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A Temporal Analysis of the Microbiota and Biofouling Development on Artificial Substrates in the Port Everglades Inlet, Florida

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HALMOS COLLEGE OF ARTS AND SCIENCES

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By

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Abstract

A pilot project was deployed in Port Everglades Inlet, Florida that aimed to evaluate the biofilm composing the microbiome on ecologically engineered artificial substrates used to build Coastal Marine Infrastructure. In April of 2017, an Articulated Concrete Block Mattress comprised of an ecological engineered concrete substrate and a standard smooth surface control substrates were compared. This study will provide a profile on the microbiome community on artificial substrates within Port Everglades Inlet on bio-enhancing concrete-based solutions in our Coastal Marine Infrastructure. To study the microbial community, the 16s rRNA technology was used in Illumina's high-throughput DNA sequencing. Samples were collected once a month from December 2017 to November 2018. Total read count of 7.8 million were produced which yielded 10,251 Amplicon Sequence Variants. Results indicated a homogenous composition over most of the study site for both alpha and beta diversity. Differences in beta diversity were seen when comparing the different types of surface area. There were moderate and significant differences from the analysis of similarity ($R = 0.133$, $p = 0.001$) for all surface areas. Species diversity varied by season but only slightly. The environmental metadata that had an impact on the microbial community was temperature, conductivity, and pH. Increased microbial abundance was seen in the late summer months, which is likely to be expected with the increased precipitation and temperature at that time of year. This study will help characterize the microbial communities composing the biofilms and can also be used as baseline for the surrounding coastal marine environment.

Keywords: Microbiome, 16S rRNA, Articulated Concrete Block Mattresses, Port Everglades Inlet, Illumina, ECOncrete ®

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In Memoriam

This research is dedicated in loving memory to Dr. Shimrit Perkol-Finkel, who was a pioneer in the advancement of marine science for women and for future generations. May she rest in peace.

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Introduction

Coastal Habitats

The 825 miles of sandy coastline bordering the Atlantic Ocean, the Straits of Florida and the Gulf of Mexico are one of Florida's most valuable and cherished natural resources. Florida's beaches and coastal zones serve several important functions and are characterized by well-defined boundaries that include freshwater, brackish-water, and saltwater. Mangroves, estuaries, or manmade inlets represent transition zones and are vital to maintaining the health of Florida's coastal environments and economy. Port Everglades Inlet (PEI), located in Fort Lauderdale, Florida is a man-made, deep-water, dredged port located along the southeastern coast of Florida. (NOS, 2011). Directly east of PEI is the Florida Reef Tract: the only living coral barrier reef in the continental United States. (NOAA, 2018; Stanley et al, 2017) as well as several fishing piers, recreational beaches, and watersport areas (Stamates et al., 2013).

Port Everglades Inlet and Florida's Ports

Port Everglades Inlet is a man-made deep dredge seaport that was established in 1927 (NOS, 2011; Stauble, 1993). PEI is located on the east coast of Florida situated in three municipalities: Fort Lauderdale, Dania Beach, and Hollywood (NOS, 2011). This highly engineered port is 641 meters in length by 295 meters wide with a depth of 13 meters (Stauble, 1993). The mean tidal wave is 0.49 meters in height and a mean tidal range of 0.79 meters. The prominent winds travel from the southeast to east and can travel at speeds greater than 17 knots and average 7 knots (NOS, 2011). Weather conditions vary with average high temperatures at 32o C to low temperatures of 16o C with the mean of 25o C (NOS, 2011). The annual precipitation is 14.51 inches per year, falling within roughly 94 days, of which 60% of that occurs in the summer months of June through November (NOS, 2011). Port Everglades generates nearly 30 billion dollars of revenue through a various combination of cargo ships, cruise lines, petroleum and other revenue producing enterprises (NOS, 2011). Total economic activity for 2017 as measured in revenue was $30,410,780 dollars with 230,747 jobs maintained making PEI one of the most active ports in the United States (NOS, 2011).

Although the economic impacts are massive, the increased maritime ship traffic has a large risk association to sensitive marine habitats (Walker et al., 2012). Located directly offshore from the PEI is a major US coral reef tract (Staley et al., 2017). The growth and port development can

have detrimental effects on coral reef systems that take thousands of years to form (Walker et al., 2012). The development includes dredging and blasting of the adjacent coral reef habitat to enable vessel access. Also included is burial of debris, to place spoils or build infrastructure that can have irreparable damage to the marine community. According to Walker and colleagues (2012), the impacts to coral reef habitats in Southeast Florida (SEF) are extensive, with 83.3% stemming from the creation of the three major Ports: Port of Miami, Port Everglades and Port of Palm Beach in the late 1920's. The habitat impact includes 260.3 hectares of habitat encompassing some 6.8 M coral reefs greater than 2cm with 9.7 hectares of live coverage (Walker et al., 2012). Total adjusted impact area for PEI was estimated to be 32.1%: buried (23.1%), dredged (7.6%), and groundings (1.4%), respectively. Currently, all three ports are planning expansions to provide accommodations for the next generation of supertankers. Specifically, PEI has a master plan to dredge an additional 8 hectares of coral reef habitat (Walker et al., 2008).

Port Everglades Inlet Navigational Improvements Project received federal authorization in December of 2016 for the U.S. Army Corps of Engineers to move forward with the deepening and widening of the Ports channels as part of the Water Infrastructure Improvements for the Nation (WIIN) Act. (https://www.usace.army). Over the past eight decades, 22 improvement projects to PEI have been classified as jetty realignments, jetty rehabilitations, and port channel dredging. Approximately 6,525,300 cubic yards were removed from the immediate area for these projects and deposited into locations that included: offshore, upland, ocean/beach, north of channel, unknown and beach, respectively. (Florida Department of Environmental Protection, 2018). The recent impacts have been monitored through feasibility studies, engineering evaluations and cost analysis for PEI by Olsen Associates in 2003, 2004, 2007 and 2014 (Florida Department of Environmental Protection, 2018). These studies have led to the adoption of implementation strategies for PEI Management Plan that minimizes impact to the environment and are subject to further evaluations.

Studies from the impacts directly related to the dredging project in the Port of Miami, Florida and the adjacent Florida reef ecosystems have been difficult to quantify (Cunning et al., 2019; Miller et al., 2016). However, using a spatially statistical approach, obtained through multiple independent datasets, Cunning and colleagues validated direct quantitative links between dredging related sediment plumes, not regional disturbances (bleaching or disease) to be the observed impacts on the reef ecosystem (Cunning et al., 2016). These dredging activities occur

along a 25 km segment, (10 km south of the port channel and 15 km north) resulted in a 10-100-fold increase in sediment cover in the Florida Reef Tract. Additionally, it is estimated that over one million corals were lost (Cunning et al., 2016). The severity of the impacts far exceeded pre-dredging predictions and should be used for mitigation, monitoring, and adaptive management to avoid comparable impacts to future dredging projects (Cunning et al., 2019; Miller et al., 2016).

Port Expansions and Dredging

Port deepening and widening developments continue across the globe to accommodate Neo-Panamax ships that were added to the fleet after the expansion of the Panama Canal in 2016 (Ashe, 2018). The shallow water ports along the eastern seaboard in the United States have dredging projects that have been completed with several additional proposed for completion in the future (Cunning et al., 2019). These ports are located adjacent to coral reef ecosystems. Based on the fragility of the coral reefs systems and the extensive decline, environmental impacts and best practices need to be monitored in lieu of the all the proposed dredging (Cunning et al., 2019).

Nearly 60% of the human population is concentrated in coastal areas worldwide, residing less than 100 km from the shoreline (Vitousek et al., 1997). This population growth includes infrastructure that has led to port development that facilitates large maritime vessels for a global freight transport system and increases in the cruise ship industry (Veronneau et al., 2011). Since 1900, maritime shipping has increased from 30,000 total vessels to 90,000 total vessels and the trend is expected to continue (Corbett et al., 2009). There is a constant influence in PEI from cruise ships, cargo ships, naval ships, and recreational boats (Banks et al., 2008). This constant volume of traffic along with the large amount of water that discharges twice daily with the tides from PEI has been considered a point source of pollution to the offshore marine environments in Florida. The discharge from the port development can have detrimental effects to related benthic communities. (NOS, 2011; Stauble,1993; Walker et al., 2012).

Thus, along with shorelines being compromised, natural habitats are compromised as well. One possible solution to mitigate the impacts of port development is to utilize biologically enhancing concrete-based solutions in our coastal marine infrastructure (CMI). CMI enhancement would include tide pool armors, seawalls, armor blocks, bio-active walls, and bio- enhanced eco-mats that have a Nature-Inclusive Designs (NID; Perkol-Finkel & Sella, 2017; Sella et al.,2021). The objective is to design structures that represent the complexity of natural habitats (Riera et al.,

2018). The CMI would then facilitate an eco-friendlier habitat integrated by the design and construction of the product (Perkol-Finkel et al., 2017; Perkol-Finkle & Sella, 2015; Sella et al.,2021). The components of the enhancement of the articulated concrete block mattresses (ACBM's) have been developed by ECOncrete® Tech Ltd., an international company based out of Israel. ECOncrete's innovative bio-enhancing concrete additives and science-based designs have been scientifically tested to add value both biological and ecological to the CMI.

(Perkol-Finkel et al., 2017; Perkol-Finkle & Sella, 2015; Sella et al., 2021). Despite the increase in hardened coastlines and infrastructure, our understanding of microbiome community on CMI is limited (Connell & Glasby, 1999; Dugan et al., 2011). Microbial biofilm communities are the first colonizers on the new infrastructure and allow for further succession of the community. These biofilms inhabit manmade surfaces naturally. Some knowledge exists about the functions and the specific roles that these assemblages play in the marine ecosystems, but more is needed (Connell & Glasby, 1999) The few studies that have assessed marine growth on CMI found assemblages that differ significantly from those of adjacent natural habitats (Lam et al., 2009). These assemblages are less diverse and frequently dominated by invasive species (Glasby et al., 2007). Recently, another approach has emerged that incorporates ecological engineering to enhance the CMI infrastructure both ecologically and biologically (Bergen et al., 2001). These improvements include design and texture characteristics, that invite more abundant and diverse species assemblages (Goff, 2010; Wiecek, 2009). Results have been correlated to biogenic buildup, which is a natural process that recruits engineering species like barnacles, oysters, serpulid worms and corals that deposit calcium carbonate ($CaCO_3$) skeletons onto the enhanced structures that produces a beneficial and natural habitat to various organisms. These structural enhancements and ecosystem benefits for coastal infrastructure are demonstrated using bio-enhanced products and recognized as an advantage (Perkol-Finkel & Sella, 2014; Sella & Perkol-Finkel, 2015; Sella et al., 2021).

Microbiomes

Microbiomes and biofilms on human-built structures can still be influenced by natural phenomena, including pervasive microbes which live in communities. The microbes living in these intricate communities (also known as 'microbiomes') display a wide range and variation of composition from ecosystem to ecosystem (Pall, 2013; Pekarova et al., 2009). The variety and

composition of the bacteria in the microbiomes largely reflects the health of the ecosystem and is essential in providing a thorough representation and understanding of these environments (Stanley et al., 2014).

These microbiomes composing the biofilms typically start with the bonding of bacterial cells that modify the physicochemical properties making it easier for colonizers like cyanobacteria, algae, and protist. (DeCarvalho, 2017). The incorporation of macromolecules to the surface area starts within minutes of substrate immersion, the bacterial colonization starts within hours, and then unicellular eukaryotes like protozoa, diatoms and yeast appear on the substrates within a week (Wahl, 1989). Bacteria have been found to be the most significant microbe on marine infrastructure that establish the structure and function of the mature biofilm. (Dang & Lovell, 2016). These biofilms inhabit man-made surfaces effortlessly. Along with microorganisms, bacteria are accountable for microfouling that enables the larger and more abundant organisms such as mussels, barnacles, and algae (DeCarvalho, 2017). This crucial process impacts the resilience of the new colonies and recoveries presented by harsh marine environments.

Previous studies in our microbiology and genetics laboratory have been conducted for multiple marine environments, organisms, and research. The microbiology and genetics laboratory at Nova Southeastern University Halmos College of Arts and Sciences (formerly HC Natural Science and Oceanography NSU HCNSO) has used high throughput DNA sequencing of 16S rRNA markers to provide knowledge of PEI microbiomes along with a comprehensive view of the microbial ecology (Campbell et al., 2015; Lopez et al., 2021; O'Connell et al., 2018). This has been achieved by innovations and tools that were able to depict microbiomes through the 16S gene.

