

SMALL-SCALE SETTLEMENT PATTERNS OF THE OYSTER *CRASSOSTREA VIRGINICA* ON A CONSTRUCTED INTERTIDAL REEF

Ian K. Bartol and Roger Mann

ABSTRACT

The construction of three-dimensional, intertidal reefs resembling those widely present during colonial times in the Chesapeake Bay, but now absent due to years of overharvesting, may provide a more ecologically advantageous environment for oyster settlement and subsequent survival than present subtidal, two-dimensional habitats. We examined settlement processes on a constructed, 210 x 30 m intertidal reef composed of oyster shell. The reef was destructively and non-destructively sampled weekly throughout the summer and fall at tidal heights ranging from 30 cm above to 90 cm below mean low water (MLW) and at two substrate levels (reef surface and 10 cm below the reef surface). Settlement at the surface of the reef community and within the reef interstices down to depths of 10 cm was statistically similar, and settlement was generally greatest subtidally; however, there were localized areas within the reef community where conditions were beneficial for intertidal settlement and where differences in intertidal/subtidal settlement rates were not detectable. These results suggest that microscale variations in tidal elevation and substrate depth strongly affect settlement processes and should not be ignored when constructing reefs.

When colonists arrived in the Chesapeake Bay region during the 1600s, they encountered a Bay ecosystem rich in intertidal reef communities. These unmistakable biological features, which proliferated in the Chesapeake Bay and tributaries during the last half of the Holocene interglacial, were important self-renewing food sources for early settlers and Native Americans alike (Hargis and Haven, 1998). As the economic value of the oyster *Crassostrea virginica* began to be realized in the mid 1800s, however, commercial exploitation of the resource began. Years of subsequent overharvesting has transformed these once massive, aerially exposed communities to mere subtidal, "footprint" structures which have significantly less vertical dimensionality and habitat heterogeneity. Disease, environmental degradation, and poor resource management in the last half century have exacerbated this degeneration.

Today Virginia's oyster population is less than 1% of what it was just 35 yrs ago (Wesson et al., 1998). Many of the rejuvenation efforts that were successful in the past and that involved the spreading of relatively thin veneers of shell over coastal and estuarine bottom for larval attachment no longer facilitate the development of healthy and viable adult oyster communities. This may in part be because these efforts revolve around re-creating habitats produced and shaped by man, rather than focusing on emulating the natural, three-dimensional, intertidal communities present during colonial times. Since oysters in the Chesapeake Bay resided in intertidal communities for centuries and were able to withstand significant environmental and biological stresses, there is probably an ecological and evolutionary advantage to intertidal, aggregational reef existence in the Bay and a return to it may help rejuvenate ailing oyster stocks.

We know little about the colonization and ecology of *C. virginica* on intertidal reefs in the Chesapeake Bay because of their absence for over a century. Thus, we constructed a

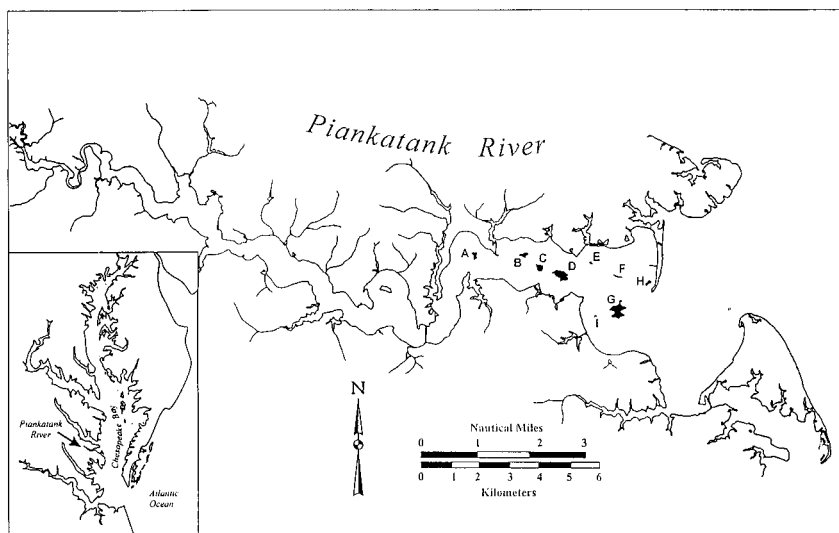


Figure 1. Map of Piankatank River showing the site of reef construction (D) and location of other nearby oyster reefs (A, B, C, E, F, G, H, I).

210 x 30 m intertidal reef to study *C. virginica* settlement - the irreversible adherence of larvae to the substrate and the first step in the colonization process. We paid particular attention to the spatial effects of elevation relative to mean low water (MLW) because tidal height is a major factor distinguishing intertidal reef environments from current subtidal habitats. We also examined settlement patterns at the surface of the reef shells and within the interstices of the reef at depths of 10 cm along the tidal gradient to determine if microscale differences in substrate depth play a critical role in settlement processes and should be considered when constructing artificial reefs.

METHODS

STUDY SITE. — This study was conducted in the Piankatank River, a sub-estuary of the Chesapeake Bay located in Virginia, at a site which once supported a highly productive intertidal reef system (Fig. 1). However, at the time of reef construction, the site contained only a remnant shell footprint of the pre-existing natural reef. The Piankatank River is ideal for artificial reef construction because it harbors a sustainable adult oyster population, has high annual oyster settlement (Morales-Alamo and Mann, 1997), and there is no commercial oyster fishery and virtually no industry or agricultural development within the watershed. Tidal range at this site is small (mean range = 36 cm), but local meteorological events, wind in particular, often dramatically alter this range from 0 to 1.25 m. The site is relatively shallow (1-3 m), and consists of a sand and shell bottom. Water temperature at this site varies from 0.5-30° C throughout the year, and salinity ranges from 8-20‰ annually.

