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**Intertidal Oyster Reefs as a Tool for Estuarine Rehabilitation
and Rejuvenation of the Virginia Oyster Fishery**

submitted to:

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Intertidal Oyster Reefs as a Tool for Estuarine Environmental Rehabilitation and Rejuvenation of the Virginia Oyster Fishery.

A General Introduction to the Project and this Report.

The oyster *Crassostrea virginica* is recognized as both a keystone organism in the ecology of the Chesapeake Bay and the focus of a substantial commercial fishery. Oyster reefs developed in recent geological time as the current Chesapeake Bay was inundated by rising sea level. By early Colonial times oyster reefs had become significant geological and biological features of the Bay - they were also major navigation hazards. Continuing harvest pressure since Colonial times have resulted in the transformation and degradation of the oyster reefs to subtidal "footprints" of former reefs that maintain drastically reduced populations of oysters. Reef degradation has undoubtedly been exacerbated by companion environmental degradation and an historical lack of consideration for water quality and natural resource management. Statements concerning over fishing by John Mercer Brooks over 100 years ago fell on deaf ears, but are now appreciated, if not entirely heeded. The past three decades have been defined by decline in the fishery production and the oyster resource under the added insult of two protistan parasites, *Perkinsus marinus* ("Dermo") and *Haplosporidium nelsoni* ("MSX"). Since the disease organisms are active throughout most of the growing range of the oyster there have been few sanctuaries in which to plant oysters or in which naturally occurring oysters could be found in appreciable quantities. Indeed, these parasites have effectively eliminated oysters from many sections of the Bay. Despite over 30 years of disease activity the native oysters have developed neither tolerance nor absolute resistance to these diseases, and do not exhibit any recovery in disease endemic areas in Virginia. The oyster fishery is in severe decline and there is a recognized and urgent need to restore the oyster resource: not just for the commercial fishery but also to provide both the benthic filter feeder that is so pivotal to the ecology of the Bay (see discussion by Newell, 1989; Mann, Burreson and Baker, 1992) and the physical structure which provides habitat for a multitude of species, including many of commercial interest.

The Secretary of Natural Resources for Virginia, in conjunction with the Commissioner of Marine Resources, appointed a Blue Ribbon Panel in the Fall of 1991 to develop a comprehensive plan for restoration of the Virginia oyster resource and fishery. Among the recommendations of the panel was a proposal to investigate the construction of oyster reefs identical to those present in the Bay before Colonial settlement - the acknowledged "structure" of the oyster community in its undisturbed (by man) form. The rationale supporting this proposal was quite simple - create an optimal physical environment and the oyster will settle and grow in a markedly improved manner compared to that observed on the current subtidal "reefs" or "rocks". A consideration of the temporal relief from continual predation pressure afforded by an intertidal location supports this rationale. Indeed, a simple observation of the distribution of oysters surviving on bridge and pier pilings in the Bay - about the only remaining oyster populations undisturbed by harvesting - reveals that the vast majority of surviving oysters in terms of size and number are intertidal. In all fairness it is important to note that the Blue Ribbon Panel was and is not the only body to suggest the value of intertidal reefs as a option to assist in restoration of the oyster resource. Such discussions have been widespread in the academic community and management agencies, often with participation of federal agencies; however, the concept lacked critical evaluation in the field. This project was designed to effect that evaluation.

The program began in May of 1993 with the construction of an intertidal oyster reef in the Piankatank River, Virginia with funds supplied by the Shellfish Replenishment Program of the Virginia Marine Resources Commission (VMRC). Monitoring and experimental work on the site were initiated shortly thereafter by faculty, staff and students of the Virginia Institute of Marine Science (VIMS) and the School of Marine Science, Virginia Institute of Marine Science (SMS/VIMS), College of William and Mary. Work continued with combined support of the aforementioned state agencies until September 30, 1993. After this date the Coastal Resources Management Program, administered by the Commonwealth of Virginia Department of Environmental Quality (DEQ), provided additional funding for the period October 1, 1993 through September 30, 1995. This document constitutes the final report for activities effected under DEQ funds.

The report is offered as a series of concise sections addressing aspects of recruitment, growth, mortality, and disease prevalence and intensity in oysters on the constructed reef system. Each section is purposefully written as a "stand alone" document to minimize the requirement for cross reference. Prior to those sections it is appropriate to offer background details relevant to the study site and reef construction.

The Piankatank River has not supported a commercial oyster fishery for over a decade; however, it has been the site of a successful seed oyster program managed by the VMRC shellfish replenishment plan, currently under the direction of one of the investigators (Wesson). A limited number of "rocks" have had applications on a regular basis with subsequent harvest of the settled seed after one or two summers of exposure (the summer being the period of oyster settlement). The temporal and spatial nature of settlement is well documented by a continuing program at VIMS under the direction of a second investigator (Mann). Oyster spat (juvenile and newly settled oysters) counts of up to 1000 individuals per bushel of shell are commonplace in seed oyster dredging from these maintained and managed areas. The footprints of the former reefs are well documented from both historical sources (Baylor Surveys), recent surveys (Haven and co-workers in the early 1980's, all material on file at both VIMS and VMRC), and continuing work by the VMRC staff. The reefs are not uniform in shape, and are clearly site specific and related to local circulation. For the current project we eliminated a costly (in dollars and time) study of optimal size and shape in relation to hydrography by simply using the naturally evolved shape illustrated in the historical footprint. The lack of a continuing commercial presence, the proven history of the site as one of good settlement, the comparatively pristine environment at the site (there is essentially no industrial and very little agricultural development in the Piankatank watershed - even residential density is low), the strongly supportive attitude of waterfront residents to environmentally sound management, and the inclusion of the study site in the National Estuarine Reserve System combined to make this a unique and attractive site for the continuing study.

There have been continuing monitoring efforts in the Piankatank for a number of years prior to initiation of the current project. These are important because they provide a long term data set that supports use of the site and provide a solid comparative background for new information collected in the current project. VIMS maintains a program to describe temporal and spatial settlement of oysters in the Virginia subestuaries of the Chesapeake Bay throughout the summer months from June through late September. At weekly intervals shell strings, twelve clean oyster shells of a standard size suspended on a galvanized wire, are deployed at selected sites. Upon retrieval the number of newly settled oyster spat are counted and the mean presented as an index of competent to settle oyster larvae present. Temperature and salinity data are collected at all sites at weekly intervals. The four sites in the Piankatank River (see Figure 1), at Three Branches, Burton's Point, Palace Bar, and Ginney Point, are all within close proximity to the study site. Data is reported in terms of a biweekly newsletter to industry and state agencies, and an annual report through the VIMS / Sea Grant Advisory Service Office. The historical data set is

Figure 1: General location of all extant oyster reefs and shell plantings (effected by the VMRC Replenishment Program) within the Piankatank River. The location of the oldest of the constructed reefs, immediately north of Roane Point, is marked R. The environmental instrument package for weather and tide data is at the eastern tip of the reef. The remaining reefs, progressing in a downstream direction, are A: Ginney Point, B: Island Bar, C: Palace Bar, D: Blands Point, E: Herring Rock, F: Cape Toone, G: Stove Point, H: Cape Toone inshore (small lumps). Oyster spatfall monitoring stations are located at Ginney Point, Palace Bar, Burtons Point (labelled I, traditionally used as shell plant on hard sand but not a natural reef), and Three Branches (labelled J in the lee of Gwynns Island on a former shell plant).

PIANKATANK RIVER
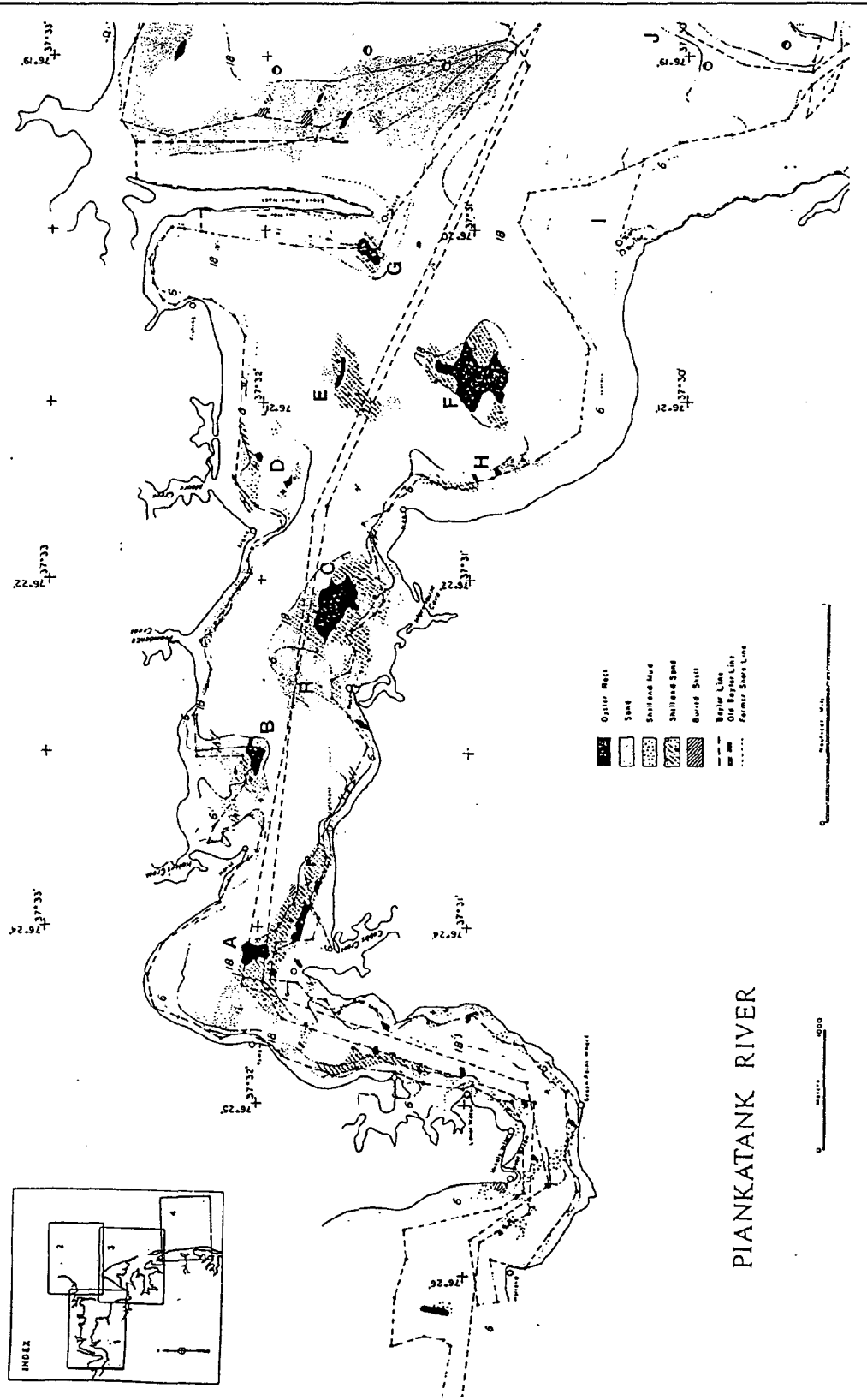
maintained on the VIMS computers. A further benthic dredge survey is effected in the Fall of each year immediately prior to opening of the commercial season on October 1st. The purpose is to assess cumulative settlement and survival of young of the year spat from the prior summer, and size distribution and total oyster count of the larger size classes. Again, data are reported through the VIMS / Sea Grant Advisory Service Office. These efforts continued during the period of the current program and will continue after its completion.

The reef for the current project was constructed in the lower Piankatank River by mass deployment of clean, fossil shell from a barge at high tide. The adjacent areas are used in "traditional" subtidal shell planting by the VMRC replenishment Program. The reef covers an area of approximately 100 X 1000 feet (30 X 300 m), is elevated to five feet (1.6 m) above the current bottom, has between one and two feet (0.3 - 0.6 m) exposed at low tide, and has a convex cross sectional profile. Appropriate permits for reef construction and guidelines for marking the reef perimeter (for navigational purposes) were obtained from local wetlands boards, VMRC, the U.S. Army Corps of Engineers, and the Coast Guard. The reef is appropriately marked with large pilings at its upstream and downstream extremities, and by buoys at regular intervals along its perimeter. It is important to emphasize that the reef was constructed on a bottom that had been cleaned of resident oysters by dredging, and that no "seeding" of the reef was attempted. The reef surface was, therefore, only to be populated by natural settlement from the surrounding environment and no residual oysters containing potential infections of either *Perkinsus marinus* or MSX were present at the beginning of the study. The projected costs of construction, $110,000, was supplied by the VMRC Replenishment Program from state funds.

Acknowledgments

As mentioned earlier, this project was effected as a joint effort of SMS/VIMS and VMRC. Although the project proposal had three investigators - Mann, Perkins and Wesson - other individuals also contributed to its success, and their efforts are gratefully acknowledged.

Ian Bartol, graduate research assistant at SMS/VIMS, used these studies as the basis of his M.A. thesis work. Ian was the functional manager of this program on a day to day basis. His work was tireless and of the highest quality. He defended his thesis in January of 1995, and continued to work on the project until completion of this report - to which he is a major contributor and acknowledged equal investigator on the cover page. A copy of Ian's bound thesis was provided to DEQ shortly after the successful defense. Ian is currently pursuing a Ph.D. degree at SMS / VIMS.

Kenneth Walker of VIMS acted research vessel captain throughout the duration of the program. He maintained a the highest standards of safety in the field, and was always accommodating to the vagaries of research timetables, tides and weather. Juanita Walker provided a unique set of eyes and years of skill to the assessment of oyster disease in the collected material. Paul Hershberg provided support in the field and in the analysis of *Perkinsus*. A number of staff and students also braved the Piankatank elements, notably Ray Morales, Soraya Moein, Juli Harding and Sandra Brooke. Finally Aswani Volety assisted in final collation of disease data.

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Small-scale patterns of recruitment on a constructed intertidal reef: the role of spatial refugia

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ABSTRACT

Traditional oyster repletion activities have utilized a two-dimensional approach to shell (substrate) deployment to attain maximal coverage in subtidal locations with little consideration for optimal thickness of deployed shell and tidal elevation. We report observations on settlement and mortality patterns of oysters on a three-dimensional structure, a constructed oyster reef in the Piankatank River, Virginia, from June of 1993 through September of 1994. The reef was constructed entirely of oyster shell on the footprint of an historical reef, and extended from 2.5 m below MLW to 0.75 m above MLW. The footprint covered an area approximately 150 x 30 m, with numerous sections, varying from 2 to 20 m^2 in area, exposed at low tide. In both intertidal and subtidal locations settlement was monitored both at the surface of the reef shells and within the interstices of the reef at depths of 10 cm. Settlement was greater in subtidal locations, and no difference in settlement intensity between surface and sub-surface environments was detected. Survivorship rates along the intertidal-subtidal continuum varied temporally but were highest at MLW for most of the year. Oysters which attached to subsurface substrate benefited primarily from refugia from temperature extremes in intertidal locations and from relief from predation in subtidal environments. We suggest the moderation of these biological and physical stresses within the reef interstices is instrumental in increased survival: even minor submergence within the reef provides relief from scorching summer and freezing winter air temperatures and furnishes protection from predators, most notably crabs and flatworms. In practical terms these results proffer an important lesson: reef tidal elevation and substrate thickness both provide microscale refugia for settlement and survival of early oyster life history stages.

INTRODUCTION

Traditional oyster replenishment programs have focused on spreading thin veneers of substrate suitable for larval settlement over coastal and estuarine bottoms or over foundations of less ideal substrates to maximize area coverage. In general, such activities have been driven by the practicality of deploying very large volumes of shell, a commodity of increasing value, at greatest cost efficiency and with reasonable speed, usually with the subsequent intent of retrieving either juvenile (seed) oysters or market size oysters. The end product of this approach, a two-dimensional subtidal carpet of available substrate, has little resemblance to the intricate, three-dimensional reef communities which often extended out of the water at low tide and which oysters once formed naturally in the Chesapeake Bay before man's intervention. In light of rapidly declining oyster stocks in the Chesapeake Bay, a concerted effort to re-establish natural oyster communities by constructing artificial reefs has been made by repletion agencies. The ultimate goal of such projects is to rejuvenate dwindling local oyster populations.

Presently, we know little about constructing reefs which are most advantageous for oyster settlement and survival. From the cumulative literature on oyster biology, we know that reefs grew by accretion over time periods of hundreds to thousands of years in a process aided substantially by the preferred settlement of metamorphically competent oyster larvae on shells of the adult oyster. We also know that the physical environment, in the form of currents, tides, and sedimentary forces, practically dictate the perimeter size and the features of the reef. However, we remain ignorant in a number of details, and as a result

there are a number of practical questions, fundamental to an organized approach to reef construction, which are without answers. For example, for a known location what size and shape should the reef be, and can we obtain guidance on this question from current "footprints" of formerly intertidal reefs? Is tidal elevation an important factor to consider when constructing reefs? Given that shell is a valuable commodity, can other substrates be used to construct reefs? How thick should substrate layers be and how should they be applied?

In this study, we focus on the issues of substrate thickness and tidal elevation. The interstices of a "natural" reef system provide both physical and biological refugia. Do subsurface environments of constructed reefs provide similar benefits and if so to what extent are these benefits quantitatively important? Do oysters even settle beneath the reef surface in these constructed environments? "Natural" reefs also have some vertical dimension, which allows for the settlement and subsequent survival of dense populations of oysters at distinct bands along the tidal continuum. Does tidal elevation play a vital role in the survivorship of oysters on artificial reefs? Do oysters settle in higher numbers at tidal heights where they benefit from refugia? We address these questions by measuring settlement and post-settlement mortality of *Crassostrea virginica* at two substrate levels (reef surface and 10 cm below reef surface) and at various tidal heights ranging from +30 cm above mean low water (mid/high intertidal zone) to -90 cm below MLW (mid subtidal zone) on a constructed intertidal reef.

STUDY SITE AND EXPERIMENTAL PROCEDURES

The location selected for this study was a sandbar known as Palace Bar in the Piankatank River, Virginia. This site once supported a highly productive intertidal reef system, but at the time of reef construction was completely devoid of live oysters. Prior to construction, the site was dredged clean of any live material to minimize residual infective material containing *Perkinsus marinus*. Water temperature at the site varied from 0.5 - 30° C, salinity ranged from 8 -20 ppt, and tidal range was small (mean range = 36 cm).

The reef was constructed by the deployment of aged oyster shells off barges using a high pressure hose. The shells were discharged in an area approximately 150 x 30 m, which were the approximate footprint dimensions of the historical reef. After completion, the reef consisted of numerous sections, varying from 2 -20 m^2 in area, exposed at low tide, and extended from 2.5 m below mean low water (MLW) to 0.75 m above MLW. The majority of the reef, however, did not extend much deeper than 1.0 m below MLW or much higher than 0.35 m above MLW.

The reef was sampled in both 1993 and 1994. During the 1993 sampling period 2 of the 12 principal intertidal hummocks were focused on: one on the reef periphery completely exposed to wave action and currents and a second situated near the middle of the reef partially shielded from wave action and currents. These mounds were sampled using a transect approach, whereby samples were collected along upstream and downstream transects on each of the two mounds during each period of sampling. Transects were carefully marked on the reef to prevent resampling. Along each transect four tidal heights were considered: 30 cm above MLW, MLW, 45 cm below MLW, and 90 cm below MLW.

During the 1994 sampling period, after data from the previous year were analyzed and we had a preliminary understanding of the reef system, a randomized approach was used which was more geographically expansive and statistically powerful. In this method, eight mounds were partitioned into 64 x 20 cm plots using rope and reinforced bars, and experimental sites were selected randomly across the mounds. Four of the 12 primary hillocks were not considered because ice scouring during the '93-'94 winter eroded the mound apices, resulting in the loss of substantial intertidal substrate. In this randomized approach, three tidal heights were considered: 25 cm above MLW, MLW, and 90 cm

below MLW. The high intertidal height was lowered slightly to accommodate as many intertidal mounds as possible in the sampling procedure, and one of the subtidal heights, 45 cm below MLW, was eliminated to incorporate more replication. In addition to tidal height another factor, substrate level, was considered. To document the effects of substrate level, samples were collected both at the reef surface and 10 cm below the reef surface.

During both years of sampling, non-destructive and destructive sampling were employed from June through September to assess settlement and early recruitment within the reef ecosystem. Non-destructive sampling involved the weekly placement of oyster shells in open-topped, 64 x 20 cm, rubber coated 1 inch wire mesh trays secured to the reef surface by reinforced bars. In 1993 a surface layer of 20 shells was placed weekly in single level trays which were fixed spatially to the reef at all four tidal height designations along upstream and downstream transects at each of the two mounds. The concave and convex side of all 20 shells within individual cages were examined for recently settled oyster larvae (spat) using a dissecting microscope, and a spat total per cage was recorded. In 1994 three-tiered trays containing 30 shell upper and lower levels, which were spaced 10 cm apart, and a 40 shell intermediate level were buried into the reef substrate until the upper level was even with the reef surface. Each week trays were placed at four distinct, randomly selected plots chosen at all three tidal heights. Both surfaces of shell found in the upper and lower tiers were examined for spat, and a surface layer spat total and a deep layer spat total were recorded at all 12 weekly selected plots.

Destructive sampling involved the weekly placement of 64 x 20 cm quadrats on the reef surface, the removal of a layer of shell, and the subsequent examination of both shell surfaces for spat. This sampling technique provided an index of cumulative spatfall on the actual reef substrate and accounted for any early post-settlement mortality losses. In 1993 the quadrats were placed at all four tidal heights along upstream and downstream transects chosen on each of the two mounds. To prevent resampling, successive samples collected over time were taken along transects which were immediately adjacent to previously sampled transects. During this period only a surface layer spat total per plot was calculated. Plots used in 1994 destructive sampling were selected randomly across all eight remaining intertidal mounds. As with 1994 non-destructive samples, four plots were selected randomly each week at all three tidal heights. At each plot, a surface layer of shell and a layer 10 cm beneath the reef surface, easily distinguishable from the surface layer by its brown detrital film, was extracted and examined for spat. This allowed for the calculation of both weekly surface and weekly deep spat totals for all 12 plots.

To determine if oysters which settled along these spatial gradients would survive, oysters of various age classes were tracked throughout the fall, winter, and summer months. On August 12, 1993, oyster larvae were set on clean oyster shells in densities of 5-25 spat per shell at the Virginia Institute of Marine Science Oyster Hatchery. Shells containing spat were placed in Vexar mesh bags (100 shells per bag), and spat were reared in hatchery systems to sizes comparable to oysters found on the reef. On September 26, 1993 the mesh bags were placed on the reef at the same 4 tidal heights designations used in the 1993 settlement monitoring program along two distinct transects on each of the two mounds. On October 14 and November 11, 1993, and May 5, 1994 25 shells were haphazardly selected from each bag, which was shaken vigorously prior to selection, and shells were photographed with an Olympus OM camera equipped with a 50 mm macro lens. Recent spat scars on each shell were noted and proportional mortalities (# scars per shell / # scars per shell + # live oysters) were calculated.

Over the summer of 1994, a different method which considered all intertidal mounds at the reef site and two year classes of oysters was used to document mortality. One year class consisted of hatchery oysters set on oyster shell on May 16, 1994 in the VIMS Oyster Hatchery, whereas the other year class consisted of a well mixed sample of oysters used in the previous experiment. For each year class, 30 oysters present

collectively on 15 randomly picked shells were numbered using paint markers and were placed on either the upper or lower level of 32 x 20 cm, three-tiered, 1 inch mesh cages. Both upper and lower levels, which were 10 cm apart, were filled with shell containing live oysters, but the middle level was filled with 20 shells devoid of live organisms. To keep densities within the 15 shell assemblages as constant as possible, the physical removal of oysters in high density communities was sometimes necessary.

At each of the three tidal heights considered in the 1994 settlement study, eight plots were selected randomly for each year class, and cages were buried into the reef substrate until the upper layer was even with the reef surface. The cages were held in place with a reinforced rod. Photographs of labeled oysters were taken in the field with a Nikonos V camera equipped with a close-up lens and focusing frame at 28 day intervals in June, July, August, and September. To enhance photographic clarity and reduce fouling, a 3 HP gasoline powered Homelite water pump was used in the field to clean labeled oysters and cages. A proportional mortality value per layer of each cage was computed for each sampling interval.

Statistical analysis:

The argument may be made that 1993 settlement and mortality samples collected over time were not independent, since successive samples were taken from either spatially fixed areas, spatially connected plots, or from the same population of organisms. To account for this, analyses of variance (ANOVA) with repeated measures on time were performed on each data set. Multivariate repeated measures were performed on settlement data, and because of missing values, univariate repeated measures were performed on mortality data. To satisfy assumptions of homogeneity, all settlement data were log $(x + 1)$ transformed and proportional mortality data were arcsine transformed. When no significant interactions between the within factor, time, and any other factor were detected, 3-way fixed factor (factors: tidal height, mound, and time) ANOVAs were performed. Significant main effects were examined using Student-Newman-Keuls (SNK) tests.

Linear correlation's were performed on surface and deep samples collected in the 1994 settlement and mortality studies to determine if a relationship existed between the two substrate levels. If no significant relationship was detected in the correlation analysis, substrate level was treated as a factor in further statistical procedures. When significant relationships were detected, paired sample t-tests were used to determine if differences existed between surface and deep samples. A mean value for surface and deep data was calculated when no significant difference between the substrate levels was detected, and further analyses were performed on these mean values.

ANOVAs were run on 1994 non-destructive and destructive log $(x+1)$ transformed settlement data, and all differences between means were revealed using SNK multiple comparison tests. Multivariate repeated measures ANOVAs were performed on arcsine transformed mortality data collected in 1994. All significant between factor effects were analyzed using SNK multiple comparison tests, whereas significant within factor effects were examined using Newman-Keuls procedure (pp. 527-528, Winer 1991).

RESULTS AND DISCUSSION

The majority of data analyzed in this study suggest that small-scale spatial changes, such as 30 cm shifts in tidal elevation or 10 cm changes in substrate depth, strongly influence the processes of oyster settlement and post-settlement survival. Rather than go into an exhaustive examination of the data, we feel that it would be more constructive, and hopefully more interesting, to present representative examples from the data which illustrate and reinforce key points related to microscale effects. For a more comprehensive treatment of the data, please see Bartol and Mann (1996 a & b, in prep).

Settlement of oyster larvae in a constructed reef environment is heavily dependent on the tidal elevation of the reef substrate. Within the shallow water (< 3 m) reef system considered in this study, settlement increased with tidal depth. This is most clearly seen in the non-destructive settlement studies, where settlement intensities both in 1993 and 1994 were greatest at -90 cm (Figure 1). This finding is consistent with several other studies conducted in non-reef environments. For example, greater subtidal settlement rates have been documented by McDougall (1942) using unglazed hearth tiles, by Chestnut and Fahy (1953) using shellstrings, and by Roegner and Mann (1990) using hatchery-reared larvae exposed to field conditions in microcosms. Nichy and Menzel (1967), who placed oysters on clothmats of mesh within a reef ecosystem, also observed greater settlement/early recruitment subtidally.

The higher rates of subtidal settlement observed in this study were likely a result of several factors. Submergence time may have been one. Oyster larvae in the water column were exposed to subtidal substrates substantially longer than to intertidal substrates, and as a result, had a wider time window in which to set. Submergence time alone, however, did not account for the observed differential settlement. Kenny et al. (1990) and Roegner (1989) found that settlement intensities are not direct functions of submergence time, especially in the high intertidal zone and the low subtidal zone. Vertical segregation of oyster larvae in the water column also may have contributed to elevated subtidal sets because oyster late stage pediveliger larvae are more abundant near the benthos than at the surface or within the midwater region (Carriker 1951, Kunkle 1957, Haskin 1964, and Baker 1994). Furthermore, because late stage competent to set larvae are photonegative at the time of settlement (Ritchie and Menzel 1969, Nelson 1953) and prefer areas of lower wave energy (Ortega 1981), they may have actively sought subtidal habitats where light intensities and wave stress are reduced.

Surprisingly, no significant differences in settlement were detected between surface and deep substrates at any of the tidal heights considered (Paired t-tests > .05). One concern, however, is that low settlement rates (mean weekly destructive/non-destructive settlement over a three-week settlement period = 0.5 - 3.5 spat per 30 shells) may have dramatically lowered the statistical power of the paired t-test. Although this may be a true, a thorough examination of the of the data sets revealed that there was no trend in greater settlement by layer. For example, of the 36 destructive samples collected, settlement was greater at the surface 7 times, greater below the surface 9 times, and equal 20 times!

Oyster larvae may have settled 10 cm beneath the reef for a number of reasons. Some of the oyster larvae may have actively attached to subsurface substrate because again they prefer darkened conditions when setting (Ritchie and Menzel 1969) and areas of reduced wave action (Ortega 1981), but also because they seek out environments where flow is low, crevices are abundant, and substrates are not heavily fouled (Bushek 1988, Michener and Kenny 1991). Reef shells found below the surface layer were considerably less infested with algal growth and barnacles. It is also feasible that because water currents are substantially reduced beneath the reef surface, the interstices serve as sediment traps and entrain larvae. Although it is not clear from this study what mechanism, active and/or passive transport of larvae, is responsible for sub-surface settlement, it is clear that larvae are capable of settling within the reef interstices and are not impeded by shell down to depths of 10 cm. This is quite remarkable considering that there may be 20 or more shells layers within the 10 cm space.

Although oyster larvae are capable of settling beneath the reef surface, do they survive in these environments? Results from this study suggest that oysters not only survive in these environments, but survive better there during certain times of the year. For example, oysters reared in 1993 which resided at the reef surface at the 25 cm tidal height experienced significantly higher mortalities than oysters residing below the surface from mid June through mid July (Figure 2). During this period air temperatures were the highest

of the year, averaging just over 28° C. It is likely that oysters beneath the reef surface benefited from a shading effect from overlying oysters and shell, and as a result resided in a cooler, moister, more hospitable environment than their surface dwelling cousins. In natural reefs oysters grow vertically in highly populous clusters, and this colonial existence provides refuge from solar radiation for all oysters in the community. Since dense assemblages of vertically growing oysters takes many years to become established, sub-surface residence may be critical for the survival of intertidal oysters residing in constructed reef systems.

