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An Experimental Evaluation Of The Effects Of Scale On Oyster Reef Restoration

Mark W. Luckenbach
Virginia Institute of Marine Science

Paige G. Ross
Virginia Institute of Marine Science

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Final Report

**AN EXPERIMENTAL EVALUATION OF THE
EFFECTS OF SCALE ON OYSTER REEF
RESTORATION**

Submitted to:

Dr. William Rickards
Virginia Sea Grant Consortium
University of Virginia
Rugby Road
Charlottesville, VA

Submitted by:

Mark W. Luckenbach and Paige G. Ross
Eastern Shore Laboratory
Virginia Institute of Marine Science
College of William and Mary
Wachapreague, VA 23480

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Project Summary

We investigated the effects of spatial scale, ranging from 10's of meters to several km's, on the development of oyster populations and other reef-associated organisms during the early stage of reef restoration. Employing a block design, experimental reefs were constructed at four sites in the lower Rappahannock River, VA. We tested the effects of sites (scale $\approx 1 - 10$ km), reef sizes (scale ≈ 100 m) and locations within reef (scale ≈ 10 m) on the settlement, survival and growth of oysters and on the abundance, biomass, species richness and diversity of developing communities utilizing the reefs.

The project provides descriptive data about the developing reefs, both in terms of their physical characteristics and the early succession of species to colonize the reefs. We test numerous hypotheses related to temporal and spatial development of the reefs, and we explore some of the relationships between the development of oyster populations, the abundance and biomass of other species, and the species richness and diversity of reef-associate fauna. We also pose and test several specific *a priori* hypotheses related to the effects of scale on the development of oyster populations and reef communities.

Our results reveal both temporal and spatial variation across all of the scales investigated and point to some of the complexities associated with ecological restoration on these scales. Most striking are the differences that occurred in the development of reefs separated by only a few km's. Some variation in reef development was related to the specific size of the reefs that were built, though most of our *a priori* predictions in this regard were not supported. Although there was considerable spatial variation within a reef in the abundance of most species collected, attempts to partition that variation with respect to specific intra-reef locations were generally unsuccessful.

These reef are still in the early stages of development and we caution that it may be too soon to evaluate most of the effects of scale on their eventual structure. This study does, however, provide insights into the early development of the reefs and some of the processes structuring that development. Our findings emphasize the importance of understanding scale-related variation in evaluating and enhancing the success of ecological restoration of the magnitude of that being attempted with oyster reef habitat restoration in the Chesapeake Bay.

Acknowledgments

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We would like to express our thanks to Alan Birch, Janet Nestlerode and Brent Parks for their extensive help in carrying out field sampling, sample processing and data organization. G. Arnold, A. Bartholemew, K. Delano, B. Gammish, J. Gascoigne, E. Hinchey, C. Morrison, K. Segerblum, L. Sorabella and M. Southworth also provided field data collection help that was much appreciated.

Additionally, Stephanie Bonniwell, Al Curry and Susan Spears provided broad assistance with oyster hatchery expertise, sample processing and data organization. C. Cackowski also assisted with hatchery activities and Lisa Calvo oversaw processing oyster disease samples, all of which were very helpful.

We would also like to thank Reade Bonniwell for assistance on myriad technical aspects of undertaking such a large and diverse research project.

Finally, we thank Scott Kaufman for allowing us use of Regent Point Marina for mooring our boat and as a staging area. This facilitated the implementation of this study immeasurably.

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INTRODUCTION

It is now widely recognized that restoration of oyster reef habitat in mid-Atlantic estuaries is requisite for restoring oyster fisheries and recovering lost ecological services. In the Chesapeake Bay region, the Chesapeake Bay Program (a state-federal partnership) has adopted a goal of a 10-fold increase in oyster standing stocks 2010 and Virginia established in 2001 the Oyster Heritage Program which seeks to rehabilitate reef habitats and enhance oyster fisheries. The basic approach of each of these programs is to establish self-sustaining reef sanctuaries that provide valuable ecological functions, such as benthic-pelagic coupling and support of increased diversity and production of macrobenthos and finfish, as well as providing oyster spawning stock to support adjacent harvest areas. While this commitment to restoring these habitats is laudable, there is much that we still do not know about how to properly restore these habitats and the specific ecological functions associated with them.

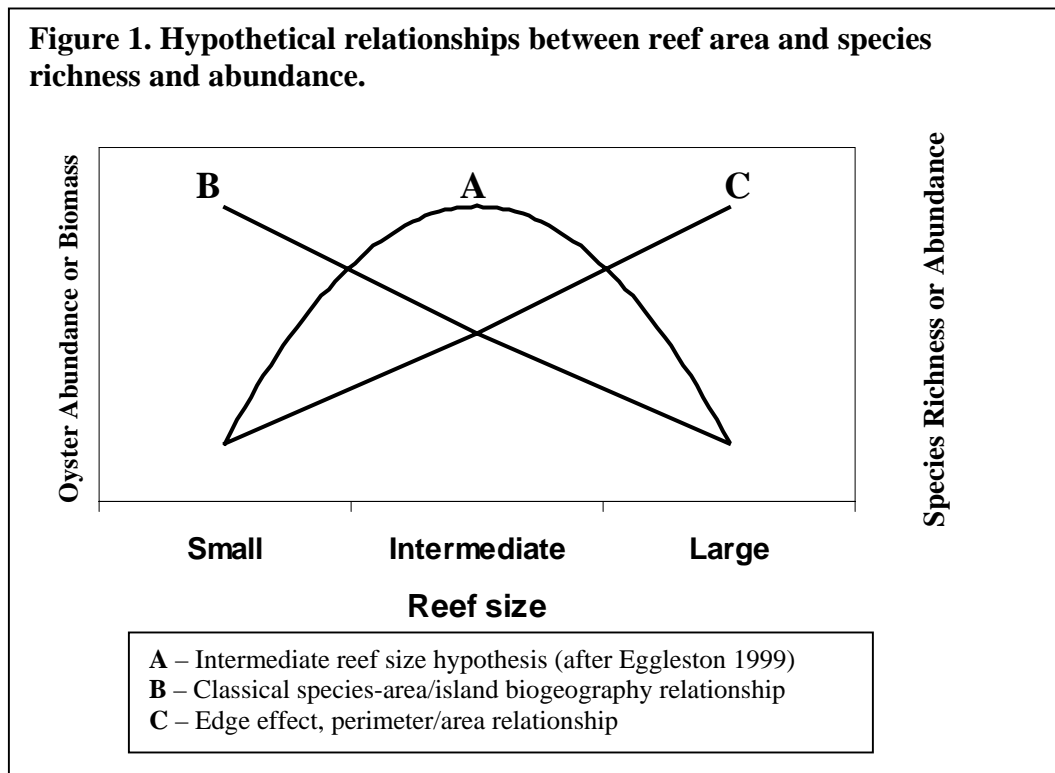
In a management context, there are only three things that can be done to restore native oyster reefs: place substrate (usually oyster shell) on the seafloor, restrict harvest, and (when recruitment limitation is evident) add brood stock. Recent evidence has revealed the importance of establishing proper vertical relief and interstitial space in the initial placement of substrate (Lenihan et al. 1996; Lenihan and Peterson 1998; Bartol and Mann 1999; Bartol et al. 1999; O'Beirn et al. 2000). A third component of reef architecture that has yet to be investigated is the aerial extent or scale of the reef. In terrestrial conservation biology this topic has often been characterized as the SLOSS (Single Large or Several Small) debate, but is more generally about optimizing the scale of a bio-reserve or sanctuary to achieve the desired ecological benefits. For oyster reef restoration, we believe that this is a critical issue because shell substrate is limiting and expensive. Thus, we need to know how to best

allocate this resource to restore ecologically functional habitats and support sustainable fisheries.

Both empirical studies and theoretical considerations lead to divergent predictions about the most appropriate scale for targeting oyster reef restoration. For oysters, larval attraction (Turner et al. 1994), flow modifications (Kennedy and Sanford 1999), food depletion (Dame 1999; Harsh and Luckenbach 1999), predation rates and refugia may all vary with reef size and oyster abundance, but details of this variation have not been investigated. Eggleston (1999) predicted that oyster abundance should follow a parabolic distribution with reef size, the greatest densities being found at intermediate reef size (see curve A in Figure 1), but this assertion remains untested. In a resource management context variations in oyster abundance with reef size can be exploited to optimize the total number of oysters supported by a particular restoration effort. That is, a fixed volume of shell can be allocated to building one large or several small reefs.

The diversity of species supported by an oyster reef is also likely to vary with scale. Classical species-area relationships (Gleason 1925) and particularly from islands (e.g., Diamond and May 1981) support the notion that the numbers of resident species varies in a positive fashion with area (curve B in Figure 1). Eggleston (1999) argues for a different pattern with greatest species richness at intermediate reef size (curve A in Figure 1). Few data are available on use of oyster reefs by mobile fish species (see Coen et al. 1999a, b and Lenihan et al. 1998 for important exceptions), but available data from the lower Chesapeake Bay suggest that a preponderance of individuals, if not species, for transient species are associated with reef edges (M. Luckenbach, F. O'Beirn, J. Nestlerode, J. Harding,

unpublished observations), leading to the prediction that abundance of these species on an area-normalized basis should be greatest on small reefs (curve C in Figure1). Again, the



important relationships for restoration efforts will be those normalized to area (or shell volume in the reef), because they indicate how scarce shell resources can be allocated to optimize the desired end product. Our research group has observed a positive relationship between the density of oysters and the species richness and abundance of resident and transient assemblages associated with experimental reefs near the Chesapeake Bay mouth (O’Beirn et al. 2000; Nestlerode et al. in prep), but these results shed no light on the role of reef size or spatial scale on associated assemblages. Thus, there are a number of reasonable alternative hypotheses relating reef size to biodiversity of associated assemblages. Distinguishing between these hypotheses, as well as the null hypothesis that species richness and abundance do not vary with reef size, and evaluating the mechanisms involved is crucial to the design of oyster reefs as bio-reserves.

Restoration of oyster reefs is receiving unprecedented attention in Virginia and other east coast and Gulf states (Luckenbach et al. 1999). Yet, restoration at this level is new and there remains much that we do not know about how to most effectively meet our goals. Further, oyster shell and other suitable substrate are in short supply and/or too expensive. We need to better understand how to most effectively use these resources to build effective bio-reserves that maximize the success of oyster populations and support diverse communities. This can only be done properly in large-scale field experiments.

This research report details the establishment of just such a large-scale field experiment in the Rappahannock River, Virginia, and reports on the early development of oyster populations and reef-associated assemblages in relation to reef size. By describing spatial patterns of oysters and other biota across the reefs, these studies suggest mechanisms that may be controlling the early development of reef communities.

OBJECTIVES

Our overall object in this research was to clarify how the spatial scale (patch size) of constructed oyster reef bases affects the early development of the reefs and associated assemblages, so that we might better understand how to create oyster reef sanctuaries and bio-reserves. We tested specific mechanistic-based hypotheses related to the development of oyster populations on the reefs and evaluated alternative models of biodiversity-reef size relationships. Specific questions and hypotheses are identified in a later section beginning on page 9.

STUDY AREA

This study was carried out at four sites in the lower portion of the Rappahannock River, Virginia, USA, which is a tributary of the Chesapeake Bay (Figure 2; latitude and longitude coordinates for each site are given in Appendix I). Historically, this area was considered a highly productive oyster area with extensive natural reefs (Hargis 1999). The area is a mix of state-owned and privately-leased bottom that has previously supported a substantial oyster industry, based both upon harvesting wild oysters and transplanting seed oysters to private leases. Specific sites chosen for the study were deemed to have been historically highly productive and, therefore, important to overall oyster reef restoration in the vicinity.

This portion of the Rappahannock River has a 0.4 m average tidal range and the average maximum current is 0.3 m/s. It is a mesohaline tributary with a 10-year average salinity of 16 ppt in the vicinity of the study reefs. Water temperature and salinity data are available from the Chesapeake Bay Program's fixed monitoring station LE 3.4, located midway between the Drumming Ground and Temple Bay sites, for the study period (Fig. 3) (<http://www.chesapeakebay.net/data/index.htm>). Due to low rainfall during 2001-2002 salinity averaged 19 psu and fluctuated seasonally from 13.9-22.2 psu with differences between surface and bottom salinity typically < 3 psu.

Fig. 2 Study area. A. Lower Chesapeake Bay with location of study outlined in the box. B. Lower Rappahannock River study site with the individual reefs indicated.

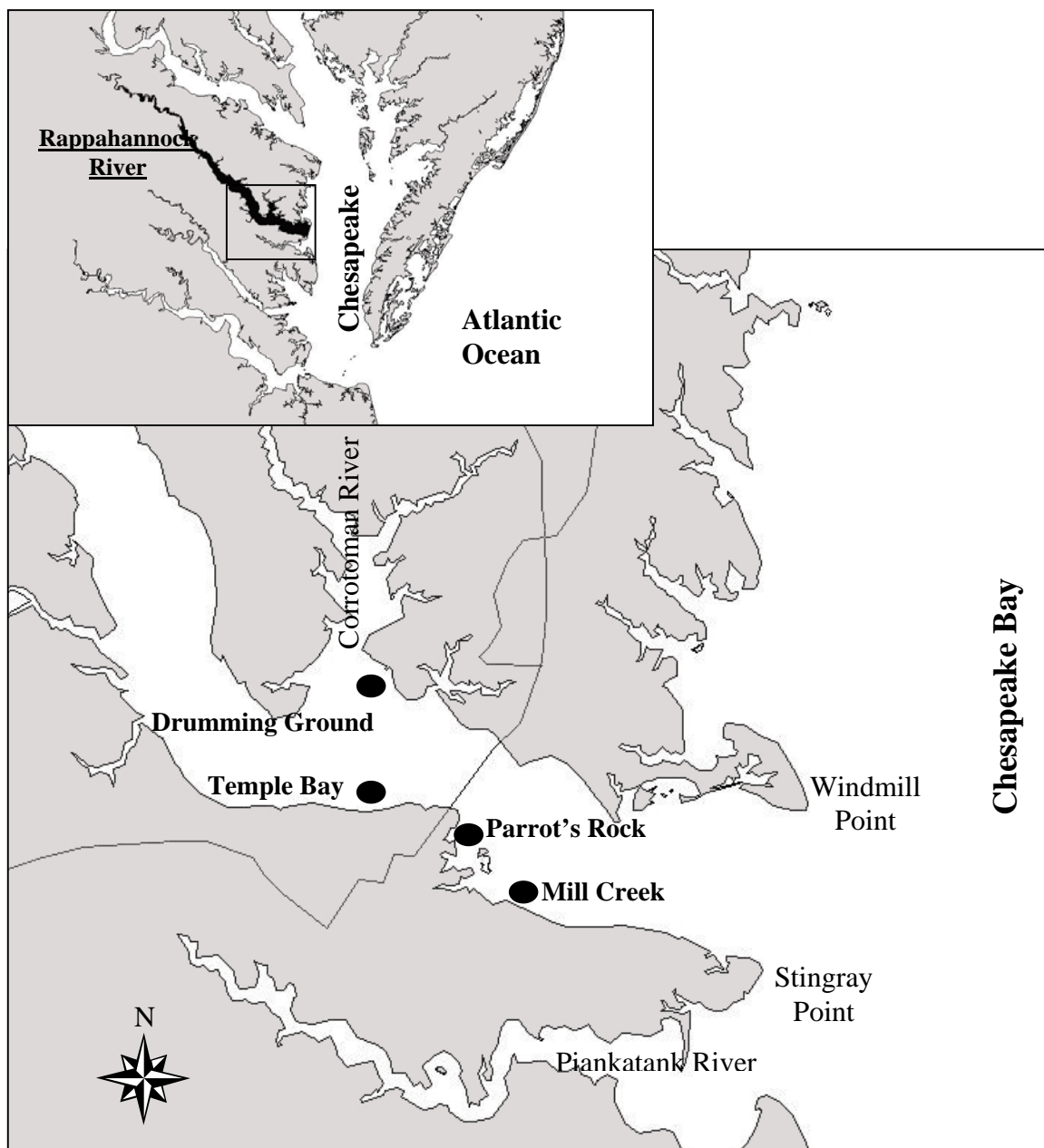
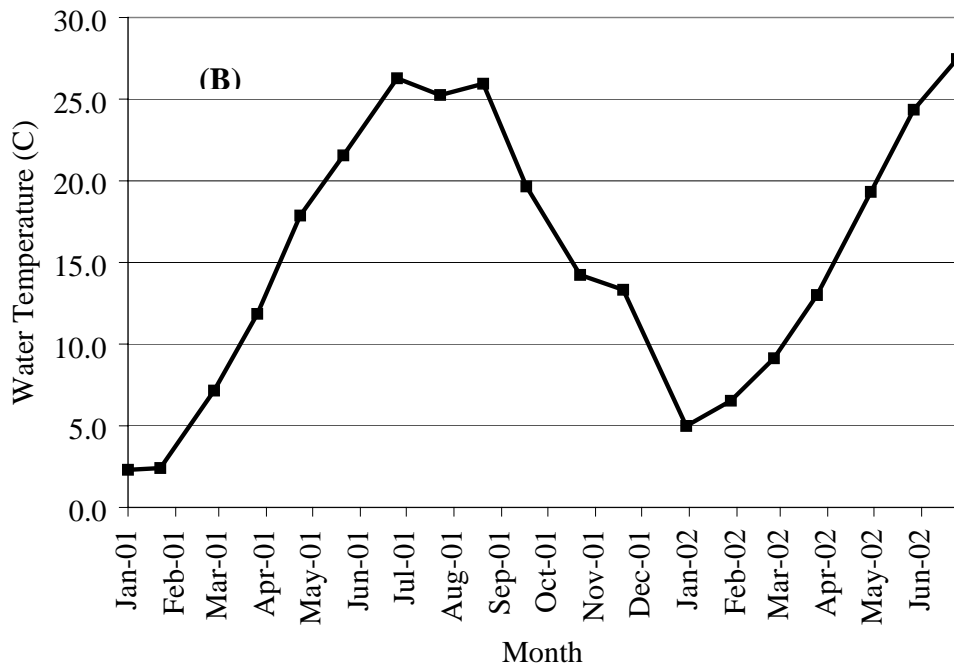
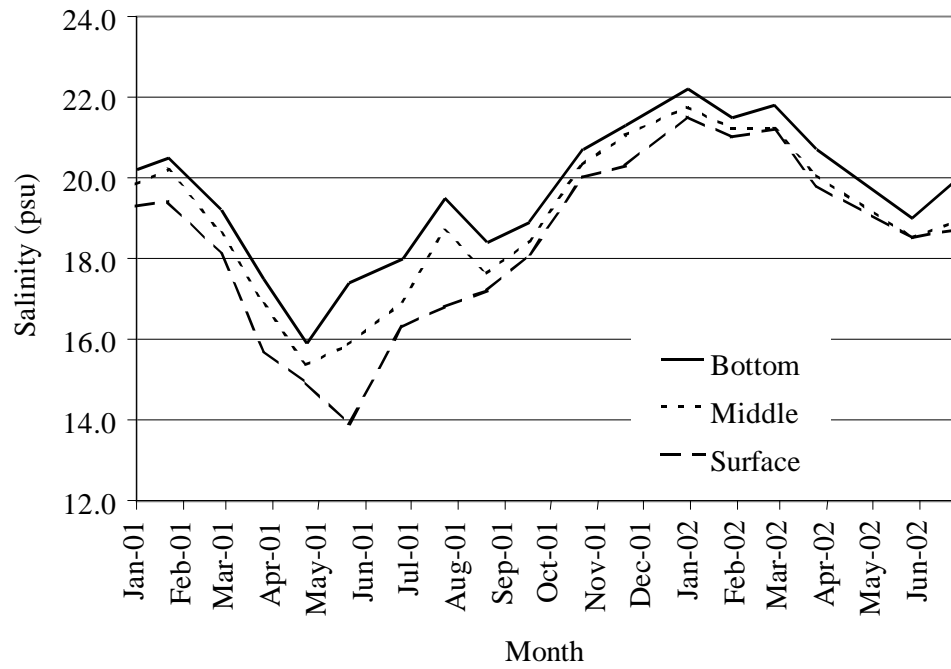


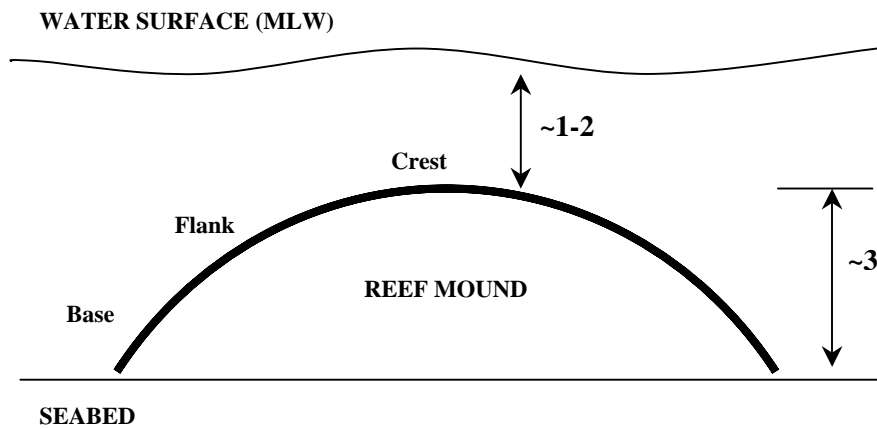
Figure 3. (A) Salinity (psu) and (B) temperature (°C) as reported by the Chesapeake Bay Program (<http://www.chesapeakebay.net/data/index.htm>) for sampling station LE 3.4 which is directly between Drumming Ground and Temple Bay sites (Figure 2).



