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Crassostrea virginica on Constructed Intertidal Oyster Reefs: The Effects of Tidal Height and Substrate Level on Settlement, Growth, and Mortality

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CRASSOSTREA VIRGINICA ON CONSTRUCTED INTERTIDAL OYSTER REEFS: EFFECTS OF TIDAL HEIGHT AND SUBSTRATE LEVEL ON SETTLEMENT, GROWTH, AND MORTALITY

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Ian K. Bartol
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APPROVAL SHEET

This thesis is submitted in partial fulfillment of
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To my parents, Anne and Curt, for their love, support, and wisdom.

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Abstract

During precolonial times intertidal oyster reefs were unmistakable geological and biological features of the Chesapeake Bay, but today aerially exposed reefs are absent from the Bay largely because of commercial exploitation, disease, and environmental degradation. It has been suggested that intertidal reef environments are ecologically advantageous for oysters and that a return to intertidal oyster reef environments in Virginia may help rejuvenate the ailing oyster population. In this study, I examine intertidal oyster reef ecology by assessing settlement, growth, and survivorship of *Crassostrea virginica* at intertidal and subtidal heights and at two substrate levels on an artificially constructed intertidal reef in the Piankatank River, a subestuary of the Chesapeake Bay.

The reef was approximately 150 x 30 m and was constructed by the deployment of aged oyster shells off barges using high pressure hoses. To monitor settlement on the reef, weekly non-destructive and destructive samples were collected at tidal heights ranging from +30 cm above mean low water (MLW) to -90 cm below MLW and at two substrate levels (reef surface and 10 cm below the reef surface). Larval counts from both samples were subsequently recorded. Growth and survivorship assessment involved monthly destructive sampling of naturally-set reef oysters and monthly photosampling of two year classes of hatchery-reared oysters placed in cages on the reef. Oysters considered in both the destructive reef study and hatchery-reared study were either extracted or placed on the reef at the same tidal heights and substrate layers sampled during the settlement study. Shell heights of naturally-set oysters and shell areas and mortalities of individually marked hatchery oysters were recorded.

Settlement and early recruitment were greatest subtidally and did not differ according to substrate level. Growth was also greatest subtidally, but unlike settlement findings, was disproportionate according to substrate level. Oysters grew faster below the surface in the high intertidal zone, at the surface in the subtidal zone, and grew most rapidly from mid-August through mid-September. Contrary to settlement and growth results, survivorship was not maximized subtidally throughout the year. During the summer and fall survivorship was maximized at MLW. Only during an unusually harsh winter were mortality rates lowest subtidally, and this was only applicable to oysters found at the surface level. Furthermore, residence below the surface was an important refuge from physical conditions, such as freezing temperatures and intense solar radiation, and from biological forces, such as predation and colonization by algae and endemic organisms, during certain times of the year.

These results suggest that although building artificial intertidal reefs will not enhance settlement and growth, it may enhance survival, especially once oysters become older and begin aggregating in colonies. During the interim from initial settlement to dense colonization, the underlying environment may play a vital role in reef development. Oysters residing below the surface may persist when conditions are unusually severe because they live in a more stable and buffered habitat and may serve to rejuvenate overlying immature communities.

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INTRODUCTION

Background

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 precolonial Chesapeake Bay (Marshall 1954, Hargis and Haven 1988, Mann et al. 1991). These biological formations, which proliferated in the Chesapeake waters and tributaries at the beginning of the Holocene during the world-wide rise in sea-level (Haven and Whitcomb 1983), were well marked navigational hazards on early nautical maps and were even integral components of festive celebrations. Middens and recorded history reveal that Native Americans and colonists alike harvested oysters from these environments and feasted on this seemingly endless delicacy during commemorative times (Haven et al. 1978). The extensive communities of fish and crab species that these reefs supported were undoubtedly important sources of food as well.

As the economic value of the oyster, *Crassostrea virginica*, began to be realized in the mid to late 1800s, commercial exploitation of the resource began. *Crassostrea virginica* was harvested for not only for its culinary appeal but also its shell, which was used in construction, in the production of calcium tablets and lime, and as feed for livestock (Bahr and Lanier 1981, Kennedy and Breisch 1981, Haven et al. 1978). Continual oyster harvesting over the past 140 years in Virginia has resulted in the transformation of all oyster reefs from protruding aerial exposed features to mere low-lying subtidal mounds. In fact, Marshall (1954) found that as early as 1870 emergent intertidal oyster shoals were absent from the James River, the site of the most productive oyster grounds in Virginia. This degeneration has been exacerbated by the arrival of two protistan parasites, *Haplosporidium nelsoni* (MSX) and *Perkinus marinus* (Dermo), and environmental degradation coupled with poor resource management.

Commercial exploitation, disease, and environmental degradation have resulted in a drastic decline in the oyster fishery since the turn of the century. In 1880 total oyster production in Virginia was 6.8 million bushels and rose to 7.6 million bushels in 1904. This total fell to 3.3 million bushels during the 1960-61 season, 1.2 million bushels during the 1980-81 season, and was as low as 43,000 bushels during the 1990-91 season (Hargis and Haven 1988, Mann et al. 1991). Presently, total oyster production is at an all time low of 31,000 bushels and continues to decline (J. Wesson, pers. comm.). This decline in oyster abundance and harvesting yield has been catastrophic for the industry.

The effect that a diminished number of oysters has on the ecology of the Chesapeake Bay may be of greater concern than the economic implications. *C. virginica* is a dominant filter feeder which comprises an important link in the cycling of nutrients and energy (Dame et al. 1984). By filtering microplankton and particulate matter, oysters reduce the impact of eutrophication and improve water quality. Newell (1989) estimated that the oyster population in the Chesapeake Bay once had the capacity to filter the water of the entire bay in 3.3 days. Today, oyster stocks probably take more than a year to accomplish this task.

Although the problem of oyster decline and its implications are now obvious, a solution for rejuvenating the ailing oyster population is not. A number of tactics have been introduced and investigated, ranging from the development of disease tolerant stocks to the culturing of oysters in low salinity areas. Although many of the approaches have had limited success, no one solution that can adequately deal with current economic and ecological needs has emerged. One frequently proposed, but critically unevaluated approach for increasing oyster stocks, is the construction of intertidal oyster reefs, environments that oysters have resided in naturally for centuries. In Virginia, many of the surviving oysters are found in the intertidal zone on bridges and pier pilings (habitats undisturbed by harvesting), and on the South Atlantic Coast of the United States, 95% of *C. virginica* reside in the intertidal zone on oyster reefs (Gracy and Keith 1972, Dame 1979). This suggests that the intertidal zone is ecologically advantageous, and that a return to an intertidal oyster reef environment in Virginia may be extremely beneficial for the survival of oysters in the Chesapeake Bay. Intertidal oyster reef environments may provide temporal relief from predation, suitable current velocities for filtration, lowered rates of fouling and siltation, and harsh environmental conditions for disease, and therefore may be ideal habitats for *C. virginica* communities (Bahr and Lanier 1981, Crosby et al. 1991). However, prior to this study, the examination of intertidal oyster reef ecology and the assessment of intertidal reefs as habitat for *C. virginica* has not been performed in the Chesapeake Bay, mainly because of an absence of intertidal oyster reefs for over a century.

In this study, a sizable artificial reef (150 m x 30 m) was constructed which provided a unique opportunity to monitor oysters in an intertidal reef environment within the Chesapeake Bay ecosystem. To assess oyster ecology at the reef and to evaluate intertidal reefs as habitat for oysters, oyster settlement (the irreversible adherence of planktonic oyster larvae to the substratum), growth, and survivorship were examined. Since elevation relative to the water level is a major factor that distinguishes intertidal reef environments from current subtidal habitats, it was important to examine settlement, growth, and mortality as a function of tidal height.

In a preliminary reef study, described here, it was found that another factor, substrate level (i.e. reef surface level, level 10 cm below the reef surface, etc.), should be considered as well. The large interstitial pores between shells in the reef environment allow for settlement, growth, and survival of oysters below the surface layer of shells. In climates such as Virginia where ice and intense heat are both seasonally present in the intertidal zone, these underlying, protected, moist areas may be advantageous, if not critical, for intertidal oyster communities. The predator and algal refuge provided by these habitats may be beneficial as well, not only for intertidal but also subtidal oysters. Research in this realm of reef dynamics is also directly applicable to present oyster restoration projects which involve the construction of artificial reefs. Generally, the proposed reefs are to be composed of crushed clam shell, tile, or mounds of sediment capped with a thin shell layer. These substrates are too dense to allow for sub-surface oyster community development, however. Therefore, research in the area of reef substrate level ecology may provide useful insight as to whether the above proposals are predestined to fail.

The pressing need to explore oyster ecology on intertidal reef environments as a function of tidal height and substrate level should be clear. In this study, I attempt to do just that by critically evaluating intertidal oyster reef environments in Virginia as habitat for *C. virginica.* Specifically, I assess settlement, growth, and survivorship of oysters at intertidal and subtidal heights and at 2 substrate levels (surface and 10 cm below the surface) on an artificially constructed intertidal reef.

Organism

The eastern oyster, *Crassostrea virginica* (Gmelin 1791), is found from Nova Scotia to Florida on the east coast of North America, and is present throughout the Gulf of Mexico; there are even reports as far south as Brazil (Gunter 1951). The eastern oyster is resident to habitats of wide ranging depth (0-40 ft), salinity (5-30‰), air temperature (0-40° C), water temperature (0-30° C or higher) and substrate type (rock to mud), and favors areas where there are relatively steady flows of water and low sedimentation (Galtsoff 1964). Diatoms, flagellates, and desmids compose the main diet of this filter feeding mollusk, but studies have shown that particulate organic matter is an important source of food as well (Hanson and Snyder 1979). *C. virginica* is generally oviparous (egg-laying) and dioecious (with separate sexes), but once a year some members of a given local population may change their gender from male to female (protandry) or female to male (protygymy). There are even very rare cases of hermaphroditic oysters (Galtsoff 1964).

The reproductive process of oysters is extremely sensitive to fluctuations in the aquatic environment. Minor changes in external conditions, such as temperature increases and spring bloom shifts, may translate into dramatic alterations in reproductive and larval cycles (Hoffman et al. 1992). Thus, the following description of reproductive behavior should be viewed as a generalized guideline, not as the inevitable process. At a young age (2-3 months) and size (1 cm) gonadal maturation can but does not necessarily occur (Abbe 1986) . Spawning can occur anytime after gonadal maturation. Spawning of *C. virginica* is triggered by temperature (20-25° C) (Galtsoff 1964) or salinity cues (> 10 ‰) (Abbe 1986) and subsequent fertilization occurs via chance encounters of eggs and sperm. Larval development then ensues, progressing from the trocophere stage through the veliger to the pediveliger stage. Oyster larvae stay in the plankton for approximately 2-3 weeks, but this time may increase substantially if conditions are not ideal (Abbe 1986). Upon finding suitable substrate, pediveligers cement to the selected medium and forfeit all pelagic motility. This process of irreversible adherence of planktonic larvae to the substratum is called settlement (Keough and Downes 1982, Luckenbach 1984, Connell 1985, Bushek 1988). Following settlement the organism reorganizes its structure and physiology from the larval form to that of a juvenile, which is often referred to as "spat". The transition from the juvenile stage to the adult stage is not a clear-cut process. Oysters become adults when they are sexually mature. However, it is difficult to determine the onset of sexual maturity in oysters, because it may occur anytime from 2 months to 2 years and at varied sizes. Thus instead of classifying oysters as juveniles or adults, ecologists generally characterize oysters by their size.

Intertidal Oyster Reefs

When left unaltered by humans, *C. virginica* found in the Chesapeake Bay forms intertidal oyster reefs similar to existing reefs found from the eastern shore of Maryland to Florida and in the Gulf of Mexico. These reefs are composed of oyster shells, mud, and live oysters, and provide habitat for a diverse array of invertebrates and fish. Intertidal oyster reefs may range from small scattered clumps to massive solid mounds up to many kilometers in length (Dame 1971). Although reef substrate often extends into the subtidal zone, the majority of oysters on existing intertidal reefs reside in the intertidal zone. This is likely a result of reduced predation pressure, low rates of sedimentation and biodeposition, more ideal current velocities, and less competition for space (Crosby et al. 1991). Within large reef communities, oysters tend to persist more at the perimeter of the reef than at the center, where biodeposition and sedimentation are often high.

High rates of sedimentation and biodeposition below the upper surface layer of the established reefs make residence in lower layers difficult. In Georgia, these high rates of sedimentation and biodeposition confine oysters to the 5 to 10 cm upper layer (Bahr and Lanier 1981). However, in other geographic areas this survivorship band may be markedly different as a result of different climatic factors, hydrodynamic conditions, and stage of the reef development. The upper layer (5 to 10 cm in Georgia) of intertidal substrate and oysters has a greenish color, characteristic of algal growth, and may dry out as a result of intense solar radiation. Below the upper layer, there is an underlying shell layer (intermediate layer) which is consistently moist and reddish-brown in color. The reddish-brown color may be attributed to a thin detrital layer covering each shell. Oysters and shell which comprise the intermediate layer lack the algal film characteristic of the upper layer (Bahr and Lanier 1981). Farther below the intermediate layer lies a silver-black layer, which derives its characteristic color from large quantities of ferrous sulfide produced in a highly anaerobic environment (Wiedermann 1971). This layer is devoid of live oysters, a feature which distinguishes it from overlying layers.

The development and decline of intertidal oyster reefs have four main stages: initial colonization, clustering, accretion, and senescence (see Bahr and Lanier, 1981). Initial colonization consists of the settlement and subsequent growth of single and small cluster oysters within the lower subtidal or intertidal zone. For this stage to occur sand, firm mud, clay, or any hard object such as fallen trees, bushes, branches, old shells, etc., must be present. Grave (1905) suggested that since suitable substrate is more abundant near the shoreline, this stage quite often begins near land. With time new generations of oyster larvae will settle and attach themselves to other live oysters and dead shell surfaces, forming 3-7 generation clusters. This is known as the clustering phase of reef development. Many of the older oysters found at the base of these clusters die as a result of overcrowding and burial, but their shells remain and serve as a foundation to the cluster. The accretionary phase sets in when clusters increase in size through settlement of additional spat and eventually coalesce, resulting in both horizontal and vertical growth as sea-level rises. The intertidal oyster reefs of precolonial Virginia began this process 6500-9000 years ago when tributaries of the Chesapeake Bay were flooded (Haven and Whitcomb, 1983). Reef accretion may produce three distinct reef types: fringe reefs, which are parallel to the shoreline and often located near the flank of the main channel axis; string reefs, which are perpendicular to the nearest shore and to the direction of tidal currents; and patch reefs, which have an irregular shape and often grow far from shore (Stenzel 1971). The final stage of reef formation, the senescent stage, occurs when the majority of oysters residing in the upper tier of the reef can no longer accrete vertically to keep up with sea level rise. Generally, oysters nestled within the central region of the reef die first, leaving a population of oysters around the fringes of the reef. Grave (1905) suggested that with time, the barrier central "flatland" surface becomes built up with thicker accumulations of sand, mud, and shell debris and eventually becomes colonized by *Spartina.* The end result is the transformation of the oyster reef to an oyster marsh island (similar to Little Egg Island in Georgia) which is surrounded by a thin band of intertidal oysters.

Intertidal oyster reefs are beneficial for a number of reasons. By stabilizing and elevating sediment, reefs increase wetland development. Reef organisms augment primary production by reducing turbidity, mineralizing organic carbon, and releasing nitrogen and phosphorous in usable forms. Oyster reefs also are invaluable mechanisms of nutrient retention and recycling (Dame et al. 1992, Dame and Libes 1993) and serve as integral coupling links between benthic and pelagic systems (Chrzanowski et al. 1986). Intertidal oyster reefs support a multitude of diverse organisms besides oysters, such as barnacles, anemones, polychaetes, amphipods, bryozoans, flatworms, and commercially valuable crabs, mussels, clams, and fish (Wells 1961, Larsen 1985, Bahr 1976). These organisms along with oysters collectively serve as important food sources for transient predators, such as birds and terrestrial animals. This intricate network of organisms culminates into one of the highest energy flux heterotrophic systems known (Dame, 1976).

The benefits that intertidal oyster reef environments provide for *C. virginica* are considerable. The intertidal zone provides more suitable substrate for settlement, greater current velocities, and less siltation for *C. virginica* than subtidal non-reef environments (Bahr and Lanier 1981, Crosby et al. 1991). Temporal relief from predation and decreased fouling within the intertidal zone are also advantageous for the eastern oyster and may result in higher survival and faster growth (Arakawa 1980, Bahr and Lanier 1981, Burrell 1986, Gibbons and Chu 1988). Oyster survival may also increase in the intertidal zone because the harsh environmental stress of intertidal existence, which *C. virginica* is well adapted to, may be less hospitable for disease organisms.

The porous nature of reef environments themselves may prove to be advantageous for oysters in both intertidal and subtidal habitats. Interstitial pores within the reef allow water currents to permeate the underlying layers and permit oysters to expand vertically and horizontally. These two factors allow oysters to settle, grow, and survive below the surface layer. The underlying layers may be beneficial for several reasons. First, they have significantly reduced rates of algal growth, which may enhance oyster survival in both intertidal and subtidal reef habitats. Second, these layers furnish refuge from predation, which is also beneficial for oysters in both intertidal and subtidal reef environments. Third, residence below the surface provides buffering from extreme environmental conditions, such as ice and intense heat or collisional impacts from flotsam, waves, and ice. Before describing an experiment to test whether tidal height and substrate layer influence settlement, growth, and survival on oyster reefs, however, it is important to examine what is currently known about these processes.

Settlement

Settlement processes of sessile organisms on rocky intertidal substrates is an area of research that has often intrigued marine ecologists. The accessible nature of these environments along with the fact that sedentary organisms are cooperative during sampling make this area of study attractive. Therefore, it is not surprising that rocky intertidal settlement research is widespread. Much research has focused on how the magnitude and location of sedentary larval settlement are influenced by pre-settlement processes, such as fluctuations in larval supply or planktonic zonation (Grosberg 1982, Keough 1983, Underwood and Denley 1984, Caffey 1985, Connell 1985, Gaines et al. 1985, Menge and Sutherland 1987, Roughgarden et al. 1988) and chemical cues (Crisp 1967, Raimondi 1988), and post-settlement processes, such as competition and predation (Connell 1972, Paine 1974, Menge 1976, Lubchenco and Menge 1978, Peterson 1979, Grosberg 1981, Osman et al. 1989) and physical and environmental stress (Menge 1976, Lubchenco and Menge 1978, Underwood et al. 1983, Wethey 1983).

Settlement research on oysters living on hard substrates in intertidal and subtidal zones is extensive as well. It is known that *C. virginica* settlement occurs during a well defined summer period in estuaries throughout the eastern oyster's range. The length of this period is quite consistent geographically except at the southernmost end, where it increases somewhat (Kennedy 1986). The onset and duration of the settlement season vary little among year (Kenny et al. 1990) and are often triggered by either water temperature (McNulty 1953, Lutz et al. 1970, Hidu and Haskin 1971) or chemical cues (Crisp 1967, Hidu et al. 1978, Bonar et al. 1990, Fitt et al. 1990, Tamburri et al. 1992). Multiple settlement peaks are typical throughout the season (McNulty 1953, Loosanoff 1966, Shaw 1969, Hidu 1978, Hayes and Menzel 1981, Haven and Fritz 1985), and settlement is gregarious (settle in colonies) (Hidu 1969, Hidu and Haskin 1971), light sensitive (Nelson 1953, Ritchie and Menzel 1969), and substrate selective (Haven and Fritz 1985, Mann et al. 1990). Although the timing of these peaks is somewhat predictable, the intensity of the peaks is not (Shaw 1969, Kenny et al. 1990, Kennedy 1986). This is because settlement intensity is the result of the interplay of a number of factors, namely water quality, hydrodynamic conditions, salinity, dissolved oxygen, water temperature, pollutants, predators, food, and disease (Abbe 1986, Kennedy 1986).

In contrast to research examining rocky intertidal organisms such as barnacles and polychaete worms (Caffey 1985, Dayton 1971, Connell 1972, 1975, Underwood 1978, Grosberg 1982, Raimondi 1988, Wethey 1983), there are few studies examining where oyster larvae settle along a vertical gradient from the intertidal to the subtidal zone at time scales short enough to allow for the accurate documentation of settlement. Many of the early settlement studies, such as Galtsoff and Luce (1930), Loosanoff (1932), and Mackin (1946), were performed on scales of a month or more. Luckenbach (1984) and Connell (1985) stressed that time scales should be short enough to distinguish between settlement and post-settlement mortality. Given that Roegner (1991) found high oyster mortality during the first week after settlement, settlement studies performed at intervals of a month or more probably incorporate a substantial post-settlement mortality period and actually measure recruitment rather than settlement. Recruitment refers to the number of settlers which have survived a period of time defined by the researcher. Measuring actual settlement in the field would require sampling at intervals of hours rather than weeks. Sampling at this rate is extremely labor intensive, costly, and as Connell (1985) found not always necessary. A reasonable compromise between hourly and monthly sampling is weekly or biweekly sampling, which should provide meaningful data on settlement.

Interestingly, when the limited body of research that examines oyster settlement weekly or biweekly is considered, two things become apparent: (1) there is no consensus of where oyster larvae settle along a vertical gradient, and (2) little is known about settlement on sloping, reef substrates. McDougall (1942), Chestnut and Fahy (1952, 1953), Nichy and Menzel (1967), and Roegner and Mann (1990) found greatest settlement subtidally; however, Hidu and Haskin (1971), McNulty (1953), Kenny et al. (1990), and Michener and Kenny (1991), either found greatest settlement intertidally or found no differences in settlement as a function of tidal elevation. Of these studies, only Michener and Kenny (1991) attempted to document settlement within a reef ecosystem. Furthermore, the majority of the studies involved the utilization of suspended substrates, such as shell strings or collection plates, vertically oriented structures, such as pilings, or in the case of Michener and Kenny's study, plastic tubes with lengthwise grooves. Studies utilizing these collection mediums fail to provide an accurate appraisal of settlement on natural, sloping, reef topography that extends from the subtidal to the intertidal zone. Hydrodynamic alteration by such sloping aerially exposed structures has been documented by Bahr and Lanier (1981), and thus it is likely that there are substantial discrepancies in settlement between sloping and vertically oriented substrates.

There is even less known about where oysters settle within the three dimensional array of the substrate itself; in fact, there are no studies which examine this phenomena. Examining settlement as a function of substrate level may be thought of as a meaningless exercise, since many of the substrates oysters settle on, such as pier pilings, bridges, or navigational buoys, have no penetrable dimension. However, oyster reefs, the most important habitat of oysters, do provide this dimension. The fact that oysters are present in underlying layers of present oyster reefs suggests that oyster larvae do penetrate and settle deep within the substrate. The question that now needs to be addressed is "to what extent does settlement occur in subsurface layers?"

Growth

Once oyster larvae have affixed themselves to a substrate, they undergo an energetic growth process. A number of studies have examined bivalve growth rates in subtidal environments (Shaw 1966, Duggan 1973, Manzi et al. 1977, Grant et al. 1990), at varied distances from shore (Ambrose et al. 1980, Dayton et al. 1989), at different latitudes (Ansell 1968), at different salinities and water temperature (Shaw 1966, Pratt and Campbell 1956), and at different food concentrations (Pratt and Campbell 1956). Studies have also examined intertidal versus subtidal bivalve growth rates (Eldridge et al. 1979, Breed-Willeke and Hancock 1980, Petersen and Black 1987).

Studies quantifying oyster growth rates in relation to exposure level are lacking, however. The majority of growth studies on oysters have been performed on large oysters in either the subtidal zone (Loosanoff and Nomejko 1949, Butler 1955, Andrews and McHugh 1957, Shaw and Merrill 1966) or the intertidal zone (Copeland and Hoese 1966, Dame 1971). Of the studies that have examined growth along tidal gradients, there is some debate over where oysters grow most proficiently. Ingle and Dawson (1952), Burrell (1982), and Roegner (1991) found that subtidal *C. virginica* grow at faster rates than intertidal oysters, and Michael and Chew (1976) and Sumner (1981) found similar results for the Pacific oyster, *Crassostrea gigas*, despite excessive fouling of subtidal trays. However, Spencer and Gough (1978) were unable to detect a difference in growth of either *Crassostrea gigas* or *Ostrea edulis* held subtidally and in the low intertidal zone, and Littlewood (1988) found best survival and growth of *Crassostrea rhizophorae* at mid intertidal levels rather than at subtidal levels. Gillmor (1982) and Crosby et al. (1991) presented evidence that *C. virginica* situated at intertidal depths are capable of as fast or faster growth per unit immersion time as oysters residing at subtidal depths, and concluded that there is a degree of optimality associated with periodic aerial exposure. As with settlement research, many of these studies were performed in the lab or in the field using suspended trays or plates and fail to provide representative documentation of growth in reef habitats.

There are no studies that directly address oyster growth as a function of substrate level, and thus much of the knowledge in this realm of research is speculative. Bahr (1976) found slow growth of individual oysters in densely packed established reefs and felt this was because lateral space was limited. This finding suggests that growth in underlying layers may be sluggish due to spatial constraints. Conversely, Copeland and Hoese (1966) found that instead of being slow, growth in established, dense reefs is impressive but erratic, which suggests that growth in underlying crowded layers may not necessarily be slower than growth at the surface. Bahr and Lanier (1981) hypothesized that the sharper growing edges of oysters in the upper layer as compared to oysters in underlying layers is indicative of faster growth, but they have no documentation of this. Galtsoff (1964) believed the high rates of biodeposition and sedimentation below the surface may hamper growth. However, Galtsoff does not directly address oyster reef environments where large quantities of sediment and biodeposits may gravitate through pores in the shell substrate and become deposited several feet below the surface layer.

Mortality

The vertical range an intertidal sessile organism occupies is a function of both physical and biological factors. The upper intertidal survivorship boundary is dominated largely by physical factors, whereas the lower boundary is determined predominantly by biological factors (Connell 1972, Lubchenco and Menge 1978, Wethey 1983). Within the intertidal zone, the physical factors which help shape the upper limit of residence are thermal shock, desiccation, solar irradiation, lowered immersion times, and physical disruption (i.e. wave action, collisions with flotsam) (Lewis 1977, Newell 1979, Wethey 1983). Predation and competition are the dominant biological factors shaping the lower distribution of intertidal organisms (Connell 1972, Paine 1979, Menge 1976, Menge 1983, Lubchenco and Menge 1978, Lubchenco 1980, Connell 1985).

C. virginica living in the intertidal zone are very adaptive and can tolerate physical stresses more readily than most other intertidal inhabitants. This allows the eastern oyster to reside high in the intertidal zone. Oysters are able to aerially respire and are capable of complete shell closure, a valuable trait that allows oysters to isolate themselves completely from hostile environmental conditions. During periods of shell closure oysters, using stored glycogen, switch from aerobic to anaerobic respiration. By using the preferred substrate glycogen in anaerobic metabolic pathways, oysters can survive prolonged periods of hypoxia experienced during both low tide stands and periods of behaviorally induced environmental isolation (de Zwaan 1983). Efficient use of this ability may be limited to older oysters, however. Galtsoff (1964), Holland and Spencer (1973), Mann and Gallager (1985), and Widdows et al. (1989) presented evidence that oysters less than one year old allocate less of their energy to glycogen storage than older oysters, and as a result are less resistant to physical conditions because they cannot employ anaerobic respiration as efficiently. The oyster's ability to aerobically respire and isolate itself from the external environment along with its overall hardiness allow oysters to tolerate internal temperatures over 50°C, withstand freezing air temperatures, survive periodic intense wave action, and survive for days or even weeks without exposure to water (Galtsoff 1964). Oysters are not entirely impervious to external conditions, however. Roegner (1989) found high mortalities of juvenile oysters at the mid to high intertidal zone in Virginia when summer temperatures rose above 30°C. Similarly, Nichy and Menzel (1967) found high mortality rates of adult intertidal oysters in Florida when air temperatures rose above 30°C and internal temperatures were as high as 37.6°C. Ortega (1981) not only found high intertidal mortality as a result of high air temperatures but also attributed high intertidal oyster fatalities to wave stress.

Competition and predation rather than physical processes dominate at the lower vertical limit of an intertidal organism's range. Due to uninterrupted water coverage, a greater suite of competitors and predators are found in the subtidal zone than are present in the intertidal zone (Nichy and Menzel 1967, Burrell 1986, Chestnut and Fahy 1953, McNulty 1953). In the subtidal or lower intertidal zone, *C. virginica* must compete for space and food with barnacles, mussels, bryozoans, ascidians, worms, amphipods, hydroids, and tunicates (Kennedy 1980, Ortega 1981, Abbe and Yates 1982, Galtsoff 1964, Osman et al. 1989, Roegner 1989, Baker 1994) and is preyed upon by oyster drills, sponges, sea stars, flatworms, crabs, and fish (Abbe 1986, Galtsoff 1964, Eggleston 1990 a, b, c, Littlewood and Marsbe 1990, Osman et al. 1989). The relationship between the presence of these organisms and oyster survivorship is generally clear; the greater the prevalence of these competitors and predators, the higher the incidence of mortality.

Although physical forces are more dominate in shaping the upper range and biological forces are more instrumental in defining the lower range, both forces take part in defining the upper and lower limits of oysters. Sand scouring (Kennedy and Breisch 1981), current flows (Galtsoff 1964), and burial by fine sediment and biodeposition (Haven and Morales-Alamo 1966, Lund 1957) are physical forces that may play integral roles in defining the lower boundary of the intertidal oyster's range. Similarly, competition and predation may influence the upper end of an organism's range (Bahr and Lanier 1981, Carriker 1955). Stresses attributed to humans, such as hydrologic alteration, toxic discharge, overharvesting, and disease may also disrupt the oysters at both ends of their range. Of these forces, disease may be the most deleterious.

Haplosporidium nelsoni and *Perkinsus marinus* are two diseases that have wreaked the greatest havoc on oyster stocks. Incidence of *H. nelsoni* first became apparent in the Chesapeake Bay in 1959 and has been responsible for mass mortalities of *C. virginica* ever since (Ford and Haskin 1987, Andrews 1982). This disease seriously drains energy reserves (Barber et al. 1988), hinders metabolic processes (Newell and Barber 1988, Barber et al. 1991), interferes with feeding processes (Newell 1985), and reduces reproductive capacity (Ford and Figuras 1988). *H. nelsoni* is pathogenic above 15‰, present but benign between 12 and 15‰, and absent at less than 12‰ (Haskin and Ford 1982, Ford 1985). *P. marinus* is a fungal disease which reached peak distribution in the Chesapeake Bay in 1986 and 1987 as a result of continuing drought conditions (Burreson and Andrews 1988). *P marinus* is tolerant to a wide range of salinities (Ragone and Burreson 1993) but is most problematic during periods of low precipitation when salinities exceed 18‰ (Abbe 1992). *P. marinus* does not develop to lethal limits until approximately two years of age (Burreson 1991, Paynter and Burreson 1991).

Physical and biological forces act on intertidal oysters and shape where they will survive along an intertidal / subtidal gradient. The magnitude and influence of each force varies dependent upon time and geographic setting. For example, in southern high salinity areas, McDougall (1942), Chestnut and Fahy (1953), and Marshall (1954) all found mortality by predation in the subtidal zone to be considerably higher than in the intertidal zone and concluded predators prevent survival of oysters below mean low water. Nichy and Menzel (1967), who attempted to document mortality within a reef setting in Florida, found similar results. Arakawa (1980) in Georgia and Littlewood (1988) in Jamaica found higher survival for oysters situated in the intertidal zone and attributed it to not only lowered predation pressure but also lowered rates of fouling. Furthermore, Kenny (1990) found similar results in South Carolina and attributed it to lowered predation pressure, lowered rates of siltation, and less competition for space. All of these studies were performed in South Carolina, Georgia, or Florida where climates are substantially warmer than in Virginia, and thus might not be representative of temperate climates. Roegner (1989) did examine oyster mortality as a function of tidal height in Virginia, but was unable to detect a consistent trend. In the first series of tests, Roegner found higher mortality in the upper intertidal and low littoral zone than in the subtidal zone. This result was attributed to extremely high temperatures observed during the study period. However, in another series of studies conducted in the fall, Roegner found greater survivorship in the intertidal zone than in the subtidal zone. Unfortunately, no data were collected during the winter months.

Physical and biological forces may also all play instrumental roles in shaping survivorship within the reef substrate itself. Lund (1957) and Haven and Morales (1967) found biodeposition of oysters significantly increased sediment deposition rates by as much as 7 to 8 times. These elevated rates of deposition along with decreased current flows may be highly detrimental to oysters residing below the surface. In fact, Bahr and Lanier (1981) found that biodeposits and silt often bury oysters in underlying layers of established reefs. However, burial may not be a significant problem on newly formed artificial reefs where oyster density at the surface is still low and sedimentation is not heavy. In fact, residence in these lower levels may actually elevate survivorship because this underlying environment may provide protection from desiccation and solar radiation. From a biological standpoint, these underlying layers may provide refuge from predation and algal growth, which may further enhance survival. These are only speculations, however. We can only hypothesize the effect of substrate depth on survivorship because there is no research in this area.

Studies examining how tidal height or substrate layer affect disease incidence are limited. Littlewood et al. (1992) found that aerial exposure does not significantly affect *H. nelsoni* parasitism. But this study failed to furnish a comparison with subtidal *H. nelsoni* incidence. Although incidence may not have differed significantly among the aerially exposed tidal heights, it may have been significantly lower than in the subtidal zone. Burrell et al. (1984), in a study on the effect of tidal exposure on *P. marinus* prevalence, also found non-significant results; however, they caution that temporal and spatial variation may have influenced the results. Clearly, additional research is warranted. Aerial exposure has been shown to limit other water-born protozoan parasites in oysters. For instance, incidence of the parasite, *Mikrocytos roughlei,* in *C. virginica* decreases with increased aerial exposure (Farley et al. 1988). Furthermore, there is nothing known of how disease changes with substrate depth. Although it is clear that research needs to be done in these areas, the effects of disease are not directly examined in this study. This study focuses on early reef colonization processes when oysters are all less than 1.5 years old, an age when disease has not reached lethal levels in the majority of organisms.

Conclusion

It is evident that there is an intriguing void in the research on settlement, growth, and survival of oysters as a function of tidal height and substrate level. There is even less known about these processes on oyster reefs which extend from the intertidal to the subtidal zone. Many of the researchers who have examined reef oysters have utilized plates, shell strings, or suspended cages because tracking and examining oysters in reef settings are difficult. However, when these substrates are used, results are not entirely representative of conditions that exist within the reef ecosystem, and all of these methods fail to address the question of substrate level. This study provided a unique opportunity to sample a reef during its early development when sampling is highly feasible. This was accomplished by first constructing an intertidal reef structure to which oysters could attach and grow and monitoring early colonization processes on it shortly after it was built. The reef was composed mostly of oyster shell which is the foundation of oyster reefs which exist today, and thus was representative of a reef structure found in nature--just one not yet inhabited.

In this paper, I present data collected on the artificially constructed reef in two separate studies: a pilot study and a primary study. The objectives of the pilot study were to develop an understanding, either through experimental tests or field observations, of the effects of certain factors on oyster settlement, growth, and mortality at the reef site, and to determine which reef sampling techniques are most practical and representative of reef conditions. The main objective of the primary study, which incorporated knowledge gained in the pilot study and was the primary focus of this paper, was to document oyster settlement, growth, and survival processes within a reef ecosystem along a tidal gradient and at varied substrate levels. Two lesser objectives of the primary study were to document how all of the above factors and processes varied with time and how settlement, growth, and mortality at the reef site were influenced by water movement and/or oyster microscale density.

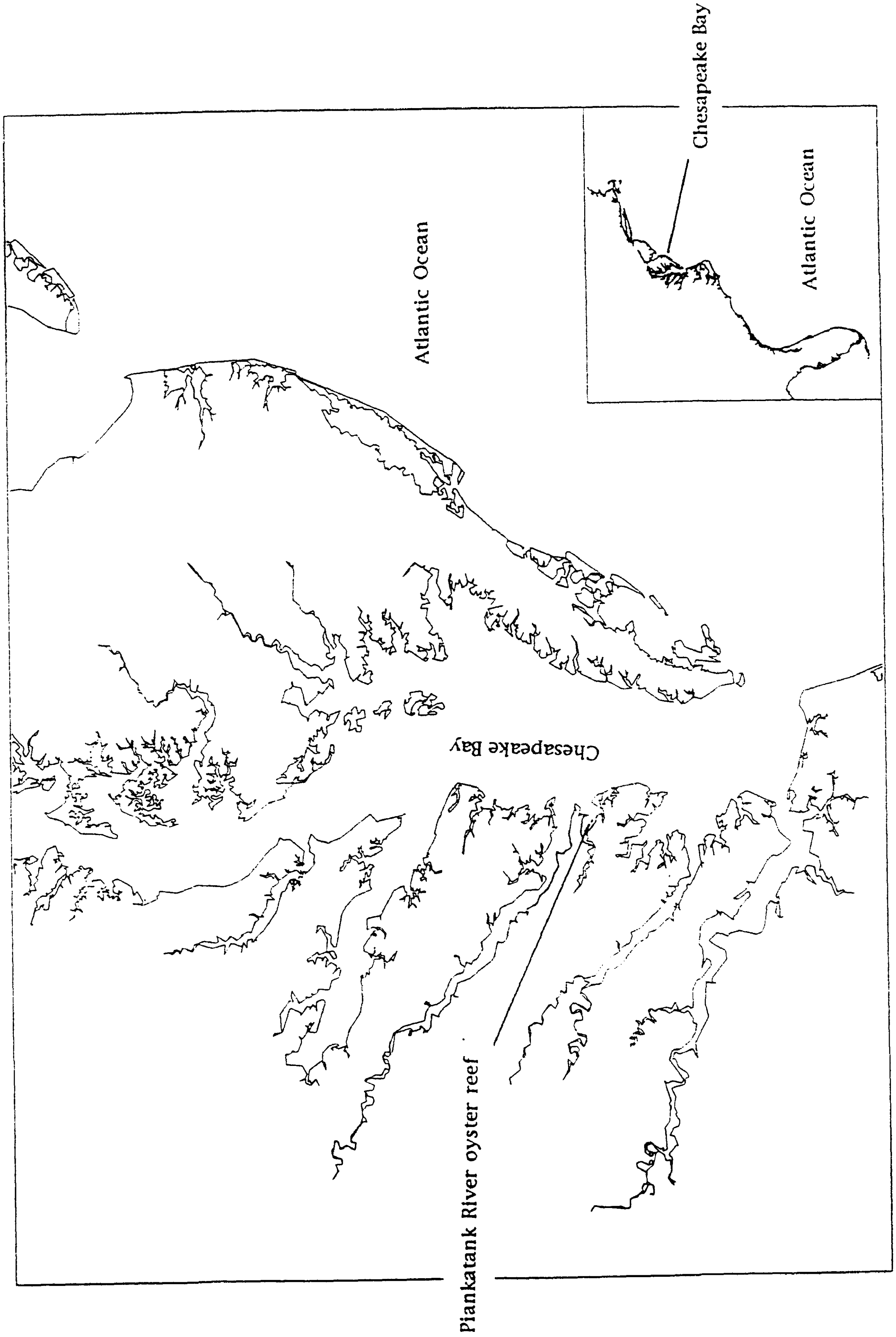
MATERIALS AND METHODS

Study Site

The location selected for this study was a sandbar known as Palace Bar in the Piankatank River, a sub-estuary of the Chesapeake Bay, located in Virginia (Figure 1). This site was chosen for a number of reasons. First, during colonial times this area supported an intertidal reef system. This is important because the establishment and subsequent maintenance of intertidal oyster reef communities depends on a specific set of interacting physical and biological processes, and will only persist when these conditions are met (Bahr and Lanier 1981). Since this site supported oyster reefs in the past, the conditions are probably favorable for oyster reefs today. Second, oyster larval abundance and "strikes" (larval attachment to substrate) are consistently high in the Piankatank River each year as documented in the VIMS Spatfall surveys. Furthermore, this area lacks a commercial oyster fishery presence and is relatively pristine--there is virtually no industry or agricultural development and residential density is low.

Tidal range at this site was small (mean range = 36 cm) and local meteorological events, wind in particular, had a substantial effect on this range. Water depth at the reef site ranged from 1 to 3 meters below mean low water (MLW) and current flow was substantial, especially in the intertidal zone. During the course of this study water temperature varied from .5 - 30°C and salinity fluctuated from 8-18 ‰.

Figure 1. Map of reef site, Piankatank River, Chesapeake Bay, Virginia.


