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Early performance of constructed oyster reefs in Great Bay, New Hampshire

Mark K. Capone

University of New Hampshire, Durham

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**EARLY PERFORMANCE OF CONSTRUCTED OYSTER REEFS IN
GREAT BAY, NH**

BY

MARK K. CAPONE
BA Vassar College, 2001

THESIS

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Master of Science

in

Zoology

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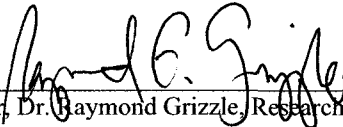
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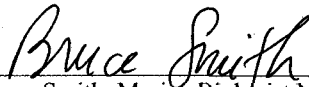
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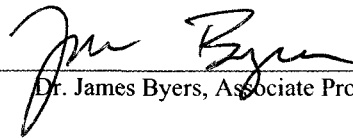
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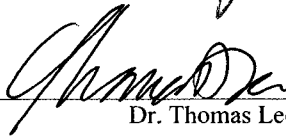
Thesis Director, Dr. Raymond Grizzle, Research Associate Professor of Zoology



Bruce Smith, Marine Biologist New Hampshire Fish and Game



Dr. James Byers, Associate Professor of Zoology



Dr. Thomas Lee, Associate Professor of Natural Resources

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ABSTRACT

EARLY PERFORMANCE OF CONSTRUCTED OYSTER REEFS IN GREAT BAY, NH

By

Mark K. Capone

University of New Hampshire, May, 2008

Several oyster reefs were constructed in Great Bay, New Hampshire using remotely-set oysters. A single large reef treatment and a cluster of several small reefs treatment were utilized to test hypotheses relevant to oyster restoration design, and to monitor early restoration reef performance. There was no significant difference in oyster size, density, and recruitment between two experimental reef structures, with both reef types having high survival and fast growth rates for the 2- year study. Both experimental reef structures had significantly higher recruitment rates than natural reefs in 2006, a year of relatively high recruitment ($p < 0.05$), and elevated yet not significantly higher recruitment rates in 2005, a weak recruitment year ($p = 0.078$). *In situ* fluorometry data showed that a restored reef can significantly impact chlorophyll-a levels in overlying water within two years of reef construction. Individual oyster clearance rates ranged from 1.87 L/hr – 2.41L/hr.

CHAPTER 1

BACKGROUND INFORMATION

Introduction

The current study is composed of three sections. Each section poses questions relevant to oyster restoration in Great Bay, NH (Fig. 1) and to restoration ecology in general. Chapter 2 describes the first 2 years of development of experimental reefs with different structures, specifically looking at the effects of reef size and structural orientation on restoration success by comparing single large reefs (single, 6 m diameter) to clusters of several small reefs (four, 3 m diameter). Chapter 3 describes a 2-year natural oyster recruitment experiment at three scales and provides data and analysis relevant to future site selection and structural design of restoration reefs. Chapter 4 describes a new *in situ* protocol to measure seston uptake or clearance rates (the amount of water filtered by oysters per unit time) of restored and natural oyster reefs. *In situ* seston uptake measurement may provide a relatively inexpensive means for resource managers to quantify the success of restoration projects. Combined, these three experiments track the early performance (from construction to 2 years age) of experimental restoration reefs. To appreciate the significance of the current study, one needs to have a basic understanding of the natural history of oysters, and a synthesis of relevant studies has been provided in the remainder of this chapter.

General Ecology of Oysters

The eastern oyster, *Crassostrea virginica* (Gmelin), inhabits coastal marine and estuarine waters of the eastern North American coast from New Brunswick, Canada to the Gulf of Mexico (Galstoff 1964). Oysters are sessile organisms occurring either singly, in clumps or clusters or forming aggregations called reefs. Throughout their range oysters live predominantly in the subtidal zone, with the exception of regions in the Southeast from Virginia into the east coast of Florida where oysters predominantly inhabit the intertidal (Burrell 1986).

Oysters are important both economically and ecologically. Designated a “keystone” species, oysters provide habitat for numerous fish and invertebrate species (Bahr and Lanier 1981, Zimmerman et al. 1989, Coen et al. 1999, Harding and Mann 2001), link pelagic and benthic food webs (Newell and Jordan 1983, Dame et al. 1984), and improve water quality by reducing sediment and nutrient concentrations in the water column (Mann 2000, Nelson et al. 2004). A history of sustenance and commercial exploitation in addition to disease and habitat degradation has led to a decline in oysters throughout their range. This decline, coupled with the increasing recognition of the ecological importance of oyster reefs, has prompted a focus on oyster reef restoration.

The eastern oyster, like many sessile marine invertebrates, exhibits a bipartite life cycle with pelagic larvae and sessile adults. Adult oysters are broadcast spawning, protandric organisms (Coe 1943), with young individuals functioning as males and older individuals as females (Andrews 1979). Although oysters generally switch from male to female as they age, this process can be reversed (Needler 1942) with oysters reproducing as females one year and then as males the next. Spawning events are correlated with

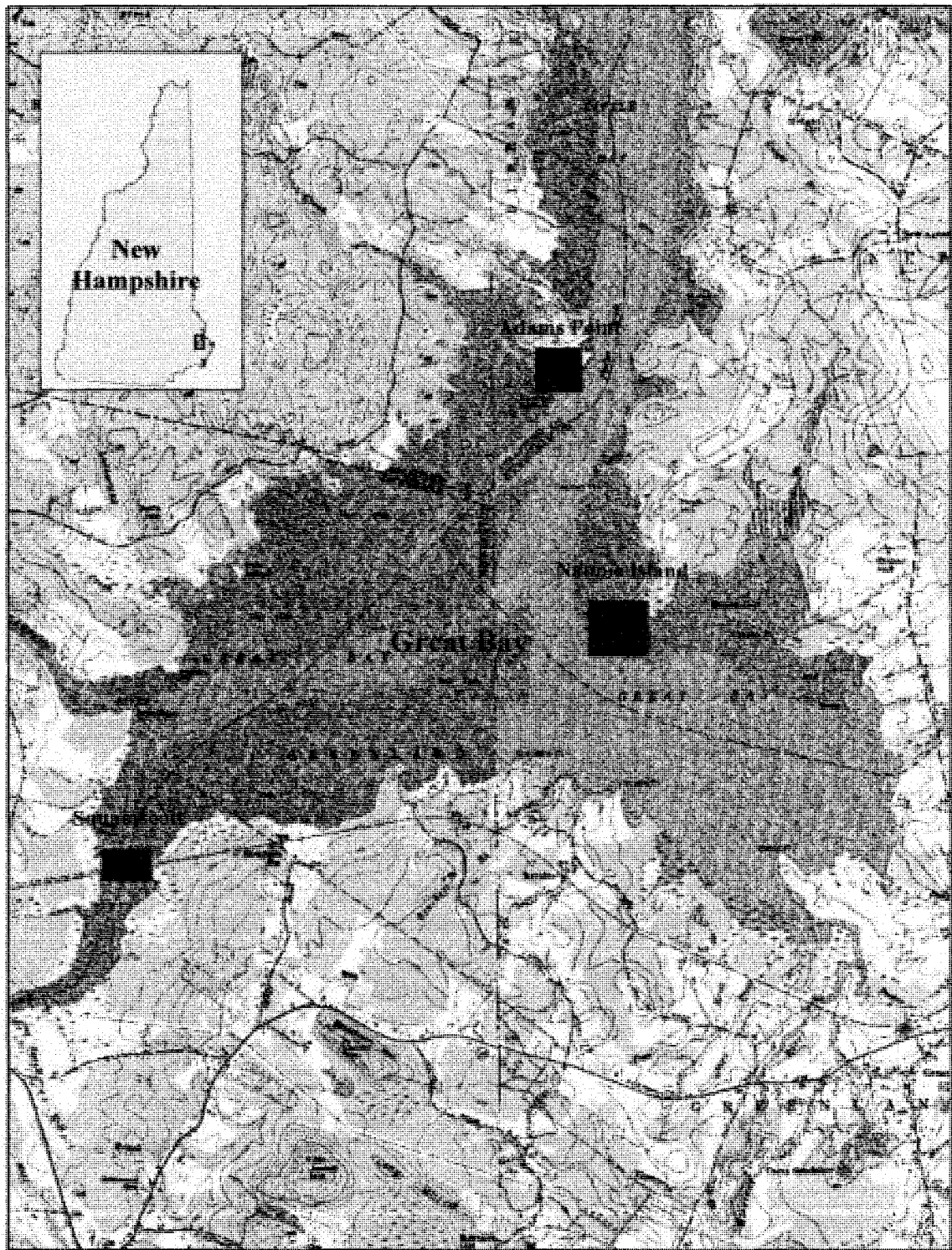


Fig. 1. Major oyster reefs in Great Bay, NH.

optimal conditions for larval growth; high water temperatures and adequate planktonic food availability (Thompson et al. 1996). The interaction of salinity, temperature, and pheromones (Geise 1959, Sastry 1975, Thompson et al. 1996), in addition to adult condition, provide a cue for initial gamete release. Eggs are fertilized externally and develop into larvae. Larvae exist in the pelagic zone and are distributed passively by water movement and perhaps to some extent actively through swimming or sinking in response to environmental cues for the duration of their 2-3 week development period. The larval stage ends with settlement, which is culminated by metamorphosis. During metamorphosis larvae cement their left valve to a substrate and become permanently attached (Harper 1992).

Oysters require hard substrate for colonization. Oyster habitat is commonly rocky-bottom; however, firm mud is also suitable provided that some hard substrate is initially present (wood, bottles, rock, shell etc.) for larval settlement. Oysters provide substrate for further larval settlement as settlement is enhanced by bacterial films on the surface of oyster shells (Weiner et al. 1985, 1989). In this manner, oysters form dense reef aggregations with a high degree of vertical relief and spatial complexity as newly settled oysters attach and cement together older oysters and remnant shells.

The environmental tolerances of oysters vary with development, as larvae and spat have lower tolerances than adult oysters. Adult oysters, as exhibited by their wide range and intertidal and subtidal distributions, are tolerant of a variety of environmental conditions. Oysters are subject to extreme temperature conditions, ranging from winter water temperatures as low as -2° C in northern New England and Canada (Galstoff 1964, Loosanoff 1965), although growth ceases below about 8° C (Price et al. 1975), to

intertidal temperatures as high as 49.5° C in the Gulf of Mexico (Ingle et al. 1971). Rate of temperature change has a greater impact on oyster survival than temperature extremes (Shumway 1996), as rapid temperature reductions and temperature increases (Fingerman and Fairbanks 1957) cause high mortalities at temperature levels known to be tolerated by oysters. Oysters occur in regions with salinities that range from 0 to 42.5 ppt (Ingle and Dawson 1950). Optimal salinities for growth range from 14 to 30 ppt (Moore 1900, Galstoff 1964, Castagna and Chanley 1973). Extended exposure to low salinities (below 2 ppt) associated with spring floods can be fatal (Andrew et al. 1959, Burrell 1977). Extended exposure to high salinities, in general, lead to reduced growth and fecundity (Shumway 1996), although exceptions do exist (Breuer 1962) suggesting the existence of multiple physiological races each adapted to local conditions (Menzel 1955, Andrews 1979). Oyster resilience to low dissolved oxygen is highly dependent on temperature as oxygen consumption increases with temperature. However, oysters can survive for as long as 3 days anaerobically (Galstoff 1964) and persist in dissolved oxygen conditions as low as 1 ppm (Andrews 1982).

In regions with appropriate environmental conditions and adequate larval supply, oyster reefs or beds will form as generation after generation of oysters cement to each other. Oyster habitat shape and area can be variable (Winslow 1881), in some instances forming high vertical relief reefs extending from the seafloor near to or above the mean low water mark. In areas of moderate to heavy fishing effort, reefs will have a lower profile and are often described as beds.

Exploitation

Human exploitation of the eastern oyster predates European colonization, as is evidenced by extensive shellfish middens excavated along the Atlantic coast of the United States (Lunz, 1938, Snow 1972, Braun 1974, and Brennan 1974 & 1976). The ecosystem effects of prehistoric oyster exploitation can only be estimated from a detailed analysis of these midden sites. Radiocarbon dating of shells from Hudson estuary sites in New York show that Amerind Indians harvested oysters nearly 7,000 years ago (Brennan 1974), but intensive shellfish exploitation throughout the Northeast coast probably did not occur until 2,000 BP (Snow 1972). Studies at archaeological sites of mid-Holocene hunter-gatherers in Europe have shown that marine foods were a major component of early coastal peoples' diets (Mannino and Thomas 2002).

