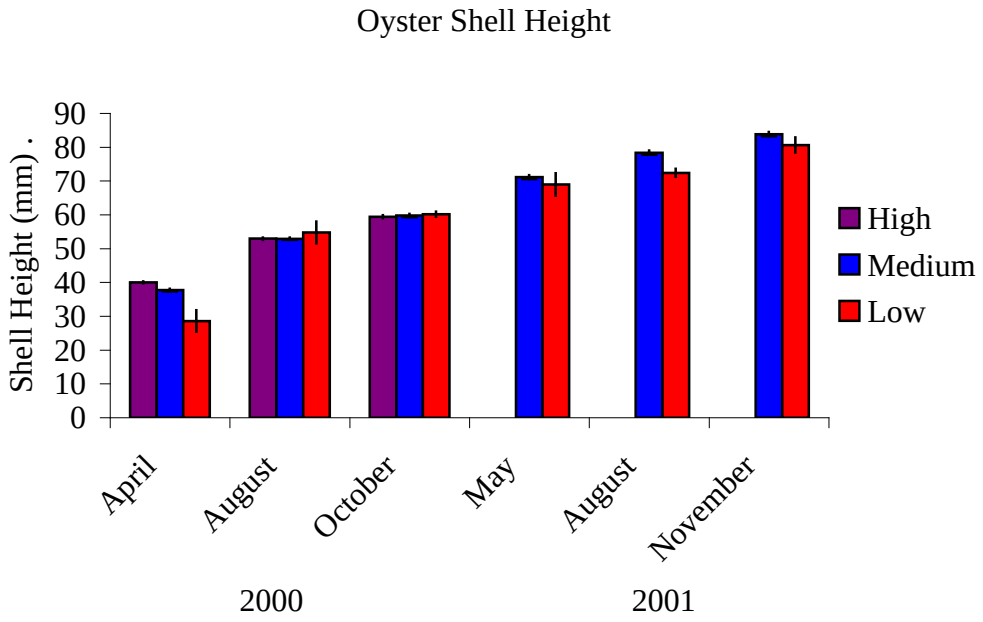

Figure 6. Graph of oyster shell height by site density for each sampling. With the exception of April 2000 ($F_{2, 1676} = 15.5$, $p < 0.0001$) and August 2001 ($F_{1, 610}$) = 21.1, $p < 0.0001$), oyster shell height is not different among site density groups.

As a result of this lack of effect due to density of oysters per site and due to the heterogeneous nature of the density of oysters at the project site, quadrat samples were used as the experimental unit, rather than sites. Quadrat samples were blocked into four groups based on density, perhaps providing a finer method of analysis to determine the effect of density of oyster growth. Density groups were assigned as follows,

Density group	Oysters per meter^2
Zero	< 64
Low	64 – 185
Medium	186 – 369
High	> 369

Between November 2000 and May 2001, a substantial and unexplained mortality event occurred, resulting in a decline in the density of oysters across all density groups ($t_{df=1}$ = 23.57, $p < 0.0001$). The average decline in oyster density was 33%, with the mean density of oysters falling from 620(± 8.0) oysters/m^2 in the fall of 2000 to 260 (± 13) oysters/m^2 in the spring of 2001. This mortality was not due to disease. Until this point, no oysters sampled tested positive for *P. marinus* or *Haplosporidium nelsoni*, the parasite that causes MSX disease, since neither was detected as of May 2001.

Although water quality data from the summer indicated poor conditions for oyster growth, it is unlikely that low dissolved oxygen led to this winter mortality, as no other mortality event of this magnitude was observed during the duration of the study. Although density of oysters declined throughout the experimental area, zero, low and medium density groups were still found among the quadrat grabs taken in the second growing season.

Oyster Growth

Shell height increased through the first and second growing seasons (Figure 7). Tukey's HSD confirmed that shell heights among the spring, summer and fall samples were significantly different in both 2000 and 2001

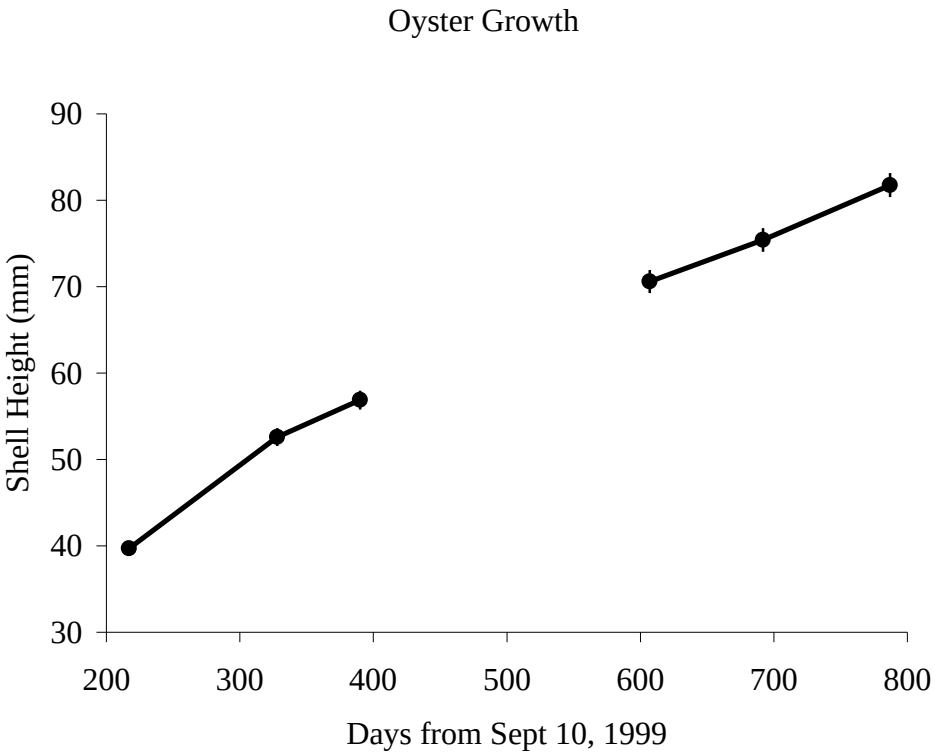

Figure 7. Growth of oysters at Kitts Marsh for the 2000 and 2001 growing seasons. Oyster growth slowed in the second year as expected, yet oysters achieved a mean shell height of 83.6 (±0.61) mm by November 2001.