Chesapeake Bay
Atlantic Ocean
Atlantic Ocean
Chesapeake Bay
Piankatank River oyster reef

Reef Construction

The artificial intertidal reef was constructed by the mass deployment of cleaned, aged oyster shells off barges using high pressure hoses. This process was performed by the Virginia Marine Resource Commission (VMRC) in June of 1993. Shells used in the construction were discharged in an area approximately 150 x 30 meters in dimension. This area was marked by buoys along its east and west flanks and by two pier pilings at its north and south extremities. Approximately 22 shell mounds aerially exposed at mean low water (MLW) and about 50 subtidal mounds comprised the reef system when it was first constructed. Many of the intertidal mounds were small (< 5 m of aerially exposed diameter at MLW) and some, near the northern boundary, were composed exclusively of crushed clam shells instead of oyster shell. After the 1993-1994 winter, it quickly became apparent that the reef was not a static structure. During this period, several mounds coalesced to form large mounds. Others underwent "mound decapitation", whereby mound peaks were dislodged by ice and deposited subtidally. The result of these displacement processes was a reduction in the number of hillocks exposed during mean low water from 22 to 12.

Methodology

This study was conducted during 2 distinct sampling periods. In the first sampling period (June '93 through May '94), a pilot study was performed to identify which parameters (i.e. mound site, plot orientation, time, and/or tidal height) most affected settlement, growth, and mortality of oysters on the reef and to determine which methods of sampling were most appropriate. Due to the large number of parameters and time constraints, substrate depth was not examined directly and only a limited section of the reef environment was sampled. During the second sampling session (June '94 through September '94), after data from the first period could be processed and certain parameters eliminated, a more geographically expansive design, which considered substrate level, was used.

Sampling Session I (Pilot study)

Settlement

Settlement monitoring of the reef began shortly after construction in mid-June. Initially, two aerially exposed mounds were selected for this study: one near the southern periphery and a second situated in the middle of the reef. The peripheral mound was completely exposed to wave action and currents and was covered with a thick layer of algal growth, whereas the middle mound was shielded from wave energy and currents and was relatively free of algal film. These mounds were chosen because they represented the two most extreme reef environments (high energy with significant algal growth and partially protected with light algal growth), and thus enhanced the probability of detecting a mound location effect if one was present.

At each of the mounds two transects were established, each situated in the most extreme orientations: upstream and downstream. The rationale for using the two most extreme orientations again was to elevate the chance of detecting a significant difference among orientations. Along each transect, four tidal heights were selected (30 cm above MLW, MLW, 45 cm below MLW, and 90 cm below MLW). These heights extended from the high intertidal to the low subtidal zone (water depth near the 2 mounds rarely exceeded 1 m during low tide). Weekly non-destructive and destructive sampling of the reef was performed from June through September. Non-destructive sampling involved the placement of 20 clean oyster shells in 64 x 20 cm open-topped trays secured to the reef surface by reinforced bars at each of the four selected tidal heights along the two transects on each of the two mounds. The shells were arranged within the trays to resemble an approximate surface layer, but no effort was made to manipulate shell orientation (i.e. all shells concave side up, all shells concave side down, etc.) since natural, reef shells were haphazardly ordered. Each week shells were retrieved and replaced with new, clean shells. Both sides of the shells were then examined for spat (recently settled or juvenile oysters) using a dissecting scope. Both the concave and convex sides of the shell were considered because Michener and Kenny (1991) found that settlement pulses may go undetected if only one side of the spat collector is considered. After all 20 shells were examined, a spat total per tray was computed. This provided a weekly index of spat settlement.

Weekly destructive sampling was performed to obtain a cumulative index of spatfall minus any early post-settlement mortality. Each week a 64 x 20 cm quadrat (same dimensions as trays) was placed on the reef surface at each of the four tidal heights on the upstream and downstream orientations of each of the two mounds. To prevent resampling the same area, positions of the quadrats were shifted over one quadrat length each week. An approximate surface layer of shells (generally 20 - 30) was removed and examined at the lab. Both shell surfaces were considered. A standardized spat count per 20 shell quadrat sample then was calculated. Shellstrings suspended from pilings at the north and south reef extremities to a depth of 90 cm below MLW were used to measure settlement as well. Shellstrings consisted of 12 single valve oyster shells, each with a hole drilled through the center and threaded onto galvanized wire. The shellstrings were deployed and retrieved weekly, and the concave and convex sides of the ten intermediate shells were examined. A spat total per shell was then computed.

Size class and mortality

Size class and mortality assessment began after the settlement season in October 1993, when oysters could be distinguished with the naked eye and were therefore easy to examine quickly and accurately. Samples were collected October 7, November 4, December 2, and May 26. The months of January through April were omitted because of inclement weather. Sampling involved the placement of quadrats (identical to quadrats used in the settlement study) at the 16 stations along the 4 shifting transects previously described. A surface sample of shells containing juvenile oysters was removed and returned to the lab. Shell heights of 50 oysters were recorded and an overall mean shell height was computed for each of the 16 quadrat stations. Initially, spat mortality, defined as gaping, non-responsive spat or recent dislodgment scars, was recorded for each sample. However, it soon became apparent that samples could not be processed rapidly enough to avoid post-field mortality, and recent mortalities were hard to discern on the heavily fouled shells. Thus, this method of mortality assessment was discontinued.

Due to uncertainty over whether significant spatfall would occur, destructive size class and mortality sampling were supplemented with the monitoring of hatchery-reared oysters placed on the reef. At the VIMS Oyster Hatchery on August 12, 1993, oyster larvae were set on 1600 clean oyster shells (30 - 70 larvae per shell). Shells containing spat were placed in plastic Vexar mesh bags (100 shells per bag), and spat were reared in hatchery systems to sizes comparable to oysters found on the reef. Before deployment into the field, the number of hatchery spat used was estimated by a spat count of 400 randomly selected shells (25% of the total number of shells), and an initial mean size was calculated based on shell height measurements of spat found on 25 randomly selected shells (1025 spat in all). On September 26, 1993 the mesh bags were placed on the reef at the same plots that the non-destructive settlement trays had been placed along the 4 transects. On October 14, November 11, and May 5, 25 oyster shells were haphazardly selected from each bag (which was shaken vigorously prior to shell selection), and the shells were photographed on slide film with an Olympus OM camera equipped with a 50 mm macro lens. The concave, shiny side and convex, rough side of each shell were photographed. However, due to difficulty in deciphering the subtle, translucent boundary of spat valves against the dark background of the rough shell side, spat affixed to this side were eliminated from consideration. Slides were first placed on the stage of a dissecting scope fitted with a video camera, and images were observed on a Sony 13 inch video monitor. Shell boundaries were traced using a mouse pad and digitizing tablet, and shell areas were calculated and stored on a 386 MHz Compaq computer, running an optical pattern recognition software package manufactured by Biosonics Inc (1988). Recent spat scars on each shell were noted and proportional mortalities (# of scars per shell / # scars per shell +

live oysters) were calculated. Mean shell areas and proportional mortalities per 25 shell sample then were computed for each sampling date.

Physical Parameters

During all sampling runs, water temperature, salinity, and secchi disk readings were collected . Air temperature, approximate wind speed and direction, cloud cover, and tide conditions were all noted. Detailed field observations were recorded as well.

Statistical Analysis

This pilot study was designed to develop an idea of what factors were most influential in shaping the processes of settlement, growth, and mortality, not to document precisely the effects of these factors, and thus some liberties were taken in its design. One such shortcoming of the pilot study was that it incorporated insufficient replication to consider many higher order interactions. Because higher order interactions could not be quantified, all between factor interaction terms were assumed to be zero, and differences among means were entirely attributable to the effects of the experimental factors operating independently of one another (Underwood 1981). Although this was a large assumption, it must be stressed that results of these analyses were only used as a starting point for determining which factors should be examined more thoroughly in the primary study. Another consideration in the analysis of the data was that in both the non-destructive settlement and hatchery-reared oyster size class/mortality studies, successive samples were taken from either spatially fixed areas or from the same population of organisms. These samples are clearly not independent and should not be analyzed as such if pseudoreplication is to be avoided (Hurlbert 1984). Furthermore, both destructive settlement and size class samples taken at different times may not be entirely independent, since new plots selected each week or month were spatially connected to plots selected during the previous sampling period.

As a result of the above conditions, 4-way fixed-factor (factors: tidal height, time, mound site, and plot orientation) analyses of variance (ANOVA) with repeated measures on time and blocking on tidal height, mound, and orientation were run on each data set. Multivariate repeated measures analyses were performed on all data except hatchery-reared oyster shell areas, which were analyzed using univariate repeated measures analyses because of missing values. Between subject variances were tested for homogeneity using Cochran's C-test (Winer et al. 1991), and within subject variances were tested using the F max statistic detailed in Winer et al. (1991). To satisfy assumptions of homogeneity, all settlement data was log (x +1) transformed and proportional mortality data was arcsine transformed. Greenhouse-Geisser and Hunyh-Feldt probabilities were calculated to measure the extent to which the correlations of the observations violated the validity of the p-values computed in the repeated measures analyses, which were based on the F distribution. These adjusted p-values were not found to deviate substantially from p-values derived from the F distribution, and thus will not be presented.

In cases where significant interactions between the within factor, time, and any other factor were detected, lower level ANOVAs were performed on data collected at each sampling time and significant main factor effects were examined using Student-Newman-Keuls (SNK) multiple comparison tests. Lower level repeated measures were not run for each level of the other factor involved in the interaction, however, because low degrees of freedom (as a result of limited replication) precluded a meaningful analysis (Winer et al. 1991). When no significant interactions between the within factor, time, and any other factor were detected, 4-way fixed-factor ANOVAs with blocking on all factors were performed. Significant main effects were examined using SNK tests.

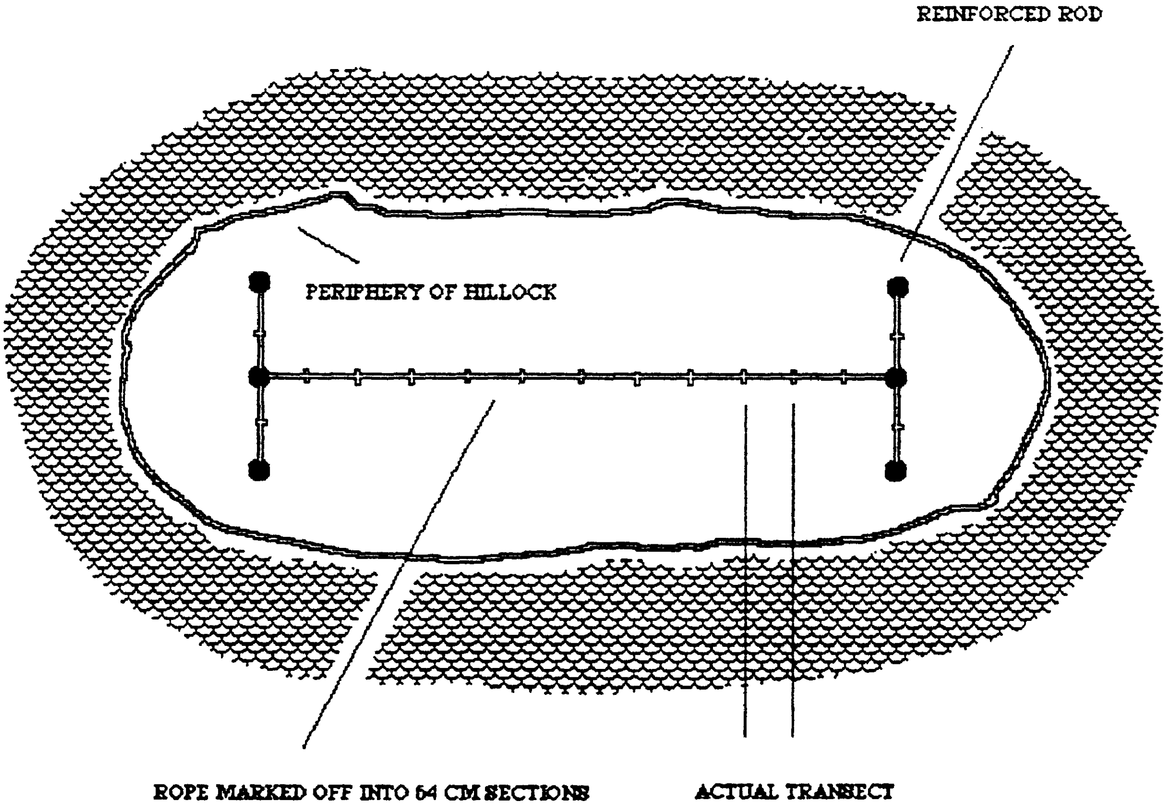
Sampling Session II (Primary study)

Based on the results of the pilot study, it was apparent that mound position and plot orientation were not as critical as tidal height and time in shaping oyster settlement, growth, and mortality (see results and discussion of pilot study). Thus, mound position and plot orientation were eliminated. This reduction in factors reduced sampling time and allowed for the development of a more comprehensive and geographically expansive sampling design (a design that considered most intertidally exposed mounds). The additional sampling effort also allowed for the investigation of another factor, substrate layer, which may be a critical variable influencing settlement, growth, and mortality at the reef site.

In the second sampling session, 8 of the 12 remaining intertidal mounds at the reef site were considered. Four mounds were eliminated because they barely protruded above the water surface at MLW, and thus did not allow for the examination of heights above MLW. Each of the 8 mounds, which varied in size and orientation, was partitioned into 64 cm transects that encircled each mound (Figure 2). This was accomplished by first establishing at what elevation on each mound the highest intertidal height to be sampled was located. Two reinforced bars were then pounded into the reef surface at this level along the longitudinal axis of each mound. This axis was easily discernible since all 8 mounds were approximately elliptical in dimension. A rope divided into 64 cm sections using colored tape was tied to the reinforced bars. This created a set of transects on each side of the longitudinal axis. To avoid omitting the ends of the mounds in the sampling scheme, two additional axes that ran transversely to the longitudinal axis were established at each reinforced bar. Additional reinforced rods were then driven into the reef along these axes at the highest sampling elevation. A rope marked into 64 cm sections was tied to each set of reinforced bars establishing sets of transects off each end of the mound.

In contrast to the first sampling period, only 3 tidal heights were considered during this sampling period: 25 cm above MLW, MLW, and 90 cm below MLW. The high intertidal height was lowered slightly (by 5 cm) to accommodate as many intertidal mounds as possible. One of the subtidal heights, 45 cm below MLW, was eliminated to incorporate more replication and because settlement, growth, and mortality at 45 cm below MLW were not found to be appreciably different than at 90 cm below MLW. At each of the 8 mounds,

Figure 2. Diagram of the division of a sample mound found at the Piankatank reef site into transects. Shaded circles represent reinforced rods pounded into the reef at the +25 cm tidal height (highest tidal height considered) and lines represent rope marked off every 64 cm.


REINFORCED ROD
PERIPHERY OF HILLOCK
ROPE MARKED OFF INTO 64 CM SECTIONS
ACTUAL TRANSECT

the 3 heights were marked with reference stakes so that they could be located expediently within any given 64 cm transect.

Settlement

Plots chosen for the settlement study were selected randomly across all 8 mounds. This was accomplished by first labeling ping pong balls with all of the transect designations from each of the 8 mounds, placing the balls in a basket, shaking the basket, and randomly selecting 4 balls. This procedure was repeated for each of the three fixed tidal heights and performed weekly to generate randomly selected replicate plots for all of the weeks of sampling from June 1, 1994 through October 1, 1994. At all 12 weekly selected plots, a 64 x 20 cm open-topped, 1-inch plastic coated tray separated into 3 tiers was buried into the reef substrate until the upper tier was level with the reef surface. The tray was secured to the reef using a reinforced bar. The upper and lower tiers, which were separated by exactly 10 cm, both contained 30 clean oysters shells, whereas the intermediate layer contained 40 shells and served as a complete border for the upper and lower layers. The 30 shell surface and bottom layers were examined for spat using a dissecting microscope and a spat total per layer was computed. Both concave and convex shell sides were considered. A sample size of 30 shells was used because asymptotic plots of spat data collected in the pilot study indicated that approximately 30 shells were needed to accurately calculate settlement intensity. A spat count for the intermediate layer was not calculated because shells comprising this layer served solely as a barrier between surface and deep tray levels. Forty shells were used in this layer because they formed a relatively uniform layer free of large gaps.

In addition to the weekly settlement tray samples, 3 shellstrings (wires containing 12 shells used to detect spat) were placed on the reef. One was located on a pier piling at the northern extremity of the reef, another at a piling at the southern extremity of the reef, and the final shellstring was placed on a subtidal mound in the middle of the reef. All shellstrings were suspended in the water column to a depth of 90 cm below MLW. These shellstrings along with 100 haphazardly selected shells from the settlement trays were examined for spat the day of sampling using a dissecting scope. If no spat were detected, no further examination was conducted. However, if any spat were detected all of the settlement tray samples were examined, and a destructive sampling regimentation was initiated the following day.

Destructive sampling was conducted from July 15 through August 18, 1994 and involved the weekly random selection of 4 replicate plots for each of the three fixed heights, using the method described earlier. At each plot, a 64 x 20 cm quadrat was placed on the reef surface and a surface layer of 30 shells was removed. After the surface layer was taken, a ruler was inserted into the reef surface, and reef substrate was extracted until the (- 10) cm layer of shells was visible. This layer was easily distinguishable from the surface layer due to a characteristic brown detrital film covering the shells. Thirty shells were subsequently taken from the reef at this depth. Both shell layers were examined for spat and a spat total per 30 shell layer was recorded.

Growth and Mortality

During the pilot study, only size class estimates and approximate mortality could be determined because individual oysters were not tracked. To document growth and acquire more precise mortality data, a design which allowed for the following of individual oysters was employed. In this design, 2 year classes of oysters were considered. The younger year class of oysters consisted of hatchery oyster larvae set on the concave, smooth side of 720 oyster shells on May 16, 1994 in the VIMS Oyster Hatchery. After settlement, the shells were grouped into 48 assemblages each containing 15 shells. Within each assemblage, every shell was labeled with a number from 1 to 15 using paint and permanent marker. Oyster spat were counted to make sure at least 30 organisms were present collectively on the smooth surface of shells in each assemblage. Excess organisms which crowded test oysters or oysters that were oriented vertically, and thus difficult to measure in a 2 dimensional photograph, were harvested. An attempt was made to keep oyster densities (# of oysters per shell) within each assemblage as uniform as possible. However, it was impossible to make densities identical due to the high degree of variability in the settlement behavior of the hatchery oyster larvae.

The older year class of oysters consisted of hatchery oysters reared on August 12, 1993 in the VIMS Oyster Hatchery as part of the pilot study. Oysters comprising this year class were all taken from a well mixed sample of oysters that had been situated either 45 or 90 cm below MLW on the Piankatank oyster reef. As with the first year class of oysters, 720 shells were marked with numbers from 1 to 15 using permanent and paint markers until 48 assemblages of 15 shells had been labeled. Oysters were subsequently counted to make sure at least 30 oysters were present collectively on the smooth surface of the 15 shells comprising each grouping. Excess oysters that crowded test subjects considerably or that were oriented vertically were extracted, and again an attempt was made to maintain approximately uniform oyster densities within each assemblage. Considerably more oysters were removed from shell for the 1993 year class than for the 1994 year class of oysters because of greater initial setting densities.

For both year classes of oysters, 8 plots for each of the 3 fixed heights (the same heights used in the settlement study) were selected randomly using the "ping pong" method described earlier. Within each year class, all 24 plots were assigned 2 numbers (one designating the surface substrate level, the other the deep substrate level) from 1 through 48. All assemblages within each year class were then assigned a number from 1-48 using a random number table, and were labeled with the corresponding plot and layer information.

Photographs of all shells, in sets of 3, were taken for both year classes of oysters, and the shell areas of 30 oysters in each assemblage (48 total assemblages per year class) were calculated using the optical pattern recognition software and hardware described earlier. A map of the spat on each marked shell was constructed by hand to allow for individual oysters to be distinguished and tracked in future study. Initial densities for oysters belonging to the 1994 year class (x=2.85 ± .35 SE spat per shell) and 1993 year class (x=2.95 ± .48 SE spat per shell) were not found to be significantly different across tidal height or substrate layer (2-factor ANOVA for 1994 oysters; tidal height : F=.074, df=2,42,p=.940; substrate level: F= .41. df=1,42, p=.513; 2-factor ANOVA for 1993 oysters; tidal height: F=.181, df= 2,42, p=.672; substrate level: F=.507, df=1,42, p=.606). Similarly, initial shell areas for 1994 oysters (x=1.18 ± .10 SE cm^2) and 1993 oysters (x=4.3 ± .52 SE cm^2) examined across tidal height and time were not found to significantly differ (2-factor ANOVA for 1994 oysters; tidal height: F=.071, df=1,42, p=.7916; substrate level: F=.014, df=1,42, p=.9077; 2 factor ANOVA for 1993 oysters; tidal height: F=.945, df=2,42, p=.397; substrate level: F=.695, df=1,42, p=.409).

Twenty-four cages, 32 x 20 cm, were constructed out of plastic coated 1 inch chicken wire for each of the 2 year classes, and each cage was given a plot label. Every cage contained 3 levels, the upper and lower of which were spaced 10 cm apart. The upper and lower levels were both filled with the appropriate shell assemblages, and the upper layer was enclosed by chicken wire. The middle level was filled with 20 shells devoid of spat to serve as a boundary between the upper and lower layers.

The 1993 and 1994 year class oyster cages were deployed into the field on June 14 and June 20, respectively. The cages were buried into the reef substrate at their designated plots so that the upper layer was even with the reef surface and held in place by a reinforced rod. On July 12 and August 9 for the 1993 year class and on July 19 and August 16 for the 1994 year class, the cages were retrieved and placed on board a boat. All cages were cleaned using a 3 HP gasoline powered Homelite water pump, opened up, and emptied into labeled buckets. To maximize photographic clarity and to ensure accurate mortality data, each shell was scrubbed thoroughly and gaping, non-responsive spat present on the shell were removed, leaving highly visible scars. Shells were photographed in sets of 3 on slide film using a Nikonos V waterproof camera equipped with a close-up lens and focusing frame. Oysters were then returned to their appropriate cages, which were secured shut with cable ties. All cages were returned to their designated plots later that day. This study was terminated on September 6 for the '93 year class and September 13 for the '94 year class, at which time all oysters were recovered, returned to VIMS, cleaned, and photographed.

All slides were processed using the Biosonics image analyzing system described earlier. Each of the 30 individual spat found within the shell assemblages was tracked, and individual growth was recorded using the formula:

$$\text{GPD} = \frac{A_{(t)} - A_{(t-1)}}{d}$$

where GPD = growth per day (mm x mm / day)

A = shell area (mm x mm)

t = sampling time

d = days within sampling interval

A mean growth value expressed in mm^2 / day then was computed for each layer of each cage for each sampling interval. Proportional mortality per layer of each cage for each sampling interval was computed as well, using the formula:

$$\text{PM} = \frac{S_t}{L_t + S_t}$$

where PM = proportional mortality

S = # of scars

L = # live oysters

t = time of sampling

Size Class and Density of Reef Oysters

In addition to measuring growth rates and mortality of hatchery-reared oysters, monthly size class and plot density measurements of naturally-set reef oysters were recorded from June through October. Each month 4 plots were selected randomly at each of the 3 tidal heights. A 64 x 20 cm quadrat was placed at each plot and a surface layer of 30 shells was extracted. A deep sample of 30 shells was also taken 10 cm below the surface. Shell heights of all oysters at each substrate level and densities expressed as the number of oysters present per 30 shell quadrat sample were recorded.

Physical Parameters

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 both the non-destructive settlement study and hatchery-reared oyster growth/mortality 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 height 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.

Statistical Analysis

Correlation coefficients were computed for all surface and deep samples taken in the destructive settlement study, the non-destructive settlement study, the growth study, the mortality study, the destructive reef size class study, and the destructive reef density study to determine if a relationship existed between the two levels. Coefficients were compared to values in a critical coefficient table (Zar, 1984), and if no significant relationship was detected, substrate level was treated as a factor in further analyses.

When significant relationships were detected, paired sample t-tests were performed. In cases where new sets of random plots were selected with each unit of time (settlement studies, reef size class study, and reef density study), paired sample t-tests were run on all data as a function of either tidal height or time. This could not be done for growth or mortality studies, however, since the same organisms were sampled throughout time. Instead mean values were computed over the sampling period, and these values were analyzed using paired t-tests as a function of the 3 tidal heights. To be certain mean values were representative of either growth or mortality during the 3 intervals of time, values were plotted against time for each tidal height.

Mean values for surface and deep samples were calculated when no significant differences were detected between the substrate levels, and further analyses were run on these mean values. If any significant difference among the levels was detected for a study, separate analyses were performed on each substrate level.

Factorial ANOVAs were performed on non-destructive and destructive settlement data and destructive reef density data. Settlement data was log $(x+1)$ transformed to meet assumptions of normality and homogeneity of variance, but density data was not because it was found to be approximately normal and had homogeneous variances (Cochrans's C test, $p > .05$). When significant interactions were detected, lower level ANOVAs were run on each level of the factors involved in the interaction. All differences between means were revealed using SNK multiple comparison tests.

An analysis of covariance (ANCOVA) was performed on destructive size class data using tidal elevation as a factor and time as a regressor. Data were found to be normally distributed and variances were homogeneous (Cochran's C test, $p > .05$). The tidal elevation by time interaction term was found to be non-significant, and thus the assumption of equality of slopes was met.

Multivariate repeated measures analyses of variance were performed on growth and mortality data for each year class of oyster studied. To satisfy the subject within group and subject between group homogeneity of variance assumptions, proportional mortality data was arcsine transformed. It was not necessary to manipulate growth data, however. Greenhouse-Geisser and Hunyh-Feldt probabilities were calculated to measure the extent to which the correlations of the observations violated the validity of the p-values computed in the repeated measures analyses, which were based on the F distribution. These adjusted p-values were not found to deviate substantially from p-values derived from the F distribution, and thus will not be presented. When interactions were present in the models, separate ANOVAs were run on data collected at each time interval and lower level repeated measures analyses were performed on each level of the other factor involved in the interaction. 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).

To compliment repeated measures analyses and paired t-tests performed on growth, linear regressions of oyster shell area on time were run for both year classes of oysters found at each tidal height and each substrate level. Plots of residuals versus time revealed that the data exhibited heteroscedasticity, and thus all data were log transformed to achieve homoscedasticity. All slopes within each year class were compared using the method explained in Zar (1984) pp. 300 - 301, and multiple comparisons between slopes were detected using Tukey's Test.

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. To determine if a functional dependence of growth and mortality on water movement was present, separate linear regressions of growth on water movement and arcsine transformed proportional mortality on water movement were performed for each year class, for each substrate level, and for each tidal height. Finally, to determine if a dependence of growth and mortality on density (# of oysters per shell) existed, linear regressions of growth and arcsine transformed proportional mortality on density were performed separately for each year class, for each substrate level, for each tidal height, and for each sampling date. The assumptions of the regressions were met as determined by residual analysis (Zar 1984).

RESULTS

Pilot Study

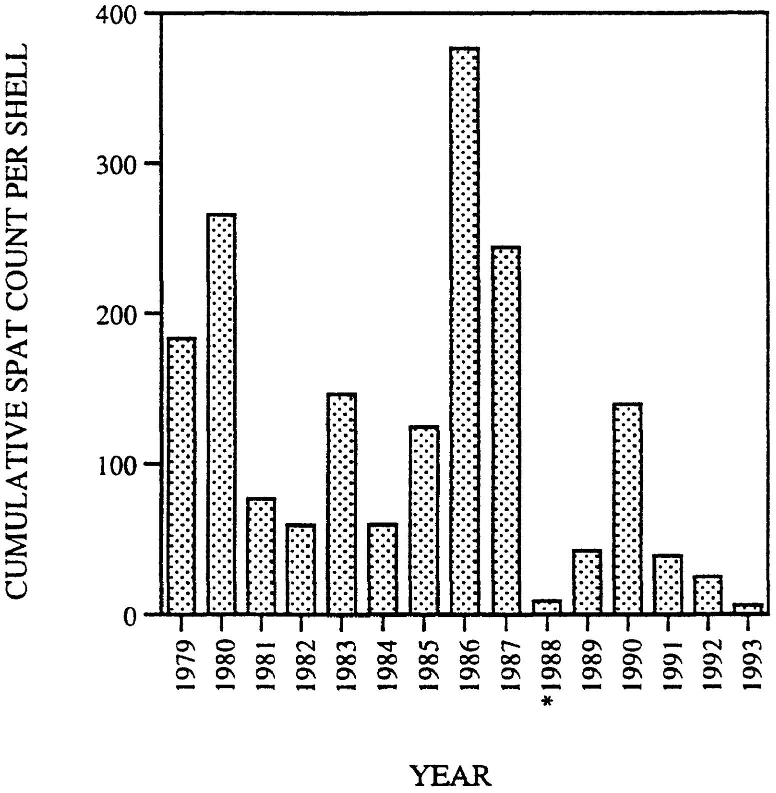
Settlement

The settlement season at the reef site lasted six weeks; spat detection began the week of August 12 - 19, and remnants of settlement were detected through the week of September 16 - 23, 1993. Cumulative spat counts per shell detected on shellstrings throughout the 1993 Summer at Palace Bar were the lowest in 14 years (Figure 3). Repeated measures ANOVAs performed on both non-destructive and destructive samples revealed that there were no significant interactive effects between the within factor, time, and any other main factor ($p > .05$) (Appendix 1a, b). Although the non-destructive time by tidal height interaction was not statistically significant ($p = .078$, Appendix 1a), it was clear that a greater percentage of the total spat detected settled during the first two weeks of the settlement season at the +30 cm tidal height than at any other tidal height (Figures 4).

As a result of the absence of significant interactions, 4-way fixed-factor ANOVAs with interaction terms assumed to be zero were performed on both samples. These analyses indicated that settlement was highly dependent on tidal height and time both on clean shell (non-destructive samples) and on reef shells (destructive samples) (Appendix 1c, d, respectively). Settlement was greatest subtidally; settlement at the -90 cm tidal height was significantly greater than at MLW and +30 cm, and settlement at the -45 cm tidal height was significantly greater than at +30 cm for both methods of sampling (Figure 5). For the destructive study settlement generally increased with time, whereas in the non-destructive study settlement was relatively constant during the first 3 weeks of the settlement season, but then decreased to significantly lower levels in weeks 5 and 6 (Figure 6).

There was a clear discrepancy between spat counts recorded from non-destructive and destructive samples. In week 1 of the settlement season (August 12 - 19), when spat counts between the two methods should be approximately equal because of no previous settlement, destructive sample spat counts were lower than non-destructive spat counts, and in week 6 of the settlement season (September 16 - 23), when only negligible amounts of spat were detected on non-destructive samples, a dramatic increase in settlement intensity was documented on destructive samples (Figure 6). A comparison of each of the three methods of spat detection deployed at the -90 cm tidal height revealed that shellstrings accumulated more spat per shell than either shells placed in non-destructive trays or shells sampled destructively from the reef (Figure 7).

Figure 3. Cumulative *C. virginica* spat counts per shell detected on shellstrings at Palace Bar, VA from 1979 to 1993 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 4. Percentage of total *C. virginica* spat set detected on non-destructive samples during the first two weeks of settlement season at four tidal heights at the Piankatank River reef site. The settlement season lasted six weeks beginning August 12 and ending September 23, 1993. Error bars denote +1 standard error.

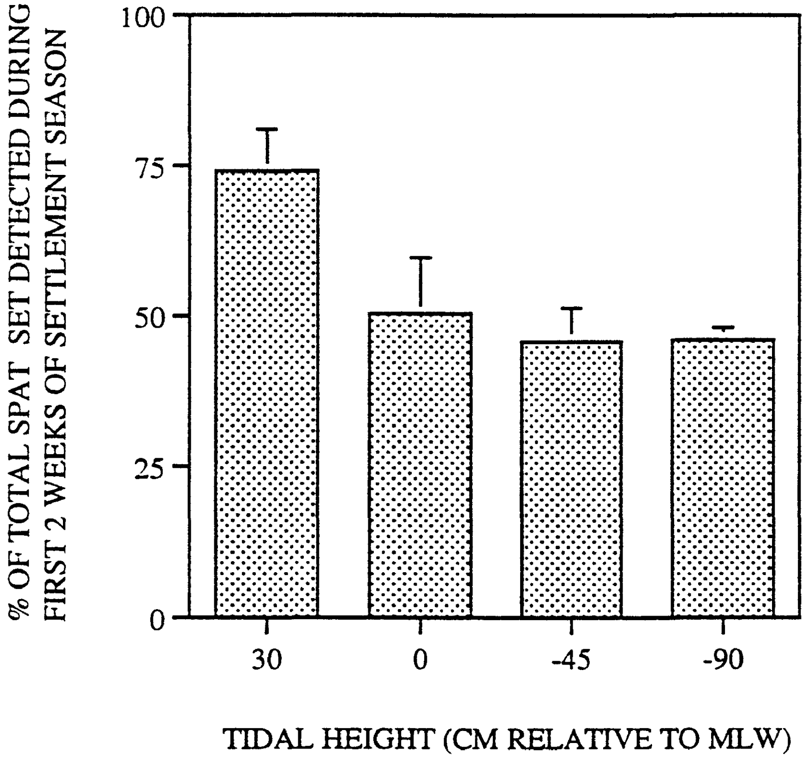
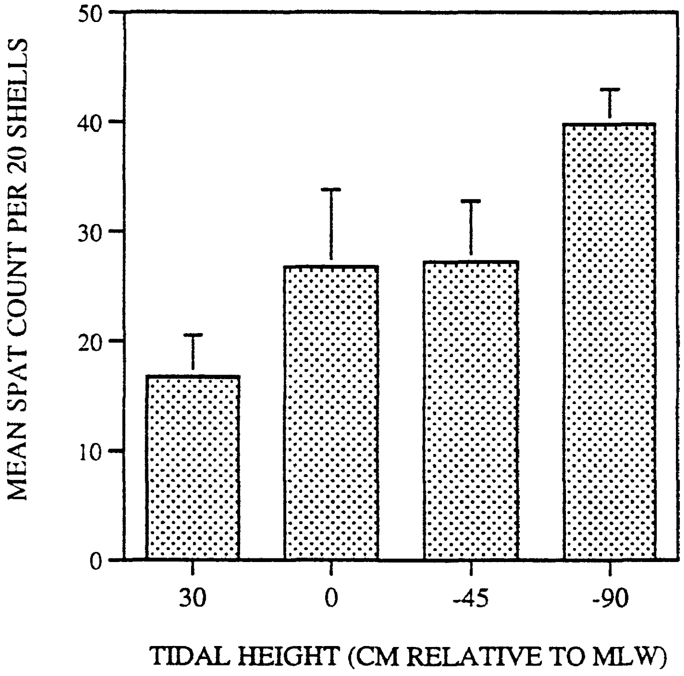

% OF TOTAL SPAT SET DETECTED DURING FIRST 2 WEEKS OF SETTLEMENT SEASON
100
75
50
25
0
30
0
-45
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)

Figure 5. Mean *C. virginica* spat counts per 20 shells over the settlement season for four tidal heights recorded from (a) non-destructive samples and (B) destructive samples. Error bars denote +1 standard error.

A) NON-DESTRUCTIVE SAMPLING



B) DESTRUCTIVE SAMPLING

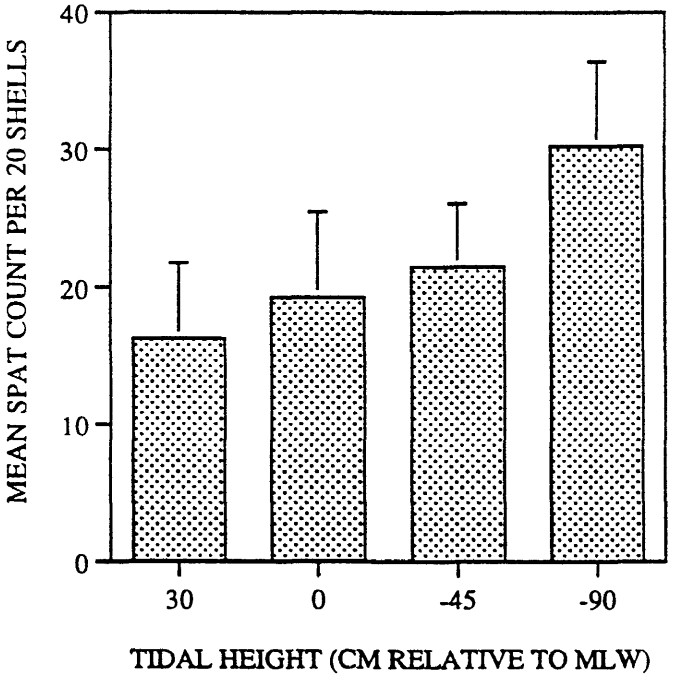
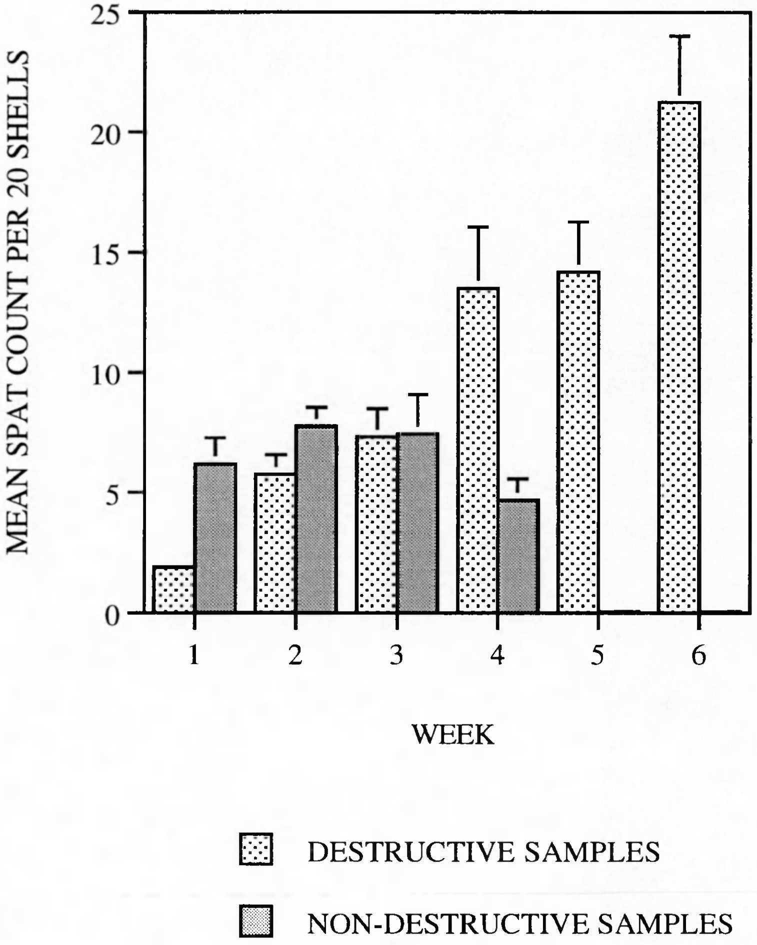
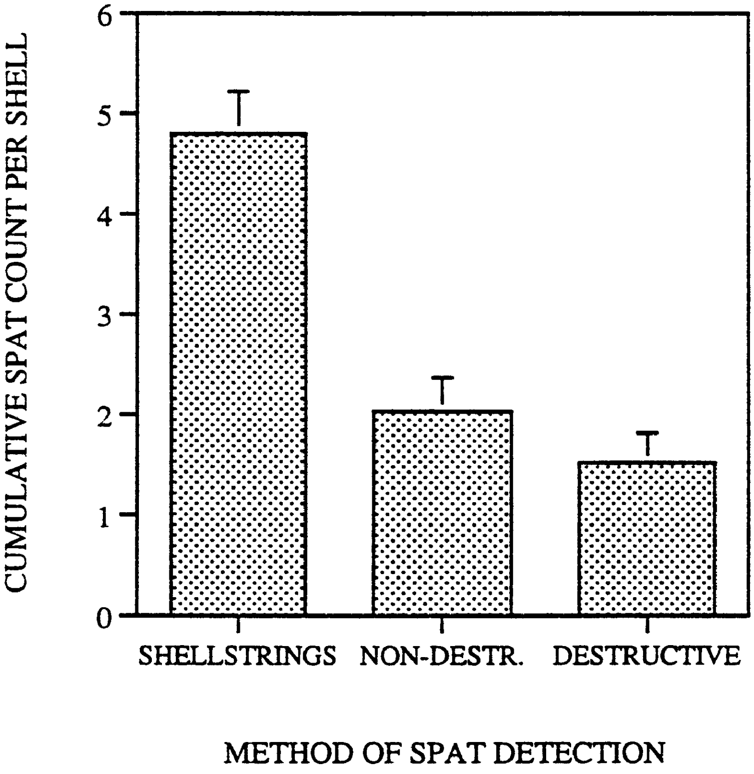


Figure 6. Mean weekly *C. virginica* spat counts for both destructive and non-destructive samples collected from August 12 through September 23, 1993. Error bars denote +1 standard error.