REEF CONSTRUCTION. — The reef was constructed by the Virginia Marine Resource Commission (VMRC) using a high pressure water cannon and barges carrying aged oyster shells. The shells were jettisoned from barges using the cannon's high velocity water stream and broadcast over a 210 x 30 m area, which were the approximate footprint dimensions of the pre-existing reef system. After completion, the reef consisted of numerous hummocks varying from 2 to 20 m² in area exposed at low tide. Although the constructed reef ranged from 0.5 m above to 3 m below MLW, the

vast majority of the hummocks did not protrude higher than 0.35 m above MLW or extend deeper than 1.0 m below MLW.

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

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

During both years of sampling, non-destructive and destructive methods were employed from June through September to assess settlement and early recruitment within the reef ecosystem. Non-destructive sampling involved the weekly placement of oyster shells in open-topped, 64 x 20 cm, rubber coated, 1-in wire mesh trays secured to the reef surface by reinforced bars. In 1993, a surface layer of 20 shells was placed in single level trays. The trays were situated at all four tidal height designations along two spatially separate transects at each of two hummocks. The concave and convex side of all 20 shells within the individual trays were examined for recently settled oyster larvae (spat) using a dissecting microscope (x 125), and a spat total per tray was recorded. In 1994, 3-tiered trays containing 30 shell upper and lower levels, which were spaced 10 cm apart, and a 40 shell intermediate level were used. Each week four plots were selected randomly at each of the three tidal heights. At every plot, a 3-tiered tray was buried into the reef until the upper shell layer was even with the reef surface. Both the concave and convex sides of shell found in the upper and lower tiers were examined for spat using a dissecting scope, and a surface and deep layer spat total were recorded at all 12 weekly selected plots.

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

In addition to non-destructive and destructive samples, shellstring samples were collected weekly from June through September during both years of sampling. Shellstrings consist of 12 single valve oyster shells, each with a hole drilled through the center, threaded onto galvanized wire. Only the 10 intermediate shells are considered because the top and bottom shells of the shellstring have a tendency to collect large numbers of recently settled juveniles (spat), leading to unrepresentative spat estimates. The shellstrings were suspended from pilings located at the east (downstream) and west (upstream) reef extremities at a depth of 90 cm below MLW. Water depth was approximately 4 m below MLW at the east reef piling and 2 m below MLW at the west reef piling. Both shell surfaces were examined and a spat total per shell was calculated so that comparisons could be made with non-destructive and destructive subtidal samples.

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 estimate of current flow at the reef site, we set out chlorine tablets housed in 20 cm x 20 cm mesh cages and held 10 cm above the reef bed by reinforced rods during both neap and spring tides. Cages were placed at plots sampled in the non-destructive settlement study. The chlorine tablets were weighed, deployed in the field for 48 h, and weighed again. Mean 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 combined relative estimate of the magnitude of both wave intensity and flow rates. Chlorine tablets were used as a surrogate measure for flow because turbulent diffusion, the major force driving the dissolution rate in the field, in the benthic boundary layer at a given bottom roughness varies in a positive fashion with current speed (Wildish and Kristmanson, 1997). It was assumed that the flow speed derived from the dissolution rate of chlorine tablets placed 10 cm above the reef would be proportional to flow conditions at the surface and 10 cm below.

STATISTICAL ANALYSIS. — The argument may be made that 1993 data collected over time were not independent, since successive samples were taken from either spatially fixed areas or spatially connected plots. Thus, analysis of variance (ANOVA) with repeated measures on time were performed on both the 1993 non-destructive and destructive data sets. To satisfy the assumptions of homogeneity, all settlement data were $\log(x + 1)$ transformed. When no significant interactions were detected between time and any other factor, 3-way fixed factor (factors: tidal height, time, mound) ANOVAs were performed. Significant main and interactive effects were examined using Student-Newman-Keuls (SNK) tests.

Linear correlations were performed first on surface and deep samples collected in the 1994 settlement study to assess the degree of dependence between the two substrate levels. Significant relationships were detected; therefore, paired sample t-tests were used to determine if differences existed between surface and deep samples. A mean value for surface and deep data was calculated when no significant difference between the substrate levels was detected, and further analyses were performed on these mean values. ANOVAs were run on 1994 $\log(x + 1)$ transformed, non-destructive and destructive settlement data, and all differences between the means were revealed using SNK multiple comparison tests. Furthermore, to determine if a functional dependence existed between settlement and water movement, linear regressions of $\log(x + 1)$ transformed non-destructive settlement on chlorine tablet dissolution rate were performed for each tidal height. The assumptions of regressions were met as determined by residual analysis (Zar, 1984).