Oyster growth, as determined by a regression of live length of oysters over time, was 0.117 (± 0.0037) mm/day or approximately 3.5 mm/month for the first growing season (Figure 8), lower than expected for the site. In a previous study at a nearby site in the Patuxent River, oyster growth was 7.8 mm/month for the first growing season (Paynter 2001). In our study, shell height of oysters was related to season, with oysters increasing in size from spring to fall, ($F_{1, 6423} = 2964.3$, $p < 0.0001$), was related to year, with oysters growing from 2000 to 2001 ($F_{1, 6423} = 17.6$, $p < 0.0001$), and was related to the interaction of season and year ($F_{1, 6423} = 120.7$, $p < 0.0001$). In 2001, oysters grew at a rate of 0.067 (± 0.0061) mm/day or 2.0 mm/month, (Figure 8). The second year growth was more

typical of the 1.2 mm/month second year growth rate noted at similar sites in the Patuxent River (Paynter, 2001).

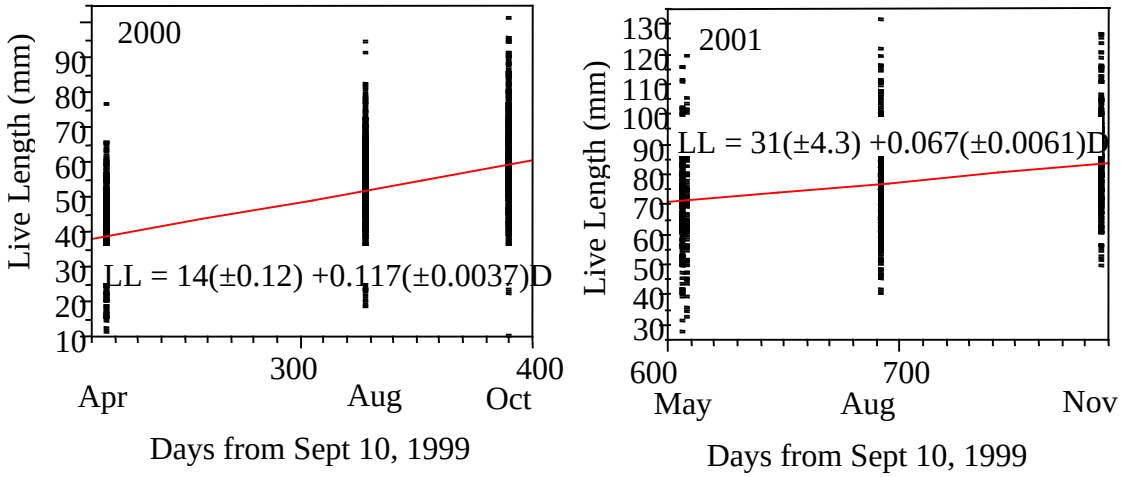

Figure 8. Shell height (live length) of oysters was regressed against time. In 2000, oyster growth was higher than in 2001, as expected.

Although the growth of oysters in the first year of the study was lower than expected, the site did yield oysters with a mean shell height of 59.2 (±0.43) mm by October. In November of the following year, mean shell height of oysters had increased to 83.4 (±0.76) mm, with nearly 75% of oysters greater than 75mm, or market size, (Figure 9). This indicates that oysters planted in the high densities observed in this experiment are capable of achieving market size in two growing seasons.

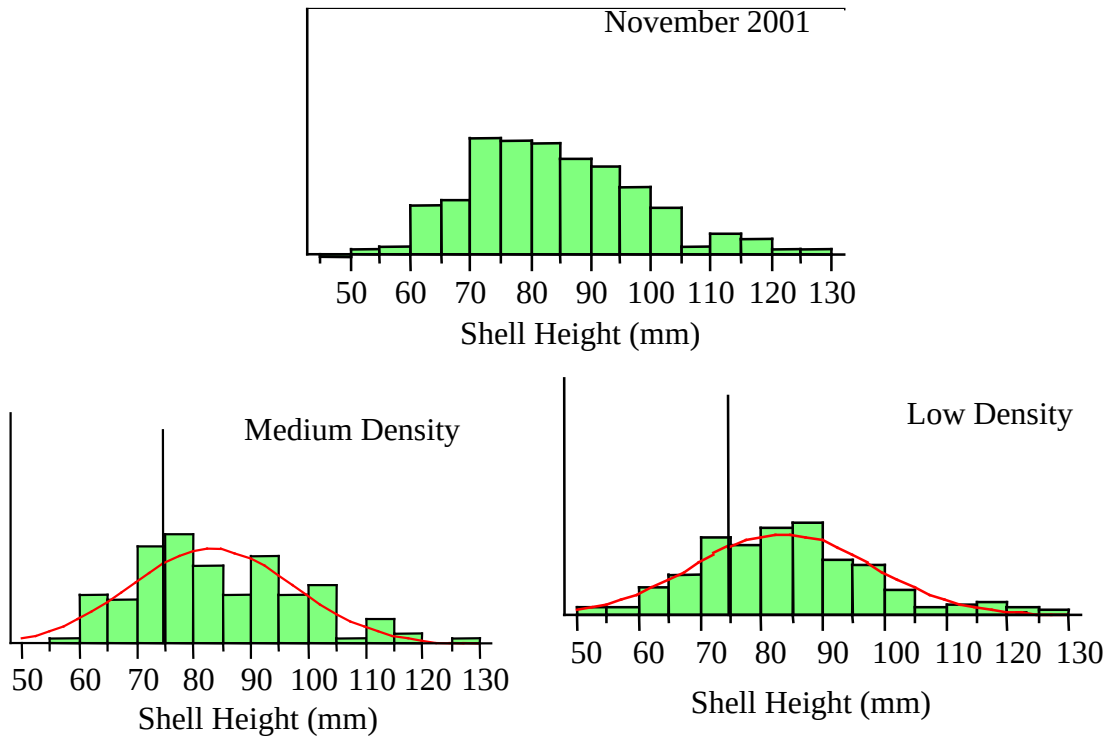

Figure 9. Shell height distribution of oysters collected in November 2001. 75% of the oysters were market size (~75 mm) or larger. Distribution of oysters in Medium and Low-density quadrats were similar.