The 16S rRNA gene marker to characterize microbiomes

Carl Woese and colleagues pioneered the use of the ubiquitous 16S small subunit ribosomal (SSU rRNA) gene in the late 1980's as a taxonomic tool for bacterial systematics (Woese, 1987). Ribosomal RNA is found in every living cell that requires protein translation, and thus qualifies as a universal molecule. The gene's utility has since revolutionized the field and now forms the foundation for the current three Domain classifications of life into Bacteria, Archaea and Eukaryote. The molecular approach also circumvents the need to culture bacteria for identifications (Easson & Lopez, 2018). To categorize the number and types of species in a given habitat, an operational taxonomic unit (OTU) or more recently Amplicon Sequence Variants of

16S rRNA can be established, after using universal primers to amplify and sequence hypervariable 16S rRNA regions. This approach has been adopted in this study, with details described in the Methods.

This study aims characterize the microbiome in Port Everglades Inlet (PEI) on CMI . The measurement of marine microbes, a dominant organism in the world's shorelines contribute to 98% of the biomass on CMI and within our water column (Thompson et al, 2017). This study will provide a more comprehensive insight to the microbiome community in Port Everglades Inlet on artificial substrates.

Objectives and Hypothesis

In this study, the microbial communities that exist in PEI on artificial substrates will be analyzed using high-throughput DNA sequencing on Illumina's MiSeq platform. The 16S amplicon library analysis will be used to obtain a comprehensive study. The purpose of this research is to determine the microbial population on the ACBM's and on various artificial substrates. These measurements will be recorded based on various manmade surfaces, season (wet and dry), and water chemistry for samples taken once per month for one full year. To validate these objectives, the following hypotheses were formulated:

1. There will be an increase in alpha and beta diversity of the microbial communities composing the biofilms from the treatment concrete substrate compared to the control concrete substrate.
2. There will be an increase in alpha and beta diversity when comparing the microbial communities on the different substrate's surfaces.
3. There will be an increase in beta diversity of the microbial communities composing the biofilms on all substrate surfaces during the wet and dry season.
4. Water chemistry (phosphate and nitrate) will have a positive correlation to changes in the relative abundances of the microbial community composing the biofilm on all surfaces.

ESRI Image, 2015

Figure 1. Port Everglades Inlet in Fort Lauderdale, Florida.

Methods

Sample Locations

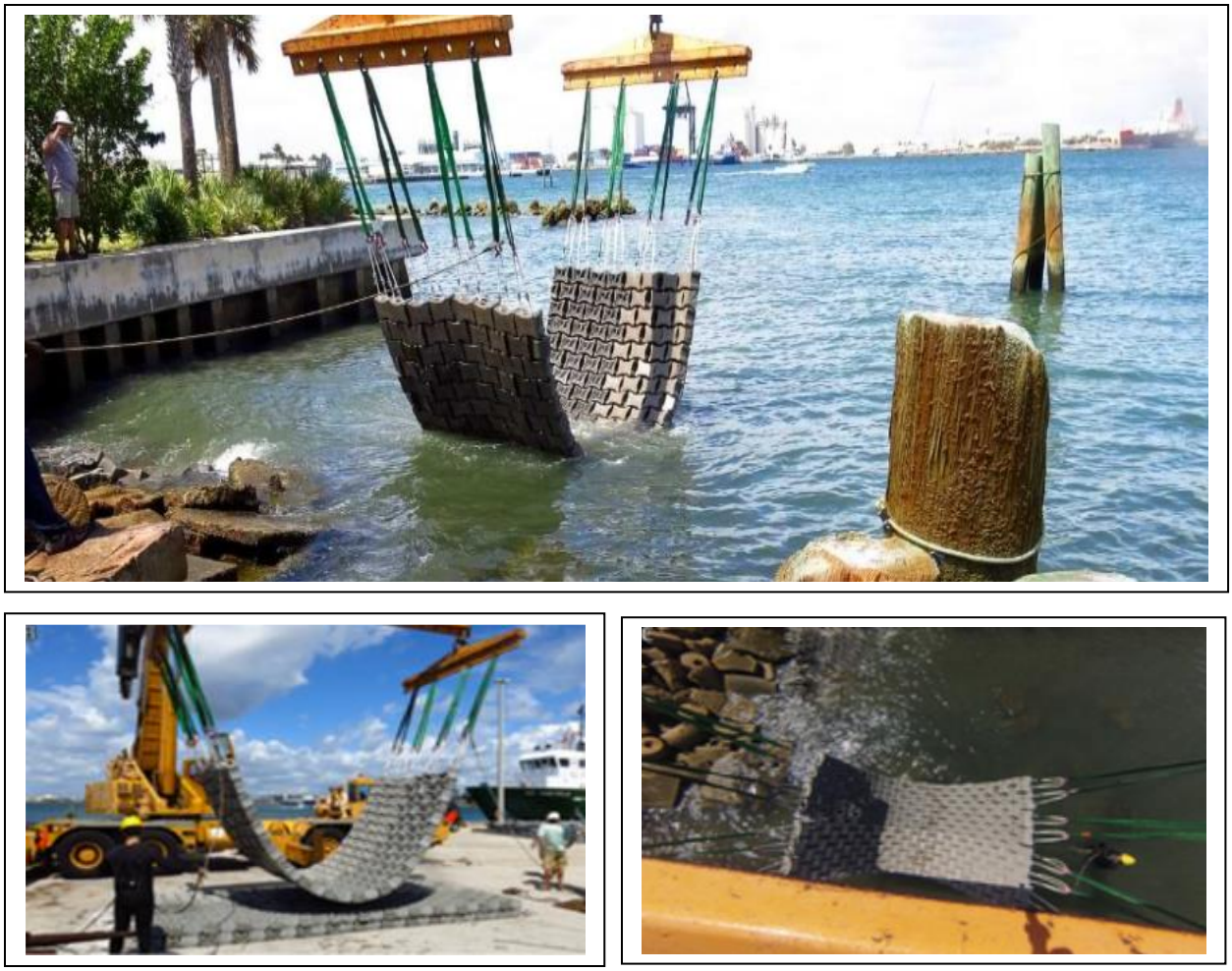
Table 1. All sites and their field locations sampled in this study. Each site has a replication of 12 representing each month sampled over a one-year period for a total of N=92.

Sample Number	Location Name	Latitude	Longitude	Extra Analysis
TPE1	ECOncrete block-inside	26.091669	-80.111588	Water chemistry
TPE2	ECOncrete block-surface	26.091669	-80.111588	Water chemistry
TPC1	Control block	26.091669	-80.111588	Water chemistry
TPSW	Manmade concrete seawall	26.091596	-80.111739	
TPRW	Manmade rip rap rubble structure	26.091447	-80.111937	
TPWP	Manmade three-set nautical wooden piling	26.091693	-80.111782	
TPEP1	Water sample mattress	26.091669	-80.111588	Water chemistry
TPEP2	Water sample rubble structure	26.091447	-80.111937	
TPEP3	Water sample wooden piling	26.091693	-80.111782	

Google Image,2017

Figure 2. Aerial view of pilot location in Port Everglades Inlet.

Aerial view of the pilot sample site at Port Everglades Inlet: Latitude 26.0937, Longitude -80.1247. The red arrow denotes the location of the Articulated Concrete Block Mattress installation. Three additional artificial structures are located within 500 meters of the pilot site location; a vertical concrete seawall, a three-set nautical wooden piling stump, and a manmade rock wall structure that served as a baseline for the study.



Sella et al., 2021

Figure 3. April 2017 deployment of Articulated Concrete Block Mattress into Port Everglades Inlet.

Articulated Concrete Block Mattress (ACBM) Deployment

The four ACBM's were deployed in April of 2017 in PEI. The location consisted of the shoreline between Nova Southeastern University's Halmos College of Arts and Sciences and the South Florida Ocean Measurement Facility, (SFOMF), Naval Surface Warfare Center, Carderock Division (Figure 2). Each ACBM is comprised of 203 units (30x24x15cm) and 26 half units (15x24x15cm). Respectively, the ACBM's consist of one half of the ECOncrete® blocks (treatment), and the other half of the standard block (control). The textured blocks are made of a concrete composition admixture (ECOncrete®) that is patented (Pub. No.: US 2015/0366170 A1). The physical properties include surface roughness and a macro three-dimensional design. The

control block units are made of Portland cement that has standard marine surface chemistry and design. These blocks are attached with stainless steel cables and polypropylene rope. The ACBM's were installed by crane (Figure 3) and were placed on the shoreline at the Mean Higher High Water (MHHW) line to ensure that the ACBM's were exposed to both intertidal and subtidal environments (Figure 5).

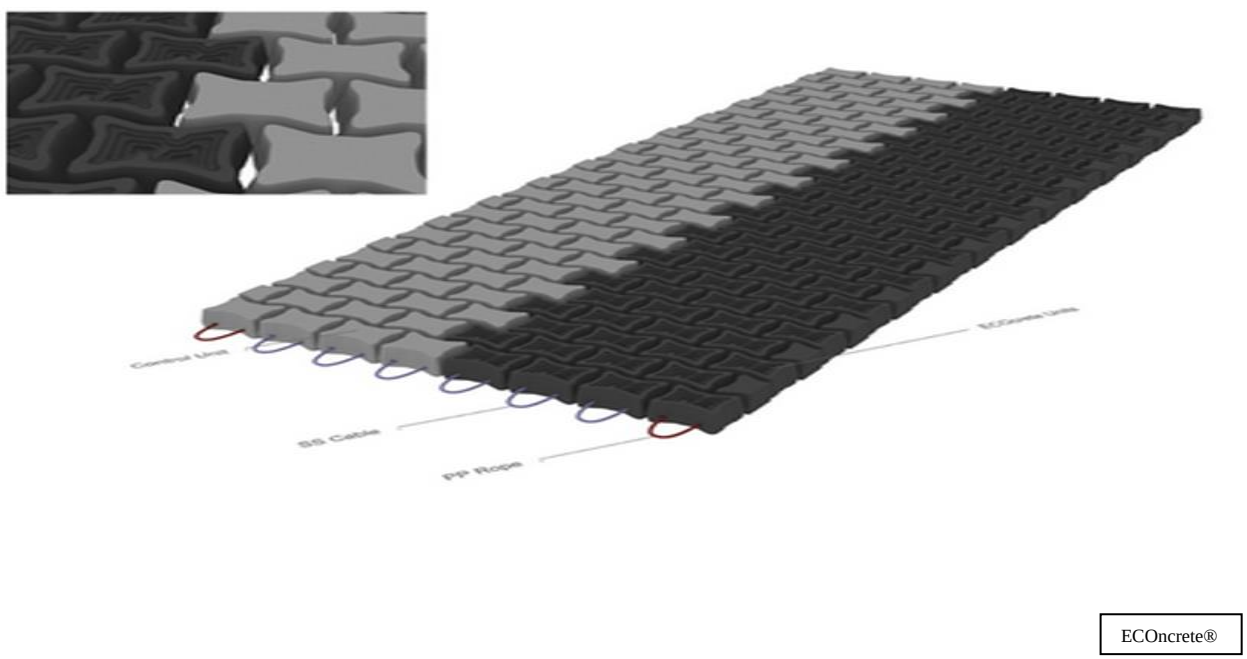


Figure 4. Illustration of ECOncrete® Articulated Concrete Block Mattress Prototype

Each individual mattress is comprised of 203 units (30x24x15cm), and 26 half units (15x24x15cm), with a total weight of ~4,000 Kg. Half of each ACBM consist of the textured ECOncrete® blocks (treatment) and the other half consist of the standard block (control).