RESULTS

1993. — Settlement lasted 6 wks in 1993 beginning the week of July 29 and ending the week of September 9, and settlement intensity was low overall (Fig. 2). Although traces of settlement were detected in non-destructive samples throughout the 6-wk settlement period, spat counts in weeks 5 and 6 were so low (< 0.02 spat per shell) that we eliminated them in our statistical analysis. Conversely, in destructive samples, dramatic increases in

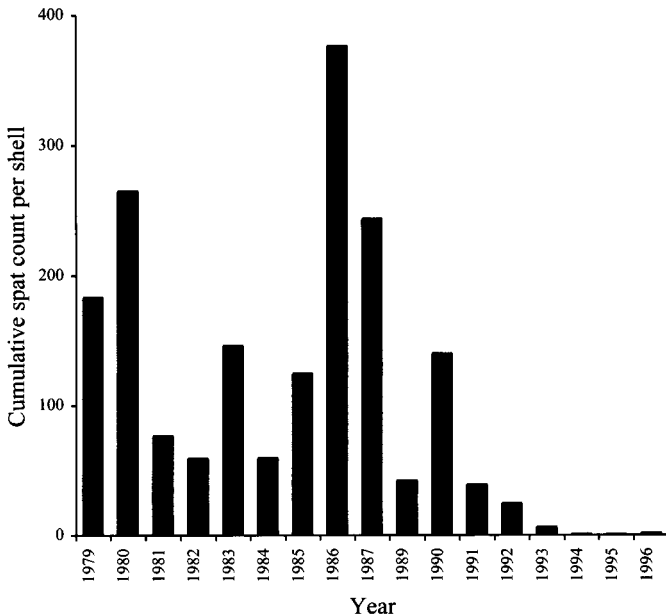


Figure 2. Cumulative oyster spat counts per shell detected on shellstrings at the reef site from 1979 - 1996 recorded in the Virginia Institute of Marine Science (VIMS) spatfall reports. Settlement data for 1988 was incomplete and thus was not included in the figure.

settlement were observed during these periods, and thus these weeks were included in destructive sample statistical tests.

No significant interactive effects between the within factor, time, and any other factor were detected when 1993 destructive and non-destructive data were analyzed using repeated measures analysis; thus, subsequent 3-way ANOVAs were performed. These analyses revealed that in non-destructive samples tidal height had a significant impact on settlement (Table 1A), with settlement being greatest at the -90 cm tidal height (Fig. 3). In destructive samples, where the entire 6-wk settlement period was considered, time had a significant impact on settlement (Table 1B). In general, destructive sample spat counts increased with time. This is quite different than settlement patterns observed in non-destructive samples where settlement magnitudes were not detectably different during the first 4 wks of the settlement season, but decreased dramatically in weeks 5 and 6 (Fig. 4). Destructive and non-destructive spat counts were also very different during week 1 of the settlement period when no previous settlement events had occurred (non-destructive = 4.8 ± 0.82 S.E. spat per 20 shells, destructive = 2.1 ± 0.17 S.E. spat per 20 shells). A mound x tidal height interaction was present in destructive samples. High spat counts at the +30 cm tidal height on hummock A (mound perpendicular to prevailing currents) prevented the detection of significant differences in settlement by tidal height and produced this interaction (Fig. 5A). Conversely, on hummock B (mound parallel to prevailing currents) where +30 cm spat counts were significantly lower than on hummock A, significantly greater settlement occurred at -45 and -90 cm than at +30 cm (Fig 5B).

1994. — The 1994 settlement season lasted only 3 wks beginning the week of 15 July and ending the week of 28 July. Spat counts detected on shellstrings throughout the 1994

Table 1A. ANOVA of 1993 non-destructive settlement data.

Source	df	Sum of squares	Mean square	F-value	P-value
Mound	1	0.078	0.078	1.059	0.3111
Tidal height	3	2.147	0.716	9.692	0.0001
Time	3	0.573	0.191	2.588	0.0701
Mound x tidal height	3	0.516	0.172	2.331	0.0928
Mound x time	3	0.127	0.042	0.572	0.6377
Tidal height x time	9	0.819	0.091	1.232	0.3108
Mound x tidal height x time	9	0.989	0.110	1.489	0.1942
Residual	32	2.362	0.074		

Table 1B. ANOVA of 1993 destructive settlement data.

Source	df	Sum of squares	Mean square	F-value	P-value
Mound	1	0.043	0.043	0.648	0.4248
Tidal height	3	1.207	0.402	6.017	0.0015
Time	5	7.583	1.517	22.690	0.0001
Mound x tidal height	3	0.582	0.194	2.903	0.0443
Mound x time	5	0.287	0.057	0.858	0.5164
Tidal height x time	15	0.878	0.059	0.875	0.5942
Mound x tidal height x time	15	0.423	0.028	0.422	0.9648
Residual	48	3.208	0.067		

sampling session were the lowest recorded in the last 18 yrs, with mean cumulative spat counts of less than 1 spat per shell over the entire settlement season (Fig. 2). Unfortunately, these low settlement magnitudes together with the short settlement season precluded a meaningful comparison of intertidal spat counts recorded on hummock A with intertidal counts on the other hummocks. Field observations did reveal, however, that noticeably more intertidal oysters were present on hummock A than at any of the other seven hummocks present at the site.

Significant correlations ($P < 0.001$) between surface and deep substrate levels were detected in both non-destructive and destructive samples, and thus paired t-tests were used to examine the effects of substrate level. Based on these tests, no significant differences in settlement were detected between surface and deep layers at any of the tidal heights in either the non-destructive or destructive samples ($P > 0.05$, Table 2).

In the non-destructive study, ANOVAs performed on surface/deep settlement means revealed that both tidal height and time influenced settlement (Table 3A). Settlement intensity was greatest at the -90 cm tidal height and during the week of July 21-28 (Figs. 6A, 6B). Settlement was influenced significantly by time and tidal height in the destructive study as well, but a significant time x tidal height interaction confounded the effects (Table 3B). This interaction was a result of spat counts being significantly greater at the -90 cm tidal height only during the weeks July 21-28 and July 28-August 4. During the first week of the settlement season, settlement magnitudes were so low across all 3 tidal heights that no significant differences were detectable.