MEAN SPAT COUNT PER 20 SHELLS
25
20
15
10
5
0
1
2
3
4
5
6
WEEK
DESTRUCTIVE SAMPLES
NON-DESTRUCTIVE SAMPLES

Figure 7. A comparison of cumulative *C. virginica* spat totals per shell at the -90 cm tidal height detected on shellstrings, non-destructive tray samples, and destructive reef samples from August 12 through September 23, 1993. Error bars denote +1 standard error.


CUMULATIVE SPAT COUNT PER SHELL
0
1
2
3
4
5
6
SHELLSTRINGS
NON-DESTR.
DESTRUCTIVE
METHOD OF SPAT DETECTION

Area and Size Measurements

Due to the absence of significant interactive effects in the univariate repeated measures ANOVA of hatchery-reared oyster shell areas (Appendix 2a), a 4-way fixed-factor ANOVA devoid of interactions was run. In this analysis and subsequent SNK tests, shell areas were found to increase with increasing tidal depth and time (Appendix 2b). When shell areas were viewed graphically, it was apparent that shell area accretion slowed during the months of mid-November through early May (days 56 -231) (Figure 8).

A significant tidal height x time interaction was detected when oyster shell heights were analyzed using multivariate repeated measures procedures (Appendix 2c). When examined graphically this interaction became clear. Oysters at the +30 cm tidal height initially were as large or larger than oysters at subtidal heights, but as time progressed, oysters at +30 cm tidal height did not grow as rapidly as subtidal oysters and were surpassed in size (Figure 9). Lower level ANOVAs and SNK multiple comparison tests, used to analyze each component of time and decouple the tidal height x time interaction, supported this observation (Appendix 2d). As was found with shell areas, shell height enlargements were retarded during the months of December through May (days 56 - 231) (Figure 10).

Figure 8. Mean shell areas of C. *virginica* situated at four tidal heights measured over time (day 0 = September 26, day 28 = October 14, day 56 = November 11, and day 231 = May 5). Error bars denote ±1 standard error.

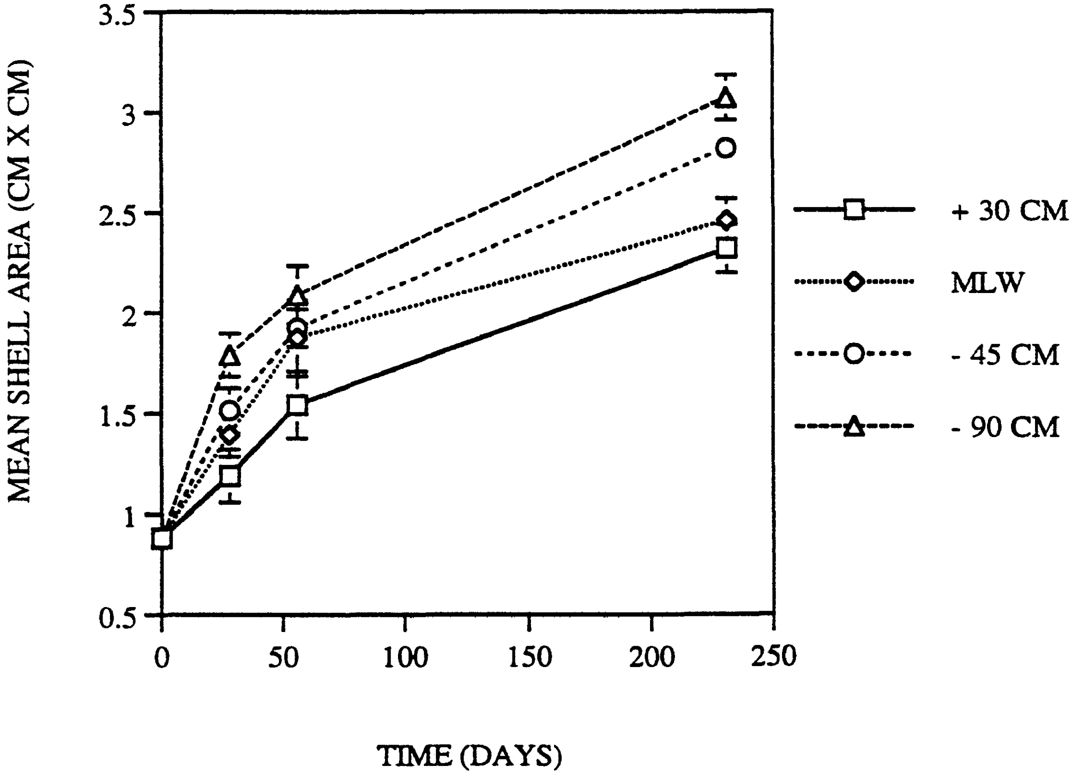

3.5
3
2.5
2
1.5
1
0.5
MEAN SHELL AREA (CM X CM)
0
50
100
150
200
250
TIME (DAYS)
+ 30 CM
MLW
- 45 CM
- 90 CM

Figure 9. Mean shell heights of *C. virginica* situated at four tidal heights examined from October 7, 1993 to May 26, 1994. Error bars denote ±1 standard error.

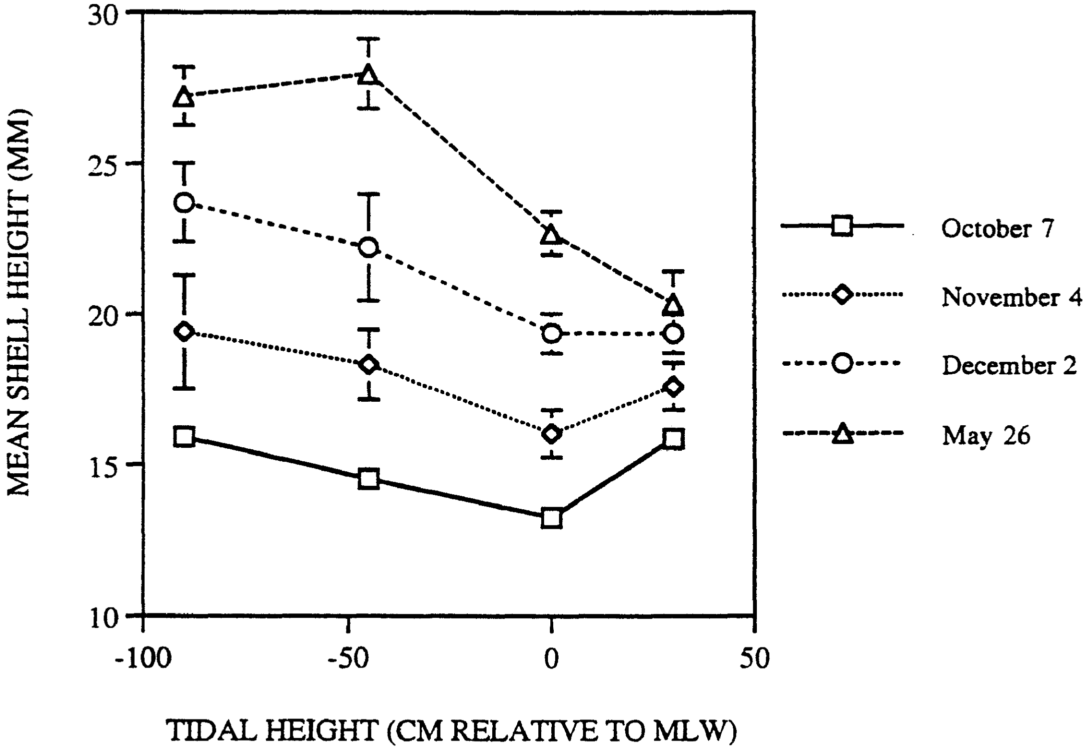
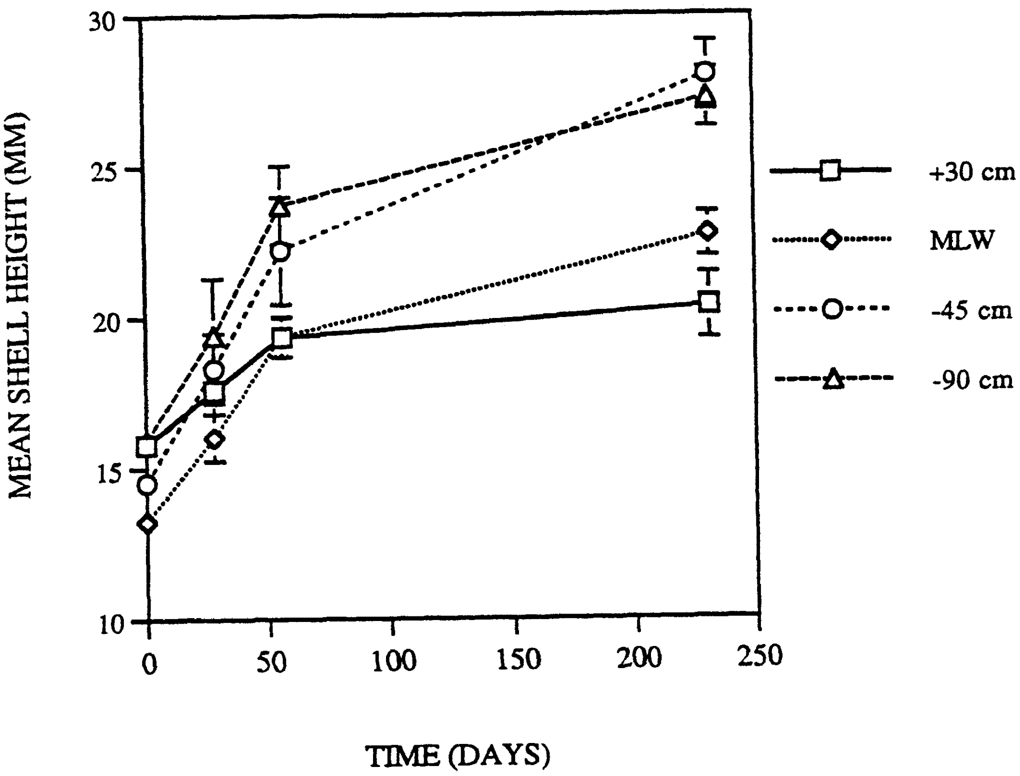

30
25
20
15
10
-100
-50
0
50
MEAN SHELL HEIGHT (MM)
TIDAL HEIGHT (CM RELATIVE TO MLW)
October 7
November 4
December 2
May 26

Figure 10. Mean shell heights of *C. virginica* situated at four tidal heights measured over time (day 0 = October 7, day 28 = November 4, day 56 = December 2, day 231 = May 26). Error bars denote ±1 standard error.


MEAN SHELL HEIGHT (MM)
30
25
20
15
10
0
50
100
150
200
250
TIME (DAYS)
+30 cm
MLW
-45 cm
-90 cm

Mortality

Both tidal height and time had a significant effect on mortality but mound site and orientation did not (Appendix 3a); however, the time by tidal height interaction effects were significant, precluding direct conclusions about the main effects (Underwood 1981). When this interaction was plotted, it appeared as if mortality was lowest at MLW during the early sampling intervals (September 16 - October 14 and October 14 - November 11), but then during the final sampling interval (November 11 - May 5) mortality at MLW as well as at + 30 cm increased dramatically (Figure 11). Separate analyses performed on each sampling time interval revealed that from September through October none of the factors had a significant effect on mortality, from October through November mortality was significantly lower at MLW, and from November through May mortality was greater at the intertidal depths than at the subtidal depths (Appendix 3b).

Physical Parameters

Salinity, water temperature, and secchi disk data collected during sampling runs from June 17 through December 2, 1993 are illustrated in Figures 12 - 14. Data were not collected over the winter. However, air and water temperature measurements recorded at the VIMS weather station, which is in the vicinity of the reef site, revealed that air temperatures dropped below freezing 28 times from December - March and water temperature dropped to a low of .59°C on January 17, 1994.

Figure 11. Mean percent mortalities of hatchery-reared *C. virginica* situated at four tidal heights sampled from September through May. Error bars denote +1 standard error.

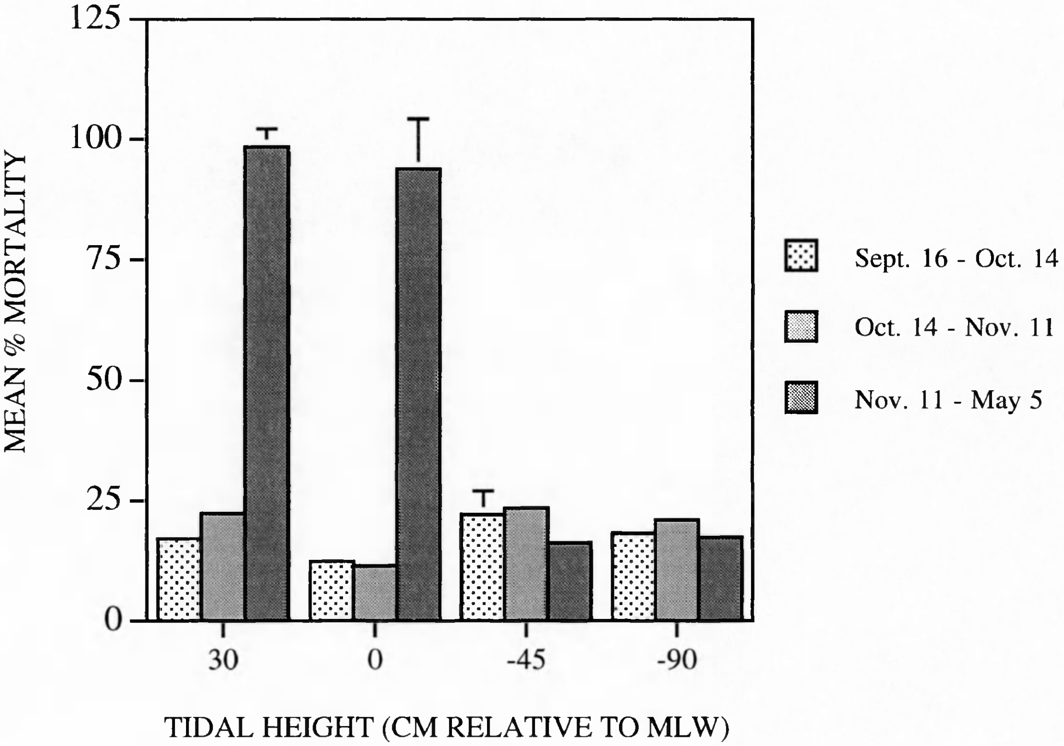
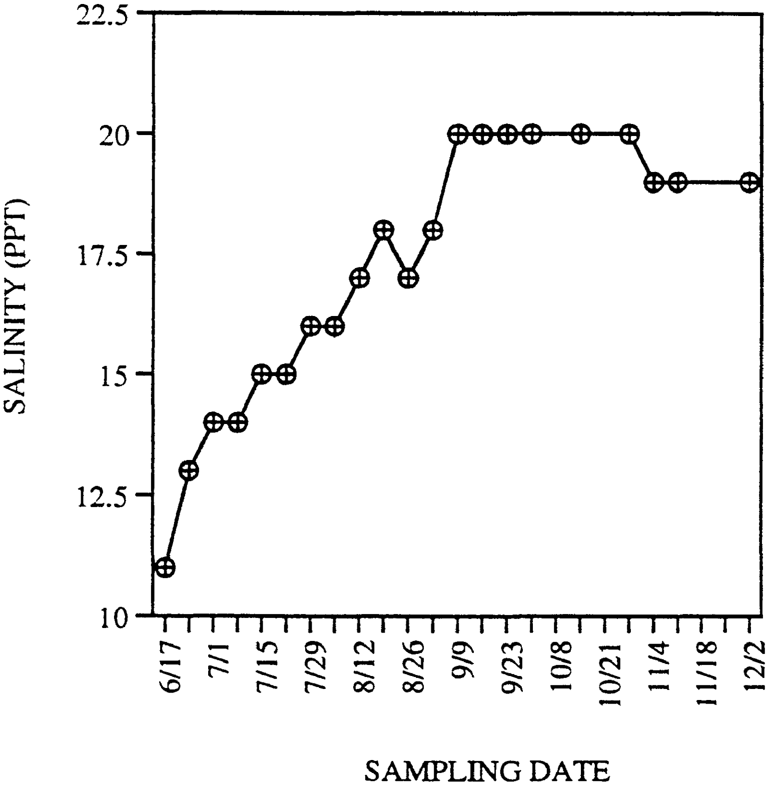

125
100
75
50
25
0
MEAN % MORTALITY
30
0
-45
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
Sept. 16 - Oct. 14
Oct. 14 - Nov. 11
Nov. 11 - May 5

Figure 12. Salinities at the Piankatank River reef site from June 17 through December 2, 1993.



22.5
20
17.5
15
12.5
10
SALINITY (PPT)
6/17
7/1
7/15
7/29
8/12
8/26
9/9
9/23
10/8
10/21
11/4
11/18
12/2
SAMPLING DATE

Figure 13. Water temperatures at the Piankatank River reef site from June 17 through December 2, 1993.

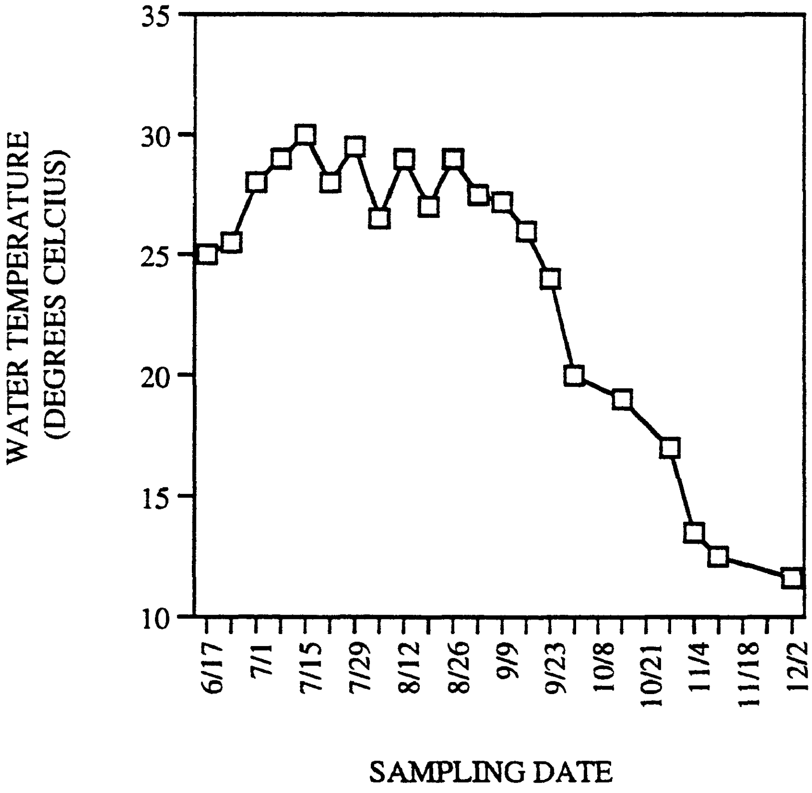
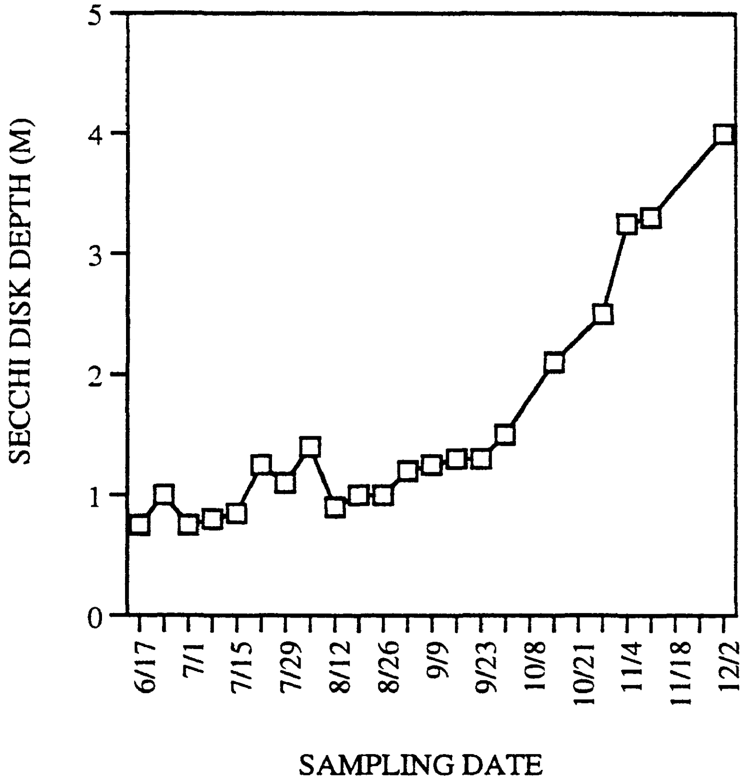

35
30
25
20
15
10
WATER TEMPERATURE
(DEGREES CELCIUS)
6/17
7/1
7/15
7/29
8/12
8/26
9/9
9/23
10/8
10/21
11/4
11/18
12/2
SAMPLING DATE

Figure 14. Secchi disk measurements at the Piankatank River reef site from June 17 through December 2, 1993. The y-axis refers to depth (m) below the water surface where the disk was still detectable.


SECCHI DISK DEPTH (M)
0
1
2
3
4
5
6/17
7/1
7/15
7/29
8/12
8/26
9/9
9/23
10/8
10/21
11/4
11/18
12/2
SAMPLING DATE

Primary Study

Relationship between Surface and Deep Substrate Levels

Significant correlations ($p < .001$) between surface and deep substrate levels were detected for the majority of experiments, with the exception of studies examining mortality (Appendix 4). Since no relationship was detected between surface and deep layers in the mortality studies, substrate level was treated as a main factor in mortality repeated measures ANOVAs. All other substrate level effects were examined using paired t-tests.

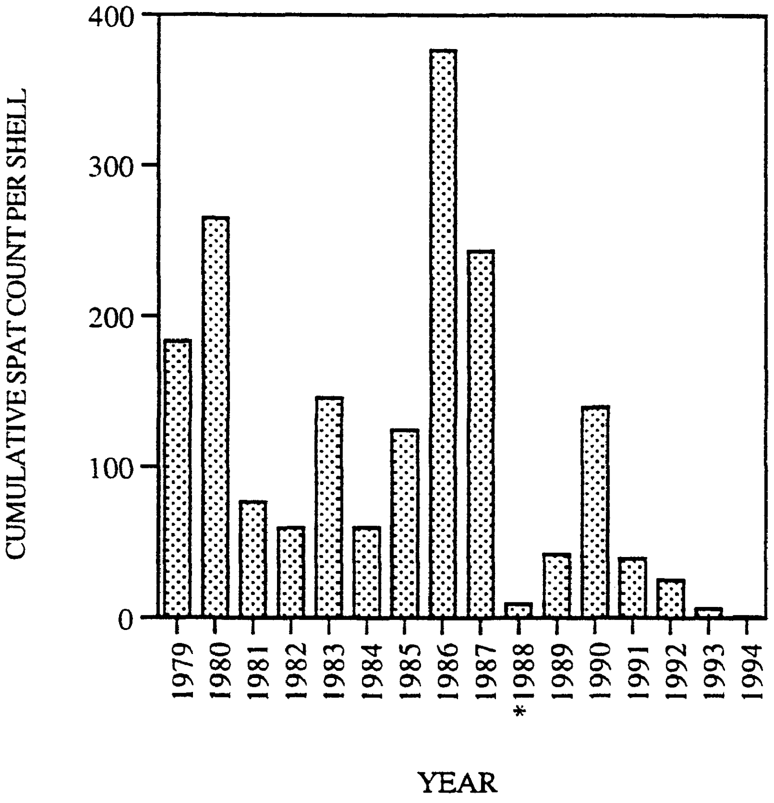
Settlement

The settlement season began July 15 and ended August 4, 1994 and was the lowest in terms of magnitude in the last 16 years in Virginia (< 1 cumulative spat per shell detected on shellstrings throughout the summer) (Figure 15). No significant differences in settlement were detected between the surface and deep layers in either the non-destructive or the destructive study when analyzed by each level of time or tidal height ($p > .05$) (Appendix 5a). In the non-destructive study, ANOVAs performed on surface/deep settlement means revealed that both tidal height and time influenced settlement (Appendix 5b). Settlement intensity peaked the week of July 21 - 28 and was greatest at the -90 cm tidal height (Figure 16).

Settlement was influenced significantly by week and tidal height in the destructive study as well, but a significant week by tidal height interaction confounded the effects (Appendix 5c). A graphical depiction of this interaction illustrated that differences in settlement between the tidal heights were more pronounced during weeks 2 and 3 (July 21 - 28 and July 28 - August 4) than during week 1 (July 15 - 21) (Figure 17). This interpretation was confirmed by lower level ANOVAs, which found settlement to be greatest subtidally only during weeks 2 (July 21 - 28) and 3 (July 28 - August 4) as result of a dramatic increase in settlement intensity at the -90 cm tidal height (Appendix 5d, e).

A graphical comparison of spat detected on destructively sampled reef shells and spat counts from non-destructive samples revealed an obvious disparity between the two methods. When cumulative non-destructive spat counts and destructive spat counts from weeks 4 and 5 (August 4 - 11 and August 11 - 18) (when destructive spat counts attained an asymptotic value) were compared, it was clear that cumulative non-destructive spat counts were consistently higher than destructive spat counts (Figure 18). Spat count differences ranged from 1 spat per 30 shells in the intertidal zone to 3.5 spat per 30 shells in the subtidal zone. Of the three methods used to detect spat at the -90 cm tidal height, the greatest amount of spat settled on shellstrings during the 1994 Summer, as was the case during the 1993 Summer (Figure 19).

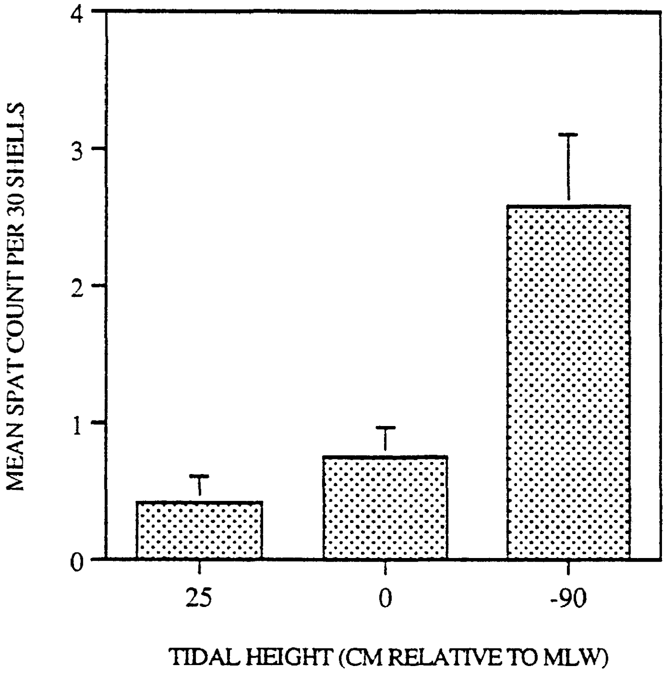
Figure 15. Cumulative *C. virginica* spat counts per shell detected on shellstrings at Palace Bar, VA from 1979-1994 recorded in the VIMS Spatfall Surveys. Spat counts were recorded for only a portion of the 1988 settlement season.



*Spat counts for 1988 reflect an incomplete set of data.

Figure 16. Mean *C. virginica* spat counts per 30 shells calculated from non-destructive settlement 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 in both graphs denote +1 standard error.

A)


MEAN SPAT COUNT PER 30 SHELLS
0
1
2
3
4
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)

B)

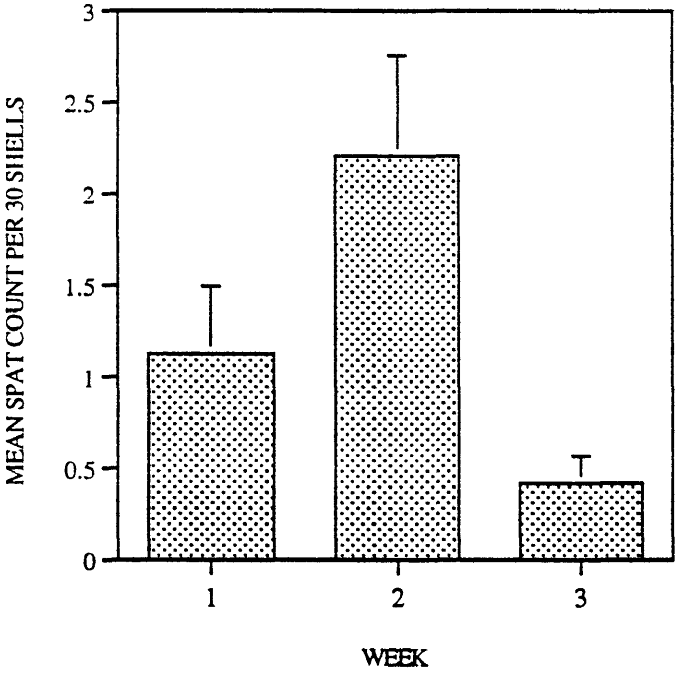

MEAN SPAT COUNT PER 30 SHELLS
0
0.5
1
1.5
2
2.5
3
1
2
3
WEEK

Figure 17. Mean *C. virginica* spat counts per 30 shells recorded from destructive samples examined by tidal height and week. The tidal heights considered were: 25 cm above MLW, MLW, and 90 cm below MLW. Error bars denote ±1 standard error.

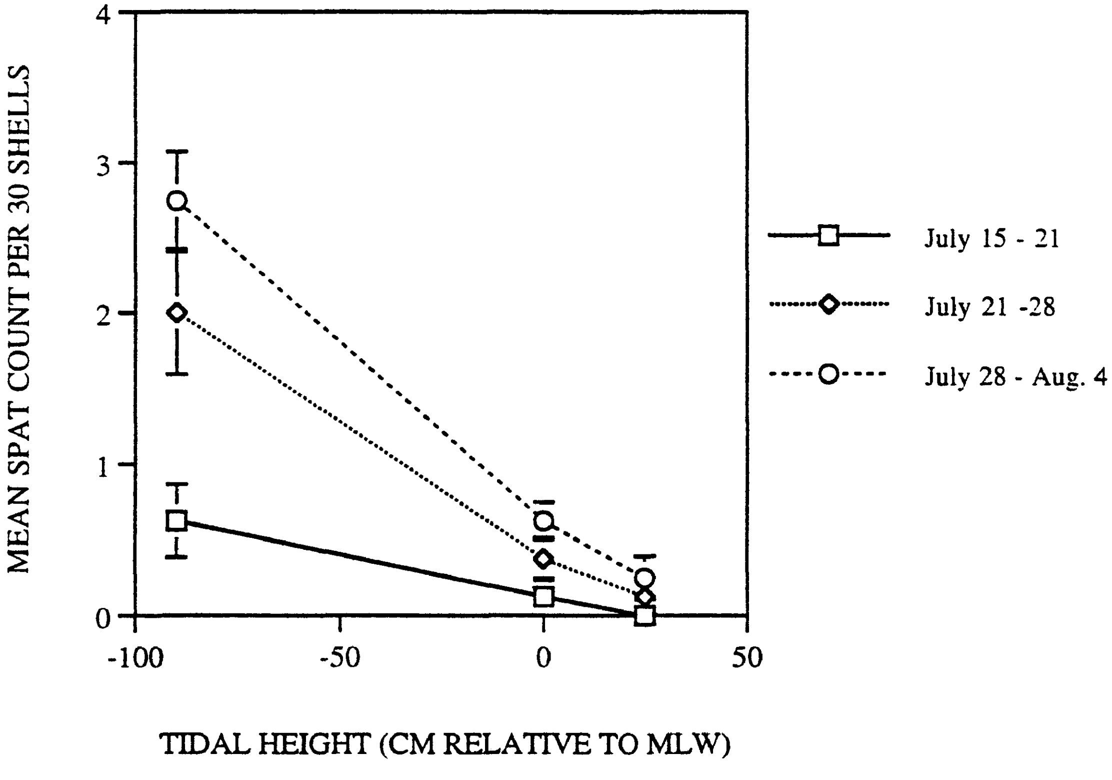
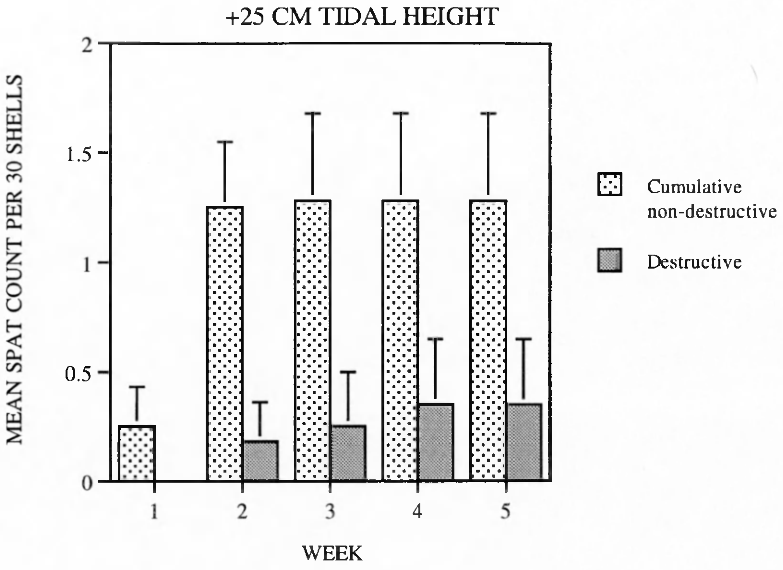
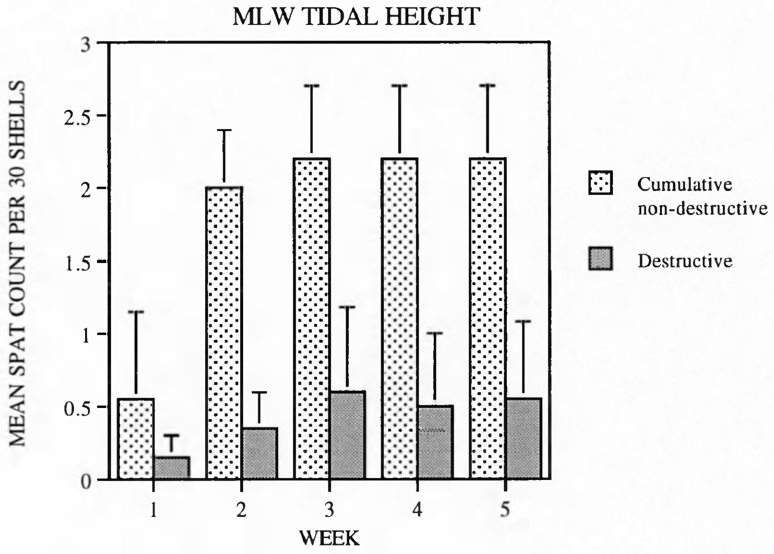

MEAN SPAT COUNT PER 30 SHELLS
4
3
2
1
0
-100
-50
0
50
TIDAL HEIGHT (CM RELATIVE TO MLW)
July 15 - 21
July 21 -28
July 28 - Aug. 4

Figure 18. Comparison of cumulative non-destructive and destructive *C. virginica* spat counts at three tidal heights from the onset of settlement to two weeks after the settlement season (week 1 = July 15-21, week 2 = July 21-28, week 3 = July 28 - August 4, week 4 = August 4-11, and week 5 = August 11-18). Error bars denote +1 standard error.


+25 CM TIDAL HEIGHT


MLW TIDAL HEIGHT

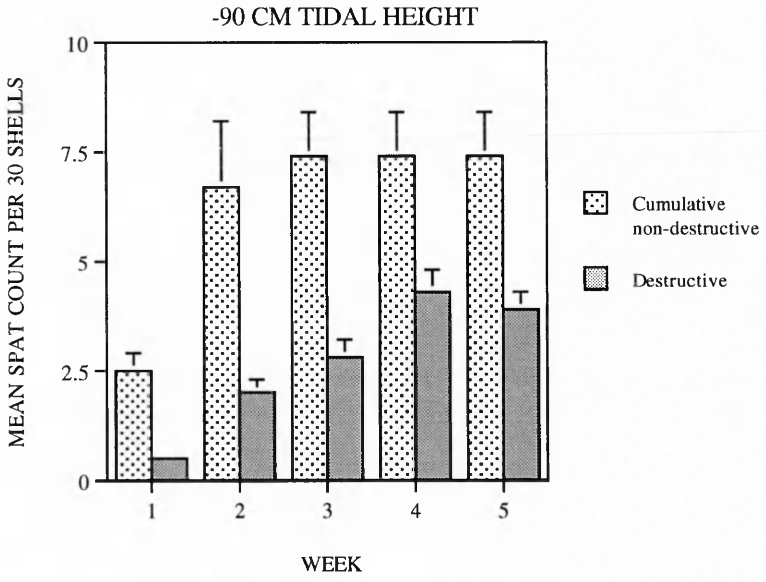
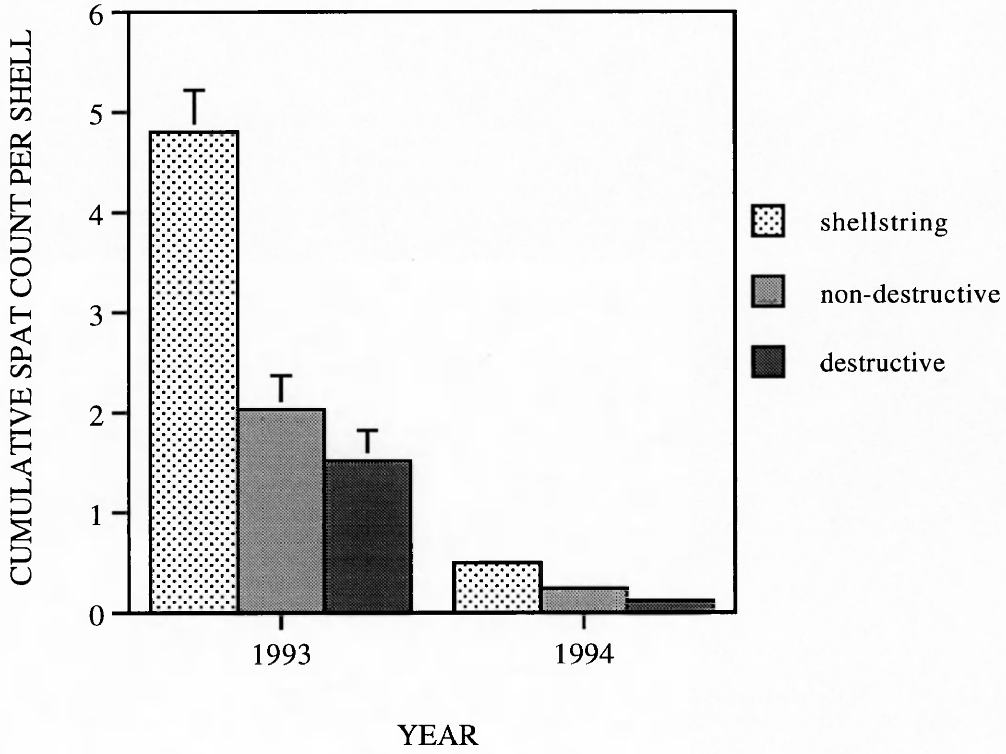

-90 CM TIDAL HEIGHT

Figure 19. 1993 and 1994 cumulative *C. virginica* spat counts per shell at the -90 cm tidal height detected on shellstrings, non-destructive samples, and destructive samples. Error bars denote +1 standard error.


CUMULATIVE SPAT COUNT PER SHELL
6
5
4
3
2
1
0
1993
1994
YEAR
shellstring
non-destructive
destructive

Growth

Significant differences in mean growth between surface and deep levels were detected when both the '93 and '94 year classes were analyzed separately by tidal height ($p < .05$) (Appendix 6a). For both year classes, growth at the + 25 cm tidal height was greater at the deep layer than at the surface layer, and growth at the -90 cm tidal height was greater at the surface layer than at the deep layer. A graphical depiction of growth at the +25 cm and -90 cm tidal heights during each of the 3 sampling periods illustrated that these growth differences were consistent over time (Figure 20).

