2014 Cook Point Oyster Sanctuary Survey: Paynter Lab, University of Maryland

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Executive Summary

The Cook Point oyster sanctuary in the Choptank River, MD is a site that offers unique experimental opportunities. In addition to a region of flat shell with large mounds of shell, there are seven reefs that were constructed of granite with or without an overlying shell veneer, and range from one to three feet in height. These artificial reefs were planted at known spat densities in 2011, which allows direct comparisons between the construction treatments. In the fall of 2014, Dr. Kennedy Paynter's laboratory at the University of Maryland analyzed growth, survival, and reproduction of the oyster populations. In addition, a basic macrofaunal biodiversity assessment was conducted at and between all sites.

The lowest survival, oyster number and biomass density readings for any substrate class occurred on unrestored sand sites, with an average of 6 oysters/m^2. Richness and density of species other than oysters was likewise much lower on sand than on restored reef substrates. In contrast, the restored experimental reefs fared much better, with oyster densities averaging 73 oysters/m^2. We found that comparisons between constructed reefs and shell-only sites were difficult to make, as the shell sites had very different planting histories than the granite-based reefs.

Within the constructed reefs, reef heights between 1 and 3 feet showed no significant oyster population differences. In contrast, significantly more oysters were found on granite plus shell reefs than on granite-only reefs, with the first averaging 91 oysters/m^2, and the latter 49 oysters/m^2. Additionally, granite-only reefs had higher densities of organisms that may compete with oysters for substrate, including anemones, tunicates and hydroids.

Dermo disease in Cook Point oysters was high during this study and had increased since a 2012 sampling. Dead oysters (boxes and gapers) made up a somewhat larger fraction of the population than oysters in other tributaries, and current levels of the disease are approaching levels expected to cause metabolic stress and potentially death. Further monitoring is advised.

This study clearly demonstrates the ecological and fishery advantages of using hard substrates as a base for oyster reef restoration. Overall, the current populations of oysters on reefs constructed and monitored by USACE at Cook Point are healthy and dense. Further monitoring is strongly recommended to ensure the success of this and future restoration efforts.

Introduction:

The Cook Point Sanctuary in the Choptank River, Maryland consists of seven oyster reefs totaling 8.5 acres, constructed and planted in 2011. The substrate used for these reefs was granite with or without a shell veneer. The original bottom substrate at Cook Point is sand, which surrounds the artificial reefs (Figure 1). The adjacent sanctuary area contains substrate and plantings from a variety of organizations within the last decade. These include oyster shell mounds built in 1998, surrounded by a flat shell site created in 2006. The 2006 flat shell and 1998 shell mound sites planted by DNR and sand areas in between alternate substrate USACE reefs were used as reference sites in this investigation. Table 1 shows each of the sampling sites included in this study, along with substrate height, total acreage, construction year, and plant year of each site.

This study evaluated the overall health of the Cook Point oyster reefs and compared the effect of different reef heights and substrate types on oyster growth, survival, and recruitment. Reef-associated epifauna were examined. Water quality was measured, and photographs were taken whenever permitted by visibility. Finally, oysters were tested for Dermo disease.

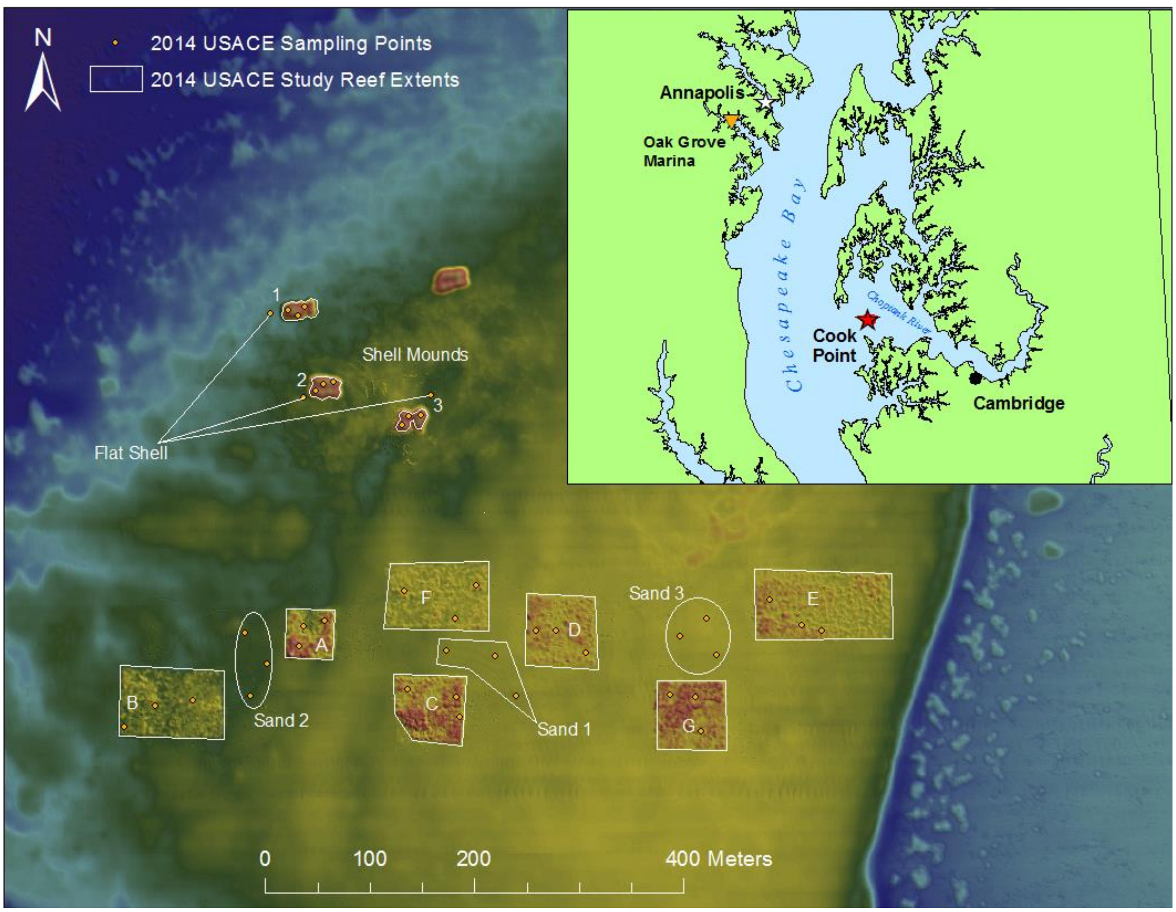

Figure 1: Location of USACE oyster reefs in the Cook Point Sanctuary. Inset shows the geographic location of the Cook Point site in the Lower Choptank River. The background bathymetry layer shows the relative depth around the sanctuary reefs in 2011. Locations of the USACE reefs are outlined in white. Points sampled by Paynter Lab divers are marked with orange dots.

Table 1: Attributes of sampling locations at Cook Point. Granite to shell ratios are approximate as given by USACE data. Reef heights are given exactly for alternate substrate reefs, while heights for control sites were not feasible to measure beyond >1 ft or ≤1 ft. Heights for the shell mound sites varied between 4 and 9 ft above the adjacent flat shell reefs; note that flat shell and shell mound sites were planted in different years than the control reefs and sand sites. Initial densities of spat-on-shell plantings are given.

Site	Acreage	Construction Year	Substrate	Reef Height (ft)	Analyzed Shell : Granite Ratio	Plant Year	# of Plantings	Initial Spat/m^2
A	0.5	2011	Granite/Shell	3	1 : 4	2011	3	1214
B	1.5	2011	Granite/Shell	1	6 : 7	2011	3	1214
C	1	2011	Granite/Shell	2	1 : 4	2011	3	976
D	1	2011	Granite/Shell	1	1 : 2	2011	3	976
E	2	2011	Granite	1	0 : 1	2011	2	698
F	1.5	2011	Granite	1	0 : 1	2011	3	976
G	1	2011	Granite	2	0 : 1	2011	2	698
Mound 1	0.15	1998	Shell Mound	>3	-	2012-2013	3	652
Mound 2	0.15	1998	Shell Mound	>3	-	2009-2010, 2012-2013	4	1426
Mound 3	0.15	1998	Shell Mound	>3	-	2009-2010	4	1223
Sand 1	*	*	Sand	≤1	-	2011	3	976
Sand 2	*	*	Sand	≤1	-	2011	3	1214
Sand 3	*	*	Sand	≤1	-	2011	2	698
Flat Shell	0.5	2006	Shell	≤1	-	2009-2010, 2012-2013	2-4	652-1223

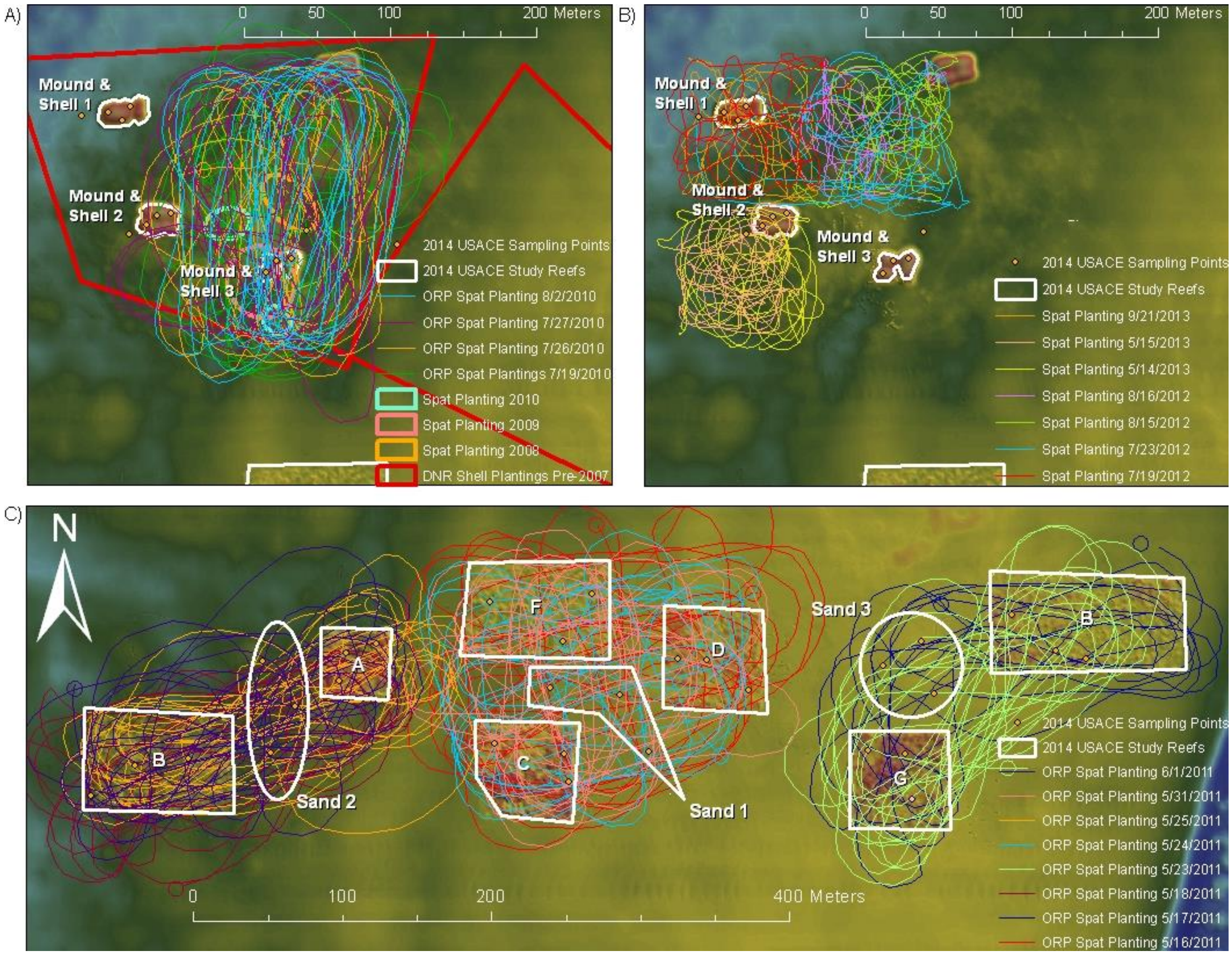

Figure 2: Spat on shell plantings on Cook Point site. As before, sampling reefs are outlined in white, while sampling points are orange dots. A) Plantings at shell and mound control sites, 2008-2010. The red outline shows the extent of the flat shell, while tracklines are from 2010 plantings. B) Plantings at shell and mound control sites in 2012 and 2013. This figure shows an identical geographical extent as (A). C) Plantings over USACE alternate substrate reefs. All reefs were planted in 2011.

Methods:

Prior to beginning fieldwork, 25m x 25m grids were created for each sampling area using ArcMap 10.2, and three centroid points were randomly selected. Sampling occurred between 17 October and 25 November 2014. In the field, each of these pre-selected points was marked as a sampling point by dive buoys with the aid of ArcPad-equipped field computers (Figure 1). Within 2m of each point, Paynter Lab divers placed four 0.25 m^2 quadrats, and sampled all oysters and select substrate on bottom within (Figure 2). These oyster and substrate samples were placed in bags lined with 1mm mesh.

Divers recorded depth, visibility, fouling degree, observed substrate composition and bottom penetration, as well as relative epifauna presence and density for each site (Appendix I, II). Underwater photographs were taken at each site. At each site, surface technicians measured water temperature, salinity, and dissolved oxygen, both at the surface and at depth, using a Yellow Spring Instruments, Inc. (YSI) 6600 V2 multimeter probe. Water samples were collected and transported to the laboratory for measurement of pH.

