REMOTE SET OF *CRASSOSTREA VIRGINICA* AS A POTENTIAL MEANS FOR PUBLIC STOCK ENHANCEMENT IN ALABAMA, AND THE ASSESSMENT OF LARVAL TANK SETTING DISTRIBUTIONS

by

David M. Lappin Jr.

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Approved by

William C. Walton, Chair, Associate Professor Extension Specialist, School of Fisheries, Aquaculture and Aquatic Sciences
Mathew J. Catalano, Assistant Professor, School of Fisheries, Aquaculture and Aquatic Sciences
Terrill R. Hanson, Professor Extension Specialist, School of Fisheries, Aquaculture and Aquatic Sciences

ABSTRACT

The eastern oyster (*Crassosstrea virginca*) has been a widely studied and influential species for its economic impacts, benefits to local habitat and fauna, and its assistance in restoration. Hatchery reared larvae play an important role in remote set spat on shell, and farming. Understanding the spatial distributions of spat in setting tanks is critical to evaluating setting success and maximizing the value of the larvae added. This study indicates that both horizontal and vertical distributions play important roles in tank setting success. Once the spat on shell is deployed, it is important to consider different planting strategies based on the size and density of spat. Results indicate that there are negligible contributions to growing spat to larger sizes or deploying at higher densities for the ranges tested in this study. Storm events and predation throughout the study highlight the importance of site selection. Overall survival rates indicated that remote set could be a viable strategy for natural population enhancement in Alabama, such that site selection is made a top priority. A comprehensive budget analysis investigated the total costs for remote set planting, as well as the potential return value based on survival rates of the spat on shell.

ACKNOWLEDGEMENTS

I would firstly like to thank Bill Walton who has helped to guide me in every aspect throughout my time at Auburn and the Auburn University Shellfish Lab. From field sampling to assistance with my thesis, I could not have asked for a better advisor to lead me into the world of shellfish. I would also like to thank Scott Rikard for mentoring me on everything hatchery related, and for his help in the collection of all my field samples. Additionally, I want to thank Glen Chaplin who has spent countless hours on the boat helping me to collect my field samples. A special thanks to Jason Hermann for his help with setting and his advisement for many parts of this thesis, as well as all staff at the Alabama Marine Resource Division for all of their assistance in tank setting and deployment. I would also like to thank all the members of my committee, Matt Catalano and Terry Hanson, who were instrumental in the completion of my thesis. Lastly, I would like to thank all the members of the Auburn University Shellfish Lab, Sarah Betbeze, Kevin Landry, Victoria Pruente, Pandora Wadsworth, Megan Griffith, Caitlin Robitaille, Chris Cochrane for their assistance in procuring larvae, and for their advice along the way.

I would like to dedicate this thesis to my grandparents for sparking my love for the outdoors, my parents for always supporting me, my sister for convincing me to follow my passions, and Sam for sticking with me the whole way through.

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CHAPTER 1: THESIS INTRODUCTION AND GENERAL OVERVIEW

Introduction:

Hatchery reared oyster larvae play an important role in a varied range of industries in the Gulf of Mexico and throughout the globe. Commercial production of viable, hatchery raised oyster larvae, helps to drive the continuation of commercial oyster aquaculture industry. In accordance with NOAAs most recent reports in 2015, an estimated 15,115 metric tons of cultured oyster meat were reported to be distributed to dealers within the United States alone (National Marine Fisheries Service 2016).

Washington State leads the country in the production of Pacific oysters (*Crassostrea gigas*), among other bivalves (Washington Sea Grant 2015). Shellfish aquaculture directly and indirectly employs more than 3,200 people and generates at least $270 million in economic contribution (Washington Sea Grant 2015). By comparison, the wild harvest shellfishery is valued at approximately $40 million. Washington is reported to have produced 8,793,138 lbs. of Pacific oysters in 2013 (Washington Shellfish Initiative 2011). Virginia leads the production of the Eastern oyster (*Crassostrea virginica*), with a total value of $18.5 million in 2017 (Virginia Shellfish Aquaculture and Situation Outlook Report 2017). This is a rapidly growing sector of Virginia's shellfish aquaculture industry and is largely controlled by a system of vertically integrated private hatcheries (Virginia Shellfish Aquaculture and Situation Outlook Report 2017). In order to successfully drive oyster production via aquaculture, hatcheries must be able to keep up with farmer and nursery demands.

In addition to the commercial production of oyster larvae, hatchery reared larvae may also support various research and restoration efforts. For example, growing concern for coastal erosion and sea level rise have led researchers to test oysters as a potential means for stabilizing shorelines. Classic examples of materials used in attempts to minimize erosion include rock, metal, and concrete (Hillyer et al. 1997). Alternatively, living oyster reefs provide three dimensional structures that double as a natural form of habitat. These "ecosystem engineers" as defined by (Jones et al. 1994) provide habitat, as well as various ecosystem services, to indigenous organisms in their region. Ecosystem services provided by oyster reefs extend past ecological benefits as they can act as natural breakwaters to mitigate high energy waves and shoreline erosion. Studies along the Louisiana coastline showed significant decreases in shoreline retreat for areas planted with shell cultch (Piazza et al. 2005). Comparable studies in Mobile Bay found that "living shorelines" greatly increased the diversity and abundance of mobile invertebrates and fishes; however, compression over time due to the lack of reef support reduced its ability to act as a breakwater barrier (Scyphers et al. 2011). It was postulated that increased rigidity in the initial reefs would have allowed the reef to "cement" and thus act as an efficient barrier.

Historically, Alabama's reef restoration efforts have been predominantly driven by shell plantings to support the commercial public fishery. Multiple legislative acts were passed through the 1900's to regulate and continue these plantings. Originally, oyster buyers were required to replant 50% of the shells removed. In 1987 these regulations were amended so that buyers could pay the state a fee to cover the cost of the shell and the planting (Wallace et al. 1999). Current Alabama law has a required fee which is determined by the quantity of sacks of oysters purchased. All fees are to be consolidated in a fund specifically designated for the purposes of replanting shell cultch and managing public reefs in Alabama waters.

Funding for replanting of oyster reefs has been critical given the historical decline of oyster reefs in the Mobile Bay region. NOAAs annual commercial landings of *C. virginica* have reported significant drops since early 2000 (National Marine Fisheries Service 2018). This may in part be due to stricter regulation; however, firmer regulation of commercial take was required to combat the loss throughout the years. Though spat settlement was recorded on a variety of Mobile Bay reefs (Saoud et al. 2000), the existing population stocks are not as significant as historical counts suggest.

For circumstances in which shell cultch can provide the ground work for habitat protection and or restoration, it has been suggested that setting oyster larvae on the shell cultch before deployment may increase its benefits. This process, known as “remote-set”, is the setting of oyster larvae to a desired cultch and the planting of such cultch in environments for further growth. This process relies on hatchery reared larvae and is often used as a primary or secondary source of harvest for farmers (and is common in Washington State) and for restoration efforts (e.g, restoration efforts in Maryland).

Remote setting on larger cultch, often recycled oyster shell, requires a less costly and less labor-intensive process than single set oysters, but typically results in clusters. This is more practical for on-bottom culture operations. These oysters may be selected for the half shell market; however, inconsistencies in the shape and quality of the adult oysters may be more appropriate for shucking meats. As such, this may be a viable method for half shell production, but it is more than likely to be the most cost-effective method for meat production. This strategy can also prove to be beneficial for restoration purposes. Distributing spat on shell to existing reefs may booster natural stocks and assist in restoration or reef recovery efforts.

Multiple studies and manuals have been published pertaining to the ideal setting environment for spat on shell. Further discussion on ideal setting locations, optimal environmental conditions, and tank designs can be found in Supan (1987), Wallace et al. (2008), and Congrove et al. (2009). While many of these studies have highlighted important environmental conditions and tank set ups, there has been little formal work identifying setting distributions across tanks. Maximizing the setting efficiency will result in higher numbers of spat per supplied oyster larvae. Since oyster larvae are typically purchased from hatcheries, it is important to maximize the value of the supplied larvae by creating the most efficient setting schemes. This is particularly important for farmers concerned with increasing their profit margins. This is important for single set oysters, but it is equally important for clustered oysters. Increased setting efficiency and survivability of spat may lead to denser clusters of adults. This would lead to larger numbers of harvestable oysters for a farmer, or larger numbers of functioning adults for restoration purposes.

Critically, there is brief mention of established protocols for assessing setting efficiency. Typically, these manuals call for collecting a certain number of shells from different sections or depths and determining an average spat per shell (Supan 1987, Congrove et al. 2009). While these methods call for samples to be taken from across different distributions, there is little data to support how the tank distributions may vary. When working with remote setting systems, it is essential that operators accurately measure setting efficiency as this assessment establishes the initial ‘inventory’ upon which all subsequent calculations depend. If oysters set in particular distribution pattern, protocols for assessing setting efficiency need to take these into account.

In this tank study, the vertical and horizontal distribution of set oysters was tested to determine if there is any preference by larvae to set at different heights and locations in the water column. The testing was done using “setting sticks”, as described in methods, to ensure that the experiments were completed using a standard method of measurement. Given the variability in the sizes and shapes of shell cultch, it was important to establish a standard method for testing the tanks efficiency and setting distributions.

While understanding the setting distributions across setting tanks will help to assess the setting efficiency, it is equally important to understand the dynamics of planted spat on shell to maximize its benefits once deployed. First off, the type of cultch used may play an important role in its success post planting. There are a variety of cultch options when considering remote setting, however; for bottom planting, oyster shells provide some of the best cultch given their weight and broad surface area that keep them anchored to the bottom (Bohn et al. 1993). Aforementioned, oysters act as ecosystem engineers by providing reef habitat (Jones et al. 1994) and studies have revealed that restored intertidal oyster reefs have significantly improved the presence of resident marine fauna (Grabowski et al. 2005, Scyphers et al. 2011). Additional studies in this region indicated that artificially created reefs were able to become functionally equivalent to their natural counter parts in short windows of time (Meyer et al. 2000). With these considerations in mind, it is possible that these may be viable strategies as a means for public stock enhancement in Alabama, and in the Gulf of Mexico. In addition to public stock enhancement, remote-set shell plantings could provide habitat for resident species and boost overall ecosystem health.

In addition to the importance of the type of cultch, the way in which spat on cultch are planted may affect planting success. Based on prior work, there is a reasonable expectation that both the size of the spat when they are deployed, as well as the densities at which they are deployed will play important roles in their ability to survive post planting (Eggleston 1990). In this study, we conducted rigorous, small-scale experiments to determine if there are optimal sizes and/or optimal densities for spat on shell planting. The ultimate goal was to gain a greater understanding for remote-set methods and its potential for success in this region.

The results of this field study could have important implications regarding the success of this method, and its expansion in the future as a stock enhancement tool and possibly for private commercial culture. As mentioned before, there is an associated cost to planting spat on shell, with costs varying with different planting strategies. The addition of the larvae, plus the labor involved in the remote set process costs considerably more than traditional shell clutching. Because of this, it is important to understand the return value of this method. Results from the field study experiments in combination with an assessment of the overall cost of the process may lend some insight to the worth of this methodology. The field study results may indicate whether or not this will be an effective method, while the budget analysis will provide a more accurate estimate for future costs.

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CHAPTER 2: ASSESSMENT OF SETTING EFFICIENCY AND LARVAL TANK DISTRIBUTIONS OF *CRASSOSTREA VIRGINICA*

Introduction:

Hatchery reared oyster larvae play an important role in a varied range of industries in the Gulf of Mexico and around the globe. Commercial production of viable, hatchery raised oyster larvae helps to drive the continuation of commercial oyster aquaculture industry. Hatchery reared larvae are cultured, set, and raised using an assortment of strategies that support aquaculture, restoration, and natural population enhancement. Additionally, they can be used for a variety of research projects requiring cultured larvae for which alternative collection methods of the same volume would be difficult or impossible.

A variety of setting methods have been documented and tested in different regions. The specific goals for the production of the oysters may influence the strategy implemented in setting. Single set oysters require setting on a micro-cultch and produce individual spat. These spat can be raised in a nursery setting to a desired size before being distributed to farmers for grow-out. This is a costly strategy for both time and labor but is particularly important for off-bottom operations. Typically, oysters raised in this manner demand a higher price on the market and are more often reserved for the half-shell market.

Remote setting on larger cultch, often recycled oyster shell, requires a less costly and less labor-intensive process than single set oysters, but typically results in clusters. This is more practical for on-bottom culture operations. These oysters may be selected for the half shell market; however, inconsistencies in the shape and quality of the adult oysters may be more appropriate for shucking meats. As such, this may be a viable method for half shell production, but it is more than likely to be the most cost-effective method for meat production. This strategy can also prove to be beneficial for restoration purposes. Distributing spat on shell to existing reefs may booster natural stocks and assist in restoration or reef recovery efforts.

Multiple studies and manuals have been published pertaining to the ideal setting environment for spat on shell. Further discussion on ideal setting locations, optimal environmental conditions, and tank designs can be found in Supan (1987), Wallace et al. (2008), and Congrove et al. (2009). While many of these studies have highlighted important environmental conditions and tank set ups, there has been little formal work identifying setting distributions across tanks. Maximizing the setting efficiency will result in higher numbers of spat per supplied oyster larvae. Since oyster larvae are typically purchased from hatcheries, it is important to maximize the value of the supplied larvae by creating the most efficient setting schemes. This is particularly important for farmers concerned with increasing their profit margins. This is important for single set oysters, but it is equally important for clustered oysters. Increased setting efficiency and survivability of spat may lead to denser clusters of adults. This would lead to larger numbers of harvestable oysters for a farmer, or larger numbers of functioning adults for restoration purposes.

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In this tank study, the vertical and horizontal distribution of set oysters was tested to determine if there is any preference by larvae to set at different heights and locations in the water column. The testing was done using “setting sticks”, as described in methods, to ensure that the experiments were completed using a standard method of measurement. Given the variability in the sizes and shapes of shell cultch, it was important to establish a standard method for testing the tanks efficiency and setting distributions.

Methods:

Setting:

Setting Tank Set Up:

Three tanks were constructed at the Alabama Marine Resource Division (MRD) on Dauphin Island, Alabama. All tanks were outdoor and thus, were exposed to natural environmental conditions throughout setting periods. Tarp covered the tanks during the setting periods to reduce the input from rain and direct sunlight. A 10-micron cartridge filter was used to fill tanks and to keep tanks on continuous flow after a static setting period of three days. The intake was located on site and drew water from the channel in Little Dauphin Bay next to the tank array. Air lines were constructed in a grid format on the bottom of the tank using PVC. The cages were able to nest between the grids to create an evenly distributed airflow.

Each tank was capable of holding 20 (3’High x 3’Long x 1’Wide) cages (60 total). The cages were filled with roughly 400lbs of shell cultch per cage. They were then washed to remove as much silt and debris as possible before loading the filled cages into a tank with a fork lift. Filled tanks were left to soak for at least three days prior to the addition of eyed larvae so the shell cultch could accumulate an appropriate biofilm (Supan 1987, Wallace et al. 2008, Congrove et al. 2009).

Setting Stick Set up:

Two types of setting sticks were constructed and used in experiments to monitor setting. In both instances, the goals were to accurately capture the distribution of spat set across the tank while maintaining a consistent and standardized method of measurement. The first type, referred to as Setting Stick A, consisted of a ½” PVC pipe fitted with 3 segments of French tubing (made by *Poly-chor Plastic Industries Ltd*.) vertically distributed (High, Middle, Low). The ½” French tube segments were 10 cm each and were secured (parallel to the water surface) to the PVC such that the “High” placement was located 15cm below the surface of the shell level in the cages. The “Low” placement was located 15cm above the bottom of the cage, and the middle was secured evenly between the “High” and “Low” (35 cm from the bottom and 35cm from the surface of the shells). 9 of these setting sticks were distributed across each tank, for each setting period. Three sticks were placed on the 4th cage in from the end, 3 were placed on the 10th cage, and 3 were placed on the 17th cage (Figure 1). In this manner, the sticks were evenly distributed across the tank and captured the scope of the tank. There were 9 replicates per vertical position and 9 replicates per horizontal placement in each of the tank settings.

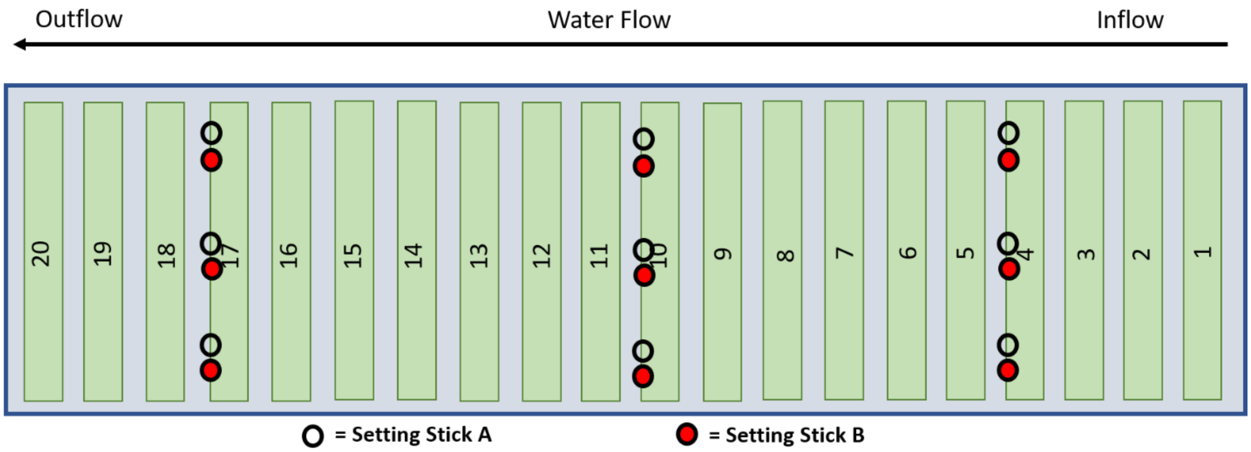

Figure 1. Overhead view of 1 of 3 set tanks at the MRD. Each cage within the tank is numbered 1 – 20. Arrow indicates the direction of water flow during flow through periods such that cage 1 is located at the inflow and cage 20 is located at the outflow. Open dots indicate the position of Setting Sticks A and red filled indicate position of Setting Sticks B.