Oyster Health

Condition index, a ratio of oyster tissue weight to interior empty shell volume, indicated that the oysters were healthy through the first year of the study. This was true of oysters planted at any density, ($F_{3, 309}$ = 1.8, p = 0.1535). Oyster condition was related to season ($F_{2, 309}$ = 6.3, p = 0.0020) and the interaction of season and density ($F_{6, 309}$ = 2.2, p = 0.0472) in 2000. In 2001, oyster condition was again related to season ($F_{2, 285}$ = 4.0, p = 0.0202), but was no longer related to the interaction of density and season ($F_{4, 285}$ = 0.44, p = 0.7767). Condition of oysters typically follows a seasonal pattern, resulting in oysters with higher condition in the spring and fall than in the summer. This depressed condition during the summer months is often due to spawning activity of oysters (Korringa 1952).

In the first growing season, oyster condition decreased from spring to summer, but did not increase in the fall as would be expected, (Figure 10).

A priori contrasts of oyster condition in 2000, showed a difference from April to August, (t_{309} = 3.02, p= 0.0027), but no change from August to October, (t_{309} = 0.35, p = 0.7292). It is possible that poor water quality conditions, such as low dissolved oxygen, at the site during the first growing season reduced the expected increase in oyster condition, leading to an inability to detect a difference in oyster condition between the August and October 2000 samples.

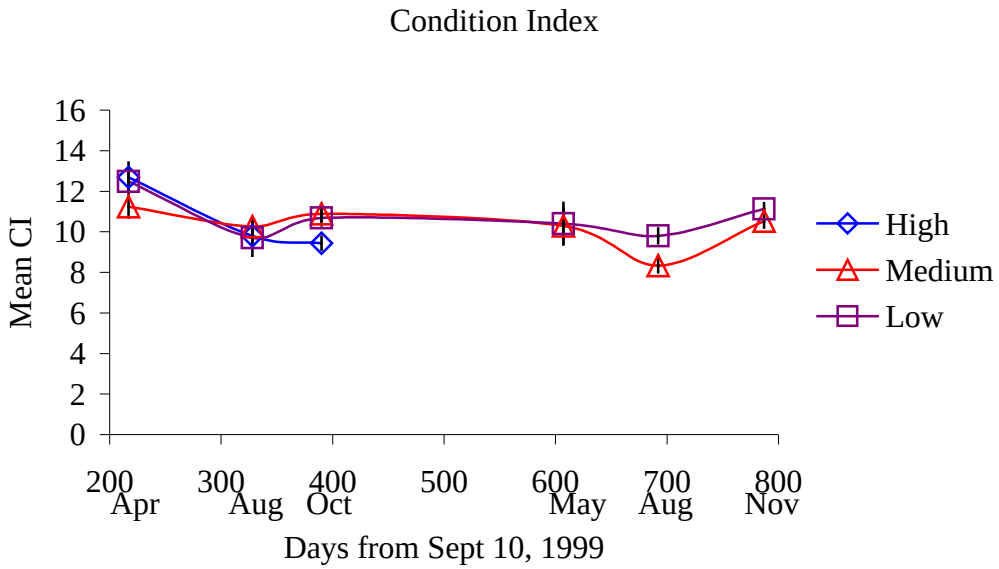

Figure 10. Graph of mean oyster condition for the 2000 – 2001 study period. Oyster condition followed a typical pattern, dropping in the summer and increasing into the fall.

In 2001, condition of oysters followed the typical pattern, declining from spring to summer, and then increased through the fall, (Figure 10). *A priori* comparisons of oysters sampled in the spring versus those sampled in the summer and of oysters sampled in the summer versus those sampled in the fall revealed no difference in oyster condition

from May to August 2001, (t_{285} = 1.8, p = 0.0686), but did indicate a difference in oyster condition from August to November, (t_{285} = 2.8, p = 0.0053). Most importantly, as in the first growing season, oyster condition did not differ among quadrat density groups within a sampling event, ($F_{2, 291}$ = 2.5, p = 0.0843).

Disease

In April 2000, a subset of each sample containing oysters was processed to determine prevalence and intensity of *P. marinus* infection. Of the oysters tested, all were found to be pathogen free. This process was repeated with the August and October 2000 samples collected. The oysters tested in these sub-samples were also free from *P. marinus* infection. Thus, the oysters in the study remained pathogen free through October of the first growing season.

In the May and August 2001 sampling periods, sub-samples of oysters collected were also found to be pathogen free. Although salinities in the second year of the study were higher than expected, *P. marinus* infection in oysters was not detected until the fall of the second growing season, later than would be expected given the high salinity conditions present during the study. The November 2001 sub-samples contained oysters with positive infections for *P. marinus*, indicating that oysters acquired the infection between August and November of the second growing season.