Each collected oyster was measured to the millimeter, and designated as live, box, or gaper. Boxes were considered to be the empty shells with intact hinges, while gapers had decaying soft oyster tissue remaining inside. Oyster height is defined as the distance from the shell hinge to the furthest edge of the shell, measured in mm. For the sake of this study, naturally-set oysters were defined as smaller than 60mm. This was intended to include oysters that were younger than the 2011 hatchery planting, in this case considered to be oysters less than two years old. In prior studies, more than 95% of oysters two years old or younger were shown to be no larger than 60 mm (Coakley 2004, Paynter unpub. data), and thus, for the sake of this analysis, all oysters ≤60 mm were counted as spat sets from 2012 and 2013.

We gathered oysters for Dermo (*Perkinsus marinus)* disease testing. Thirty oysters each from the C, D, F, Mound 2, Shell 1, and Sand 1 sites were analyzed via the Burreson (2009) modification to Ray's fluid thioglycollate culture method. This procedure produces two values: prevalence and intensity. Dermo prevalence is the percentage of oysters in a sample that contains *P. marinus*. Intensity measures how densely infected with parasites an oyster population is by averaging parasite densities across individual oysters.

Statistical analysis was conducted using JMP 11 and Microsoft Excel, with mapping done in ESRI ArcGIS 10.2. Significant differences in numerical oyster density, biomass density, and average oyster length were examined using ANOVA with Tukey's range test. All average results reported in this document are of the form Mean Number (Standard Error of Mean) unless otherwise specified. Individual dry tissue biomasses were calculated using the equation $m_B = 3 \times 10^{-5} \times l_o^{2.3512}$, where m_B is biomass in grams and l_o is oyster live length in mm (Liddell 2007). This formula was derived and confirmed from repeated studies of Chesapeake Bay *C. virginica*.

ANOVA results are shown in diamond plots, where sample means are shown as a line in each diamond's center, with the width of this line proportional to the sample size. Top and bottom points of the diamond represent 95% confidence intervals for the sample population. The grand mean of all values is represented by the grey horizontal line across the entire plot; this mean is displayed in all JMP categorical comparisons given in this report.

Results:

Overview

Figure 3 shows oyster density and oyster biomass density at all surveyed points. Broadly, sand points had low densities, shell mounds had moderate densities, and flat shell and artificial reefs had higher densities.

A)

B)

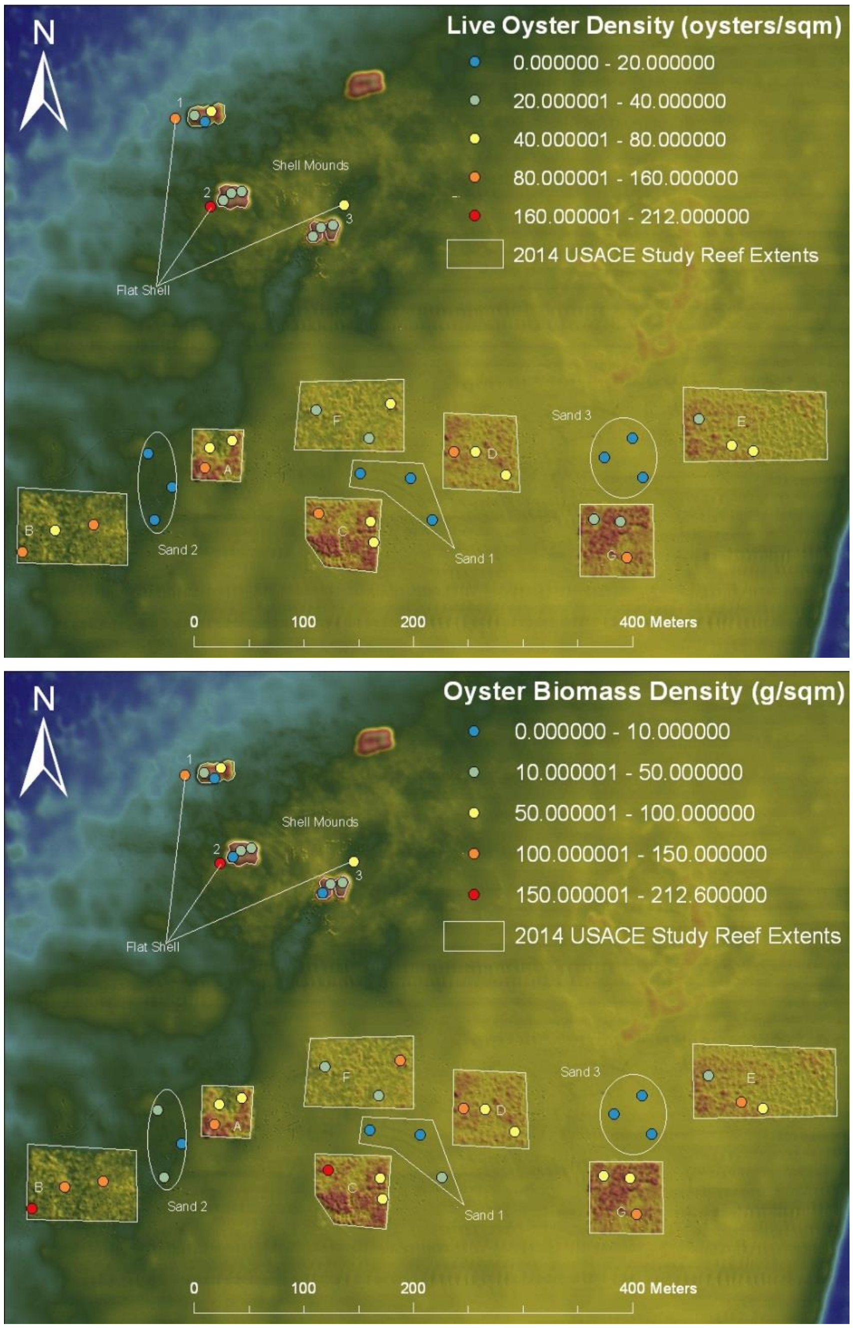

Figure 3: Oyster densities and biomass densities at all sampled points. A) Live oyster density (oysters/m^2). B) Oyster biomass density (g/m^2). Both densities were generally greater at sites with harder structure and more shell.

Site Substrate

<u>Overview</u>

We first analyzed oyster populations between different substrate types. Of sites planted in 2011, granite with shell reefs had the highest oyster densities and survival rates of any USACE reef, while unrestored sand reef sites had the lowest populations. Granite with shell reefs contained significantly more oysters than granite reefs, although the oyster mass per area of these sites was not statistically different. While flat shell and shell mound sites were planted more recently, and are thus difficult to compare to the granite-based reefs, we found that flat shell sites were very dense, but this result is expected from the more recent planting. Sand sites had uniformly low densities.

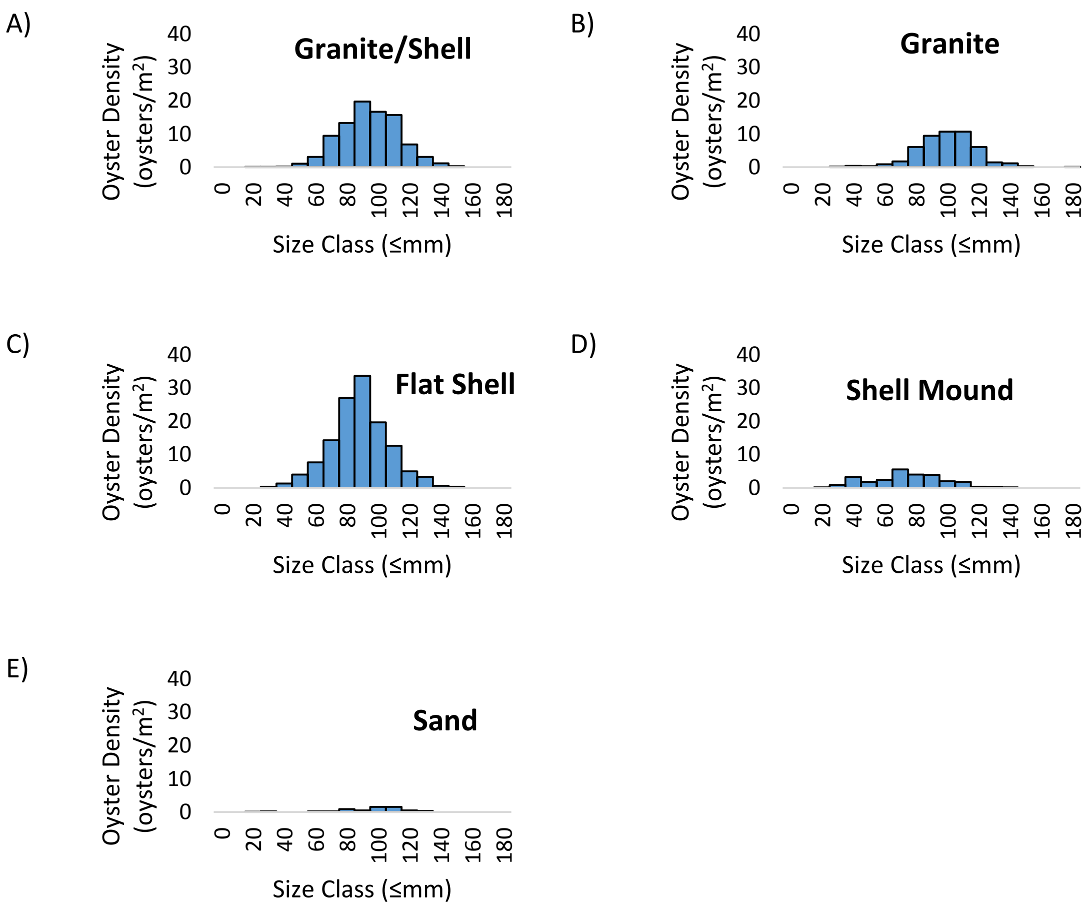

Figure 4: Live oyster lengths, with each bar representing the average number of oysters in a given size class per m^2. Flat shell reefs yielded the highest oyster density, followed by granite with shell, granite, shell mound, and sand reefs in order of decreasing density. With the exception of shell mound reef oysters, live lengths were very similar between sites. Shell mound oysters and, to a limited extent flat shell reef oysters were smaller than those of other reefs.

Table 3: Overview of oyster metrics analyzed by site substrate and reef height in Mean±SEM.

Site Substrate	Live Oyster Density (oysters/ m^2)	Biomass Density (g/m^2)	Mean Live Oyster Length (mm)	Spat Density ($spat/m^2$)	Spat Proportion (% Live)	Dead Length (mm)	% Dead	Survival (% of Initial)	Fouling Coverage (%)
Granite/Shell	91±8	114.4±12.7	90±1	5±1	6±1	82±2	10±2	8.4±1.0	31±8
Granite	49±8	71.3±10.5	95±1	2±1	3±1	92±6	9±3	6.5±1.2	87±8
Shell Mound	28±5	20.9±6.6	70±1	9±2	41±10	71±6	11±3	3.0±1.2	18±8
Flat Shell	130±38	140.1±34.1	84±1	13±9	9±4	75±7	15±5	15.7±2.1	13±13
Sand	6±2	7.9±2.8	91±3	0.6±0.4	10±9	86±7	28±9	0.6±1.2	53±8

Live Length

Figure 5 shows the same live oyster lengths as in Figure 4, compared directly to each other with diamond plot ANOVA results. Confirming the patterns seen in Figure 5, shell mound oysters were the smallest on average at 70±1 mm, while granite reefs were the largest at 95±1 mm. Oysters on shell mounds were significantly smaller than oysters on granite, granite and shell, and sand. Flat shell and shell mound sites were both planted up to two years after the other sites and did not have different mean live lengths from each other.

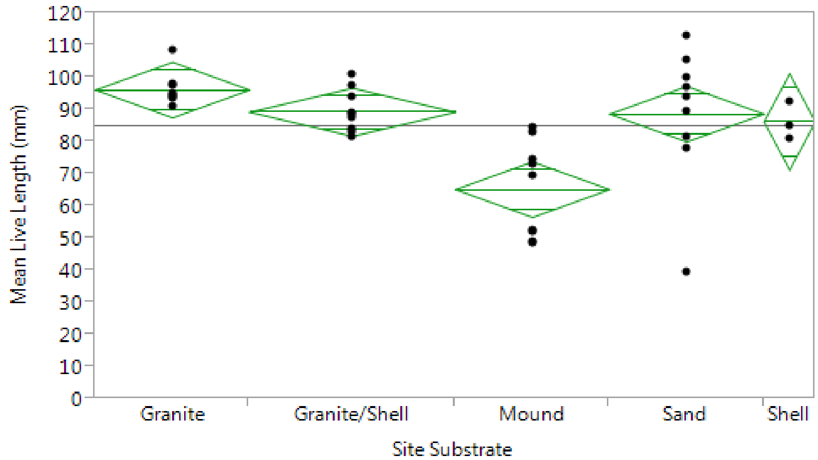

Figure 5: Comparison of mean live length of oysters across all substrate classes in USACE reef restoration study area. Only oysters at the shell mound points were significantly different in mean length from any other class, being statistically separable from oysters on granite, granite with shell, and sand points.

Table 4: Comparative p-values in Tukey's t-tests between average oyster live lengths on different reef substrates in the study area. The first two rows are the sites being compared, while the third row is the p-value. Values in bold are significant to <0.01.

Substrate 1	Sand	Sand	Sand	Sand	Shell	Shell	Shell	Mound	Mound	Granite
Substrate 2	Shell	Mound	Granite	Granite/ Shell	Mound	Granite	Granite/ Shell	Granite	Granite/ Shell	Granite/ Shell
Live Length (mm)	0.9986	**0.0035**	0.7277	1.0	0.1197	0.7742	0.9962	**<.0001**	**0.0012**	0.7379

Oyster & Biomass Density, Percent Survival:

We examined both oyster density (number of oysters per m^2) and biomass density (g dry weight per m^2) on each substrate. These values, shown in Figure 6, were generated using the known area of the sampling quadrats. Oyster biomass density was calculated using the relationship between live oyster lengths and dry biomass. Additionally, we compared the initial planting densities with the surveyed oyster densities to generate a percent survival.

