ABSTRACT

Title of Thesis: **EVALUATING SUBSTRATE REHABILITATION TECHNIQUES FOR BOTTOM CULTURE OF THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) IN CHESAPEAKE BAY**

Jessie Todd Long, Master of Science, 2020

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Marine Estuarine and Environmental Science

The degradation of Chesapeake Bay bottom conditions and oyster beds over the past century from habitat destruction, overharvesting, disease, and sedimentation have resulted in many areas that are detrimental for healthy oyster populations. In leased oyster aquaculture areas, unsuitable bottom characteristics result in suboptimal survival. Although the addition of oyster shell as substrate has been a common practice for building new oyster beds, the current high cost and lack of available shell can make this approach impractical. The goal of this study was to measure the effects of new and traditional bottom rehabilitation techniques (harrowing and shell addition) on oyster survival and growth on three distinct bottom types. The data revealed that treatments, whether singularly or in combination, were insignificant in respect to oyster size and survival across all bottom types. However, the observed bottom type had a significant effect on the percentage of oyster survival.

EVALUATING SUBSTRATE REHABILITATION TECHNIQUES FOR BOTTOM CULTURE OF THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) IN CHESAPEAKE BAY

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2020

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Dedication

I would like to dedicate this thesis to my mother and father, whose unending love and encouragement has driven me through every phase of life. Their constant support has allowed me to pursue my goals and continue my education.

Acknowledgments

First and foremost, I want to express my sincere gratitude to my supervisor, Dr. Jeff Cornwell, who stepped in and supported me throughout my graduate experience and continuously gave me sound advice. When problems surfaced, his encouragement, guidance, and expertise made this thesis possible. A second big thank you goes to the rest of my committee, Don Webster, whose support and extensive oyster aquaculture knowledge shaped me into a better writer and researcher, and Dr. Louis Plough who saved the day by completing my committee. Other outstanding UMCES faculty include, Dr. Jacob Cram, who always had time for my unending statistical questions and bettered my understanding of R. My appreciation extends further than this acknowledgment can express, thank you all.

The Horn Point Hatchery is one of a kind. To Steph, Jeff, Julie, Stacey, Steven, Alex, Alicia, and of course Bob, your friendship, knowledge, support, mentorship, and kindness shaped my experience at UMCES for the better. I always felt right at home in the hatchery. Without my crew, I would not have been able to process all those oyster samples. I am so thankful to have shared many laughs, lunches and weekends with you all.

Additionally, I would like to thank Maryland Industrial Partnerships for their generous grant that made my graduate studies possible. Last, but not least, a thank you to Shannon Hood, whose friendship I cherish. She is always willing to listen, lend a hand or share her wisdom.

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Glossary

Scarred Oyster- An area on a shell where an oyster was once attached, but leaves only an outer ring to aid in determining time of mortality.

Gaping Oyster- A pair of shells still attached by a ligament, either empty or some tissue remains indicating recent oyster mortality.

Introduction

Background Information

The oyster (*Crassostrea virginica)* industry was a commercial powerhouse in Chesapeake Bay during the 1880's, producing almost 27 million bushels each year (MacKenzie Jr., 1996, Rothschild 1994). The steady decline to 5.9 million bushels harvested annually in the early 1990's has been attributed to overfishing, habitat destruction, sedimentation, poor water quality, and disease (Figure 1. Rothschild, 1994) (MacKenzie Jr., 1996 & 2007, Grabowski and Peterson, 2007, Kennedy et al. 2011).

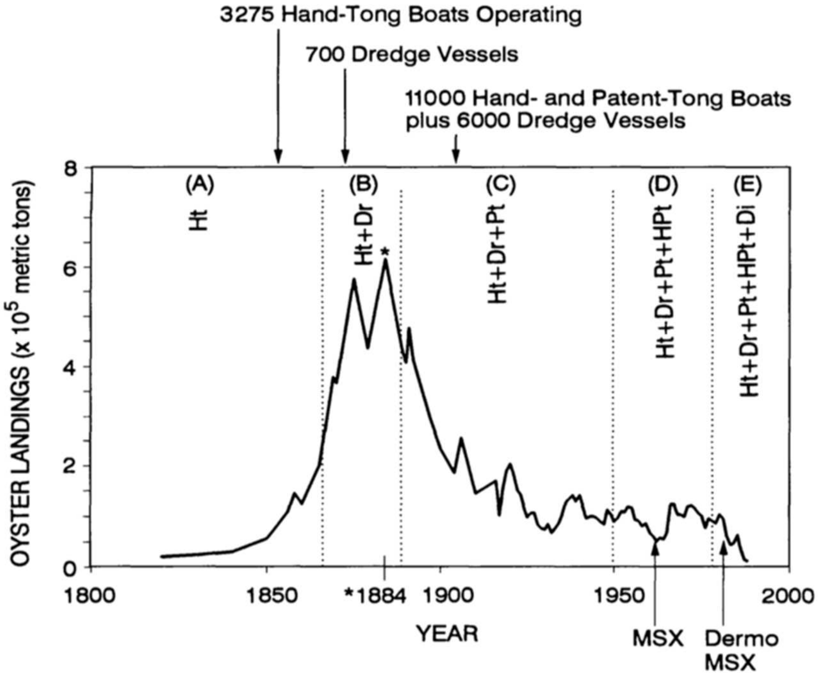

Figure 1. Time series of Maryland, USA, oyster landings (source: Maryland Department of Natural Resources). Panel segments show corresponding evolution of oyster fishing gears: (A) use of hand tongs (Ht); (B) introduction of dredges (Dr) (*note production peak 1884); (C) introduction of patent tongs (Pt) which corresponds with the beginning of the catch decline; (D) introduction of the hydraulic patent tong (HPt) in 1950 and date when disease was first recorded; (E) the addition of diver harvesting (Di) in 1980 (Rothschild, 1994).

In addition to the oyster's historic economic value in the Chesapeake Bay, their reef-like structures and filter feeding ability make them a keystone species and ecosystem engineers (Wilberg et al. 2013, Rossi-Snook et al. 2010). The effective management of abundant eastern oyster populations are required to ensure future viability of the oyster industry and to restore associated ecosystem services (Beck et al. 2011).

Oysters form biogenic reefs due to their ability to grow vertically and create upright clusters (Colden et al. 2017, Grabowski and Peterson, 2007). The over-harvesting of oysters damaged the physical integrity of oyster bars through the use of tongs and dredges (Rothschild, 1994; Mercaldo-Allen and Goldberg, 2011) and has contributed to the decline in oyster biomass. “Bagless dredging”, considered at one time to be a form of rehabilitation by turning over shells to separate them from fine-grained substrate without removing them, appeared to provide less substrate to oyster recruitment than before the dredging (Homer, 2017). The excess suspension of sediments increased turbidity, created sediment plumes and disrupted the benthic habitat (Mercaldo-Allen and Goldberg, 2011). Culling, “separating the oysters from other things brought up by a dredge”, can be beneficial to oyster beds by scattering oysters to extend the bar, but also inadvertently kills the smaller oysters (Ingersoll, 1881; OAC, 2016). However, removing excessive amounts of oysters and shell degrades a population to a state where sedimentation covers previously productive beds and decreases the amount of available habitat (Rothschild, 1994; Brooks, 1891). When vertical structure is diminished, the ratio of debris to living oysters increases (Luckenbach, 1999). Older oyster generations are the foundation for new larval settlement; without hard substrate, larvae have no place for attachment (Turner et al, 1994, Luckenbach, 1999). If reef height is below a certain threshold, beds progress towards degradation and require active intervention (Colden et al, 2017; Schulte et al, 2009). The restoration and rehabilitation of oyster beds impaired by mud deposition is necessary for continued provision of economic and environmental services.

Reasoning for Rehabilitating Oyster Leases

Although oyster aquaculture was introduced into the Chesapeake Bay in the 1830's, seed mismanagement and disease in recent decades has played a large part in its decline (Krantz and Otto, 1981). In 2009, shellfish leasing laws were revised to encourage the development of the shellfish industry in the state of Maryland (Maryland Oyster Advisory Commission, 2009; Webster 2009 & 2019). The Oyster Advisory Commission, tasked with providing advice on matters related to oysters, subsequently recommended that the sustainable future of the oyster industry would be enhanced by consolidating the lease application process to the Maryland Department of Natural Resources instead of being fragmented into other agencies (Maryland Department of Natural Resources, 2016). This action was designed to encourage the expansion of the aquaculture industry by shortening the approval time for both Submerged Land and Water Column Leases (Maryland Department of Natural Resources, 2016; Webster et al, 2019). Maryland oyster aquaculture harvests have been increasing in recent years (Figure 2), with bottom culture providing both the largest acreage and harvest in the state (Department of Natural Resources, 2019). In 2019, bottom culture comprised 77% of the

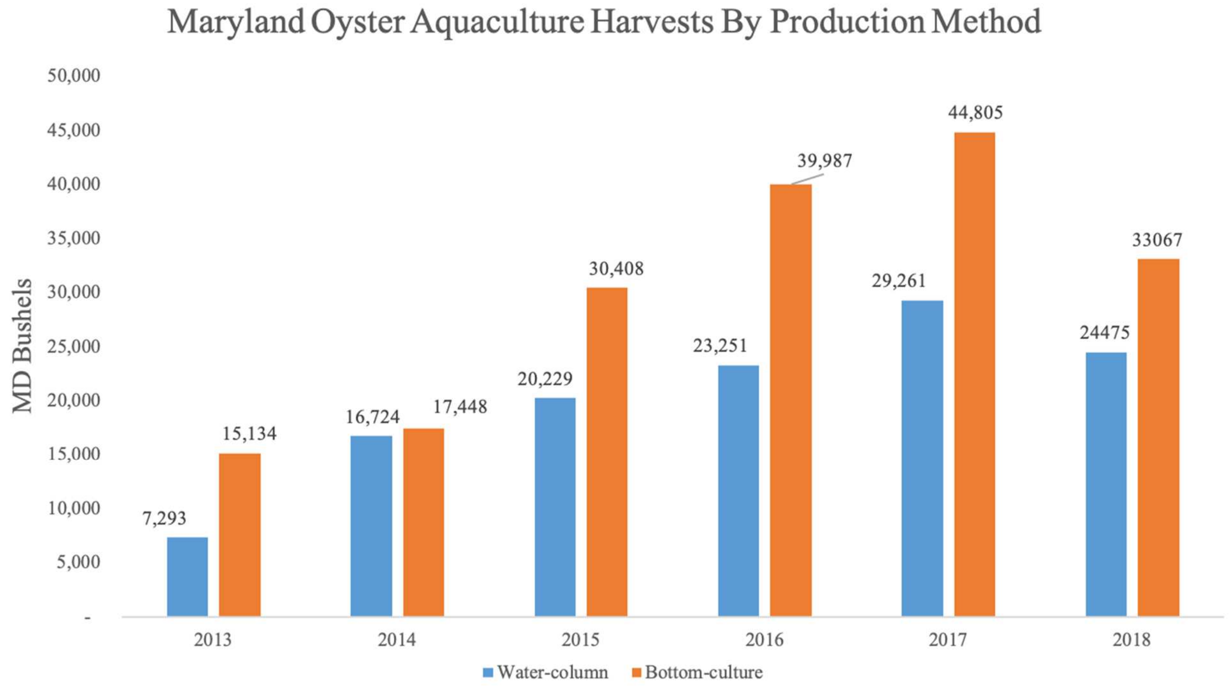

Figure 2. Maryland Aquaculture Oyster Harvest 2013-2018 by lease type. Data courtesy of Maryland Department of Natural Resources.

leased aquaculture areas with 353 of the 455 leases being Submerged Land Leases used for bottom culture of spat on shell (Maryland Aquaculture Coordinating Council, 2019).

Remote setting is a method where oyster larvae produced in a hatchery are set on clean, containerized, aged shell, or other setting material known as cultch (Bohn et al, 1995; Meritt et al, 2011). Upon settlement, larvae are referred to as “spat on shell”, which can be grown in bags and cages in the water column or planted on the bottom (Bohn et al, 1995; Meritt et al, 2011, Webster et al, 2019). Farmers using bottom production plant their spat on shell on leases to sell product to the shucking plants market or cull for sale to raw bars (Maryland Department of Natural Resources, 2016). The state requirement that new public leaseholds be devoid of oysters has the unfortunate consequence of often limiting leased grounds to suboptimal areas unfit for growing oysters without rehabilitation using expensive and scarce oyster shell as substrate (Webster and Meritt, 1988). While revised leasing laws have created opportunities for leaseholders, poor quality and often muddy bottom conditions present ongoing challenges for bottom culture.

With new leases being authorized yearly (Figure 3), Maryland's oyster aquaculture industry is expected to grow; therefore stabilized ground management is needed to support spat on shell plantings. High sediment loads and low reef height density on permitted leases (Rothschild, 1994; Colden et al, 2017) result in challenges to bottom culture in the first few years of application. The accumulation of sediment and drift algae negatively impact young sessile organisms like oysters, as their gills are susceptible to clogging which results in suffocation (Thomsen et al 2006; Ortega and Sutherland, 1992). Suitable bottom characteristics include hard compacted sediment such as sand and clay that can support oyster spat on shell (Webster and Meritt, 1988; Galtsoff, 1964). Fine sediments and mud tend to shift with currents and move or bury shell (Webster and Meritt, 1988). At high concentrations, suspended sediments can negatively affect growth and survival of larval shellfish (Wilber and Clark, 2001 & 2010). Traditionally, cultch is planted on bottom to create a surface for seed oysters and prevent suffocation from silty sediments (Webster and Meritt, 1988). Although oyster shell has been traditionally the primary cultch material used to harden beds, high costs and scarcity of the material has made this method uneconomic (Powell, 2007; Webster and Meritt, 1988). Some grow-out operations would rather process shucked oyster shell into spat on shell, rather than planting old shell as a hardening substrate (Meritt and

Webster, 2019). New methods to restore oyster beds without supplementing oyster shell would be an advantage to bottom culture.