REEF DESIGN AND CONSTRUCTION

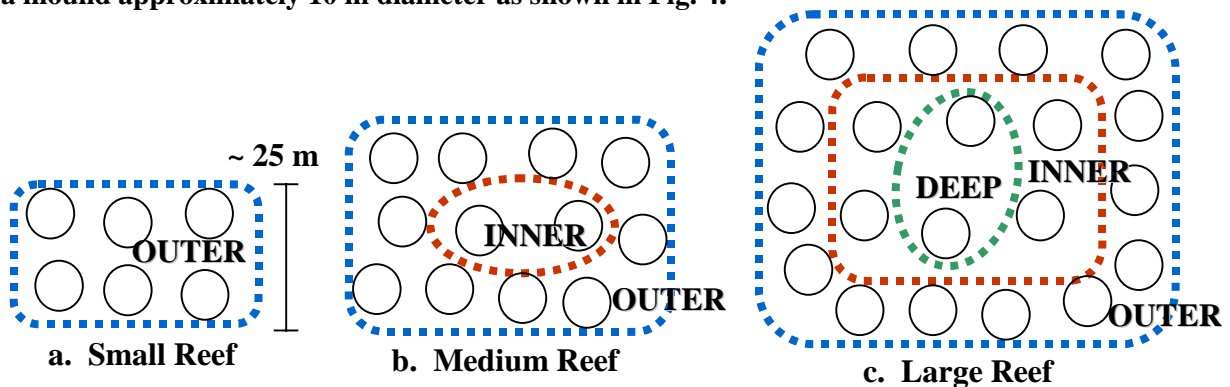
As part of Virginia’s Oyster Heritage Program, the Virginia Marine Resources Commission created the reef bases for this study during August 2000. High relief reef bases were constructed by placing shell piles as shown in Fig. 4 in arrays as shown in Fig. 5. Core material for individual mounds was comprised of surf clam (*Spisula solidissima*) shell

Figure 4. Generalized side view of an individual shell mound.



that was capped off with a veneer (~15 – 20 cm) of clean oyster shell. Materials were barged to reef sites and deployed via a crane and bucket rig. Reefs ranged in size from approximately 400 m² to 8000 m². Overall, this created ‘upside-down egg carton’ shaped

Figure 5. Generalized aerial footprint of reefs denoting intra-reef locations. Each circle represents a mound approximately 10 m diameter as shown in Fig. 4.



sub-tidal reefs. Reef crests were generally 3 m above the seabed and 1-2 m below the water surface at mean low water (Figure 4). Intra-reef locations were designated in relation to distance from reef edge along longitudinal axes (e.g., Figure 5).

SPECIFIC QUESTIONS AND HYPOTHESES

Oyster population development – Restoration of oyster reefs is not synonymous with placing settlement substrate on the seafloor. Development of self-sustaining oyster populations are required to “grow” the biogenic reef structures. Towards that end it is crucial that the architecture of the reef bases be optimized for oyster population development. Eggleston’s (1999) intermediate reef size hypothesis predicts a parabolic relationship between reef size and abundance of oysters (curve A in Figure 1). We sought to determine the actual shape of this relationship by testing several of the specific mechanisms involved: recruitment, survival and growth.

(1) *How does oyster recruitment vary across a reef and with reef size?* Recruitment reflects both settlement and early post-settlement survival. For settlement, we predict that low larval abundances (relative to historical patterns) will contribute to depletion of larvae over larger reef surfaces. Specifically, we hypothesize that settlement patterns would be as follows:

H1: Settlement rates decline with distance away from the edge of the reef crest towards the reef interior. $S_o = M_o = L_o > M_i = L_i > L_d$, where S, M and L refer to small, medium and large reef, respectively, and the subscripts, o, i & d, refer to intra-reef positions outer, inner and deep inner, respectively.

H2: On an area-normalized basis settlement rates vary inversely with reef size.

S>M>L

(2) How do predation rates on oysters vary across reefs and on reefs of varying scale?

Oyster reefs can provide habitat for a number of oyster predators, including juvenile and adult blue crabs and several species of mud crabs. Other invertebrate oyster predators (drills, starfish and flatworms) are not expected to be present in the mesohaline environments of the Rappahannock River where this study will be conducted. Colonization of the reefs by oyster predators is expected to be associated with encounter rate, which on an area-normalized basis should be related to perimeter area. Further, we expect that habitat heterogeneity, and thus refugia from predation will increase with reef size such that:

H3: Oyster mortality from predation will decrease with distance away from the edge of the reef crest. $S_o=M_o>M_i=L_i>L_d$.

Hence,

H4: Averaged over the reef, mortality rates for oysters from predation will be negatively related to reef size. S>M>L

(3) How do growth rates of oysters vary across reefs and on reefs of varying scale?

Reef structures physically alter flow parameters in a number of ways, including flow acceleration and deceleration in different areas and turbulence generation, which can lead to variations in the seston abundance field available to oysters. Additionally, oysters, through their filtration, alter seston concentrations and may contribute to food depletion. The net effect of these impacts on the supply of food to oysters will vary depending upon the

particulars of the flow regime, seston abundance fields and oyster abundances. Following the development of sufficient oyster populations on the reefs we hypothesize that seston depletion will occur across the medium and large reefs such that oyster growth rates are affected.

H5: Oyster growth rates will vary negatively with sufficient distance from the reef edge. $S_0=M_0=M_i=L_0=L_i>L_d$

Consequently,

H6: Averaged across the reef crests, oyster growth rates will be reduced on the largest reef size. $S=M>L$.

The resultant patterns of oyster abundance and population size structure across reef types will depend upon the relative strengths of each of the above mechanisms as well as other factors, such as sedimentation rates and disease mortality, which will not be experimentally investigated in this study. A likely pattern resulting from these mechanisms is provided by Eggleston's intermediate patch size model (curve A in Figure 1) in which oyster abundance (and more appropriately biomass) is maximized at intermediate reef sizes, but other patterns are possible, including those represented by curves B & C in Figure 1.

H7: Oyster abundance and biomass peak at intermediate reef size. $M>S=L$

Biodiversity – The expectation that oyster reefs provide important habitat for other species is a central tenet of current efforts to restore and protect these habitats. However, as noted above, the relationship between reef size and the numbers of species and individuals that it can support is unknown.

(4) *How does species richness and abundance for resident assemblages vary across reefs and on reefs of varying scale?* As with oysters, the result of this will depend upon the combination of recruitment, survival and growth that may vary in a variety of ways with scale for different organisms. Though resident reef biota encompass a wide array of taxa, including macroalgae, sessile invertebrates (e.g., tunicates, hydroids and bryozoans), xanthid crabs and fishes (blennies, gobies and toadfish), we expect that two factors will have the greatest influence on these processes. (i) Many species will be positively associated with oyster abundance, which is predicted to be greatest on intermediate sized reefs. (ii) Large reefs, on the other hand, are expected to provide greater habitat heterogeneity, including areas of high and low oyster density, and therefore could be expected to support more species. Though either scenario is possible, we expect that the latter will hold sway and that on an area-normalized basis large reefs will support a more diverse and abundant resident biota.

H8: Within reefs diversity and abundance of resident biota will be positively correlated with oyster abundance (or biomass). $S_o = M_o = L_o > M_i = L_i > L_d$

However, across the entire reef large reefs will support a greater total number of resident species.

H9: Total species richness of resident biota on reefs will increase with reef size.

S<M<L

(5) *How does species richness and abundance of transient fauna associated with reefs vary across reefs and with reef size?* A great many motile organisms may be associated with oyster reefs on a time variant basis, including larval, juvenile and adult finfish and zooplankton (e.g., Breitburg 1999; Coen et al. 1999a; Harding and Mann 1999). Though all of these groups may be ecologically important, for the purposes of our study we will focus on adult finfish and blue crabs (*Callinectes sapidus*). For motile species encounter rate with a reef will be a partial function of the perimeter size, which on an area-normalized basis will be greatest for small reefs. Furthermore, the reef edge provides an ecotone at the boundary of the reef and the open water/soft sediment environment that would be expected to support a high diversity of species. Thus, we predict:

H10: Abundances of transient finfish and blue crabs will be greatest at reef edges and decrease with distance into the reef. $S_o=M_o=L_o>M_i=L_i>L_d$

H11: On an area-normalized basis abundances of transient finfish and blue crabs will vary inversely with reef size. $S>M>L$.

METHODS

Physical Characterization of the Reefs

The actual size and configuration of the reefs in the study (4 sites x 3 reef sizes/site) necessarily varied from the idealized design presented above. Practical considerations including water depth, current velocity and weather conditions affected the ability of the marine contractors employed to construct the reef bases to implement the design. Therefore, prior to initiating biological sampling we mapped each reef using a Marine Sonics Technology® side-scan sonar system that links a patented PC-based survey system with a 300 kHz towfish to provide high-resolution digital sonar data that permits detailed bathymetry to be coupled with precise navigational positioning. The towfish is towed just above the seafloor and emits narrow focused beams of sound perpendicular to the direction of motion. The pulses are reflected off the seafloor and objects, such as reefs, and the signal strengths of the echoes are recorded. The system enables wide tracts of seafloor to be viewed and mapped. We employed this system during spring 2001 to map the actual size and shape of the experimental reefs and refine the locations of our proposed experimental sites. We also used these maps to evaluate which of the reefs met our design criteria and to allocate samples as indicated above.

Three reefs of different aerial extent were built at each site. However, based on our design criteria, two of these were eliminated from the study resulting in two sites with three reef sizes and two with only two sizes (Table 1 & Figure 6). At Parrot's Rock the largest reef only met our size criteria for a medium reef, while at Mill Creek the large reef was not capped off with oyster shell. Additionally, specific details of the physical characteristics of individual reef arrays will be discussed in the results section.

Figure 6. Generalized layout of reef arrays. Spatial orientations of individual mounds and different reef sizes at each site are indicated in relation to compass directions noted along the margins. Mounds used as replicates for various samplings are indicated in black. Intra-reef locations are delineated with dotted lines.

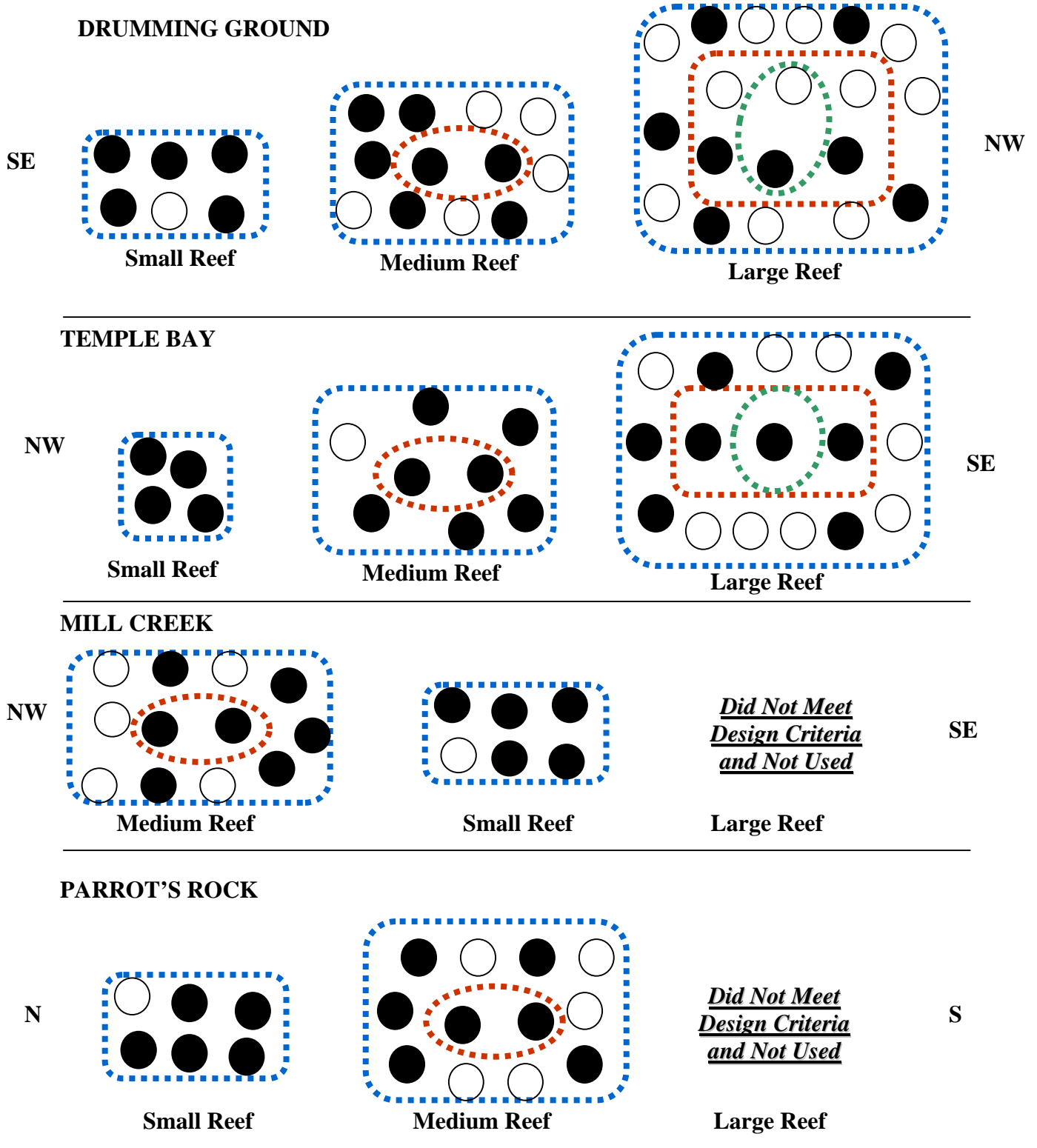


Table 1. Reef size treatments location and intra-reef locations established at each site.

Reef Size	Intra-reef Location	Site			
		Drumming Ground	Mill Creek	Parrot's Rock	Temple Bay
Large	Outer	+	-	-	+
	Inner	+	-	-	+
	Deep Inner	+	-	-	+
Medium	Outer	+	+	+	+
	Inner	+	+	+	+
Small	Outer	+	+	+	+

During spring 2001 we conducted Acoustic Doppler Current Profile (ADCP) surveys around the reefs during flood and ebbs on both spring and neap tidal cycles using a towed ADCP unit to produce current vector maps. Operating in bottom tracking mode, the ADCP measured current velocity underway from a moving boat. Vertical profiles of current data were measured with vertical resolution of 25 cm or smaller. These measurements were intended to provide a general description of the flow field around the reefs, rather than a more detailed mapping. At this stage we are not posing any specific hypotheses related to reef size and flow characteristics, rather these measurements were made in the event that they might aid in the interpretation of the results from the various biological sampling described below.

Additionally, we collected samples of reef material from randomly selected representative quadrat samples (described below) and measured the surface area of individual particles to compare between reef sites. Ten shells from each sample were

haphazardly selected. Digital images of these particles were then processed using Image Pro Plus image analysis software and one-sided surface area was estimated (mm^2). Data from samples was pooled to compare particle size between reef sites.

Oyster Settlement

Roger Mann at VIMS runs an oyster spatfall survey that maintains stations in the Rappahannock River from June through October (<http://www.vims.edu/mollusc/monorestitution/monoyster.htm>). This survey is appropriate for establishing the timing and relative magnitudes of oyster settlement between years and across tributaries; however, it is inadequate to estimate absolute settlement abundances on an individual reef or to assess

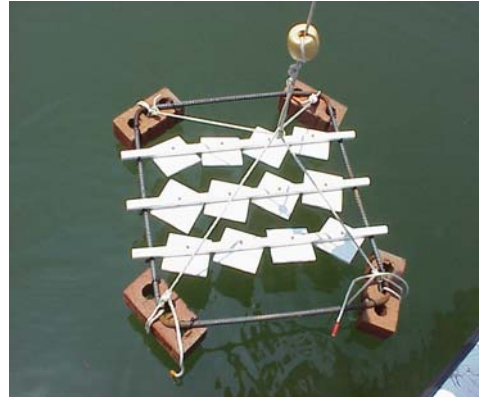
patterns in relation to reef size. Therefore, to test our hypotheses **H1** and **H2** we determined

patterns of oyster settlement at reef surfaces by deploying settlement panels constructed of 4" x 4" ceramic tiles mounted on PVC arms and steel frames 5 cm above the reef surface (Figure 7).

Replicate tile arrays were deployed on each intra-reef location and reef size combination at each

reef site during the entire recruitment period between during July – September 2001 and 2002. The numbers of replicate tile arrays allocated to each reef size and intra-reef location varied between years (see Appendix II). Tiles remained on reefs between 1-3 weeks after which time they were retrieved and replaced by new tile panels (See Appendix III for specific deployment dates and durations). After retrieval settlement tiles were transported to

Fig. 7. Array of ceramic tiles used to assay oyster settlement on the reefs.



the laboratory and the unglazed side examined under a dissecting scope for newly settled juvenile oysters. Over the course of the study, over 5,500 tiles were analyzed for newly recruited oysters.

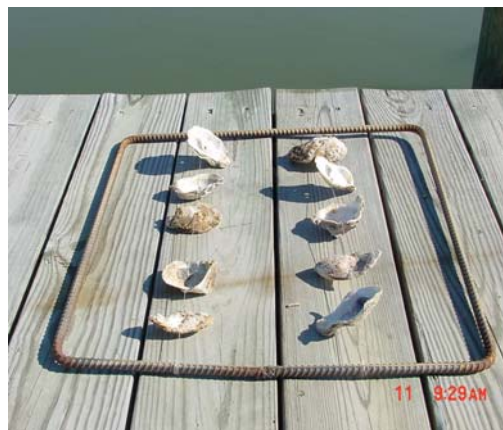
Oyster Mortality and Growth Rates

Hypotheses related to oyster mortality (**H3** and **H4**) and growth rates (**H5** and **H6**) were tested using hatchery-produced oysters deployed onto the reefs. Oysters were spawned and reared at the VIMS Eastern Shore Laboratory in Wachapreague, VA. Eyed-larvae were introduced into tanks containing filtered (1- μ nominal pore diameter) and uv irradiated seawater and clean, single oyster shells, and allowed to settle and metamorphose. Post-

settlement oysters were maintained in aerated seawater, with every other day water changes, and fed daily on a mixture of cultured algae for several weeks before being transferred to flow-through seawater tables. The oysters were maintained in this system until they reached an

approximate average size of 5 mm shell height (longest hinge-lip distance) and then they were transferred to a field nursery system until they attained appropriate sizes for use in the experiments (see Results section for specifics on sizes used seasonally). After the field nursery stages, ten of these shells were tethered to 0.30 m² reinforcing bar frames using heavy duty monofilament line through pre-drilled holes in the shells (Figure 8). Prior to

Fig. 8 “Predation frames”—Ten tethered shells with attached juvenile oysters.



deployment juvenile oysters on the shells were manipulated to achieve a total density of 38–42 oysters per frame. Shell heights of all oysters were measured to the nearest mm.

Replicate frames were deployed on the crests of reefs of each size and each intra-reef location during summer and fall 2001 and spring and summer 2002 (Table 2). The total numbers of frames deployed at each time varied depending upon the availability of appropriate sized oysters; values are given in Table 2. The numbers of frames allocated to each intra-reef location and reef size for each time are given in Appendix IV.

Table 2. Oyster growth and mortality experiments. Deployment and retrieval dates, duration, total frames deployed and retrieved.

Experiment	Date Deployed	Date Retrieved	# Days in Field	# Frames Deployed	# Frames Retrieved	% Recovered
Summer 2001	7/6/01	8/30/01	55	54	45	83
Fall 2001	8/30/01	10/23/01	54	44	39	89
Spring 2002	4/26/02	6/11/02	45	54	48	89
Summer 2002	6/18/02	8/28/02	61	54	46	85

Duration of the deployments varied between 45 and 61 days (Table 2) after which time the frames were retrieved by divers or via a tethered float at the surface. Some frames were lost during these experiments, presumably eroded off the reefs, but the recovery rate was $\geq 83\%$ across all experiments (Table 2). Surviving oysters were enumerated and measured after retrieval. Frames deployed during summer 2001 were retrieved, all oysters counted and measured, then re-deployed for the fall 2001 experiment. During the course of these experiments over 6,800 oysters were deployed to field experiments.

Disease diagnostics

Additionally, in August 2002, 25 oysters from each site were tested to assess the prevalence and intensity of several important oyster diseases. Oysters that were sampled were natural recruits to each site (i.e. not oysters that we had reared and deployed).

Perkinsus marinus infections were diagnosed with Ray's Thioglycollate medium assays (Ray 1952). *Haplosporidium nelsoni* infections were diagnosed using standard histological techniques (Burreson et al. 1988). All disease diagnoses were performed by the VIMS Shellfish Pathology Laboratory.

Oyster Abundance and Biomass

Oyster abundance and biomass were estimated from replicate 25 cm x 25 cm quadrat samples excavated from the reef surfaces by divers, collecting all reef material to a depth of 10 cm. Samples were collected during July 2001, October 2001 and July 2002 with additional limited sampling in October 2002 (Appendix V details number of replicate samples that were taken from each reef size and location). Live oysters and articulated shells of dead oysters (henceforth referred to as "boxes") were enumerated and measured. A sub-sample of 133 of these oysters covering the full size range was selected and ash-free dry tissue weight measured. A best-fit power function was then computed relating shell height to ash-free dry tissue weight. This relationship was then used to compute biomass for all oysters sampled. These data were used to evaluate **H7** relating oyster abundance and biomass to reef size.

Epifaunal Community

Epifaunal communities on the reefs were characterized from the same quadrature samples described above. All organisms in the quadrature samples were identified to the lowest practical taxon and enumerated. These data were used to test hypotheses **H8** and **H9**.

From the October 2001 sample, standard size and dry tissue biomass were determined from a sub-sample of oysters, barnacles and ribbed mussels covering the entire size range encountered for each species. Individuals were dried to a constant weight at 90°C and ashed at 538 °C for 5 hours to determine ash-free dry weight (AFDW). Size-weight regressions were constructed using linear and power functions as appropriate. Over 135,000 organisms were enumerated and over 8,500 were used in constructing size-weight regressions.

Motile Resident Organisms

We sampled small resident mobile fishes and crustaceans using substrate baskets embedded in the reef. Thirty cm diameter PVC pipe was cut into 15 cm lengths and one end covered with 1 mm plastic mesh. Three 15 cm diameter ovals were made along the midline of this PCV ring and also covered with 1 mm mesh (Figure 9). Baskets were

Figure 9. Substrate basket used for sampling reef resident fish and decapods.



then filled with clean oyster shells similar to those used in the reef construction and buried by divers flush with the reef surface. The mesh bottom and holes in the sides permitted the exchange of interstitial pore water with the surrounding reef, while the basket allowed the

retrieval of intact samples which retain mobile reef residents such as blennies, gobies and mud crabs.

During April 2001, 189 baskets were deployed onto each reef site, size and intra-reef locations (Table 3). The reef surface was excavated and the baskets planted flush with the reef surface. Divers retrieved replicate baskets in July 2001, October 2001 and July 2002; unfortunately, some baskets were lost during the course of the study (see Appendix V for numbers of replicates retrieved at each location). Upon retrieval, baskets were transported to the laboratory for processing. All motile organisms in the baskets were removed and fixed initially with an isotonic histological fixative (Normalin) and then transferred to 70% ethanol for storage. Organisms were later identified to the lowest practical taxon, enumerated, and, where appropriate, measured.

