COLONIZATION OF HARD-SUBSTRATE INVERTEBRATE COMMUNITIES ON VARIOUS MATERIALS IN GALVESTON BAY, TEXAS

An Undergraduate Research Scholars Thesis
by
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Submitted to the LAUNCH: Undergraduate Research office at
Texas A&M University
in partial fulfillment of requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Faculty Research Advisor: Dr. Laura Jurgens

May 2021

Major: Marine Biology

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ABSTRACT

Colonization of Hard-substrate Invertebrate Communities on Various Materials in Galveston Bay, Texas

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Hard-substrate organisms such as oysters and barnacles reduce coastal erosion, improve water quality, and promote biodiversity. However, hard-substrate communities are threatened by dredging, sedimentation, invasive species, and coastal development. Engineered structures such as pilings, bulkheads, and seawalls simplify the environment and change the composition of available substrates. Because of these differences, engineered structures often do not support the same community as the unmodified environment. By building marine structures from materials that enhance settlement, it may be possible to mitigate their impact and use marine engineering for environmental restoration. Panels made from five common marine engineering materials, wood, steel, PVC, and two different cement mixes, one containing fly ash and silica fume (CM1), the other containing ground granulated blast furnace slag (CM2), were deployed in Galveston Bay. Invertebrate colonization was monitored for three months, and resulting communities were compared at the end of deployment. CM1 panels had significantly greater cover than steel early in the observation period, and all CM1 panels had live cover ≥95% at the

end of deployment. CM1 panels had significantly greater richness than wood and were significantly more diverse than CM2 panels. Furthermore, the materials appeared to form unique and different communities. These results demonstrate that hard substrate organisms show a settling preference for different construction materials. It may be advisable for marine engineering structures in Galveston Bay to be made using cement mixtures similar to CM1 to promote the biodiversity of hard-substrate organisms.

DEDICATION

To my family for supporting me throughout the research process

ACKNOWLEDGEMENTS

Contributors

I would like to thank my faculty advisor, Dr. Laura Jurgens, and her lab manager, Melissa Ciesielski, for their guidance and support throughout the course of my research.

I would also like to thank Jonah Lumpe with J Tex Fabrication and Welding for generously cutting and preparing the steel plate required for this research experiment.

Finally, thanks to my aunt for her encouragement and to my mom for her patience, love, and support.

Funding Sources

This undergraduate research was supported by the Aggies Commit to Excellence Scholar program. The contents of this undergraduate research project are solely the responsibility of the author and do not necessarily represent the official views of Texas A&M University.

NOMENCLATURE

ASTM	American Society for Testing and Materials
CFFA	Class F Fly Ash
CM1	Cement Mix 1
CM2	Cement Mix 2
GGBFS	Ground Granulated Blast Furnace Slag
HSD	Honestly Significant Difference
PVC	Polyvinyl chloride
RFP	Request for proposal
SF	Silica Fume
TAMUG	Texas A&M University at Galveston
TC	Total Cover
TxDOT	Texas Department of Transportation

1. INTRODUCTION

1.1 Hard-substrate Marine Invertebrate Communities

Hard-substrate marine invertebrate communities are composed of various sessile or sedentary organisms, many of which are suspension feeders (Wahl, 2009b). These communities can be highly diverse and include organisms such as ascidians, barnacles, bivalves, bryozoans, cnidarians, sponges, and various tube-dwelling polychaetes (Wahl, 2009b). In subtidal habitats, hard-substrate invertebrate communities dominate vertical surfaces and overhangs composed of minerals, such as rocky outcroppings, biologic material, such as live bivalves, or anthropogenic structures, such as seawalls (Davis, 2009; Glasby & Connell, 2001). When growing over other living organisms, hard-substrate invertebrates may be referred to as epibionts, and when growing over anthropogenic structures, they may be referred to as fouling organisms (Wahl, 2009b).

Every exposed surface in the marine environment begins the process of fouling, the colonization of organisms onto submerged, solid surfaces (Wahl, 2009a). Fouling occurs in four main stages (Wahl, 2009a; Richmond & Seed 1991; Wahl, 1989). Within seconds to minutes of submersion, surfaces spontaneously adsorb dissolved compounds like proteins and polysaccharides (Wahl, 2009a; Wahl, 1989). Within hours, bacteria adhere to the surface, forming a biofilm, and over the course of several days, unicellular eukaryotes such as protozoans, yeasts, and diatoms colonize the surface (Wahl, 2009a; Richmond & Seed 1991; Wahl, 1989). Finally, after several weeks, multicellular eukaryotes such as planktonic larvae and algal spores attach to the surface (Wahl, 1989). In the first two stages, physical principles such as Brownian motion, water currents, Van-der-Waal forces, and electrostatic interactions mainly control the supply of macromolecules and bacteria as well as the process of attachment to a surface (Wahl, 2009b; Wahl, 1989). However, in the last two stages, unicellular and multicellular eukaryotes are more capable of selecting and attaching to a substrate themselves (Wahl, 2009b; Wahl, 1989).

As this community develops, five factors influence its composition: surface characteristics, interspecific competition and facilitation, larval supply, surface area and disturbances (Osman 1977). 1) Larvae may be selective of what surfaces on which they will attach (Osman 1977). For instance, biofilm presence (and the community forming it) along with surface texture, rugosity, and material composition may influence what species attach to a substrate and their relative abundance (Myan et al., 2013; Davis, 2009; Wahl, 2009a; Anderson & Underwood, 1994; Schmidt & Warner, 1989; Wahl, 1984; Crisp & Ryland, 1960; Pomerat & Weiss, 1946). 2) The presence of specific species may facilitate or inhibit recruitment by modifying surface topography or by competing with or preying on other organisms (Wahl, 1989; Dean, 1981; Dean & Hurd, 1980; Connell & Slatyer, 1977; Osman, 1977; Dayton, 1971). 3) Larval supply varies locally and seasonally, directly affecting what species will grow on a surface at any instance (Davis, 2009; Underwood & Anderson, 1994; Osman, 1977; Sutherland, 1974). 4) The size of a substrate affects community development as a larger surface area allows for a greater number of larvae to encounter the substrate randomly (Davis, 2009; Osman, 1977). Together, factors 3 and 4 demonstrate that marine substrates may be seen as “islands” in the MacArthur and Wilson (1967) equilibrium model (Davis, 2009; Anderson & Underwood, 1994; Osman, 1977; Schoener, 1974). 5) Physical damage or disturbance randomly removes members of the community (Osman, 1977).

1.2 Value of Hard-substrate Communities

Many hard-substrate marine invertebrates are valuable ecosystem engineers in coastal environments. An ecosystem engineer is an organism that causes a significant change to its environment by creating, modifying, or maintaining habitats (Jones et al., 1994). Bivalves, such as oysters and mussels, are significant autogenic engineers, meaning they produce physical structures in the environment (Jones et al., 2010; Jones et al., 1994). Calcified materials like living and dead shells or the tubes of polychaetes are hard, persistent structures that increase the complexity of a habitat, providing prey with refuge areas, acting as a surface for epibionts to grow on, and protecting the coastline against erosion (Grabowski & Peterson, 2007; Hastings et al., 2007; Gutiérrez, 2003; Stewart et al., 1999; Jones et al., 1994). Overall, structure-building invertebrates like bivalves, bryozoans, tube-dwelling polychaetes and barnacles promote diversity by creating a habitat for a range of sessile and mobile organisms (Grabowski & Peterson, 2007; Hastings et al., 2007).

Besides providing structure to the environment, bivalves and many filter-feeding organisms significantly modify water quality. Bivalves filter particulates from the water and separate them into nutritive and non-nutritive material (Newell et al., 2005; Kautsky & Evans, 1987). The nutritious matter is digested and densely packed into mucus-bound feces, while the non-nutritious matter is rejected as looser pseudofeces (Newell et al., 2005; Kautsky & Evans, 1987). By removing sediment from the water and packing it into larger aggregates, filter feeders increase the settling rate of particles and decrease turbidity (Newell et al., 2005; Newell & Koch, 2004; Widdows et al., 1998; Kautsky & Evans, 1987). When filter feeders, such as bivalves, reduce the abundance of suspended particulates, they may also aid in the restoration of seagrass beds damaged by sedimentation (Newell & Koch, 2004). Suspension feeding organisms also influence the planktonic population by removing phytoplankton, zooplankton, bacteria, and organic matter from the water column (Riisgård & Larsen, 2010). The process of pulling particulates, living organisms, and organic material from the water column and packing them into feces or pseudofeces also sequesters nitrogen, phosphorous, and carbon (Grabowski & Peterson, 2007; Newell et al., 2005; Kautsky & Evans, 1987). In regions that suffer from eutrophication or algal blooms, consuming phytoplankton and sequestering nutrients is a valuable ecosystem service (Grabowski & Peterson, 2007; Newell et al., 2005; Kautsky & Evans, 1987)

1.3 Changes to Hard-substrate Communities

Despite their environmental benefit, hard-substrate communities, such as oyster reefs, have suffered from significant degradation (Beck et al., 2011). In a review of 144 bays from 40 ecoregions, Beck et al. (2011) found that 70% of bays have lost more than 90% of their historic oyster reef abundance. Many processes have led to these significant losses, including disease, competition from invasive species, overharvesting, dredging, sedimentation, poor water quality, hypoxia, and habitat loss (Beck et al., 2011; Bulleri & Chapman, 2010; Cerrano & Bavestrello, 2009; Grabowski & Peterson, 2007; Lenihan & Peterson, 1998).