Figure 3. Commercial shellfish lease applications received and issued by the State of Maryland from 2010-2019. Data courtesy of Maryland Department of Natural Resources.

This study was an applied research endeavor attempting to solve a practical problem and aid future decision making in the bottom culture industry. Some approaches used in this study include: identifying a study site; initial assessment and manipulation of the site; observing and assessing changes within the system and; quantifying the effects on planted oyster spat on shell. This methodology attempts to understand the changes and effects within this singular system, but to consider them in a broader scope relevant to other areas in Chesapeake Bay.

Goals and Objectives

Historically, oyster farming has been based on tradition, opinion and observation rather than science-based guidelines. The variability of Chesapeake Bay bottom conditions, in conjunction with a growing aquaculture industry, suggests that the development of effective management strategies that can improve bottom culture would strongly benefit the aquaculture industry. The use of alternative substrate rehabilitation techniques requires research to verify the efficacy of new approaches. The goal of this study is to gain insight on how distinct bottom characteristics affect oyster mortality and growth. A key component is to determine if active substrate management can improve sub-optimal grounds that are used for planting oyster spat on shell. The general absence of published work on the success of on-bottom culture makes this research important beyond the oyster grounds used for this study.

Materials and Methods

Study Site

The experiment site was an oyster lease used for commercial aquaculture on the Big Annemessex River, located off the Tangier Sound (Figure 4) in Maryland's Chesapeake Bay. Prior to treatments, bottom surveys were conducted by SCUBA divers on target areas within the 24.4-hectare lease, to pinpoint 0.40 ha plots that fit defined criteria based on specific bottom properties (Figures 5, 6, 7). Sites were classified as *poor* (mud), *intermediate* (buried shell) and *good* (exposed shell). Natural bottom substrates were identified as follows:

Mud Shell: primarily mud bottom with bits of shell hash mixed within the mud substrate

Mud Sand: layer of mud sitting on top of compacted sand bottom

Buried Shell: layer of mud with whole sized oyster shell substrate buried underneath

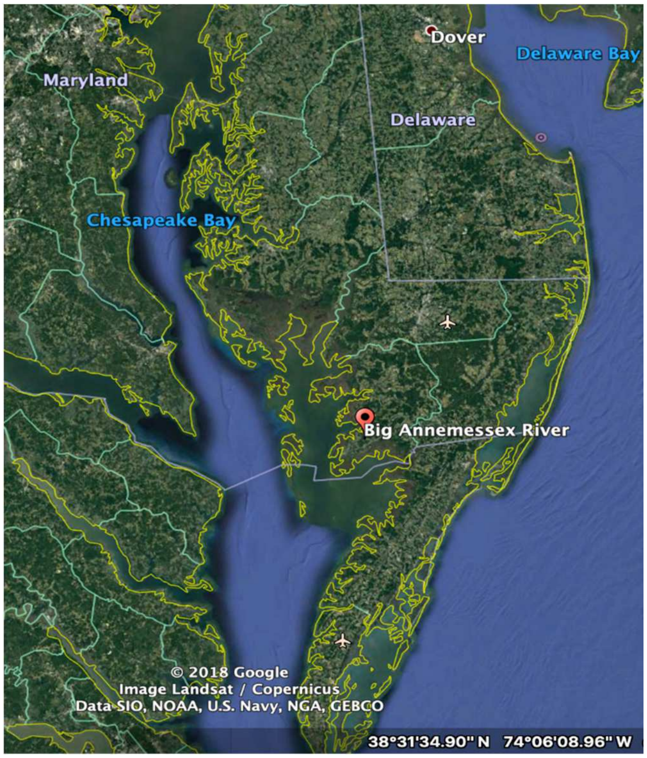

Figure 5. Map highlighting the Big Annemessex River located off the Tangier Sound in Chesapeake Bay. (Google Maps, n.d. Web).

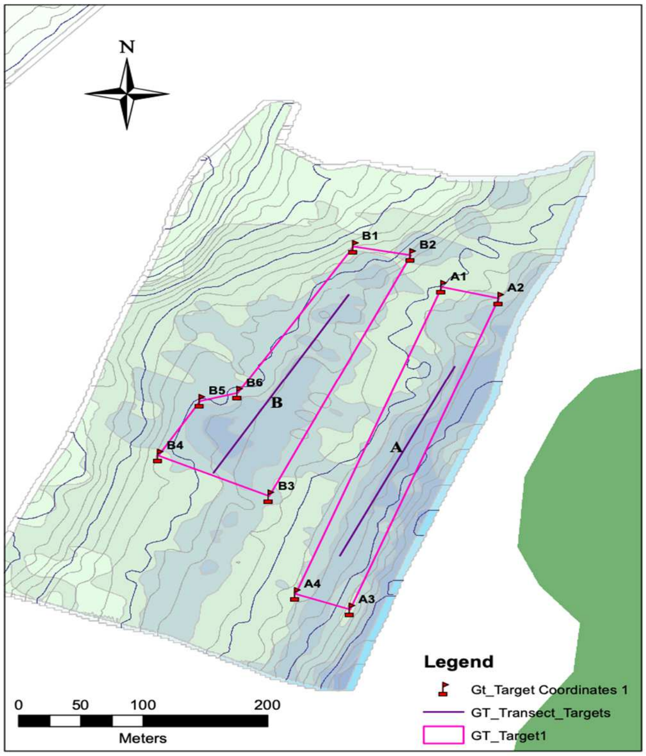

Figure 4. Survey area 1 with ground truthing target areas A and B located on the Big Annemessex River.

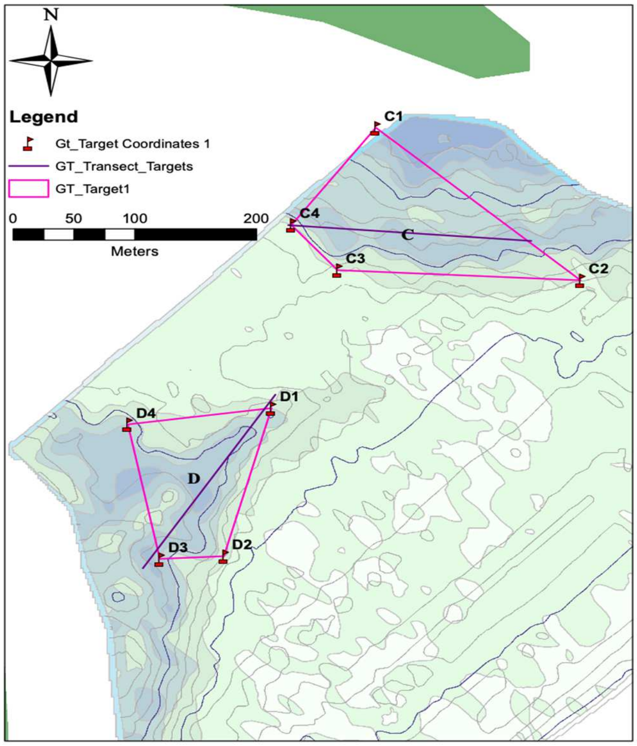

Figure 6. Survey area 2 with ground truthing target areas C and D located on the Big Annemessex River.

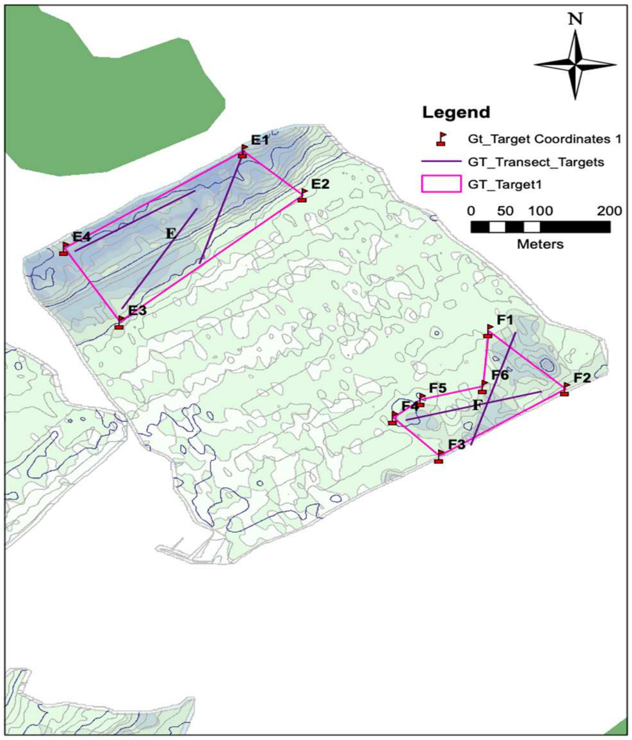

Figure 7. Survey area 3 with ground truthing target areas E and F located on the Big Annemessex River.

Ground truthing site surveys were conducted before bottom manipulation and throughout the oyster grow-out period to monitor on-bottom variations.

Treatments

Each of the three plot types - mud sand, mud shell, and buried shell - received two bottom rehabilitation treatments which were applied individually and in tandem. The first treatment included a new harrowing technique (Figure 8), which disrupts the top layer of sediment from the bottom and suspends it in the water column, where it moves off site during ebb tide. The harrow was 3.66 x1.83 meters long, and estimated to weigh between 204.12 - 272.16 kilograms and was towed behind a 16.76-meter barge. The amount of time towing the harrow was based on the darkness of the sediment plume being raised,

Figure 8. The harrow suspended from a vessel by a davit and winch.

which varied between plots. As the sediment plume became lighter, it was assumed the majority of the silt had been suspended off the plot, which indicated time to retrieve the harrow. The second, more traditional, treatment involved a shell technique where old oyster shells that had been cleaned were planted on their respective plot. Plots treated with shells received 3,150 bushels, which approximately totaled 8 cm of shell on two of the 0.30-hectare plots. In total, four treatments were applied to 0.10 ha of both the mud sand and mud shell plots, which included a control (no rehabilitation), a harrow technique, a shell technique, and a combination of harrow and shell application (Figure 9). Based on the first ground truthing assessment, it was decided that the buried shell plot would not receive a shell treatment because there was sufficient shell substrate from a previous, but unsuccessful planting. The opposite side of the lease was treated, but unplanted to survey for natural recruitment.

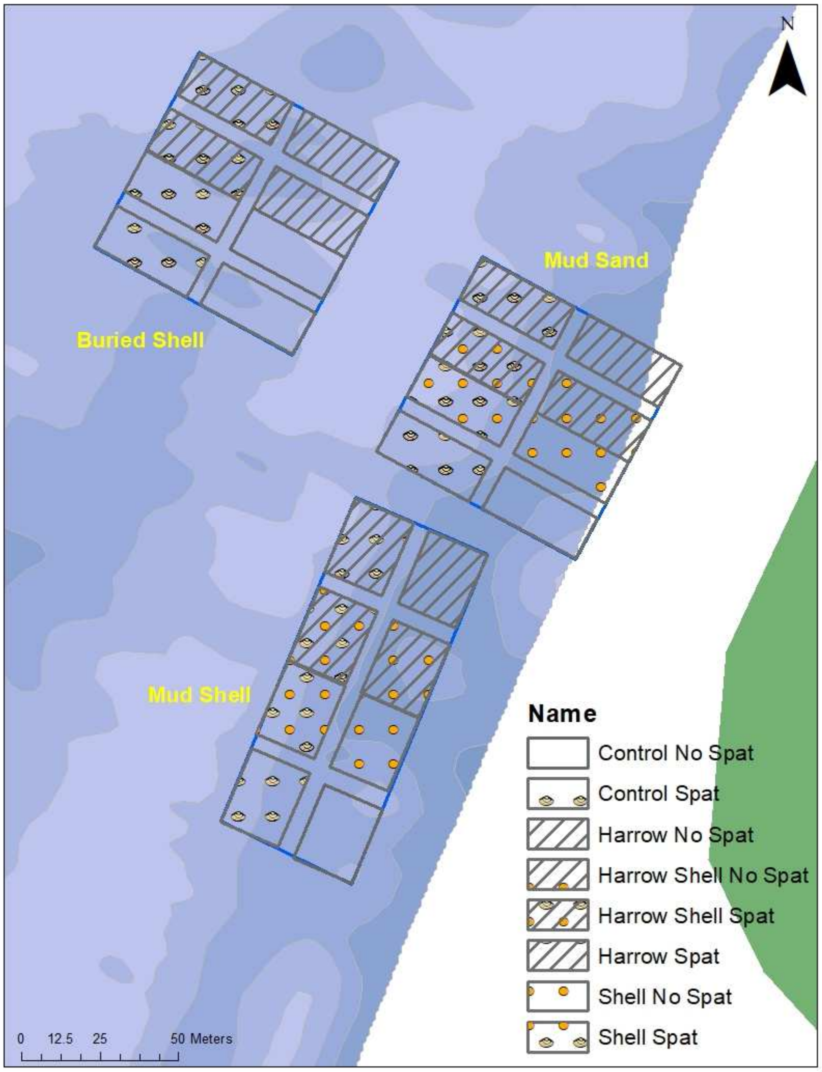

Figure 9. The three individual plots, mud shell, mud sand and buried shell, that have four treatments, control, harrow, shell, and harrow shell, applied to seeded (spat on shell) and non-seeded (no spat on shell) sides.