Mannino and Thomas (2002) asserted that prehistoric peoples were capable of overexploiting shallow marine resources such as oysters and other shellfish. Recent ecological studies support this hypothesis as it has been shown that small-scale harvesting can have a significant effect on target shellfish species (McLachlan et al. 1996). Shell size and relative shell abundances of preferred or optimal species within middens have been used to determine levels of exploitation (Hockey and Bosman 1986). Middens from numerous New England sites show a shift in the relative importance of different shellfish species to prehistoric hunter-gatherer diets. The utilization of oysters peaked approximately 1,500 years ago for both Martha's Vineyard (Ritchie 1969) and Boston Harbor (Braun 1974) and subsequently declined in both areas to be replaced by the soft shell clam, *Mya arenaria*. This shift could be due to cultural preferences or overfishing of oyster, as the infaunal soft shell clam requires greater effort and time than epibenthic

oysters to harvest. Optimal foraging theory (MacArthur and Pianka 1966) supports the latter explanation.

Early accounts of eastern oysters by European explorers and colonists elaborate on the abundance and extent of oyster reefs along the coasts of the New World. Francis Louis Michel stated in 1702:

The abundance of oysters is incredible. There are whole banks of them, so that the ships must avoid them. A sloop, which was to land us at Kings Creek, struck an oyster bed, where we had to wait about two hours for the tide.

The first commercial fishing of the eastern oyster started in the Hudson River Estuary in the early 1600s (Ingersoll 1881). Early colonial harvesters utilized hand tongs and later patent tongs and small canoes, which allowed the exploitation of deeper water. Oysters appeared at first to be an inexhaustible resource, but it was clear to some that high levels of exploitation could not be maintained:

In North America the oysters are so fine and cheap that they are eaten daily by all classes. Hence they are now, and have been for a long time, a real means of subsistence for the people... but as the number of consumers increases in America the price will also surely advance and then there will arise a desire to fish the banks more severely than hitherto and if they do not accept in time the unfortunate experience of the oyster culturist of Europe, they will surely find their oyster beds impoverished for having defied the biocenotic laws. (Karl Mobius quoted by Sweet 1941)

The northern estuaries succumbed to fishing pressure first, likely owing to higher human population densities and physical factors influencing the distribution and reproduction of oysters. New England and New York oyster fisheries collapsed in the late 19th century (Kirby 2004). The collapse of northern oyster populations put additional pressure on southern estuaries, which were relied on for “seed” oysters to be transported to

impoverished waters. Declines in oyster densities caused by exploitation were further compounded by habitat degradation and the onset of two oyster diseases: Dermo (*Perkinsus marinus*) in the 1940s and MSX (*Haplosporidium nelsoni*) in the late 1950s (Ford and Tripp 1996).

Restoration

The first American restoration efforts consisted of transporting oysters from areas of abundance to depleted areas. This early restoration attempt occurred in the 1840s (Sweet 1941) and continues to the present. Following the practice of transferring oysters to depleted regions, fishermen began to notice that seed oysters would occasionally catch sets of young oysters. This led to the first recorded experimental planting of oyster shell as a means to attract oyster spat in 1847 in Norwalk, Connecticut (Sweet 1941). Shell planting spread rapidly throughout the northern estuaries as a relatively inexpensive means of increasing fisheries yield. As with the transfer of seed oysters, shell planting remains a popular method for restoring oysters, but these methods rely on either the presence of high density oyster beds or the natural transport of larvae to restoration sites, two requisites which may not be present in exploited regions (Hargis and Haven 1999). A third method, remote setting, has recently gained popularity in estuaries with low or inconsistent spat set. Remote setting involves the use of hatchery produced oyster larvae to restore depleted areas (Castagna et al. 1996). Oyster larvae are set on cultch material in large tanks and the resulting “spat-on-cultch” are transferred to the desired restoration location.

The success of oyster restoration for fisheries has varied from region to region depending on the technique utilized and the local physical and biotic conditions (Paynter 1999), with success measured as an increase in fishery yield. The success of oyster restoration for ecological function is not as easily quantified. Oyster reefs have gained increased attention for the ecosystem services they provide (Breitburg et al. 1995, Coen and Luckenbach 2000), including habitat complexity (Breitburg et al. 1995, Lenihan et al. 2001), and water quality improvement through filtration (Newell 1988, Newell et al. 2002). The science and practice of oyster restoration for ecological function is in its infancy and a large amount of money is spent throughout the east coast of the United States on ecological restoration with few quantifiable successes (Hargis and Haven 1988, Frankenberg 1995). Researchers and resource managers are struggling to identify the most effective restoration strategies to enhance ecological function as well as means for quantifying the success and value of non-fishery based restoration.

This thesis addresses three topics relevant to effective restoration strategies and the quantification of restoration success. I provide descriptive data for early constructed reef processes and test a number of hypotheses in a two-year study of two constructed reef structures. I monitor oyster recruitment for two-years at several scales critical to the proper site selection and structural design of future restoration work in Great Bay, NH. Lastly, I describe and utilize a new method to quantify water quality improvement, an ecological function that is commonly associated with oyster restoration.

CHAPTER 2

EXPERIMENTAL REEF DESIGN, CONSTRUCTION, AND DEVELOPMENT

Introduction

Unexploited oyster reefs have a biogenic structure far different from reefs or beds found in areas that either are currently or were historically known to sustain a commercial or heavy recreational fishery (Hargis and Haven 1999). Unexploited reefs are characterized by a complex, three-dimensional structure, extending as much as two meters from the substrate towards the water surface and in some regions breaking the water surface at low tide (Kennedy and Sanford 1999). These reefs are comprised of an outer veneer of live and growing oysters over a base and center of shell formed by previous generations of oysters. The three-dimensional structure is easily disrupted by mechanical and tong harvest, leaving habitat of a distinctly different morphology and perhaps function. Exploited oyster reefs, also called beds, because of their flat morphology, often exist as a mix of live oysters lying flat or orientated vertically as singles or small clumps and shell on the substrate.

High relief, three-dimensional reef structures provide a number of benefits to oysters and associated organisms through physical-biological coupling especially increased flow and seston flux (Lenihan 1999). The influence of physical structure on biological populations has received a great deal of attention both in terrestrial and marine settings (Bell et al. 1991). Lessons from unexploited oyster reefs' structure could be

applied to the construction of restoration reefs; however, there have been few studies to quantify the success of constructed oyster reefs of different structures. Lenihan (1999), in a large-scale field experiment, found that constructed oyster reefs with higher vertical height had increased growth and survival as well as less susceptibility to anoxic events.

Although oyster larvae will settle on many different kinds of substrates, oyster shell is a preferred substrate for oyster larval recruitment (Crisp 1967, Veitch and Hidu 1971) and supports greater oyster growth and survival than some other available shell types in constructed reefs (Nestlerode et al. 2007). Unfortunately, oyster shell has become a limited resource because of use for other purposes such as lime and as a base material for roads and driveways. Therefore, it is critical for restoration scientists to determine how to optimize their limited resources to restore the greatest amount of habitat with self-sustaining reefs.

This chapter describes an experiment designed to compare large constructed reefs with multiple smaller reefs of equal total area. This question is a restoration ecology extension of the Single Large or Several Small (SLOSS) debate regarding the design of reserve systems that followed the publication of MacArthur and Wilson's (1967) work "The Theory of Island Biogeography." This text brought the relationship between population dynamics and habitat spatial characteristics to the forefront of ecological thinking, and continues to guide the actions of conservation planners and restoration scientists to this day.

Methods and Materials

Experimental Design

The growth and survival of remotely-set oysters on two constructed reef treatments near Nannie Island within Great Bay, NH were compared in this study. Natural oyster recruitment was compared between two constructed reef treatments and two control treatments, harvested natural reef and unharvested natural reef. In addition, oyster size and density were compared between all treatments to assess how successfully constructed reefs establish and maintain oyster assemblages compared to natural reefs.

Eight experimental reef plots (25 m x 25 m) were sited on existing degraded oyster habitat, areas with few living oysters and no topographic relief. Existing natural oysters were removed from experimental plots (control plots were unaltered) with rakes and by hand picking. Four large (6 m diameter) and four clusters of four small (each 3 m diameter) restoration reefs were constructed, with a single large reef or a cluster of four small reefs in each plot (see Fig. 2 and 3). Each restoration reef was composed of a crushed granite base-layer covered with a veneer of live remotely-set oysters on cultch.

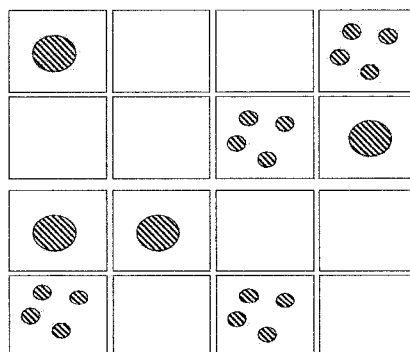


Fig. 2. Experimental design for this study. These 16 , 25 x 25m plots were located in a “no harvest” area. Shaded circles represent single large and several small constructed reefs. Four of the 8 empty or control plots were randomly selected to be unharvested control treatment replicates.

A single-factor (reef type), four treatment experimental design was utilized in this study. The four treatments: single large constructed reef, several small constructed reef, harvested control reef, and unharvested control reef, were each replicated four times (Fig. 2), with each replicate having four subsamples. The unharvested control reef samples were randomly taken in 25 x 25 m plots within the closed for harvest area. Harvested control samples were randomly taken in 25 x 25 m plots outside, but adjacent to the closed for harvest area.

The dependent variables, growth and survival of remotely-set oysters, and oyster recruitment, were compared between the single large constructed reef and the several small constructed reef treatments. The dependent variables, oyster size and oyster density were compared across all treatments. The dependent variable, recruitment, the number of oysters naturally setting on reefs, was also compared across all treatments.

Data were analyzed using JMP 5 software. Analysis of variance (ANOVA) was used to compare means. *A posteriori* Students t-test was used when significant differences occurred between means.

Remote Setting

Remote setting techniques developed at Jackson Estuarine Laboratory (see Grizzle et al. 2003) based on standardized protocols (Castagna et al. 1996, Supan et al. 1999) were used to promote the setting of 10 million oyster larvae (fast-growth broodstock, from Damariscotta River, Maine spawned by Muscongus Bay Aquaculture). Two, 2,000-gallon tanks were filled with filtered seawater (Fig. 4). Concrete blocks were broken into irregular pieces (<20 cm in longest dimension) and washed with a power

washer to remove sediment. Bags were made with Vexar mesh (1 cm openings) and cable ties, and filled with broken concrete pieces to provide substrate (“cultch”) for oyster larvae settlement. Mesh cultch bags were then hung in tanks for 4 days prior to the addition of larvae to allow for the growth of biofilm on the cultch material.

Eyed-larvae (15-20 days old) were shipped in a cooled container wrapped in a moist mesh towel. Larvae were separated into approximately two equal groups and added to the two tanks. Fine mesh nets were used twice daily to sample for the presence of larvae in the water column. Sampled larvae were viewed under 30x magnification using a microscope and stomach content was assessed to determine if food was limited. Algal paste was added to tanks when a majority of sampled larvae stomachs were empty. Most larvae had set by 7/6/04, as was determined by a scarcity of larvae in water column samples. This was verified by the analysis of cultch material under microscope.

Nursery

Oyster spat-on-cultch were transferred by boat to a nursery raft near Jackson Laboratory on 7/9/04. Vexar bags were suspended in the water column on 2-inch diameter steel pipes from 7/9/04 to 9/15/04 in a high flow environment. Vexar bags were checked weekly and cleaned of fouling organisms, as fouling tends to clog mesh opening and stifle water flow to the growing oysters.

Reef Construction

Approximately 32 m³ of crushed granite (<5 cm in longest dimension) was transferred by barge to the experimental plot sites. Crushed granite was dropped to form

reef mounds of the desired diameters (6 m or 3 m) with approximate vertical relief of 0.2 m. The four reefs in the several small treatment were separated by approximately 1 m. Remotely set oysters (spat-on-cultch) were spread over the crushed granite base-layer by hand to maintain consistent densities.