Table 5: Comparative p-values in Tukey's t-tests between different reef substrates for oyster population density, oyster biomass density, and percent survival. The first two rows are the sites being compared. Values in bold are significant to <0.01, values in italics to <0.05.

Substrate 1	Sand	Sand	Sand	Sand	Shell	Shell	Shell	Mound	Mound	Granite
Substrate 2	Shell	Mound	Granite	Granite/ Shell	Mound	Granite	Granite/ Shell	Granite	Granite/ Shell	Granite/ Shell
Live Oyster Density (oysters/m^2)	**<.0001**	0.4869	*0.0184*	**<.0001**	**<.0001**	**0.001**	0.224	0.4818	**<.0001**	*0.0141*
Biomass Density (g/m^2)	**<.0001**	0.9355	**0.0047**	**<.0001**	**0.0001**	*0.046*	0.7965	*0.0356*	**<.0001**	0.0649
% Survival	**<.0001**	0.6064	*0.0095*	**0.0001**	**<.0001**	**0.0038**	*0.0246*	0.2477	*0.0119*	0.7444

Figure 6A shows that oysters were least dense in number on sand. Sand sites had 6±2 oysters/m^2, significantly fewer than on any other substrate except for shell mound (Table 5). The high density of 91±8 oysters/m^2 on granite with shell reefs was statistically similar to flat shell populations. Granite reef oysters were found at a density intermediate to those on other substrates, 49±8/m^2. Oyster numbers were highest in the shell substrate control group at 130±38 oysters/m^2.

Total oyster biomass density (Figure 6B) has a similar pattern of higher density in hard, shelly substrate reefs. Sand control sites contained significantly less biomass than all but those on shell mound, 7.9±2.8 g/m^2 on average. Flat shell and granite with shell reefs produced similar oyster biomass density, with 140.1±34.1 g/m^2 on flat shell and 114.4±12.7 g/m^2 on granite with shell. Granite reefs had similar biomass density to granite with shell, but less dense than flat shell reef (71.3±10.5 g/m^2).

When we examine the same sites' percent survival since initial planting (Figure 6C), oysters on granite with shell reefs still have higher survival (8.4±1.0) than on granite alone (6.5±1.2), but they are not significantly different. As in other comparison types, sand sites have the lowest survival (0.6±1.2).

The general patterns mentioned above do not hold for the shell mound reef populations. Mound site live oyster density of 28±5 /m^2 and oyster biomass density of 20.9±6.6 g/m^2 are only statistically similar to sand and, for the former, granite sites.

The current density and biomass standards for successful restoration are a minimum threshold of 15 oysters/m^2, and 15 g/m^2, with a target goal of 50 oysters/m^2 and 50 g/m^2.The constructed reefs at Cook Point meet or surpass these values. In previous studies, we have observed survival frequently below 5% in two-year-old oysters, so the values measured in this study are very encouraging. **Both live oyster density and biomass density are higher on reefs with granite and shell reefs than on granite alone. Percent survival is not statistically different between the sites. Constructed sites had far more oysters than sand sites by any metric.**

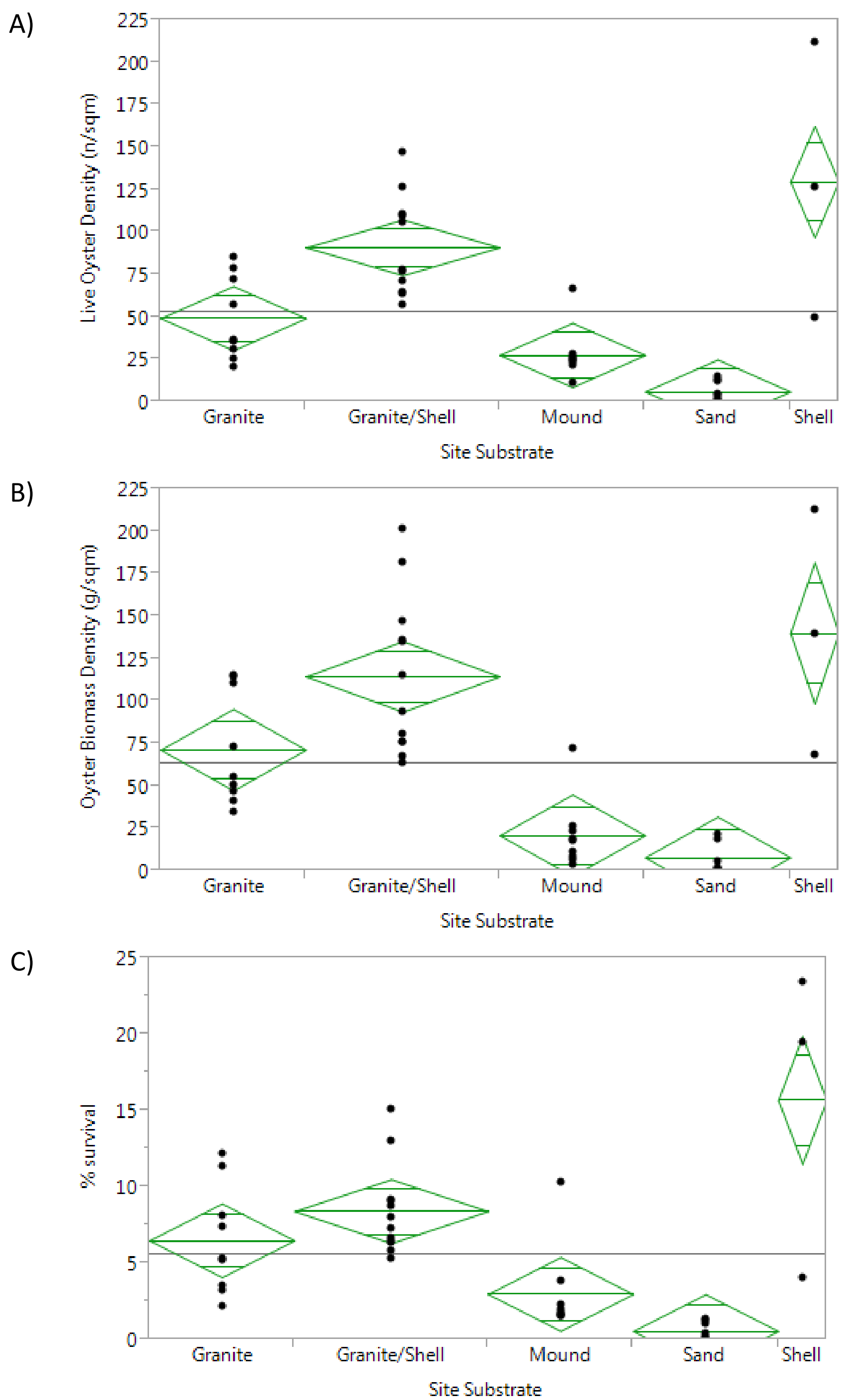

Figure 6: A) Mean oyster density per m^2, B) Mean oyster biomass density in g/m^2. C) Percent survival of oysters. The number and biomass of oysters on sand and shell mound sites were significantly lower than those of any other site, except for density on granite reefs. Oyster density and biomass density on shell and granite with shell reefs were highest and not statistically different from each other, while granite reefs were lower than both shell-containing reefs in number density and shell reefs in biomass density. An order of magnitude lower survival rate is seen on sand compared to the other substrates. A marginal superiority of oyster survival rates on granite with shell based reefs is seen compared to granite-only reefs.

Dead Length & Prevalence:

We surveyed dead oysters to investigate recent mortality on each substrate. Only two gapers were found at any location throughout the study, and thus they were combined in analysis with boxes to form a total dead sample. The presence of small dead oysters would indicate that mortality was occurring before young oysters reached their adult size. A high proportion of dead oysters in a population, expressed as a percent of the total sample, would indicate a higher mortality rate.

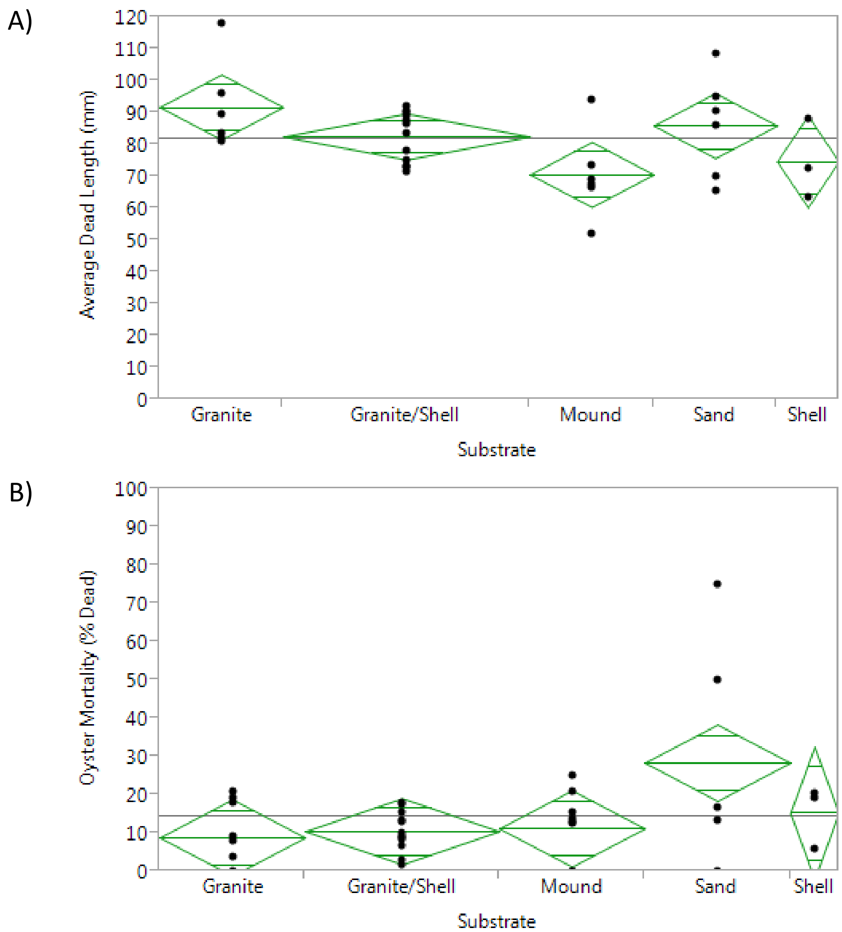

Figure 7: A) Mean length of dead (box and gaper) oysters and B) Percent of total oysters that were dead. Only dead oyster samples located on granite and shell mound reefs had significant differences in average length. Although average dead percentage on sand reefs was elevated, no statistically significant difference was found between different substrate reefs. Dead oysters were generally smaller on average than live oysters on every substrate, although not significantly so (Figure 5).

Table 6: Comparative p-values in Tukey's t-tests between average dead oyster length and percentage of dead oysters out of all sampled on different reef substrates in the study area. The first two rows are the sites being compared. Values in italics are significant to <0.05.

Substrate 1	Sand	Sand	Sand	Sand	Shell	Shell	Shell	Mound	Mound	Granite
Substrate 2	Shell	Mound	Granite	Granite/ Shell	Mound	Granite	Granite/ Shell	Granite	Granite/ Shell	Granite/ Shell
Dead Length (mm)	0.6888	0.2168	0.9209	0.9791	0.9893	0.3011	0.8555	*0.0408*	0.315	0.5606
Oyster Mortality (% Dead)	0.6728	0.1201	0.0573	0.0652	0.9937	0.965	0.9866	0.997	1	0.9991

Figure 7A shows the average length of dead oysters found on different substrate reefs. Mean dead oyster lengths were comparable between most sites. The only statistically significant difference was between shell mound and granite reefs (p = 0.0408). This is likely due the earlier planting date of the mound site, which would cause most dead oysters there to be younger and smaller than those on granite reefs. Lengths of dead oysters ranked the same by substrate as live oyster lengths, though dead oysters were generally smaller than live oysters. Dead oysters on granite reefs were largest (92±6 mm), followed by sand (86±7 mm), granite with shell (82±2 mm), flat shell (75±7 mm), and shell mound reef oysters (71±4 mm). Again, flat shell and shell mound were planted more recently than the other sites.

Figure 7B displays the percentage of all collected oysters that were dead per substrate. While the oyster mortality rate on sand of 28±9% was higher than at any other site, there was no statistical difference between any of the groups. Mortality rates at the other sites, ranging from 9±3% on granite to 15±5% on flat shell, are all near or better than the 15% expected from prior studies for a healthy oyster bar planted at least two years prior (Paynter et al. 2014).

Post-Planting Spat Density and Proportion

We investigated the presence of spat set from after 2011 at each reef to determine whether populations on specific substrates included younger oysters, especially naturally-set oysters. Based on prior studies and the plant date of the majority of sites, spat from these years were defined as oysters ≤60 mm long.