The second version of the setting sticks, Setting Stick B, was a single length of French Tubing. This method was added after the first two tank sets and was only included in 8 tank sets (Table. 1). This design was meant to allow for spat to settle on any portion of the stick. In similar fashion to the vertically distributed setting sticks, 9 lengths of French Tubing were distributed across the tank. Setting Stick B pipes were zip-tied directly adjacent to the Setting Stick A set ups (Figure 2). For both Setting Sticks A and B, pipes would be placed in the tanks 3 days before larvae were added to allow for a biofilm to establish. Sticks would remain in the tanks for the duration of the larval setting periods (3 days static, 7 days flow through).

Table 1. Inclusion of Setting stick method for associated tank set. Each range of dates for larval addition are shown, and the dates for which the setting sticks were assessed are shown.

Addition of Larvae Dates	Assessment Dates	Setting Stick A	Setting Stick B
9/22/2017	10/2/2016	Yes	No
5/26/17 - 5/31/17	6/11/2017	Yes	No
6/5/2017	6/15/2017	Yes	Yes
6/13/2017	6/23/2017	Yes	Yes
7/27/2017	8/7/2017	Yes	Yes
7/28/17 - 7/29/17	8/8/2017	Yes	Yes
7/30/2017	8/8/2017	Yes	Yes
9/19/2017	9/29/2017	Yes	Yes
9/20/2017	9/30/2017	Yes	Yes
9/21/2017	10/1/2017	Yes	Yes

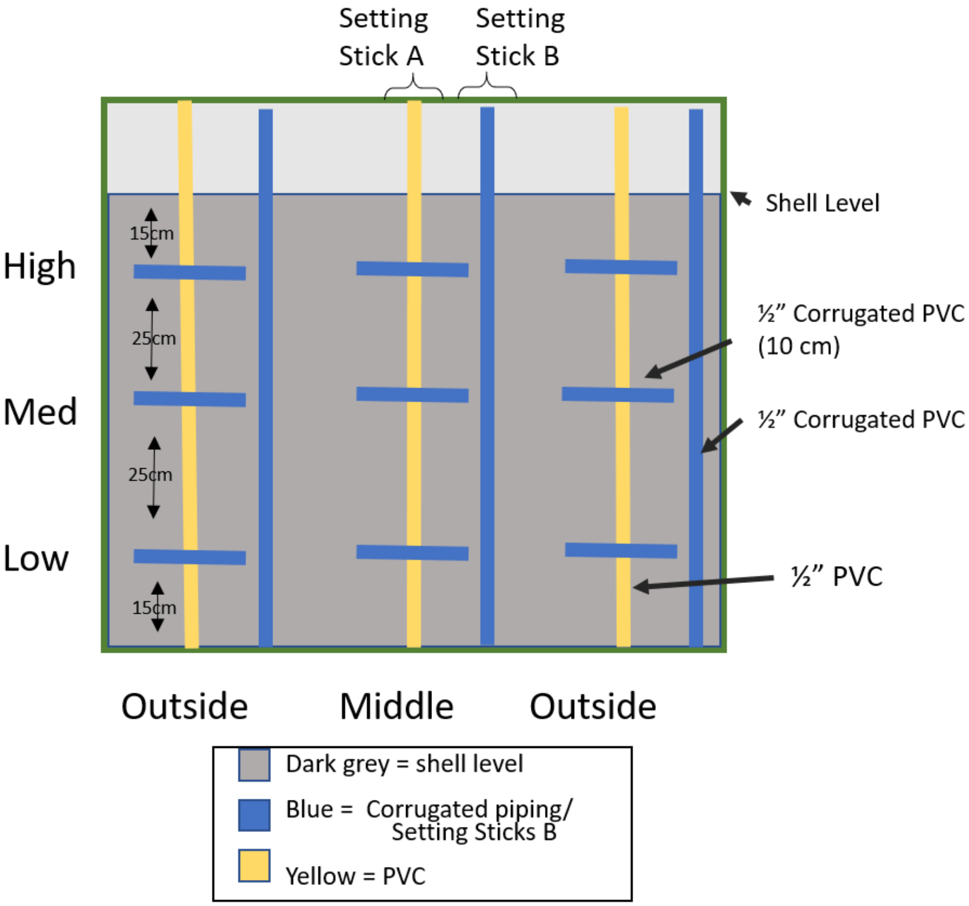

Figure 2. A profile view of the setting stick set up is shown. The two types of setting sticks are displayed adjacent to one another as they were in the tanks. Materials are described. The shell level in grey shows that the shell did not completely fill cages.

Setting Period:

Hatchery-reared eyed larvae were procured from the Auburn University Shellfish Lab from 10/2/2016 – 10/1/2017 (Table 1). Collected larvae were added to MRD set tanks in increments of 5 million per tank. It was attempted in all trials to supply the eyed larvae in increments of 5 million, however; this was dependent on the ability to produce large volumes of viable larvae at one time. In some cases, larvae were refrigerated for no more than one day in order to amass a full 5 million. In a few trials, a full 5 million was not possible within 2 days and thus, the larvae were added incrementally over several days. Setting sticks were placed in 10 total tank sets (Table 1), and data was recorded for each.

Larvae were fed a commercial algae paste, Reed Mariculture Inc.'s Shellfish Diet 1800®, over a three-day static period. Feedings would occur in the mornings and at night. A fully stocked tank (5 million) received 50ml of algae paste in both the morning and the evening. The feeding rates were adjusted accordingly if the larvae were added incrementally. In the instance in which the larvae were added over longer periods, water changes were required. Larval tanks went a maximum of three days before either a water change or a switch to flow through.

Flow Through Period:

After a 3-day static period, the systems were switched to flow through. The systems remained in flow through for 1 week before sampling and deployment. Once attached to the shell cultch as spat, it was no longer necessary to feed the tanks with algae paste. The incoming water from the channel had sufficient amounts of food to allow for further growth and development within the system.

Sampling:

Setting sticks were removed from each tank after the full setting period was complete. Sticks were taken to the lab and assessed. Counts were taken and recorded for each of the vertical (High, Middle, Low), and horizontal combinations (Inflow, Middle, Outflow) for Setting Sticks A. Averages across trials were taken for each of the vertical positions. For Setting Sticks B, measurements were taken for the distance of spat in relation to the bottom of each stick. In this manner, the vertical distribution was not defined to a limited number of positions. The vertical distribution was noted as the density of the spat as a function of depth.

Statistical analysis:

For Setting Sticks A, all sticks were treated as subsamples and the tank sets were treated as blocking factors. Given the many counts of zero in the data frame, the spat counts were analyzed using a generalized linear model with a negative binomial sampling distribution. The negative binomial was selected over the Poisson distribution because the variance in spat counts greatly exceeded the mean. All terms were included for vertical and horizontal positions (with three levels of each factor) and allowed for interactions. Post-hoc comparison of treatment means was completed using a Tukey-Test to determine significant differences ($p \leq 0.5$).

For Setting Sticks B, a Kernel Density Estimator was used to model the distribution of data points across all possible vertical positions. In addition to the density plots, each individual measurement (176 data points) was graphed as a scatter plot. Given that there was only one variable measurement (distance from the bottom), data were plotted against a numeric string (1-176) such that the data points were spread evenly throughout the plot. Data from this analysis were combined for all set tanks in the experiment. All statistical analysis was completed using RStudio (RStudio Team 2016).

Results:

Setting Sticks A:

Across all 10 tanks sets, 415 spat were counted on the French tube segments. Variability was noted between the tanks, but since the individual tank sets were treated as blocking factors, this was not of particular interest. There was a significant effect ($p < 0.01$) of the vertical position on the mean predicted counts of spat (Figure 3). A post-hoc Tukey Test indicated that the number of spat found on the lowest position was significantly higher ($p < 0.001$) than the number found on the highest vertical position. There were no significant differences ($p< 0.05$) between the middle position and either the low or high vertical positions. Additionally, there was a significant effect ($p < 0.01$) of the horizontal position on the mean predicted counts of spat (Figure 4). A post hoc Tukey test indicated that there was significantly fewer spat observed nearest to the Inflow when compared to both the Middle ($p = 0.02$) and Outflow positions ($p < 0.01$). There were no significant interactions noted between the horizontal and vertical positions ($p = 0.51$).

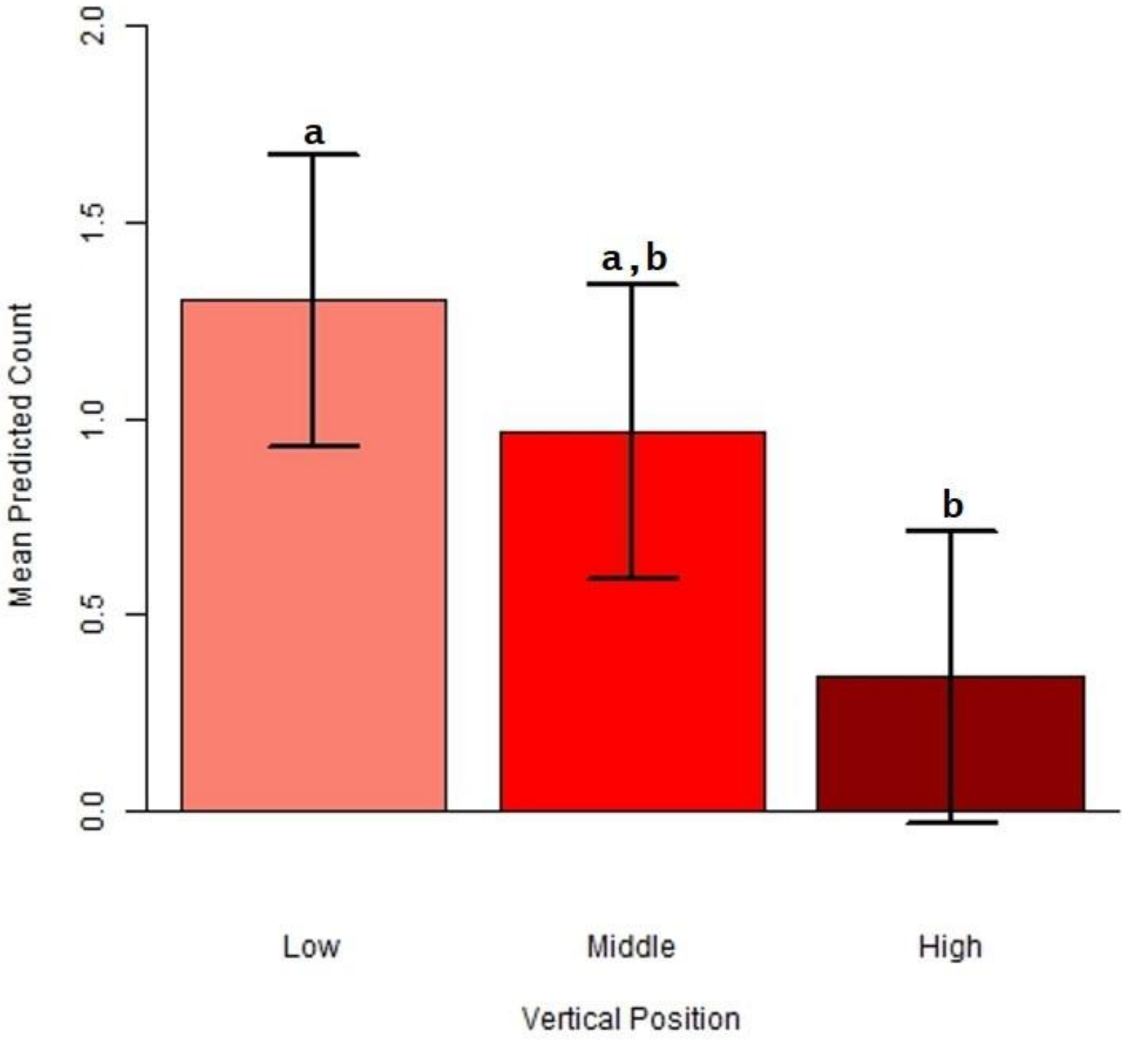

Figure 3. The mean predicted spat counts (± SEM) for each vertical positioning are shown. Groups that share a superscript are not significantly different ($p < 0.05$) from one and other.

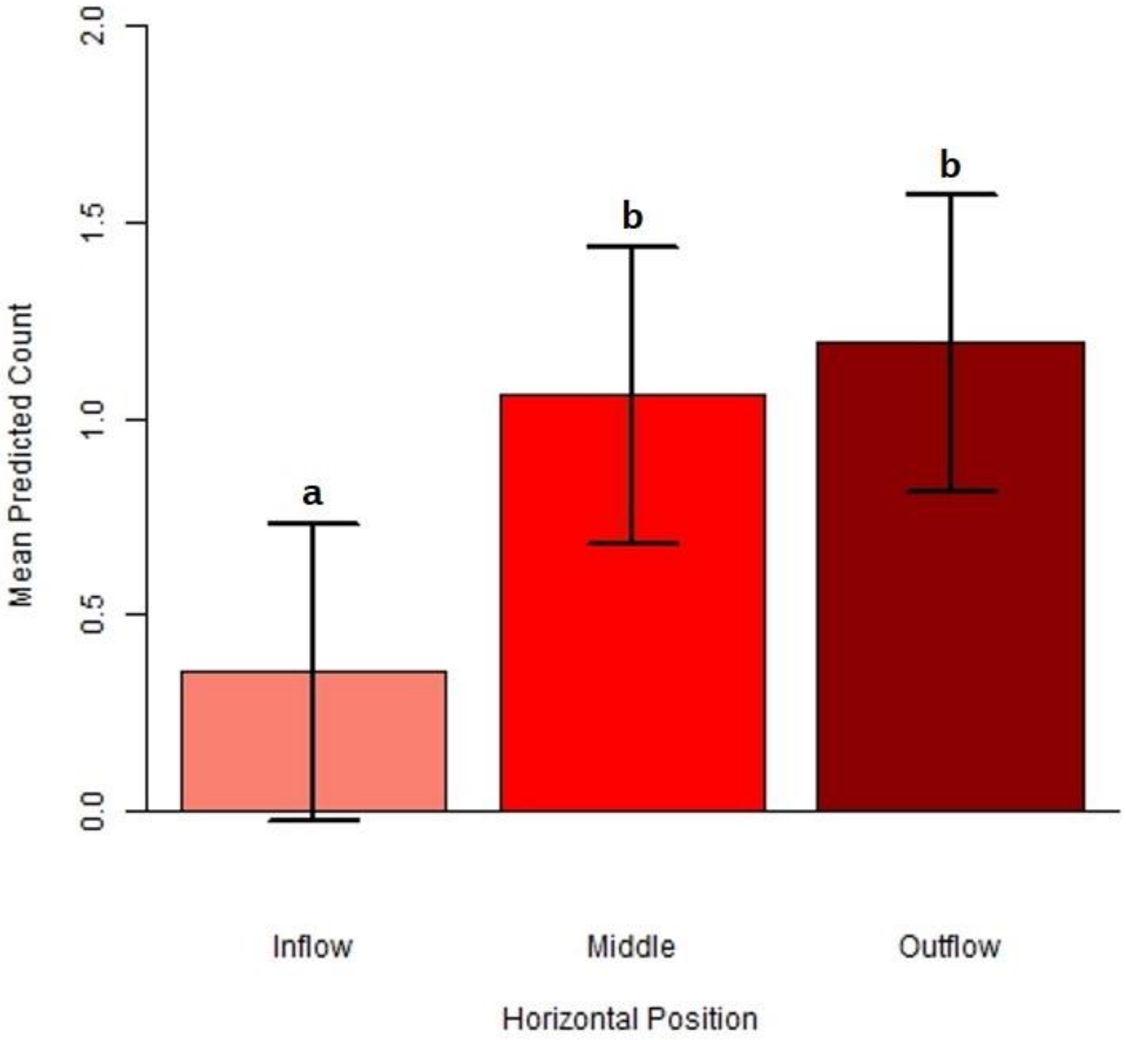

Figure 4. The mean predicted spat counts (± SEM) for each horizontal positioning are shown. Groups that share a superscript are not significantly different ($p < 0.05$) from one and other.

Setting Sticks B:

Across all tank sets, there were 176 observed spat. The mean distance from the bottom was 19.13 cm (± 17.21). Of all the measurements, 65.9 % fell below the mean (Figure 5). The smoothed kernel density distribution indicated that the highest probabilities of finding spat occurred on the lower 25% of the setting stick (Figure 6).