Reef Protection

Restoration reefs were constructed in an area previously open to recreational harvest. To protect restored oyster reefs, and to minimize human disturbance to the experimental restoration plots, letters were sent to New Hampshire recreational shellfish license holders notifying them of the restoration and subsequent harvest closure. A sign indicating the experimental plots closed to harvest was posted in the center of the area and marker buoys were set at the corners.

Field Methods

Constructed reefs, the harvested control reef, and the unharvested control reef (see Figs. 2 and 3) were sampled in the spring and fall over two years: 28 October 2004, 1 June 2005, 24 October 2005, and 28 June 2006. During each of the four sampling periods, four replicate 0.10 m² quadrats (selected randomly by assigning a number scheme to each reef area and choosing numbers from a random number table) were excavated (oysters and hard substrate) by snorkelers. All live oysters were counted, and shell height was measured to the nearest millimeter with Vernier calipers. Excavated material was then returned to reef locations.

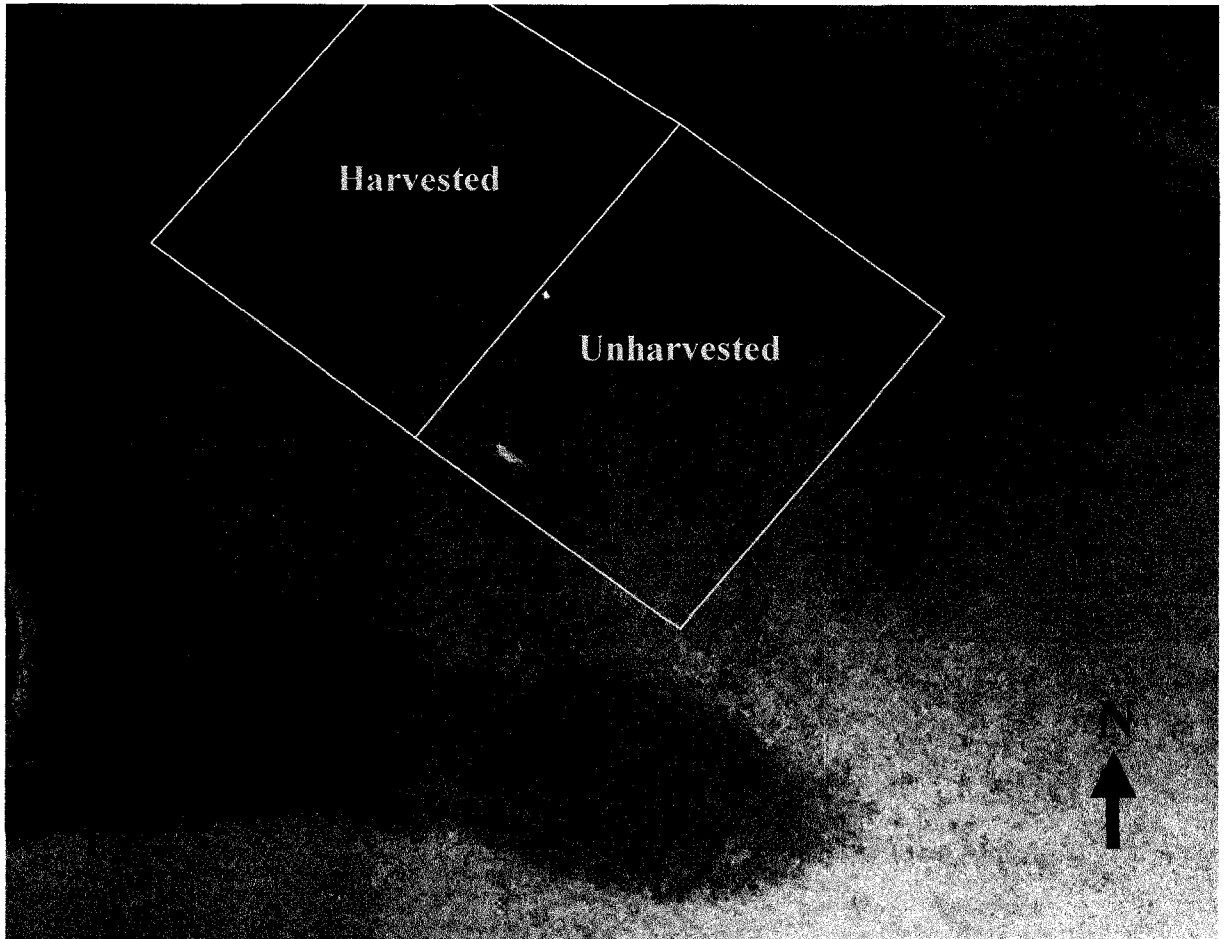


Fig. 3. Aerial view of experimental oyster reefs. The unharvested area box highlights the location of four 6m diameter reefs and four groups of four 3m diameter reefs. Larger dark circular shapes are the single large reefs and the grouping of smaller dark shapes are the clusters of several small reefs. The harvested area box highlights the harvested control area, the region where natural oyster reefs are exploited by recreational fishery. Also visible in the photograph is a section of Nannie Island in the lower left and Woodman Point in the upper right.

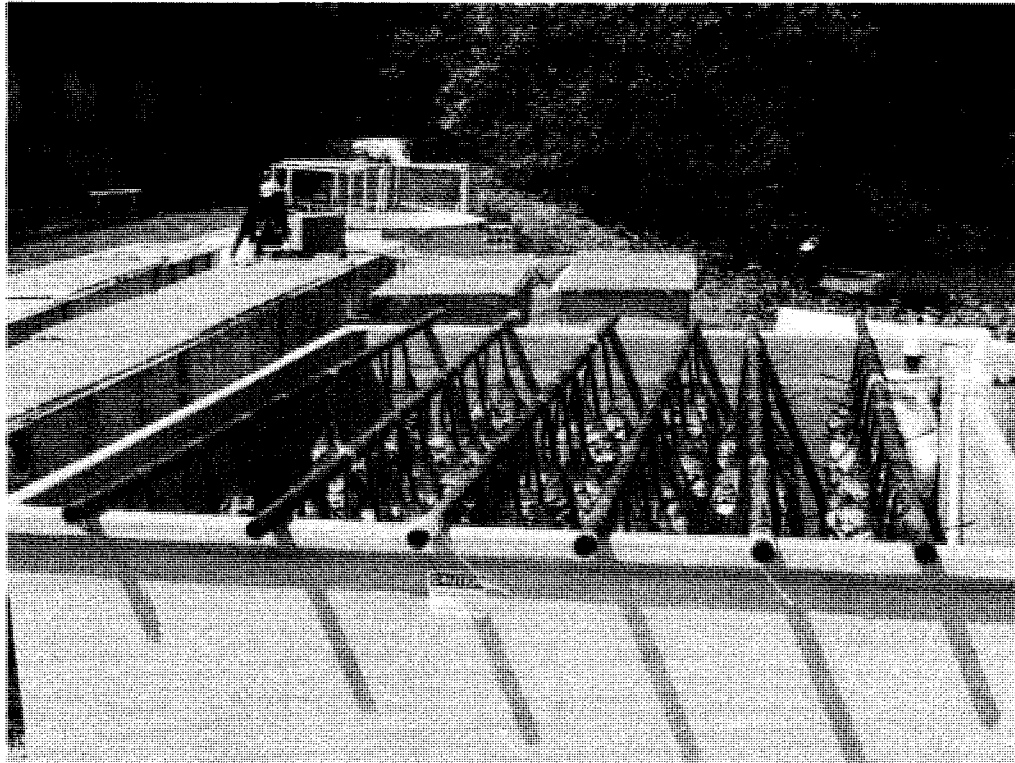


Fig. 4. One of two, 2,000-gallon remote setting tanks. Notice orange Vexar bags, filled with cultch hanging on steel bars.

Results

Initial spat densities for single large and several small experimental treatment reefs were far greater than harvested and unharvested control treatments (see Table 1, Fig. 5). Experimental reef densities remained constant for 13 months after initial seeding. Between 13 and 20.5 months post-spat seeding, both experimental reef treatments experienced high mortalities. There were no significant density differences between the single large and several small treatments throughout the duration of this study (Tables 1 and A 1, Fig. 6), however experimental reefs both had significantly higher densities than the harvested and unharvested control reef from 8.5 months post-spat seeding till the end of the study (Tables 1, and A 2-4).

Table 1. Mean oyster densities for reef treatments over time (error units = 1 standard error).

Mean Oyster Density (oysters/ m ²)				
Treatment	Months, post-spat seeding			
	1.5	8.5	13	20.5
Single Large	131.25 ± 78.54	138.00 ± 44.28	135.50 ± 56.03	61.88 ± 19.56
Several Small	173.13 ± 31.89	165.50 ± 26.20	153.50 ± 36.35	60.00 ± 17.11
Harvested		55.00 ± 13.38	66.88 ± 9.21	10.00 ± 3.68
Unharvested		20.00 ± 9.76	9.38 ± 3.44	18.13 ± 6.24

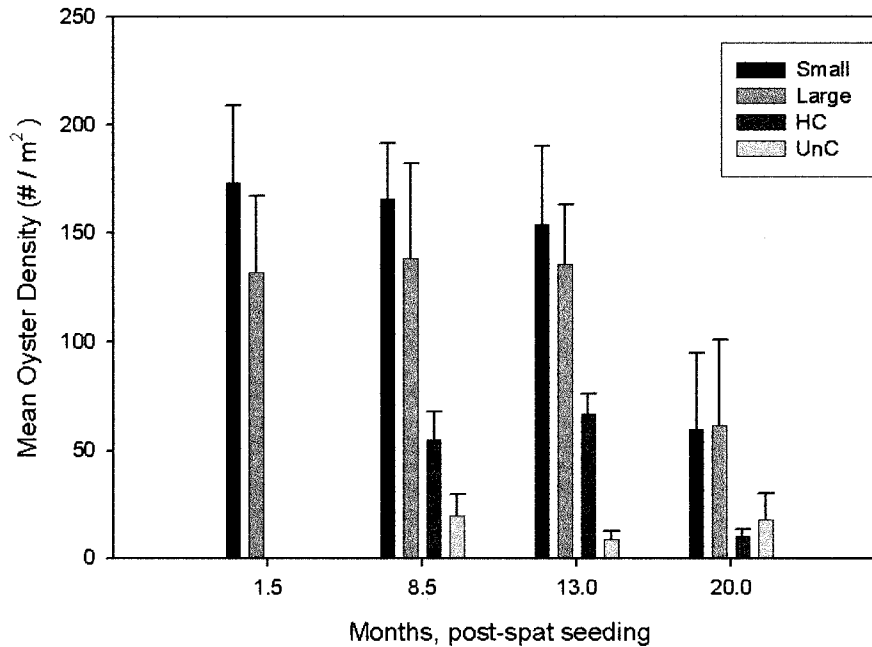


Fig. 5. Mean total oyster densities from October 2004 (1.5 months post-spat seeding) to June 2006 (20 months post-spat seeding) for reef treatments: several small, single large, harvested control (HC), and unharvested control (UnC). (Error bars = 1 standard error).

Oysters on experimental treatment reefs reached sizes comparable to nearby natural reefs within 20.5 months (Table 2, Fig. 6). Mean shell height for experimental treatment reefs did not increase between October and June sampling dates for either year.

Table 2. Mean oyster shell heights for reef treatments over time (error units = 1 standard error).