In November 2001, twenty-one of the thirty-six samples collected contained oysters and were tested for *P.* marinus. Four of the sub-samples tested were positive for *P. marinus* infection. Two of the sub-samples were from low-density quadrats; one was from a medium-density quadrat, and the final was from a zero-density quadrat. The sub-sample

collected from the zero density quadrat contained a single oyster, which had a light infection. This oyster may have been errantly planted on the site, as it was a single large oyster, rather than a cluster of several oysters as was typical of the hatchery-reared oysters that were used for the study. The other sub-samples were comprised of ten oysters each (see methods). The two low-density quadrat sub-samples each had one infected oyster, (one very light and one heavy), yielding a 10% disease prevalence within the low-density quadrat samples. Weighted infection intensities were 0.05 and 0.5 respectively for the low-density quadrat samples. The medium-density quadrat sub-sample contained four infected oysters, (three light and one heavy), resulting in a 40% disease prevalence and 0.8 weighted infection intensity within the medium density quadrat sample.

With the exception of the single oyster from a zero density quadrat grab, the sub- samples containing infected oysters were from plots that were initially high in density of oysters. Two of the three sub-samples from plot F contained infected oysters and were the only sub-samples noted during the study period to reveal heavy infection intensities. The prevalence of infected oysters on plot F was 19%, as compared to plot B, another formerly high-density plot, which had a plot disease prevalence of 3%. Overall plot infection intensities were low, 0.5 and 0.02 for sites F and B respectively, but may represent the genesis of disease within the plot.

Oyster Reef Community

At the April 2000 sampling, it was evident that few organisms had colonized the oyster or shell plots, providing a relatively clean surface for epifaunal settlement during the coming warm season. Abundance of epifaunal organisms was recorded for each sample

at every date. The following organisms were recorded at least once throughout the course of the study, Amphipoda (Gammarus spp.), *Balanus eburneus, Balanus improvisus, Chasomodes bosquianus, Diadumene leucolena, Eurypanopeus depressus, Gobiesox strumosus, Gobisoma bosci, Hypsoblennius hentzi, Ischadium recurvum, Macoma balthica, Membranipora tenuis, Molgula manhattensis, Nereis succinea, Opsanus tau, Panopeus herbstii, Rhithropanopeus harrisii, and Stylochus ellipticus*. Due to the low sample size of many of these organisms, only a few were analyzed statistically. Results from barnacle (*Balanus eburneus* and *Balanus improvisus*), mussel (*Ischadium recurvum*) and anemone (*Diadumene leucolena*) data analysis are presented below.

Barnacle abundance

Barnacle abundance increased throughout the first growing season and was related to the density of oysters per quadrat (Figure 11). Between April and August of 2000, a barnacle recruitment event (set) occurred at the project site. Barnacle densities across all quadrats increased from a mean of 1.0 (±1.9) barnacle per quadrat in the spring to a mean of 57 (±9.3) barnacles per quadrat in the summer. Due to heteroscedasticity in the data, the analysis was weighted to allow for four variance groups. In 2000, higher barnacle abundances were noted in quadrats with zero or low densities of oysters, ($F_{3, 96} = 7.9$, $p < 0.0001$). The abundance of barnacles also increased throughout the growing season, indicating more than one settlement event, ($F_{2, 96} = 31.5$, $p < 0.0001$). *A priori* comparisons were made for the summer and fall barnacle data among the density groups with and without oysters.

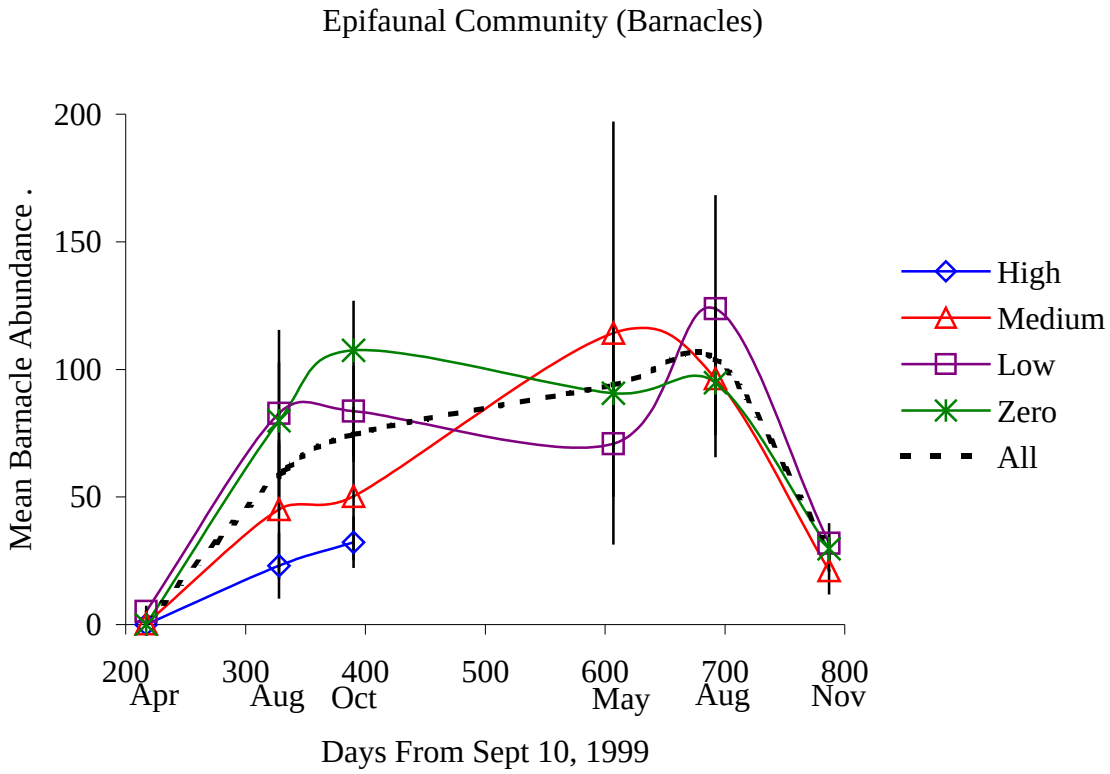

Figure 11. Barnacle abundance by time for specific density groups and all groups combined. Barnacle abundance increased throughout the first growing season, but declined from the summer to fall of the second season. Barnacle abundance was negatively correlated to oyster density throughout the first growing season. During the second growing season, barnacle abundance was not related to density of oysters.