A further example of beneficial subsurface residence is found at the -90 cm tidal height. At this height, significantly higher surface mortalities were detected for 1993 reared oysters over the entire three month summer sampling session (Figure 3). Although the two most deleterious predators, oyster drills and starfish, were absent at the reef site because of low salinities, the flatworm *Stylochus elliptus*, the mud crabs *Panopeus herbstii, Eurypanopeus depressus, and Rhithropanopeus harrisii*, and the blue crab *Callinectes sapidus* were present, and all are known to contribute to substantial oyster mortalities (Landers and Rhodes 1970, Abbe 1986, Littlewood 1988, Eggleston 1990, and Baker 1994). These predators were found within cages at surface and deep layers at all tidal heights, but were most abundant at the reef surface and at subtidal depths based on field observations. Flatworms and mud crabs were probably the most deleterious because they were highly abundant at the study site and were not restricted by the mesh of the experimental cages. Although adult blue crabs may not have been able to enter the cages, they were able to prey upon the numerous oysters which grew through the cage mesh.

Of the three tidal heights examined, surface residing oysters survived best at MLW throughout the summer (June-September). For example, oysters belonging to the 1994 year class and dwelling at the MLW tidal height had a cumulative percent mortality of 12% over the summer compared with mortalities of 22% and 23% recorded at the +25 cm and the -90 cm tidal heights, respectively (Figure 4a). Interestingly, higher MLW survival was not observed for oysters residing below the surface. In fact, beneath the reef surface, there was no detectable difference in mortality along the intertidal-subtidal continuum. This may because physical and biological environments within the interstices are relatively constant and stable regardless of tidal elevation.

During the fall oysters situated at MLW had a cumulative percent mortality of 13%, which again was significantly lower than mortalities recorded at other tidal heights (Figure 4b). Oysters residing at MLW during the summer and fall probably experienced less predation pressure than subtidal oysters as a consequence of aerial exposure, but did not suffer from significant heat and respiratory stress like mid to high intertidal oysters because of shorter emergence times. This is consistent with the findings of McDougall (1942), Chestnut and Fahy (1953), McNulty (1953), Nichy and Menzel (1967), Arakawa (1980), and Littlewood (1988), all of who have found high oyster survival in the mid to low intertidal zone as a result of lowered predation pressure, physical stresses, sedimentation, and/or competition for space.

Oysters situated at MLW did not fare as well during the winter months. Mortality rates at MLW and higher in the intertidal zone were 95-100% , whereas mortality rates at the -45 and -90 cm tidal heights were on the order of 25% (Figure 5). These mortality rates, especially at MLW, were likely atypical and a result of the coincidence of an unusually brutal winter and the presence of a young population of oysters (oysters were 4 months old at the onset of the winter). From December of '93 through March of '94 air temperatures dropped below freezing 28 days, which is very unusual for Virginia. Oysters less than 1 years old are especially vulnerable to freezing conditions because they put much of their energy into growth and maintenance rather than into the storage of glycogen, a preferred substrate for anaerobic respiration, and thus are less capable of environmental isolation (Mann and Gallager 1985, Widdows et al. 1989). In a separate study conducted

by the authors over the '94-'95 winter, oysters of a similar age (5 months) and oysters 15 months residing at MLW experienced winter mortalities between 15 and 20%. This is evidence that the mortality rates observed over the '93 - '94 winter were exceedingly high.

It should be made clear that the above mortalities only reflect oysters at the surface substrate layer, since oysters beneath the reef surface were not measured during the winter periods. It was interesting to note, however, that one cage buried 15 cm beneath the reef surface in the intertidal zone during the '93 -'94 winter, which is not depicted in the graph, had mortalities of 50%. This is substantially lower than intertidal mortalities recorded at the surface. Furthermore, visual inspections of "natural set" oysters in underlying intertidal environments revealed higher below surface survival. These observations suggest that residence below the reef surface may not only provide refugia from high temperatures and predators during the summer and fall, but may also provide relief from ice and wind during the winter months.

To recap briefly, settlement and early recruitment of oyster larvae are greatest subtidally, and settlement intensities at the reef surface and 10 cm below the surface are similar. During the summer and fall, survivorship after substrate attachment is maximized at MLW; during the winter, mortality of surface dwelling intertidal oysters may be substantial. Survivorship patterns may differ on a smaller spatial scale as well. Submergence 10 cm within the reef provides an important refuge both for intertidal oysters during periods of peak solar exposure (June/July) and for subtidal oysters during periods of intense predation pressure (summer and fall). Furthermore, there is some evidence to suggest that sub-surface residence may be beneficial for oysters living in the intertidal zone during the winter months.

In practical terms these results proffer an important lesson: microscale variability should not be ignored when constructing reef systems. Adding merely 1 m of vertical topography onto a constructed reef system so that it may extend marginally out of water at low tide may elevate survival ability substantially, especially if the addition of substrate provides a spatial refuge from intense predation and fouling. This was clearly demonstrated in this study during the summer and fall when mortality rates were lowest at MLW. Unfortunately since mortalities recorded over the winter were a product of unusual circumstances, this study fails to provide a representative comparison between summer, fall, and winter mortalities, which, of course, would be useful in determining whether summer/fall survivorship benefits outweigh mortality losses over the winter. As a result, we cannot provide a definitive answer as to whether building intertidal reefs will maximize survival. Nonetheless, we have shown that tidal elevation does affect settlement and post-settlement survival and that determining the tidal elevation at which recruitment is maximized for a given geographic setting before deciding on a reef elevation is a necessary exercise if survivorship is to be maximized. Substrate depth also should be considered. The veneer level of shell over a base substrate in reef construction should be thick enough to provide microscale refugia for settlement and survival of early life history stages. Based on the results of this study, the substrate should be at least 10 cm thick and allow for subsurface colonization. Finally, the most important advice we offer to reef builders is to be aware that the issues of settlement and mortality in relation to biological and physical environments are determined by microscale variability rather than larger scale uniformity, and the macroscale patterns observed in the field are the sum of these microscale events.

Acknowledgments.

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Table 1. Paired t-tests performed on surface and deep substrate layer spat counts for non-destructive and destructive samples. Separate analyses were performed on each tidal elevation.

NON-DESTRUCTIVE SAMPLES

Tidal height	Mean difference (surface-deep)	Degrees of freedom	t-value	p-value
+ 25 cm	-.333	11	-.886	.3944
MLW	-.083	11	-.321	.7545
-90 cm	.333	11	.549	.5940

DESTRUCTIVE SAMPLES

Tidal height	Mean difference (surface-deep)	Degrees of freedom	t-value	p-value
+25 cm	-.083	11	-.561	.5863
MLW	.083	11	.432	.6742
-90 cm	-.500	11	-1.149	.2750

Figure 1. Mean *C. virginica* spat counts recorded in the 1993 and 1994 non-destructive settlement studies. Error bars denote +1 S.E.
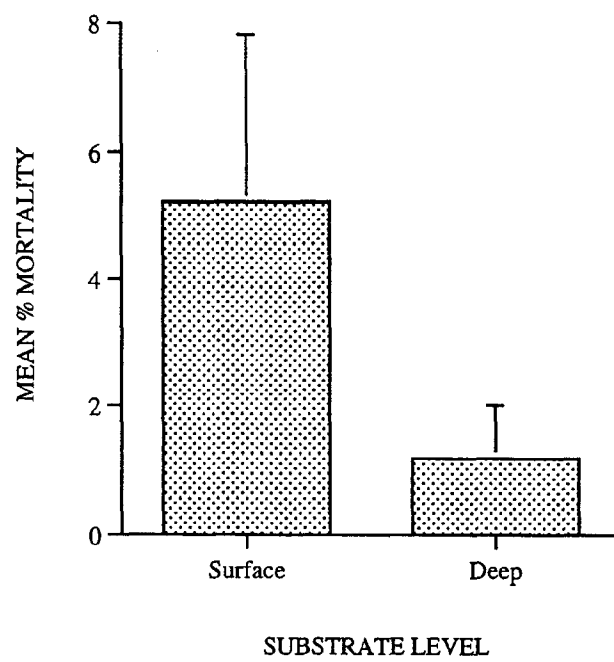
1993

MEAN SPAT PER 20 SHELLS

TIDAL HEIGHT (CM RELATIVE TO MLW)
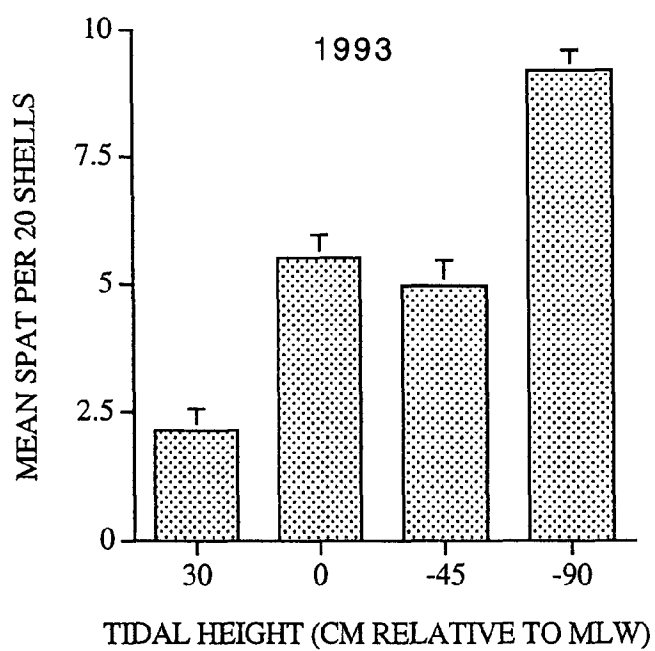
* 3-Factor ANOVA; F= 9.69, df= 3, 32, p < .0001

1994

MEAN SPAT COUNT PER 30 SHELLS

TIDAL HEIGHT (CM RELATIVE TO MLW)
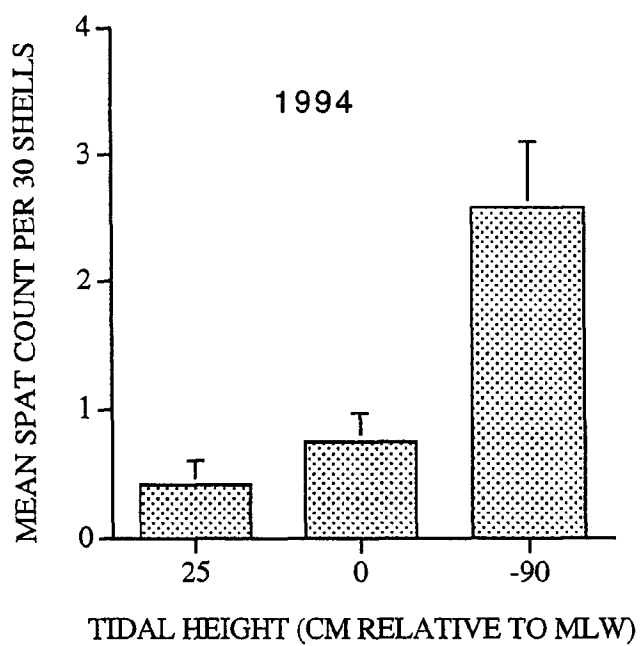
*2-Factor ANOVA; F= 26.20, df= 2, 27, p< .0001

Figure 2. Mean percent mortalities of *C. virginica* reared in 1993 residing at the + 25 cm tidal height during the June/July sampling period. Error bars denote +1 S.E.

*1-Factor ANOVA; F=4.857, df=1,12, p=.0478

Figure 3. Mean cumulative percent mortalities from June through September, 1994 for the '93 year class oysters residing at the -90 cm tidal height. Error bars denote +1 S.E.
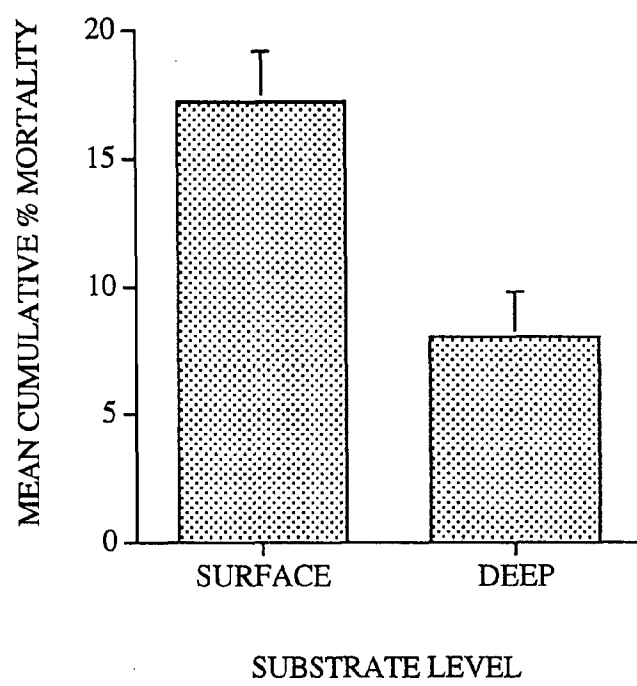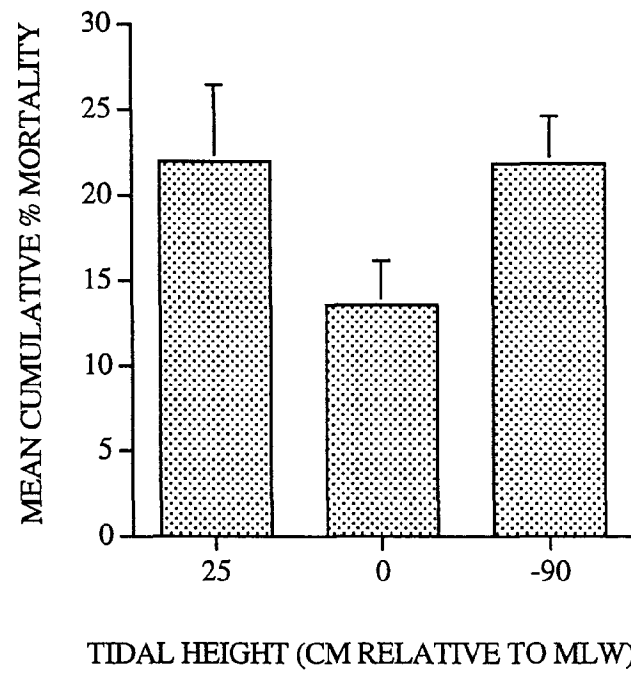
Figure 4. Cumulative percent mortalities for A) the '94 year class of oysters from June
- September, 1994 and B) 3-week old oysters from September - November,
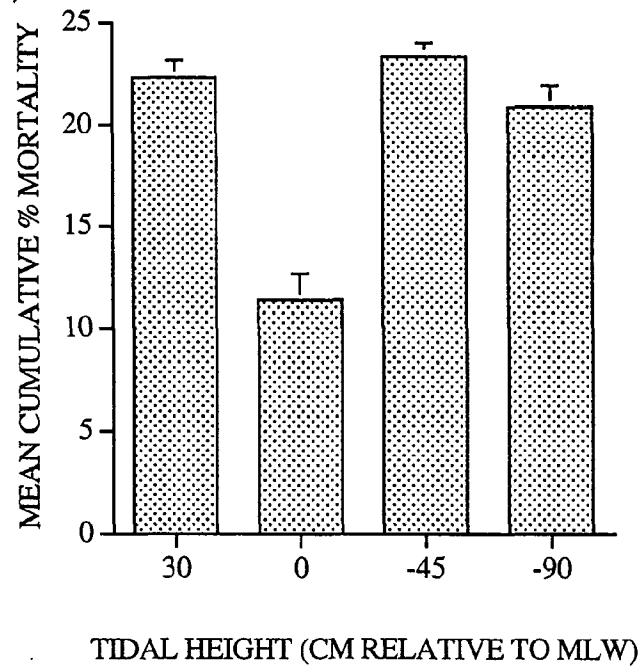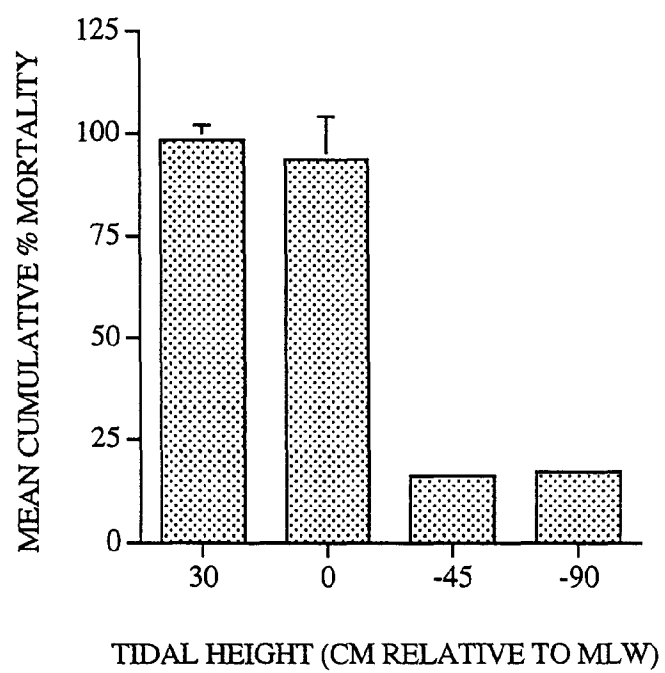1993. Error bars denote +1 S.E.

A)

B)

Figure 5. Cumulative percent mortality of juvenile oysters from November, 1993 -
May, 1994. Error bars denote +1 S.E.

MEAN CUMULATIVE % MORTALITY

TIDAL HEIGHT (CM RELATIVE TO MLW)

Small-scale Settlement Patterns of the Oyster *Crassostrea virginica* on a Constructed Intertidal Reef

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ABSTRACT

The construction of artificial reefs resembling those widely present during colonial times in the Chesapeake Bay, but now absent due to years of overharvesting, may provide a more ecologically advantageous environment for oyster settlement and subsequent survival than present subtidal, two-dimensional habitats. We examined settlement processes on a constructed, 150 x 30 m intertidal reef composed of oyster shell. The reef was destructively and non-destructively sampled weekly at tidal heights ranging from 30 cm above to 90 cm below mean low water (MLW) and at two substrate levels (reef surface and 10 cm below the reef surface). Oysters settled in comparable magnitudes at the surface of the reef community and within the reef interstices down to depths of 10 cm. Furthermore, settlement was generally greatest subtidally; however, there were localized areas within the reef community where conditions were beneficial for intertidal settlement and where intertidal/subtidal settlement rates were not significantly different. These results suggest that microscale variations in tidal elevation and substrate depth strongly affect settlement processes and should not be ignored when constructing reefs.

INTRODUCTION

When colonists arrived in the Chesapeake Bay region during the 1600s, they encountered a Bay ecosystem littered with intertidal reefs. These unmistakable biological features, which proliferated in the Chesapeake Bay and tributaries during the last half of the Holocene interglacial, were important self-renewing food sources for early settlers and Native Americans alike (Hargis and Haven 1995). As the economic value of the oyster *Crassostrea virginica* began to be realized in the mid 1800s, however, commercial exploitation of the resource began. Years of subsequent overharvesting has transformed these once massive, aerial exposed communities to mere subtidal, "footprint" structures which have significantly less vertical dimension and habitat heterogeneity. Disease, environmental degradation, and poor resource management in the last half century have expedited this degeneration.

Today, Virginia's oyster population is less than 1% of what it was just 35 years ago (Wesson et al. 1995). Many of the past rejuvenation efforts, which often have involved the spreading of relatively thin veneers of shell over coastal and estuarine bottom for larval attachment, now fail to produce healthy and viable adult oyster communities. These efforts may be ineffective because they revolve around re-creating habitats produced and shaped by man, rather than focusing on emulating the natural, three-dimensional, intertidal communities present during colonial times. Since oysters in the Chesapeake Bay resided in intertidal communities for centuries and were able to withstand significant environmental and biological stresses, there is probably an ecological and evolutionary advantage to intertidal, colonial reef existence in the Bay and a return to it may help rejuvenate ailing oyster stocks.

We know little about the colonization and ecology of *C. virginica* on intertidal reefs in the Chesapeake Bay because of their absence for over a century. Thus, we constructed a 150 x 30 m intertidal reef to study *C. virginica* settlement -- the first step in the colonization process. We paid particular attention to the effects of elevation relative to mean low water

(MLW) because tidal elevation is a major factor distinguishing intertidal reef environments from current subtidal habitats. We also examined settlement patterns at the surface of the reef shells and within the interstices of the reef at depths of 10 cm along the tidal gradient to determine if substrate depth is a factor to consider when constructing artificial reefs.

MATERIALS AND METHODS

This study was conducted in the Piankatank River, a subestuary of the Chesapeake Bay located in Virginia, at a site which once supported a highly productive intertidal reef system, but at the time of reef construction was completely devoid of live oysters. At this site water temperature varied from 0.5 - 30 C, salinity ranged from 8 - 20 ppt, and tidal range was small (mean range = 36 cm).

The reef was constructed by the Virginia Marine Resource Commission (VMRC) by spraying aged oyster shells off barges with a high pressure water canyon. The shells were broadcast over an area approximately 150 x 30 m, which were the approximate footprint dimensions of the pre-existing reef system. After completion, the reef consisted of numerous hummocks varying from 2 to 20 m^2 in area exposed at low tide. Although the constructed reef ranged from 0.5 m above to 3 m below MLW, the vast majority of the hummocks did not extend much higher than 0.35 m above MLW or much deeper than 1.0 m below MLW.

The constructed reef was sampled in both 1993 and 1994. During the 1993 sampling period 2 of the 12 principal intertidal hummocks were the focus of study: one on the reef periphery perpendicular to prevailing currents and unprotected from wave action and currents and a second situated near the middle of the reef parallel to prevailing currents and partially shielded from wave action and currents. These mounds were sampled using a transect approach, whereby samples were collected along 2 transects at tidal heights of 30 cm above MLW, MLW, 45 cm below MLW, and 90 cm below MLW.

During the 1994 sampling period, after data from the previous year were analyzed and we had a preliminary understanding of the reef system, a randomized approach was used which was more geographically expansive and statistically powerful. In this method a series of reinforced bars were driven into the reef substrate on 8 of the 12 aerial exposed hummocks or mounds and connected with rope so as to partition the mounds into 64 x 20 cm plots. This grid system encompassed reef area from the base to the crest of each mound. Experimental sites were selected randomly across the hummocks. Four of the 12 hummocks were eliminated because ice scouring during the '93 -'94 winter eroded the mound apexes, resulting in the loss of substantial intertidal substrate. In this randomized approach, tidal heights of 25 cm above MLW, MLW, and 90 cm below MLW were considered. The high intertidal height was lowered slightly in 1994 to accommodate as many intertidal mounds as possible in the sampling procedure, and one of the subtidal heights, 45 cm below MLW, was eliminated to incorporate more replication. Furthermore, another factor, substrate level or depth within the substrate, was considered. To document the effects of substrate level, samples were collected both at the reef surface and 10 cm below the reef surface.

During both years of sampling, non-destructive and destructive sampling were employed from June through September to assess settlement and early recruitment within the reef ecosystem. Non-destructive sampling involved the weekly placement of oyster shells in open-topped, 64 x 20 cm, rubber coated 1 inch wire mesh trays secured to the reef surface by reinforced bars. In 1993 a surface layer of 20 shells was placed in single level trays. The trays were situated at all 4 tidal height designations along 2 spatially separated transects at each of two mounds. The concave and convex side of all 20 shells within the individual trays were examined for recently settled oyster larvae (spat) using a dissecting microscope, and a spat total per tray was recorded. In 1994, three-tiered trays containing 30 shell upper and lower levels, which were spaced 10 cm apart, and a 40 shell

intermediate level were used. Each week 4 plots were selected randomly for each of the 3 tidal heights. At each plot a three-tiered tray was buried into the reef substrate to a depth where the upper layer of shell was even with the reef surface. Both surfaces of shell found in the upper and lower tiers were examined for spat, and a surface and deep layer spat total were recorded at all 12 weekly selected plots.

Destructive sampling involved the weekly placement of 64 x 20 cm quadrats on the reef surface, the removal of layer of shell, and the subsequent examination of both shell surfaces for spat. This sampling technique provided an index of cumulative spatfall on the actual reef substrate minus any early post-settlement mortality losses. Thus, this method provided an estimate of early recruitment--the number of larvae which had survived from settlement to the time of sampling (1-6 weeks later). In 1993 the quadrats were placed at all 4 tidal height designations along 2 spatially distinct transects on each of the 2 mounds. To prevent re-sampling, successive samples collected over time were taken immediately adjacent to previously sampled plots. During this period only surface layer samples were excavated and recorded. Plots used in the 1994 destructive sampling were selected randomly across the 8 principal intertidal mounds. As with the 1994 non-destructive samples, 4 plots were selected randomly each week at all 3 tidal heights. At each plot, a surface shell layer and a layer 10 cm beneath the reef surface, easily distinguishable from the surface layer by its brown detrital film, was extracted and examined for spat. This allowed for the calculation of both weekly surface and weekly deep spat totals for all 12 plots.

In addition to non-destructive and destructive samples, shellstring samples were collected weekly from June through September during both years of sampling. Shellstrings consist of 12 single valve oyster shells, each with a hole drilled through the center, threaded onto galvanized wire. Only the 10 intermediate shells are considered because the top and bottom shells of the shellstring have a tendency to collect large numbers of spat, leading to unrepresentative high spat estimates. The shellstrings were suspended from pilings located at the north and south reef extremities to a depth of 90 cm below MLW. Both shell surfaces were examined and a spat total per shell was calculated so that comparisons could be made with non-destructive and destructive samples.

A number of physical variables were measured during this sampling session to aid in assessing the above processes. Water temperature, salinity, and secchi depth readings were recorded each week. To develop an idea of current flow at the reef site, chlorine tablets housed in 20 cm x 20 cm mesh cages and held 10 cm above the bottom by reinforced rods were deployed during both neap and spring tides. Cages were placed at plots sampled in the non-destructive settlement study. The chlorine tablets were weighed, deployed in the field for 48 hours, and weighed again. Differences in chlorine tablet mass were compared within each tidal weight to construct, in the case of subtidal plots, a framework of relative flow rates and, in the case of intertidal plots, a model of both wave intensity and flow rates. Chlorine tablets were used as a surrogate measure for flow because turbulent diffusion, the major force driving the dissolution rate in the field, in the benthic boundary layer at a given bottom roughness varies in a positive fashion with current speed. It was assumed that the flow speed derived from the dissolution rate of chlorine tablets placed 10 cm above the reef would be proportional to flow conditions at the surface and 10 cm below.

Statistical Analysis

The argument may be made that 1993 data collected over time were not independent, since successive samples were taken from either spatially fixed areas or spatially connected plots. Thus, analysis of variance (ANOVA) with repeated measures on time were performed on both the 1993 non-destructive and destructive data sets. To satisfy the assumptions of homogeneity, all settlement data were log $(x + 1)$ transformed. When

no significant interactions were detected, 3-way fixed factor (factors: tidal height, time, mound) ANOVAs were performed. Significant main effects were examined using Student-Newman-Keuls (SNK) tests.

Linear correlation's were performed first on surface and deep samples collected in the 1994 settlement study to determine if a relationship existed between the two substrate levels. Significant relationships were detected, therefore paired sample t-tests were used to determine if differences existed surface and deep samples. A mean value for surface and deep data was calculated when no significant difference between the substrate levels was detected, and further analyses were performed on these mean values. ANOVAs were run on 1994 log (x + 1) transformed non-destructive and destructive settlement data, and all differences between the means were revealed using SNK multiple comparison tests. Furthermore, to determine if a functional dependence existed between settlement and water movement, linear regressions of log (x + 1) transformed non-destructive settlement on water movement were performed for each tidal height. The assumptions of regressions were met as determined by residual analysis (Zar 1984).

RESULTS

1993

Settlement lasted 6 weeks in 1993 beginning the week of August 5 and ending the week of September 16, and settlement intensity was low overall (Figure 1). Although the settlement period lasted 6 weeks, non-destructive spat counts recorded during weeks 5 and 6 of the settlement period were so small that they were eliminated from statistical models. Conversely, dramatic increases in destructive settlement magnitudes were observed during these periods, and thus these weeks were included in destructive sample statistical tests.

No significant interactive effects between the within factor, time, and any other factor were detected when 1993 destructive and non-destructive data were analyzed using repeated measures analysis; thus, subsequent 3-way ANOVAs were performed. These analyses revealed that in non-destructive samples only tidal height had a significant impact on settlement (Table 1a), with settlement being greatest at the -90 cm tidal height (Figure 2). In destructive samples, where the entire 6-week settlement period was considered, time had a significant impact on settlement (Table 1b). In general, destructive sample spat counts increased with time, which is quite different from the pattern observed in non-destructive samples where spat counts decreased dramatically in weeks 5 and 6 (Figure 3). Destructive and non-destructive spat counts were also very different during week 1 of the settlement period when no previous settlement had occurred. A mound x tidal height interaction was present in destructive samples. This interaction was a product of spat counts at the +25 cm tidal height on mound A (mound perpendicular to prevailing currents) preventing the detection of significant differences in settlement by tidal height (Figure 5). Conversely, on mound B (mound parallel to prevailing currents) where +25 cm spat counts were significantly lower than on mound A, significantly greater settlement occurred at MLW and -90 cm.

1994

The 1994 settlement season lasted only 3 weeks beginning the week of July 15 and ending the week of July 28. Spat counts detected on shellstrings throughout the 1993 sampling session were the lowest recorded in the last 17 years, with mean cumulative spat counts of less than 1 spat per shell over the entire settlement season (Figure 1). Unfortunately, these low settlement magnitudes together with the short settlement season precluded a meaningful comparison of intertidal spat counts recorded on mound A with intertidal counts on the other mounds. Field observations did reveal, however, that noticeably more intertidal oysters were present on mound A than at any of the other 8 mounds present at the site.

Significant correlation's (p<.001) between surface and deep substrate levels were detected in both non-destructive and destructive samples, and thus paired t-tests were used to examine the effects of substrate level. Based on these tests, no significant differences in settlement were detected between surface and deep layers at any of the tidal heights in either the non-destructive or destructive samples (p>.05, Table 2).

In the non-destructive study, ANOVAs performed on surface/deep settlement means revealed that both tidal height and time influenced settlement (Table 3a). Settlement intensity was greatest at the -90 cm tidal height and peaked the week of July 21-28 (Figure 5). Settlement was influenced significantly by time and tidal height in the destructive study as well, but a significant time x tidal height interaction confounded the effects (Table 3b). This interaction was a result of spat counts being significantly greater at the -90 cm tidal height only during the weeks July 21-28 and July 28 - August 4. During the first week of the settlement season, settlement magnitudes were so low across all 3 tidal heights that no significant differences were detectable.