Hard-substrate habitats have been lost and fragmented by increasing urbanization, which modifies the native environment and introduces new structures such as breakwaters, groins, riprap, jetties, seawalls, bulkheads, pilings, and floating docks (Bulleri & Chapman, 2010; Davis et al., 2002; Connell, 2001). Research spanning across multiple locations has demonstrated repeatedly that communities may vary on different marine structures and are often significantly different from the native community (Bulleri & Chapman, 2010; Bulleri, 2005; Bulleri & Chapman, 2004; Davis et al., 2002; Holloway & Connell, 2002; Connell, 2001; Attrill et al., 1999). This may be caused by the simplification of the environment and changes to the composition of the available substrates (Layman et al., 2014; Bulleri & Chapman, 2010; Holloway & Connell, 2002; Attrill et al., 1999). Engineered coastlines feature structures made from concrete, wood, stone, metals, plastics, and fiberglass (Bulleri & Chapman, 2010). The materials used may affect the composition or abundance of the community and may even limit the benefits of ecosystem services such as water filtration (Layman et al., 2014; Bulleri & Chapman, 2010; Attrill et al., 1999). For instance, Layman et al. (2014) determined that if all the dock pilings in the Loxahatchee River, Florida, were made from concrete, the quantity of water filtered by the organisms growing on them could exceed that of the local oyster reefs. This demonstrates that proper planning and investment into marine infrastructure can not only mitigate damage but also act as a restorative force for heavily modified coasts.

1.4 Monitoring Hard-substrate Communities

Researchers have monitored multiple qualities of hard-substrate communities across the globe. With relatively simple methods, it is possible to determine what organisms are in the larval pool at different depths and times, identify invasive species, understand how marine organisms respond to environmental changes, study predator-prey interactions, and understand the processes behind recruitment (Jurgens et al., 2017; Underwood & Anderson, 1994; Mook, 1983; Osman, 1977). Researchers commonly study hard-substrate communities by submerging clean panels, often called recruitment panels or colonization plates underwater. After a period, the panels are collected and qualities such as species coverage, diversity and biomass are measured. However, the procedures for recruitment studies are not standardized; panel size, material, and more may vary. Of the articles examined, materials used in recruitment experiments typically fall into three categories: plastics like PVC, minerals or mineral substitutes like concrete, or a type of wood like pine (Table 1). Panels are generally cut to approximately 100-250 cm^2 and hung vertically or face down.

Table 1: Parameters of a variety of fouling experiments

Panel Material	Surveyed Area (cm^2)	Location	Author(s)
Polyvinyl chloride (PVC)	196*	Flamenco Island, Panama	Jurgens et al. (2017)
	196*	Galapagos Islands, Ecuador	Calder et al. (2019)
	100*	São Sebastião Island, Brazil	Oricchio et al. (2016)
Plexiglas	100	Heligoland & Düne Islands, Germany	Harms & Anger (1983)
Asbestos-cement	100*	Broadkill River Estuary, DE, USA	Dean (1981)
	600	Izmir Bay, Turkey	Kocak et al. (1999)
	225*	Kilkieran Bay, Ireland	Shin (1981)
Concrete	225*	Sydney Harbour, Australia	Glasby & Connell (2001)
	100*	Quibray Bay, Australia	Anderson & Underwood (1997)
Ceramic	225*	Indian River Lagoon, FL, USA	Mook (1983)
	232	Duke Marine Lab, NC, USA	Sutherland (1974)
Slate	14.5 & 103	Woods Hole, MA, USA	Osman (1977)
Asbestos	150	Chesapeake Bay, VA, USA	Calder & Brehmer (1967)
Pine Wood	464.52*	Pearl Harbor, HI, USA	McCain (1975)

Note: I only selected experiments that used a single material for recruitment panels. A material with multiple articles nested within it demonstrates that it appeared repeatedly in the sampled literature.

* I calculated the surveyed area from the dimensions and experimental description.

Table 2: Examples of experiments comparing recruitment on different materials

Panel Material	Surveyed Area (cm^2)	Location	Author(s)
Concrete cement	100*	Quibray Bay, Australia	Anderson & Underwood (1994)
Aluminum 5083	100*		
Fiberglass with Gelcoat	100*		
Marine plywood	100*		
Carbon Steel	625*	Langstone Harbour, UK	Schmidt & Warner (1989)
Perspex	625*		
Marine grade douglas fir plywood	516.13*	Monterey Harbor, CA, USA	Haderlie (1968)
Asbestos	516.13*		
Polypropylene	225*	Mactan Island, Philippines	Olalia et al. (2009)
Wood	225*		
Sandstone	169	Middle Harbour, Australia	Glasby (2000)
Concrete	169		
Marine plywood	169		
4 glass textures	516.13‡	Miami, FL, USA	Pomerat and Weiss (1946)
5 plastics	†‡		
9 woods	†‡		
7 metals	†‡		
6 steel coatings	†‡		
7 wood coatings	†‡		
5 miscellaneous materials	†‡		

Note: This is a non-comprehensive selection of articles, which focused on comparing the development of fouling communities on different materials.

* I calculated the surveyed area from the dimensions and experimental description

† No data

‡ The authors corrected their community measurements to an area of 144 in^2 (929.03 cm^2)

In comparative experiments (Table 2), researchers construct panels from two or more materials and compare the communities that develop on them using measures such as coverage, richness, diversity, wet biomass, or dry biomass. They may also contrast the abundance of specific organisms in the community to determine if different materials inhibit or facilitate the growth of a single species. Many materials have been tested; however, few studies have compared the effect of substrate material on the community in the Gulf of Mexico region.

By performing these experiments on different materials, it becomes easier to compare recruitment studies and makes it possible to identify an ideal material that researchers can use as a standard for future research. Furthermore, comparative studies may reveal how the use of different construction materials in the marine environment affects community development in estuaries like Galveston Bay.

1.5 Objectives and Focus

I will determine if the hard-substrate invertebrate community develops differently on substrates made from different materials commonly used in Galveston, Texas: PVC, oak wood, steel, and two unique cement mixes. The steel type and cement mixes were chosen based on the standard construction specifications for the city of Galveston, local construction proposals, and the mix design of a marine friendly cement: ECOncrete®. I chose these materials for two reasons. 1) Fouling experiments often use PVC, cement, or wood. It is valuable to determine if one material recruits organisms more effectively than another does. This may facilitate comparison between experiments. 2) Marine construction projects often use PVC, wood, steel, or cement for structures like bulkheads and pilings. If one material recruits organisms most effectively, that material could be prioritized over others to mitigate the damages of habitat loss. I hypothesized that natural materials like wood and materials that mimic minerals like cement would recruit organisms more quickly than PVC or steel and have a richer and more diverse community.

2. METHODS

2.1 Study Area

I conducted this study along a floating dock at the Texas A&M University at Galveston (TAMUG) boat basin (29°18'48.4"N 94°49'00.9"W), located on Pelican Island. The TAMUG boat basin is part of the Galveston Ship Channel, which is connected to Galveston Bay. At 384,000 acres, Galveston Bay is the largest estuary along the Texas Gulf Coast and the seventh-largest estuary in the contiguous United States (Sage, 2002; McKinney et al., 1989). A 1993 estimation indicated that bulkheads and docks alone take up 10% of the Galveston Bay coastline (Ward, 1993). In 2013, up to 19% of the Galveston Bay shoreline and associated tributaries were classified as "developed" (*State of the bay*, 2020).

2.2 Panel Fabrication

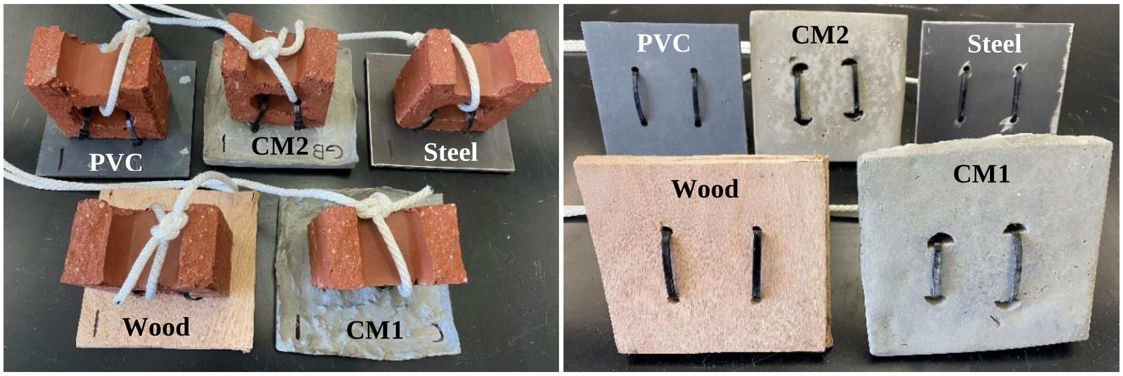

Figure 1: Examples of experimental panels. The image on the left depicts panels attached to a weight (brick), which were suspended face down from a rope at a depth of 1±0.5m. The image on the right shows the face of the panel analyzed for invertebrate colonization. The materials are organized in the photos from left to right and top to bottom: PVC, CM2, steel, wood, and CM1.