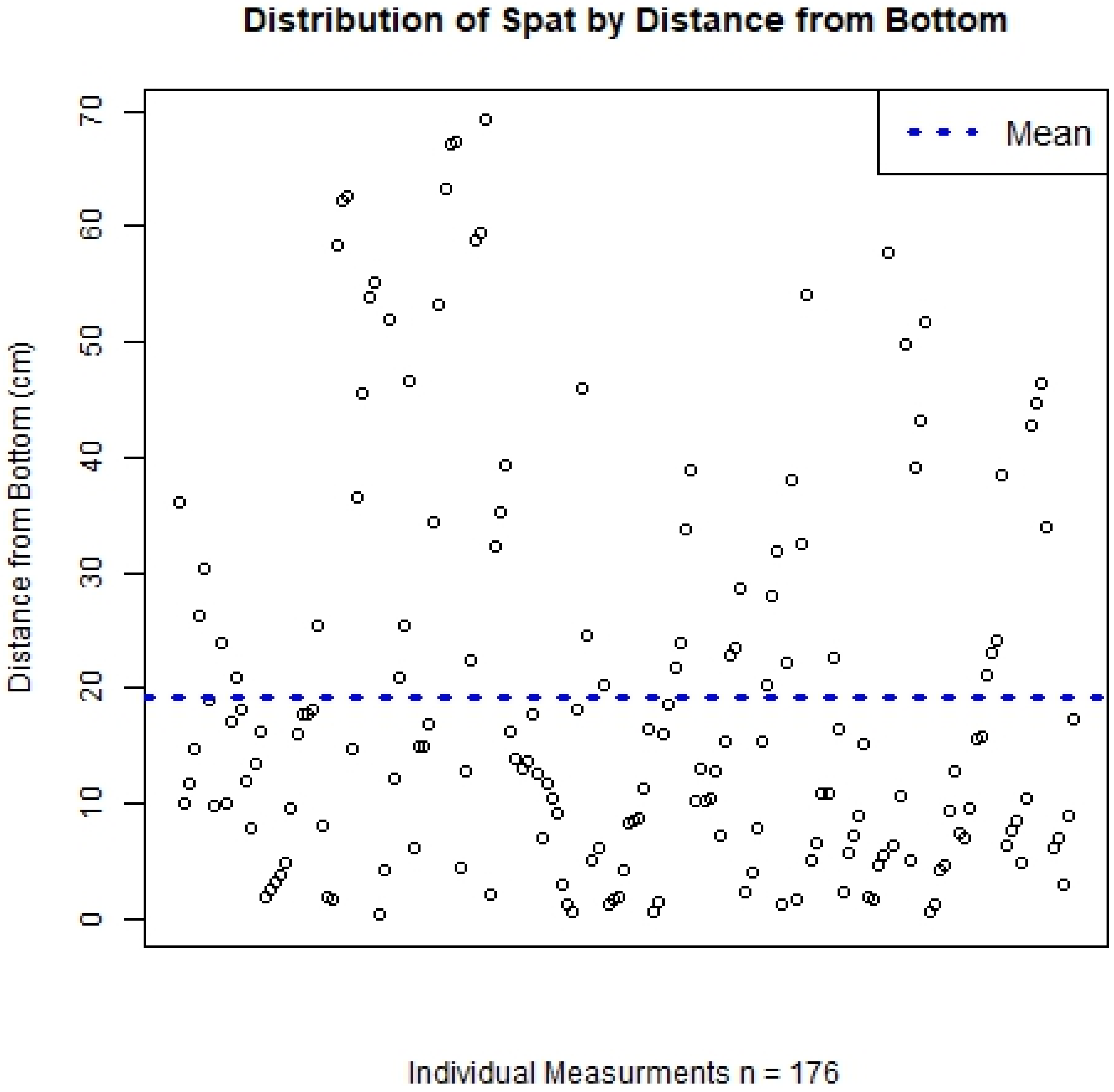

Figure 5. The measurement (cm) from the bottom of oyster spat on setting sticks B is shown in this graphic. Each individual spat (n =176) was graphed against an arbitrary character string to spread out data points. The mean distance (19.13 (cm)) from the bottom is displayed as the dashed horizontal line. 65.9% of measurements fell below the mean line.

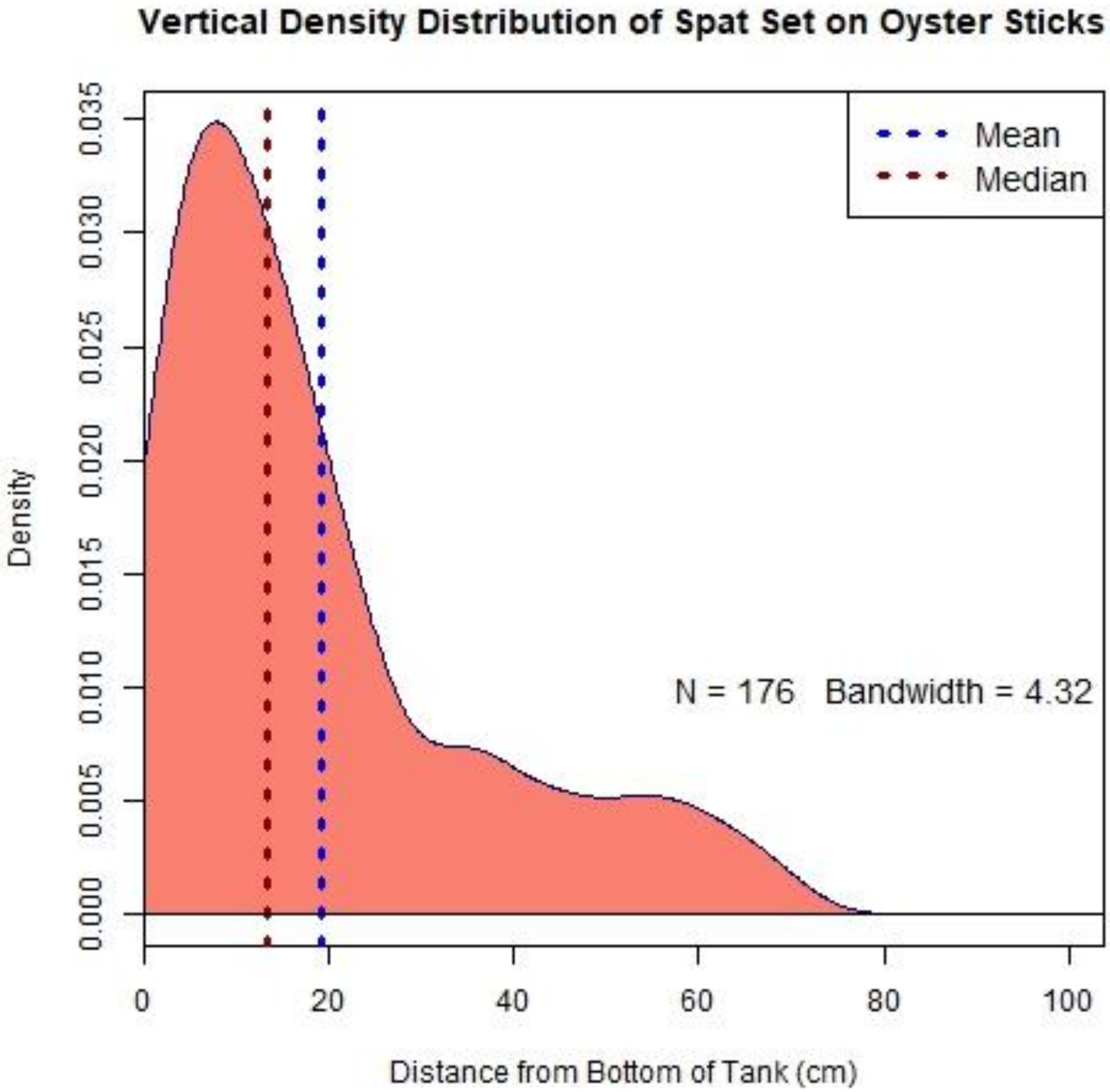

Figure 6. A smoothed Kernel density distribution (n = 176, Bandwidth = 4.32) of the distance from the bottom is shown. Both the mean and median values of all measurements is overlaid for reference. The distribution is positively skewed to the right with the highest concentrations of values falling below the mean value.

Discussion:

Across all 10 tanks sets, 415 spat were counted on the French tube segments of Setting Sticks A. Firstly, the mean predicted counts differed significantly ($p < 0.001$) between tank sets. While differences among tanks was not the focus of this study, and instead treated as a random block factor, differences among tanks appeared to be important. Only a finite amount of tank sets could be completed in this study, and for that reason, it was decided that we should remain consistent in the chosen methods (Setting Sticks A, B) to maximize the results. Continually changing the methodology in an attempt to increase spat collection would have likely been less useful. In the future, a spat collector known as "Chinese Hats" may be a more successful spat collection method (Figure 7). These spat collectors have conical layers that increase settable surface area and are designed to attract spat. Identical methods could be used with these collectors such that specific vertical ranges could be assessed as low, medium, and high ranges.

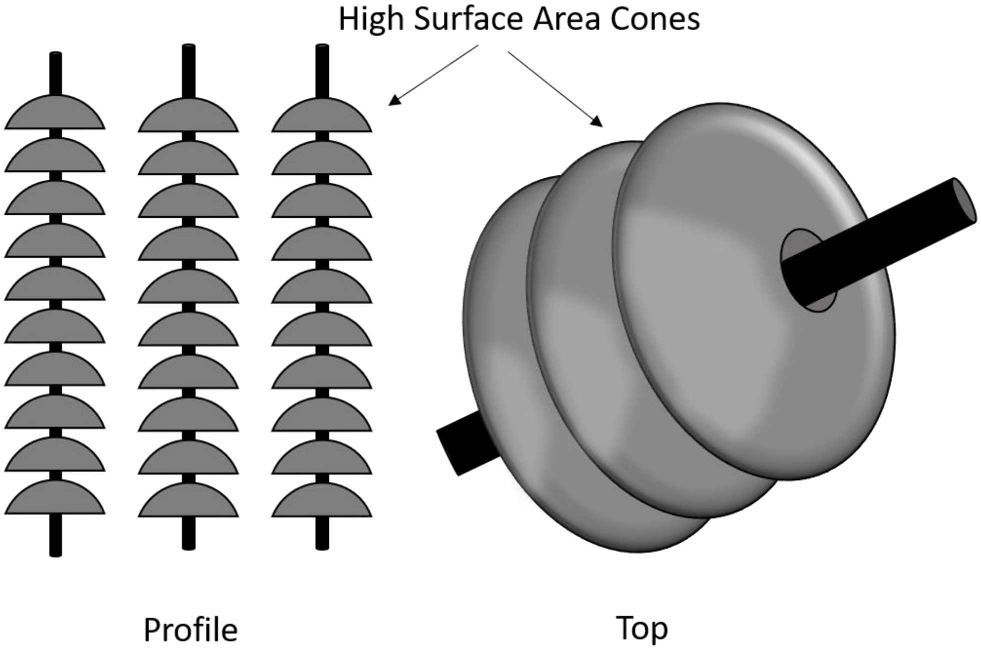

Figure 7. High surface area conical spat collectors, "Chinese Hats".

In addition to tank sets, there was a significant effect of vertical position ($p < 0.01$), with a clear pattern of higher abundances of oyster spat lower in the tank. A post-hoc Tukey Test determined that there was a significant difference ($p < 0.001$) between the Low and the High positions. These data suggests that there is a tendency for the larvae to set lower in the water column of the set tanks. The positively skewed density distribution for Setting Sticks B (Figure 6) also supports these conclusions; of all the oyster spat, the majority (65.9%) collected on Setting Sticks B fell below the mean average of 19.13 (cm) from the bottom of the tank, or within the bottom 20% of the available vertical distribution (Figure 5).

A possible explanation for this trend would be that the larvae prefer darker environments (Kennedy 1980, Nelson 1953, Chesnut 1968, Ritchie and Menzel 1969). This conclusion would agree with (Kennedy, 1980) who determined that increased turbidity in the Chesapeake River system lead to decreased light intensity, and thus reduced the need for spat to settle on the shaded underside of shell cultch. Additional studies (Nelson 1953, Chesnut 1968, Ritchie and Menzel 1969) support the hypothesis that spat typically settle in areas of reduced light intensity. This aversion to high light intensity environments lends important implications to efficient setting tank systems.

In this study, light tarps covered the setting tanks following the recommendations of Supan (1987). Light plastic is suggested as an alternative cover material by Wallace et al. (2008). While these materials reduce the intensity of light, they do not completely eliminate light from the system. Further studies testing the percentage reduction of light intensity (from uncovered to completely dark) could uncover optimal light allowances in set tanks. This has important implications for spat on shell since an even distribution of spat across shell is most desirable. This also highlights the importance of stratifying shell samples when completing setting assessments. To determine a more accurate assessment of setting efficiency, and to procure a more accurate initial count of spat on shell, cultch samples must be taken from the cages at multiple levels in the water column. Neglecting to sample in this manner would likely reduce the overall accuracy of the estimates.

Like the vertical position, the horizontal position within the tank was determined to have a significant effect ($p < 0.01$) on the counts of spat. The outflow and middle portions of the tank had significantly more spat than the inflow. This finding may point to some degree of survivability in relation to the water flow of the tanks. During the 7-day flow through period, raw water was drawn from Little Dauphin Island bay and circulated through the tanks. It would seem intuitive that the spat closest to the inflow of water would have greater access to food and thus possibly display increased survival however, the first few cages closest to the inflow displayed a higher degree of sedimentation and silt accumulation. Despite undergoing some filtration, the raw water still deposited some degree of mud within the system. The sediment settled within the first few cages and there was reduced sedimentation closer to the outflow.

In this manner, it is possible that the first few cages of shell had greater sedimentation issues than cages further from the inflow which may ultimately have decreased spat survival in this area. This highlights that settlement in this study occurred not immediately after the settlement period of 3 days, but also included an additional 7 days for additional growth. A solution to the issue of losses due to sedimentation could be to increase the filtration of incoming water, but one must be careful not to reduce food availability in the process. There may not be a viable solution for this issue given the tank design. This distribution of spat may simply have to be accounted for in this setting system design.

Conclusions:

This study supports the commonly accepted theory that larval settlement is dependent on light. While previous studies suggest that light aversion is a typical behavior in the wild (Kennedy 1980), this study indicates that similar behaviors are relevant in setting tank systems. Significantly more spat settled lower in the water column away from light sources. As such, this highlights the importance of sampling methods when assessing the success of setting tanks. This suggests that sampling by the operator must include some degree of stratification within the shell cultch. Depending on the dimensions of the cages in question, this could be slightly variable; however, the sampling should always include shell from a range of vertical positions. This methodology will better represent the vertical distributions of the larvae and thus return a more accurate assessment of setting efficiency within the tank.

Additionally, accounting for uneven distributions due to sedimentation may be important when considering tank designs and system set ups. This suggests that horizontal distributions are equally important to consider when sampling. In this study, setting sticks were placed at the inflow, middle, and outflow to determine horizontal distributions. Sampling methods for shell should emulate similar patterns to encapsulate potential variation throughout tank sections. Overall, it is important that operators consider their tank design and complete their sampling in a manner that best describes the full spectrum of horizontal and vertical distributions throughout the tank.

In addition to the sampling recommendations, it is recommended that a similar approach to the setting sticks be taken as a means for setting tank assessment. While it may not be possible to compare the shell samples directly to the setting sticks, the sticks provide a consistent measure across tank sets. Individual variability in shell shape makes shell sampling a difficult method to gauge success in tanks. The setting sticks provide a consistent surface for spat collection and remove variability that the shell cannot. Data collection from these set sticks provides valuable insight to tank setting dynamics and can be utilized to increase accuracy in sampling methods.

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CHAPTER 3: ASSESSMENT OF REMOTE SET AS A VIABLE MEANS FOR POPULATION ENHANCEMENT IN ALABAMA PUBLIC REEFS

Introduction:

Hatchery reared oyster larvae play an important role in a varied range of industries in the Gulf of Mexico and throughout the globe. Commercial production of viable, hatchery raised oyster larvae, helps to drive the continuation of commercial oyster aquaculture industry. In accordance with NOAAs most recent reports in 2015, an estimated 15,115 metric tons of cultured oyster meat were reported to be distributed to dealers within the United States alone (National Marine Fisheries Service 2016).

Washington State leads the country in the production of Pacific oysters (*Crassostrea gigas*), among other bivalves (Washington Sea Grant 2015). Shellfish aquaculture directly and indirectly employs more than 3,200 people and generates at least $270 million in economic contribution (Washington Sea Grant 2015). By comparison, the wild harvest shellfishery is valued at approximately $40 million. Washington is reported to have produced 8,793,138 lbs. of Pacific oysters in 2013 (Washington Shellfish Initiative 2011). Virginia leads the production of the Eastern oyster (*Crassostrea virginica*), with a total value of $18.5 million in 2017 (Virginia Shellfish Aquaculture and Situation Outlook Report 2017). This is a rapidly growing sector of Virginia's shellfish aquaculture industry and is largely controlled by a system of vertically integrated private hatcheries (Virginia Shellfish Aquaculture and Situation Outlook Report 2017). In order to successfully drive oyster production via aquaculture, hatcheries must be able to keep up with farmer and nursery demands.

In addition to the commercial production of oyster larvae, hatchery reared larvae may also support various research and restoration efforts. For example, growing concern for coastal erosion and sea level rise have led researchers to test oysters as a potential means for stabilizing shorelines. Classic examples of materials used in attempts to minimize erosion include rock, metal, and concrete (Hillyer et al. 1997). Alternatively, living oyster reefs provide three dimensional structures that double as a natural form of habitat. These "ecosystem engineers" as defined by (Jones et al. 1994) provide habitat, as well as various ecosystem services, to indigenous organisms in their region. Ecosystem services provided by oyster reefs extend past ecological benefits as they can act as natural breakwaters to mitigate high energy waves and shoreline erosion. Studies along the Louisiana coastline showed significant decreases in shoreline retreat for areas planted with shell cultch (Piazza et al. 2005). Comparable studies in Mobile Bay found that "living shorelines" greatly increased the diversity and abundance of mobile invertebrates and fishes; however, compression over time due to the lack of reef support reduced its ability to act as a breakwater barrier (Scyphers et al. 2011). It was postulated that increased rigidity in the initial reefs would have allowed the reef to "cement" and thus act as an efficient barrier.