Table 3. Numbers of replicate substrate baskets deployed at each *Site, Reef size and Intra-reef location* during April 2001. (See Appendix V for numbers retrieved during subsequent sampling.)

Reef Size	Intra-reef Location	Site			
		DG	MC	PR	TB
Large	Outer	15	-	-	15
	Inner	6	-	-	6
	Deep Inner	3	-	-	3
Medium	Outer	15	15	15	15
	Inner	6	6	6	6
Small	Outer	15	15	15	15

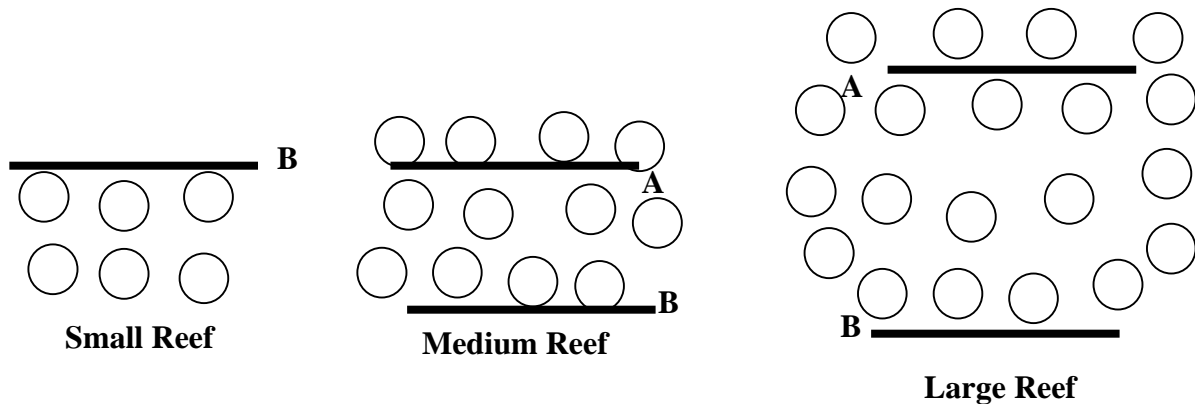
Transient Organisms

Characterizing the assemblage of motile organisms that utilize the reefs as refuge and foraging sites poses a significant challenge. No single sampling approach is sufficient to

characterize all species utilizing the reef. For the purpose of testing **H10** and **H11**, we collected data using gill nets and diver observations. Additionally, we used an otter trawl to characterize some of the other transient species present at the reefs for the purpose of providing background information. However, this technique was not utilized to test scale effects.

Gill Netting – Anchored monofilament gill nets were precisely deployed for 3 hr at both inner and outer reef locations (Figure 10). Nets measured 9 m long by 3 m high and were rigged to fish from the seabed up (i.e., sinking rigged net). During 2001, 7.5 cm (3”) and 12.5 cm (5”) stretch mesh nets were used. Because the larger mesh caught very little (the

Figure 10. Location of possible “inner” (A) vs. “outer” (B) gill net sets and diver transects on large, medium and small reefs using a generalized footprint from Drumming Ground study site.



mesh size was too large for the fish present during sampling), 6.3 cm (2.5”) and 7.5 cm (3”) were used during 2002. Nets were set at all outer and inner locations during a given deployment (2 nets each per large and medium reef and 1 net per small reef). Sets were repeated so that all locations were sampled with both mesh sizes during both flood and ebb tidal cycles within sampling periods. Nets were randomly allocated to specific locations

and a total of 31 sets were done at each site x reef size x intra-reef location combination. After 3 hr, nets were retrieved and fish were identified, enumerated, measured and released away from the reefs. In some cases, due to high catches, processing of samples had to be undertaken after all nets were harvested and taken to a remote location. In these instances, most fish were not released alive. Although the majority of gill net sampling occurred between dawn and dusk because of logistical and safety reasons, one sample effort that included all scale treatments was undertaken during the night.

Diver Observations - Divers swam 15.25 m transects along the long axis of the reefs at several locations relative to the reef (Figure 10). Transect lines consisted of cord weighted at each end. Additionally, one weight was tethered to a buoy on the surface to facilitate divers finding the beginning of a transect line without disturbing it. These transect lines were deployed >2 hr prior to actual data collection. “Inner” and “outer” transects were paired on large and medium reefs, while only an “outer” transect was deployed on small reefs. Divers recorded the species and number of fish and crabs observed. Over a 5-day period each reef in the study was surveyed twice in this manner, once during flood tide and once during ebb tide. Diver observations were conducted in June 2001 and 2002 and in August 2001.

Trawl Samples – A small 4.9 m bottom-fishing otter trawl was towed to sample finfish not caught in other gear to provide further background information on transient finfish using reefs. Paired tows, one along and one across the longest reef matrix axis were timed and performed on both flood and ebb tidal cycles. At each reef site another set of similarly paired tows were performed away from the reef arrays to get a sense of any potential “at-reef” vs. “away-from-reef” differences in species composition or abundance.

These data were not used to evaluate the effects of reef scale other than that of geographical location within the river system.

Statistical Analyses

Three main effects relating to scale were built into the experimental design for this study: 4 *Sites*; several *Reef sizes* at each site (2 sites had 3 reef sizes and 2 sites only had 2 reef sizes due to construction issues); and *Intra-reef location* within each reef size (3 within large reefs, 2 within medium reefs and 1 within small reefs). Individual mounds within the intra-reef designations were randomly selected for placement replicate gear and samples (see Figure 6). Where appropriate we used *Date* as a main effect to address inter-annual variation or seasonality. Therefore, in most cases, we used four-way full factorial ANOVA's with *Date*, *Site*, *Reef size* and *Intra-reef location* as fixed factors. When significant interactions of main effects were observed, we performed separate multi-way full factorial ANOVA to further analyze such relationships. To test some of our *a priori* hypotheses, we combined *Reef size* and *Intra-reef location* designations to identify specific *Treatments* indicated in hypotheses 1, 3, 5, 8 & 10 (e.g. S_o, M_i, L_d). Percent data were arcsine transformed prior to analysis (Sokal and Rohlf, 1997). All statistical tests were run using SAS™.

RESULTS

Physical Characterization of Reefs

Digital sonar data coupled to differential GPS were used to generate detailed bathymetry maps of each reef site. A typical map resulting from this is shown in Figure 11. Together with aerial photographs of each of the reef sites (e.g. Figure 12) we used these bathymetry maps to produce working maps of each reef site such as those in Figure 6.

Figure 11. Composite map of Parrot's Rock Reefs constructed from side scan sonar images.

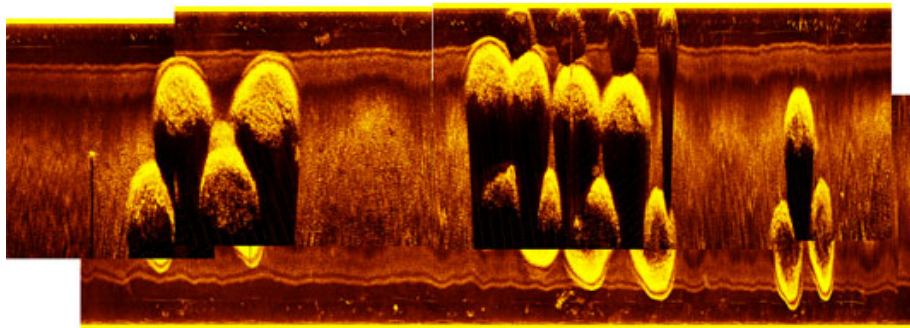
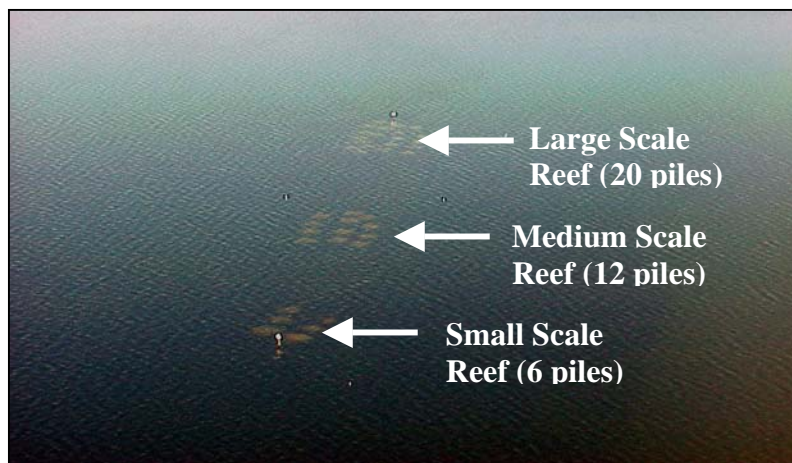


Figure 12. Aerial photograph of Drumming Ground Reefs taken at low tide.



Acoustic Doppler Current Profiles taken around the reefs revealed complex flow patterns that varied throughout the tidal cycle (Appendix VI). These data were not used to evaluate any of the specific hypotheses, but rather to provide preliminary information that might be used to generate future hypotheses related to oyster settlement and growth.

Surface area of individual reef substrate particles varied between reef sites, with larger particles on the Parrot’s Rock and Mill Creek reef sites ($p < 0.0001$; Table 4). It is unclear

whether these differences were a function of shell source or potentially differential erosive forces. We did observe small quantities of clamshell in some quadrat samples,

Table 4. One-sided surface area of individual reef substrate particles and Percent interstitial volume from each reef site. Values are means and (SE). NS = no differences between means ($p > 0.05$); means with different letters are significantly different.		
Site	Surface Area (mm)	Interstitial Volume (%)
Parrot’s Rock	3,827 (220) ^A	79.3 (0.1) ^{NS}
Mill Creek	3,386 (192) ^A	81.3 (0.1)
Drumming Ground	1,377 (192) ^B	82.0 (0.0)
Temple Bay	1,246 (192) ^B	81.8 (0.1)

indicating the potential translocation of the oyster shell veneer. This emphasizes the point that scale effects observed between reef sites may be a function of the physical characteristics of reefs in addition to geographical differences. Because interstitial space has previously been shown to be related to oyster survival on restored reefs (O’Beirn et al. 2000), we estimated interstitial volume on these reefs as water volume displaced by a standard area of reef material. No differences in interstitial volume were observed between materials from the different reef sites.

Reef Biota

A total of 62 taxa were collected at reefs during this study (Table 5). While most organisms were identified to species, it was only practical to classify others to broader taxonomic groups given the scope of this study. Because of the limits of the sampling gear we employed for this study, certain community components were underrepresented (e.g. small transient finfish). Therefore, the organisms we collected during this study likely do not represent a complete accounting of all taxa utilizing the reef.

Table 5. Species collected via all sample techniques during 2001-2002.

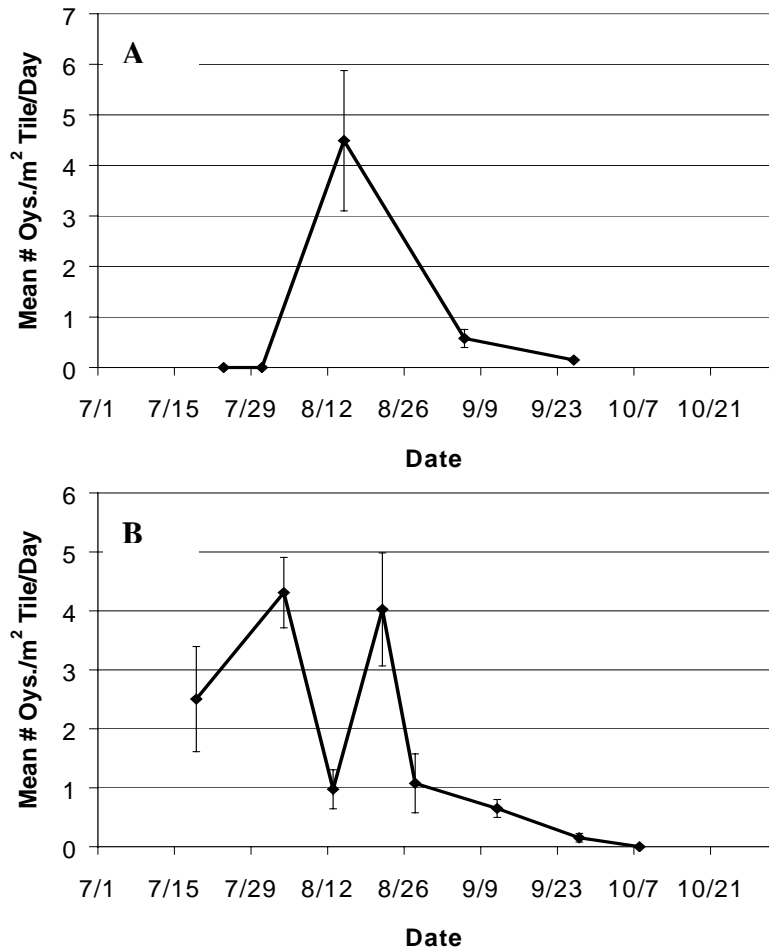
FINFISH		CRUSTACEANS	
<i>Anchoa mitchilli</i>	Bay Anchovy	<i>Balanus spp.</i>	Barnacles
<i>Anguilla rostrata</i>	American Eel	<i>Callinectes sapidus</i>	Blue Crab
<i>Archosargus probatocephalus</i>	Sheepshead	<i>Neopanope sayi</i>	Equal-clawed Mud Crab
<i>Brevoortia tyrannus</i>	Menhaden	<i>Eurypanopeus depressus</i>	Flatbacked Mud Crab
<i>Centropristis striata</i>	Black Sea Bass	<i>Gammarus sp.</i>	Amphipods
<i>Chaetodipterus faber</i>	Spadefish	<i>Palaemonetes spp.</i>	Grass Shrimp
<i>Chasmodes bosquianus</i>	Striped Blenny	<i>Panopeus herbstii</i>	Black-fingered Mud Crab
<i>Cynoscion regalis</i>	Weakfish	PORIFERA	
<i>Dasyatis say</i>	Bluntnosed Stingray	<i>Cliona celata</i>	Boring Sponge
<i>Dorosoma cepedianum</i>	Gizzard Shad	<i>Microciona prolifera</i>	Redbeard Sponge
<i>Echeneis neucratoides</i>	White-Fin Sharksucker	<i>Halichondria bowerbanki</i>	Bread Sponge
<i>Gobiesox strumosus</i>	Skilletfish	TUNICATES	
<i>Gobiosoma bosci</i>	Naked Goby	<i>Aplidium sp.</i>	Constellation Tunicate
<i>Hippocampus erectus</i>	Lined Seahorse	<i>Molgula manhattensis</i>	Sea Squirt/Grape
<i>Hypsoblennius hentzi</i>	Feather Blenny	ANNELIDS	
<i>Leiostomus xanthurus</i>	Spot	<i>Nereis succinea</i>	Clam Worm
<i>Membras martinica</i>	Rough Silverside	<i>Hydroides dianthus</i>	White Tubeworm
<i>Micropogonias undulates</i>	Croaker	PLATYHELMINTHES	
<i>Morone americanus</i>	White Perch	<i>Oligoclado floridanus</i>	Variable Flatworm
<i>Morone saxatilis</i>	Striped Bass	BRYOZOANS	
<i>Opisthonema oglinum</i>	Thread Herring	<i>Membranipora tenuis</i>	White Crust
<i>Opsanus tau</i>	Oyster Toad	CNIDARIANS	
<i>Paralichthys dentatus</i>	Summer Flounder	<i>Diadumene leucolena</i>	Ghost Anemone
<i>Pomatomus saltatrix</i>	Bluefish	MACROALGAE	
<i>Rhinoptera bonasus</i>	Cownosed Ray	<u>Polysiphonia</u>	
<i>Sciaenops ocellatusd</i>	Red Drum	<u>Enteromorpha</u>	
<i>Sphoeroides maculatus</i>	Northern Puffer	<i>Ulva</i>	
<i>Syngnathus floridae</i>	Dusky Pipefish		
<i>Synodus foetens</i>	Inshore Lizardfish		
<i>Trinectes maculatus</i>	Hogchoker		
MOLLUSCS			
<i>Anadara ovalis</i>	Blood Ark		
<i>Boonea impressa</i>	Incised Odostone		
<i>Crassostrea virginica</i>	Eastern Oyster		
<i>Doriopsilla phorpa</i>	Lemon Drop Sea Slug		
<i>Geukensia demissa</i>	Atlantic Ribbed Mussel		
<i>Macoma balthica</i>	Baltic Macoma		
<i>Mulinia lateralis</i>	Dwarf Surf Clam		
<i>Mya arenaria</i>	Steamer Clam		
<i>Mytilus edulis</i>	Blue Mussel		
<i>Nassarius vibex</i>	Eastern Mud Snail		
<i>Petricola pholadiformis</i>	False Angel Wing		
<i>Tellina sp.</i>	Tellins		

Oyster settlement

Oyster settlement data were normalized for the varying deployment durations (Appendix III) and are reported as the number of oysters per m^2 tile surface per day. In 2001 oyster settlement was monitored with bi-weekly deployment and retrieval of tile arrays; in 2002 we increased the frequency of sampling to weekly, but lost some spatial resolution by combining inner and outer location, but not deep inner, samples. Three-way full-factorial ANOVA (including year, site and reef size as factors, but not intra-reef location) revealed that overall oyster settlement was greater ($p=0.0055$) in 2002 than in 2001 ($x=1.76$, $SE=0.22$ and

$x=1.04$, $SE=0.29$, respectively), but also showed a significant interaction between year and site (Appendix VII). Subsequent analyses were performed using three-way ANOVA's (site \times reef size \times intra-reef location) for each year separately (Appendix VIII). In 2001, settlement was first recorded in late-July, peaked during early-August and diminished to

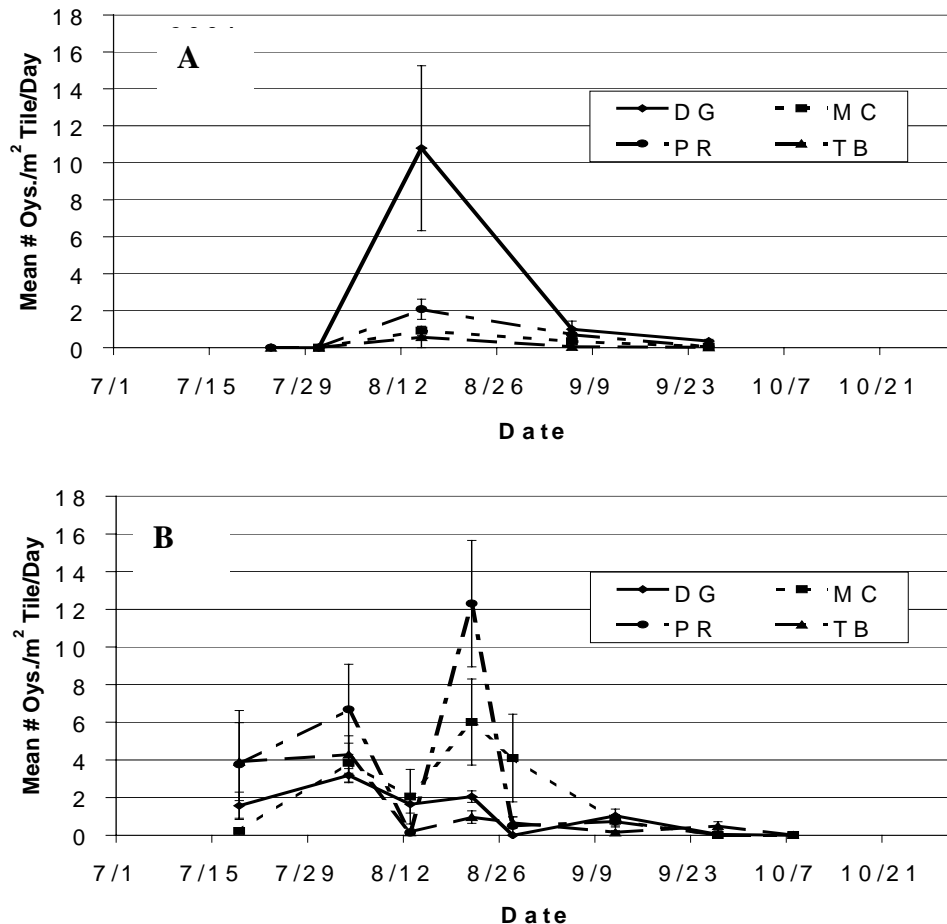
Figure 13. Overall mean (\pm SE) oyster settlement pooled data during (A) 2001 and (B) 2002 measured using settlement tiles.



near zero by late-September (Figure 13A). Settlement was observed earlier in 2002 (Figure 13B), possibly due to increased salinity resulting from low. In 2002 settlement was already occurring by our first sample in mid-July, peaking from early to mid-August and nearing zero by late-September (Figure 13B). Recruitment in 2001 appears to have one peak compared to two peaks in 2002. However, this pattern may be an artifact of the higher sampling frequency in 2002.

Settlement timing was comparable at all four reef sites in 2001 (Figure 14A). The highest settlement peak was observed at the Drumming Ground reefs, followed by the Parrot Rock reef that had a higher settlement peak than the other two sites. Oyster stock

Figure 14. Mean oyster settlement (+/- SE) pooled by reef sites during (A) 2001 and (B) 2002 as sampled by settlement tiles.



assessments carried out by VIMS in fall 2001 also noted very high juvenile oyster abundance at Drumming Ground and, to a lesser extent, Parrot Rock (Southworth et al. 2002). In 2002, settlement timing followed similar patterns at all four sites, with Parrot Rock and Mill Creek having higher settlement than the other sites, especially during the late-August peak. Three-way ANOVA's revealed no significant differences between sites in total oyster settlement over the entire period during 2001 (Appendix VIIIA). During 2002 there was a significant effect of site on oyster settlement, with Parrot's rock having the highest settlement levels (Appendix VIIIB). Oyster stock assessments carried out by VIMS in fall 2002 also noted high juvenile oyster abundance at Parrot's Rock relative to other reef sites in the River system (Southworth et al. 2003).

Oyster settlement was variable across reef size and intra-reef locations (Table 6); however, no significant differences in oyster settlement were observed with respect to either reef size or intra-reef location for either both years combined (Appendix VII) or for years analyzed separately (Appendix VIII).