I fabricated 20 panels using five materials (CM1, CM2, steel, PVC, and wood; details below) with four replicates of each (Figure 1). The panels had a length and width of approximately 14 cm with four evenly spaced holes used to suspend them during deployment. I sanded each panel to 250 grit to ensure they shared an even and similarly textured surface and attached them to a weight to aid in suspending them in the water column.

I designed Cement Mix 1 (CM1) using the city of Galveston's standard construction specifications, section 16421, along with the mix options provided by TxDOT's Item 421 on hydraulic cement concrete. CM1 used mix design option 3 in the two previously mentioned documents so that it contained the proper silica fume (SF) and class F fly ash (CFFA) content for class C or H concrete. CM1 contained 4-5% SF, 14-17% CFFA, and 78-82% Portland cement type I/II by mass. This mix is similar to the proportions used for concrete pilings and bridge substructures. I did not add aggregate, sand, chemical admixtures, or air-entraining admixtures for simplicity and to ensure the final surface was even. Fly ash, the fine residue left over from coal combustion, and SF are pozzolanic mineral admixtures (Lothenbach et al., 2011). SF is nearly entirely composed of SiO_2 while, CFFA is composed of high concentrations of SiO_2 and Al_2O_3 (Lothenbach et al., 2011)

I designed Cement Mix 2 (CM2) to be similar to the products of the ECOncrete® Company. The general description of their concrete mix is provided in Technical Memo #0817. CM2 contained 50% ground granulated blast furnace slag (GGBFS) and 50% Portland cement type I/II by mass. This is the maximum GGBFS described by ECOncrete® and allowable by mix design option 3. CM2 did not contain aggregate, sand, chemical admixtures, or air-entraining admixtures for simplicity and to ensure the final surface was even. GGBFS is a mineral admixture produced as a byproduct of pig iron manufacturing (Lothenbach et al., 2011). GGBFS is a pozzolan that contains less SiO_2 and more CaO than both CFFA and SF (Lothenbach et al., 2011).

The other materials were made from steel, PVC and wood. I designed the steel panels based on the steel sheet piling and open pipe piles specified in RFP #20-03 for Legas Drive Bulkhead, a construction project in an estuarine environment in Galveston, Texas. Steel panels were fabricated from ASTM A572 Grade 50 steel. These panels are representative of uncoated steel sheet piles or steel pilings with a worn-down coating. I chose to construct panels from gray polyvinyl chloride (PVC) due to its long-term use for sheet piling and as a protective wrap for wooden piles (Dutta and Vaidya 2003). Finally, I prepared panels from untreated oak wood. This is representative of native wooden debris or untreated wooden piles.

2.3 Analysis of Panel Surface Area

Due to differences in fabrication methods, the sizes of the panels varied slightly. Panel size may influence community measures such as richness or diversity and would directly affect the wet and dry biomass at the end of the measurement period. This made it necessary to calculate the surface area and determine the significance of the difference for future analysis. I measured the height and width of each panel to the nearest millimeter. I then used the height and width to find the area of the panels and the diagonal distance or the distance from one corner to another. I photographed the panels and used these digital images to measure each panels’ surface area with the software ImageJ®. The measurements were calibrated using the diagonal distance across the panel to better compensate for variations in photo angle. I also measured the area of the holes and subtracted this from the previously calculated panel area to find the true surface area. I performed a log transformation on the area values to improve normality and equality of variance of the data. To determine whether panel area was significantly different by material, I conducted an ANOVA followed by a post-hoc Tukey’s honestly significant difference (HSD) test.

2.4 Data Collection

2.4.1 Community Development

I deployed the panels from November 10, 2020, to February 10, 2021, a period of 3 months. The panels were suspended face down at a depth of 1±0.5m. I randomly selected where to suspend each panel along the floating dock at the TAMUG boat basin.

Every two weeks, I photographed the panels while out of the water, and took general notes on community composition. I then selected the highest quality photo for each panel and analyzed the photographs. I used ImageJ® to calculate the proportion of the surface area covered by organisms, referred to as total cover (TC). I calibrated these measurements using the diagonal distance across the panel to better compensate for variations in photo angle. TC did not differentiate between live or dead growth; it simply represented the surface area that had been successfully colonized at one point.

2.4.2 Final Community

At the end of the three-month period, the panels were retrieved, and analyzed in the lab at Texas A&M University at Galveston. I photographed the panels, scraped the sides and back clean, and weighed them. While waiting for analysis, the panels were stored in aerated seawater. I observed the front of the panel under a stereoscope, identified the organisms based on their morphology and taxonomy, and then visually estimated their percent live cover. An organism estimated to cover <1% of the panel was recorded as covering 0.1%. The organisms were categorized under the following morpho-taxa: anemones, barnacles, calcified-encrusting bryozoans, gelatinous-encrusting bryozoans, hydroids, kamptozoans, mussels, oysters, sabellids, serpulids, soft bryozoans, spionids, sponges, and tunicates. When added together the cover of individual organisms may be greater than 100% due to organisms growing over one another. I also visually estimated the total live cover, which I defined as the proportion of the panel covered in living organisms. Once the panels were analyzed, I scraped the front of them into a pre-weighed drying pan and dried the contents at 60°C. I weighed the cleaned panels again and used the difference in weight before and after scraping to calculate the wet biomass. The dried scrapings were then weighed to produce dry biomass.

2.5 Data Analysis

2.5.1 Community Development

All data analysis used R version 4.0.2. I performed a repeated-measures ANOVA for weeks 2-12 to determine if a significant difference in the TC existed between the materials. Based on these results, I analyzed the difference in TC for each week I observed the panels. I performed a log transformation on weeks 2, 4, and 6 to improve normality and equality of variance. Transformation was not necessary or did not improve normality for weeks 8, 10, and 12. I used an ANOVA and Tukey's HSD test for weeks 2, 4, 6, 8, and 12. For weeks 6, 8, and 12, I reduced the alpha significance level to 0.025 due to moderate deviations from normality. For weeks 10 and 13, data transformation was insufficient to meet the assumptions of a parametric test, so I used a Kruskal Wallis test followed by a Dunn Test with a Bonferroni correction.

2.5.2 Final Community

I calculated the richness and Shannon-Wiener index of diversity for each panel using the coverage of the morpho-taxa identified. I compared live cover across the materials using a Welch's ANOVA since variance was unequal. I compared richness using an ANOVA and Tukey HSD test. I log-transformed the Shannon-Wiener index of diversity to improve normality, and compared the transformed diversity data using a Welch's ANOVA and a post-hoc Games-

Howell test with the alpha significance level reduced to 0.025. I then used an ANOVA to analyze wet and dry biomass.

To assess the differences in community composition, I compared the live cover of the four morpho-taxa present on all the materials. Calcified encrusting bryozoans were compared using a Welch's ANOVA and barnacle, hydroid, and gelatinous bryozoan cover was compared using a Kruskal Wallis test followed by a Dunn test with a Bonferroni correction.

Due to the differences in surface area between materials, I graphed the quantities: live cover, richness, and the Shannon-Wiener index of diversity against the surface area and plotted a linear regression for each material. I used these graphs to identify if panel size was correlated with these community measures.

3. RESULTS

3.1 Community Development

In general, CM1, CM2 and PVC panels were colonized more quickly than the other materials, community composition varied over time, and at least one panel from all the materials approached 100% cover by the end of the three-month period. Calcified encrusting bryozoans and barnacles were present on most of the panels by the first two weeks. The encrusting bryozoan population consistently increased in percent cover over time for all panels. However, during weeks 8, 10, and 12, CM1 and CM2 panels had a similar pattern of scraping around their edges, likely produced from predation (Figure 2). Within the first 2 weeks, the steel panels showed signs of corrosion, and by week 6, bryozoans had begun growing over the rust.

Figure 2: Notice the repeated scrape marks around the perimeter of this CM2 panel at 8 weeks. This same pattern was seen on multiple other CM1 and CM2 panels. The fibrous organisms, which appear to be hydroids along the left upper edge and around the lower left hole, fell below 1% cover before the end of deployment. Hydroid populations decreased on all panels after 10 weeks.

By week 4, two CM1 panels, one CM2 panel, and one wood panel were colonized with stringy, clear, soft-bodied organisms, which were presumed to be hydroids but which were not examined under a microscope. These organisms reached a maximum abundance at week 10 and were visually estimated to cover up to 70% of a CM1 panel at one time by growing over the encrusting bryozoan population. After week 10, the hydroid population decreased greatly, and by week 13, their population was only up to 15% cover on one wood panel.