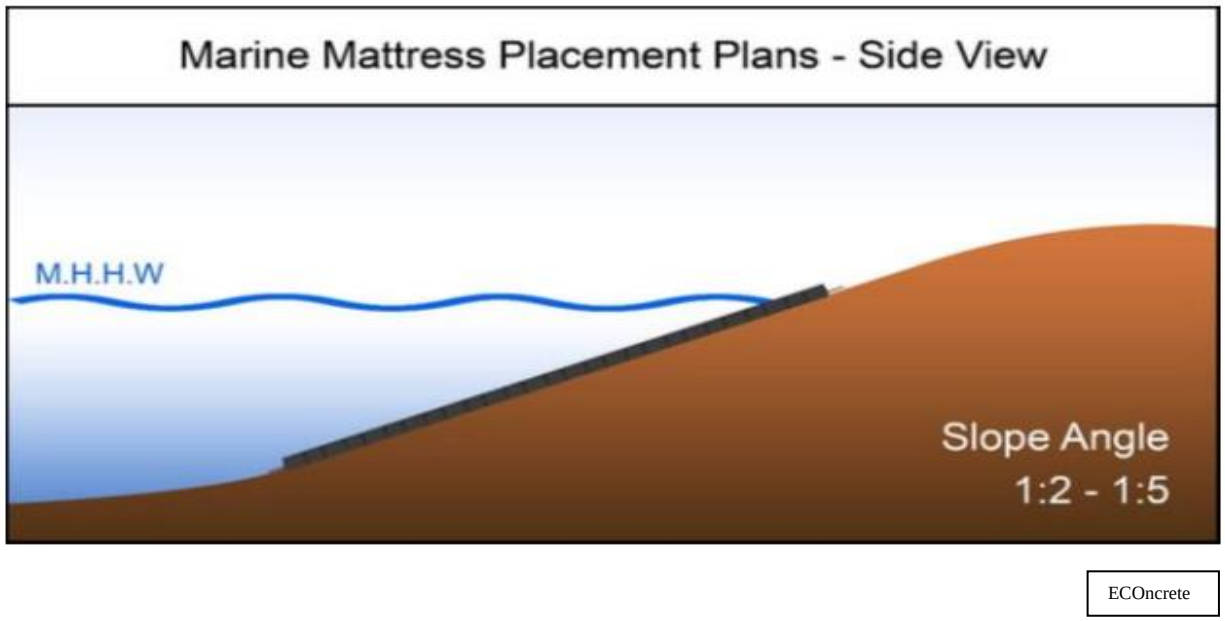


Figure 5. Side view of deploying ECOncrete® Articulated Concrete Block Mattress. Mean Higher High Water, (MHHW).

On Site Biological Monitoring and Sample Collection

Biomass and seawater samples were collected from PEI located in Broward County, Florida areas shown in Table 1 and Figure 1. A total of 24 blocks that consist of 12 ECOncrete blocks, and 12 control blocks were cleared of all existing coverage of turf, algae and growth with a paint scraper and a wire brush was used to scrub the surfaces clean. Three additional manmade control site locations had all existing material removed as well. These sites consist of similar depth and represent the immediate ecosystem habitat: a manmade concrete vertical seawall, a three-set nautical wooden piling stump and a manmade rock wall structure. During the months of December 2017 through November 2018, 12 time point collections were conducted on mattress #3 and at each of the control locations. A total of 72 samples were collected throughout the year: with each time point equaling two samples from the ECOncrete® block treatment, one from the control block and one from each of the three manmade control sites. The biomass samples were collected in a polyethylene sterile Whirl-Pak ® then placed in ice coolers and transported to the laboratory within a two-hour period. All samples were stored at a -80 °C storage unit until DNA extractions were completed.

The seawater samples were collected monthly for one full year in PEI located in Broward County. The sea water samples correspond in location to ACBM #3, the seawall and the wooden

piling location. A total of 36 samples were collected over the year. These water samples were collected in one-liter sterile polyethylene bottles, stored in ice coolers, and transported to the laboratory within 2 hours of collection. The one-liter seawater samples were filtered using a vacuum filtration system with a Pall GN-6 Metrical® μ grid 47 mm, 0.45 μm membrane filter using a vacuum pump (Hobbie & Jaspers, 1977; Knight et al., 2012). The filters will be stored in a – 80 °C storage unit until DNA extractions can be completed.

Microbial DNA Extraction

DNA was extracted using the Qiagen DNeasy PowerSoil Kit adhering to the Qiagen protocol (Qiagen, USA, #47016). All extractions are verified with a 0.5% agarose gel that runs for 45 minutes at 75V (Lee et al., 2012). The Electrophoresis uses a ladder that has a set molecule size to compare DNA fragments that have been extracted. After the success of genomic DNA, a polymerase chain reaction (PCR) was run to ensure DNA amplification. This amplification uses 16s specific PCR primers 806R and 515F with the Platinum Hot Start PCR 2X Master Mix (Invitrogen, USA). These primers yield a ~300 bp length fragment (Caporaso et al., 2012). DNA is stored in a -20° C until ready for sequencing.

Illumina High Throughput Metagenomic Sequencing

Using the Earth Microbiome Project (EMP) protocols, the amplicons of the 16S rRNA were sequenced (Thompson et al., 2017). Using the Illumina MiSeq platform protocols the amplicon PCR followed the EMP protocols using the 806R, 515F primers and the Platinum Hot Start PCR 2X Master Mix designed for the 16S rRNA V3 and V4 amplicon regions. The PCR was performed using the initial denaturing step of 94ºC for three minutes. Next, the initial denaturing step was followed by denaturation at 94ºC for 45 seconds, annealing at 50ºC for 60 seconds. Lastly, an extension cycle at 72ºC for 90 seconds. These steps are repeated 30 times where the reactions were then held at a 4ºC indefinitely. Amplification is verified by performing gel electrophoresis using a 0.% agarose gel This PCR product is then cleaned using Ampure XP beads and a magnetic plate as summarized in the 16S metagenomic library prep guide (Illumina, 2013). Then further verification of proper DNA concentrations was completed with the Qubit 2.0 High Sensitivity Fluorometry instrument (Life Technologies, USA, model #Q32866). A final quality control verification is performed using the Agilent Bio-analyzer Tape Station 2200 (Agilent Technologies,

USA, model #G2991AA). This final product is then loaded onto the Illumina MiSeq system for the 16S metagenomics at 500 cycles sequencing that adheres to the final library pooling protocol (Illumina, 2013).

Water Quality and Monitoring

Water quality monitoring was conducted monthly at the same time of the in-situ field sample collections. The YSI digital handheld device (model # 606950) took measurements in dissolved oxygen (mg/L), salinity, pH, conductivity (mS/cm), and temperature (°C). (YSI Incorporated, USA). These samples were collected in the water column monthly at the 12 time point periods for one full year (see Table 2). To monitor trends over time, three separate Hobo loggers were deployed into PEI. The location consisted of the bottom of the ACBM #3 in the intertidal zone of PEI. Initial deployment dates for all three devices were 07/18/2017, including: a conductivity logger, model #U-24-002, a pressure and temperature logger model # U201, and a dissolved oxygen logger model # U-26-001 (Onset Computer Corporation, USA). Each device collected data every 15 minutes for approximately 30 days when data was offloaded and then redeployed. To assess the water chemistry, water samples were collected using sterile, acid-washed syringes then placed in acid-washed PVC containers by filtering through disposable hydrophilic PVDF 0.22 micrometer Millipore filters. Water samples were tested with a portable Hach DR900 multiparameter colorimeter for NO_3^- using Hach Nitraver5 reagent pillows, and PO_4^{3-} using Hach Phosver3 reagent pillows (Hach Company, USA).

Statistical Analysis

Output of the DNA sequences was analyzed using the open-source software, Quantitative Insights into Microbial Ecology (QIIME2 version 2021.8). The software is used to analyze data, create histograms that compare samples within the data set, and to help establish if there are core sets of organisms that represent certain habitats (Caporaso et al., 2010). Sequences were quality-filtered to remove chimeras. Following taxonomic classification, microbial alpha and beta diversity for the sequenced samples can be processed using R Studio (an open-source software) and Primer-E v7 to verify that the complete range of the microbial community was portrayed in the samples (Clarke & Gorley, 2015). Alpha diversity is species richness and evenness whereas beta diversity is the difference in community composition (Jankowski et al., 2009). Analysis of

alpha diversity will be quantified through a Shannon Diversity Index, Inverse Simpson Index, and an analysis of variance (ANOVA). Analysis of beta diversity will be quantified through Bray-Curtis similarity values. A Non-metric Multidimensional Scaling plot (NMDS), a BEST overlay of environmental variables combined with a NMDS, and an analysis of similarity (ANOSIM) will also be used for the analysis of beta diversity

All microbial data was compared with physical data collected at the time of sample site extractions. Significant correlations were assessed to determine if water quality variables and microbial composition are similar (Campbell et al., 2015). Using R Studio and Primer 7, further statistical analysis was used to assess and to validate findings. Regression analysis was used to compare a single response, dependent variable to one or more responses and independent variables. Specifically, the regression analysis was used to compare the correlation between the water chemistry and the bacteria assemblages in the samples collected in PEI. Thus, allowing for conclusions on the influences of water chemistry on the microbial community's abundance and assemblage.

Results

Chemical and Environmental Data Analysis

Chemical and environmental analysis was completed for the water samples for each specific time point for one full year. The results for the collected profiles are shown in Table 2. Temperature readings ranged from 21.36 °C in January 2018 to 45.37 °C in August 2018 from the surface readings collected from the YSI handheld device. Both temperature and conductivity displayed seasonal fluctuations (Figure 6 and Figure 7). The average temperature readings recorded from the Hobo logger situated at a depth of approximately two meters (~2 m) was January 2018 = 23.28 °C, March 2018 = 23.62 °C and November 2018 = 26.78 °C (the dry season months) compared to August 2018 = 30.3 °C, June 2018 = 31.08 °C and July 2018 = 31.73 °C (the wet season months). Conductivity averaged for all months at 49.96 (mS/cm). Dissolved oxygen ranged from 137.4% to 235.6%. The pH (average = 8.40), phosphate (average = 0.06), and nitrate (average = 0.59) all indicated to be stable.

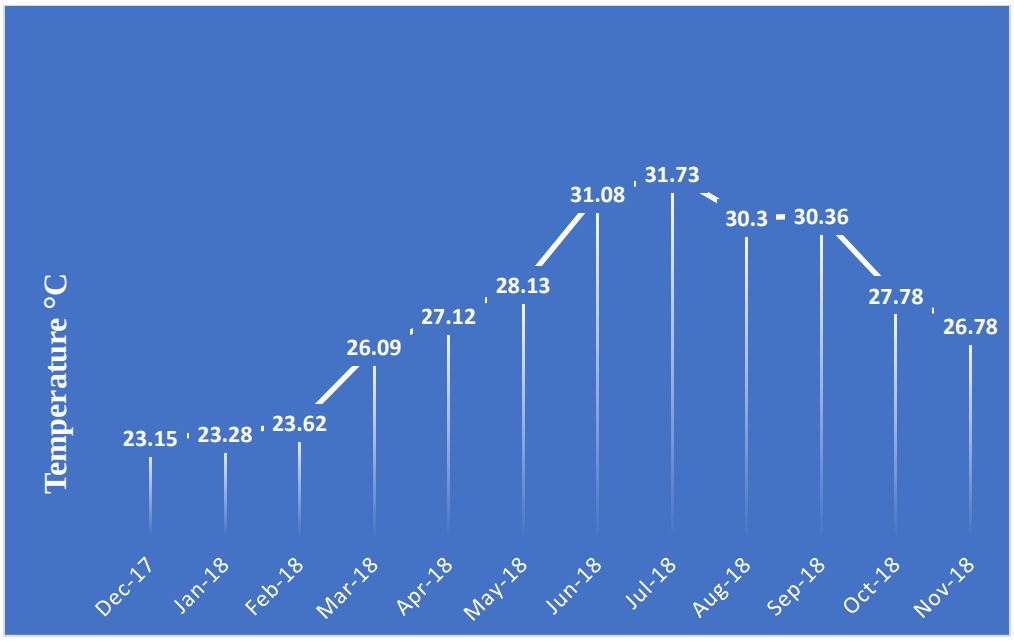


Figure 6. Line graph showing average temperatures in degrees Celsius for each month between December 2017 and November 2018 acquired from the Hobo temperature logger located on mattress 3.

Figure 7. Depicting conductivity (mS/cm) readings for each month between December 2017 and November 2018 acquired from the YSI digital handheld device.

Table 2. Results of environmental and chemical data collected over a one-year period between December 2017 and November 2018. Samples were collected once a month from the YSI digital handheld device. Water samples were processed using the portable Hach DR900 multiparameter colorimeter.