1993 Year Class

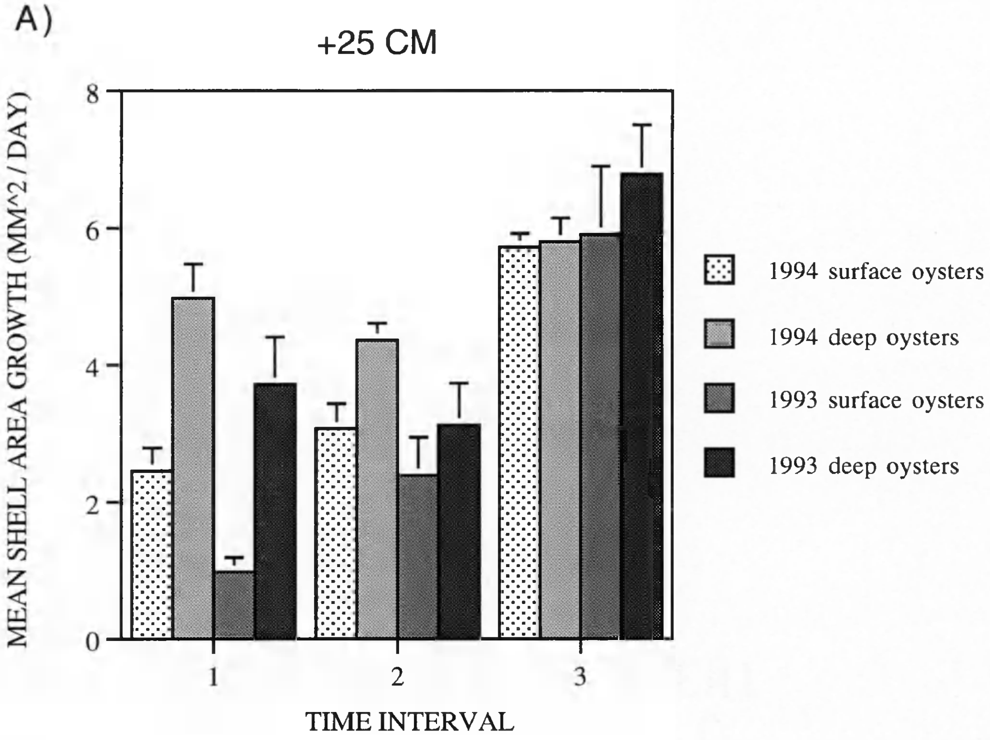
Growth for the 1993 oysters residing at the surface was dependent on tidal height and time (Appendix 6b). SNK multiple comparison tests revealed that oyster growth at tidal heights of -90 cm and MLW were greater than growth at +25 cm (Figure 21a), and Newman-Keuls tests revealed that growth was greatest across all tidal heights during the last time interval sampled (mid-August through mid-September) (Figure 22a). Although the interaction between time and tidal height was not significant at the alpha level = .05, the fact that the p-value was equal to .058 merited a graphical examination of the interaction. When this was done, it was apparent that oyster growth at the -90 cm tidal height decreased during the second sampling period to a level similar to that observed at MLW (Figure 23a).

When the 1993 oysters residing at the deep layer were examined, growth was not found to be dependent on tidal height (Appendix 6c, Figure 21b). However, growth at the deep layer was found to increase significantly during the third time interval sampled (mid-August through mid-September), as observed in the surface layer (Figure 22b). A trend in reduced growth during the second sampling session (mid-July through mid-August) was also apparent (Figure 23b).

Regressions of shell area on time for 1993 oysters residing at the 3 tidal heights and 2 substrate layers were all significant ($p < .001$) (Appendix 6d, Figures 24 - 26). Slopes were found to be significantly different ($F_{calc.}=(12.34) > F_{.05(1),5,172} = 2.26$) and results of Tukey's test of slopes were supportive of data already presented. At the +25 cm tidal height growth was greater below the surface, and at the -90 cm tidal height was greater at the surface. Furthermore, of the oysters that resided at the surface, growth was greater at MLW and -90 cm than at +25 cm.

Figure 20. Mean shell area growth of *C. virginica* reared in 1993 and 1994 at surface and deep substrate levels and at tidal heights of +25 cm and -90 cm relative to MLW for the three sampling intervals (time interval 1 = June/July sampling period, time interval 2 = July/August sampling period, and time interval 3 = August/September sampling period). Error bars denote +1 standard error.

A)


+25 CM
MEAN SHELL AREA GROWTH (MM^2 / DAY)
0
2
4
6
8
1
2
3
TIME INTERVAL
1994 surface oysters
1994 deep oysters
1993 surface oysters
1993 deep oysters

B)

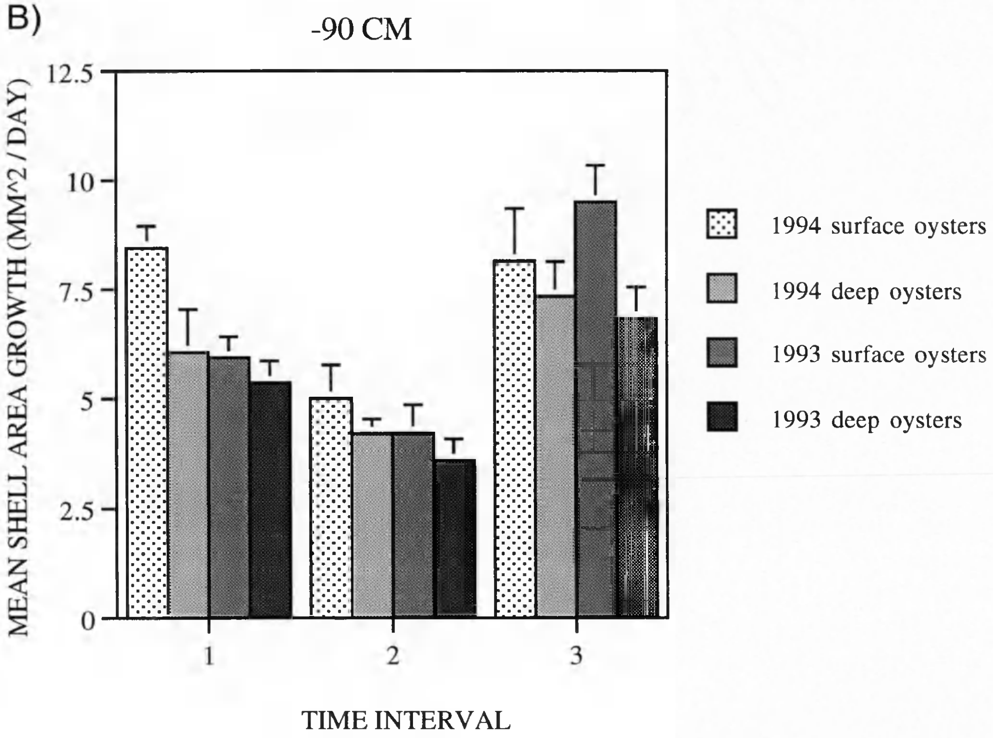
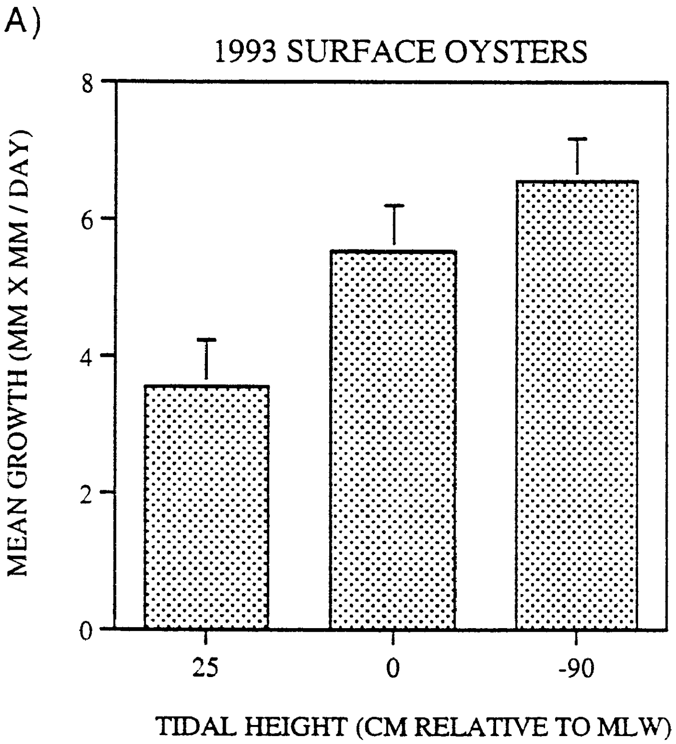

-90 CM
MEAN SHELL AREA GROWTH (MM^2 / DAY)
0
2.5
5
7.5
10
12.5
1
2
3
TIME INTERVAL
1994 surface oysters
1994 deep oysters
1993 surface oysters
1993 deep oysters

Figure 21. Mean growth of 1993 reared *C. virginica* residing at different tidal heights and found at the (A) reef surface and (B) 10 cm below the reef surface. Error bars denote +1 standard error.


A)
1993 SURFACE OYSTERS
MEAN GROWTH (MM X MM / DAY)
0
2
4
6
8
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)

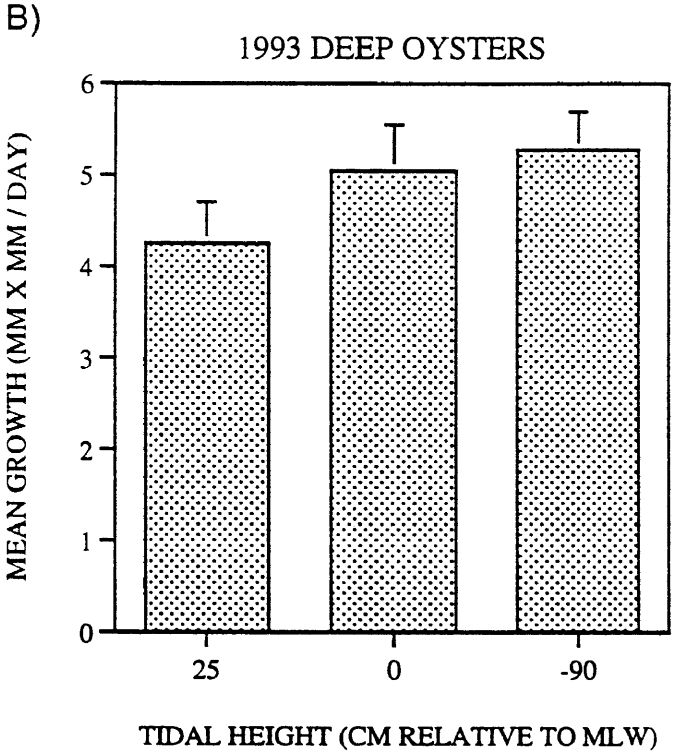
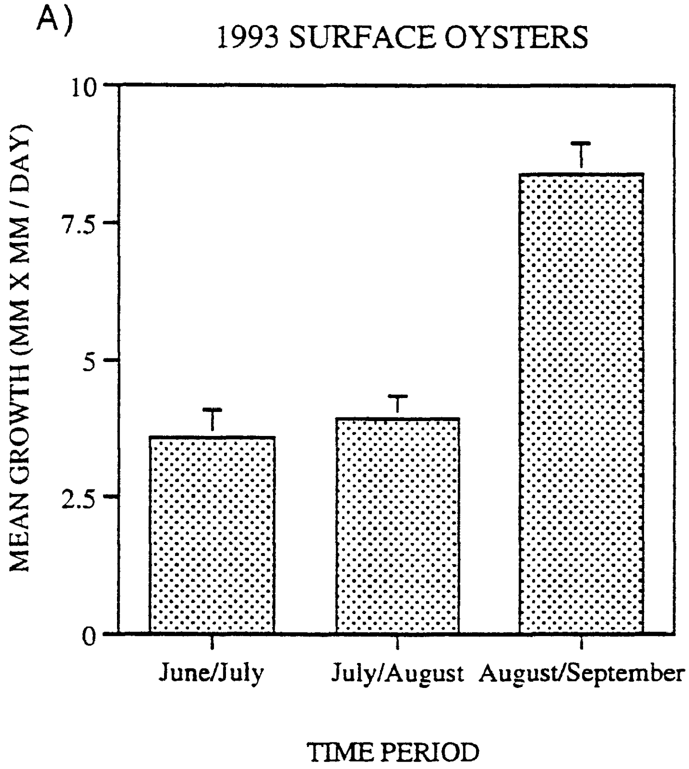

B)
1993 DEEP OYSTERS
MEAN GROWTH (MM X MM / DAY)
0
1
2
3
4
5
6
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)

Figure 22. Mean growth of 1993 reared *C. virginica* during the three sampling periods at the (A) reef surface and (B) 10 cm below the reef surface. Error bars denote +1 standard error.

A)


1993 SURFACE OYSTERS
MEAN GROWTH (MM X MM / DAY)
10
7.5
5
2.5
0
June/July
July/August
August/September
TIME PERIOD

B)

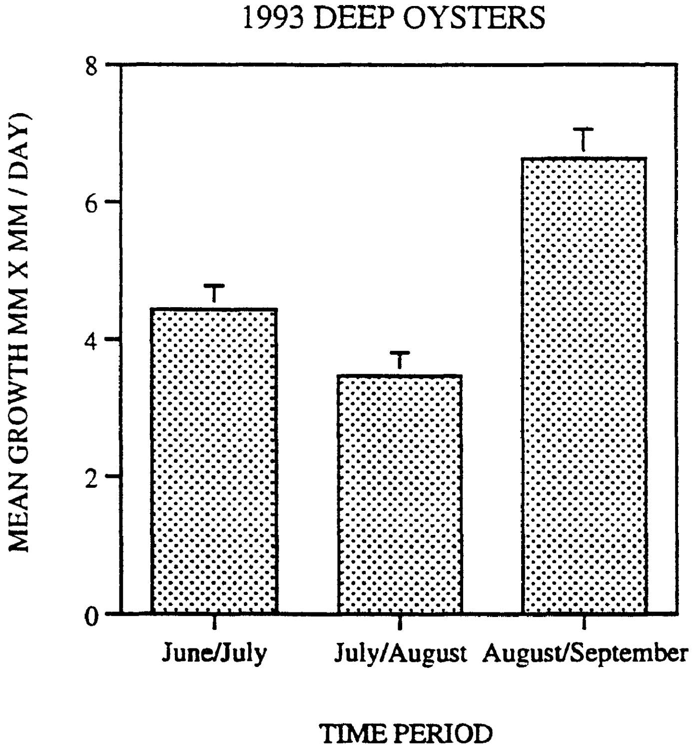

1993 DEEP OYSTERS
MEAN GROWTH MM X MM / DAY)
8
6
4
2
0
June/July
July/August
August/September
TIME PERIOD

Figure 23. Mean growth of 1993 reared *C. virginica* examined by tidal height, time period, and substrate level. (A) depicts oysters at the reef surface and (B) depicts oysters found 10 cm below the reef surface. Error bars denote +1 standard error.

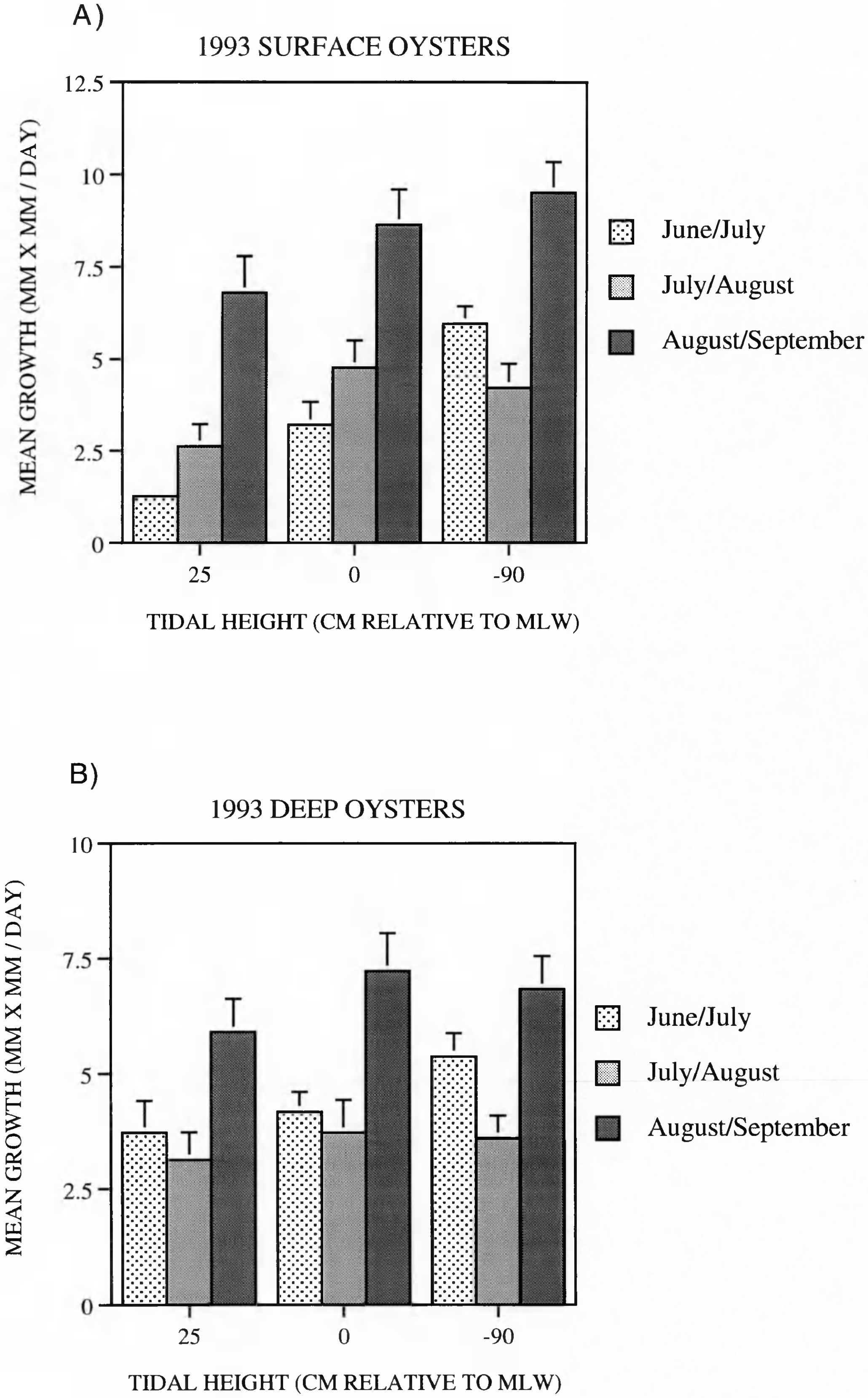
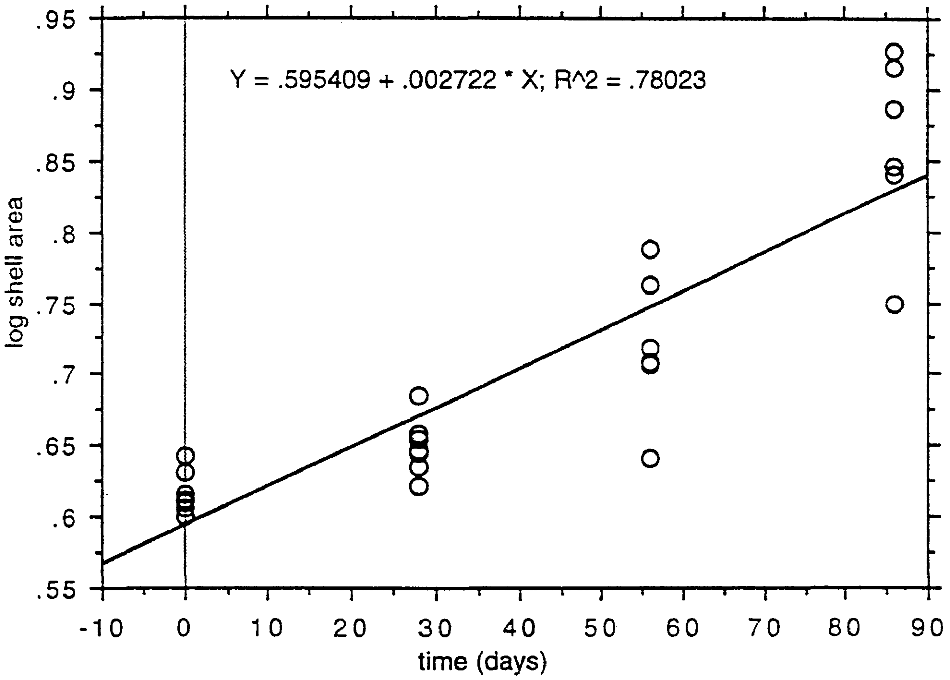

A)
1993 SURFACE OYSTERS
MEAN GROWTH (MM X MM / DAY)
12.5
10
7.5
5
2.5
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
June/July
July/August
August/September
B)
1993 DEEP OYSTERS
MEAN GROWTH (MM X MM / DAY)
10
7.5
5
2.5
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
June/July
July/August
August/September

Figure 24. Regressions of shell area on time for 1993 reared *C. virginica* residing at the +25 cm tidal height (A) on the reef surface and (b) 10 cm below the reef surface. The time period considered was June 14 - August 9, 1994.

A) 1993 SURFACE OYSTERS RESIDING AT THE +25 CM TIDAL HEIGHT



B) 1993 DEEP OYSTERS RESIDING AT +25 CM TIDAL HEIGHT

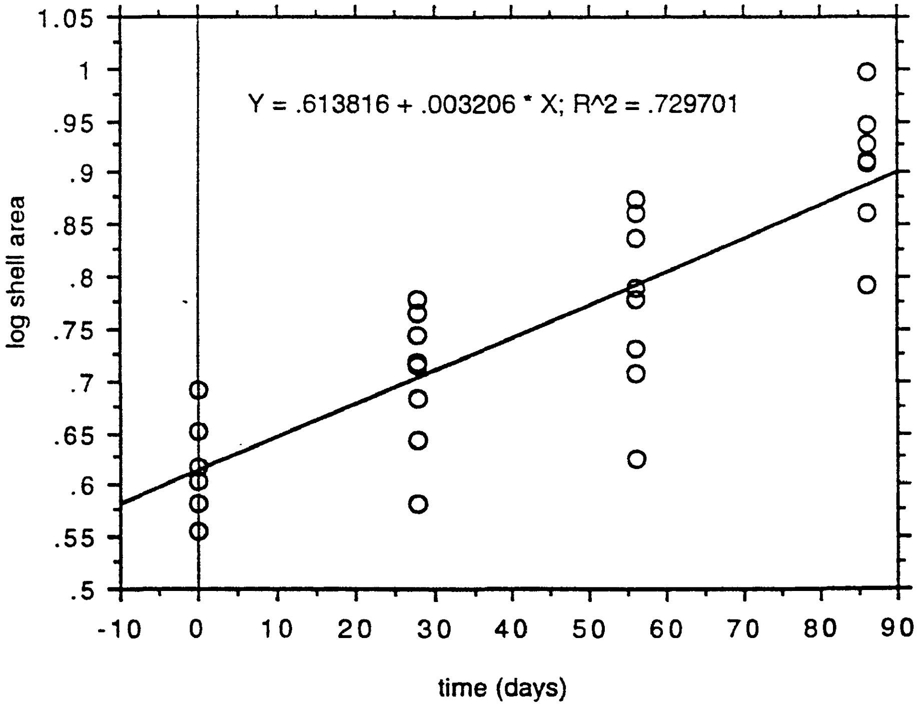
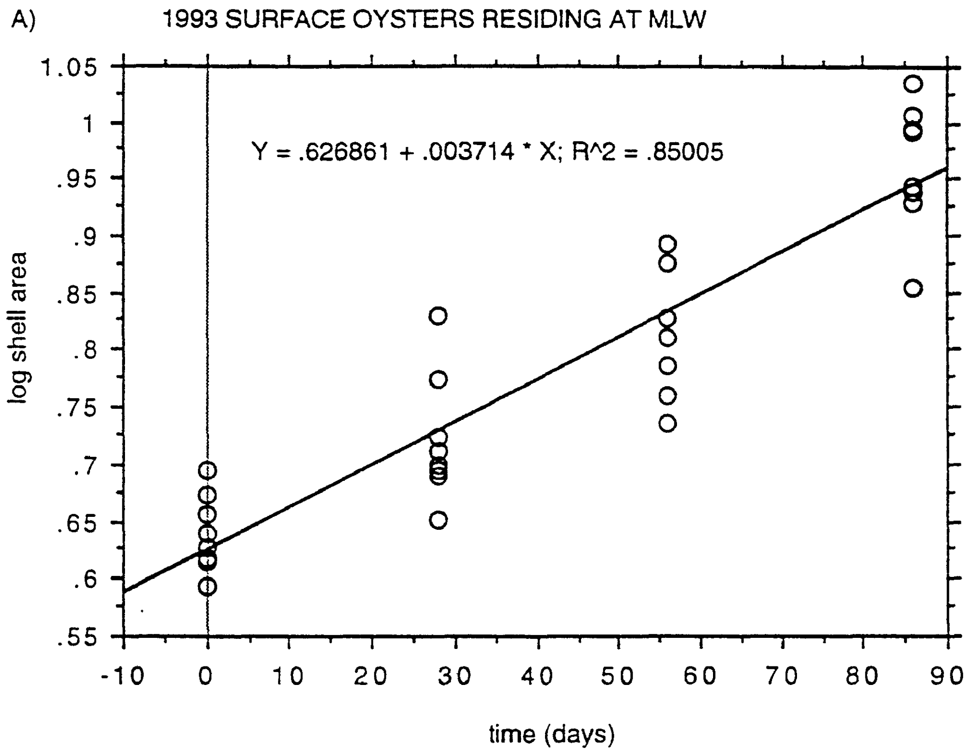


Figure 25. Regressions of shell area on time for 1993 reared C. *virginica* residing at the MLW tidal height (A) on the reef surface and (b) 10 cm below the reef surface. The time period considered was June 14 - August 9, 1994.


A)
1993 SURFACE OYSTERS RESIDING AT MLW
Y = .626861 + .003714 * X; R^2 = .85005
log shell area
1.05
1
.95
.9
.85
.8
.75
.7
.65
.6
.55
-10
0
10
20
30
40
50
60
70
80
90
time (days)

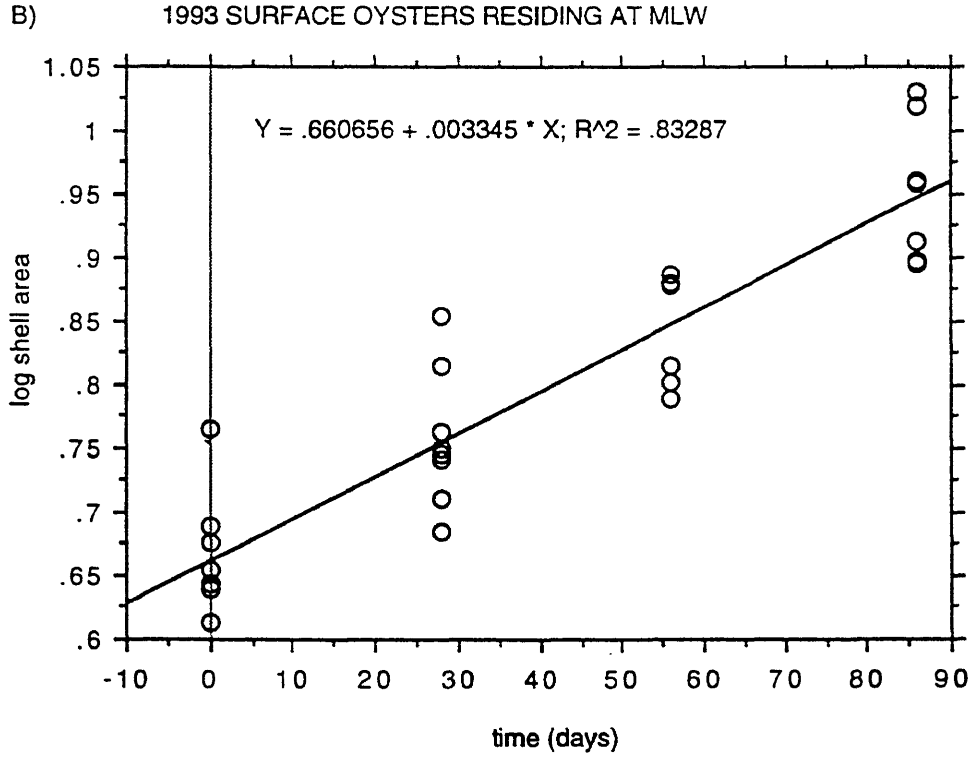
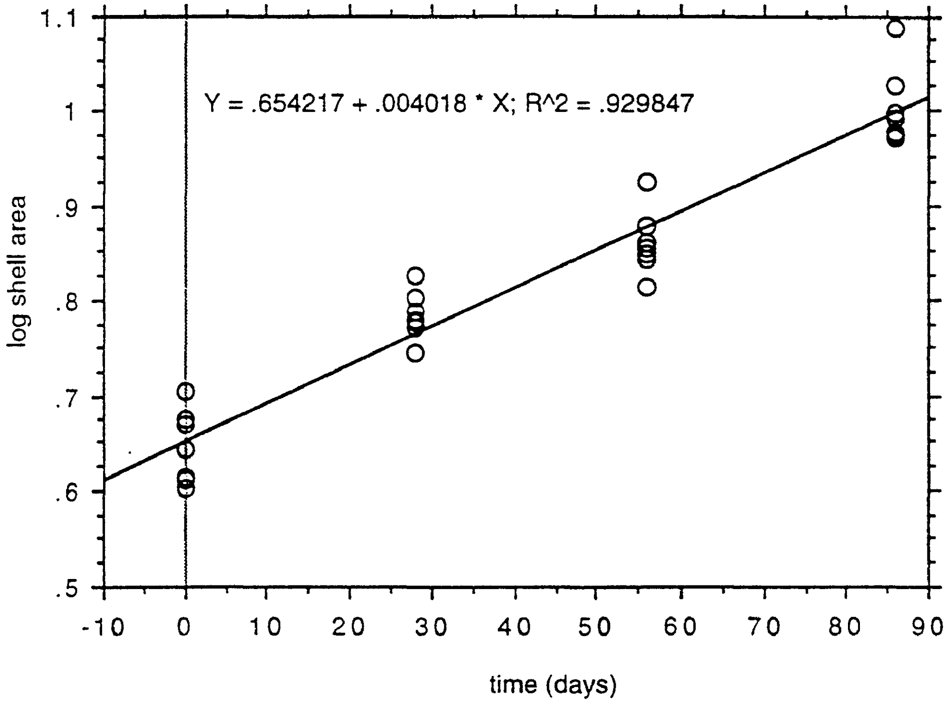
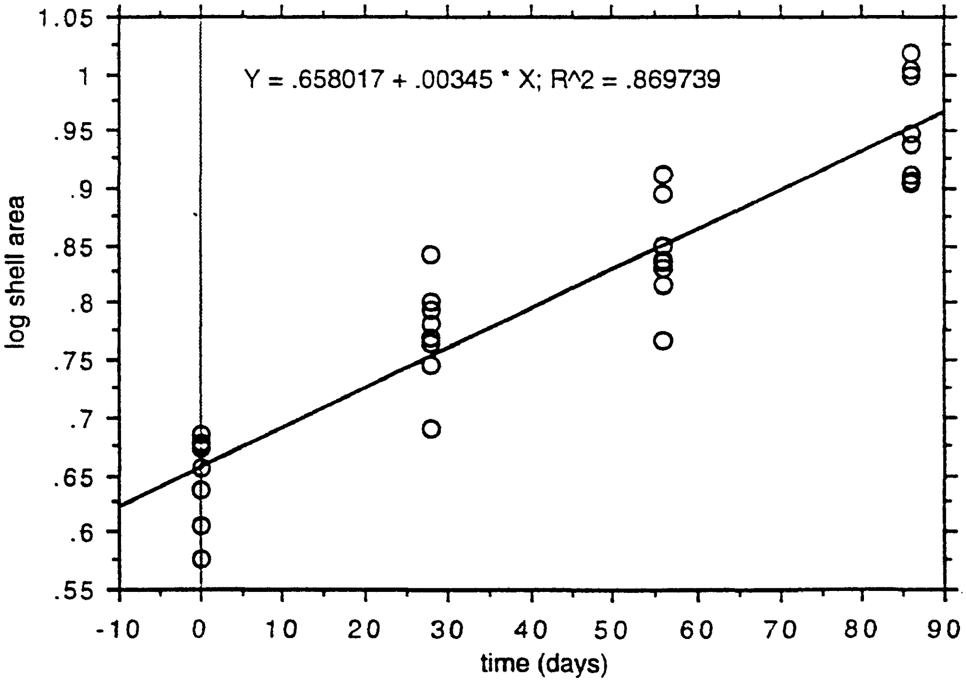

B)
1993 SURFACE OYSTERS RESIDING AT MLW
Y = .660656 + .003345 * X; R^2 = .83287
log shell area
1.05
1
.95
.9
.85
.8
.75
.7
.65
.6
-10
0
10
20
30
40
50
60
70
80
90
time (days)

Figure 26. Regressions of shell area on time for 1993 reared *C. virginica* residing at the -90 cm tidal height (A) on the reef surface and (B) 10 cm below the reef surface. The time period considered was June 14 - August 9, 1994.

A) 1993 SURFACE OYSTERS RESIDING AT THE -90 CM TIDAL HEIGHT



B) 1993 DEEP OYSTERS RESIDING AT THE -90 CM TIDAL HEIGHT



1994 Year Class

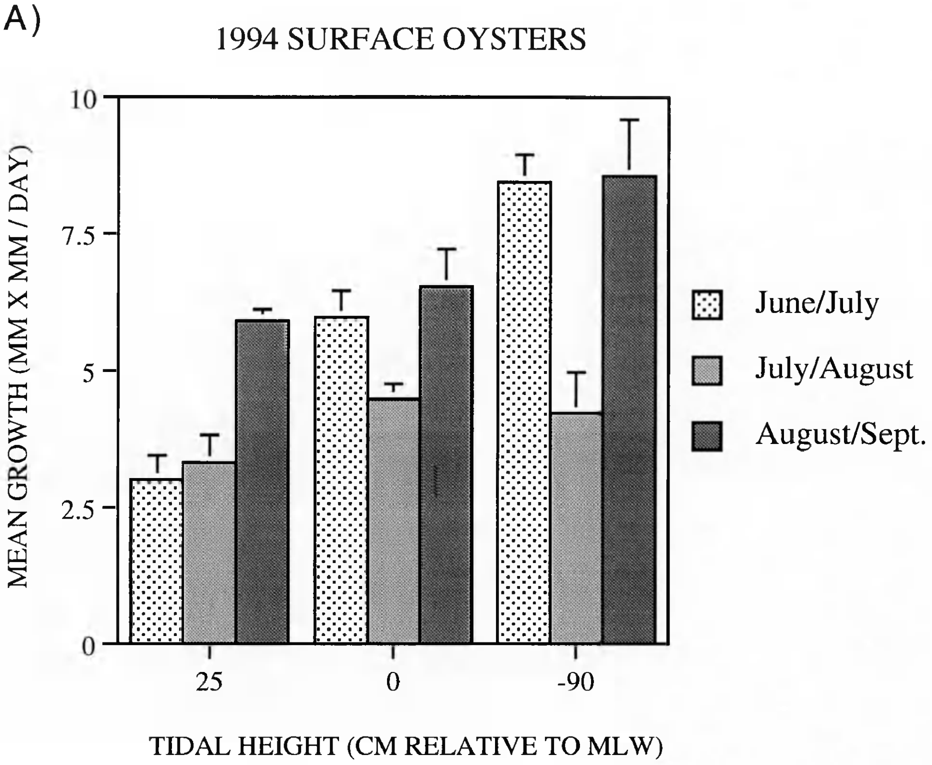
Repeated measures ANOVA performed on growth of 1994 oysters residing at the surface layer revealed that a significant time by tidal height interaction was present (p = .003, Appendix 6e). Separate ANOVAs and SNK tests run on each time interval revealed that during the June/July sampling period oyster growth was greater with increasing tidal depth, and during the August/September sampling period oyster growth at the -90 cm tidal height was greater than growth at the +25 cm tidal height (Appendix 6f, Figure 27a). No significant difference in growth as a function of tidal height was detected during the July/August time period. Separate repeated measures analyses performed on each tidal height revealed that growth was highly dependent on time for all three tidal heights sampled (Appendix 6g). Newman-Keuls tests indicated that growth for oysters situated at the +25 cm tidal height was greatest during the August/September period and growth for oysters situated at either MLW or -90 cm was lowest during the July/August period (Figure 27a).

Within the deep layer, 1994 oyster growth was dependent on tidal height and time (Appendix 6h). Growth was greatest at the -90 cm tidal height (Figure 28) and was the lowest during the July/August period (Figure 27b).

Regressions of shell area on time for 1994 oysters residing at the three tidal heights and two substrate layers were all significant (p<.001) (Appendix 6i, Figures 29 -31). Slopes were found to be significantly different ($F_{calc.} = 2.42 > F_{.05(1),5,170} = 2.26$) and results of Tukey's test of slopes were consistent with data already presented. At the +25 cm tidal height slopes were greater below the surface, and at the -90 cm tidal height slopes were greater at the surface; overall, slopes were greatest subtidally.

Figure 27. Mean growth of 1994 reared *C. virginica* examined by tidal height, time period, and substrate level. (A) depicts oysters at the reef surface and (B) depicts oysters found 10 cm below the reef surface. Error bars denote +1 standard error.

A)


1994 SURFACE OYSTERS
MEAN GROWTH (MM X MM / DAY)
10
7.5
5
2.5
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
June/July
July/August
August/Sept.

B)

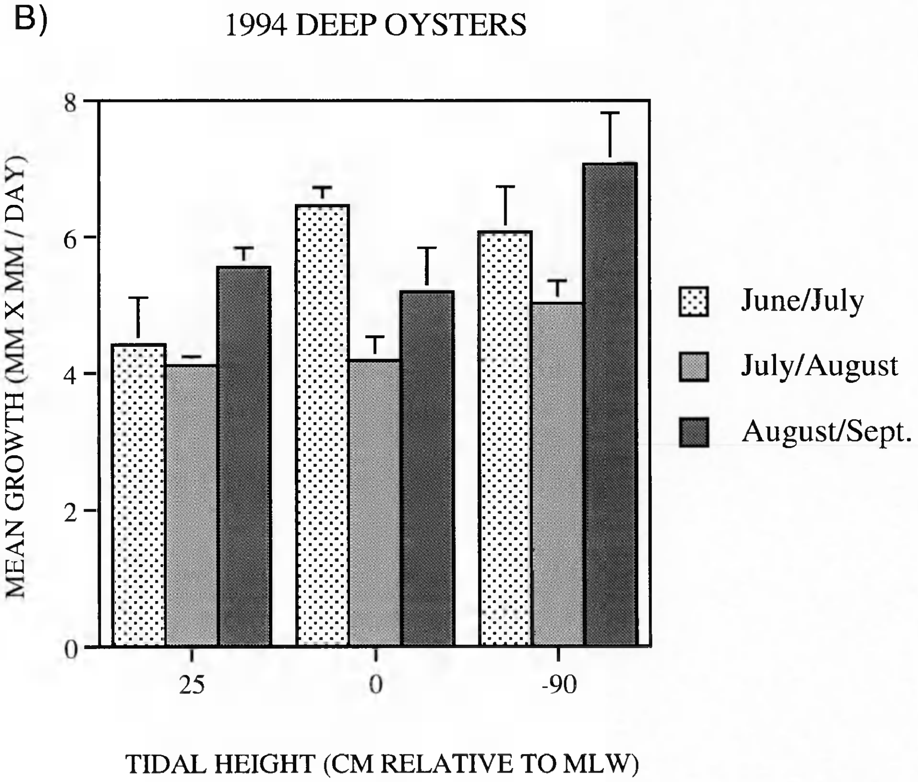

1994 DEEP OYSTERS
MEAN GROWTH (MM X MM / DAY)
8
6
4
2
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
June/July
July/August
August/Sept.

Figure 28. Growth of 1994 reared *C. virginica* residing 10 cm below the reef surface at three tidal heights. Error bars denote +1 standard error.

