5-2021

Cathodically Protected Steel Mesh as an Environmentally Friendly Material for Oyster Reef Restoration

Afanasy Melnikov

Cathodically Protected Steel Mesh as an Environmentally Friendly Material for Oyster Reef Restoration

by
Afanasy Melnikov

Bachelor of Science
Organismal Biology and Ecology
Middle Tennessee State University
2016

A thesis submitted to the Department of Ocean Engineering and Marine Sciences of Florida Institute of Technology
in partial fulfillment of the
requirements for the degree of

Master of Science
in
Biological Oceanography

Melbourne, Florida
May 2021

We the undersigned committee hereby approve the attached thesis, " Cathodically Protected Steel Mesh as an Environmentally Friendly Material for Oyster Reef Restoration" by Afanasy Melnikov

---

Kelli Z. Hunsucker, Ph.D.
Assistant Professor
Ocean Engineering and Marine Sciences
Major Advisor

Spencer Fire, Ph.D.
Assistant Professor
Ocean Engineering and Marine Sciences
Committee Member

Geoffrey Swain, Ph.D.
Professor
Ocean Engineering and Marine Sciences
Committee Member

Richard B. Aronson, Ph.D.
Professor and Department Head
Ocean Engineering and Marine Sciences

Abstract

Cathodically Protected Steel Mesh as an Environmentally Friendly Material for Oyster Reef Restoration

by

Afanasy Melnikov

Major Advisor: Kelli Z. Hunsucker, Ph.D.

Plastic mesh is widely used in oyster reef restoration efforts worldwide, including the Indian River Lagoon (IRL) estuary in Florida. Plastic degrades overtime, releasing microplastics and nanoplastics particles into the environment and causing harm to the marine ecosystem. Thus, there is an ongoing need to find an alternative eco-friendly material. Cathodically protected steel, a possible alternate substrate for such restoration, involves an electrical current running through steel to create an impressed cathodic system. This results in the formation of chalk on the steel, similar to the limestone (calcium carbonate) base of reefs. A benefit of this process is that the low current stimulates mineral growth and may aid in the shell formation of calcareous organisms, thus increasing the settlement and growth of oysters and other marine organisms. The aim of this thesis was to address the use of steel mats in the IRL for oyster reef restoration. Specifically, a two-part study was designed to determine the impact of cathodically protected steel on oyster growth and benthic organism biodiversity.

The first study measured the effect of three current treatments (low, medium and high) on growth and survival rates of the Eastern Oyster *Crassostrea virginica*. Replicate steel boxes for each of the electrical currents and control plastic boxes housed eight triploid oysters (25.6 mm – 43.3 mm) per box. Oyster growth was assessed by weekly weight and length measurements , as well as mortality counts. At the end of the 8-week experiment, a significant difference was found for the length growth rate among the steel treatments ($p < 0.05$). The highest length growth rate of 7.7% was observed with the high current treatment. The high current treatment and the low current treatment boxes had 90% survival rate compared to the medium current treatment and plastic controls which had a 75% survival rate.

The second study monitored cathodically protected steel mats and plastic mats over the course of 21-weeks at three testing sites in the IRL. Oyster settlement and biodiversity associated with the mats was conducted with weekly assessments. At the end of the experiment, 70 individual oysters were observed on the steel mats compared to 18 oysters on plastic mats at one of the test sites. The diversity on steel mats had a significant difference ($p < 0.05$) among test sites demonstrating the importance of ecological and environmental parameters for the success of the steel mats.

Overall, the research supported the idea that the steel mats can be used as an environmentally friendly alternative material for oyster reef restoration. The steel and plastic mats had comparable results for oyster growth and recruitment, as well as the biodiversity of sessile and mobile organisms. The chalking on electrical stimulated steel mats created a suitable substrate for the oysters and other hard and soft benthic organisms to grow not only on the dead oyster shells but also directly on the steel mats.

Table of Contents

Abstract	iii
Table of Contents	v
List of Figures	vii
List of Tables	ix
Acknowledgements	x
Dedication	xi
1.0 Background	1
2.0 Research Questions and Hypotheses	7
2.1 Research Questions	7
2.2 Hypotheses	7
3.0 The Effect of Current on Oyster Growth & Survival	9
3.1 Introduction	9
3.2 Methodology	11
3.3 Results	18
3.4 Discussion	31
4.0 Biodiversity Associated with Cathodically Protected Steel Mats	34
4.1 Introduction	34
4.2 Methodology	36
4.3 Results	42

Table of Contents (cont.)

4.4 Discussion	66
5.0 Conclusion and Recommendation for Future Work	72
References	74
Appendix A	83
Appendix B	86
Appendix C	87

List of Figures

Figure 1.1	1
Figure 1.2	2
Figure 1.3	3
Figure 3.1	11
Figure 3.2	12
Figure 3.3	14
Figure 3.4	15
Figure 3.5	16
Figure 3.6	17
Figure 3.7	18
Figure 3.8	21
Figure 3.9	25
Figure 3.10	27
Figure 3.11	30
Figure 4.1	38
Figure 4.2	39
Figure 4.3	40
Figure 4.4	43
Figure 4.5	44

List of Figures (cont.)