Comparison of spat detection methods

There was a clear discrepancy in spat counts between the 3 sampling methods even though they were all deployed at a depth of 90 cm below MLW. Clearly more spat settled on shellstrings, which were replaced weekly and suspended in the water column, than on shells placed at weekly intervals in trays fixed to the reef or on shells sampled destructively from the reef (Figure 6). Furthermore, non-destructive samples appeared to collect more spat than destructive samples.

Physical Parameters

Salinity, water temperature, and secchi disk readings recorded during both years of sampling are presented graphically in Figure 7. In 1993 settlement occurred during a minor decrease in water clarity (August 5 - 12), whereas the onset of settlement in 1994 coincided with a rise in water clarity (July 15 - 21) and water temperature (July 15). Flow rate was greatest at the reef crests and lowest at the reef bases, and there was no linear dependence of settlement on water flow (p > .234, R^2 < .231).

DISCUSSION

The short, unimodal settlement events recorded in this study coupled with low overall settlement magnitudes are indicative of a rapidly declining broodstock population in the Chesapeake Bay, which at present is showing little sign of rejuvenation (Morales-Alamo and Mann 1995). Bimodal peaks in settlement were recorded not long ago in the Chesapeake Bay, and it was not uncommon to detect settlement from June through October at the Piankatank reef site (Virginia Institute of Marine Science annual oyster spatfall surveys, unpublished data for the period 1970-1995). In 1986, cumulative spat counts at the site were 376.5 spat/shell, whereas now they are at 0.9 spat/shell . Although there are probably a number of reasons for this devastating decline ranging from disease to poor water quality, years of overharvesting where ecologically advantageous intertidal reef communities were degraded to mere subtidal footprints undoubtedly weakened oyster stocks considerably. Hargis (1995) offers the opinion that overharvesting was the principal instigator of the demise of the Chesapeake oyster. Although this is debatable, there is widespread agreement that oyster reefs are the most optimal ecosystems for oysters and we need to learn more about oyster ecology on them.

Based on two years of settlement monitoring on a constructed intertidal oyster reef, it was clear that tidal elevation had a large impact on settlement and early recruitment. The higher rates of settlement recorded in the subtidal zone than in the intertidal zone are consistent with several other studies conducted in non-reef environments, such as McDougall (1942) where unglazed hearth tiles were used as substrate, Chestnut and Fahy

(1953) where shellstrings were utilized, and Roegner and Mann (1990) where hatchery-reared larvae exposed to field conditions in microcosms were considered. Nichy and Menzel (1967), who placed oysters on clothmats of mesh within a reef ecosystem, also observed greater settlement/early recruitment subtidally.

The high rates of subtidal settlement/early recruitment observed throughout most of this study were likely a result of a number of factors. Submergence time, for instance, may have contributed to settlement discrepancies observed at different tidal heights. Oyster larvae in the water column were exposed to subtidal substrates substantially longer than to intertidal substrates, and as a result, had a wider time window in which to set. Submergence time alone, however, did not account for the observed differences in settlement. Kenny et al. (1990) and Roegner (1989) found that settlement intensities were not direct functions of submergence time, especially in the high intertidal zone where settlement was lower than predicted and the low subtidal zone where settlement was higher than predicted. Another factor contributing to elevated subtidal sets may have been vertical segregation of oyster larvae within the water column because oyster late stage pediveliger larvae are more abundant near the benthos than at the surface or within the midwater region (Carriker 1951, Kunkle 1957, Haskin 1964, and Baker 1994). Furthermore, because late stage competent to set oyster larvae are photonegative at the time of settlement (Cole & Knight-Jones 1939, Ritchie & Menzel 1969, Shaw et al. 1970) and prefer areas of lower wave energy (Ortega 1981, Abbe 1986 Bushek 1988), they may have actively sought subtidal habitats where light intensities and wave stress are reduced.

Although settlement was maximized subtidally for most of the study, the lack of significant settlement differences between tidal heights at mound A during the 1993 season suggests that this is not always the case in artificial reef environments. Mound A is the most unique hummock comprising the reef system. It is more than twice as large, in terms of substrate aerial exposed at low tide, as any of the mounds at the site, is the first mound exposed to tidal influx, is the only mound oriented completely perpendicular to prevailing currents, and experiences the most intense wave action. These factors, especially wave action which kept intertidal substrate clean, may have contributed to high intertidal sets. In 1994, we had hoped to compare sets on mound A with sets on the seven other mounds at the reef site to determine if intertidal settlement intensities were indeed higher at mound A. Unfortunately, spat counts were so low and the settlement season was so abbreviated that no meaningful comparisons could be made. The highest abundance of intertidal oysters clearly occurs on mound A based on field observations, which certainly suggests that conditions at mound A are conducive for intertidal settlement. Conversely, results from this study and field observations indicate that subtidal settlement is no higher at mound A than at any of the mounds.

Several other studies have found that settlement/early recruitment is not always maximized subtidally. Hidu and Haskin (1972) found that although settlement was greatest subtidally 1/2 mile offshore at a transitional slope region where tidal flats merge with deep water, settlement was greatest intertidally in shallow water near the shore. They attributed the high intertidal sets inshore to rapid rises in water temperature as seawater passed over heated intertidal substrates and to the presence of dense intertidal adult populations which released chemical cues. McNulty (1953), using bags of shell left in the field for two weeks, found higher settlement/early recruitment in the intertidal zone than in the subtidal zone, and Kenny et al. (1990), using asbestos plates sampled every two weeks in the summer and four weeks in the winter, found settlement to be similar from 70 cm above mean low water in the intertidal zone to 30 cm below mean low water in the subtidal zone. McNulty and Kenny et al. attributed the high rates of settlement/early recruitment in the intertidal zone to less predation and more desirable substrate in the intertidal zone.

The lack of detectable differences in settlement/early recruitment (1-3 weeks) between surface and deep substrates at any of the tidal heights considered was admittedly

unexpected. Our first reaction was that the low settlement rates recorded in this study dramatically lowered the power of the statistical tests. Although this may be true, a graphical re-examination of the data by layer revealed no trend in greater settlement for either substrate depth. Thus, we concluded that larval oyster settlement was not impeded by shell down to depths of 10 cm on artificial reefs composed of oyster shell. Unfortunately, there are no studies on the settlement of sessile organisms as a function of substrate depth to which this study can be compared. The fact that adult oysters are found in greater number at the surface of established reefs (Bahr and Lanier 1981), however, suggests that settlement patterns and adult ranges may deviate from one another. This was documented by Roegner (1989) and Kenny et al. (1990), who both found discrepancies in the ranges of settlers and adults.

There are several reasons why oyster larvae may select substrate within the interstices of the reef. Some larvae may settle on sub-surface substrate because again they prefer darkened conditions when setting (Cole & Knight-Jones 1939, Ritchie & Menzel 1969, Shaw et al. 1970) and areas of reduced wave action (Ortega 1981, Abbe 1986), but also because they seek out environments such as the reef interstices where flow is low, crevices are abundant, and substrates are not heavily fouled (Bushek 1988, Michener & Kenny 1991, Morales-Alamo and Mann 1990). Fouling by algae and encrusting barnacles beneath the surface was considerably less than that found at the reef surface. A final explanation for the observed sub-surface settlement may be that the interstices, where flow was substantially less than that found at the surface, may have served as sediment traps and entrained larvae. Although it is not clear from this study which active or passive transport mechanism(s) is/are responsible for sub-surface settlement, it is clear that larvae are capable of settling within the fabric of the reef and are not impeded by shell down to depths of 10 cm. This is quite remarkable considering there may be 20 or more shell layers within the 10 cm space.

The fact that settlement estimates from shellstrings, a frequently used method of estimating oyster abundance's, were greater than both destructive and non-destructive samples suggests that suspended shellstrings overestimate settlement on sloping reef bottoms. Baker (1994) also found shellstrings to be unreliable predictors of settlement magnitudes on adjacent substrates. Even though shellstrings fail to provide accurate assessment of oyster settlement on actual reef topography, they are efficient and reliable predictors of the presence of late-stage pediveligers at a given site. This was evident by the fact that the beginning dates of the settlement season coincided exactly with the detection of spat in destructive and non-destructive samples. Furthermore, when compared with shellstrings suspended at other sites, they may be useful tools for determining relative settlement intensities.

A direct comparison between the two methods to predict post-settlement mortality rates could not be made because settlement rates during week 1 of the settlement season were dissimilar between destructive and non-destructive samples when no previous settlement had occurred. The discrepancy in settlement rates during week 1 was probably a product of two factors: substrate differences and difficulties in spat detection. The higher degree of fouling and colonization on reef shells compared with that found on shells placed in trays on a weekly basis may have contributed to lower destructive sample settlement rates. Bryozoans, colonial ascidians, and certain barnacles, organisms all present at the reef site, considerably reduce settlement and survival of oysters (Ortega and Sutherland 1989). Difficulties in identifying spat on heavily fouled reef shells was probably another factor contributing to lower destructive spat counts. During the early weeks of settlement, spat were small and difficult to detect on the heavily fouled reef shells, but when spat became larger (weeks 5 and 6 of the 1993 study), they were more visible and thus spat counts were higher. This was evident by the substantial increases in settlement found in

the destructive samples during weeks 5 and 6 while only negligible amounts of spat were detected on non-destructively sampled shells and shellstrings.

Mass spawning of oysters occurs at temperatures between 22°C and 23°C (Galtsoff 1964), and larvae generally mature in two to three weeks (Abbe 1986); however, the current results indicate that settlement did not occur until at least 8 weeks after water temperatures first rose to this range in 1993 and after 5 weeks during the 1994 sampling period. The onset of the '93 and '94 settlement seasons did not coincide with any change in salinity either. The '93 settlement season occurred shortly after a minor drop in water clarity, and the '94 settlement began during a rise in water clarity. The coincidence of initial settlement and high turbidity in 1993 was surprising given that Calabrese & Davis (1966) and Davis and Hidu (1969) both found suspended sediment loads in the water column to be detrimental to larval development and settlement. The results of this study suggested that neither water temperature, nor salinity, nor clear water conditions alone can explain fully the onset of settlement. Instead settlement is triggered by the interaction of numerous factors such as water temperature, salinity, dissolved oxygen, suspended sediments, food supply, pollutants, availability of substratum, hydrodynamic factors, light, and other organisms (Abbe 1986).

Settlement intensity examined at each tidal height/substrate level combination was not found to be dependent on water movement. The lack of dependence of settlement on water movement may appear somewhat surprising, given that several researchers have found settlement to be high in areas of low to moderate flow. For instance, Nelson (1921) and Roughley (1933) found high concentrations of swimming and setting oyster larvae in eddies and areas of slack water. Hidu and Haskin (1971) found high settlement in Delaware Bay at sharp transition zones between high and low current velocities, and concluded that this was a result of concentrations of larval falling out of suspension as water flow decreased. Furthermore, in Galveston Bay, Bushek (1988) found oysters preferentially settled nearshore on pier pilings where current velocities were low. One exception to the above studies is found in Carriker (1959), who found settlement to be greatest near faster currents in a sheltered salt-water pond.

The observed independence of settlement and water flow may be a result of two factors. First, the velocities considered in this experiment may not have varied dramatically enough for a strong functional relationship to be observed. Second, microscale flow conditions may have had a larger impact on settlement intensities than mean flow rates calculated 10 cm above the reef bed. Oyster larvae setting on the underside of surface substrates and within the fabric of the reef probably experienced dramatically different flow regimes than those measured 10 cm above the benthos. Microspatial flow measurements need to be measured to accurately determine if larval settlement is dependent on flow in an environment such as an artificial reef where flow rates are highly variable on a small scale.

Although none of the physical factors investigated were reliable predictors of the onset of settlement and settlement intensity did not appear to be strongly correlated with flow rate, we did learn that vertical elevation relative to both the reef surface and MLW influences settlement. Oysters settle not just at the surface of reef communities but settle in comparable numbers within the reef interstices down to depths of 10 cm. This finding is significant because presently there is debate over which substrates should be used for reef construction. Many of the proposed reefs are to be composed of crushed clam shell, tile, or mounds of sediment capped with a thin shell layer. When these substrates are used, sub-surface interstitial space is limited, precluding oyster community development below the surface. The sub-surface environment provides biological and physical refugia for oysters and may be very important for survival in developmentally young, artificial reef communities (Bartol and Mann, in prep). Furthermore, settlement on artificial reefs is generally greatest subtidally; however, there may be localized areas within the community where conditions are beneficial for intertidal settlement and intertidal/subtidal settlement

rates are comparable. These zones may be important for oyster reef development because they allow for the rapid establishment of intertidal and not just subtidal environments. The establishment of oysters in heterogeneous intertidal/subtidal environments, habitats they once thrived in naturally, assuredly is beneficial for dwindling oyster populations.

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Table 1a. ANOVA of non-destructive settlement data.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	.078	.078	1.059	.3111
tidal height	3	2.147	.716	9.692	.0001
time	3	.573	.191	2.588	.0701
mound * tidal height	3	.516	.172	2.331	.0928
mound * time	3	.127	.042	.572	.6377
tidal height * time	9	.819	.091	1.232	.3108
mound * tidal height * time	9	.989	.110	1.489	.1942
Residual	32	2.362	.074		

Dependent: non-destructive settlement log (x+1)) transformed

Table 1b. ANOVA of destructive settlement data

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	.043	.043	.648	.4248
tidal height	3	1.207	.402	6.017	.0015
time	5	7.583	1.517	22.690	.0001
mound * tidal height	3	.582	.194	2.903	.0443
tidal height * time	15	.878	.059	.875	.5942
mound * time	5	.287	.057	.858	.5164
mound * tidal height * time	15	.423	.028	.422	.9648
Residual	48	3.208	.067		

Dependent: destructive settlement log (x+1) transformed

Table 2. Paired t-tests performed on surface and deep substrate layer spat counts for non-destructive and destructive samples. Separate analyses were performed on each tidal elevation.

NON-DESTRUCTIVE SAMPLES

Tidal height	Mean difference (surface-deep)	Degrees of freedom	t-value	p-value
+ 25 cm	-.333	11	-.886	.3944
MLW	-.083	11	-.321	.7545
-90 cm	.333	11	.549	.5940

DESTRUCTIVE SAMPLES

Tidal height	Mean difference (surface-deep)	Degrees of freedom	t-value	p-value
+25 cm	-.083	11	-.561	.5863
MLW	.083	11	.432	.6742
-90 cm	-.500	11	-1.149	.2750

Table 3a. ANOVA of log (x+1) transformed spat counts measured in the non-destructive settlement study.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
week	2	.607	.303	15.807	.0001
tidal height	2	1.006	.503	26.204	.0001
week * tidal height	4	.038	.010	.499	.7366
Residual	27	.518	.019		

Dependent: log (x+1) transformed mean spat count

Table 3b. ANOVA of log (x+1) transformed spat counts measured in the destructive settlement study.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
week	2	.295	.148	15.156	.0001
tidal height	2	.832	.416	42.709	.0001
week * tidal height	4	.129	.032	3.299	.0252
Residual	27	.263	.010		

Dependent: Log (x+1) transformed mean spat count

Figure 1. Cumulative oyster spat counts per shell detected on shellstrings at Palace Bar, VA from 1979 - 1994 recorded in the VIMS Spatfall Surveys. Spat counts were recorded for only a portion of the 1988 settlement season.
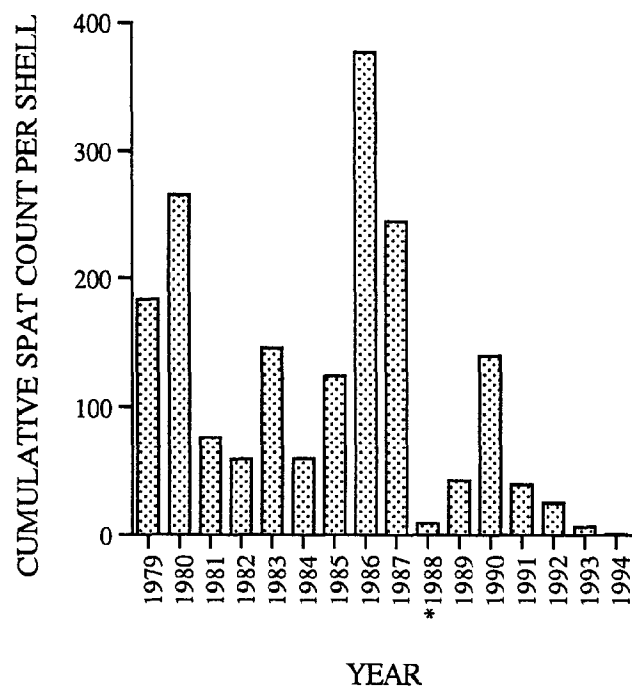
*Spat counts for 1988 reflect an incomplete set of data.

Figure 2. Mean oyster spat counts per 30 shells recorded in the non-destructive study. Error bars denote +1 S.E.

NON-DESTRUCTIVE SETTLEMENT DATA
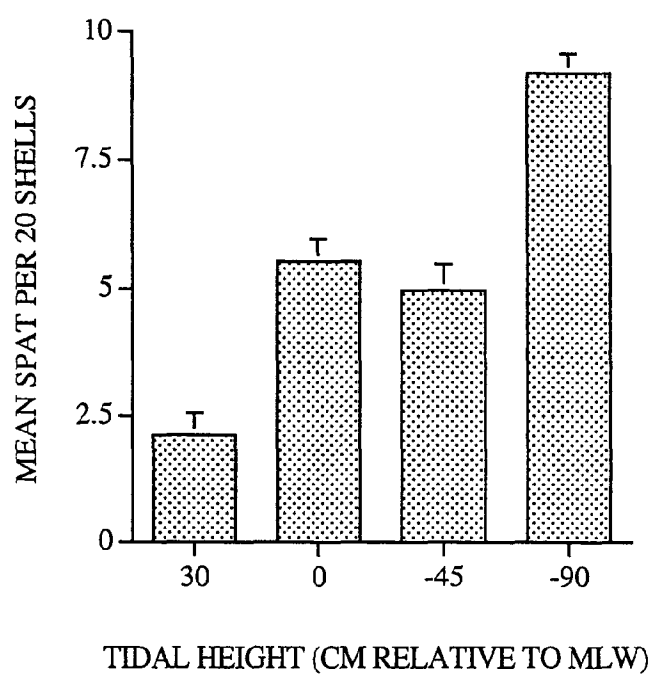
Figure 3. Mean weekly spat counts for both destructive and non-destructive samples
collected from August 12 through September 23, 1993. Error bars denote
+1 S.E.
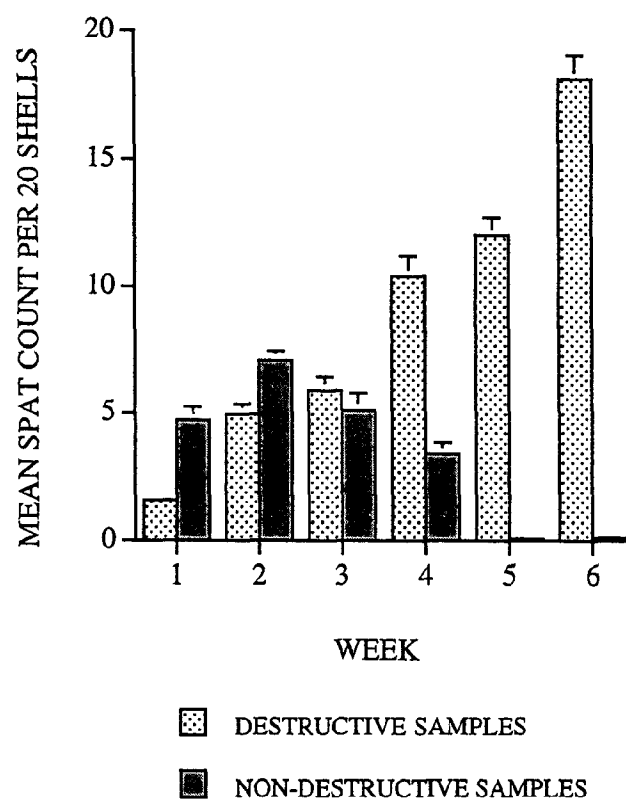
Figure 4. Mean spat counts recorded on mound A and B during the 1993 settlement
season. Error bars denote +1 S.E.

DESTRUCTIVE SETTLEMENT DATA COLLECTED ON MOUND A

MEAN SPAT COUNT PER 20 SHELLS

TIDAL HEIGHT (CM RELATIVE TO MLW)

DESTRUCTIVE SETTLEMENT DATA COLLECTED ON MOUND B

MEAN SPAT COUNT PER 20 SHELLS

TIDAL HEIGHT (CM RELATIVE TO MLW)
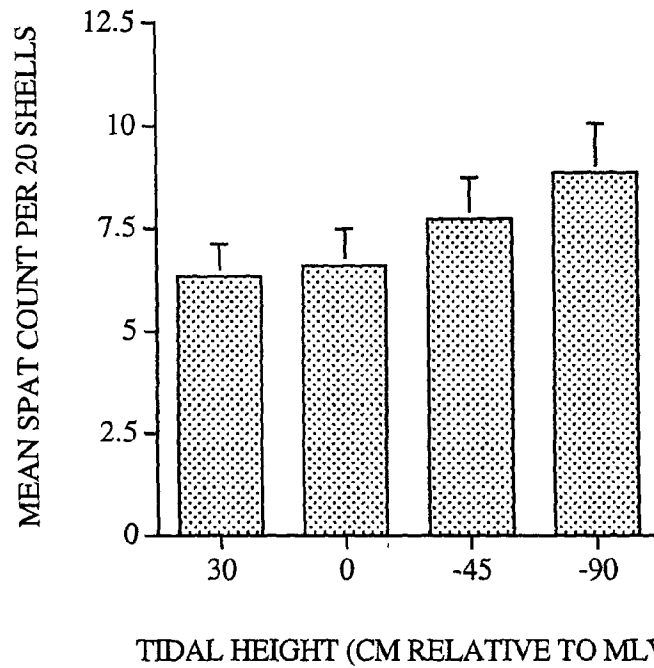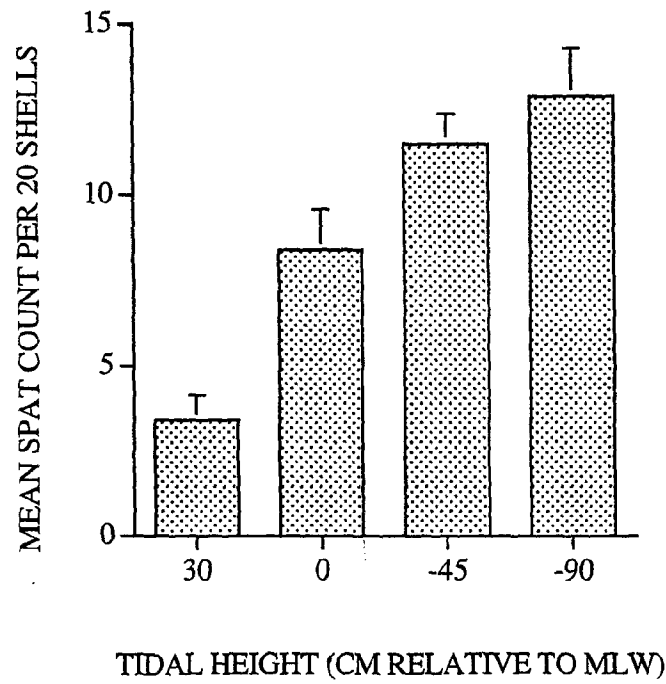
Figure 5. Mean spat counts per 30 shells calculated from non-destructive samples for (A) each of the three tidal heights and for (B) each week of sampling (week 1 = July 15-21, week 2 = July 21 - 28, and week 3 = July 28 - August 4, 1994). Error bars denote +1 S.E.

A)
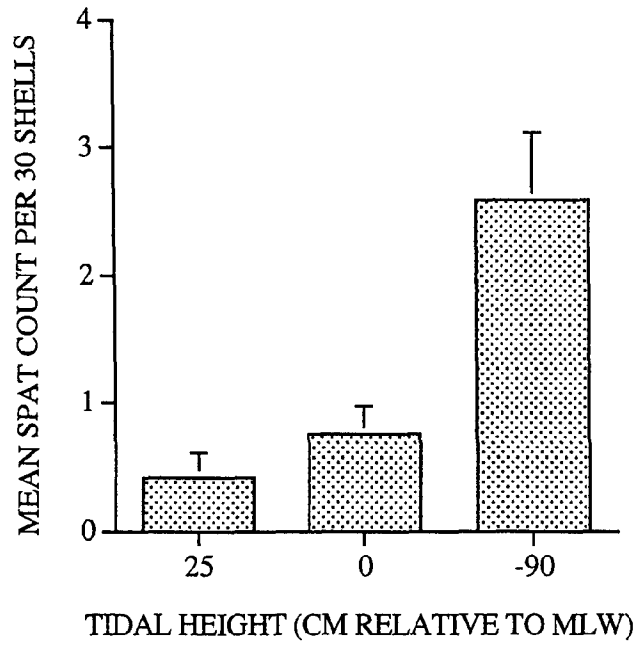
B)
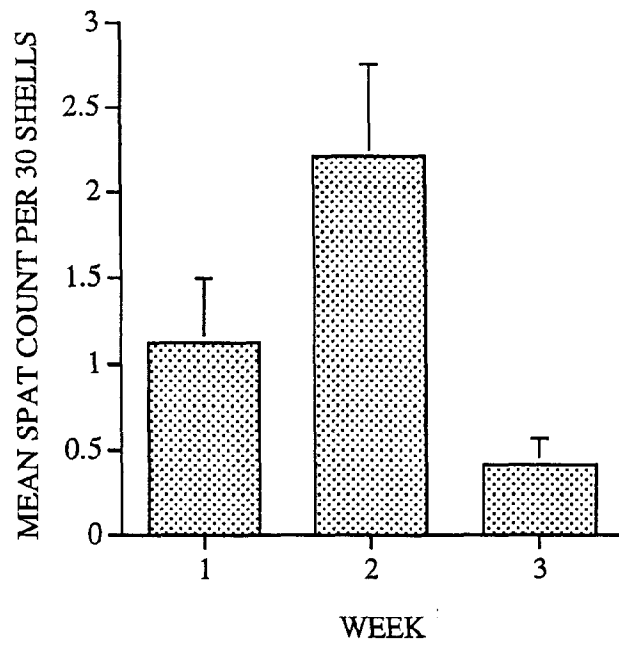
Figure 6. Cumulative spat counts per shell at the -90 cm tidal height detected on shellstrings, non-destructive samples, and destructive samples in 1993 and 1994. Error bars denote +1 S.E.
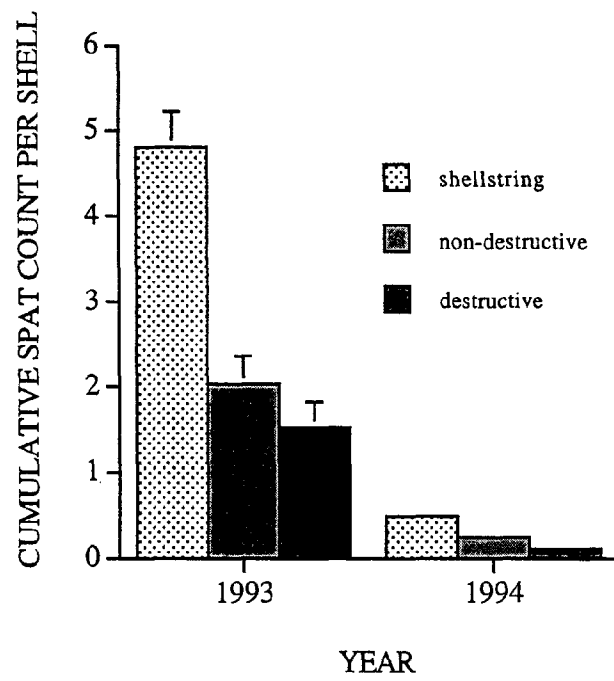
Figure 7. Salinity, secchi disk readings, and water temperatures recorded at the reef
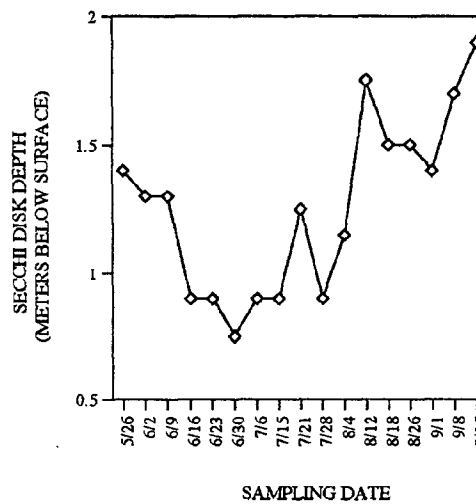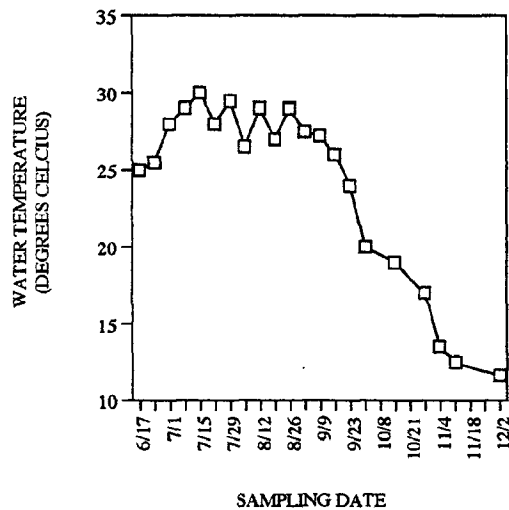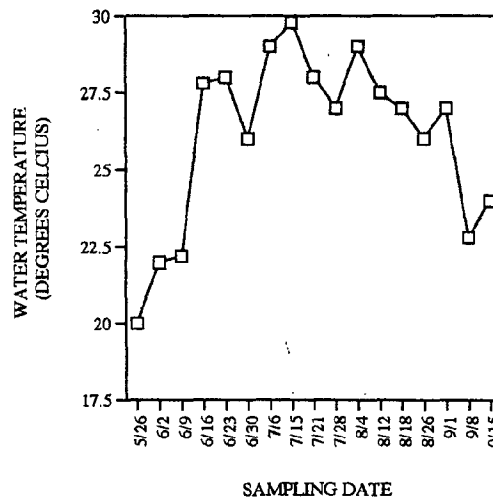site during 1993 and 1994.
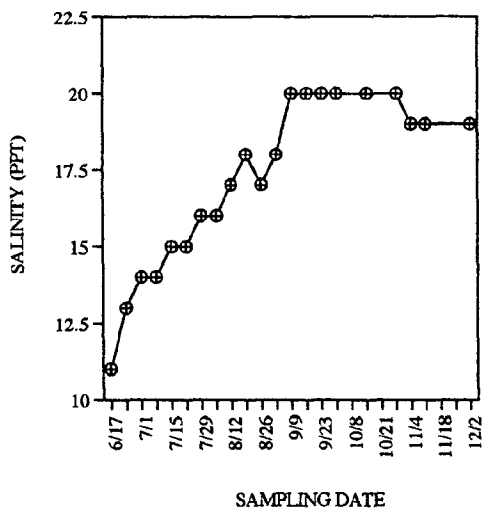
1993
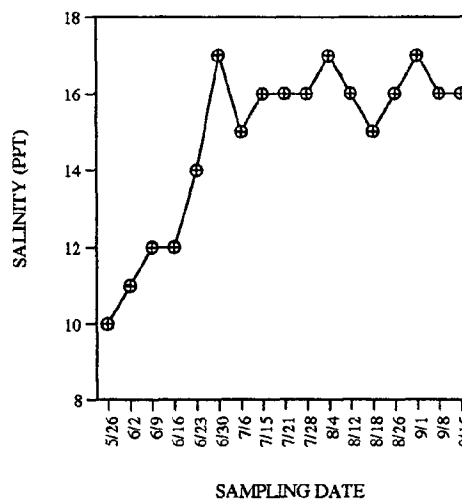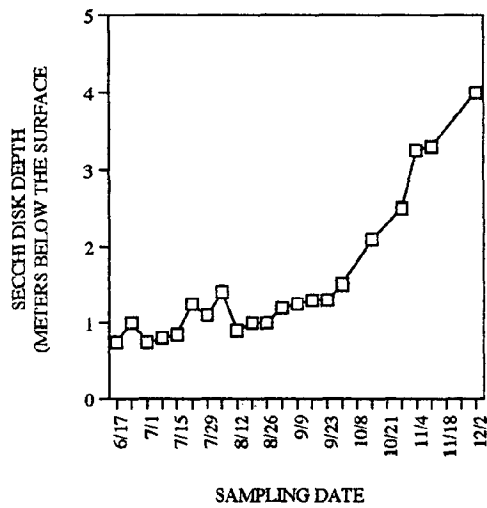
1994

The Importance of Small-scale Spatial and Temporal Variation in *C. virginica* Growth and Mortality on Constructed Intertidal Reefs

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ABSTRACT

The spreading of thin veneers of shell over coastal and estuarine bottoms, which provides hard substrate for oyster settlement and subsequent colonization, no longer guarantees the establishment of *C. virginica* communities in the Chesapeake Bay largely because of the presence of endemic diseases. The construction of intertidal reefs resembling those present in the Bay during colonial times, but now absent largely because of overharvesting, may provide a more ecologically suitable environment for oyster growth and survival than present, two-dimensional habitats. We constructed a 150 x 30 m intertidal reef, and during three, 28-day sampling periods in the summer, examined growth and mortality of two year classes of oysters placed in cages at three tidal heights (25 cm above MLW (mid intertidal zone), MLW, and 90 cm below MLW (low subtidal)) and at two substrate depths (reef surface and 10 cm below the reef surface). Oysters grew faster below than at the surface in the mid intertidal zone, and grew faster at the surface than below in subtidal environments. Growth was greatest subtidally and oysters at all tidal elevations grew well from mid August through mid September. In the intertidal zone, residence below the reef surface provided relief from heat and respiratory stress during periods of peak solar exposure, and in the subtidal zone, sub-surface residence provided refugia during periods of high predation. These results suggest that small-scale spatial and temporal factors have a large impact on oyster reef ecology and should be considered when constructing reefs.