To test this, contrasts of barnacle abundance within quadrats of high and medium oyster density were compared to quadrats containing low and zero densities of oysters. In the fall of 2000, abundance of barnacles was negatively related with density of oysters, ($t_{df=2}$ = -2.82, p = 0.0058). This relationship was examined further to find that barnacle abundance within quadrats containing high and medium densities of oysters had fewer barnacles than quadrats containing low and zero densities of oysters, (t_{96} = -2.56, p < 0.0119). The absence of oysters, or the presence of only a low density of oysters may have allowed for greater barnacle colonization of the shell present at the project site.

Barnacle abundance continued to increase into spring of the second year. In May 2001, the barnacle density per quadrat increased from a mean of 63 (±11.5) barnacles per quadrat in October 2000 to a mean of 100 (±28) barnacles per quadrat, (Figure 11). The barnacle set that resulted in this increase may have occurred prior to the October sampling, yet the small size of the newly set animals could have been undetected during the processing of the sample. In the second year of the study, barnacle abundance was no longer correlated with oyster density, as in the first year. Analysis of the data for 2001 indicated that barnacle abundance did not change among quadrats containing different densities of oysters, ($F_{3, 102} = 0.10$, $p = 0.9623$). Barnacle abundance did change throughout the second year, ($F_{2, 102} = 9.8$, $p = 0.0001$), but rather than increasing as in the first growing season, barnacle abundance declined from summer to fall 2001 (Table 7).

Mussel abundance

The abundance of mussels increased throughout the study period (ANOVA, $F_{1, 214} = 158.7$, $p < 0.0001$, Figure 12).

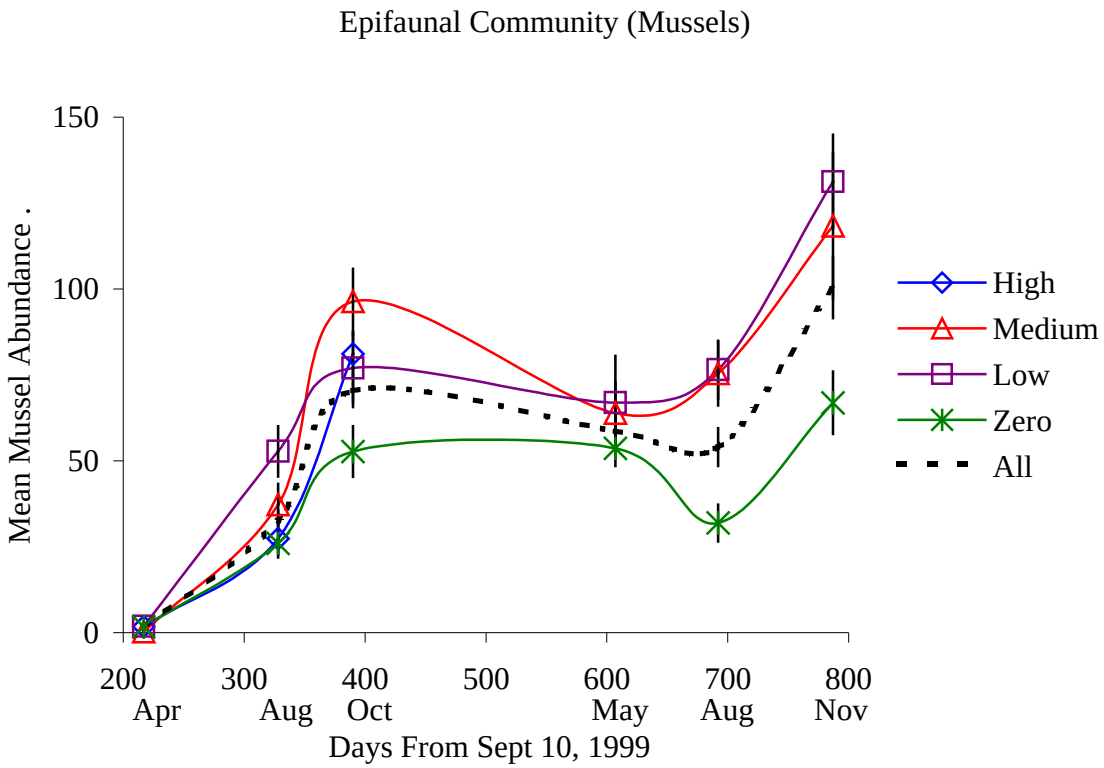

Figure 12. Mussel abundance by time for specific density groups and all groups combined. Mussel abundance increased throughout the study period. Quadrats containing oysters had greater mussel abundance throughout the study.

In 2000 the number of mussels per quadrat increased from a mean of 1.4 (±0.85) mussels per quadrat in the spring to a mean of 77 (±3.5) mussels per quadrat in the fall, (Figure 12). Tukey's HSD confirmed that mussel abundance increased at each sampling in 2000, indicating that mussel abundance was different among the spring, summer and fall samples for 2000.

In addition to increasing throughout the first year of the study, mussel abundance was related to the density of oysters, ($F_{3, 96} = 5.9$ $p = 0.0010$). *A priori* contrasts revealed that mussel abundance increased within quadrats containing oysters, i.e. high, medium and low quadrats contained greater numbers of mussels than zero density quadrats. In the fall, quadrats with oysters had greater mussel abundance than quadrats without oysters (t_{96}

= 4.8 $p < 0.0001$). This relationship was further examined to reveal that high and medium oyster density quadrats containing more mussels than low and zero density quadrats, ($t_{96} = 3.10$, $p = 0.0026$).