At week 6, an oyster was identified on a CM1 panel, and serpulids were identified on CM1 and CM2 panels. These species had a relatively low and stable surface cover throughout the deployment period.

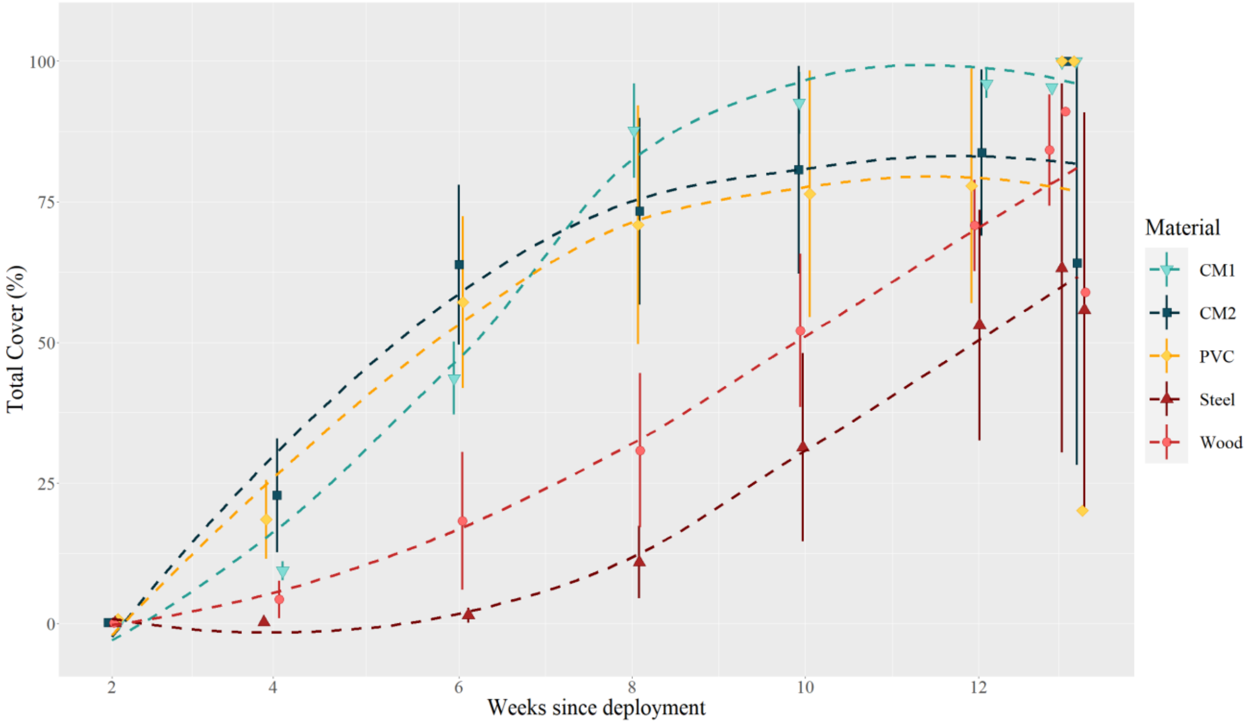

Figure 3: The materials had unique growth curves when I plotted total cover against time with a smoothed regression. It should be noted that the data points are jittered to ease visualization. From top down, the materials are organized in the key: CM1, CM2, PVC, steel, and wood (N= 4 replicates per material). In this order, they are represented by a triangle facing downward, a square, a diamond, a triangle facing upward, and a circle.

The growth curves produced when percent cover was plotted against time varied considerably based on material (Figure 3). The materials CM1, CM2, and PVC typically had a greater percent cover than wood or steel early in the deployment period. The repeated measures ANOVA determined that the percent cover between the materials was significantly different from weeks 2 through 12 (F = 3.220; df = 4, 15; p = .0474; Table A1). At weeks 2, 10, 12, and 13, the total cover (TC) was not significantly different between materials. At week 4 (F = 7.320; df = 4,15; p = 0.00178), 6 (F = 14.61; df = 4,15; p = 4.66E-5), and 8 (F = 5.102; df = 4,15; p = 0.00849) the TC was significantly different between materials based on the results of an ANOVA. Tukey's HSD test found that CM1, CM2, and PVC had significantly greater TC than steel panels at weeks 4 and 6 (Table A2; Table A3). At week 8, Tukey's HSD identified that only CM1 had significantly greater cover than steel (Table A4). This is likely due to the small sample size and the fact that both PVC and CM2 had an outlier with a low TC. For a similar reason, wood was never significantly lower than CM1, CM2, or PVC. Despite its low median and mean early in the deployment, it had one outlier with a high TC.

3.2 Final Community

3.2.1 Live Cover and Biomass

While scraping the materials, I removed a significant quantity of rust from the steel panels along with the hard-substrate community. Steel was therefore not included in the biomass analysis because an accurate biomass could not be determined. CM1 had the highest mean and median live cover with the lowest variance (Figure 4). All panels had a wet biomass within 0.01-0.09 g/cm^2 and a dry biomass within 0.002-0.02 g/cm^2 (Figure 5). The average wet biomass was 0.059 g/cm^2 (590g/m^2), and the average dry biomass was 0.009 g/cm^2 (90 g/m^2). Live cover, wet biomass and dry biomass were not significantly different between materials.

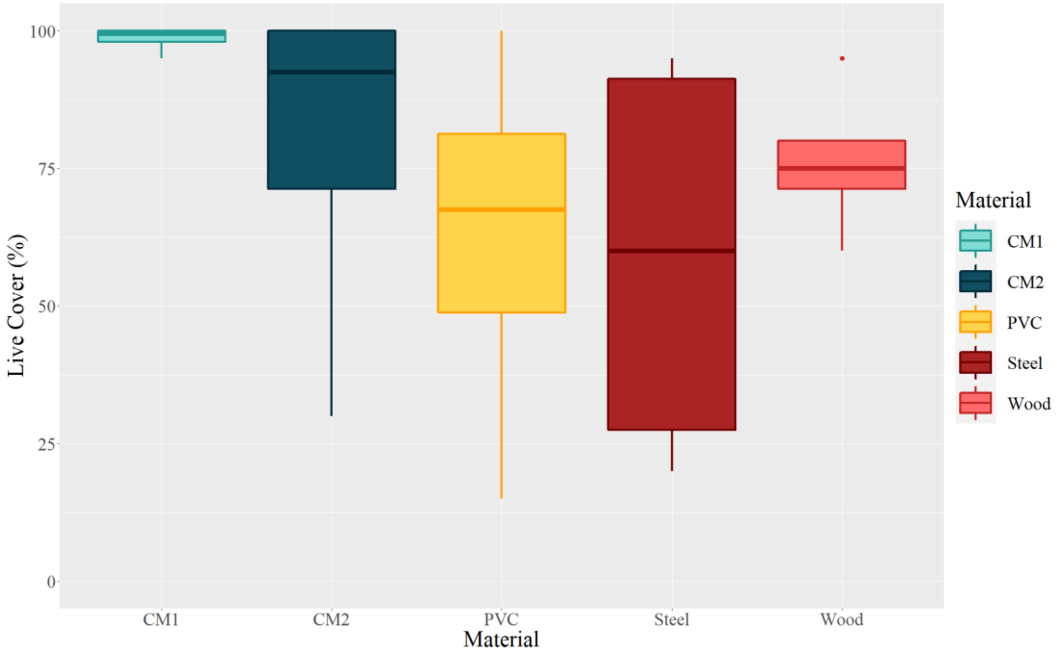

Figure 4: The mean and median percent live cover was greatest on panels constructed from CM1, and CM1 had the lowest variance in cover. There was no significant difference in live cover between materials. From left to right in the figure and top down in the key, the materials are organized: CM1, CM2, PVC, steel, and wood.