INTRODUCTION

Artificial reefs provide structure and spatial complexity within marine ecosystems and facilitate the establishment of diverse communities of organisms. The construction of artificial reefs for habitat restoration purposes has increased dramatically worldwide, with reef projects in Australia, Japan, Southeast Asia, the Caribbean, the eastern and northern Mediterranean basins, the Pacific Islands, and North America (Seaman et al. 1989). Many of these projects have been conducted to enhance local fish resources, and as a result much of the artificial reef research pertains to fish ecology (Turner et al. 1969, Buckley 1989, Grant et al. 1982, Grove 1982, Bohnsack and Sutherland 1985, Relini et al. 1986, Bombace et al. 1990, Beets and Hixon 1994, Bortone et al. 1994, Fabi and Fiorentini 1994). Recently, several studies have been conducted to determine the effect of artificial structures on lobster communities (Davis 1987, Cruz et al. 1987, Eggleston et al. 1990, Lozano-Alvarez et al. 1994, Barshaw and Spanier 1994) and sessile corals, invertebrates and algae (Riemers and Branden 1994, Downing et al. 1985, Fitzhardinge and Bailey-Brock 1989, Hixon and Brostoff 1985, Bombace et al. 1994, Relini et al. 1994); however, few studies have examined how artificial reefs can serve as tools for enhancing oyster populations.

Traditionally, oyster rejuvenation efforts in the Chesapeake Bay have involved the deployment of cultch, generally oyster or clam shell, off barges in subtidal locations with the subsequent intent of retrieving either juvenile (seed) or market size oysters which attach to the cultch. Shells were often broadcast over large areas to maximize coverage because of the increasing cost of available substrate, producing subtidal carpets of shell which lacked vertical relief. These 2-dimensional "artificial reefs" substantially enhance settlement, the

irreversible adherence of oyster larvae to the substrate, by providing clean, hard substrate for attachment, especially when planted at the correct time (Manning 1952, Abbe 1988, Morales-Alamo and Mann 1990). In the past these procedures facilitated the establishment of adult oyster communities; however, in the Chesapeake Bay where disease is highly problematic, these efforts no longer guarantee adult oyster development. This may in part be because this two-dimensional approach fails to accurately simulate 3-dimensional reef communities formed naturally in the Chesapeake Bay before man's intervention.

During colonial times intertidal oyster reefs were unmistakable geological and biological features of the Bay landscape. In the early 1800's there were approximately 10 miles of intertidal reef in the James River, a subestuary of the Chesapeake Bay (Hargis, pers. comm.). As the economic value of the oyster began to be realized in the mid to late 1800s commercial exploitation of the resource began. Years of subsequent harvesting resulted in the transformation of all of these protruding, aerially exposed features in the Bay to mere low-lying subtidal "footprints" of pre-existing intertidal reefs. This degeneration was exacerbated by the arrival of two protistan parasites, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo), and environmental degradation.

The rationale for re-creating colonial intertidal reef systems for the rejuvenation of oyster populations is simple; since oysters in the Chesapeake Bay resided in extensive intertidal reef communities before man's intervention, these environments are probably ecologically and evolutionary advantageous and a return to them may elevate survival rates. Therefore, we constructed a 150 x 30 m intertidal reef and monitored growth and mortality of *C. virginica* within the reef ecosystem. In this study we took a micro rather than a macro approach to reef ecology, in which we examined growth and mortality within a narrow tidal range (25 cm above to 90 cm below MLW), at different levels within the reef structure (at the reef surface and 10 cm below the reef surface), and during three, 28-day sampling intervals in the summer. We investigated whether small-scale spatial and temporal variation have an effect on *C. virginica* growth and mortality in constructed reef settings and whether these variations should be considered in future rejuvenation efforts.

STUDY SITE

This study was conducted in the Piankatank River, a sub-estuary of the Chesapeake Bay located in Virginia, at a site which once supported a highly productive intertidal reef system but at the time of reef construction was devoid of live oysters. The Piankatank River is ideal for artificial reef construction because generally there is a high abundance of oyster larvae (Morales and Mann 1995), and there is no commercial oyster fishery and virtually no industry or agricultural development within the watershed. Tidal range at this site is small (mean range = 36 cm); however, local meteorological events, wind in particular, often dramatically alter this range from 0 to 1.25 m. The site is relatively shallow (1-3 meters), and consists of a sandy bottom. During the course of this study water temperature at the study site varied from 0.5 - 30 °C and salinity fluctuated from 8-20 ppt.

MATERIALS AND METHODS

The reef was constructed in June 1993 by the Virginia Marine Resource Commission (VMRC). The construction procedure involved the deployment of aged oyster shells off barges using a high pressure water cannon. The shells were broadcast over an area approximately 150 m x 30 m, which were the approximate footprint dimensions of the pre-existing reef system. When this study was initiated, the constructed reef consisted of 12 intertidal hummocks. Only eight of these hummocks were considered for this study because the remaining four did not protrude sufficiently above the water surface to allow for the consideration of heights above mean low water (MLW) in the sampling design.

Tidal heights of 25 cm above MLW (mid intertidal zone), MLW, and 90 cm below MLW (low subtidal zone) were considered. At each of the 8 mounds, which varied in size and orientation, reinforced bars were driven into the reef shells at all three tidal height designations. This allowed for the expedient location of the tidal elevations during sampling. Substrate found at each tidal height on all eight mounds was marked and partitioned into 64 x 20 cm plots using rope and reinforced bars. This resulted in the formation of three distinct bands of substrate (one for each height) which potentially could be sampled on each of the eight mounds.

To document growth and mortality, two year classes of oysters, both of which were set and reared for several weeks in the Virginia Institute of Marine Science (VMS) Oyster Hatchery, were considered. One year class consisted of oysters set on oyster shell on May 16, 1994 and reared in hatchery systems for three weeks, whereas the other year class consisted of oysters set on oyster shell on August 12, 1993, reared in hatchery systems for three weeks, and placed in subtidal cages on the Piankatank Reef until the commencement of this study. On June 1, 1995 oysters comprising each year class were retrieved from either the field or the hatchery and mixed thoroughly. For each year class, the cultch shells were grouped into 48, 15-shell assemblages. The concave, smooth surface of the 15 shells within each grouping were subsequently marked using paint and permanent markers. We were careful to make sure at least 30 oysters were present collectively on the labeled sides of shells comprising each assemblage. To keep densities as constant as possible, the physical removal of oysters in high density communities was sometimes necessary. Overall densities were 2.85 ± .35 S.E. oysters per shell for the 1994 year class and 2.95 ± .48 S.E. oysters per shell for the 1993 year class.

The labeled sides of shells comprising each 15-shell assemblage were photographed on slide film using a Nikonos V camera equipped with a close-up lens and focusing frame. The shell areas of 30 oysters in each assemblage (48 total assemblages per year class) were calculated using an image analysis system manufactured by Biosonics Inc. (1988), and a map of the oysters on each shell was constructed so that they could be tracked in future study. Initial shell areas for 1994 and 1993 oysters were 1.18 ± .10 S.E. cm^2 and 4.3 ± .52 S.E. cm^2, respectively. After being photographed, the 15 shell units were placed on either the upper or lower level of labeled 32 x 20 cm, three-tiered, 1 inch mesh cages. The upper and lower levels were 10 cm apart and separated by an intermediate level of 20 oysterless shells.

For both year classes, eight plots were selected randomly at each of the three tidal heights. The '93 year class and '94 year class oyster cages were deployed at their designated plots on June 14 and June 20, 1994, respectively. The cages were buried into the reef substrate until the upper layer of oysters was level with the reef surface and held in place with reinforced bars. At 28 day intervals in July, August, and September, oysters were removed from the cages, cleaned using a 3 HP gasoline powered Homelite water pump, and photographed with the Nikonos V camera. Using the Biosonics image analysis system, a mean growth value for each 15 shell assemblage was computed over all three, 28-day sampling intervals. This was accomplished by determining the shell areas of all 30 tests oysters within each grouping, calculating a mean shell area for the assemblage, and subtracting this value from the mean shell area at the beginning of the period. The areas were expressed in mm^2/day. For each 28-day sampling interval a mean proportional mortality value (# dead oysters at the end of the 28-day interval/#live oysters at the beginning of the interval) was also computed.

Physical Parameters

Each week throughout the study, water temperature, salinity, and secchi depth readings were recorded at the reef site. After completion of the study, chlorine tablets housed in 20 cm x 20 cm mesh cages held 10 cm above the reef substrate were deployed during both neap and spring tides at all of the sampled plots. The chlorine tablets were

weighed, deployed in the field for 48 hours, and weighed again. Differences in chlorine tablet mass at each tidal height were compared to construct in the case of subtidal plots, a framework of relative flow rates, and in the case of intertidal plots, a combined relative estimate of the magnitude of both flow rates and wave intensity. Chlorine tablets were used because they were a cost-effective method of obtaining flow information. The theory behind using chlorine tablets as a surrogate measure of flow is that turbulent diffusion, the major force driving the dissolution rate of the tablets in the field, in the benthic boundary layer at a given bottom roughness will vary in a positive fashion with current speed.

Statistical Analysis

Correlation coefficients were computed for all surface/deep sample pairs and compared with values in a critical coefficient table to determine if a relationship existed between surface and deep levels (Zar 1984). When no significant relationship was detected for a data set, surface and deep samples collected at a given plot were treated independently. Conversely, when significant relationships were detected, paired sample t-tests were performed to decipher significant differences. These tests were performed separately by height on mean values computed over the entire sampling period.

Multivariate repeated measures analyses of variance (ANOVA) were performed separately by year class on growth and mortality data. To satisfy the subject within group and subject between group homogeneity of variance assumptions, proportional mortality data were arcsine transformed; however, it was not necessary to manipulate growth data. When interactions were detected, lower-level ANOVAs and/or repeated measures analyses were performed. All significant between factor effects were analyzed using SNK multiple comparison tests and significant within factor main effects were examined using Newman-Keuls procedure (pp. 527-528, Winer 1991).

Linear regressions of growth on water movement were performed to determine if a functional dependence was present. Analyses were performed separately by each year class, substrate level position, and tidal elevation. The assumptions of the regressions were met as determined by residual analysis (Zar 1984).

RESULTS
Growth

1993 Oysters

Significant differences in growth rates between substrate levels were detected at two of the three tidal heights examined (Table 1). At +25 cm growth was greater 10 cm below the surface than at the surface, and at -90 cm growth was greater at the surface than below. These discrepancies were consistent throughout the three sampling periods.

Growth for the 1993 oysters residing at the surface was dependent on tidal height and time (Table 2a). Surface oyster growth at tidal heights of -90 cm and MLW was greater than growth at +25 cm, and growth was greatest at all heights during the August/September sampling period (Figure 1a, 2a). Surface residing oysters situated at the -90 cm tidal height also experienced a visible decrease in growth during the July/August sampling period, although this decline was not statistically significant (time x tidal height = .058) (Figure 3a).

No difference in growth across the three tidal heights was detected for oysters residing below the surface within the reef interstices (Table 2b); however, growth at the deep layer increased significantly at all tidal heights during the August/September sampling period, as was observed at the surface (Figure 2b). A trend in reduced growth during the July/August sampling period was also apparent (Figure 2b).

1994 Oysters

As was the case with the 1993 year class of oysters, growth at +25 cm was greater 10 cm below the surface than at the surface, and growth at -90 cm was greater at the

surface than in sub-surface environments (Table 1). These differences were consistent throughout the 3 sampling periods. No difference in growth by substrate level was detected at MLW.

A significant time x tidal height interaction was present when the 1994 surface residing oysters were analyzed (p=.003) (Table 3a). Further analyses revealed that during two of the three sampling periods growth was greatest subtidally. During the June/July period growth increased significantly with tidal depth, and during the August/September period growth at the -90 cm was greater than growth at +25 cm (Figure 2c). Greater subtidal growth was not, however, found during the July/August period, because of a significant decline in growth at MLW and -90 cm (Figure 2c). Furthermore, growth rates were high during the August/September period across all tidal heights, especially at +25 cm where growth was significantly greater during this time. Below the reef surface within the fabric of the reef, oyster growth was dependent on tidal height and time (Table 3b). Growth was greatest at -90 cm and was lowest during the July/August period (Figure 2d, 3).

Mortality
1993 Oysters

A tidal height x layer and tidal height x time interaction both were detected when mortality data from the 1993 year class were analyzed (Table 4a). Lower level analyses performed to decouple these interactions revealed several interesting findings. At MLW, oysters residing in the interstices of the reef experienced greater mortalities than oysters at the surface substrate level, but at -90 cm, oysters residing at the surface had the greatest mortalities (Figure 4). Of the oysters that resided at the surface, those that were situated at MLW had significantly lower mortalities than those situated at +25 cm or -90 cm (Figure 4). During the August/September sampling period, the highest mortalities across both substrate depths were detected at the -90 cm tidal height, whereas during the June/July sampling period, the highest surface mortalities occurred at +25 cm (Figure 5). Furthermore, during the June/July period significantly higher mortalities were recorded at the surface than beneath the surface for oysters situated at +25 cm (Figure 6).

1994 Oysters

A significant time x tidal height interaction and a nearly significant time x tidal height x substrate level interaction (p=.066) were detected when mortality data from the 1994 year class were analyzed (Table 4b). The low p-value in the three-way interaction was in part a product of higher surface than below surface mortalities during the June/July period for oysters at +25 cm (Figure 7). Lower level analyses revealed that the time x tidal height interaction was a product of mortality during the June/July period being greatest at +25 cm and during the August/September period being greatest at -90 cm (Figure 8).

Although mortality was not found to be significantly lower at any of the three tidal heights, a graphical depiction of cumulative percent mortality throughout the sampling period revealed a definite trend of lower mortality at the MLW tidal height (Figure 9). Overall mean cumulative mortality ranged from 14.2% to 23.4%, which was higher than that documented for the older, 1993 oysters.

Physical Factors

Growth of 1993 and 1994 oysters was independent of water flow (p > .05 ; r^2< .30). Salinity, water temperature, and secchi disk readings recorded during the sampling period are presented graphically in Figure 10. During the first sampling period, which began June 14 for 1993 oysters and June 20 for 1994 oysters, there was a rapid increase in salinity. Salinity in subsequent periods, however, was relatively constant, fluctuating between only 15‰ and 17‰. Water temperatures during the first two sampling periods (mid June / mid July and mid July / mid August) ranged from 26°-30°C, and during last

sampling period (mid August / mid September) decreased considerably to 22.5°C. Water clarity also improved during the last sampling period, which began August 9 for 1993 oysters and August 16 for 1994 oysters. Mean air temperatures were greatest during the June/July period and lowest during the August/September period for both year classes.

DISCUSSION

The elevated rates of growth detected below the reef surface at +25 cm for both year classes was probably a product of both increased submergence times and a reduction in stressful environmental conditions. Since oysters residing in the deep layer were lower in vertical elevation, they were inundated longer by tidal flow, and as a result could filter feed for longer periods of time and achieve greater sizes. The attainment of larger size in oysters with increased submergence time has been demonstrated by Ingle and Dawson (1952), Burrell (1982), and Roegner (1989). Oysters dwelling in these underlying habitats also were shaded from direct sunlight and wind , and thus were less susceptible to desiccation and heat stress. Bahr (1976) found temperatures 6 cm beneath the surface at an intertidal oyster reef community at Sapelo Island, Georgia, to be 7° C lower than at the surface even in October. The reduction in atmospheric stress allowed oysters to invest more energy in growth and less in environmental resistance.

Higher subsurface intertidal rates of growth may not occur in all intertidal reef communities, however. Bahr and Lanier (1981) found surface residing oysters in intertidal reef communities in the South Atlantic to have sharper growing edges than oysters in lower layers and felt this was indicative of faster growth at the surface. This finding may have been because Bahr and Lanier studied a mature, established reef community, where flow and spatial constraints below the surface inhibited subsurface growth. Dense assemblages of oysters, present at the surface of established reef communities increase sedimentation rates by as much as 8 times (Lund 1957, Haven and Morales-Alamo 1967). These high rates of biodeposition coupled with well established surface fouling communities severely restrict flow in underlying layers, making growth more difficult. Furthermore, the stationary nature of established substrate, which is a product of the clustering of generations of oysters, makes growth more arduous in underlying layers where space is limited. Flow and space problems are not as problematic in newly constructed or developing reef communities, however, because biomass is still low, substrate is still movable, and interstitial spaces are abundant.

Contrary to what was found at +25 cm, oysters from both year classes situated at - 90 cm grew faster at the reef surface than below. This reversal in growth optimization was largely because subtidal oysters within the interstices did not benefit from longer submergence times (both subtidal substrate levels were submerged constantly) and more favorable environmental conditions (there was no difference in refugia from atmospheric stresses) like intertidal oysters. Instead, oysters residing below the surface in the subtidal zone lived in an environment where flow was somewhat limiting. Unlike the intertidal zone, where fouling was low and water motion energetic, surface algae and colonizing infauna were highly abundant in the subtidal zone and overall flow rates were low. These factors reduced the amount of flow reaching oysters in underlying layers and resulted in slower growth rates.

Oyster growth in relation to tidal height is quantified in several studies, most of which have found growth to be greatest in the subtidal zone. Loosanoff (1932), Ingle and Dawson (1952), Burrell (1982), and Roegner (1989) as mentioned earlier all found greatest growth subtidally for C. virginica, and Sumner (1981) and Roland and Albreit (1986) found similar results for C. gigas. Gillmor (1982) and Crosby et al. (1991) presented evidence that C. virginica held in the intertidal zone under certain levels of aerial exposure were capable of growing as fast or faster per unit immersion time as subtidal oysters. The reason for this is that periodicity in feeding allows for the more efficient processing of crude fiber. In both studies, however, overall growth rates per day were still greatest

subtidally because of longer submergence times. In contrast to the above studies, Littlewood (1988), working with *C. rhizophorae* in Jamaica, found growth in the low intertidal zone was greater than growth at subtidal depths, and Spencer and Gough (1978) were unable to detect a difference in growth of either *C. gigas* or *O. edulis* held subtidally and in the low intertidal zone.

Most of the results of this study suggest that oyster growth in artificial reef environments is maximized subtidally. This may be largely due to longer feeding times provided by constant submergence (Peterson & Black 1987); however, growth is not simply a direct function of submergence time. Kenny et al. (1991) found that intertidal oysters exhibited double the expected decrease in growth than that predicted by immersion time alone, and Peterson and Black (1988) found that growth rates of a variety of suspension feeding bivalves found in both the high intertidal and low subtidal were not directly proportional to submergence time. Within the intertidal zone, the metabolic stress associated with emergence may have played a role as well (Dame 1972, Newell 1979). As a result of harsh environmental conditions in the intertidal zone, intertidal oysters allocate more energy to environmental resistance and less to somatic growth than subtidal oysters (Dame 1972, Newell 1979). This translates into reduced growth intertidally.

While significantly greater subtidal growth was detected in most of the data collected, no significant growth difference between tidal heights was observed for 1993 oysters residing below the surface. This was not unexpected for differences in submergence time and environmental stress, the major reasons for growth discrepancies along the tidal gradient, were less pronounced in subtidal and intertidal sub-surface environments. In the intertidal zone, oysters in sub-surface environments were lower in vertical elevation, allowing for longer submergence and feeding times than surface dwelling intertidal oysters. Sub-surface intertidal oysters also could afford to invest energy in growth since the shaded, moist underlying environment was more hospitable than the surface. These factors lead to faster intertidal growth. Conversely, flow restrictions in sub-surface subtidal habitats caused by overlying fouling communities and sluggish flow rates lowered growth rates below MLW. This convergence in intertidal and subtidal growth rates made growth differences less detectable. Interestingly, growth was greatest subtidally for the 1994 year class residing beneath the surface. This suggests that the benefits of longer submergence times may outweigh the disadvantages of restricted flow and that submergence time is an important factor to consider when assessing growth rates.

The depressions in growth during the July/August period observed across both year classes was perplexing. A dramatic decrease in growth like this is often correlated with some environmental condition or physiological event. But the data collected in this study supplied no insight as to which mechanism caused the depression. No drastic fluctuations in water temperatures or salinity occurred during this period, and although a brief peak in water clarity took place, water clarity was often higher during the August/September period when growth was often greatest. The decrease in growth did coincide with peak spawning time (June through August (Galtsoff 1964)), in which a shift is made from totally somatic growth to gametic and somatic growth. A prerequisite for this shift is sexual maturity. Oysters may develop functional gonads at a young age (2 to 3 months) and small size (less than 1 cm in height) (Galtsoff 1964). The 1994 oysters were only 1.5 months old by the onset of the growth depression period, and thus it is highly unlikely that growth reductions were attributable to gametic energy reallocation. Conversely, this metabolic shift may be applicable to 1993 oysters, which were about 11 months in July. However, some other cause which applies to both year classes is more probable. The depression in growth may have been attributable to the fluctuation of some environmental factor not measured in the experiment, such as dissolved oxygen or phytoplankton assemblages, or may have been a result of a combination of a number of factors.

Many oysters considered in this study grew most rapidly during the August/September period, and all oysters grew well during this time. Salinity probably did

not account for this since it remained relatively constant throughout the sampling period. But lower water temperatures and reduced sediment loads in the water column, which were evident from secchi disk readings, may have. Galtsoff (1964) suggested that optimal filter feeding of oysters occurs between 25 - 26 ° C, water temperatures most often encountered during the August/September period. These temperatures may have elevated growth rates. Furthermore, turbidity, which was substantially lower during the August/September period, is highly detrimental to oyster pumping rates, and is closely coupled with growth since oysters are filter feeders (Galtsoff 1964). Elevated growth rates during this period for 1993 oysters may have also been partly due to a shift from somatic and reproductive growth to simply somatic growth.

The observed independence of growth on water motion in the subtidal zone, where water motion measurements were a reflection of flow rates, may have been because growth, especially below the reef surface, was more closely coupled with food depletion on a microscale than with mean flow speed above the reef bed. Loosanoff (1958) and Walne (1972) demonstrated that growth increases with elevated levels of flow. Grizzle et al. (1992) found that growth decreases with increased current flow in one study which focused on current velocities from 0 - 10 cm/sec, and in another study which considered velocities from 0 - 5 cm/sec found maximal growth at 1 cm/sec. In all of these studies, however, individual oysters in a flume rather than a patch of oysters in the field were considered. At the level of a group of non-siphonate bivalves localized food depletion rather than flow rate may have a greater influence on growth. This is because at the relatively quiescent region near the bed, especially within the fabric of the reef, clearance rates probably exceed the influx of new particles, creating zones of low food concentration. These zones which are influenced not only by flow rate but also resident species density and reef topography assuredly had a large impact on growth. Malouf and Breese (1977) and Wilson et al. (1992) came to a similar conclusion, and emphasized that bivalve growth in the field is more closely correlated with food availability in the water column than with current velocity.

At intertidal plots, where water movement measurements were a reflection of not only flow rate but wave action as well, growth was not dependent on water motion either. Within the intertidal zone one might expect oysters, especially in sub-surface environments, to benefit from more intense water movement because hydrologic forces serve to flush out fecal wastes and replenish the stagnant environment with fresh, nutrient rich seawater. We did not see a positive relationship between water motion and growth, though. Nor did we see a detrimental effect of wave action on growth as observed on highly energetic coasts (Ortega 1981) largely because wave energy at our relatively protected experimental site was not sufficient to be deleterious. We feel that the lack of dependence was again a product of growth being more closely coupled with the microscale environment than with flow rate or wave action 10 cm above the reef bed. Food patchiness, while not as significant a problem as in the subtidal zone where water movement is reduced substantially, still likely had a larger impact on growth than flow rate or wave action. When completely submerged, intertidal oysters which have been aerially exposed for a period often filter feed at higher rates than subtidal oysters which are continually submerge. These elevated feeding rates may cause clearance rates to exceed particle influx rapidly, especially within subsurface environments.

Environmental buffering beneath the reef surface was the primary reason why higher mortality was observed at the surface than below at +25 cm during the June/July period, the interval with the highest mean air temperature (28.2 ° C). In established, natural intertidal oyster reefs such as those found on the coasts of Georgia and South Carolina, oysters at the reef surface are clustered and tend to grow vertically so that neighboring oysters may provide shade and protection from the sun and drying winds. At the constructed reef surface, however, where a colonial adult oyster community had not yet been established, there was little relief from the harsh effects of solar exposure and high air

temperatures, factors which contribute to oyster mortalities in the intertidal zone. Intertidal oyster mortalities in environments which experience intense solar exposure but offer little shade are not uncommon, especially when oysters are young. In Virginia from June through July, Roegner (1989) found a mortality rates of > 75 % for unshaded juvenile oysters held in the mid to high intertidal zone, and in South Carolina, Crosby et al. (1991) and Michener and Kenny (1991) reported summer intertidal mortalities of 80 - 90% for oysters set on exposed asbestos plates. Oysters residing below the surface in the intertidal zone were shielded from atmospheric stresses. Overlying reef shell coupled with longer periods of submergence (as a result of being slightly lower in vertical elevation) made the underlying environment cooler and moister than surface habitats, and lowered both heat stress and evaporative water loss.

The fact that mortality of 1993 oysters found at -90 cm was higher at the reef surface than below throughout the 3 month sampling period suggests that residence within the reef interstices may be advantageous in the subtidal zone as well. Oysters in sub-surface subtidal environments benefited from a reduction in predation pressure. The two most prominent oysters predators in the Chesapeake Bay, the oyster drills, *Urosalpinx cinera* and *Eupleura caudata*, and the seastar, *Asterias forbesi*, were absent at the reef site because of low salinities. However, a number of predators were present such as the flatworm *Stylochus ellipticus*, the mud crabs *Panopeus herbstii*, *Eurypanopeus depressus, and Rhithropanopeus harrisii*, and the blue crab *Callinectes sapidus*. Flatworms near oyster populations cause significant mortalities even though the extent of the damage is unknown (Landers and Rhodes 1970, Morales et al. 1988, Abbe 1986, Littlewood 1988, Baker 1994), mud crabs prey upon small oysters (Abbe 1986, Baker 1994), causing mortalities as high as high as 50% (Mackenzie 1981), and predation by blue crabs (Carriker 1955, Abbe 1986, Roegner 1989, Eggleston 1990a, b, c) is well documented.