Historically, Alabama's reef restoration efforts have been predominantly driven by shell plantings to support the commercial public fishery. Multiple legislative acts were passed through the 1900's to regulate and continue these plantings. Originally, oyster buyers were required to replant 50% of the shells removed. In 1987 these regulations were amended so that buyers could pay the state a fee to cover the cost of the shell and the planting (Wallace et al. 1999). Current Alabama law has a required fee which is determined by the quantity of sacks of oysters purchased. All fees are to be consolidated in a fund specifically designated for the purposes of replanting shell cultch and managing public reefs in Alabama waters.

Funding for replanting of oyster reefs has been critical given the historical decline of oyster reefs in the Mobile Bay region. NOAAs annual commercial landings of *C. virginica* have reported significant drops since early 2000 (National Marine Fisheries Service 2018). This may in part be due to stricter regulation; however, firmer regulation of commercial take was required to combat the loss throughout the years. Though spat settlement was recorded on a variety of Mobile Bay reefs (Saoud et al. 2000), the existing population stocks are not as significant as historical counts suggest.

For circumstances in which shell cultch can provide the ground work for habitat protection and or restoration, it has been suggested that setting oyster larvae on the shell cultch before deployment may increase its benefits. This process, known as “remote-set”, is the setting of oyster larvae to a desired cultch and the planting of such cultch in environments for further growth. This process relies on hatchery reared larvae and is often used as a primary or secondary source of harvest for farmers (and is common in Washington State) and for restoration efforts (e.g, restoration efforts in Maryland).

Remote setting on larger cultch, often recycled oyster shell, requires a less costly and less labor-intensive process than single set oysters, but typically results in clusters. This is more practical for on-bottom culture operations. These oysters may be selected for the half shell market; however, inconsistencies in the shape and quality of the adult oysters may be more appropriate for shucking meats. As such, this may be a viable method for half shell production, but it is more than likely to be the most cost-effective method for meat production. This strategy can also prove to be beneficial for restoration purposes. Distributing spat on shell to existing reefs may booster natural stocks and assist in restoration or reef recovery efforts.

With declining population stocks, potential improvements needed to be studied for reef restoration, and the Alabama Marine Resources Division (MRD) requested a formal study of different planting strategies for spat on shell as a potential stock enhancement tool (C. Blankenship, pers. comm.). As such, the intent of this study was to determine if the augmentation of traditional shell plantings with spat on shell was a potential means for population enhancement in Mobile Bay. Small-scale experimental treatments, using remote-set, tested multiple historically active oyster reefs. Within the small-scale experiments, variable sizes and densities of spat on shell were tested to determine the most appropriate and effective planting strategies. The ultimate goal was to gain a greater understanding for remote-set methods and its potential for success in this region.

Methods:

Setting:

Setting Tank Set Up:

Three tanks were constructed at the Alabama Marine Resource Division (MRD) on Dauphin Island, Al. All tanks were outdoor and thus, were exposed to natural environmental conditions throughout setting periods. Tarp covered the tanks during the setting periods to reduce the input from rain and direct sunlight. A 10-micron cartridge filter was used to fill tanks and to keep tanks on continuous flow after a static setting period of three days. The intake was located on site and drew water from the channel in Little Dauphin Bay next to the tank array. Air lines were constructed in a grid format on the bottom of the tank using PVC. The cages were able to nest between the grids to create an evenly distributed airflow.

Each tank was capable of holding 20 (3’High x 3’Long x 1’Wide) cages (60 total). The cages were filled with roughly 400lbs of shell cultch per cage. They were then washed to remove as much silt and debris as possible before loading the filled cages into a tank with a fork lift. Filled tanks were left to soak for at least three days prior to the addition of eyed larvae so the shell cultch could accumulate an appropriate biofilm (Supan 1987, Wallace et al. 2008, Congrove et al. 2009).

Setting Period:

Hatchery-reared eyed larvae were procured from the Auburn University Shellfish Lab from 8/19/2016 – 10/1/2017. Collected larvae were added to MRD set tanks in increments of 5 million per tank. It was attempted in all trials to supply the eyed larvae in increments of 5 million, however; this was dependent on the ability to produce large volumes of viable larvae at one time. In some cases, larvae were refrigerated for no more than one day in order to amass a full 5 million. In a few trials, a full 5 million was not possible within 2 days and thus, the larvae were added incrementally over several days. The maximum amount of days needed to set occurred in the first attempt (August 5-11, 2016), spanning 6 days.

Larvae were fed a commercial algae paste, Reed Mariculture Inc.’s Shellfish Diet 1800®, over a three-day static period. Feedings would occur in the mornings and at night. A fully stocked tank (5 million) received 50ml of algae paste in both the morning and the evening. The feeding rates were adjusted accordingly if the larvae were added incrementally. In the instance in which the larvae were added over longer periods, water changes were required. Larval tanks went a maximum of three days before either a water change or a switch to flow through.

Flow Through Period:

After a 3-day static period, the systems were switched to flow through. The systems remained in flow through for 1 week before sampling and deployment. Once attached to the shell cultch as spat, it was no longer necessary to feed the tanks with algae paste. The incoming water from the channel had sufficient amounts of food to allow for further growth and development within the system.

Sampling and Analysis of Setting Efficiency:

After a week of flow-through conditions, samples of the shell were collected for analysis of setting efficiency. To promote representative samples, two shells from each of the 20 cages were collected and brought to the lab for counting. Shells were selected by digging 6-10 inches below the surface shell level and haphazardly selecting two shells without regard to the presence or absence of spat. This was to ensure randomness, and that the shells selected represented, to some degree, shells from the inner portion of the cages.

Using a dissecting scope, each shell was examined and the number of spat were counted and recorded. In addition to the counts, two size measurements were recorded randomly from each shell (if spat were present). Averages across the tank were determined and were later used as pre-deployment reference points. Furthermore, these counts helped to determine reasonable estimates for the number of total spat across the tank, and industry-accepted standard estimates for the setting efficiency.

Survival and Growth Experiments:

Experimental Design:

The design of the study was a two-factor field study constructed to test two factors under the control of resource managers: planting density and size. Three planting densities (10, 50 and 100/ft^2, designated hereafter as Low, Medium and High), and 3 different nursery durations (as a proxy for size classes) were deployed at 3 separate times (referred to as deployments A, B and C). The study, therefore, was a 3 densities x 3 size class factor design, with 4 replicates (yielding a total of 36 experimental units per planting).

Site Selection:

Sites for the plantings were selected in consultation with the MRD such that the experiments were conducted in areas of interest, or areas that were consistent with large-scale plantings underway or planned by MRD. Additionally, sites with pre-existing MRD shell plantings were chosen, so that the experiments were conducted in areas with existing oyster reefs. Plantings I and II were located south of Cedar Point, while Planting III was located farther north into Mobile Bay on White House Reef (Figure 1).

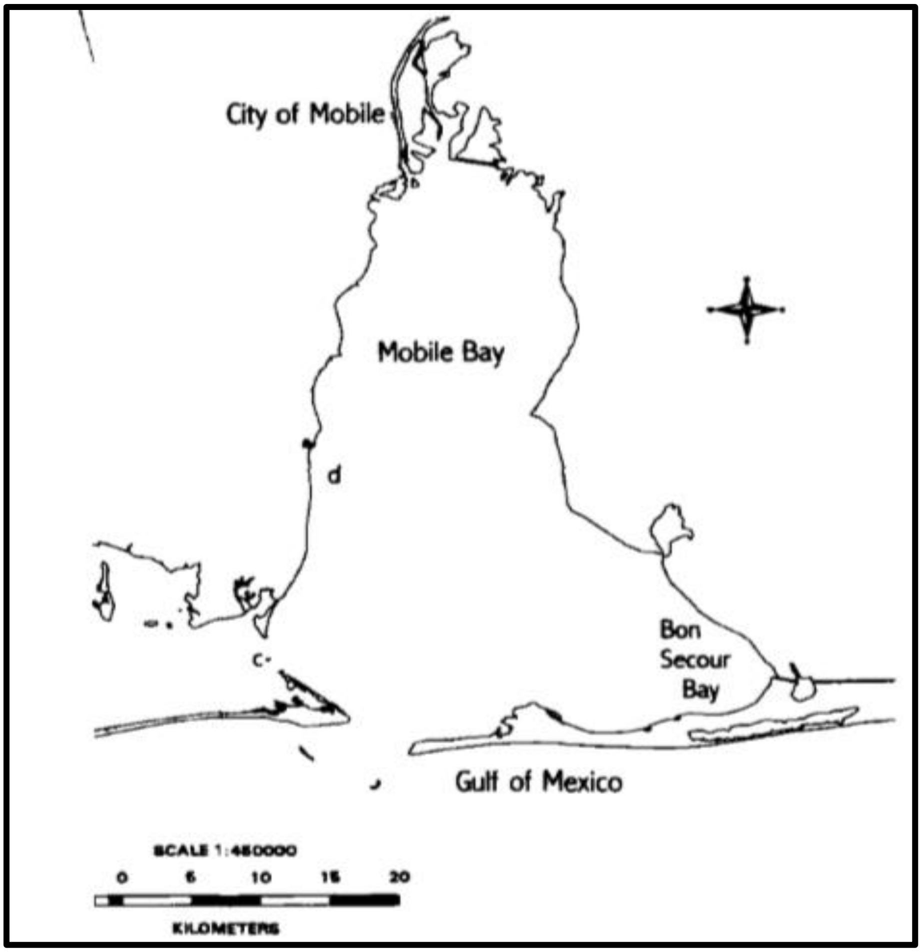

Figure 1. Mobile Bay Alabama and Study Sites**.** Point d = White House Reef. Point c = Cedar Point Reef. Original Image Source (Saoud et al. 2000).

Tray Array Design and Arrangement:

Spat on shell were placed in vinyl-coated wire trays (3’ x 3’ x 4” with x mesh) for field deployments. The trays were open at the top and not elevated such that the bottom of the tray was flush with the sediment when placed in the field. This design was preferred as it mimicked a more natural setting for the deployed spat on shell.

Trays were set up in two separate ways in different plantings (I, II, III), though both set ups were nearly identical apart from the number of rows in the array. Planting (I) included an additional replicate and thus required 3 rows of 15 trays (Figure 2). When one of the treatment replicates were dropped, the array was consolidated to two rows of 18 in Plantings II and III (Figure 3, Figure 4). This method was logistically simpler for the divers and for the over-all deployment. In both cases, the trays were aligned so that there was 0.5' in between each. Each of the rows of trays ran along a 3/8", braided polyester rope line which was anchored at either end by 18-inch earth anchors. The purpose of the line was to ensure that the trays were arranged linearly and to guide divers during sampling. Trays were secured by two 3/8" rebar stakes in opposite corners. Each tray was fitted with an identifiable cow ear tag. These tags assisted in deployments and in sampling considering the particularly limited visibility in the water.

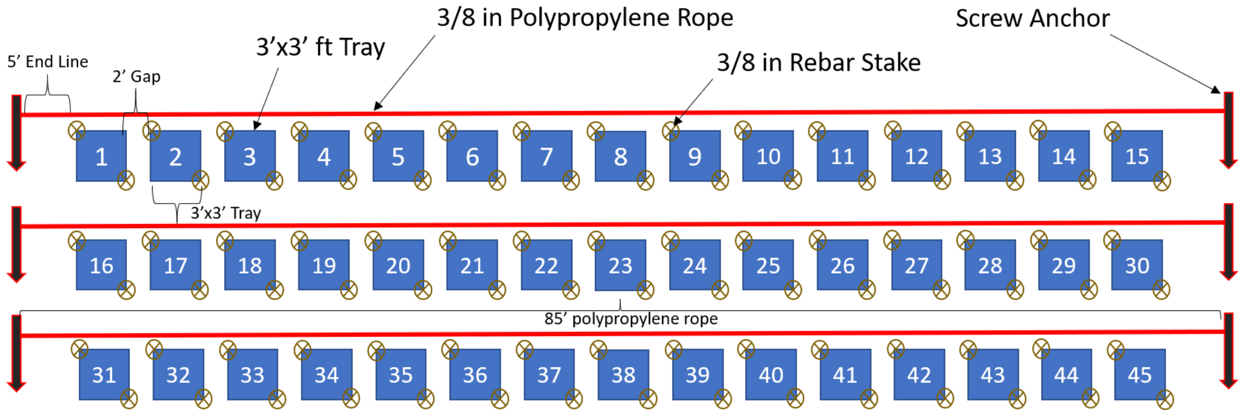

Figure 2. An overview of the tray array for Planting I is shown. In this planting, 3 rows were required.

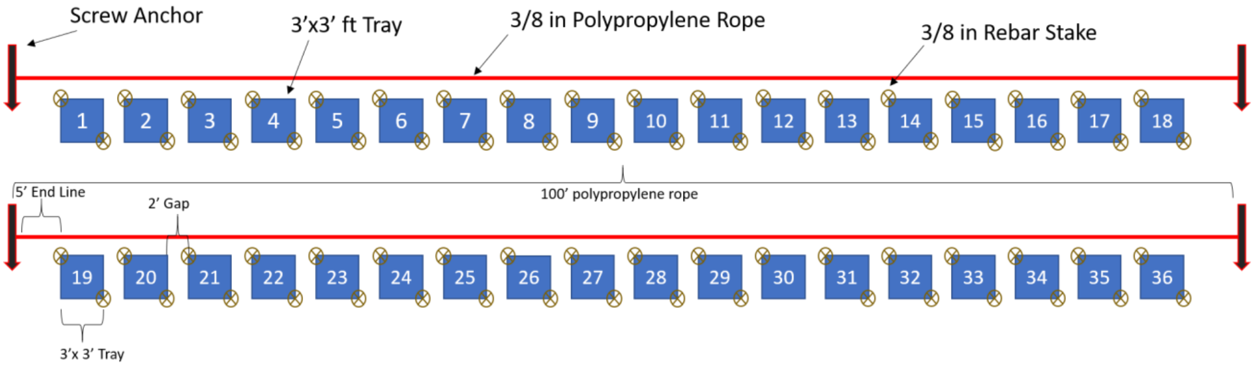

Figure 3. An overview of the tray array for Plantings II and III is shown. In these plantings, 2 rows were required.

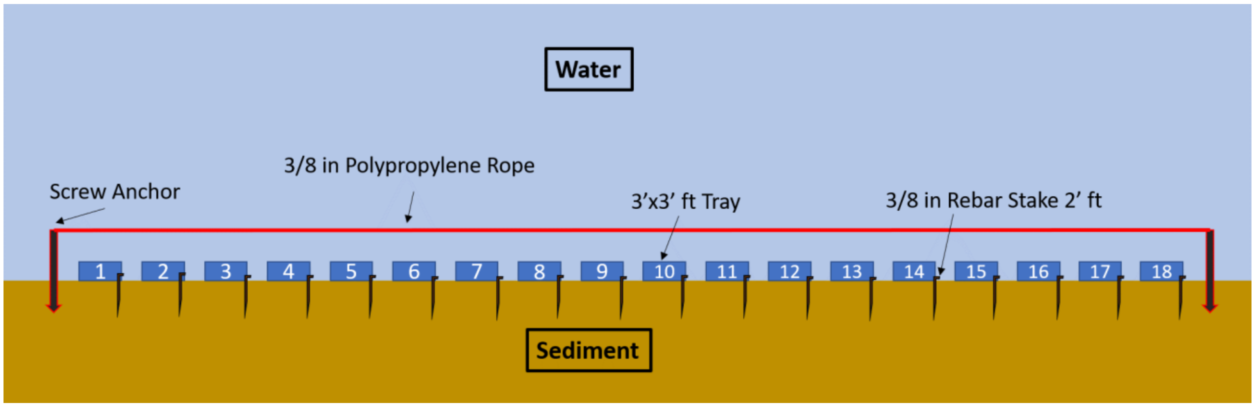

Figure 4. A profile view of the tray array for Plantings II and III is shown. The profile view for Planting I was set up identically except that trays were aligned in rows of 15 unlike the rows of 18 (shown).

Deployment:

After the completion of 7 days in a flow-through system, shells were removed and bagged for small-scale deployments. The number of shells stocked per tray was determined by the averages across the tanks such that there would be sufficient amounts for all density and size combinations. Nursery time was used as a proxy for size such that longer nursery periods coincided with larger sizes of spat. The three nursery periods were 0 weeks, 2 weeks, and 4 weeks (± 3 days), post setting period. These three size classes were referred to as deployments (A, B, C) respectively. The first size class (Deployment A) of spat on shell were deployed in randomly selected trays within the week following their removal from the tank systems. The arrangement of the different treatment combinations was selected using a random number generator to ensure that there was no bias within the array. The remaining oysters were placed in 6mm BST^{TM} bags and taken to the Auburn University Shellfish Lab farm site in Portersville Bay to continue the nursery period before deployments. Subsequent deployments (B, and C) were completed in the same manner after 2 and 4 weeks respectfully. Within each deployment, 3 separate control bags were placed in randomly selected trays and contained 20 aged shells. There were no live spat or other organisms present on the control bags prior to deployment.