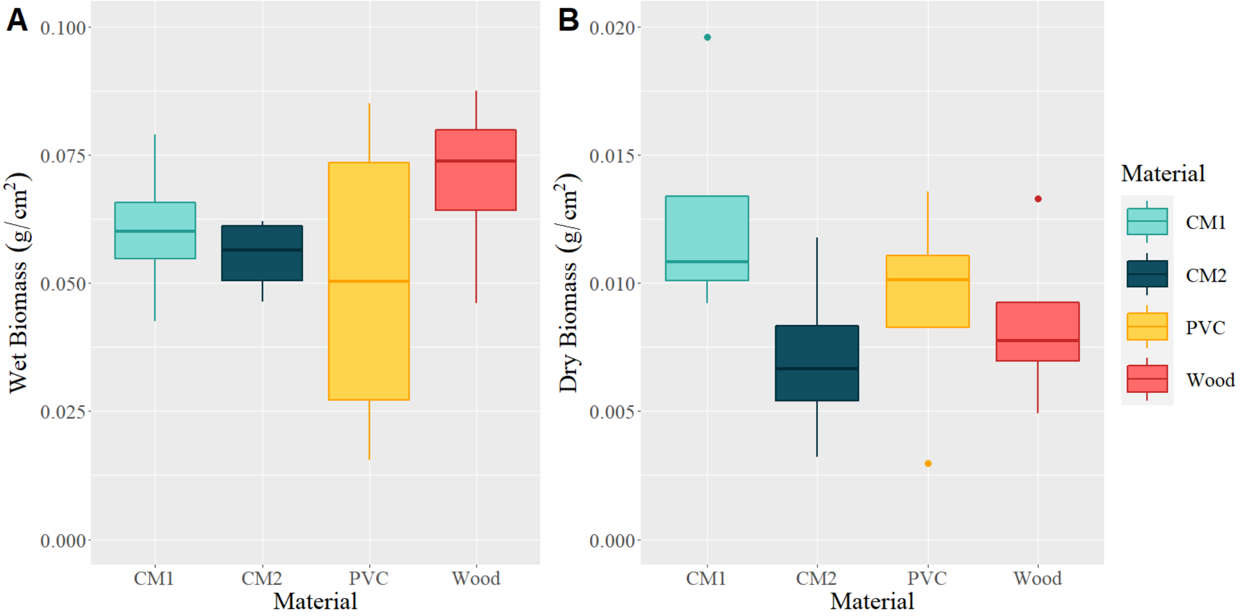

Figure 5: A) Wet biomass was not significantly different between materials. B) Dry biomass was not significantly different between materials. Steel was removed from the analysis. Rust was collected with the hard-substrate community so an accurate biomass could not be determined. From left to right in the figures and top down in the key, the materials are organized: CM1, CM2, PVC, and wood.

3.2.2 Richness, Diversity, and Community

Richness was significantly different between materials (F = 3.587; df = 4,15; p = 0.0304). A post-hocTukey's HSD test indicated that the richness was significantly different between wood and CM1 panels (Figure 6; Table A5).

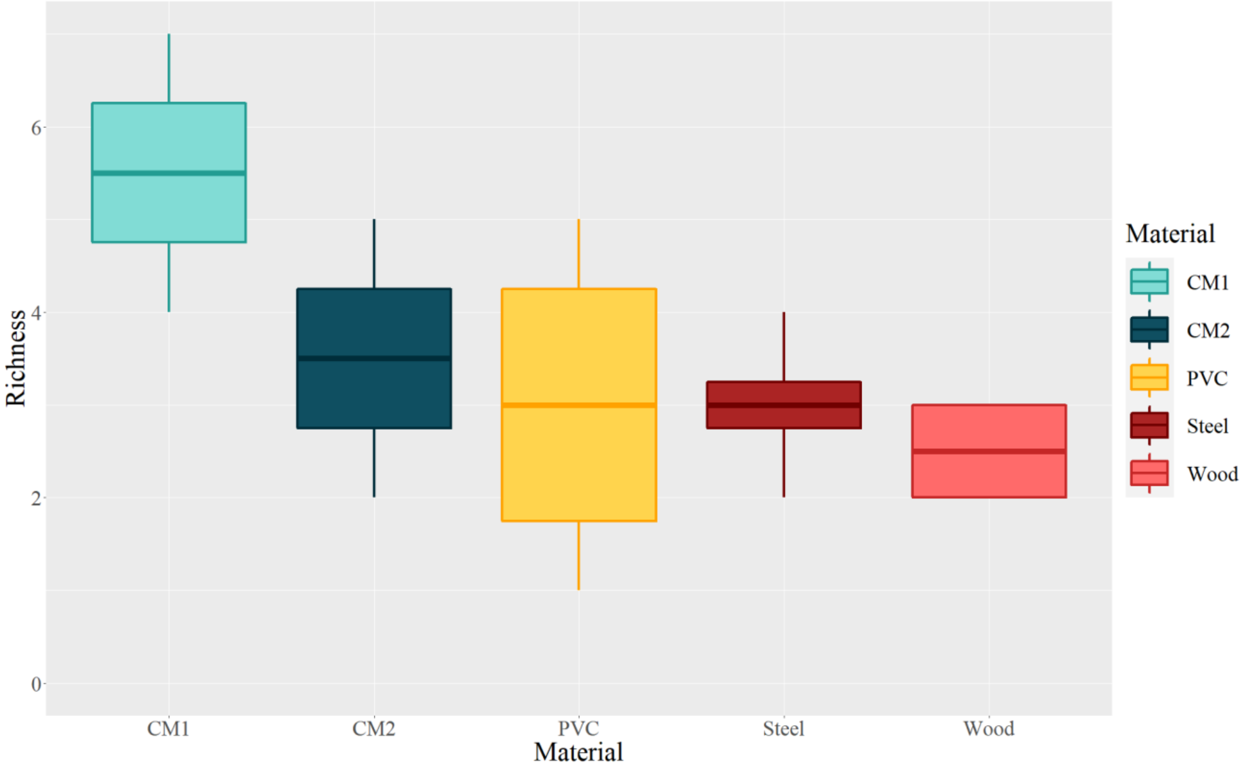

Figure 6: The community on CM1 panels was significantly richer than on wood. From left to right in the figure and top down in the key, the materials are organized: CM1, CM2, PVC, steel, and wood

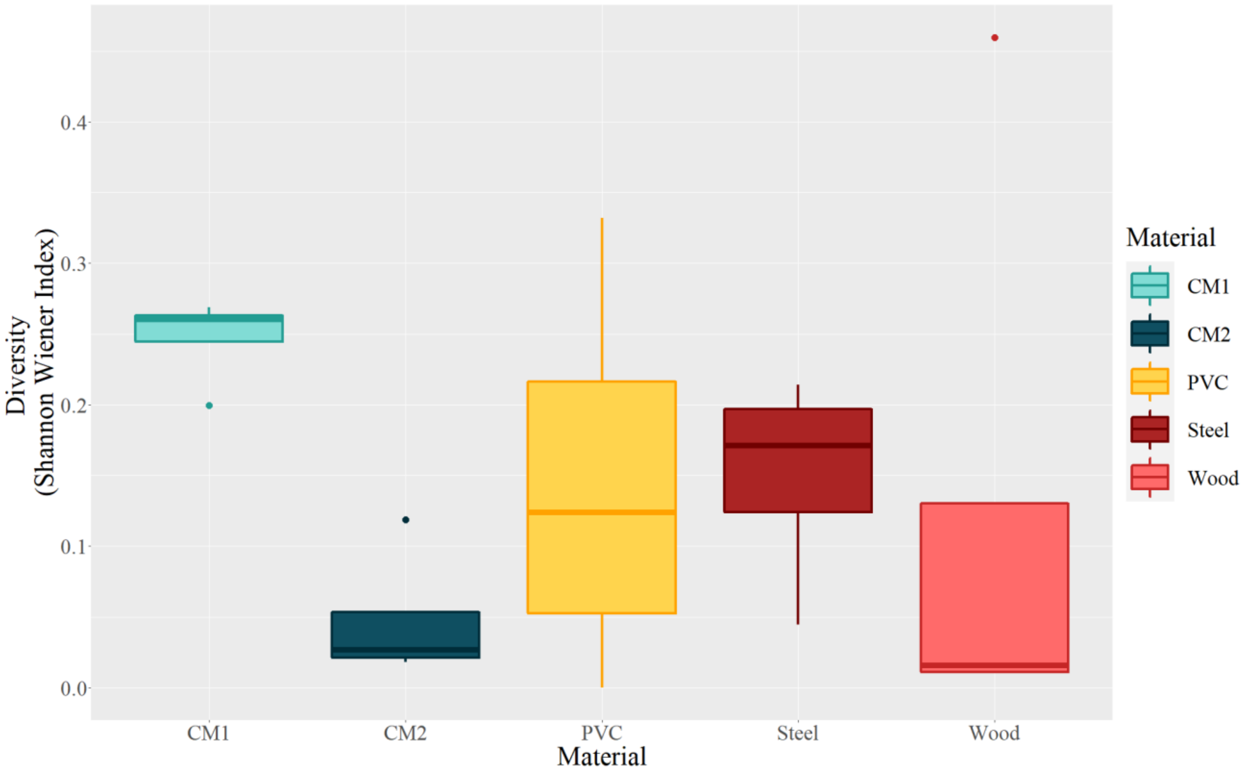

Figure 7: CM1 had a significantly more diverse community than CM2. From left to right in the figure and top down in the key, the materials are organized: CM1, CM2, PVC, steel, and wood.

CM1 panels had the greatest mean and median diversity with the lowest variance (Figure 7). Wood generally had a low diversity, except for one outlier, which had the highest diversity of any panel. The Welch's ANOVA determined that the diversity values were significantly different between materials (F = 9.336; df = 4,6.913; p = 0.00640). The Games-Howell post-hoc test determined that CM1 panels had significantly greater diversity than CM2 (Table A6).