Flatworms, mud crabs, and blue crabs were found within cages at surface and deep substrate layers and at all tidal heights, but were most abundant at surface substrate layers and subtidal depths based on field observations. Flatworms and mud crabs were probably most deleterious for they were not restricted by the mesh of the experimental cages. Flatworms and mud crabs both were observed within the valves of recently dead oysters, and in photographs, we noticed the presence of numerous boring holes in test oyster shells. Blue crabs, which were only able to enter cages during juvenile developmental stages, were less problematic. Even though entry into the cages was restricted to smaller crabs, predation on caged oysters may not have been limited to juvenile forms. Numerous oysters were found growing through the cage mesh and were exposed to the surrounding environment. These oysters were freely accessible to larger predators in the area. Furthermore, field observations revealed that blue crabs often laid flat on the tops of cages and extended their claws inward into the interior of the cages. Although no direct predation of blue crabs on oysters was observed during these maneuvers, this behavior may have allowed larger crabs to feed on caged oysters. The higher rates of subtidal surface mortality in 1993 oysters together with greater observed predator abundance's suggests that the above predators preferred to prey upon oysters that were highly accessible and readily available, rather than burrow through the reef topography to expose and prey upon oysters in the underlying layers.

The lack of a detectable differences in subtidal surface/sub-surface mortality for 1994 oysters was likely a product of the young oyster's reduced tolerance to sedimentation and hypoxic conditions. Sedimentation was greater in the deep substrate layer than at the surface. Although this sedimentation was not substantial and had little effect on the 1993 oysters, it may have contributed to mortality of the smaller 1993 oysters found in lower layers. Even low/moderate sediment rates bury and subsequently kill small oysters (Mackenzie 1981, Abbe 1986). Furthermore, periodic conditions of hypoxia may occur in deep layers because flow is low and often restricted. When hypoxic conditions are present,

oysters need to isolate themselves from their surroundings and if necessary, switch from aerobic to anaerobic respiration. This switch may be more difficult for oysters less than 1 year of age because young oysters put much of their energy into growth and maintenance rather than in the storage of glycogen, the preferred substrate for anaerobic respiration (Galtsoff 1964). As a result young oysters cannot employ anaerobic respiration as efficiently as older oysters which have larger glycogen reserves. Mann and Gallager (1985) and Holland and Spencer (1973) found that this is highly probable, given that nutrient reserves are small and polysaccharides account for only a small proportion of the total energy reserves during development. Thus, the benefits of reduced predation for subtidal subsurface residence probably were eradicated by the young oyster's inability to tolerate sedimentation and periods of hypoxia.

Of the three tidal heights considered, 1993 oysters living at the surface survived best at MLW; when examined over the entire sampling period, 1994 surface residing oysters clearly demonstrated the lowest mortality at MLW. Oysters at MLW experience less predation, sedimentation, and fouling than oysters in subtidal habitats and encounter less severe atmospheric conditions and diminished respiratory stress than oysters in higher intertidal regions. Thus, oysters at MLW experience the best of both worlds; they benefit from lowered predation pressure and fouling as a consequence of aerial exposure, but do not suffer from severe heat and respiratory stress because they are not exposed for extended periods of time.

The advantages of surface residence at MLW are similar to the benefits of sub-surface residence; oysters receive a refuge from predation and experience environmental buffering. Unlike oysters within the reef interstices, however, surface residing oysters do not have to contend with restricted water flow or hypoxic conditions, which may both contribute to mortality. Consequently, at MLW mortality was lower at the surface than below.

The fact that the highest mortalities were detected at +25 cm during the June/July period and at -90 cm during the August/September interval suggests that physical factors dominated during the initial sampling session but biological factors became more pronounced in the final session. During the June/July period when air temperature were at their highest, mortality from heat stress in the intertidal zone was more prominent than mortality from predation and fouling in the subtidal zone. Thus, greater mortalities were detected in the intertidal zone. However, from mid-August through mid-September, when air temperatures were reduced (mean air temperature = 24.5 - 25.1 °C) and predators and subtidal algal growth became more prevalent, biological factors dominated and mortalities became highest in the subtidal zone. During the final sampling period there were greater sitings, both in photographs and in the field, of flatworms. Baker (1994), in a study conducted in August in the York River, Chesapeake Bay, Virginia found numerous flatworms to be red in color, the color of a stain used to mark test oysters, and concluded that predation by flatworms was substantial during this time. Elevated flatworm sitings also coincided with an influx of blue crabs at the reef site, which may have contributed to elevated mortality rates. Water temperatures during the early part of this period hovered around 26-27 °C. At these temperatures crabs are voracious feeders and exhibit type II inversely density dependent predation, whereby partial prey refuges found at lower temperatures are eliminated (Eggleston 1990 a). In addition to predation, algal growth may have been a source of mortality. *Enteromorpha* grew prolifically in the subtidal zone during the August/September period, and may have disrupted both pumping rates and respiratory functions.

Often when we seek to evaluate large systems like an intertidal reef ecosystem or a series of reef structures, we think in terms of the big picture. Will these reefs enhance survival of the depleted oyster population? What influence do these systems have on the Chesapeake Bay? What is the most cost-effective method of reef construction. These questions are important and are often the reason for conducting a specific project.

However, in evaluating and answering these questions we often loose track of the small-scale variability inherent within the system and simply focus on the larger scale uniformity. This study demonstrates that microscale spatial (cms) and temporal (months within a season) factors have a profound impact on the underpinnings of an artificial reef ecosystem. Residence merely 10 cm beneath the reef substrate may enhance growth at a particular tidal elevations and substantially elevate survival during periods of intense solar exposure or predation. Likewise, a shift of 30 cm in tidal elevation may move an oyster from an environment which is highly conducive for growth and survivorship to an environment which is highly stressful during certain times of the year.

With these factors in mind, our advice to oyster reef builders is to consider using substrate porous enough to allow for sub-surface colonization. This will significantly increase the amount of available habitat per m^2 of substrate and will provide valuable refugia from physical and biological stresses. This is especially important in newly constructed artificial reefs where significant biomass at the reef surface, helpful in the buffering of outside stresses, has not yet been established. Reef builders should also be aware of the effects of tidal elevation. Before a reef is constructed, builders should determine which height along the tidal continuum provides the most advantageous compromise of desirable factors (i.e. settlement, growth, survivorship, etc.). At the Piankatank reef site, where the goal was to maximize growth but at the same time minimize mortality, the low intertidal/high subtidal zone was the most advantageous region for the summer months. Proposed reefs should them be constructed so that there is suitable substrate within the desirable tidal range. Finally, temporal variation should not be overlooked. This study has demonstrated that in a matter of weeks, substantial changes in growth and mortality may occur within a reef ecosystem. Reefs are constantly in a state of flux not just over years/decades but also over weeks/days, and it is these microtemporal and microspatial variations that produce the large-scale patterns that we observe in the field

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Table 1. Paired t-tests peformed on mean growth of oysters residing at the reef surface and 10 cm below the reef surface from June through September. Separate analyses were performed on each tidal height for both the 1993 and 1994 year classes.

Paired t-tests run on surface and deep layers for the 1993 oysters.

Tidal height	Mean difference (surface-deep)	Degrees of freedom	t-value	p-value
+25 cm	-1.44	6	-3.518	.0126
MLW	.137	6	.206	.8417
-90 cm	2.193	7	2.531	.0392

Paired t-tests run on surface and deep layers for the 1994 oysters.

Tidal height	Mean difference (surface-deep)	Degrees of freedom	t-value	p-value
+25 cm	-1.218	7	-3.066	.0182
MLW	.381	6	.898	.4037
-90 cm	1.165	7	4.286	.0036

Table 2a. Multivariate repeated measures ANOVA performed on growth data
from the 1993 year class of oysters residing at the reef surface.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TIDAL HEIGHT	2	88.926	44.463	7.820	.0039
Subject(Group)	17	96.658	5.686		
TIME	2	287.401	143.701	61.380	.0001
TIME * TIDAL HEIGHT	4	23.727	5.932	2.534	.0581
TIME * Subject(Group)	34	79.599	2.341		

Dependent: GROWTH

Table 2 b. Multivariate repeated measures ANOVA performed on growth data
from the 1993 year class of oysters residing 10 cm below the reef surface.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TIDAL HEIGHT	2	13.523	6.762	1.559	.2349
Subject(Group)	20	86.753	4.338		
TIME	2	121.867	60.934	23.460	.0001
TIME * TIDAL HEIGHT	4	6.566	1.641	.632	.6426
TIME * Subject(Group)	40	103.893	2.597		

Dependent: GROWTH

Table 3a. Multivariate repeated measures ANOVA performed on growth data from the 1994 year class of oysters residing at the reef surface.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	107.804	53.902	15.058	.0001
Subject(Group)	20	71.595	3.580		
time	2	104.308	52.154	21.707	.0001
time * tidal height	4	47.054	11.763	4.896	.0026
time * Subject(Group)	40	96.103	2.403		

Dependent: growth (mm/ day)

Table 3b. Multivariate repeated measures ANOVA performed on growth data from the 1994 year class of oysters residing 10 cm below the reef surface.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	22.190	11.095	5.336	.0139
Subject(Group)	20	41.585	2.079		
time	2	28.898	14.449	7.197	.0021
time * tidal height	4	15.052	3.763	1.874	.1338
time * Subject(Group)	40	80.308	2.008		

Dependent: growth

Table 4a. Multivariate repeated measures ANOVA performed on arcsine transformed mortality data collected from the 1993 year class of oysters.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TIDAL HEIGHT	2	400.739	200.370	4.019	.0257
LAYER	1	.028	.028	.001	.9811
TIDAL HEIGHT * LAYER	2	717.397	358.698	7.194	.0021
Subject(Group)	40	1994.427	49.861		
TIME	2	931.755	465.878	12.660	.0001
TIME * TIDAL HEIGHT	4	453.722	113.430	3.082	.0205
TIME * LAYER	2	36.603	18.302	.497	.6100
TIME * TIDAL HEIGHT * LAYER	4	264.045	66.011	1.794	.1382
TIME * Subject(Group)	80	2943.926	36.799		

Dependent: MORTALITY

Table 4b. Multivariate repeated measures ANOVA performed on arcsine transformed mortality data from the 1994 year class of oysters.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	343.135	171.567	1.656	.2037
layer	1	11.369	11.369	.110	.7422
tidal height * layer	2	18.367	9.183	.089	.9153
Subject(Group)	40	4143.799	103.595		
time	2	588.868	294.434	4.403	.0153
time * tidal height	4	1066.695	266.674	3.988	.0053
time * layer	2	205.856	102.928	1.539	.2208
time * tidal height * layer	4	614.294	153.574	2.297	.0662
time * Subject(Group)	80	5349.560	66.870		

Dependent: mortality

Figure 1. Mean growth rates examined by tidal elevation for the 1993 year class
oysters found at (A) the reef surface and (B) 10 cm below the reef surface. Error
bars denote +1 S.E.

A)

1993 SURFACE OYSTERS

B)

1993 DEEP OYSTERS
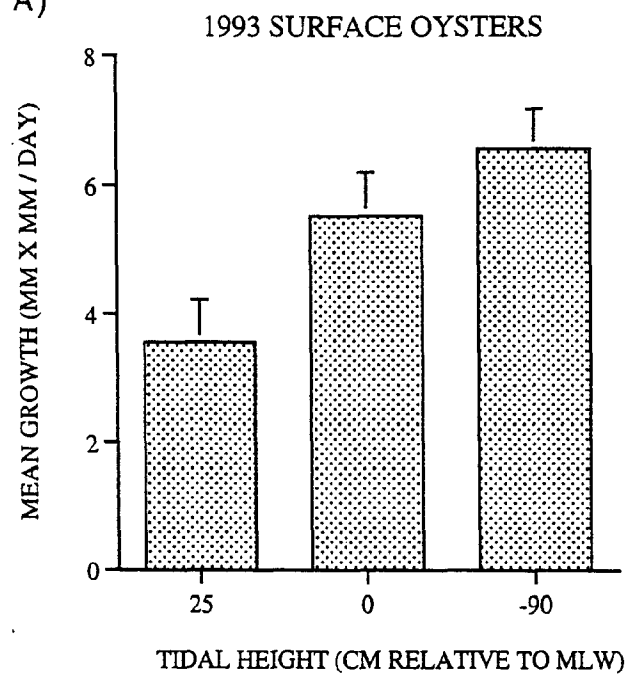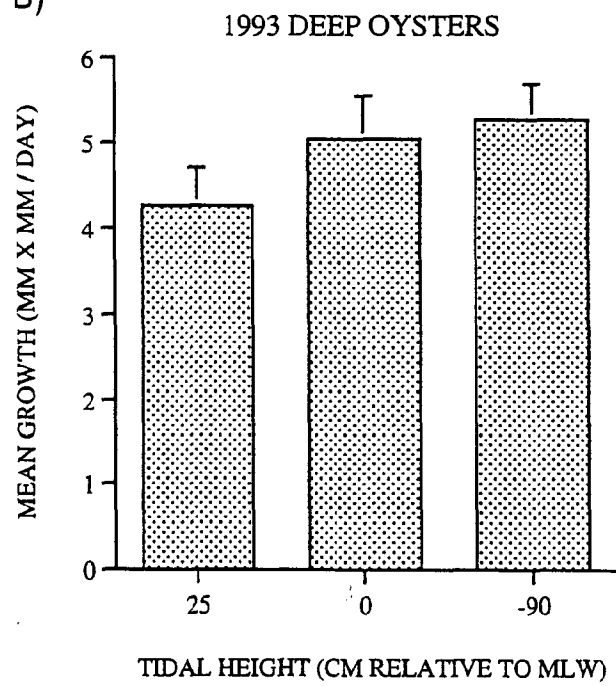
Figure 2. Mean growth rates examined by tidal height and sampling period for (A) the 1993 year of oysters residing at the reef surface, (B) the 1993 year class of oysters residing 10 cm below the surface, (C) the 1994 year class of oysters residing at the surface, and (D) the 1994 year class of oysters residing 10 cm below the surface. Error bars denote +1 S.E.

A) 1993 SURFACE OYSTERS

C) 1994 SURFACE OYSTERS

B) 1993 DEEP OYSTERS

D) 1994 DEEP OYSTERS

MEAN GROWTH (MM X MM / DAY)

TIDAL HEIGHT (CM RELATIVE TO MLW)
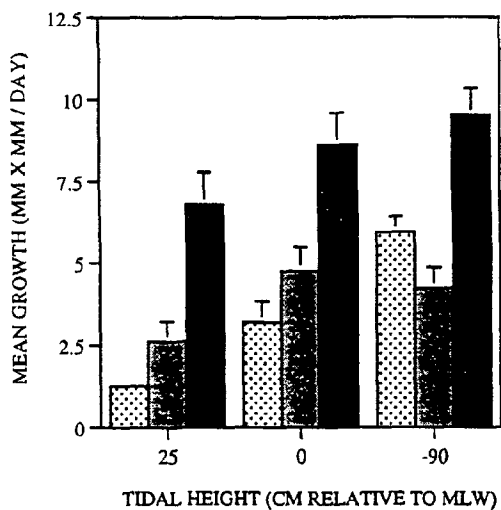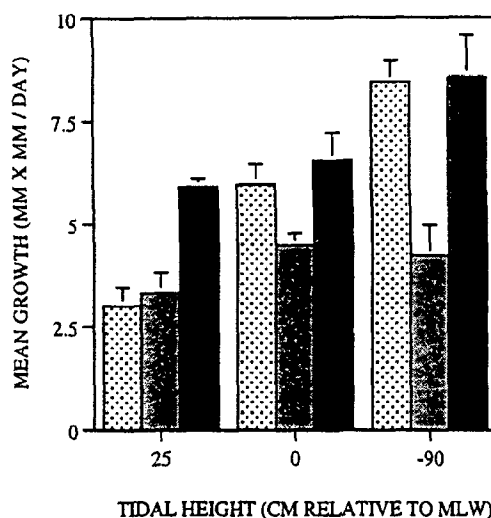
☐ June/July

▨ July/August

■ August/September
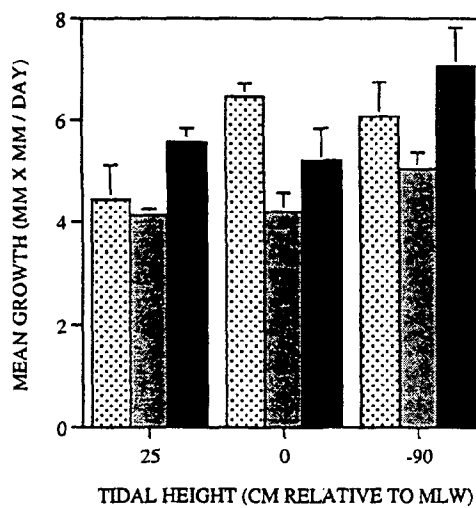
Figure 3. Mean growth rates examined by tidal height of the 1994 year class of
oysters residing below the reef surface. Error bars denote +1 S.E.
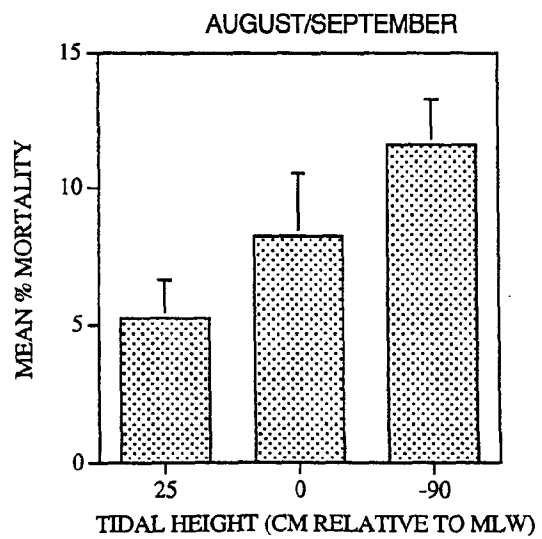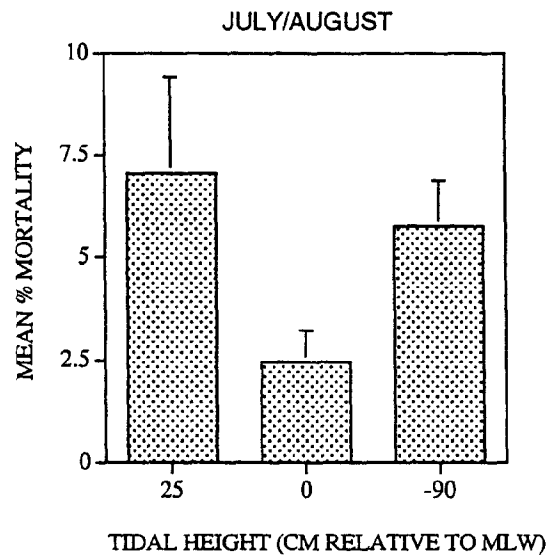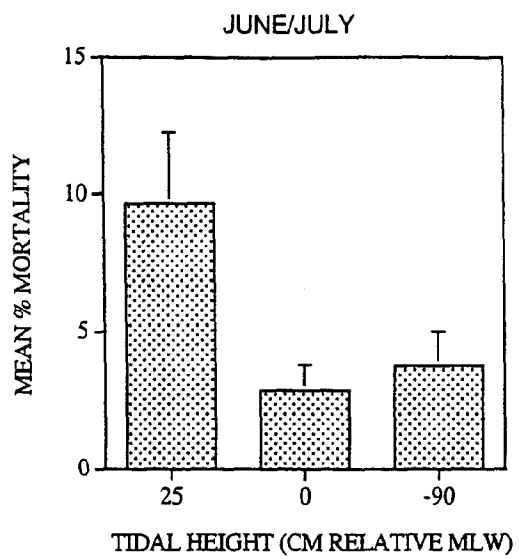
1994 DEEP OYSTERS
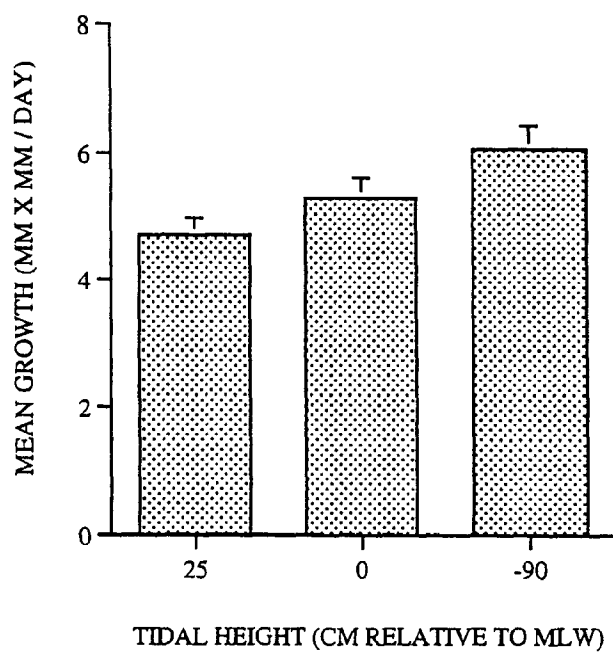
Figure 4. Mean cumulative percent mortalities of the 1993 year class of oysters
from June through September, 1994. Error bars denote +1 S.E.

MEAN CUMULATIVE % MORTALITY OVER 3 MONTH PERIOD

TIDAL HEIGHT (CM RELATIVE TO MLW)

SURFACE LEVEL

DEEP LEVEL
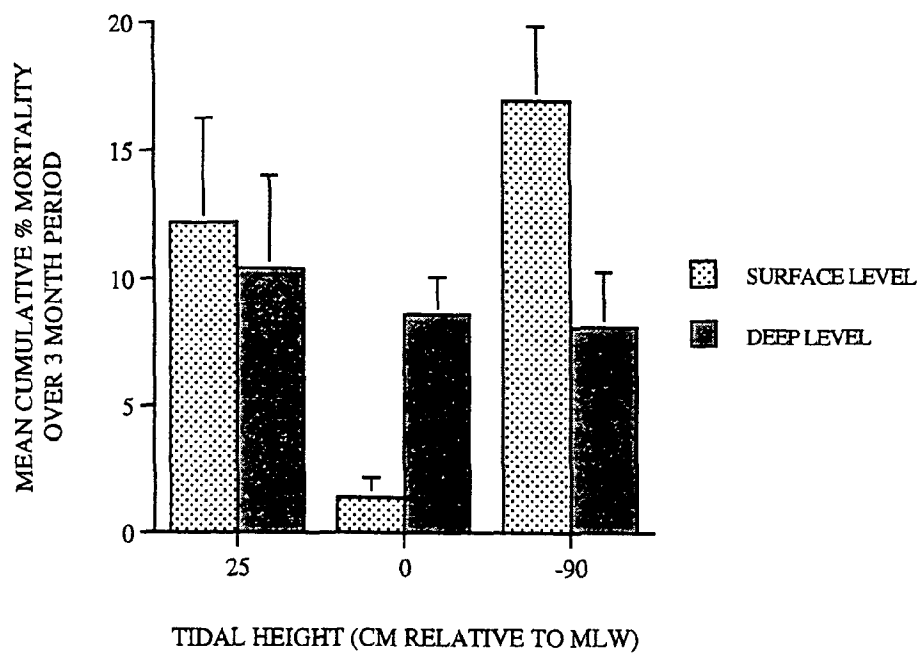
Figure 5. Mean monthly percent mortality for the the 1993 year class of oysters
        examined by sampling period and tidal height. Error bars denote +1 S.E.

A)

JUNE / JULY

B)

JULY / AUGUST

C)

AUGUST / SEPTEMBER
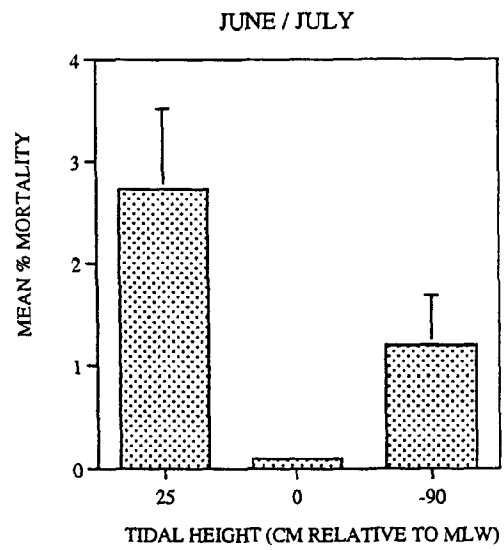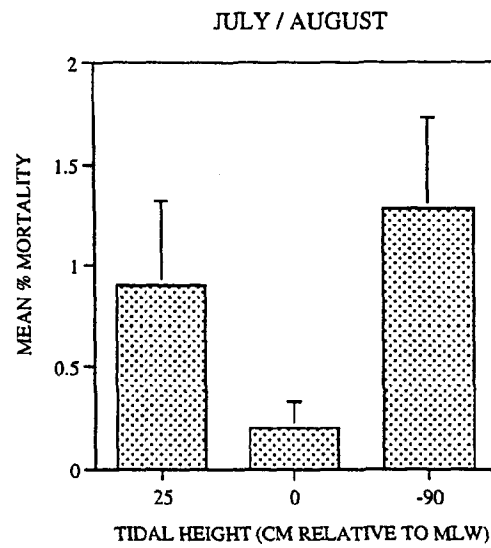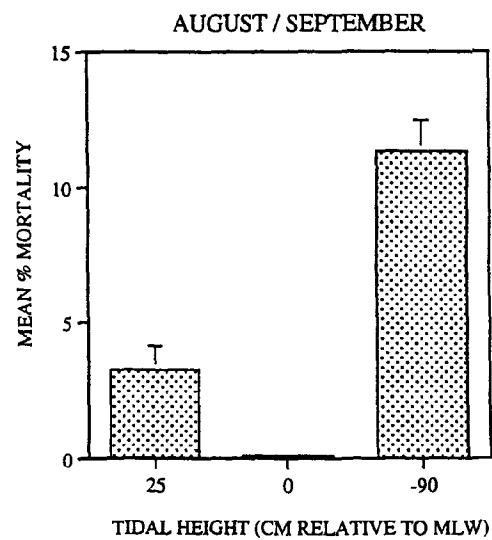
Figure 6. Mean percent mortality at the +25 cm tidal height during the June/July
sampling period for the 1993 year class oysters. Error bars denote +1 S.E.
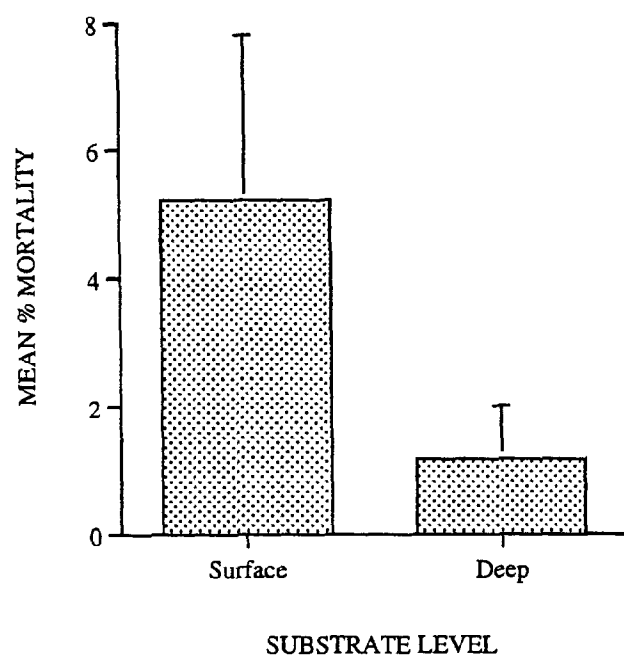
Figure 7. Mean percent mortality at the +25 cm tidal height during the June/July
sampling period for the 1994 year class oysters. Error bars denote +1 S.E.
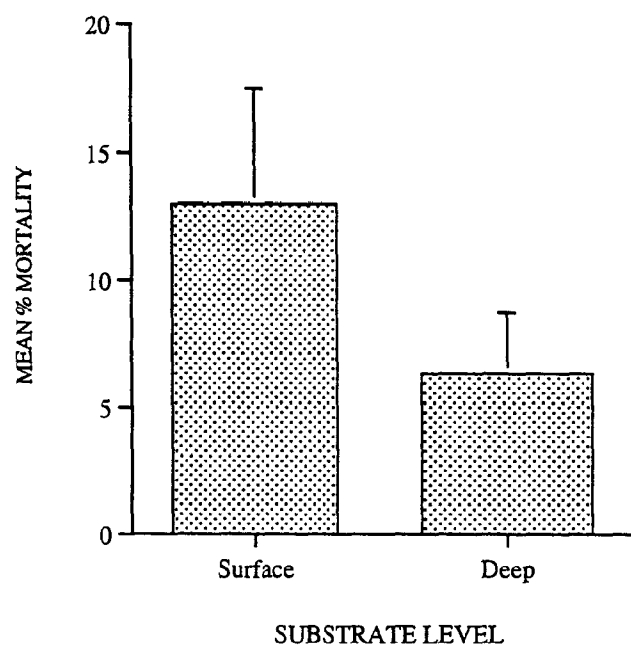
Figure 8. Mean percent mortality for the 1994 year class oysters examined by tidal height and sampling period. Error bars denote +1 S.E.

JUNE/JULY

MEAN % MORTALITY vs TIDAL HEIGHT (CM RELATIVE MLW)

JULY/AUGUST

MEAN % MORTALITY vs TIDAL HEIGHT (CM RELATIVE TO MLW)

AUGUST/SEPTEMBER

MEAN % MORTALITY vs TIDAL HEIGHT (CM RELATIVE TO MLW)

Figure 9. Mean cumulative percent mortality for the 1994 year class oysters from June
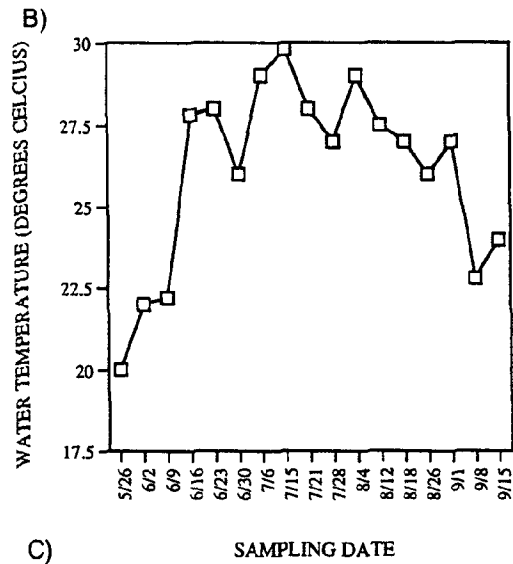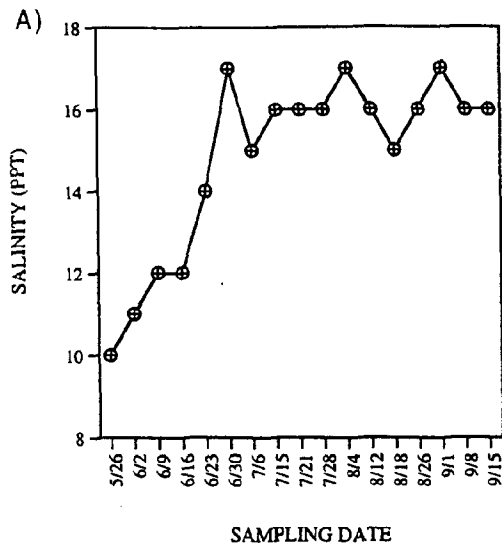through September, 1994. Error bars denote +1 S.E.