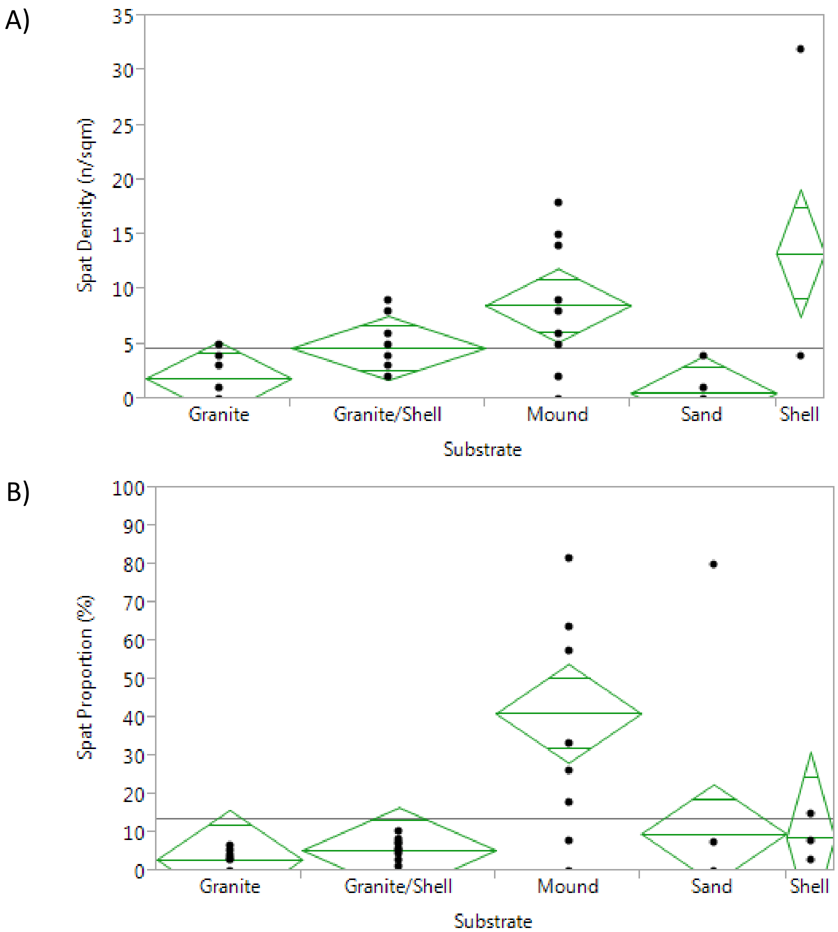

Figure 8: A) Number of spat per m^2 on different substrates in the USACE reef restoration study area. Two identical densities of 4 spat set/m^2 were seen on flat shell substrate, totaling three points. The third point is higher than any other in the study, and a significant difference. The percent of live oysters classified as post-2011 spat was significantly higher on shell mounds and flat shell than on any other substrate—these sites were planted in 2012 and 2013. No other significant differences in spat density or proportion were found.

Table 7: Comparative p-values in Tukey's t-tests between densities of spat set-sized oysters and percentage of spat out of all live oysters on different reef substrates in the study area. The first two rows are the sites being compared. Values in bold are significant to <0.01, values in italics to <0.05.

Substrate 1	Sand	Sand	Sand	Sand	Shell	Shell	Shell	Mound	Mound	Granite
Substrate 2	Shell	Mound	Granite	Granite/ Shell	Mound	Granite	Granite/ Shell	Granite	Granite/ Shell	Granite/ Shell
Spat (≤60 mm) Density (oysters/m^2)	**0.0039**	*0.0131*	0.979	0.3503	0.6076	*0.0118*	0.0741	0.0535	0.4058	0.7142
Spat Proportion (% Live)	1	*0.0106*	0.9446	0.9859	0.1028	0.9914	0.9988	**0.0013**	**0.0013**	0.9985

Figure 8A examines the number spat per unit area on the study substrates. Overall, harder substrates with more shell had the most spat set, with the descending order of density being flat shell, shell mound, granite with shell, granite, and sand reefs. Significant differences were discovered between spat numbers on shell mounds (9±2/m^2) and on sand (0.6±0.4/m^2), as well as between flat shell (13±9/m^2) and both sand and granite (2±1/m^2). All other comparisons were statistically similar, with spat set on granite with shell reefs occurring at an intermediate frequency of 5±1 per m^2.

The percentage of live oysters classified as spat set on different substrates is displayed in Figure 8B. These results indicate that shell mounds held a significantly greater proportion of spat-sized oysters than any other substrate reefs. Flat shell (9±3%), granite with shell (5±1%), granite (3±1%), and sand reefs (10±9%) all were composed of 10% or less spat set, and were not significantly different by these values. The shell mound reef spat set proportion was much higher at 41±10%. Only on flat shell reefs, where points were sampled near to those of the shell mounds and in a limited number that elevated the margins of error, was the spat set percentage not statistically different from the shell mound.

This result in particular draws into question the use of the shell mound and flat shell sites as experimental controls. These sites were planted at the same time (2012-2013) after the 2011 USACE reef plantings, and thus a large number of oysters would be expected to fit into a category of "spat". Furthermore, the small populations on shell mounds are unexpected, and do not seem to be explained by natural mortality, as these sites do not have elevated rates of boxes. Please see the Discussion and Conclusions section for more thoughts on these sites.

Epifauna

Divers identified benthic macrofauna on the Cook Point site, and estimated their prevalence. Organisms were qualified as none, few, some, or many at each point. At least 17 total benthic groups were identified, with up to nine found at any one point. Appendix 1 displays the sightings of the most common benthic organisms found, along with the total number of benthic groups found. The three most common readily verified organisms were barnacles, mussels, and gobies, found at 90%, 87.5%, and 85% of all sampling points, respectively. Mud crabs (72.5%), tunicates (67.5%), and hydroids (65%) comprised the next most frequent organismal groups, while bryozoans (50%) and anemones (47.5%) were located at about half of all points. A series of groups identified less frequently due to their greater mobility, rarity, or difficulty of confirmation is detailed in Appendix 3.

Figure 9 shows species richness, sorted both by substrate (panel A) and by reef height (panel B). Richness was significantly lower on sand substrate than on shell mound and granite reefs ($p = 0.0083$ and $p = 0.0391$). No differences were found in other comparisons.

A t-test of the number of benthic groups of organisms present showed no significant difference in how many groups were seen at different reef heights, while grouping by substrate found differences between sand and both shell mound ($p = 0.0077$) and granite ($p = 0.0382$) biotic groups. Looking at community composition, less than half of sand substrate points contained mussels (3 of 7 points), hydroids (3 of 7 points), and bryozoans (2 of 7 points). No anemones were found at any of the sampled granite with shell points, while the tunicates and hydroids were found at their smallest percentage of points at these points (both 33.3%). In contrast, divers located bryozoans at a higher percentage, 75%, within the granite and shell reefs than at any other site.

While these results are not comparable to previous studies using quantitative macrofauna trays, higher species richness and density on restored reefs agree with findings of Rodney & Paynter (2006).

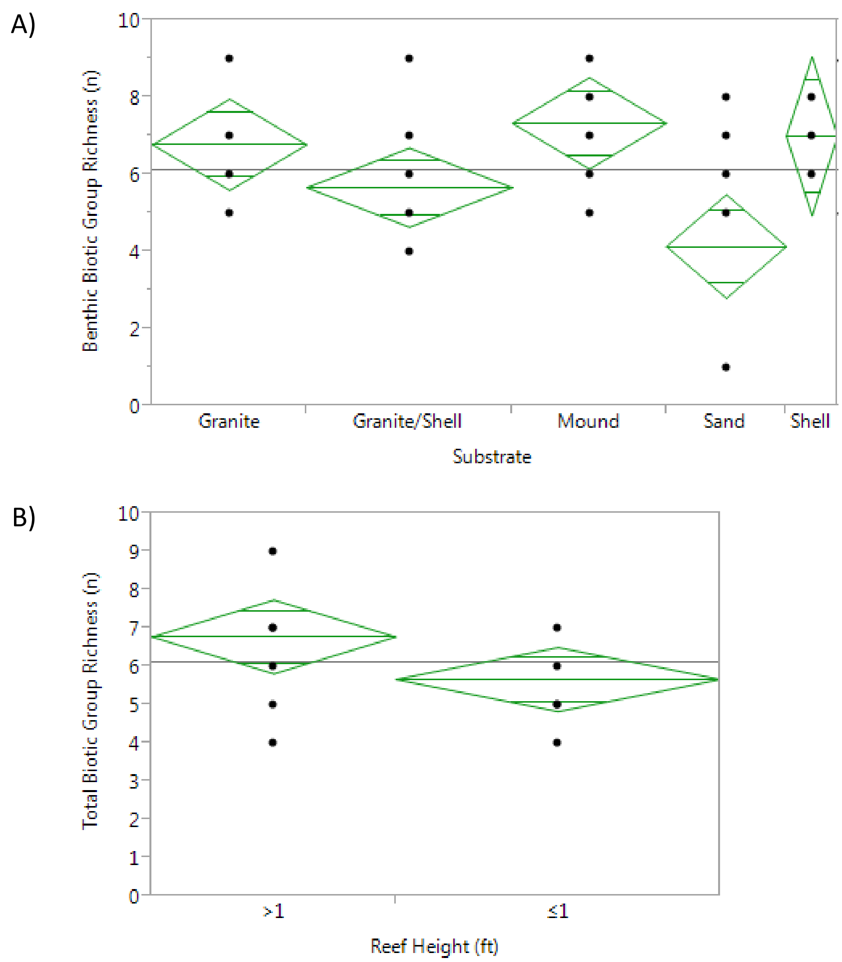

Figure 9: Overall richness by presence of different benthic biotic taxonomic groups across different A) substrate classes and B) reef height within the USACE alternate substrate experiment. Ecological richness was significantly lower on sand substrate reefs than on shell mound and granite reefs (p = 0.0083 and p = 0.0391). No significant differences were found between other substrate reefs or reefs of different height.

Sedimentation

Brief analysis of sedimentation on Cook Point survey sites highlights the high spatial and time variability of sediment coverage on oyster bars throughout the Chesapeake Bay. Beyond the near complete coverage of sand sites by the sediment that defines an unrestored reef, patterns of sedimentation were irregular across substrate sites. 24% of non-sand points surveyed showed some degree of sedimentation, with every substrate class represented. The flat shell (2 of 3 points) and shell mound (3 of 9) control sites both presented with higher relative frequency and density of sedimentation than USACE sites (1 of 9 on granite, 2 of 12 on granite with shell). Whether this result is a function of site substrate or site construction age is not clear at this time. Any sedimentation result should be viewed with caution due to the extreme hour-to-hour variability of sediment flow throughout Chesapeake Bay sites such Cook Point. Any effect, from weather to natural cycling flows, may potentially shift sediment across the estuarine floor, and a larger study would be required to determine sedimentation patterns in greater detail.

Epibiont Coverage

We examined the extent of reef coverage by non-oyster sessile organisms as an estimated percent of the total area. These values were reported by divers, and were composed of organisms that may compete with oysters for resources, specifically mussels, barnacles, anemones, hydroids, tunicates, and bryozoans. Coverage on two points was not recorded, although photographic records indicate that these points had coverage similar to other granite with shell points.

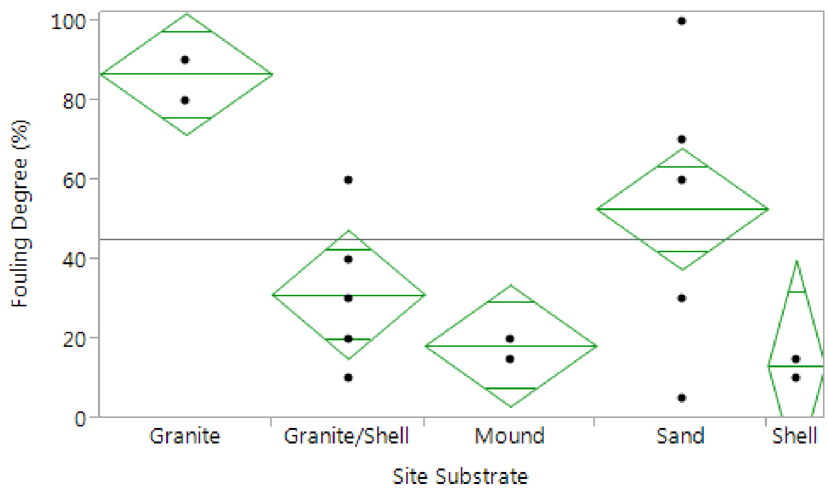

Figure 10: Percent extent of oyster reef coverage by other sessile organisms on reefs specified in the USACE Cook Point study. Coverage was significantly higher on granite substrate than on any other substrate, with anemones, hydroids, and tunicates the primary contributors to this result. Reefs with shell in their base all had less coverage. Results on sand were highly variable, particularly where tunicates had completely colonized isolated shells.

Table 9: Comparative p-values in Tukey's t-tests between extents of fouling organism coverage on different reef substrates in the study area. The first two rows are the sites being compared. Values in bold are significant to <0.01, values in italics to <0.05.

Substrate 1	Sand	Sand	Sand	Sand	Shell	Shell	Shell	Mound	Mound	Granite
Substrate 2	Shell	Mound	Granite	Granite/ Shell	Mound	Granite	Granite/ Shell	Granite	Granite/ Shell	Granite/ Shell
Fouling Degree	0.0886	*0.0213*	*0.0242*	0.3045	0.9972	**0.0002**	0.7656	**<.0001**	0.7628	**0.0001**

Figure 10 shows the sessile organism coverage of reef substrate for each type of substrate. Granite reefs contained a significantly higher coverage than any other substrate at 87±8%. Sand sites were difficult to quantify, as neither oysters nor sessile fauna occurred on the sand itself. While granite with shell reefs had an intermediate average fouling percentage of 31±8%, this was not significantly different from the 18±8% or 13±13% seen on mound and shell reefs, respectively. **Overall, granite reefs had the most sessile organism coverage, followed by sand, granite with shell, shell mounds, and flat shell.**

Shell : Granite Ratio

When originally constructed, the alternate substrate reefs were made by USACE with different fractions of shell and granite as their base. We examined the oyster population metrics on USACE reefs A-G by shell to granite ratio. All metrics investigated for the site substrate portion of this study were calculated again here, chiefly live oyster and biomass density, average live and dead oyster lengths, spat set density, and percentage of dead oysters.

Table 10: Quantities of alternate substrate used in the construction of USACE experimental reef in cubic yards and approximate ratios of shell to granite at each reef.