We tested **H1** relating settlement rates to distance from the reef edge by treating specific reef size x intra-reef location combinations as treatments and running separate two-way ANOVA's for each summer's data using treatment and site as factors. Our *a priori* hypothesis and observed results are shown in Table 7. Since *Outer* and *Inner* intra-reef samples were combined in 2002, we tested a modified version of our original hypothesis with the second year data. **H2** relating settlement rates to reef size was tested using the full data set from both years (Appendix VII) and separately for each year (Appendix VIII). In each case we were unable to reject the null hypothesis of no effect of reef size on oyster settlement (Table 8).

Table 6. Oyster recruitment onto settlement tile arrays by Reef size and Intra-reef location. Values are means (SE) for oysters m⁻² d⁻¹ for (A) Summer 2001 and (B) Summer 2002.

(A)

Reef Size	Intra-reef Location	Site			
		DG	MC	PR	TB
Large	Outer	0.87 (0.35)	-	-	0.40 (0.24)
	Inner	0.52 (0.22)	-	-	0.06 (0.06)
	Deep Inner	2.12 (1.49)	-	-	0.00 (0.00)
Medium	Outer	1.15 (0.73)	0.45 (0.21)	0.46 (0.21)	1.05 (0.56)
	Inner	2.96 (2.83)	0.19 (0.14)	0.74 (0.53)	1.67 (1.05)
Small	Outer	3.96 (2.89)	0.20 (0.08)	0.54 (0.24)	0.44 (0.24)

(B)

Reef Size	Intra-reef Location	Site			
		DG	MC	PR	TB
Large	Outer/Inner	1.45 (0.38)	-	-	1.53 (0.57)
	Deep Inner	1.08 (0.41)	-	-	2.19 (1.48)
Medium	Outer/Inner	1.14 (0.30)	2.56 (0.92)	2.95 (1.23)	1.49 (0.48)
Small	Outer/Inner	1.06 (0.30)	1.69 (0.55)	3.31 (1.31)	0.51 (0.17)

Table 7. Test of H1: Settlement rates decline with distance away from the edge of the reef towards the reef interior. (Here and henceforth observed treatment results in the hypothesis tests are listed in the rank ordering of highest to lowest values, non-significantly different treatments are connected by an “=”, significantly different values are indicated by “>”).

<u>Predicted</u>	<u>Observed 2001</u>	<u>Observed 2002</u>
$S_o = M_o = L_o > M_i = L_i > L_d,$	$L_d = M_i = S_o = M_o = L_o = L_i$	$M_{oi} = L_d = S_{oi} = L_{oi}$

Table 8. Test of H2: Settlement rates decline with increasing reef size.

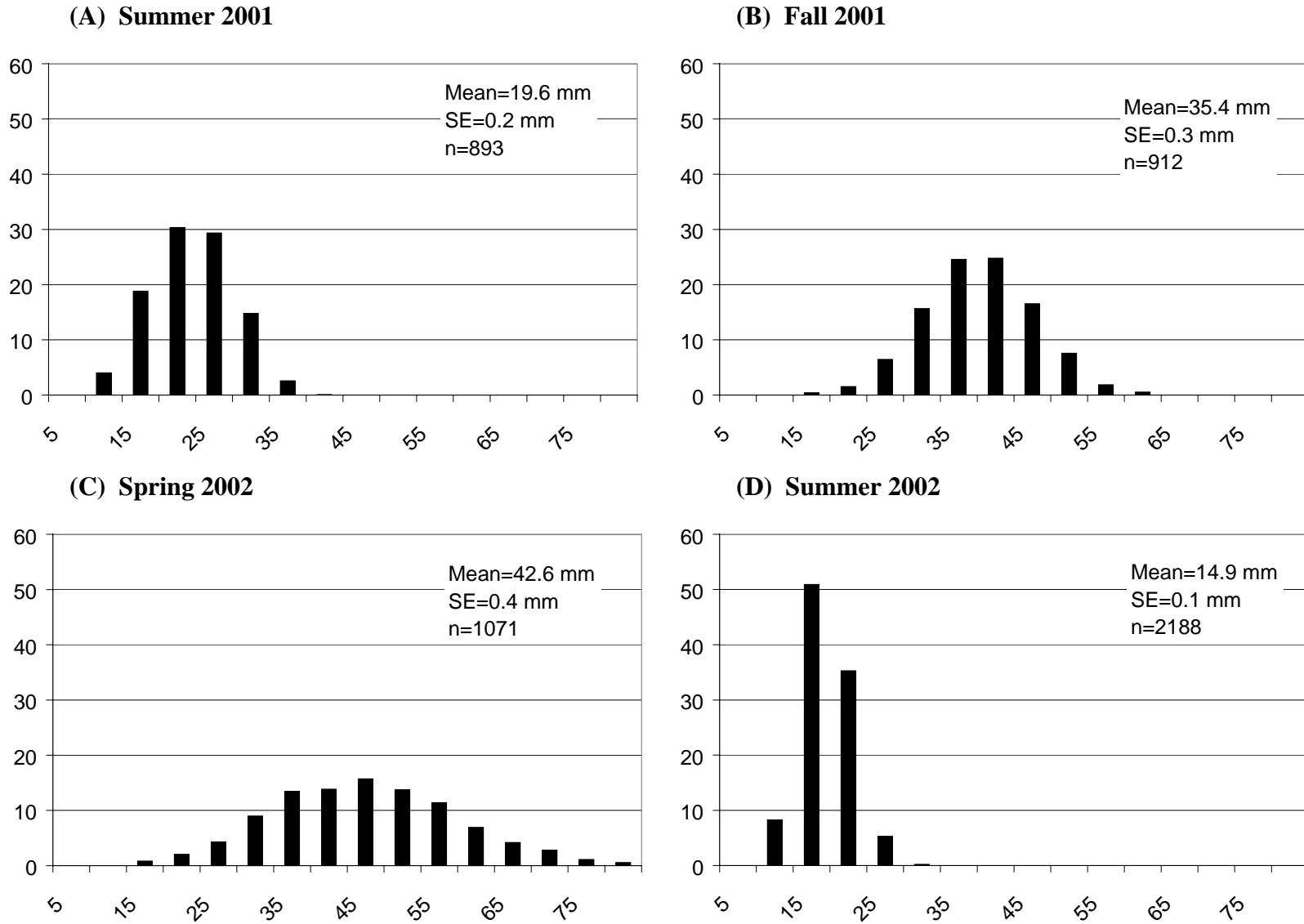
<u>Predicted</u>	<u>Observed, both years combined</u>	<u>Observed 2001</u>	<u>Observed 2002</u>
$S > M > L,$	$S = M = L$	$S = M = L$	$S = M = L$

Oyster Mortality and Growth

Four groups of experiments were deployed over several seasons during the study, summer 2001, fall 2001, spring 2002 and summer 2002 (see Appendix IV for dates and duration of deployment). As noted above, different sized oysters were used during different seasons to measure oyster mortality and growth, generally reflecting the size of oysters present on the reefs at that time (Figure 15). Both mortality and growth were standardized by unit time (day), as separate experiments remained in the field for varying amounts of time. They are reported as percent mortality/day and shell height increase (mm)/day, respectively.

Different patterns of oyster mortality were observed in relation to different scales. Four-way ANOVA with *Date*, *Site*, *Reef size* and *Intra-reef location* as factors revealed significant effects of date ($p=0.0033$) and site ($p=0.0063$) on oyster mortality (Appendix IX). None of the other factors or interaction terms had a significant effect on oyster mortality (Appendix IX).

Figure 15. Initial size frequency distribution (%) of oysters deployed for mortality and growth experiments for each sample period.



As expected, seasonal differences were observed for oyster mortality rates (Table 9, Appendices IX). Mortality was significantly lower during spring 2002 than other sample times ($p=0.0033$), which were similar (Table 9).

Table 9. Initial and final shell heights and mean mortality rates by season. Values are means (SE).

Sample	Shell height (mm) at Deployment	Shell height (mm) at Retrieval	Mortality Rate (# • d ⁻¹)
Summer 2001	19.6 (0.2)	35.4 (0.2)	0.32 (0.03) ^A
Fall 2001	35.4 (0.2)	45.9 (0.3)	0.21 (0.03) ^A
Spring 2002	42.6 (0.4)	47.0 (0.4)	0.15 (0.03) ^B
Summer 2002	14.5 (0.1)	34.6 (0.2)	0.32 (0.04) ^A

Means with different letters are significantly different ($p<0.05$). Individual columns were compared separately.

Variation in oyster mortality across the various spatial scales is summarized in Table 10. Across all of the spatial scales examined, only *Site* had a significant effect on mortality rate, with Mill Creek having a higher rate than the other sites.

Table 10. Oyster mortality (% day⁻¹) at different reef scales (data pooled for all sample times). Reef site, reef size and location groupings were statistically analyzed separately.

		% Daily Mortality
Reef Site	Parrot's Rock	0.22 (0.03) ^A
	Drumming Ground	0.23 (0.02) ^A
	Mill Creek	0.35 (0.05) ^B
	Temple Bay	0.16 (0.02) ^A
Reef Size	Small	0.27 (0.03) ^{NS}
	Medium	0.28 (0.02)
	Large	0.19 (0.02)
Location	Deep	0.31 (0.08) ^{NS}
	Inner	0.22 (0.03)
	Outer	0.26 (0.02)

NS=means not significantly different; means with different letters are significantly different ($p<0.05$).

Our *a priori* prediction relating predation on oysters to distance from the reef edge (**H3**) posited declining predation rates with increasing distance from the edge. We tested this hypothesis by treating specific reef size x intra-reef location combinations as treatments and running a three-way ANOVA with *Date*, *Site* and *Treatment* as factors. This analysis resulted in significant main effects of *Date* ($p=0.00017$) and *Site* (0.0009), as observed in the four-way ANOVA, as well as a significant interaction between *Date* and *Treatment* ($p=0.0382$) (Appendix X). Because of the significant interaction, we further partitioned the dataset by date and ran separate two-way ANOVA's with *Site* and *Treatment* as main effects. In only one of these ANOVA's (the Summer 2001 data) did we find a significant ($p=0.0179$) effect of treatment (*Reef size / Intra-reef location* combination) on oyster mortality. During this sampling period the *Deep Inner* samples from the large reefs were represented by a single sample, so our inferences are tentative and indicated by “ \geq ” rather than “ $>$ ” below. (Table 11).

Table 11. Test of H3: Predation rate declines with distance from the reef edge.

<u>Predicted</u>	<u>Observed</u>
$S_o=M_o=L_o>M_i=L_i>L_d$,	Summer 01: $M_o=S_o=M_i=L_i=L_o\geq L_d$
	Fall 01: $M_o=L_i=L_o=S_o=L_d=M_i$
	Spring 02: $L_d=M_i=M_o=L_o=S_o=L_i$
	Summer 02: $L_d=M_i=S_o=M_o=L_o=L_i$

We further hypothesized that this increased edge-effect predation would result in higher overall predation rates on smaller reefs and that predation rate would decline with increasing reef size (Table 12). The reef size factor in the four-way ANOVA (Appendix IX)

provides a test of this hypothesis. We were unable to reject the null hypothesis of equal mortality rates across reef size ($p=0.9325$) and thus the observed pattern again varies from the predicted one (Table 12).

Table 12. Test of H4: Predation rate declines with increasing reef size

<u>Predicted</u>	<u>Observed</u>
S>M>L	M=S=L

Oyster growth rate averaged across all spatial scales varied over time, with the lowest growth observed during the spring 2002 deployment period (Figure 16, Table 13), corresponding to the period with the lowest mean seawater temperature. Multiple comparisons test reveals that growth rate varied significantly among all of the deployment periods.

Figure 16. Mean oyster growth rate (bars) and water temperature (line) for the four seasonal deployments

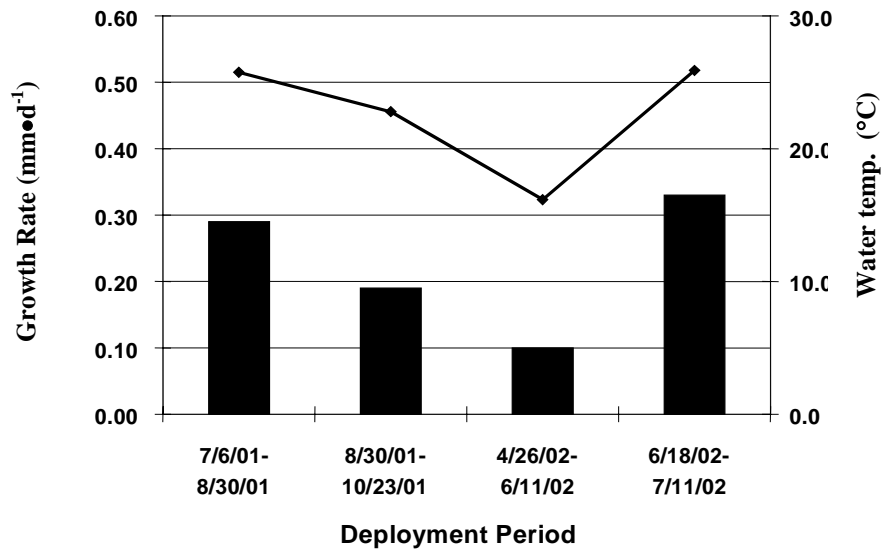


Table 13. Initial and final shell heights and mean growth rates by season. Values are means and (SE).

Sample	Mean Oyster Shell height (mm) at Deployment	Mean Oyster Shell height (mm) at Retrieval	Growth Rate (mm • d⁻¹)
Summer 2001	19.6 (0.2)	35.4 (0.2)	0.29 (0.01) ^B
Fall 2001	35.4 (0.2)	45.9 (0.3)	0.19 (0.01) ^C
Spring 2002	42.6 (0.4)	47.0 (0.4)	0.10 (0.01) ^D
Summer 2002	14.5 (0.1)	34.6 (0.2)	0.33 (0.01) ^A

Means with different letters are significantly different ($p < 0.05$). Individual columns were compared separately.

Four-way ANOVA revealed significant variation in oyster growth rates across deployment dates and sites; however, there was a significant sample date-site interaction ($p = 0.0307$) (Appendix XI). Separate three-way ANOVA's were then run for each date with *Site*, *Reef size* and *Intra-reef location* as factors. No differences were observed between *Sites* in summer 2001 and fall 2001; however, significant variation in growth rate was observed between *Sites* during spring 2002 ($p = 0.0401$) and summer 2002 ($p = 0.0025$); Table 14 A). No differences were found at smaller scales of reef size and intra-reef location (Table 14 B).

Table 14. Mean (SE) oyster growth (mm shell height•day⁻¹) for different reef scales.

(A) Reef site comparison with each season separate due to a significant *Date x Site* interaction. Statistical notation refers to each column separately.

	Summer 2001	Fall 2001	Spring 2002	Summer 2002
Parrot's Rock	0.30 (0.01) ^{NS}	0.16 (0.02) ^{NS}	0.15 (0.02) ^A	0.35 (0.01) ^A
Drumming Ground	0.28 (0.01)	0.21 (0.01)	0.08 (0.01) ^B	0.31 (0.01) ^B
Mill Creek	0.27 (0.02)	0.16 (0.02)	0.09 (0.02) ^{A,B}	0.33 (0.01) ^{A,B}
Temple Bay	0.29 (0.02)	0.20 (0.02)	0.09 (0.01) ^{A,B}	0.35 (0.01) ^A

(B) Reef size and Intra-reef location comparisons for both years pooled.

Reef Size	Small	0.23 (0.01) ^{NS}
	Medium	0.23 (0.01)
	Large	0.21 (0.01)
Location	Deep	0.24 (0.03) ^{NS}
	Inner	0.23 (0.01)
	Outer	0.22 (0.01)

NS=means not significantly different and means with different letters are significantly different (p<0.05). *Reef size* and *Location* were analyzed separately.

Our *a priori* prediction relating oyster growth rate to distance from the reef edge (**H5**) suggested growth rates would be reduced at the *Deep Inner* location relative to other intra-reef locations (Table 15). We tested this hypothesis by treating specific *Reef size x Intra-reef location* combinations as treatments and running a three-way ANOVA with *Date*, *Site* and *Treatment* as factors. Significant effects of *Date*, *Site* and *Date x Site Interaction*,

but not *Treatment* ($p=0.9569$) were observed (Appendix XII). Thus, we again failed to reject the null hypothesis and our *a priori* predictions are not supported (Table 15).

Table 15. Test of H5: Growth rate reduced at deep inner reef location

<u>Predicted</u>	<u>Observed</u>
$S_o=M_o=L_o=M_i=L_i>L_d$	$L_d=M_i=S_o=L_i=M_o=L_o$

We also hypothesized that mean growth rate averaged across all intra-reef locations would be reduced on the large reefs relative to the small and medium reefs (Table 16). Although the rank ordering of the observed means fit this prediction, *Size* was not a significant factor ($p=0.7497$) in the four-way ANOVA and our predictions were not supported (Appendix XI and Table 16).

Table 16. Test of H6: Mean growth rate across the reef declines with reef size

<u>Predicted</u>	<u>Observed</u>
$S=M>L$	$S=M=L$

Disease

We did not pose any specific hypotheses relating oyster diseases and reef scale, but we sampled oysters on the reef to determine disease status during the late Summer 2002. Both MSX and Dermo were found to be present at modes, though the prevalences and intensities were generally low to moderate (Table 17). At three of the reef sites we did find 1 oyster out of 25 sampled had high intensity infections of MSX. The observed levels of disease were not expected to cause widespread mortality to oysters during the course of these experiments.

TABLE 17. Disease causing organism prevalence and intensity in oysters sampled from each reef site. H=high, M=medium, L=low.

Site	H. nelsoni		P. marinus	
	Prevalence %	Intensity H-M-L	Prevalence %	Intensity H-M-L
Drumming Ground	12	0-2-1	4	0-1-1
Mill Creek	24	1-0-5	12	0-1-2
Parrot Rock	28	1-0-6	12	0-2-1
Temple Bay	16	1-0-3	12	0-0-3

Oyster Abundance and Biomass

Diver-collected quadrat samples provided data on the abundance, shell height and biomass of live oysters on the reefs. We also enumerated and measured “box” shells, which are dead oysters with valves still articulated. Appendix XIII provides details of sample dates and numbers of samples. No oysters were present in the summer 2001 sample and too few samples were collected in fall 2002, so only oysters sampled during the fall 2001 and summer 2002 dates were used for statistical comparisons.

Four-way ANOVA testing the effects of *Date*, *Site*, *Size* and *Intra-reef location* on oyster abundance revealed a significant main effect for *Site* ($p=0.0001$), but there were significant *Date x Site* and *Date x Intra-reef location* interactions (Appendix XIV). We then conducted the appropriate lower-level ANOVA’s for each date separately. Table 18 summarizes the effects of various scales on oyster abundance on the reefs. During the Fall 2001 sampling Drumming Ground Reef had significantly higher oyster densities than the other reef sites; however, by Summer 2002 Drumming Ground, Temple Bay and Parrot’s Rock reefs all had similar oyster densities and the Mill Creek Reef had significantly lower

densities (Table 18 A). When the data were pooled across years and sites to test for the effects of *Size* no significant differences in oyster abundance were observed (Table 18 B).

Analyzed separately by date, *Outer* reef locations had greater oyster abundances than *Inner* locations in Fall 2001, but no differences were observed in Summer 2002 (Table 18 C).

Table 18. Mean (and SE) oyster abundance (# • m⁻²) from quadrat samples for different reef scales. Note that no oysters were present in the Summer 2001 quadrat samples and are excluded from this summary. NS denotes means that are not significantly different and means with different letters are significantly different (p<0.05).

(A) Site comparison with each year separate due to a significant date x reef site interaction. Statistical notation refers to each column separately.

		Fall 2001	Summer 2002
Site	Parrot's Rock	176.0 (29.48) ^B	230.7 (23.99) ^A
	Drumming Ground	529.6 (58.53) ^A	267.0 (34.74) ^A
	Mill Creek	162.7 (26.84) ^B	76.8 (18.13) ^B
	Temple Bay	317.3 (30.62) ^B	276.9 (48.49) ^A

(B) Reef size comparison pooled for both years and all sites.

Reef Size	Small	280.9 (35.63) ^{NS}
	Medium	246.9 (26.02)
	Large	341.7 (40.15)

(C) Intra-reef location comparison with each year separate due to a significant date x location interaction. Statistical notation refers to each column separately.

		Fall 2001	Summer 2002
Location	Deep	N/A	N/A
	Inner	219.6 (53.98) ^A	218.2 (32.67) ^{NS}
	Outer	355.9 (33.99) ^B	214.9 (22.71)

Size distributions of oysters measured in the quadrature samples showed one year class in Fall 2001 and two in Summer 2002 (Figure 17) as expected. Further differences between *Sites* were also evident (Figure 18).

Figure 17 . Size frequency distribution of oysters collected in quadrature samples (pooled data) during (A) Fall 2001 and (B) Summer 2002.