COMPARISON OF SPAT DETECTION METHODS. — There was a clear discrepancy in spat counts between the 3 sampling methods even though they were all deployed at a depth of 90 cm below MLW. Clearly more spat settled on shellstrings, which were replaced weekly and suspended in the water column, than on shells placed at weekly intervals in trays fixed to

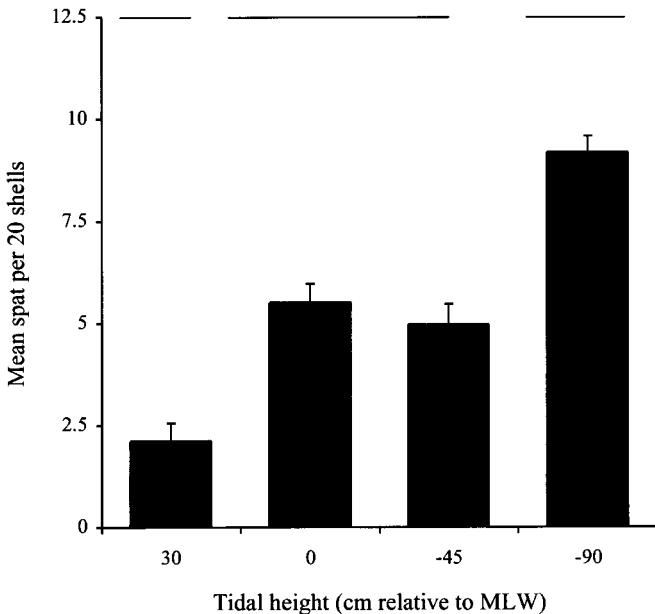


Figure 3. Mean oyster spat counts per 20 shells recorded in the 1993 non-destructive study. Error bars denote +1 SE and lines above the bars represent results from Student-Newman-Keuls (SNK) tests.

the reef (non-destructive samples) or on shells sampled destructively from the reef (destructive samples) (Fig. 7). Furthermore, non-destructive samples appeared to collect more spat than destructive samples.

PHYSICAL PARAMETERS. — During both years of sampling, settlement occurred when or shortly after salinities approached 16‰, water temperatures reached 30° C, and water clarity rose (Fig. 8). Flow rate was greatest at the reef crests and lowest at the reef bases, and there was no linear dependence of settlement on water flow ($P > 0.234$, $r^2 < 0.231$). Furthermore, no optimal flow rate for settlement was apparent.

DISCUSSION

The short, unimodal settlement events recorded in this study coupled with low overall settlement magnitudes are indicative of a rapidly declining broodstock population in the Chesapeake Bay, which at present is showing little sign of rejuvenation (Morales-Alamo and Mann, 1997). Bimodal peaks in settlement were recorded not long ago in the Chesapeake Bay, and it was not uncommon to detect settlement from June through October at the Piankatank reef site (Virginia Institute of Marine Science Annual Oyster Spatfall Surveys, unpublished data for the period 1970-1997). In 1986, cumulative spat counts at the site were 376.5 spat/shell, whereas now they are at 1.6 spat/shell. Although there are probably a number of reasons for this devastating decline ranging from disease to poor water quality, years of overharvesting where ecologically advantageous intertidal reef communities were degraded to mere subtidal footprints undoubtedly weakened oyster stocks considerably. Hargis and Haven (1997) offer the opinion that overharvesting was the principal instigator of the demise of the Chesapeake oyster. Although this is debat-

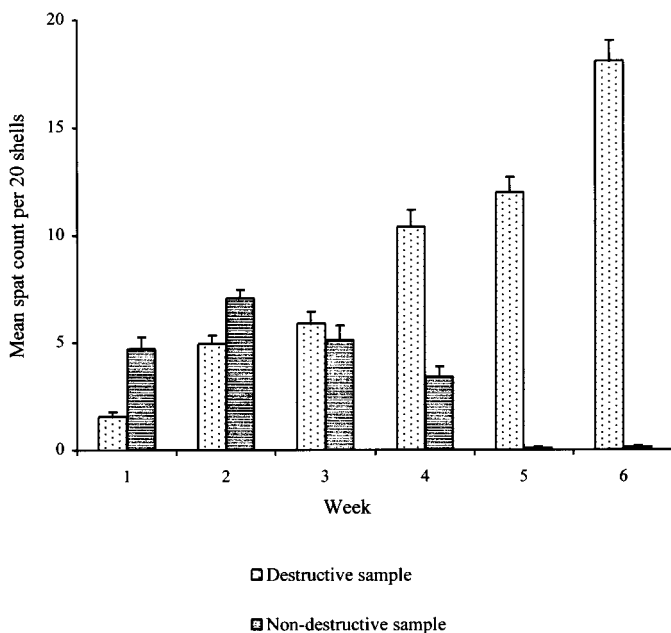


Figure 4. Mean weekly spat counts for both destructive and non-destructive samples collected from July 29 through September 16, 1993. Error bars denote +1 SE

able, there is widespread agreement that oyster reefs are the most optimal ecosystems for oysters and we need to learn more about oyster ecology on them.