Upon completion of the treatments, *Crassostrea virginica* larvae were placed in a setting system at Metompkin Bay Oyster Company in Crisfield, Maryland to produce spat on shell. The shell in fourteen tanks was set with ~2.5 million larvae each, with the intent of a planting target of 4 million spat per 0.4 ha. Samples were removed and setting efficiencies were calculated ~3 days after the tanks were set. Individual spat from 10 shells were counted under a microscope with average spat per shell recorded and the total number of live spat in the tank estimated. Planting counts were taken 6-7 days after setting and prior to placement on the lease. The mud sand plot received 1,604,281 spat on shell, while the mud shell plot had 2,982,031 and the buried shell plot had 3,575,000 planted respectively. Figure 10 illustrates how spat on shell deployment was conducted to reduce the amount of variability between plantings due to maneuvering the vessel.

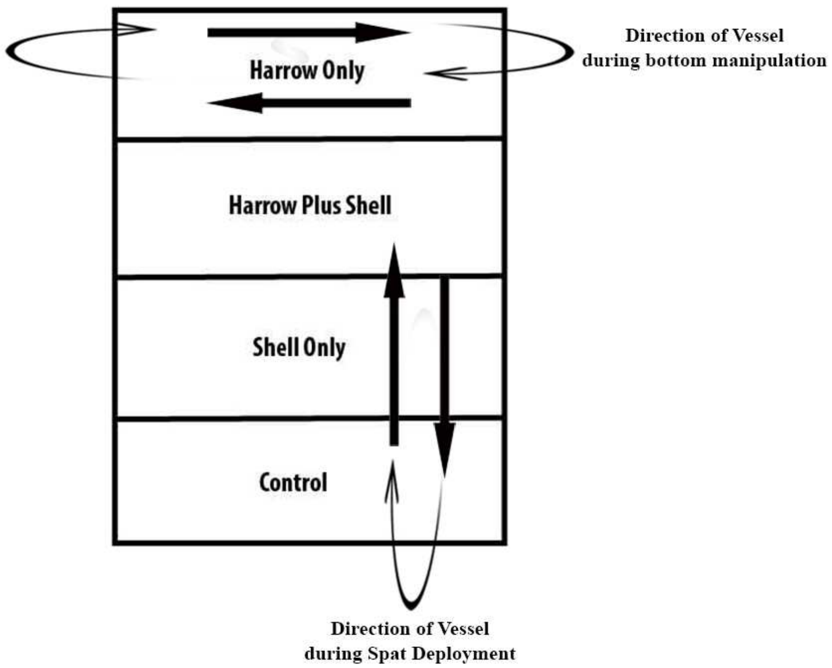

Figure 10. The direction of the vessel when applying treatment manipulations or spat on shell plantings.

Sampling Procedures

The treatments and spat on shell plantings were completed between June and August 2018. Field observations and sample collections were made using SCUBA divers, with the first, second, and third sampling in October 2018, April 2019, and September 2019 respectively. Each of the three plots were sampled along a 200 meter transect run through the seeded and non-seeded sides. During transect sampling, tag numbers were recorded to identify the boundaries of each treated subsection. Buoys were used as visual aids to keep the boat within each treated plot. Within the 4 boundaries (Figure 9), 3 sample points were randomly selected to collect ten oyster shells. In total, 360 shells were collected, with 120 shells collected in each seeded portion of the three plots. The non-seeded side was also sampled, with ten shells collected where possible, since not all treatments had shell added. Samples were taken to Horn Point Laboratory to record and measure size and counts of live, dead, scarred and gaping oysters.

Topography and bottom conditions were recorded during each transect sampling. Shell exposure was indicated as being either *full*, *some*, *little*, *very little*, or none using criteria developed by the Paynter lab (Ken Paynter, University of Maryland, pers. comm.). Bottom substrate samples identified by the diver were classified as whole oyster shell, loose shell with spat, loose shell without spat, shell hash, and mud. Bottom penetration to indicate ground hardness was measured qualitatively by pressing a hand into the bottom and noting the depth it was able to penetrate and recording *hard* (0 cm), *knuckle* (5 cm), *finger* (10 cm), and *hand* (20 cm).

Calculations

Upon planting the spat on shell, the setting efficiency for each tank (n=10) and planting counts (n=30) were calculated using the formulas:

$$\left(\frac{\left(\frac{Total\ Live\ Spat\ Counted}{\#\ Shells\ Sampled}\right)\times(\#Shells\ Planted)}{(\#\ Larvae\ Set\ in\ Tank(s))}\right)\times 100 = Setting\ Efficiency$$

$$\left(\left(\frac{Total\ Live\ Spat\ Counted}{\#\ Shells\ Sampled}\right)\times(\#Shells\ Planted)\right)=Planting\ Count$$

This verified that the percentage of spat settlement was adequate (>15%) and quantified the initial amount of oyster spat on shell that was planted (Meritt et al, 2011). When the oyster shell samples were collected by the dive team (n=30), live counts were documented on each treatment plot per bottom type, so the percentage of living oysters at each time of sampling could be calculated from the number of oyster spat on shell that were initially planted (Meritt et al, 2011).

$$\left(\frac{(Live\ Count\ @\ Time\ Sampled)(\#ShellsPlanted)}{(Average\ Spat\ per\ Shell\ Deployed)(\#\ Shells\ Planted)}\right)x\ 100$$
$$=Percent\ Survival$$

Statistical Analyses

Statistical analysis of differences in mean size and percent survival among bottom types and treatments between time points were evaluated in the computer software program R (version 3.6.2). The normality of the data was checked with a Shapiro-Wilk test, confirming the non-normal distribution (p-value<2.2e-16) of the size and percent survival data for treatments and bottom type. Using the FSA package in R, the Kruskal-Wallis test is a rank-based, non-parametric alternative to a one-way ANOVA to assess if there are statistically significant differences between the bottom types based on size and percent survival. Subsequently, a post-hoc analysis was preformed using the Dunn's test of multiple comparisons to identify which treatment pairs among the groups were significantly different.

Results

The extreme salinity decline observed in large portions of the Bay in 2019 did not affect the Big Annamessex River. Using observation from the Maryland Department of Natural Resources, the salinity oysters were exposed to was at or above 8.70 ppt in February 2019 (Figure 11), values suggesting salinity did not limit the survival and growth of the oysters (Loosanoff, 1965, Shumway, 1996). The near-market size oysters sampled in September 2019 tested negative for Dermo (*Perkinsus marinus*) at the University of Maryland, College Park (Paynter, unpublished data), so disease did not play a factor in the results.

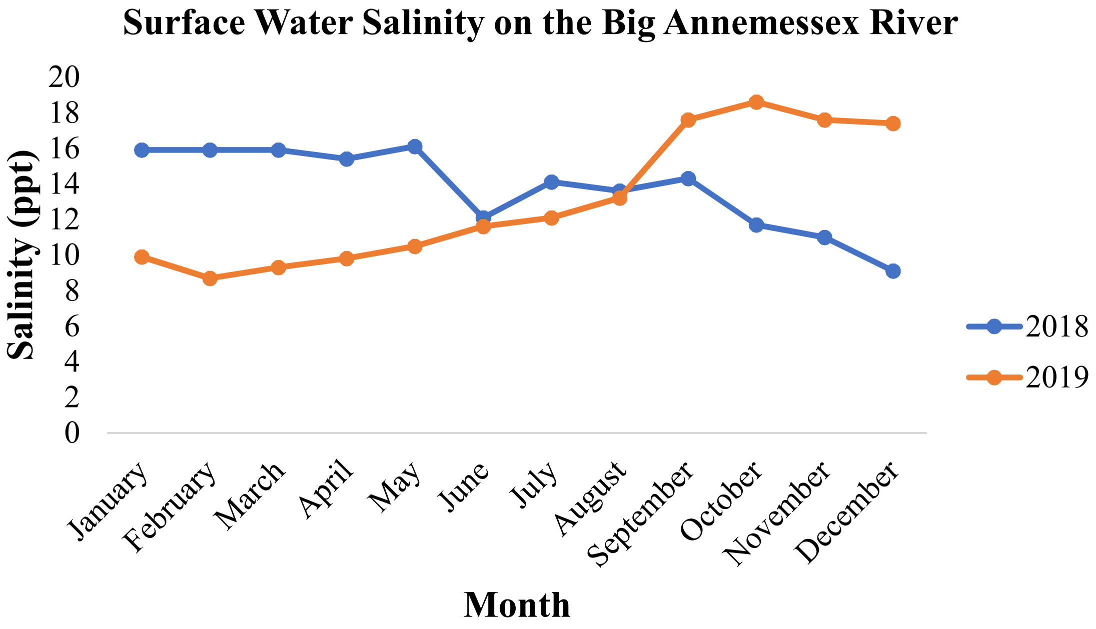

Figure 11. The monthly surface water salinity on the Big Annemessex River collected by the Maryland Department of Natural Resources for 2018 and 2019.

<u>Survival: Treatments</u>

The effect of treatments had on mean percent survival per each bottom type varied between the different sampling time points. The Kruskal-Wallis Rank Sum test (α<0.05) revealed that at Time Point One, treatments were significant on mud sand and mud shell bottom types, at Time Point Two, treatments were significant on all three bottom types, while at Time Point Three, mud shell was the only bottom type where treatments were significant (Table 1). This analysis reveals the inconsistency of treatment effects over time across the different bottom types.

Table 1. Summary of Kruskal-Wallis Rank Sum Test for mean percent survival on bottom type

Bottom Type	**Time Point**	**Rank Sum Statistics**	**P-Value**	**FDR**
Mud Sand	1	11.15	0.011	0.025
Mud Shell	1	8.61	0.035	0.053
Mud Sand	2	19.10	0.00026	0.0022
Mud Shell	2	11.52	0.0092	0.025
Buried Shell	2	12.18	0.00048	0.0022
Mud Shell	3	8.76	0.033	0.053

Dunn's test of multiple comparisons with a Benjamini-Hochberg adjustment and Wilcoxon rank sum test (for *Buried Shell* bottom specifically) confirmed the specifics of which treatment comparisons were significant (p<0.05) at the different time points (Table 2-3). Table 4 is a list of summary statistics that support Dunn's test and show which treatments had higher mean percent survival. The only treatments that were significantly different from each other on the mud sand bottom type at Time Point One were shell (123.5% ± 14.91 (SE)), with the highest mean survival percentage and control (66.42% ± 9.27 (SE)) having the lowest. On the mud shell bottom at Time Point One, the harrow shell treatment (47.26% ± 5.28(SE)) had highest survival, which was significantly different from the shell treatment (29.97% ± 5.26 (SE)). At Time Point Two on the mud sand bottom, the harrow treatment (96.42% ± 13.066 (SE)) had significantly higher survival than control (50.00% ± 6.92 (SE)), shell (58.57% ± 12.20(SE)), and harrow shell (41.10% ± 11.42(SE)). Survival on the harrow treatment (40.35% ± 7.91 (SE)) was significantly higher than harrow shell (11.14% ± 2.31(SE)) on the mud shell bottom at Time Point Two. On the buried shell bottom at Time Point Two, the harrow treatment (19.87% ± 2.77 (SE)) had significantly higher survival than the control (10.10% ± 2.16 (SE)). The only significant treatment comparisons at Time Point Three were on mud shell bottom were control (38.42% ± 4.074 (SE)) having higher survival than shell (24.59% ± 3.40 (SE)).

Table 2. Summary of significant (p<0.05) Dunn's Kruskal-Wallis Multiple Comparison tests for comparison of mean percent survival of treatments on different bottom types.

Bottom Type	Time Point	kw.p.value	Comparison	Z	P.unadj	P.adj
Mud Sand	1	0.011	Control - Harrow	-0.79	4.31E-01	0.86
Mud Sand	1	0.011	Control - Harrow Shell	-1.61	1.06E-01	0.32
Mud Sand	1	0.011	Harrow - Harrow Shell	-0.39	6.93E-01	0.69
Mud Sand	1	0.011	Control - Shell	-3.31	9.43E-04	0.0057
Mud Sand	1	0.011	Harrow - Shell	-1.63	1.02E-01	0.41
Mud Sand	1	0.011	Harrow Shell - Shell	-1.69	9.06E-02	0.45
Mud Shell	1	0.035	Control - Harrow	0.040	9.68E-01	0.97
Mud Shell	1	0.035	Control - Harrow Shell	-2.00056	4.54E-02	0.18
Mud Shell	1	0.035	Harrow - Harrow Shell	-2.040	4.13E-02	0.21
Mud Shell	1	0.035	Control - Shell	0.80	4.25E-01	1.00
Mud Shell	1	0.035	Harrow - Shell	0.76	4.48E-01	0.90
Mud Shell	1	0.035	Harrow Shell - Shell	2.80	5.13E-03	0.031
Mud Sand	2	0.00026	Control - Harrow	-2.41	1.61E-02	0.064
Mud Sand	2	0.00026	Control - Harrow Shell	1.86	6.28E-02	0.19
Mud Sand	2	0.00026	Harrow - Harrow Shell	4.26	1.98E-05	0.00012
Mud Sand	2	0.00026	Control - Shell	0.55	5.86E-01	0.59
Mud Sand	2	0.00026	Harrow - Shell	2.95	3.17E-03	0.016
Mud Sand	2	0.00026	Harrow Shell - Shell	-1.31	1.88E-01	0.38
Mud Shell	2	0.0092	Control - Harrow	-2.12	3.39E-02	0.17
Mud Shell	2	0.0092	Control - Harrow Shell	1.23	2.18E-01	0.44
Mud Shell	2	0.0092	Harrow - Harrow Shell	3.35	7.97E-04	0.0048
Mud Shell	2	0.0092	Control - Shell	-0.23	8.20E-01	0.82
Mud Shell	2	0.0092	Harrow - Shell	1.89	5.83E-02	0.23

Mud Shell	2	0.0092	Harrow Shell - Shell	-1.46	1.44E-01	0.43
Mud Shell	3	0.033	Control - Harrow	1.11	2.65E-01	0.80
Mud Shell	3	0.033	Control - Harrow Shell	2.16	3.07E-02	0.15
Mud Shell	3	0.033	Harrow - Harrow Shell	1.047	2.95E-01	0.59
Mud Shell	3	0.033	Control - Shell	2.74	6.08E-03	0.037
Mud Shell	3	0.033	Harrow - Shell	1.62	1.03E-01	0.41
Mud Shell	3	0.033	Harrow Shell - Shell	0.58	5.60E-01	0.56

Table 3. Wilcoxon Rank Sum Test summary for the comparison of treatments on the Buried Shell bottom type.