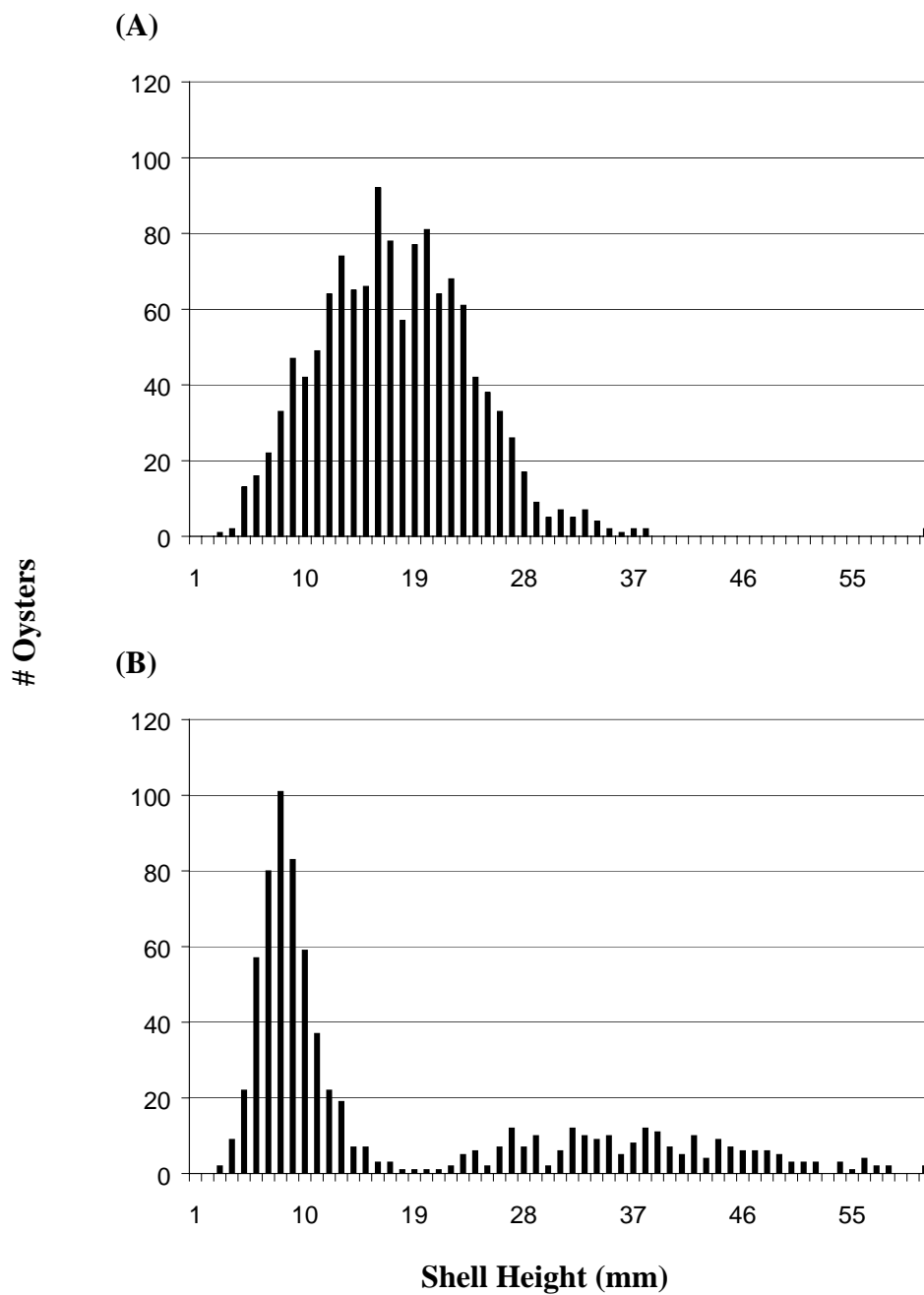
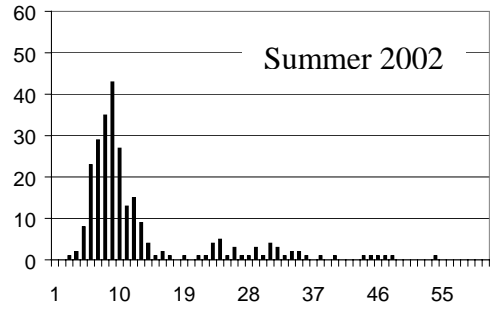
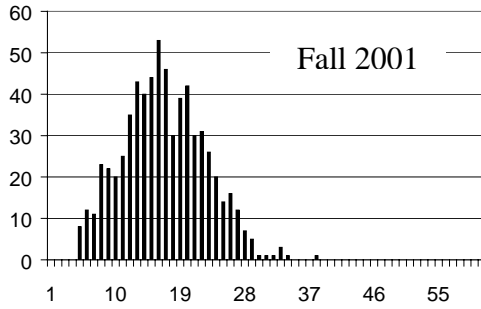
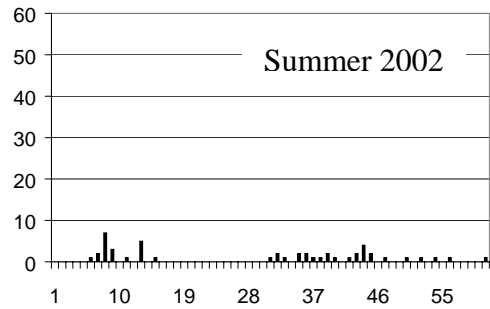
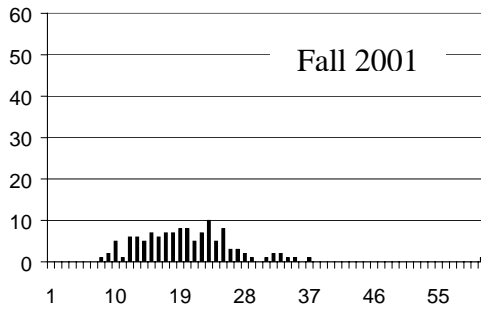


Figure 18. Size frequency distribution of oysters collected in quadrat samples at the 4 experimental reef sites during Fall 2001 and Summer 2002.

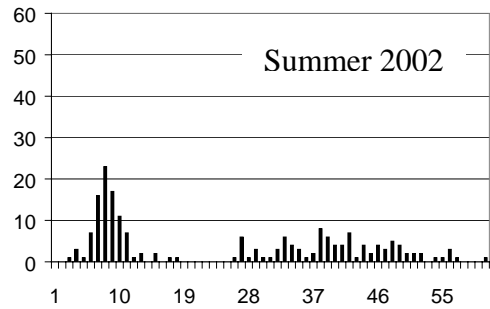
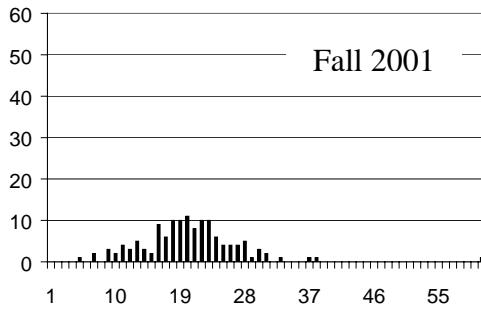
(A) Drumming Ground



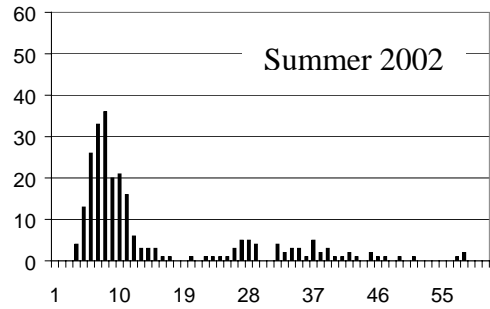
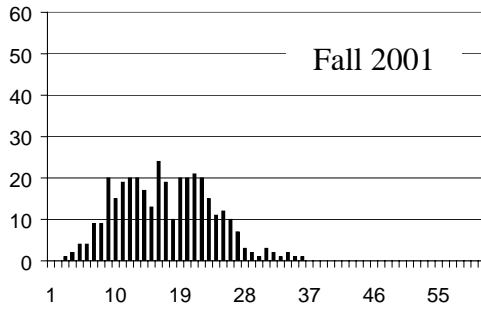
(B) Mill Creek



(C) Parrot's Rock



(D) Temple Bay



We computed a regression between shell height and ash-free dry weight from oysters collected in the Rappahannock River during this study (Appendix XV) and used the equation $\text{BIOMASS (mg)} = 0.007 \cdot \text{SHELL HEIGHT}^{2.8614}$ to estimate the biomass of all oysters collected in the quadrat samples.

Four-way ANOVA testing the effects of *Date*, *Site*, *Size* and *Intra-reef location* on oyster biomass had significant *Date x Site*, *Date x Intra-reef location*, *Site x Size* and *Date x Site x Intra-reef location* interactions (Appendix XV). We, therefore, analyzed for the effects of *Site*, *Size* and *Intra-reef location* on oyster biomass using three-way ANOVA's for each date separately. Different patterns of oyster biomass were observed in relation to reef scale (Table 19). No significant differences were observed for any reef scale for fall 2001 samples. However, significant differences between reef sites ($p=0.0015$) and intra-reef locations ($p=0.0373$) were recorded for summer 2002 (Table 19 A). Reefs at Parrot's Rock had significantly greater oyster biomass than the other reefs in Summer 2002. Also, inner reef locations had higher oyster biomass than outer reef sites. Additionally, there was a significant interaction between *Site x Reef size* interaction, resulting from the fact that Temple Bay had significantly higher oyster biomass on the medium reef (Table 19 B).

Table 19. Mean (and SE) oyster biomass ($\text{g} \cdot \text{m}^{-2}$) from quadrat samples for different reef scales. Note that, as in Table 16, no oysters were present during the Summer 2001 sampling. NS=means not significantly different and means with different letters are significantly different ($p < 0.05$).

(A) Site, Reef size and Intra-reef location effects by year.

		Fall 2001	Summer 2002
Site	Parrot's Rock	8.9 (1.5) ^{NS}	37.1 (8.7) ^A
	Drumming Ground	14.6 (1.8)	7.3 (1.8) ^B
	Mill Creek	8.3 (2.9)	15.3 (6.5) ^B
	Temple Bay	9.6 (1.0)	17.2 (5.9) ^B
Reef Size	Small	11.8 (2.1) ^{NS}	See Table 19 B below for a breakdown of 2002 Reef size data
	Medium	10.1 (1.3)	
	Large	10.9 (1.6)	
Location	Deep	N/A	N/A
	Inner	7.1 (1.3) ^{NS}	27.8 (7.8) ^A
	Outer	11.6 (1.1)	15.1 (3.3) ^B

(B) Reef size x Site effects on biomass for Summer 2002.

		Drumming Ground	Mill Creek	Parrot's Rock	Temple Bay
Reef Size	Small	9.4 (4.3)	4.5 (2.5)	46.9 (15.8)	9.6 (8.3) ^B
	Medium	3.6 (1.5)	22.5 (9.9) ^{NS}	28.9 (9.0) ^{NS}	42.0 (7.3) ^A
	Large	8.6 (3.0) ^{NS}	-	-	3.2 (1.6) ^B

We had predicted that oyster abundance and biomass, which represents a composite of recruitment, growth and survival on the reefs, would be greatest at the intermediate reef size (Table 20). For oyster abundance we were able to test this directly using the four-way ANOVA (Appendix XIV) and the observed pattern differed from our predictions (Table 20). Because of the numerous significant interaction terms noted above, it was necessary to test this hypothesis separately by date and by site (in summer 2002). The results show a variety of patterns with only the Temple Bay Reef in summer 2002 following the predicted pattern.

Table 20. Test of H7: Oyster abundance and biomass greatest at intermediate reef size. Observed biomass patterns during Summer 2002 are reported for each reef site separately (DG=Drumming Ground, MC=Mill Creek, PR=Parrot’s Rock, TB=Temple Bay).

Predicted	Observed for Abundance	Observed for Biomass	
		Fall 2001	Summer 2002 Site
M > S = L	L = S = M	S = L = M	DG: S=L=M MC: M=S PR: S=M TB: M>S=L

The density of dead, yet still articulated oysters (“boxes” • m⁻²), increased significantly (p=0.0005) from 2001 to 2002 (x=7.0, SE=1.7 and x=13.5, SE=2.9, respectively). No significant differences were seen at any reef scale during the fall 2001 sample, whereas differences were observed in 2002 for reef site, reef size and intra-reef location effects (p=0.0026, p=0.0020 and p=0.0204, respectively). The number of boxes is not only a function of mortality, but also of abundance (i.e. when mortality is equal, areas with more oysters should have more boxes). Reef scale patterns exhibited by oyster box abundance do not appear to be similar to abundance and mortality data presented earlier. It is important to note that box abundance was generally low across the board. Similar

patterns between such variables may well evolve as the oyster population further develops on these reefs.

Epifaunal Community

Over 137,000 individual sessile organisms (exclusive of oysters) were counted and identified to 15 different taxa (Table 21), with an additional 103 organisms in nine incidental (i.e. non-sessile) species in quadrat samples (Table 22). The numerically dominant species in these samples (exclusive of oysters, which were discussed above) were barnacles (*Balanus* spp.), sea squirts (*Molgula manhattensis*), the white tubeworm (*Hydroides dianthus*) and ribbed mussels (*Geukensia demissa*). Biomass for *Balanus* spp. and *G. demissa* were estimated from empirically-derived regressions below.

$$\mathbf{Balanus\ spp.:}\quad y=0.00001x^{2.8915}, \quad (\text{Eq. 1})$$

where x is barnacle basal diameter (mm) and
y is ash-free dry tissue biomass (g).

$$\mathbf{G. demissa :}\quad y=0.00002x^{2.7305}, \quad (\text{Eq. 2})$$

where x is shell height (mm) and
y is ash-free dry tissue biomass (g).

Abundance ($\# \bullet \text{m}^{-2}$), biomass density ($\text{g} \bullet \text{m}^{-2}$) and mean size (mm) for these taxa are shown together with the same values for oysters in Table 23. Densities of each of these species, with the exception of *G. demissa*, varied between sampling periods; however, biomass did not vary significantly over time (Table 23).

Table 21. Epifaunal species collected in quadrat samples. + indicates that a species was present in a given category.

Species/Group	Total Abundance	Summer 2001	Fall 2001	Summer 2002	Drumming Ground	Temple Bay	Parrot's Rock	Mill Creek	Small	Medium	Large	Deep Inner	Inner	Outer
Barnacle (<i>Balanus spp.</i>)	128,474	+	+	+	+	+	+	+	+	+	+	+	+	+
Sea squirt (<i>Molgula manhattensis</i>)	2,180	+	+	+	+	+	+	+	+	+	+	+	+	+
White tube worm (<i>Hydroides dianthus</i>)	2,131		+	+	+	+	+	+	+	+	+	+	+	+
Eastern Oyster (<i>Crassostrea virginica</i>)	2,006	+	+	+	+	+	+	+	+	+	+	+	+	+
Ribbed mussel (<i>Geukensia demissa</i>)	1,942	+	+	+	+	+	+	+	+	+	+	+	+	+
White Crust (<i>Membranipora tenuis</i>)	147	+	+	+	+	+	+	+	+	+	+	+	+	+
Sea Anemone (<i>Diadumene leucolena</i>)	116	+	+	+	+	+	+	+	+	+	+	+	+	+
Blue mussel (<i>Mytilus edulis</i>)	57	+			+	+	+		+	+	+	+	+	+
Boring Sponge (<i>Cliona celata</i>)	12		+		+	+	+		+	+	+		+	+
Red Beard Sponge (<i>Microciona prolifera</i>)	11			+	+		+		+	+	+		+	+
Bread Sponge (<i>Halichondria bowerbanki</i>)	2			+			+	+	+					+
Colonial Tunicate (<i>Aplidium</i>)	1		+				+		+					+
Enteromorpha	-	+	+		+		+	+	+	+	+	+	+	+
Sea lettuce (<i>Ulva sp.</i>)	-	+	+		+		+			+	+		+	+
Polysiphonia	-	+	+		+		+	+	+	+	+			+
Totals	137,079	10	12	9	13	9	15	10	14	13	13	9	12	15

Table 22. Non-epifaunal incidental species collected in quadrat samples. + indicates that a species was present in a given category.

Species/Group	Total Abundance	Summer 2001	Fall 2001	Summer 2002	Drumming Ground	Temple Bay	Parrot's Rock	Mill Creek	Small	Medium	Large	Deep Inner	Inner	Outer
Lemon Drop Sea Slug (<i>Doriopsilla phorpa</i>)	76	+		+	+	+	+		+	+	+	+	+	+
Flatworm (Platyhelminthes)	7	+		+	+	+	+			+	+		+	+
Baltic Macoma (<i>Macoma balthica</i>)	6		+		+	+			+	+	+		+	+
Steamer Clam (<i>Mya arenaria</i>)	4	+			+	+			+	+			+	+
Dwarf Surf Clam (<i>Mulinia lateralis</i>)	4	+	+	+	+	+				+	+		+	+
False Angel Wing (<i>Petricola pholadiformis</i>)	3	+			+	+					+			+
Blood Ark (<i>Anadara ovalis</i>)	1			+		+					+		+	
Incised Odostome (<i>Boonea impressa</i>)	1		+		+					+				+
Common Eastern Mud Snail (<i>Nassarius vibex</i>)	1		+					+		+				+
Totals	103	5	4	4	7	7	2	1	3	7	6	1	6	8

Table 23. Mean temporal abundance (# • m⁻²), dry tissue biomass (g • m⁻²) and size (mm) for epifaunal species sampled in quadrats. Notation for means follows standard statistical notation and refer to vertical comparisons within each column (NS=no significant difference, *=p<0.05, **=p<0.01, capital letters refer to multiple comparison groupings with similar letters denoting means that do not differ significantly).

	<i>Crassostrea virginica</i> ^a			<i>Balanus</i> spp. ^a			<i>Geukensia demissa</i> ^b			<i>Molgula manhattensis</i>	<i>Hydroides dianthus</i>
	Abun. (# • m ⁻²)	Biomass (g • m ⁻²)	Size (mm)	Abun. (# • m ⁻²)	Biomass (g • m ⁻²)	Size (mm)	Abun. (# • m ⁻²)	Biomass (g • m ⁻²)	Size (mm)	Abun. (# • m ⁻²)	Abun. (# • m ⁻²)
Summer 2001	0 ^B	n/a	n/a	14,530 ^A	n/a	n/a	226 ^{NS}	n/a	n/a	455 ^A	0 ^C
Fall 2001	329 ^A	10.8 ^{**}	17.1 ^{NS}	11,861 ^B	32.0 ^{NS}	6.4 ^{**}	202	16.7 ^{NS}	20.1 ^{NS}	106 ^B	90 ^B
Summer 2002	224 ^A	18.1	16.8	8,064 ^C	22.5	6.1	87	6.1	18.9	2 ^C	548 ^A

Abundances of barnacles varied both temporally and spatially on the reefs (Table 24).

Drumming Ground Reef consistently had the lowest abundance of barnacles, though the

Table 24. Mean (and SE) for barnacle abundance (# • m⁻²) from quadrat samples. NS=means not significantly different and means with different letters are significantly different (p<0.05).

(A) *Site, Reef size and Intra-reef location* effects by year.

Scale		Summer 2001	Fall 2001	Summer 2002
Reef Site	Parrot's Rock	15,228 (772) ^A	15,870 (1,882) ^{A, B}	18,457 (2,721) ^A
	Drumming Ground	12,508 (836) ^B	3,232 (602) ^C	1,400 (209) ^C
	Mill Creek	15,360 (640) ^A	21,537 (2,995) ^A	6,382 (1,737) ^{B, C}
	Temple Bay	15,759 (241) ^A	12,324 (1514) ^B	7,788 (1548) ^B
Reef Size	Small	1,4389 (678) ^{NS}	12,776 (1,964) ^{NS}	See Table 22 B below for a breakdown of 2002 Reef size data
	Medium	15,227 (448)	13,745 (1,954)	
	Large	13,521 (939)	7,594 (1,934)	
Location	Deep	11,384 (4,616) ^{NS}	4,480 (2,880) ^{NS}	-
	Inner	14,059 (1,060)	10,945 (2,952)	7,063 (2,028) ^{NS}
	Outer	14,764 (385)	12,368 (1,336)	8,315 (1,470)

(B) Effect of *Reef size and Site* for Summer 2002.

		Drumming Ground	Mill Creek	Parrot's Rock	Temple Bay
Reef Size	Small	1,315 (450)	3,172 (938)	24,323 (5,354)	10,442 (1,154) ^A
	Medium	1,568 (313)	8,522 (2,534) ^{NS}	1,4267 (,1616) ^{NS}	10,796 (4,078) ^A
	Large	1,331 (379) ^{NS}	-	-	4,456 (1,187) ^B

rank ordering of the other sites varied between sampling dates (Table 24 A). There were no effects of *Reef size* or *Intra-reef location* on barnacle abundance during either of the 2001 sampling events (Table 24 A); however, during Summer 2002 there was significant interaction between *Site* and *Reef size*. Subsequent analyses by *Site* revealed significant effects of *Reef size* only at the Temple Bay Reef, where the Large reef had significantly fewer barnacles (Table 24 B). Analysis of barnacle biomass per area of reef revealed significant differences between reef sites in Fall 2001, but no other temporal or spatial effects (Table 25).

Table 25. Mean (and SE) for barnacle biomass by *Date, Site, Reef size* and *Intra-reef location* from quadrature samples

		Fall 2001	Summer 2002
Reef Site	Parrot's Rock	43.40 (6.21) ^{A, B}	63.87 (24.06) ^{NS}
	Drumming Ground	7.50 (1.83) ^C	2.73 (0.55)
	Mill Creek	68.21 (10.82) ^A	20.48 (5.94)
	Temple Bay	27.62 (4.03) ^{B, C}	12.73 (2.35)
Reef Size	Small	37.58 (6.95) ^{NS}	37.78 (18.37) ^{NS}
	Medium	36.49 (6.49)	20.73 (3.55)
	Large	17.95 (5.03)	5.35 (1.54)
Location	Deep	8.35 (5.92) ^{NS}	14.75 ^{N/A 1}
	Inner	23.42 (8.37)	15.83 (3.89) ^{NS}
	Outer	34.71 (4.42)	24.98 (8.50)

For the sea squirt *Molgula manhattensis* both temporal and spatial differences in abundance were also observed (Table 26). *M. manhattensis* were abundant at all reef sites except Mill Creek during Summer 2001, with an abundance at Drumming Ground Reef of over 1,000 individuals • m⁻². However, by Summer 2002 sea squirts were absent from Drumming Ground and Temple Bay reefs and rare at the other two. No significant effects of *Reef size* or *Intra-reef location* were observed.

Table 26. Mean (and SE) for sea squirt abundance by *Date, Site, Reef size* and *Intra-reef location* from quadrat samples. NS=means not significantly different and means with different letters are significantly different (p<0.05).

		Summer 2001	Fall 2001	Summer 2002
Reef Site	Parrot's Rock	111 (25) ^B	144 (23) ^A	5 (2) ^{NS}
	Drumming Ground	1,076 (199) ^A	113 (34) ^{A, B}	0 ^{NS}
	Mill Creek	15 (8) ^B	11 (4) ^B	6 (4) ^{NS}
	Temple Bay	288 (31) ^B	135 (25) ^A	0 ^{NS}
Reef Size	Small	289 (69) ^{NS}	74 (20) ^{NS}	0 ^{NS}
	Medium	490 (162)	114 (22)	6 (2)
	Large	593 (165)	129 (38)	0
Location	Deep	224 (64) ^{NS}	144 (96) ^{NS}	0 ^{NS}
	Inner	577 (269)	94 (35)	1 (1)
	Outer	436 (90)	106 (17)	3 (1)

The tube-building polychaete worm *Hydroides dianthus* also showed considerable variation in abundance across temporal and spatial scales (Table 27). The temporal pattern for this species was just the reverse of that seen for sea squirts; no *H. dianthus* were

collected in the Summer 2001 samples, but this species was present on all reefs in substantial numbers during the Fall 2001 and Summer 2002 sampling event (Table 27). Densities of this worm reached a peak in this study on the Mill Creek reef during Summer 2002.

Table 27. Mean (and SE) for *Hydroides dianthus* abundance by Date, Site, Reef size and Intra-reef location from quadrat samples. NS=means not significantly different and means with different letters are significantly different (p<0.05).