Based on 2 yrs of settlement monitoring on a constructed intertidal oyster reef, it was clear that tidal elevation had a large impact on settlement and early recruitment. The higher rates of subtidal settlement recorded here are consistent with several other studies conducted in non-reef environments, such as McDougall (1942) where unglazed hearth tiles were used as substrate, Chestnut and Fahy (1953) where clam shells suspended in baskets were utilized, and Roegner and Mann (1990) where hatchery-reared larvae exposed to field conditions in microcosms were considered. Nichy and Menzel (1967), who placed oysters on clothmats of mesh within a reef ecosystem, also observed greater settlement/early recruitment subtidally.

The high rates of subtidal settlement/early recruitment observed throughout most of this study were likely a result of a number of factors. Submergence time, for instance, may have contributed to settlement discrepancies observed at different tidal heights. Oyster larvae in the water column were exposed to subtidal substrates substantially longer than to intertidal substrates, and as a result, had a wider time window in which to set. Submergence time alone, however, did not account for the observed differences in settlement. Kenny et al. (1990) found that settlement intensity is not a direct function of submergence time, especially in the high intertidal zone where settlement is lower than predicted and the low intertidal zone where settlement is higher than predicted. Another factor contributing to elevated subtidal sets may have been vertical segregation of oyster larvae within the water column. Oyster late stage pediveliger larvae are more abundant near the benthos than at the surface or within the midwater region (Carriker, 1951; Kunkle, 1957; Baker,

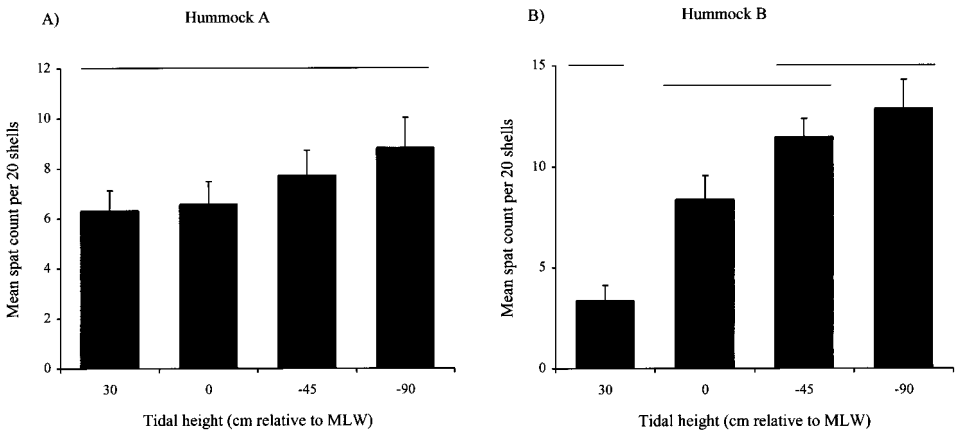


Figure 5. Mean spat counts recorded on (A) hummock A and (B) hummock B during the 1993 settlement season. Error bars denote +1 SE and lines above the bars represent results of SNK tests.

1994), and this may have contributed to high subtidal settlement. Furthermore, because late stage competent to set oyster larvae are negatively phototactic (Cole and Knight-Jones, 1939; Ritchie and Menzel, 1969; Shaw et al., 1970) and prefer areas of low wave energy (Ortega, 1981; Abbe, 1986), they may have actively sought subtidal habitats where light intensities and wave stress are reduced.

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

Results of several other studies suggest that settlement/early recruitment is not always maximized subtidally, as was the case at hummock A. Hidu and Haskin (1971) found that although settlement was greatest subtidally 1/2 mi offshore at a transitional slope region where tidal flats merge with deep water, settlement was greatest intertidally in shallow water near the shore. They attributed the high intertidal sets inshore to rapid rises in water temperature as seawater passed over heated intertidal substrates and to the presence of dense intertidal adult populations which released chemical cues. McNulty (1953), using bags of shell left in the field for 2 wks, found higher settlement/early recruitment in the

Table 2. Paired t-tests performed on surface and deep substrate layer spat counts for 1994 non-destructive and destructive samples. Separate analyses were performed for each tidal elevation.

	Tidal height	Degrees of freedom	t-value	p-value
Non-destructive samples	+25 cm	11	-0.886	0.3944
	MLW	11	-0.321	0.7545
	-90 cm	11	0.549	0.5940
Destructive samples	+25 cm	11	-0.561	0.5863
	MLW	11	0.432	0.6742
	-90 cm	11	-1.149	0.2750

intertidal zone than in the subtidal zone, and Kenny et al. (1990), using asbestos plates sampled every 2 wks in the summer and 4 wks 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. Kenny et al. concluded that subsequent post-settlement mortality was responsible for the confinement of oyster reefs to the intertidal zone in south-eastern coastal areas of the United States.

The lack of detectable differences in settlement/early recruitment (1-3 wks) between surface and deep substrates at any of the tidal heights considered was admittedly unexpected. Our first reaction was that the low settlement rates recorded in this study dramatically lowered the power of the statistical tests. Although this may be true, a graphical re-examination of the data by layer revealed no trend in greater settlement for either substrate depth at any of the tidal heights considered. Thus, we concluded that larval oyster settlement was not impeded by shell down to depths of 10 cm on artificial reefs composed of oyster shell. The fact that adult oysters are found in greater numbers at the surface of established reefs (Bahr and Lanier, 1981) suggests that settlement patterns and adult ranges may deviate from one another. Such discrepancies in the ranges of recently settled larvae and adults are not uncommon in oysters (see Roegner, 1989; Kenny et al., 1990).

