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Examining the effect of interstitial space on Eastern oysters (Crassostrea virginica): applications of photogrammetry and three-dimensional modeling

Bailie Lavan

Examining the effect of interstitial space on Eastern oysters (*Crassostrea virginica*): applications of photogrammetry and three-dimensional modeling

Bailie Lavan

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Committee Chair: Dr. Patrice Ludwig

Committee Members:

Dr. Christine May

Dr. Bryan Cage

Dedication

I would like to dedicate this thesis to my grandmother, Karen. She has been my biggest supporter throughout my life, no matter how great of a distance separates us. Thanks, Grandma!

Acknowledgements

I would first like to acknowledge my advisor, Dr. Patrice Ludwig, for being an incredible mentor throughout my time at JMU. Her unwavering support, wealth of knowledge, and guiding hand have made me a better student, a better scientist, and a better person. I would also like to acknowledge my committee members, Dr. Christine May and Dr. Bryan Cage, for never hesitating to share their vast knowledge with me. I would not have been able to achieve making this thesis the best science it could be without their assistance. Next, I would like to thank Brent James of Lynnhaven River Now for collaborating with me on this project and for being able to use his property for my field site. Through this relationship I developed, Brent has shared his relentless passion of oysters with me over the past year and has been an incredible source of knowledge and advice. Next, I would like to acknowledge the James Madison University Biology Department for supporting my research by providing transportation to my field site four hours away and allowing me travel to national conferences to share my work. I would like to thank my fellow biology graduate students for assisting me in field work even in the middle of winter, and for being an exceptional academic family to lean on. I would also like to thank my incredible support system, Erik and Tyler, for being an unwavering source of encouragement. Finally, I would like to thank my parents for never failing to be understanding, incredibly supportive and encouraging, and for always showing me how proud they are.

Thank you all so much, I could not have accomplished this without you!

Table of contents

List of figures

Abstract

Global oyster populations have decreased by as much as 99% in the past century. Oysters are known ecosystem engineers, providing benthic habitat for macrofauna, linking benthic and pelagic food webs, improving water quality, and mitigating shoreline erosion. Restoration efforts are critical in re-establishing native oyster populations. In the Chesapeake Bay, where oyster loss is primarily due to severe over harvest, artificial substrates with geometric shapes are widely used in restoration efforts. However, natural oyster reefs form emergent shapes with a high degree of aggregation and many interstitial spaces (three-dimensional volumetric spaces between oysters within a reef). The lack of interstitial space in artificial substrates contrasted with the presence of interstitial spaces in natural reefs led to the research question: Is there an amount of interstitial space which facilitates oyster recruitment and survival? Previous studies have hypothesized the importance of interstitial space in oyster reefs; however, current research lacks practical and effective methodology for measuring interstitial space of any ecosystem. We implemented a field study to observe the direct effect of interstitial space on oyster recruitment and survival using a concrete artificial oyster reef. Additionally, we used photogrammetry and three-dimensional digital modeling to develop a method for measuring interstitial space of the concrete artificial oyster reef used in the field. We found there to be significantly greater oyster recruitment and survival on substrates with 50 – 100 cm^3 interstitial space per 50 mm^2 surface area. This is the first study to directly examine the effect of interstitial space on oyster recruitment and survival. This is also the first study to develop a practical methodology for measuring interstitial space which may be transferred for use in other systems, as photogrammetry and three-dimensional modeling are not limited to oyster reef ecosystems. Filling these knowledge gaps will have positive impacts on oyster reef restoration and other ecosystems in which interstitial space is hypothesized to play a critical role.

I. Effect of interstitial space on oyster recruitment and survival

Abstract

Many oyster restoration efforts are focused around the addition of substrate to marine ecosystems. These substrates provide larval oysters with a firm surface to recruit and settle to. While natural oyster shell is undoubtedly the most effective substrate for larval oyster recruitment, the loss of natural shell is imminent due to continued overharvest of mature oyster populations. The loss of natural shell has led to the development of artificial substrates, such as concrete pyramids, castles, or domes. In contrast, natural oyster reefs exhibit a high number of interstitial spaces. We hypothesized that oyster recruitment and survival correlate with interstitial space. To examine the effect of interstitial space on oyster recruitment and survival, we conducted a field experiment from May 2018 - January 2019 in the Lynnhaven River located in the Chesapeake Bay using an artificial substrate designed with three treatments of interstitial space. We found oyster survival to be up to 57% greater on the intermediate interstitial space treatment, relative to treatments with high or low interstitial space. Incorporating intermediate interstitial space into artificial substrates used for oyster restoration may increase population growth. Intermediate interstitial space provides juvenile oyster spat with adequate room for growth and refuge from predation. Restoring native oyster populations to even a fraction of their historic populations may have significant positive impacts on marine ecosystems and marine food web dynamics, as well as the oyster fishing industry and coastal economies.

Introduction

Oysters are a keystone species providing ecosystem services to marine communities allowing other organisms to thrive. These “ecosystem engineers” link the benthic and pelagic food webs (Newell, 1988; Rick et al., 2016). Many benthic organisms and macroinvertebrates, such as oyster toad fishes, aquatic worms, marine barnacles and mussels, use oyster reefs as substrate for growth, habitat, and protection from predation. Oyster reefs provide benthic prey protection from their pelagic and benthic predators, thus attracting predators to the reef as well. Therefore, oyster reefs have the ability to support multiple trophic levels and form the basis of their own ecosystem; similar to ecosystems formed around tropical coral reefs.

As a keystone species, one critical ecosystem service provided by oysters is their ability to improve water quality through filter feeding as they filter toxins out of the water (Newell, 1988). Studies have shown that oysters are able to reduce suspended sediment (species dependent), detritus, and particulate-bound nutrients in the water column through active filter feeding (Gerritsen et al., 1994; Nelson, 2004). Additionally, due to their reef structure intertidal oysters may reduce shoreline erosion by as much as 0.15 m/month (Piazza et al., 2005; Taylor & Bushek, 2008; Hossain et al., 2013; Lindquist et al., 2014). These ecosystem services are important components when examining the health of shorelines worldwide.

Oysters are equally as important to many human populations as they are to marine ecosystems. Oyster reefs provide food, jobs and revenue (as oyster harvests) to many coastal populations. A loss in oyster reefs directly translates to a loss in income for many human communities; over the past 40 years Maryland and Virginia have seen a loss of more than $4 billion due to the decline in oyster harvesting industries (Krantz & Merritt, 1977; Rick et al., 2016). Restoring oyster populations to sustainable levels will have positive impacts on coastal populations.

The Tragedy of the Commons

Oyster populations around the world, including the Chesapeake Bay, have been devastated by severe overfishing, disease, and competition from invasive species. The Chesapeake Bay is the largest estuary in the continental United States, located in the mid-Atlantic region bordering Maryland, Virginia, and the District of Columbia (Rick et al., 2016) (Figure 1). In 1880, oyster production in the Chesapeake Bay totaled more than the oyster output of the rest of the world combined, exporting 400,000- 600,000 tons annually (Alford, 1973). Oyster production in the Chesapeake Bay quickly began to decline as output totals displayed a 50% reduction by the early 1900’s, and a 98% reduction by the early 1990’s (Rothschild, 1994).

A leading cause for the historic decline of Chesapeake Bay oyster populations is that oyster reefs in Maryland and Virginia were common property resources (Alford, 1973). The oyster bars were open to the public for fishing and harvest, and therefore had no true ownership. This lack of ownership led to the decreased health and sustainability of the oyster reefs because size, condition, or catch limits were not imposed (Alford, 1973; Hardin, 1968). Additionally, common ownership led to the degradation of oyster bars in the Chesapeake Bay through the lack of maintenance and restoration (Alford, 1973). The Tragedy of the Commons was the first documented major threat to the Chesapeake Bay oyster population and there have since been others.

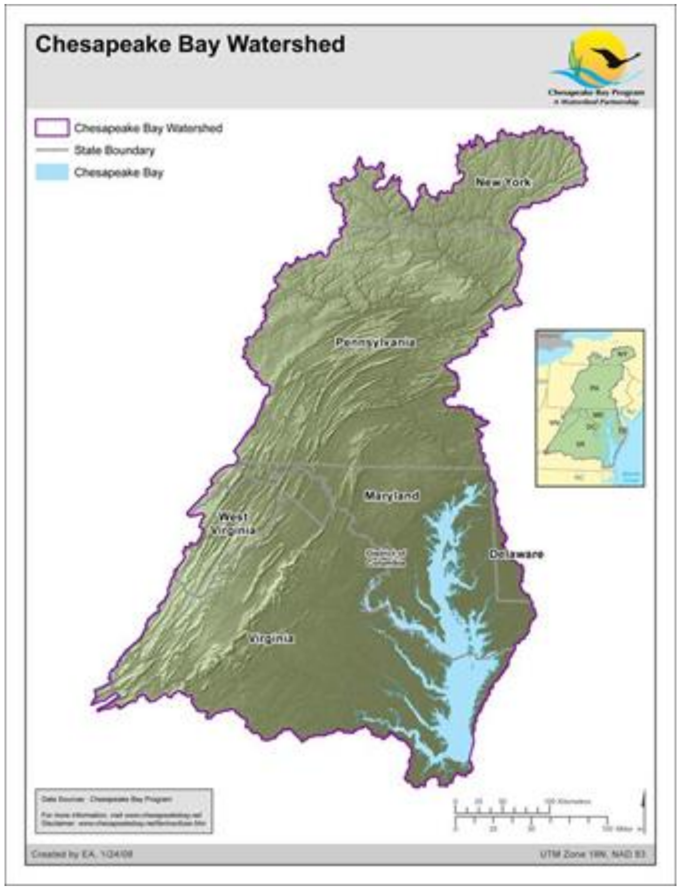

Figure 1. Map of the Chesapeake Bay watershed.
https://www.chesapeakebay.net/what/maps/P240

Threats

Eastern oysters continue to face threats to their health, survival, and sustainability. Sustainable global oyster reef populations will decline because of the predicted increase in global surface temperature and decrease in oceanic pH due to global climate change (GCC). GCC is primarily driven by the addition of large amounts of excess carbon dioxide released into the atmosphere through increased anthropogenic activity (Gattuso et al., 2012). One of the most notable side effects of this increase in released carbon dioxide is the phenomenon known as ocean acidification. The world's oceans have long been known to be a carbon dioxide sink; however, the increase in atmospheric carbon dioxide has led to an increase in the ocean's absorption as well

(Roberts et al., 2014) (Figure 2). Ocean acidification leads to increased dissolved inorganic carbon and bicarbonate which decreases oceanic pH and leads to decreased levels of carbonate, which calcifying organisms, like the Eastern oyster, rely upon to produce their calcium carbonate based shells (Gattuso et al., 2012) (Figure 3). Consequently, the loss of carbonate due to ocean acidification makes growth difficult for these organisms, while the simultaneous reduction of pH leads to shell deterioration (Sanford et al., 2013).

Increased pollution has led to a steady decline in water quality, causing strong summer anoxia. This anoxia decreases bivalve settlement by as much as 75%, as well as reducing growth and survival (Newell, 1988; Baker & Mann). Severe changes in water quality can greatly increase susceptibility of native populations to competition by invasive snails (Sanford et al., 2013). While the environment plays a key role in oyster reef health, reefs are also subject to degradation by multiple anthropogenic factors.

When oysters are harvested, it is not just the meat that is taken. Historic and continued overharvesting contribute to reef degradation because natural shell is not replaced after being removed from the reefs (Soniat et al., 2014). It is hypothesized that if natural shell were returned following fishing efforts, oyster reef sustainability would improve. Some effort has been made to replace natural shell through restaurant recycling programs and smaller similar efforts, but these are simply inefficient for reef sustainability and significant population growth. Additionally, naturally occurring factors have become reasons for shell and reef degradation, such as increased frequency of disease outbreaks, biodegradation or erosion, and sedimentation which blocks proper water and nutrient flow (Soniat et al., 2014). Returning oyster reefs to a healthy state and maintaining sustainability of reefs is key to successful restoration.

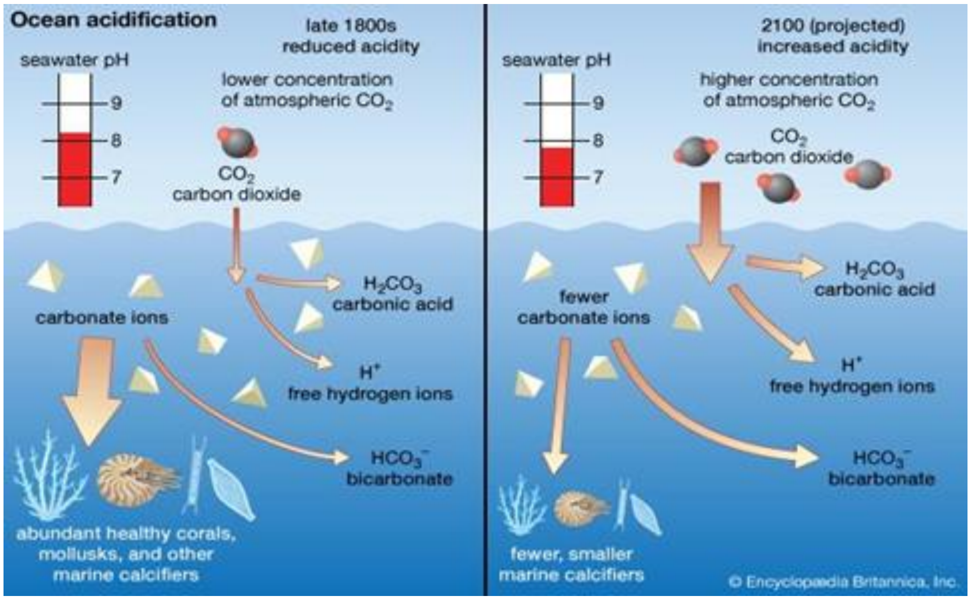

Figure 2. Differences in effects of ocean acidification from late 1800's to projected in 2100. https://www.britannica.com/science/ocean-acidification