Mean Shell Height (mm)				
Treatment	Months, post-spat seeding			
	1.5	8.5	13	20.5
Single Large	24.33 ± 1.08	23.28 ± 1.31	58.80 ± 0.67	65.88 ± 4.25
Several Small	24.23 ± 0.65	23.58 ± 0.33	59.53 ± 0.79	60.43 ± 2.12
Harvested		66.73 ± 1.47	66.70 ± 1.48	78.98 ± 1.13
Unharvested		69.80 ± 1.35	73.68 ± 3.05	68.05 ± 22.73

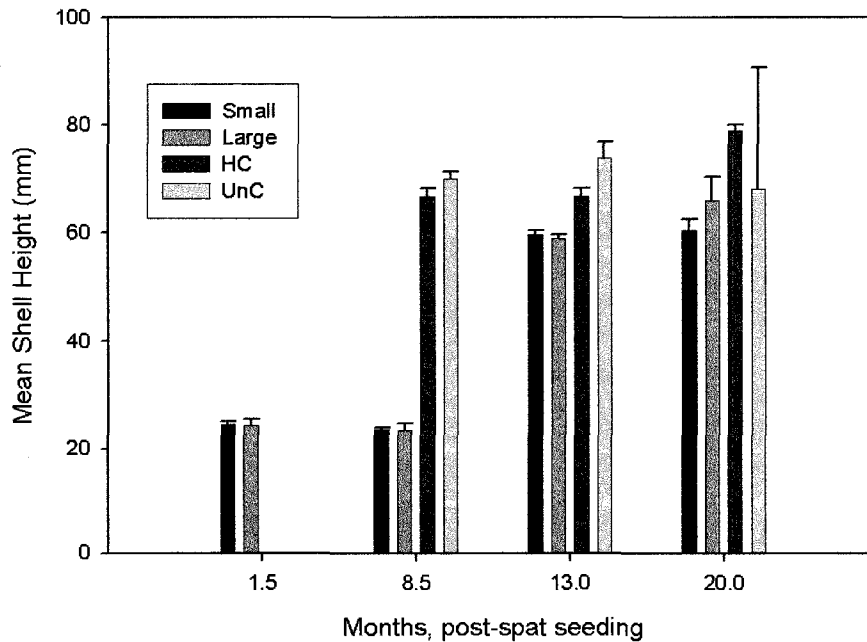


Fig. 6. Mean oyster size or shell height from October 2004 (1.5 months post-spat seeding) to June 2006 (20 months post-spat seeding) for reef treatments (Error bars = 1 standard error).

Natural spat set was significantly higher on both experimental treatment reefs than either harvested or unharvested control treatment reefs ($p < 0.05$, Table A10, Fig. 7). Spat set was measured during the October 2005 sampling period.

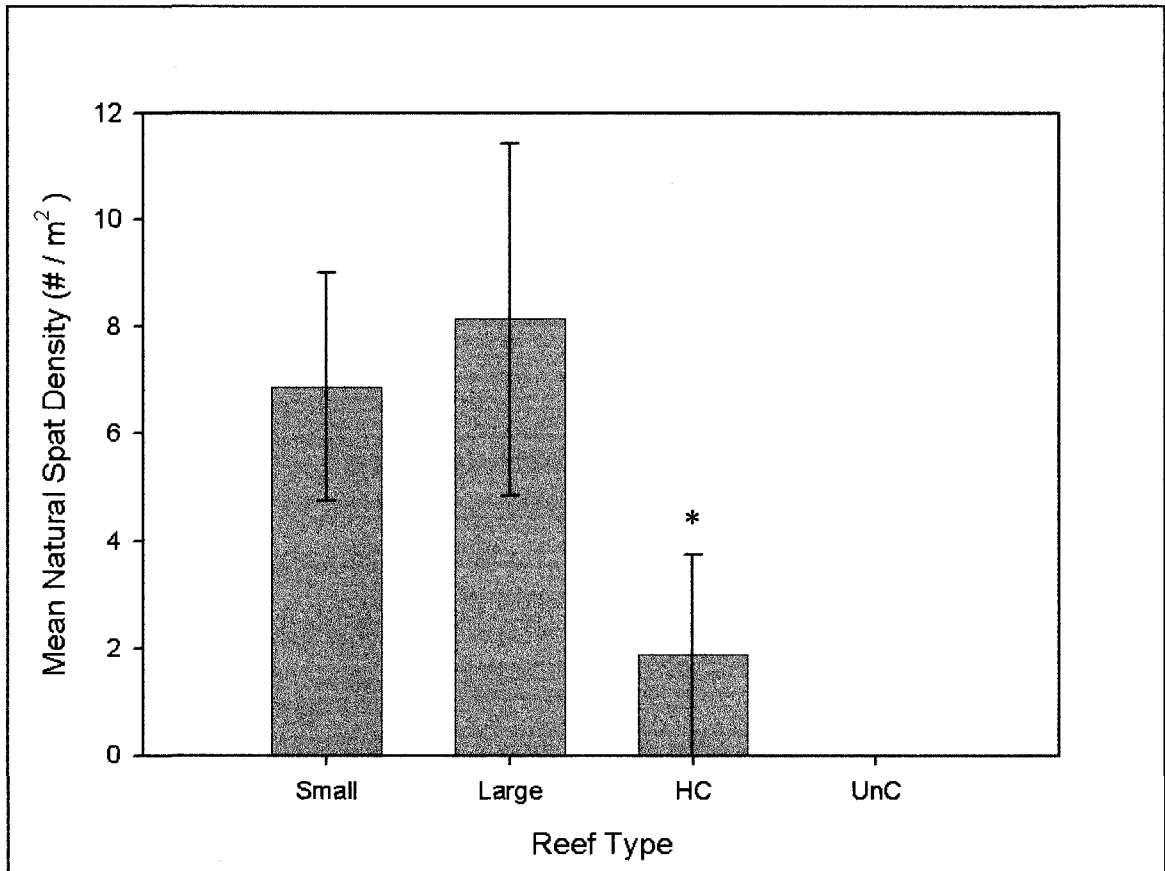


Fig. 7. Mean recruitment, oyster spat (< 40 mm shell height) based on quadrat sampling method October 2005 (Error bars = 1 standard error). Asterisk indicates $p < 0.05$.

Discussion

The present study found no differences in oyster density, mean shell height and spat density between the two experimental constructed reef treatments after two years of development. These results do not necessarily indicate that reef structure has no effect on

early reef processes; they do however show that at the spatial and temporal scales examined in this study, the diameter of restored reefs does not significantly affect the early reef processes studied. Reef structure, specifically the size and configuration of restored reefs, requires more attention. Only one other study has examined the optimal structure for early restoration reef performance, finding a non-significant trend of increased spat density as reef size was reduced from a maximum surface area of 8,000 m² down to 400m² (Luckenbach and Ross 2003). The reefs examined in this study were much smaller and are more representative of reef sizes in Great Bay restoration efforts.

The single large and several small restored experimental reefs had extremely high early survival rates, showing both reef structures to be viable options for future restoration work in Great Bay. In the first 13.5 months after construction, nearly 100% of restored oysters survived. Between 13.5 and 20 months post-construction survival rates dropped to near 40% for the restored reefs, perhaps owing to disease or extremely low salinities associated with high rainfall in the spring of 2006.

Total oyster densities on both restored experimental reefs far exceeded adjacent harvested and unharvested control reefs throughout the 2-year duration of this experiment. Restored oysters on both experimental treatments exhibited fast growth rates and the mean shell heights of the two experimental treatments approached sizes comparable to natural reefs only 13.5 months post-construction. Oyster growth only occurred between the June and October sampling dates. As oyster growth rates are strongly affected by temperature (Butler 1953), it is not surprising that increases in mean size only occurred during a 5 month window from June to October. Loosanoff and Nomejko (1949) found similar limitations in growth season, although slightly extended in

the comparatively warmer Long Island Sound where most oysters showed growth increases during 6 or 7 months.

Both experimental reefs received significantly higher density of spat set than did nearby natural reefs as determined by quadrat sampling. Although, experimental reefs exceeded harvested and unharvested natural reefs in natural spat set, the relatively low spat set (mean values <10 spat/ m^2) found even on these reefs highlights the need for remotely-set oysters in the restoration of habitat in Great Bay. Great Bay, like many northern estuaries (Prytherch 1929, Medcof 1939 and 1955), has a single recruitment peak following one peak spawning period. This is in sharp contrast to many estuaries from Virginia south into the Gulf of Mexico that have numerous spawning events and recruitment occurring continuously from April to November (Ingle 1951, McNulty 1953, Butler 1965, and Kenny et al. 1990). In areas of high recruitment, such as these, the use of remotely-set oysters in restoration projects may not be necessary beyond the addition of clean cultch material. The latitudinal difference in oyster spawning frequency and recruitment highlights the need for different management strategies for this organism throughout its range.

The quadrat methodology used in this study is not ideal for comparative spat settlement studies. As discussed earlier, oyster settlement often requires clean hard substrate, and hard substrate availability appears to be a confounding factor in the current study. For this reason we cannot conclude from this data that experimental reefs affected spat settlement. It is possible that the observed trends in spatfall are simply due to more available hard substrate in the experimental reefs. Underwater observation of natural (control reefs) and restored experimental reefs did in fact reveal an obvious difference in

the availability of hard substrate, as natural reefs were often characterized by sparse distribution of single live oysters, shell fragments, and few articulated shells (dead oysters with both valves still attached, also called “boxes”) on a firm mud bottom. In contrast, restored experimental reefs offered an abundance of hard settlement surfaces in the form of crushed granite, cement blocks, and live oysters. The confounding variable, substrate availability, was removed in a separate; more controlled spat recruitment experiment discussed in the following chapter.

The restored experimental reefs in this study were all constructed to have base layers with approximately 20 centimeters of vertical relief above the substrate. Vertical relief may affect a number of physical variables critical to oyster survival and recruitment by raising reefs into swifter water (Lenihan 1999, Lenihan et al. 1999, Kennedy and Sanford 1999). In the Bellamy River, Great Bay, restoration reefs were constructed by placing remotely set oysters attached to cultch material directly onto the substrate. These oyster reefs experienced high mortality rates over the course of the first 12 months apparently due to smothering by sediment (Grizzle et al. 2006). The current study was not designed to test the effects of reef height on early reef performance, but it is likely that reef vertical relief influenced the success of the restoration project in a manner consistent to previous restoration studies (Lenihan 1999). It must be noted, however, the use of crushed granite as a base layer increases the materials and associated labor costs and may therefore be prohibitive in some situations.

CHAPTER 3

RECRUITMENT STUDIES IN GREAT BAY WITH IMPLICATIONS FOR RESTORATION SITE SELECTION AND DESIGN

Introduction

Self-sustainable restoration reefs require suitable natural spat settlement. Oyster recruitment has been shown to vary spatially and temporally at almost all scales (Nelson 1903), with shells lying side-by-side on a bed often differing dramatically in spat numbers (Nelson 1909), and regions receiving good sets only 8 years out of 57 (Loosanoff 1974). However, it also has been noted that some regions within estuaries consistently have higher recruitment than others (Kennedy 1980). Powell et al. (1995) showed that reef location was the single most important factor in determining accretion, or loss of oyster reefs, in Galveston Bay. Likewise, the success or failure of future restoration work in Great Bay, NH may depend on proper site selection. Restoration site selection has received increased attention in recent years (Kennedy and Sanford 1999), and a number of criteria have been established to aid restoration site planning (Chesapeake Bay OMP 2000). The current state of site selection for restoration within Great Bay involves locating and restoring areas that historically supported oyster populations. Historic resource maps, in addition to bottom video survey, have been used to locate these regions in Great Bay. It is at least preliminarily assumed that if oyster reefs existed prior to harvesting, disease, and deleterious environmental impacts

associated with increased nutrient and sediment inputs, then natural processes in that area would be suitable for reef development. However, it is possible that the initial decline of such areas was caused by changes in water circulation, or sedimentation, and decreased the natural delivery of larvae and spat, making these sites unsuitable for self-sustainable restoration (Kennedy and Sanford 1999, Mann and Evans 2004).

Crassostrea virginica is a broadcast spawning, protandric species, with younger oysters predominantly releasing sperm and older larger oysters releasing eggs. Adult female oysters produce millions of eggs to be shed into the water column (Cox and Mann 1992). The release of gametes is synchronized in local oyster populations to increase fertilization success and consequently maximize the energy spent producing gametes (Levitan 1991, Hay 1997). Environmental cues in the water column, sensed as water is filtered through the gills, induce the release of gametes, and the presence of gametes further stimulates nearby oysters to spawn (Kennedy et al. 1996). An increase in water temperature is one known cue for spawning (Nelson 1928, Galtsoff 1964), although the presence of phytoplankton blooms, and more specifically algal ectocrine cues, also serve to influence spawning (Nelson 1955, 1957; Geise and Kanatani 1987). Reproduction can occur throughout the year in subtropical regions, but in temperate regions mass spawning occurs in the warmest months, generally when water temperatures are above 22° C (Galtsoff 1964). In Great Bay, NH spawning predominantly occurs in the months of July and August (Ayer et al. 1970).