Date	High Tide(HT) Low Tide(LT)	Temp °C	Conductivity (mS/cm)	Salinity (Sal)	Dissolved oxygen (DO%)	pH	Total Phosphate	Total Nitrate
12/14/17	Time after HT	25.70	48.28	31.00	230.70	8.84	0.05	0.90
01/17/18	Time after HT	21.36	43.04	30.06	215.30	8.35	0.06	0.70
02/13/18	Time after HT	25.06	52.14	34.27	194.10	8.20	0.03	0.50
03/20/18	Time after HT	24.08	53.26	35.88	235.60	8.33	0.05	0.70
04/20/18	Time after HT	27.11	57.23	36.39	221.00	8.30	0.06	0.80
05/17/18	Time after HT	26.65	51.72	32.86	187.70	8.40	0.04	0.80
06/21/18	Time after HT	30.37	47.58	27.62	200.70	8.39	0.03	0.60
07/21/18	Time after HT	29.97	49.87	29.35	198.30	8.49	0.15	0.60
08/16/18	Time after HT	45.37	30.71	12.94	189.06	8.02	0.05	0.70
09/16/18	Time after HT	26.54	55.93	35.91	209.10	8.47	0.08	0.13
10/21/18	Time after HT	27.90	56.04	34.94	192.60	8.54	0.08	0.05
11/21/18	Time after HT	24.58	53.81	35.80	137.40	8.52	0.07	0.60

MiSeq sequencing output

A total of 108 samples were collected monthly from PEI from December 2017 to November 2018 for 16S rRNA amplicon sequencing. Six samples were removed from the sample set because of low PCR product. An additional 10 samples were removed from the sample set analysis, because sequencing efforts only yielded 10,000 reads per sample. The final sample set after the quality control and sequencing process included 92 samples (Table 3). The final total number of raw DNA sequences generated from the MiSeq platform was 7,816,848. The average number of reads per sample was 86,854, with the minimum number of reads equaling 11,605 and the maximum number of reads equaling 561,592.

Table 3. Sample ID's and the Number of reads per sample.

Sample	# Of MiSeq Reads	Date	Time point	Sample Type	Sample location
141217t1ep2	141,463	12/14/17	1	water	seawall
141217t1ep3	183,193	12/14/17	1	water	wood pile
141217t1sw	19,511	12/14/17	1	biofilm	seawall
141217t1wp	17,304	12/14/17	1	biofilm	wood pile
170118t2ep1	201,118	01/17/18	2	water	mattress 3
170118t2ep2	139,109	01/17/18	2	water	seawall
170118t2ep3	138,288	01/17/18	2	biofilm	wood pile
170118t2c1	24,108	01/17/18	2	biofilm	control block
170118t2e1	20,549	01/17/18	2	biofilm	treatment block
170118t2wp	21,288	01/17/18	2	biofilm	wood pile
130218t3ep1	17,908	02/13/18	3	water	mattress 3
130218t3ep2	25,491	02/13/18	3	water	seawall
130218t3c1	49,216	02/13/18	3	biofilm	control block
130218t3e1	156,019	02/13/18	3	biofilm	treatment block
130218t3sw	102,830	02/13/18	3	biofilm	seawall
130218t3rw	89,157	02/13/18	3	biofilm	rock wall
130218t3wp	82,082	02/13/18	3	biofilm	wood pile
200318t4ep1	84,752	03/20/18	4	water	mattress 3
200318t4ep2	68,809	03/20/18	4	water	seawall
200318t4ep3	63,783	03/20/18	4	water	wood pile
200318t4c1	35,100	03/20/18	4	biofilm	control block
200318t4e1	84,752	03/20/18	4	biofilm	treatment block
200318t4e2	81,292	03/20/18	4	biofilm	treatment block
200318t4sw	64,453	03/20/18	4	biofilm	seawall
200318t4rw	69,742	03/20/18	4	biofilm	rock wall
200418t5ep1	111,074	04/20/18	5	water	mattress 3
200418t5ep2	105,333	04/20/18	5	water	seawall
200418t5ep3	128,313	04/20/18	5	water	wood pile
200418t5c1	136,861	04/20/18	5	biofilm	control block
200418t5e1	60,906	04/20/18	5	biofilm	treatment block
200418t5e2	76,032	04/20/18	5	biofilm	treatment block
200418t5sw	90,603	04/20/18	5	biofilm	seawall
200418t5rw	85,050	04/20/18	5	biofilm	rock wall
200418t5wp	118,667	04/20/18	5	biofilm	wood pile

170518t6ep2	80,846	05/17/18	6	water	seawall
170518t6ep3	52,950	05/17/18	6	water	wood pile
170518t6e1	59,237	05/17/18	6	biofilm	treatment block
170518t6e2	22,742	05/17/18	6	biofilm	treatment block
170518t6rw	67,600	05/17/18	6	biofilm	rock wall
170518t6wp	97,782	05/17/18	6	biofilm	wood pile
210618t7ep1	56,910	06/12/18	7	water	mattress 3
210618t7ep2	74,619	06/12/18	7	water	seawall
210618t7ep3	78,152	06/12/18	7	water	wood pile
210618t7c1	82,623	06/12/18	7	biofilm	control block
210618t7e1	107,463	06/12/18	7	biofilm	treatment block
210618t7e2	49,312	06/12/18	7	biofilm	treatment block
210618t7sw	81,004	06/12/18	7	biofilm	seawall
210618t7rw	124,766	06/12/18	7	biofilm	rock wall
210618t7wp	34,032	06/12/18	7	biofilm	wood pile
210718t8ep1	51,562	07/21/18	8	water	mattress 3
210718t8ep2	90,603	07/21/18	8	water	seawall
210718t8ep3	81,727	07/21/18	8	water	wood pile
210718t8c1	84,734	07/21/18	8	biofilm	control block
210718t8e1	59,687	07/21/18	8	biofilm	treatment block
210718t8e2	62,268	07/21/18	8	biofilm	treatment block
210718t8sw	92,663	07/21/18	8	biofilm	seawall
210718t8rw	23,317	07/21/18	8	biofilm	rock wall
210718t8wp	43,403	07/21/18	8	biofilm	wood pile
160818t9ep1	126,260	08/16/18	9	water	mattress 3
160818t9ep2	61,048	08/16/18	9	water	seawall
160818t9ep3	561,592	08/16/18	9	water	wood pile
160818t9c1	55,328	08/16/18	9	biofilm	control block
160818t9e1	100,394	08/16/18	9	biofilm	treatment block
160818t9e2	97,048	08/16/18	9	biofilm	treatment block
160818t9sw	78,598	08/16/18	9	biofilm	seawall
160818t9rw	63,615	08/16/18	9	biofilm	rock wall
160818t9wp	96,277	08/16/18	9	biofilm	wood pile
160918t10ep1	12,670	09/16/18	10	water	mattress 3
160918t10ep3	160,519	09/16/18	10	water	wood pile
160918t10c1	84,973	09/16/18	10	biofilm	control block
160918t10e2	125,888	09/16/18	10	biofilm	treatment block
211018t11sw	63,974	09/16/18	10	biofilm	seawall

160918t10rw	63,646	09/16/18	10	biofilm	rock wall
160918t10wp	70,000	09/16/18	10	biofilm	wood pile
211018t11ep1	113,755	10/21/18	11	water	mattress 3
211018t11ep2	108,529	10/21/18	11	water	seawall
211018t11ep3	86,775	10/21/18	11	water	wood pile
211018t11c1	90,603	10/21/18	11	biofilm	control block
211018t11e1	96,003	10/21/18	11	biofilm	treatment block
211018t11e2	49,718	10/21/18	11	biofilm	treatment block
211018t11rw	107,620	10/21/18	11	biofilm	rock wall
211018t11wp	145,878	10/21/18	11	biofilm	wood pile
211118t12ep1	11,605	11/21/18	12	water	mattress 3
211118t12ep2	111,639	11/21/18	12	water	seawall
211118t12c1	52,243	11/21/18	12	biofilm	control block
211118t12e1	75,489	11/21/18	12	biofilm	treatment block
211118t12e2	74,305	11/21/18	12	biofilm	treatment block
211118t12sw	85,474	11/21/18	12	biofilm	seawall
211118t12rw	130,266	11/21/18	12	biofilm	rock wall
211118t12wp	100,958	11/21/18	12	biofilm	wood pile
Total reads	7,816,848				
Average reads	86,854				

Key
Date: Day, month, year
t; indicating the time point 1-12 for each month
ep1; water sample collection mattress 3 location
ep2; water sample collection seawall location
ep3; water sample location wood pile location
c1; biofilm sample collection control block
e1; biofilm sample collection treatment block, top surface
e2; biofilm sample collection treatment block, inside surface
sw; biofilm sample collection vertical seawall
rw; biofilm sample collection rock wall
wp; biofilm sample collection wood pile
Example: 141217t1ep2 date; 12/14/2017 t; (1) indicating the first sample month ep2; water sample collection seawall location

Relative Abundance

Relative abundance of amplicon sequence variants (ASVs) for all samples were calculated at the Phylum and Family levels (Figure 8 and Figure 9). The top five taxa at the Phylum level comprise of Proteobacteria, Cyanobacteria, Acidiobacteria, Chloroflexi, and Bacteroidetes for both substrate and water samples. SIMPER analysis revealed an average similarity of all samples at 86.60% at the family level. The summed average of each individual factor indicates a presence of Flavobacteriaceae with a contribution of 38.84%. Yet to be cultivated cells in the environment accounts for 20.98% of the population with 11.35% represented by Mitochondria. Dominant taxa at the family level in the water samples are Cyanobiaceae (14.71%), Flavobacteriaceae (6.72%), and Rhodobacteraceae (5.72%). Dominant taxa at the family level for the treatment and control substrates are Rhodobacteraceae (5.37%, 5.37%), Flavobacteriaceae (5.31%, 5.73%), and Saprospiraceae (5.26%, 4.95%). There was not a significant difference in the microbial community diversity of the biofilm in the treatment concrete substate verses the control concrete substrate (ANOSIM, R = -0.041, p = 0.809).

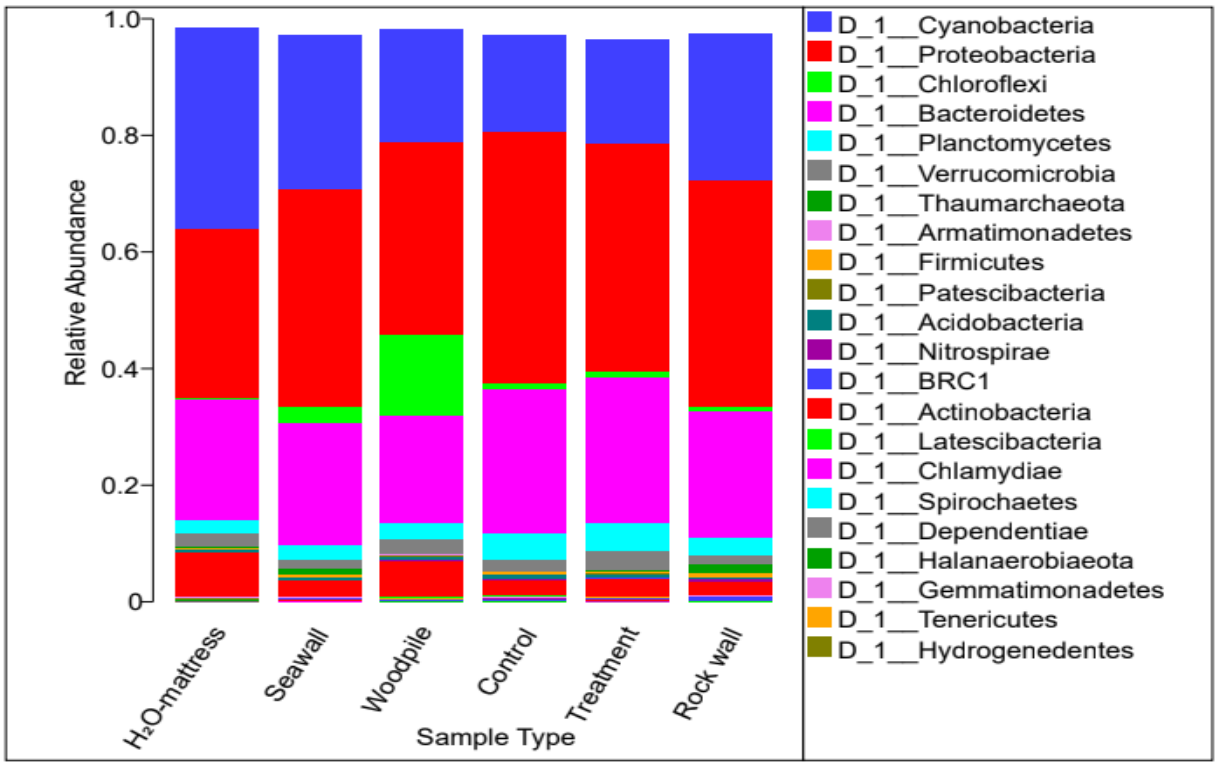


Figure 8. Stacked bar chart showing relative abundance at Phylum taxonomic level for all samples. Seawall, wood-pile and rock-wall served as a baseline. Treatment denotes the enhanced treatment substrate and control denotes the standard concrete substrate.