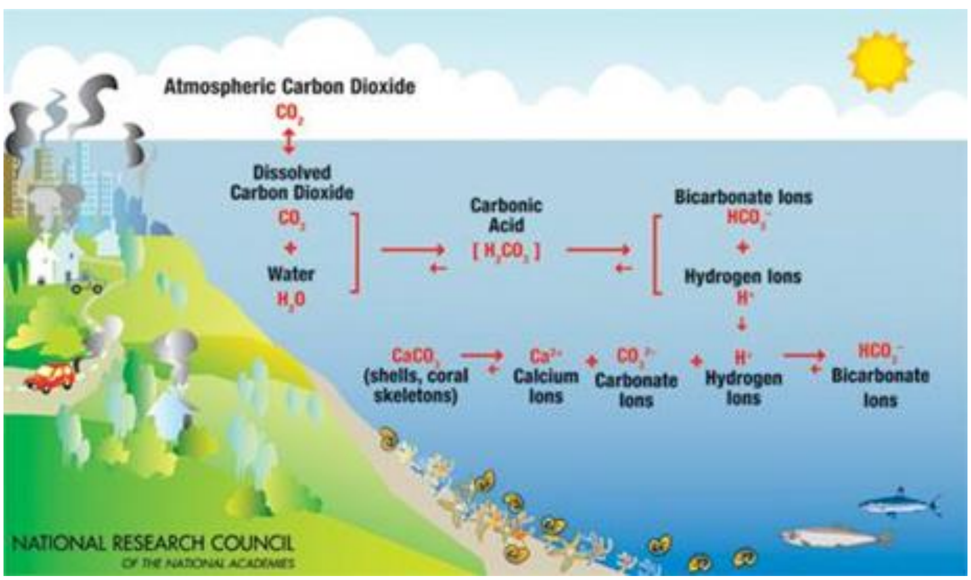

Figure 3 Chemical equation of ocean acidification as it relates to the part of the environment the reaction occurs in. http://www.beachapedia.org/Ocean_Acidification

Restoration efforts in the Chesapeake Bay

Previous oyster restoration efforts in the Chesapeake Bay can be described in two ways: construction of oyster habitat to recreate three-dimensional oyster reefs, and management of the remaining fishery to improve brood stock (Brumbaugh et al., 2000). In the late 1990's, hatchery-produced brood stock were transplanted onto constructed reefs in designated sanctuaries harbored from fishing and damaging recreational activities (Brumbaugh et al., 2000). The transplanted spat remained in the sanctuary for the following years, however; they did not increase recruitment rates in the surrounding rivers (Brumbaugh et al., 2000). This system of transplanting hatchery stock is still widely used today. While this method has been shown to improve the directly transplanted reefs, it is labor intensive, costly, and not producing large scale results. Rather than transplanting brood stock, larvae from mature oysters in the Chesapeake Bay need adequate substrate, perhaps in the form of constructed habitat, to settle and survive on in order to further facilitate the improvement of oyster recruitment rates.

Substrate Needs

Oysters have a life history similar to that of most bivalves in that they have both a free-swimming larval stage before they metamorphose, settle, and grow through a spat phase to become a sessile adult oyster (Newell, 1988) (Figure 4). Late-stage larvae (pedivegiler) can still move and sample small areas of space but quickly become sessile for the remainder of its life (Figure 4). Thus, substrate type becomes particularly important when examining oyster reef dynamics. Oysters that find and settle on firm substrate are more likely to survive because they are at a reduced risk of substrate erosion (Newell, 1988). Oysters settling on erosion-prone substrates may not survive long enough to reproduce because the substrate is easily washed away or covered by sediment. Oysters are biogenic; they form reefs out of their own biomass (Soniat et al., 2014). Juvenile oyster spat recruit to the adult oyster shells and settle, using the shell of adult oysters that have died as substrate for attachment. The mortality of older oysters enhances oyster reef growth. Therefore, even the shells of dead oysters are needed to support multiple generations of growth (Soniat et al., 2014). Thus, the removal of shells through overfishing is harmful to reef sustainability.

While the need for firm substrate is important to support multiple generations of oysters, proper water and nutrient flow is equally important to the sustainability of the reef (Soniat et al., 2014). Because oysters form reefs out of their own biomass, shell maintenance is crucial to reef sustainability (Soniat et al., 2014). Accretion of oysters is limited by predation, recruitment rate, harvest, ocean acidification, and disease.

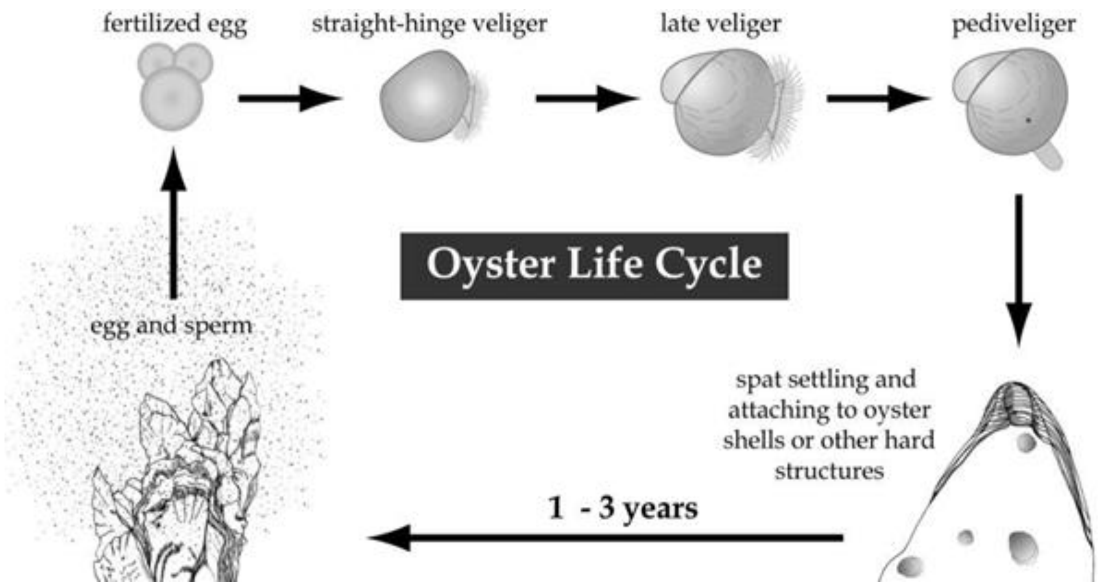

Figure 4. Eastern oyster (Crassostrea virginica) life cycle.
http://score.dnr.sc.gov/deep.php?subject=2&topic=15

Interstitial space in context of oyster reefs

Considering that oysters create the reef out of their own biomass, it follows that the shape of the reef is emergent. However, there are properties that every reef has, such as the space between shells, interstitial space. Interstitial space is cited as playing a critical role in many ecosystems (Humphries et al., 2011; Martin et al., 2012; O'Beirn et al., 2000); however, its effect is seldom explicitly quantitatively tested (Hesterberg et al., 2017; Nestlerode et al., 2007; Warfe et al., 2008). Interstitial space can be defined simply as the space between objects: for example, the space between cells in the body. In the current study, interstitial space is defined as the space between oysters on a particular oyster reef. Space influences the flow of water through an oyster reef, which in turn can affect recruitment either positively or negatively dependent upon the amount of available space (Martin et al., 2012). However, this definition requires an understanding of what is meant by the word “space.”

“Space” is the volumetric area between oysters, and is created by oysters adjacency. The physical arrangement of elements in a structure defines its three-dimensional structural complexity (Humphries et al., 2011). Space and complexity of a structure are dependent; large spaces often define little complexity and vice versa. Combining these definitions can give a concise concept of interstitial space: more concisely, interstitial spaces are the volumetric gaps between elements of a structure (Kim, 2018). As the structural complexity of an object increases, the object’s interstitial space increases, while the physical volume of the space decreases (Figure 5,6). There are a few studies in which an increase in complexity leads to an increase in ecosystem abundance and diversity. For example, interstitial space in complex habitats in a dense marsh system has shown to lead to a greater diversity and abundance of organisms due to decreased stress of predation, decreased competition, increased food availability, increased resource and niche availability, and increased refuge and surface area for living that results as an increase in interstitial space, or structural complexity (Bartholomew et al., 2000).

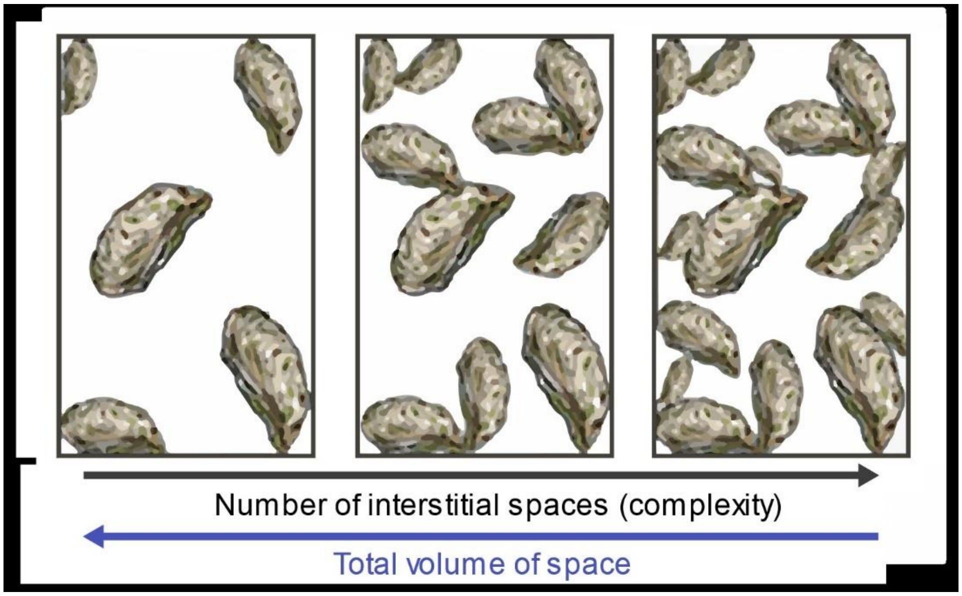

Figure 5. Complexity of interstitial space increases as volume of space decreases

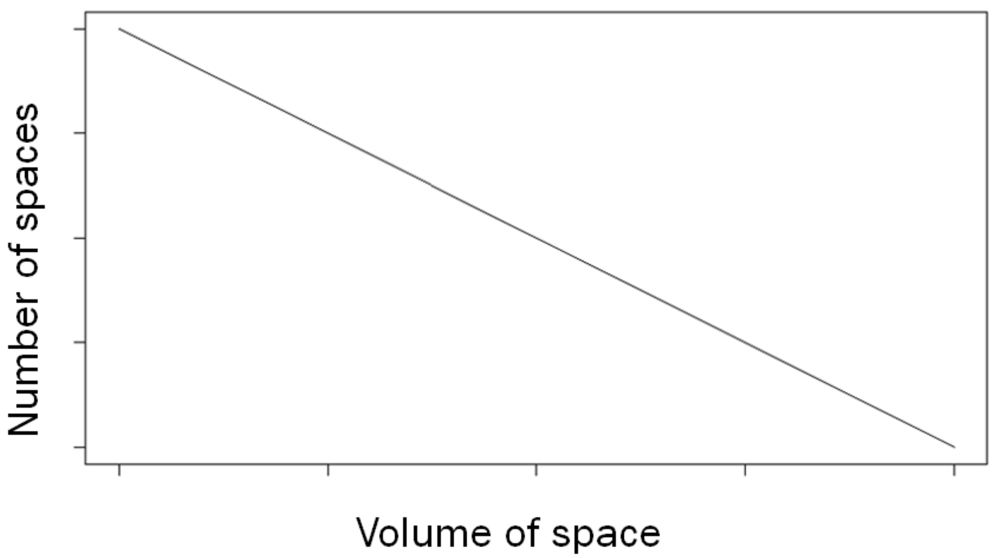

Figure 6. The general relationship between the number of interstitial spaces and the total volume of space.

An important ecological impact of an increase in interstitial space is the change in predator-prey dynamics. For example, small interstitial spaces disrupt predator-prey interactions through top-down control because it inhibits predator access to prey by means of the predator being too large to physically access the space (Grabowski, 2004). The change in predator-prey interactions by means of changing interstitial space has been emphasized in multiple ecosystems. There is a positive correlation between the survival of bay scallops residing in the interstitial spaces of seagrasses, and the habitat complexity of the seagrass bed. (Carroll et al., 2015). There is a positive correlation between the size of salamanders surviving in the interstitial spaces of rocks in stream-beds and the size of the interstitial spaces (Martin et al., 2012). Each of the previous examples depicts a population using a space as a resource, but what happens when the population is both the creator and user of the space?

As ecosystem engineers, oysters build the physical system used by themselves and by other species; oysters build the reef and create interstitial space in this capacity. The importance of interstitial space in oyster reef ecosystems has been hypothesized but it has never been studied directly. An indirect study of the effect of interstitial space (defined as habitat complexity) on oyster reefs discovered that oyster toadfish (*Opsanus tau*) were indirectly responsible for the mortality rate of spat by controlling mud crab abundances via predation on low interstitial space reefs (Grabowski, 2004). This indirect study of oyster mortality demonstrated that increased habitat complexity can modify multiple trophic interactions, rather than simply affecting one direct predator-prey relationship. This principle may be applied to oysters directly: juvenile oysters with adequate availability of interstitial space will be safer from predators, leading to an increased probability of survival. An increase in chance of survival leads to an increase in oyster population size, thus incorporating interstitial space into oyster reef restoration efforts should be an imperative.

Artificial oyster reefs

An additional concern in oyster reef restoration is the lack of natural shell available for these efforts. In the late 1800’s after being harvested, shells were used for lime and road building material (Alford, 1973). These activities removed natural substrate from the system, so without artificial reef substrate large quantities of adult oysters would need to die to compensate for the loss of natural shell to fishing (Soniat et al., 2014). Natural shell is by far the most effective substrate to facilitate oyster recruitment and growth, but the loss of natural substrate is imminent. Current restoration efforts are shifting to artificial reef substrates in order to compensate for the loss of natural oyster shell reefs (Theuerkauf et al., 2015). Commonly researched artificial substrate types and structures being researched are concrete, porcelain, limestone, and river rock (George et al., 2015).

Some researchers have explored the use of substrate from other sessile, aquatic organisms, such as surf clamshell. Results from a study investigating recruitment differences surf clamshell and natural oyster shell, showed that natural oyster shell offered better growth, survival, and habitat complexity for oyster settlement (Nestlerode et al., 2007). The authors suggest that the increase in survival and habitat complexity on the natural oyster shell reefs was due to the increased availability of interstitial space that is not offered by other artificial substrates (Nestlerode et al., 2007). Artificial reefs that are replacing natural reefs are typically constructed as oyster castles, oyster pyramids, or oyster domes (Theuerkauf et al., 2015). These substrates are efficient to construct but do not take the shape of natural oyster reefs (Figure 7). Perhaps, an artificial substrate that more closely resembles the shape of a reef will increase the rate of natural recruitment and reef building. One way to make the artificial substrate more realistic is to incorporate interstitial space. The current research aims to investigate the effect of incorporating interstitial space in artificial oyster substrates to increase oyster spat recruitment and oyster population growth.