Site	Granite Volume (y^3)	Shell Volume (y^3)	Shell : Granite Ratio	# points sampled
A	2411	621	1 Shell : 4 Granite	3
B	2446	2102	6 Shell : 7 Granite	3
C	3241	804	1 Shell : 4 Granite	3
D	1616	824	1 Shell : 2 Granite	3
E	3226	-	0 Shell : 1 Granite	3
F	2416	-	0 Shell : 1 Granite	3
G	3226	-	0 Shell : 1 Granite	3

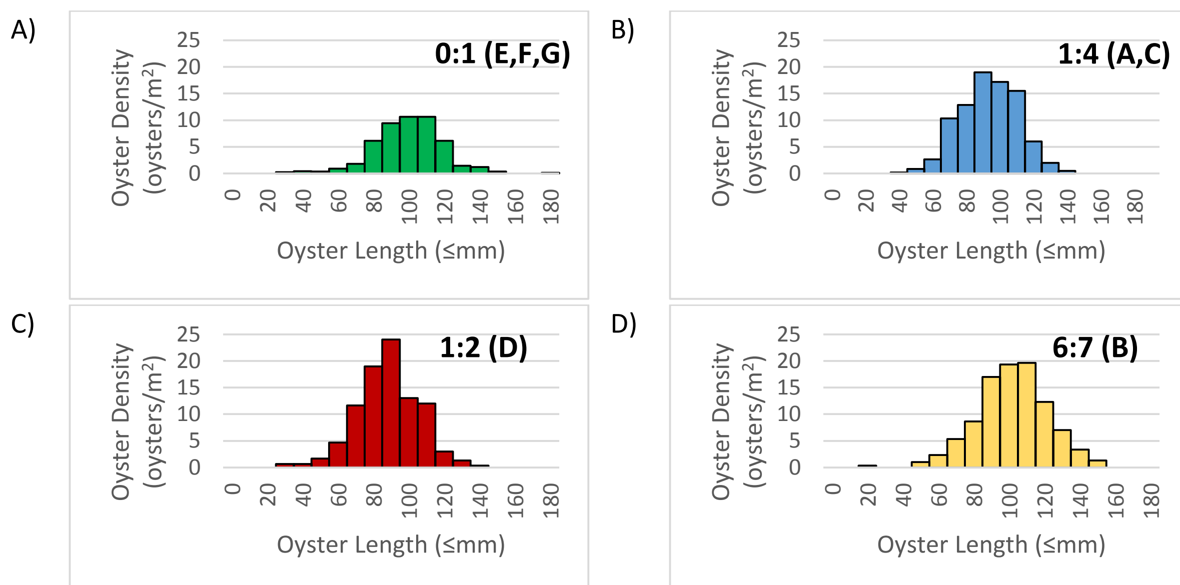

Figure 11: Live oyster lengths, with each bar representing the average number of oysters in a given size class per m^2 on A) reefs E, F and G, made only of granite, B) reefs A and C with ratio 1:4, C) reef D with ratio 1:2, and D) reef B with ratio 6:7.

Figure 11 shows histograms of oyster length density, for each ratio of shell to granite. Reefs with intermediate proportions of shell base (A, C, and D) contained significantly smaller oysters than the granite reefs or the higher shell ratio reef B. This coincided with an elevated density and proportion of spat set oysters on reefs A, C, and D, seen in Figure 8. Since the planting dates for these sites are separated by less than three weeks, oyster age does not explain this result. No other significant relationships were found.

Table 11: Oyster metrics analyzed by shell to granite ratio on reefs A-G. All values are rounded to the nearest integer except in sparse populations.

Shell : Granite Ratio	Reef Names	Live Oysters /m^2)	Biomass Density (g/m^2)	Live Oyster Length (mm)	Spat /m^2	Spat Proportion (% Live)	Dead Height (mm)	Oyster Mortality (% Dead)	Survival (%)	Coverage (%)
0	E, F, G	50±9	71.3±13.4	96±1	2±1	3±1	92±4	9±2	6±1	87±4
1:4	A, C	87±11	106.1±16.4	88±2	4±1	5±1	83±4	10±3	8±1	28±6
1:2	D	92±16	97.5±23.2	84±2	8±1	9±1	78±6	9±4	9±2	37±7
6:7	B	98±16	148.1±23.2	97±2	4±1	4±1	85±6	13±4	8±2	N/A

Table 12: Comparative p-values in Tukey's t-tests between oyster metrics of different shell-to-granite ratio. Lower epibiont coverage on the highest reefs produced the only significantly different results, and these are confounded by the absence of 3 foot high granite reefs in analysis. Significance to p<0.01 is indicated in bold, and to p<0.05 indicated in italics.

Compared Granite to Shell Ratios	0 vs 1:4	0 vs 1:2	0 vs 6:7	1:4 vs 1:2	1:4 Vs 6:7	1:2 vs 6:7
Live Oyster Density (oysters/m^2)	0.0879	0.1422	0.0823	0.9942	0.9490	0.9945
Biomass Density (g/m^2)	0.3827	0.7647	*0.0480*	0.9899	0.4731	0.4365
Mean Live Length (mm)	*0.0105*	**0.0019**	0.9733	0.4706	*0.0305*	**0.0051**
Spat (≤60 mm) Density (oysters/m^2)	0.4186	**0.0011**	0.4417	*0.0222*	0.9928	0.0828
Spat Proportion (% Live)	0.6498	*0.0121*	0.9752	0.1021	0.9614	0.0866
Dead Length (mm)	0.5372	0.2936	0.8231	0.8840	0.9940	0.8292
Oyster Mortality (% Dead)	0.9981	1	0.8317	0.9998	0.9068	0.9123
Percent Survival (%)	0.8125	0.5719	0.8995	0.9393	1	0.9573
Epibiont Coverage (%)	**<0.0001**	**<0.0001**	N/A	0.6139	N/A	N/A

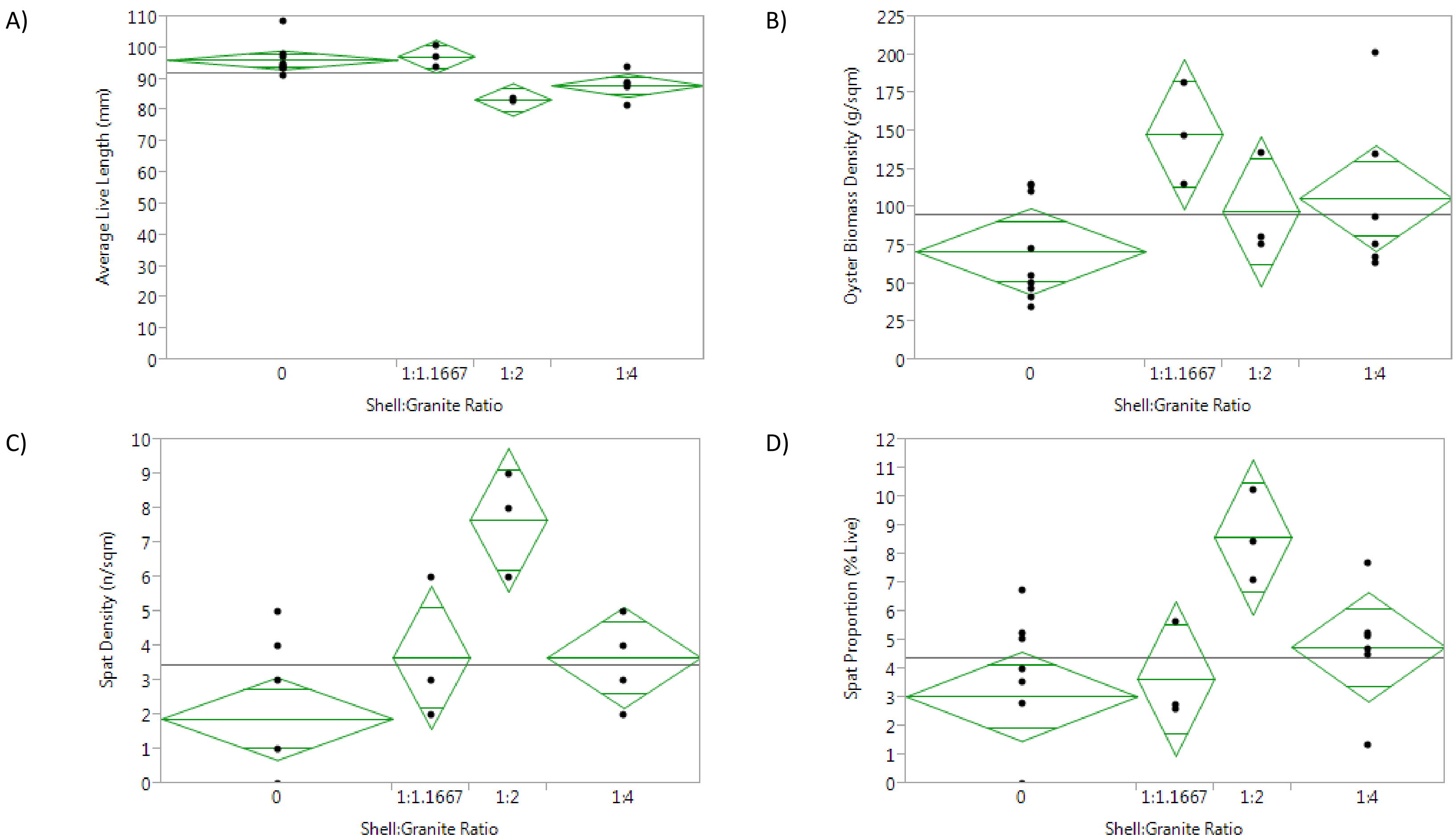

Figure 12: A) mean oyster live length, B) mean individual oyster biomass, C) spat present per m^2, and D) percentage of spat on reefs with different ratios of granite to shell. In these graphs, the data comparison groups listed with a 1:1.1667 shell:granite ratio are the 6:7 reef B, while those with a ratio of 0 are the granite reefs E-G. The 1:2 and 1:4 ratio reefs A, C, and D showed significantly lower average oyster hieght than 6:7 (reef B) and granite only reefs E, F, and G. 1:2 reef D also had a higher total number of spat than any other shell ratio group and a higher proportion of spat than the granite reefs E-G.

E)

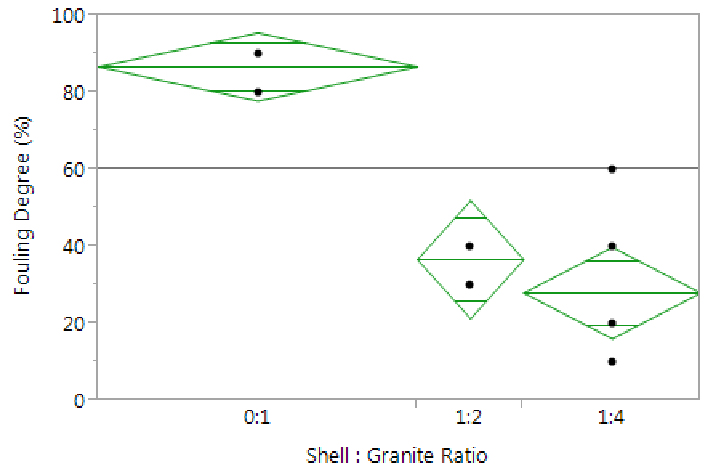

Figure 12 Continued: E) coverage by epibionts by shell-to-granite ratio of substrate on USACE constructed test reefs. Granite reefs were signficantly more covered than either granite with shell reef class measured. Epibiont coverage was not recorded on 6:7 shell:granite ratio reef B. While subjective variations in coverage reporting from different divers are not necessarily excluded from this data set, no significant result presented that could be explained by this cause. This conclusion of error elimination is further supported by photographic verification (Figure 14)

Figure 12 displays results that did contain significant differences between reefs with varied shell-to-granite composition, while Table 11 displays all population metrics by shell-to-granite ratio. Oyster biomass was more than twice as high on 6:7 ratio reef B than on the granite reefs E-G, with 148.1±23.2 g/m^2 compared to 71.3±13.4 g/m^2 on average ($p=0.0480$). The average live oyster hights for the 1:2 mix reef D (84±2 mm) and 1:4 mix reefs A and C (88±2 mm) are significant lower than those of the granite reef E-G (96±1) mm) and 6:7 mix reef B (97±2 mm). Spat-sized oysters were located in higher density (8±1/m^2) and proportion (9±1%) on the 1:2 reef D than on other sites. This density result was significantly higher than that for all other reefs, while the proportion was significantly greater than for the granite only reefs E-G (3±1%).

Overall, granite-only reefs had lower oyster, biomass, and spat set oyster densities and higher epibiont coverage than other reefs. Reef B, which had the most shell in its base substrate, had the highest oyster and biomass densities of any USACE site and an intermediate density and proportion of spat, indicating superiority of high shell-to-granite proportions in oyster reef substrates.