Oysters have a larval period of 2 to 3 weeks depending on food supply and water temperature (Galtsoff 1964). During this period larvae feed on phytoplankton, detritus and bacteria (Kennedy et al. 1996). Oyster larvae are relatively weak swimmers,

reported to swim horizontally at speeds of 0.7- 2 mm/s in moving water (Mileikovsky 1973). However, oyster larvae have been observed sinking or swimming downward at higher speeds in response to physical and chemical cues (Tamburri et al. 1992), suggesting larval behavior could influence macroscale transport by sinking to take advantage of different hydrodynamic conditions at different water depths (Hidu and Haskin 1978; Scheltema 1986; Mann et al 1991). It is unclear whether passive or active transport plays a larger role in macroscale larval transport.

The gregarious nature of oyster settlement (Cole and Knight-Jones 1939), immediately suggests some degree of active transport during settlement. In contrast to macroscale level where larvae may primarily be dispersed as passive particles, larval behavior in response to physical and chemical cues plays a large role prior to settlement at the meso and microscale level (meters down to centimeters).

Ultimately the settlement location of oyster larvae is dependent on a combination of both larval behavior and physical processes (Mullineaux and Butman 1990, Butman and Grassle 1992, Grassle et al. 1992, Snelgrove et al. 1993), the strength of these factors being more or less apparent depending on the scale studied. I investigated recruitment at the kilometer, meter, and centimeter spatial scales to determine spat settlement over the course of two years in the Great Bay estuary. The kilometer scale tested the effect of site selection on recruitment. The meter scale tested the effect of reef structure and location within reef on recruitment. And lastly, the centimeter scale tested the effect of subtle differences in vertical height on recruitment. Such information is needed for optimal restoration reef design and site location.

Methods and Materials

Recruitment was studied at several scales within Great Bay, NH during 2005 and 2006. Spat samplers were deployed to two constructed experimental reef treatments at Nannie Island and nearby natural reef controls. In addition, oyster recruitment was monitored within Great Bay proper at three of the largest natural, oyster reefs: Nannie Island, Adams Point, and Squamscott River (see Fig. 1) to determine whether recruitment differed between different areas. In 2006 the effect of vertical height (0cm, 5cm, 10cm, 15cm) on recruitment was examined.

Sampler Design

Samplers were constructed with mesh bagging (1.5 cm openings) attached to wood stakes (2.5 x 2.5 cm cross section) cut to lengths between 50 and 80 cm (Fig. 8). Each mesh bag was filled with approximately 25 oyster shells (filled bags were approximately 30 x 20 x 4 cm) and was held in place with opposing wooden dowels hammered into drill holes in wood stakes. Because shells varied in size and were often fragmented, a standardized volume was used to determine the amount of shell in each bag (~2300 cm³).

Experimental Design

Centimeter Scale. A single-factor experimental design was utilized to determine the effect of vertical height on recruitment with the following four treatments: sampler height of 0 cm, 5 cm, 10 cm, and 15 cm. The data were analyzed using JMP 5 software.

Analysis of variance (ANOVA) tests were used to compare means. A *posteriori* Student t-test was used when significant differences occurred between means.

Meter Scale. A single-factor experimental design was utilized to determine the effect of reef structure on recruitment with the following four reef treatments: single large constructed reef, several small constructed reef, between several small, constructed reef, and natural reef. To test for the effect of location within a constructed reef on recruitment, another single-factor experimental design was used with the following four location treatments: single large core, single large edge, several small core, and several small edge. Data were analyzed using JMP 5 software. Analysis of variance (ANOVA) tests were used to compare means. A *posteriori* Student t-test was used when significant differences occurred between means.

Kilometer Scale. A t-test was used to compare Great Bay recruitment in 2005 and 2006; each site (Nannie Island, Adams Point, and Squamscott) was used as a replicate providing a sample size of three. A single-factor experimental design was utilized to determine the effect of site location on recruitment. Data was analyzed using JMP 5 software. Analysis of variance (ANOVA) tests were used to compare means. A *posteriori* Student t-test was used when significant differences occurred between means.

Field Methods

Deployment and Retrieval. The timing of sampler deployment was determined by weekly gonad analysis of Great Bay oysters. Adult oysters, in lots of ten, were collected by hand or with hand tongs from either Nannie Island or Adams Point and the oysters were dissected. Gonad appearance, i.e., size and color, was assessed visually to estimate the timing of local spawning events (see Ayer et al. 1970). Spawning was determined to have occurred when a majority of dissected oysters had thin, clear gonad regions. We attempted to deploy samplers approximately 3-4 weeks prior to larval settlement, or 1 week prior to oyster spawning (2-3 week larval duration). This time frame was selected to allow for the growth of a biofilm, but not long enough for significant sediment accumulation or heavy settlement of potential space competitors.

Samplers were deployed by pushing, or hammering, sampler stakes into substrate. Shell-filled mesh bags were deployed flush to the substrate for 0cm treatment. For 5, 10, and 15 cm samplers, opposing wooden dowels were used to secure mesh bags so that the majority of shells within the bag were held at the desired vertical height.

In 2005, 188 samplers were deployed from 8/4/05 to 8/5/05. Samplers were retrieved on 9/12/05 and 9/13/05. In 2006, 280 samplers were deployed from 6/29/06 to 7/5/06. Samplers were retrieved from 8/17/06 to 8/19/06. After retrieval, samplers were kept in large tanks filled with filtered seawater until spat were counted. Shells were then removed from bags, cleaned with a seawater rinse and all oyster spat were counted and recorded.

Centimeter and Kilometer Scale. Spat samplers were deployed to the natural oyster reefs at Nannie Island, Adams Point, and Squamscott River during both 2005 and 2006. In 2006, additional spat samplers were placed at each area with the shell filled mesh bag of the samplers raised to 5, 10, and 15 cm vertical height above the substrate to determine the effect vertical height has on recruitment.

Meter Scale. Four spat samplers were placed flush with substrate on each constructed reef replicate in 2005 and 2006. This accounted for 32 total samplers each year (4 single large reef replicates x 4 samplers and 4 several small reef replicates x 4 samplers). For the single large treatment reefs, two spat samplers were placed on the outer edge of the reef, less than 0.5 m from the edge of the constructed reef. Two additional spat samplers were placed in the core region of each single large reef, within 0.5 m of the center of the reef. A single spat sampler was placed on each of the four small reefs that comprised each of the several small reef treatments. Two of these samplers were placed in edge habitat, less than 0.5 m from the edge of the constructed reef and two samplers were placed in core habitat, less than 0.5 m from the center of the constructed reef.

Four spat samplers were placed flush to the substrate haphazardly between the four small reefs comprising each of the several small treatment replicates to determine the effect several small reefs have on recruitment to the area they surround. Spat samplers were also deployed to random locations in the adjacent natural reef area.

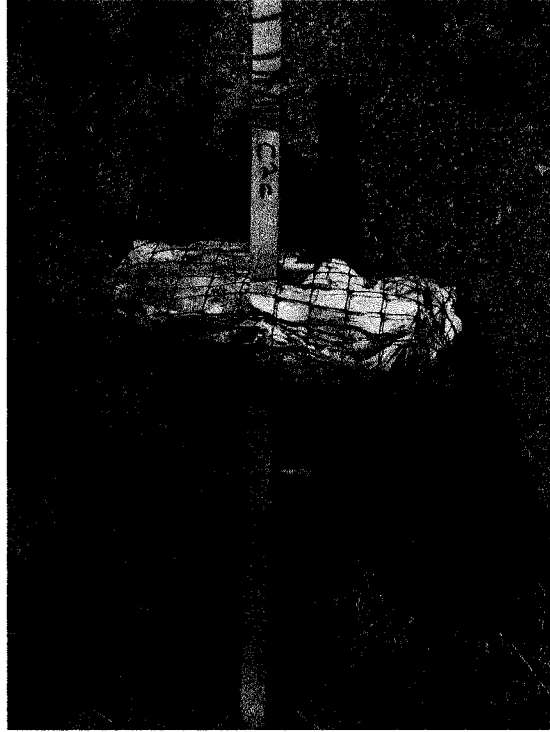


Fig. 8. Oyster spat sampler. Notice wooden dowels used to secure mesh bag and to appropriate placement depth. Small market buoys were attached to facilitate sampler retrieval.

Results

Centimeter Scale Experiment

There were no significant differences in recruits per shell at different vertical height treatments. There were more recruits at 0 cm (substrate level), 8.56 recruits/shell, than other treatments (Fig. 9). For the 5, 10, and 15 cm vertical treatments, there was a statistically non-significant trend of increasing recruitment with increasing vertical height (Table A11).

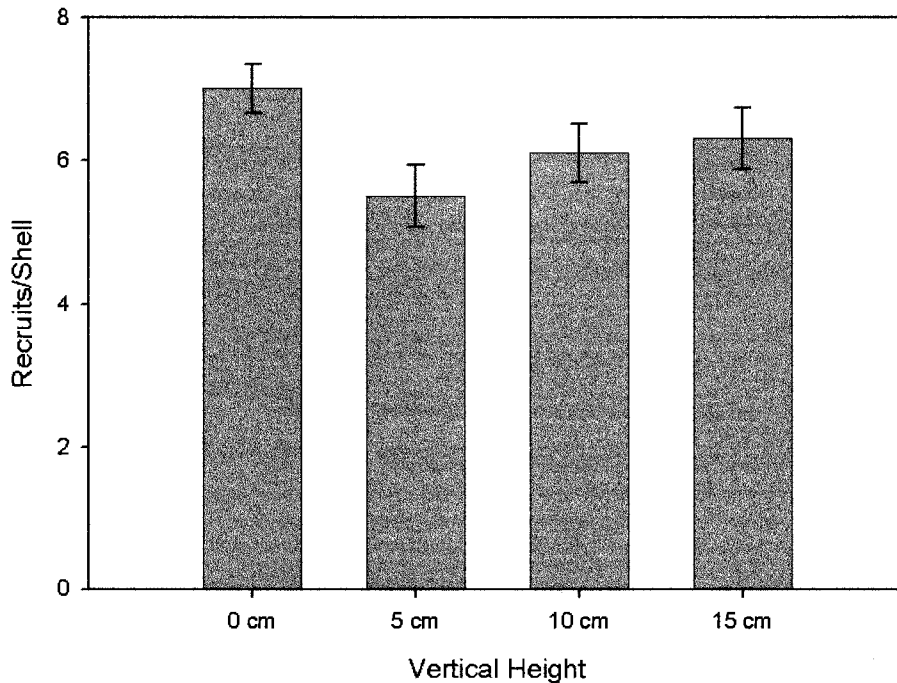


Fig. 9. Recruitment for samplers placed at different vertical heights within Great Bay (Error bars = 1 standard error).

Meter Scale Experiment

Recruitment was not significantly different for any treatments in 2005 ($p = 0.0778$, Table A12, Fig. 10). In 2006, recruitment was significantly greater in both the Single Large and the Several Small treatments than the natural treatment ($p < 0.05$, Table A13, Fig. 10). Recruitment was higher for the between several small treatment than the natural reef both years; however there was no significant difference between the two treatments (Fig. 10). Edge and core habitat on constructed reefs did not have significantly different recruitment either year ($p > 0.05$, Table A14 and A15, Fig. 11).