Figure 7. Examples of artificial oyster reef substrates.

Methods

Substrate construction

Artificial oyster reef habitat was constructed to mimic natural oyster habitat. Two iterations of a standard Portland cement mixture were used to create the artificial reef habitat. The first iteration was a mixture of Portland cement and limestone sand from a local quarry (http://www.frazierquarry.com) in a 2: 2: 1 ratio of cement: limestone sand: water. The second iteration included the addition of powdered Magnesium to the first iteration in a 2: 2: 1: 0.1 ratio of cement: limestone sand: water: magnesium. Field studies showed that the magnesium supplement had no effect on recruitment or survival in field trials (Elder, 2018). Based on this result we assume no effect of Mg present in eight of the tiles used in this field study.

Concrete squares were poured with dimensions of 40 cm x 40 cm x 5 cm. Using a spatial template, previously cast concrete shells were placed vertically in the slab to a depth of roughly 3 cm as the tile was drying. The template was used to standardize the tiles within a treatment so that the arrangement of the “shells” would not be a factor within the treatments. Concrete shells were constructed by combining Portland cement, limestone sand, and water in a 2:2:1 ratio, pouring the mixture into shell molds, and leaving to dry for 24 hours (Elder, 2018) Fifteen tiles each with 25 artificial shells were created with 3 levels of interstitial space qualitatively identified as “low,” “medium,” and “high” interstitial space (5 tiles of each for a total of 15 tiles) (Figure 8). The 25 shells of each treatment were arranged differently to create the three levels of interstitial space. A template was used to place the concrete oysters in a similar arrangement within a treatment. The resulting shell arrangements occupied different amounts of surface area on the top face of the tile between treatments: the high space treatment occupied 1600 cm^2 surface area, the intermediate space treatment occupied 897 cm^2 surface area, and the low space treatment occupied 563 cm^2 surface area. A qualitative assessment of the three treatments was necessary because there was not a method of measuring interstitial space available when the tiles were created. The tiles cured outside (in Harrisonburg, VA) for a year before they were placed in the water.

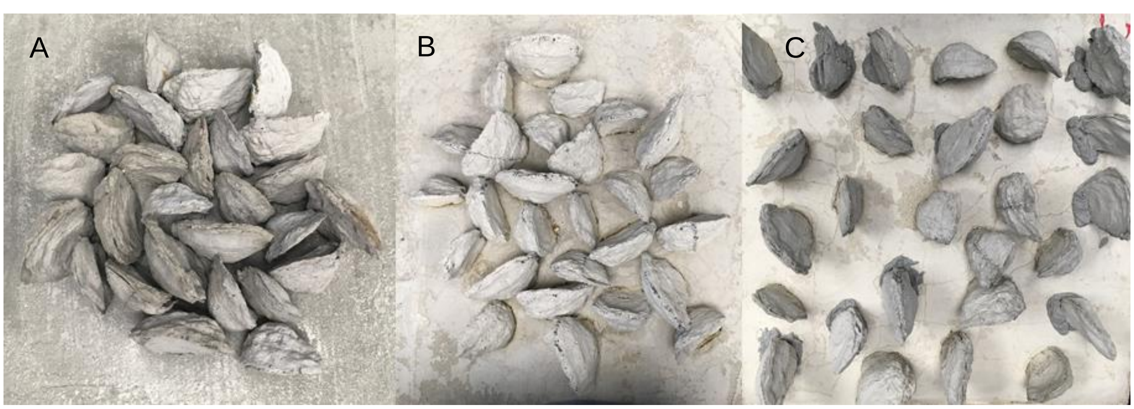

Figure 8. Concrete tiles with artificial shells created with 3 levels of interstitial space: (a) low, (b) intermediate, and (c) high respectively.

Location

The field site for this study was located at Sandy Point in Lynnhaven Inlet, Virginia Beach, VA (Figure 9). Field site access and permitting was granted through collaboration with Lynnhaven River Now. The Sandy Point field site had a sandy bottom with little to no additional substrate (i.e. rocks, gravel, or mud). Anecdotally, tidal fluctuation and wave action is moderate with an occasional increase in wave action from boat traffic. No major storms occurred during the study period. This field site had an added benefit of being known as a substrate-limited site rather than a recruitment-limited site; meaning that we could assume oyster spat would settle to our artificial reef substrates. This information was gathered through collaboration with Lynnhaven River Now from their long-term oyster data in the Lynnhaven inlet.

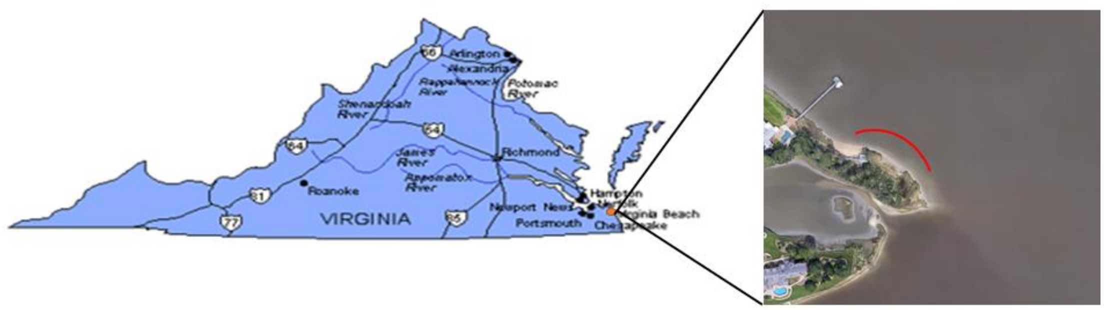

Figure 9. Field site location at Sandy point, Lynnhaven inlet, Virginia Beach, Va. GPS Coordinates: 36.8767, -76.0706

Data collection

Concrete tiles were deployed at the field site on June 2, 2018. The tiles were placed in a randomized order in 2 rows with approximately 1 m between each tile around the mean low tide line (Figure 10). Data collection was conducted during 2018 - 2019 on July 2 2018, August 7 2018, August 24 2018, September 23 2018, October 13 2018, November 3 2018, and January 5 2018. Tiles were removed from the water and gently scrubbed for 5 minutes to remove excess sediment, biofilm, algae, and large barnacles in order to increase visibility of oysters. The process of removing fouling organisms from the oyster substrate is common methodology used in the oyster research field (Calvo et al., 2000; Nelson et a., 2004). Each tile was examined and spat counts were recorded. Spat was recorded as any live oyster seen at the time of data collection. Low treatment substrates were often examined for longer due to their tighter spatial arrangement being more difficult to examine. It was noted if the oyster was “inside” or “outside” of the spatial arrangement (Figure 11). Oysters within the spatial arrangement had the treatment of interstitial space. Oysters recorded outside of the spatial arrangement were part of the zero-space treatment because they were subject to no levels of interstitial space. At least once during the season, photos of each tile were taken in the field for use in the photogrammetry portion of the study.

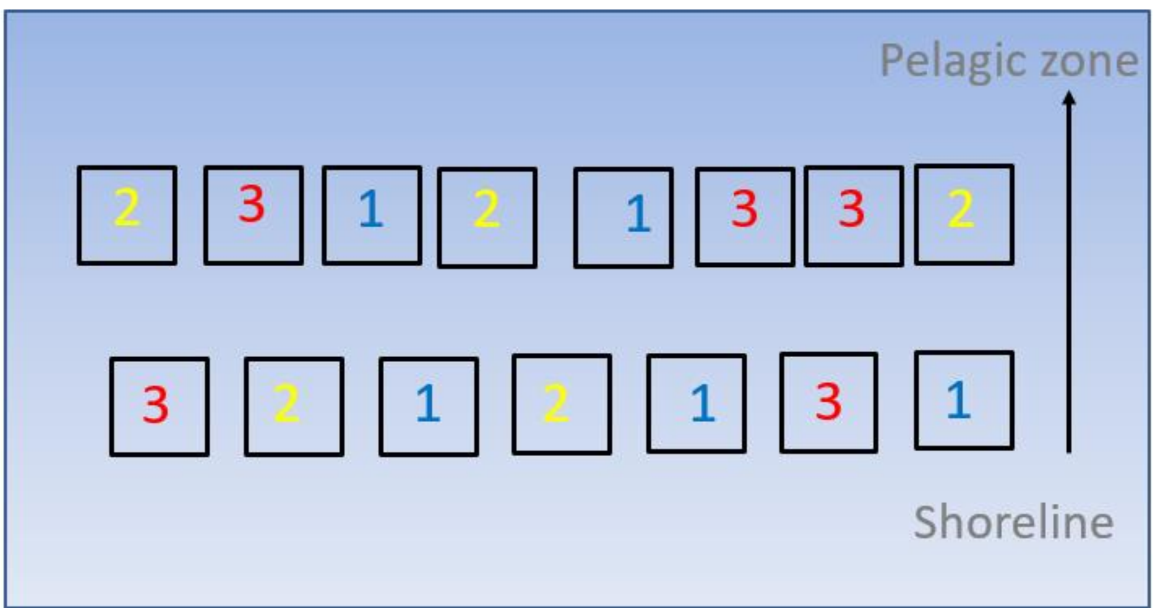

Figure 10. Order of tile placement at field site. The numbers correspond to high (3), medium (2), and low (1) interstitial space treatments. There was approximately 1 m between each tile.

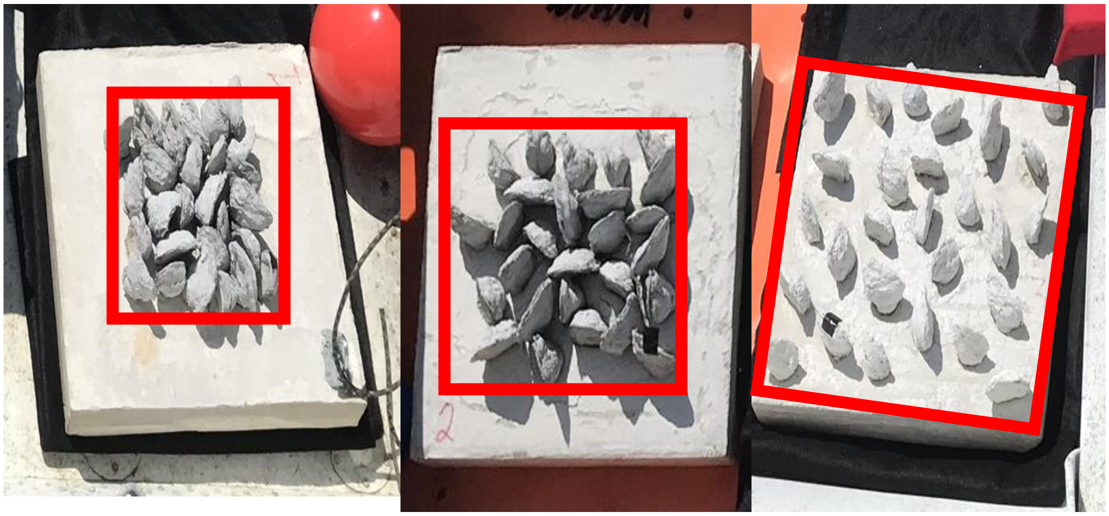

Figure 11. Interstitial space treatments (low, intermediate, and high respectively). Red outline denotes the extent of the spatial arrangement. Spat recorded within the red line were noted as being "inside" the spatial arrangement and spat recorded beyond the arrangement are "outside" of the spatial arrangement.

Analysis of oyster spat preference

All statistical analyses were performed using RStudio (version 1.1.463) (Affero General Public License). We tested the hypothesis of equal recruitment between treatments in the field. Interstitial space was the independent variable and spat count was the response variable of recruitment. Interstitial space is qualitatively defined as “low,” “intermediate,” “high,” and “zero space” making it a categorical variable. Descriptive statistics of spat count per treatment including summary statistics of mean, median, and mode were calculated. Boxplots display final spat count distribution trends between treatments. The Shapiro-Wilk test was used to test for normality of spat counts (R package: stats); the Levene test was used to test for equal variances (R package: dplyr). We assume that larvae show preference for an amount of interstitial space according to the number of spat that settle on a particular tile (Garshelis, 2000). Chi-square analysis was used to test for differences in larval preferences for space based on the final spat count (R package: Mosaic).

Roughly half (n=8) of the substrates we used were constructed with an additional magnesium supplement to the concrete mixture. The magnesium tiles weathered poorly in comparison to the standard concrete treatments making it difficult to use them for measures of the volume of interstitial space. A previous study reported no significant difference in recruitment between the magnesium and standard concrete treatments, so we assume no effect of the Mg on oyster settlement (Elder, 2018). We tested whether erosion effected recruitment between the Mg and regular concrete tiles using a chi-square test.

The design of the tiles created differences in the total amount of the tile surface occupied by the shells; either distributed across the entire surface of the tile or concentrated to create many small interstitial spaces, or an intermediate distribution of the shells. All three treatments were created with 25 artificial shells; however, the arrangement of the 25 shells differed in surface area between treatments (Figure 11). We are most interested in the response variable of volume of space created by the arrangement of shells in these treatments, so it is important that we test for the potential impact due to the amount of occupied tile surface area. We determined the surface area of tile occupied by the spatial arrangement of the shells (low = 563 cm^2, intermediate = 897 cm^2, high = 1600cm^2). The surface area of the zero space treatment was determined by subtracting the surface area of the spatial arrangement from the total surface area of the tile, including the sides and bottom (total surface area = 4000 cm^2). We used final spat count as the response variable that indicates the impact of interstitial space so we also used these counts to calculate spat density. An ANOVA was used to test the null hypothesis for larval settlement preference (no preference) between the three treatments (R package: stats).

Each interstitial space treatment (low, intermediate, and high) was replicated five times (for a total of 15 tiles). The zero space treatment contained 15 replicates due to each of the 15 interstitial space treatment tiles containing surface area with no interstitial space. Even the low space treatment tiles had surface area available (the sides and bottom of the tile itself. Total final spat count data for the 15 zero space replicates was averaged and the average was multiplied by 5 to adjust for comparison to the treatments with 5 replicates.