Bottom Type	**Time Point**	**P-value**	**Comparison**
Buried Shell	2	0.00049	Control-Harrow

Table 4. Summary statistics for percent survival of the three different bottom types and their respective treatments.

Bottom Type	**Treatment**	**Mean**	**SD**	**SQN**	**SE**	**Time Point**
Buried Shell	Control	21.22	22.69	7.62	2.98	1
Buried Shell	Harrow	22.98	25.13	7.68	3.27	1
Mud Sand	Control	66.42	50.75	5.48	9.27	1
Mud Sand	Harrow	72.07	34.91	3.32	10.53	1
Mud Sand	Harrow Shell	79.50	44.76	5.48	8.17	1
Mud Sand	Shell	123.56	81.68	5.48	14.91	1
Mud Shell	Control	32.66	23.85	5.48	4.35	1
Mud Shell	Harrow	35.73	32.37	5.48	5.91	1
Mud Shell	Harrow Shell	47.26	28.93	5.48	5.28	1
Mud Shell	Shell	29.97	28.81	5.48	5.26	1
Buried Shell	Control	10.10	16.74	7.75	2.16	2
Buried Shell	Harrow	19.87	21.45	7.75	2.77	2
Mud Sand	Control	50.00	37.89	5.48	6.92	2
Mud Sand	Harrow	96.42	71.56	5.48	13.066	2
Mud Sand	Harrow Shell	41.10	62.53	5.48	11.42	2
Mud Sand	Shell	58.57	66.80	5.48	12.20	2
Mud Shell	Control	25.74	35.26	5.48	6.44	2
Mud Shell	Harrow	40.35	43.32	5.48	7.91	2
Mud Shell	Harrow Shell	11.14	12.66	5.48	2.31	2
Mud Shell	Shell	19.98	20.065	5.48	3.66	2
Buried Shell	Control	7.69	10.44	7.75	1.35	3
Buried Shell	Harrow	6.41	6.48	6.71	0.97	3
Mud Sand	Control	61.42	47.99	5.48	8.76	3

Mud Sand	Harrow	53.57	33.64	5.48	6.14	3
Mud Sand	Harrow Shell	56.81	50.079	5.48	9.14	3
Mud Sand	Shell	53.57	43.13	5.48	7.87	3
Mud Shell	Control	38.42	22.31	5.48	4.074	3
Mud Shell	Harrow	33.045	23.59	5.48	4.31	3
Mud Shell	Harrow Shell	26.90	19.93	5.48	3.64	3
Mud Shell	Shell	24.59	18.60	5.48	3.40	3

Although there are significant treatments on all three bottom types at each time point, there is no collective trend that shows one rehabilitation treatment causing higher survival on any bottom type than the rest over time (Figure 12, 13 and 14).

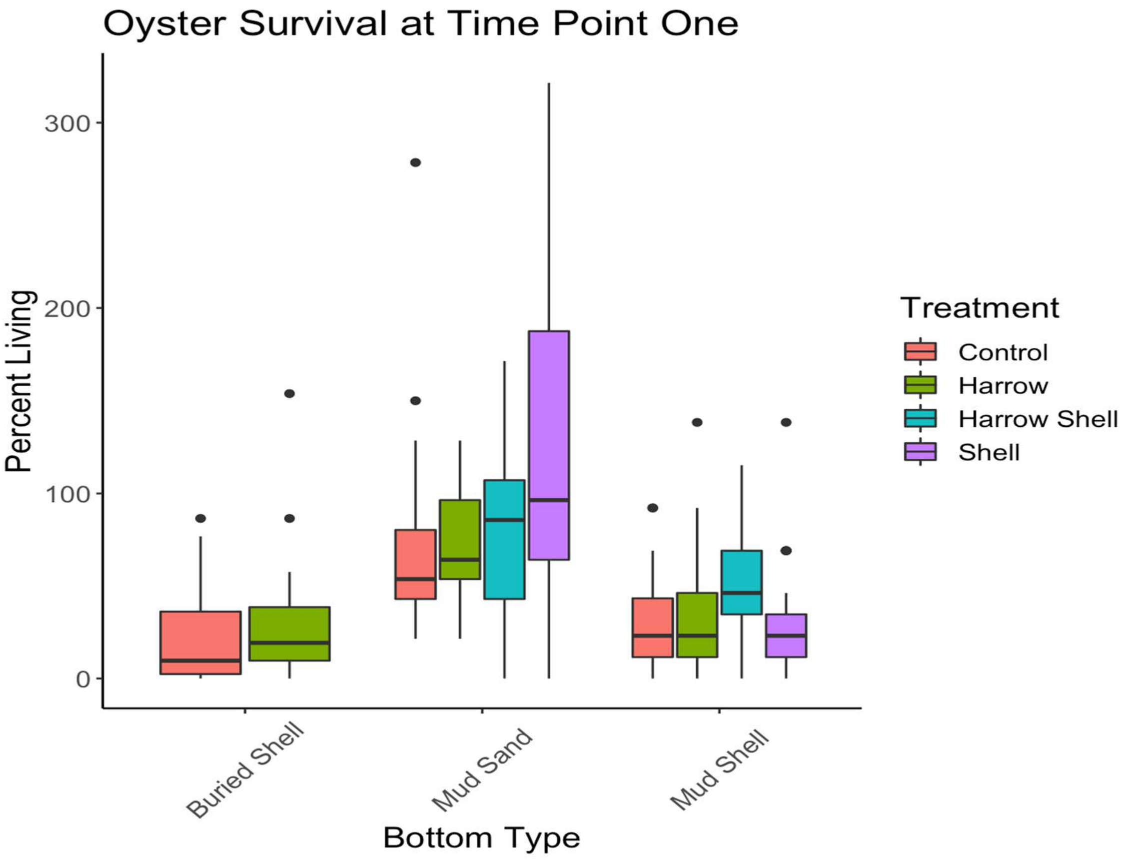

Figure 12. The percentage of living oysters on each bottom type per each treatment at the first time point.

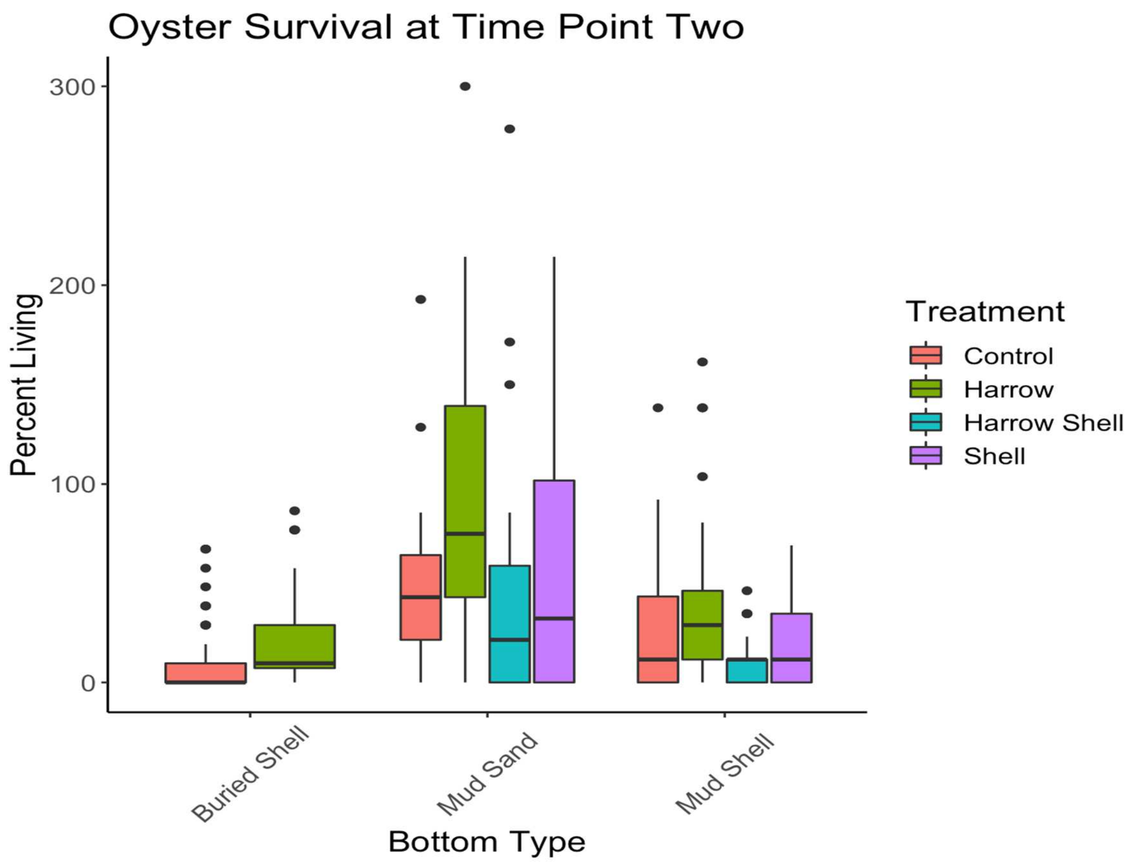

Figure 13. The percentage of living oysters on each bottom type per each treatment at the second time point.

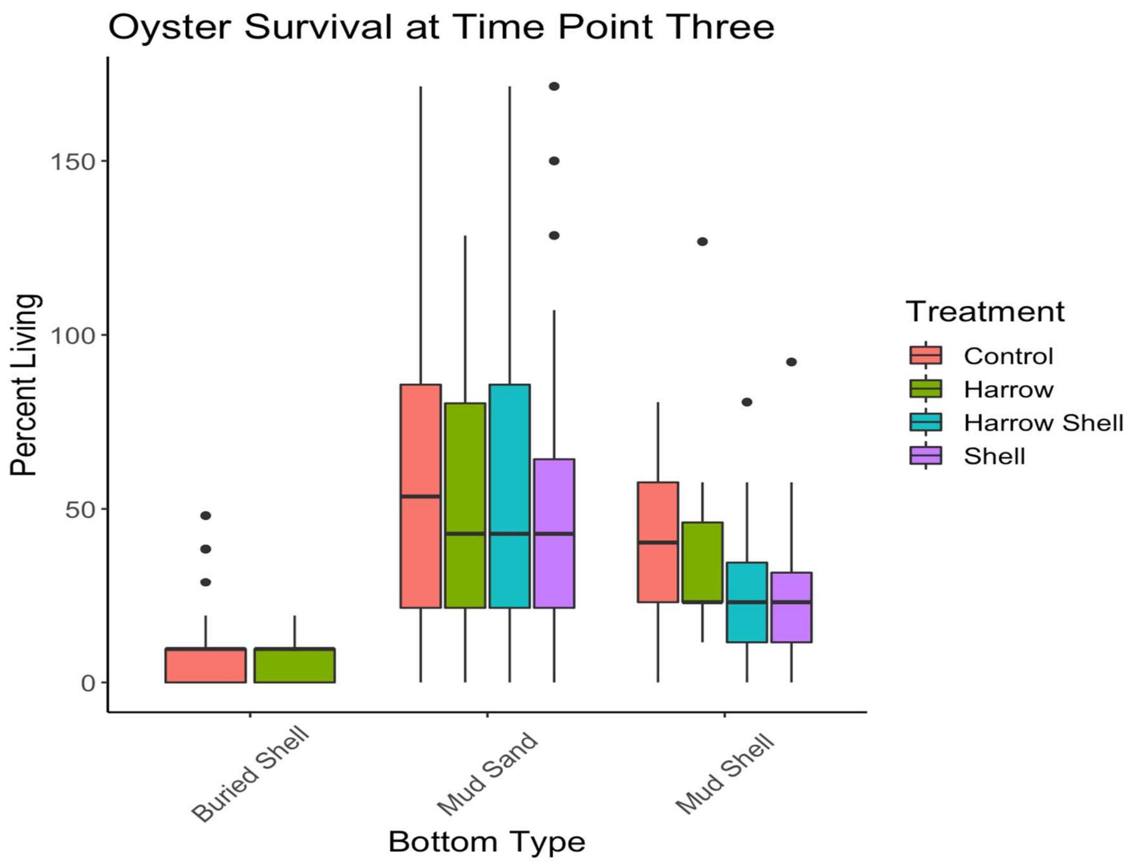

Figure 14. The percentage of living oysters on each bottom type per each treatment at the third time point.

Survival: Bottom Type

A Kruskal-Wallis rank sum test revealed that survival based solely on bottom condition was significantly different between the bottom types at time point three (p-value <0.05). The Dunn's test of multiple comparison with a Benjamini-Hochberg adjustment confirmed different mean percent survival rates from each bottom (p-value <0.05) (Table 5); mud sand had 56.34% (± 3.99 (SE)) survival, while mud shell had 30.74% (± 1.97 (SE)) and buried shell had 7.14% (± 0.873 (SE)) (Table 6). The best bottom type for oyster survival in this particular study was mud sand, with mud shell having intermediate survival and buried shell having the poorest (Figure 15).

Table 5. Summary of Dunn's test of multiple comparisons at time point three.

Comparison	Z	P.unadj	P.adj
Buried Shell-Mud Sand	-11.88	1.50e-32	4.50e-32
Buried Shell-Mud Shell	-9.25	2.25e-20	3.37e-20
Mud Sand-Mud Shell	2.72	6.47e-03	6.47e-03

Table 6. Summary statistics for bottom type only at time point three.