MEAN CUMULATIVE % MORTALITY

SURFACE

DEEP

TIDAL HEIGHT (CM RELATIVE TO MLW)
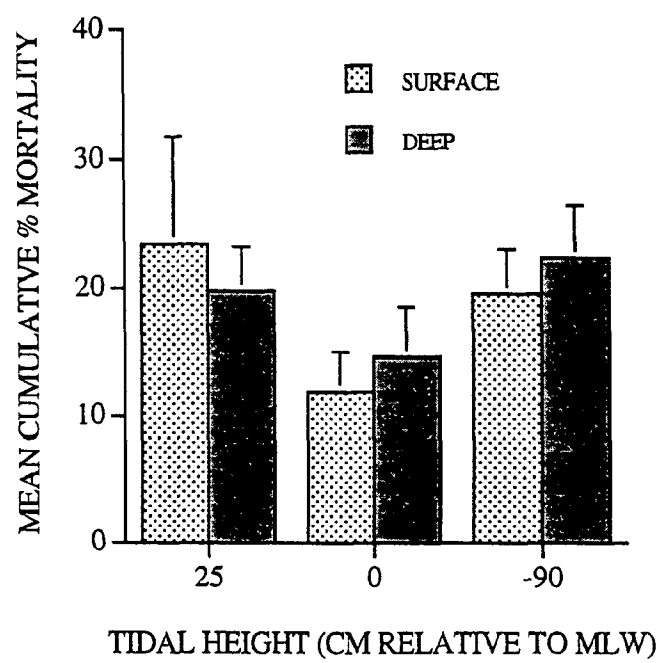
Figure 10. Salinity, water temperatures, and secchi disk readings recorded at the
Piankatank reef site during the 1994 summer.

A)

SALINITY (PPT) vs SAMPLING DATE

B)

WATER TEMPERATURE (DEGREES CELCIUS) vs SAMPLING DATE

C)

SECCHI DISK DEPTH (METERS BELOW SURFACE) vs SAMPLING DATE
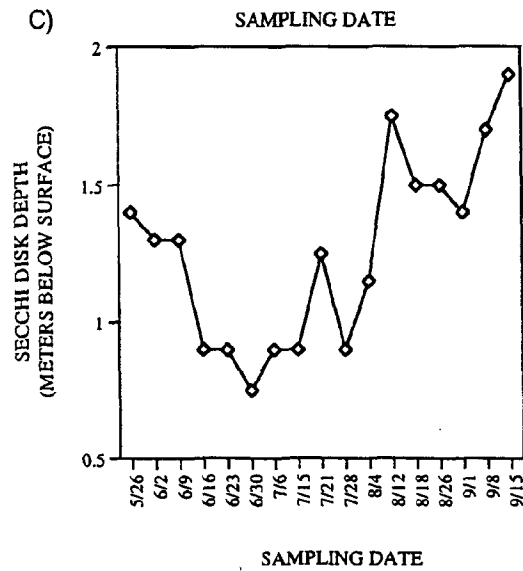
Temporal and Spatial Patterns of Growth and Mortality on a Constructed Intertidal Reef: Results from a Year-Long Study

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ABSTRACT

During pre-colonial times intertidal oyster reefs were unmistakable geological and biological features of the Chesapeake Bay, but today aerially exposed reefs are absent in the Bay largely because of commercial exploitation, disease, and environmental degradation. Reconstructing three-dimensional oyster reef habitats is one frequently proposed but critically unevaluated approach for rejuvenating ailing oyster populations. In this study, a sizable intertidal reef was constructed in the Piankatank River, Virginia, and growth and mortality of two year classes of hatchery-reared *C. virginica* placed at three tidal elevations (25 cm above MLW, MLW, and 90 cm below MLW) were monitored for a period of a year. To develop an overview of mortality of naturally-set oysters at the reef site, three annual surveys of oyster density at the three tidal elevations were performed. Furthermore, we present results from a short mortality study conducted over an unusually harsh winter, in which oysters were placed in bags on the reef at various tidal elevations. The results indicated that there is considerable spatial and temporal variation in growth and mortality at the reef, but that when these variations are summed over a year's time, growth is greatest subtidally and mortality is lowest at MLW, provided weather conditions are not unusually harsh. More importantly, however, density measurements collected annually and mortality measurements collected over a year's time suggest that survivorship is still low in reef settings regardless of tidal elevation. Reefs, especially those constructed of porous substrate and having some vertical dimensionality, still may be advantageous for oyster survival because mortality is still likely lower in reefs than in non-reef settings, but based on data collected here, it will likely take time before we see a dramatic rebound in oyster stocks as a result of reef construction.

INTRODUCTION

Intertidal oyster reefs, structures aerially exposed during low tide and composed of dense assemblages of live oysters, oyster shell, various invertebrate fauna, and mud, were unmistakable geological and biological features of the pre-colonial Chesapeake Bay. These complex habitats, which proliferated in the Chesapeake Bay and tributaries during the last half of the Holocene interglacial, were important self-renewing food sources for early settlers and Native Americans alike (Hargis and Haven 1995). As the economic value of the oyster *Crassostrea virginica* began to be realized in the mid 1800s, however, commercial exploitation of the resource began. Years of subsequent overharvesting has transformed these once massive, aerially exposed communities to mere subtidal, "footprint" structures which have significantly less vertical dimensionality and habitat heterogeneity. Disease, environmental degradation, and poor resource management in the last half century have expedited this degeneration.

Today, Virginia's oyster population is less than 1% of what it was just 35 years ago (Wesson et al. 1995) and is in severe jeopardy of collapse. One frequently proposed, but critically unevaluated approach for increasing oyster stocks, is the construction of intertidal oyster reefs, environments oysters resided in naturally before man's intervention. The rationale here is simple; since oysters in the Chesapeake Bay lived in intertidal communities for centuries and were able to withstand significant environmental and biological stresses, there is likely an ecological and evolutionary advantage to intertidal, colonial reef existence in the Bay, and a return to it may help rejuvenate ailing oyster stocks.

As a result of the absence of intertidal oyster reefs in the Chespaeake Bay for over 100 years, we know little about intertidal oyster reef ecology in temperate estuaries. In this study, we examined *Crassostrea virginica* growth and mortality on a constructed intertidal reef in the Chesapeake Bay to assess both the oyster's capacity to withstand environmental and biological stresses and its ability to develop in a temperate estuary, where presently intertidal reefs are largely absent. Specifically, we measured growth and mortality of two year classes of oysters placed at three tidal elevations (25 cm above MLW, MLW, and 90 cm below MLW) on the constructed reef for a period of a year.

STUDY SITE

This study was conducted in the Piankatank River, a sub-estuary of the Chesapeake Bay located in Virginia, at a site which once supported a highly productive intertidal reef system but at the time of reef construction was devoid of live oysters. The Piankatank River is ideal for artificial reef construction because generally there is a high abundance of oyster larvae (Morales and Mann 1995), and there is no commercial oyster fishery and virtually no industry or agricultural development. Tidal range at this site is small (mean range = 36 cm). However, local meteorological events, wind in particular, often dramatically alter this range from 0 to 1.25 m. The site is relatively shallow (1-3 meters), and consists of a sandy bottom. During the course of this study water temperature at the study site varied from 0.5 - 30 °C and salinity fluctuated from 8-20 ppt.

MATERIALS AND METHODS

The reef was constructed in June 1993 by the Virginia Marine Resource Commission (VMRC). The construction procedure involved the deployment of aged oyster shells off barges using a high pressure water cannon. The shells were broadcast over an area approximately 150 m x 30 m, which were the approximate footprint dimensions of the pre-existing reef system. When this study was initiated, the constructed reef consisted of 12 intertidal hummocks. Only eight of these hummocks were considered for this study, however, because the remaining four did not protrude sufficiently above the water surface to allow for the consideration of heights above mean low water (MLW).

Two year classes of oysters were considered, both of which were reared originally in the Virginia Institute of Marine Science (VIMS) Oyster Hatchery. One year class of oysters was set on oyster shell on May 16, 1994, whereas the other was set on oyster shell on August 12, 1993. The oysters belonging to each year class were placed in cages on the Piankatank reef after spending approximately 3 weeks in the Hatchery.

The '93 year class was used in an experiment to develop a preliminary understanding of winter mortality at the reef site. For this experiment a total of 100 cultch shells containing live oysters was placed in each of 12 Vexar mesh bags. On each of two mounds, which were vastly different in orientation, size, fouling, and flow conditions, a bag of oysters was placed at tidal heights of 30 cm above MLW, MLW, 45 cm below MLW, and 90 cm below MLW along two, spatially distinct transects. The bags were placed on the reef in November, 1993, and in May, 1994 the bags were retrieved, shaken vigorously, and opened. Twenty-five cultch shells were removed and photographed in sets of 5 using an Olympus OM camera and a focusing boom. Spat scars on each cultch shell were noted in the photographs and mean proportional mortalities (# of scars per shell / # scars per shell + live oysters) were calculated for each bag of oysters.

After this experiment the oysters were placed in cages and returned to the reef site, where they were joined by the newly reared '94 year class. On October 10, 1994, all the oysters were retrieved from the reef and brought back to the lab. At this time, oysters were removed from the cultch shells until only 1 oyster remained on each shell. Using a drill press, a hole, through which 1 of 5 color-coded labels was attached, was excised into 600 individual cultch shells (300 per year class). Shell heights were recorded, and five oysters,

each containing a different colored label, were placed into each of 120, numbered, 15 cm x 15 cm cages.

Instead of considering four tidal heights as was the case in the preliminary study, three tidal heights (25 cm above MLW, MLW, and 90 cm below MLW) were investigated for the remainder of the experiment. The high intertidal height was lowered slightly to accommodate all eight intertidal mounds in the sampling procedure, and one of the subtidal heights, 45 cm below MLW, was eliminated to incorporate more replication. At each of the eight mounds, all reef substrate falling within the 3 tidal ranges was partitioned into 64 x 20 cm sections using rope and reinforced bars.

For both the '93 and '94 year classes of oysters, four plots were selected randomly at each of the three tidal heights. Reinforced bars were driven into the reef substrate at each plot on October 18, 1994 and served to anchor 5, 15 x 15 cm cages, all containing color coded oysters from the same year class, to the reef surface. At 28 day intervals from October, 1994 through October, 1995, with the exception of the December through April when no measurements were taken, oyster shell heights were collected. Since each oyster could be defined by its color tag and cage number, individual oysters were tracked and we were able to calculate monthly growth rates and proportional mortalities (# oysters dead/ # oysters alive at the beginning of the 28-day interval). Mean growth rates and proportional mortalities were computed for each quintuple of cages for all sampling times.

In addition to mortality measurements collected during the '93 - '94 winter and growth and mortality measurements collected from October '94 - October '95, a yearly survey of oyster density on the reef was conducted. This survey began in September '93, shortly after the first settlement event was detected on the reef, and ended in September '95. This information served to compliment data collected during the first two studies and provided a generalized overview of naturally-set oyster survivorship on the reef. In early September of '93, '94, and '95, four 64 x 20 cm plots were selected randomly at each of the three tidal heights. A 64 x 20 cm quadrat was placed at each plot and a surface layer of 30 shells was extracted. Densities, expressed as the number of oysters present per 30 shell quadrat sample, were recorded, and mean densities per tidal height were calculated for each of the three years.

Multivariate repeated measures analyses of variance (ANOVA) were performed separately by year class on growth and mortality data. To satisfy the subject within group and subject between group homogeneity of variance assumptions, proportional mortality data were arcsine transformed. However, it was not necessary to manipulate growth data. When interactions were detected, lower-level ANOVAs and/or repeated measures analyses were performed. All significant between factor effects were analyzed using SNK multiple comparison tests and significant within factor main effects were examined using Newman-Keuls procedure (pp. 527-528, Winer 1991).

RESULTS

Significant time x tidal height interactions were detected in both the 1994 and 1993 year class oysters when growth was analyzed (2-factor ANOVA; 1993 oysters: $F = 5.92$, $df = 12, 48$, $p < .0001$; 1994 oysters: $F = 5.58$, $df = 12, 54$, $p < .0001$). Lower level analyses revealed that these interactions occurred because growth was not maximized at any one tidal height consistently throughout the experiment. For the 1993 year class oysters, growth was greatest at the -90 cm tidal height during the October-November and November-May periods, and during the August-September period growth was significantly greater at -90 cm than at +25 cm (Figure 1A). However, during the May-June period growth was maximized at MLW, and in the remaining periods (June - August and September - October) there was no difference in growth according to tidal height, primarily because of low growth at the -90 cm tidal height (Figure 1A). For the 1994 year class oysters, growth was greatest at -90 cm during the October-November, November-May, and September - October periods, and during the August-September period growth at both

MLW and -90 cm was greater than at +25 cm (Figure 1B). No significant difference in growth according to tidal height was detected in the May-June, June-July, or July-August periods again because of a significant drop in growth at the -90 cm tidal height.

Shell height measurements recorded at the end of 1 year in the field indicated that the largest shell heights were achieved subtidally. For the 1993 year class, shell height measurements were 55.3 ± 2.8 S.E. mm at the +25 cm tidal height, 58.1 ± 1.9 S.E. mm at the MLW tidal height, and 64.0 ± 1.6 S.E. mm at the -90 cm tidal height. For the 1994 year class, final shell height measurements were 44.7 ± 1.3 S.E. mm at the +25 cm tidal height , 50.5 ± 3.1 S.E. mm at the MLW tidal height, and 55.9 ± 3.0 S.E. mm at the -90 cm tidal height (Figure 2).

When mortality data were analyzed a significant time x tidal height interaction was detected as well for both year classes (2-factor ANOVA; 1993 oysters: $F = 9.58$, $df = 12$, 54, $p < .0001$; 1994 oysters: $F = 5.33$, $df = 12$, 54, $p < .0001$). This was also attributed to variations over time as to where along the tidal gradient survivorship was maximized. During the November - May period mortality of 1993 oysters was highest at +25 cm and lowest at -90 cm, during the May - June period mortality was greater at -90 cm than at MLW, during the July - August and August - September period mortality was lowest at +25 cm, and during the September - October period mortality was lowest at MLW (Figure 3A). For the 1994 year class oysters, mortality was greatest at +25 cm during the November - May period, greatest at -90 cm during the July - August period, and lowest at MLW during the August - September period (Figure 3B).

Cumulative percent mortality over the 1 year sampling period was lowest at MLW. Figure 4, which depicts conservative estimates of cumulative mortality, shows that for 1994 oysters, mortality rates were 76.1 ± 2.4 S.E. % at +25 cm, 61.3 ± 4.1 S.E. % at MLW, and 78.2 ± 9.3 S.E. % at -90 cm. For 1993 oysters, mortality rates were 55.0 ± 7.0 S.E. % at +25 cm, 51.4 ± 7.5 S.E. % at MLW, and 63.4 ± 4.8 S.E. % at -90 cm after 1 year's time. These values are conservative because we assumed that oysters lost throughout the study survived. If the assumption that missing oysters did not survive is made, these estimates would be 7 - 15% higher, but the trend of lowest mortality at MLW would remain.

During the initial mortality experiment conducted over the '93 - '94 winter, mortality rates at MLW and higher in the intertidal zone were 95 - 100%. Mortality rates during this same period were only on the order of 20 - 25% for oysters residing in the subtidal zone at depths of -45 cm and -90 cm. Substantially lower mortalities at all tidal heights were recorded during the '94 - '95 winter. The 1993 year class oysters had a mean mortality of 35.8 ± 7.1 S.E. % at +25 cm, 15.1 ± 4.4 S.E. % at MLW, and 1.0 ± 1.0 % at -90 cm during this period, and 1994 oysters had a mean mortality of 57.5 ± 5.7 S.E. % at +25 cm, 20.9 ± 6.4 S.E. % at MLW, and 4 ± 1.2 S.E. % at -90 cm.

Density measurements collected annually at the Piankatank reef are illustrated in Figure 5. We could not extrapolate mortality rates directly from the data because of yearly settlement events, but it is clear from the graph that considerable mortalities occurred within the intertidal zone from 1993 to 1994 and across all tidal heights from 1994 to 1995.

DISCUSSION

The results of this study suggest that there is substantial temporal and spatial variation in growth and mortality of oysters on constructed reefs, and when these variations are summed over a year or years the macroscale patterns observed in the field are generated. Over the '94 - '95 growth study, growth was not greatest subtidally at each sampling period, but significantly greater subtidal growth from August through May was substantial enough for the largest shell heights to be detected at -90 cm after a year's time. This occurred despite periods when significant growth differences were undetectable across tidal heights and periods of significantly greater intertidal growth from May - August. Faster subtidal than intertidal oyster growth has also been found in an earlier study conducted on the reef (see preceding manuscript) and by Loosanoff (1932), Ingle and

Dawson (1952), Burrell (1982), Roegner (1989), Sumner (1981), and Roland and Albrect (1986).

We suspect the low subtidal growth rates from May to August, when growth at other tidal heights matched or surpassed growth at -90 cm, was a product of the allocation of more energy towards gonadal tissue production and reproduction. Sexual maturity in *Crassostrea virginica* happens after at least 3 months of age, and peak spawning generally occurs from June through August, when water temperatures fall within optimal ranges for reproduction (Galtsoff 1964, Abbe 1986). In May, oysters belonging to the '93 and '94 year classes were 13 months and 22 months, respectively. Thus, they were old enough to undergo somatic/reproductive growth reallocation. Oysters in the intertidal zone may have delayed or shortened the period of high reproductive energy allocation so that they could put more energy into their own growth and survival. This may have been necessary, especially at +25 cm, to withstand the high physical stresses associated with periods of aerial exposure. Oysters situated at MLW, which encounter much less physical stress and experience longer submergence times than those at +25 cm, may have been able to take greater advantage of periods devoted predominantly to somatic growth than oysters residing higher in the intertidal zone. As a result, growth at MLW was high at either the beginning or end of the spawning period, even surpassing that observed at -90 cm.

Significantly lower rates of mortality at MLW during the August-September and/or September-August periods coupled with relatively low mortalities in the remaining months allowed oysters at MLW to experience the lowest mortality during the '94 - '95 study. High mortality rates at +25 cm from November through May, when air temperatures were low, and during the June-July, August-September, and September-October periods, when air temperatures were high, contributed to the high overall mortality rates at +25 cm. Similarly, high subtidal mortality rates from June through October, when mortality from predation and subtidal fouling were elevated, generated high overall mortality rates at -90 cm. An elevated incidence of disease for oysters residing at -90 cm relative to oysters situated higher in the intertidal zone may have contributed to high subtidal mortalities as well (see disease discussion).

The low mortality rates at MLW relative to those detected at +25 cm and -90 cm probably were a result of these oysters experiencing less predation, fouling, and possibly disease incidence than oysters in subtidal habitats and encountering less severe atmospheric conditions than oysters in higher intertidal regions. Oysters at MLW, therefore, experience the best of both worlds; they benefit from lowered predation pressure, fouling, and disease as a consequence of aerial exposure, but do not suffer from severe heat, cold, or respiratory stress because they are not exposed for extended periods of time. High survivorship at MLW was also detected in the study conducted over the summer of 1994 (see previous manuscript).

The high rates of mortality detected at +25 cm and at MLW over the '93 - '94 winter together with the substantial drop in oyster density in the intertidal zone from 1993 - 1994 suggest that survivorship may not always be maximized at MLW. These exorbitant mortality rates, especially at MLW, were likely atypical and a result of the coincidence of an unusually brutal winter and the presence of a young population of oysters (oysters were 4 months old at the onset of the winter). From December of '93 through March of '94, air temperatures dropped below freezing 28 days, which is very unusual for Virginia. Oysters less than 1 years old are especially vulnerable to freezing conditions because they put much of their energy into growth and maintenance rather than into the storage of glycogen, a preferred substrate for anaerobic respiration, and thus are less capable of environmental isolation (Mann and Gallager 1985, Widdows et al. 1989). The substantially lower mortalities detected during the '94 - '95 winter, especially at MLW, give weight to the argument that the '93 - '94 mortalities were indeed unusual.

Although the intertidal mortality rates over the '93 - '94 winter are atypical and survivorship at the reef during a normal year when older oysters are present in the reef

community is high at MLW, the high cumulative mortalities and low density figures recorded at all tidal heights are disturbing. Oyster which survive to 5 months of age and 14 months of age experience mortalites of at least 50% (based on conservative estimates) by the time they reach 17 and 26 months, respectively, during an average year in terms of weather even if they are situated at the most optimal tidal depth for survival (MLW). If you factor in unusually harsh weather and begin with younger oysters, which are more susceptible to predation and environmental stress, these mortalties will be even higher.

Mortality rates in reef settings are still likely to be lower than in non-reef settings, especially if the reefs have vertical dimensionality, but unfortunately, based on the results of this study these differences are not so substantial that they lead to dramatically higher oyster survivorship. Two years after the only significant settlement event on the reef, which occurred in August '93, oyster densities in the subtidal zone where settlement intensities were highest went from 45.1 oysters per 30 shells to 3.1 oysters per 30 shells. These densities are higher than densities on adjacent, 2-dimensional beds of shell, where < 0.5 oysters were present per 30 shells, but nonetheless are distressingly low. Based on the results presented in the previous manuscript, oyster densities may be increased somewhat by constructing the reef out of substrate porous enough to allow for sub-surface colonization. But even these efforts may not guarantee the establishment of immediate adult oyster communities because mortalities in the interstitial environment may be considerable as well. Presently, with the lack of more cost-effective alternatives, the construction of 3-dimensional oyster reefs which provide spatial complexity and enhance survivorship may be the best alternative for rejuvenating oyster stocks; the results of this study suggest, however, that a dramatic rebound in oyster stocks as a result of reef construction may take many years.

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Figure 1. Mean shell growth from October '94 through October '95 for A) oysters reared in 1993 and B) oysters reared in 1994. Error bars denote +1 S.E.
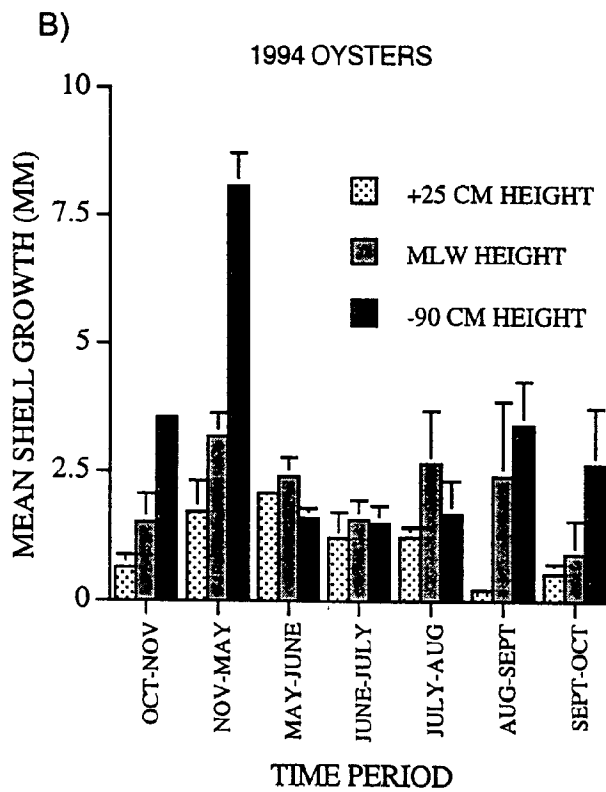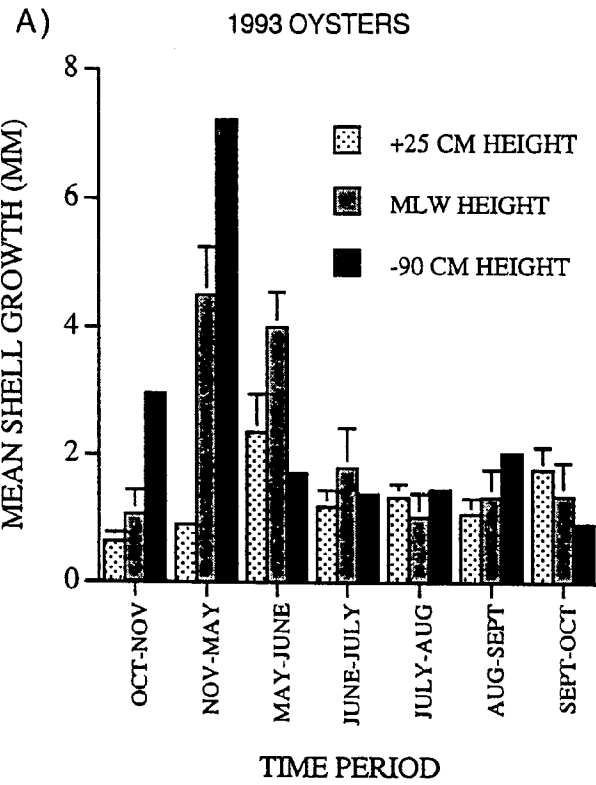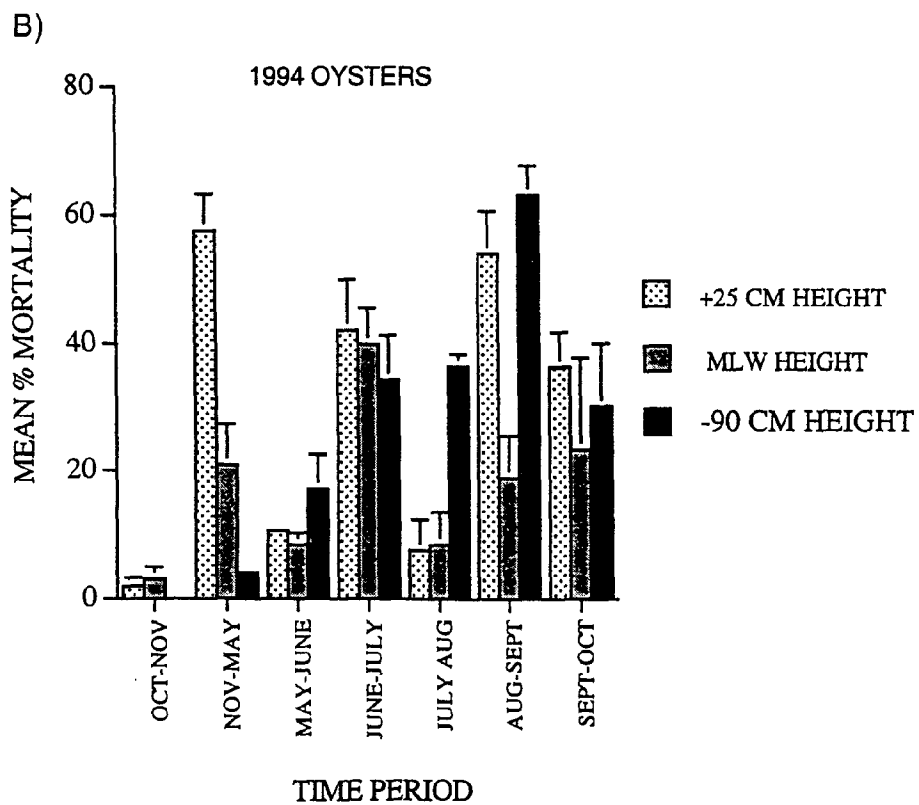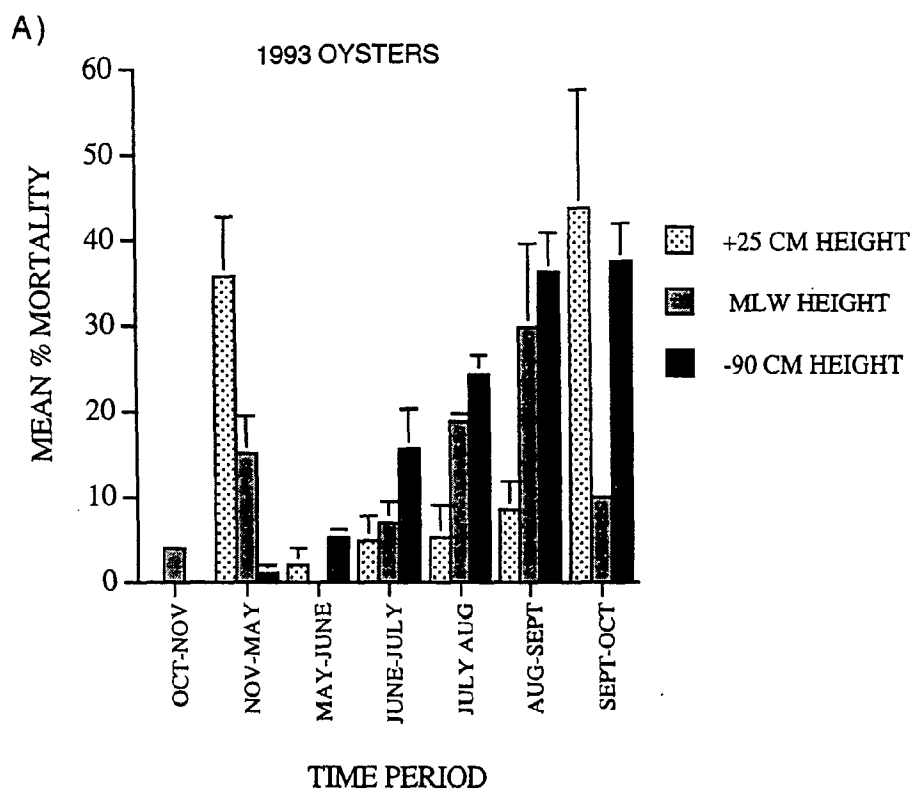
A) 1993 OYSTERS

MEAN SHELL GROWTH (MM)

+25 CM HEIGHT
MLW HEIGHT
-90 CM HEIGHT

TIME PERIOD

OCT-NOV
NOV-MAY
MAY-JUNE
JUNE-JULY
JULY-AUG
AUG-SEPT
SEPT-OCT

B) 1994 OYSTERS

MEAN SHELL GROWTH (MM)

+25 CM HEIGHT
MLW HEIGHT
-90 CM HEIGHT

TIME PERIOD

OCT-NOV
NOV-MAY
MAY-JUNE
JUNE-JULY
JULY-AUG
AUG-SEPT
SEPT-OCT

Figure 2. Final shell heights of the 1993 and 1994 year class oysters after being placed on
the reef at 3 different tidal elevations for a year. Error bars denote ± 1 S.E.
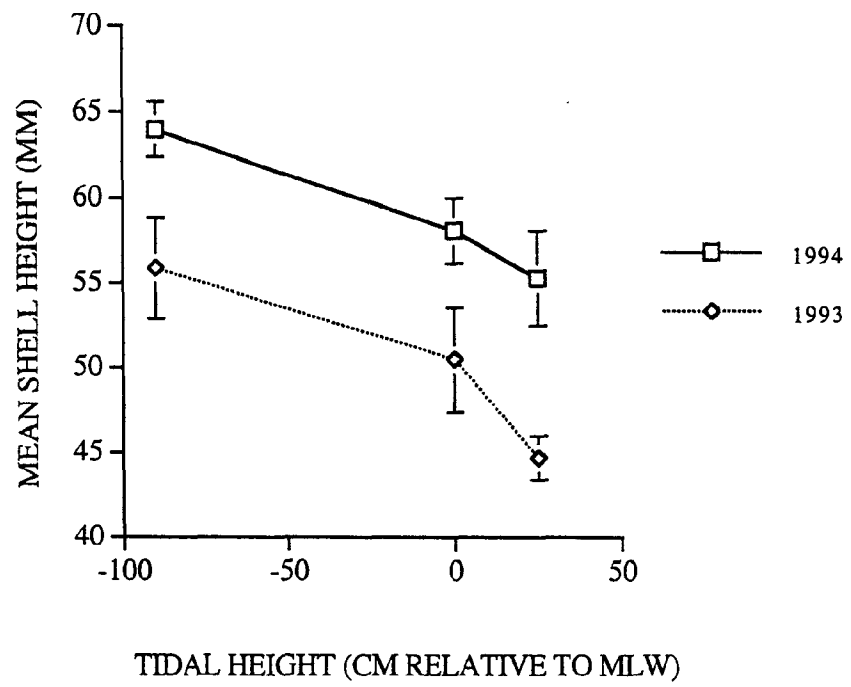
Figure 3. Mean percent mortalities from October '94 through October '95 for A) oysters reared in 1993 and B) oysters reared in 1994. Error bars denote +1 S.E.