The different substrates formed unique communities (Figure 8). At the end of the observation period, calcified encrusting bryozoans covered the greatest proportion of the panels. The difference in encrusting bryozoan cover was not significant, and it followed similar trends to the live cover analysis. CM1 panels included nine different morpho-taxa, while all other materials included five or fewer. CM1 and CM2 did not have significantly different richness, but the CM2 community was mostly composed of calcified encrusting bryozoans, causing the significantly lower diversity. Finally, barnacles and calcified encrusting bryozoans dominated the community on steel panels.

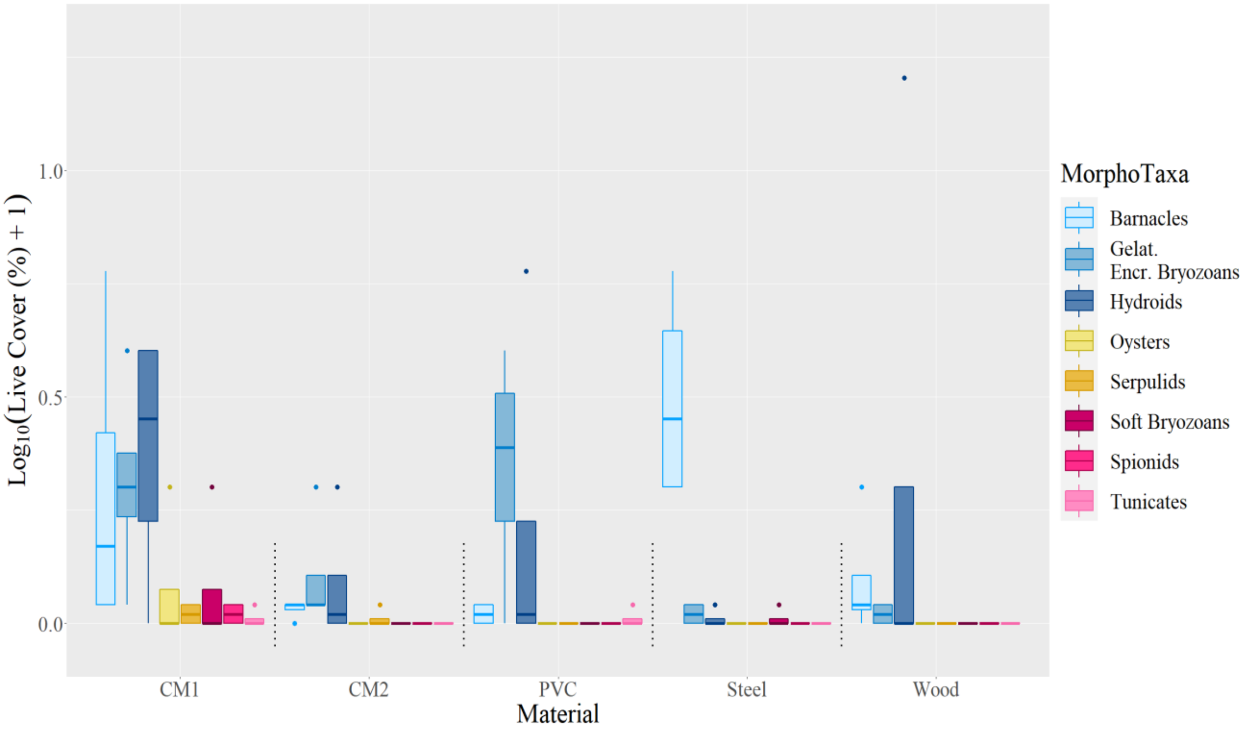

Figure 8: To allow for better visual comparison, calcified encrusting bryozoans were removed from the figure and the live cover was graphed using a log scale. CM1 had the greatest number of morpho-taxa, and steel panels had a significantly greater barnacle cover than PVC panels. From top to bottom in the key the morpho-taxa are organized: Barnacles, Gelatinous encrusting bryozoans, hydroids, oysters, serpulids, soft bryozoans, spionids, and tunicates.

Barnacle cover was significantly different across the materials based on the Kruskal Wallis test (Chi-squared = 10.998; df = 4; p = .0266). Barnacle cover on steel was significantly greater than on PVC but not significantly different from the other materials (Table A7). The mean and median cover of gelatinous encrusting bryozoans was greatest on PVC, but it was not significantly different across the materials. A single wooden panel had the maximum hydroid cover, and CM1 panels had the greatest median hydroid cover. However, the difference in hydroid cover between materials was not significant.

3.3 Interaction between panel size and community measures

All panels fell within a range of 178-200 cm^2 (Figure 9). The panels were significantly different sizes, based on the ANOVA (F = 6.575; df = 4,15; p = 0.0029). Tukey's HSD determined that CM1 panels were significantly larger than PVC, steel, and wood (Table A8). The surface area of the panels was not found to be correlated with the community measures of live cover, richness, and diversity within the materials (Figure 10). However, across all the panels, richness generally increased as panel size increased. This trend may be related to panel size or may be explained by the material.

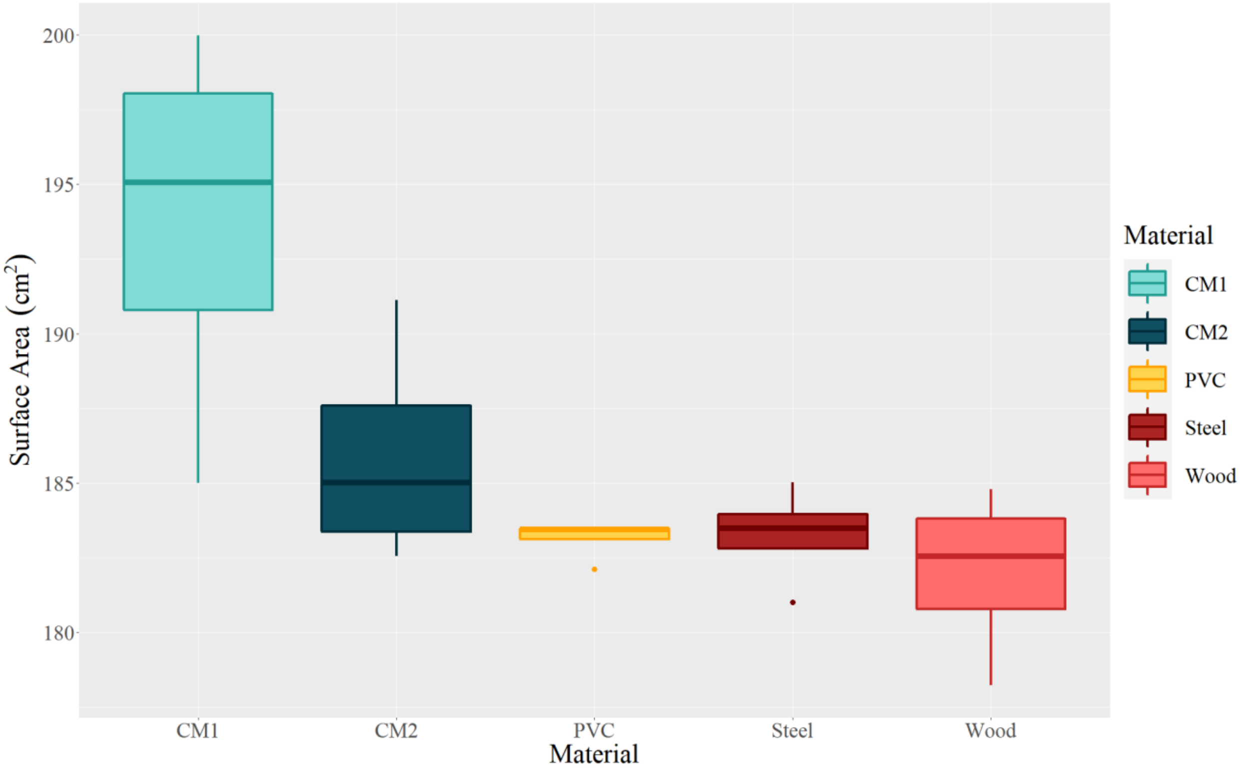

Figure 9: CM1 panels were significantly larger than PVC, steel and wood panels. From left to right in the figure and top down in the key, the materials are organized: CM1, CM2, PVC, steel, and wood.