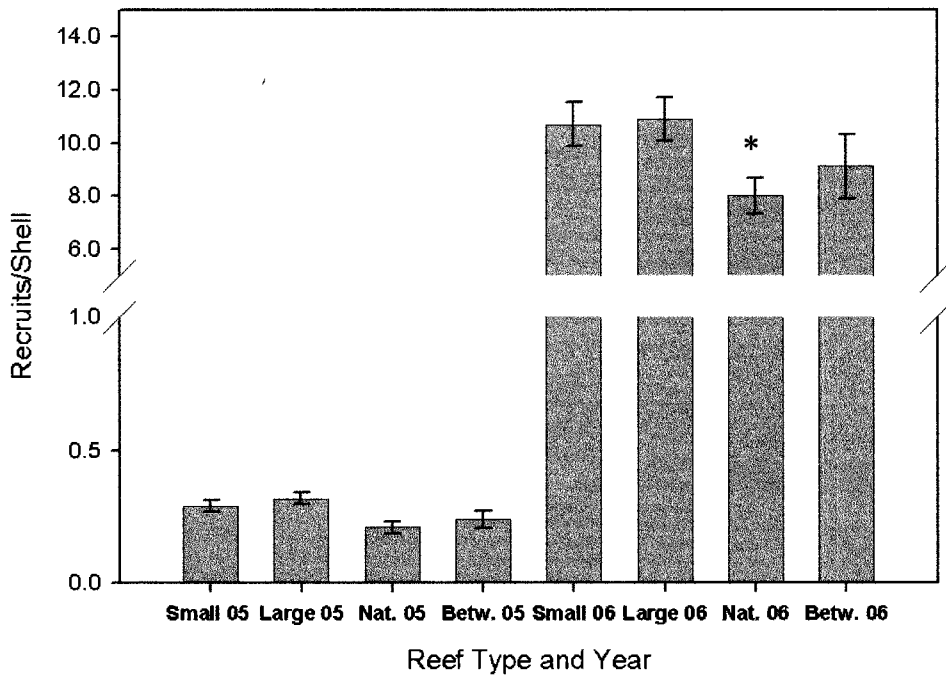


Fig. 10. Recruitment for experimental reef types during 2005 and 2006 (Error bars = 1 standard error). Asterisk indicates $p < 0.05$.

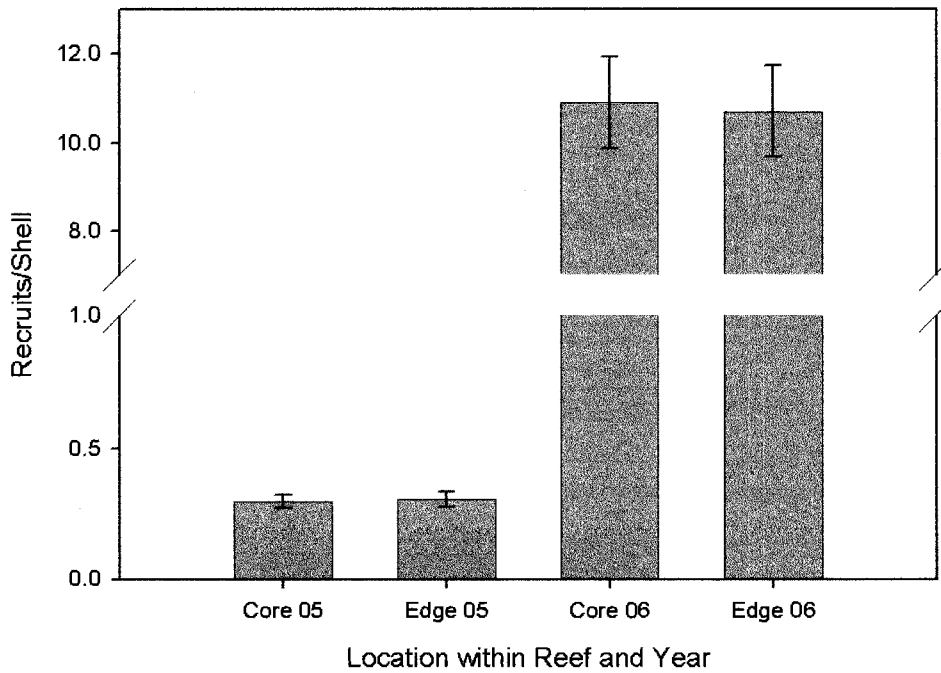


Fig. 11. Recruitment for edge (outer 0.5 m of constructed reef) and core (area within 0.5 m of the center of constructed reef) habitats (Error bars = 1 standard error).

Kilometer Scale Experiment

Recruitment differed significantly from summer 2005 to summer 2006 for Great Bay (comprised of the three aforementioned reefs), with a greater than three-fold increase in recruitment from 2005 to 2006 (Table A16, Fig. 12). Mean oyster recruitment (recruits/shell) differed significantly between Squamscott reef and the other two Great Bay reefs, Nannie Island and Adams Point. During 2005, Squamscott reef had significantly more recruits ($p < 0.05$, Table A17, Fig. 13) than either Nannie Island or Adams Point. The following year, Squamscott had significantly fewer recruits ($p < 0.05$, Table A18, Fig. 13) than either Nannie Island or Adams Point.

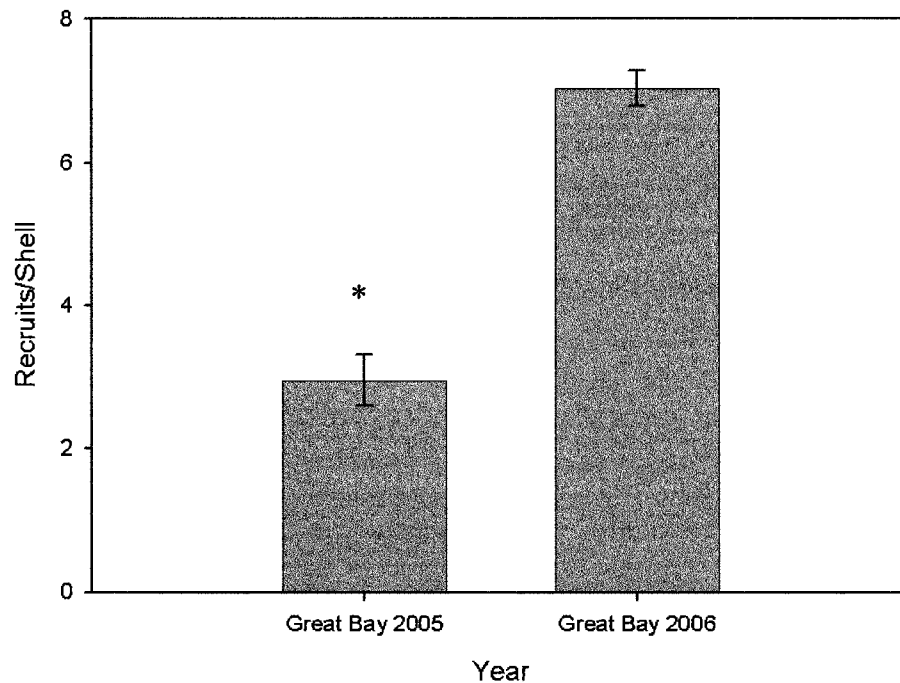


Fig. 12. Recruitment for Great Bay in 2005 and 2006 with three natural reefs combined (Error bars = 1 standard error). Asterisk indicates $p < 0.05$.

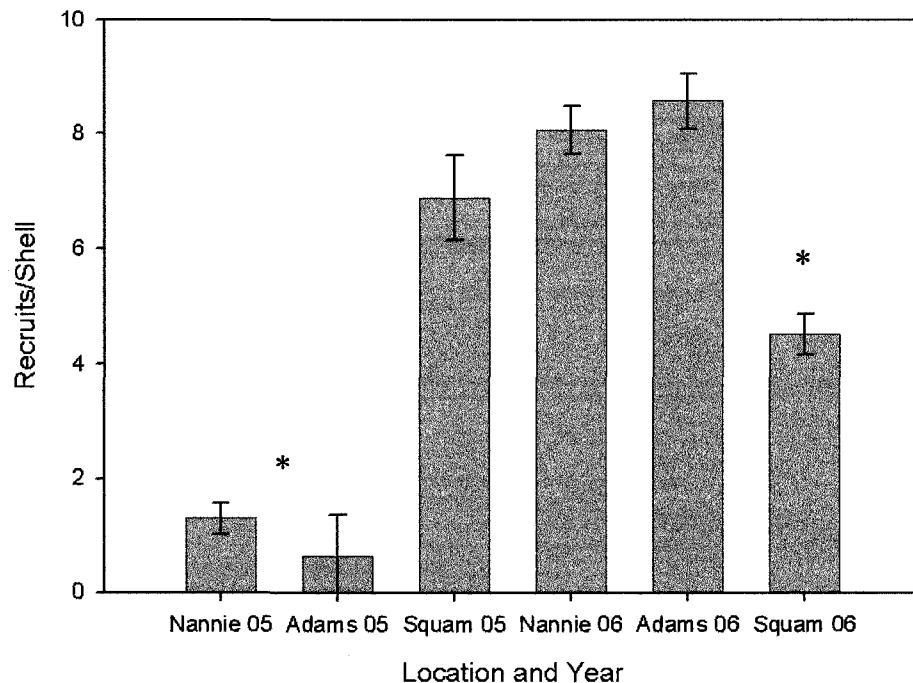


Fig. 13. Recruitment for three reefs (Nannie Island, Adams Point, and Squamscott reef) within Great Bay during 2005 and 2006 (Error bars = 1 standard error). Asterisk indicates $p < 0.05$.

Discussion

Recruitment within Great Bay, NH displayed the high degree of variability characteristic of recruitment studies (Shumway 1996). However, trends were apparent at all three scales studied. The continued use of spat sampler methodology to monitor oyster recruitment in Great Bay is a necessary component to oyster resource management.

In previous years, replicate quadrat samples have been used to determine spat set in Great Bay (Smith 1999 and 2000). This method gives an accurate assessment of living oyster densities, but may not accurately assess the suitability of an area for restoration as it does not take into account that oyster recruitment depends on the availability of hard

substrate. It is possible with the quadrat method fails to find recruits during low to moderate spatfall years simply because there is a lack of clean hard substrate in a given area. The spat sampling bag methodology provides equal recruitment surface for each sample.

Great Bay, within the Great Bay Estuary System, is extremely small in comparison to other Atlantic Coast estuaries such as Long Island Sound or Chesapeake Bay. The three reefs in this study are less than 4 kilometers away from each other. However, reef proximity did not result in similar recruitment rates for either year of this study. In 2005, an average recruitment year for Great Bay relative based on NH Fish and Game historical data (Smith 1999 and 2000), both Nannie Island and Adams Point received similar recruit densities, and the Squamscott reef received more than three times the recruits than did the neighboring two reefs. This trend was reversed in 2006, a relatively high recruitment year, where Nannie Island and Adams Point again received similar recruit densities. However, this year the Squamscott reef received nearly half the recruit density than the other two reefs. It is difficult to make generalizations based on two years of data; however, when these results are coupled with New Hampshire Fish and Game oyster monitoring results (Smith 1999 and 2000), it becomes clear that Squamscott reef consistently receives a different amount of oyster recruits than the two other Great Bay reefs. This suggests that Squamscott reef receives recruits from a separate source reef than do Nannie Island or Adams Point. This assumption is supported by observations of similar recruitment onset dates for both Nannie Island and Adams Point reefs and a 1 week delayed onset of recruitment for the Squamscott reef in 2006 (pers. obs.).

It is also of interest that although both Nannie Island and Adams Point received significantly higher recruit densities in 2006 than 2005, Squamscott reef received a similar recruit density both years. Consistent year-to-year recruitment into the Squamscott reef should result in greater population stability than would be expected in either of the highly variable Nannie Island or Adams Point reefs. Further monitoring of the Squamscott reef is necessary. If this location consistently has high recruitment rates, density-dependent processes could limit adult population size. If this were in fact true, the Squamscott reef would be an excellent “spat donor reef” for future restoration projects, as the removal of spat would simply lower density-dependent mortality.

The second scale investigated in this study is pertinent to the structural design of future restoration projects. The following questions were addressed in the meter scale portion of this study:

1. Does reef structure affect recruitment?
2. Does location within reef affect recruitment?
3. Do several small reefs affect recruitment in the area they surround?

The two alternative reef structures explored in this study, single large or several small, exhibited no significant differences in recruitment either year. Perhaps the size and arrangement differences in the experimental reefs were not sufficient to determine how different structures influence recruitment. Additionally, location within experimental reefs did not affect recruitment. It is interesting that reef edges exhibited wider variation than did core reef habitat, perhaps owing to the wider variation in flow conditions experienced by reef edges (eg. Sides verse ebb and flood edges).

Recruitment was higher in the natural reef areas between the clusters of several small reefs than in natural reef distant from experimental reefs. This increased recruitment level was apparent in both 2005 and 2006, however it was not statistically significant. This observation warrants further study, for if the presence of multiple reef structures enhances recruitment in the regions they surround, then large areas could be more efficiently enhanced by the creation of many small reef structures.