Bottom Type	Mean	SD	SQN	SE	Time Point
Buried Shell	7.14	8.95	10.25	0.87	3
Mud Sand	56.34	43.73	10.95	3.99	3
Mud Shell	30.74	21.62	10.95	1.98	3

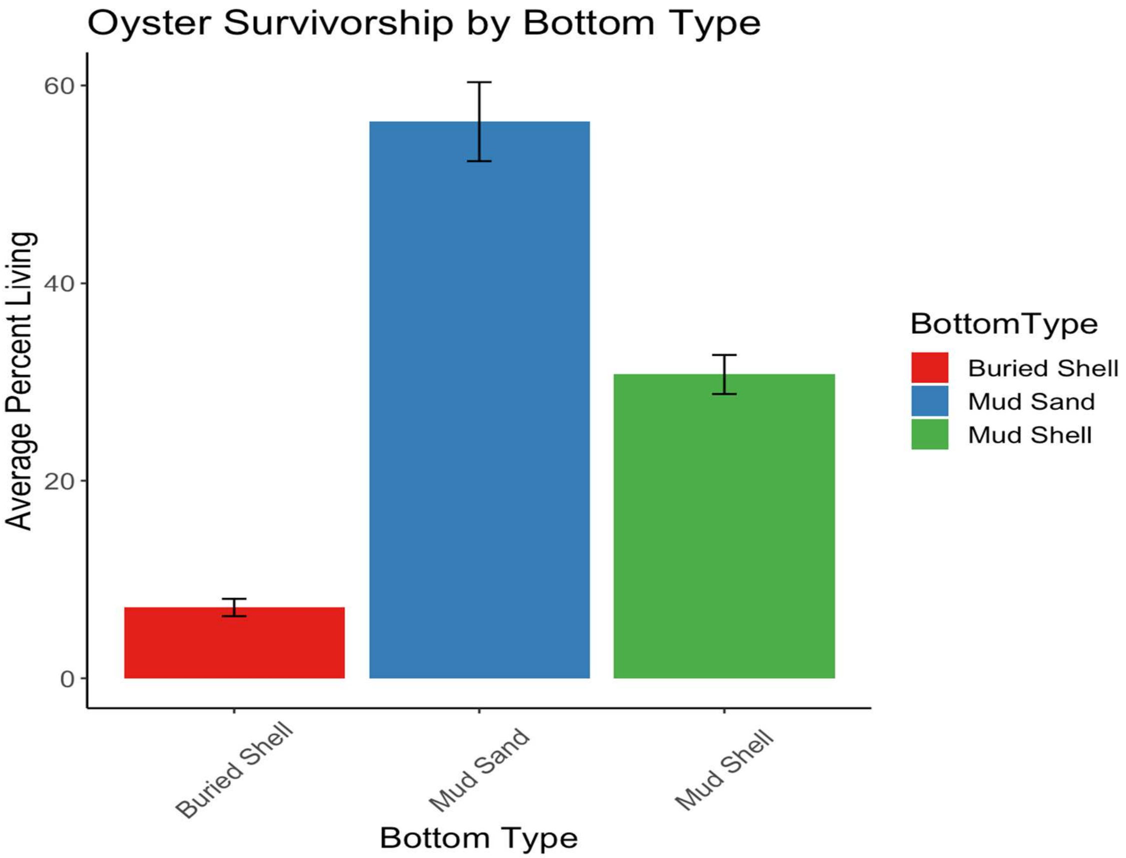

Figure 15. Bar plot representing the survival percentage of oysters from the three different bottom types at time point three.

Size

The effect of treatments had on the size (mm) of the oysters on each bottom type varied between the different sampling time points. The Kruskal-Wallis Rank Sum test ($\alpha<0.05$) revealed which bottom types had treatments that were significantly differently from each other (p-value<0.05) (Table 7). This analysis reveals the inconsistency of treatment effect on each of the bottom types. At Time Point One, mud sand and mud shell both had significant treatment effects, while at Time Point Two only mud sand was affected and at the last time point mud shell was the single bottom type with effective treatments. There were some treatments that were effective at certain times (Figure 16, 17 and 18) and Dunn's test of multiple comparisons (Table 8) revealed which treatment comparisons were effective on which bottom type at each of the time points. The summary statistics of the average size is shown in Table 9 to pinpoint the significant treatment from the comparisons.

At the first time point on the mud sand bottom, the control (24.3 ± 0 0.74 (SE)) had significantly smaller sized oysters than the shell (27.08 ± 0.47 (SE)), and harrow shell (26.51 ± 0.61 (SE)) treatments. On the mud shell bottom at the first time point the oysters on harrow shell (20.81 ± 0.58 (SE)) were significantly smaller than the control (23.21 ± 0.78 (SE)). At Time Point Two, the control (30.43 ± 1.16 (SE)) had significantly smaller oysters than harrow (35.73 ± 0.80 (SE)), harrow shell (35.43 ± 1.18 (SE)), and shell (36.73 ± 1.045 (SE)) on mud sand bottom. At the last time point, the mud shell bottom had smaller oysters on the harrow shell treatment (44.29 ± 1.010 (SE)) versus the control (50.32 ± 1.041(SE)), as well as a significant difference between the harrow (49.77 ± 0.95 (SE)) and the harrow shell (44.29 ± 1.010 (SE)). In the end, there was no single treatment that was more effective on any bottom type than the others pertaining to the size of the oysters over time (Figure 19).

Table 7. Summary of Kruskal-Wallis Rank Sum Test for mean size (mm) on bottom type.

Bottom Type	**Time Point**	**Rank Sum Statistic**	**P-Value**	**FDR**
Mud Sand	1	12.15	0.0069	0.021
Mud Shell	1	10.41	0.0154	0.035
Mud Sand	2	16.065	0.0011	0.0050
Mud Shell	3	17.23	0.00064	0.0050

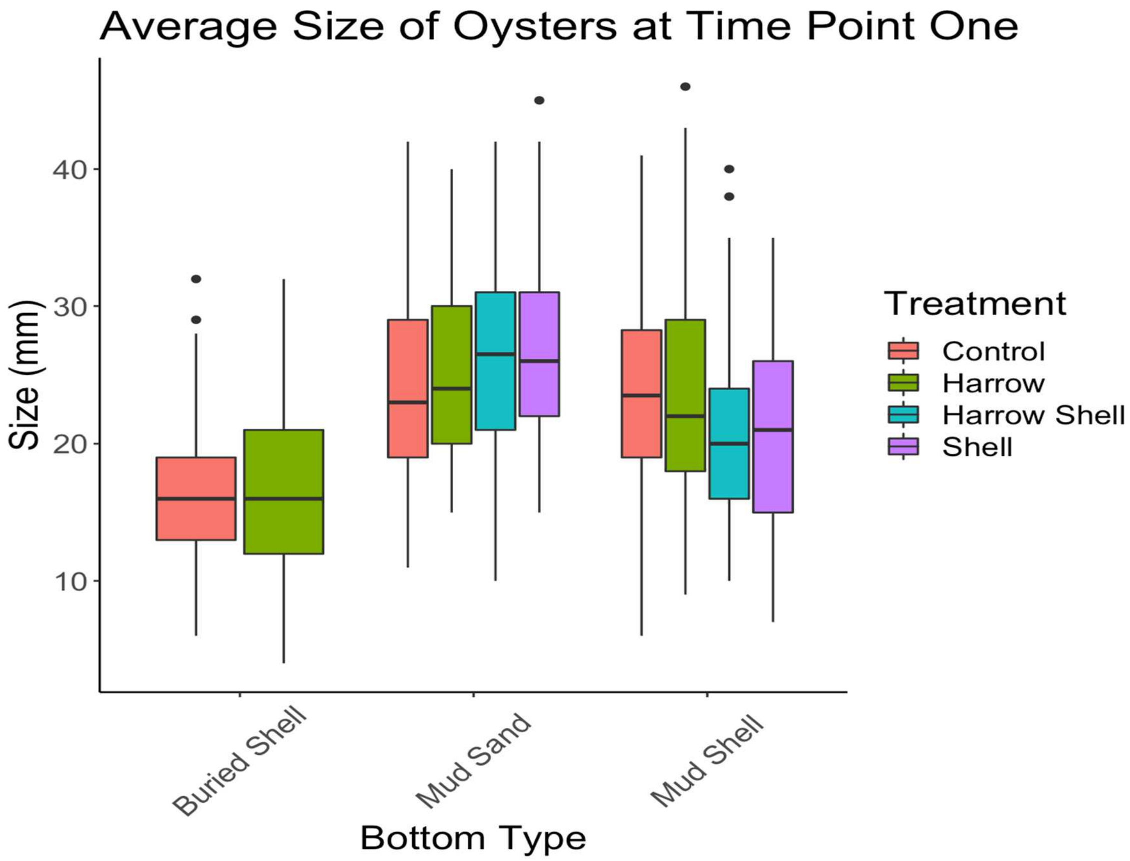

Figure 16. The size (mm) of living oysters on each bottom type per each treatment at the first time point.

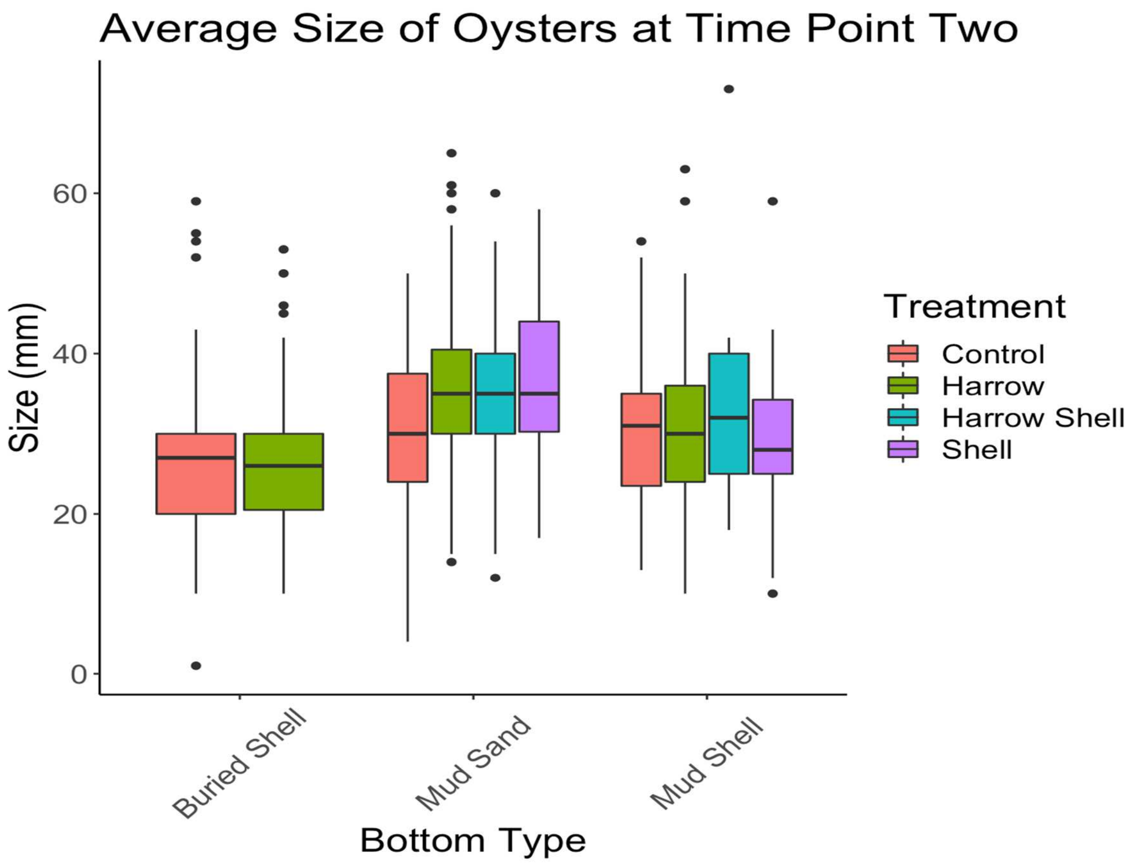

Figure 17. The size (mm) of living oysters on each bottom type per each treatment at the second time point.

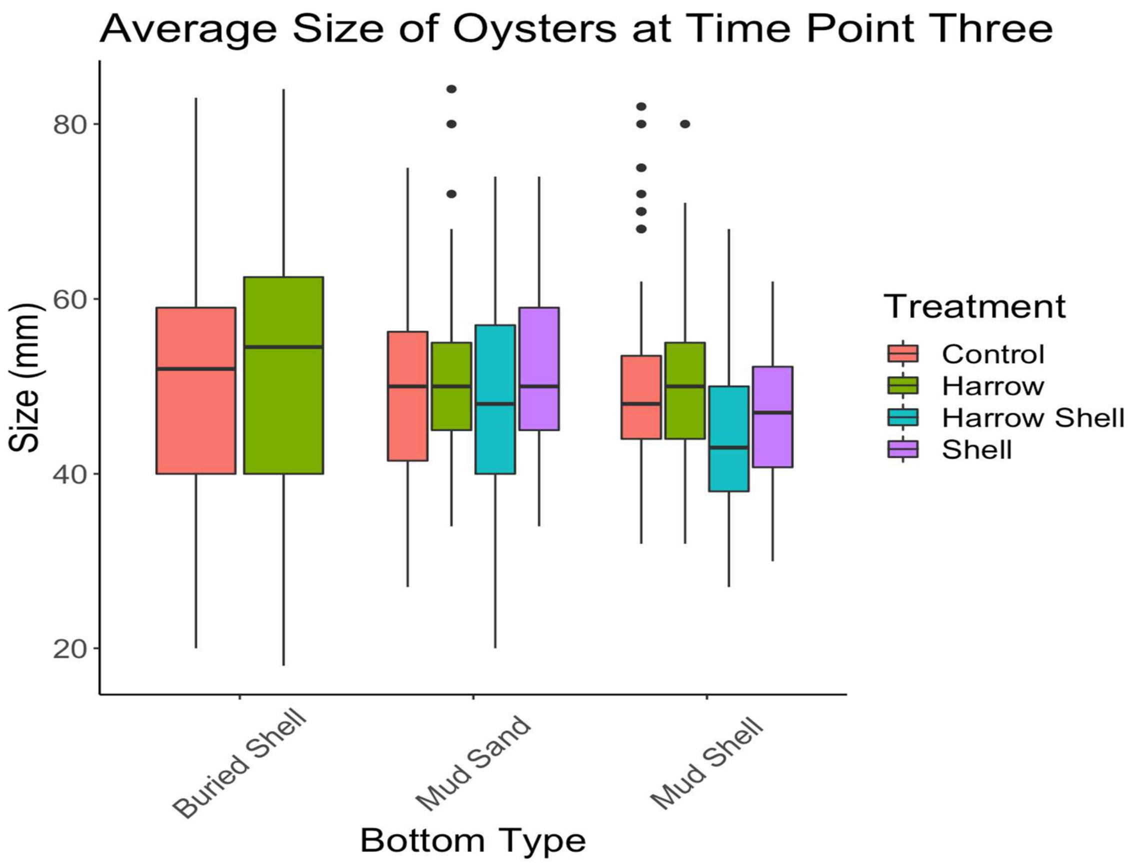

Figure 18. The size (mm) of living oysters on each bottom type per each treatment at the third time point.