Although there are no studies on the settlement of sessile organisms as a function of substrate depth, there is evidence to suggest that settling larvae actively seek out preferable habitats. Upon setting, barnacles and sponges select small sediment depressions, pits, and crevices where shear is low (Crisp, 1967; Wethey, 1984, 1986; Bergron and Bourget, 1986; Keen, 1987; Chabot and Bourget, 1988). Oysters demonstrate a similar preference for crevice microhabitats (Michener and Kenny, 1991), as well as for darkened conditions when setting (Cole and Knight-Jones, 1939; Ritchie and Menzel, 1969; Shaw et al., 1970), areas of reduced wave action (Ortega, 1981; Abbe, 1986), environments where flow is low (Bushek, 1988), and substrates which are not heavily fouled (Abbe, 1986; Morales-Alamo and Mann, 1990). Some oyster larvae may have actively sought out substrate deep within the reef interstices because the reef interstices provide a plethora of microhabitats and offer sheltered, shaded conditions, reduced flows, and less fouled substrates. Settlement inducing compounds released by adult oysters (Bayne, 1969; Hidu et al., 1978; Tamburri et al., 1992; Zimmer-Faust and Tamburri, 1994) and in biofilms on oyster shell surfaces (Bonar et al., 1986; Fitt et al., 1989, 1990; Tamburri et al., 1992) were likely more concentrated within the fabric of the reef than at surface, and this too may have contributed to subsurface settlement. Furthermore, despite the ability of oyster larvae to regulate their position in the vertical dimension (generally by accelerating sink-

Table 3A. ANOVA of 1994 non-destructive settlement data.

Source	df	Sum of Squares	Mean Square	F-Value	P-Value
Time	2	0.607	0.303	15.807	0.0001
Tidal height	2	1.006	0.503	26.204	0.0001
Week x tidal height	4	0.038	0.010	0.499	0.7366
Residual	27	0.518	0.019		

Table 3B. ANOVA of 1994 destructive settlement data.

Source	df	Sum of squares	Mean square	F-value	P-value
Time	2	0.295	0.148	15.156	0.0001
Tidal height	2	0.832	0.416	42.709	0.0001
Week x tidal height	4	0.129	0.032	3.299	0.0252
Residual	27	0.263	0.010		

ing rates) (Tamburri et al., 1992), oyster larvae simply may have become entrained in underlying reef layers. Low flows, wave action, and an intricate maze of substrate may have been sufficient to prevent weak swimming larvae from navigating vertically to the reef surface. Although it is not clear from this study which active or passive transport mechanism(s) are responsible for subsurface settlement, it is clear that larvae are capable of settling within the fabric of the reef and are not impeded by shell down to depths of 10 cm. This is quite remarkable considering there may be 20 or more shell layers within the 10 cm space.

The fact that settlement estimates from shellstrings, a frequently used method of estimating oyster abundance, were greater than both destructive and non-destructive samples suggests that suspended shellstrings overestimate settlement on sloping reef bottoms. Baker (1994) also found shellstrings to be unreliable predictors of settlement magnitudes on adjacent substrates. Even though shellstrings fail to provide an accurate assessment of oyster settlement on actual reef topography, they are efficient and reliable predictors of the presence of late-stage pediveligers at a given site. This was evident by the fact that the onset of settlement was detected simultaneously on all three substrate mediums during both years of sampling. Furthermore, when compared with shellstrings suspended at other sites, they may be useful tools for determining relative settlement intensities.

A direct comparison could not be made between destructive and non-destructive methods to predict early post-settlement mortality rates, as was originally intended. This is because settlement rates on the two mediums during week 1 of the settlement season, when no previous settlement had occurred, were dissimilar. This discrepancy was probably a product of two factors: substrate differences and difficulties in spat detection. The higher degree of fouling and colonization on reef shells compared with that found on shells placed in trays on a weekly basis may have contributed to lower destructive sample settlement rates. This is because bryozoans, colonial ascidians, algal growth, and high sedimentation, organisms and conditions all present at the reef site, considerably reduce settlement of oysters (Abbe, 1986; Ortega and Sutherland, 1992; Osman et al., 1989). Difficulties in identifying spat on heavily fouled reef shells was probably another factor contributing to lower destructive spat counts. During the early weeks of settlement, spat

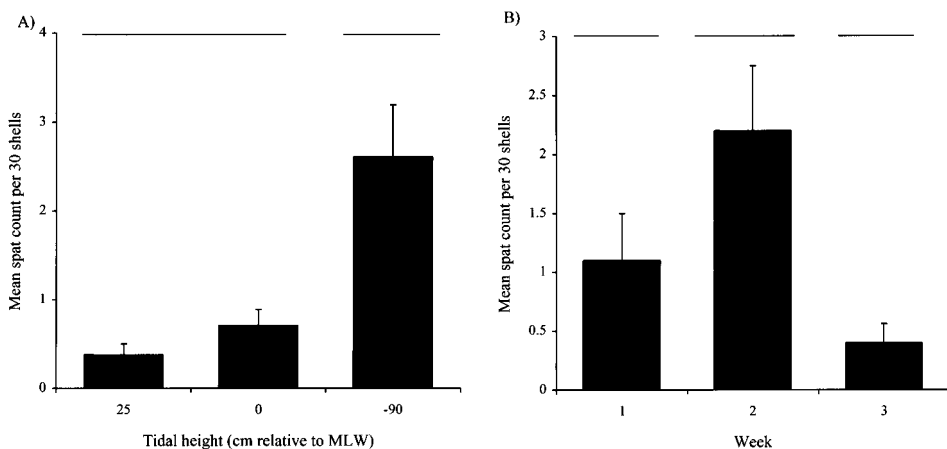


Figure 6. Mean spat counts per 30 shells calculated from non-destructive samples for (A) each of the three tidal heights and for (B) each week of sampling (week 1 = July 15-21, week 2 = July 21-28, and week 3 = July 28-August 4, 1994). Error bars denote +1 SE and lines above the bars represent results of SNK tests.

were small and difficult to detect on the heavily fouled reef shells, but when spat became larger (weeks 5 and 6 of the 1993 study), they were more visible and thus spat counts were higher. This was readily apparent when settlement intensities on destructively sampled shells increased substantially during weeks 5 and 6 while only negligible amounts of spat were detected on non-destructively sampled shells and shellstrings.