Sampling Methods:

Collection of Field Samples:

Within a planting, treatments were destructively sampled at two separate times, designated as First Sample and Second Sample. For Planting I samples were taken one month (December 8, 201) and three months (February 14, 2017) after the last deployment (November 11, 2016). After observing the results from Planting I, it was determined that shorter-term samplings might reveal more dynamics after deployment. Accordingly, Planting II was sampled two weeks (August 25, 2017) and six weeks (September 26, 2017) after the final deployment. Due to a hurricane, sampling was delayed for the first sampling in Planting III to 3.5 weeks (October 11, 2017) with the second sampling at six weeks post-deployment (October 26, 2017).

At each sampling, trays were randomly selected such that two replicates of each treatment were destructively sampled at each time point. Specific trays were identified by the diver by the cow ear tags placed on each tray at the beginning of the trials. Samples were collected into polypropylene mesh potato sacks. These sacks were labeled with the corresponding ID tag found on each tray. The loaded sacks of oysters were attached via shark clip to a main line with a buoy (Figure 5). Once all samples had been collected, the main line was pulled into the boat along with all of the attached bags. This allowed for the shell cultch, and all associated fauna, to remain in the water. Once removed from the water, samples were loaded into coolers and brought back to the lab for analysis. Analysis of the samples took a considerable amount of time, and so, during this period, samples were placed in a flow-through system to reduce oyster mortality.

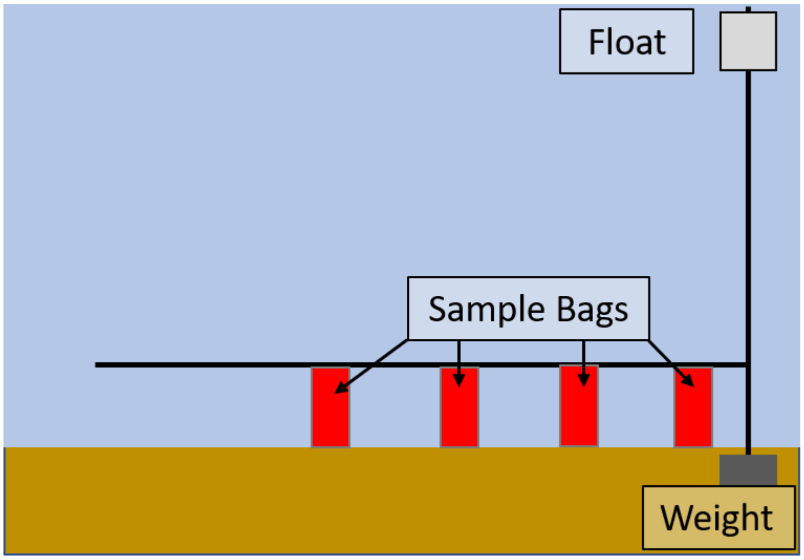

Figure 5. The sampling method set up is shown. The sample bags are attached the weighted line. Each sample bag contained a unique identification tag that corresponded with the appropriate treatment tray. All lines were equipped with a floatation device for easy retrieval.

Analysis of the samples:

Samples were assessed by individually examining each shell from each treatment. A count of all live spat were taken, as well as a measurement of each of their sizes. In addition to live spat, scars and dead spat were measured. All spat were categorized as live spat, scar present only, dead with shell present, or dead with evidence of oyster drill predation. Each measured and counted shell was identified and recorded for its corresponding treatment conditions.

Statistical Analysis:

Due to the differences in sampling among the plantings, each of the three experimental plantings was analyzed separately. The variability in the seasons and environmental conditions made the plantings inconsistent, and thus it would have been challenging to draw any conclusions from comparisons across them. Given the complications, the methods for each planting's statistical analysis is explained separately. All statistical analysis was completed using the program RStudio (RStudio Team 2016).

Planting I – Cedar Point Reef:

There was nearly 100% mortality observed in Planting I, largely as a result of heavy predation by southern oyster drills (*Thais haemastoma floridana)*. With very few live spat, nearly 100% of the counts were marked as zero and almost no size measurements could be made.

Planting II – Cedar Point Reef:

All counts and sizes of spat were recorded along with the density treatment (Low, Medium, High), Deployment (A, B, C), replicate, and sample time (First, Second). In this analysis, the counts and average sizes of the spat associated with individual shells were considered to be subsamples, where trays were considered replicates. As such, all subsamples were averaged to develop a mean count and mean size of live spat for each replicate tray of each treatment at each sample time. The analysis across each of the groups was ultimately made at the replicate level.

Analysis of Variance (ANOVA) tests were used to determine significant differences across densities, deployments and sample times for both the average counts and the average sizes of the spat. Each of the interaction terms was tested against the counts and the sizes separately. A post-hoc analysis was completed in both cases with a Tukey Test to assess all pairwise comparisons where the factors were found to be significant.

A secondary analysis was completed in which the assumed natural-set spat was removed from the dataset to allow for comparisons to be made with only the hatchery-reared remote-set oysters. The control shell bags were used to determine the mean sizes of natural spat associated with each treatment and sample time. Data points in each treatment that fell below the (*Mean + 1 Standard Deviation*) of the associated controls were removed from the data frame as these were assumed to be naturally set spat. The remaining data underwent the identical analysis as described above. ANOVA tests were used to compare across treatments types for both the average counts and the average sizes. A post-hoc analysis was completed in both cases with a Tukey Test to assess all pairwise comparisons where the factors were found to be significant.

Planting III – White House Reef:

Similar to Planting I, this planting experienced heavy mortality, though this time from a hurricane affecting the study site. Many of the treatments partially or wholly lost shell from the replicate trays, while others were covered in sediment. Additionally, the cause of mortality could not be accurately determined, and so the counts of the live spat were likely to be inaccurate estimates of the effects of the tested factors. The loss of shell, and the loss of treatments rendered this planting unsuccessful and thus no further analysis was completed.

Results:

Planting I – Cedar Point Reef:

This planting experienced nearly 100% mortality. Of the 1099 individual shell subsamples assessed from the First sample, only 46 live spat were found across all treatments. Notably, 30 (65.2%) of the spat were found in a single treatment (Deployment C, High density, Replicate 1).

The average size of the spat before each deployment differed significantly ($p < 0.001$) and was 7.89 ± 2.61 mm, 16.72 ± 4.28 mm, and 25.74 ± 6.86 mm for deployments A, B, and C respectively. Of all of the spat, 44/46 (95.65%) fell below the average (± the standard deviation) of the size at deployment, and are assumed to be natural set since it is unlikely that the spat did not grow for an entire month. This suggests that a very small percentage (2/46 or 4.3%) of the remote-set oysters survived to this time point, regardless of treatment.

There were 820 individual shell subsamples assessed at the Second sample, and only 3 spat found. The sizes of the three spat were 9.66mm, 8.42mm, and 8.39mm. All three individuals fell below the average sizes at deployment, and given that 2 months had passed, they were assumed to be natural set, suggesting that mortality of remote-set oysters was virtually 100% in this planting across all treatments.

Planting II – Cedar Point Reef:

Overall, oyster survival was much greater in Planting II in comparison to Plantings I and III. Across all treatments and sample times, excluding the controls, there were 2,401 observed spat. There was no significant effect of density alone or in interaction with other factors ($P \geq 0.28$), on the average live count of spat (Figure 6). There was, however, a significant interaction ($P = 0.039$) noted between sample time and deployment on the average spat counts (Figure 7). A post-hoc Tukey Test was used to interpret all pairwise comparisons. At the First Sample Time, the average live count of spat tended to increase from Deployment A to C, with significant differences between Deployment A and C ($p < 0.001$). By the Second Sample Time, however, there were no differences among Deployments ($p \geq 0.1$), and the average number was significantly lower than Deployment C at the First Sample Time ($p < 0.01$). There were no other significant interactions (Table 1). Looking at the overall survival rate (mean counts by deployment for each sample time), differences were found at the first sample time, but not at the second. At the First sample time, deployment A and C had a 10.08%, and 29.70% survival rate respectfully. The overall mean survival rate for the first sample was 20.13%. There were no significant differences in the second sample time, and the overall mean survival was 12.55%.

A significant three-way interaction ($p = 0.04$) was noted between the size of the spat and all three of the tested variables: density, deployment, sample time (Figure 8, Table 2). A post-hoc Tukey test was used to compare all possible pairwise comparisons. In the second sample time, the low-density treatment in deployment B significantly differed ($p = 0.005$) from the medium density treatment of deployment C. There were no other significant pairwise comparisons noted in the post-hoc analysis.

Table 1. ANOVA results for average counts of spat in response to explanatory variables for all data.

Explanatory Variable	p-Value	Degrees of Freedom	F -Ratio
Sample Time	<0.01 *	1	14.2
Deployment	<0.001*	2	14.2
Density	0.42	2	0.9
Sample Time x Deployment	0.04 *	2	3.9
Sample Time x Density	0.33	2	1.2
Deployment x Density	0.33	4	1.2
Sample Time x Deployment x Density	0.29	4	1.4

Significance ($p < 0.05$) for treatment types is signified by (*)

Table 2. ANOVA results for average sizes of spat in response to explanatory variables for all data.

Explanatory Variable	p-Value	Degrees of Freedom	F -Ratio
Sample Time	0.07	1	3.6
Deployment	0.75	2	0.3
Density	0.30	2	1.4
Sample Time x Deployment	0.03 *	2	4.3
Sample Time x Density	<0.01 *	2	6.5
Deployment x Density	0.36	4	1.2
Sample Time x Deployment x Density	0.04 *	4	3.2

Significance ($p < 0.05$) for treatment types is signified by (*)

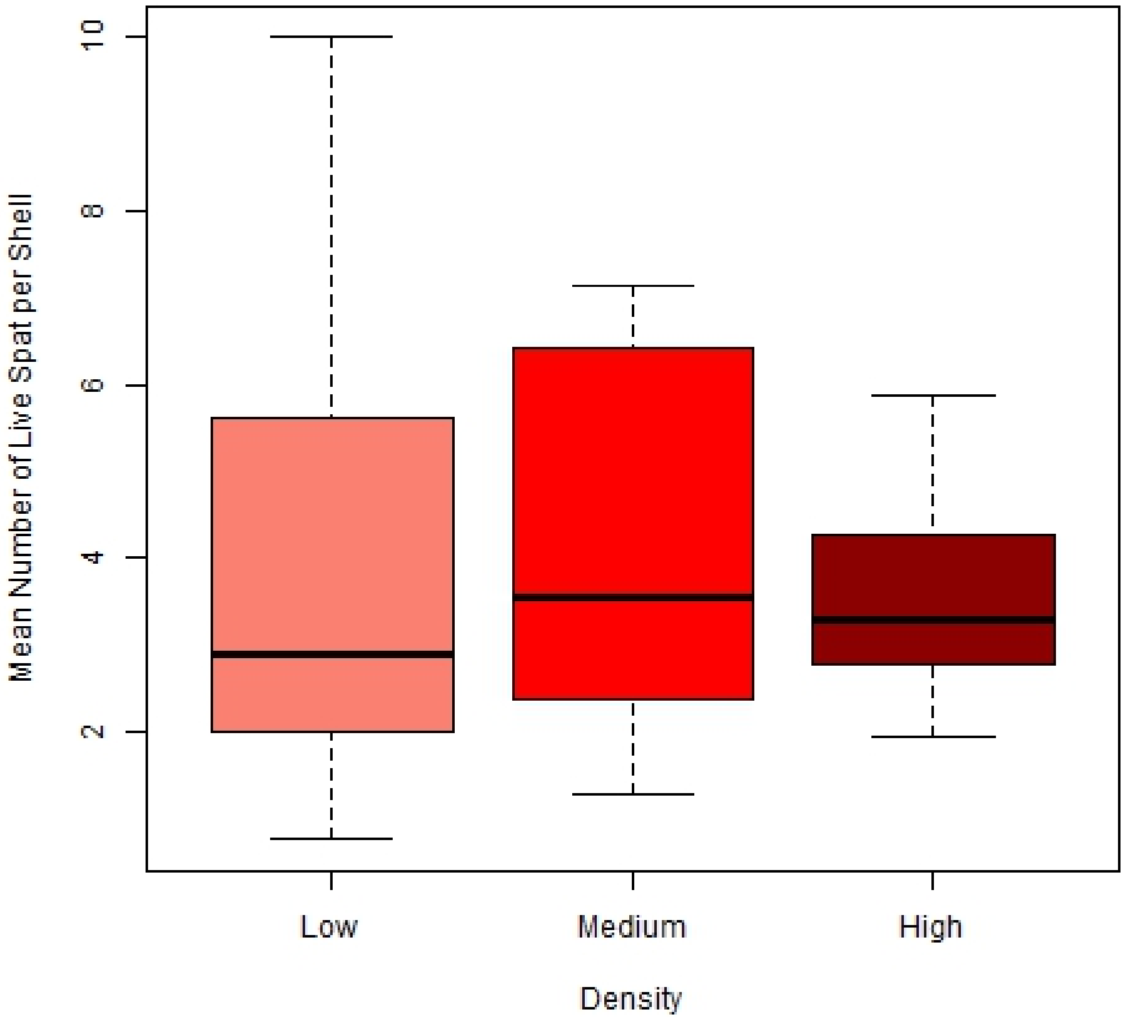

Figure 6: [Count x Density] For All Data - The average counts of spat on shell did not vary among density treatments (Low, Medium and High). The treatments low, medium, and high were held at 10, 50, and 100 spat/ft^2 respectively. There were no significant differences ($p = 0.42$) between the density treatments. The midline represents the median value while the upper and lower limits of the box represent the third and first quartiles respectively. Whiskers extend up to 1.5 times the interquartile range. Data outside of this range are represented individually as points.

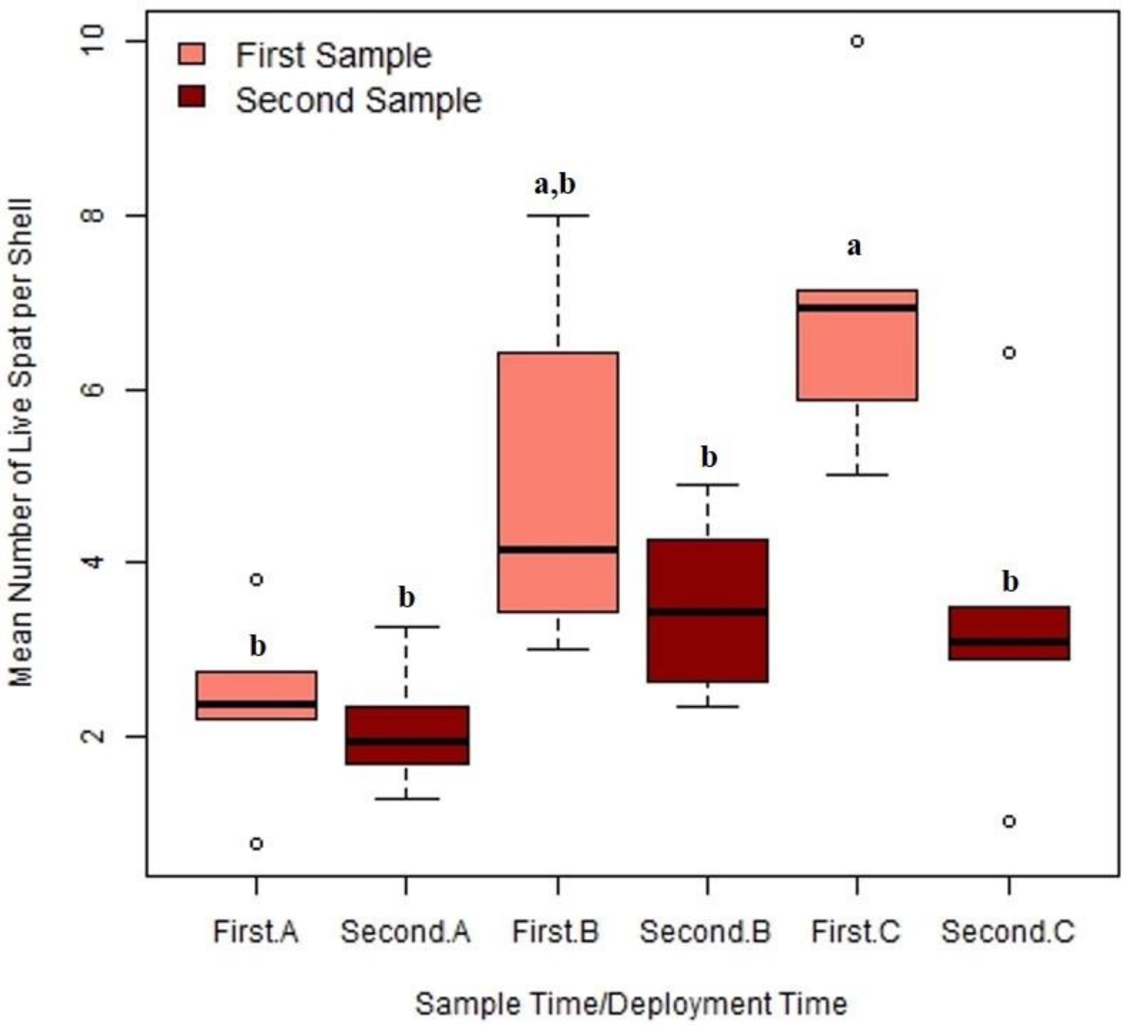

Figure 7: **[Count x (Sample Time x Deployment)] For All Data -** The average counts of spat on shell were related to the interaction between sample time (First, Second) and deployment (A, B, C). Groups that share a superscript are not significant ($p < 0.05$) from one and other. The midline represents the median value while the upper and lower limits of the box represent the third and first quartiles respectively. Whiskers extend up to 1.5 times the interquartile range. Data outside of this range are represented individually as points.