Results

The current research aims to examine the effect of interstitial space on oyster recruitment and survival on an artificial substrate visually resembling a natural oyster reef. Recruitment, total number of live oysters observed per tile, was measured for the treatments (zero space, low, intermediate, and high interstitial space) on the eight collection dates from May 2018 - January 2019. All treatments showed a similar pattern in recruitment with a large peak in late August and early September (Figure 12); however, recruitment was variable between treatments throughout the data collection period. Almost twice as many total spat settled on the intermediate treatment tiles (total for all replicates = 52) relative to the low (total for all replicates = 35), high (total for all replicates = 29), and zero space (average total for 5 replicates = 34) interstitial space treatments (Figure 13).

Realized recruitment (final spat count) is defined as the number of living spat recorded during the final data collection period (January 5, 2019) after the tiles had been in the field for approximately 30 weeks. Final spat count and all statistical analyses are reported for the recruitment within the study region of each treatment to emphasize the focus on the differences in interstitial space between treatments. Final spat count on the intermediate treatment was statistically significantly greater than the final spat count on the low or high treatments (Chi-square analysis, X^2=8.54, df=3, p = 0.0365). The intermediate treatment showed a 57% (n=52) greater final spat count relative to the high treatment (n=29), and 39% greater final spat count relative to the low treatment (n=35) and zero space (n=34) (Figure 13). Distribution of final spat counts varied greatly between treatment replicates ranging between zero and 18 (Figure 14). Visual examination of the data distribution showed that there are outliers for final spat counts (Figure 14) in the low and intermediate treatments. Data analyzed when removing these points showed a similar pattern, thus outliers were removed for statistical analyses (Figure 15). Two outliers were removed; one from a low treatment (n=16) and one from an intermediate treatment (n=0). The low treatment outlier was on a tile that exhibited extreme erosion toward the middle of the data collection period, thus no longer having the spatial arrangement necessary to be deemed part of the low treatment. The intermediate treatment outlier was on a tile that experienced heavy sedimentation for 2 - 4 weeks during the data collection period. Prior to this sedimentation period, the treatment replicate had a higher recruitment value (n=22), thus sedimentation may have caused the loss of the previous oyster spat recruits.

Spat density (spat count/cm^2) was examined to ensure that the effect of interstitial space was not a function of the amount of surface area a treatment occupied on the tile. The low interstitial space treatment (n= 0.012 spat/cm^2) exhibited equal final spat density relative to the intermediate treatment (n= 0.011 spat/cm^2), and 109% greater final spat density relative to the high treatment (n=0.004 spat/cm^2) (Figure 16). The zero space treatment exhibited equal spat density relative to the high treatment (n=0.003 spat/cm^2). The low and intermediate treatments had a significantly greater spat density relative to the high and zero space treatments (ANOVA, n=5, df = 1, F-value = 13.98, p = 0.0009).

We observed greater erosion on tiles with Mg added relative to the tiles constructed with standard concrete (Figure 17). We tested for the potential impact of substrate type (Mg or standard concrete) on the final spat count as a way to determine if the erosion influenced our results. A chi-square test showed no significant difference between the final spat counts on tiles with Mg (n=103) and standard concrete (n=114) substrates (Chi-square, X^2=0.187, df=1, p = 0.67) (Figure 18).

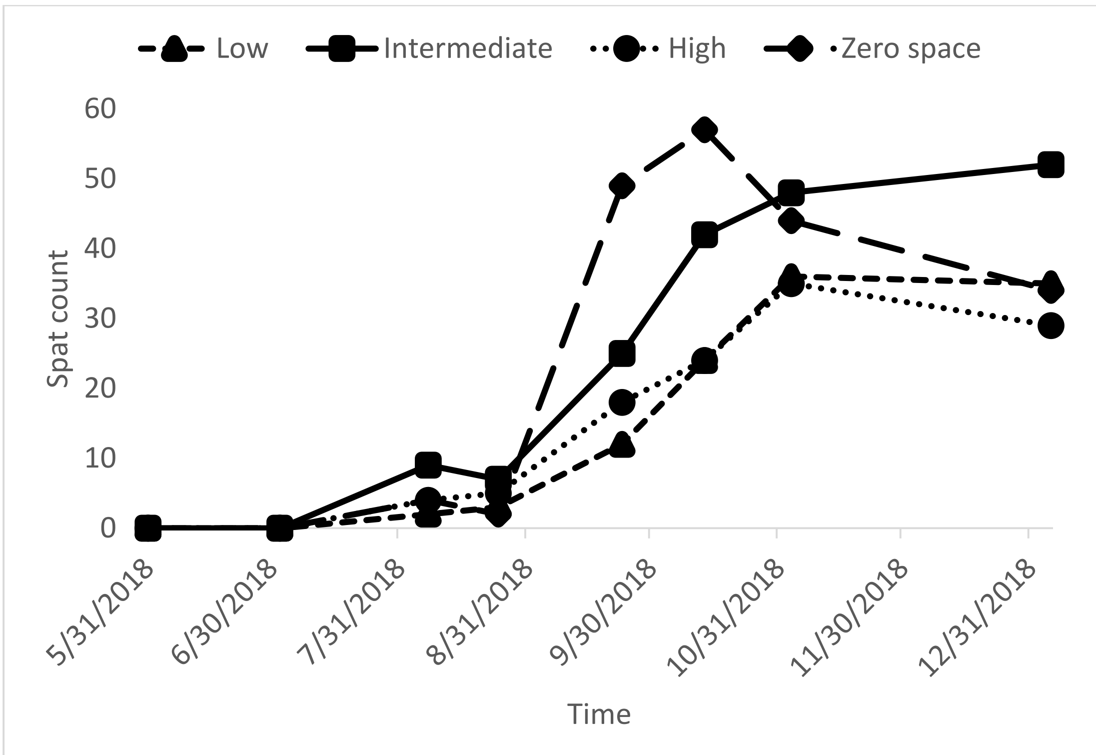

Figure 12. Recruitment of each treatment recorded from May 2018 - January 2019.

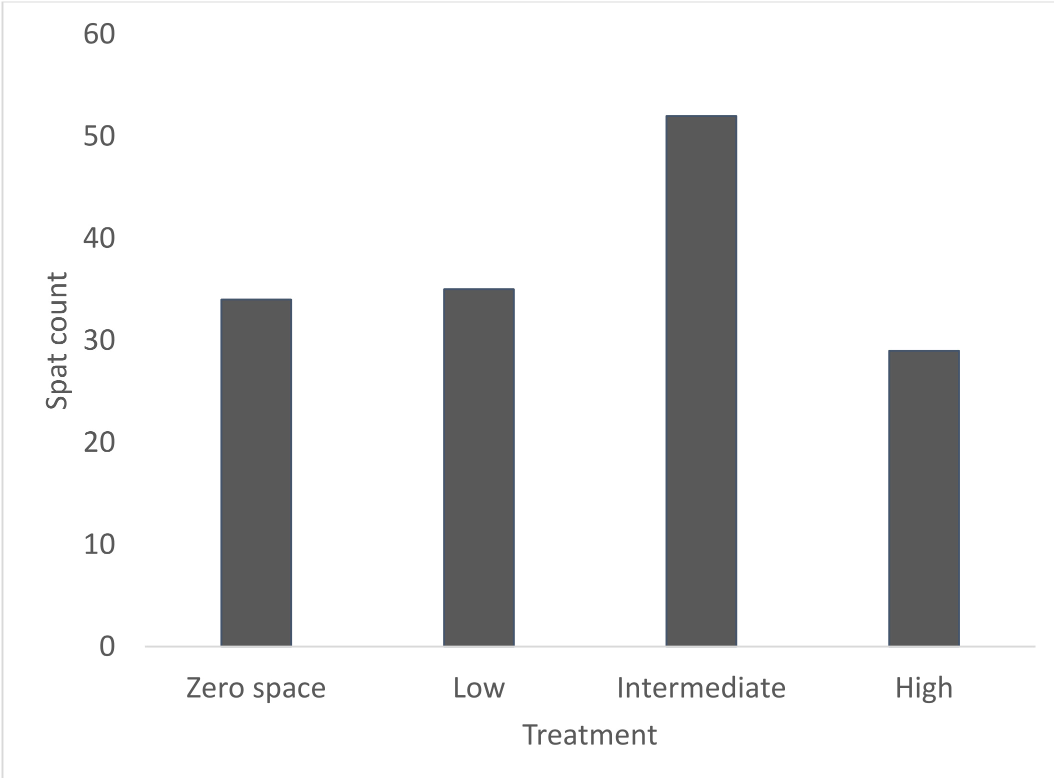

Figure 13. Total final spat count is defined as the number of living spat found on each treatment during the final data collection period.

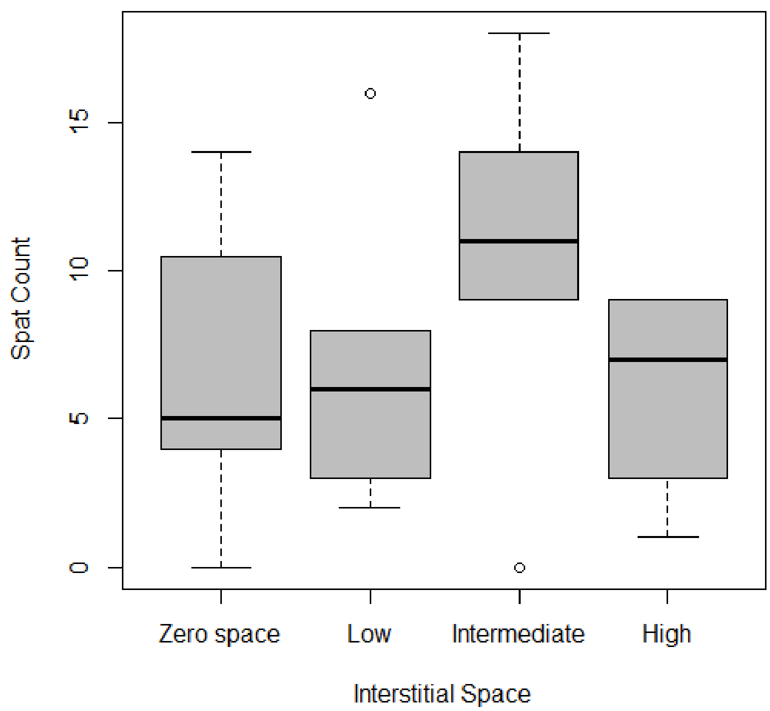

Figure 14. Boxplot reporting initial distribution of final spat counts between interstitial space treatments. Each boxplot contains 5 replicates of each treatment.

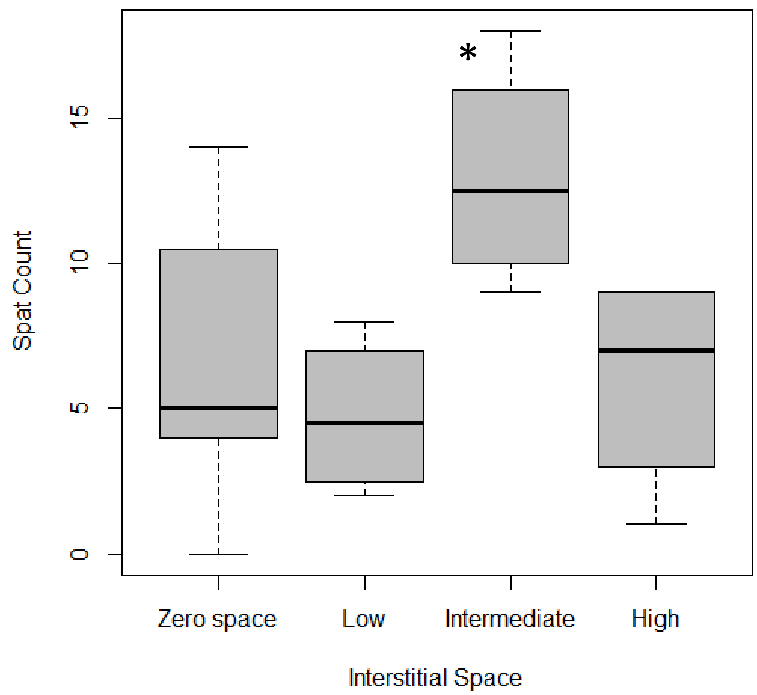

Figure 15. Boxplot reporting the distribution of final spat counts between interstitial space treatments after removing any initial outliers. Chi-square analysis, X^2=8.54, df=3, p = 0.0365

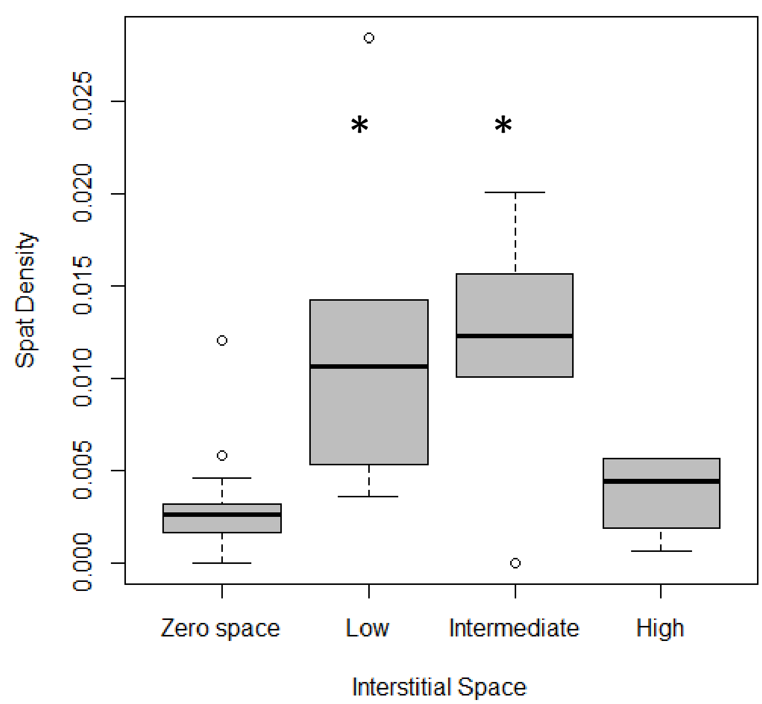

Figure 16. Boxplot displaying distribution of final spat count density as a factor of surface area between treatments (ANOVA, n=5, df = 1, F-value = 13.98, p = 0.0009). Surface area is defined as the extent of the surface area of the interstitial space arrangement for each treatment.

Figure 17. (Left) substrate constructed with magnesium supplement observed greater erosion than the substrate constructed with standard concrete (right).