		Fall 2001	Summer 2002
Reef Site	Parrot's Rock	71 (9) ^{NS}	571 (117) ^{A, B}
	Drumming Ground	83 (10)	284 (40) ^B
	Mill Creek	115 (15)	950 (296) ^A
	Temple Bay	94 (12)	564 (74) ^{A, B}
Reef Size	Small	96 (11) ^{NS}	439 (75) ^{NS}
	Medium	89 (9)	694 (152)
	Large	84 (12)	459 (74)
Location	Deep	80 (32) ^{NS}	-
	Inner	81 (12)	656 (218) ^{NS}
	Outer	92 (7)	512 (74)

For ribbed mussel (*Geukensia demissa*) abundances we observed significant interactions between *Date*, *Site* and *Reef size*, so lower level ANOVA's were used to evaluate temporal and spatial effects. Parrot's Rock Reef consistently had the highest abundances of ribbed mussels, but abundances declined there as well as on the other reefs over time (Table 28 A). No effects of *Reef size* were observed during Fall 2001 or Summer 2002, but during Summer 2001 there was a significant effect of reef size observed at the Temple Bay Reef (Table 28 B). We also did not find any significant effect of *Intra-reef location* on the abundance of ribbed mussels on the reefs (Table 28 A).

Table 28. Mean (and SE) for *Geukensia demissa* abundance from quadrat samples by (A) Date, Site, Reef size and Intra-reef location and (B) Site, Reef size and Intra-reef location for Summer 2001. NS=means not significantly different and means with different letters are significantly different (p<0.05).

(A)

		Summer 2001	Fall 2001	Summer 2002
Reef Site	Parrot's Rock	900 (200) ^A	861 (144) ^A	367 (107) ^A
	Drumming Ground	60 (15) ^B	30 (9) ^B	1 (1) ^B
	Mill Creek	113 (89) ^B	35 (10) ^B	5 (3) ^B
	Temple Bay	37 (8) ^B	63 (13) ^B	6 (3) ^B
Reef Size	Small	See Table 28 B below for a further breakdown of these data	301 (106) ^{NS}	158 (84) ^{NS}
	Medium		229 (82)	82 (36)
	Large		38 (11)	4 (3)
Location	Deep	-	32 (32) ^{NS}	-
	Inner	254 (124) ^{NS}	193 (127)	76 (45) ^{NS}
	Outer	228 (70)	210 (56)	95 (41)

(B)

		Drumming Ground	Mill Creek	Parrot's Rock	Temple Bay
Reef Size	Small	42 (8)	249 (210)	1312 (388)	80 (22) ^A
	Medium	69 (28)	16 (9) ^{NS}	606 (142) [*]	40 (10) ^{A, B}
	Large	64 (28) ^{NS}	-	-	14 (5) ^B

The only significant variations in *G. demissa* biomass were between *Date*, with none found during Summer 2001 and peak biomass during Fall 2001, and *Site*, with Parrot's Rock Reef having significantly greater biomass than the other reefs (Table 29).

Table 29. Mean (and SE) for ribbed mussel biomass by *Date*, *Site*, *Reef size* and *Intra-reef location* from quadrat samples. NS=means not significantly different and means with different letters are significantly different ($p < 0.05$).

		Fall 2001	Summer 2002
Reef Site	Parrot's Rock	85.42 (19.73) ^A	27.3252 (12.1993) ^A
	Drumming Ground	0.0619 (0.0833) ^B	0.0002 (0.0002) ^B
	Mill Creek	0.04365 (0.0231) ^B	0.0007 (0.0006) ^B
	Temple Bay	0.4185 (0.1806) ^B	0.0163 (0.0110) ^B
Reef Size	Small	27.4696 (13.3013) ^{NS}	14.0166 (9.0117) ^{NS}
	Medium	18.9187 (8.8053)	3.6390 (1.9958)
	Large	0.1009 (0.0381)	0.0082 (0.0079)
Location	Deep	0.0749 (0.0749) ^{NS}	-
	Inner	16.7144 (12.8897)	3.0851 (2.0724) ^{NS}
	Outer	17.3410 (6.5825)	7.3267 (4.1757)

Each of the dominant species, with the exception of tubeworms, decreased during the Summer 2002 sampling period (Figure 19). However, overall abundance (excluding barnacles) showed an insignificant increase over time, as did diversity of epifauna collected in the quadrat samples (Figure 29).

Figure 19. Mean abundance for several epifaunal species for the three sample periods.

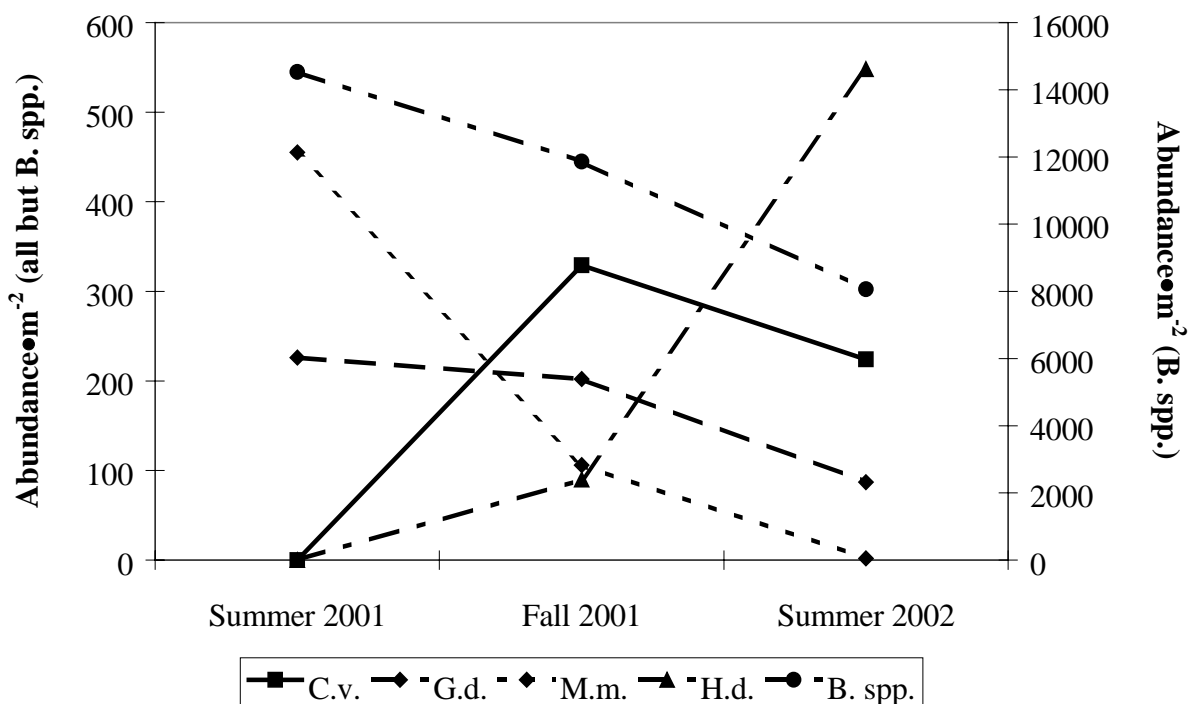
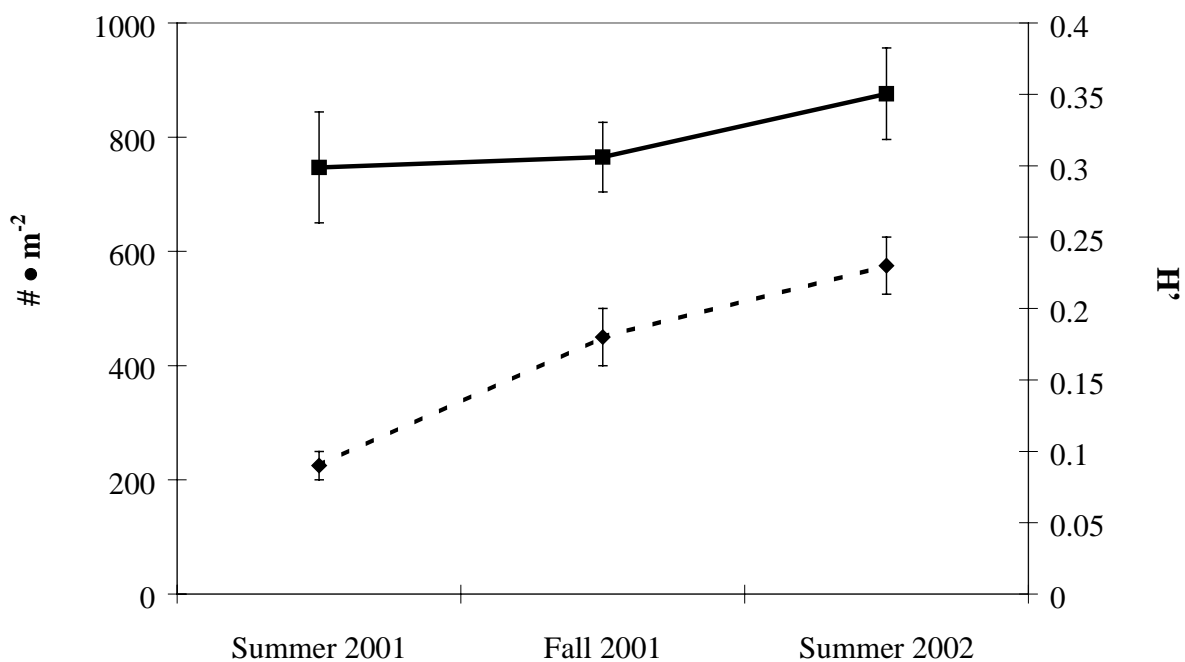


Figure 20. Mean total abundance, excluding barnacles (solid line) and Shannon-Weiner diversity index, H' , (broken line) for epifauna collected in quadrat samples during three sample periods. Bars represent \pm SE.



Motile Resident Organisms

Resident Finfish

Replicate baskets were retrieved in summer 2001, fall 2001 and summer 2002. Appendix XVII details sample deployment and retrieval dates and recovery efficiencies; only 38% of baskets were retrieved for the summer 2002 time period, but recovery efficiencies for the other periods were $\geq 95\%$. By summer 2002 many of the substrate baskets had eroded out of the reef matrix and in some cases were found capsized after tumbling down the reef mounds. We expected some losses of gear after being deployed for nearly 18 months, but did not anticipate this situation. Additionally, some finfish samples from this sample period were unusable due to problems in fixation. Therefore, only samples from 2001 were used for the following data analysis.

Overall, 8 finfish species were collected in substrate baskets (Table 30). Five of these species are generally regarded as full-time reef residents (*sensu* Breitburg 1999): skilletfish (*Gobiosox strumosus*), naked goby (*Gobiosoma bosci*), feather blenny (*Hypsoblennius hentzi*), striped blenny (*Chasmodes bosquianus*) and oyster toadfish (*Opsanus tau*). Based on the number of individuals caught, only skilletfish and naked gobies were analyzed separately. The other species were included in total abundance and diversity measures

It is important to note that no oyster population was present at the time of the summer 2001 sampling, but had developed somewhat by the fall 2001 sampling. Previous studies have indicated that resident finfish and live oyster populations are intimately correlated. Given this fact, we expected to see temporal differences in resident finfish

populations and chose *a priori* to analyze both sample dates separately with regard to reef scale effects.

Table 30. Total numbers of each fish species captured in the substrate baskets. + indicates that a species was present in the specified category.

Species	Total Abundance	Drumming Ground	Temple Bay	Parrot's Rock	Mill Creek	Small	Medium	Large	Deep Inner	Inner	Outer
Skilletfish (<i>Gobiesox strumosus</i>)	824	+	+	+	+	+	+	+	+	+	+
Naked Goby (<i>Gobiosoma boscii</i>)	568	+	+	+	+	+	+	+	+	+	+
Feather Blenny (<i>Hypsoblennius hentzi</i>)	57	+	+	+	+	+	+	+	+	+	+
Striped Blenny (<i>Chasmodes bosquianus</i>)	27	+	+	+	+	+	+	+	+	+	+
Oyster Toadfish (<i>Opsanus tau</i>)	18	+	+	+	+		+	+	+	+	+
American Eel (<i>Anguilla rostrata</i>)	11	+	+				+	+	+	+	+
Northern Pipefish (<i>Syngnathus fuscus</i>)	3	+					+			+	+
Dusky Pipefish (<i>Syngnathus floridae</i>)	2	+				+		+			+
Totals	1,510	8	6	5	5	5	7	7	6	7	8

Different patterns of resident finfish abundance were observed in relation to reef scale. Skilletfish abundance was significantly different between *Sites* for both samples ($p=0.0001$ and $p=0.0003$), but was variable at smaller scales depending on the sample date (Table 31). Medium size reefs had significantly fewer skilletfish than either large or small reefs during Fall 2001 and similar, but non-significant, pattern during Summer 2001. The

deep inner reef location (found on large reefs only) had significantly more skillettfish in Summer 2001 and a similar non-significant trend in Fall 2001 (Table 31).

Table 31. Skillettfish abundance, mean and (SE) # • m⁻², from substrate basket samples by Date, Site, Reef size and Intra-reef location. NS=means not significantly different and means with different letters are significantly different (p<0.05).

		Summer 2001	Fall 2001
Reef Site	Parrot's Rock	71 (9) ^B	62 (7) ^B
	Drumming Ground	114 (11) ^A	115 (8) ^A
	Mill Creek	45 (8) ^B	68 (7) ^B
	Temple Bay	130 (9) ^A	88 (7) ^B
Reef Size	Small	94 (9) ^{NS}	97 (8) ^A
	Medium	83 (10)	70 (7) ^B
	Large	124 (11)	107 (7) ^A
Location	Deep	212 (34) ^A	116 (7) ^{NS}
	Inner	90 (12) ^B	92 (14)
	Outer	94 (7) ^B	86 (5)

There was a significant *Site x Reef size* interaction effect on naked goby abundance in the summer 2001 sample (p=0.0035), but no differences at any scale for the fall 2001 sample (Table 32). When the Summer 2001 goby abundance data were analyzed separately by *Site*, significant differences between *Reef sizes* were only seen at the Drumming Ground site, where the small reef had significantly higher abundances of naked gobies than the other reef sizes (p=0.0056, Table 32 B).

Table 32. Goby abundance, mean and (SE) # • m⁻², from substrate basket samples. NS=means not significantly different and means with different letters are significantly different (p<0.05).

(A) By Date, Site, Reef size and Intra-reef location

		Summer 2001	Fall 2001
Reef Site	Parrot's Rock	74 (14) ^{NS}	74 (10) ^{NS}
	Drumming Ground	71 (23)	45 (5)
	Mill Creek	115 (18)	70 (10)
	Temple Bay	43 (8)	43 (6)
Reef Size	Small	See Table 32 B below for a breakdown of this Reef size data	63 (9) ^{NS}
	Medium		56 (6)
	Large		44 (4)
Location	Deep	14 (14) ^{NS}	27 (0) ^{NS}
	Inner	58 (15)	54 (9)
	Outer	78 (11)	57 (5)

(B) Reef size x Site for Summer 2001 data only.

		Drumming Ground	Mill Creek	Parrot's Rock	Temple Bay
	Small	200 (64) ^A	99 (24)	63 (21)	58 (31)
Reef Size	Medium	35 (10) ^B	127 (27) ^{NS}	82 (19) ^{NS}	39 (13)
	Large	21 (10) ^B	-	-	39 (10) ^{NS}

When grouped together, resident fish abundance followed patterns similar to skillettfish with lower abundances on medium size reefs in Fall 2001 (Table 33 A). As with

naked goby abundance, there was a significant interaction between *Site* and *Reef size* in the Fall 2001 total resident finfish data; separate analyses by *Site* revealed that abundances were lower on the small reef at Drumming Ground only (Table 33 B).

Table 33. Resident finfish, mean and (SE) # • m⁻², from substrate basket samples. NS=means not significantly different and means with different letters are significantly different (p<0.05).

(A) By Date, Site, Reef size and Intra-reef location

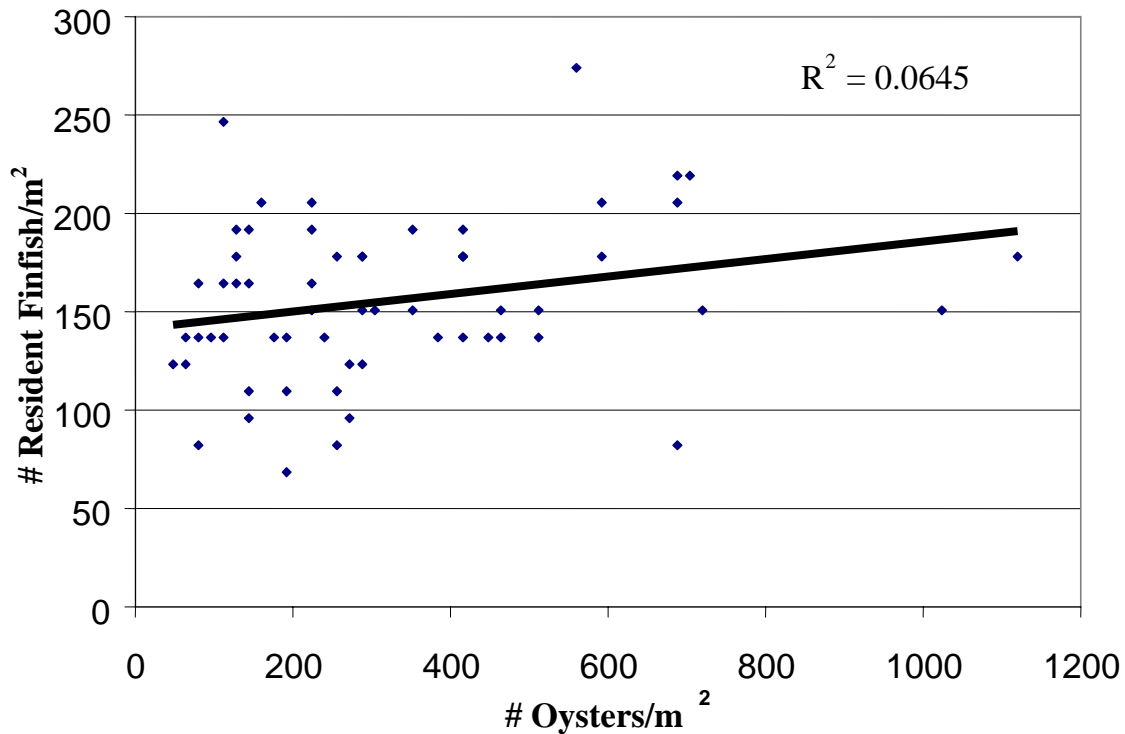
		Summer 2001	Fall 2001
Reef Site	Parrot's Rock	154 (18) ^{NS}	143 (9) ^{NS}
	Drumming Ground	200 (25)	177 (9)
	Mill Creek	174 (17)	150 (13)
	Temple Bay	177 (12)	143 (9)
Reef Size	Small	See Table 33 B below for a breakdown of this Reef size data	169 (10) ^A
	Medium		140 (8) ^B
	Large		166 (8) ^A
Location	Deep	226 (48) ^{NS}	164 (14) ^{NS}
	Inner	159 (13)	166 (12)
	Outer	183 (12)	153 (6)

(B) Reef size x Site for Summer 2001 data only.

		Drumming Ground	Mill Creek	Parrot's Rock	Temple Bay
Reef Size	Small	323 (73) ^A	175 ((25)	162 (32)	178 (29)
	Medium	174 (21) ^B	172. (25) ^{NS}	149 (22) ^{NS}	164 (22)
	Large	146 (14) ^B	-	-	187 (16) ^{NS}

We regressed resident finfish abundances from substrate baskets against oyster abundances from paired quadrat samples and found that a non-significant amount of variation was explained by the relationship (Figure 21). The oyster population may be too low at this point to be heavily affecting resident finfish abundance. This may change as larger and more complex oyster populations develop.

Figure 21. Linear regression of total resident fish abundance against oyster abundance.



Xanthid crabs

Mud crabs (Xanthidae) were also collected using substrate baskets. All abundance data are expressed as # • m⁻² and all size measurements refer to carapace width (mm). As noted above, replicate baskets were sampled in summer 2001, fall 2001 and summer 2002. Appendix XVII details sample deployment and retrieval dates and recovery efficiencies.

Overall, three Xanthid species were collected in substrate baskets: *Eurypanopeus depressus*, *Panopeus herbstii* and *Neopanope sayi*. *E. depressus* was the dominant species, accounting for ~98% of crabs, while *N. sayi* was rare. All three species were grouped together for data analysis as a functional group due to similar life history characteristics relative to the scope of this study.

Four-way ANOVA of Xanthid abundance revealed significant interactions between *Date* and *Site*, thus each date was analyzed separately (Table 34). The only significant

Table 34. Xanthid crab abundance, mean and (SE) # • m⁻², from substrate basket samples. NS=means not significantly different and means with different letters are significantly different (p<0.05).

		Summer 2001	Fall 2001	Summer 2002
Reef Site	Parrot's Rock	658 (81) ^B	2,067 (105) ^{B, C}	1,380 (369) ^{NS}
	Drumming Ground	350 (38) ^C	2,572 (194) ^{A, B}	1,748 (201)
	Mill Creek	1,017 (111) ^A	3,089 (194) ^A	1,613 (181)
	Temple Bay	442 (28) ^{B, C}	1,473 (102) ^C	1,201 (374)
Reef Size	Small	681 (81) ^{NS}	2,630 (247) ^{NS}	1,324 (310) ^{NS}
	Medium	610 (71)	2,194 (146)	1,661 (164)
	Large	353 (31)	1,992 (158)	1,669 (219)
Location	Deep	377 (21) ^{NS}	1,959 (370) ^{NS}	-
	Inner	535 (85)	2,261 (209)	1,745 (165) ^{NS}
	Outer	581 (52)	2,300 (133)	1,496 (165)

scale effect observed from these analyses was between *Sites* during Summer 2001 and Fall 2001, when Mill Creek consistently had the greatest abundance of Xanthid crabs, but the other sites varied between times (Table 34).

Other Motile Residents

In addition to finfish and Xanthid crabs, several other groups of organisms were also collected using substrate baskets. Gammarid amphipods and the polychaete *Nereis succinea* were the dominant groups collected (Table 35) and were used for statistical analysis. Flatworms (Platyhelminthes) and grass shrimp (*Palaemonetes*) were uncommon and isopods, blue crabs (*C. sapidus*) and nematodes were rare. These groups were not analyzed statistically. It is important to note that this technique, using substrate baskets with 1 mm

Table 35. Total numbers of other motile organisms captured in the substrate baskets. + indicates that a species was present in the specified category.