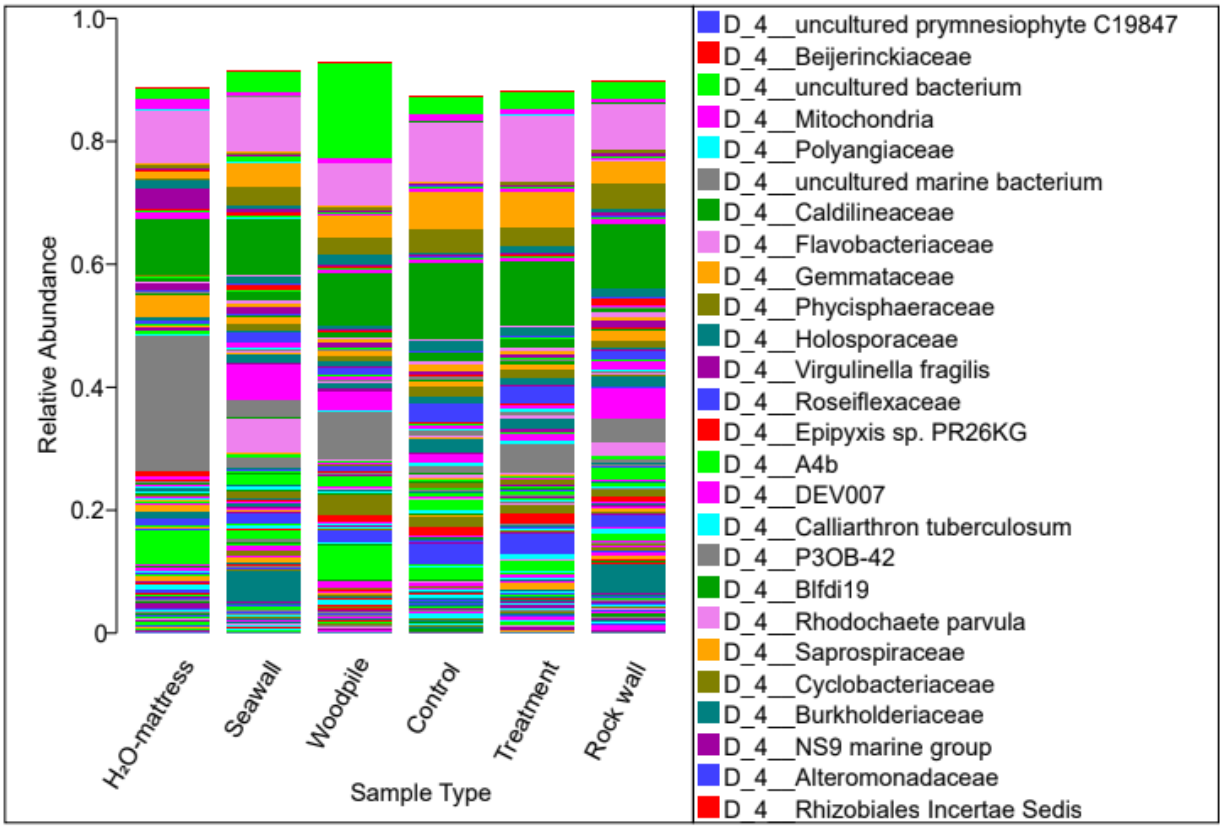


Figure 9. Stacked bar chart showing relative abundance at the Family taxonomic level for all samples. Seawall, wood-pile and rock-wall served as a baseline. Treatment denotes the enhanced treatment substrate and control denotes the standard concrete substrate.

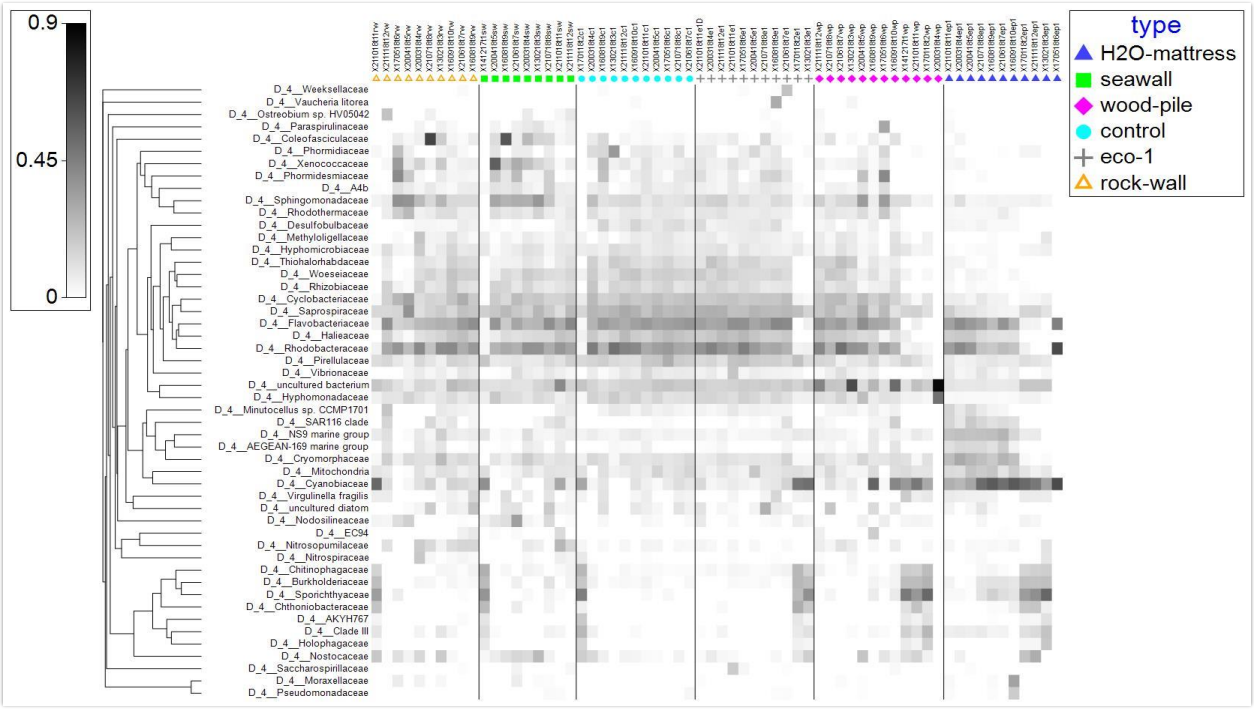


Figure 10. Heat map at the Family taxonomic level of the microbial community composing the biofilms for all sample locations in Port Everglades Inlet. Darker colors indicate greater abundance. Using a cluster analysis (to the left of the figure) the tree represents how a bacterial species is related to another species. Seawall, wood-pile and rock-wall served as a baseline. Eco-1 denotes the enhanced treatment substrate and control denotes the standard concrete substrate.

Bacterial microbiome in Port Everglades Inlet

Port Everglades Inlet composition for the microbiome communities composing the biofilms were analyzed through both alpha and beta diversity in the community population. Alpha diversity is richness and evenness of the community. Beta diversity is used as a measure to compare samples to each other and solves the question of community similarity for differences. Quality sorting of all amplicon sequence variants (ASV) was performed, and community richness and diversity were calculated for all samples.

Alpha Diversity

Figures 11 and 12 display a box plot comparison of alpha diversity for all samples collected in PEI. Both Shannon Index and Inverse Simpson box plats are displayed (Figure11 and 12). The sample sets yielded similar composition. This was validated by running an ANOVA test followed by a Tukey test for multiple comparisons. The mean sample value was significantly

different between the control and the wood pile samples (p = 0.034, 95% confidence interval (C.I.) = [0.005, 0.294]). There was no statistically significant difference in mean sample values between treatment and the H20 mattress samples (p = 0.177), between treatment and seawall samples (p = 0.151), between treatment and the wood pile samples (p = -0.093) or between treatment and the rock wall samples (p = 0.455). All other pair wise comparisons of the mean resulted in no significant differences (Figure 11 and 12).

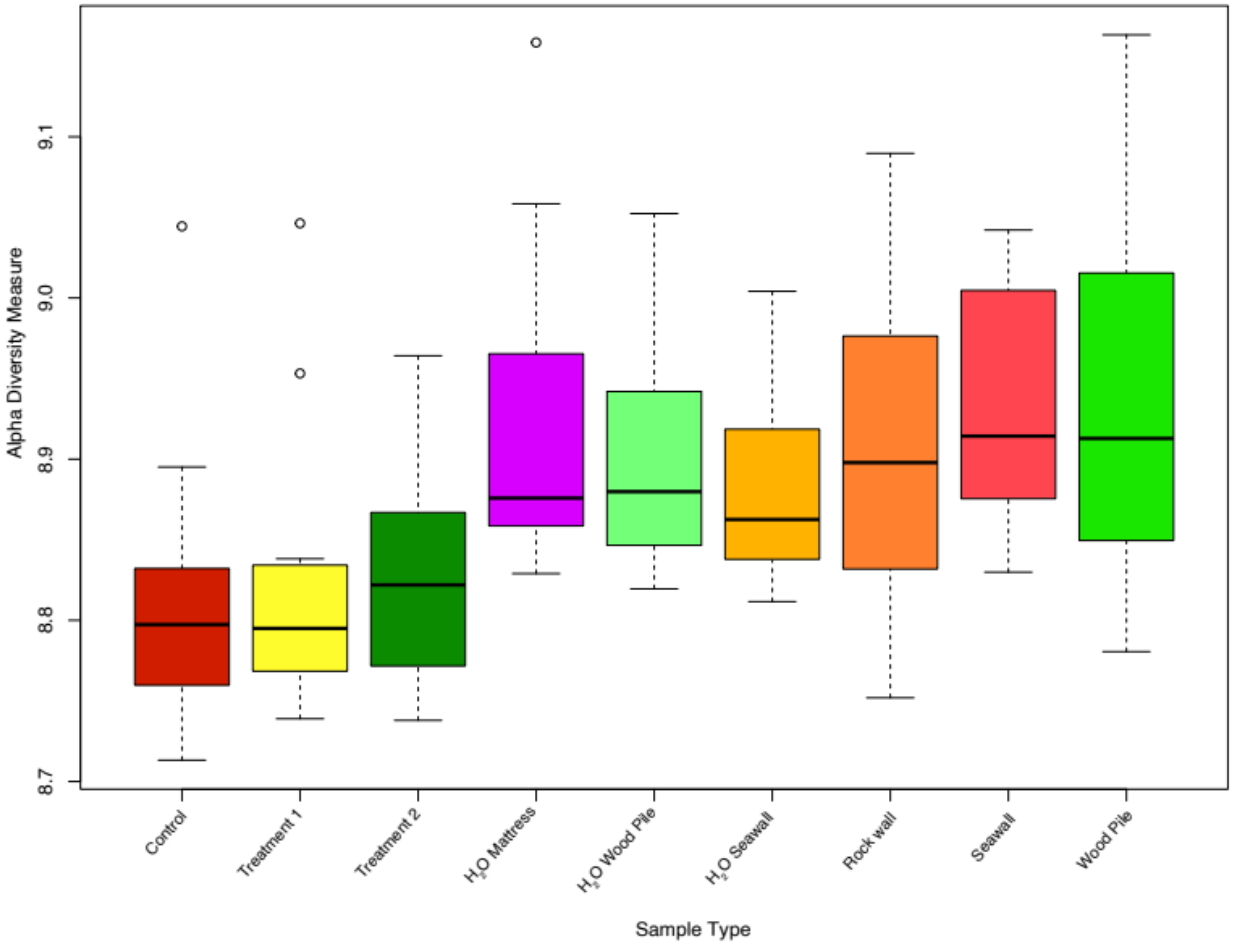


Figure 11. Alpha diversity box plot with Shannon Index depicting the microbiome communities composing the biofilms. The Shannon index seeks to measure the diversity of the species. Seawall, wood-pile and rock-wall served as a baseline. Treatment 1 and Treatment 2 denotes the enhanced treatment substrates and control denotes the standard concrete substrate.