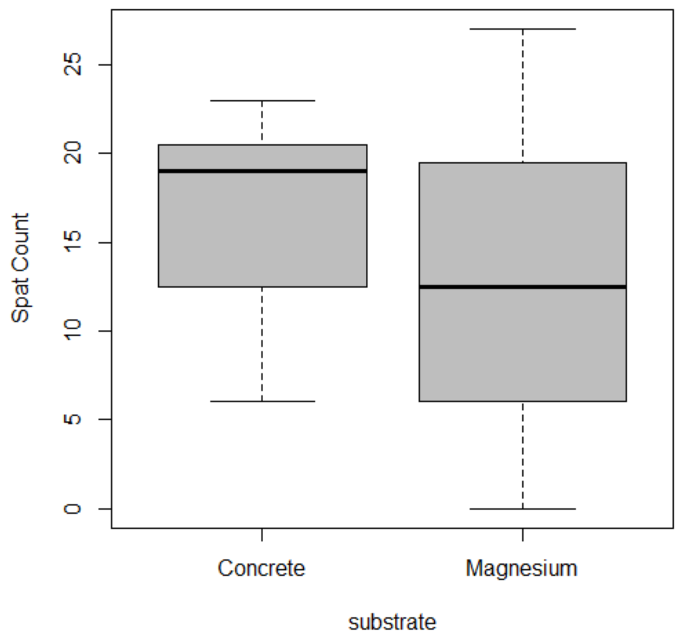

Figure 18. Boxplot reporting distribution of final spat counts between substrates constructed with standard concrete and magnesium supplements. Chi-square analysis, X^2=0.18, df=1, p = 0.67

Discussion

The goal of this research was to determine the effect of interstitial space on oyster spat recruitment and survival. We found that recruitment and survival were statistically greatest on substrates designed with intermediate amounts of interstitial space (Figures 13, 15). Our findings support the inclusion of intermediate-size interstitial spaces in artificial oyster substrates used for restoration efforts in order to significantly increase oyster spat survival. Tiles with intermediate-size interstitial spaces showed 57% greater final spat count when compared to substrates with significantly larger spaces. Additionally, when compared to substrates with smaller available spaces, intermediate-size interstitial spaces increased final spat count by 39%. The significant increase in final spat count of oysters on substrates with intermediate-size interstitial spaces could translate to a positive impact on oyster population growth because these spaces offer juvenile oysters adequate room for growth (relative to small spaces) and refuge from predation (relative to large spaces) (see below). An increasing oyster population has positive implications for the health of the surrounding marine ecosystem, the health and diversity of benthic organisms, and potential positive implications for coastal economies (Sarker et al., 2018).

The arrangement of the 25 shells on the tiles created different amounts of surface area used by the shells high (1600 cm^2), intermediate (897 cm^2), and low (563 cm^2). The final spat density (count/cm^2) of the low and intermediate treatments was significantly greater than the high and zero space treatments. These results suggest that differences we see in final spat count and spat density are not due to the surface area of the tiles occupied by the shell arrangement. If oyster recruitment and survival was a function of surface area, we would expect to see significantly more oysters on the high space treatment, given that it has the greatest amount of surface area occupied by the 25 shells. This result also supports the need for some amount of interstitial space being that the spat density of the low and intermediate treatments are higher than the spat density of the zero space treatment.

Our results may be explained by the interstitial spaces providing refuge for juvenile oysters (Bartol & Mann, 1997; Bartol et al., 1997; Coen & Luckenbach, 2000, Grabowski & Powers, 2004; Nestlerode et al., 2007; Humphries et al., 2011(a);

Humphries et al., 2011(b); Hill & Weissburg, 2013). The importance of this trait to the survival of young spat suggests that the angle at which oysters grow could be under selection to optimize space; the oysters that grow at particular angles, creating optimal interstitial spaces, create reefs that support further reef accretion. In this way, interstitial space may be optimized. The mechanism of optimal interstitial space is known to operate in multiple ecological systems (O'Beirn et al., 2000; Martin, 2012; Carroll, 2015) including stream beds, coral ecosystems, reef fishes, and even benthic invertebrate microfauna (Swedmark, 1964; Grigg, 1994; Gayraud & Philippe, 2001). Interstitial space follows the ecological principle of the 'Goldilocks effect,' in which the trait of interest will optimally fall within the two extremes of its range (Kerr & Feldman, 2007; Heimpel & Asplen, 2011; Lloyd et al., 2011).

We observed a bimodal pattern of recruitment throughout the data collection season. Our overall recruitment data exhibited a larger recruitment peak in the fall 2018 (September - October) with a smaller recruitment peak earlier in the summer 2018 (June - July) (Figure 12). This pattern aligns with the known pattern of recruitment of the genus Crassostrea (*Crassostrea virginica* and *Crassostrea madrasensis*) with peaks in the early summer and fall seasons (Furukawa & Linton, 1968; Hidu & Haskin, 1971; Roegner & Mann, 1995; Amin et al., 2007; Narvaez et al., 2012; Parker et al., 2013).

The intermediate amount of interstitial space in oyster reef substrate creates complex habitat, primarily due to the emergent and aggregated nature of settling oysters (Wade et al., 2018). The habitat complexity within oyster reefs offers niche space to benthic organisms, allowing oyster reefs to bridge the gap between benthic and pelagic food webs (Newell, 1988; Hassain et al., 2013; Rick et al., 2016). Therefore, a reduction of bivalve density reduces habitat complexity which leads to a decrease in benthic macrofaunal biodiversity (Dame et al., 2002). A change in benthic macrofaunal biodiversity may lead to dynamic changes in predator-prey interactions within the oyster reef (Grabowski, 2004; Grabowski et al., 2008). While we did not directly quantify macrofaunal diversity between the treatments of our study, we observed that the intermediate interstitial space treatment frequently had higher sightings of blue crabs, mud crabs, gobies, polychaetes, and periwinkle snails. Anecdotally, the biodiversity was much lower on the high interstitial space treatment substrates; however, the only predatory Chesapeake blue crabs (*Callinectes sapidus*) observed were found on the high treatment. The low-space treatment substrates also showed lower diversity relative to the species diversity on the intermediate-space treatment substrates, likely due to the spaces being too small for organisms to inhabit. Further research of the interstitial space oyster substrate should quantify biodiversity differences between treatments in order to examine any direct effect of interstitial space on benthic macrofaunal biodiversity. If intermediate interstitial space has a significant positive impact on biodiversity, in addition to the known significant positive effect on oyster survival gathered from our study, then the inclusion of optimal interstitial space in oyster restoration efforts will almost immediately increase biodiversity as well as supporting oyster reef growth.

This study spawned many additional future questions regarding interstitial space and oyster reef restoration. We followed the practice of removing benthic fouling organisms from our oyster substrates which is the current accepted practice in oyster research. This practice is necessary in order to make the oyster spat visible to researchers; however, it is not known how this practice may influence the community on the oyster substrate. Barnacles are a known predator of oyster spat, therefore removing barnacles may skew data in favor of the oysters which may not occur in an uninterrupted system. Additionally, removing algae, biofilm, and sediment greatly increases visibility of oyster spat. While this is necessary for the researcher to record data, it may increase visibility of the oyster spat by a predator. Future research should examine the impact of removing benthic fouling organisms from oyster communities on research outcomes.

Future research may also examine the possibility of developing a methodology for visualizing oyster spat without disturbing the natural system.

Although individual oysters were not tracked throughout the study period, we did not observe a pattern of edge effects on our artificial oyster substrate. However, it is rational to hypothesize oysters who settle on the edge of the substrate may be more susceptible to predation than oysters settling within the substrate. The possibility of an edge effect should be more closely examined when further developing interstitial space substrates for oyster restoration. Additionally, water flow and wave action may play an important role in how dependent an oyster community is upon interstitial space. For example, oyster spat in habitat with considerably higher wave action may benefit more greatly from more interstitial space than oyster spat in habitats with less wave action. Comparable studies should be conducted in field sites with differing wave energies to examine the effect of wave action on oyster spat need for interstitial space.

In summary, our work emphasizes the importance of incorporating an optimal amount of interstitial space into artificial substrates used for oyster reef restoration. While many previous studies have hypothesized the importance of interstitial space, this is the first study directly quantifying the effect of interstitial space on oysters. Improving the growth and survival of oyster populations has positive implications for the surrounding marine ecosystem. A significant increase in oyster populations may improve water quality of the marine ecosystem and improve interactions between benthic and pelagic food webs. Optimal interstitial space between oysters may also improve the habitat complexity on oyster reefs and lead to an increase in biodiversity within oyster reef ecosystems. The inclusion of interstitial space in oyster reef restoration may have an overall positive impact on all aspects of coastal ecosystems. While most of the reefs in the Chesapeake Bay are under management protocols now, we are still working to mitigate the historical impact of the Tragedy of the Commons.

II. Using photogrammetry to measure interstitial space

Abstract

Habitat complexity is widely recognized as a crucial component for maintaining biodiversity of many ecological systems. A key component of habitat complexity is interstitial space, or the volumetric gaps between elements within a structure. While multiple ecological studies have emphasized the importance of interstitial space within a system, there is currently no developed methodology that is practical for use in the field and transferable across systems. Using photogrammetry and three-dimensional digital modeling of an artificial oyster reef substrate, we developed a method for measuring interstitial space of oyster reefs. We found complexity and volume of space to have an inverse linear correlation. We developed an index of interstitial space based on volume of space and complexity to reflect the importance of these elements. We found that examining a proportion of interstitial space as volume of space per individual may be a practical method for examining the effect of interstitial space on a response variable (i.e. oyster spat recruitment). The method of measuring interstitial space developed in this study will be applicable to many ecological systems as photogrammetry and three-dimensional modeling are not system-specific processes and will fill acknowledged gaps in many ecological studies. This method may be used to better inform our knowledge of habitat complexity and species interactions within and across systems.

Introduction

Interstitial space is everywhere

Interstitial space plays an important role in many ecosystems (O'Beirn et al., 2000; Humphries et al., 2011; Martin et al., 2012). Interstitial space is subject to natural selection and follows the ecological principle of the ‘Goldilocks effect;’ where a trait of interest will optimally fall within the two extremes of its range (Kerr, 2007; Heimpel and Asplen, 2011; Lloyd et al., 2011). It is reasonable to think that oyster reefs with an optimal amount of space will have greater net productivity than oyster reefs that are too crowded or too sparse. Optimal interstitial space in a structure has the ability to give individuals in the system increased access to resources, increased refuge from predation, and increased niche availability. The “space” between objects or organisms (interstitial space) incorporates the physical volume of the area as well as the physical arrangement, or complexity, of the space (Humphries, 2011). More concisely, interstitial spaces are the volumetric gaps between elements of a structure (Kim, 2018). Structural complexity accounts for the full three-dimensional arrangement of the structure, whereas rugosity is typically viewed as a two-dimensional view of amplitude in the height of the object (Figure 19). In oyster studies, rugosity is used to assess flow turbulence and vertical accretion of settlement (Colden et al., 2016). However, structural complexity is needed to assess recruitment within interstitial spaces because recruitment does not only occur on the two-dimensional surface of the oyster reef. As the structural complexity of an object increases, the physical volume of the space decreases. Likewise, the volume of interstitial space of the object decreases as the number of interstitial spaces in the object increases (Figure 20).

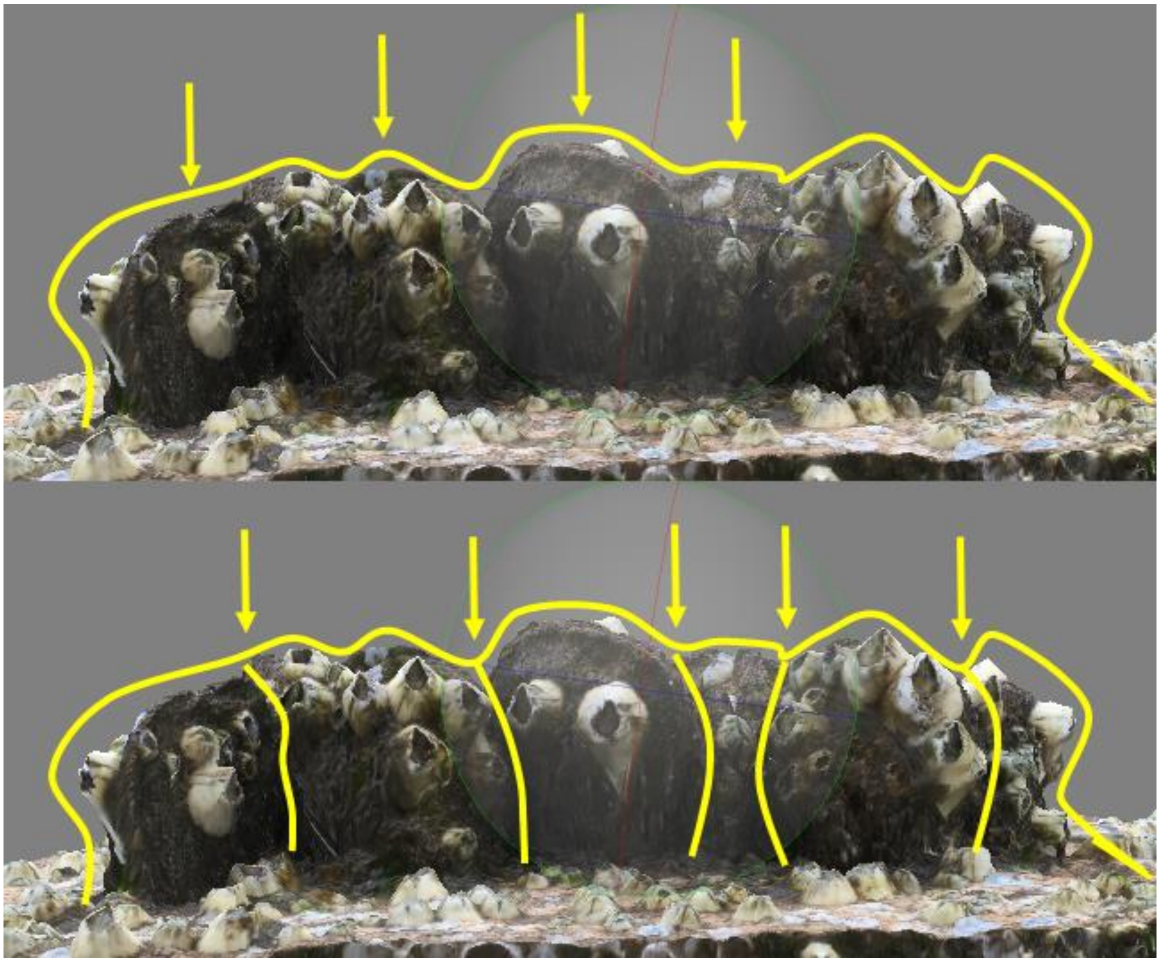

Figure 19. Above: rugosity is the two-dimensional view of amplitude (indicated by arrows) in the height of the oyster reef. Below: Complexity is a three-dimensional view of the arrangement of the oyster reef, this accounts for the amplitude in height of the oysters and the volumetric gaps between oysters as indicated by the arrows.