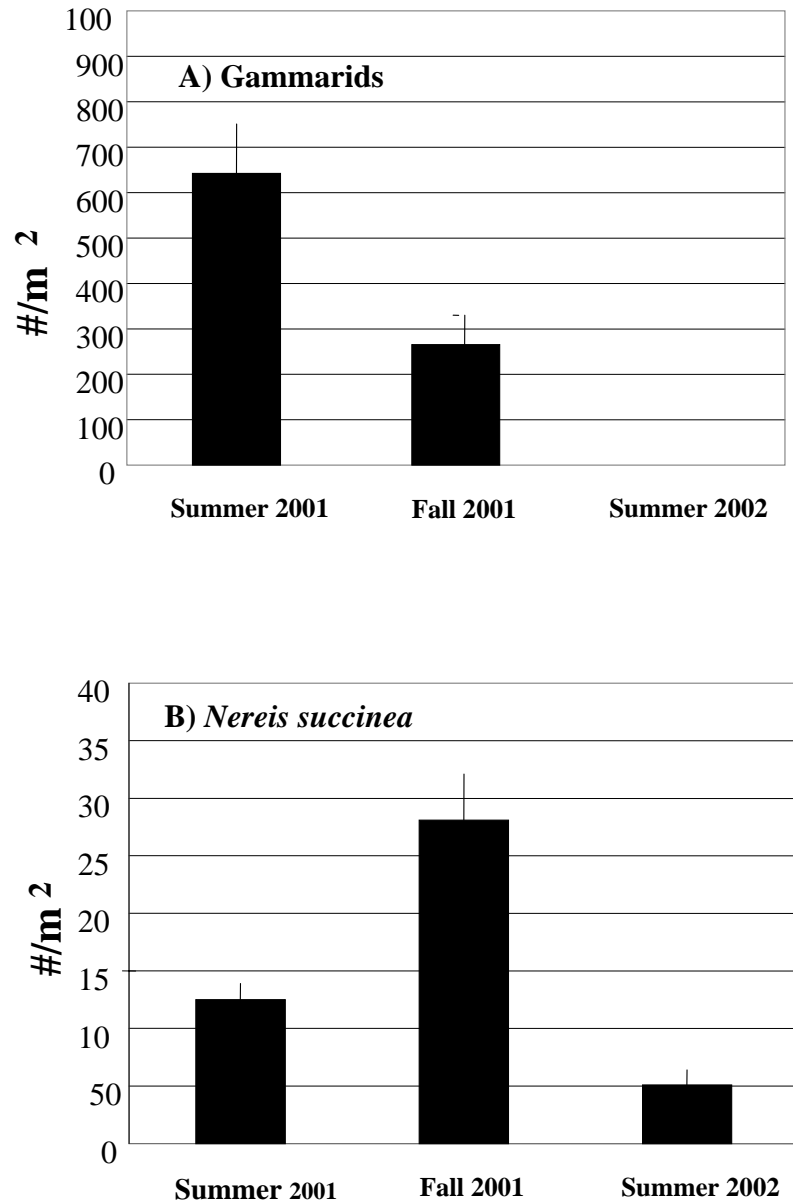
Species	Total Abundance	Drumming Ground	Temple Bay	Parrot's Rock	Mill Creek	Small	Medium	Large	Deep Inner	Inner	Other
Amphipods (<i>Gammaridae</i>)	4,070	+	+	+	+	+	+	+	+	+	+
Polychaete (<i>Nereis succinea</i>)	1,886	+	+	+	+	+	+	+	+	+	+
Flatworm (<i>Oligoclado floridanus</i>)	218	+	+	+	+	+	+	+		+	+
Grass Shrimp (<i>Palaemonetes sp.</i>)	37	+		+	+	+	+	+		+	+
Isopod (<i>Sphaeroma sp.</i>)	6	+	+				+	+			+
Blue Crab (<i>Callinectes sapidus</i>)	3	+		+		+					+
Nematods	1	+				+					+
Totals	6,221	7	4	5	4	6	5	5	2	4	7

mesh bottoms, is not quantitatively ideal for some of these groups. Gammarids and *N. succinea* could escape via mesh openings, for example. Additionally, although small blue crabs could be captured, they often fled before baskets could be secured. However, with regard to temporal and scale evaluations, we expected such impacts to be similar throughout the study and, therefore, conducive to relative comparisons.

Both Gammarid and *N.*

succinea abundances differed between sample dates (p=0.0009 and p= 0.0073, respectively). Declines over time were observed for both groups, although *N. succinea* abundance initially increased (Figure 22). Interestingly, these declining temporal trends are similar to those observed for several species of epifauna collected in quadrat samples (Figure 19). No gammarids were collected in summer 2002 samples.

Figure 22. Abundances of (A) gammarid amphipods and (B) *Nereis succinea* in substrate baskets



Gammarid abundance was significantly different between *Sites* during Summer 2001 only ($p=0.0003$; Table 35). During that sampling period very high numbers of gammarids were collected on the Drumming Ground Reef; however, in Fall 2001 the density of amphipods collected on this reef was comparable to that on the other reefs (Table 36).

Table 36. Gammarid amphipod abundance, mean and (SE) # • m⁻², from substrate basket samples. NS=means not significantly different and means with different letters are significantly different ($p<0.05$).

(A) By Date and Site

	Summer 2001	Fall 2001
Parrot's Rock	70 (38) B	211 (70) NS
Drumming Ground	1,324 (234) A	342 (126)
Mill Creek	14 (8) B	417 (226)
Temple Bay	686 (138) B	55 (24)

(B) By Reef size and Intra-reef location

	Small	388 (110) NS
Reef Size	Medium	312 (79)
	Large	496 (118)
	Deep	743 (662) NS
Location	Inner	304 (83)
	Outer	391 (68)

Mean abundance of *N. succinea* ranged from 115 individuals \bullet m⁻² at the Temple Bay Reefs to 253 individuals \bullet m⁻² at the Mill Creek Reefs, but no significant differences were observed at any reef scale for this species (Table 37).

Table 37. *Nereis succinea* abundance, mean and (SE) # \bullet m⁻², from substrate basket samples. NS=means not significantly different ($p>0.05$).

	Parrot's Rock	145 (22) ^{NS}
Reef Site	Drumming Ground	194 (26)
	Mill Creek	253 (68)
	Temple Bay	115 (26)
	Small	219 (41) ^{NS}
Reef Size	Medium	168 (29)
	Large	143 (22)
	Deep	182 (68) ^{NS}
Location	Inner	129 (26)
	Outer	190 (24)

We examined the effects of reef scale on resident biodiversity by combining the data from the quadrature samples (which sampled sessile epibenthos) with the substrate basket samples (which sampled motile epibenthos) and normalizing for area sampled. Because of the low recovery rate for substrate baskets described above, our analyses were limited to Summer 2001 and Fall 2001 data.

Our *a priori* hypothesis **H8** predicted that species richness and diversity would decrease away from the reef edge towards the center (Table 38). To test this hypothesis we combined *Reef size* and *Intra-reef locations* as before to define specific treatments and we tested the effects of *Date*, *Site* and *Treatment* in three-way, full-factorial ANOVA's for both species richness (Appendix XVIII) and Shannon-Wiener Diversity Index (Appendix XIX). These analyses revealed significant *Date* and *Site* effects for both parameters and a significant *Date* x *Site* interaction for species richness. There was, however, no significant effect treatment and we are unable to reject the null hypothesis of no difference in these parameters with distance from the reef edge (Table 38).

Table 38. Test of H8: Biodiversity (# of species and H') will be greatest at the reef edge and decrease with distance into the interior. (Treatment designations are as before and the rank ordering of treatments indicates the rank ordering of observed means; “=” indicates means that are not significantly different and “>” indicates differences among means.)

<u>Predicted</u>	<u>Observed for Species Richness</u>	<u>Observed for H'</u>
$S_o=M_o=L_o>M_i=L_i>L_d$	$L_o=M_i=L_d=S_o=L_i=M_o$	$L_d=L_i=L_o=S_o=M_i=M_o$

To test **H9**, that species richness and diversity vary with reef size (Table 39), we began with four-way ANOVA's investigating the effects of *Date*, *Site*, *Reef size* and *Intra-reef location* on both species richness and H'. These analyses revealed significant interactions between *Date* and several of the other main effects, so we conducted separate three-way ANOVA's with data from each date for species richness (Appendix XX) and diversity (Appendix XXI). In each case the effect of reef size was not significant. Thus, the null hypothesis of equal biodiversity among reef sizes could not be rejected (Table 39).

Table 39. Test of H9: Biodiversity (# of species and H') will be greatest on large reefs and decrease with reef size.

Predicted	Observed for Species Richness		Observed for H'	
	Summer 2001	Fall 2001	Summer 2001	Fall 2001
L > M > S	L = M = S	M = S = L	L = S = M	L = S = M

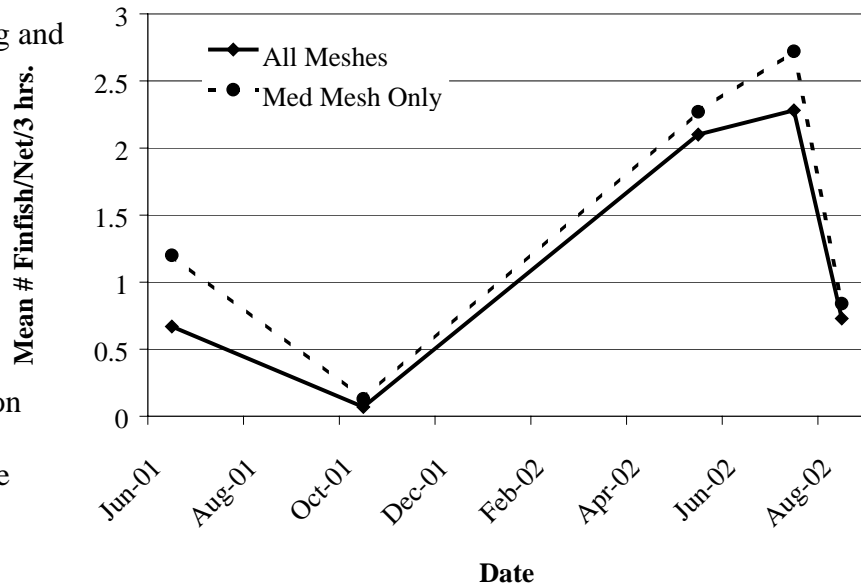
Transient Finfish

Gill net samples

Overall, 16 finfish species were collected in gill nets throughout the study (Table 40). Four of these species comprised >90% of samples and were subsequently analyzed statistically: menhaden (*Brevoortia tyrannus*), Atlantic croaker (*Micropogonias undulatus*), white perch (*Morone americanus*) and striped bass (*Morone saxatilis*). Abundances of other species were too small to yield meaningful comparisons in a full-factorial model.

As expected, transient finfish abundance was temporally variable (Fig 23), with most fish caught during the spring and early summer 2002. Therefore, we chose to conduct statistical examinations of the effects of spatial scale on these time periods. Size

Figure 23. Mean fish abundance caught in gill nets throughout the study. Medium mesh (3" stretch) was used for both years, while additionally, 5" was used in 2001 and 2.5" was used in 2002.



information by species is drawn from data pooled for all sample dates to provide basic descriptions of the fish classes encountered throughout the study.

Table 40. Total numbers of other motile organisms captured in the substrate baskets. + indicates that a species was present in the specified category.

Species	Total Abundance	Drumming Ground	Temple Bay	Parrot's Rock	Mill Creek	Small	Medium	Large	Inner	Outer
Menhaden (<i>Brevoortia tyrannus</i>)	426	+	+	+	+	+	+	+	+	+
Croaker (<i>Micropogonias undulatas</i>)	351	+	+	+	+	+	+	+	+	+
White Perch (<i>Morone americanus</i>)	221	+	+	+	+	+	+	+	+	+
Striped Bass (<i>Morone saxatilis</i>)	100	+	+	+	+	+	+	+	+	+
Spot (<i>Leiostomus xanthurus</i>)	60	+	+	+	+	+	+	+	+	+
Bluefish (<i>Pomatomus saltatrix</i>)	12		+	+	+	+	+	+	+	+
Summer Flounder (<i>Paralichthys dentatus</i>)	8	+		+	+	+	+	+	+	+
Red Drum (<i>Sciaenops ocellatus</i>)	5			+		+	+		+	+
Cownose Ray (<i>Rhinoptera bonasus</i>)	2	+					+	+		+
Sheepshead (<i>Archosargus probatocephalus</i>)	2	+						+	+	
Thread Herring (<i>Opisthonema oglinum</i>)	2			+			+		+	
Weakfish (<i>Cynoscion regalis</i>)	2			+	+	+	+			+
Gizzard Shad (<i>Dorosoma cepedianum</i>)	1			+			+		+	
Inshore Lizardfish (<i>Synodus foetens</i>)	1		+				+		+	
Sharksucker (<i>Echeneis neucratoides</i>)	1	+						+		+
Spadefish (<i>Chaetodipterus faber</i>)	1			+			+		+	
Totals	1,195	9	7	12	8	9	14	10	13	11

Generally, similar patterns of finfish abundance were observed in relation to reef scale for total fish abundance and individual species abundances (Table 41). *Micropogonias undulatus* abundance, however, was significantly different between intra-reef locations ($p=0.0309$). Significantly more *M. undulatus* were captured on the outer portions of reefs than on inner portions.

Table 41. Transient finfish abundance, mean and (SE) # • net⁻¹ • 3 hr⁻¹, captured in gill nets. NS=means not significantly different and means with different letters are significantly different ($p<0.05$).

		Total Finfish	Croaker (<i>M. undulatus</i>)	Menhaden (<i>B. tyrannus</i>)	White Perch (<i>M. mericanus</i>)	Striped Bass (<i>M. saxatilis</i>)
Reef Site	Parrot's Rock	5.5 (1.4) ^{NS}	0.9 (0.2) ^{NS}	2.8 (0.7) ^{NS}	1.3 (0.7) ^{NS}	0.1 (0.1) ^{NS}
	Drumming Ground	2.5 (0.5)	1.2 (0.2)	0.5 (0.2)	0.3 (0.1)	0.3 (0.1)
	Mill Creek	4.2 (1.2)	1.2 (0.2)	2.0 (0.9)	0.5 (0.3)	0.3 (0.1)
	Temple Bay	3.7 (0.8)	1.0 (0.1)	1.2 (0.4)	0.9 (0.3)	0.3 (0.1)
Reef Size	Small	4.7 (0.9) ^{NS}	1.4 (0.2) ^{NS}	2.0 (0.6) ^{NS}	0.6 (0.2) ^{NS}	0.3 (0.1) ^{NS}
	Medium	3.8 (0.7)	1.0 (0.1)	1.4 (0.4)	0.9 (0.3)	0.2 (0.1)
	Large	2.7 (0.7)	1.1 (0.2)	0.7 (0.3)	0.5 (0.2)	0.3 (0.1)
Location	Inner	2.7 (0.6) ^{NS}	0.7 (0.1) ^A	0.8 (0.2) ^{NS}	0.7 (0.3) ^{NS}	0.2 (0.1) ^{NS}
	Outer	4.3 (0.6)	1.3 (0.1) ^B	1.7 (0.4)	0.7 (0.2)	0.3 (0.1)

Although not analyzed statistically, total lengths of finfish (cm) are summarized in Table 42. We emphasized that these size ranges were likely heavily influenced by the gill net mesh sizes employed for sampling. Smaller fish of these species were almost certainly present, but not sampled. Larger fish were also potentially present, but not caught in the gill nets.

Table 42. Total length (cm) of fish collected in gillnets (all mesh sizes).

Species	#	Min.	Max.	Mean Size	SE
Menhaden (<i>Brevoortia tyrannus</i>)	423	19.0	40.8	28.3	0.2
Croaker (<i>Micropogonias undulates</i>)	340	19.0	45.0	30.8	0.2
White Perch (<i>Morone americanus</i>)	201	17.8	27.6	22.6	0.1
Striped Bass (<i>Morone saxatilis</i>)	101	24.2	43.8	32.3	0.4
Spot (<i>Leiostomus xanthurus</i>)	56	16.2	28.5	22.9	0.3
Bluefish (<i>Pomatomus saltatrix</i>)	12	21.7	48.8	37.1	2.3
Summer Flounder (<i>Paralichtys dentatus</i>)	7	16.5	38.5	26.9	3.5
Red Drum (<i>Sciaenops ocellatus</i>)	5	25.1	31.0	27.7	1.2
Sheepshead (<i>Archosargus probatocephalus</i>)	2	18.0	19.6	18.8	0.8
Thread Herring (<i>Opisthonema oglinum</i>)	2	22.5	22.7	22.6	0.1
Weakfish (<i>Cynoscion regalis</i>)	2	22.0	41.5	31.8	9.7
Gizzard Shad (<i>Dorosoma cepedianum</i>)	1	41.1	41.1	41.1	-
Inshore Lizardfish (<i>Synodus foetens</i>)	1	20.3	20.3	20.3	-
White-Fin Sharksucker (<i>Echeneis neucratooides</i>)	1	54.6	54.6	54.6	-
Spadefish (<i>Chaetodipterus faber</i>)	1	39.0	39.0	39.0	-
Total	1,155	-	-	-	-

Cumulative species richness and diversity (Shannon-Weiner Diversity Index) for the entire study are reported in Table 43. Statistical analysis of these parameters was limited to May and July 2002 samples, similar to preceding analyses. Species richness and diversity were significantly different between these two sample dates ($p=0.0098$ and $p=0.0370$,

respectively). Both richness and diversity increased from May ($x=1.1$, $SE=0.1$ and 0.11 , $SE=0.01$) to July ($x=1.6$, $SE=0.1$ and 0.15 , $SE=0.2$) and most likely indicate seasonality of the transient finfish population. No significant differences in either community metric were observed in relation to reef scale.

Table 43. Mean (and SE) cumulative species richness and diversity index (Shannon Weiner) for gillnet data by Site, Reef size and Intra-reef location (pooled data from all time periods).

		Richness	Diversity
Site	Parrot's Rock	8.00 (1.53)	0.63 (0.04)
	Drumming Ground	6.40 (0.24)	0.64 (0.03)
	Mill Creek	6.33 (1.20)	0.55 (0.02)
	Temple Bay	5.20 (0.37)	0.63 (0.02)
Reef Size	Small	6.50 (0.29)	0.61 (0.01)
	Medium	6.62 (0.75)	0.63 (0.03)
	Large	5.50 (0.64)	0.61 (0.05)
Location	Inner	6.33 (0.99)	0.66 (0.03)
	Outer	6.30 (0.37)	0.59 (0.02)

It is important to note several things with regard to these transient species in the context of this study. Given the high mobility of the finfish in question, individual reef complexes (e.g. the three reef sizes at Drumming Ground) may be functionally acting as one big reef. In this case, our designation of different reef sizes may be rendered meaningless.

Diver Surveys

Diver surveys were utilized to sample transient organisms on the reefs that were not adequately sampled by the gill nets. Surveys were conducted during June 2001, August 2001 and June 2002. Surveys were initially expected to gather data on transient finfish and crabs. However, while some transient species were observed, resident organisms such as gobies, blennies and Xanthids dominated samples. Because these species were sampled in a more quantitatively rigorous manner by substrate baskets (reported earlier), we chose to not analyze these groups using survey data. Furthermore, abundances of other finfish species were too low to yield meaningful comparisons. As a result, blue crabs (*C. sapidus*) were the only species analyzed statistically for this technique.

Overall, 9 finfish and 2 decapod species/groups were observed in the diver transects (Table 44). Blue crab abundance decreased over time during the study ($p < 0.0001$; Fig 24).

Figure 24. Mean blue crab (*Callinectes sapidus*) abundance (+/- SE) based on diver surveys.

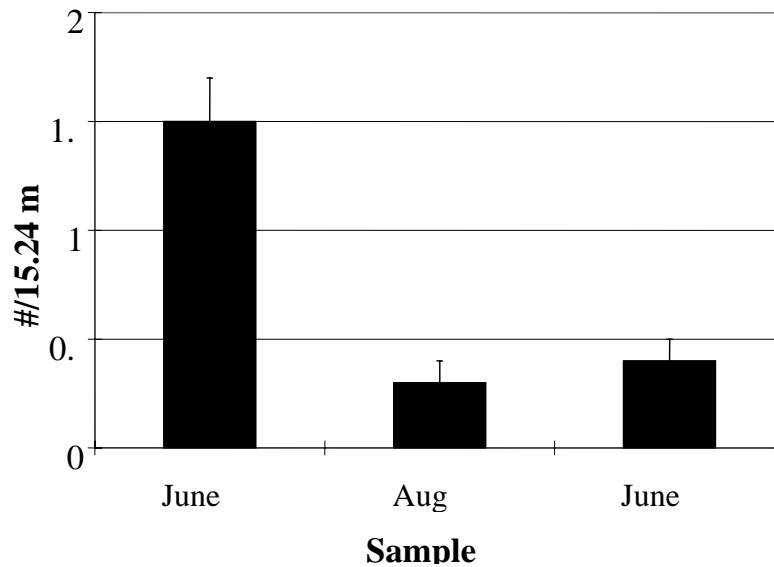


Table 44. Organisms identified from diver surveys, total numbers observed and presence at specified locations.

Species	Total Abundance	Drumming Ground	Temple Bay	Parrot's Rock	Mill Creek	Small	Medium	Large	Inner	Outer
Goby (<i>Gobiosoma</i> spp.)	3,507	+	+	+	+	+	+	+	+	+
Blenny (<i>Bleniidae</i>)	276	+	+	+	+	+	+	+	+	+
Skilletfish (<i>Gobiesox strumosus</i>)	38	+	+	+	+	+	+	+	+	+
Oyster Toadfish (<i>Opsanus tau</i>)	8	+		+	+		+	+	+	+
Pipefish (<i>Syngnathus</i> spp.)	4	+	+		+		+	+	+	+
Flatfish (<i>Pleuronectiformes</i>)	3	+		+			+		+	+
Black Sea Bass (<i>Centropristis striata</i>)	3		+		+	+				+
Northern Puffer (<i>Sphoeroides maculatus</i>)	2	+	+			+		+		+
American Eel (<i>Anguilla rostrata</i>)	1			+			+		+	
Finfish Totals	3,842	7	6	6	6	5	7	6	7	8
Blue Crab (<i>Callinectes sapidus</i>) (< 2.5 cm carapace width) (2.5-5.0 cm carapace width)	119 (37) (68)	+	+	+	+	+	+	+	+	+
Mud Crab (<i>Xanthidae</i>)	476	+	+	+	+	+	+	+	+	+
Unknown Crab (Could not distinguish group)	212	+	+	+	+	+	+	+	+	+
Crab Totals	807	3	3	3	3	3	3	3	3	3

Abundance of blue crabs was significantly different between reef sizes, with *C. sapidus* numbers increasing with larger reef sizes ($p=0.0297$) (Table 45). No significant differences were observed at other reef scales.