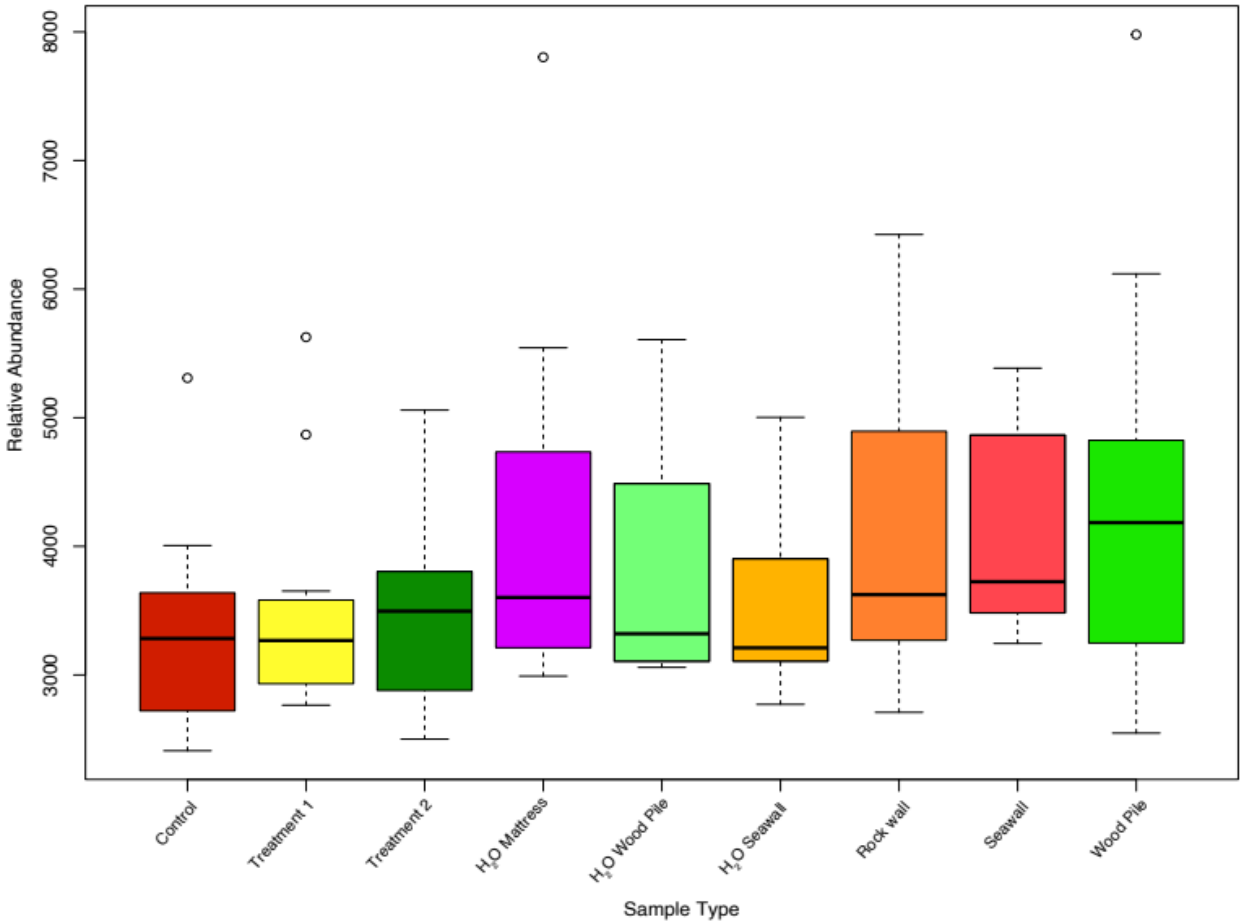


Figure 12. Alpha diversity box plot with Inverse Simpson depicting the microbiome communities composing the biofilms. The Inverse Simpson indicates the richness in the community with uniform evenness that has the same level of diversity. Seawall, wood-pile and rock-wall served as a baseline. Treatment 1 and Treatment 2 denotes the enhanced treatment substrates and control denotes the standard concrete substrate.

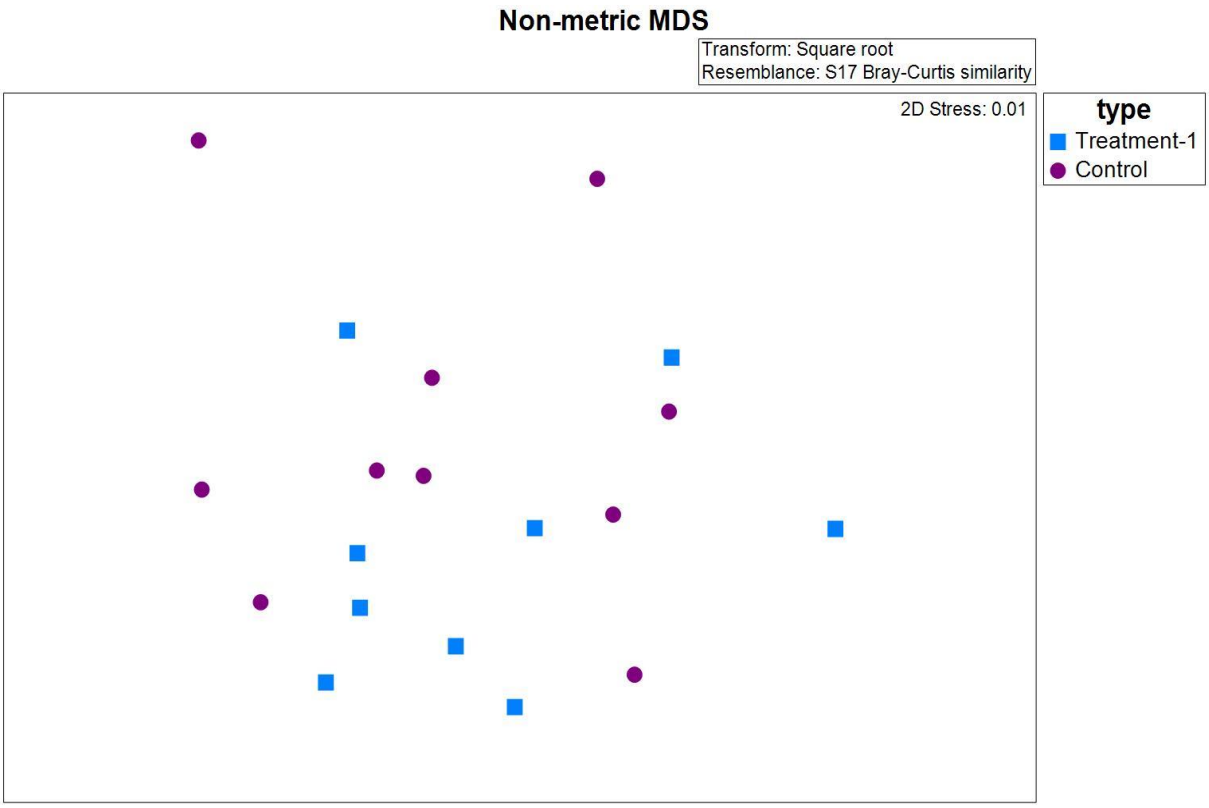


Figure 13. Non-metric Multidimensional Scaling (NMDS) of treatment substrate verses control substrate using the Bray-Curtis similarities. Treatment-1 denotes the enhanced treatment substrate and control denotes the standard concrete substrate.

Beta Diversity

Beta diversity is used as a measure to compare samples to each other and solves the question of community composition. Beta diversity looks at the ratio between the local species and measures the distance or dissimilarity between each sample set (Jankowski et al., 2009). There was not a significant difference in the microbial community diversity of the biofilm in the treatment concrete substate verses the control concrete substrate (ANOSIM, R = -0.041, p = 0.809, Figure 13).

When considering the five manmade surface areas, there was a weak and significant difference between the microbial community composing the biofilms for all surface areas (ANOSIM, R = 0.133, p = 0.001, Figure 14). Additionally, there were statistically significant differences between the groups when considering pairwise comparisons. There were moderate and significant differences for the treatment-seawall surface (ANOSIM, R = 0.343, p = 0.002), treatment-woodpile surface (ANOSIM, R = 0.124, p = 0.05), and the treatment-rock wall surface

(ANOSIM, R = 0.178, p = 0.001). Moreover, there were moderate and significant differences from the control-seawall surface (ANOSIM, R = 0.453, p = 0.002), the control-rock wall surface (ANOSIM, R = 0.23, p = 0.001) and the control-woodpile surface (ANOSIM, R = 0.197 p = 0.008).

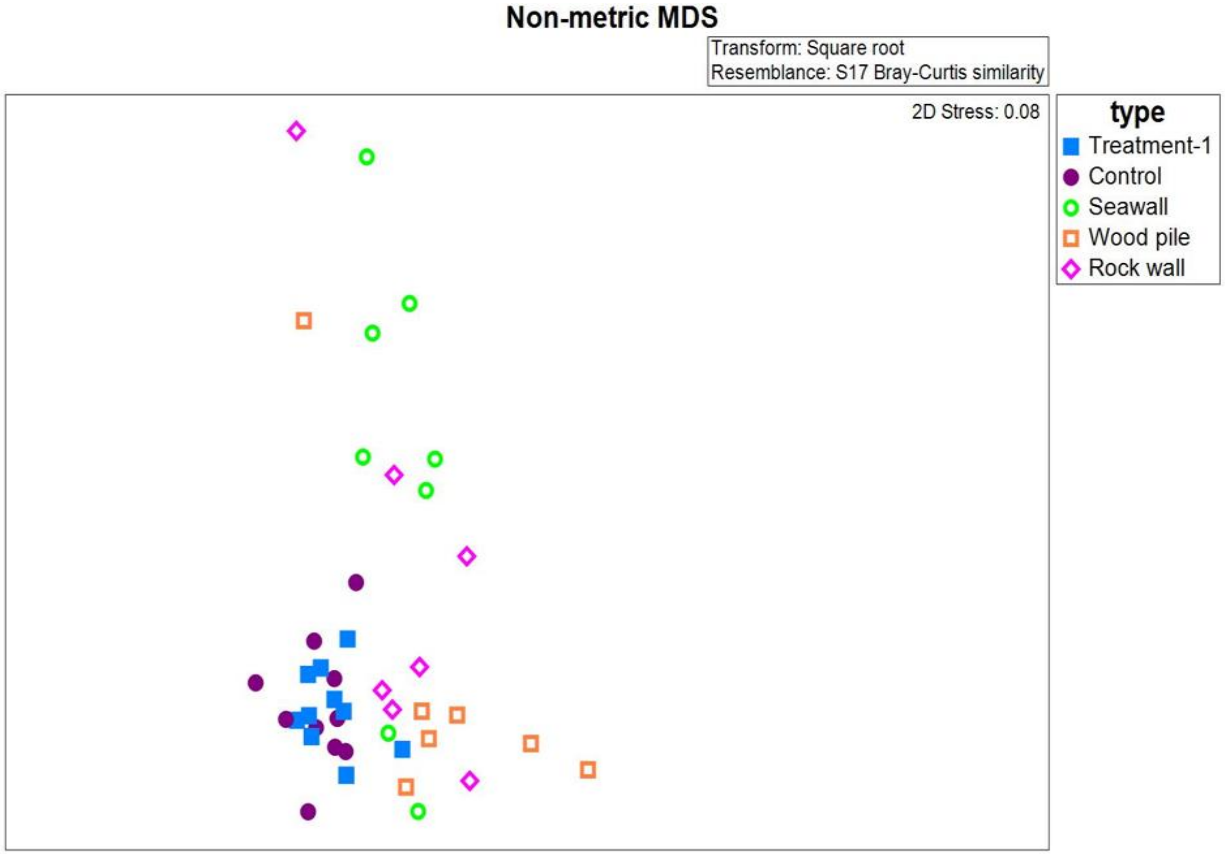


Figure 14. NMDS plot of all surface areas of the microbial community composing the biofilms using the Bray-Curtis similarities. Seawall, wood-pile and rock-wall served as a baseline. Treatment-1 denotes the enhanced treatment substrate and control denotes the standard concrete substrate.