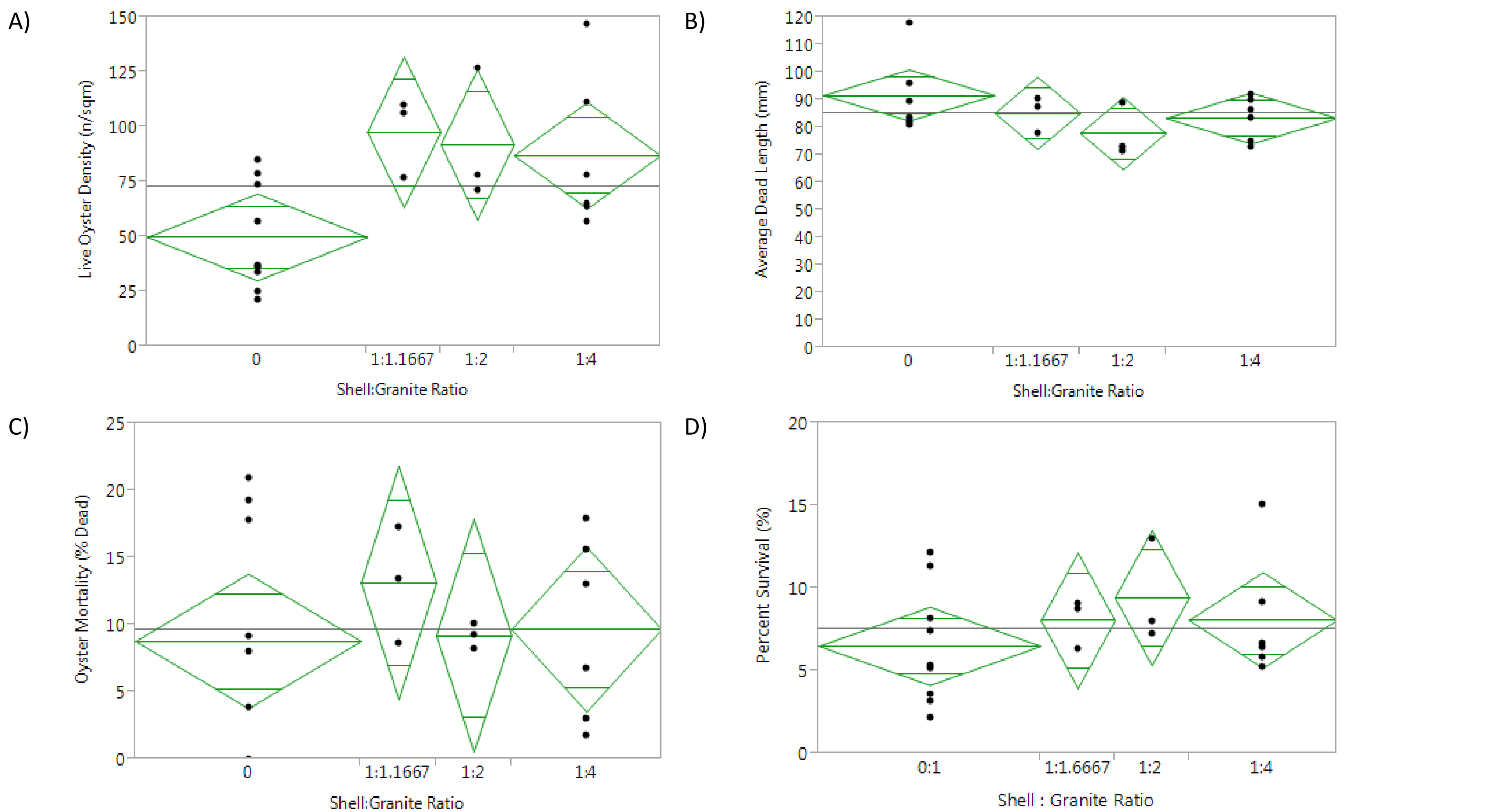

Figure 13: Oyster population comparisons between alternate substrate reefs with different shell:granite ratios. A) Live oysters/m^2. B) Average length of dead oysters in mm. C) Percent mortality of oysters as given by the number of boxes and gapers divided by the total number of live and dead oysters found. D) Percentage of oysters surviving from the initial 2011 plantings. No significant differences between groups were seen in these comparisons.

Photos

Below are characteristic photographs of each substrate type.

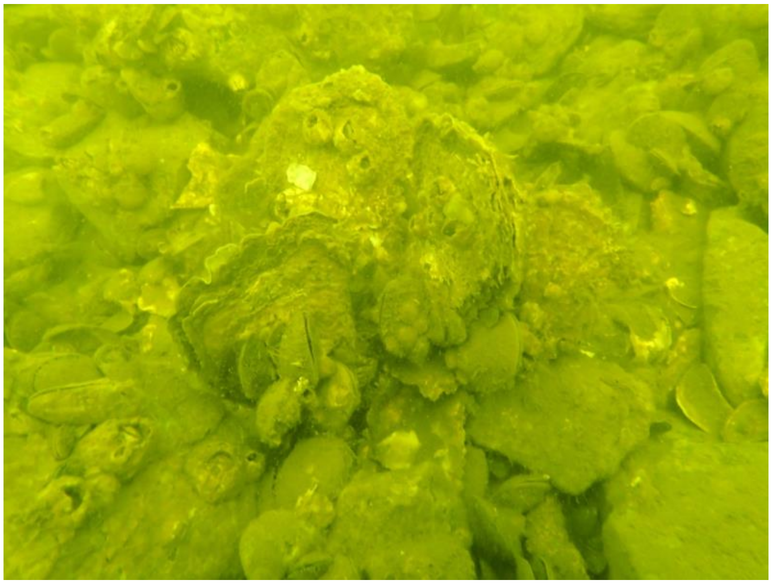

Figure 14A: Granite-only reef G. The characteristic mussels, tunicates, and anemones are visible, as are large pieces of granite.

B) Granite with shell reef B. While other sessile organisms were present, they occurred less frequently.

C) Sand site.

D) Shell Mound 2.

E) Flat shell 3.

Reef Height

We examined the impact of reef height on oyster populations on experimental reefs. These ranged from 1 to 3 feet above the original depth contour. With the reefs grouped by height, we investigated the same factors as previously: count and biomass density of oysters, live and dead oyster lengths, dead oyster density and proportion, and density and proportion of spat-sized oysters. Table 10 shows the mean and SEM values for numerical and biomass density, average live and dead (box and gaper) length, spat set numbers and percentages at 1-3 foot reef height, percent survival from the initial planted population, and epibiont coverage.

We initially grouped the reefs into two groups: one foot tall reefs, and taller reefs. As seen in Figure 15, **both reef height categories had much higher survival and oyster populations than sand, but were almost identical to each other.**

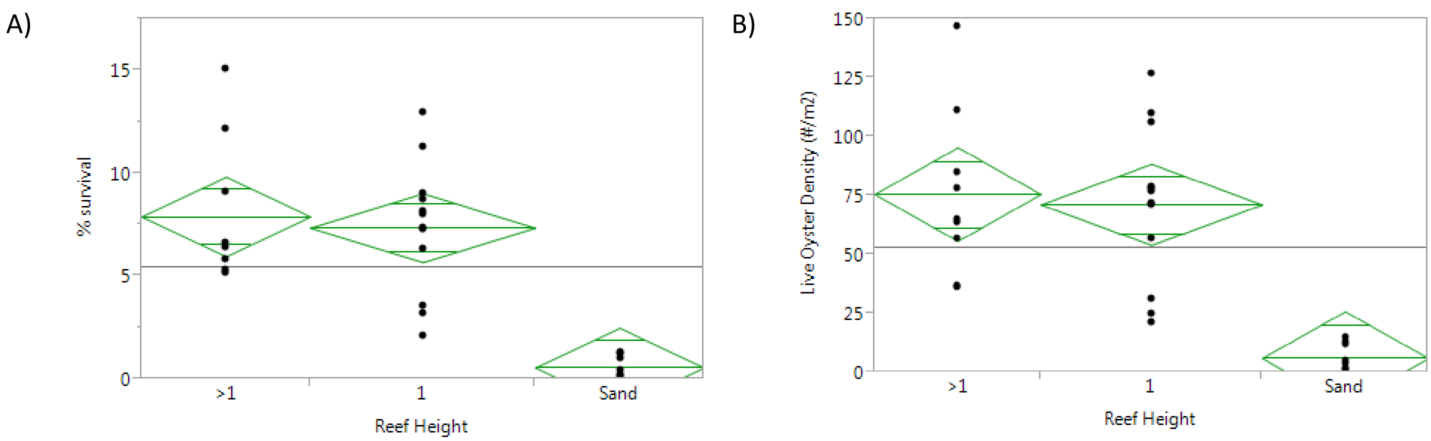

Figure 15. Oyster survival and density on >1 ft experimental reefs, 1 ft reefs, and sand. Experimental reefs performed much better than sand sites, and almost identically to each other. Reefs taller than 1 ft were A, C and G, while 1 ft tall reefs were C, D, E and F.

Given the range of values found within each group, we also tried splitting the reefs into one foot, two foot and three foot tall categories, which are addressed in the rest of this section.

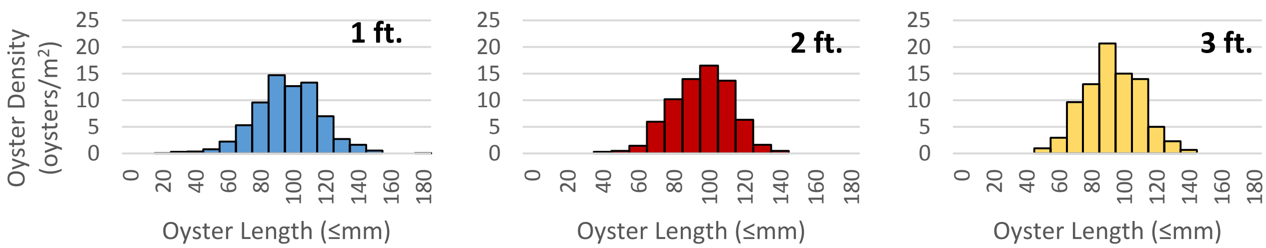

Figure 16: Histograms of live length values for oyster populations at USACE-constructed reef sites located at A) 1 foot, B) 2 foot, and C) 3 foot height above initial bottom depth. Histograms are scaled to total oyster density. No discernible differences were found between oyster density and length metrics between reef heights.

Table 10: Oyster metrics analyzed by reef height in Mean±SEM. All oyster numbers, percentages, and lengths are rounded to the nearest integer to reflect reading accuracy except in sparse populations.

Reef Height (ft)	Reef Names	Live Oysters /m^2	Biomass Density (g/m^2)	Live Oyster Length (mm)	Spat /m^2	Spat Proportion (% Live)	Dead Length (mm)	Oyster Mortality (% Dead)	Survival (% Initial)	Epibiont Coverage (%)
1	B, D, E, F	72±10	96.5±13.9	94±2	4±1	5±1	87±3	12±2	7±1	69±9
2	C, G	71±14	92.1±19.6	91±3	2±1	3±1	82±7	5±3	8±1	70±12
3	A	84±20	101.3±27.8	88±4	4±1	5±2	85±7	9±4	7±2	20±15

Table 11: Comparative p-values in Tukey's t-tests of population metrics between different reef heights. Significance to p<0.05 is indicated in italics.

Reef Heights being Compared	1 ft vs 2 ft	1 ft vs 3 ft	2 ft vs 3 ft
Live Oyster Density (oysters/m^2)	0.9997	0.8399	0.8562
Biomass Density (g/m^2)	0.9817	0.9871	0.9608
Live Height (mm)	0.8071	0.4647	0.7906
Spat /m^2	0.4366	0.9986	0.6255
Spat Set Proportion (% Live)	0.6056	0.9996	0.7888
Dead Height (mm)	0.8167	0.9562	0.9644
Oyster Mortality (% Dead)	0.085	0.7474	0.6219
Survival (%)	0.8231	0.9816	0.8270
Epibiont Coverage (%)	0.9967	*0.0325*	*0.0464*

As seen in the population density histograms in Figure 16, no differences were noted for oyster population metrics between the USACE reefs of different heights. While a significant difference was seen in epibiont coverage between three foot and shorter reefs, this is likely due to three-foot reefs all having a shell veneer. Across the study locations and the heights surveyed (1-3 feet), **reef height does not appear to have a significant impact on oyster population dynamics.**

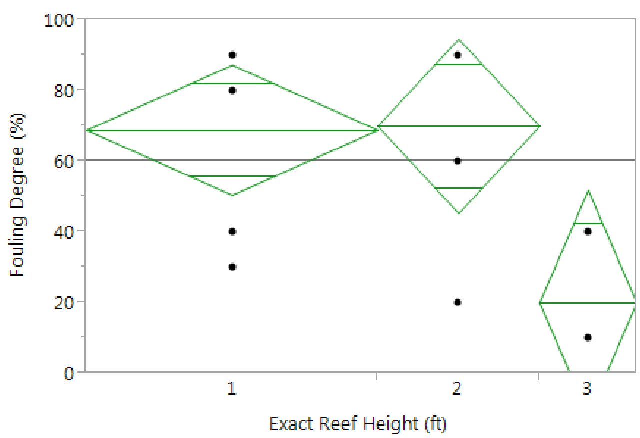

Figure 17. Percent coverage by epibionts on reefs of different heights. This coverage was significantly lower on 3 ft high reefs, although those reefs all are granite with shell substrate.