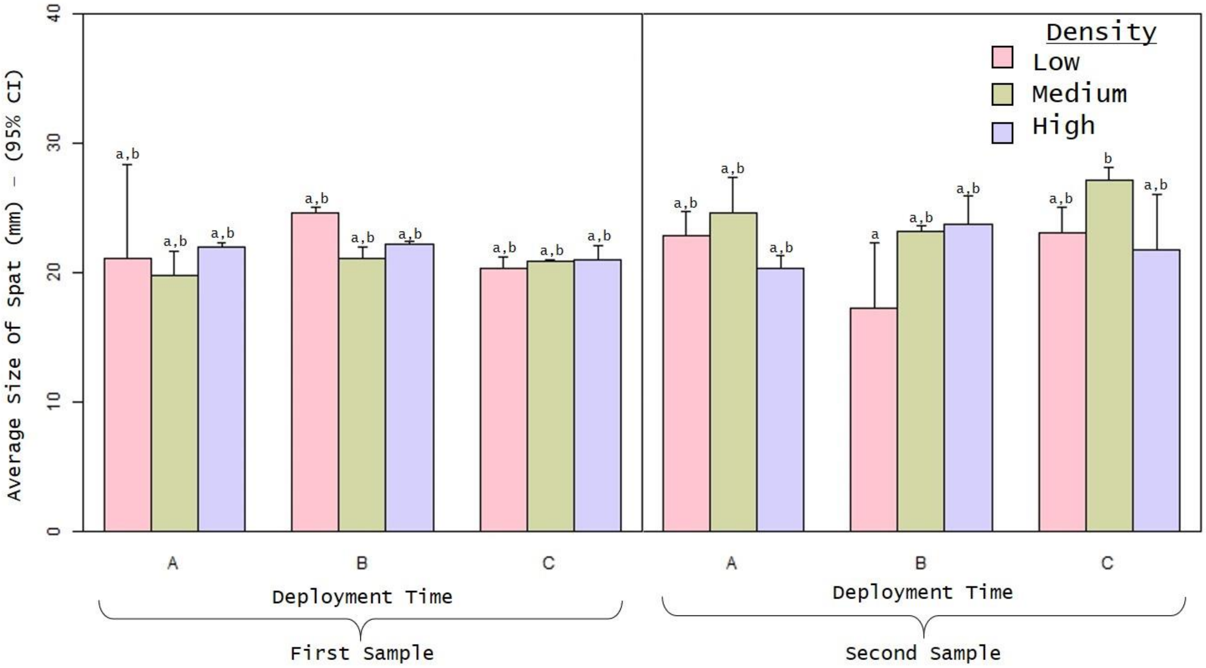

Figure 8: 3 [Size x (Sample Time x Deployment x Density)] For All Data - For the average size of the spat, a significant 3-way interaction was noted between Sample Time, Deployment Time, and Density ($p = 0.04$). A post-hoc Tukey Test indicated that there was a significant difference ($p < 0.01$) between (Sample Time B/Deployment B/Density Low) and (Sample Time B/ Deployment C/ Density Medium). There were no other significant pairwise comparisons. Groups that share a superscript are not considered to be significant ($p \leq 0.05$). Error bars show the 95% confidence interval.

After removing the oysters that were assumed to be natural set from the data frame, a secondary analysis was conducted. Across all treatments and sample times, excluding the controls, there were 2,005 observed spat (with 396 spat designated as likely to be natural set, or 16.5% of the initial total).

Again, there was no significant effect of density (Figure 9), alone or in interaction with other factors ($p \geq 0.62$). The average counts per shell, also again, were significantly affected by the interaction ($p = 0.04$) between sampling times and between deployment times (Figure 10). At the First Sample Time, the average live count of spat tended to increase from Deployment A to C, with significant differences between Deployment A and C ($p < 0.001$). By the Second Sample Time, however, there were no differences among Deployments ($p \geq 0.76$), and the average number was significantly lower than Deployment C at the First Sample Time ($p \leq 0.01$). At the first sample time, deployment A and C had a 9.15%%, and 29.70% survival rate respectfully. The overall mean survival rate for the first sample was 19.15%. There were no significant differences in the second sample time, and the overall mean survival was 12.55%.

For the average sizes of the spat, there was no significant effect of density (Figure 11), alone or in interaction with other factors ($p \geq 0.28$). Furthermore, there was an interaction between Sample Time and Deployment ($p = 0.05$). Within the First Sample Time, Deployment B had significantly larger spat than Deployment C ($p = 0.01$), but neither differed from Deployment A, which was intermediate ($p > 0.25$). At the Second Sample Time, however, there was a trend for average spat size to decrease from Deployment A to C, with A and C differing significantly ($p = 0.02$). Additionally, all the spat at the Second Sample Time, regardless of Deployment, were larger than the spat at the First Sample Time ($p < 0.001$).

Table 3. ANOVA results for average counts of spat after removal of assumed natural set in response to explanatory variables.

Explanatory Variable	p-Value	Degrees of Freedom	F -Ratio
Sample Time	<0.001 *	1	17.0
Deployment	<0.001 *	2	10.3
Density	0.49	2	0.5
Sample Time x Deployment	0.04 *	2	3.7
Sample Time x Density	0.56	2	0.6
Deployment x Density	0.65	4	0.6
Sample Time x Deployment x Density	0.86	4	0.3

Significance ($p < 0.05$) for treatment types is signified by (*)

Table 4. ANOVA results for average sizes of spat after removal of assumed natural set in response to explanatory variables.

Explanatory Variable	p-Value	Degrees of Freedom	F -Ratio
Sample Time	<0.001 *	1	152.0
Deployment	0.001 *	2	10.3
Density	0.28	2	1.4
Sample Time x Deployment	0.05 *	2	3.5
Sample Time x Density	0.41	2	0.9
Deployment x Density	0.64	4	0.6
Sample Time x Deployment x Density	0.42	4	1.0

Significance ($p < 0.05$) for treatment types is signified by (*)

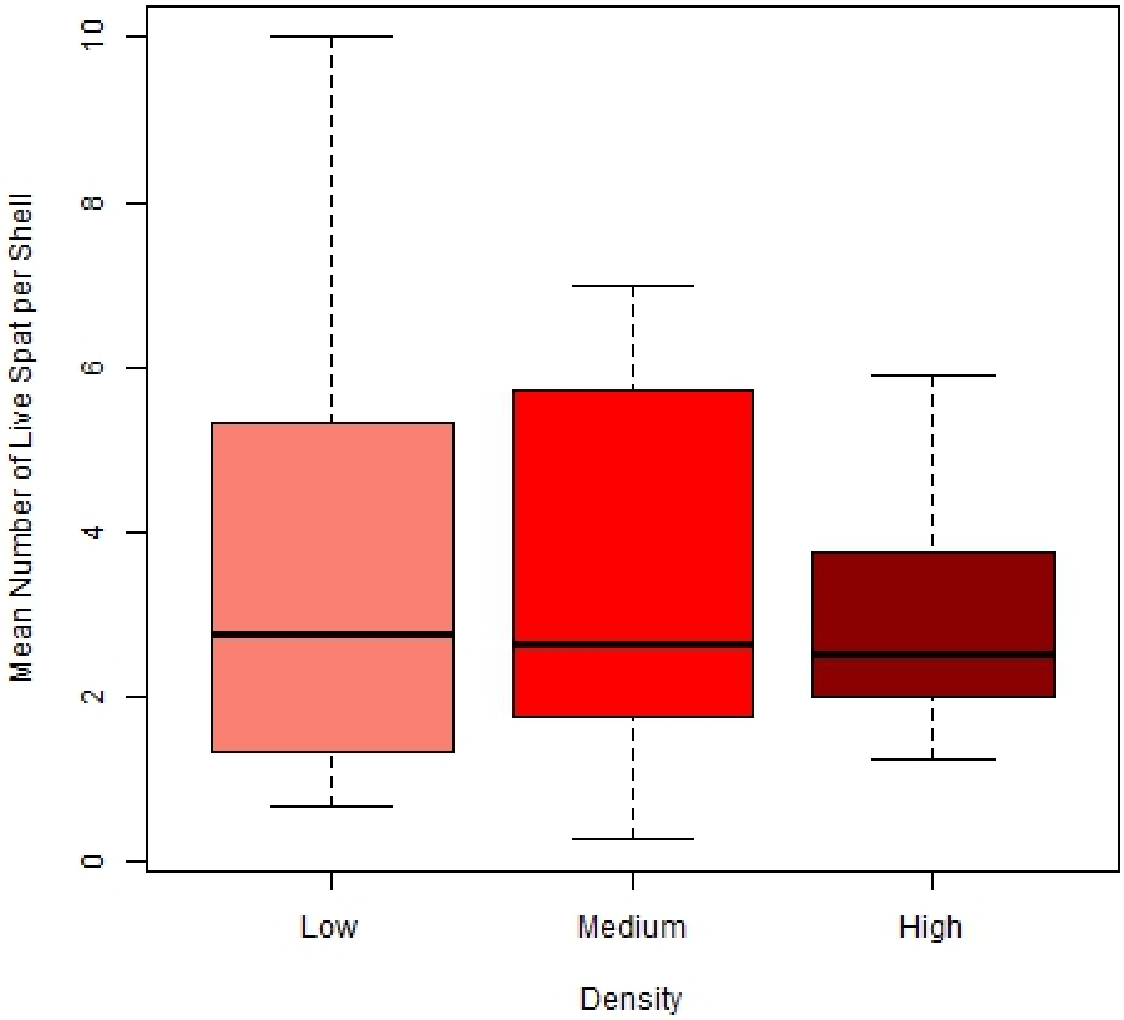

Figure 9: [Count x Density] For Data with Natural Set Removed - The average counts of spat on shell are shown density treatments (Low, Medium and High) after the natural-spat had been removed from the data frame. The treatments low, medium, and high were held at 10, 50, and 100 spat/ft^2 respectively. There were no significant differences (p = 0.49) between the density treatments. The midline represents the median value while the upper and lower limits of the box represent the third and first quartiles respectively. Whiskers extend up to 1.5 times the interquartile range. Data outside of this range is represented individually as points.

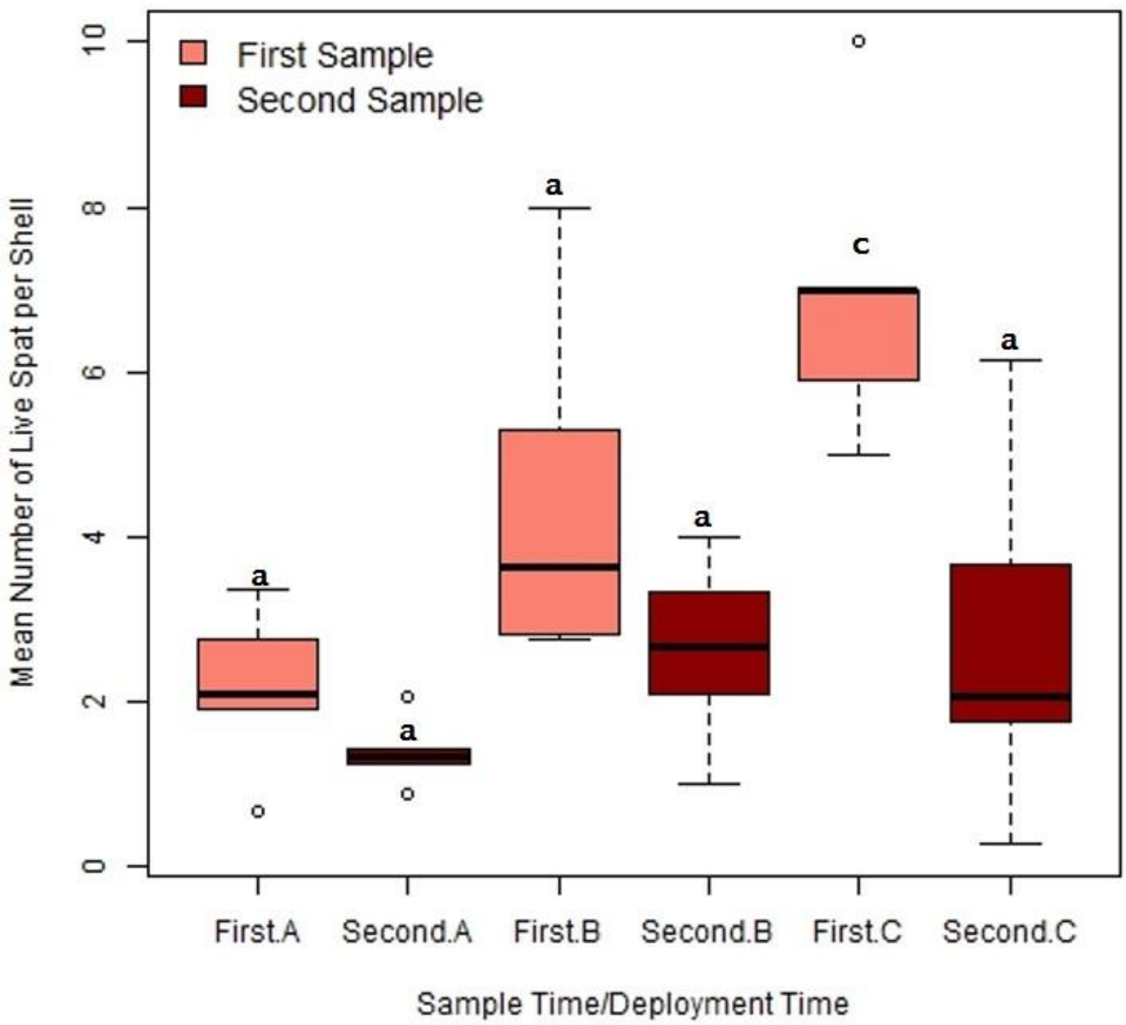

Figure 10: [Count x (Sample Time x Deployment)] For Data with Natural Set Removed - The average counts of spat on shell are shown for both sample time (First/Second) and deployment time (A, B, C) after natural-set had been removed from the data frame. Groups that share a superscript are not significant ($p < 0.05$) from one and other. The midline represents the median value while the upper and lower limits of the box represent the third and first quartiles respectively. Whiskers extend up to 1.5 times the interquartile range. Data outside of this range is represented individually as points.

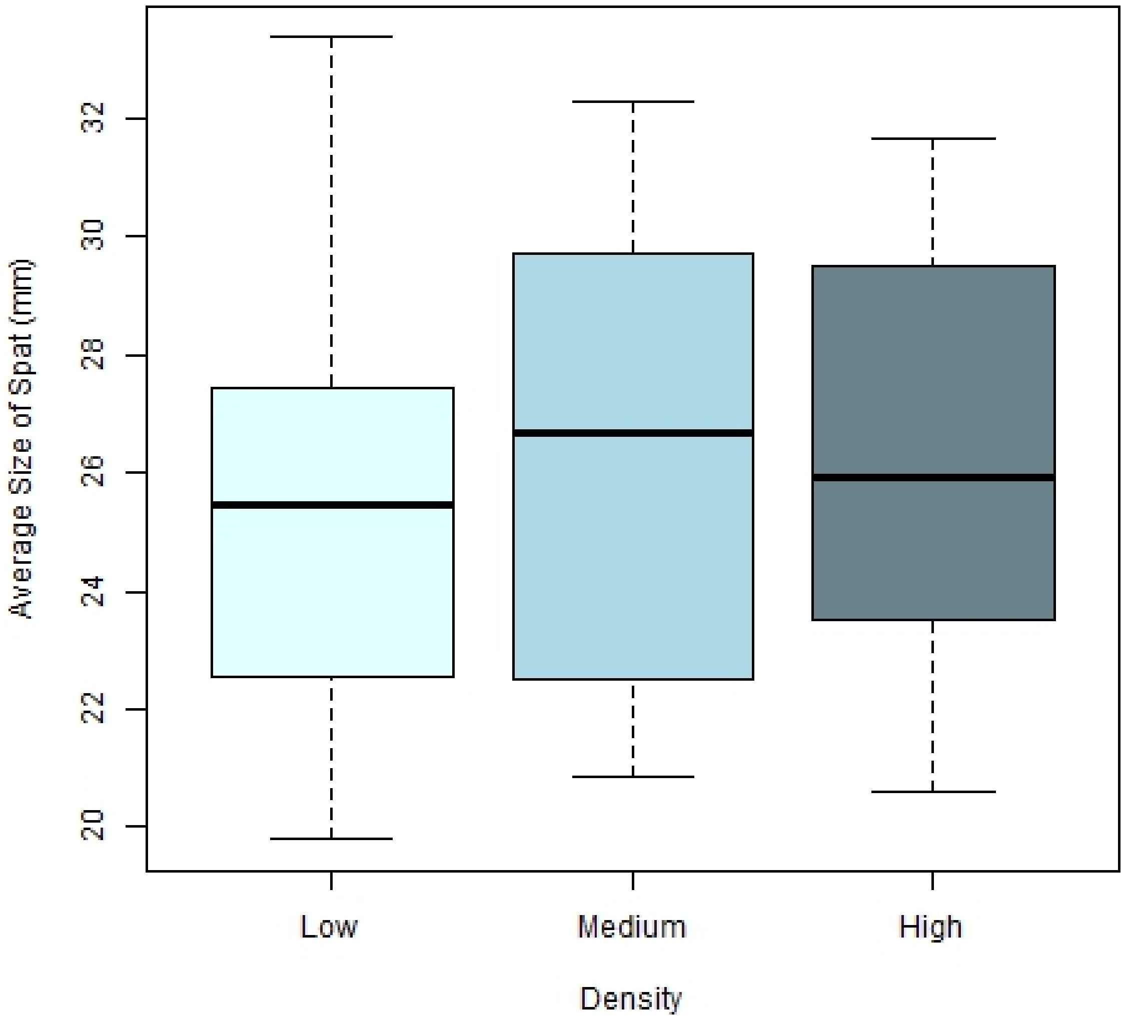

Figure 11: [Size x Density] For Data with Natural Set Removed - The average sizes of spat are shown density treatments (Low, Medium and High) after the natural-spat had been removed from the data frame. The treatments low, medium, and high were held at 10, 50, and 100 spat/ft^2 respectively. There were no significant differences (p = 0.28) between the density treatments. The midline represents the median value while the upper and lower limits of the box represent the third and first quartiles respectively. Whiskers extend up to 1.5 times the interquartile range. Data outside of this range is represented individually as points.