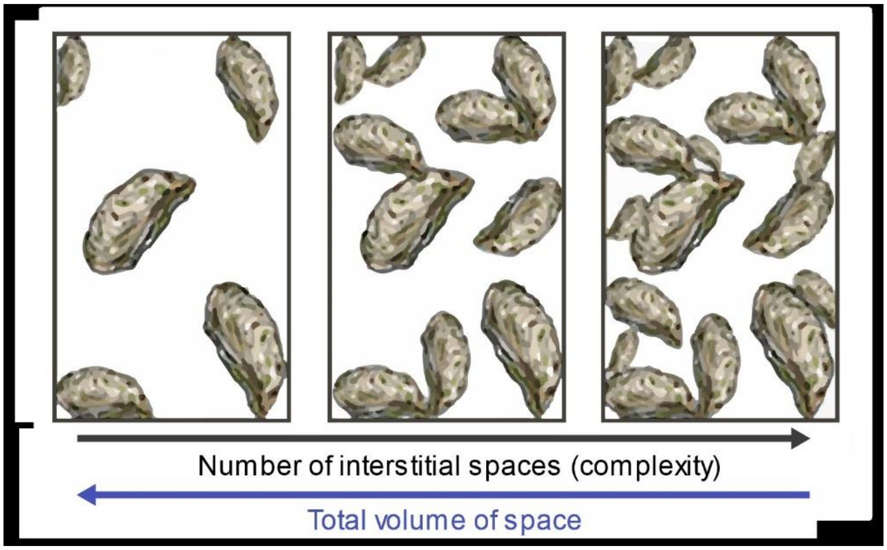

Figure 20. As the total volume of the space decreases, the number of spaces increases.

Interstitial space concept

Interstitial space can be seen at every biological level, spanning from cellular function to entire ecosystems. Within the body of a multicellular organism, interstitial compartment space is the space that surrounds individual cells and is filled with interstitial fluid (Guyton et al., 1971). The interstitial compartment space is crucial to the body's ability to perform cellular processes, such as bringing oxygen and nutrients to the cell. Organisms can use or create interstitial space. Studies across multiple ecological communities have supported the hypothesis of an optimal amount of interstitial space that best facilitates organism interactions within the system (Martin, 2012; Carroll, 2015; O'Beirn et al., 2000).

Predator-prey interactions are a logical starting point for examining how interstitial space between organisms can influence the ecology of a system. Interstitial space offers refuge for benthic organisms and refuge for recruitment with less predation (Kovalenko et al., 2012). If we assume that size of the spaces in a system is proportional to the average size of the organisms using the spaces (Figure 21) in a simple, three trophic-level model in which there are prey, target species, and predators, we can provide a conceptual framework for this idea. In this situation, size is relative to the target organism's size. "Small"-size spaces can contain an abundance of prey for the target organism but would be inaccessible by the target organism due to its size constraints. Thus, "small" spaces provide access to prey but may limit growth. "Large" size spaces may provide more space for growth for the target species but also may have an abundance of predators with scarcity of prey. "Large" spaces also offer fewer niches, providing little refuge from potential intraspecific. "Intermediate" size spaces theoretically provide the highest survival rate for the target organism due to being accessible to smaller prey, while also offering refuge from larger predators. In comparison to "large" interstitial spaces, "intermediate" spaces provide increased niche space which decreases competition between organisms and increases biodiversity of the ecosystem. This principle of interstitial space has been suggested to operate in multiple ecological communities, discussed below.

A study that examined salamanders living in spaces between rocks in a stream bed implemented a 3 x 6 matrix of enclosures in the stream with three different substrate sizes (small, medium, and large substrate) (Martin, 2012). The authors assume that small substrates create small interstitial spaces and large substrates create large interstitial spaces. More salamanders were found in the "medium" substrate size treatments relative to the other substrate size treatments. This result is attributed to the physical constraints of the substrate size: the salamanders cannot fit into the "small", prey-rich substrate, and the "large" substrate exhibits prey scarcity and predator abundance (Martin, 2012). This study supports the concept that interstitial space size influences the community structure and predator-prey interactions.

Another study explored the impact of interstitial space by examining the relationship between bay scallop survival and habitat complexity in sea grasses (Carroll, 2015). In this study, habitat complexity in sea grasses is synonymous with the available interstitial space between the grasses. This study utilized mesocosm lab experiments using seagrass mimics with four levels of habitat complexity. The study resulted in increased bay scallop survival in more complex habitats than when no sea grass was present. Additionally, when testing survival of bay scallops in the presence of different predators within the model, the researchers determined that predator identity (likely due to size) is an important factor in determining the relationship between prey survival and habitat complexity. This study supports the observation that interstitial space needs to be examined within the context of the study organism.

A third study focused on interstitial space examined the relationship between habitat complexity of oyster reefs and community dynamics. The combined effects of toadfish presence and habitat complexity of an oyster reef on mud crab mortality were tested in a laboratory study. The study found that more complex reefs, showed increased oyster survival via reducing predation by mud crabs (Grabowski et al., 2004). Complexity was inferred as increased number of spaces and increased depth of the reef. The study concluded that the effect of habitat complexity on the dynamics of the overall trophic cascade is dependent upon whether the physical complexity of the structure offers refuge for predator-prey pairs, thus supporting the importance of interstitial space on ecosystem dynamics. Collectively, these studies offer support for the ecological importance of interstitial space; however, none of them effectively measured the physical volume of the interstitial space.

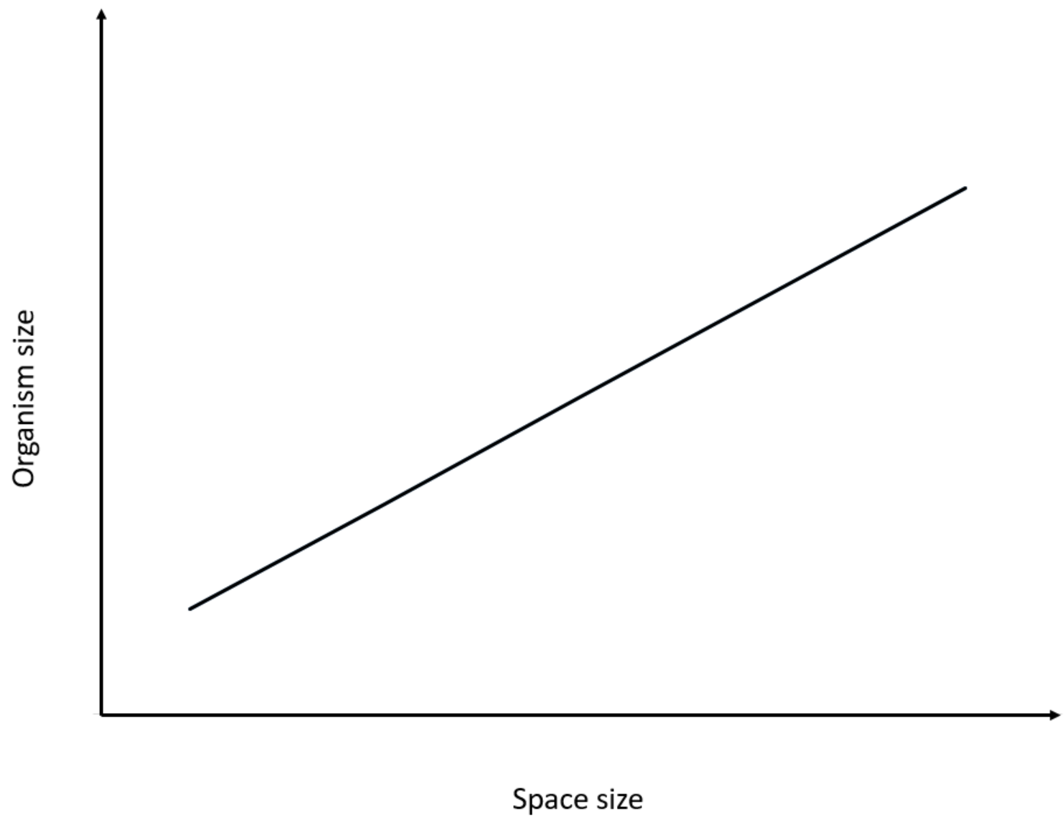

Figure 21. General relationship between organism size and space size.

Previous measures of interstitial space

Most studies exploring interstitial space lack measurement of the actual physical space. When studies do measure the space, their measurements are tedious, ineffective in the field, and do not transfer across taxa. One reason that there is currently no single way to measure interstitial space is because it is three-dimensional. (Hesterberg et al., 2017). The single published study that directly measured interstitial space of oyster reefs used 3D-printed oyster shells, clay and water displacement to measure volume, and angle measurements of individual shell attachment to the reef (Hesterberg et al., 2017). This method is extremely time intensive and is not possible to perform in the field. A qualitative index comparing macroinvertebrate abundance to habitat complexity of macrophytes was also developed; but, it cannot be easily applied to other systems (Warfe et al., 2008). Bartholomew (2000) created a dimensionless habitat complexity index by dividing the average space (width) size by predator size. This index measures the proportion of the space size relative to the predator size, but it does not directly measure the interstitial space size because it only accounts for the width of the space rather than the total volume of the space. Additionally, because these reefs were constructed using PVC in a laboratory setting, this method is difficult to replicate and ineffective in the field.

Developing a practical method for measuring interstitial space of oyster reefs could have a positive impact on the field of oyster restoration and long-term sustainability of oyster populations. Artificial reefs that are more effective at improving the survivorship of juvenile oysters will increase the chance of survival for the entire reef. Interstitial space can be incorporated into artificial reef construction in order to make reefs follow a more natural reef shape, in comparison to the geometric artificial reef shapes currently used in restoration. The technology to measure interstitial space has not been readily available in the past but is becoming more accessible allowing us to creatively measure interstitial space in the field. Using a combination of photogrammetry and three-dimensional digital modeling, we could measure interstitial space of existing oyster reefs in the field.

Photogrammetry

Photogrammetry is a method that uses photography for surveying and mapping to measure distances between objects or points on a map. It was first developed in 1984 to map topography through the use of satellite imagery (Wheeler, 2016). Photogrammetry incorporates multiple images in order to create three-dimensional digital models of a subject. The difference between photogrammetry and photography can be compared to the difference between a three-dimensional and two-dimensional movie. Three-dimensional movies utilize multiple camera angles in order to allow the human brain to reconstruct the original three-dimensional image the movie is depicting (Wheeler, 2016). Photogrammetry uses multiple photos from different camera angles to recreate an image with all angles, which would have been lost if only given from one angle (Wheeler, 2016). Through the use of multiple photos and angles to create three-dimensional digital models, photogrammetry can accurately depict topography, distance, and elevation. Today photogrammetry is used in a variety of fields: the historic preservation of statues and artifacts, aerial surveys in engineering and architecture, advancement of virtual and artificial reality, and mapping in biology. Using photogrammetry in biology and ecology allows researchers to examine habitats and substrates, like oyster reefs, from perspectives that would otherwise be lost in two-dimensions.

Corals are the most popular marine system in which photogrammetry is currently used. Photogrammetry of corals allows researchers to measure the surface area and volume of the coral, as well as visualizing the coral in a 360° view (Guitterrez, 2015). This method works well for corals because it is possible to get a complete view of the point of attachment of the coral. One preliminary laboratory study using photogrammetry to study interstitial space on oyster reefs showed a relationship between oyster interstitial space size (measured using geomorphometric analysis) and predator (crab) size (Kim, 2018). This study examined oysters in the lab and utilized photogrammetry of individual oyster shells and small clusters of oysters. While this is an important first step, the ultimate goal is to make the measurements in the field.

The objective of the current study is to develop a practical method for measuring interstitial space of oyster reefs using photogrammetry. The first step of an ultimate goal of taking these measurements in the field is to use photogrammetry on constructed oyster tiles to measure the space. This method will give insight into measuring interstitial space and conducting photogrammetry in a field setting which has not been previously explored.

Methods

Substrate construction

Artificial oyster reef habitat was constructed to mimic natural oyster habitat. Two iterations of a standard Portland cement mixture were used to create the artificial reef habitat: a standard mixture of Portland cement and limestone sand from a local quarry (http://www.frazierquarry.com), and a mixture including the addition of powdered Magnesium to the first iteration. Concrete slabs were poured with dimensions of 40 cm x 40 cm x 5 cm. Using a spatial template, previously cast concrete shells were placed vertically in the slab to a depth of roughly 3 cm as the tile was drying. The template was used to standardize the tiles within a treatment so that the arrangement of the "shells" would not be a factor within the treatments. Concrete shells were constructed by combining the cement mixture, pouring the mixture into shell molds, and leaving to dry for 24 hours (Elder, 2018). Fifteen tiles each with 25 artificial shells were created with three levels of interstitial space qualitatively identified as "low," "medium," and "high" interstitial space (Figure 22).

Methodology development

The use of photogrammetry and three-dimensional digital modeling to measure interstitial space was inspired by the use of photogrammetry of coral reefs (Gutierrez, 2015). Consultation with experts, Dr. Luis Guiterrez and specialists from Autodesk

Netfabb, affirmed the decision to use photogrammetry and three-dimensional modeling to potentially measure interstitial space of oyster reefs. Interstitial space has two defining components: the volume of space within the structure and the arrangement (complexity) of the structure. Photogrammetry and three-dimensional modeling were used to separately measure volume of space and complexity of the oyster substrates. These measurements were then combined to examine the measure of interstitial space.

Three-dimensional modeling

Approximately 100 photos of each tile were taken approximately 0.25 m above the tile encompassing an aerial view and all side views of the tile (Canon 550d, 18 M resolution). Photos of each tile were imported into Agisoft Photoscan Standard (Educational standard license, Windows x 64 bit) to build a three-dimensional model of the concrete tile (Figure 23) (protocol described in Appendix B). Meshmixer 3.5 (2017 Autodesk Meshmixer, General Public license, Windows X 64 bit) was then used to fill any digital holes in the model. Due to the complexity of the oysters' shape, photography of all angles was difficult and small holes in the model were inevitable. Each entire model took approximately 24 hours to create and the process was repeated for each tile. Autodesk Netfabb Standard 2018 (Windows x 64 bit) was then used to measure the interstitial space of the model (Figure 24).