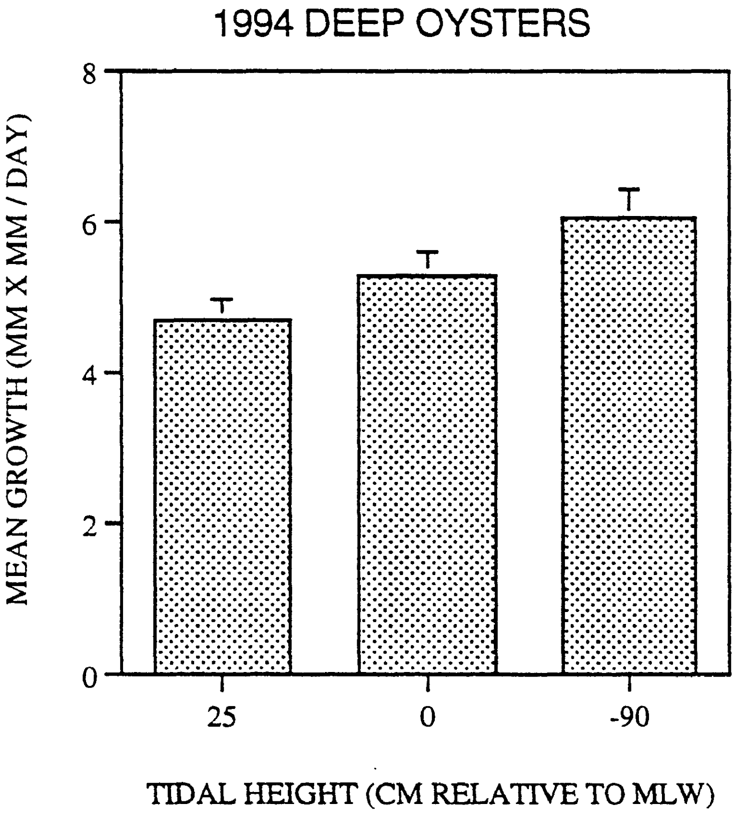
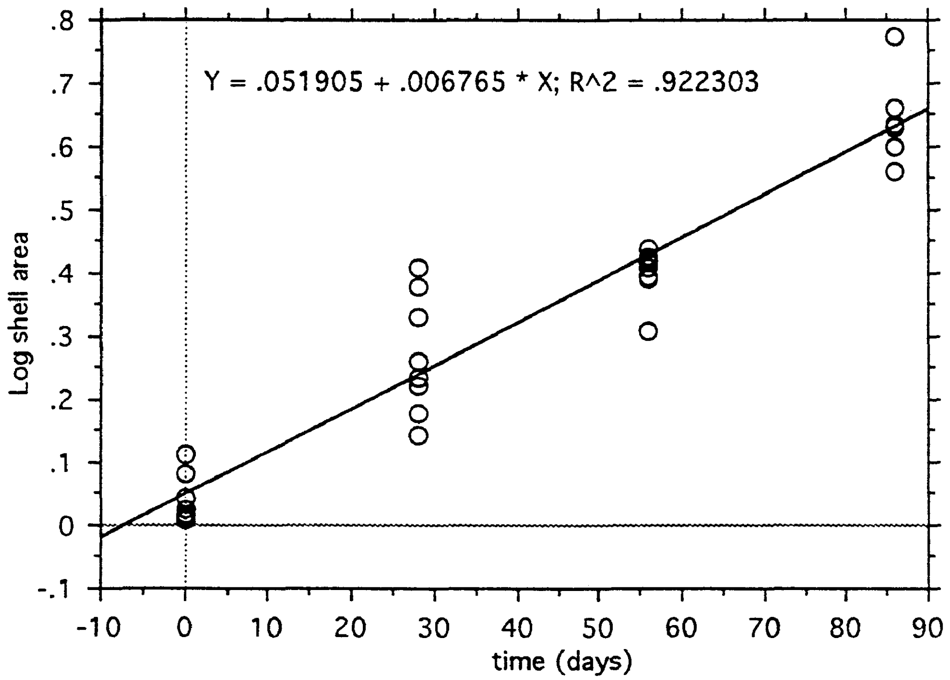

1994 DEEP OYSTERS
MEAN GROWTH (MM X MM / DAY)
0
2
4
6
8
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)

Figure 29. Regressions of shell area on time for 1994 reared *C. virginica* residing at the +25 cm tidal height (A) on the reef surface and (B) 10 cm below the reef surface. The time period considered was June 20 - September 13, 1994.

A)

1994 SURFACE OYSTERS RESIDING AT THE +25 CM TIDAL HEIGHT



B)

1994 DEEP OYSTERS RESIDING AT THE +25 CM TIDAL HEIGHT

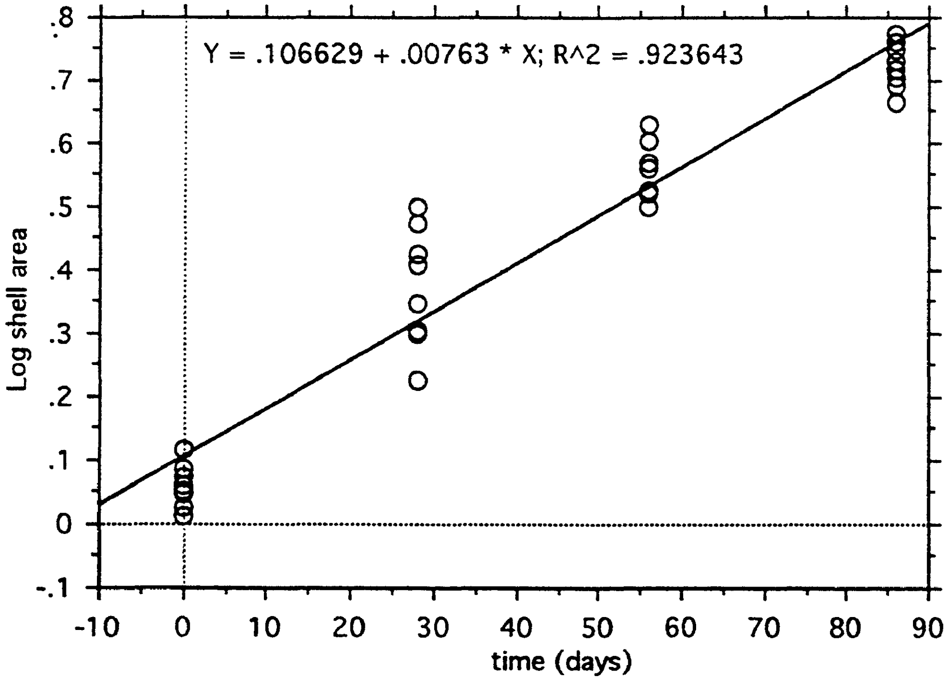
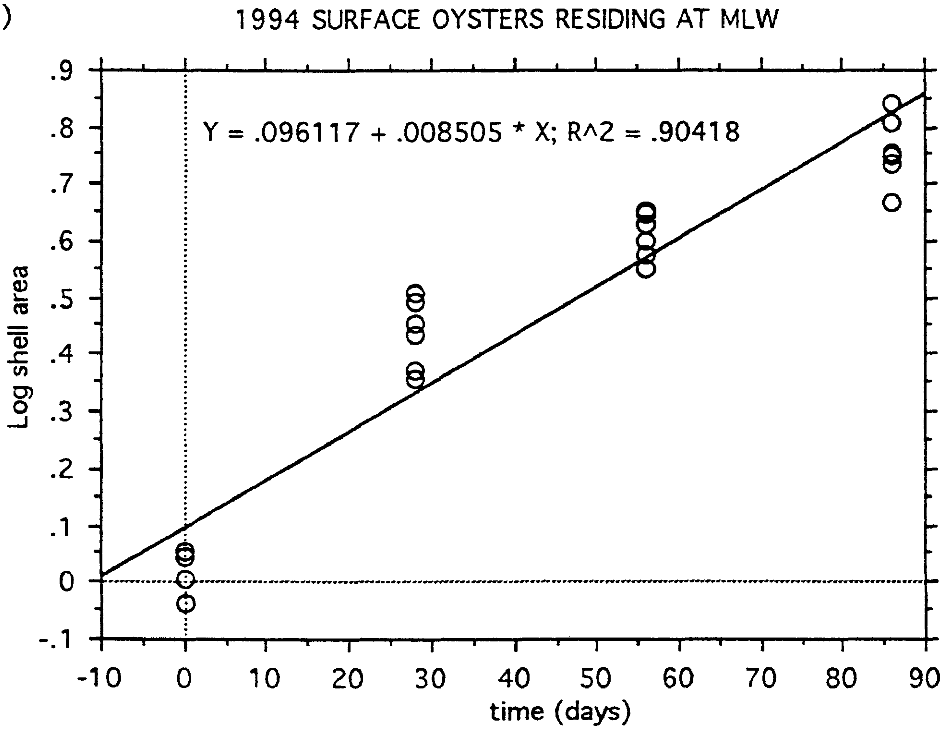


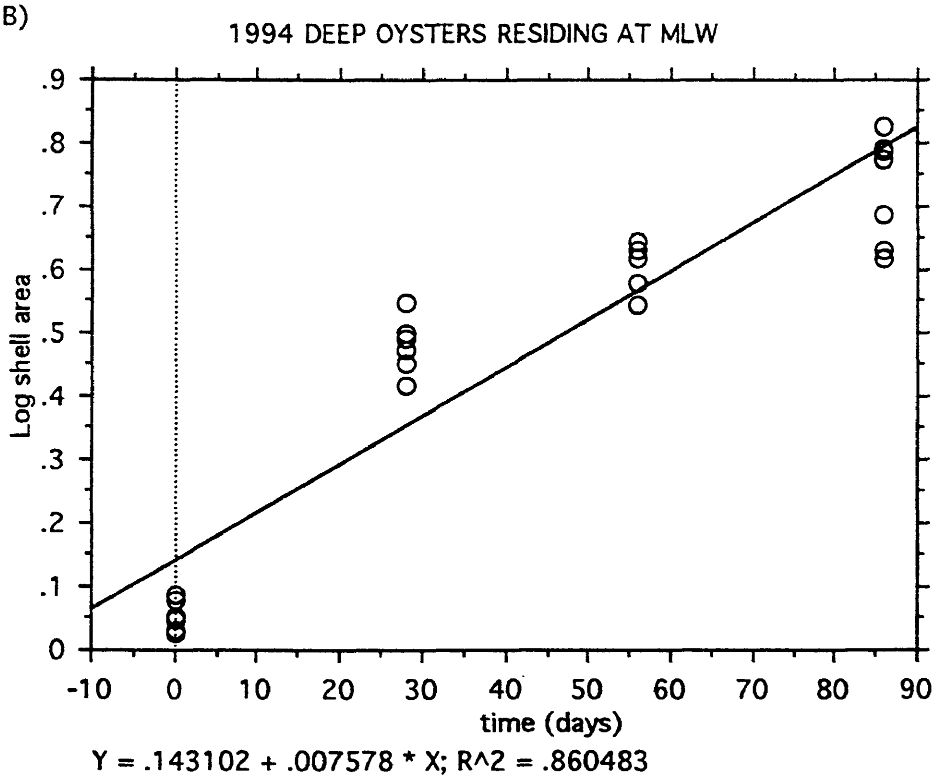
Figure 30. Regressions of shell area on time for 1994 reared *C. virginica* residing at the MLW tidal height (A) on the reef surface and (B) 10 cm below the reef surface. The time period considered was June 20 - September 13, 1994.

A)



1994 SURFACE OYSTERS RESIDING AT MLW

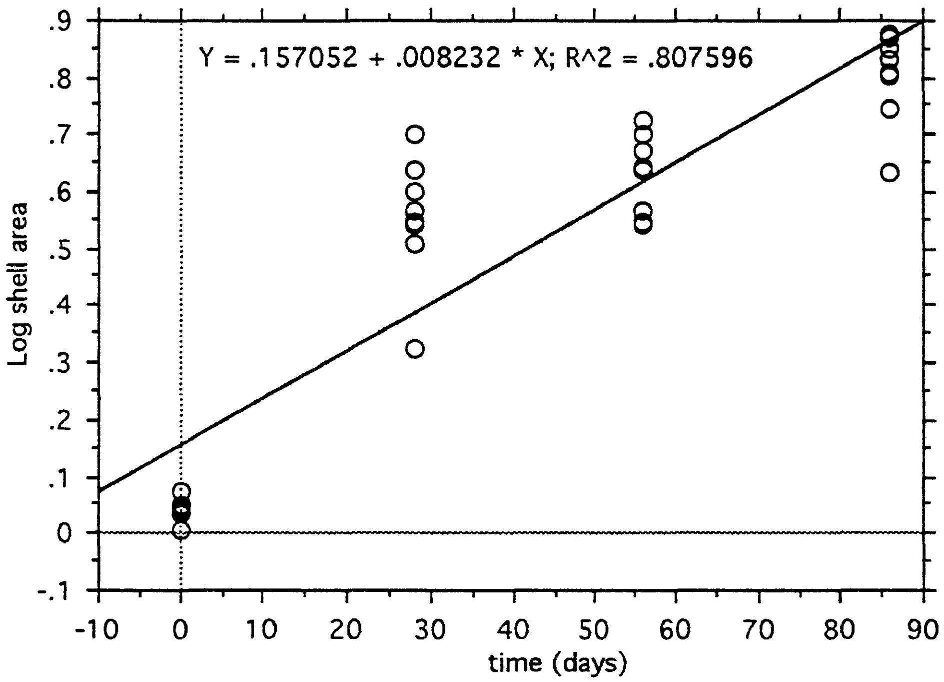
B)



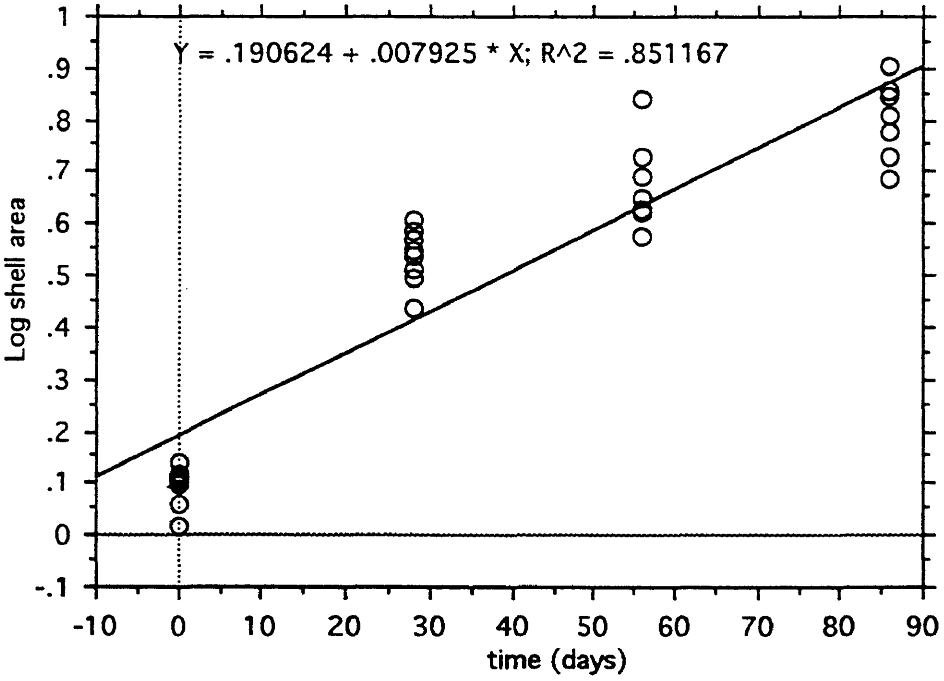
1994 DEEP OYSTERS RESIDING AT MLW

Figure 31. Regressions of shell area on time for 1994 reared C. *virginica* residing at the -90 cm tidal height (A) on the reef surface and (B) 10 cm below the reef surface. The time period considered was June 20 - September 13, 1994.

A)


1994 SURFACE OYSTERS RESIDING AT THE -90 CM TIDAL HEIGHT

B)


1994 DEEP OYSTERS RESIDING AT THE -90 CM TIDAL HEIGHT

Mortality

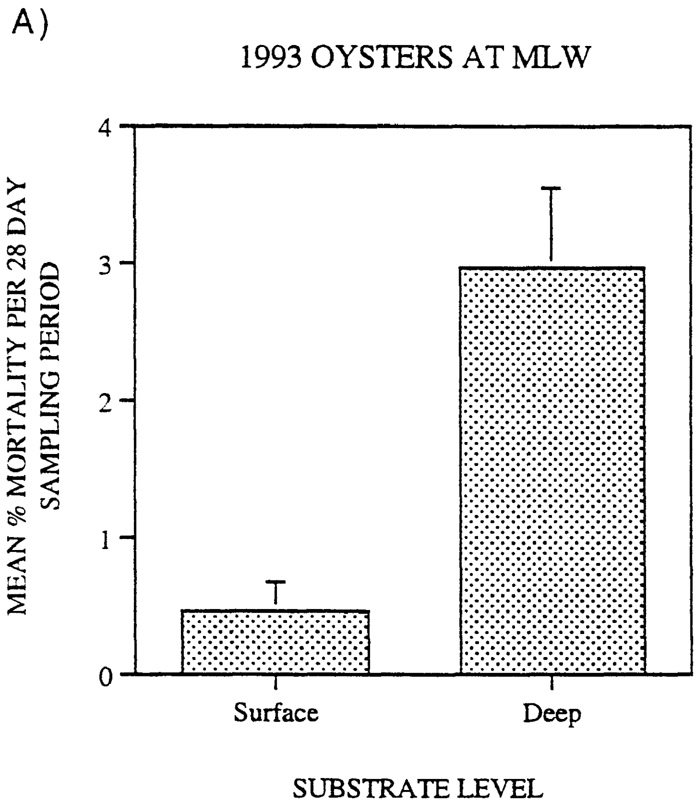
1993 Year Class

A significant tidal height by time interaction was detected when mortality data from the 1993 year class of oysters were analyzed using repeated measures analysis (Appendix 7a). Separate repeated measures analyses run on each tidal height to decouple the interaction revealed that at the MLW tidal height oysters residing in the deep level experienced greater mortality than oysters at the surface, but at the -90 cm tidal height, oysters residing at the surface layer had the greatest mortalities (Appendix 7b, Figure 32). No significant differences at the +25 cm tidal height were detected, however. Furthermore, at the -90 cm tidal height, greater mortalities occurred during the last time interval sampled (August/September) (Figure 33).

When separate ANOVAs were performed on each time interval to examine the effect of time in the tidal height by time interaction, an additional interaction of tidal height by layer was detected at two (June/July and August/September) of the three time intervals (Appendix 7c). During the July/August interval when no interactions were detected, mortality was found to be independent of both tidal height and substrate level. Separate analyses performed on the other two intervals confirmed results already presented; mortality was higher below the reef surface than at the reef surface at MLW, and mortality was higher at than below the reef surface at the -90 cm tidal height (Appendix 7d). In addition to these findings, lower level ANOVAs revealed that during the June/July interval mortality of oysters at the +25 cm tidal height was higher at the surface than in underlying layers (Appendix 7d, Figure 34), and of the oysters that resided at the surface, oysters at MLW had the least mortalities (Appendix 7e, Figure 35). Furthermore, at the surface, mortality was highest at the +25 tidal height during the June/July period and at the -90 cm tidal height during the August/September period (Figure 35).

To develop an idea of overall mortality on the reef at each tidal height and substrate level, cumulative mortalities for the three month sampling period were plotted (Figure 36). Mortality was relatively low overall, reaching a maximum of only 17% at the -90 cm tidal height, and was lowest at the surface at MLW.

Figure 32. Mean percent mortalities of 1993 reared *C. virginica* found at surface and deep (10 cm below reef surface) substrate levels at (A) MLW and (B) -90 cm below MLW. Error bars denote +1 standard error.


A)
1993 OYSTERS AT MLW
MEAN % MORTALITY PER 28 DAY SAMPLING PERIOD
0
1
2
3
4
Surface
Deep
SUBSTRATE LEVEL

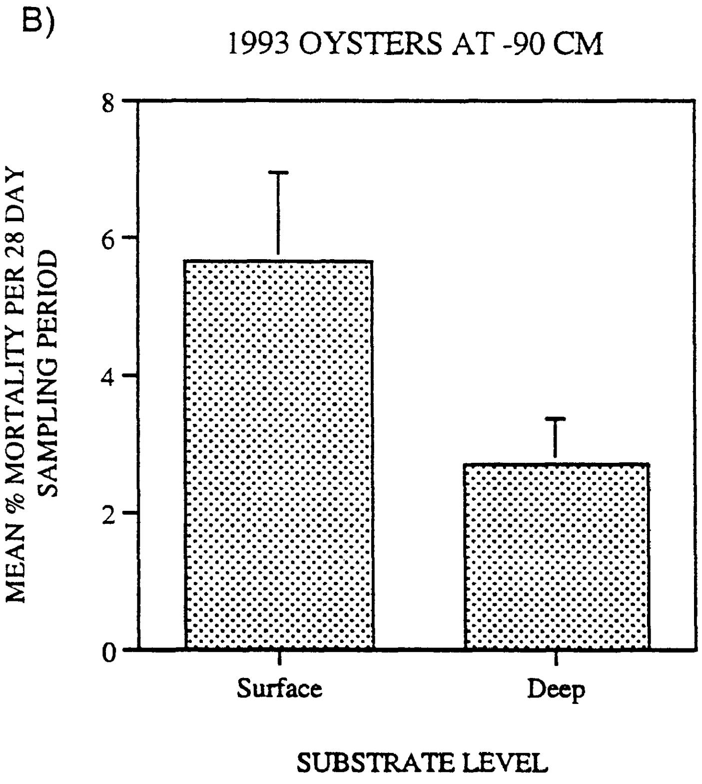

B)
1993 OYSTERS AT -90 CM
MEAN % MORTALITY PER 28 DAY SAMPLING PERIOD
0
2
4
6
8
Surface
Deep
SUBSTRATE LEVEL

Figure 33. Mean percent mortalities for 1993 reared *C. virginica* examined by time. Error bars denote +1 standard error.

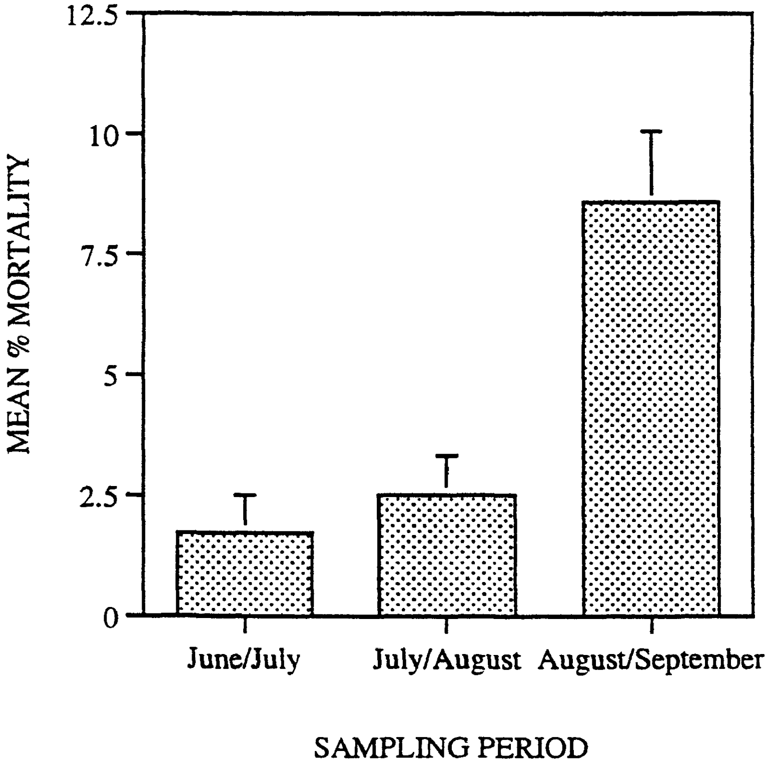
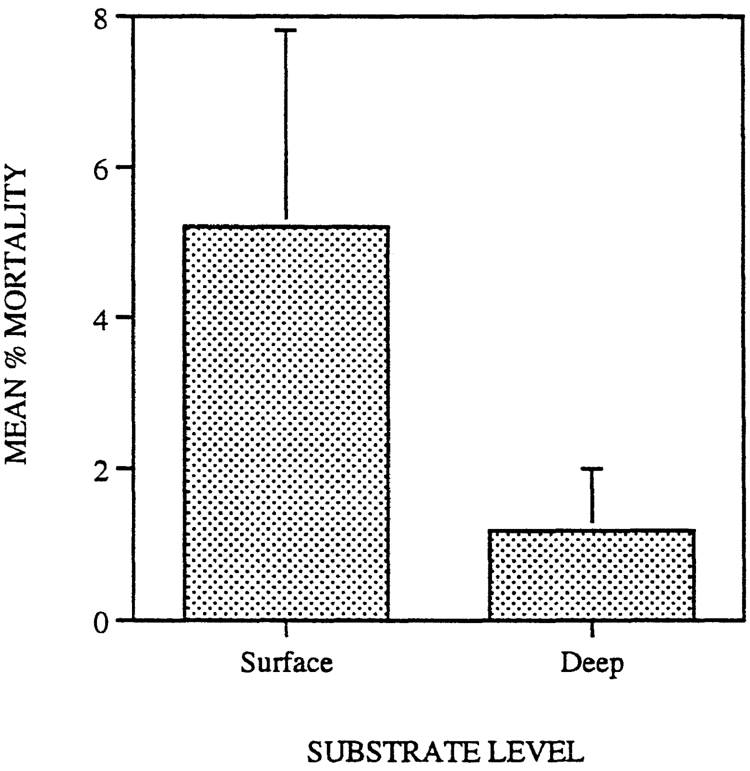

12.5
10
7.5
5
2.5
0
MEAN % MORTALITY
June/July
July/August
August/September
SAMPLING PERIOD

Figure 34. Mean percent mortalities examined by substrate level of 1993 reared *C. virginica* residing at the +25 cm tidal height during the June/July sampling period. Error bars denote +1 standard error.



8
6
4
2
0
MEAN % MORTALITY
Surface
Deep
SUBSTRATE LEVEL

Figure 35. Mean percent mortalities examined by tidal height and sampling period of 1993 reared *C. virginica* found at the reef surface. Error bars denote +1 standard error.

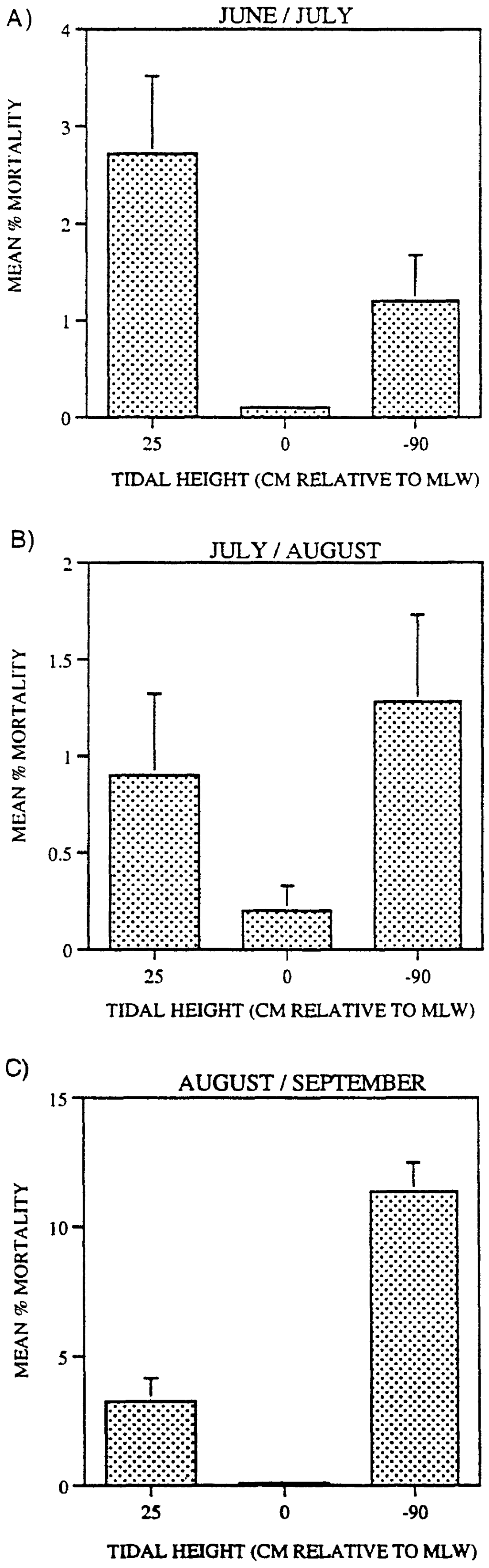
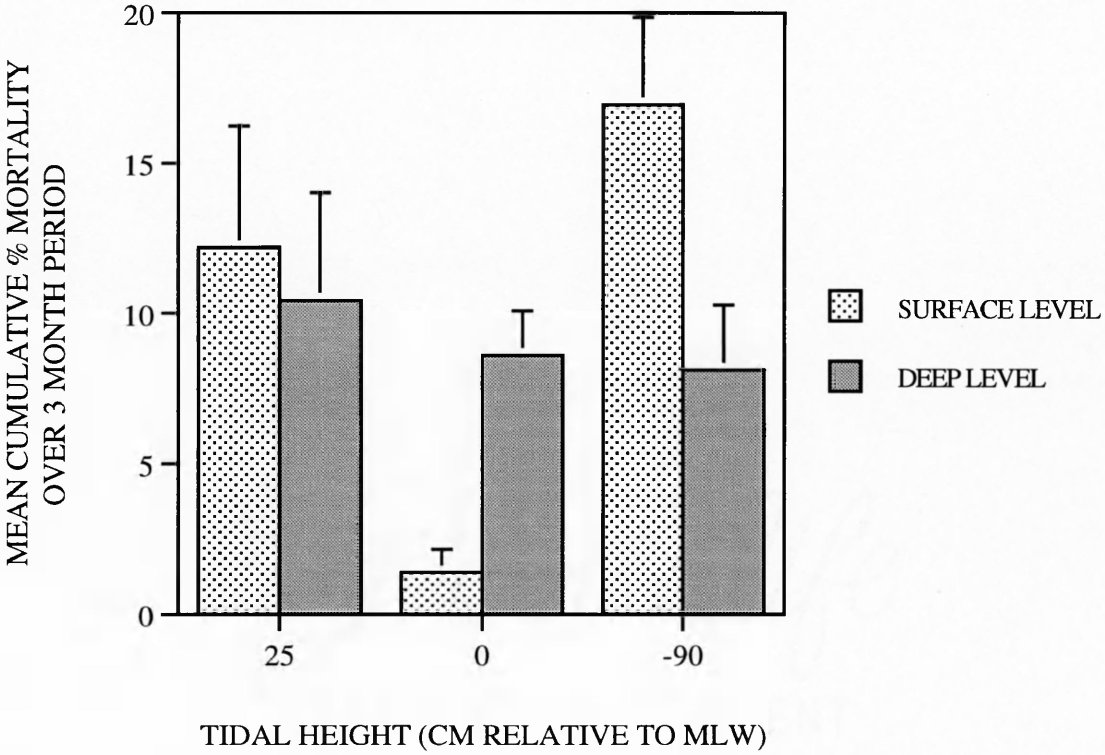

A)
JUNE / JULY
MEAN % MORTALITY
4
3
2
1
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
B)
JULY / AUGUST
MEAN % MORTALITY
2
1.5
1
0.5
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
C)
AUGUST / SEPTEMBER
MEAN % MORTALITY
15
10
5
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)

Figure 36. Mean cumulative percent mortalities for 1993 reared *C. virginica* found on or below the reef surface at three tidal heights. Cumulative percent mortality was computed from June 14 through September 6, 1994. Error bars denote +1 standard error.


MEAN CUMULATIVE % MORTALITY
OVER 3 MONTH PERIOD
20
15
10
5
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
SURFACE LEVEL
DEEP LEVEL

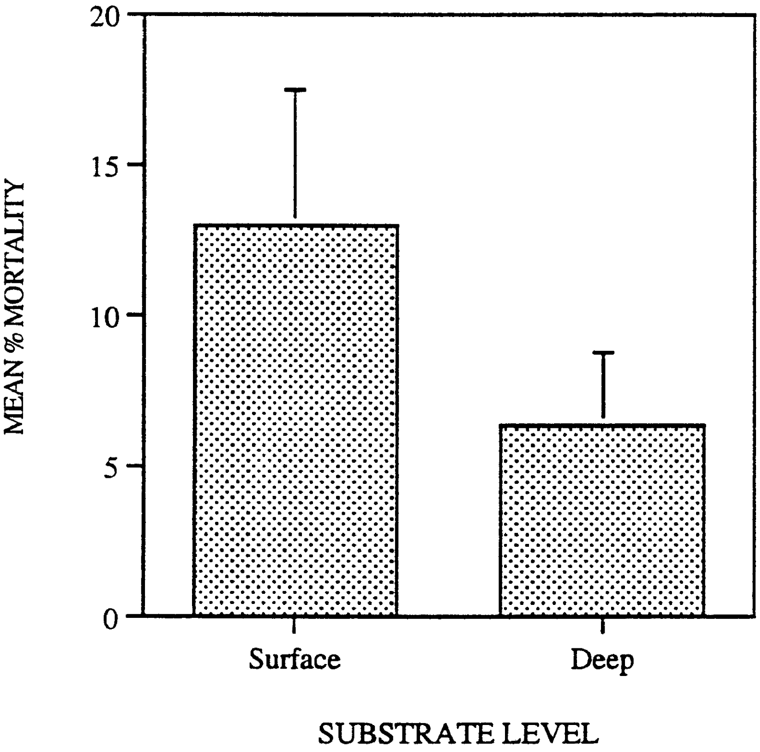
1994 Year Class

A significant time by tidal height interaction was detected when a repeated measures ANOVA was performed on the 1994 oysters (Appendix 7f). Thus, it was necessary to run separate analyses on each level of this interaction. However, before the results of these tests are presented, an examination of the time by tidal height by layer interaction (p = .066) is in order, because this interaction was close to the .05 alpha level. When this was done there appeared to be a trend of greater mortality at the surface layer than below the surface at the +25 cm tidal height during the June/July period (Figure 37).

When separate repeated measures were performed on each tidal height, mortality was found to be greater during the August/September period for oysters found at MLW and -90 cm tidal heights and during the June/July sampling period for oysters found at the + 25 cm tidal height (Appendix 7h, Figure 38). Furthermore, when each time component was examined separately to help decouple the tidal height by time interaction, mortality was found to be highest at the +25 cm tidal height during time interval 1 (June - July) and highest at the -90 cm tidal height during time interval 3 (August - September) (Appendix 7g, Figure 38). No significant difference in mortality was detected during the second sampling period.

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 that there was a definite trend of lower mortality at the MLW tidal height (Figure 39). Overall mean cumulative mortality ranged from 14.2% to 23.4%, which was higher than that documented for the older, 1993 oysters (Figure 40).

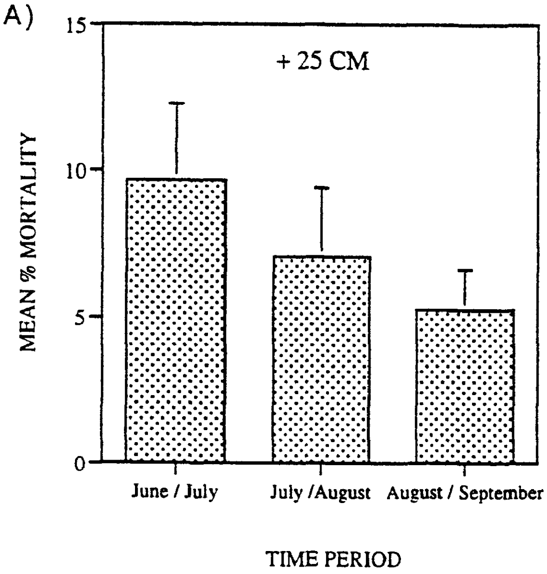
Figure 37. Mean percent mortalities examined by substrate level of 1994 reared *C. virginica* residing at the +25 cm tidal height during the June/July sampling period. Error bars denote +1 standard error.



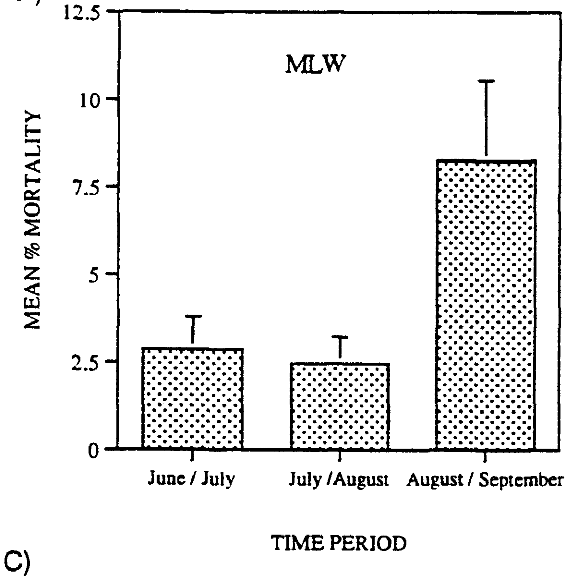
20
15
10
5
0
MEAN % MORTALITY
Surface
Deep
SUBSTRATE LEVEL

Figure 38. Mean percent mortalities examined by tidal height and sampling period of 1994 reared *C. virginica*. Error bars denote +1 standard error.

A)


+ 25 CM
MEAN % MORTALITY
15
10
5
0
June / July
July /August
August / September
TIME PERIOD

B)


MLW
MEAN % MORTALITY
12.5
10
7.5
5
2.5
0
June / July
July /August
August / September
TIME PERIOD

C)

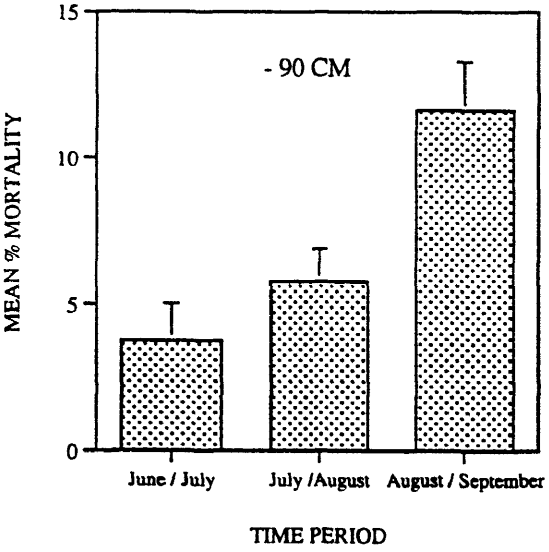

- 90 CM
MEAN % MORTALITY
15
10
5
0
June / July
July /August
August / September
TIME PERIOD

Figure 39. Mean cumulative percent mortalities of 1994 reared *C. virginica* found at three tidal heights. Error bars denote +1 standard error.

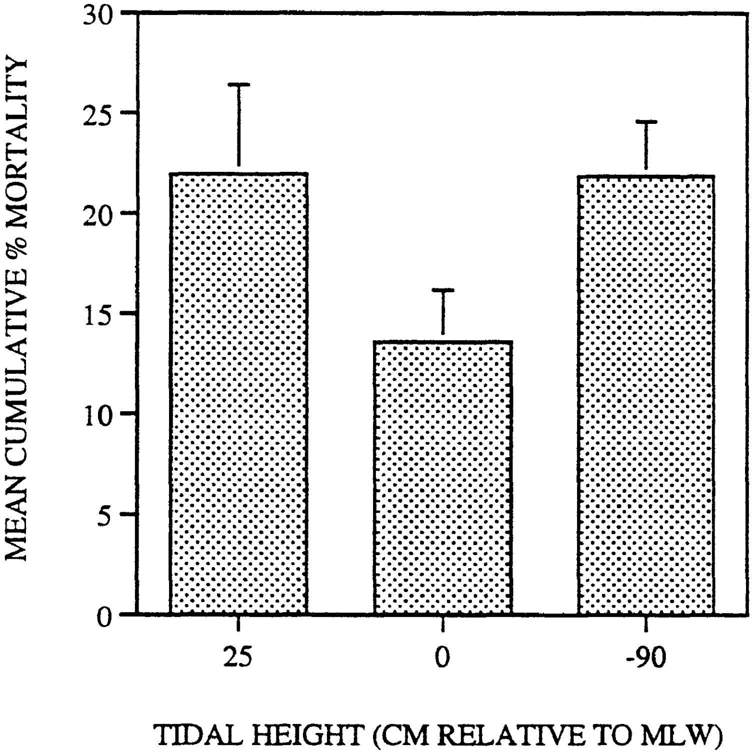
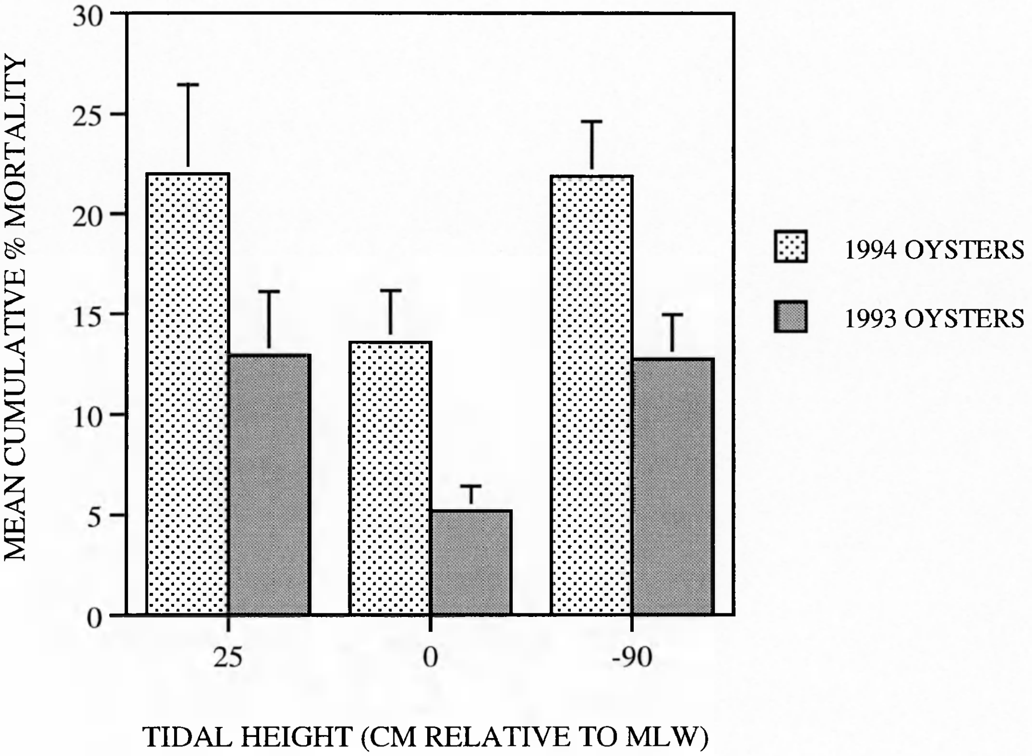

MEAN CUMULATIVE % MORTALITY
30
25
20
15
10
5
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)

Figure 40. Comparison of mean cumulative percent mortalities of 1993 and 1994 reared *C. virginica* found at three tidal heights. Error bars denote +1 standard error.