In 2005, a relatively weak recruitment year, the single large and single small treatments received more recruits than the natural area, although this result was not statistically significant. Both single large and several small experimental treatments received significantly more recruits than did adjacent natural areas in 2006, a relatively strong recruitment year. This result is likely due to the increased vertical height associated with experimental reefs. Spat samplers placed on experimental reefs are elevated on average 20 cm above the substrate. Reef height has been shown to affect flow conditions and subsequently biotic interactions (Lenihan 1999). Sedimentation could have played a role in the observed differences, however, spat samplers were intentionally deployed within weeks of a predicted settlement event to prevent the accumulation of sediment, and differences in sediment cover were not noticed during sampler retrieval (pers. obs.).

Location within experimental reefs did not affect recruitment. Samplers in reef cores and edges received similar recruitment densities both years. Variation was much higher for samplers located in edge habitat, potentially owing to the greater variation of physical conditions present in edge habitat. In a similar study, Luckenbach and Ross (2003) attributed a lack of significant differences between edge and core reef portions to

an abundance of recruits during the time period studied. They suggested that in years of low recruitment, larval depletion would lead to greater recruit densities at the edges of reefs (area of first contact) and lower densities at the cores. Different recruit sampling methods prevents a direct comparison of recruitment with Luckenbach and Ross (2003), however, no differences were found between edge in and core in the current study in both a year of high recruitment and one of low recruitment. Perhaps much larger reefs would be necessary to observe within reef recruitment trends caused by larval depletion.

The centimeter scale component of this study provides a glimpse into the complexities of oyster recruitment. The highest amount of recruitment was observed on the substrate level samplers, followed by the samplers elevated 15 cm. This result perhaps highlights the complex interplay between biological behavior and physical conditions that dictate oyster recruitment. Eyed-larvae, oyster larvae ready to settle, are negatively phototactic (Kennedy 1996), and will actively swim or sink when exposed to light. This behavior draws late-stage larvae near to the substrate (Kennedy 1996) and also explains the preponderance of recruits found on the underside (shaded area) of settlement plates and shells in numerous studies (Nelson 1953, Ritchie and Menzel 1969). Negative phototaxis is a behavior likely contributed to the results of higher substrate level recruitment found in this study. Physical conditions account for the increased recruitment rates at the 15 cm spat samplers. In a passive particle study sampling gear captured the highest number of particles in areas with the highest flow speed (Wood and Hargis 1971). Elevated 15 cm samplers were higher in the benthic boundary layer where water flow is less influenced by the seafloor drag and faster than water closer to the substrate (Vogel 1994).

CHAPTER 4

CONSTRUCTED REEF PERFORMANCE: EFFECTS ON WATER QUALITY

Introduction

The success of fisheries-based oyster reef restoration is easily quantified. If a restored area leads to increased oyster harvest, then the restoration was successful. Ecological restoration for ecosystem services is not so easily measured. Water quality benefits associated with bivalve filtration are often cited as an argument for the restoration of oyster habitat; however few studies have quantified this effect in the field.

A report on New Hampshire water quality found few indicators of eutrophication in Great Bay Estuary (Langan 2000). However, intense algae blooms and periodic hypoxic conditions have been noted in freshwater and tidal portions of some of the rivers feeding into the Great Bay Estuary (Jones and Langan 1996) and as development continues, eutrophication impacts can be expected to increase. Increased oyster densities within Great Bay could reduce phytoplankton populations and lower the risk of eutrophication. In fact, water quality improvements are often cited as reason for oyster restoration in Great Bay.

Clearance rates of bivalve populations in the field are highly variable and can be influenced by a number of factors including seston quality (Cranford and Hargrave 1994), water temperature (Bayne et al. 1977), salinity (Widdows 1985), pollution

(Widdows and Donkin 1992), and energy requirements related to reproductive activity (Newell and Thompson 1984, Kreeger 1993, Kreeger et al. 1995). Numerous studies based on feeding experiments conducted in optimal laboratory conditions have asserted that dense bivalve assemblages can control phytoplankton concentrations (Cloern 1982, Officer et al. 1982, Nichols 1985, and Dame 1993). However, few field data exist to demonstrate this under natural conditions.

The current study compared the differences in seston removal by a constructed oyster reef to a nearby natural reef of equal size but differing in oyster density and size. *In situ* fluorometers were used to measure seston uptake over a one year-old constructed reef, and then again for the same reef after an additional year of growth and a heavy spatfall to determine whether and how long after construction a reef can provide ecological benefits similar to a natural reef.

Methods and Materials

The removal of chl- a, or seston uptake, through filtration by restored and natural oyster reefs at Nannie Island, Great Bay Estuary was measured in this study. A single large constructed reef (see chapter 2) was measured over the course of two years (2005-2006) and natural reefs were measured during the summer of 2006.

In Situ Fluorometer Instruments

This study utilized two *in situ* fluorometer instruments designed by Dr. Raymond Grizzle (Fig. 14). Each system is comprised of a Seapoint Sensors fluorometer (Model SCF), and a multimeter/datalogger. The fluorometer probe is housed in a 5-cm ID PVC

pipe that is attached to a 2-mm thick stainless steel metal bottom plate (see Grizzle et al. 2006). The PVC pipe has been cut to allow water to flow through the fluorometer probe. A clamp secures the sensor in the desired height and allows the sensor height to be adjusted by loosening and tightening the clamp. The bottom plate allows the system to rest evenly on the bottom and a fin attached to the back of the bottom plate lines the sensor directly with the flow upon deployment. Above the probe, a second plate is secured to shade the fluorometer from direct sunlight.

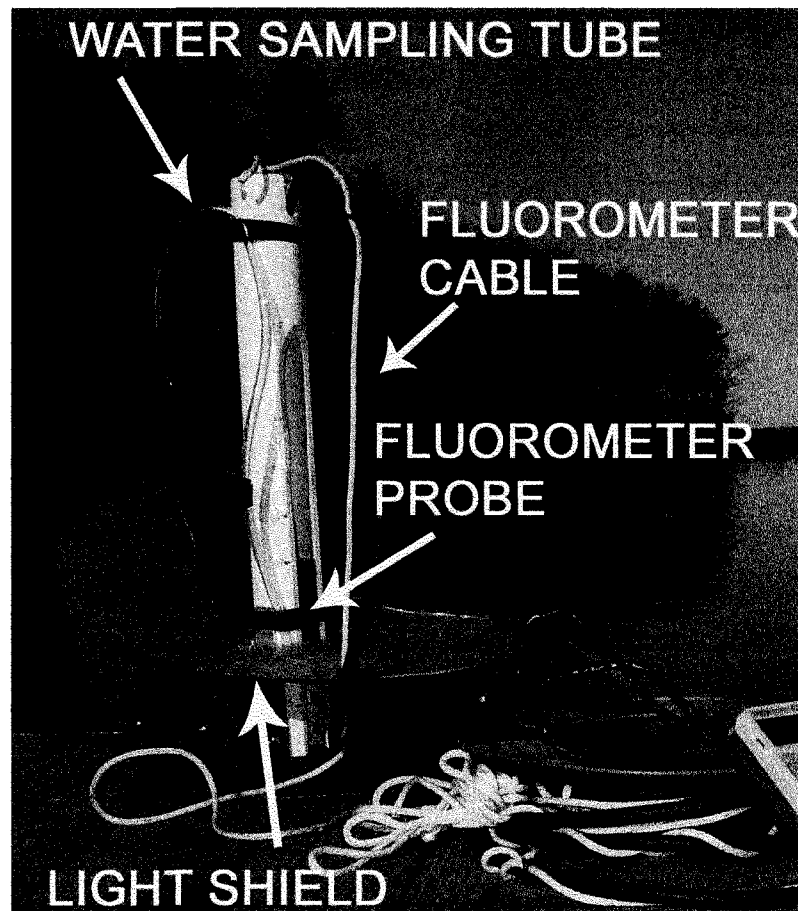


Fig. 14. *In situ* fluorometer ready for deployment.

Deployment and *in situ* measurement with these systems involves placing one instrument on the upstream edge of the target reef and the second instrument on the

downstream edge of the target reef (this was done for one single large reef and one natural reef). *In situ* fluorometer sensors were set approximately 10 cm from the substrate, as height at which previous field studies had determined water to be well mixed (Grizzle pers. comm.), yet not shallow enough that sunlight would interfere with the sensor. Prior to and after all *in situ* measurements taken in this study, both instruments were placed side by side on the bottom (Fig. 15) for at least 5 minutes of data logging to verify the calibration of instruments. If the side by side fluorometers were producing discrepant measurements, the data acquisition was aborted and the fluorometers were sent to Seapoint Sensors for recalibration.



Fig. 15. *In situ* fluorometers deployed for a side-by-side calibration check on a newly constructed restoration reef near Nannie Island, Great Bay.

Data Acquisition

Fluorometry data were measured in millivolts which are directly related to chlorophyll-a (Grizzle et al. 2006). Reefs were measured in 2005 and 2006 between June and October (cold water temperatures cause oysters to cease feeding during the remaining six months of the year). Sampling durations ranged from 30 minutes to 150 minutes. In 2005, data were logged manually taking 5 upstream and downstream fluorometer measurements every five minutes. In 2006, data were logged directly to a storage device and readings were recorded every 20-30 seconds. In addition to fluorometer data, the length and width of each reef was measured using transect tape. Depth and flow speed and at 10 cm above substrate were measured every 10 minutes throughout the sampling duration using a marked pvc pole and a Marsh-McBirney 201 EM current meter. Flow direction was measured every 10 minutes by releasing a piece of bread into the water. This allowed me to verify that the fluorometers were both in line with the primary flow direction. Replicate 0.1 m² quadrats were sampled on each treatment reef, shell height and mean densities were determined for live oysters.

Data Processing

Data were separated into 15 minute segments for analysis to best capture the changing flow speed and water depth characteristics (see Fig. 19-60). Fluorometry data acquired from 10 cm above substrate is assumed to be representative of the overlying water column at flow speeds measured in this study (see Grizzle et al. 2006 and Grizzle et al. *in prep*). Mean water depths and flow speeds were determined for each segment by

taking the average of 3 measurements taken over 30 minutes. Mean percent of water column cleared (change in mean chl-a between the upstream and downstream meters divided by mean upstream chl-a), mean water depth, and mean flow speeds for each 15-minute segment were used to calculate clearance rates in liters per hour. Liters per hour clearance rate was determined using the following formula:

1. Flow speed (m/hr) x water depth (m) x reef width (m) x percent of water cleared = clearance rate (m³/hr)

2. Clearance rate in m³/hr x 1000 = Clearance rate (L/hr)

Clearance rate per individual organism was determined by dividing reef clearance rate by the number of oysters in the reef.

Data Analysis

All sampling dates were combined for each treatment reef and temporally matched upstream and downstream chlorophyll-a measurements were analyzed using a paired t-test (JMP 5). Inferential statistics were not used to detect clearance rate differences between the treatment reefs. The pseudo-replicates used in this study, same two reefs (restored and natural), were measured repeatedly over time and are therefore not statistically independent. For this reason, classical inferential statistical methods were not used.

Results

In situ fluorometers were deployed on 15 separate days. Four days of data were discarded due to equipment failure or calibration problems. Of the remaining 11 days, 2 measured the 1st year restored reef (2005), 5 the 2nd year restored reef (2006), and 4 the

natural reef (2006). These sampling days resulted in approximately 4 hours of data for the 1st year restored reef, 4.5 hours for the 2nd year restored reef, and 2 hours for the natural reef. These data were separated into 16, 18, and 8 – fifteen minute data sets for the 1st year restored reef, 2nd year restored reef, and natural reef respectively (see Fig. 16 for data output example).

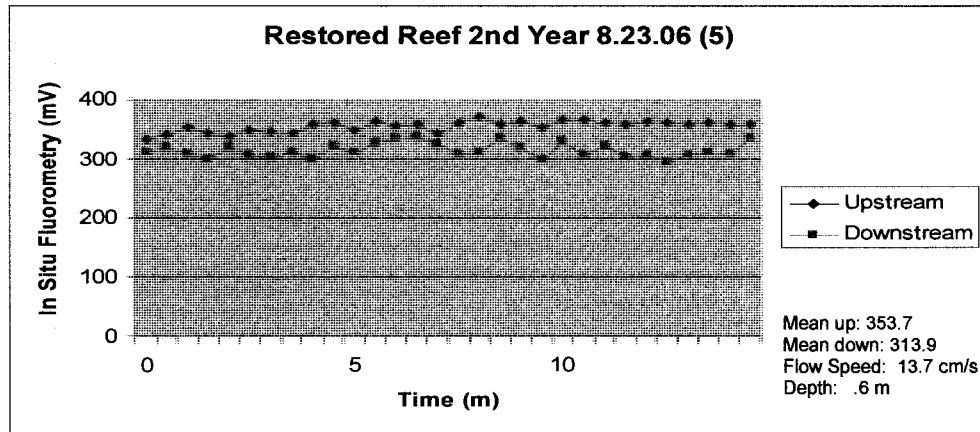


Fig. 16. Fluorometry results for 2nd year restored reef. Mean upstream and downstream values, flow speed and depth are displayed.