Mussel abundance declined during the winter of 2000 – 2001, falling from a mean of 77 (±3.5) mussels per quadrat in October 2000 to a mean of 62 (±5.3) mussels per quadrat in May 2001(Figure 12). This decline was likely due to a winter related mortality event. Through the summer 2001, mussel abundance remained constant, but then increased to a mean of 105 (±7.0) mussels per quadrat in the fall, ($t_{105} = -4.99$, $p < 0.0001$), (Figure 12).

Anemone abundance

As with barnacle and mussel abundance data, anemone abundance increased throughout the first year of the study, from a mean of 6 (±2.3) anemones per quadrat in April to a mean of 14 (±2.2) anemones per quadrat by October, (Figure 13).

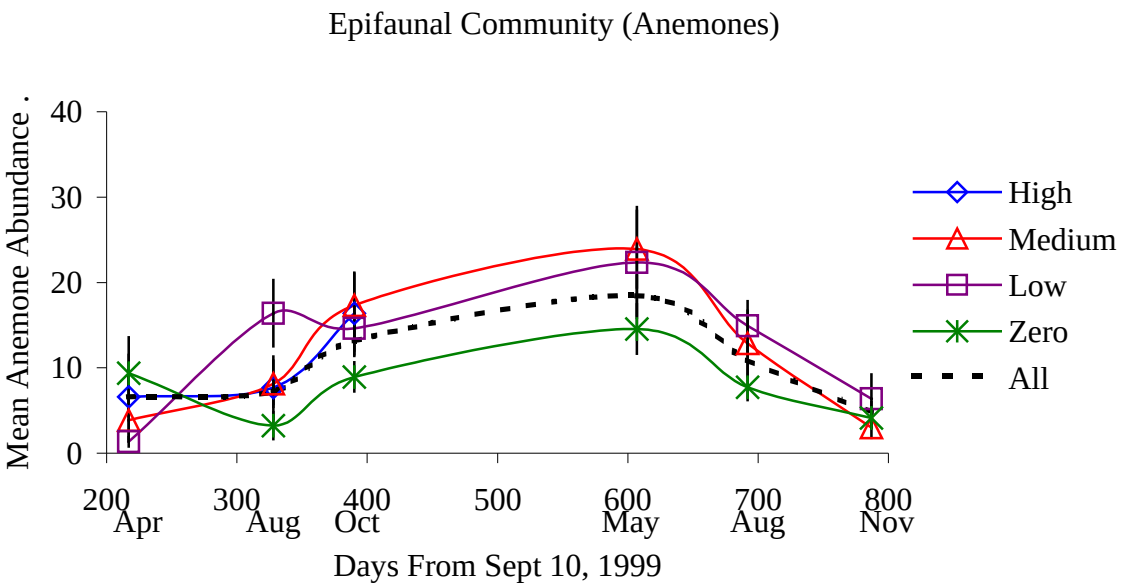

Figure 13. Anemone abundance for the study period. Quadrats containing oysters yielded greater numbers of anemones from summer 2000 through summer 2001.

Although the number of anemones sampled per quadrat was low, the log of anemone abundance increased over time for 2000 ($R^2 = 0.21$, $F_{2, 105} = 14.2$, $p < 0.0001$, Figure 14). A regression of the log of anemone abundance versus time indicated that anemone abundance within quadrats containing oysters increased throughout 2000 (Figure 14).

Throughout the second year of the study, the log of anemone abundance decreased ($R^2 = 0.29$, $F_{2, 105} = 21.6$, $p < 0.0001$) across all quadrats (Figure 14). This drop in anemone abundance was not related to the density of oysters per quadrat, ($F_{3, 102} = 2.29$, $p = 0.0829$).

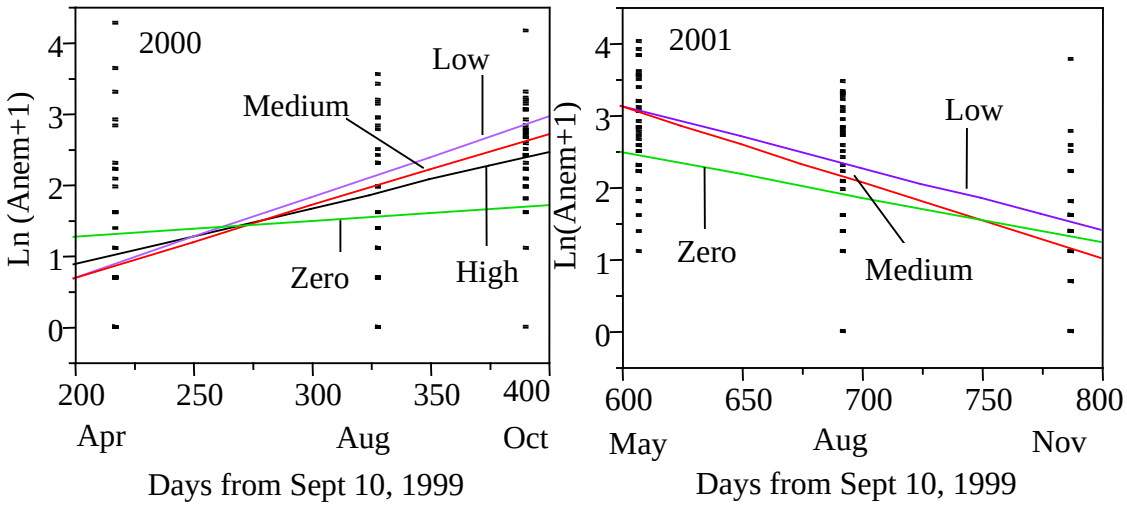

Figure 14. Anemone abundance was regressed against time. In 2000 anemone abundance increased among density groups containing oysters, while in 2001 anemone abundance decreased across all density groups.