Figure 22. Constructed artificial oyster substrate with low, intermediate, and high interstitial space respectively.

Figure 23. Aerial view screenshot of three-dimensional digital model created using Agisoft Photoscan Standard. The faint overlay is a part of the modeling program and may be ignored.

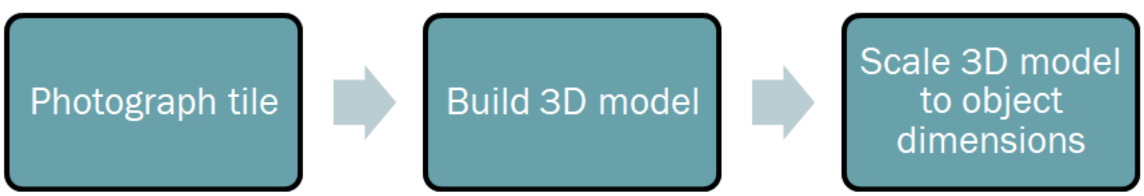

Figure 24. General workflow of photogrammetry methods.

Measuring interstitial space

After creating the three-dimensional model, the middle 150 mm^2 radius of the surface area of the model was defined as a basis for comparison between treatment. This minimum area represents a circle scaled from its center to a point on a horizontal line tangent to the circle and marking the outer edge of shell on the tiles with the lowest interstitial space. This ensures that comparisons between treatments are void of unoccupied surface area. The 150 mm^2 radius was chosen due to being the radius of the spatial arrangement of the low interstitial space treatment. This standardization of 150 mm^2 radius was necessary because the total amount of the square tile that the artificial oyster shells occupied was different depending on the space treatment (Figure 25). The circle created with this radius was used for all of the volume and crowding measurements. Each focal circle was overlaid with a 50 mm^2 grid. Five complete grid squares from each tile were randomly selected to measure volume. Then, the volume of the five grid squares on a tile were averaged to give a mean volume of the tile (Figure 26).

Populations that aggregate, like oyster populations, typically experience nonrandom spatial distribution which causes two individuals of the same population to experience different levels of competition (Iwao, 1976; Orensanz et al., 1998; Wade, 2018). Mean crowding accounts for the degree of spatial clustering of individuals in relation to competition for resources (Lloyd, 1976; Wade, 2018). In short, mean crowding measures density from the perspective of the individual, rather than the density of the population (Figure 27). Crowding was measured by identifying the number of oysters physically touching each other within each randomly selected grid square. The values of crowding were measured for each of the 5 selected grid squares of each substrate and averaged to give a mean crowding of the tile. The values of crowding for each tile within a treatment were averaged to give a mean crowding for the treatment.

Being that there is no currently adopted method for measuring three-dimensional spaces in an ecological system, measuring volume of space within the artificial oyster reef proved to be a non-trivial problem. We used the five grids that were randomly selected in each tile from the interstitial space measurements to calculate the volume of space in each square. Each grid square fully within the circumference of the 1500 m^2 radius was assigned a number. A random number generator was then used to randomly select the five grids used for measurement. Measuring each acceptable square was not feasible; determining the volume of one square took approximately 2 hours, and each area had 16 acceptable squares, which means a total of 32 hours would be required to measure one substrate. The volume of space within each randomly selected square was calculated by subtracting the volume of the model in the square, from the volume of a solid three-dimensional cube created with the same length, width, and height dimensions as the model (Figure 28). The resulting three-dimensional model depicts a physical representation of the space within the initial model (Figure 28). The volume of the resulting model is equivalent to the volume of space within the grid square of the artificial substrate; the volume of the negative space. This process was repeated for each of the 5 grid squares and the resulting space volumes were averaged to give a mean volume of space for each substrate. The values for volume of space for each substrate within a treatment were averaged to give a mean volume of space for the treatment.

Figure 25. Photographs of treatments of interstitial space low, intermediate, and high respectively.

Figure 26. Three-dimensional model with outlined grid squares. Randomly selected grid squares (blue) used to measure volume of space and crowding.

Figure 27. Three-dimensional model output of treatments. Left: High interstitial space treatment with a population density of 25 artificial oyster shells and a mean crowding of 0.27. Right: Low interstitial space treatment with a population density of 25 and a mean crowding of 4.13.

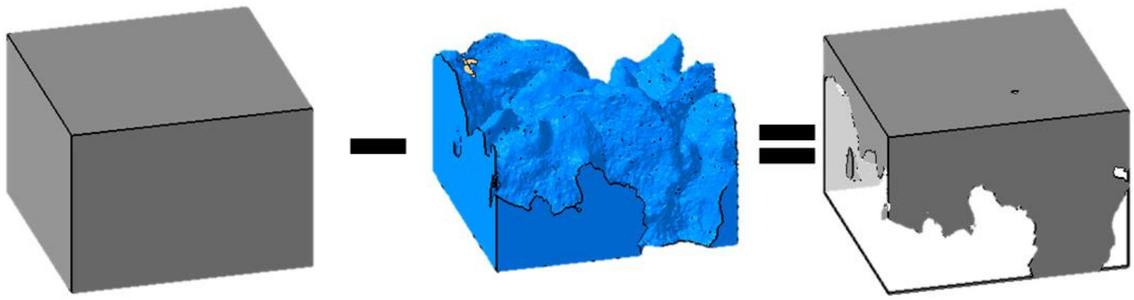

Figure 28. Generalized workflow for calculating volume of space of a randomly selected grid square of the oyster substrate. The three-dimensional model sample (blue) is subtracted from a solid cube (grey) with equal dimensions to result in a model (grey and white) with the volume of space available in the oyster substrate.

Interstitial space index

The two major elements of interstitial space are the structure's volume of space and the complexity of the structure. Combining the volume of space of each tile with the average crowding of each oyster tile allowed an index of interstitial space to be created that displays the correlation between volume of space and crowding. Discounting the mean volume of space for each tile by the mean crowding of each tile resulted in an 'interstitial space value' for each oyster tile. The resulting interstitial space values depict space as a function of crowding in terms of volume of space per individual oyster.

$$\textbf{Interstitial Space (cm}^3\textbf{ / Individual) = (Volume of space cm}^3\textbf{) / (Mean crowding)}$$

Statistical Analysis

All statistical analyses were performed using RStudio (version 1.1.463) (Affero General Public License). We tested to make sure that the interstitial space treatments were indeed different using a chi-square test (R package: Mosaic). We tested for difference between interstitial space values between treatments, volume of space between treatments, and crowding between treatments. Once we determined the treatments were indeed different, we used a chi-square test to determine if habitat preference for final spat count was different between the treatments.

Results

The current research aims to develop a practical method for measuring interstitial space using photogrammetry. Interstitial space is a function of both volume of space and the complexity of the structure. In order to measure interstitial space both of these elements were measured separately. All results are reported for the substrates constructed with the standard concrete mixture because the tiles made with the magnesium additive deteriorated. The mean volume of space is reported for five 50 mm^2 boxes of surface area for each of three treatments. The mean volume of the high interstitial space treatment (mean = 166.87 cm^3) was 25% greater than the low space treatment (mean = 109.14 cm^3) and 42% greater than the intermediate space treatment (mean = 130.03 cm^3). Volume of space values between treatments are statistically significant (chi-squared analysis, X^2=6.10, df=2, p = 0.047) (Figure 29). Crowding is reported as the mean crowding for each treatment (low = 3, intermediate = 2, high = 0.87) and are not statistically significant (chi-squared analysis, X^2=0.977, df=2, p = 0.61) (Figure 30). Crowding and volume of space are inversely correlated (linear regression, R^2= 0.98, $p > 0.05$) (Figure 31). A correlation of crowding vs volume of space vs final spat count of oysters shows the greatest final oyster spat count at an intermediate amount of crowding and an intermediate amount of space (Figure 32).

Since interstitial space is a function of both volume of space and the complexity of the object, we generated an index. The index of interstitial space reports the volume of space as a function of crowding. Generating a proportion of interstitial space (reported as volume of space per individual) generates a statistical difference between treatments (chi-squared analysis, X^2=62.12, df=2, $p < 0.01$). The mean interstitial space value for the high treatment was 149% greater than the low treatment and 118% greater than the intermediate. Correlating final spat count of oysters and interstitial space value displays the highest survivorship at an intermediate interstitial space value.

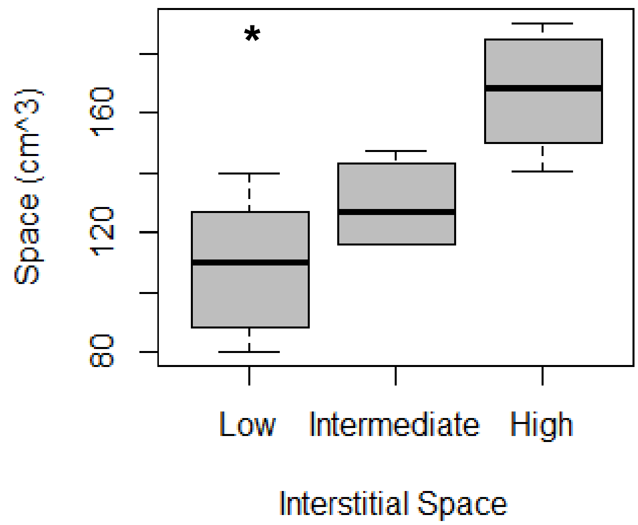

Figure 29. Boxplot displaying the distribution of interstitial space volume between treatments. Chi-squared analysis, X^2=6.10, df=2, p = 0.047

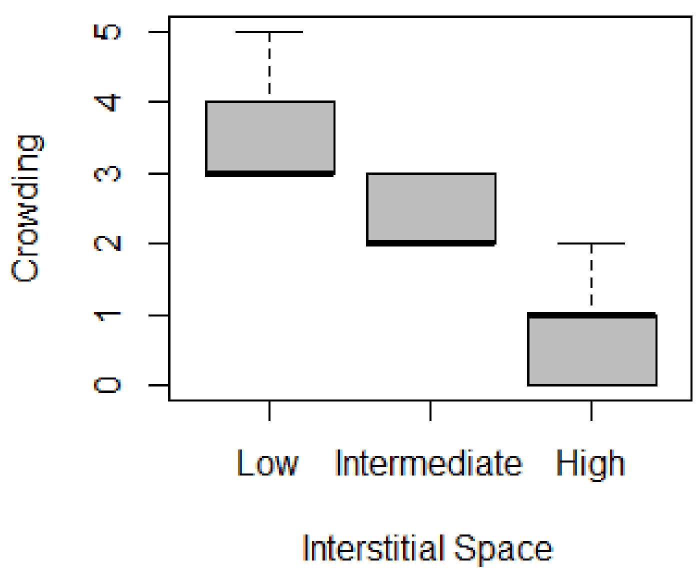

Figure 30. Boxplot displaying the distribution of crowding between treatments. Chi-squared analysis, X^2=0.977, df=2, p = 0.61

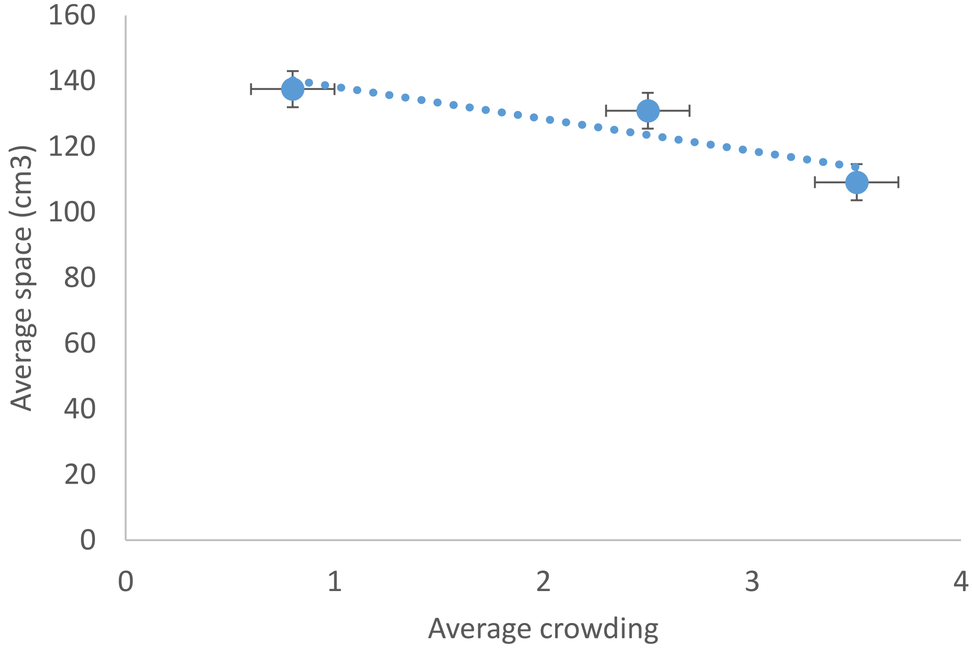

Figure 31. Average crowding (SE = 0.2) vs average volume of space (SE = 5.5) for each treatment. Linear regression analysis generated R^2 = 0.98. Average space is shown for each treatment. Average space for each treatment is generated by calculating the mean of all volume of space values (n=5) for each substrate of the treatment.

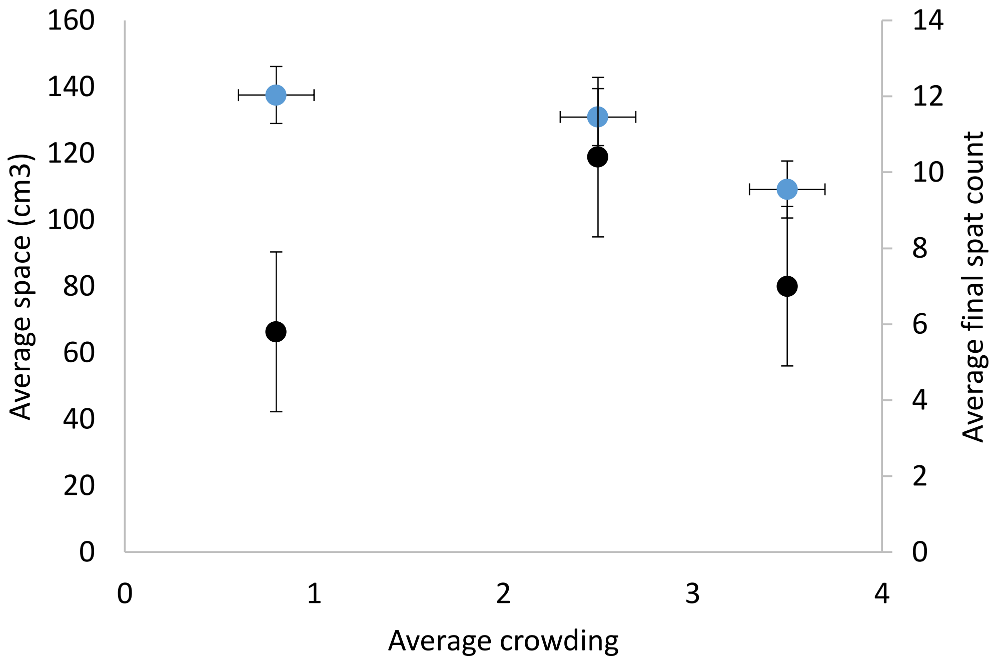

Figure 32. Average crowding (SE = 0.2) vs average volume of space (SE = 5.5) vs average final spat count (SD = 2.1) for each treatment. Average space vs average crowding represented in blue, average final spat count represented in black.