MEAN CUMULATIVE % MORTALITY
30
25
20
15
10
5
0
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
1994 OYSTERS
1993 OYSTERS

Destructive Reef Samples

Shell heights of oysters collected from the reef did not differ between the two substrate levels (Appendix 8a). Results of ANCOVA analysis revealed that both tidal height and the covariate, time, significantly influenced shell height measurements (Appendix 8b). SNK multiple comparison tests performed on tidal height detected significantly larger heights with increased tidal depth. On the final sampling date (October 13), mean shell height at +25 cm was 33.6 mm ± .8 SE, at MLW was 43.6 mm ± 1.1 SE, and at -90 cm was 47.4 mm ± 2.4 SE (Figure 41). Since no oysters were present when the reef was constructed and the settlement season began on the reef August 12, 1993, these oysters were no older than 14 months.

Density at the +25 cm tidal elevation was greater at the deep substrate layer than at the surface layer, and density at the -90 cm tidal elevation was greater at the surface layer than at the deep layer (Appendix 8c). A graphical depiction of density at the +25 cm and -90 cm tidal heights at each sampling date revealed that the differences detected by paired t-tests were consistent with trends observed over time (Figure 42). Thus, the difference in layers could not be attributed to a large disparity observed at any one particular time. Separate ANOVAs performed on each substrate layer, revealed that density was dependent on tidal height for both substrate levels (Appendix 8d). SNK multiple comparison tests revealed that densities were greatest at the -90 cm tidal height (Figure 43).

Figure 41. Mean shell heights of *C. virginica* found at three tidal heights collected from the Piankatank reef on October 13, 1994. Since the earliest detection of oyster spat on the reef was August 12, 1993, these oysters were no older than 14 months. Error bars denote +1 standard error.

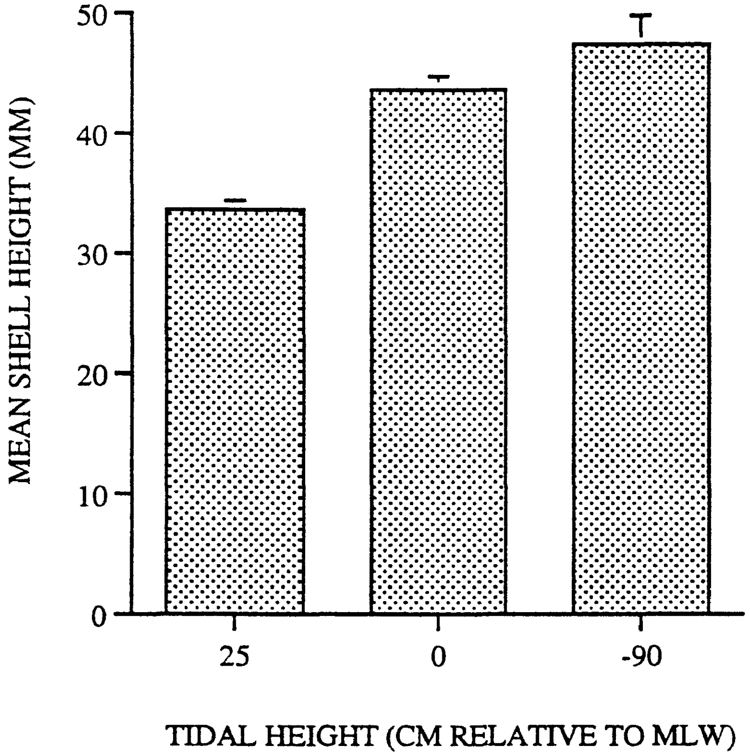
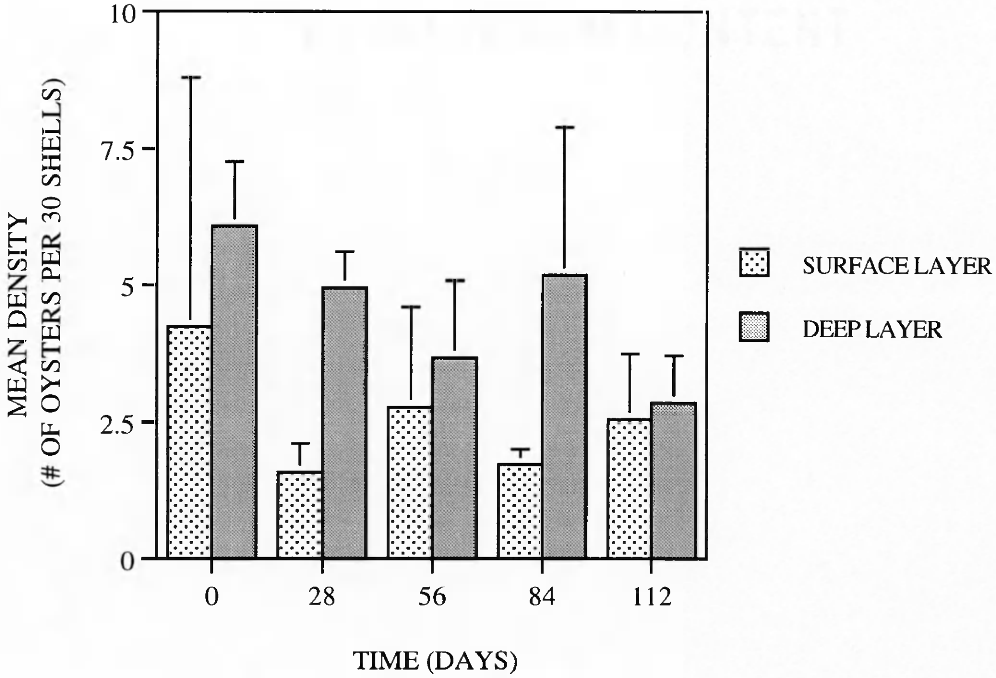

50
40
30
20
10
0
MEAN SHELL HEIGHT (MM)
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)

Figure 42. Surface and deep substrate level *C. virginica* mean densities at the Piankatank reef from June 23 through October 13, 1994 collected at (A) the +25 cm tidal height and (B) the -90 cm tidal height. Error bars denote +1 standard error.

A)

+ 25 CM


MEAN DENSITY
(# OF OYSTERS PER 30 SHELLS)
10
7.5
5
2.5
0
0
28
56
84
112
TIME (DAYS)
SURFACE LAYER
DEEP LAYER

B)

- 90 CM

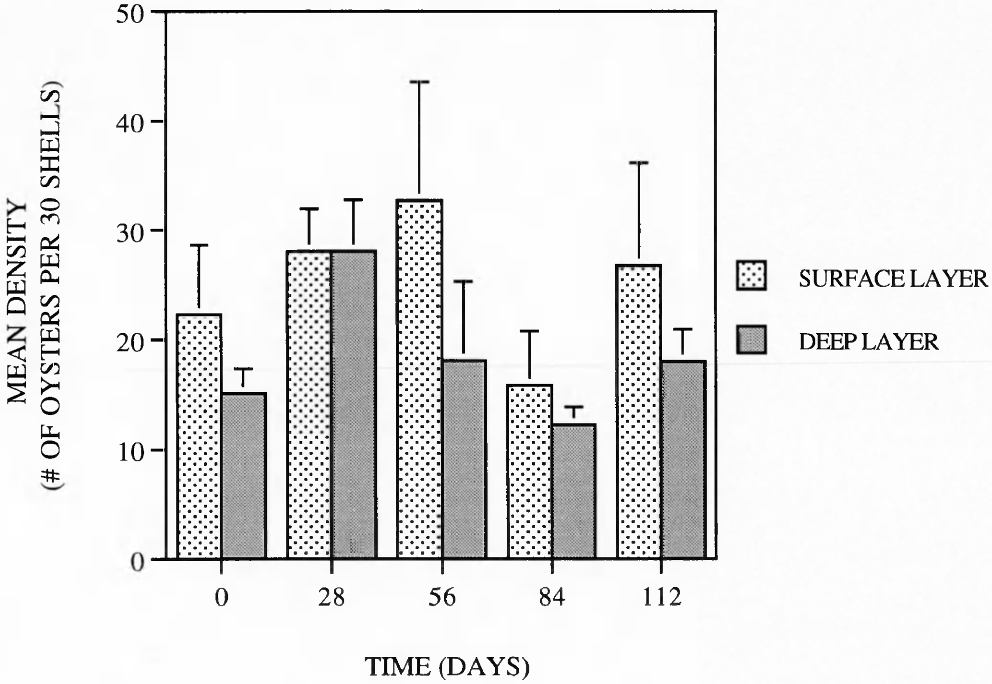
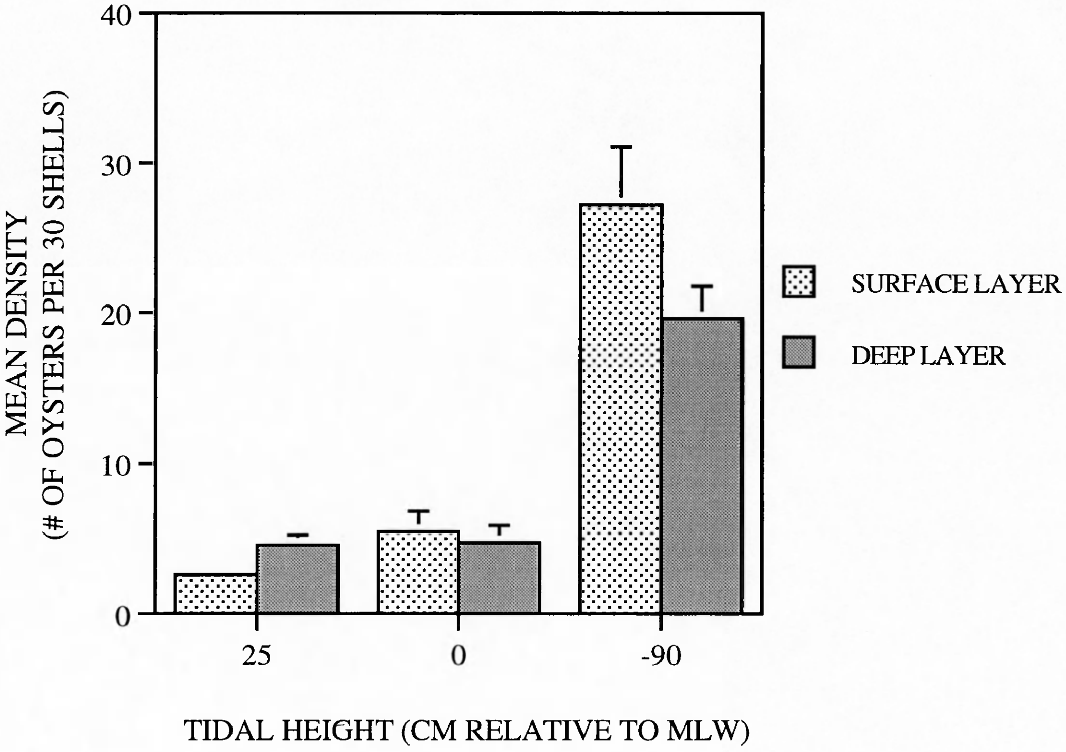

MEAN DENSITY
(# OF OYSTERS PER 30 SHELLS)
50
40
30
20
10
0
0
28
56
84
112
TIME (DAYS)
SURFACE LAYER
DEEP LAYER

Figure 43. Mean surface and deep substrate layer densities of *C. virrginica* found at three tidal heights. Error bars denote +1 standard error.


40
30
20
10
0
MEAN DENSITY
(# OF OYSTERS PER 30 SHELLS)
25
0
-90
TIDAL HEIGHT (CM RELATIVE TO MLW)
SURFACE LAYER
DEEP LAYER

Effects of Flow Rate and/or Density on Settlement, Growth, and Mortality

Settlement was not dependent on water flow ($p > .05$; $r^2 < .25$) (Appendix 9a). Growth of 1993 and 1994 oysters were not dependent on water flow ($p > .05$; $r^2 < .30$), and mortality of 1993 and 1994 oysters did not have a functional dependence on water flow for the majority of cases (Appendix 9b). Finally, growth and mortality of 1993 and 1994 were not functionally dependent on density for the majority of cases (Appendix 9c), and there was no trend of increased significance or r^2 values with time.

Physical Factors

Salinity, water temperature, and secchi disk readings recorded during the sampling period are presented graphically in Figures 44 - 46. During the first sampling period of the hatchery-reared growth/mortality studies which began either June 14 (for 1993 oysters) or June 20 (for 1994 oysters), a rapid increase in salinity was recorded. But salinity in subsequent periods was relatively constant, fluctuating between only 15 and 17‰. Water temperature during the first two periods of the growth/mortality studies (June 14 - August 16) ranged from 26° - 30°C, and during the last period (August 9 - September 13) decreased considerably to 22.5°C. Higher water clarity was also observed during the last sampling period, which began August 9 for 1993 oysters and August 16 for 1994 oysters. For studies conducted on both year classes, mean air temperatures were greatest during the first sampling period and lowest during the last sampling period (Figure 47).

Figure 44. Salinities at the Piankatank River reef site from May 26 through September 15, 1994.

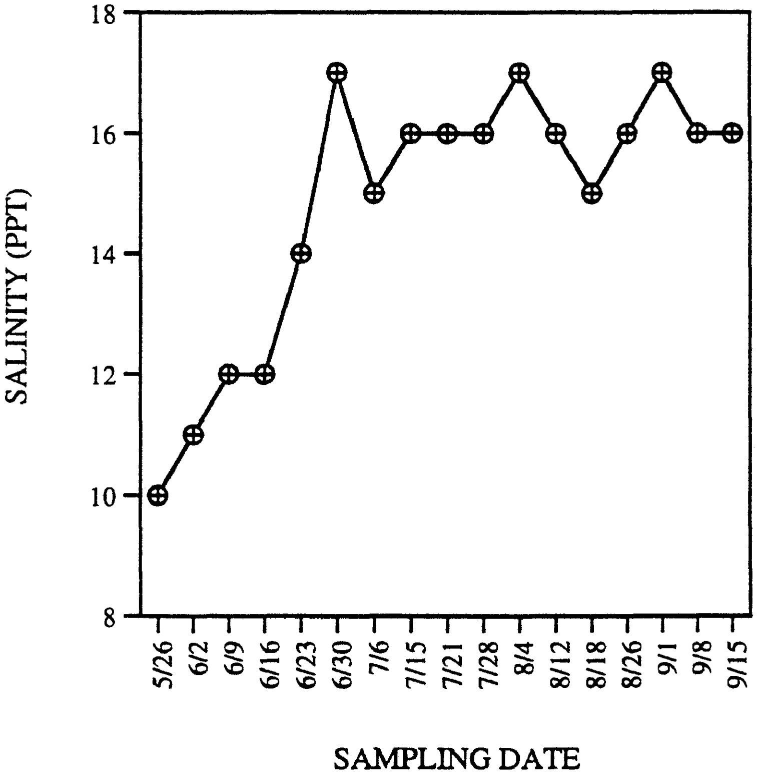
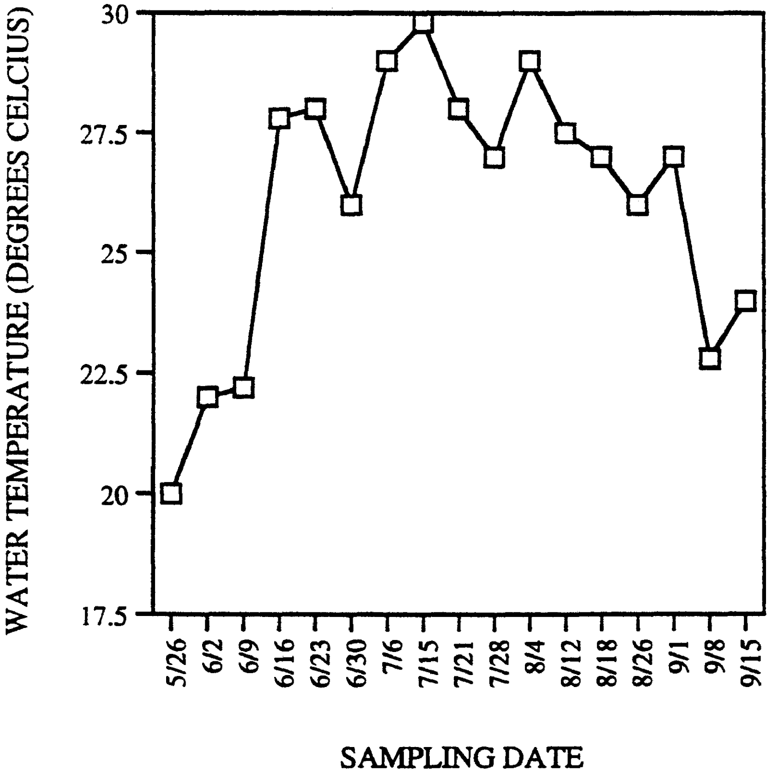

18
16
14
12
10
8
SALINITY (PPT)
5/26
6/2
6/9
6/16
6/23
6/30
7/6
7/15
7/21
7/28
8/4
8/12
8/18
8/26
9/1
9/8
9/15
SAMPLING DATE

Figure 45. Water temperatures at the Piankatank River reef site from May 26 through September 15, 1994.



WATER TEMPERATURE (DEGREES CELCIUS)
30
27.5
25
22.5
20
17.5
5/26
6/2
6/9
6/16
6/23
6/30
7/6
7/15
7/21
7/28
8/4
8/12
8/18
8/26
9/1
9/8
9/15
SAMPLING DATE

Figure 46. Secchi disk measurements at the Piankatank River reef site from May 26 through September 15, 1994. The y-axis refers to depth (m) below the water surface where the disks was still detectable.

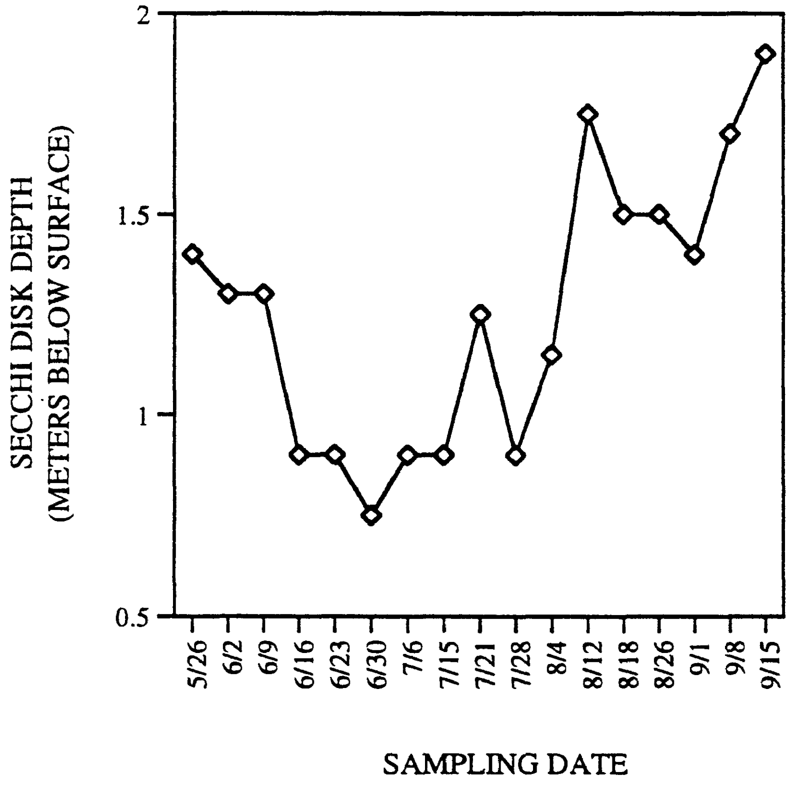
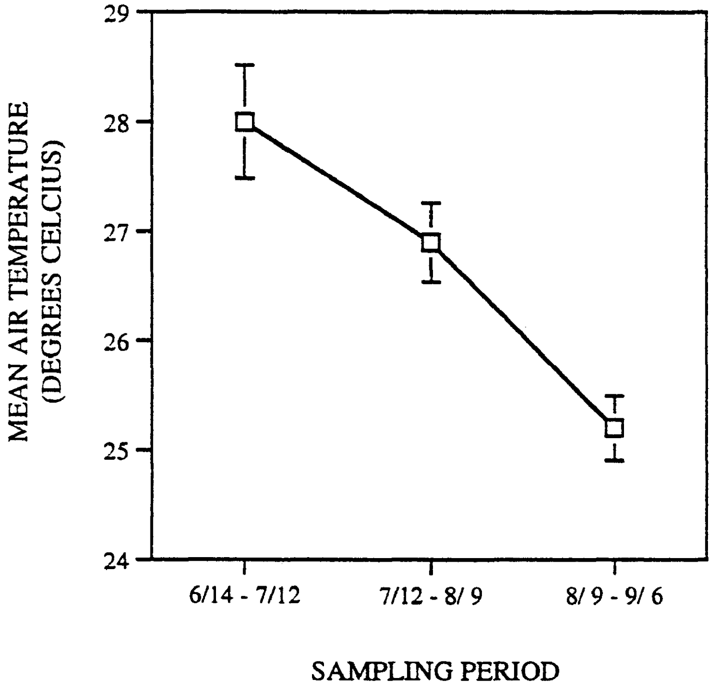
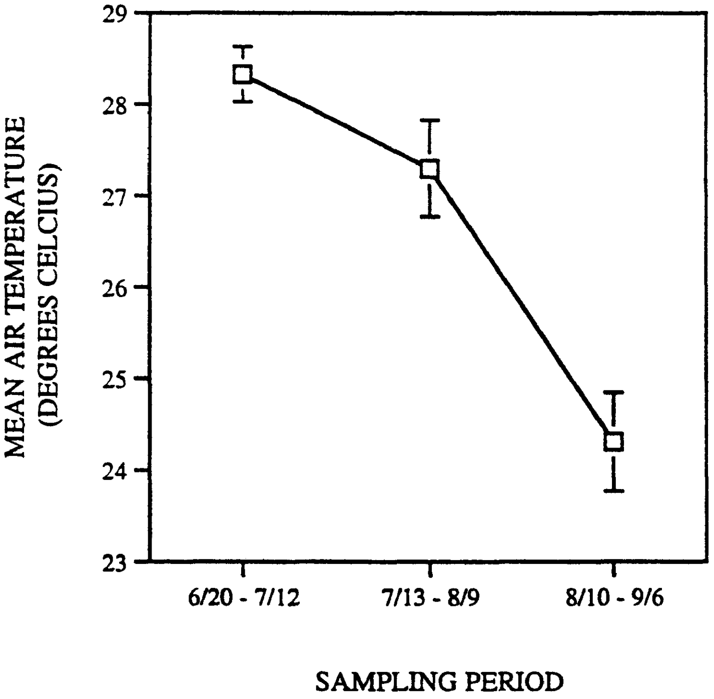

SECCHI DISK DEPTH
(METERS BELOW SURFACE)
2
1.5
1
0.5
5/26
6/2
6/9
6/16
6/23
6/30
7/6
7/15
7/21
7/28
8/4
8/12
8/18
8/26
9/1
9/8
9/15
SAMPLING DATE

Figure 47. Mean air temperatures for the three sampling periods considered in (A) the 1993 hatchery-reared oyster growth/mortality cage study and (B) the 1994 hatchery-reared oyster growth/mortality cage study. Error bars denote ±1 standard error.

A)


MEAN AIR TEMPERATURE
(DEGREES CELCIUS)
29
28
27
26
25
24
6/14 - 7/12
7/12 - 8/ 9
8/ 9 - 9/ 6
SAMPLING PERIOD

B)


MEAN AIR TEMPERATURE
(DEGREES CELCIUS)
29
28
27
26
25
24
23
6/20 - 7/12
7/13 - 8/9
8/10 - 9/6
SAMPLING PERIOD

DISCUSSION

Pilot Study

A number of researchers have reported on the effects of tidal elevation, time, and a variety of microspatial factors on oyster settlement (Chestnut and Fahy 1952, 1953, McNulty 1953, Nichy and Menzel 1967, Bushek 1988, Roegner and Mann 1990, Kenny et al. 1990, Michener and Kenny 1991, Ortega and Sutherland 1992, Tamburri et al. 1992), growth (Bahr 1976, Spencer et al. 1978, MacKenzie 1981, Littlewood 1988, Roegner 1989, Morales and Mann 1990, Crosby et al. 1991), and survivorship (Nichy and Menzel 1967, Spencer et al. 1978, MacKenzie 1981, Ortega 1981, Roegner 1989, Littlewood et al. 1992). However, research in these areas performed on reefs, the most important habitat for oysters, is surprisingly limited. When this small body of research is examined, it quickly becomes apparent that the effects of tidal elevation, time, and microspatial factors are highly variable depending on geographic location, sampling time, and size of reef community considered. It also becomes quite evident that a wide range of sampling techniques, many of which are artificial, are utilized in these studies. Although artificial forms of monitoring oysters in reef systems are more efficient and practical, they may not be representative of natural ecosystems. In fact, faced with the realization that their "artificial" reef samples deviated dramatically from nature, Dayton et al. (1989) stressed that a "proper study of bivalves on reefs would be fascinating but very difficult".

The objectives of this study were twofold. First, I wanted to develop an idea of what effect a variety of factors had on settlement, growth, and mortality of oysters at the Piankatank River reef site. Second, I wanted to determine which sampling methods were not only practical and accurate, but also the most representative of natural reef ecosystems. Since the objective of the pilot study was not to precisely document the effects of certain factors on oyster processes, a detailed explanation of the data will not be discussed here. Comprehensive explanations of critical factors will be covered in the discussion of the primary study, where factors were tested more expansively. Only data collected during months not considered in the primary study or data anomalies will be discussed thoroughly.

Settlement

Several factors appeared to influence settlement. Settlement was highly dependent on tidal height and time; spat counts were highest subtidally and settlement intensity increased over time for destructive samples and decreased four weeks into the settlement season for non-destructive samples. Conversely, settlement was not dependent on plot orientation or mound location. Aside from tidal height and time, two additional elements detected during field observations appeared to influence settlement. One element was substrate level. It appeared as if more spat settled on shell six inches below the reef surface, where substrate was free of algal growth, than at the reef surface. It would be of interest in future study to determine experimentally if oyster larvae actively seek out these less-fouled lower layers and settle in higher densities below the reef surface. A second element was flow rate. It appeared that settlement was higher in areas of moderate to significant flow than in areas of low flow and that flow rate, more so than mound location or plot orientation, influenced settlement patterns. Therefore, it may be useful to incorporate flow rate in future research as well.

A comparison between destructive and non-destructive settlement samples revealed some key differences. Significant incremental increases in settlement were detected with each week of sampling for destructive samples but not for non-destructive samples. This was not surprising considering destructive sampling provided a cumulative index of settlement, whereas non-destructive sampling provided a weekly index of settlement. It was also clear that higher settlement rates were present in the non-destructive samples. This may have occurred for two reasons. First, destructive sampling factored in a longer post-settlement mortality period. Second, the substrate provided in non-destructive samples may have been more favorable because shell substrate was placed in the field for only a week, and thus was less fouled with algae, sedimentation, and colonization organisms. If post-settlement effects dominate and differences in settlement between the two substrates are minimal and/or fairly constant, than post-settlement mortality predictions are highly feasible. Therefore, the first question that needs to be addressed is: was the discrepancy in spat counts between the two methods more a product of post-settlement mortality or more a result of discordant initial settlement rates on the two substrates?

This question was addressed in an analogous study by Ortega and Sutherland (1992), in which oyster recruitment was examined using both mats of shell replaced weekly and mats of shell unmanipulated during the settlement season. They concluded that fouling due to algal overgrowth and sedimentation, rather than post-settlement mortality, was the main cause of reduced settlement on the unmanipulated mats. Unfortunately, this same determination could not be made in this study because spat detection for destructive samples was more erroneous than for non-destructive samples. This was evident by the fact that when negligible amounts of spat were detected on non-destructive shells and shellstrings during week 6, substantial increases in settlement were found on destructive reef shells. This result suggests that when spat were small, detection was difficult on heavily fouled reef shells, but when spat became larger (weeks 5 and 6), they were more detectable and thus spat counts were higher.

Although accurate detection of spat on heavily fouled reef shells is probably unachievable during the early weeks of settlement, destructive sampling should not be eliminated because it provides an index of actual recruitment (number of surviving settlers after a well defined period of time) on the natural reef substrate which is not supplied by more artificial methods. Non-destructive sampling should not be omitted either. Although non-destructive sampling fails to provide a realistic appraisal of settlement on the natural reef surface, it does provide precise estimates of weekly settlement and is useful in determining when settlement begins and ends.

In future study, however, additional destructive samples should be collected and processed after settlement is no longer detected, and this should be continued until spat counts level out or decline (as a result of post-settlement mortality). Collecting additional samples will not only provide a more accurate measurement of settlement/recruitment on the reef substrate, since spat in additional samples will be larger and more detectable, but also will provide an estimate of post-settlement mortality because weekly destructive spat counts can be compared when no settlement is occurring. This estimate will be useful in determining whether discrepancies in the settlement samples are attributed more to substrate selectivity or to post-settlement mortality. When this is known, useful comparisons can be made between destructive and non-destructive settlement samples.

Cumulative subtidal spat counts per shell computed from shellstrings were greater than both destructive and cumulative non-destructive subtidal spat counts per shell. The fact that spat counts were higher on shellstrings than non-destructive samples that also utilized cleaned shell suggests that shellstrings, which are suspended from pilings, overestimate settlement on sloping reef bottoms. Baker (1994) also found shellstrings to be unreliable predictors of settlement magnitudes on adjacent substrates. However, even though shellstrings fail to provide accurate assessments of actual oyster sets on reef topography, they are efficient and reliable predictors of the presence of competent to settle larvae at the study site. This was evident by the fact that beginning and ending dates of the settlement season predicted by shellstrings coincided exactly with detection of spat on non-destructive and destructive samples.

Size Class/Growth Assessment

Tidal height and time, rather than mound position and plot orientation, surfaced as being the critical factors shaping shell area and shell height. Shell areas of hatchery-reared oysters were found to increase with both tidal depth and time. The effects that tidal height and time had on shell heights were less straightforward and are worthy of a more in depth discussion. During the first month (October) of destructive reef sampling, shell heights of oysters found at the +30 cm tidal height were equal or greater than oysters residing in the subtidal zone. This finding was probably a product of age discrepancies in the oysters found at different tidal heights. A higher percentage of the total number of oysters found at the +30 cm tidal height settled during the first two weeks of the settlement season than at any other height. Thus, the population of oysters at the +30 cm tidal height consisted of a greater percentage of older, larger oysters, and as a result, size data collected during the initial sampling period reflected this difference in age. But as time progressed, oyster residing at deeper tidal depths grew faster and soon attained larger shell heights.

A retardation in both shell area and shell height accretion was detected from December through April. This was not surprising. Growth and feeding of *C. virginica* are inhibited below 8°C, at which temperature the oyster hibernates (Galtsoff 1964). Water temperature fell below 8°C on December 12 and did not rise above this temperature until late March. Thus, during the five months from December through April, oysters were able to grow only during a part of December and all of April. Growth during the months of December, April, and May are also quite slow in the mid-Atlantic region (MacKenzie 1981).

In addition to tidal height and time, substrate level and flow rate may be important factors to consider when assessing oyster growth. Within the intertidal zone, oysters below the reef surface appeared to grow faster, as was evident by sharper peripheral shell edges, than oysters at the surface. This observation suggests that underlying layers may provide an environment highly conducive for oyster growth. Although no obvious relationship between size and flow speed was observed in the field, it may be useful to consider how flow affects growth in future studies. Oysters are filter feeders and thus are dependent on flow to some degree for nourishment. Given that there appeared to be a wide gradient of current regimes at the reef site, flow rate may be an important factor to consider when measuring growth in future studies at this reef system.

In the course of this study, it became apparent that there were several weaknesses in methods used to assess growth. First, many reef oysters grew in highly irregular patterns in response to neighboring oysters, barnacles, or substrate shell attributes, and measuring only shell height was not the most representative measure of growth. Irregular shapes could be measured using the photosample/digitization method described earlier. However, the major shortcoming of this approach was that oysters oriented vertically could not be measured accurately using two-dimensional photography. Furthermore, hatchery oysters used in the digitization study were too densely aggregated on the shells and were not representative of oyster densities found on the reef. Finally, neither method provided a precise measure of growth since the same individual organisms were not tracked throughout time.

The most accurate method for documenting oyster growth on reefs would be to photograph labeled (and thus trackable) oysters situated both horizontally on some flat plane of the shell and in densities comparable to those found on the reef. Shell areas could then be documented using the optical pattern recognition system. Although this method is very precise, it is also expensive and time consuming and may not be feasible for all growth assessment studies. However, if shell heights are used as a parameter for monitoring the growth of oysters in dense communities such as oyster reefs, the limitations of this method should be considered.

Mortality

This study revealed that mortality was lowest at the MLW tidal height from September through October (although not statistically significant) and from October through November (statistically significant), and that this tidal height provided a more favorable environment for oysters than either the +25 cm or the -90 cm tidal heights during the fall. The survivorship of sessile intertidal organisms in the upper intertidal zone is dominated largely by physical factors, whereas the lower vertical limit is determined predominately by biological factors (Connell 1972). It appears that in this study, the MLW tidal height provided the best balance between these physical and biological factors during the fall. In the subtidal zone oysters must compete for space and food with a suite of organisms such as barnacles, bryozoans, worms, amphipods, hydroids, and tunicates (Kennedy 1980, Abbe and Yates 1982, Galtsoff 1964, Osman et al. 1989) and may be preyed upon by oyster drills, sea stars, flatworms, crabs and fish (Galtsoff 1964, MacKenzie 1981, Abbe 1986, Eggleston 1990 a,b,c). Although these biological forces probably have the greatest impact on mortality in the subtidal zone, oysters in this region also have to contend with the sand scoring (Kennedy and Breisch 1981) and burial by fine sediment and biodeposition (Lund 1957, Haven and Morales-Alamo 1966). All of the above forces are reduced in the intertidal zone. However in the intertidal zone, desiccation stress (Bahr and Lanier 1981, Roegner 1989), rapid temperature fluctuations (Galtsoff 1964, Nichy and Menzel 1967, Newell 1979), wave action (Ortega 1981), and birds and terrestrial organisms (Bahr and Lanier 1981) are all detrimental to oyster survival. During the Fall of '93, oysters residing at the MLW mark may have achieved some refuge from subtidal predators, competitors, and physical stresses because of emergence, but because of only moderate aerial exposure, did not have to withstand harsh atmospheric conditions for prolonged periods of time.

If physical conditions are too severe and oysters are still relatively young, however, oysters living at the MLW tidal height may experience high mortalities even though they are only exposed for a short period of time. This was clearly evident by the high mortality rates at +30 cm above MLW and MLW observed in this study over the winter. These mortalities may be attributed to an unusually harsh winter, in which air temperatures dropped below freezing 28 times from December through March, and to the fact that oysters were no older than five months old. Oysters are unable to survive several hours of exposure to below freezing air temperatures, especially during the first year of development (Galtsoff 1964). Galtsoff found oysters to be especially vulnerable to freezing conditions during their first year because they put much of their energy into growth and maintenance rather than the storage of glycogen, a preferred substrate for anaerobic respiration, and thus were less capable of environmental isolation.

The devastating effect that freezing air temperatures had on intertidal oysters was obvious in January, shortly after a spell of freezing air temperatures. Oysters within the intertidal zone on the surface of the reef gaped widely and many were embedded in ice. Of the eight bags of hatchery-reared oysters placed on the reef in the intertidal zone, only one bag contained oysters with mortality rates below 85%. This bag of oysters, which had a mortality rate of 49.2%, was actually buried 6 cm below the reef surface whereas the other bags were totally exposed. Furthermore, an examination of reef oysters in these underlying layers revealed that considerably more oysters were alive there than at the surface. This finding implies that oysters in underlying layers may have gained refuge from ice and wind during the winter months, which may have resulted in higher rates of survival. Thus, in addition to tidal height and time, substrate layer is an important factor to consider when examining mortality of reef oysters.

The inexact method used to assess mortality together with the examination of oysters in unrepresentative densities were major shortcomings of the mortality study. Mortality rates were documented by first recording the number of clean, easily distinguishable oyster scars found on the concave, shiny sides of 25 shells haphazardly sampled at each plot, and then dividing that value by the total number of live oysters plus oyster scars found on the concave sides of the shells. This provided a proportional mortality value for each plot. For this method to be accurate, all dead oysters must leave an obvious scar. This was a major assumption, considering many of the subtidal shells were heavily fouled and many of the scars may not have been easily discernible. Furthermore, oysters considered in this study were more densely aggregated than oysters found on the reef. Higher densities may have inflated intraspecific competition, and thus mortality estimates of hatchery oysters may have been unrepresentative of mortality found on the reef. This study could be improved considerably by tracking the fate of individually marked organisms situated on shell in densities similar to those found on the reef. This would allow for the precise and representative documentation of oyster mortality.

Conclusion

Tidal height and time clearly accounted for a greater proportion of the variation in settlement, growth, and mortality than either mound location or plot orientation. Thus, tidal height and time are the most instrumental factors (of the factors tested directly) influencing the above processes and should be addressed in future study to precisely document their effects. From the results of this study, all that can be concluded is that tidal height and time significantly influence settlement, growth, and mortality on two mounds within the entire reef ecosystem. It is of greater interest to determine what effect these factors have over the entire intertidal reef community, and therefore future study should be more geographically expansive.

During the course of this experiment, several other factors surfaced as being worthy of further examination. One factor was substrate level, or depth within the reef topography. Observations during the settlement, growth, and mortality studies suggested that reef habitat below the surface layer may play an important, if not vital, role in the reef ecosystem. Flow rate, rather than mound location and plot orientation, also may be an important factor to consider. There were several areas at the reef site where flow was minimal and oyster settlement appeared to be greatly reduced. Since oysters are filter feeders, it is also highly feasible that growth and mortality may be influenced by flow rate as well. Density, which in all likelihood influenced size and mortality measurements, should also be considered.

Future study should incorporate a number of modifications in the sampling procedure. It was clear from the present settlement study that each method of sampling used had its limitations. However, each method also made a valuable contribution to recruitment assessment, and when considered collectively were quite informative. Although shellstrings overestimated spat counts, they were reliable, efficient tools for detecting competent to settle larvae. Non-destructive sampling provided an accurate weekly measure of spat set on the reef under ideal substrate conditions, whereas destructive sampling provided a measure of actual short-term recruitment at the reef site. Results from this study suggest that early detection of spat on destructive reef shells may be difficult, and thus early settlement estimates should be considered with caution. Furthermore, it is important to continue destructive sampling after the settlement season until spat counts either attain an asymptotic value or decline. This is necessary to provide an accurate estimate of recruitment on the reef structure and to develop an idea of post-settlement survival rates. Of the two methods considered to assess growth, the photosample/digitization method was more accurate than simply measuring shell heights because it accounted for irregular shell growth. However, if this method is used, oysters oriented vertically should be eliminated from consideration, and oyster densities used in the study should resemble densities found on the reef. In addition, for growth to be documented most precisely, individual oysters should be tracked. This procedure of marking and tracking oysters as well as making sure representative oyster densities are used should be followed for survivorship assessment as well, so that the most precise and representative estimate of mortality can be measured.

Primary Study

Based on the results of the pilot study, it was clear that tidal height and time play instrumental roles in shaping settlement, growth, and mortality of oysters at two localized mounds and that substrate layer, flow rate, and density may influence these processes as well. However, all of these factors could not be investigated extensively in the follow-up study because resources were limited. A good example of a factor that could not be examined thoroughly was time. Even though time was found to play a critical role in oyster ecology, it was not feasible to investigate the effects of time over a year or more or even over six months, given budgetary and time constraints. Thus, the effects of time were only considered over a three month period from June through September. Furthermore, the effects of flow rate and density, although very intriguing, were not of primary concern in this study, and thus were not examined thoroughly.