Settlement at the reef site occurred when or shortly after water temperatures reached 30° C, salinities approached 16‰, and water clarity rose; however, no single parameter could be used to reliably predict the onset of settlement. It is difficult to use any one physical factor as a predictor of settlement because the timing of settlement events depend on a number of variables, including not only water temperature, salinity, and water quality, but also dissolved oxygen, food supplies, substrate availability, predation, and flow conditions (Abbe, 1986; Kennedy, 1986). Water temperature is generally the best predictor of settlement (Kenny et al., 1990; McNulty, 1953), whereas salinity often has little effect on settlement within broad limits (Hidu and Haskin, 1971; Haven and Fritz, 1985). Turbidity may be deleterious to oyster larvae (Davis and Hidu 1969) and if the suspended silts and sediment within the water column accumulate on substrates, they may deter settlement (Abbe, 1986; Morales-Alamo and Mann, 1990). However, there is little known about how short term (1-2 wks) changes in water clarity affect oyster settlement.

The exact role of flow velocity in settlement is yet to be determined. Based on flume studies and indirect field observation, bivalve larvae appear to demonstrate a preference for low flows. Jonsson et al. (1992) found that when flow speeds exceed 10 cm s⁻¹ in a flume bivalve larvae (*Cerastoderma edule*) are resuspended into the water column by penetrating eddies when attempting to "land", preventing settlement from occurring. Butman et al. (1988) found high rates of *Mercenaria mercenaria* settlement in low flows generated in a flume, Bushek (1988) demonstrated that *C. virginica* preferably settle nearshore on pier pilings where current velocities are low, and Hidu and Haskin (1971) found high settlement in Delaware Bay at a sharp transition zone between high and low

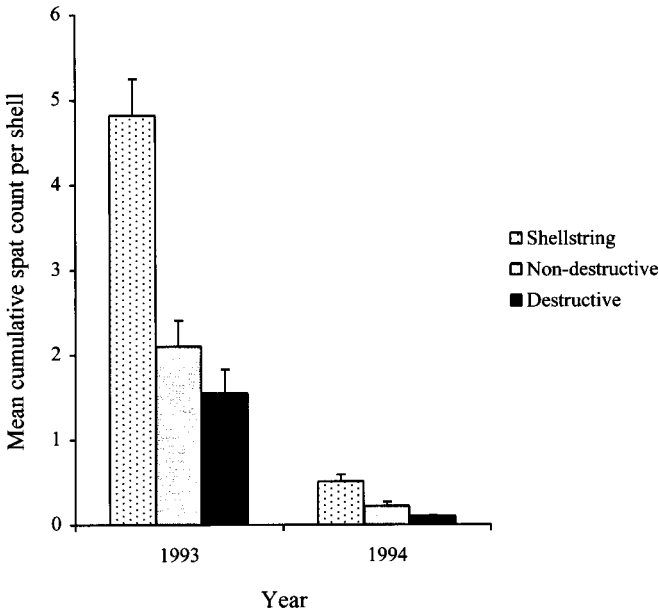


Figure 7. Mean cumulative spat counts per shell at the -90 cm tidal height detected on shellstrings, non-destructive samples, and destructive samples in 1993 and 1994. Error bars denote +1 S.E.

current velocities. On the other hand, polychaetes avoid settling in low flows ($5\text{--}10\text{ cm s}^{-1}$), and in the field settle in the highest flows for which attachment and substratum exploration are physically possible (Pawlick and Butman, 1993). Barnacle cyprids reject substrates more frequently in high flows (10 cm s^{-1}) than low flows (5 cm s^{-1}) (Mullineaux and Butman, 1991). But Mullineaux and Butman explain that a higher rejection rate does not necessarily result in lower settlement, since contact rate is higher in fast than slow flows. Thus, passive transport processes play a large role in bringing cyprids to the substrate, but once there they prefer to settle in areas of low shear such as in crevices and pits (Crisp, 1967; Wethey, 1986).

The results of this study unfortunately fail to elucidate the relationship between settlement and flow velocity. No linear dependence of settlement on flow was observed, nor was any optimal flow velocity for settlement apparent. This may be a result of two factors. First, the velocities considered within each tidal range may not have varied dramatically enough for relationships to be observed. Most of the studies focusing on the effects of flow on settlement are conducted in flumes, allowing for the consideration of a wide gradient of flows. In this study, however, flow 10 cm above the reef surface in the subtidal zone did not exceed 10 cm s^{-1} . Although flows were faster in the intertidal zone as a result of flood and ebb tides, large differences in relative flow rates and wave action were not observed across the hummocks (with the exception of hummock A where flow and wave action were generally high). Second, microscale flow conditions may have had a larger impact on settlement intensities than mean flow rates calculated 10 cm above the reef bed. Oyster larvae setting on the underside of surface substrates and within the fabric of the reef probably experienced dramatically different flow regimes than those