Table 8. Summary of significant (p<0.05) Dunn's Kruskal-Wallis Multiple Comparison tests for comparison of mean oyster size between treatments on different bottom types.

Bottom Type	**Time Point**	**kw.p.value**	**Comparison**	**Z**	**P.unadj**	**P.adj**
Mud Sand	1	0.0069	Control - Harrow	-0.68	0.50	0.10
Mud Sand	1	0.0069	Control - Harrow Shell	-2.61	0.0091	0.046
Mud Sand	1	0.0069	Harrow - Harrow Shell	-1.18	0.24	0.72
Mud Sand	1	0.0069	Control - Shell	-3.26	0.0011	0.0066
Mud Sand	1	0.0069	Harrow - Shell	-1.59	0.11	0.45
Mud Sand	1	0.0069	Harrow Shell - Shell	-0.61	0.54	0.54
Mud Shell	1	0.015	Control - Harrow	0.56	0.58	0.58
Mud Shell	1	0.015	Control - Harrow Shell	2.80	0.0050	0.03
Mud Shell	1	0.015	Harrow - Harrow Shell	2.37	0.018	0.090
Mud Shell	1	0.015	Control - Shell	1.92	0.055	0.22
Mud Shell	1	0.015	Harrow - Shell	1.48	0.14	0.42
Mud Shell	1	0.015	Harrow Shell - Shell	-0.60	0.55	1
Mud Sand	2	0.0011	Control - Harrow	-3.47	0.00052	0.0026
Mud Sand	2	0.0011	Control - Harrow Shell	-2.89	0.0038	0.015
Mud Sand	2	0.0011	Harrow - Harrow Shell	-0.015	0.99	0.99
Mud Sand	2	0.0011	Control - Shell	-3.60	0.00031	0.0019
Mud Sand	2	0.0011	Harrow - Shell	-0.56	0.58	1
Mud Sand	2	0.0011	Harrow Shell - Shell	-0.44	0.66	1
Mud Shell	3	0.00064	Control - Harrow	-0.23	0.81	0.81
Mud Shell	3	0.00064	Control - Harrow Shell	3.57	0.00036	0.0018
Mud Shell	3	0.00064	Harrow - Harrow Shell	3.67	0.00024	0.0016
Mud Shell	3	0.00064	Control - Shell	1.49	0.14	0.27
Mud Shell	3	0.00064	Harrow - Shell	1.65	0.10	0.29
Mud Shell	3	0.00064	Harrow Shell - Shell	-1.77	0.076	0.30

Table 9. Summary statistics for the average size (mm) of living oysters on the different bottom types per treatment at the three time points.

Bottom Type	**Treatment**	**Mean**	**SD**	**SQN**	**SE**	**Time Point**
Buried Shell	Control	16.36	4.66	11.22	0.42	1
Buried Shell	Harrow	16.81	5.68	11.83	0.48	1
Mud Sand	Control	24.30	7.16	9.70	0.74	1
Mud Sand	Harrow	25.22	6.57	6.082	1.080	1
Mud Sand	Harrow Shell	26.51	7.14	11.66	0.61	1
Mud Sand	Shell	27.08	6.18	13.00	0.47	1
Mud Shell	Control	23.21	7.15	9.167	0.78	1
Mud Shell	Harrow	23.038	6.99	10.20	0.69	1

Mud Shell	Harrow Shell	20.81	6.46	11.09	0.58	1
Mud Shell	Shell	21.00	6.92	8.49	0.82	1
Buried Shell	Control	27.21	10.93	7.94	1.38	2
Buried Shell	Harrow	26.33	7.97	11.27	0.71	2
Mud Sand	Control	30.43	9.49	8.19	1.16	2
Mud Sand	Harrow	35.73	9.42	11.79	0.80	2
Mud Sand	Harrow Shell	35.43	8.95	7.62	1.18	2
Mud Sand	Shell	36.73	9.46	9.06	1.045	2
Mud Shell	Control	29.70	8.76	8.19	1.070	2
Mud Shell	Harrow	30.33	9.21	10.25	0.90	2
Mud Shell	Harrow Shell	32.48	10.49	5.39	1.95	2
Mud Shell	Shell	28.79	8.93	7.21	1.24	2
Buried Shell	Control	50.73	14.07	7.00	2.011	3
Buried Shell	Harrow	52.22	15.11	5.66	2.67	3
Mud Sand	Control	49.24	10.38	9.38	1.11	3
Mud Sand	Harrow	51.00	8.92	8.66	1.031	3
Mud Sand	Harrow Shell	47.74	11.33	9.00	1.26	3
Mud Sand	Shell	51.64	9.55	8.66	1.10	3
Mud Shell	Control	50.32	10.41	10.00	1.041	3
Mud Shell	Harrow	49.77	8.78	9.27	0.95	3
Mud Shell	Harrow Shell	44.29	9.20	8.37	1.010	3
Mud Shell	Shell	47.050	7.27	7.75	0.94	3

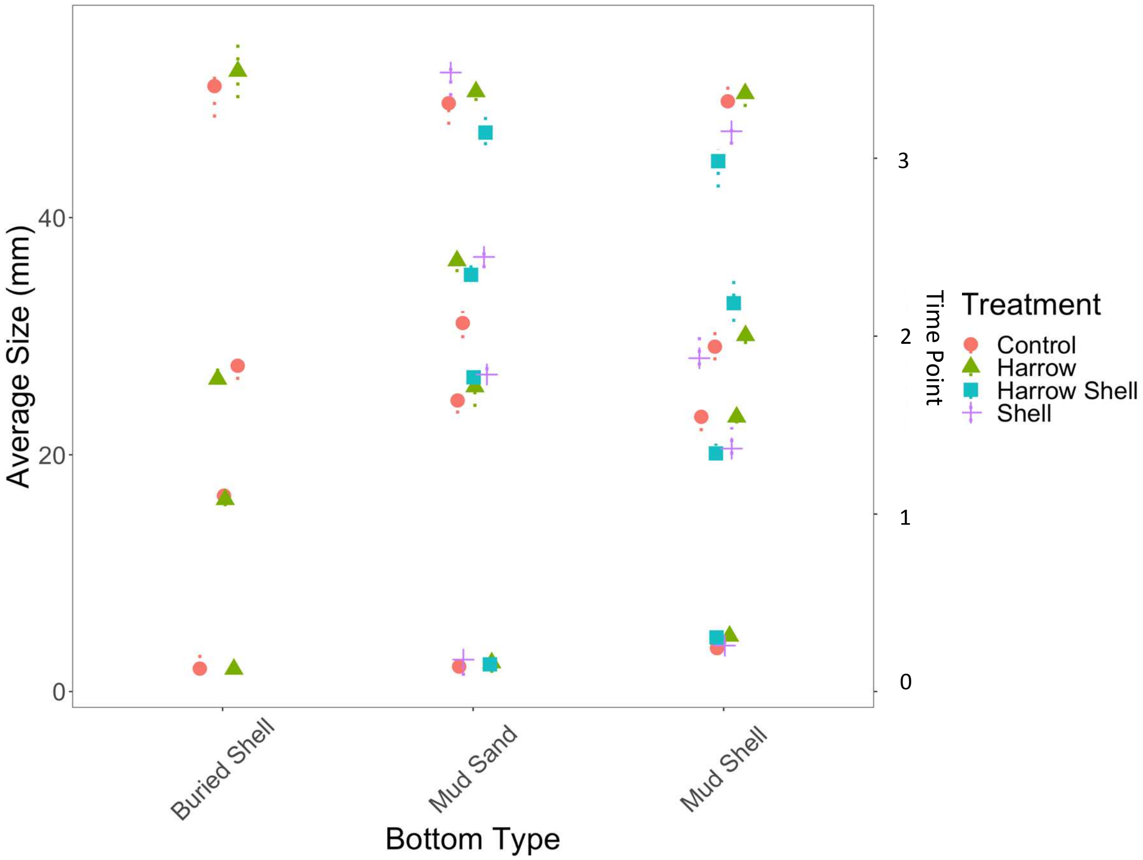

Figure 19. Scatterplot indicating the average size (mm) of oysters at a given time point based on treatment and bottom type.

Ground Truthing Surveys

The quantitative data from each bottom survey had high variability. Due to the high variability between the different treatments and the insignificance of treatment effect on both survival and size of oysters, the surveys were analyzed for trends based on the bottom type as a whole.

Mud Sand (Table 10 & 11)

Overall, the mud sand bottom type had a lot of visible spat on shell across all four treatments. Both surveys noted a good amount of visible exposed shell. The hardness of the bottom as measured by hand penetration varied within each treatment section; the October 2018 survey had mostly hand (20 cm) penetration followed by much of the hard bottom (0 cm) sampled on April 2019. The first substrate observed on both surveys was mostly loose shell and patchiness was noted throughout. Although not all treatment sections received a shell substrate layer, it was evident shell had been pushed over to other treatment sections by physical processes such as currents. On the September 2019 sampling, the divers were unable to complete the ground truthing survey for the mud sand bottom type due to an equipment malfunction.

Table 10. Ground truthing survey for the seeded and non-seeded sides of the mud sand plot for the October 2018 sampling.

Big Annemessex River	**10/9/18**	**Mud Sand Plot**

Transect	Point	Exposed Shell	Substrate 1	Substrate 2	Substrate 3	Penetration	Comments
Control	6	Fully	Loose Shell			Hand (20 cm)	Mostly spat on shell
Control	9	Very Little	Mud			Knuckle (5 cm)	
Control	10	Some	Loose Shell	Loose Shell	Mud	Knuckle (5 cm)	
Shell	16	Fully	Loose Shell	Shell Hash		Hand (20 cm)	
Shell	17	Fully	Loose Shell			Hand (20 cm)	
Shell	22	Fully	Loose Shell			Hand (20 cm)	
Harrow Shell	30	Fully	Loose Shell			Hand (20 cm)	
Harrow Shell	35	Some	Loose Shell	Mud		Hand (20 cm)	
Harrow Shell	37	Some	Loose Shell	Mud		Hand (20 cm)	
Harrow	43	Very Little	Mud	Loose Shell		Knuckle (5 cm)	
Harrow	45	Zero	Mud			Hand (20 cm)	
Harrow	49	Zero	Mud			Hand (20 cm)	

Table 11. Ground truthing survey for the seeded and non-seeded sides of the mud sand plot for the April 2019 sampling.

Big Annemessex	**4/30/2019**	**Mud Sand Plot**

Transect	**Point**	**Exposed Shell**	**Substrate 1**	**Substrate 2**	**Substrate 3**	**Penetration**	**Comments**
Control	14	None	Mud	Shell Hash	Loose Shell	Finger (10 cm)	
Control	17	Very Little	Loose Shell	Shell Hash		Finger (10 cm)	
Control	18	Very Little	Mud	Loose Shell		Finger (10 cm)	
Shell	27	Fully	Loose Shell			Hard (0 cm)	
Shell	29	Fully	Loose Shell			Hard (0 cm)	
Shell	30	Fully	Loose Shell			Hard (0 cm)	
Harrow Shell	33	Fully	Loose Shell	Shell Hash		Hard (0 cm)	
Harrow Shell	34	Some	Loose Shell	Shell Hash	Mud	Knuckle (5 cm)	
Harrow Shell	38	Fully	Loose Shell			Hard (0 cm)	
Harrow	44	Some	Loose Shell	Mud	Shell Hash	Knuckle (5 cm)	
Harrow	47	Fully	Loose Shell			Hard (0 cm)	
Harrow	45	Some	Loose Shell	Mud	Shell Hash	Knuckle (5 cm)	Lots of Spat

Mud Shell (Tables 12-14)

The majority of the mud sand bottom had areas where there was some shell exposure or full shell exposure at the April and September 2019 surveys. The main first substrate across all treatments was loose shell, likely from planted shell with spat mortality or from shell blown over from other treatments. The bottom trend across all three surveys was either knuckle (5 cm) penetration or hard bottom (0 cm), but it was also noted there was a layer of silt and sediment on top of the first layer of loose shell. Patchiness of shell distribution was also noted throughout.

Table 12. Ground truthing survey for the seeded and non-seeded sides of the mud shell plot for the October 2018 sampling.