The factors of most interest were tidal height, because aerial exposure is the most prominent factor distinguishing this reef community from present subtidal reefs in Virginia, and substrate layer, because residence below the surface may play a vital role in the early colonization and establishment of artificial reefs in temperate climates. Therefore, this study was designed to investigate extensively the effects of tidal height and substrate layer on settlement, growth, and mortality of oysters over the entire artificially constructed intertidal reef system. To a lesser extent, I considered how the effects of tidal height and substrate layer, as well as oyster settlement, growth, and mortality, varied with time over a summer period. The effects of flow rate and density on settlement, growth, and mortality were examined briefly as well.

Settlement and Early Post-settlement Mortality

The results of this study demonstrated that oyster settlement intensity was similar at and 10 cm below the surface layer of reef communities, irrespective of whether reef substrate consisted of clean, non-fouled oyster shell or moderately fouled natural shell bottom. This was unexpected considering the deep substrate level at times was buried beneath 15 layers of shell (destructive samples), and thus was not easily accessible to oyster larvae. To my knowledge there are no studies on the settlement of sessile organisms as a function of depth within the substrate to which this study can be compared. However, the fact that adult oysters are found in greater numbers at the surface of established reefs (Bahr and Lanier 1981, Galtsoff 1967) 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 that discrepancies in ranges of settlers and adults were indicative of differences in post-settlement mortality. Post-settlement mortality comparisons between the two layers will be discussed shortly, but now it is of interest to consider why oyster larvae settled in similar numbers at both the surface and at less accessible layers below.

Whenever a tests fails to reject a null hypothesis, it is important to consider statistical power (1-ß), or the probability of not making a type II error (Peterman 1990). Considering that settlement at the reef site was dramatically lower than it has been in recent years and that paired t-tests revealed no significant differences among the two layers, it is important to consider statistical power. The mean statistical power of the paired t-test for detecting differences of 3 spat per 30 shells was .99. If one considers during moderate years of settlement in the Piankantank River hundreds or even thousands of spat settle per 30 shells, these tests appear quite powerful. However, this year settlement rates were dramatically low. In fact, mean weekly spat counts throughout the three week settlement period in the intertidal zone for both destructive and non-destructive samples were less than 1 spat per 30 shells, and at subtidal depths, ranged from 2 to 3 spat per 30 shells! The power of paired t-tests for detecting differences of 1 and 2 spat per 30 shells was .54 and .78, respectively. Thus, as a result of low settlement rates in the intertidal zone, even if spat counts between the layers were dissimilar, this difference would be rarely 2 or more, and because of low statistical power within this range, may not be detected. Therefore, caution should be used when interpreting intertidal results. At the -90 cm tidal height, the t-tests were more powerful because spat counts were greater and spat count differences (if they existed) would be in all likelihood greater as well. However, discretion still should be exercised when evaluating the results.

Results of power analyses suggest that a lack of statistical power may have been the primary reason settlement differences between the layers were not observed. However, I am not entirely convinced that low power was the cause of the lack of differences. Of the 36 non-destructive samples collected, settlement was greater at the surface 10 times, greater below the surface 10 times, and equal 16 times. Of the 36 destructive samples collected, settlement was greater at the surface 7 times, greater below the surface 9 times, and equal 20 times! These results strongly suggest that no consistent trend in settlement between the surface and deep layers was present.

Another tempting explanation for the lack of differences observed is that planktonic oyster larvae were transported into the immediate vicinity of surface and deep substrates at approximately equal rates by hydrodynamic forces. This may have been true in the intertidal zone where wave action facilitates water movement into the deep layer, but is unlikely in the subtidal zone. As larvae approach the surface layer of subtidal substrates, flow is dramatically reduced as a result of shear stresses within the benthic boundary layer. As a result of reductions in flow rate, no strong hydrodynamic forces, such as waves, drove oyster larvae deep below the surface layer. Therefore, for larvae to have settled in underlying, not readily approachable areas, they may have been demonstrating some photaxic or geotaxic response or some other behavioral tendency.

Oyster larvae actively engage in free-swimming searching behavior prior to settlement (Nelson 1924, Cole and Knight-Jones 1939, Cranfield 1973). If oyster larvae are capable of selecting favorable habitats and did not attach in the greatest quantities to the most readily available substrates (surface shell layers), then it may be argued that surface substrates were not ideal. Some oyster larvae may actively seek substrate far below the surface because they prefer darkened conditions when setting (Cole and Knight-Jones 1939, Nelson 1953, Ritchie and Menzel 1969, Shaw et al. 1970), areas of reduced wave action (Ortega 1981, Abbe 1986), and environments where flow is low and crevices are abundant (Bushek 1988, Michener and Kenny 1991). Shells in the deep layers were also free of algal growth, unlike the surface layers, and as a result some oysters may have chosen to settle below the surface on these cleaner substrates. Galtsoff (1964) felt that locating clean substrate is the most important concern of oyster larvae.

Although oysters larvae are capable to some degree of swimming, they by no means have complete control over where they will end up settling. Late-stage pediveliger larvae have high specific gravity, making them rather cumbersome swimmers, and often require currents to aid them in locomotion. It is highly feasible that once oyster larvae enter the interstices of the reef they have difficulty exiting due to reduced currents, and as a result the underlying layers serve as sediment traps. Thus, although hydrodynamic forces may not drive considerable amounts of larvae into the interstitial pores, they may serve to entrain larvae once they arrive. This larval entrainment may have been another mechanism contributing to settlement below the surface.

The higher quantities of spat observed in the subtidal zone for both the destructive and non-destructive samples suggest that both settlement and early recruitment (1 to 3 weeks) on the reef structure are greatest subtidally. This finding is consistent with the results of the pilot study and several other studies that examined settlement/early recruitment. For example, greater subtidal settlement was documented by McDougall (1942) using unglazed hearth tiles, by Chestnut and Fahy (1953) using shellstrings, by Nichy and Menzel (1967) using clothmats of mesh, and by Roegner and Mann (1990) using hatchery-reared larvae exposed to field conditions in microcosms.

All of the research does not indicate that settlement is always maximized subtidally, however. Hidu and Haskin (1971) 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 passes over heated intertidal substrates and to the presence of dense intertidal adult oyster populations which release chemical cues. McNulty (1953), using bags of shell left in the field for two weeks, found higher settlement 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. Comparisons between the latter two studies with the ones mentioned above should be made cautiously, however. In all of the studies discussed earlier sampling for the majority of the experiment was conducted weekly. Conversely, in the last two studies, samples were collected at intervals of 2 weeks or more. These periods may have been long enough for significant post-settlement mortality to take affect, which as Luckenbach (1984), Connell (1985), and Roegner (1991) stressed, may dramatically disguise settlement distributions of sessile invertebrates.

The higher rates of subtidal settlement observed in this study were likely a result of a number of factors. Submergence time may have played a large role in shaping settlement along the vertical gradient. Oyster larvae in the water column were exposed to subtidal substrates substantially longer than to intertidal substrates, and as a result may have set in higher quantities subtidally. Submergence time alone, however, did not account for the observed differential 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. Vertical segregation of oyster larvae within the water column may have contributed to elevated subtidal sets as well. Carriker (1951), Kunkle (1957), Haskin (1964), and Baker (1994) all found that oyster late state pedeviliger larvae in the water column are more abundant near the benthos than at the surface or within the mid-water region. Late stage competent to set oyster larvae also may have actively sought subtidal habitats where light intensities and wave stress are lower because they are photonegative at the time of settlement (Cole and Knight-Jones 1939, Ritchie and Menzel 1969) and prefer areas of lower wave energy (Ortega 1981, Abbe 1986, Bushek 1988). Chemical cues from adult oysters and barnacles, which were both more abundant in the subtidal zone, may have encouraged subtidal settlement as well. Oyster larvae may be induced to settle by waterborne pheromones or metabolites released from adults (Hidu and Haskin 1971, Hidu et al. 1978, Tamburri et al. 1992), conchiolin matrixes and shell periostracums of live oysters (Crisp and Meadows 1963, Crisp 1967), bacterial supernatants on live oyster shell (Bonar et al. 1990, Fitt et al. 1990, Tamburri et al. 1992), and by the presence of certain barnacles (Osman et al. 1989). These cues often result in gregarious settlement (settlement of oysters in dense aggregations often near established populations) which is widely documented (Crisp and Knight-Jones 1953, Hidu and Haskin 1971, Bushek 1988).

Results of both the primary and pilot studies indicate that only a single peak settlement event occurred over the summer rather than a bimodal peak of settlement often reported in other studies (McNulty 1953, Loosanoff 1966, Shaw 1969, Hidu 1978, Hayes and Menzel 1981, Haven and Fritz 1985, Kenny et al. 1990). The 1994 settlement season was also shorter (lasting only 3 weeks) and lower in terms of magnitude (< .25 cumulative spat per shell on non-destructive reef shells, < .6 cumulative spat on shellstrings) than any other season in the last 16 years (VIMS Spatfall Annual Reports). These low settlement figures together with statewide reports of reduced settlement (1994 VIMS Spatfall Survey) are strongly indicative of a rapidly declining oyster population weakened by disease, overharvesting, poor water quality, and predation (Hargis and Haven 1988, Mann et al. 1991).

Although the settlement period lasted 3 weeks, the majority of settlement occurred during a 2-week period from July 15 to July 28, and only negligible amounts of oyster larvae set the following week. As was discovered in the pilot study, early detection of spat on reef shells is difficult and early destructive spat counts provide a poor representation of settlement patterns during the early settlement period. This precluded a direct comparison of destructive reef samples and non-destructive tray samples during the peak settlement period (July 15 to July 28). However, after July 28 when settlement diminished and spat became larger and thus more detectable, measurements of spat on reef shells became more reliable and comparisons between the two methods could be made.

When a comparison between destructive and cumulative non-destructive spat counts was made, it was clear that non-destructive spat estimates were greater than destructive spat counts. However, mean spatfall detected on the reef at intertidal and subtidal depths for both cumulative non-destructive and destructive methods only ranged from .25 - 7 spat per 30 shells, and differences between the methods were only on the order of 1 - 3.5 spat per 30 shells. With such low settlement intensities and differences, it is difficult to determine whether settlement discrepancies were more attributable to post-settlement mortality or pre-settlement substrate preferences. This is because either mechanism could completely account for such small disparities.

Keough and Downes (1982), Luckenbach (1984), and Connell (1985) demonstrated that post-settlement mortality rates of sessile invertebrates may be very high shortly after settlement, which may dramatically reduce recruitment. In regards to oysters, high early mortality has been alluded to by Chestnut and Fahy (1952), MacKenzie (1981), and Michener and Kenny (1991). Roegner (1991) directly measured early post-settlement mortality and found a Type I mortality curve for recently settled hatchery-reared oyster larvae, in which mortality was high during the first week after settlement (73%) but dramatically decreased in subsequent weeks. Baker (1994) found that Roegner's early mortality estimates were unusually high when they were compared with other sets of data collected in the same area. Baker computed his own early post-settlement estimates by combining several sets of oyster mortality data and using regression analysis to derive a daily oyster mortality rate, which he computed to be 6.5% during the early post-settlement period. Based on this value mortality should be on the order of 45.5% after 7 days and 91% after 14 days. In this study, the destructive sample spat counts underestimated recruitment predictions by non-destructive samples by 50% subtidally and 70 - 80% intertidally. Therefore, based on Baker's and Roegner's estimates, early post-settlement mortality may have been a major reason for the observed discrepancies in spat counts.

On the other hand, the high post-settlement mortalities reported in Roegner and Baker's studies may not be representative of post-settlement mortality at the Piankatank reef, and another mechanism may be the cause of the discrepancies in spat counts between the two methods. The fact that only small differences in destructive spat counts were detected after July 28, when spat counts were a reflection of recruitment 1 - 3 weeks post-settlement, suggests that early post-settlement mortality at the reef site was not substantial and may not have contributed significantly to the disparity in spat counts Instead, the higher degree of fouling and colonization found on the reef shells may have been the cause. In an analogous study, Ortega and Sutherland (1992) found that shells attached to Vexar mesh mats retrieved weekly to examine spat, but returned to the field uncleaned, have significantly lower weekly spat densities than mats of shell that are changed each week. They attributed this disparity to algal turf attachment and sedimentation on the uncleaned shell mats. Resident infauna on reef substrate may have also deterred settlement on reef shells collected in destructive samples. Osman et al. (1989) found that few oysters are able to settle and survive on bryozoans, colonial ascidians, and certain barnacles. These organisms were all present on reef shells, and thus may have reduced settlement rates.

Effects of Physical Parameters on Settlement

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 results indicated that settlement did not occur until five weeks after water temperatures fell within this range. The onset of settlement did not coincide with any change in salinity or turbidity either, but peak settlement did occur during a rise in water clarity.

This is consistent with Calabrese and Davis (1966) and Davis and Hidu (1969), who found that rates of larval settlement and development are elevated when sediment loads in the water column are reduced. The results of this study suggest that neither water temperature, nor salinity, nor water clarity 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 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 documented heaviest settlement 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 larvae dropping out of suspension as water flow decreased. Furthermore, in Galveston Bay, Texas, Bushek (1988) found oysters preferentially settle nearshore on pier pilings where current velocities are 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. However, in this study, only a narrow range of currents was considered. Currents that were defined as high in Carriker's study corresponded to low/moderate current velocities in the studies mentioned above.

The observed independence of settlement and water flow may have been 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, the methods used to detect flow/water movement in this study in all likelihood did not accurately reflect water flow conditions at the point of larval attachment. Oyster larvae setting on the underside of surface substrates or 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 recorded 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.

Growth

Oysters of both year classes residing at the +25 cm tidal height grew at a greater rate below the reef surface than at the reef surface. In a report on intertidal oyster reefs of the South Atlantic Coast, Bahr and Lanier (1981) found that oysters at the surface of established intertidal reefs have sharper growing edges than those in lower layers, and felt that this was indicative of faster growth. This observation appears to contradict what was found in this study. However, this apparent contradiction may be resolved by considering differences in the developmental stages of the reefs surveyed in each study. Bahr and Lanier examined mature intertidal reef systems with dense aggregations of oysters at the surface layer, whereas I examined an artificial reef in its early stages of development when dense aggregations of oysters were not yet established. This distinction is notable because dense aggregations of oysters may seriously alter the underlying environment.
Lund (1957) and Haven and Morales (1967) found that biodeposition of oysters significantly increase sediment biodeposition rates by as much as seven to eight times. In established reefs where surface oysters are present in high densities, this biodeposition may fill interstitial pores, which allow high water flow, and reduce growth of underlying oysters.

In addition to biodeposition, sedimentation and siltation may also inhibit growth and elevate mortality (Galtsoff 1964, MacKenzie 1981). Dame et al. (1992) and Dame and Libes (1993) found accumulations of large quantities of sediment and silt on oyster reefs, especially within the interstitial pores. Although this may be true in established reefs where sediment can accumulate over years and in areas where sedimentation is high, sedimentation and siltation were not major problems below the surface of this reef because the reef was both very young and located in an area where sedimentation was low.

Flow limitations and spatial constraints may not have been major detriments to growth in underlying layers at the +25 cm tidal height, either. Water movement within the intertidal zone was very swift and energetic which allowed for the penetration of water deep below the surface, and algal growth and sedimentation, which may limit water access to underlying layers, was not heavy. Furthermore, Bahr (1976) found that growth of individual oysters in dense aggregations is slow when space is limited, suggesting that oyster growth in underlying layers should be reduced. This may not have been observed because oysters in underlying layers were able to create space by shifting overlying shell as they grew. In established reefs, much of the substrate is fixed and connected because of extensive clustering of generations of oysters. However, the reef examined in this study consisted of loose shell which could be easily dislocated. Thus, largely because this reef was in its early stages of development, oysters in lower layers were able to grow and survive.

Enhanced growth below the surface at the +25 cm tidal height was likely a product of both increased submergence times and less stressful environmental conditions. Since oysters residing in the deep layer were 10 cm below the surface layer in vertical elevation, they were inundated longer by tidal flow, and as a result could filter feed for longer periods of time and achieve larger 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 less susceptible to desiccation and heat stress. Bahr (1976) confirmed this at an intertidal oyster reef community at Sapelo Island, Georgia, where he found temperatures in October to be 7°C lower 6 cm beneath the surface than at the surface. This less stressful underlying habitat allowed oysters to put more energy into growth and less into environmental resistance. Therefore, as a result of both longer submergence times and environmental buffering, oysters in this study were able to grow faster than oysters at the surface at the +25 cm tidal height.

Contrary to what was found at the +25 cm tidal height, oysters from both year classes situated at the -90 cm tidal height grew fastest at the surface level. This reversal of growth optimization was largely due to the fact that oysters below the reef surface in the subtidal zone no longer benefited from longer submergence times (both subtidal layers were submerged constantly) and more environmentally favorable conditions (subtidal oysters were not exposed to harsh atmospheric stresses). Instead, oysters residing below the surface in the subtidal zone probably even lived in a less advantageous environment for growth than oysters at the surface. This was because unlike the intertidal zone where flow was energetic and swift, overall water movement in the subtidal zone was slow and gentle and less capable of penetrating the reef substrate. Furthermore, flow may have been more restricted below the surface in the subtidal zone than in the intertidal zone as well due to greater abundances of algae and colonizing infauna. Although oysters and shells to which they were attached found in the lower tier of subtidal cages had less algal growth and colonizing organisms than oysters in the upper tier, they may have been worse off. This is because for water to arrive at the lower tier of the cage, it had to pass through a heavily fouled surface layer as well as several intermediate shell layers containing sediment, infauna, and algal canopies. As a result, water accessibility to the lower layers was greatly restricted.

Oysters require a steady exchange of water to grow optimally (Galtsoff 1964). As a result of reduced and restricted flow, a steady, free exchange of water may not have always reached oysters below the surface in the subtidal zone, resulting in lowered and/or erratic growth. Furthermore, oysters at the surface had more unoccupied space to exploit than oysters in underlying layers and were able to extend vertically into the water column to access greater and less turbulent flows.

Oyster growth in relation to tidal height is quantified in several studies, most of which have found growth to be fastest in the subtidal zone. Loosanoff (1932), Ingle and Dawson (1952), Burrell (1982), and Roegner (1989) all found fastest growth subtidally for *C. virginica,* and Sumner (1981) found similar results for *C. gigas*. Gilmor (1982) and Crosby et al. (1991) presented evidence that *C. virginica* held in the intertidal zone under certain levels of aerial exposure is 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 faster growth in the intertidal zone than 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.

Many of the results of this study suggest that oysters growth in reef environments is greatest subtidally. At the surface substrate layer, 1993 oysters grew faster at heights of -90 cm and MLW than at +25 cm, and 1994 oysters grew faster with increasing tidal depth during sampling period 1 (June/July) and faster at -90 cm than at +25 cm during sampling period 3 (August/September). Within the deep layer, growth was greatest subtidally for the 1994 year class. Enhanced growth rates in the subtidal zone may be largely due to longer feeding times provided by constant submergence (Peterson and Black 1987). However, growth is not simply a direct function of submergence time. Crosby 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 zone were not directly proportional to submergence time. Within the intertidal zone, the metabolic stress associated with emergence may have played a role in reducing growth as well. 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.

Although significantly greater subtidal growth was detected for most of the oysters studied, no significant growth difference between tidal heights was observed for 1993 oysters residing below the surface. This finding may have been attributed to two factors. First, although growth appeared to be lower (though not always statistically) in the intertidal zone than in the subtidal zone for all samples collected, growth differences were maximized in the surface level samples and minimized in the deep level samples. This is clearly evident when one considers that growth of oysters dwelling at the +25 cm tidal height (the height at which growth was slowest) was greater at the deep level than at the surface level, and growth of oysters residing at the -90 cm tidal height (the height at which growth was fastest) was greater at the surface level than at the deep level. As a result, growth differences of oysters residing beneath the reef surface were more constricted than growth differences at the surface, and this made growth discrepancies harder to detect. The fact that the statistically significant probability values for surface layer comparisons in the 1994 year class were substantially lower than for the deep layer comparisons illustrates this point. The second reason for an absence of significant differences was that differences in growth were harder to detect for the 1993 year class than for the 1994 year class. Overall growth expressed in mm^2/day appeared to be similar between the two year classes and even may have been greater for the 1994 year class. Since oysters belonging to the 1993 year class were older and substantially larger than oysters belonging to the 1994 year class, proportional growth was correspondingly much smaller, and this made growth increments less perceivable.

It was intriguing to consider growth at the deep layer for the 1994 year class. The degree of sedimentation, amount of algal growth, and number of colonizing organisms found on oysters and shell within the deep layer at the -90 cm tidal height was only slightly higher than that found on oysters and shell within the deep layer at the +25 cm tidal height.

This is logical given that both habitats were isolated and protected from physical conditions that existed at the reef surface. However, the overlying environments were considerably different. Sedimentation, algal growth, and colonization were all greater at the surface layer of -90 cm habitats than at the surface layer of +25 cm habitats. This fact coupled with reduced overall water movements at the -90 cm tidal height suggest that water flow was more restricted within the deep layers at subtidal depths, which should have suppressed growth. On the other hand submergence times, and thus feeding times, were greatest subtidally, which should have also elevated growth rates. The fact that growth was greatest subtidally for the 1994 year class found in the deep layer suggests that the benefits of longer submergence time outweighed the disadvantages of restricted flow.

The depressions in growth rate from July through August observed across both year classes were perplexing. A dramatic decrease in growth rate 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 fastest. The decrease in growth rate 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. However, the 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 rate 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 factor which applies to both year classes is more likely the cause of the observed growth rate reductions.

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. Or the depression may have been an artifact of the method used to detect growth. During the first sampling interval (June/July) growth was clearly discernible as a lateral, band of newly deposited translucent shell at the periphery of the oyster. During the second sampling interval (July/August), the band width of new translucent shell was dramatically reduced for those oysters that experienced rapid growth during period 1 (generally oysters at MLW and -90 cm). Visual inspection of photographs during the second sampling session revealed that shell added during the first sampling period was no longer translucent but was thicker and layered. This shell layering pattern was indicative of growth in the vertical dimension that was not considered in the calculation of growth. Furthermore, there were also a number of cases of vertical curvature of newly deposited shell during the second sampling interval which could not be measured with the two dimensional system used. These observations suggest that lateral shell accretion may have decreased during the second sampling period for oysters residing at MLW and -90 cm, but overall growth may not have. The results also suggest that growth of oysters at the shell edge may not be linear during periods of intense grow-out, but instead may resemble a "stair-step" pattern. Erratic reef oyster growth was also detected by Copeland and Hoese (1966).

Most oysters considered in this study grew most rapidly during the last sampling period (mid-August through mid-September). Salinity probably did not account for this elevation in growth rate since it remained relatively constant throughout the sampling period. Increased growth may have been instead a product of both lowered water temperature and reduced sediment load in the water column, which was evident from secchi disk readings. Galtsoff (1964) suggested that optimal filter feeding of oysters occurs between 25 - 26 ° C. Water temperatures fell within this range more often during the last sampling period than at any other period. This fact may have contributed to elevated growth rates. Turbidity is highly detrimental to oyster pumping rates, which is closely coupled with growth since oysters are filter feeders (Galtsoff 1964). Thus, gills were able to pump water more efficiently during the last sampling period when turbidity was reduced, and as a result growth rates were increased. Elevated growth rates during this period for 1993 oysters may have also been partly due to a shift in growth from somatic and reproductive to simply somatic.

Effects of Water Motion and Density on Growth

The observed independence of growth and water motion in the subtidal zone, where water motion measurements were a reflection of flow rates, may have been because growth was more closely coupled with localized food depletion than with flow speed. Loosanoff (1958), Walne (1972), and Grizzle et al. (1992) all have documented relationships between oyster growth and water velocity. Loosanoff (1958) and Walne (1972) demonstrated that growth increases with elevated levels of flow. Whereas Grizzle et al. (1992), in one study which focused on current velocities from 0 - 10 cm / sec, found that growth decreases with increased current flow, and in another study which considered velocities from 0 - 5 cm/sec, found maximal growth at 1 cm/sec. In all of the 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 often 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 concentration 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 the intertidal plots, where water movement measurements were a reflection of both flow rate and wave action, growth was not dependent on water motion either. This lack of dependence may be partially explained by the reasoning used above; localized food depletion rather than flow speed was more closely coupled with growth. However, unlike measurements in the subtidal zone, the effects of wave action need to be considered. Ortega (1981) found oysters survive and grow poorly in areas of high wave energy. But Ortega considered high wave energy sites directly exposed to the Atlantic Ocean, whereas this study only considered a subestuary of the Chesapeake Bay where waves were smaller and less energetic. Since wave energy at the Piankatank reef site was not substantial wave action probably had little effect on growth.

It also should be pointed out that the lack of dependence of growth on water movement for oysters residing at any tidal height or substrate layer may have been attributed to inaccurate measurements of water movement at the microspatial level. The dissolution rate of chlorine tablets was 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 bed would be proportional to flow conditions at the surface and 10 cm below. This may have been an incorrect assumption, since reef topography at and below the reef surface may have dramatically disrupted water motion.

Peterson and Beal (1989) found that the suspension-feeding bivalve, *Mercenaria mercenaria,* exhibits depressions in individual growth when density is increased. Density dependent growth was also detected in the bivalves *Anomalocardia sqamosa, Circe lenticularis, Katelysia scalarina, Katelysia rhytiphora, and Mercenaria mercenaria* (Eldridge et al. 1979, Peterson 1982, Peterson and Black 1987, Peterson and Black 1988). Aside from space limitation, Peterson and Beal (1989) believed a reason for these depressions in growth was the limitation of local resources by high density patches of bivalves. The density independent growth found for the majority of oysters considered in this study appears to contradict the above findings. However in all of the above studies that examine bivalves, densities were manipulated by factors of 4, 6, and 8. Densities in this study did not vary throughout the experiment by more than a factor of 2. In fact, care was taken at the beginning of the experiment to keep densities as constant as possible because the effects of density were not of primary concern in this study. As a result of the small gradient of densities used, density dependent effects were not as pronounced and thus less detectable. Density dependent effects are also probably less dominant on oyster growth than on the growth of bivalves examined above because oysters are able to shift their principal axes of growth (Galtsoff 1964). This plasticity allows oysters to grow irregularly when neighbors are in close proximity, and is essential for growth in densely packed reef systems (Bahr and Lanier 1981). Finally, the observed independence of growth and density may have been a result of the consideration of low overall densities, which seldom exceeded 5 spat per shell.

Mortality

During the mid-June to mid-July period, 1993 oysters residing at the +25 cm tidal height had significantly higher mortalities at the reef surface than below the surface, and 1994 oysters exhibited a clear trend of higher surface mortality. Excessive heat stress, evaporative water loss, and respiratory inhibition were the main reasons for these findings. Air temperatures from mid-June through mid-July often rose above 30° C and averaged 28.2° C, the highest mean air temperature of the three periods examined. Oysters at the surface had no protection from high air temperatures and direct solar exposure, as oysters in the lower layers did, and as a result experienced greater desiccation stress. Similarly, Roegner (1989) found high rates of mortality for exposed, unshaded oysters held in the mid to high intertidal zone in Virginia from June through July, when air temperatures often rose above 30°C. In South Carolina, Crosby et al. (1991) and Michener and Kenny (1991) reported high mortalities as well for oysters set on asbestos plates, which had no penetrable dimension and thus protection from solar exposure.

Oysters living beneath the surface at the +25 cm tidal height were shielded from direct solar radiation and wind by overlying shell, and were submerged for longer periods since they were lower in vertical elevation. These factors made underlying environments cooler and moister than surface habitats and lowered both heat stress and evaporative water loss. This was demonstrated by Bahr (1976) who found temperatures in October to be 7° C lower and relative humidity to be higher below the surface at an intertidal oyster reef in Georgia.

Shading and retention of moisture are extremely important for oysters living in the intertidal zone. In fact, oysters in South Atlantic reef communities are able to survive high summer temperatures at the surface substrate layer by growing vertically and in clusters, which provides a shading effect from sun and drying wind (Bahr and Lanier 1981). Since dense assemblages of vertically growing oysters were not yet established on the Piankatank reef, oysters at the surface did not benefit from this shading effect. Thus, the best refuge from atmospheric stresses was residence below the reef surface. This was illustrated when a cage of 1993 oysters at the +25 cm tidal height was elevated, presumably by a boat collision, to a tidal height of +40 cm. Oysters within the surface tier of this cage all perished during the first month, probably because they could not tolerate high temperatures and limited submergence times. However, oysters in the deep layer had 100% survivorship through the first month and 93% survivorship throughout the entire sampling season.

Aside from heat and evaporative stress from solar exposure, respiratory stress may have been a source of mortality differences at the +25 cm tidal height during the first sampling period as well. Oysters beneath the reef surface were submerged for longer periods of time than oyster at the surface as a result of being 10 cm lower in vertical elevation, and thus were not required to respire anaerobically as often as oysters at the surface. Although 10 cm may not seem like a substantial amount, it may have translated into substantial differences in submergence times because the mean range at the reef site was only 36 cm. Nichy and Menzel (1967), Bahr and Lanier (1981), and Michner and Kenny (1991) have all suggested that respiratory stress from prolonged exposure periods contributes to intertidal mortality. Since oysters at the surface were aerially exposed for longer periods, they probably experienced higher mortalities from respiratory stress than oysters in underlying layers.

The fact that mortality for the 1993 oysters found at the -90 cm tidal height was greater at the surface than at the deep layers throughout the sampling period suggests that the deep layers may provide some refuge from fouling and/or predation. Fouling reductions were probably not the main reason for mortality differences between the layers, however. Although algal growth and colonization were greater at the surface layer than at the deep layer, results from the growth study indicated that fouling may have been more deleterious for oysters in deep layers than for those at the surface. Oysters in deep layers were not only below the heavy fouled surface layer but were also below 2-3 layers (intermediate layers) of moderately fouled shells, all of which heavily restricted water flow and reduced growth (and possibly contributed to some mortality). Thus, fouling reductions were probably not as instrumental in the lowering of mortality within the deep layer as predation pressure differences.

The two most prominent oyster predators found in the Chesapeake Bay, the oyster drills, *Urosapinx cinerea* and *Eupleura caudata* , and the seastar, *Asterias forbesi* , were absent at the reef site because of low salinities. Oyster drills generally cannot tolerate salinities below 18-20‰ (Carriker 1955, Galtsoff 1964) and seastars are generally restricted to the mouth of the Chesapeake Bay where salinities do not decrease below 16-18‰ (Galtsoff 1964). During this study salinities at the Piankatank reef often fell below 16 ‰ and thus prevented the intrusion of these two predators. As a result of the absence of these two key predators, overall oyster mortalities at the reef site were substantially lower than those recorded in other studies, such as those performed by MacKenzie (1981) and Roegner (1989).

Although the two most deleterious oyster predators were absent at the reef site, a number of other predators were observed. These potential predators included: the flatworm *Stylochus elliptus*, the mud crabs *Panopeus herbstii, Eurypanopeus depressus,* and *Rhithropanopeus harrisii*; the blue crab *Callinectes sapidus*; and the oyster toadfish *Opsanus tau.* Flatworms near oyster populations cause significant mortalities even though the extent of damage is unknown (Landers and Rhodes 1970, Morales et al. 1988, Abbe 1986, Baker 1994). Littlewood (1988) suggested that flatworms elevate mortality of oysters by either directly preying upon them or by suffocating them with mucous. Mud crabs prey upon small oysters (Abbe 1986, Baker 1994) causing mortalities as high as 50% (Mackenzie 1981), and predation by blue crabs (Carriker 1955, Abbe 1986, Roegner 1989, Eggleston 1990a,b,c) and oyster toadfish (Abbe 1986) are well documented.

Flatworms, mud crabs, blue crabs, and oyster toadfish were found within cages at surface and deep layers and at all tidal heights, but were most abundant at surface substrate layers and subtidal depths based on field observations. Of the four potential predators, flatworms and mud crabs were probably the most deleterious because they were not restricted by the experimental cages. Blue crabs and toadfish, which were only able to enter cages during juvenile developmental stages, were less problematic. However, even though entry into cages was restricted to smaller blue crabs and oyster toadfish, 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, 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 finding that mortality rates were higher at the surface level suggests that the above predators preferred to prey upon oysters that were highly visible and readily available, rather than burrowing through the reef topography to expose and prey upon oysters in underlying layers. This hypothesis is consistent with greater observed predator abundances at the surface.

The fact that no significant difference or trend across substrate levels was observed at the -90 cm tidal height for the 1994 year class of oysters was probably a product of the young oyster's reduced tolerance to sedimentation and temporarily hypoxic conditions. Sedimentation was greater in the deep layer than the surface layer. 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. Burial by sedimentation is a major source of mortality loss for small oysters because sedimentation rates do not have to be that high to completely cover small oysters (MacKenzie 1981, Abbe 1986). Furthermore, periodic conditions of hypoxia may occur in deep layers because flow is low and often restricted beneath the surface. When hypoxic conditions are present, oysters need to isolate themselves from their surroundings and if necessary, switch from aerobic to anaerobic respiration. This switch in respiration may be more difficult for younger oysters. Galtsoff (1964) suggested that spat less than one year old put much of their energy into growth and maintenance rather than the storage of glycogen, the preferred substrate for anaerobic respiration. 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 oyster development. Thus, the benefits of refuge from predation in the deep layer at the -90 cm tidal height were eradicated by the young oyster's reduced physiological tolerances.

Of the three tidal heights considered, 1993 oysters living at the surface survived best at MLW, primarily because this tidal height provided the best balance of detrimental physical and biological factors. At MLW oysters benefited from lowered subtidal predation pressure as a consequence of aerial exposure, but did not suffer from severe heat and respiratory stress because they were not exposed for extended periods of time. Dayton (1971), Connell (1972), Paine (1974), Menge (1976), Lubcheno and Menge (1978), Underwood (1978), Grosberg (1982), and Wethey (1983) also found that certain tidal heights along a vertical gradient in the rocky intertidal region provide the best balance of physical and biological conditions for sessile invertebrates In regards to oysters, McDougall (1942), Chestnut and Fahy (1953), Marshall (1954), Nichy and Menzel (1967), Arakawa (1980), and Littlewood (1988) all have found that survival in the mid to low intertidal zone is most optimal due to lower predation pressure, physical stresses, and/or sedimentation. Thus, the advantages that oysters at the surface had in the intertidal region were comparable to those found below the surface (predator refuge and environmental buffering). Unlike oysters within the deep layer, however, oysters at the surface did not have to contend with restricted water flow or hypoxic conditions, which may both contribute to mortality (Bahr and Lanier 1981, Abbe 1986). Consequently, at the MLW tidal height, mortality was lower at the surface rather than below the reef surface.

Although mortality was reduced significantly at MLW for oysters living at the surface, no differences in mortality across the three tidal heights were detected at the deep layer. This finding was not unexpected. The two factors, desiccation stress and predation, that contributed to high surface mortalities observed at the +25 cm and -90 cm tidal heights were minimized within the deep layers, as was discussed previously. As a result, mortalities within the deep layer at tidal heights of +25 cm and -90 cm were dramatically reduced and mortality differences at the three tidal heights were no longer detectable.

A graphical examination of cumulative mortalities throughout the summer for 1994 oysters revealed a clear trend in reduced mortality at MLW, even though no significant mortality differences were detected. This suggests that MLW is not only optimal for older oysters, but is advantageous for young oysters as well. The reduced efficiency with which younger oysters tolerate sedimentation and anaerobically respire may have elevated mortalities at MLW and made mortality differences between the tidal heights, as well as between layers, less detectable.

These lowered physiological tolerances along with increased rates of predation were the main reasons higher mortalities were observed for the 1994 year class of oysters than for the 1993 year class. As mentioned earlier, young oysters have lowered tolerances to sedimentation, reduced flow, and temporarily hypoxic conditions. Younger, smaller oysters are also more susceptible to predation. Flatworms, blue crabs, and mud crabs, predators found in high numbers at the reef site, all preferentially prey on smaller oysters (MacKenzie 1981, Abbe 1986, Littlewood 1988, Eggleston 1990a).

Oysters belonging to the 1993 year class and residing at the reef surface and oysters belonging to the 1994 year class and dwelling at both surface and deep levels experienced the highest mortality at the +25 cm tidal height during the June/July interval and at the -90 cm tidal height during the August/September interval. These results suggest that physical factors dominated during the first sampling session (June/July) and biological factors were more pronounced during the final sampling session (August/September). During the first sampling session, mean air temperature for both year class studies was 28 - 28.3°C, which was the highest air temperature measured of the three periods. This high air temperature contributed to greater mortalities in the intertidal zone as a result of heat stress, evaporative water loss, and respiratory inhibition than predation and fouling caused in the subtidal 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 abundant, biological factors dominated and mortalities became highest in the subtidal zone. During the August/September sampling interval, there were greater sitings, both in photographs and in the field, of flatworms. Baker (1994), in a study conducted in the York River, Chesapeake Bay, Virginia felt predation of oysters by flatworms could be quite extensive. Although direct predation was not observed, Baker found numerous flatworms to be red in color, which was the color of a stain used to mark test oysters, and deduced that predation by these flatworms was substantial. Elevated flatworm sitings also coincided with an influx of blue crabs at the reef site. The predation effect of blue crabs was probably substantial. Water temperatures during the early part of this sampling period hovered around 26-27°C. At these temperatures, crabs exhibit type II inversely density dependent predation, whereby partial prey refuges found at lower temperatures are eliminated (Eggleston 1990a). Much of the predation probably occurred within the 15 - 35 mm range since oysters begin exhibiting prey refuge at sizes greater than 35 mm (Eggleston 1990b). In addition to predation, algal growth may have been a source of mortality. *Enteromorpha* and an unidentified brown algae grew most prolifically in the subtidal zone during the August/September period, and may have disrupted both pumping rates and respiratory functions.

The lack of detectable differences for 1993 and 1994 oysters found at different substrate layers and tidal heights during the July/August sampling period was not entirely understood. This result may have occurred because neither physical factors, which shaped mortality in the intertidal zone, nor biological factors, which shaped mortality in the subtidal zone, were dominant. During this sampling period, greater numbers of predators were observed at the reef site than were found in the previous sampling period (June/August), but mortality losses caused by predation did not dominate as they did during the last sampling period (August/September) because predators were not as abundant. Although mean air temperature was quite high (27 - 27.5°C) during this period, mortality from dessication stress did not dominate either. This may have been due in part to extensive algal canopies. During, the first week of July, *Enteromorpha* growth became heavy on the aerially exposed caps of mounds which made up the reef structure. This growth may have provided some protection from intense solar radiation experienced during the months of July and August. Long strands of algal growth, as was found on the reef, are often useful resources in rocky intertidal habitats because they retain moisture and provide shade from solar exposure (Newell 1979, Wethey 1983). In addition, intertidal mortalities may not have been as high during this season as during the first session because many weaker, less adapted oysters already were eliminated. All oysters do not have the same capacity to withstand aerial exposure, and thus die off periods where maladapted oysters are "weeded out" often occur during the early exposure to the intertidal environment (Crosby et al. 1991).

Effects of Water Movement and Density on Mortality

The reasons used to explain why growth was independent of flow may be used to explain why mortality was independent of flow: (1) mortality was more closely coupled with localized food depletion than with flow rate; (2) wave energy in the intertidal zone was not strong enough to be detrimental to survival; (3) and/or water movement measurements were inaccurate at the microscale level.

An attempt was made to keep overall number of oysters per layer and microscale density (i.e. number of oysters per shell) as uniform as possible at the beginning of the experiment. Although no significant differences were found in mean microscale density across heights and substrate layers, the numbers of oysters per shell did differ within each of the 8 replicate assemblages for each tidal height for each layer. Thus it was of interest to determine within each replicate assemblage if density had an effect on mortality. The effect of density on mortality has been examined for a number of sessile invertebrates using linear regression analysis, in which mortality may be either density-dependent (positive slope), density-independent (zero slope), or inverse density-dependent (negative slope) (Connell 1985, Davis 1988, McQuinnes and Davis 1989). Roegner (1991) went one step further and hypothesized that with time, as oysters become larger, oyster mortality will become more density dependent because space and resources become increasingly limited. As was the case with this experiment, Roegner (1991) found density independent mortality through time. Roegner felt the reason for the lack of density dependence was an initial mortality event that reduced oyster densities dramatically and constricted the range of densities used. This initial mortality peak was not observed in this study, but initial densities were not that high or variable to begin with, ranging from one to eight spat per shell for either year class considered (versus .72 - 14.12 spat /cm^2 used by Roegner). Thus, density-dependent mortality may have been difficult to detect. Furthermore, the lack of density dependent mortality may be a result of the oyster's ability to change its axis of growth. When oysters are found in high densities, they may shift their growth axis to wherever there is free space, which reduces intraspecific competition.