Sedimentation

Throughout the study, diver observations of the bottom indicated sedimentation of the sites at a rate higher than expected. Although no quantitative measurements were made, observations indicated that sites with low or no densities of oysters had moderate amounts of silt accumulation on and among oyster shells. Heavy sedimentation was noted on high-density sites, especially in between the shells of oyster clumps. Diver observations as well as discoloration of oyster clumps due to anoxic sediments, indicated that by the fall of the first year, many of the oysters from high-density sites were half buried in sediment. Oysters did not show any affect of sedimentation on growth, as all sites were similar in shell height and condition index, yet sedimentation of sites varied. Abundance of fouling organisms may have been affected by sedimentation, especially the abundance of anemones and barnacles (see results in the oyster reef community section).

Conclusion / Discussion

The Kitts Marsh project was designed to test the hypothesis that oyster growth would be reduced above a certain density of oysters planted on the bottom. In addition to growth limitation, the study was designed to test whether the acquisition and development of *P. mariuns* infection in oysters was related to the density of oysters planted on the bottom. Specifically, plots planted with higher densities of oysters were expected to have higher prevalence and intensity of *P. mariuns* infection. The development of the epifaunal community associated with oyster reefs was also investigated during this project. Plots containing greater numbers of oysters were expected to have greater epifaunal abundance.

Water quality data collected at the site indicated lower than expected dissolved oxygen levels throughout August 2000 and from July through September 2001. Low dissolved oxygen may have stressed oysters and impacted their condition, growth, or both. Condition indices for oysters did drop from spring to summer, but given the normal seasonal variation in oyster condition (Korringa 1952), this is not surprising. It is possible that poor water quality conditions increased this decline in oyster condition. Oyster growth was lower than expected in the first growing season, but appeared more typical in year two. The reduced growth was not clearly related to low dissolved oxygen, but it may have played a role in reducing oyster growth. As expected, dissolved oxygen levels improved into the fall of 2001 reducing the potential stress on oysters at the project site.

Drought conditions throughout the region affected mean salinity levels, which were higher than normal. As oysters were free from infection with *P. marinus* throughout

much of the 2001 sampling season, this high salinity did not affect the prevalence of *P. marinus*, but may have contributed to the infections detected in the November 2001 sample. The growth rate for oysters was lower than expected in the first growing season in spite of the higher than normal salinities for the region. Although low dissolved oxygen during the summer months may have offset the potential benefit of high salinity in terms of growth rate, there were not enough combinations of dissolved oxygen and salinity to test the effect of salinity and dissolved oxygen on growth of oysters.

Water temperature at the project site followed a seasonal pattern. In 2001, bottom water temperature declined throughout the fall, yet into November, bottom water temperature remained above 10°C, the average temperature noted by Paynter and DiMichele (1990) at which oyster growth ceases in the Chesapeake estuary. This moderate bottom water temperature may have allowed oysters to continue growing into November.

The densities of oysters used in the study were representative of natural populations, such as those found colonizing oyster reefs in Virginia. Intertidal reefs constructed in Virginia and colonized by the natural settlement of oysters showed densities of up to 834 oysters/m^2 (O'Beirn et al 2000). The Kitts Marsh study contained densities of oysters higher than the 0 – 250 oysters/m^2 found on harvested natural oyster bars in the Maryland portion of the estuary (Vanisko et al 2002).

In this experiment, high density plots of oysters varied from 500 to 900 oysters/m^2. At these densities, density-dependent mechanisms should have resulted in a reduction of oyster growth, yet oysters grown at median densities of up to 450 oysters/m^2 displayed

maximal growth rates during the first growing season. One quadrat sampled during the study indicated densities of oysters greater than 1200 oysters/m^2, yet no difference in growth was observed between these oysters and others sampled from quadrats containing lower densities of oysters. When compared to a previous study of oyster growth in the Patuxent River, the 3.5 mm/month (±0.11) growth rate of oysters during the first growing season at the Kitts Marsh site was less than half of the 8 mm/month (±1.4) first year growth rate of oysters grown at an adjacent site in 1997, (Paynter 2001). An historic study in the Patuxent River, showed growth rates for oysters varying from 3-6 mm/month (Beaven 1952). Other studies in the Chesapeake estuary, which measured the growth of oysters in trays determined rates as high as 8.3 to 16.7 mm/month, (Paynter and Mallonee 1990, Paynter and DiMichele 1990). While it is unreasonable to expect oysters grown on the bottom to match the growth rates of animals grown in an aquaculture setting, the first year growth rate of oysters at Kitts Marsh was lower than expected. This cannot be explained by disease pressure either since samples tested were found to be free from infection throughout the first growing season.

The apparent winter growth between October 2000 and May 2001 was likely due to growth of oysters in the fall 2000, after the October sampling. Oyster growth resumed in 2001, again with no reduction in growth due to the density of oysters. The growth rate of oysters in 2001 was lower than the previous year, yet this second year growth rate for oysters was 2.0 mm/month, versus the 1.2 mm/month second year growth rate reported for oysters grown elsewhere in the Patuxent River in previous years (Paynter 2001). Poor water quality conditions seen in August 2000 were also noted for the summer 2001, yet the growth rate of oysters was more typical for second year animals. By the end of the

second growing season, oysters had reached a mean shell height of 83.4 mm (±0.76), greater than the marketable size of 75 mm. Even more remarkable was that 75% of the oysters on the bottom had reached marketable size in two years rather than the three to four year period typically accepted for the Chesapeake estuary (Beaven 1952, Hargis and Haven 1988).