Big Annemessex River	**10/9/2018**	**Mud Shell Plot**

Transect	Point	Exposed Shell	Substrate 1	Substrate 2	Substrate 3	Penetration	Comments
Control	1	Zero	Mud			Knuckle (5 cm)	Layer of silt on top of shells
Control	13	Some	Loose Shell	Mud		Knuckle (5 cm)	
Control	23	Very Little	Mud	Loose Shell		Knuckle (5 cm)	
Shell	28	Some	Mud			Knuckle (5 cm)	
Shell	36	Fully	Loose Shell			Hand (20 cm)	Shell was spat covered
Harrow	64						
Harrow	66	Some					

Table 13. Ground truthing survey for the seeded and non-seeded sides of the mud shell plot for the April 2019 sampling.

Big Annemessex River	**4/30/19**	**Mud Shell Plot**

Transect	Point	Exposed Shell	Substrate 1	Substrate 2	Substrate 3	Penetration	Comments
Control	20	Fully	Loose Shell	Shell Hash		Hard (0 cm)	
Control	24	Some	Loose Shell	Mud	Shell Hash	Knuckle (5 cm)	
Control	32	Some	Loose Shell	Mud	Shell Hash	Knuckle	No adults, just spat/lots of crabs
Shell	38	Fully	Loose Shell	Shell Hash	Mud	Hard (0 cm)	
Shell	40	Fully	Loose Shell	Shell Hash	Mud	Hard (0 cm)	
Shell	42	Fully	Loose Shell	Mud	Shell	Hard (0 cm)	
Harrow Shell	46	Fully	Shell			Hard (0 cm)	
Harrow Shell	52	Fully	Loose Shell	Shell Hash	Mud	Hard (0 cm)	Lots of crabs
Harrow Shell	58	Fully	Loose Shell			Hard (0 cm)	Silt layer on top
Harrow	69	Very Little	Mud	Loose Shell		Finger (10 cm)	
Harrow	73	Fully	Loose Shell	Mud		Hard (0 cm)	Lots of crabs
Harrow	75	Fully	Loose Shell	Shell Hash		Hard (0 cm)	

Table 14. Ground truthing survey for the seeded and non-seeded sides of the mud shell plot for the September 2019 sampling.

Big Annemessex River	**9/29/2019**	**Mud Shell Plot**

Transect	**Point**	**Exposed Shell**	**Substrate 1**	**Substrate 2**	**Substrate 3**	**Penetration**	**Comments**
Control	1	Zero	Mud				
Control	7	Some	Loose Shell	Mud	Shell Hash	Hard (0 cm)	½ inch sediment on top
Control	9	Very	Oyster	Mud		Finger (10 cm)	
Control	11	Zero	Mud				
Shell							
Shell	26	Very	Loose Shell			Hard (0 cm)	Recent Predation
Harrow Shell	32	Some	Loose Shell	Mud		Hard (0 cm)	
Harrow Shell	44	Some	Loose Shell	Oysters		Hard (0 cm)	
Harrow Shell	45	Fully	Loose Shell	Oysters		Hard (0 cm)	
Harrow	52	Some	Loose Shell	Mud		Knuckle (5 cm)	Lot of loose shell
Harrow	59	Some					
Harrow	60						½ inch sediment

Buried Shell (Tables 15-17)

The exposed shell on bottom of the buried shell plot was extremely variable; coverage ranged from being fully exposed in some areas, with patchy and unexposed areas as well. The majority of the plot had a dominant substrate of loose shell, but there was patchiness and ~ 1.27 cm of mud covering that top layer, which was noted on each of the three surveys. The penetration trend on bottom could be explained as mainly knuckle (5 cm) finger (10 cm), or hand (20 cm).

Table 15. Ground truthing surveys for the seeded and non-seeded sides of the buried shell plot for the October 2018 sampling.

Big Annemessex River	**10/9/18**	**Buried Shell Plot**

Transect	**Point**	**Exposed Shell**	**Substrate 1**	**Substrate 2**	**Substrate 3**	**Penetration**	**Comments**
Control	15	Full	Loose Shell	Mud		Hand (0 cm)	Mud on top
Control	17	Some	Loose Shell	Mud		Knuckle (5 cm)	
Control	20	Some	Loose Shell	Mud	Shell Hash	Knuckle (5 cm)	
Control	26	Full	Loose Shell			Hand (0 cm)	
Control	28	Some	Loose Shell	Mud	Shell Hash	Knuckle (5 cm)	
Control	29	Full	Loose Shell			Hand (0 cm)	
Harrow	33	Very Little	Mud	Loose Shell		Finger (10 cm)	Mud on top
Harrow	34	Very Little	Mud	Loose Shell		Finger (10 cm)	
Harrow	36	Full	Loose Shell			Hand (0 cm)	
Harrow	40	Full	Loose Shell			Hand (0 cm)	
Harrow	41	Full	Loose Shell			Hand (0 cm)	
Harrow	42	Full	Loose Shell			Hand (0 cm)	

Table 16. Ground truthing surveys for the seeded and non-seeded sides of the buried shell plot for the April 2019 sampling.

Big Annemessex	**4/30/2019**	**Buried Shell**

Transect	**Point**	**Exposed Shell**	**Substrate 1**	**Substrate 2**	**Substrate 3**	**Penetration**	**Comments**
Control	10	Fully	Loose Shell	Mud		Hard (0 cm)	*transect ran close to the edge of where the oysters were planted
Control	13	Some	Loose Shell	Mud		Knuckle (5 cm)	
Control	15	Some	Loose Shell	Mud	Shell Hash	Knuckle (5 cm)	
Control	21	Fully	Loose Shell	Mud		Hard (0 cm)	
Control	22	Some	Loose Shell	Mud		Knuckle (5 cm)	
Control	26	Fully	Loose Shell	Mud		Hard (0 cm)	
Harrow	30	Some	Loose Shell	Mud	Shell Hash	Knuckle (5 cm)	
Harrow	32	Fully	Loose Shell	Mud		Hard (0 cm)	
Harrow	34	Some	Loose Shell	Mud	Shell Hash	Knuckle (5 cm)	
Harrow	38	Very Little	Mud	Loose Shell		Finger (10 cm)	Mud on top
Harrow	41	Fully	Loose Shell	Mud		Hard (0 cm)	
Harrow	43	Very Little	Mud	Loose Shell		Finger (10 cm)	

Table 17. Ground truthing surveys for the seeded and non-seeded sides of the buried shell plot for the September 2019 sampling.

Big Annemessex River	**9/29/2019**	**Buried Shell**

Transect	**Point**	**Exposed Shell**	**Substrate 1**	**Substrate 2**	**Substrate 3**	**Penetration**	**Comments**

Control	8	Very Little	Mud	Loose Shell		Finger (10 cm)	
Control	10	Very Little	Loose Shell				
Control	12						
Shell	15	Some	Loose Shell	Mud			
Shell	17	Very Little	Mud	Loose Shell	Shell Hash	Finger (10 cm)	
Shell	20	Fully	Loose Shell			Hard (0 cm)	
Harrow Shell	26	Fully	Loose Shell			Hard (0 cm)	½ inch mud
Harrow Shell	27	Some	Loose Mud	Shell Hash		Knuckle (5 cm)	
Harrow Shell	32	Fully		Shell		Hard (0 cm)	
Harrow	36	Some	Shell	Mud		Knuckle (5 cm)	
Harrow	39						
Harrow	41	Fully	Loose Shell			Hard (0 cm)	

Discussion

Increased sedimentation and altered oyster reef habitats are challenges affecting the success of bottom oyster aquaculture (Colden et al 2017; Langland and Cronin, 2006; Wilber et al 2010). As oyster aquaculture increases in the state of Maryland, incorporating new sediment removal techniques is essential to refine best management practices (Department of Natural Resources, 2019; Webster 2009). The study was the first to examine different bottom types under a variety of rehabilitation treatments. The harrowing technique which disrupted and suspended the top layer of sediment was compared to a traditional shell technique where the bottom was hardened with oyster cultch. The data revealed that the treatments, whether singularly or in combination, were insignificant in respect to oyster size and survival. This confirms the null hypothesis that harrow treatments had no effect across all bottom types. However, pre-existing bottom type explained the observed variance when evaluating the percentage of oyster survival. The harrowing technique used in the experiment was a new, exploratory approach that had not been used for oyster bed rehabilitation in Chesapeake Bay. Across all three bottom types, there was no significant relationship between oyster survival and the harrowing application. The treatment was applied before the spat on shell were planted, in a manner that the suspended sediment in the water column drifted down current on ebb tide. Conversely, it is unknown what the rate of sedimentation is or how fast the current flows on these specific sites. While the sediment was initially removed from the lease, a layer of mud and sediment was observed on top of both the buried sand and mud shell plots at the October 2018 and April 2019 survey date. Both of these did poorer in respect to survival when compared to the mud sand site. The harrowing was initially able to remove the top sediment layer, but over time it may not have been enough to support the spat on shell planting on plots with a softer base layer or with a higher sedimentation rate. Even the combination treatment of harrow and shell provided no improvement in higher survival. Patchiness of shell distribution and settling of sediments may be part of the cause.

The current mapping tool used by Maryland farmers is an ArcGIS substrate layer that characterizes bottom type based on Maryland Department of Natural Resource's Acoustic Bay Bottom Survey (MDNR). This tool designates areas by cultch, mud, sand, leased bottom, hard bottom, mud with cultch, and sand with cultch. This data used in this assessment was collected between 1974 to 1983 and is likely to be outdated. This substrate layer tool is applicable to farmers planning their leases and would be extremely useful if updated. These results clearly show the need for a more efficient version of the Chesapeake Bay bottom layer to aid farmers in future aquaculture endeavors.

The traditional shell treatment portion of the experimental design was based on guidance on the stabilization of oyster grounds provided by Maryland Sea Grant Extension (Webster and Meritt, 1988). Customarily, a bed being stabilized gets sufficient shell planted on it to have a 2.5 cm layer above the base ground; the amount added is dependent on bottom texture. To cover one hectare with 2.5 cm of cultch requires 5,535 Maryland oyster bushels. This project used 9,900 bushels of shell to cover three, 30.2 ha sites with 7.6 cm of shell (2,200 bushels/0.4 ha-2.54cm x 7.6 cm @ 0.2 ha/site). Figure 10 depicts how the shell was deployed in a horizontal, clockwise manor with the shell being washed off the side of the vessel by a pressured water hose. The boat's track pattern was mapped with a GPS tracking tool to attempt even distribution within the two target areas. The same tracking was used when planting the spat on shell, but in a horizontal deployment across all target areas to more evenly distribute shell across treatments (Figure 10). Ground truthing surveys revealed a patchy distribution of cultch, which could have been an effect of shell deployment logistics. The spat on shell may not have been placed precisely on top of the cultch layer, which could have factored into the less successful bottom types that had softer sediment like the buried shell and mud shell bottoms. Shell addition can create complexity and harden a muddy bottom, but uneven distribution and low reef height may have limited success in this experiment (Powell, 2007, Colden et al, 2017). Wesson, Mann and Luckenbach (1999) stated that if reef profiles are too low, cultch restoration will be ineffective unless the entire reef elevation is raised.

While these results were surprising, it raises the question of how effective current shell treatment practices are when being used to harden beds in the bottom culture industry. Shell application rates vary in every setting due to differences in equipment, location and need. However, ensuring the evenness of the initial spread of cultch is difficult due to the lack of visibility, currents within the Chesapeake Bay, and maneuverability of the boat. Figure 20 is a picture of the pathway (dotted pink line) the boat took within the two plots that received shell treatment. Shell was only washed off when the boat was within the target areas. Although the dotted line covers a significant portion of the plot, untreated patches remained upon which spat on shell was planted. This study’s observations suggest that current shell application practices are unreliable and that further studies on shell density application in different settings are warranted within Chesapeake Bay (Webster and Meritt, 1988).

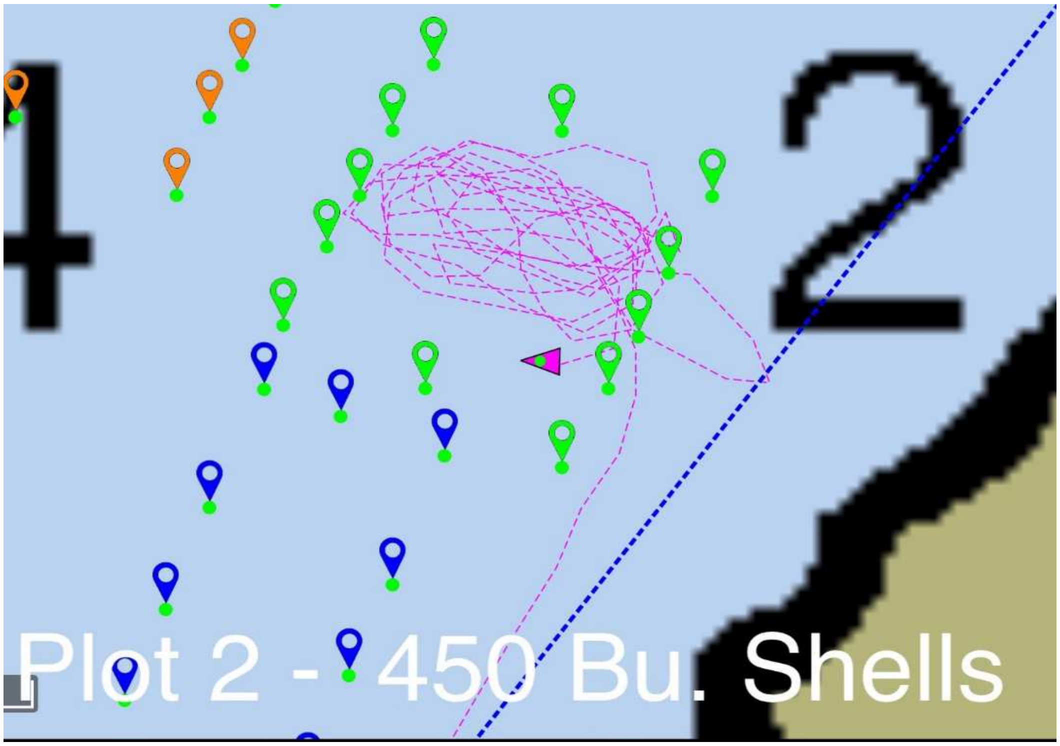

Figure 20. Picture of the vessel's pathway during the cultch planting process on the mud sand plot within the two shell treatment sites.