Comparison between Growth/Mortality Cage Study and Destructive Reef Study

Based on the results of the cage study which indicated that growth was greatest below the surface at the +25 cm tidal height and at the surface at the -90 cm tidal height, oyster shell heights on the reef were expected to be correspondingly greater at these substrate layer/height combinations. However, no differences in shell height were found. The major reason for this inconsistency may have been that the parameters and techniques used to document shell height were dramatically different than those used to detect growth. Shell heights measurements were less precise than shell area measurements used in the cage study for three reasons. First, shell height measurements did not reflect irregular shell shapes frequently observed at the reef site as effectively as shell area measurements. Second, in contrast to the cage studies where individual oysters were tracked throughout the study, destructive sample measurements were collected from different sets of randomly selected organisms each week. This resulted in the incorporation of greater variation. Third, because of shortages of intertidal oysters (as a result of high winter mortalities), mean shell height measurements collected at each intertidal plot were based on lower than ideal numbers of oysters. As a result, estimates of the mean were less accurate.

Although shell height measurements from the reef samples were not in accord with growth measurements recorded in the cage studies, density measurements from the reef were. Greater numbers of oysters per 30 shells were observed below the surface at the +25 cm tidal height and on the surface at the -90 cm tidal height , which were the same substrate level/tidal height combinations that coincided with maximum growth. Results of the settlement study suggest that these density differences were not a result of differential settlement, since settlement intensities were not found to vary by substrate level. Mortality measurements collected throughout the summer could not entirely account for these reef density differences either. There was evidence that oysters from both year classes residing at the +25 cm tidal height survived better below the surface than at the surface during certain periods. However, survivorship for 1993 oysters residing at the -90 cm tidal height was found to be lower at the surface than beneath the reef surface for two of the sampling periods, and no significant differences between substrate layers at the -90 cm tidal height were detected for the 1994 oysters.

The inconsistency between reef density and mortality findings may have been because different temporal scales were considered in each study. Michener and Kenny (1991) stressed that when and how frequently oysters are sampled greatly influences results of recruitment experiments. The density measurements were based on what has happened over a year's time, whereas the mortality study was based on what was observed over a three month period in the summer. Extended periods of sedimentation and fouling in the subtidal zone, which could not be detected in the three month study, may have contributed to higher mortalities below the surface. Although the data were inconsistent in suggesting whether surface or deep layers are most advantageous in the subtidal zone, there was much evidence to suggest that residence below the surface at the +25 cm tidal height is most optimal.

Shell height measurements of reef oysters, which were greatest subtidally, were consistent with data presented previously. Furthermore, it was interesting to note that growth of oysters over the period of 12 - 14 months at the reef site was dramatic at both intertidal and subtidal heights. Intertidal oysters reached a mean height of 43.6 mm, whereas subtidal oysters had a mean height of 47.1 mm. These shell heights are substantially larger than shell heights of oysters of comparable age found in non-reef settings in the James River, another subestuary of the Chesapeake Bay (R. Morales, pers. comm.). Although research which compares growth in reef and non-reef environments needs to be performed, this preliminary comparison suggests that growth may be elevated in reef environments.

Conclusion

This study has provided a wealth of information regarding the ecology of oysters on intertidal reefs, an area of research which is underdeveloped in the literature. This study demonstrated that both settlement and early recruitment, which have declined drastically in recent years, were greatest subtidally at the reef site and did not differ at the surface and 10 cm below the surface throughout the settlement season. Growth and shell height measurements were also found to be greatest subtidally, mainly because of longer submergence times. But unlike settlement findings, growth was found to be disproportionate according to substrate layer. Both year classes of oysters examined grew faster below the reef surface at the +25 cm tidal height primarily because of longer submergence times and less stressful conditions, and grew faster at the reef surface at the -90 cm tidal height primarily because of greater unrestricted flow. Oysters of both year classes grew best during the August to September sampling interval and those that grew fastest (oysters at MLW and -90 cm), demonstrated somewhat erratic peripheral shell accretion, having periods of extensive, lateral growth at the shell edge, followed by periods of vertical shell layering.

Contrary to the settlement and growth results, survivorship was not maximized subtidally throughout the year. During the summer, 1993 oysters had significantly greater survival at the MLW tidal height and 1994 oysters showed a clear trend of reduced mortality at MLW. Furthermore, the pilot study demonstrated survivorship may be maximized at MLW during the fall as well. Only during the winter were mortality rates lowest subtidally, and this was only applicable to oysters found at the surface since no deep layer samples were collected. Observations during the pilot study and data collected during the primary study demonstrated that residence below the surface was an important refuge from physical conditions, such as freezing temperatures and intense solar radiation and from biological forces, such as predation and colonization by algae and endemic organisms during certain times of the year. During the winter and early summer at the reef site physical forces tended to dominate, with freezing air temperatures being the major source of mortality in the winter and high air temperatures being the major contributor to mortality in the summer. When temperatures were not excessively hot or cold during the late summer and fall, biological forces such as predation, fouling, and colonization tended to govern mortality losses.

Surprisingly density and flow were not found to influence oyster dynamics at the reef system. The lack of dependence of settlement, growth, and mortality on water movement may have been a result of two factors: (1) water movements as a result of either current flow or wave action were within the tolerance range of oysters and thus had little effect on oyster processes; (2) or flow did have an effect but only at the microspatial level which was not measured in this experiment. The independence of growth and mortality and water movement may be explained further by the fact that growth and mortality of groups of oysters in the field are more closely coupled with localized food depletion than with flow rate. In addition, the lack of dependence of density on growth and survival may have been because a limited range of densities were considered and the densities that were used were quite low. This lack of dependence also may have been due to the oyster's ability to change its growth axis, allowing it to coexist with other oysters in very dense aggregations, which is essential for oyster reef formation.

There are several implications of the ecological data for the utilization of artificial intertidal reef structures to rejuvenate oyster stocks in the Chesapeake Bay. Building artificial reefs which are aerially exposed will not elevate the magnitude of settling oyster larvae nor will it lead to more rapid oyster growth, since settlement and growth were maximized subtidally. But at the same time, settlement and growth in the low intertidal zone (MLW) are not so low that they preclude the establishment of oyster communities. Building artificial intertidal reefs may actually enhance survival. During most of the year, oysters residing at MLW enjoy greater survivorship than oysters in higher intertidal and subtidal reef habitats because of an optimal balance between atmospheric stresses, fouling, and predation pressure. The high winter mortalities recorded in this study were probably atypical and likely a result of the combination of an unusually harsh winter, a population of young oysters no older than 5 months, and an immature reef community. After one or more typical winters, older oysters which are more tolerant of low air temperatures can be established in the intertidal zone and begin aggregating in colonies. Once colonies are established, the survival benefits of intertidal existence may be fully maximized. Oysters in dense intertidal reef communities were able to flourish in the Chesapeake Bay for centuries before man's intervention and were capable of withstanding harsh environmental stresses.

When dense aggregations of oysters are not yet established on artificial reefs, residence in underlying layers may play a vital role in reef development. Even though oysters in their early stages of development may be decimated at the surface by physical and biological forces throughout the year, oysters in underlying layers of the reef often persist. These oysters residing beneath the reef surface serve as a steady base population that can spawn and rejuvenate surface communities. This process may continue until a solid, highly aggregated population of oysters, which can provide both physical and biological buffering, is established. Although this process was most dramatic in the intertidal zone at the Piankatank reef, it may occur in the subtidal zone as well.

As a result of low settlement and unpredictable atmospheric conditions, the establishment of intertidal reef communities may take years, which is not what management agencies would like to hear given the critical state of the oyster industry. However, if future research suggests that the high winter intertidal mortalities observed in this study were in fact atypical and if the results of the reef disease study performed in conjunction with this study indicate that not only is mortality from physical and biological forces reduced in the intertidal zone, but mortality from disease is minimized as well, it may be in the best interest of managers to put resources into building intertidal reefs even though their establishment may take years. If the results of the disease study do not suggest that there is a difference in disease incidence as a function of tidal height, it may be still beneficial to utilize reefs as rejuvenation tools. In the Piankatank River, the reef site is truly an "oasis" of oysters and marine life in a river relatively devoid of oysters. Its success to this point strongly suggests that oysters are benefiting from reef existence. If future reefs are constructed they should be built of substrate porous enough to allow for settlement below the surface, so that base oyster populations can be established there. Furthermore, natural and artificial reefs should be left unaltered for years, so that established, mature oyster communities which have higher resistance to environmental conditions can form.

Appendix 1. Analyses performed on settlement data collected during the pilot study.

Appendix 1a. Multivariate repeated measures ANOVA performed on log (x+1) transformed spat counts recorded in the non-destructive settlement study.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	.039	.039	.320	.5839
orientation	1	.001	.001	.005	.9464
tidal height	3	1.621	.540	4.433	.0316
Subject(Group)	10	1.219	.122		
time	5	11.821	2.364	42.620	.0001
time * mound	5	.171	.034	.618	.6865
time * orientation	5	.164	.033	.592	.7061
time * tidal height	15	1.429	.095	1.718	.0775
time * Subject(Group)	50	2.774	.055		

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

Appendix 1b. Multivariate repeated measures ANOVA performed on log (X+1) transformed spat counts recorded in the destructive settlement study.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	.043	.043	.311	.5894
orientation	1	.276	.276	1.984	.1893
tidal height	3	1.207	.402	2.888	.0887
Subject(Group)	10	1.393	.139		
time	5	7.583	1.517	34.666	.0001
time * mound	5	.287	.057	1.310	.2748
time * orientation	5	.357	.071	1.634	.1684
time * tidal height	15	.878	.059	1.337	.2164
time * Subject(Group)	50	2.188	.044		

Dependent: Destructive Settlement (log x+1) transformed

Appendix 1c. 4-way ANOVA and Student-Newman-Keuls tests 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
mound	1	.039	.039	.576	.4498
orientation	1	.001	.001	.009	.9265
tidal height	3	1.621	.540	7.977	.0001
time	5	11.821	2.364	34.904	.0001
Residual	85	5.757	.068		

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

Student-Newman-Keuls Tests

tidal height:
-90 cm > MLW > +30 cm
-45 cm > +30 cm
time:
week 1, week 2, week 3 > week 6, week 5
week 2 > week 4 > week 5, week 6

Appendix 1d. 4-way ANOVA and Student-Newman-Keuls tests of log (X+1) transformed spat counts measured in the destructive settlement study.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	.043	.043	.722	.3980
orientation	1	.020	.020	.326	.5817
tidal height	3	1.207	.402	6.701	.0004
time	5	7.583	1.517	25.269	.0001
Residual	85	5.102	.060		

Dependent: destructive settlement log (x+1) transformed

Student-Newman-Keuls Tests

tidal height:

-90 cm, -45 cm > +30 cm
-90 cm > MLW

time:

week 6 > week 4 > week 3 > week 2 > week 1
week 5 > week 3

Appendix 2. Analyses performed on size class data collected during the pilot study.

Appendix 2a. Univariate repeated measures ANOVA performed on shell areas of hatchery-reared oysters.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Mound	1	.081	.081	.707	.4200
Orientation	1	2.153E-4	2.153E-4	.002	.9663
tidal height	3	2.187	.729	6.352	.0110
Subject(Group)	10	1.148	.115		
Month	2	4.080	2.040	60.397	.0001
Month * Mound	2	.073	.036	1.077	.3671
Month * Orientation	2	.174	.087	2.572	.1118
Month * tidal height	6	.346	.058	1.706	.1921
Month * Subject(Group)	14	.473	.034		

Dependent: Area

Appendix 2b. 4-way ANOVA with interaction terms assumed to be zero performed on shell areas of hatchery-reared oysters. Results of Student-Newman-Keuls tests are displayed below.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Mound	1	.031	.031	.531	.4713
Orientation	1	.019	.019	.323	.5735
Tidal height	3	1.915	.638	10.923	.0001
Time	2	8.990	4.495	76.935	.0001
Residual	34	1.987	.058		

Dependent: Area

Student-Newman-Keuls Tests

Tidal height:

-90 cm > MLW > +30 cm

Time:

December - May > November - December > October - November

Appendix 2c. Multivariate repeated measures ANOVA performed on shell heights of destructively sampled oysters.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	1.126	1.126	.148	.7085
orientation	1	8.187	8.187	1.076	.3240
tidal height	3	161.100	53.700	7.059	.0079
Subject(Group)	10	76.078	7.608		
time	3	838.502	279.501	89.458	.0001
time * mound	3	8.005	2.668	.854	.4755
time * orientation	3	5.413	1.804	.577	.6343
time * tidal height	9	98.980	10.998	3.520	.0044
time * Subject(Group)	30	93.731	3.124		

Dependent: shell height

Appendix 2d. ANOVAs and Student-Newman-Keuls tests of shell heights performed on each month of destructive sampling.

ANOVA and Student-Newman-Keuls tests performed on September samples.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	.090	.090	.381	.5509
orientation	1	.062	.062	.265	.6182
tidal height	3	19.262	6.421	27.178	.0001
Residual	10	2.363	.236		

Dependent: Size (month 1)

Student-Newman-Keuls Tests

Tidal height: +30 cm, -90 cm > -45 cm > MLW

ANOVA performed on October samples.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	5.244	5.244	.860	.3755
orientation	1	7.618	7.618	1.250	.2897
tidal height	3	24.030	8.010	1.314	.3235
Residual	10	60.950	6.095		

Dependent: Size (month 2)

ANOVA performed on November samples.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	2.081	2.081	.337	.5744
orientation	1	4.940	4.940	.800	.3921
tidal height	3	56.782	18.927	3.065	.0780
Residual	10	61.744	6.174		

Dependent: Size (month 3)

Appendix 2d. (cont.)

ANOVA and Student-Newman-Keuls tests performed on May samples.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	1.716	1.716	.383	.5496
orientation	1	.980	.980	.219	.6498
tidal height	3	160.006	53.335	11.918	.0012
Residual	10	44.752	4.475		

Dependent: Size (month 4)

Student-Newman-Keuls Tests

Tidal height: -90 cm, -45 cm > +30 cm, MLW

Appendix 3. Analyses performed on mortality data collected during the pilot study.

Appendix 3a. Multivariate repeated measures ANOVA performed on arcsine transformed mortality data.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	.778	.778	.010	.9222
orientation	1	9.514	9.514	.123	.7334
tidal height	3	3121.344	1040.448	13.418	.0008
Subject(Group)	10	775.439	77.544		
time	2	7395.753	3697.876	61.845	.0001
time * mound	2	350.395	175.198	2.930	.0766
time * orientation	2	54.355	27.177	.455	.6411
time * tidal height	6	9249.805	1541.634	25.783	.0001
time * Subject(Group)	20	1195.856	59.793		

Dependent: mortality

Appendix 3b. ANOVAs and Student-Newman-Keuls tests of arcsine transformed proportional mortality performed by sampling interval.

Results of ANOVA performed on September - October samples.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	131.618	131.618	2.725	.1298
orientation	1	11.441	11.441	.237	.6370
tidal height	3	113.692	37.897	.785	.5293
Residual	10	483.057	48.306		

Dependent: Mortality(month 1)

Results of ANOVA and Student-Newman-Keuls tests performed on October - November samples

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	2.950	2.950	.251	.6270
orientation	1	2.831	2.831	.241	.6340
tidal height	3	214.308	71.436	6.086	.0126
Residual	10	117.372	11.737		

Dependent: Mortality (Month 2)

Student-Newman-Keuls tests

Tidal height: +30 cm, -45 cm, -90 cm > MLW

Results of ANOVA and Student-Newman-Keuls tests performed on November - May samples.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
mound	1	216.605	216.605	1.580	.2373
orientation	1	49.597	49.597	.362	.5609
tidal height	3	12043.149	4014.383	29.284	.0001
Residual	10	1370.867	137.087		

Dependent: Mortality (Month 3)

Student-Newman-Keuls Tests

Tidal height: +30 cm, MLW > -45 cm, -90 cm

Appendix 4. Correlation analyses performed on data collected during the primary study.

Appendix 4. Linear correlations performed on surface and deep substrate layers for each experiment in the primary study.

Experiment	correlation coefficient	critical value	P-value
Destructive Settlement	.63	.329	< .001
Non-Destructive Settlement	.62	.329	< .001
1993 Growth Study	.45	.239	< .001
1993 Mortality Study	.076	.223	.543
1994 Growth Study	.69	.232	< .001
1994 Mortality Study	.089	.232	.523
Reef Size Class Study	.78	.259	< .001
Reef Plot Density Study	.76	.254	<. 001

Appendix 5. Analyses performed on settlement data collected during the primary study.

Appendix 5a. Paired t-tests performed on surface and deep substrate layer spat counts for both non-destructive and destructive samples. Separate analyses were performed as a function of tidal height and time.

Paired t-tests performed on surface and deep layers for non-destructive settlement samples examined by tidal height.

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

Paired t-tests performed on surface and deep layers for non-destructive settlement samples examined by week.

week	Mean difference (surface-deep)	Degrees of freedom	t-value	p-value
1	.083	11	.266	.7949
2	-.083	11	-.123	.9046
3	-.083	11	-.432	.6742

Paired t-tests performed on surface and deep samples for destructive settlement samples examined by tidal height.

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

Appendix 5a. (Cont.)

Paired t-tests performed on surface and deep layers for destructive settlement samples examined by week.

Week	Mean difference (surface-deep)	Degrees of freedom	t-value	p-value
1	.167	11	.804	.4382
2	-.083	11	-.290	.7774
3	-.250	11	-.672	.5152

Appendix 5b. ANOVA and Student-Newman-Keuls tests 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) transfomed mean spat count

Student-Newman-Keuls Tests

week:

July 21 - July 28 > July 15 - July 21 > July 28 - August 4

tidal height:

-90 cm > MLW, +25 cm

Appendix 5c. ANOVA performed on 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

Appendix 5d. ANOVAs and Student-Newman-Keuls tests of log (x +1) transformed spat counts for each tidal height sampled during destructive sampling.

Results of 1-factor ANOVA performed on Log (x+1) transformed spat counts collected at the +25 cm tidal height.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
week	2	.016	.008	1.286	.3227
Residual	9	.054	.006		

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

Results of 1-factor ANOVA performed on log (x+1) transformed spat counts collected at MLW.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
week	2	.053	.027	2.413	.1449
Residual	9	.100	.011		

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

Results of 1-factor ANOVA and Student-Newman-Keuls tests performed on spat counts collected at the -90 cm tidal height.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
week	2	.355	.178	14.634	.0015
Residual	9	.109	.012		

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

Results of Student-Newman-Keuls Tests

Week:
July 21 - 28, July 28 - August 4 > July 15 - 21

Appendix 5e. ANOVAs and Student-Newman-Keuls tests performed on log (x +1) transformed spat counts for each week of destructive sampling.

Results of 1-factor ANOVA performed on spat counts collected July15-21, 1994.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	.057	.029	3.713	.0667
Residual	9	.069	.008		

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

Results of 1-factor ANOVA and Student-Newman-Keuls tests performed on spat counts collected July 21-28, 1994.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	.402	.201	13.501	.0020
Residual	9	.134	.015		

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

Results of Student-Newman-Keuls Tests

Tidal height:
-90 > MLW, +25

Results of 1-factor ANOVA and Student-Newman-Keuls tests performed on spat counts collected July 28-August 4, 1994.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	.502	.251	37.678	.0001
Residual	9	.060	.007		

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

Results of Student-Newman-Keuls Tests

Tidal height:
-90 cm > +25 cm, MLW

Appendix 6. Analyses performed on growth data collected during the primary study.

Appendix 6a. Paired t-tests peformed on mean growth of oysters residing either at the reef surface or 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

Appendix 6b. Multivariate repeated measures ANOVA performed on growth data from the 1993 year class of oysters residing at the reef surface. Student-Newman-Keuls tests were used to analyze the between factor, height, and Newman-Keuls tests were used to analyze the within factor, time.

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

Student-Newman-Keuls tests on tidal height:
-90 cm, MLW > +25 cm

Newman-Keuls tests on time:
August - September > June - July, July - August

Appendix 6c. Multivariate repeated measures ANOVA performed on growth data from the 1993 year class of oysters residing 10 cm below the reef surface. Newman-Keuls tests were used to analyze the within factor, time.

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

Newman-Keuls tests on time:
August - September > June - July, July - August

Appendix 6d. Linear regressions of log transformed shell area on time for 1993 oysters residing at the three tidal heights and two substrate layers.

Surface oysters at the +25 cm tidal height:

Regression equation: y = .5954 + .0027*x
R-squared = .780

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.210	.210	95.855	<.0001
Residual	27	.059	.002		
Total	28	.270			

Deep oysters at the +25 cm tidal height:

Regression equation: y = .6138 + .0032 * x
R-squared = .730

	DF	Sum of Squa...	Mean Square	F-Value	P-Value
Regression	1	.336434	.336434	80.988027	<.0001
Residual	30	.124624	.004154		
Total	31	.461057			

Surface oysters at the MLW tidal height:

Regression equation: y = .626861 + .0037 * x
R-squared = .850

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.448837	.448837	164.398123	<.0001
Residual	29	.079175	.002730		
Total	30	.528013			

Appendix 6d. (Cont.)

Deep oysters at the MLW tidal height:

Regression equation: y = .6607 + .0033 * x
R-squared = .8329

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.341719	.341719	139.534710	<.0001
Residual	28	.068572	.002449		
Total	29	.410290			

Surface oysters at the -90 cm tidal height:

Regression equation: y = .6543 + .0040 * x
R-squared = .9321

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.462400	.462400	344.617807	<.0001
Residual	26	.034886	.001342		
Total	27	.497286			

Deep oysters at the -90 cm tidal height:

Regression equation: y = .6581 + .0035 * x
R-squared = .8740

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	.389644	.389644	200.306105	<.0001
Residual	30	.058357	.001945		
Total	31	.448002			

Appendix 6e. 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)

Appendix 6f. ANOVAs and Student-Newman-Keuls tests of growth data from the 1994 year class of oysters residing at the reef surface performed on each time interval.

Analyses performed on data from June/July sampling interval:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	118.559	59.280	33.232	.0001
Residual	20	35.677	1.784		

Dependent: growth (time interval 1)

Student-Newman-Keuls Tests

Tidal height: -90 cm > MLW > +25 cm

Analysis performed on data from the July/August sampling interval:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	5.663	2.831	1.131	.3426
Residual	20	50.091	2.505		

Dependent: growth (time interval 2)

Analyses performed on data from the August/Sept. sampling interval:

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	30.636	15.318	3.739	.0417
Residual	20	81.930	4.096		

Dependent: growth (time in...

Student-Newman-Keuls tests

Tidal height: -90 cm > +25 cm

Appendix 6g. Multivariate repeated measures ANOVAs and Newman-Keuls tests of growth data from the 1994 year class of oysters residing at the reef surface performed on each tidal height sampled.

Analyses performed on 1994 surface oysters residing at the +25 cm tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Subject	22	179.399	8.155		
time	2	107.504	53.752	16.521	.0001
time * Subject	44	143.157	3.254		

Dependent: growth (mm/ day)

Newman-Keuls tests on time:

August - September > June - July, July - August

Analyses performed on 1994 surface oysters residing at the MLW tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Subject	6	12.478	2.080		
time	2	15.771	7.886	4.601	.0329
time * Subject	12	20.567	1.714		

Dependent: growth (mm/ day)

Newman-Keuls tests on time:

June - July, August - September > July - August

Appendix 6g. (Cont.)

Analyses performed on 1994 surface oysters residing at the -90 cm tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Subject	22	179.399	8.155		
time	2	107.504	53.752	16.521	.0001
time * Subject	44	143.157	3.254		

Dependent: growth (mm/ day)

Newman-Keuls tests on time:

June - July, August - September > July - August

Appendix 6h. Multivariate repeated measures ANOVA performed on growth data from the 1994 year class of oysters residing 10 cm below the reef surface. Student-Newman-Keuls tests were used to analyze the between factor, height, and Newman-Keuls tests were used to analyze the within factor, time.

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

Student-Newman-Keuls tests on tidal height:
-90 cm > MLW, +25 cm

Newman-Keuls tests on time:
June - July, August - September > July - August

Appendix 6i. Linear regressions of log transformed shell area on time for 1993 oysters residing at the three tidal heights and two substrate layers.

Surface oysters at the +25 cm tidal height:

Regression equation: y = .0519 + .0068 * x
R-squared = .9223

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.498	1.498	356.114	<.0001
Residual	30	.126	.004		
Total	31	1.624			

Deep oysters at the +25 cm tidal height:

Regression equation: y = .1066 + .00763 * x
R-squared = .9236

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.905541	1.905541	362.889598	<.0001
Residual	30	.157531	.005251		
Total	31	2.063072			

Surface oysters at the MLW tidal height:

Regression equation: y = .0961 + .0085 * x
R-squared = .9042

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	2.071271	2.071271	245.342623	<.0001
Residual	26	.219501	.008442		
Total	27	2.290772			

Appendix 6i. (Cont.)

Deep oysters at the MLW tidal height:

Regression equation = .1431 + .0076 * x
R-squared = .8605

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	1.644623	1.644623	160.357292	<.0001
Residual	26	.266656	.010256		
Total	27	1.911279			

Surface oysters at the -90 cm tidal height:

Regression equation: y = .1571 + .0082 * x
R-squared = .808

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	2.217989	2.217989	125.921574	<.0001
Residual	30	.528422	.017614		
Total	31	2.746410			

Deep oysters at the -90 cm tidal height:

Regression equation: y = .1906 + .0079 * x
R-squared = .851

	DF	Sum of Squares	Mean Squa...	F-Value	P-Value
Regression	1	2.055614	2.055614	171.568871	<.0001
Residual	30	.359438	.011981		
Total	31	2.415053			

Appendix 7. Analyses performed on mortality data collected during the primary study.

Appendix 7a. 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

Appendix 7b. Multivariate repeated measures ANOVA of arcsine transformed mortality data collected from the 1993 year class of oysters performed by tidal height. Newman-Keuls tests were used to analyze time when it was found to be significant.

Analyses performed on 1993 oysters which resided at the +25 cm tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
LAYER	1	22.970	22.970	.260	.6191
Subject(Group)	12	1058.500	88.208		
TIME	2	103.650	51.825	.937	.4058
TIME * LAYER	2	136.870	68.435	1.237	.3082
TIME * Subject(Group)	24	1328.066	55.336		

Dependent: MORTALITY

Analyses performed on 1993 oysters which resided at the MLW tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
LAYER	1	436.391	436.391	21.747	.0004
Subject(Group)	14	280.940	20.067		
TIME	2	64.723	32.362	1.169	.3254
TIME * LAYER	2	93.406	46.703	1.687	.2034
TIME * Subject(Group)	28	775.246	27.687		

Dependent: MORTALITY

Layer: Deep > Surface

Appendix 7b. (Cont.)

Analyses performed on 1993 oysters residing at the -90 cm tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
LAYER	1	258.077	258.077	5.516	.0340
Subject(Group)	14	654.987	46.785		
TIME	2	1253.422	626.711	20.875	.0001
TIME * LAYER	2	63.131	31.566	1.051	.3628
TIME * Subject(Group)	28	840.613	30.022		

Dependent: MORTALITY

Layer: Surface > Deep

Newman-Keuls tests on time: August - September > June - July, July -August

Appendix 7c. ANOVAs of arcsine transformed mortality data from the 1993 year class of oysters performed by time period.

Analysis run on data collected during time period 1 (June - July)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TIDAL HEIGHT	2	127.585	63.793	1.804	.1778
LAYER	1	56.156	56.156	1.588	.2149
TIDAL HEIGHT * LAYER	2	328.698	164.349	4.647	.0153
Residual	40	1414.613	35.365		

Dependent: Mortality (period 1)

Analysis run on data collected during time period 2 (July - August)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TIDAL HEIGHT	2	56.623	28.312	.617	.5448
LAYER	1	11.263	11.263	.245	.6231
TIDAL HEIGHT * LAYER	2	102.988	51.494	1.122	.3358
Residual	40	1836.462	45.912		

Dependent: Mortality (period 2)

Analysis run on data collected during time period 3 (August - September)

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
TIDAL HEIGHT	2	733.774	366.887	9.943	.0003
LAYER	1	1.526	1.526	.041	.8399
TIDAL HEIGHT * LAYER	2	642.298	321.149	8.704	.0007
Residual	40	1475.938	36.898		

Dependent: Mortality (period 3)

Appendix 7d. ANOVAs of arcsine transformed 1993 mortality data collected during period 1 (June - July) and period 3 (August - September) performed by tidal height.

Analysis of oysters residing at +25 cm during period 1.

Source	df	Sum of Squares	Mean Square	F-value	P- value
layer	1	250.501	250.501	4.857	.0478
residual	12	618.879	51.573		

Layer : surface > deep

Analysis of oysters residing at MLW during period 1

Source	df	Sum of Squares	Mean Square	F-value	P- value
layer	1	48.407	48.407	1.525	.2372
residual	14	44.380	31.741		

Analysis of oysters residing at -90 cm during period 1

Source	df	Sum of Squares	Mean Square	F-value	P- value
layer	1	248.220	248.220	7.891	.0139
residual	14	440.393	31.457		

layer: surface > deep

Analysis of oysters residing at the +25 cm tidal height during period 3

Source	df	Sum of Squares	Mean Square	F-value	P- value
layer	1	3.093	3.093	.057	.8160
residual	12	656.057	54.671		

Analysis of oysters residing at the MLW tidal height during period 3

Source	df	Sum of Squares	Mean Square	F-value	P- value
layer	1	393.031	393.031	14.5	.0019
residual	14	379.488	27.106		

layer: deep > surface

Appendix 7d. (Cont.)

Analysis of oysters residing at the -90 cm tidal height during period 3

Source	df	Sum of Squares	Mean Square	F-value	P- value
layer	1	248.220	248.220	7.891	.0139
residual	14	440.393	27.106		

layer: surface > deep

Appendix 7e. ANOVAs of arcsine transformed 1993 mortality data of oysters residing at surface and deep substrate layers during period 1 (June - July) and period 3 (August - September). Student- Newman-Keuls tests were used to analyze tidal height when it was found to be significant.

Analysis of oysters residing at the surface during period 1

Source	df	Sum of Squares	Mean Square	F-value	P- value
tidal height	2	424.930	212.465	4.899	.0186
residual	20	867.357	43.368		

Student-Newman-Keuls tests

Tidal height: +25 cm, -90 cm > MLW

Analysis of oysters residing at the deep layer during period 1

Source	df	Sum of Squares	Mean Square	F-value	P- value
tidal height	2	30.335	15.168	.423	.6598
residual	24	860.496	35.854		

Analysis of oysters residing at the surface layer during period 3

Source	df	Sum of Squares	Mean Square	F-value	P- value
tidal height	2	1354.987	677.494	19.08	.0001
residual	20	770.177	35.509		

Student-Newman-Keuls tests

Tidal height: -90 cm > +25 cm > MLW

Analysis of oysters residing at the deep layer during period 3

Source	df	Sum of Squares	Mean Square	F-value	P- value
tidal height	2	154.338	77.169	1.752	.1949
residual	24	1057.265	44.053		

Appendix 7f. 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

Appendix 7g. ANOVAs and Student-Newman-Keuls tests of arcsine transformed mortality data from the 1994 year class of oysters performed by time period.

Analyses performed on mortalities from the June / July period

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	627.740	313.870	3.768	.0317
layer	1	73.914	73.914	.887	.3519
tidal height * layer	2	134.558	67.279	.808	.4530
Residual	40	3331.991	83.300		

Dependent: Mortality (time period 1)

Student-Newman-Keuls tests

tidal height: +25 cm > MLW, -90 cm

Analysis performed on mortalities from the July / August period

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	236.954	118.477	1.500	.2354
layer	1	120.302	120.302	1.523	.2243
tidal height * layer	2	174.749	87.375	1.106	.3407
Residual	40	3158.914	78.973		

Dependent: Mortality (time period 2)

Analyses performed on mortalities from the August / September period

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
tidal height	2	545.135	272.567	3.631	.0356
layer	1	13.576	13.576	.181	.6729
tidal height * layer	2	323.353	161.677	2.154	.1293
Residual	40	3002.454	75.061		

Dependent: Mortality (time period 3)

Student-Newman-Keuls tests

Tidal height: -90 cm > MLW, +25 cm

Appendix 7h. Multivariate repeated measures ANOVAs of arcsine transformed mortality data from the 1994 year class of oysters performed by tidal height.

Newman Keuls tests were used to analyze the within factor, time, when it was found to be significant.

Analyses performed on oysters residing at the +25 cm tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
layer	1	12.223	12.223	.119	.7314
Subject(Group)	44	4505.301	102.393		
time	2	564.711	282.355	3.534	.0334
time * layer	2	195.569	97.785	1.224	.2990
time * Subject(Group)	88	7030.549	79.893		

Dependent: mortality

Newman-Keuls tests

Time: June - July > July - August, August - September

Analyses performed on oysters residing at the MLW tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
layer	1	.137	.137	.002	.9675
Subject(Group)	12	952.735	79.395		
time	2	416.742	208.371	3.621	.0422
time * layer	2	281.452	140.726	2.445	.1080
time * Subject(Group)	24	1381.085	57.545		

Dependent: mortality

Newman Keuls tests

Time: August - September > July - August, June - July

Appendix 7h. (Cont.)

Analyses performed on oysters residing at the -90 cm tidal height

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
layer	1	12.223	12.223	.119	.7314
Subject(Group)	44	4505.301	102.393		
time	2	564.711	282.355	3.534	.0334
time * layer	2	195.569	97.785	1.224	.2990
time * Subject(Group)	88	7030.549	79.893		

Dependent: mortality

Newman-Keuls tests

Time: August - September > July - August > June - July

Appendix 8. Analyses performed on data collected during the destructive reef study.

Appendix 8a. Paired t-tests performed on shell heights of oysters collected from surface and deep substrate layers during destructive reef sampling. Separate analyses were performed on each tidal height and time interval.

Analyses performed by tidal height

Tidal height	Mean difference (surface-deep)	df	t-value	p-value
+25 cm	-1.490	16	-1.036	.3156
MLW	-.902	12	-.429	.6757
-90 cm	.770	19	.769	.4513

Analyses performed by time interval

Time interval	Mean difference (surface-deep)	df	t-value	p-value
1	-2.385	9	-1.547	.1562
2	-1.655	9	-.932	.3756
3	-.617	6	-.328	.7543
4	-3.939	8	-1.168	.2765
5	-5.929	6	-2.238	.0665

Appendix 8b. ANCOVA, using time interval as the regressor, performed on oyster shell heights measured at different tidal heights. Student-Newman-Keuls tests were used to analyze tidal height.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
height	2	194.509	97.255	5.341	.0079
month	1	1955.834	1955.834	107.418	.0001
month * height	2	42.665	21.333	1.172	.3182
Residual	50	910.382	18.208		

Dependent: mean shell height

Student-Newman-Keuls tests

Tidal height: -90 cm > MLW > +25 cm

Appendix 8c. Paired t-tests performed on oyster densities found at surface and deep substrate layers during destructive reef sampling. Separate analyses were performed on each tidal height and time interval.

Analyses performed by tidal height

Tidal height	Mean difference (surface-deep)	df	t-value	p-value
+25 cm	-1.973	19	-2.422	.0256
MLW	.977	17	1.238	.2326
-90 cm	6.270	19	2.108	.0486

Analyses performed by time interval

Time interval	Mean difference (surface-deep)	df	t-value	p-value
1	2.646	11	1.162	.2696
2	-2.125	11	-1.795	.1001
3	4.629	9	1.013	.3376
4	.850	11	.386	.7067
5	3.400	11	1.382	.1944

Appendix 8d. ANOVAs and Student-Newman-Keuls tests performed on oyster densities recorded at surface and deep substrate levels of the the Piankatank reef.

Surface level densities

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
month	4	411.309	102.827	.860	.4956
height	2	7002.111	3501.055	29.283	.0001
month * height	8	837.739	104.717	.876	.5442
Residual	43	5141.018	119.559		

Dependent: surface

Student-Newman-Keuls tests

tidal height: -90 cm > MLW, +25 cm

Deep level densities

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
month	4	334.300	83.575	2.067	.1018
height	2	3030.248	1515.124	37.480	.0001
month * height	8	364.638	45.580	1.128	.3647
Residual	43	1738.269	40.425		

Dependent: deep

Student-Newman-Keuls tests

tidal height: -90 cm > MLW, +25 cm

Appendix 9. Linear regressions performed to determine effects of flow rate and density.

Appendix 9a. Linear regressions of log (x+1) transformed non-destructive 1994 settlement spat counts on flow rate. Separate regressions were performed on data collected at each tidal height.

Tidal Height	p-value	r^2	slope
+25	.621	.0032	-1.312
MLW	.234	.073	2.134
-90	.821	.231	.073

Appendix 9b. Linear regressions of growth and arcsine transformed mortality on flow rate. Separate regressions were performed on data collected from each year class, substrate level, and tidal height.

Regression run on 1994 oysters residing at the surface.

		Growth			Mortality	
Tidal Height	p-value	r^2	slope	p-value	r^2	slope
+25	.5761	.073	-.801	.9114	.002	.616
MLW	.1703	.288	-1.235	.8061	.011	3.394
-90	.9933	.00001	-.011	.3344	.155	6.06

Regressions run on 1994 oysters residing beneath the surface.

		Growth			Mortality	
Tidal Height	p-value	r^2	slope	p-value	r^2	slope
+25	.2126	.245	2.469	.0829	.419	-7.461
MLW	.7989	.018	-.157	.0953	.395	18.916
-90	.1805	.277	-1.095	.5731	.086	-4.639

Regressions run on 1993 oysters residing at the surface.

		Growth			Mortality	
Tidal Height	p-value	r^2	slope	p-value	r^2	slope
+25	.234	.173	2.61	.234	.173	2.61
MLW	.652	.074	-1.114	.652	.074	-1.114
-90	.845	.003	-2.387	.845	.003	-2.387

Regressions run on 1993 oysters residing beneath the surface.

		Growth			Mortality	
Tidal Height	p-value	r^2	slope	p-value	r^2	slope
+25	.5813	.026	2.389	.4185	.169	6.090
MLW	.1914	.138	-.748	.0344	.553	-.851
-90	.4576	.040	-.553	.7487	.029	.998

Appendix 9c. Linear regressions of growth and arcsine transformed mortality on density. Separate regressions were performed on data collected from each year class, substrate level, tidal height, and time.

Regressions run on 1994 oysters residing at the surface.

			Growth			Mort.	
Tidal Height	Time Interval	p-value	r^2	slope	p-value	r^2	slope
+25	1	.967	.0003	-.042	.981	.0001	.233
	2	.927	.001	.088	.0996	.387	-12.71
	3	.718	.023	-.130	.547	.077	3.045
MLW	1	.2792	.191	-2.167	.533	.068	5.607
	2	.8118	.01	-.299	.2027	.254	-6.142
	3	.8215	.009	.479	.6212	.052	-6.58
-90	1	.6618	.041	-.899	.2187	.28	-15.455
	2	.335	.032	-1.007	.1608	.35	-9.24
	3	.0474	.578	-5.732	.9946	.0001	.04

Regressions run on 1994 oysters residing beneath the surface.

			Growth			Mort.	
Tidal Height	Time Interval	p-value	r^2	slope	p-value	r^2	slope
+25	1	.685	.036	1.922	.707	.03	-9.03
	2	.8814	.005	-.060	.0266	.659	-25.525
	3	.5706	.069	.546	.7169	.029	3.321
MLW	1	.5345	.067	.597	.956	.0005	-.649
	2	.3648	.138	.948	.8017	.011	-1.65
	3	.906	.003	-.257	.9552	.0006	.704
-90	1	.5144	.074	-1.807	.533	.068	-7.044
	2	.07	.447	4.181	.118	.356	-28.63
	3	.686	.029	1.673	.146	.317	-23.39

Regressions run on 1993 oysters residing at the surface.

Tidal Height	Time Interval	Growth			Mort.		
		p-value	r^2	slope	p-value	r^2	slope
+25	1	.7495	.022	-.399	.5430	.078	12.270
	2	.2385	.264	2.911	.3766	.158	-10.738
	3	.8198	.015	1.699	.6253	.065	-12.846
MLW	1	.349	.146	-1.195	.247	.214	2.808
	2	.335	.022	-1.268	.481	.104	2.314
	3	.357	.171	2.776	.0536	.55	7.452
-90	1	.371	.135	.622	.2110	.246	4.99
	2	.568	.069	-.867	.394	.148	5.11
	3	.0426	.594	-3.307	.686	.035	-2.63

Regressions run on 1993 oysters residing beneath the surface.

Tidal Height	Time Interval	Growth			Mort.		
		p-value	r^2	slope	p-value	r^2	slope
+25	1	.5790	.054	.924	.6963	.027	-1.820
	2	.1503	.312	2.245	.5957	.050	-5.020
	3	.6263	.051	-1.440	.5429	.065	-6.155
MLW	1	.0818	.421	-1.856	.0114	.683	11.573
	2	.6316	.049	1.157	.4499	.118	-6.544
	3	.1257	.403	-3.2139	.1467	.371	-7.101
-90	1	.0135	.666	4.026	.5135	.074	-7.298
	2	.5141	.074	1.105	.2469	.084	7.676
	3	.5385	.066	1.725	.9258	.002	.778

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