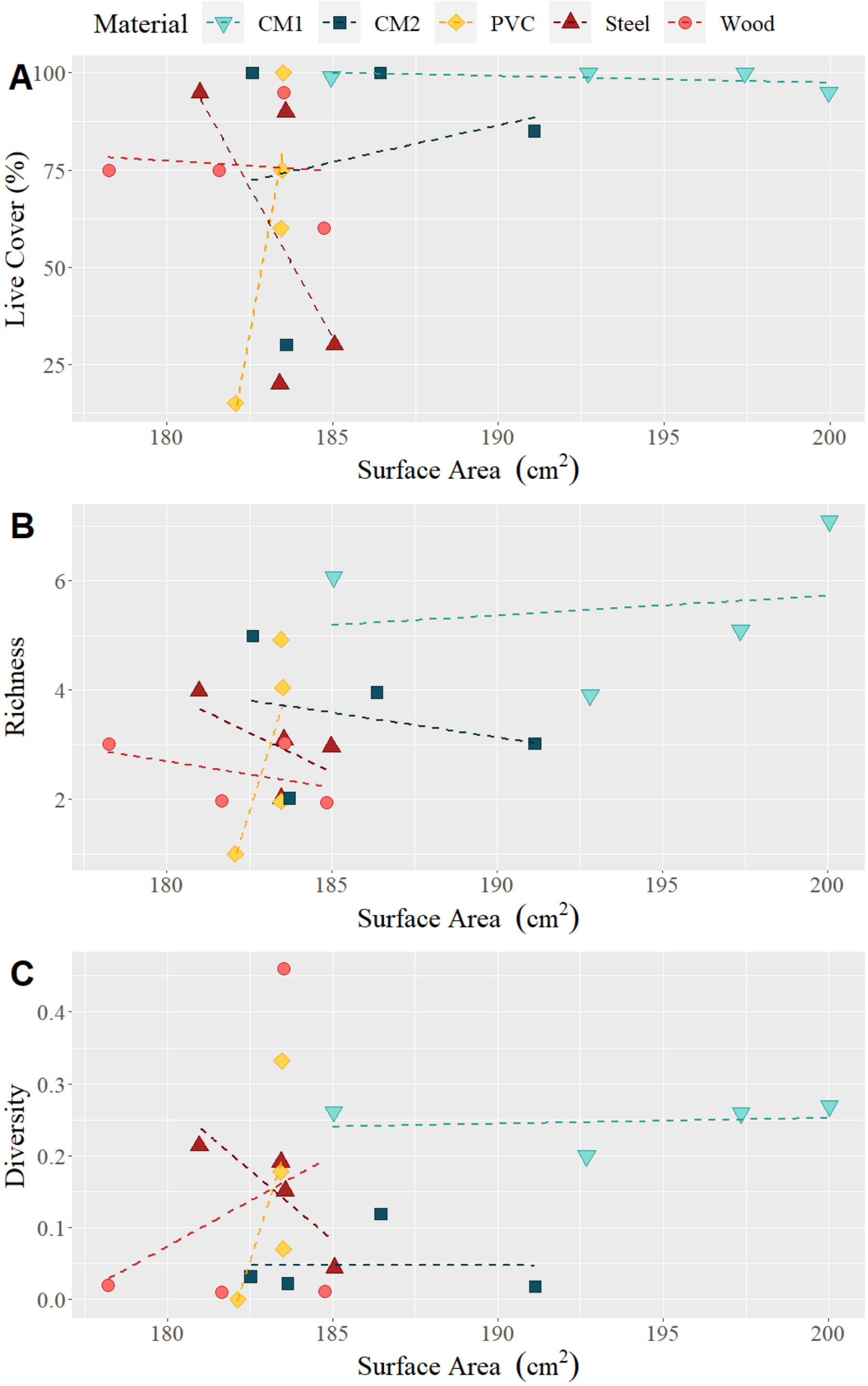

Figure 10: There was no consistent correlation between panel size and live cover, richness or diversity. Live cover, richness and the Shannon-Wiener index of diversity are graphed against panel surface area in graphs A, B, and C respectively. From left to right in the key the materials are organized: CM1, CM2, PVC, steel and wood. In that order the materials are represented by a triangle facing downward, a square, a diamond, a triangle facing upward, and a circle.

4. CONCLUSION

4.1 Effects of Material

The results of this experiment do not support my hypothesis. I hypothesized that wood and cement panels would be colonized more quickly and would have a greater richness and diversity than the other panels. Cement and wood panels were not necessarily associated with faster colonization rates or greater richness and diversity. Furthermore, although their colonization rates and, at times, their general community compositions varied, the final CM2, PVC, steel, and wood communities did not significantly differ in richness or diversity.

Panels constructed from CM1 appear to have higher and more favorable community metrics than the other materials. CM1 panels were rapidly colonized, with CM1 having a significantly greater cover than steel early in the deployment period. Although the live cover was not significantly different at the end of deployment, the CM1 panels had the greatest median and mean live cover along with the lowest variance. The live cover was always $\geq 95\%$ on CM1 panels, while all other materials, excluding wood, had at least one panel with cover $\leq 30\%$. CM1 panels had a unique community with the greatest number of morpho-taxa. They had significantly greater richness than wood panels and a significantly greater diversity than CM2 panels. CM1 shows a high level of consistency in live cover, a high recruitment rate, richness, and diversity.

Previous researchers have not compared the same materials I did, but their results produce a general trend supporting my conclusions. Anderson and Underwood (1994) found that panels constructed from concrete had a significantly greater number of Sydney Rock Oysters than plywood, fiberglass and aluminum, significantly more spirorbids and *Hexaminius sp.* barnacles than fiberglass, and aluminum, and at times, a greater bryozoan cover than the other materials. Glasby (2000) also found that the percent cover of two bryozoan species was greater on concrete panels than on wood, and concluded that wood and concrete panels supported unique and different communities. This trend has also been replicated in real-world applications. Layman et al. (2014) reported that the communities formed on concrete dock pilings have a greater water filtration capacity than those on wood or PVC wrapped piles. My results and past research demonstrate that hard bottom invertebrates appear to settle preferentially on concrete materials.

However, the settling preference of hard-substrate invertebrates for different cement mixes is not fully understood. Some marine-friendly concrete mixes such as ECOncrete® and the green artificial reef concrete proposed by Huang et al. (2016), like CM2, use ground granulated blast furnace slag (GGBFS) as the primary mineral admixture. Marine restoration projects have also taken advantage of fly ash rich cements similar to CM1. Oyster reef restorations at six locations across Galveston Bay use fly ash pellets as a hard substrate, and large fly ash blocks deployed off the Texas coast were observed to form a diverse hard-substrate and mobile community (Ansley et al., 2004; Sage & Gallaway, 2002). Cement mixes similar to CM1 and CM2 have both been used for environmentally friendly marine applications; however, experimental trials show that different cement mixes are not equally good recruitment materials. The Guidelines for Marine Artificial Reef Materials second edition states that the surface of uncured concrete is toxic to marine organisms due to its high pH. For example, experiments by Perkol-Finkel & Sella (2014) found that all of the alumina-rich concretes tested and only a third of the slag-based concretes tested performed significantly better than Portland cement in at least one recruitment measure such as inorganic biomass, and in situ or in vitro coral settlement. It is believed that the addition of pozzolanic admixtures may neutralize concrete and make it more habitable; however, there is evidence to refute this (Hsiung et al., 2020; Ansley et al., 2004). Research by McManus et al. (2018) found that a concrete mix using only Portland cement had a significantly greater richness than a cement mix containing 24% GGBFS. A cement mix containing 24% pulverized fly ash, on the other hand, was not significantly different in richness from either mix (McManus et al., 2018). It is difficult to draw conclusions due to differences in experimental design. However, it is important to note that in both of the experiments described here as well as my own, another mix at least marginally outperformed one containing GGBFS. This suggests that cement mixes with GGBFS as the only admixture may not be the best material to promote the colonization of marine organisms.

4.2 Applications

The results of this experiment would suggest that CM1 and PVC are suitable materials for the construction of recruitment panels. Despite being used in previous recruitment studies, wooden panels did not appear to be a viable option, especially for a recruitment experiment shorter than three months. Wood’s low richness, generally low diversity, and slower recruitment rate are all major contraindications. CM2 had a low diversity but a high richness and rapid recruitment rate, which, for the added difficulty of fabrication, is not enough of a benefit. Hard-substrate organisms colonized PVC panels quickly, and their richness and diversity was not significantly different from CM1. Furthermore, PVC may be easier to fabricate, and as shown previously in table 1.2, it is a standard recruitment material, allowing for comparison against past research. PVC may have two minor drawbacks, though. PVC panels had high variability in nearly every community measure, and they had significantly lower barnacle cover than steel. This indicates that they may underrepresent barnacle abundance at times. CM1 panels may not be as easy to produce as PVC, but the high consistency in live cover, rapid recruitment rate, high richness, and high diversity means that CM1 may be a valuable option for future recruitment experiments.

Cement containing CFFA and SF admixtures such as CM1 may be a better marine engineering material for Galveston Bay. The basic composition of CM1 conforms to the construction specifications of Galveston city. This means that future marine engineering projects in Galveston and possibly Texas as a whole could implement it more easily. When possible and appropriate, it may be advisable to prioritize the use of concretes based on CM1 over materials like steel, PVC, and wood. Concrete also benefits from the fact that it can be used in unique ways, which extends its function and amplifies its environmental benefits. Most modern concrete structures have a simple smooth surface, but structures like seawalls can be cast with unique textures, recessed pockets, or shelves, which increase environmental complexity (Strain et al., 2020; Ushiama et al., 2019; Perkol-Finkel et al., 2018). These modifications can increase the live cover, diversity, and richness of the hard-substrate community and benefit from being attractive environments for local fish (Strain et al., 2020; Ushiama et al., 2019; Perkol-Finkel et al., 2018). These advantages can be spread to other marine structures as well. Wood pilings can be encased in textured concrete instead of materials like fiberglass or PVC, and standard, smooth-faced breakwaters can be made with complex textured and pocketed surfaces (Perkol-Finkel & Sella, 2015; Sella & Perkol-Finkel, 2015).