One of the main concerns for hardening beds with shell addition within aquaculture is the expense of shell substrate. Although these shell treatment results showed insignificant improvement, these observations broaden the range of knowledge available to those who practice bottom culture in Chesapeake Bay. Current practices push shell off the sides of vessels, creating a patchy distribution. This study planned for 7.62 centimeters of shell per 0.2 hectare, using ~9,900 bushels of shell. If the target bed area is minimized, the boat could navigate around the plot while planting cultch and have more track lines, higher substrate area coverage, thicker beds, and less patchiness (refer to Figure 10). Zacherl et al (2015) confirmed thickness was a significant element for restoring Olympia oyster beds in Newport, California. Deployment of a thickness of 4 cm versus 12 cm showed thicker beds had significant vertical relief from deleterious sedimentation (Zacherl et al, 2015). Ground truthing surveys from our experiment found that bed penetration never exceeded 10 cm and shell height was <10 cm in all surveyed areas. When comparing to Zacherl et al, our bed height did not meet the significant parameters stated in that study; if applicable to the culture of Eastern oysters, this may help explain the lack of increased performance on the shell treated plots.

Oyster reef height and complexity are driving factors for successful restoration, but also successful oyster bottom culture (Lenihan, 1999; Colden et al, 2017; Schulte et al, 2009). Reef profiles below a certain threshold are unable to support successful spat on shell plantings when sedimentation and burial are issues at a site (Luckenbach et al, 1999; Colden et al, 2017). However, even when leases are barren and covered in sediment, the bottom type has proven to be a critical factor for increased survival in this study and in unproductive areas of the Chesapeake Bay (Theuerkauf and Lipcius, 2016). In a habitat and substrate suitability study by Theuerkauf and Lipcius (2016), muddy sand, sand, and hard bottom are listed as highly suitable habitats because reefs are less likely to subside; this is consistent with our observations. Moving forward, emphasis on bottom type identification prior to spat on shell plantings will optimize culture practice until successful sediment removal techniques are developed.

Conclusions and Recommendations

While the results of this study do not support the hypothesis that harrowing benefits oyster culture success, the need for further research into sediment removal techniques to support more successful oyster bottom culture in Chesapeake Bay is warranted. Sediment removal by way of harrow is a new method with little research or application behind it. While this study showed insignificant improvement with the harrowing, different applications or designs for sediment removal could be the focus for new research endeavors. This study applied the harrow before spat on shell planting in June 2018, but visualizations of bottom changes were not assessed until the October 2018 sampling. Future researchers may have the bottom surveyed directly after application to make sure sufficient sediment removal occurs. Equipment that includes a rotor that intensely tills the area could have a different outcome. These results should not deter future research efforts, but instead encourage other investigations into sediment removal techniques that are specific to bottom culture. In addition, understanding the importance of exogenous sources of sediments in different parts of oyster leases may be important as well.

While the addition of shell substrate has been successful for oyster bed restoration, those methods are not practical in bottom culture (Powers et al, 2009; Schulte et al, 2009). Shell substrate planted for restoration is meant to remain there while several generations of spat on shell are planted on top. However, in bottom culture, the base layer of cultch and planted oysters will get removed every time a grower is ready to harvest. This presents the continual problem of barren grounds and low reef height every time a grower plants new spat on shell. The shell application from this study did not provide sufficient benefits and such applications warrant further exploration into shell substrate planting densities within smaller areas. This study revealed the downfalls of patchy substrate distribution. While adding more shell could potentially fill the gaps and heighten the reef, it would an uneconomical choice (Powell, 2007; Webster and Meritt, 1988). Further investigation into shell application and planting densities could shed light on more practical, cost-effective methods to increase survival on bottoms that are currently less than suitable for profitable aquaculture. New best management practices that include improvement of bottom substrate are necessary for the advancement of the bottom culture industry and oyster restoration within Chesapeake Bay.

Appendix I. Supplemental Water Quality Data

Table 18. Surface water salinity for the Big Annemessex River in 2018 and 2019. The minimum, mean, and maximum are for the year 2018.

Surface Water Salinity (ppt) **Lower Eastern Shore / Big Annemessex River (ET9.1)**					
Month	**Minimum**	**Mean**	**Maximum**	**2018**	**2019**
January	9.01	15.78	20.15	15.90	9.90
February	10.37	15.08	19.17	15.90	8.70
March	9.59	14.59	19.87	15.90	9.30
April	9.72	14.55	19.04	15.40	9.80
May	10.11	14.61	18.83	16.10	10.50
June	11.08	14.75	18.45	12.10	11.60
July	11.28	14.82	18.48	14.10	12.10
August	12.40	15.45	19.24	13.60	13.20
September	12.93	16.64	20.71	14.30	17.60
October	11.70	17.45	21.06	11.70	18.60
November	11.00	16.87	20.15	11.00	17.60
December	9.10	16.14	19.94	9.10	17.40

Table 19. Surface water temperature for the Big Annemessex River in 2018 and 2019. The minimum, mean and maximum are for the year 2018.

Surface Water Temperature (° F) **Lower Eastern Shore / Big Annemessex River (ET9.1)**					
Month	**Minimum**	**Mean**	**Maximum**	**2018**	**2019**
January	30.56	39.74	49.64	37.40	46.76
February	32.36	39.92	47.66	37.40	39.38
March	37.76	47.34	56.48	43.52	44.60
April	48.56	58.55	69.80	54.68	60.26
May	58.28	66.49	74.84	74.48	68.00
June	69.44	77.92	86.36	74.12	76.28
July	78.98	83.01	88.16	82.76	87.08
August	74.12	81.19	87.98	87.98	81.14

September	68.18	74.75	80.24	78.80	75.74
October	52.70	61.79	71.60	55.94	66.56
November	41.90	51.89	60.26	60.08	46.40
December	35.42	43.23	55.22	38.48	44.60

Table 20. Bottom Water Dissolved Oxygen for the Big Annemessex River in 2018 and 2019. The minimum, mean and maximum are for the year 2018.

Bottom Water Dissolved Oxygen (mg/l) Lower Eastern Shore / Big Annemessex River (ET9.1)					
Month	**Minimum**	**Mean**	**Maximum**	**2018**	**2019**
January	10.20	11.71	14.10	12.30	11.80
February	9.70	11.82	14.30	12.30	12.80
March	9.10	11.16	13.90	12.40	11.70
April	7.00	9.31	12.00	8.90	9.70
May	5.80	7.68	9.20	7.40	7.40
June	5.20	6.64	7.60	7.00	7.60
July	4.70	6.40	7.60	6.70	6.00
August	5.20	6.43	7.20	6.90	5.90
September	5.90	7.13	8.00	7.10	6.90
October	7.10	8.55	10.20	9.70	7.70
November	8.40	10.03	12.30	9.00	10.00
December	9.10	11.11	12.80	11.90	10.50

Appendix II. A Note on Natural Recruitment

Oyster reef structure plays an important role for the next generation of oysters, as they provide the hard substrate needed for settlement (Loosanoff, 1965). Over time, the progression of oyster bed restoration creates the three-dimensional structure that supports natural oyster recruitment. However, barren beds covered in sediment within Chesapeake Bay cannot support natural recruitment due to the lack of hard surface. Little research has been conducted in regards to bottom rehabilitation for commercial use and the effects on natural recruitment. This study recorded natural recruitment found on samples taken from the non-seeded side of the three different plots to see if unsuitable habitat could be rehabilitated to support natural oyster recruitment. While the treatments in this study statistically did not have an effect on oyster size or survival, it is interesting to note that natural oyster recruitment was found at the April 2019 sampling period (Table 21). Spat on shell was not planted on this side of the plot, so there is potential for futures studies to focus on bed rehabilitation for natural recruitment using these treatment strategies.

Table 21. The number of natural oyster recruits counted on the sampled shells from the third sampling time point.

Bottom Type	**Treatment**	**Number of Natural Recruits**	**Number of Shells**
Buried Shell	Control	3	21
Buried Shell	Harrow	0	24
Buried Shell	Harrow Shell	16	30
Buried Shell	Shell	6	30
Mud Sand	Control	0	2
Mud Sand	Harrow	7	28
Mud Sand	Harrow Shell	14	27
Mud Sand	Shell	44	30
Mud Shell	Harrow	2	1
Mud Shell	Harrow Shell	2	12
Mud Shell	Shell	0	2

Appendix III. Oxygen and Nutrient Fluxes

On the same lease, an aluminum pole corer was used to take two randomly selected sediment samples from the control, harrow, and harrow shell treatment subplots on the seeded mud sand and mud shell plots in August 2019 (Figure 9). The pole corer was used from the side of the boat to collect an intact (~15 cm) sediment core sample, which was capped and placed in a cooler of water from the site. These *in situ* cores were incubated with aeration in a tub overnight while temperature, pressure and light levels remained constant so a time-course approach could be used to gather fluxes of O_2 N_2, Ar, SRP, NH_4+, and dissolved nutrients the following day to better understand the exchange of gases and solutes between water and sediment. Here, we present only the oxygen data.

Using similar methods as Owens and Cornwell (2016), the solute sampling used a 20 mL syringe to sample water from each of the sealed, incubated cores. Seven samples were collected over the course of an eight-hour period, with three in the dark, followed by three in the light with a with a transition light/dark sample taken between to have a four-point time series. Sampled water was filtered into vials and frozen at -20 °C at every time point. For Ar, N_2 and O_2 gas analysis, 10 *u*L of 50% saturated $HgCl_2$ preservative was added to 7mL of water sampled from the incubated cores. Post incubation, the water volume above the sediment core was measured, in addition to sampling the core surface (0-1cm) to analyze for chlorophyll *a*, which were also frozen to -20 °C.

The fluxes of O_2 in the aquatic sediment core samples comes from a summation of direct uptake of oxygen during anaerobic sediment decomposition, uptake during reoxidation of reduced species and autotrophic oxygen production by benthic microalgae (Cornwell et al, 2014). Table 22 is the oxygen flux data for the mud sand and buried shell plots sampled in August 2019. The flux in oxygen between the two bottom types were not statistically different (Figure 21).

Table 22. The flux of oxygen on the mud sand and buried shell plots.

Bottom Type	**O_2 Flux (umol m^{-2} h^{-1})**
Mud Sand	2751.5
Mud Sand	7315.7
Mud Sand	3680.1
Mud Sand	1256
Mud Sand	1269.7
Mud Sand	4273.4
Mud Sand	835.2
Mud Sand	1648.4
Mud Sand	778.2
Mud Sand	2204.3
Mud Sand	3402.0
Buried Shell	1485.3
Buried Shell	1052
Buried Shell	2654.2
Buried Shell	1423.8
Buried Shell	1021.1
Buried Shell	999.6
Buried Shell	2836.1
Buried Shell	1827.6
Buried Shell	1302.3
Buried Shell	1161.9
Buried Shell	807.4
Buried Shell	1918.9

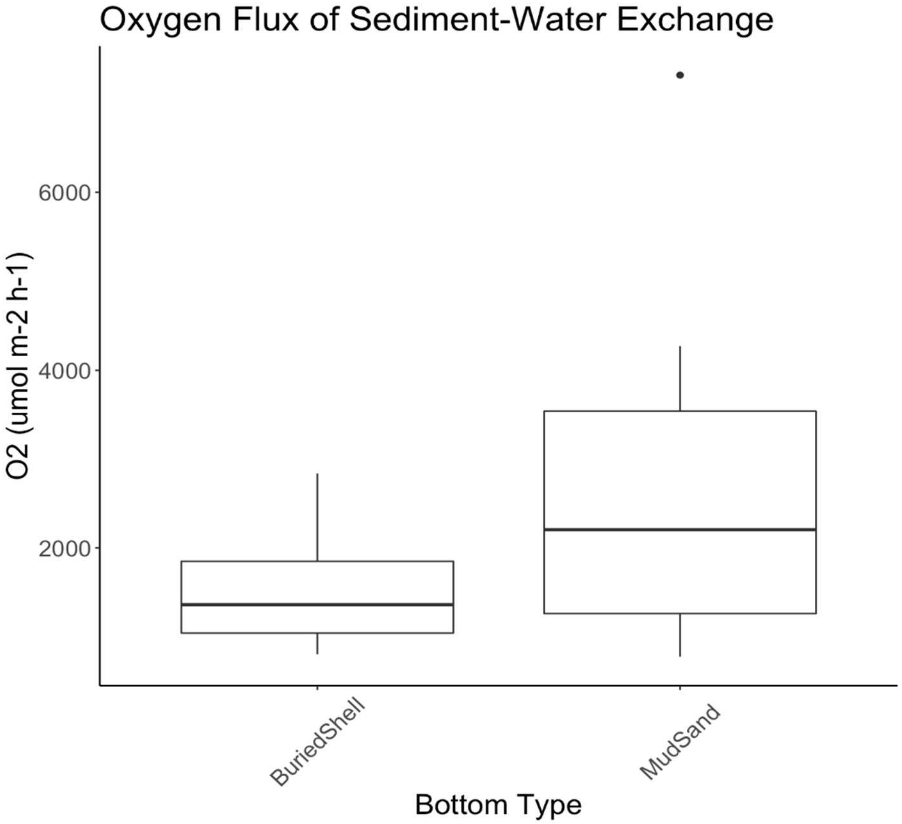

Figure 21. Boxplot representing the flux of oxygen between the buried shell and mud shell plots. Statistically, there is no difference.

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