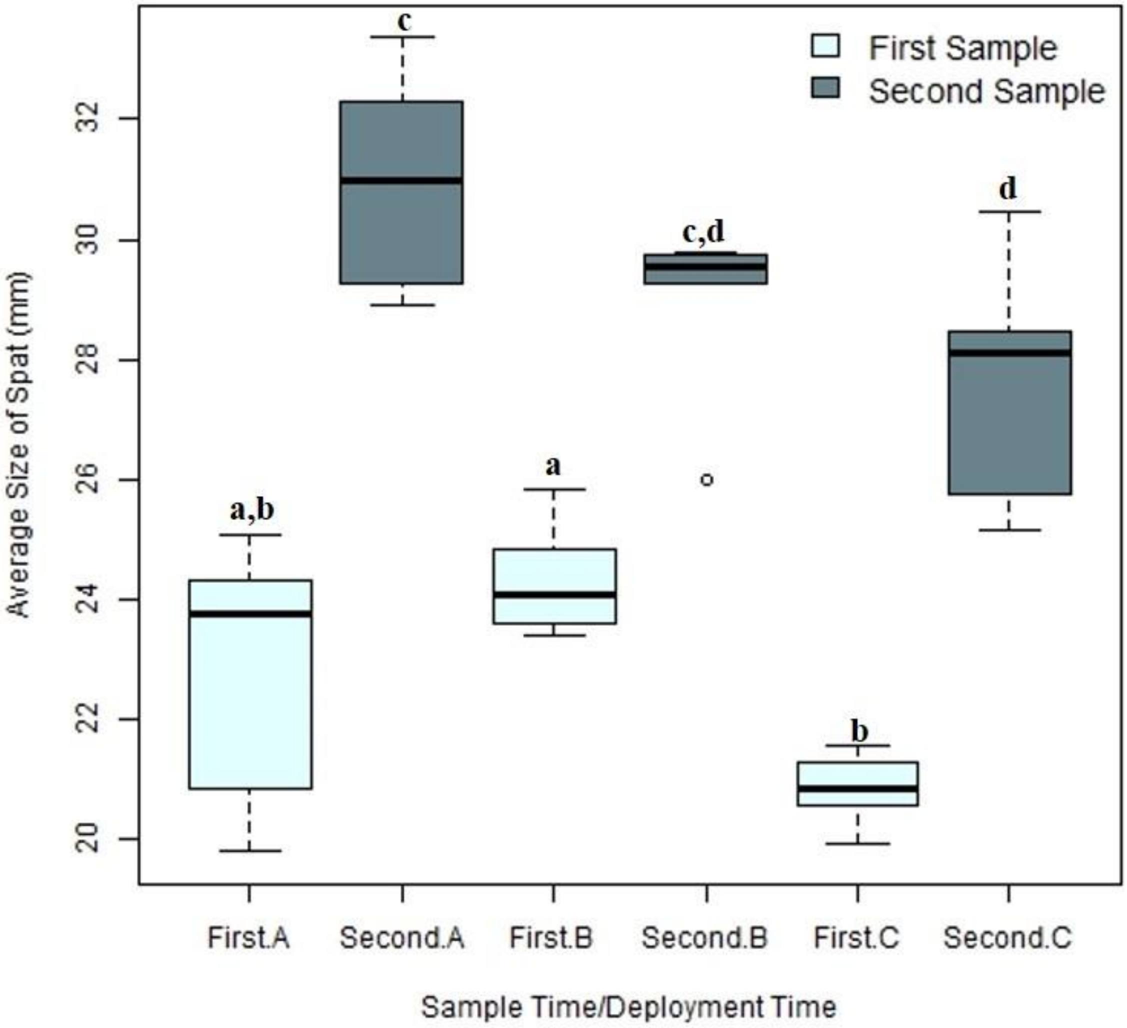

Figure 12: [Size x (Sample x Deployment)] For Data with Natural Set Removed The average sizes of spat are shown for both sample time (First/Second) and deployment time (A, B, C) after natural-set had been removed from the data frame. Groups that share a superscript are not significant ($p < 0.05$) from one and other. The midline represents the median value while the upper and lower limits of the box represent the third and first quartiles respectively. Whiskers extend up to 1.5 times the interquartile range. Data outside of this range are represented individually as points.

Planting III – White House Reef:

The effect of Hurricane Nate buried or scattered a sufficient number of replicate trays that no statistical analysis was attempted. Qualitatively, very high mortality was observed on the shell that remained, due in many cases to partial or complete burial by sediment.

Discussion:

Planting I – Cedar Point Reef:

There was nearly 100% mortality in this planting. As noted in the results, only 46 live spat were found across all treatments. Based on the average sizes before deployment, and the assumption of some degree of growth over the elapsed time, it was apparent that of the few live spat, ~ 95% were highly likely to be natural set. It is very possible, that the remaining 4 spat that did not fall below the average sizes at deployment were natural set as well. If this were the case, then 100% of the hatchery raised and remote set spat had perished one month after deployment at the first sample time. The 3 live spat found at the second sample were far below the average sizes at deployment, and were determined to be natural spat indicating that there was virtually 100% mortality after 2 months in the field. Additionally, the sharp decline in the number of spat from the first sample to the second sample indicates that the natural-set oysters were unable to survive either.

Calcium carbonate scarring from deceased spat was evident on almost all the samples assessed. These scars indicated that there were in fact spat present at one point, but the cause of death was impossible to determine with such little evidence. In some instances, the top shell of the deceased spat remained attached. In these cases, it was more likely that we were able to determine the cause of death. In almost all samples with the top shell intact, a small hole was found. These observations indicated that there was heavy predation by Southern Oyster Drills *Stramonita haemastoma* across the entirety of the planting. Southern Oyster Drills are a shell boring gastropod that predominantly feed on bivalves and shelled invertebrates. In order to circumvent the shell defenses, the oyster drills use both chemical excretions and radular scraping to bore a hole through which they may extend their proboscis and feed (Watanabe and Young, 2005). The abundance of oyster drills within the sampling bags provided further evidence for their predation.

Southern Oyster Drills thrive in higher salinity waters which is not typically the case for Mobile Bay. Regular high rainfall increases freshwater flows outward into the bay and allows the system to maintain a lower average salinity. In instances of drought, the lack of freshwater inputs into the system lead to higher average salinities. Drought conditions in early 2000's caused a large influx of oyster drills to Alabama's oyster reefs. Significant drops in harvest production from 2007 to 2008 were largely in part to heavy inundation of oyster drill predation (Waters 2010). A significant outbreak of oyster drills in Apalachicola Bay, FL between 2013 and 2015 paralleled the collapse of the oyster fishery in the region (Pusack et al., 2018). In drought conditions, the Southern Oyster Drill may pose a far greater threat to oyster reefs, or in the case of this work, the spat on shell deployments.

The failure to maintain a living population of remote set oysters in this planting highlights the importance of site selection, and the importance of predation risk assessment. For remote set to be a viable means of natural population enhancement in Alabama, a detailed assessment of the site locations is of paramount importance. Understanding the predation risks in a given location is important, as is understanding the environmental conditions that support predators. Detailed site monitoring of salinity (a critical environmental parameter for oyster drill habitat), paired with site sampling to assess potential predator abundances, may assist in determining optimal times for spat on shell deployments. Reducing the impacts of predation while spat are smaller and more vulnerable may allow time for them to grow, avert predation, and ultimately survive. Furthermore, remote set could potentially be conducted at times when predation rates are expected to be low.

Planting II – Cedar Point Reef:

This planting provided the best data to analyze the interactions between all of the treatment types, since there were spat that survived the entire trial. At the first sample time, where deployment led to significant differences, deployment A and C had a 10.08%, and 29.70% survival rate respectively. There were no significant differences in the second sample time, and the overall mean survival was 12.55%. With the removal of natural spat, the estimated survival rates dropped slightly except for deployment C. At the first sample time, deployment A and C had a 9.15%%, and 29.70% survival rate respectively. There were no significant differences in the second sample time, and the overall mean survival was 12.55%. This survival was considerably greater than both of the other plantings. In this timeframe, there were no major storm events, and far fewer oyster drills were sampled alongside the shells.

Interestingly, the densities at which the spat were planted (10, 50, 100 spat/ft^2) had no effect on the average counts once sampled (Figure 6, Figure 9). Statistically, there was no evidence that stocking at higher densities benefited or harmed the survival of the spat in each treatment. This was true both before and after the natural-set spat were removed from the data frame. This is important because it indicates that spat on shell plantings can, in theory, be more dilute (covering more ground with the same amount of shell) and still achieve the same benefits. A more detailed study focusing on large scale planting densities would have to be conducted;

however, this small-scale experimental set up supports that the lowest density (10 spat/ft^2) achieves the same benefits of higher densities. The ability to conduct remote set without regard to density allows resource managers flexibility in their decision-making about site selection.

For average counts, a significant interaction between sample time and deployment is apparent in both analyses (Figure 7, Figure 10), with the same trend. Counts of oysters are much higher on average in later deployments than the prior deployment. While this could suggest that the later deployment of the spat allowed greater survival, we note that the spat on the third deployment (Deployment C) had also been in the field for 4 weeks less than the first deployment. By the time of the second sampling (4 weeks after the first sample), this trend is no longer apparent. This result indicates that there may be short-term benefits to holding spat on shell in a protective environment to grow, but the longer-term benefits are not apparent. This suggests that there is no significant benefit to resource managers to hold spat on shell for additional time, in terms of the average number of live spat (particularly when it is noted that resource managers are interested in survival to reproductive and/or harvest size).

In the first analysis containing the natural-set spat (Figure 8), there was a significant 3 - way interaction between all tested variables (density, deployment, and sample time). The only significant pairwise comparison was determined to be between the second sample time, low density treatment in deployment B, and the medium density treatment of deployment C. While this is clearly a significant interaction, it is difficult to assess the biological significance. These differences were noted at relative extremes and were unaccompanied by any additional significant pairwise comparisons. As such, it was particularly difficult to assess the importance of this finding.

In the second analysis, two interesting trends are apparent in the sample time and deployment time interaction (Figure 12). Firstly, it is now obvious that the spat were in fact growing over time. The sizes at the first sample time were significantly smaller than those at the second sample time. Secondly, it is apparent in both sample times that the average size is lowest at the later deployment times. This is not an expected result because, it seems more intuitive that spat deployed at a later time should be equal to or larger than spat deployed at smaller sizes when sampled. One potential theory to explain this trend revolves around the protected environment used to grow them to larger sizes. During the nursery phase, it is possible that biofouling accumulation on the bag throughout the holding period had reduced water flow in and out of the bags. This reduced water flow could have restricted food availability and thus stunted their growth. It is possible that in the attempt to protect and grow the spat to larger sizes, they were inadvertently stunted. This is notable because this suggests that the additional holding time resulted not only in a lack of effect on oyster survival but also a negative effect on average size.

Planting III – Cedar Point Reef:

Hurricanes are a potential threat to all coastal systems including the Gulf of Mexico and Mobile Bay. In accordance with NOAA’s Historical Hurricane Tracks database (updated last in 2017), there have been 64 notable hurricanes and tropical storms (Figure 13) to track within 100 miles of Dauphin Island since 1900 (NOAA, 2017). In each of these events, coastal areas are at increased risk of storm surges, higher energy wave action, sedimentation, and shoreline erosion.

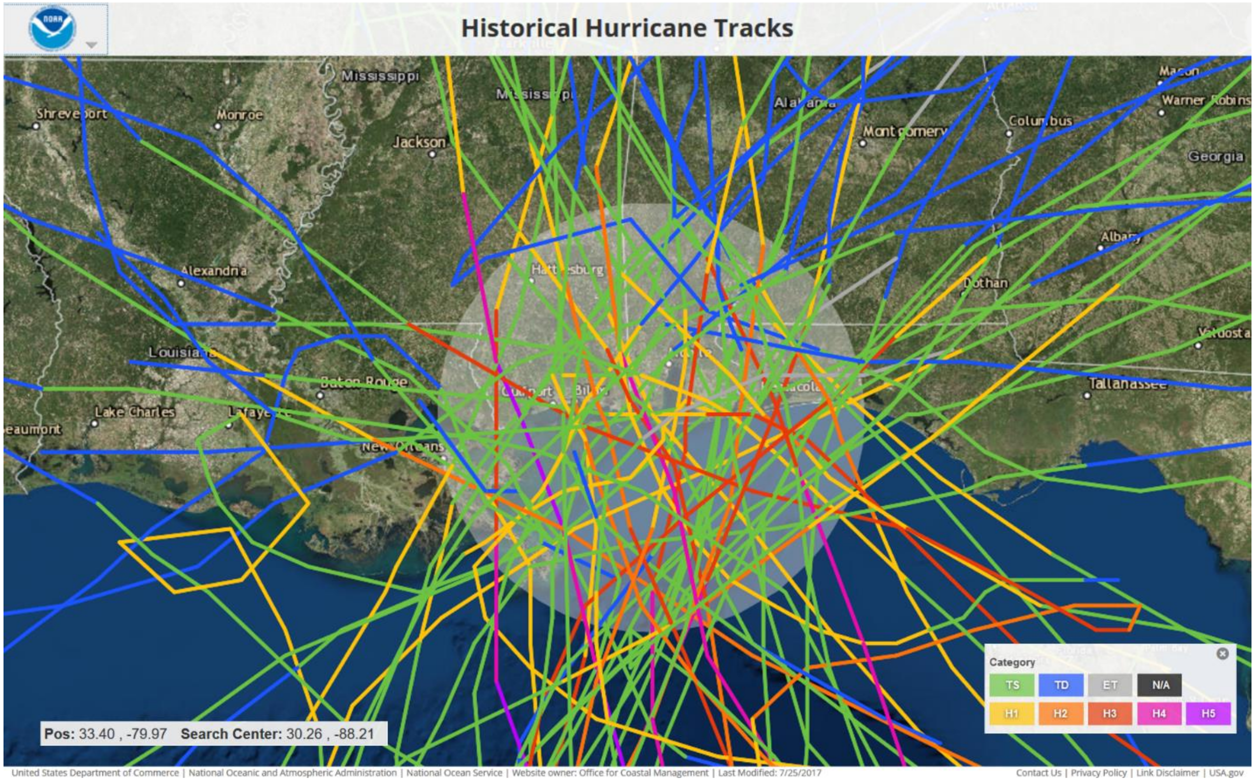

Figure 13: NOAA Historical Hurricane Tracks Since 1900 - This figure taken from NOAA's historical hurricane data base shows all of the hurricane tracks within 100 miles of the study area since 1900. The color indicates the severity of the storm ranging from "Tropical Storm" to "H5" on the Saffir-Simpson Hurricane Scale. Original Image source (NOAA 2017).

Hurricane Nate passed over Mobile Bay on Oct. 7th, 2017 as a category one hurricane (Figure 14). This was not an incredibly destructive hurricane when compared to other historical storms, but it did have considerable effects on the study area. This was one of the fastest moving hurricanes of all time and so its effects did not linger; however, the effects were great enough to cause significant damage to our planting. Ultimately, Nate's effects on Planting C were great enough to not allow any discernable significance between treatment types. The two most significant issues that this hurricane caused were shell loss via increased wave energy and sedimentation and burial of remaining shell.