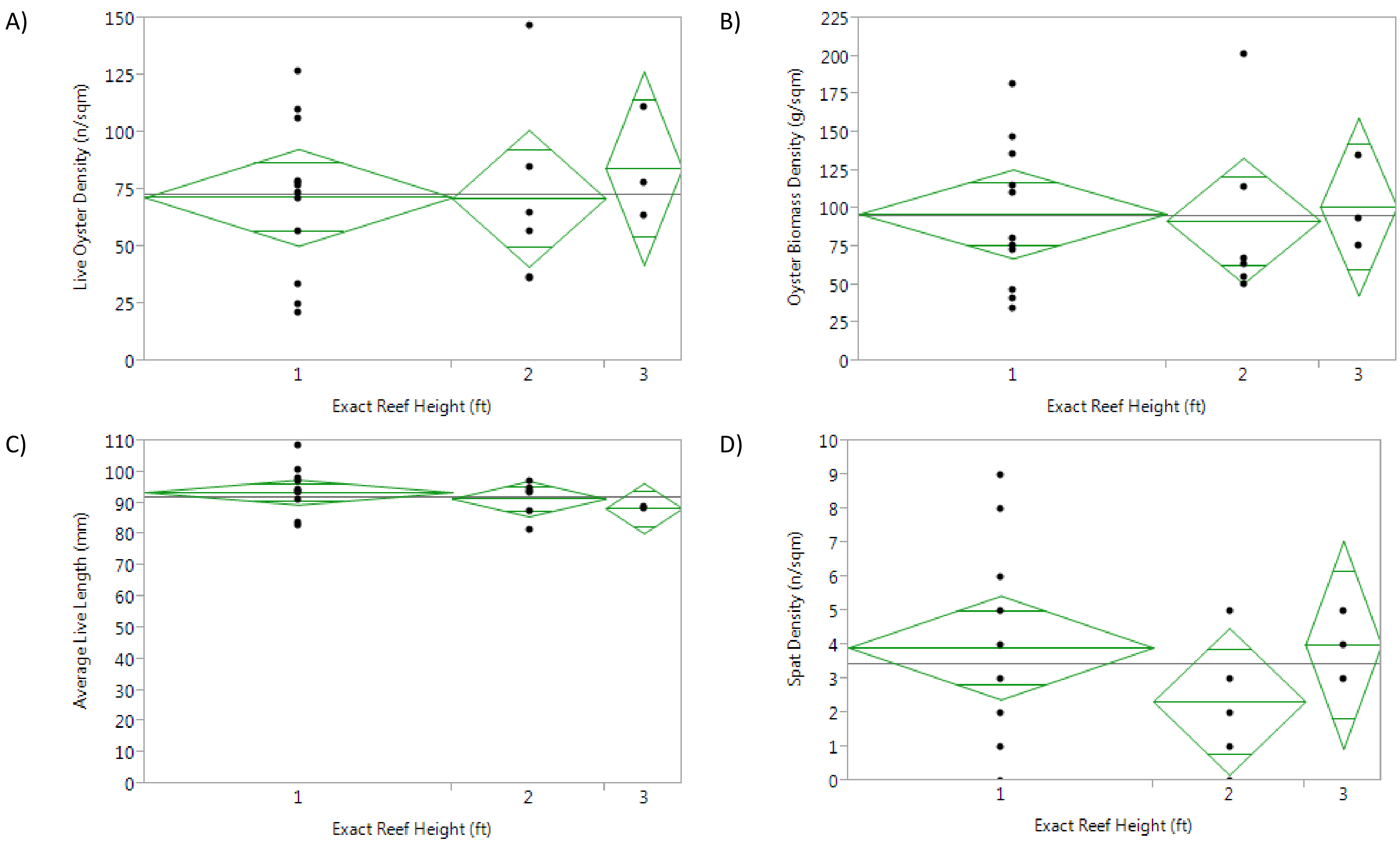

Figure 18: Oyster population metrics on reefs of different height. A) Live oyster density. B) Oyster biomass density. C) Average length of live oysters. D) Density of spat-sized oysters. No statistical differences were found in any of these metrics.

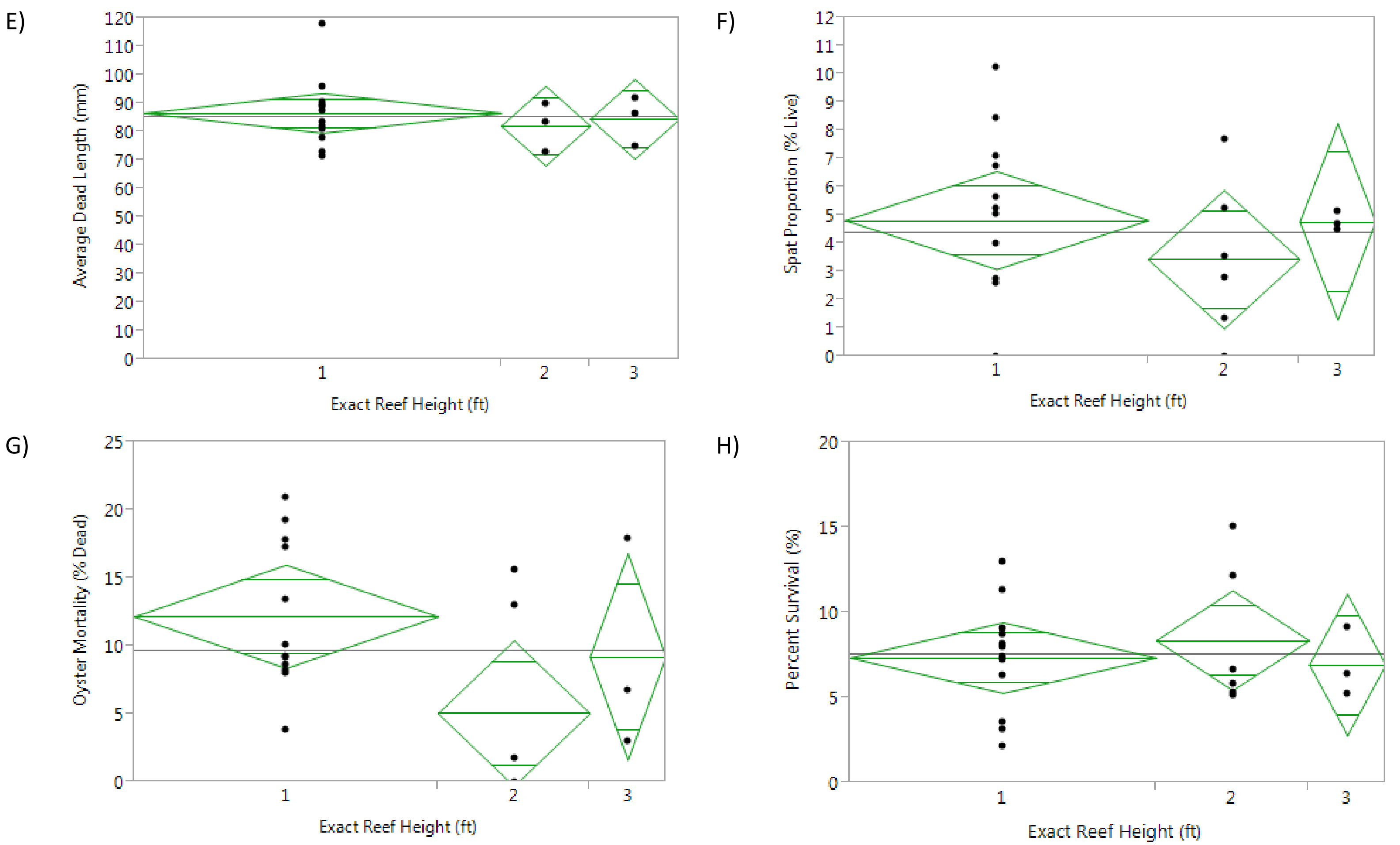

Figure 18 continued: Oyster population metrics on reefs of different heights. E) Average length of dead oysters. F) Percentage of spat in the total live sample. G) Percentage of dead oysters out of all oysters sampled. H) Percentage of oysters surviving from 2011 planting to 2014 survey. No significant differences were found in any of these metrics.

Disease Analysis

We studied the prevalence and intensity of parasitic Dermo infections in oysters from different reefs in the study area, using a 2012 survey of the same Cook Point plantings for comparison. As previously described, prevalence is the percentage of oysters that contained any Dermo, while intensity measures the severity of such infection on a 0-5 scale. Infection rates were lowest at the recently planted mound reef and highest at the sand and shell reefs.

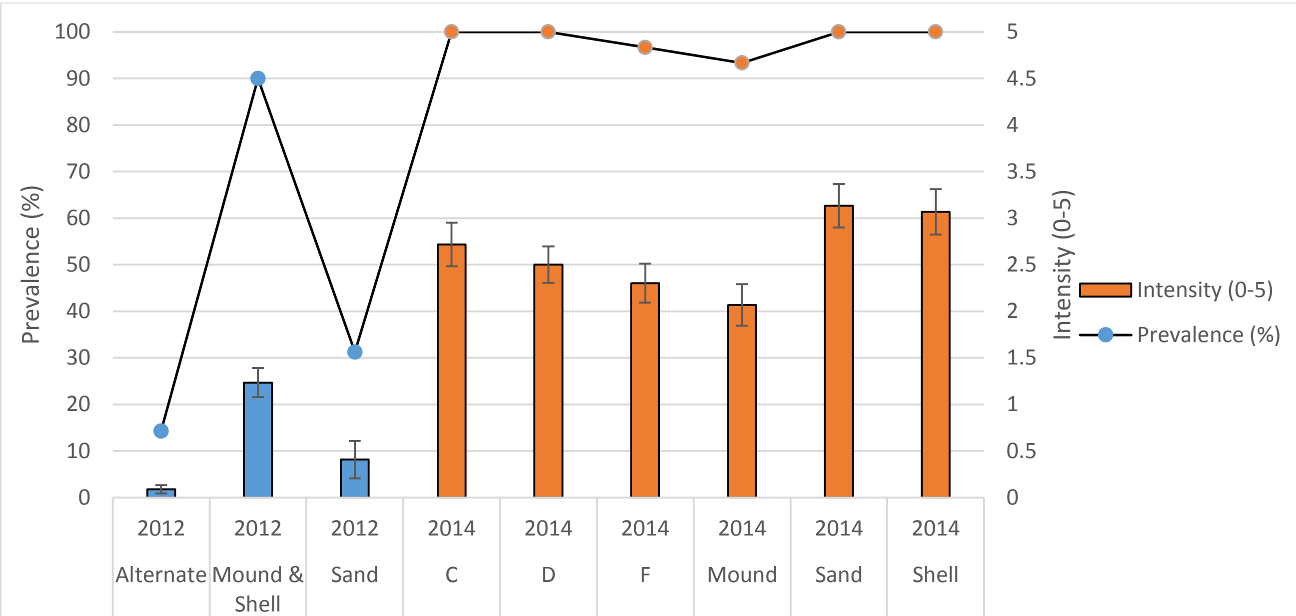

Figure 19: Intensity and prevalence of Dermo disease (*Perkinsus marinus*) infection. Columns show average intensity means. Error bars represent standard error. Prevalence is represented by points, connected by lines. Sites were surveyed in December 2012 (blue) and October-November 2014 (orange). In 2012, intensity averaged below 1. By 2014, all sites had intensities around 2.5.

Table 12 shows the complete results for disease prevalence and intensity, while Figure 19 shows the weighted intensity with standard error, as well as prevalence for each site. In 2012, Dermo prevalence and intensity were significantly higher on the mound and flat shell reefs than elsewhere (sand p-value = 0.0005, alternate substrate p-value <0.0001), but even there most infection levels were light. By 2014, the parasite was widespread at moderate intensities throughout the Cook Point Sanctuary area. For all sites surveyed in 2014, the prevalence of Dermo was 98.3±1.1%, while the intensity was 2.6±0.1, compared with 48.6±18.7% and 0.6±0.1 in 2012. Both the differences between years, and all differences on individual sites except for shell mounds are highly significant.

Table 12: Metrics for Dermo sampling from oyster in sites from the USACE study. Alternate substrate reefs are granite and granite with shell reefs designated A-G by this study. Significantly different disease intensities found in 2014 between mound reefs and reefs on flat shell and sand are indicated in bold. All differences in prevalence and intensity that were demonstrably different between 2012 and 2014 are in italics. Asterisks for prevalence and intensity of mound and flat shell sites surveyed in 2012 indicate significantly greater values than those for all other sites surveyed in that year.

	2014						2012		
Site	Mound 2	Shell 1	Sand 1	C (Granite + Shell)	D (Granite + Shell)	F (Granite Only)	Alternate	Mound & Shell	Sand
Mean Shell Length (mm)	70.43	86.17	97.33	90.13	93.97	104.63	76.29	83.40	77.88
Mean Total Weight (g)	70.07	92.29	148.61	103.16	111.86	115.54	45.49	83.83	62.83
Mean Shell Weight (g)	58.36	73.58	123.47	82.21	91.10	87.56	34.32	67.54	48.91
Negative Dermo Oysters (0)	2	0	0	0	0	1	24	3	11
Rare Dermo Oysters (0.5)	0	0	0	1	2	0	3	0	1
Light Dermo Oysters (1)	12	6	5	7	6	10	1	22	3
Moderate Dermo Oysters (3)	15	17	18	18	21	18	0	5	1
Heavy Dermo Oysters (5)	1	7	7	4	1	1	0	0	0
Dermo Prevalence (%)	93.33	100.00	100.00	100.00	100.00	96.67	*14.29*	*90.00	*31.25*
Dermo Intensity (0-5)	**2.1**	**3.1**	**3.1**	2.7	2.5	2.3	*0.04*	**1.2*	*0.4*
Intensity SEM	0.22	0.24	0.23	0.23	0.19	0.21	0.04	0.15	0.19

Across all substrates sampled in 2014, Dermo infection intensity approached or exceeded the threshold for mortality (Burreson 2009, Paynter et al 2014). Only the sand and mound sites ($p = 0.012$) and mound and shell sites ($p = 0.023$) were significantly different from each other in intensity of dermo infections in 2014. Younger oysters typically have lower disease rates, so the lower average Dermo intensity of 2.1±0.2 on the shell mound is not surprising, and not significantly higher than the mixed result from flat and mound shell sites of 2012. Infection intensity of oysters at both flat shell (3.1±0.2) and sand (3.1±0.2) both were high enough to cause extensive oyster mortality (Carnegie and Burreson 2008), and significantly increased from 2012 to 2014.

Discussion and Conclusions:

Overall, this study found that artificial reef at the Cook Point Sanctuary are supporting dense, healthy oysters and reef habitat. The following details the study results. All restored points sampled contained well over the minimum threshold of 15 oysters/m^2 and 15 g/m^2 oyster dry biomass criteria for successful restoration defined by the Oyster Metrics Workgroup (OMW).

1. This study reiterates the importance of hard substrate for oyster planting. Sand sites had far fewer oysters at less biomass and supported fewer other organisms than any other site. Oyster survival on sand is general poor due to sinking shell or sediment shifting that buries individuals, inhibiting digestion and respiration. This burial can also conceal mortality on sand, since divers are unable to see or find buried shell. Our observations are strongly supported by prior studies; hard surfaced reef substrate is known to positively impact oyster recruitment and support macrofaunal communities in the Chesapeake Bay and elsewhere (Rodney & Paynter 2006).