Reef widths were 6.0 m for the restored reef and 6.75 m for the natural reef. Depths ranged from 0.37 m to 1.57 m for the sites sampled. Flow speeds ranged from 5.6 cm/s to 15.6 cm/s. Paired t-tests revealed no significant differences between upstream and downstream water chl-a for the natural reef and restored 1st year treatment. The natural reef and restored 1st year reef cleared an average of 976 and 5,171 L/hr (Table 3). There was a significant decrease in chl-a as water flowed over the restored 2nd year treatment ($p < 0.05$) which cleared 52,209 L/hr (Table 3). Individual organism clearance rates were determined to be 2.32 L/hr, 1.87 L/hr, and 2.41 L/hr for the restored 1st year, restored 2nd year, and natural reefs respectively.

Table 3. Summary of seston uptake study. Bivalve density, size, mean flow length, water depth, and flow speed were measure in the field. Reef *in situ* and individual *in situ* clearance rates were determined by methods described above and values represent means.

Site	Mean Oyster Density (A; #/m ²)	Mean Oyster Size (Shell L, mm)	Mean Flow Length (B; m)	Mean Reef Width (C; m)	Water Depth (D; m)	Water Flow Speed (E; cm/s)	Total Water Flow Rate (CxDXE m; F; L/hr)	Mean Percent of Water Cleared (field measured; G)	Reef <i>In situ</i> Clearance Rate (FxG; E; L/hr)	Individual <i>in situ</i> Clearance Rates (E / (A xBxC); L/hr)
Nannie Natural	10	79.9	6.00	6.75	0.72	9.5	1669572	0.05%	976	2.41
Restored 1st Yr	61.9	65.9	6.00	6.00	0.95	8.0	1641600	0.32%	5171	2.32
Restored 2nd Yr	774.7	29.7	6.00	6.00	0.86	14.5	2693520	1.94%	52209	1.87

Discussion

This study shows that relatively inexpensive *in situ* fluorometers are a viable tool for monitoring the impacts oyster reefs have on chlorophyll-a in overlying water. The *in situ* fluorometry methods used in this study have been used previously to quantify seston uptake for numerous bivalve assemblages in a number of regions (Grizzle et al. 2006). This however, was the first study to determine how long after construction a restored oyster reef provides significant, measurable water quality benefits. I measured the uptake of a single 6-meter diameter restored oyster reef over the course of two years to determine how clearance rates change over time and at what point a restored reef meets or exceeds a natural reef of equal size in water quality improvement.

The natural reef, the 1st year restored reef, and the 2nd year restored reef all exhibited periods of seston uptake, periods of no change when oysters appeared to cease feeding, and periods of release (increase in chl-a at the downstream sensor relative to upstream sensor). Patterns could be discerned from the data to determine what factors

influence feeding behavior in oysters. Release periods were noted for all reefs, and appear to be caused by the resuspension of phytoplankton that had settled in the interstitial spaces of the reef and/or biodeposits from the oysters. Further monitoring is necessary to study these supposed resuspension events, however release periods seem to consistently, yet not exclusively, occur at the change from slack to flood or ebb tide. Additionally, increases in chlorophyll-a readings have been noticed in the presence of boat wakes (Dr. Ray Grizzle, unpublished data). Both of these observations support the hypothesis that release periods may be caused by some physical disturbance resuspending settled particles, although it is possible that horizontal advection of chl-a rich water cannot be ruled out.

The clearance rate of restored reefs (1st and 2nd year) exceeded that of the natural reef. The difference between the 1st year reef and the natural reef was negligible when the variation in feeding rates is considered. Neither the natural nor the 1st year restoration reduced overlying chlorophyll-a in a statistically significant manner even though both reefs had positive clearance rates. The 2nd year reef did significantly affect water quality, and exhibited a 60-fold higher clearance rate than the natural reef and a 10-fold increase from the previous year.

Individual clearance rates as determined by dividing the reef clearance rate by the number of individual oysters within the reef, yielded remarkably similar results.

Clearance rates per individual for the three treatments ranged from 1.87 to 2.41 L/hr. The 2nd year restoration reef, owing to high densities of recently settled spat, had the smallest mean shell height of the three reefs, and also the lowest individual clearance rates. These field measured clearance rates can be compared to previously determined rates from

laboratory studies. Many modeling and laboratory feeding studies (Newell and Koch 2004, Fulford et al. 2007) used larger oysters and reported oyster size data as soft tissue dry weight (DW) as opposed to shell height which was used in this study. Oyster shell heights from the current study were converted to soft tissue dry weights using a general power function ($DW = 0.00003 * \text{shell height}^{2.3952}$) developed by Ross and Luckenbach (2006) that resulted in mean dry weights of 1.08, 0.68, and 0.10 g for natural reef, 1st year constructed reef, and 2nd year constructed reef respectively. To predict clearance rates for oysters in the current study, a power function developed by Riisgard (1988) for oysters feeding in a laboratory setting under optimal conditions ($\text{Clearance rate} = 6.79 * DW^{0.73}$) was used. This resulted in predicted clearance rates of 7.18, 5.13, and 1.27 L/hr as compared to *in situ* clearance rates of 2.41, 2.32, and 1.87 L/hr for natural reef, 1st year constructed reef, and 2nd year constructed reef respectively.

It is impossible to make generalizations about the time required before restored oyster reefs provide similar water quality benefits to natural reefs from this study. Mean oyster size and to a larger extent, total number of oysters controlled reef clearance rates. The higher clearance rate exhibited by the 2nd year reef appears to be due largely to a successful recruitment year.

In situ fluorometry is a new technology. Although much progress and refinement has occurred since its inception (Grizzle et al. 2003), additional work and innovation are needed. Deploying multiple upstream and downstream fluorometers at different depths, and for extended periods (days to weeks as opposed to hours) will increase statistical power of analysis, and potentially reveal the causes of variable feeding behavior. The addition of total suspended solid (tss) meters to the apparatus would provide an additional

layer of valuable information. As stands, *in situ* fluorometry is a relatively low-cost method capable of assessing the effect of bivalve assemblages on water quality. This method has numerous potential applications from determining optimal stocking densities in shellfish farms based on seston consumption to the quantification of the ecological function of constructed or natural reefs by resource managers.

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APPENDIX

Table A1. Density comparison (t-test) for constructed reefs October 2004.

Difference	-41.88	t Ratio	-0.82775
Std Err Dif	50.59	DF	5.75759
Upper CL Dif	83.19	Prob > t	0.4408
Lower CL Dif	-166.94	Prob > t	0.7796
Confidence	0.95	Prob < t	0.2204

Table A2. Density comparison by reef type for June 2005.

Analysis of Variance

Source	DF	SS	MS	F	P
Treatment	3	56174.750	18724.9	6.4099	0.0077
Error	12	35055.000	2921.3		
C. Total	15	91229.750			

Table A3. Density comparison by reef type for October 2005.

Analysis of Variance

Source	DF	SS	MS	F	P
Treatment	3	52523.063	17507.7	7.9468	0.0035
Error	12	26437.375	2203.1		
C. Total	15	78960.438			

Table A4. Density comparison by reef type for June 2006.

Analysis of Variance

Source	DF	SS	MS	F	P
Treatment	3	8928.125	2976.04	4.0887	0.0325
Error	12	8734.375	727.86		
C. Total	15	17662.500			

Table A5. Size comparison for constructed reefs in October 2004.

Difference	0.1000	t Ratio	0.079316
Std Err Dif	1.2608	DF	4.93525
Upper CL Dif	3.3538	Prob > t	0.9399
Lower CL Dif	-3.1538	Prob > t	0.4699
Confidence	0.95	Prob < t	0.5301

Table A6. Size comparison by reef type for June 2005.
Analysis of Variance

Source	DF	SS	MS	F	<i>P</i>
Treatment	3	8060.6969	2686.90	460.5948	<.0001
Error	12	70.0025	5.83		
C. Total	15	8130.6994			

Table A7. Size comparison by reef type for October 2005.
Analysis of Variance

Source	DF	SS	MS	F	<i>P</i>
Treatment	3	584.55500	194.852	15.5162	0.0002
Error	12	150.69500	12.558		
C. Total	15	735.25000			

Table A8. Size comparison by reef type for June 2006.
Analysis of Variance

Source	DF	SS	MS	F	<i>P</i>
Treatment	3	727.6419	242.547	0.4489	0.7227
Error	12	6484.0325	540.336		
C. Total	15	7211.6744			

Table A9. Size comparison by year for Harvested Control reefs.
Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Jun-05	4	66.7250	1.3707	63.624	69.826
Jun-06	4	78.9750	1.3707	75.874	82.076
Oct-05	4	66.7000	1.3707	63.599	69.801

Std Error uses a pooled estimate of error variance

Table A10. Spat density comparison by reef type for October 2005.
Analysis of Variance

Source	DF	SS	MS	F	<i>P</i>
Treatment	3	182.42188	60.8073	3.2207	0.0613
Error	12	226.56250	18.8802		
C. Total	15	408.98438			

Table A11. Comparison of spat set for different vertical heights (0 cm, 5 cm, 10 cm, 15 cm) for Great Bay. 2006.
Analysis of Variance

Source	DF	SS	MS	F	<i>P</i>
Vert. Height	3	18.27152	6.09051	1.0492	0.3737
Error	116	673.37937	5.804999		
C. Total	116	691.65089			

Table A12. Comparison of substrate level spat set for experimental reef type (single large, several small, natural, and between several small). 2005 data.

Analysis of Variance

Source	DF	SS	MS	F	P
Type	2	0.02934742	0.009782	2.814	0.0778
Error	25	0.04869704	0.003478		
C. Total	27	0.07904446			

Table A13. Comparison of substrate level spat set for experimental reef type (single large, several small, natural, and between several small). 2006 data.

Analysis of Variance

Source	DF	SS	MS	F	P
Type	3	36.196117	12.0654	3.89606	0.025
Error	20	62.505329	3.1253		
C. Total	23	98.701446			

Table A14. Comparison (t-test) of substrate level spat set for location on constructed reefs (edge and center). 2005 data.

Difference	-0.00675	t Ratio	-0.17009
Std Err Dif	0.03968	DF	13.76258
Upper CL Dif	0.07850	Prob > t	0.8674
Lower CL Dif	-0.09200	Prob > t	0.5663
Confidence	0.95	Prob < t	0.4337

Table A15. Comparison (t-test) of substrate level spat set for location on constructed reefs (edge and center). 2006 data.

Difference	0.2300	t Ratio	0.157315
Std Err Dif	1.4620	DF	11.55855
Upper CL Dif	3.4290	Prob > t	0.8777
Lower CL Dif	-2.9690	Prob > t	0.4389
Confidence	0.95	Prob < t	0.5611

Table A16. T-test of Great Bay (3 locations combined) substrate level spat set years 2005 and 2006.

Difference	-4.7358	t Ratio	-8.75195
Std Err Dif	0.5411	DF	69.6932
Upper CL Dif	-3.6565	Prob > t	<.0001
Lower CL Dif	-5.8151	Prob > t	1.0000
Confidence	0.95	Prob < t	<.0001

Table A17. Comparison of Reef location (Nannie Island, Adams Point, Squamscott) substrate level spat set for 2005.

Analysis of Variance

Source	DF	SS	MS	F	<i>P</i>
Location	2	115.09375	57.5469	61.8519	<.0001
Error	33	30.70313	0.9304		
C. Total	35	145.79688			

Table A18. Comparison of Reef location (Nannie Island, Adams Point, Squamscott) substrate level spat set for 2006.

Analysis of Variance

Source	DF	SS	MS	F	<i>P</i>
Location	2	133.53879	66.7694	20.2405	<.0001
Error	35	115.45821	3.2988		
C. Total	37	248.99700			