Discussion

The research objective was to develop a practical method for measuring interstitial space. We have shown that photogrammetry and three-dimensional digital modeling can be used as a practical method for measuring interstitial space within an artificial oyster reef. Beneficially, the developed method is easily translated for use in other systems. In oysters, interstitial space is determined by the volume of space between the oysters and the reef's complexity (crowding in relation to oyster reefs). Our results indicate an inverse linear correlation between crowding and volume of space within the structure. Since these two traits are related, we created an index of interstitial space by discounting the volume of space by crowding to result in a proportion of interstitial space per individual to account for both traits on oyster recruitment. Correlating volume of space with crowding of each treatment increased the magnitude of difference in the interstitial spaces between treatments. The treatment that was designed to have the greatest interstitial space was effective: the mean interstitial space value for the high treatment was 149% greater than the low treatment, and 118% greater than the intermediate. Both volume of space and crowding are critical components to the amount of interstitial space, creating the need for an index of interstitial space to account for the relationship between the two elements. Developing an index of interstitial space allows researchers to more easily examine a variable of interest in relation to interstitial space without the need for multiple analyses or a secondary axis. Correlating the interstitial space index with average final spat count from our field study showed that oyster spat counts were greatest at an interstitial space of 50 – 100 cm^3/individual. The combination of optimal amount of interstitial space for oyster survival and the ability to measure this space can be used to enhance current oyster restoration efforts and research concerning oyster reef ecosystems.

We increased the practicality of our developed methodology by using free software, or software with a free version available. Additionally, no specific camera requirements are needed to photograph the substrates making the method accessible to virtually all interested researchers. There has been growing interest in the use of LIDAR in measuring habitat complexity of systems such as marshes, tidal flats, or benthic shorelines (Tuell et al., 2005; Morris et al., 2007; Choe et al., 2012; Zavalas et al., 2014). LIDAR requires expensive equipment and software programs which may not be accessible to some researchers. Additionally, many LIDAR systems produce rugosity data rather than a measure of complexity. When measuring interstitial space, complexity measurements are needed to examine the full three-dimensional area of the space (Figure 19). In comparison, rugosity only gives a measure of the complexity of the surface area of the structure (Figure 19). Due to the limitations of LIDAR technology, our method of photogrammetry and three-dimensional modeling is more practical for measuring interstitial space and more accessible for researchers.

An important caveat to this index is that the volume of space measurement requires adequate access to the system in order to take photos from multiple angles. Intertidal oyster reefs are ideal; however, if subtidal reefs can be photographed with underwater cameras this practical method for measuring interstitial space can positively impact multiple areas of study. Ecosystems such as coral reef ecosystems, stream ecosystems, habitat complexity studies, and trophic level dynamics can benefit from interstitial space measurements and allow researchers to develop a better understanding of the effect of interstitial space in other ecosystems.

To the best of our knowledge, there are no currently available methods for comparing interstitial space or complexity across ecological systems (Warfe et al., 2008;

Kovalenko et al., 2012; Hesterberg et al., 2017). All the reported methods available to measure interstitial space are species-specific or ecosystem-specific (Bartholomew et al., 2000; Warfe et al., 2008; Hesterberg et al., 2017). Additionally, the current methods are tedious and not easily transferable (Warfe et al., 2008; Hesterberg et al., 2017). However, the need for a practical and broadly applicable method has been widely emphasized. For example, sonar scanning technology is currently being used to evaluate underwater habitat of aquatic snails but missing interstitial space data regarding the underwater habitats has limited the usefulness of this information (Cholwek et al., 2000; Garner et al., 2016). The method for measuring interstitial space using photogrammetry and three-dimensional modeling developed in this study was inspired by the use of photogrammetry of coral skeletons (Guitierrez et al., 2015) and is not limited to any one ecological system.

The power of analyses was limited because of an unexpected structural issue with some of the tiles. Some of the substrates we used were constructed with an addition of magnesium to the concrete mixture. These tiles weathered more poorly than the standard concrete treatments (Figure 33). Although a previous study reported no significant difference in recruitment between magnesium and standard concrete treatments (Elder, 2018), erosion of the substrate was not a factor. Thus, the magnesium concrete substrates were unavailable for the interstitial space measurements and subsequent analyses.

In summary, our work demonstrates the initial development of a practical method for measuring interstitial space using photogrammetry and three-dimensional modeling. We have demonstrated that data collection for photogrammetry can easily be performed in the field. Interstitial space and habitat complexity is known to operate and play a key role in many ecological systems (O'Beirn et al., 2000; Martin, 2012; Carroll, 2015). The developed method of measuring interstitial space is practical for use in the field and is easily transferable to other systems. Interstitial space within other systems can be measured by applying this method of photogrammetry and three-dimensional modeling. Systems such as stream beds, mussel beds, and marsh grass habitats will likely benefit from the application of this methodology due to having substrate structures similar to oyster reefs. This method of measuring interstitial space can be used to fill the knowledge gaps surround interstitial space in multiple ecological systems in order to better inform ecosystem processes and potential management practices.

Figure 33. Substrates constructed with magnesium concrete mixture (left) exhibited greater rates of erosion than substrates constructed with standard concrete (right). Above photos taken at the time of the final data collection period (January 2019).

III. Conclusion

The overall goal of this research was to answer the question, “Is there an amount of interstitial space which facilitates oyster recruitment and survival?” Our findings support the conclusion that there is a significantly higher oyster spat count at an intermediate level of interstitial space. Incorporating the optimal amount of interstitial space for oyster recruitment and survival into artificial reef substrates used in oyster restoration efforts may enhance oyster recruitment and survival, contribute to the overall growth of oyster populations, and positively impact the surrounding oyster reef ecosystem.

Future research concerning the effect of interstitial space on oyster reefs may focus on the sound of the oyster substrate, quantifying habitat complexity and biodiversity of the oyster substrate, and the ability of the oyster substrate to disrupt wave action and reduce shoreline erosion. The three-dimensional models of oyster reef substrates generated during this research may be available upon request to interested researchers. Additionally, in the future, we hope to make the three-dimensional oyster substrate models accessible to oyster growers or coastal homeowners interested in utilizing a natural-looking artificial substrate for oyster growth. Oyster reef substrates with optimal interstitial space will improve oyster populations and improve coastal habitat conditions for benthic organisms.

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Appendix I: Tile Construction

Artificial shells were constructed by creating silicone molds of natural shell. Silicone molds were made using Mold Max 30. A natural oyster shell was half embedded in clay and placed in a plastic cup to occupy roughly half of the cup's volume. The rest of the cup was filled with Mold Max and left to dry for 24 hours before being cut and separated from the cup. The clay was removed from the shell and the mold with the attached shell were then placed in another cup and filled with Mold Max to complete the remaining half of the mold, again the mold was left to dry for 24 hours. The shell was then cut from the mold and we were left with a functioning shell mold to be filled with concrete. Natural shells for mold use were obtained from a local restaurant. Concrete shells and concrete tiles were created using one of two cement mixtures previously stated.

Appendix II: Photogrammetry

Data will be archived using Open Science Framework.Approximately 100 photos of each tile were taken from an aerial view approximately 0.25 m above the tile. Photos of each tile were imported into Agisoft Photoscan Standard (Educational standard license, Windows x 64 bit) to build a three-dimensional model of the concrete tile. Photos were aligned and used to build a dense cloud, which was then used to build a mesh followed by a textured mesh and exported as a wavefront (.obj) file (Figure 4). The exported model from Agisoft Photoscan Standard was imported into Meshmixer 3.5 and used to fill any holes in the model. Due to the complexity of the oysters' shape, photography of all angles including the bottom of the tile was difficult and small holes in the model were inevitable. After filling holes in the model, the model was again exported as a wavefront (.obj) file. The newly exported model was imported into Autodesk Netfabb Standard 2018 (Windows x 64 bit) to measure the interstitial space of the model.

Measuring interstitial space

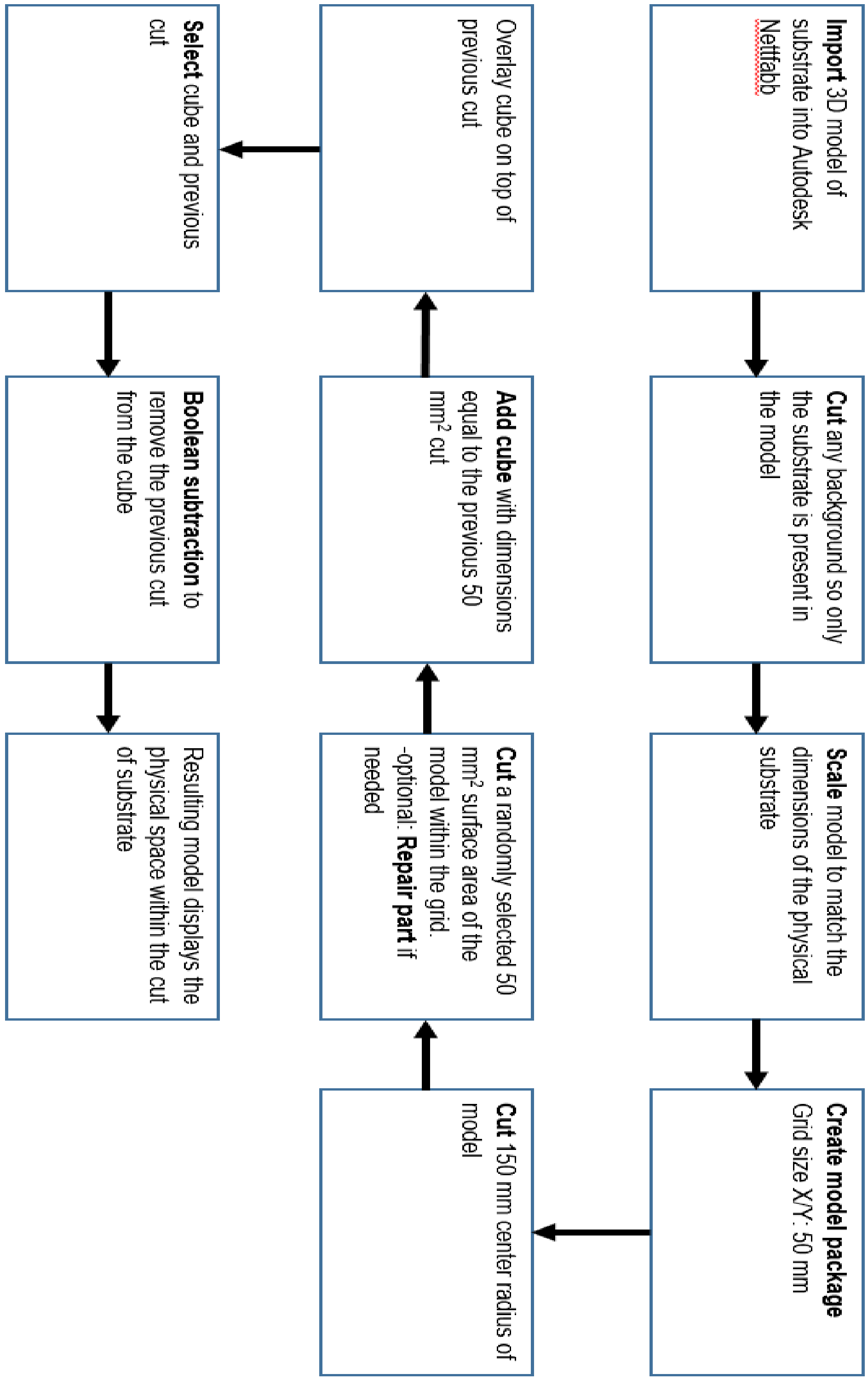

Figure 1. Flowchart depicting methods to measure one sample of interstitial space within one substrate model. Steps 6-11 were performed 5 times in total for each substrate model.

Appendix III: R Code

```
#Recruitment
#Summary stats
> tapply (Spat, Space, summary)
$`High`
$Low
$Medium

#Shapiro test
> shapiro.test(Spat) #test for normality
#Levene Test
> levene.test(Spat, Space) #test for equal variance

#Chi-square analysis
#test for significant differences between final spat counts of treatments
High<- c(1,9,9,7,3)
Intermediate<- c(0,11,18,9,14)
Low<- c(2,6,8,3,16)
boxplot(Low, Intermediate, High, xlab = "Interstitial Space", ylab = "Spat Count", col =
c("grey"), names = c("Low", "Intermediate", "High"))

spat<-matrix(c(39,39,39,29,52,35),nrow=3) #included outliers
spat
chisq.test(spat) # test for differences between spat with outliers

spat1<-matrix(c(33,33,33,29,52,19),nrow=3) #without outliers
spat1
chisq.test(spat)

#Measuring interstitial space
#linear regression analysis for crowding vs space
space<-c(109.14,137.51,166.87)
crowding<-c(3.5,2.5,0.8)
lm(space~crowding)
summary(lm(space~crowding))
plot(space~crowding)
abline(lm(space~crowding))

#volume of space
space<-matrix(c(137.84,137.84,137.84,109.14,137.51,166.87),nrow=3)
chisq.test(space) #test for differences between volume of space

#crowding
crowding<-matrix(c(2.27,2.27,2.27,3.5,2.5,0.8),nrow=3)
chisq.test(crowding) #test for differences between crowding

#interstitial space values
interstitial_space<-matrix(c(85.15,85.15,85.15,31.18,52.37,171.89),nrow=3)
chisq.test(interstitial_space) #test for significant differences between treatments
```