South Florida has two main seasons, the wet season or hurricane season which ranges from May through October and the dry season which ranges from November through April. There was no significant difference between the microbial community composing the biofilms for all samples during the wet and dry season (ANOSIM, R = 0.089, p = 0.001, Figure 15). However, there were statistically significant differences between the groups when considering pairwise comparisons. There were moderate and significant differences for the water samples (ANOSIM, R = 0.363, p =

0.03), the treatment concrete substrate samples (ANOSIM, R = 0.325, p = 0.009, Figure 16), and the control concrete substrate samples (ANOSIM, R = 0.333, p = 0.013, Figure 17).

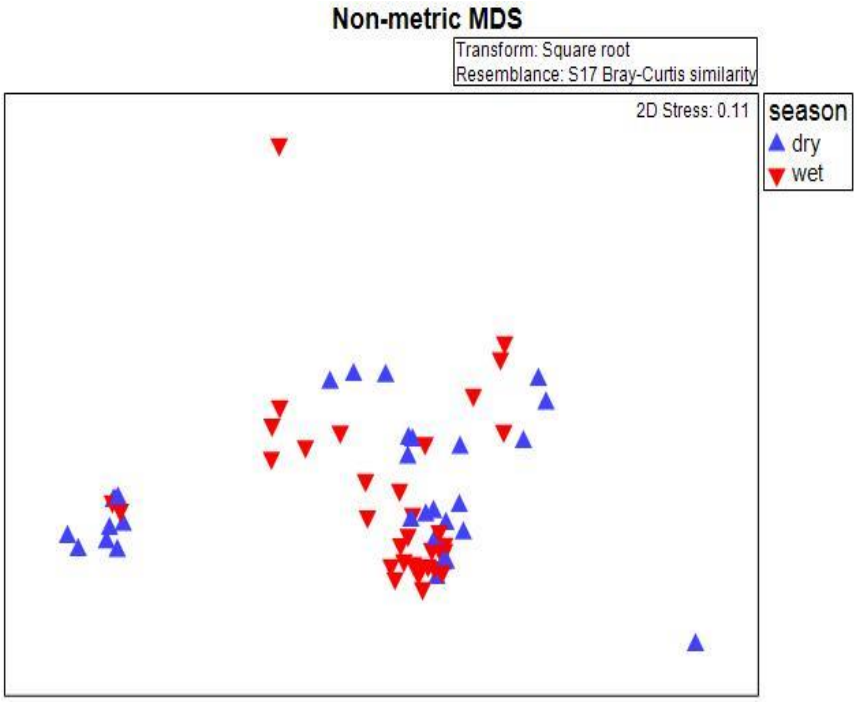


Figure 15. NMDS plot of both wet and dry season of the microbial community composing the biofilms for all sample locations using Bray-Curtis similarities.

Figure 16 and 17. NMDS plot of both wet and dry season of the microbial community composing the biofilms for the treatment concrete substrate (Figure 16, left) and the control concrete substrate (Figure 17, right) using Bray-Curtis similarities.

Water Chemistry and Environmental Parameters

Temperature, conductivity, and pH weakly correlate to diversity for microbial communities in this study (BEST, R2 = 0.117, Figure 18). The NMDS model with the BEST overlay of environmental variables explained approximately 12% of the overall sample variance with the temperature representing the most principal environmental variable, followed by conductivity and pH. The correlation method used was Spearman ranking with a Euclidean distance resemblance measure. The data is depicted in a Non-Metric Multidimensional Scaling (NMDS) plot using the BIOENV or best method in Primer. This method finds best possible rank order match between the dissimilarities derived from the environmental data (Figure 18).

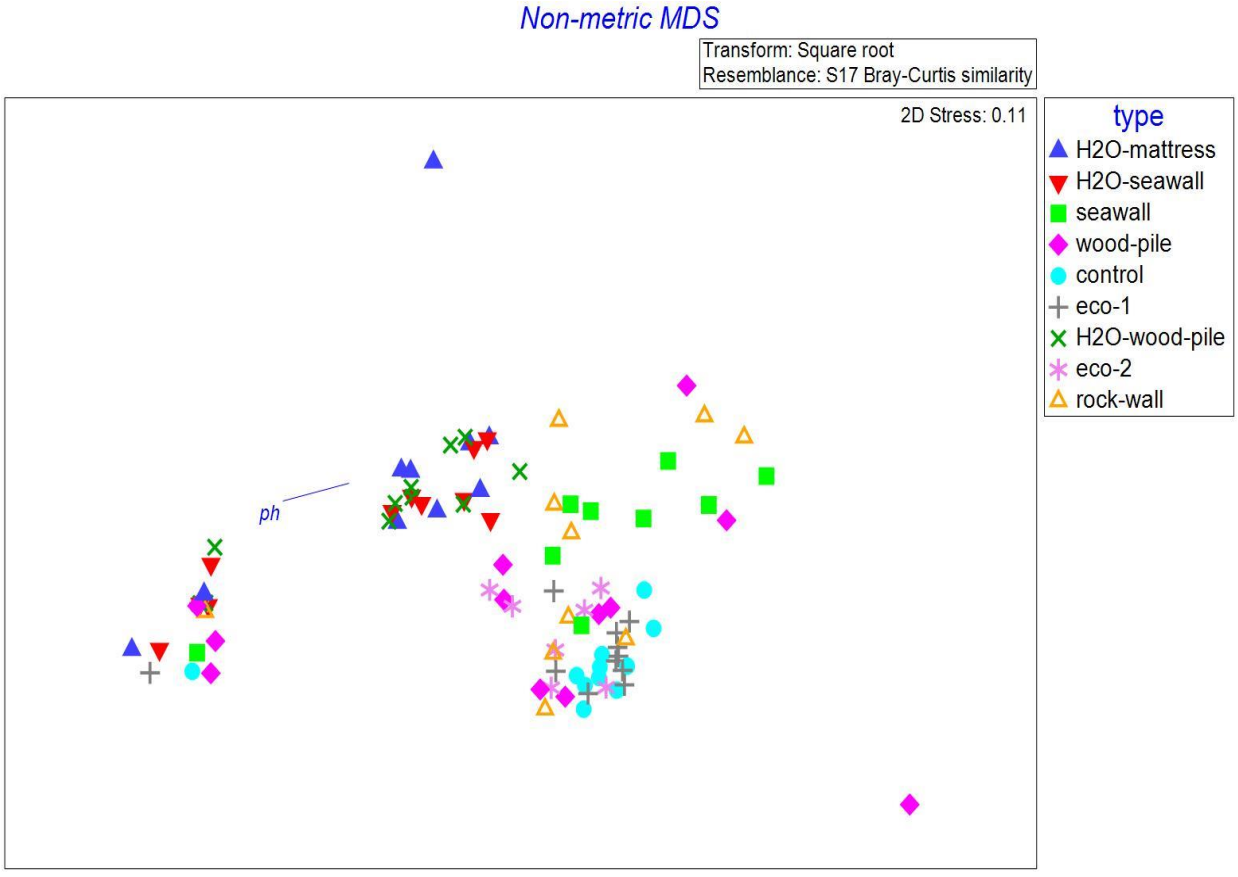


Figure 18. NMDS plot of all samples in the microbial community composing the biofilms using Bray-Curtis similarities and includes all environmental parameters. The correlation method used was Spearman ranking with a Euclidean distance resemblance measure. Seawall, wood-pile and rock-wall served as a baseline. Eco-1 and Eco-2 denote the enhanced treatment substrate and control denotes the standard concrete substrate.

DISCUSSION

The objective of this study was to characterize the microbial communities composing the biofilms on artificial substrates with modern molecular ecology methods. The initial focus was to measure the differences in alpha and beta diversity of the two different substrates that comprised the articulated concrete block mattresses, (ACBM), that were deployed into Port Everglades Inlet on a degraded shoreline location in 2017. The mattresses consisted of two types of substrates: a science-based designed concrete substrate, that is comprised of an enhanced concrete composition, surface texture and macro design (treatment), compared to the standard gray, featureless CEM-1 based concrete substrate (control). Previous studies have demonstrated ecologically engineered concrete substrates have the capability to attract more organisms and improve recruitment (Perkol-Finkel et al., 2017; Perkol-Finkel & Sella, 2015; Sella et al., 2021). These studies were conducted in controlled laboratory settings and field experiments and only observed macro-organisms. This study is unique to the ecological engineering science studies as it looks at these substrates at the microbial level. This study is the first, to the author's knowledge, that has been utilized to look at the biofilms composing the microbiome on artificial substrate constructed by ECOncrete® utilizing Illumina MiSeq DNA sequencing technology. The findings of the current study did not support the hypothesis that there will be significant difference in the microbial communities composing the biofilms between the two separate substrates located in PEI.

Port Everglades Inlet Location and Sample Collection

PEI has a major economic impact on Broward County, but it is also a major source of pollution that impacts the coastal environment, adjacent reef systems and recreational areas next to the port (Banks et al., 2008; Walker et al., 2012). Therefore, examining the microbial communities in PEI and its environmental impacts is valuable as a baseline study for best practices for future proposed dredging, the health of the inlet's ecosystem, the marine environment, and coastal areas adjacent to the inlet (Cunning et al., 2019). Additionally, examining how the performance of the CMI comprised of the treatment in the Port Everglades Inlet compared to control provides insight to future nature inclusive design projects.

Taxonomy of bacteria in the microbiome

Healthy ecosystems have a set of core taxa that are composed of microorganisms that can be found in an abundance of over 1% opposed to rare taxa that are found in less than 0.1%, and that are also present in majority of samples of a specific habitat (Bjork et al., 2018; Jiao et al., 2019; Lovejoy et al., 2006). Bjork and colleagues, (2018) have proposed that the core microbiome is the common taxa in a habitat and have taken the next step in identifying and characterization of the core microbiome. Most of all of microbiomes that have been found in environmental ecosystems are dominated by some 5-20 bacterial taxa while the remaining taxa can number in the 100's to 1000's and appear infrequently (Easson & Lopez, 2009). The latter are sometimes referred to as the "rare biosphere" (Sogin, 2006). The notion of 'everything is everywhere, but the environment selects' is being addressed through the next generation high-throughput sequencing techniques. In this study there are 12 core taxa were found in all sample locations. The most abundant phyla in all samples were Proteobacteria, Cyanobacteria, Acidiobacteria, Chloroflexi, and Bacteroidetes for both substrate and water samples. These findings are consistent with prior studies for marine coastal waters (Campbell et al., 2015; O'Connell et al., 2018).

Comparison of Alpha Diversity Indices

Alpha diversity indices were used to calculate community richness for all samples in this study, as it captures both the organismal richness of a sample and the evenness of the organisms' abundance distribution (Morgan & Huttenhower, 2012). Alpha diversity can be assessed by using the species richness estimators Shannon-Wiener and Inverse Simpson (Figure 11 and 12). Both indexes are used to measure comparable concepts of alpha diversity. The Shannon index calculates as to the lower the value the lower the alpha diversity of the community. The Simpson index is positively correlated with Shannon's index. Meaning that it shows higher values when the alpha diversity is lower, this is why we use the inverse measurement to compare the alpha diversity. Several alpha diversity measurements are used to complete the picture of the microbial community composition. The results for the Shannon and the Inverse Simpson alpha diversity indices for the microbiome were not different across all samples with similar levels of diversity between all samples with no significant differences. This can be attributed to the location of all samples being taken within a 500-meter radius. The only significant reading was the woodpile which displayed

high species diversity. The woodpile showed the most variance when compared to all of the other substrates. This was most likely because of the wood pile being the only natural, biodegradable product that was sampled.

Comparison of Beta Diversity Indices, Type and Season

In this study, all surface area substrates were assessed and compared. As well as the two seasons, (wet and dry) for the location sample site location in PEI, Florida. Beta diversity is used as a measure to compare samples to each other. Beta diversity describes the ratio between the local species and measures the distance or dissimilarity between each sample set (Jankowski et al., 2009; Morgan & Huttenhower, 2012). When comparing all the different surface samples; the enhanced treatment, the standard control, the vertical seawall, the rock wall, and the woodpile there were significant differences. However, there were similar results that grouped treatment substrate to seawall, rock wall and wood pile and control to seawall, rock wall and wood pile. It was concluded that these structures are similar in composition with comparable microbial community abundance. Similarly, Sello et al., (2021), identified 16 total taxa on both the treatment and control substrates over a 24-month period in this protype study. The differences that were identified was that there was significantly more biomass accumulation on the treatment block compared to the control blocks ($p < 0.05$) for both organic and inorganic matter. (Appendix; Figure A) Moreover, there was a trend increase in univariate parameters (species richness and biodiversity) on the treatment blocks compared to the control block only showed fluctuations. (Appendix; Figure B).