A)

1993 OYSTERS

MEAN % MORTALITY

Legend:
- +25 CM HEIGHT
- MLW HEIGHT
- -90 CM HEIGHT

Time periods: OCT-NOV, NOV-MAY, MAY-JUNE, JUNE-JULY, JULY AUG, AUG-SEPT, SEPT-OCT

TIME PERIOD

B)

1994 OYSTERS

MEAN % MORTALITY

Legend:
- +25 CM HEIGHT
- MLW HEIGHT
- -90 CM HEIGHT

Time periods: OCT-NOV, NOV-MAY, MAY-JUNE, JUNE-JULY, JULY AUG, AUG-SEPT, SEPT-OCT

TIME PERIOD

Figure 4. Cumulative percent mortality of the 1993 and 1994 year class oysters after being
placed on the reef at three tidal elevations for a year. Error bars denote ± 1 S.E.
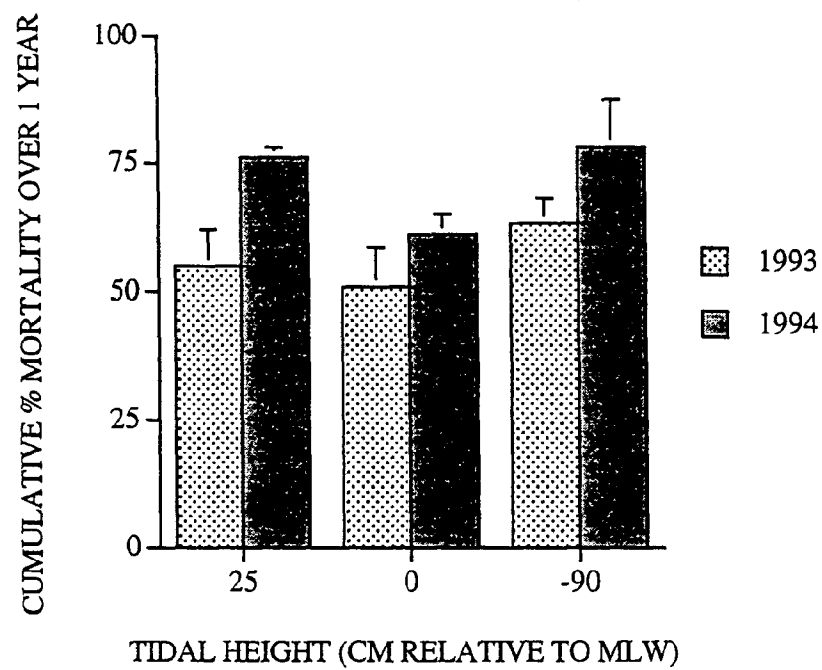
Figure 5. Oyster densities taken in September, 1993, 1994, and 1995. Error bars denote
+1 S.E.
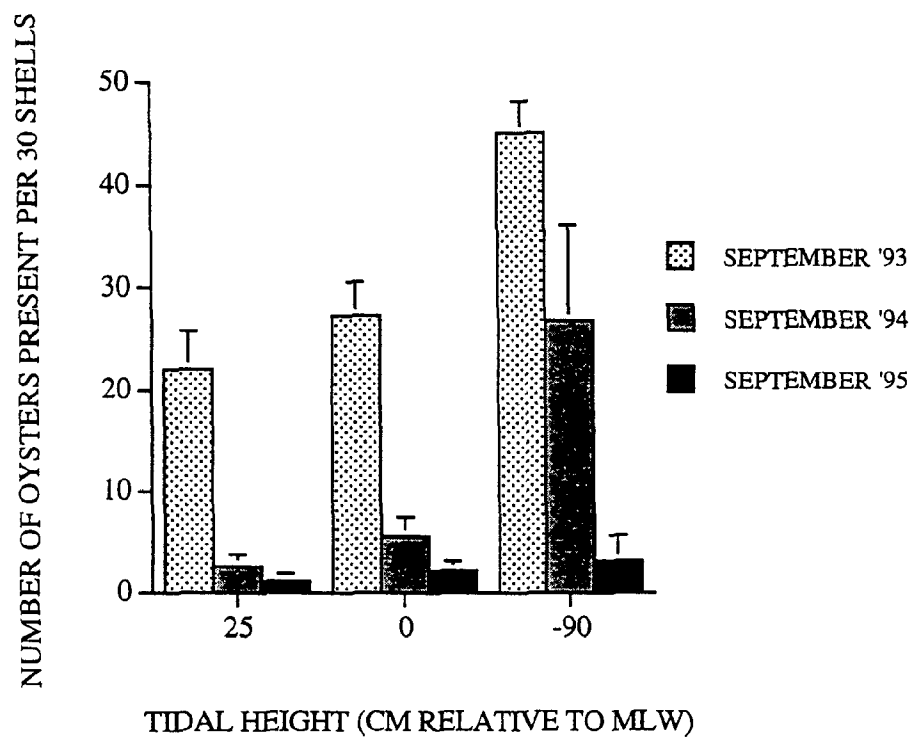
Progression of diseases caused by the oyster parasites, *Perkinsus marinus* and *Haplosporidium nelsoni*, in *Crassostrea virginica* on Constructed Intertidal Reefs.

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INTRODUCTION

From May 5, 1994 to December 14, 1995 the progression of diseases caused by the oyster parasites, *Perkinsus marinus* and *Haplosporidium nelsoni*, were evaluated by periodic sampling of oysters which set in August, 1993 on the artificial reef located in the Piankatank River. It had been previously established (Mackin, 1962; Haskin and Andrews, 1988) that oysters most often do not become infected by either parasite in the first 9 to 12 months of life; therefore, no sampling was conducted until 10 months after setting. This proved to be a reasonable assumption for the present study since the first *P. marinus* infections were not detected until 14 months after setting and the first *H. nelsoni* infections at plus 15 months (with the exception of one oyster at plus 13 months). The infections observed were recorded as a function of 1) prevalence (incidence) and intensity (weighted incidence), 2) host mortalities, 3) oyster size and age and 4) depth below mean low water at which the host oyster was found on the reef. The total number of reef oysters sampled was 3,908.

The study was conducted to determine 1) whether depth below mean low water at which the oysters resided was significant in determining to what extent the oysters became infected, 2) at what size and age the oysters became infected to levels which resulted in significant infections and mortalities, and 3) whether oysters which set in an endemic area (the Piankatank River) on an artificial reef have lower mortalities than susceptible oysters from an area in which the parasites are rarely observed (the upper James River). The latter comparison is only valid when similar age oysters are compared; therefore, the comparisons are of interest only at the end of the study. The first observations/conclusions should be of interest to individuals responsible for constructing artificial reefs and the second observations are of interest to individuals who must determine when to harvest oysters to avoid excessive losses.

METHODS

Oysters which had set on the reef were sampled every 2 to 4 weeks during the study period of May 5, 1994 to December 14, 1995. They were obtained by hand or by using oyster hand tongs, depending on the depth. Six samples of 25 oysters per sample were obtained for each sample time at two locations on the reef. The depths in the first 15 sample times were intertidal and 45 and 90 cm below mean low water. Due to the ever increasing difficulty in finding intertidal oysters and the recognition that samples from the bottom of the reef should be obtained, the intertidal sampling was discontinued and sampling was initiated from near the bottom of the reef at 167 cm for the 16th sample (February 10, 1995) until the end of the study. With respect to depth the data is analyzed in terms of ≤45 cm and ≥90 cm. The paucity of intertidal oysters is believed to have been due to deaths which occur as a result of exposure to freezing temperatures during the winter months. The base of the reef was located in 2-3 meters below mean low water.

The observations are expressed in terms of number of weeks after setting. Most of the set in 1993 occurred from August 5 to 12. To facilitate the handling of the data herein, August 12 was selected as the date of set. Whereas another primary set occurred during the study period in 1994, the set which was followed through the course of the study was the 1993 set.

As a means of comparing the progression of infections in the reef-set oysters with the progression through a population of susceptible adult oysters, 350 uninfected, susceptible adult oysters were obtained from the upper James River seed beds (Horsehead rock) and placed in plastic mesh bags on the Piankatank River reef near the sample sites at the time of the third sampling of the reef oysters (June 16, 1994). The depth of placement was about midway between the top and bottom of the reef (ca. 100 cm below mean low water). At the time of placement a sample of 25 oysters was analyzed for the presence of the two parasites, using techniques described below. No *P. marinus* or *H. nelsoni* cells were found. There was a possibility that the sampling period would occur when one or both parasites were in low numbers in the area of the reef. Therefore, in order to confirm that the parasite detection methodology was being applied properly and to check for patchiness in distribution of the parasites,, 350 James River oysters from the same population used on the Piankatank River reef were placed in plastic mesh bags in the York River behind VIMS, an area in which both diseases are known to be commonly present in high levels. The treatment of the 350 oysters held at VIMS was the same as described for the other 350 oysters. Each batch of 350 oysters was sampled until none remained. In the following spring (April 14, 1995) another batch of 350 oysters from the same James River site was placed on the reef and 350 at VIMS as in the previous year and sampled until none remained.

Accumulative mortality data was obtained by counting the number of "boxes" (shells without tissue) encountered in the course of selecting 150 oysters at the time of each sampling of the August, 1993 set. Unfortunately, because of a lack of communication, mortality data was not collected for the final 19 weeks of the study. In retrospect it may have been better to have also selected about 500 oysters on May 5, 1994 at the beginning of the study, placed them in trays or bags and sampled them for mortalities during the course of the study. However, this approach would have presented the difficulty that the oysters would have been crowded so that transmission of *P. marinus* infections would have occurred more readily and the mortality would have been higher than for oysters dispersed over the reef, yielding misleading results.

Oysters were assayed for the presence of *P. marinus* using the Ray fluid thioglycollate medium technique (Ray, 1954) in which samples of gill and digestive gland were incubated in the medium. Perkins (unpublished data) has determined that use of those organs reveals the presence of the parasite in very light or light infections more frequently than when mantle or rectal samples are used. The intensity of infections was recorded using a modification of the Mackin scale (Mackin, 1962) in which 0= no infection, 1=very light, 2=light, 3=light-moderate, 4=moderate, 5=moderate-heavy, and 6=heavy. This differs from the Mackin scale in that very light is assigned a value of 1 instead of 0.5, thus the highest 5 values used herein are one unit more than on the Mackin scale.

H. nelsoni was detected by using histological, paraffin-embedded sections stained in hematoxylin and eosin. The scale of Burreson et al. (1988) was employed in recording intensities of infections where 0= no infection, 1= cells were rare, 2= less than two cells were seen per field of view using a 40X objective, 3= 2-5 cells per field of view and 4= more than 5 cells per field of view.

The effects of depth and sampling time (age) of oyster on disease susceptibility (prevalence and intensity) to *P. marinus* and *H. nelsoni* were assessed using logistic regression analysis (Agresti, 1990).

RESULTS

The observations for *Perkinsus marinus* prevalence in oysters which had set on the Piankatank reef are summarized in Figure 1 for depths of ≤45 cm and ≥90 cm below mean low water. Weighted prevalences are summarized for the same depths in Fig. 2. Infections did not start to appear until 14 weeks into the study when the oysters were one year old. Conversion of sampling elapsed times to ages of oysters and times of year are found in Table 1. For the next 44 weeks until the oysters became a year and 10 months old the number of infected oysters ranged mostly between 15 and 35% after which the prevalences rose rapidly in the ensuing 2 months to 100% or nearly 100% where they remained until the end of the study when the oysters were almost 2 years and 5 months old. The intensities of infections during the plateau phase remained mostly below very light until the end of the plateau (plus one year, 10 months old) then rose rapidly to the levels of moderate to moderate-heavy at the age of 2 years plus 1.5 months old followed by a decline to between light and light-moderate at the end of the study.

The prevalence of *H. nelsoni* markedly different from that of *P. marinus* (Fig. 3). With the exception of one lightly infected, 50 week old oyster, the onset of *H. nelsoni* infections did not occur until the oysters were one year, 6.5 months old as opposed to the appearance of *P. marinus* in one year old oysters. Thereafter, the infection prevalences of *H. nelsoni* rose rapidly to a maximum of 45% when the oysters were 11 months old. The infections then declined precipitously to almost 0% when the oysters were 2 years, 1.5 months old followed by a slight rise which remained below 10% for the final 4 months of the study. The intensities of infections reached a peak at the age of one year, 11 months, one month before the prevalence peak was reached and declined to almost as rapidly as did the prevalences (Fig. 4).

A comparison was made between the oysters obtained at ≤45 cm and ≥90 cm below mean low water using logistic regression to analyze the differences in *P. marinus* and *H. nelsoni* infection prevalences and intensities. It was observed that *P. marinus* prevalence was significantly higher (p < 0.0001) in oysters collected from depths ≥90 cm compared to those from ≤45 cm. Prevalence significantly increased (p < 0.0001) in oysters from all depths with age of oysters, indicating that continued exposure to *P. marinus* or increasing age of oysters results in increased infection. Similar results were observed when *P. marinus* infection was expressed as weighted incidence or intensity. *P. marinus* infection intensity was significantly higher at the greater depths (p < 0.01) and significantly increased in oysters from all depths with age (p < 0.0001).

Likewise, oysters collected from ≥90 cm had a significantly higher prevalence and intensity of *H. nelsoni* infections compared to those from ≤45 cm (p < 0.0001). In addition, *H. nelsoni* prevalence and intensity increased with increasing oyster age (p < 0.0001).

From the cumulative mortality data one can see that the mortalities began at the age of one year 2 months old, 2 months after *P. marinus* infections first appeared and 6.5 months after *H. nelsoni* appeared (with the exception of one infected oyster noted above). The peak mortality value of 40% observed when the oysters were 2 years old corresponds to time at which the oysters became 100% infected with *P. marinus* and 3 weeks after the peak prevalence value for *H. nelsoni* (Fig. 5). Unfortunately mortality data is not available for the last 19 weeks of the study. After the appearance of *H. nelsoni*, it is not possible to distinguish between mortalities caused by the two pathogens, nor is it possible to distinguish those causes from other sources of mortality. This would have been possible only if approximately daily samples had been obtained in which gapers were selected for

analysis, an impossible task using the resources which were available. Less frequent sampling would have yielded shells without oyster tissues due to the predation which occurs by crabs and fish once the oysters are not able to close their valves.

Examination of the oyster size data shows that 4 weeks before the appearance of first *P. marinus* infections (10 weeks into the study vs. 14 weeks) there was a decrease in the rate of growth (Fig. 6). This resembles the findings of Paynter and Burreson (1991) where they observed a decrease in the growth rate of juvenile and adult oysters immediately after or just before infection. Since *H. nelsoni* did not appear until 7.5 months after the change in growth rate, that pathogen was not responsible. Whether the high summer temperatures (Fig. 7) or some other factor such as the oysters' food source was responsible can not be determined. The salinity was relatively constant (Fig. 7); therefore it is unlikely that it was responsible. The decline in oyster sizes after the age of 2 years (65 weeks into the study) is believed to be due to death of the larger oysters resulting from infections of the two pathogens.

The data for the adult oysters which were imported from the upper James River seed beds and which represent a disease-susceptible population is used to confirm that the two pathogens were present in the study area and in the neighboring area of the lower York River (Figs. 8-11). They were used primarily to indicate presence or absence of *H. nelsoni* since its levels fluctuate greatly, some years being nearly absent from the lower York River region. During the study period, one can see that the pathogens were prevalent and the patterns of disease organism expression was what one would expect based on the studies of Andrews (1988) and Haskin and Andrews (1988). The discontinuities in the curves seen in Figs. 8 to 11 are a result of depletion of the first stock of 350 oysters from which samples were obtained followed by replenishment with a new stock of 350 oysters.

It is interesting to note that *P. marinus* infection prevalence was the same in oysters held in the York River and at the Piankatank River reef during 1994 but in 1995 was expressed earlier and reached 100% 15 weeks before those held in the York River. One would have expected that the oysters held in the York River would have shown a stronger prevalence where the salinities ranged about 5 ppt above those in the Piankatank River and thus would have presented more favorable salinities for expression of *P. marinus*. On the other hand, in 1994 *H. nelsoni* infections were nearly non-existent in the Piankatank River reef oysters during 1994, whereas in the York River stock infections were above a prevalence of 60% during the summer and fall of 1994 (Fig. 10). These observations reaffirm the necessity of having a stock of susceptible, adult oysters present in a study of this type where juvenile and young oysters are being observed. Age of the oysters will play a role determining the prevalence and intensities of infections. In addition, the patchiness of distribution of *H. nelsoni* within the Chesapeake Bay is confirmed by the data.

In 1995 a different picture of *H. nelsoni* infection distributions in the imported, adult oysters was observed (Figs. 10 and 11). The prevalences and intensities were quite similar at the two stations with the infections appearing earlier at the York River station and lasting longer in the population. Nine more weeks of data was obtained from the York River stock because the stock at the reef was depleted by mortalities earlier, probably due to *P. marinus* infections (Figs. 8 and 9).

The reef oysters at the age of ≥2 years old can reasonably be compared to the imported oysters in terms of response to the diseases. The former is a population which set in an endemic area and thus had a chance to acquire some immunity to the two diseases, if capable of such a response. The latter was a stock of 2-4 year old (ages not known, only

estimated) susceptible oysters from an area with little or no prior exposure to the two pathogens. With respect to *P. marinus* the prevalences and intensities of infections were similar in the two groups of oysters at the reef. Therefore, one can not state that the reef-set oysters were more resistant to the pathogen. On the other hand, the *H. nelsoni* data indicates that the reef-set oysters were more resistant to those infections. Imported oysters at the reef reached a peak of 68 % infections (Fig. 10), whereas the reef oysters peaked at only 36 and 45%, depending on the depth of residence (Fig. 3). Likewise, the intensities of *H. nelsoni* infections reached a mean high of level 2 in imported oysters as opposed to 1.3 in the reef oysters. Therefore, some advantage appears to have been obtained for the reef-set oysters, if one can neglect age differences.

DISCUSSION

The interpretation of epizootiological data such as that generated in this study is difficult because of the plethora of factors which dictate to the prevalence and intensities of infections and mortalities. Most, but probably not all, of the factors are: temperature, salinity, water quality in terms of anthropogenically-derived chemicals present, density of oyster populations, residence depth in the water column, patterns of water movement, oyster age and/or size, genetic strains of oysters involved, physiological condition of the oysters as dictated by food availability (density and species composition of planktonic food organisms present) and numbers and levels of other parasitic species causing stress on the oysters. A further complication is the fact that the reservoir of *H. nelsoni* infective cells is unknown and transmission of infections is not from oyster-to-oyster as opposed to *P. marinus* where transmission is direct.

Despite these formidable complicating factors, one is able to detect patterns of oyster mortalities and oyster growth changes which can be related to the levels of *P. marinus* and *H. nelsoni* found in the oysters. The present data set is unique in that it is the first time a population of naturally set oysters of known age has been assayed *in situ* in terms of the progression of infections by *P. marinus* and *H. nelsoni* over an extended period of time, in this case for one year and 8 months. Other epizootiological studies have involved placing naturally set or hatchery set oysters of known age in containers in an endemic area or placing adult oysters of unknown ages from non-endemic or marginally endemic areas into containers in an endemic area. Placement in containers provides a greater degree of experimental control but closer proximity of oysters can lead to results different from those in naturally set populations where distances from oyster-to-oyster varies greatly.

Since infections did not appear until 3 months into the study (3 months after May 5, 1994), one can assume that the disease progressions were essentially followed from setting (August 12, 1993) until the oysters were 2 years and 4.5 months old (December 14, 1995). Considering the fact that the salinity values recorded during the first 11.5 months after setting did not go below 10 ppt. and most of the time were ≥16 ppt, it is reasonable to assume that infections did not occur prior to May 5, 1994 when sampling began. This assumption is based on results of other studies in which is was observed that when a population does become infected the infections do not disappear unless the salinity drops below 10 ppt. for an extended period of time.

The following factors are mostly considered independently and related to the existing knowledge generated from 44 years of studying the epizootiology of *P. marinus* and 37 years for *H. nelsoni* in the Chesapeake Bay. Conclusions are then presented considering all factors measured. In the following discussion, reference is made to age of

the oysters or elapsed time in the study, whichever is appropriate. The reader is referred to Table 1 for conversion of age to elapsed time to time of year.

Temperature and Time of Year-

The prevalence and intensities of infections of *P. marinus* followed generally the patterns observed in earlier studies (Andrews, 1957; 1988), i.e., a rise in the spring, peaking in October and November and declining in the winter months into spring. The temperatures had risen to the range of 25-30°C by the time at which *P. marinus* appeared in the reef oyster population (Table 1; Figs. 1 and 7). Had the oysters been placed on or pre-existed on the reef from August 12, 1993 as juveniles or adults, one would expect infections to appear earlier when the temperatures had exceeded ca. 15°C, depending on the temperatures to which the oysters had been exposed in the previous 12 months. The decline in prevalences in the colder months was gradual with unexpected and unexplained increases in January and February (2-7°C) following by minimum values being expressed in March to May when temperatures were rising from 8 to 19°C. The lag in loss of *P. marinus* infections during December to February is consistent with observations from the earlier studies cited. Intensities of infections were more nearly reflective of previous reports in that the peaks for the two depths were reached in October and November and reached a minimum in May of the following year.

As one would expect from the literature, late in the second year of life and early in the third year after temperatures had exceeded 20°C (in June), infection prevalences and intensities rose rapidly. The peak in prevalence (ca. 100%) was reached in July-August when the temperatures were 25 to 30°C and held at about 100% until the temperatures were below 15°C and the peak in intensities (>5) was reached in September when the temperature was 23°C and had declined to 2.6 by the end of the study. This is an earlier than expected time to reach the maximum prevalence value; however, the slight decline in December is when would expect the decline in prevalences to commence.

The data for *H. nelsoni* was somewhat surprising in that only one oyster was found to be infected in the first year of life (Fig. 3) and the population did not otherwise begin to show infections until the oysters were over 1.5 years old. It possible that this lag can be attributed to 1) the oysters being young and thus less susceptible as has been reported from other studies, and 2) the fact that even the susceptible, imported adult oysters did not acquire very many infections at the Piankatank River reef (Fig. 10) in the first year of reef oyster life. It was clear that *H. nelsoni* was present in strength in the nearby York River (Fig. 10), but not in the reef area, thus illustrating the patchiness in distribution of the reservoir of infective cells, at least in that part of the Chesapeake Bay. The decline in prevalence and intensity of *H. nelsoni* in reef oysters was more precipitous that has been previously reported using imported susceptible adult oysters (Haskin and Andrews, 1988), reaching a very low level by August rather than about December; however, the start of the decline was the same as previously reported.

Salinity-

Consideration of the salinity data vs. prevalences and incidences illustrates the point made in the first paragraph of this discussion concerning the complexities involved in determining which factors are responsible for limiting or encouraging expression of the disease organisms. In the first three sample periods the salinities were unfavorable for expression of the disease organisms in that it was below 15 ppt., but the lack of expression observed was probably mainly due to the young age of the oysters. Thereafter, the salinities were mostly above 15 ppt. and were highly favorable for the pathogens when the values approximated 20 ppt. and in fact that was the time period when the prevalences rose markedly. The question which can not be answered is whether the primary factor in

encouraging or permitting a rapid increase in prevalences was temperature, oyster age or salinity. Most probably the best answer is that all three played synergistic roles.

Age-
This factor has been mentioned above. Other studies have noted that oysters are refractory to acquiring infections in the first year of life and become increasingly more susceptible into the second year with significant prevalences, incidences and mortalities being observed then (Andrews, 1957). In fact that pattern was observed in the present study (Figs. 1-5). As mentioned above, the complicating factor was the low level of infection pressure from *H. nelsoni* in the first year of life at the reef.

Ray (1954) followed the progression of *P. marinus* infections through a population of young Louisiana oysters of known age and found that infections occurred as early as 9-10 weeks old but did not start a progression of ever increasing levels of infection until they were 3 months old. At the end of 12 months the infections reached a level of 37% prevalence, much lower than the susceptible, adult oysters assayed (90%). The per cent mortality and weighed incidences of the young oysters remained low for the duration of the study. The fact that these observations differ from those obtained in this study could be due to there being a different strain of *P. marinus* in Louisiana, the higher salinities found in the study site around Grand Isle, LA and the presence of larger numbers of infective cells in the water column. In addition, Perkins (unpublished data) has found that a diversity of bivalve molluscs will filter *P. marinus* cells from the ambient water and the cells can accumulate in the interstitial spaces and lumens of the tissues without multiplying thus infections, in the strictest sense, may not occur in resistant individuals. Nevertheless, it is unlikely that infective cells of *P. marinus* were absent from the waters around the Piankatank River reef in the first year after setting and some pathogen cells should have accumulated in the tissues of the young oysters; therefore, there are differences between the Louisiana and Virginia oysters.

Residence depth in water column-
Of considerable interest is the residence depth of the oysters relevant to mean low water, because the premise behind construction of artificial reefs is that the survival of oysters in the presence of *P. marinus* and *H. nelsoni* will be enhanced if they are grown in the more natural environment of an oyster shell reef off the bottom of the estuary. In fact, as stated in the Results section above, there is reason to believe that the oysters which are growing at 45 cm or less can be expected to have lower prevalences and intensities of infections of both diseases. Whether this translates to significant differences in survival of the oysters was not elucidated in this part of the reef study (see following section on mortalities).

These conclusions concerning *P. marinus* differ from those of Quick and Mackin (1971) who observed no effect of depth on prevalences from intertidal to 3m below mean low water. More significantly, their weighted incidences (intensities) data showed an **decrease** with increasing depth. Their study area was the Atlantic and Gulf of Mexico coasts of Florida. Similar observations are those of Burrell et al. (1984) who found higher prevalences and intensities of *P. marinus* in intertidal oysters than in subtidal oysters, differing from the conclusions of Mackin (1962) who observed that intertidal oysters have lower levels of infection. He speculated that this results because the oysters are not exposed to as many infective cells by virtue of the increased amount of time they are closed and not feeding as compared to subtidal oysters.

Mortalities-

Although the levels of infections which are adequate to kill oysters are not precisely known, it has been suggested that a mean level of light (level 1) for *P. marinus* in a population of oysters can be considered to signal the beginning of significant mortalities (Andrews, 1988). In studies of this type the many potential causes of mortalities can not be given relative values, but it may be significant in this study that before *H. nelsoni* became a source of mortalities (Fig. 4) and when levels of *P. marinus* were still mostly below level one, oyster mortalities had begun to climb (after the age of 58 weeks). It is suggested that when incidences of *P. marinus* reach a mean of **less than** level 1, mortalities will begin to summate at a relatively constant rate (Fig. 5). As stated, the problem with this assumption is that other sources of mortalities exist and can not be separated from those caused by *P. marinus*. Nevertheless, the accumulative mortality curve in Fig. 5 matches well with the prevalence and intensity curves of *P. marinus* (Figs. 1 and 2). After 91 weeks of oyster age the prevalence, intensity and mortality curves began to rise rapidly.

The prevalence and intensity curves for *H. nelsoni* do not match as well to the mortality curve, the complication being the earlier onset of infections at the deeper levels below mean low water. Since the mean levels of infection were mostly below level 1 (rare), it is suggested that *H. nelsoni* played a relatively minor role in contributing to mortalities.

CONCLUSIONS

1) *Perkinsus marinus* was more significant as an agent of mortalities than was *H. nelsoni*.
2) In the first 11 months of life it can be expected that only a very small (insignificant) number of oysters will become infected with the two species of pathogens on the oyster reef.
3) Assuming that temperature and salinity values approximate those of the study period, oyster mortalities from *P. marinus* can be expected to begin 13 months after setting, rising most significantly one year, 10 months after setting.
4) Residence depths ≤45 cm below mean low water were more favorable at the study site in terms of prevalences and intensities of infections than residences of ≥90 cm.
5) In the second year of oyster life, the epizootiological patterns of disease development (as a function of temperature, salinity and time of year) for *P. marinus* and *H. nelsoni* approximate those patterns which have been previously described in the literature where adult oysters were used.
6) *H. nelsoni* has a patchy distribution in the area of the York and Piankatank Rivers; therefore, adult, susceptible oysters must be used during a study such as the present one to determine whether the pathogen is present in sufficient numbers to be a factor.