4.3 Limitations

It is important to note that these results may be seasonally and locally restricted. The materials were only observed in Galveston Bay for three months during winter. The presence and abundance of different larvae in the water column vary seasonally and regionally, so the differences in the community observed here may differ as well.

Each sampling method also had limitations. The repeated surface cover measures relied on the use of photo analysis, meaning photo quality limited the accuracy of the total cover assessment. The development of a layer of detritus and the presence of organisms with transparent bodies such as hydroids or soft bryozoans also made it difficult to measure total cover. Conspicuous organisms with high contrast such as encrusting, calcified bryozoans were represented well, while poorly contrasted organisms such as hydroids may not have been. I also calculated richness and diversity using morpho-taxa groups instead of species. This method likely underestimated richness and diversity and may have made the materials appear to have a more or less similar richness and diversity than they would at the species level. The panels also had slightly different surface areas. This had the potential to affect cover, richness, and diversity. Only richness showed any overall positive correlation with surface area. While the panels were significantly different sizes, it is unlikely that the relatively small variations in size were ecologically significant.

4.4 Future considerations

This experiment can be built upon in multiple ways. The conclusions could be strengthened by performing this study with more replications and by deploying the panels during different seasons. This would sample a greater variety of organisms and determine if the trends identified here are seasonal. Future experimentation may benefit from comparing the hard-substrate communities on different cement mixes. It is easy to observe from the resources previously cited that the selective recruitment of hard-substrate organisms onto different concrete mixes has a great deal of potential. Additionally, the root causes of these variations are not well understood. There are multitudes of other mineral admixtures that may be used. The proportions and combinations of different admixtures can be modified, and an ideal mix may be identified for different regions and purposes.

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APPENDIX

Table A1: Results of the Repeated measures ANOVA for weeks 2-12

Effect	DFn	DFd	SSn	SSd	F	p	ges
Intercept	1	15	201432.46	33575.81	89.990	9.955e-08*	0.797
Material	4	15	28829.50	33575.81	3.220	4.274e-02*	0.360
Weeks	5	75	93315.77	17868.70	78.335	4.162e-11*	0.645
Material: Weeks	20	75	14247.32	17868.70	2.990	2.0143e-02*	0.2171

Note: The effect "Weeks" is representative of the number of weeks since deployment. The effects "Weeks" and "Material:Weeks" did not pass the assumption of sphericity, so the Greenhouse-Geisser correction was used.

* $p < 0.05$

Table A2: Results of Tukey's HSD test for week 4

Material	diff	lwr	upr	p. adj
CM2-CM1	0.261	-0.523	1.046	0.838
PVC-CM1	0.141	-0.644	0.925	0.980
Steel-CM1	-0.904	-1.689	-0.120	0.0204*
Wood-CM1	-0.483	-1.268	0.301	0.357
PVC-CM2	-0.121	-0.905	0.664	0.988
Steel-CM2	-1.166	-1.950	-0.381	0.00279*
Wood-CM2	-0.745	-1.529	0.0398	0.0668
Steel-PVC	-1.045	-1.829	-0.260	0.00700*
Wood-PVC	-0.624	-1.408	0.161	0.154
Wood-Steel	0.421	-0.364	1.205	0.487

Note: CM1, CM2 and PVC were found to have a significantly greater TC than steel.

* $p < 0.05$

Table A3: Results of Tukey's HSD test for week 6

Material	diff	Lwr	upr	p. adj
CM2-CM1	0.139	-0.595	0.873	0.975
PVC-CM1	0.0487	-0.685	0.782	0.100
Steel-CM1	-1.388	-2.122	-0.655	0.000269*
Wood-CM1	-0.576	-1.310	0.157	0.162
PVC-CM2	-0.0903	-0.824	0.643	0.995
Steel-CM2	-1.527	-2.261	-0.794	0.0000959*
Wood-CM2	-0.715	-1.449	0.0184	0.0577
Steel-PVC	-1.437	-2.170	-0.703	0.000186*
Wood-PVC	-0.625	-1.358	0.109	0.114
Wood-Steel	0.812	0.0784	1.546	0.0268

Note: Due to moderate deviations from normality, alpha significance was reduced to .025. CM1, CM2 and PVC were found to have a significantly greater TC than steel.

* $p < 0.025$

Table A4: Results of Tukey's HSD test for week 8

Material	diff	Lwr	upr	p. adj
CM2-CM1	-14.325	-76.851	48.200	0.952
PVC-CM1	-16.792	-79.317	45.734	0.917
Steel-CM1	-76.749	-139.274	-14.223	0.0131*
Wood-CM1	-56.854	-119.379	5.672	0.0835
PVC-CM2	-2.466	-64.992	60.0594	0.100
Steel-CM2	-62.423	-124.949	0.102	0.0504
Wood-CM2	-42.528	-105.0538	19.997	0.270
Steel-PVC	-59.957	-122.483	2.568	0.0632
Wood-PVC	-40.0621	-102.588	22.463	0.322
Wood-Steel	19.895	-42.630	82.421	0.859

Note: Due to moderate deviations from normality, alpha significance was reduced to .025. CM1 had a significantly greater TC than steel.

* $p < 0.025$

Table A5: Results of Tukey's HSD test, comparing richness

Material	diff	lwr	upr	p. adj
CM2-CM1	-2.000	-4.704	0.704	0.203
PVC-CM1	-2.500	-5.204	0.204	0.0767
Steel-CM1	-2.500	-5.204	0.204	0.0767
Wood-CM1	-3.000	-5.704	-0.296	0.0264*
PVC-CM2	-0.500	-3.204	2.204	0.977
Steel-CM2	-0.500	-3.204	2.204	0.977
Wood-CM2	-1.000	-3.704	1.704	0.782
Steel-PVC	-4.442E-16	-2.704	2.704	1.000
Wood-PVC	-0.500	-3.204	2.204	0.977
Wood-Steel	-0.500	-3.204	2.204	0.977

Note: CM1 had significantly greater richness than wood panels.

* $p < 0.05$

Table A6: Results of the Games-Howell test, comparing diversity

Material	estimate	Conf. low	Conf. high	p. adj
CM2-CM1	-0.0760	-0.122	-0.0304	0.006*
PVC-CM1	-0.0397	-0.178	0.0985	0.647
Steel-CM1	-0.0359	-0.106	0.0346	0.308
Wood-CM1	-0.0503	-0.256	0.156	0.730
PVC-CM2	0.0363	-0.0956	0.168	0.724
Steel-CM2	0.0401	-0.0287	0.109	0.277
Wood-CM2	0.0256	-0.175	0.226	0.961
Steel-PVC	0.00382	-0.124	0.131	1.000
Wood-PVC	-0.0106	-0.198	0.177	0.999
Wood-Steel	-0.0145	-0.207	0.178	0.996

Note: Due to moderate deviations from normality, alpha significance was reduced to .025. CM1 had a significantly greater richness than CM2.

* $p < 0.025$

Table A7: Results of the Dunn test, comparing barnacle cover

Material	Z	P.unadj	P.adj
CM1 - CM2	1.4853327	0.137	1.000
CM1 - PVC	1.8961694	0.0579	0.579
CM2 - PVC	0.4108367	0.681	1.000
CM1 - Steel	-0.9796875	0.327	1.000
CM2 - Steel	-2.4650202	0.0137	0.137
PVC - Steel	-2.8758569	0.00403	0.0403*
CM1 - Wood	1.0744960	0.283	1.000
CM2 - Wood	-0.4108367	0.681	1.000
PVC - Wood	-0.8216734	0.411	1.000
Steel - Wood	2.0541835	0.0400	0.400

Note: Steel had significantly greater barnacle cover than PVC panels. The p values were corrected using the Bonferroni method.

* $p < 0.05$

Table A8: Results of Tukey's HSD test, comparing panel size

Material	diff	lwr	upr	p. adj
CM2-CM1	-0.0178	-0.0365	0.000830	0.0645
PVC-CM1	-0.0243	-0.0430	-0.00569	0.00822*
Steel-CM1	-0.0241	-0.0427	-0.00541	0.00899*
Wood-CM1	-0.0270	-0.0456	-0.00834	0.00351*
PVC-CM2	-0.00652	-0.0252	0.0121	0.814
Steel-CM2	-0.00624	-0.0249	0.0124	0.836
Wood-CM2	-0.00917	-0.0278	0.00948	0.567
Steel-PVC	0.000278	-0.0184	0.0189	1.000
Wood-PVC	-0.00265	-0.0213	0.0160	0.991
Wood-Steel	-0.00293	-0.0216	0.0157	0.988

Note: CM1 panels had significantly more surface area than PVC, steel and wood panels.

* $p < 0.05$