When looking at the wet and dry season using beta diversity indices, there were no significant differences represented in the analysis between the wet and dry seasons. However, there were minor variations in the microbial community composition at the phylum level across both seasons. The changes can be seen with Cyanobacteria, where there is an increase during the wet season in relative abundance of the community and a decrease in the dry season months of relative abundance of the community. This data correlates with the cyanobacterial blooms in Florida that occur in the late summer months in both the freshwater and coastal water ecosystems (Flombaum et al., 2013). It should be noted that while we can see the seasonal change in temperature and conductivity affecting the ecosystem community, it was not significant or strong enough to have a major impact the microbial community. O'Connell et al. (2018) concluded that salinity and temperature were significant in impacting the changes in the microbial community. This study

supports the finding that a combination of pH, temperature, and conductivity weakly correlate and account for only ~12% of the effects on the microbial community.

Significance of Abundant Taxa and Correlation with Environmental Metadata

The NMDS model with the BEST overlay of environmental variables explained approximately 12% of the overall sample variance with the temperature representing the most principal environmental variable, followed by conductivity and pH. Temperature variation exhibited seasonal fluctuation that followed the typical Florida wet and dry season. Conductivity displayed an inverse correlation to the seasonal temperature change, with a striking drop in August that correlated to the very high surface temperature reading that was collected by the YSI handheld device. The readings from the pH parameter did show a weak correlation to the overall sample variance, however it was not strong enough to impact the microbial community.

Conclusion

The primary goal of this study was to characterize microbial communities by assessing differences in alpha and beta diversity between an enhanced design concrete substrate and standard concrete substrate that were deployed into Port Everglades Inlet on a degraded shoreline location in 2017. This study is the first study, to the author's knowledge, that has been utilized to look at the biofilms composing the microbiome on ECOncrete substrate utilizing Illumina MiSeq DNA sequencing technology. Results indicated that there were no significant differences in alpha diversity and beta diversity when comparing the microbial communities of the enhanced treatment substrate verses the standard control substate. This is likely a result of similar composition of both substrates. Moreover, the research was restricted to a twelve-month time period. The ideal extended research might require a 2-year or 3-year study of the biofilm development to see changes similar to what was observed in the microorganism community. Significant differences in beta diversity were seen when comparing the different types of surface areas which was consistent with the hypothesis. The woodpile showed the most variance when compared to all of the other substrates. This was most likely because of the wood pile being the only natural, biodegradable product that was sampled. Species diversity varied by season but only slightly. The environmental metadata that had an impact on the microbial community was temperature, conductivity, and pH.

Increased microbial abundance was seen in the late summer months, which is likely to be expected with the increased precipitation and temperature at that time of year. Taken together with the Sella et al., (2021) microorganism prototype study, these results provide valuable insight into future coastal marine infrastructure via 'blue' nature inclusive designs. This study can be added to the comprehensive studies that have been conducted to characterize water quality and environmental ecology in Port Everglades Inlet and surrounding waters over the last several years utilizing the Illumina MiSeq DNA sequencing technology (Campbell et al., 2015; Lopez et al., 2021; O'Connell et al., 2018). Data from this study can be used for future project management, port expansions and master planning to provide a thorough overview of Port Everglades Inlet's microbial communities on artificial substrates.

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APPENDICES

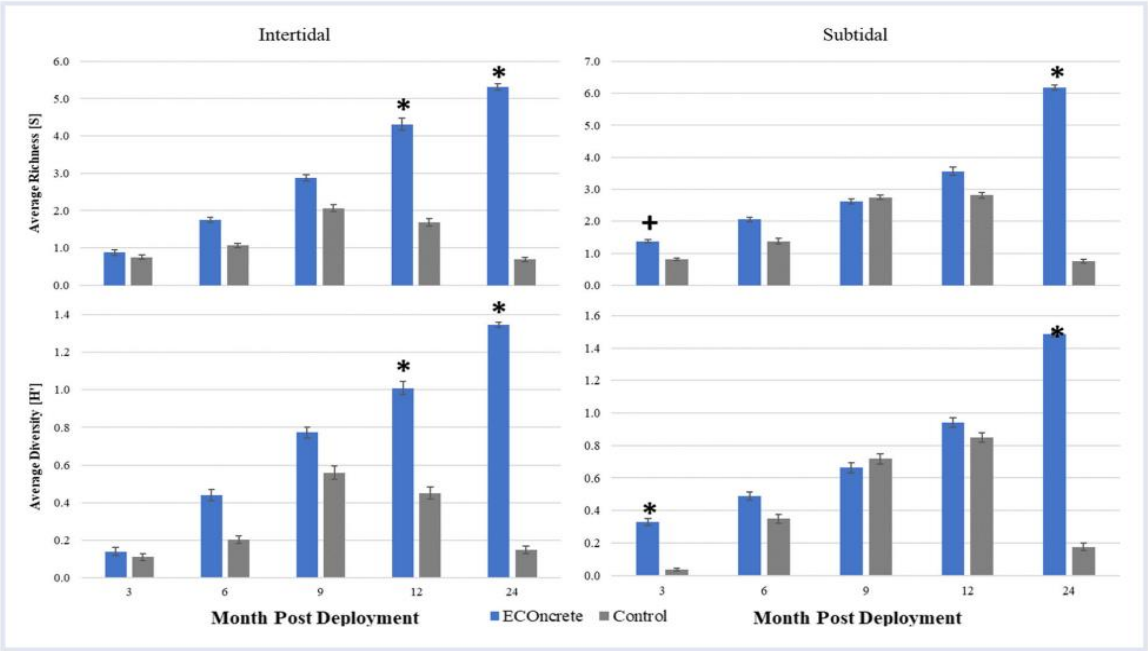

FIGURE 3 Differences in univariate parameters between ECO and control blocks at 3, 6, 9, 12, and 24 months postdeployment for intertidal (left) and subtidal (right) areas. *Significant differences ($p < 0.05$); +Marginal differences. Error bars represent the standard error

Sella et al., 2012

Figure A: Differences in the accumulation in univariate parameters when comparing the ECOncrete (treatment) to control over a 2-year period for both intertidal and subtidal conditions.

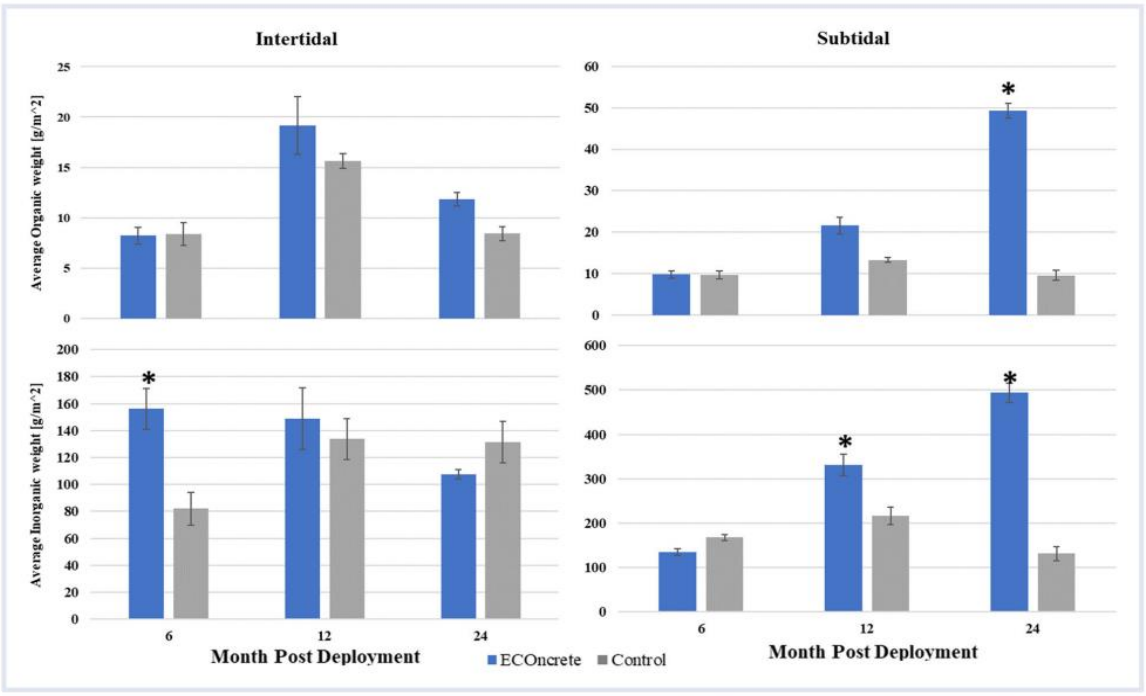

FIGURE 4 Differences in the accumulation of organic and inorganic biomasses between ECO and control blocks at 6, 12, and 24 months postdeployment for intertidal (left) and subtidal (right) areas. *Significant differences ($p < 0.05$). Error bars represent the standard error

Sella et al., 2012

Figure B. Differences in the accumulation of organic and inorganic mater when comparing the ECOncrete (treatment) to control over a 2-year period for both intertidal and subtidal conditions.

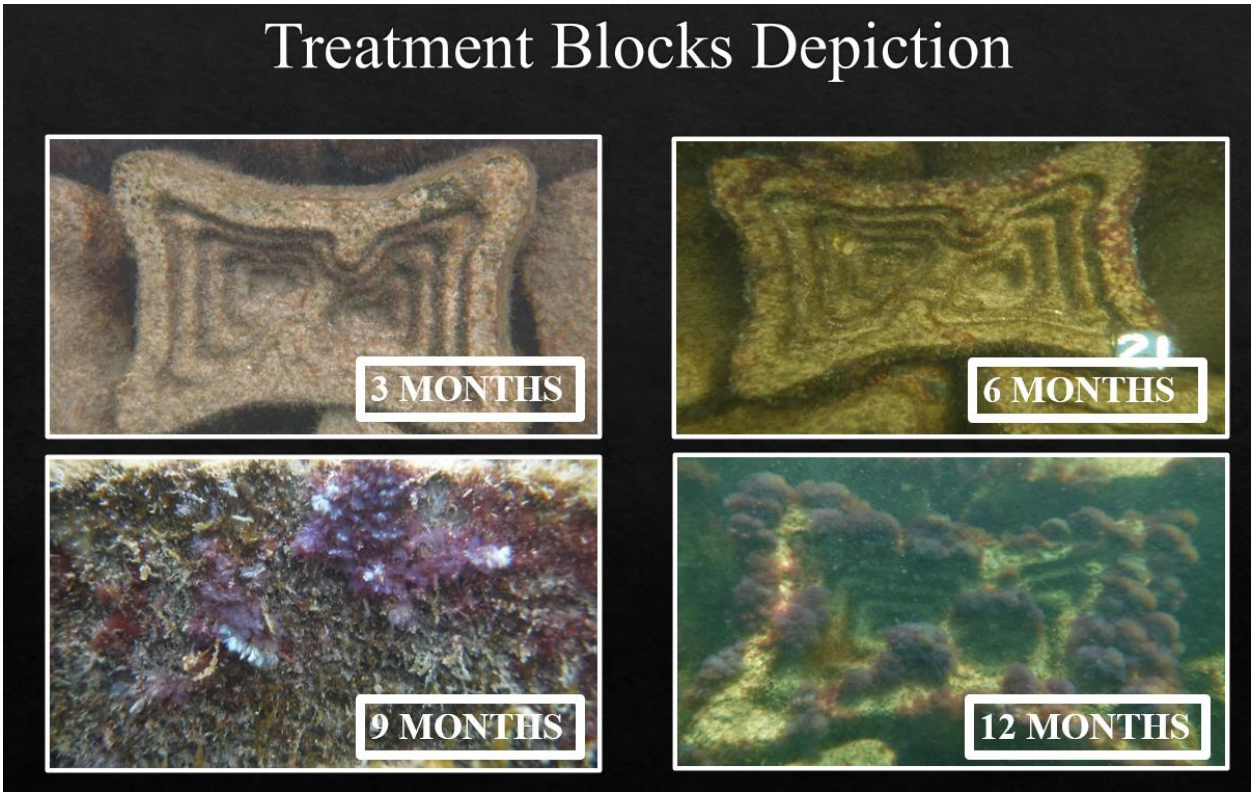

Figure C. Depiction of an ECOncrete block (treatment) the buildup of microorganisms at 3-, 6-, 9 - and 12-months post deployment.