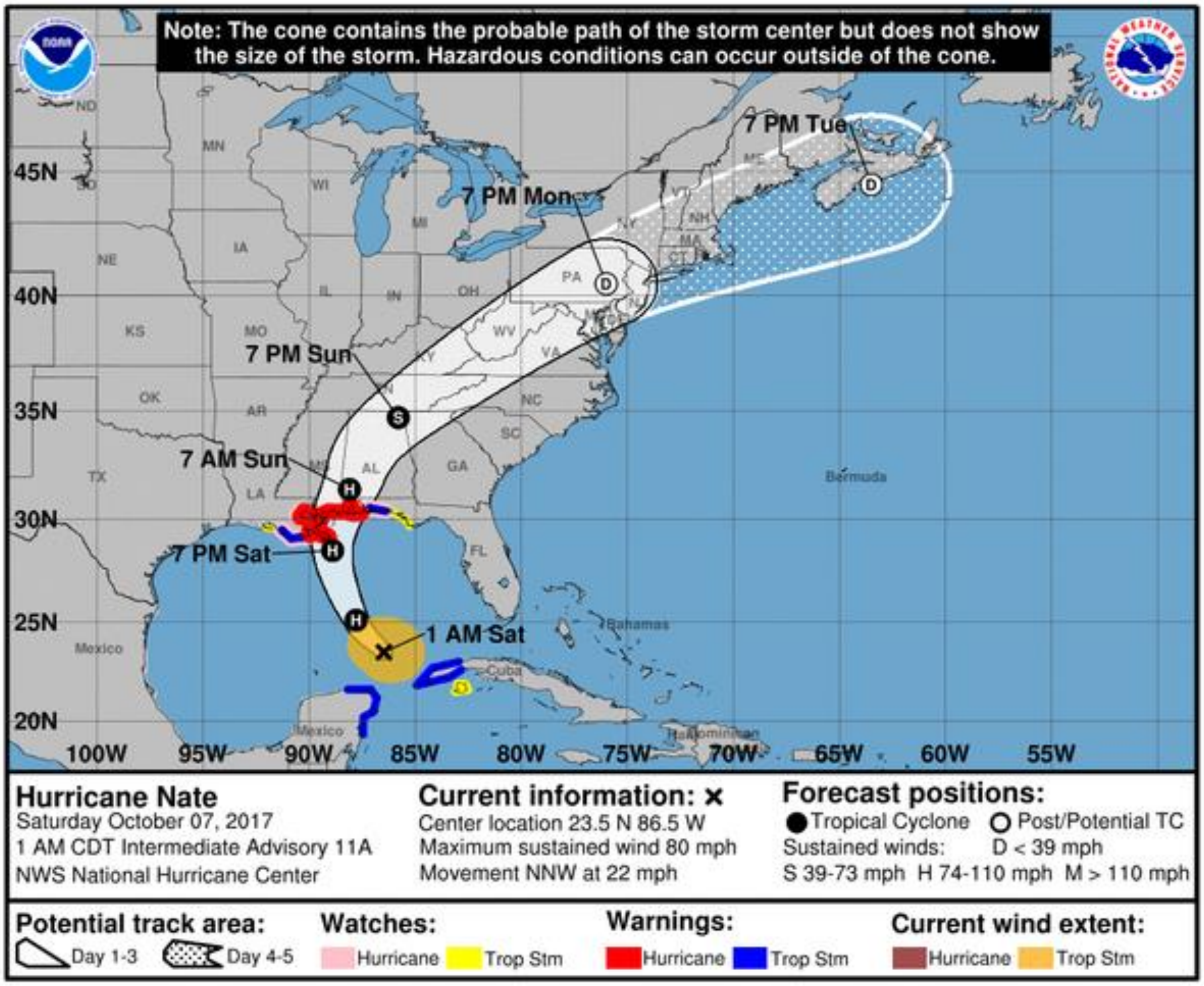

Figure 14: NOAA Historical Hurricane Track (Hurricane Nate) - This figure taken from NOAA's historical hurricane data base shows hurricane Nate passing through the study area between 7PM Oct. 6th and 7AM Oct. 7th, 2017. Original Image source (NOAA 2017).

Increased wave energy from the storm had a huge impact on the planting. This planting site was located in relatively shallow water (4-6ft) in normal circumstances. At this depth, normal wave action was not great enough to disturb the planting sites; however; the increased storm wave energy had direct effects on the benthic environments which included our experiments. Fast currents and violent waves caused the shell to be thrown from, and likely into, the trays. This was the more significant of the two issues since it physically removed subsamples from the experiment and caused the experiment to be incomplete. Given the amount of shell that had been lost in each treatment, and the number of treatments that had been lost entirely, it was impossible to determine accurate estimates that reflected the experimental effects.

The other predominant issue that affected this planting was sedimentation. This was likely caused by the increased wave energy. As with the shell, the shallow environment subjected the sediments to stresses via waves and currents. The violent wave action would have suspended sand and other particulates within the water column and then deposited them on top of the treatments, ultimately burying the shell. This sediment transport may have happened directly within the site, but it is also likely that deposited sediments may have originated from elsewhere in the bay or from river inputs. In particular, this site was located directly south of Fowl River. Suspended particulates and debris due to storm conditions on land may have been deposited into river systems and then subsequently deposited into the planting site.

Regardless of the origin of this sediment transport, the result was that it effectively suffocated the spat in our treatment. When samples were taken, it was impossible to tell the cause of death. It was possible that the spat that were buried had died before the storm, but it was equally likely that they had suffocated from burial. This further convoluted the data, and as such, the survival counts no longer reflected the true effects of the treatment.

The combined effects of increased wave energy and sedimentation exemplified the possible negative impacts of storm systems to oyster restoration efforts. Major storms are difficult to prepare or plan for due to their irregular and unpredictable nature. This again, highlights the importance of site selection to account for this possibility. Increasing the depth of selected sites may negate some of the effects of increased wave energy. Shells deposited further from surface waters are less likely to be physically transported by waves or currents. Mobile Bay is shallow by nature, but there are certainly areas for which the increased depth may enhance the success of remote set methods. Additionally, being aware of the location of riverine inputs may help to avoid some degree of sediment transport and burial in storm events.

Conclusions:

Remote-set spat on shell stands as a potentially viable method for population enhancement of public oyster reefs in Alabama so long as specific care is taken to assess site locations in advance. Of the experimental plantings, two of the three were failures, though for different reasons. Data from Planting II support survival of deployed spat over a 6-week period in the field. There is no evidence to support increased densities of spat on shell plantings, nor is there strong evidence that the additional holding time to allow the spat to grow larger before deployment led to greater survival (and, in fact, led to smaller oysters). Evidence of oyster drill predation in Planting I reveals the risk of high mortality events from predation. A careful assessment of predator risk in addition to site monitoring of environmental conditions can assist in the evasion of predation. The nature of shallow water systems exposes plantings to high energy storm events via increased wave action and sedimentation. Choosing sites with increased depths may reduce the impacts of wave action and sediment transport in storm events or other.

In the second planting, however, the survival of the spat on shell suggests that spat on shell may contribute to stock enhancement. The majority of losses were observed by the time of the first sample. While losses continued to the second sample time, the rate of decline had dramatically slowed. Notably, the survival of oysters in the first deployment did not drop between the two sample times, further suggesting that the majority of mortality was experienced within the first couple weeks of deployment. Further work needs to be done to establish the rates of survival from the time of the second sampling (e.g., six weeks) to potential harvest size.

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CHAPTER 4: BUDGET ANALYSIS AND THE ASSESSMENT OF FUTURE COSTS AND RETURN VALUE

Budget Analysis and Assessment of Future Costs

The analysis of the budget, and the projection of future costs associated with remote set methods are particularly important to this study. The indication of survival in one planting in Chapter 3 demonstrates that remote set may be a viable method for the Gulf of Mexico, and Mobile Bay in particular. To further assess the viability of remote set, future work will need to be competed. Here, however, I provide a basic budget analysis of remote set as a potential stock enhancement tool in Alabama.

Throughout the field study, hourly requirements for all aspects of the remote set process were determined with the assistance of the Alabama Marine Resource Division. From this, a sum total of the hours required could be developed for each tank set (Table 1). The total hours required (98.5 hr) per tank was 46.5 hours greater than the originally budgeted cost for the field study. An estimated 52 hours of labor was budgeted for each of the 10 tank sets. This proved to be one of the financial bottlenecks in the original budget and highlighted the importance of accurate labor estimates for future work. It should be noted that there are no cost estimations for the nursery phase discussed in Chapter 3. Results indicated, however, that there was no significant biological benefit to holding them in a nursery phase, and so it is not included in the assessment of costs as this is not a recommended strategy.

Table. 1 Component hourly labor requirements for the set up and completion of one tank set.

Activity	**Personnel Breakdown**	**Biologist Aide Hours**
Shell Loading (cages)	2 bio aides x 8 hours	16
Shell Washing	2 bio aides x 6 hours	12
Tank Loading	2 bio aides x 8 hours	16
Daily Feeding (3 days)	1 bio aide 0.25 hr feed am/pm (3 days)	1.5
Tanks Maintenance	Fine filter cleanout - 0.75 hour	0.75
	Course filter cleanout - 0.5 hour	0.5
Growth Period (7 days)	1 bio aide x 0.25-hour tank check am/pm (7 days)	4
Deployment	4 bio aides x 8 hours	32
Cleaning Tanks, air grid, cages, for next event	2 bio aides x 8 hours	16
Total hours Per Tank		98.25

In order to determine a potential yearly cost, it was simplest to determine a standardized deployment size. For the purposes of this budget analysis, all component costs were standardized to represent the cost associated with planting one full acre of spat on shell. In this manner, the total yearly costs represent a planting size of one acre planted with 1” deep coverage (Table 2). These yearly figures could be adjusted for larger or smaller deployments depending on the acreage desired for the project.

Table. 2 Predicted total yearly costs for the production of remote set spat on shell required to cover 1 acre of ground in 1 inch of shell cultch. Yearly costs are broken down into component costs and totaled..

Costs	Cost / Acre (USD)
Cultch	4,725
Vessel Usage	11,972
Labor	44,325
Eyed Larvae	27,000
Feed	2,850
Total	$90,872

The cost of cultch was estimated by prior years bid estimates per cubic yard (c.y.) of material. Bid estimates for shell cultch ranged between $50 – 75, however, deployment costs were factored into this estimate. Since deployment would be handled internally (accounted for in vessel costs), shell estimates would be considerably lower. Only the cost of cultch and a delivery fee would need to be accounted for, and so the original bid estimates were halved to $35/c.y. of cultch. The cost of the cultch was multiplied by the total cubic yards required to cover 100% of one acre in 1 inch. This was determined to be 135 c.y. per acre in accordance with the Alabama Marine Resource Division (Alabama Oyster Management Plan 2016). The total cost per acre was determined to be $4725 (Table 3). The estimate of 135 c.y./acre was also used to determine the number of tanks necessary. Each cage was capable of holding 0.38 c.y. of cultch. This was used to calculate the total tanks needed per acre (Equation 1).

Equation. 1 Calculation to determine the number of tanks required to cover one acre of bottom with spat on shell.

$$\frac{(0.38\,c.y./cage\ \ x\ \ 20\,cages/tank)}{135\,c.y./acre} = 17.76\,Tanks\ (rounded\ to\ 18)$$

Table. 3 Projections for the estimated cost of one acre's worth of spat on shell based on previous years bid estimates.

Cultch Cost	Cost of shell/c.y.	c.y. shell/ Acre	Cost Shell/Acre
	$35	135	$4725

Labor costs, as mentioned before, were determined by breaking down the elements of each tank set and determining a total hourly requirement. The hourly requirement was multiplied by an estimated hourly wage, and then multiplied by the number of tanks required for one acre of planting (Table 4).

Table. 4 Projections for the estimated labor cost associated with one acre of spat on shell planting. Labor costs are based on an estimated hourly wage which includes both salary and benefits.

Labor Costs	Hr/Tank Set	Wage/Hr	Cost/Tank Set	Cost/Acre
	98.25	$25.00	$2463	$44,325

The vessel costs were determined by day, where both the gas, maintenance, and vehicle depreciation were accounted for. Only one tanks worth of cultch (20 cages) was able to be deployed in the field per day, so the daily cost ($665.10) was equivalent to the cost per tank. Multiplying the cost per tank by the total tanks per acre determined the yearly cost (Table 5).

Table 5. Projected vessel costs associated with one acre of spat on shell planting. Cost per day is determined by the vessel cost per day in 2016-2018.

Vessel Costs	Cost/Day	Tank/day	Tank/Acre	Cost/Acre
	665.1	1	18	11,972

The last two components of the setting process were the hatchery reared eyed larvae and the associated feed needed for the 3-day setting period. Larval cost estimates were taken from current rates offered at the Auburn University Shellfish Laboratory in Dauphin Island, Alabama. The price for 1 million diploid oyster larvae (sized over 200 microns) is currently $300.00. At 5 million larvae per tank set, the cost per tank was determined to be $1500. For all the larvae needed per 1 acre of shell deployment, the total cost was determined to be $27,000 (Table 6). Larvae required feed for the first three days they were introduced to the tank. Commercially cultured algae, Reed Mariculture Inc.'s Shellfish Diet 1800®, was fed to the tank at 50 ml, 2 times a day with reference to Rikard and Walton (2012). This particular company distributes in one-quart containers which is equivalent to 946.35ml (rounded to 950 ml for calculations). One bottle was capable of feed 3.17 tanks with 57 total bottles needed per acre. Shellfish Diet 1800® is priced at $50 per 1-quart bottle and so the total feed cost per acre was determined to be $2850

(Table 7). It should be noted that this is a conservative feed estimate. Depending on larval availability, feeding may span over 4-5 days at maximum which would inherently increase the cost of feed.

Table 6. Projected larval costs associated with one acre of spat on shell planting. Costs for larvae were estimated based on prices offered at Auburn University Shellfish Lab.

Eyed Larvae Costs	Cost/Mil 2N	Eyed Larvae/Set (Mil)	Cost/Tank 2N	Cost/Acre 2N
	300	5	1500	27,000

Table 7. Projected feed costs associated with one acre of spat on shell planting. Costs were determined based off Reed Mariculture Inc.'s Shellfish Diet 1800®.

Feed Costs	Cost/Bottle	ml/bottle	Bottles/Tank	Bottles/Acre	Cost/Acre
	50	950	3.17	57	2850

Assessment of Potential Return Value

In order to estimate return value for the deployed oysters purely as potential harvest, survival rates from Chapter 3 were used in conjunction with current estimated market prices for oysters in Alabama. In this manner, the return value was a reflection of the potential number of harvestable oysters per acre. The first step was to determine the approximate number of shells per acre of deployment since the average survival in the study was based on a per shell basis (2.95 spat/shell with Natural Set (NS), 2.23 spat/shell with Removal of Natural Set (RNS)). The shell/cage was estimated by manually counting random tanks and averaging the counts. This average was extrapolated to determine the total shells required per acre (Table 8).

Table 8. Total shells required to plant one acre. Shell/Cage was determined by the average of multiple randomly selected subsamples which were manually counted.

Shells/Cage	Shells/Tank	Shells/Acre
2879.35	57,587	1,036,566

After estimating the total number of shells per acre of planting, the average spat/shell, as determined in Chapter 3, could be used to calculate a theoretical population which survived to adulthood. Using current market estimates per bushel ($42) as well as the approximate number of oysters in each bushel (200 oysters), a harvestable value of surviving adult oysters could be calculated at varying theoretical survival rates from the time of last assessment (the second sampling) to potential harvest. Estimations on return values were completed in both scenarios with natural set included and natural set removed (Table 9, Table 10). By using the total predicted yearly costs for the planting of one acre ($90,872), these calculations could be used to determine percent survival required to break even.

Table 9. Return harvest values for theoretical survival rates based on findings in Chapter 3 for data with Natural Set (NS) included. In bold is the final spat/shell counts determined in the second sampling of Chapter 3. Percentage survival noted with (*) indicates the required survival to cover initial costs.

Percentage Survival	Spat/Shell	Oyster/Acre	# Bushels	Harvest Value
5%	0.15	152,893	764	$32,108
10%	0.30	305,787	1529	$64,215
*14.15%	0.42	432,723	2164	$90,872
15%	0.44	458,680	2293	$96,323
20%	0.59	611,574	3058	$128,431
25%	0.7375	764,467	3822	$160,538
100%	**2.95**	**3,057,870**	**15,289**	**$642,153**

Table 10. Return harvest values for theoretical survival rates based on findings in Chapter 3 for data with Natural Set Removed (NSR) included. In bold is the final spat/shell counts determined in the second sampling of Chapter 3. Percentage survival noted with (*) indicates the required survival to cover initial costs.

Percentage Survival	Spat/Shell	Oyster/Acre	# Bushels	Harvest Value
5%	0.11	115,577	578	$24,271
10%	0.22	231,154	1156	$48,542
15%	0.33	346,731	1734	$72,814
*18.72%	0.42	432,723	2164	$90,872
20%	0.45	462,308	2312	$97,085
25%	0.5575	577,886	2889	$121,356
100%	**2.23**	**2,311,542**	**11,558**	**$485,424**

Conclusions:

Tracking hourly labor and expenditures throughout the study was a helpful way to plan for future costs associated with remote setting. It was important to note that some of the costs, particularly the labor costs would need to be adjusted from the original budget. The time required to set each tank was considerably higher than expected. An accurate estimate, determined through this study, can now be implemented in future financial planning. Many of the costs within the study are not likely to fluctuate considerably. The feeding costs, as well as the cultch costs, are not likely to vary from year to year, and are relatively easy to predict. The average daily vessel costs are subject to change over the years based on the price of the gas and vehicle maintenance inherent with the age of the vessel. This may be slightly harder to predict, but the average costs from the prior year are a reasonably good estimate. Larval costs are subject to change based on market demand and hatchery availability; however, given an appropriate availability of larvae, these prices should remain relatively static. In all, it is not possible to predict all future costs with certainty, but by using data collected from current studies one can make educated predictions for future project costs.

By using data collected from Chapter 3, possible return values could be collected for a range of theoretical survival rates. The range of theoretical survival percentages assessed (5-25%) represent a much more realistic scenario. The break-even points to cover the upfront costs of labor (Table 2) was determined to be 14.15% survival and 18.72% survival for NS data an NSR data respectively. Larger percentage survivals than these would result in greater harvest values than cost of deployment for one acre. It should be noted, however, that in the purposes of restoration, value can not simply be determined by harvestable populations. There are a variety of ecosystem services provided by oyster reefs, all of which add additional value to each spat surviving to adulthood. These additional values can be difficult to compute and were not the focus of the study. As such, for the purposes of this cost benefit analysis, the harvestable return value provides a tangible metric to gauge the relative success of these planting with a dollar value. Theoretically, however, these budget estimates suggest that use of remote set for spat on shell has the potential to be a worthwhile investment, particularly if site selection is improved through further work.

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