Table 45. Blue crab abundance, mean (SE) # • 15.25 m⁻¹ transect, by Site, Reef size and Intra-reef location.

Reef Site	Parrot's Rock	0.8 (0.3) ^{NS}
	Drumming Ground	0.8 (0.2)
	Mill Creek	0.8 (0.2)
	Temple Bay	0.7 (0.2)
Reef Size	Small	0.5 (0.1) ^B
	Medium	0.7 (0.1) ^{A,B}
	Large	1.2 (0.2) ^A
Location	Inner	0.9 (0.2) ^{NS}
	Outer	0.7 (0.1)

Trawl Samples

Trawl data was used to provide further background on transient species present at the different reef sites that might not have been sampled by other techniques. We were not able to conduct trawls at the scale of individual reefs or intra-reef locations. As such, statistical analysis was limited to comparing total fish abundance across *Sites* (# • 3 min tow⁻¹).

Overall, 11 species were collected in trawls which were dominated by *Anchoa mitchilli* (Table 46). We chose to focus analyses on total finfish and *A. mitchilli* abundance. No significant differences were observed between *Sites*.

Table 46. Species caught in trawl samples, total numbers caught and presence by site. + indicates that a species was collected at the site.

Species	Total Abundance	Drumming Ground	Temple Bay	Parrot's Rock	Mill Creek
Bay Anchovy (<i>Anchoa mitchilli</i>)		+	+	+	+
Spot (<i>Leiostomus xanthurus</i>)			+		
Weakfish (<i>Cynoscion regalis</i>)		+		+	+
Croaker (<i>Micropogonias undulatas</i>)			+		
Hogchoker (<i>Trinectes maculatus</i>)				+	
Flounder (<i>Paralichthys dentatus</i>)				+	+
Rough Silverside (<i>Membras martinica</i>)					+
White Perch (<i>Morone americanus</i>)			+		
Lined Seahorse (<i>Hippocampus erectus</i>)		+			
Bluntnose Stingray (<i>Dasyatis sayi</i>)				+	
Striped Bass (<i>Morone saxatilis</i>)				+	
Totals		3	4	6	4

We hypothesized that transient finfish would be found in greater abundance at the reef edges and decline in abundance towards the interior of the reefs (Table 47). This hypothesis was tested using gill net data from May and July 2002 when finfish were most abundant at the reef sites (Figure 23). As before, we combined *Reef size* and *Intra-reef location* to define *Treatments* and tested this hypothesis with a two-way ANOVA using *Site* and *Treatment* as factors; *Date* was not tested as a factor in this analysis since both sampling dates were from a similar time period with high fish abundance. We were unable to sufficiently sample the deep inner reef locations with gill nets, so our original hypothesis

(page 13) was modified (Table 47). Although there was not a statistically significant effect of *Treatment* in this analysis (Appendix XXII) the rank ordering of the observed means was consistent with our hypothesis (Table 47).

Table 47. Test of H10: Transient finfish abundance is greatest at the reef edges and decreases towards the interior.

Predicted	Observed
$S_o=M_o=L_o>M_i=L_i$	$S_o=M_o=L_o=M_i=L_i$

Based upon our predicted edge effect on transient finfish abundance, we further hypothesized that transient finfish abundance would be greatest around small reefs and would decrease with reef size (page 13, Table 48). We tested this hypothesis with a three-way ANOVA using *Site*, *Reef size* and *Intra-reef location* as factors and, as above, using only data from May and July 2002 (Appendix XXIII). Our observed means were in the same rank order as our *a priori* prediction (Table 47), but the means were not significantly different (Appendix XXIII).

Table 48. Test of H11: Transient finfish abundance is greatest at the reef edges and decreases towards the interior.

<u>Predicted</u>	<u>Observed</u>
$S > M > L$	$S = M = L$

DISCUSSION

We investigated reef development on three spatial scales relevant to current oyster restoration efforts in the Chesapeake Bay and other areas along the U.S. mid-Atlantic coast. The reefs in the Rappahannock River are still in the early stages of development, but variation across several spatial scales is evident. Understanding how this variation will ultimately affect the viability of the reefs and their ecological functions will be crucial to successful restoration. This study, which investigated the first two years of reef development, provides an initial step towards achieving that understanding.

Implementing an experiment on this scale necessarily involves compromises between design criteria and construction constraints. We attempted to establish a fully balanced design with all three reef sizes at each of the four sites. Unfortunately, all of our specifications were not met at two of the sites and this was not realized. Nevertheless, the resultant experimental reefs provided a powerful design for testing many of the hypotheses that we have posed.

The approach used to estimate oyster settlement rates in this study differed from the “shellstring” method that has been employed by the VIMS Spatfall Monitoring Program for several decades. The traditional approach uses clean oyster shells suspended on a wire in the water column. We choose to emphasize settlement at the reef surface and to describe variation on smaller spatial scales than typically addressed by the traditional method. Further, we computed oyster settlement as rate ($\# \bullet \text{m}^{-2} \bullet \text{d}^{-1}$) because our focus was on reef development rather than simply identifying the times of peak settlement. Consequently, direct comparisons of recruitment values between the methods are not appropriate.

Nevertheless, we note that Southworth et al. (2002, 2003) observed similar settlement timing in the Piankatank River, which is just south of the Rappahannock River, during 2001 and 2002. They also found that spatfall values in 2002 were the highest observed in 15 years in the region. Although we observed higher recruitment levels across most sites in 2002 than in 2001, cumulative recruitment rates at the Drumming Ground site were slightly higher in 2001.

Settlement was variable across years, sites and within sites; however, interactions between years and sites resulted in inconsistent patterns between the two years of this study. In 2001 we observed the highest level of recruitment on the Drumming Ground reefs, but in 2002 this site had the lowest observed recruitment of any of the sites. This interaction between temporal and spatial variation in recruitment success has important implications for the siting of future restoration efforts, suggesting that several years of oyster settlement data may be required in advance of selecting the most appropriate sites.

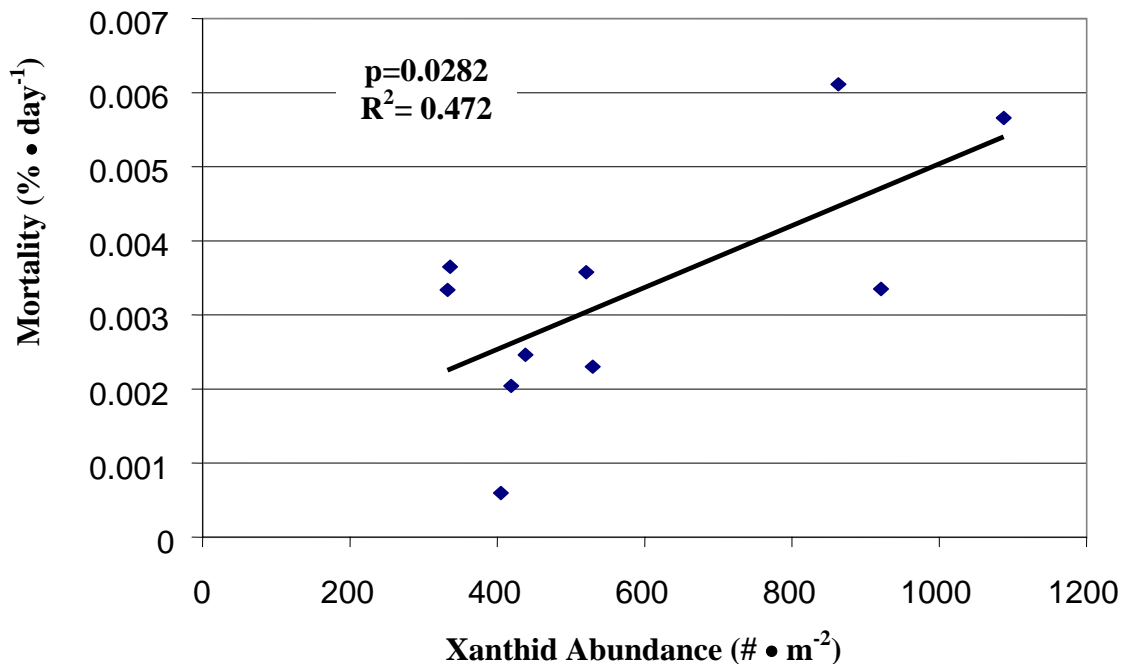
We had predicted that larval depletion would lead to a pattern of higher settlement rates near reef edges than in the interior (H1), leading to an inverse relationship between settlement density and reef size (H2). These predictions were not (strongly) supported. We did not observe any evidence of the predicted edge effects (Table 7), but the rank ordering of settlement in relation to reef size showed a consistent, though statistically non-significant, inverse relationship (Table 8). The reasons that our settlement predictions failed are not known, but include several possibilities. Larvae may not encounter reef edges and interior in a simple linear fashion. Complex flow patterns around and through the reefs (e.g. Appendix VI) may contribute to transporting larvae to the interior of reefs before they have a chance to settle at the edge. Alternatively, the depletion hypothesis may apply during

years of low larval abundance, but not during high recruitment years such as 2001 and 2002. Or, the general hypotheses may be correct, but we failed to choose the appropriate scales (e.g., the reefs may be too small to observe depletion). Finally, since we did observe a non-significant trend in relation to reef size consistent with our predictions, we may simply have had too few replicates given the high variation in settlement rates. This pattern bears investigation at other reef sites.

The “predation frames” provided an accurate assessment of oyster mortality, but they did not quantitatively distinguish between predation and other potential sources of mortality (e.g., low dissolved oxygen, harmful algae blooms, high sedimentation or disease—all of which occur in the Rappahannock River). Although this region of the Rappahannock River is known to experience seasonal low dissolved oxygen events in bottom waters in the channel, we did not observe any widespread mortality on the reefs that would be indicative of a low dissolved oxygen event. Also, this area is subject to extensive red tide blooms (caused by a mix of *Cochlodinium heterolobatum*, *Gyrodinium uncatenum*, *Gymnodinium* spp. and *Prorocentrum minimum*) during some years, that are a known cause mortality to oysters (Luckenbach et al. 1993). During 2001 and 2002 we did not observe any such large scale blooms and saw no evidence of widespread mortality resulting from any such bloom. Further, the oyster diseases MSX (caused by *Haplosporidium nelsoni*) and Dermo (caused by *Perkinsus marinus*) are also not the likely cause of the observed mortality, because the oysters that were deployed were young, the deployment times short and the observed prevalences and intensities among wild oysters on the reefs were low to moderate (Table 17).

Most of the mortality of oysters in these treatments was likely the result of predation. Evidence of crab predation (chipped, broken or crushed shell) was noted for most replicates in these experiments. Blue crabs (*Callinectes sapidus*) and mud crabs (Xanthidae) are known to be a major oyster predator in the region. Although blue crabs were sampled directly by diver transects and indirectly by gill nets, we suspect that their actual densities are underestimated by these methods. Mud crab abundances, however, were well quantified by the substrate basket samples. There was a significant ($p=0.0282$), but weak, relationship between oyster mortality and mud crab abundance in our study (Figure 25). We observed the lowest mortality rate for oysters at the Temple Bay reefs (Table 10) and this site also consistently had low xanthid crab abundance (Table 33). This suggests that mud crabs may be responsible for a portion of oyster mortality measured in this study.

Figure 25. Regression of daily oyster mortality versus xanthid crab density from paired quadrat and substrate basket samples.



Similar to Eggleston (1999), we had predicted that oyster abundance and biomass would be greatest on intermediate sized reefs. This prediction was based upon the expectant outcome of competing effects of reef size on settlement, growth and mortality of oysters. Since we did not observe consistent differences between reef size in each of these parameters across sites, it is not surprising that the resultant patterns of oyster biomass with reef size also varied with site. Only the reefs at Temple Bay exhibited the predicted pattern of oyster biomass with reef size (Table 19). The pattern at this site may have been driven by initially higher recruitment to the medium-sized reefs during the first year of the study (Table 6 A), but other factors may have contributed as well. The unbalanced design of the experiment (with all three reef sizes present at only two of the sites) may have contributed to our inability to discern a clearer pattern of oyster abundance and biomass with reef size. Furthermore, as noted above, we may simply have not chosen the proper reef sizes. It is possible that the intermediate reef size hypothesis, as proposed by Eggleston (1999), may be conceptually valid, but without more explicit definitions of reef sizes it remains difficult to test.

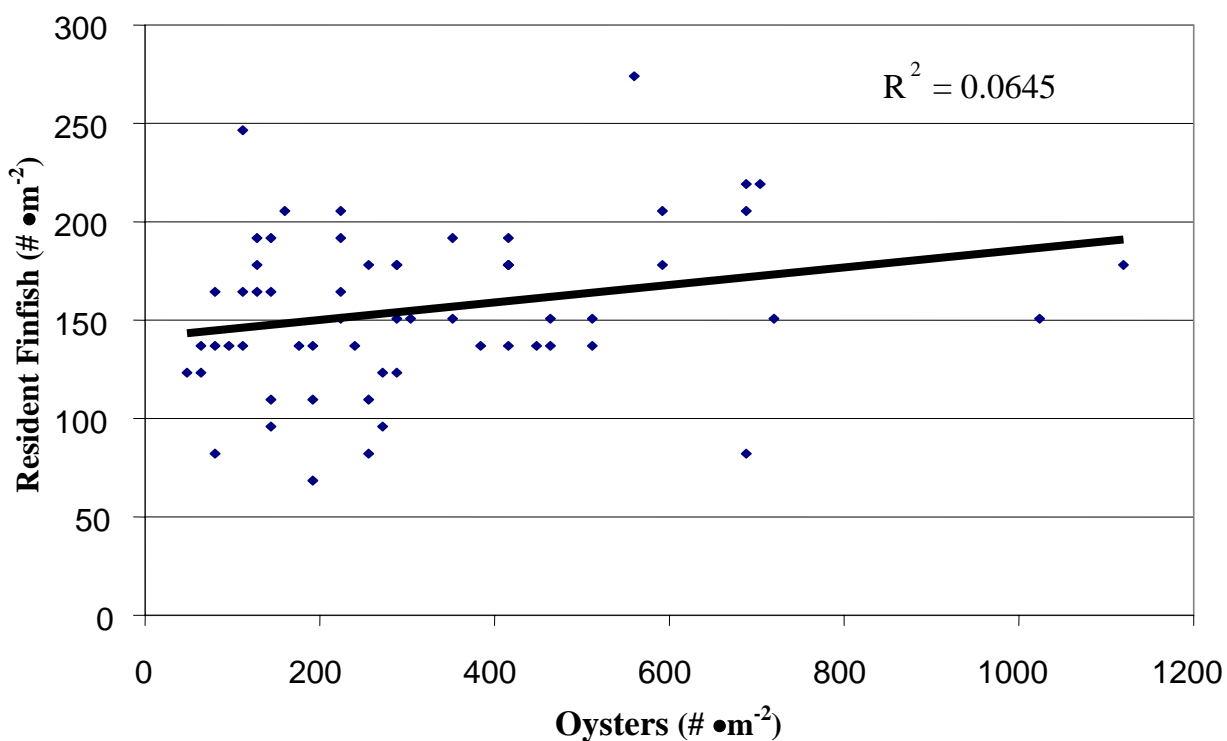
The diversity of organisms collected on the reefs in this study was comparable to those reported for other oyster reefs in the Chesapeake Bay region and throughout the Southeastern United States (Coen et al. 1999a, b; O'Beirn et al. 2000). For the dominant epifaunal species (barnacles, ribbed mussels, sea squirts and tube worms) we observed variation in abundances on several scales, often with significant interactions between temporal and spatial variables. Significant differences between reef sites were seen for most sample periods for most species ($p < 0.05$), whereas no differences were observed between intra-reef locations. In two instances, reef site x reef size interactions were documented and

in both cases the reef size differences were observed only at Temple Bay. The factors contributing to the variation in abundances on these scales are unclear and deserving of further study. Sessile epifauna not only contribute to the biodiversity and structural complexity of oyster reef communities, but they may in some cases compete with oysters for limited substrate. Thus, the factors controlling this variation as well as the direct and indirect effects of epifauna on the development of oyster reefs warrants further investigation.

Resident fish and decapods on the reefs also exhibited considerable variation over both temporal and spatial scales in this study. Both species richness and diversity of resident fish varied between sites, but the patterns relative to sites varied across years (Appendices XVIII – XXXI). Abundance of resident fishes, on the other hand, was similar across sites, but varied with reef size (Table 33). During Summer 2001 the small reef at the Drumming Ground site had approximately double the density of reef resident fish as the medium and large reefs at that site, but no such differences were observed at the other sites (Table 33 B). A few months later during Fall 2001, there was a consistent pattern of lower resident fish abundance on the medium reefs relative to the small and large reefs (Table 33 A). The causes of these differences across spatial scales are not revealed in this study, but we do note that there was a statistically non-significant trend of lower oyster abundance and biomass on the medium reefs (Tables 18 – 20) relative to the other reef sizes. We speculate that the abundances of resident fish may, in part, be controlled by the abundance and population structure of oysters on the reefs. Breitburg (1999) has suggested that the abundance of appropriate sized “boxes” (dead, but still articulated oyster shells), which serve as refuge and nesting sites for resident fishes, may be important determinants of their distribution and abundance. Our experiments were not designed to specifically test these

hypotheses, but we attempted to explore them in several ways. First, a comparison of resident fish density versus oyster density made by comparing data across all of our quadrat samples and substrate baskets did not reveal a significant relationship between resident fish and oysters (Figure 26). A similar regression between resident fish density and the density of “boxes” was also non-significant, but we point out that during the reefs and the oyster

Figure 26. Reef resident fish versus oyster density.



populations in this study are still young and that few boxes were present on the reefs (Table 49). Lastly, we note that there was a significant positive correlation between the abundance of *Gobiesox strumosus* (skilletfish) and oyster abundance and biomass on the reefs, but this pattern was not observed for the other resident fish species, total abundance, species richness or diversity (Table 50).

Table 49. Mean (and SE) number of “boxes” (dead, still articulated shells) by *Site*, *Reef size* and *Intra-reef location* for Fall 2001.

Reef Size	Intra-reef Location	Site			
		DG	MC	PR	TB
Large	Outer	6 (4)	-	-	6 (4)
	Inner	16 (0)	-	-	0 (0)
	Deep Inner	16 (n/a) ¹	-	-	0 (n/a) ¹
Medium	Outer	19 (16)	6 (4)	6 (6)	0 (0)
	Inner	8 (8)	0 (0)	0 (0)	16 (n/a) ¹
Small	Outer	10 (6)	6 (4)	0 (0)	12 (8)

We combined the data on resident reef assemblages from the quadrature and substrate samples to explore the relationships between reef scale and reef community diversity. In doing so, we did not observe the patterns that we had predicted (Tables 38 & 39). Again, there are several possible explanations for this, including the ones discussed above regarding sample size, the unbalanced design and the appropriateness of the reef sizes used in this experiment. However, it is also likely that diverse organisms included within this community (including fishes, decapods and sessile invertebrates) are subject to a diverse factors affecting their distribution and abundance that are not fully captured in our hypotheses. It would seem appropriate to refine these hypotheses to reflect different controlling factors for different taxonomic or functional groups.

Transient finfish that utilizes the reefs are difficult to quantify, due to sampling biases inherent in any gear chosen. Each of the three methods used in this study (gill nets, diver surveys and trawl samples) have their advantages and disadvantages, but together they provide a reasonably complete description of the transient species that were utilizing the

reefs during the period of this study. As noted earlier, it is likely that both the largest and most motile species and/or size classes were under represented in these samples, as were the smallest species and sizes classes of fish. The abundance and diversity of fishes collected from the Rappahannock River reefs in this study are comparable to those observed across other systems from Maryland to Texas (Coen et al. 1999a). Our specific hypotheses related to transient fish abundances in relation to reef edges and reef size were supported by observed trends that were consistent our predictions, but these trends were not statistically significant (Tables 47 & 48). Again, we interpret this lack of significance, in part, to a combination of sample size, unbalanced design and (possibly) choice of reef sizes. The most important limitation in establishing the effects of reef scale on transient fish utilization of oyster reefs was the limited duration of the study.

The reefs in the Rappahannock River were in the early stages of development during this study. Base material for the reefs were deployed in August 2000 after oyster settlement had occurred in that year. Recruitment of some species to the reefs no doubt began immediately after placement of the material on the bottom. However, with only two seasons of oyster recruitment, these shell piles are only beginning to develop into oyster reefs. Some of our research tested specific processes, such as oyster settlement, mortality and growth across different scales and those tests consistently revealed both temporal and spatial variation which should help to inform future efforts to restore oyster populations. Other parts of this research explored the development of reef-associated communities. We posed several hypotheses about scale-dependent patterns of abundance and diversity for these reef-associated communities. While we observed considerable variation in relation to scale in several of these metrics, the patterns were variable with respect to our predictions. Implicit

in our assumptions about utilization of these reefs by sessile, resident and transient species was the notion that the presence (and probably abundance, biomass, size and population structure) of oysters would be important in determining patterns of utilization across varying spatial scales. Again, therefore the early stages of development of these reefs will have a dramatic effect on the patterns that we observe. As a preliminary exploration of the possible effects of oysters on the utilization of these reefs by other species, we computed Pearson product moment correlation coefficients between several oyster population descriptors (total abundance, abundance of year class 2 and biomass) and reef community and population descriptors (species abundance, richness and diversity). The observed patterns were mixed with some species and community metrics varying significantly with oyster abundance and biomass and other not (Table 50). Importantly, the abundance of year class 2 oysters was positively correlated with more of these metrics than overall abundance or biomass, suggesting that following further development of the oyster population in subsequent years these relationships may become more evident.

Despite many years of attempts to enhance oyster populations and harvests, our understanding of the restoration ecology of oyster reefs is in its infancy (Luckenbach et al 1999). The application of basic ecological tenets to oyster restoration will be crucial to achieving success in restoring these habitats (Palmer et al. 1997). The exploration of several aspects of scale-dependence in this study attempts to provide some of that application. The research, however, needs to be extended into later stages of the reef development to more fully elucidate some of the patterns and processes controlling reef development.

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