2. Reefs with a shell veneer over granite performed better than granite-only sites, with more oysters and oyster biomass. Division of results by the ratio of granite to shell within mixed reefs supported the idea that oyster biomass density is higher on reefs with the highest proportion of shell substrate than on other reefs. These results support a higher inclusion of oyster shell in any potential reef base. We hypothesize that this may be driven by the size of the granite pieces used to construct the reefs. Anecdotally, divers have observed on

3. In contrast, we found no significant differences between oyster populations on reefs of different heights. In a restoration context, this means that more reef area can be built with less substrate, and there is no benefit to building reefs taller than one foot.

4. Shell mounds and flat shell were not very good controls for the constructed reefs. Their planting histories were very different, with many years of planting, instead of the concentrated 2011 plantings on the artificial reefs. This led to high densities of smaller oysters on flat shell sites. The shell mounds had unexpectedly low oyster densities, much lower than the surrounding flat shell. They are also much taller than any other treatment, averaging seven feet above the flat shell. We hypothesize that the low oyster densities may be due to the shell mounds gradually shifting and settling, subsuming and covering planted oysters.

5. Dermo (Perkinsus marinus) was ubiquitous throughout the study populations at moderate intensity in 2014. Disease severity had increased significantly since 2012. Compared to similarly-aged populations in the Choptank River, we found a higher percentage of boxes. As these other populations generally had lower infections, this may suggest that Dermo is killing oysters at the Cook Point site. A possible contributing factor is shown by Dr. Elizabeth North's models, which show that Cook Point is a "sink" for larvae, and so may also accumulate Dermo particles as well.

6. Epibiota was more diverse and dense on restored reef areas than on unrestored sand. Large areas of sand were completely uninhabited by other organisms. While biodiversity by richness and density were similar on all restored reefs with shell in their base, more epibionts in higher densities were present on granite-only reefs than on any other site class. The principal organisms responsible for this difference were anemones, hydroids, and tunicates, all of which can compete with oysters for resources, but also contribute to water filtering.

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Appendices

Appendix 1: Depth, visibility, fouling degree, observed substrate composition, bottom penetration, and bottom and surface temperature, salinity, and dissolved oxygen for all reef points sampled by divers.

Site - Point	Date Sampled	Depth (ft)	Visibility (ft)	Substrate 1	Substrate 2	Substrate 3	Penetration (cm)	Fouling (%)	Surface Temperature (°C)	Surface Salinity (ppt)	Surface DO (mg/L)	Bottom Temperature (°C)	Bottom Salinity (ppt)	Bottom DO (mg/L)
Mound 1-1	10/17/2014	17	6	Loose Shell	Shell Hash		0	20	19.35	15.07	8.6	19.3	15.61	8.04
Mound 1-2	10/14/2014	18	1	Loose Shell	Oyster		0	15	19.35	15.07	8.6	19.3	15.61	8.04
Mound 1-3	10/14/2014	15	7	Loose Shell	Shell Hash		0	15	19.35	15.07	8.6	19.3	15.61	8.04
Mound 2-1	10/21/2014	17	8	Loose Shell	Shell Hash	Silt	0	20	17.14	15.18	8.72	17.01	15.32	8.64
Mound 2-2	10/21/2014	17	8	Loose Shell	Shell Hash	Silt	0	20	17.14	15.18	8.72	17.01	15.32	8.64
Mound 2-3	10/21/2014	17	6	Loose Shell	Shell Hash	Silt	0	20	17.14	15.18	8.72	17.01	15.32	8.64
Mound 3-1	10/21/2014	15	6	Loose Shell	Shell Hash		0	20	17.43	15.03	8.73	17.01	15.5	8.38
Mound 3-2	10/21/2014	18	8	Loose Shell	Shell Hash		0	15	17.43	15.03	8.73	17.01	15.5	8.38
Mound 3-3	10/21/2014	17	6	Shell Hash	Loose Shell		0	20	17.43	15.03	8.73	17.01	15.5	8.38
Shell 1-1	10/17/2014	24	4.5	Loose Shell	Shell Hash	Silt	0	15	19.39	14.92	8.76	19.28	15.1	8.57
Shell 2-1	10/17/2014	23	4.5	Loose Shell	Silt	Oyster	0	15	19.43	14.66	8.71	19.44	15.5	7.94
Shell 3-1	10/21/2014	22	5	Loose Shell	Shell Hash	Silt	0	10	17.43	15.03	8.73	17.01	15.5	8.38
Sand 1-1	10/21/2014	21	5	Sand	Loose Shell		10	5	18.04	15.59	8.78	16.75	15.58	8.38
Sand 1-2	10/21/2014	21	5	Sand	Loose Shell		10	5	18.04	15.59	8.78	16.75	15.58	8.38
Sand 1-3	10/21/2014	21	5	Sand	Loose Shell		10	5	18.04	15.59	8.78	16.75	15.58	8.38

Site - Point	Date Sampled	Depth (ft)	Visibility (ft)	Substrate 1	Substrate 2	Substrate 3	Penetration (cm)	Fouling (%)	Surface Temperature (°C)	Surface Salinity (ppt)	Surface DO (mg/L)	Bottom Temperature (°C)	Bottom Salinity (ppt)	Bottom DO (mg/L)
Sand 2-1	10/27/2014	22	8	Sand	Loose Shell		5	60	16.26	14.57	9.61	15.42	15.31	9.32
Sand 2-2	10/27/2014	21	7	Sand	Loose Shell		10	30	16.26	14.57	9.61	15.42	15.31	9.32
Sand 2-3	10/27/2014	22	7	Sand	Loose Shell		10	70	16.26	14.57	9.61	15.42	15.31	9.32
Sand 3-1	11/25/2014	20	3	Sand			10	100	7.12	15.23	10.81	1.04	15.27	10.89
Sand 3-2	11/25/2014	20	1.5	Sand			10	100	7.12	15.23	10.81	1.04	15.27	10.89
Sand 3-3	11/25/2014	20	2	Sand			10	100	7.12	15.23	10.81	1.04	15.27	10.89
A-1	10/21/2014	18	7	Loose Shell	Rock		0	40	17.69	15.3	8.9	17.05	15.55	8.31
A-2	10/21/2014	20	5	Loose Shell	Silt	Rock	0	10	17.69	15.3	8.9	17.05	15.55	8.31
A-3	10/21/2014	21	5	Loose Shell	Silt	Rock	0	10	17.69	15.3	8.9	17.05	15.55	8.31
B-1	10/27/2014	21	8	Loose Shell	Oyster		0	-	16.18	14.52	9.39	15.4	14.76	9.1
B-2	10/27/2014	20	8	Oysters	Loose Shell	Rock	0	-	16.18	14.52	9.39	15.4	14.76	9.1
B-3	10/27/2014	21	7	Loose Shell	Oyster		0	-	16.18	14.52	9.39	15.4	14.76	9.1
C-1	11/4/2014	17	4	Loose Shell	Rock		0	60	12.62	15.57	9.37	12.54	15.54	9.22
C-2	11/4/2014	19	4	Loose Shell	Rock		0	-	12.62	15.57	9.37	12.54	15.54	9.22
C-3	11/4/2014	19	5	Loose Shell	Oyster		0	20	12.62	15.57	9.37	12.54	15.54	9.22
D-1	11/4/2014	20	3	Loose Shell	Oyster		0	40	12.52	15.43	9.31	12.41	15.52	9.24
D-2	11/4/2014	20	1	Loose Shell	Oyster		0	30	12.52	15.43	9.31	12.41	15.52	9.24
D-3	11/4/2014	20	3	Loose Shell	Rock		0	40	12.52	15.43	9.31	12.41	15.52	9.24

Site - Point	Date Sampled	Depth (ft)	Visibility (ft)	Substrate 1	Substrate 2	Substrate 3	Penetration (cm)	Fouling (%)	Surface Temperature (°C)	Surface Salinity (ppt)	Surface DO (mg/L)	Bottom Temperature (°C)	Bottom Salinity (ppt)	Bottom DO (mg/L)
E-1	10/31/2014	20	6	Rock			0	90	15.58	15.64	9.04	15.74	15.72	8.78
E-2	10/31/2014	19	5	Rock	Oyster		0	90	15.58	15.64	9.04	15.74	15.72	8.78
E-3	10/31/2014	19	6	Rock			0	90	15.58	15.64	9.04	15.74	15.72	8.78
F-1	11/25/2014	19	3	Rock	Sand		5	80	6.7	15.04	11.14	6.87	15.2	11.16
F-2	11/25/2014	18	1.5	Rock			0	80	6.7	15.04	11.14	6.87	15.2	11.16
F-3	11/25/2014	19	5	Rock			0	80	6.7	15.04	11.14	6.87	15.2	11.16
G-1	10/31/2014	17	6	Rock			0	90	15.54	15.11	9.62	15.38	15.28	9.6
G-2	10/31/2014	19	6	Rock	Oyster		0	90	15.54	15.11	9.62	15.38	15.28	9.6
G-3	10/31/2014	17	6	Rock			0	90	15.54	15.11	9.62	15.38	15.28	9.6

Appendix 2: Opportunistically sighted organisms during Cook Point Sanctuary surveys and the number of points on which they were sighted. Amphipod and polychaete occurrence is likely heavily underestimated by this survey, as both groups of species are found ubiquitously on oysters throughout the Chesapeake Bay using finer scale surveys (Rodney & Paynter 2006, K. Kesler unpub. data). Only one opportunistic fish sighting, a skilletfish, was made on a sand site.

Organism	Blennies	Amphipods	Polychaetes	Blue Crab	Oyster Toadfish	Skilletfish	Clam	Feather Algae	Sculpin
Sightings	10	3	5	1	3	3	1	1	1

Appendix 3: Presence of frequent sessile and limited-range organisms found by divers on reefs sampled during the alternate substrate oyster reef study. Presence within individual groups is indicated by binary response (1 = present, 0 = absent). The total number of all macroscopic benthos found on individual points is given in the right-hand column. A higher presence of organisms on restored reefs supports prior ecological findings (Rodney & Paynter 2006, K. Kesler unpub. data), with mud crabs, mussels, and fish species notably present with greater frequency on restored sites.

Site	Point	Substrate	Reef Height (ft)	Date Sampled	Mud Crabs	Gobies	Mussels	Bryozoans	Barnacles	Anemones	Hydroids	Tunicates	Total Species Richness (n)
Mound 1	1	Mound	-	10/17/14	1	1	1	0	1	1	1	0	7
Mound 1	2	Mound	-	10/14/14	1	0	1	1	1	0	0	0	5
Mound 1	3	Mound	-	10/14/14	1	1	1	0	1	1	1	0	8
Mound 2	1	Mound	-	10/15/14	1	1	1	1	1	1	1	1	8
Mound 2	2	Mound	-	10/16/14	1	1	1	0	1	1	1	1	7
Mound 2	3	Mound	-	10/17/14	1	1	1	1	1	1	1	1	8
Mound 3	1	Mound	-	10/18/14	1	1	1	1	1	1	1	1	8
Mound 3	2	Mound	-	10/19/14	1	1	1	1	1	1	0	0	6
Mound 3	3	Mound	-	10/20/14	1	1	1	1	1	1	1	1	9
Shell 1	1	Shell	-	10/21/14	1	1	1	0	1	1	1	1	7
Shell 2	1	Shell	-	10/22/14	1	1	1	0	1	1	1	1	8
Shell 3	1	Shell	-	10/23/14	1	1	1	0	1	0	1	1	6
Sand 1	1	Sand	-	10/24/14	1	1	1	1	1	1	0	1	8
Sand 2	1	Sand	-	10/25/14	0	1	1	0	1	1	1	1	6
Sand 2	2	Sand	-	10/26/14	0	1	0	1	1	1	1	1	7
Sand 2	3	Sand	-	10/27/14	0	1	1	0	1	1	1	0	5
Sand 3	1	Sand	-	10/28/14	0	0	0	0	0	0	0	1	1
Sand 3	2	Sand	-	10/29/14	0	0	0	0	0	0	0	1	1
Sand 3	3	Sand	-	10/30/14	0	0	0	0	0	0	0	1	1
A	1	Granite/Shell	>1	10/31/14	1	1	1	1	1	0	0	1	7
A	2	Granite/Shell	>1	11/1/14	0	1	1	1	1	0	1	1	7
A	3	Granite/Shell	>1	11/2/14	1	1	1	1	1	0	0	1	7
B	1	Granite/Shell	1	11/3/14	1	1	1	0	1	0	0	0	5
B	2	Granite/Shell	1	11/4/14	1	1	1	1	1	0	0	1	6
B	3	Granite/Shell	1	11/5/14	1	1	1	0	1	0	0	1	5
C	1	Granite/Shell	>1	11/6/14	1	1	1	1	1	0	1	1	9
C	2	Granite/Shell	>1	11/7/14	1	1	1	1	1	0	0	0	5
C	3	Granite/Shell	>1	11/8/14	1	1	0	1	1	0	0	0	4
D	1	Granite/Shell	1	11/9/14	0	1	1	1	1	0	1	0	5
D	2	Granite/Shell	1	11/10/14	1	1	1	0	1	0	0	0	4
D	3	Granite/Shell	1	11/11/14	0	1	1	1	0	0	1	0	4
E	1	Granite	1	11/12/14	1	1	1	1	1	0	1	1	7
E	2	Granite	1	11/13/14	0	1	1	0	1	1	1	0	5
E	3	Granite	1	11/14/14	1	1	1	1	1	0	1	1	7
F	1	Granite	1	11/15/14	0	0	1	0	1	1	1	1	7
F	2	Granite	1	11/16/14	1	1	1	0	1	1	1	1	7
F	3	Granite	1	11/17/14	1	0	1	0	1	1	1	1	6
G	1	Granite	>1	11/18/14	1	1	1	0	1	1	1	0	6
G	2	Granite	>1	11/19/14	1	1	1	1	1	0	1	1	9
G	3	Granite	>1	11/20/14	1	1	1	0	1	0	1	1	7