The ability of the oysters to grow at similar rates across all density levels tested may be due to vertical growth, which allows for oysters to avoid competition for space and increase the delivery of food. The roughness of the oyster reef increases with this vertical growth therefore increasing turbulence and reducing the benthic boundary layer above the oyster reef (Dame 1996, Lenihan 1999). The natural processes of growth and recruitment allow the oyster reef to support maximal growth conditions at high densities in the face of sedimentation. Lack of *P. marinus* in the oysters at Kitts Marsh aided in the maintenance of maximal second year growth rates. If oysters do not continue to grow or recruit to the site, this mechanism will fail to maintain the reef height above the bottom and sedimentation of the reef will lead to death of oysters and burial of the reef substrate. This is likely the case for many of the former oyster reefs in the Chesapeake estuary, (Rothschild et. al. 1994)

Typically, oysters follow a seasonal pattern for condition, decreasing in condition index from spring to summer, and then increasing from summer to fall (Korringa 1952). The drop in condition from the spring to summer is typically due to spawning of oysters, while the increase from summer to fall is due to increased fat stores. Mirroring the results of oyster growth, density of oysters did not influence oyster condition. In 2000,

oyster condition did not change from spring to summer. This may have been due to the size of oysters, a lack of spawning by the animals, or both. In the fall, oyster condition did increase as expected. An improvement in local water quality conditions, the increased size of oysters, or both may have aided this increase in oyster condition.

In the second growing season, condition of oysters followed a seasonal pattern, with oyster condition decreasing from spring to summer and then increasing into the fall. The larger size of animals, spawning of animals prior to the summer sampling, low dissolved oxygen levels during the summer months or all of these factors may have contributed to the decline in condition. Cooling fall temperatures allowed for an increase in dissolved oxygen levels and the increase in oyster condition. Densities of oysters did not influence condition during the 2001 sampling season. This may be due to the same mechanisms that allowed for the maintenance of uniform growth rates of oysters in the second growing season.

During the winter of 2000 – 2001, a density-independent mortality event reduced the overall densities of oysters across the Kitts marsh project site. In spite of this reduction in density, the intention of the experiment was preserved, as medium, low and zero density plots remained. The mean density of oysters across all plots dropped from nearly 400 oysters/m^2 in the fall of 2000 to 260 oysters/m^2 in the spring of 2001. The reduction in density of oysters was not due to *P. marinus* infection, as oysters tested negative for the pathogen through the summer of the second year.

P. marinus is found throughout the Chesapeake estuary. In the Patuxent River, Brooms Island oyster bar had *P. marinus* prevalence of 100% in 1999 and 94% in 2000, with infection intensities of 4.6 and 4.0 respectively (MD DNR 2001). Although *P. marinus* levels in the Patuxent River are generally high, the study area was not located adjacent to an active natural oyster bar. Oysters did not acquire *P. marinus* infections throughout the 2000 sampling season, nor through August 2001. In November, *P. marinus* was detected in 4 of 36 samples processed, indicating that oysters acquired infections after the August sampling. The results of this study support the work of Andrews and Ray (1988) in that oysters will not acquire *P. marinus* infections if isolated from diseased populations.

Colonization of the plots by fouling organisms occurred from April to August 2000. The development of the oyster reef community revealed an interesting relationship between oysters and barnacles. In October 2000, barnacle density was related to oyster density, with fewer barnacles present in quadrats with high densities of oysters. This may be due to physical damage to the barnacle larvae by the filtering activity of oysters, lack of settlement cues in areas containing oysters, or a combination of these and other factors.

Another barnacle set occurred between October 2000 and May 2001, although it may be possible that the settlement event did occur before the October sampling and was not detected during the processing of samples due to the small size of the post-set individuals. A large barnacle mortality event was observed across all quadrats between August and November 2001, but this decrease was independent of the presence or abundance of oysters. The reduction in barnacle abundance was likely due to the

sedimentation of the site, which reduced the available surface area, buried existing barnacles, or both.

The abundance of mussels increased throughout 2000 due to multiple settlement events. In August, the abundance of mussels was positively correlated to the presence of oysters. In October, quadrats containing high and medium densities of oysters had greater abundances of mussels than quadrats containing low or zero densities of oysters. Unlike barnacles, mussel settlement increased with the presence, and in some instances density, of oysters.

Over the winter of 2000 – 2001, mussel abundance did not change. Between August and November, a mussel set occurred, increasing the abundance of mussels across all densities of oysters. In 2001, the abundance of mussels was independent of the density of oysters. Unlike barnacles, mussels were not affected by the sedimentation.

Anemone settlement occurred between April and August as well as between August and October 2000, as indicated by the number of anemones per quadrat. Throughout 2001, anemone abundance decreased across all quadrats. Throughout the study, the abundance of anemones was independent of the density of oysters. As with the decline of barnacles in the fall of 2001, it is likely that the decline in anemone abundance was due to increased sedimentation of the plots, resulting is reduced surface area, burial of anemones or both.

Although no quantitative measurements were taken, diver observations indicated that sedimentation of the plots was high, especially in quadrats with high densities of oysters.

The growth of dense clusters of oysters is known to increase the delivery of food to the reef by altering the flow of water over the reef. Coupled with the filtering activity of oysters, the accumulation of feces and pseudofeces, and modern increased sedimentation rates, it is possible that high densities of oysters may bury themselves if no sufficient scouring or re-suspension mechanism is available (Newell 1988). Sedimentation of the reef structure may be an important mechanism in controlling epifaunal community abundance by reducing interstitial space within the oyster reef. This is likely the case with barnacles and anemones. In November 2001, both barnacle and anemone abundances declined across all quadrats. A reduction in surface area combined with burial of existing organisms would likely result in the declines noted. Interestingly, mussel abundance was not reduced in the same manner and in fact, increased.

Currently, harvestable natural oyster bars in Chesapeake estuary have densities of oysters varying from 0 to 250 oysters/m^2 (Vanisko et al 2002). If properly managed, these oyster bars could be seeded with high densities of disease free seed from either hatcheries, natural production or both, and harvested in a rotational manner, as described by Andrews and Ray (1988). This type of managed harvest would provide a greater return to the commercial fishery due to reduced mortality. In addition, a rotational system would provide greater ecological benefit to the estuary due to the higher densities of oysters and a longer residence time of these populations. Additionally, the longer residence time of oyster populations would allow for increased development of the epifaunal community, further increasing the potential ecological benefit of the reef.

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