Table 1. Time scale for sampling times used in the study. The oyster ages are estimated assuming a setting time of Aug.12, 1993

Sampling Date	Oyster Age (wks.)	Sampling Elapsed Time (wks.)
May 5, 1994	38	0
May 26	41	3
June 16	44	6
June 30	46	8
July 15	48	10
July 28	50	12
August 12	52	14
August 26	54	16
September 8	56	18
September 23	58	20
October 5	60	22
October 20	62	24
November 11	65	27
December 8	69	31
January 12, 1995	74	36
February 10	78	40
March 13	83	45
April 14	87	49
May 11	91	53
June 15	96	58
June 30	98	60
July 13	100	62
July 31	103	65
August 24	106	68
September 18	110	72
October 24	115	77
December 14	122	84

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ACKNOWLEDGEMENT

LIST OF FIGURES

Figs. 1 & 2- Prevalences (Fig. 1) and intensities (weighted incidences) (Fig. 2) of *Perkinsus marinus* infections in Piankatank River reef oysters which set in August, 1993, represented as a function of oyster age and depth of residence below mean low water ($\leq$45 cm and $\geq$90 cm).

Figs. 3 & 4- Prevalences (Fig. 3) and intensities (weighted incidences) (Fig. 4) of *Haplosporidium nelsoni* infections in Piankatank River reef oysters which set in August, 1993, represented as in Figs. 1 and 2.

Fig. 5- Cumulative mortalities observed in Piankatank River reef oysters which set in August, 1993, represented as a function of oyster age.

Fig. 6- Sizes of Piankatank River reef oysters which set in August, 1993 and which were sampled for disease studies summarized above. Sizes are presented as a function of oyster ages and depths of residence below mean low water ($\leq$45 cm and $\geq$90 cm).

Fig. 7- Temperatures and salinities of Piankatank River water at the sampling site and at the time oysters were obtained for disease studies.

Figs. 8 to 11- Prevalences and intensities (weighted incidences) of *P. marinus* and *H. nelsoni* for adult oysters imported from the upper James River and placed on the Piankatank River reef and in the York River behind the Virginia Institute of Marine Science. Two batches of 700 oysters each were placed at the sites (350 at each site) and assayed until the populations were depleted by sampling and natural mortalities. The disease organism data is expressed as a function of site and sampling time in the study.

Prevalence of P. marinus infections in Reef Oysters
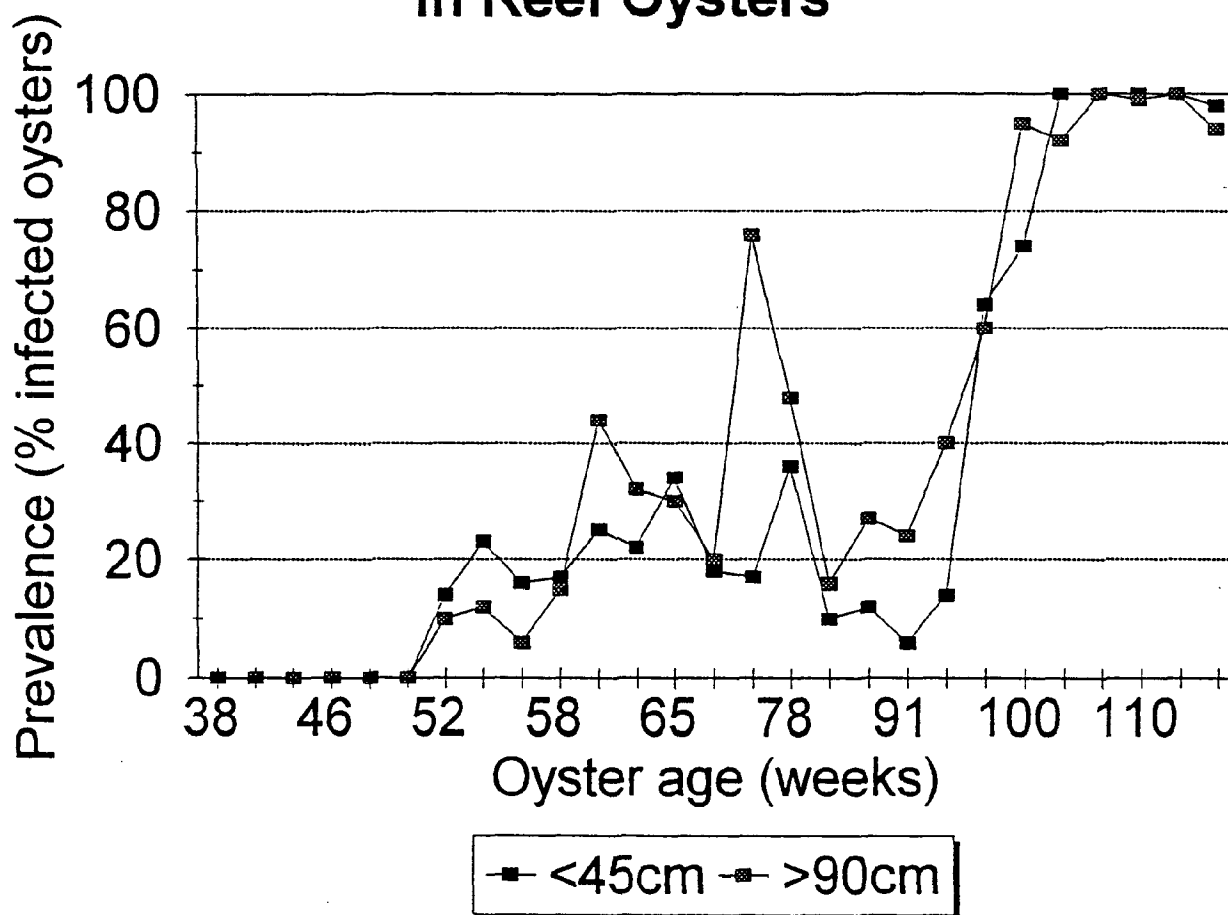
Figure 1

P. marinus infection intensities
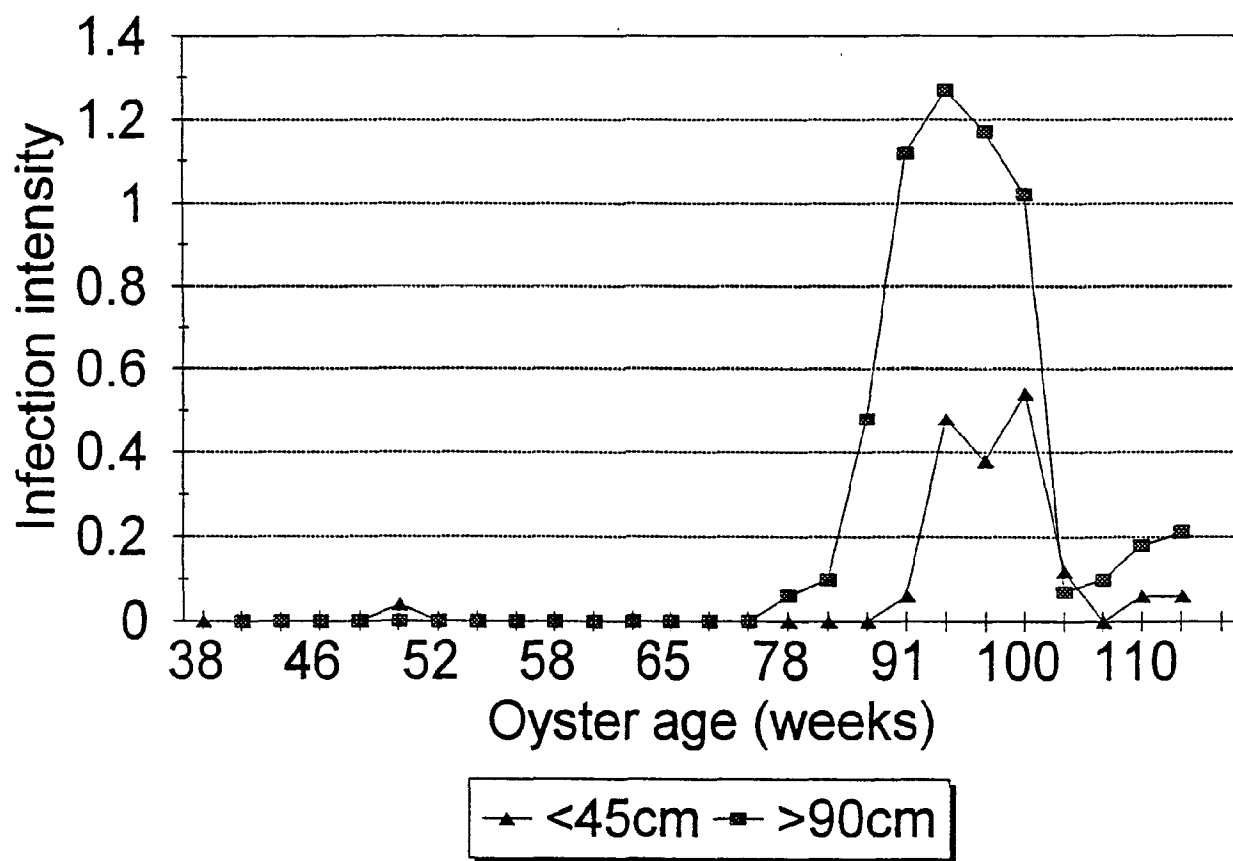in Reef Oysters

Figure 2

Figure 3

Figure 4

Mortality in Reef Oysters
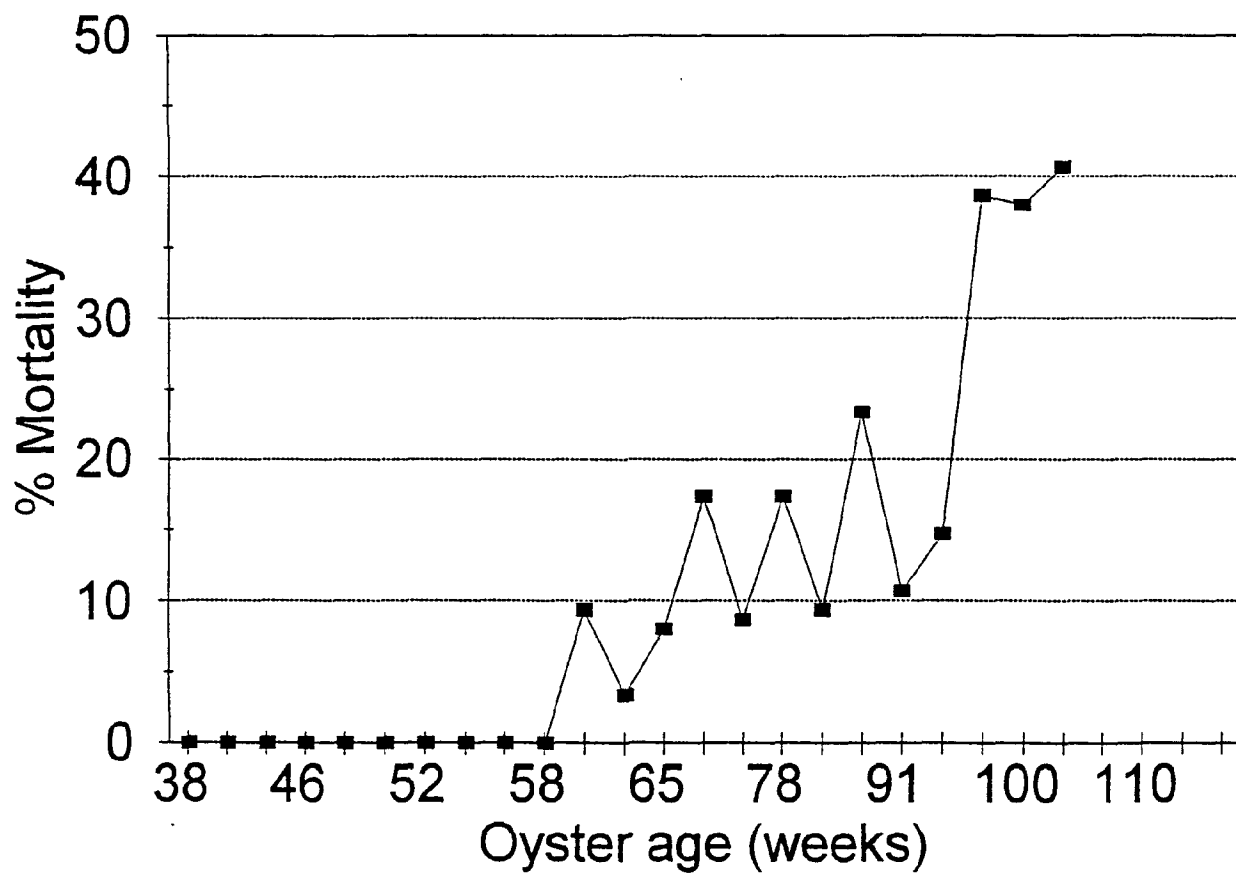
Figure 5

Sizes of Reef Oysters assayed for infections
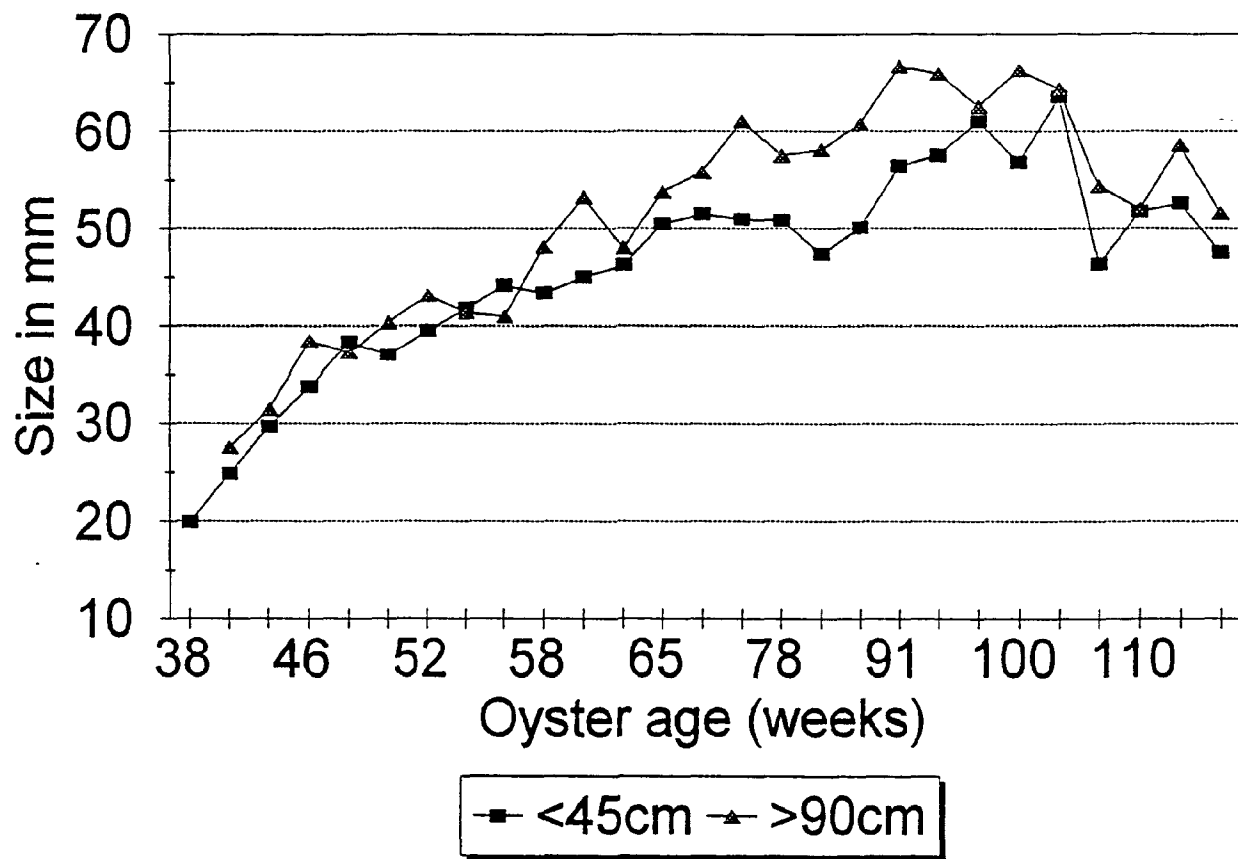
Figure 6

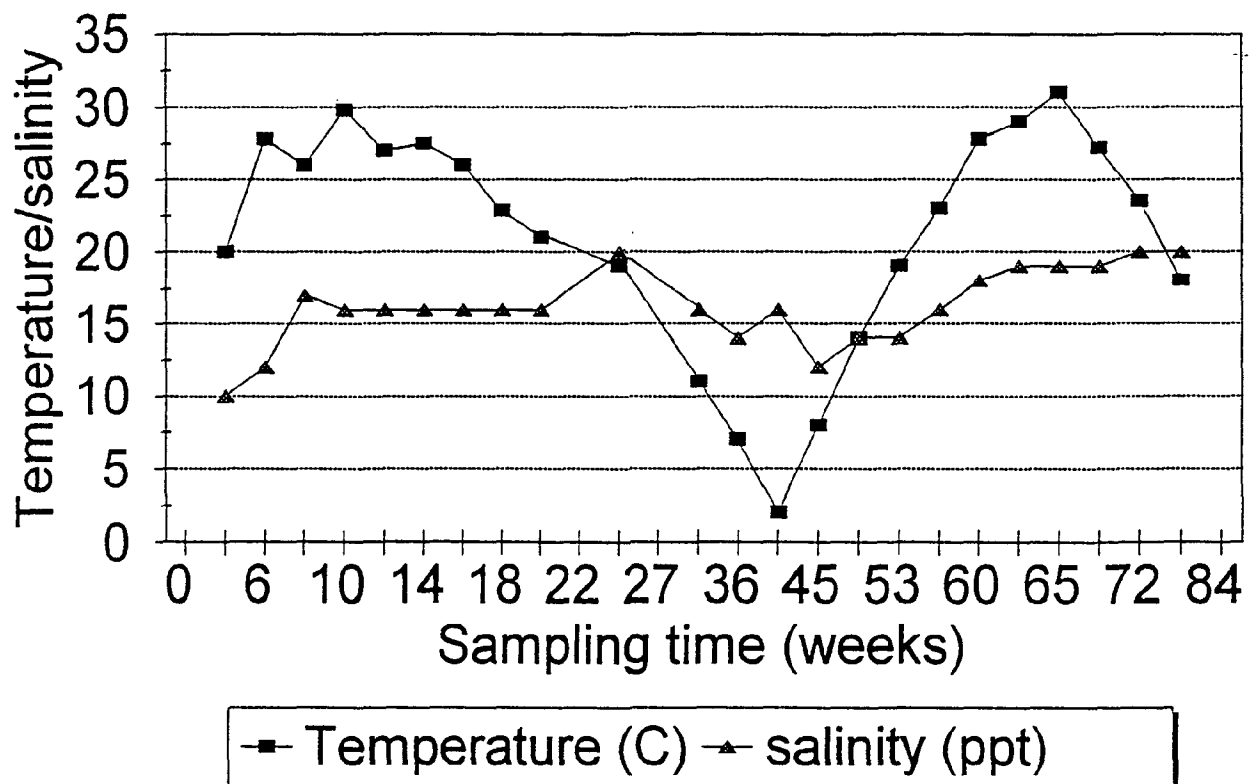**Temperature and salinity profile during the sampling period**

Figure 7

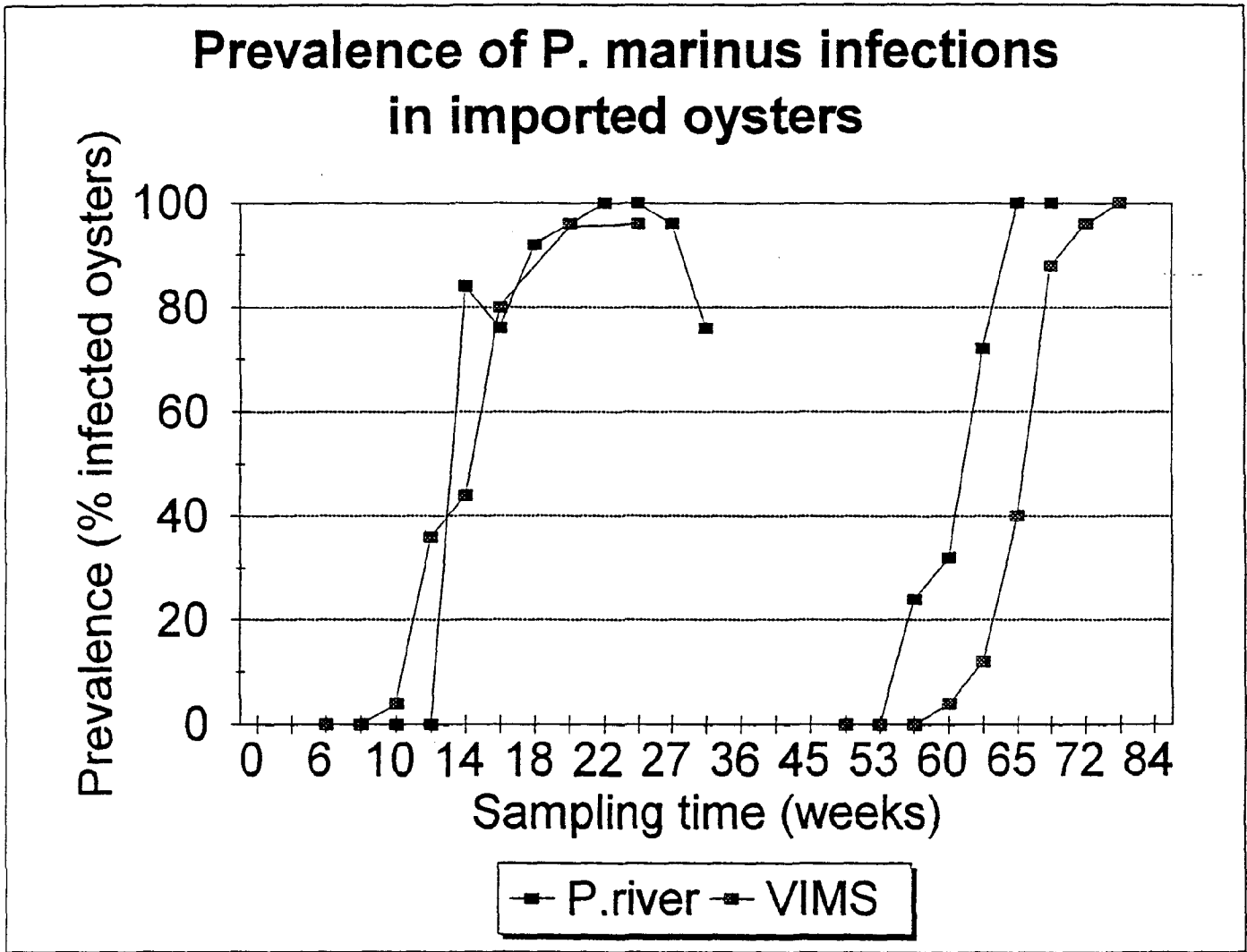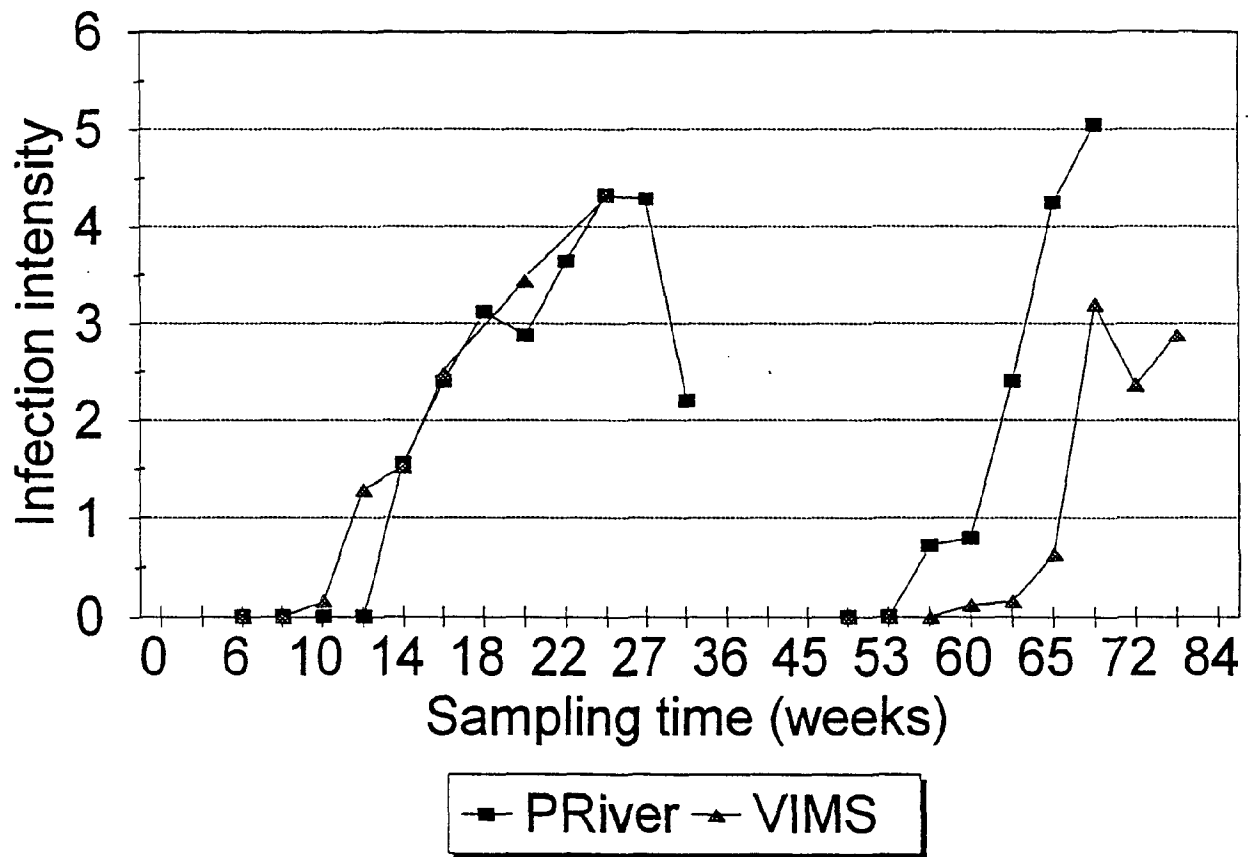Figure 8

P. marinus infection intensities in imported oysters

Figure 9

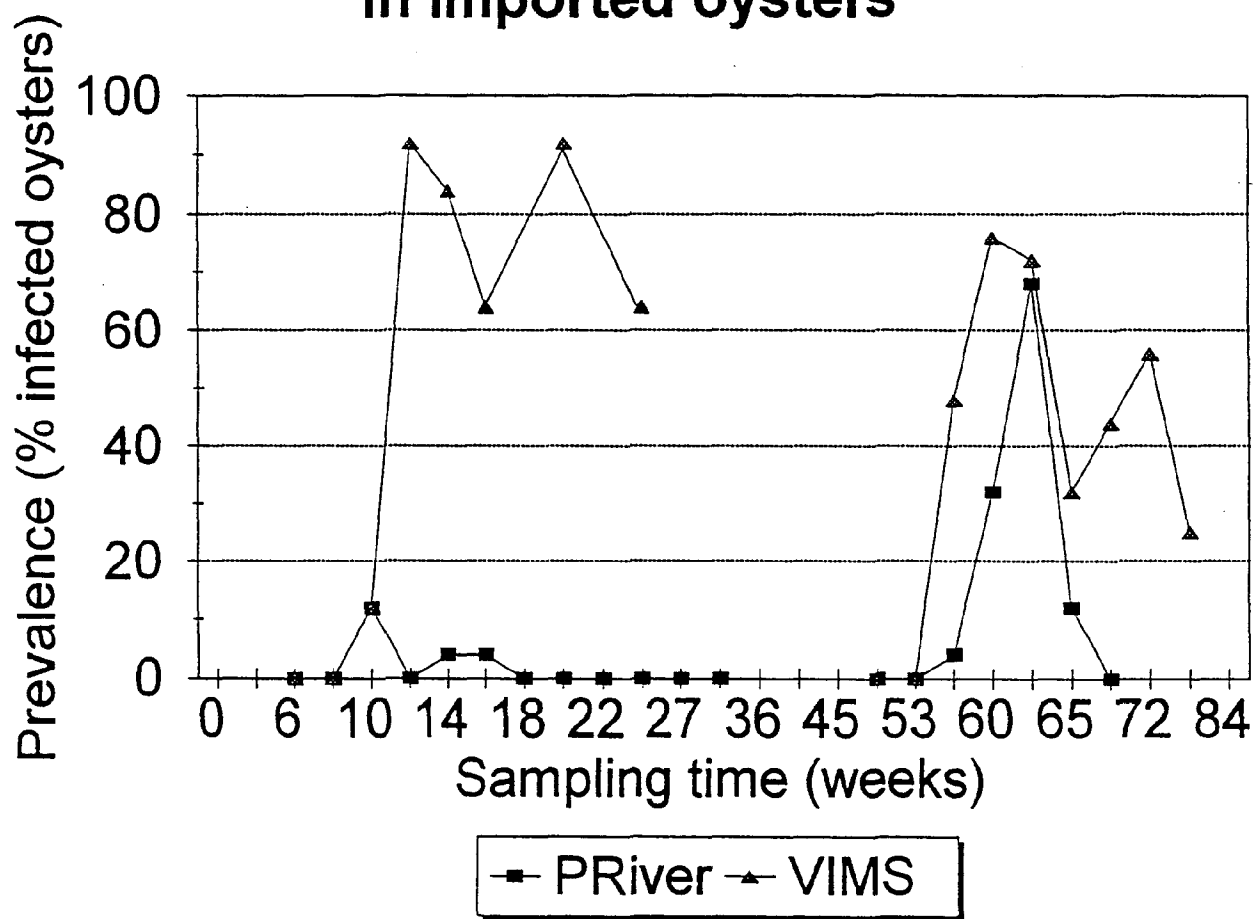Figure 10

H. nelsoni infection intensities
in imported oysters

Figure 11