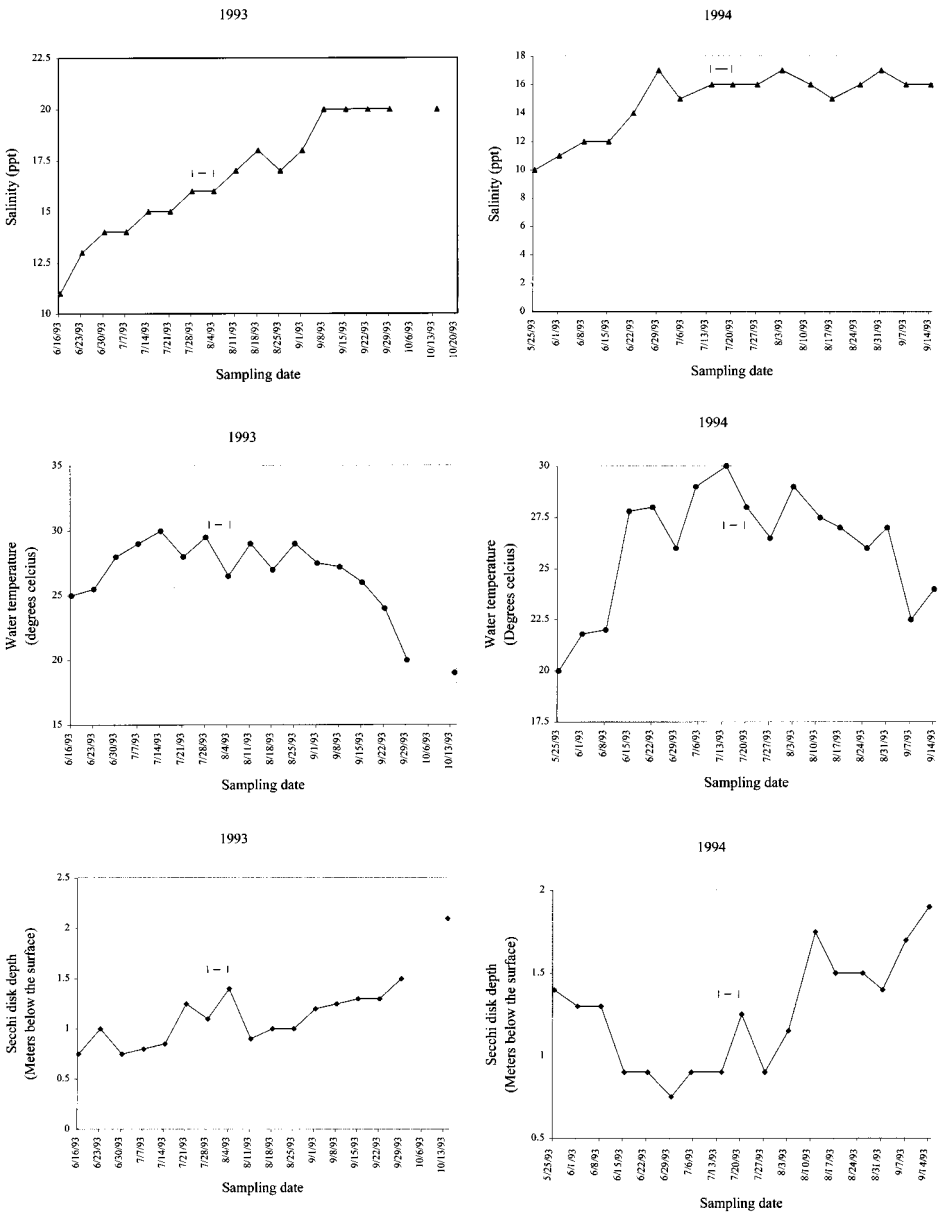


Figure 8. Salinity, Secchi disk readings, and water temperatures recorded at the reef site during 1993 and 1994. The symbol (I—I) indicates the beginning of the settlement season.

measured 10 cm above the benthos. Microspatial flow measurements at the point of larval attachment need to be measured in oyster reef environments to accurately determine the role of flow on settlement.

Although no one physical factor investigated in this study was a predictor of the onset of settlement and settlement intensity did not appear to be strongly dependent on flow rate 10 cm above the reef bed, we did learn that vertical elevation relative to both the reef

surface and MLW influences settlement. Oysters settle not just at the surface of reef communities but settle in comparable numbers within the reef interstices down to depths of 10 cm. This finding is significant because presently there is debate over which substrates should be used for reef construction in the Chesapeake Bay. Many of the proposed reefs are to be composed of crushed clam shell, tile, or mounds of sediment capped with a thin shell layer. When these substrates are used, subsurface interstitial space is limited, precluding oyster community development below the surface. The subsurface environment likely provides biological and physical refugia for oysters and may be very important for survival in developing, artificial reef communities (Bartol and Mann, 1998). Furthermore, settlement on artificial reefs is generally greatest subtidally; however, there may be localized areas within the community where conditions are beneficial for intertidal settlement and intertidal/subtidal settlement rates are comparable. These zones may be important for oyster reef development because they allow for the rapid establishment of intertidal and not just subtidal environments. The establishment of oysters in heterogeneous intertidal/subtidal environments, habitats they once thrived in naturally, assuredly is beneficial for dwindling oyster populations.

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ADDRESS: Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, P.O. Box 1346, Gloucester Point, Virginia, 23062; phone (804) 684-7305, fax (804) 684-7045, E-mail: ibartol@vims.edu.