Figure 4.6	46
Figure 4.7	47
Figure 4.8	49
Figure 4.9	52
Figure 4.10	53
Figure 4.11	54
Figure 4.12	55
Figure 4.13	56
Figure 4.14	57
Figure 4.15	60
Figure 4.16	63
Figure 4.17	64
Figure 4.18	64
Figure A1	85
Figure A2	86
Figure B1	87
Figure C1	88
Figure C2	89
Figure C3	90
Figure C4	91

List of Tables

Table 3.1	20
Table 3.2	20
Table 3.3	23
Table 3.4	23
Table 3.5	24
Table 3.6	28
Table 4.1	50
Table 4.2	59
Table 4.3	62
Table 4.4	65
Table A1	84

Acknowledgements

I would like to thank everyone who has helped make this thesis research possible:

- I want to start by thanking Dr. Hunsucker and Dr. Geoffrey Swain for all the help through CCBC lab, as well as my third committee member Dr. Spencer Fire, who all gave continuous support and guidance to me!
- Thanks to the *Lagoon Tourism Grant, Environmentally Endangered Lands, Rib City, and Indian River Oyster Company* for the ongoing support.
- And ultimately, I want to thank OEMS Department and Florida Institute of Technology for this amazing opportunity to further my knowledge in this everchanging world!

To my family:
grandparents Yuri and Alexandra,
parents Max and Larissa
and my sister Yuliya

1.0 Background

Coral and oysters live in colonies and form reefs, which are cemented together by skeletons of calcium carbonate ($CaCO_3$) produced by extracellular biomineralization. Biomineral accretion depends on the saturation of seawater with required ions, pH, and the special chemical conditions inside cells (Furla *et al.*, 2000).

Hilbertz (1970) developed a method to stimulate this mineral accretion on cathodically protected steel using a low-voltage electrical current. This technology, formerly known as Biorock, uses the electrolysis of seawater to precipitate calcium and magnesium minerals to “grow” a crystalline coating over artificial structures (Hilbertz 1975, 1979). The electrical stimulated mineral accretion (Figure 1.1) is composed largely of calcium carbonate which is similar in chemical and physical properties to reef limestones that are primarily the remains of the aragonite skeletons of corals and green calcareous algae (Hilbertz 1992).

Figure 1.1. Biorock materials grown at Ihuru, North Male Atoll, Maldives. The steel is cathodically protected to prevent corrosion and allow for the buildup of mineral accretion (Goreau 2012).

Follow-up work experimented with different structures along the Louisiana and Texas coasts and discovered that this method can aid in coral reef restoration especially in areas where coral will not grow due to excessive physical or chemical stresses (Goreau and Hilbertz 2005). An example of an artificial coral reef created through this process can be seen in Figure 1.2. It is reported that**,** on a steel cathode coral grows 2-10 times faster, healing after breakage is 2-20 times greater and settlement increases up to 1000 times (Goreau and Hilbertz 2008, 2012).

Mineral accretion methods have been used for coral and oyster reef restoration, beach erosion control, and fish habitat conservation in locations such as Indonesia (Baxti 2012), Jamaica, and Maldives (Goreau *et al.,* 2012, Goreau and Prong 2017). Several studies have also tested physiological responses of corals to electrical fields (Borell *et al*., 2009), zooxanthellae abundance in electrically stimulated corals (Goreau *at al*., 2004), coral reef and fisheries habitat restoration (Goreau 2010), and rehabilitation for cold-water coral reefs (Stromberg *et al*., 2010). Experiments with oysters show that, under

Figure 1.2. Pemuteran Bali coral restoration project in 2010 (Goreau *et al.,* 2012)

electrical stimulation, oysters grow faster and have higher survival rates (Karissa *at al*., 2012).

The mineral accretion process described above is based on electrolysis of seawater, which is also used for other applications such as plating metals, removing rust, and creating hydrogen batteries (Britannica 2002). A low voltage, direct current is applied to an electrical circuit with two metal electrodes immersed in seawater.

An example of such a system with an anode, a cathode, and a power source on a typical cathodically impressed system is shown in Figure 1.3. At the positively charged anode, electrons flow from the metal into the water and cause H_2O to break down and form oxygen gas and hydrogen ions. The surrounding area becomes a highly acidic and oxidizing environment, keeping the anode small. At the negatively charged cathode, electrons flow into the metal. The surrounding area becomes alkaline, and under these conditions, calcium and other minerals are no longer soluble in water. The minerals

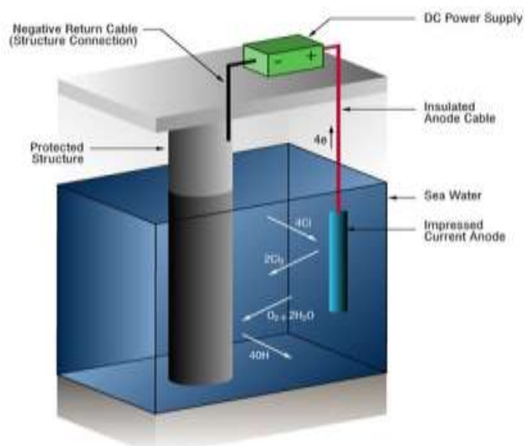


Figure 1.3. An example of an impressed current cathodic protection system (Baxter and Britten 2007)

precipitate from the water and accumulate on the cathode. This mineral accretion is a poor electrical conductor and protects the metal cathode from rusting and corrosion. Steel is usually used for cathodes since it permits water to flow though the structure like in the natural coral reefs (Goreau 2017).

The physical properties of the mineral accretion depend on electrical current parameters. Higher current densities result in faster accumulation, but create a weaker material dominated by Mg $(OH)_2$ (brucite). Lower current densities produce slower deposition rates but result in a harder material dominated by $CaCO_3$ (aragonite). The chemical reactions which result in the deposition of minerals are described below (Hilbertz 1992).

The oxidation reaction at the anode creates an acidic environment and a release of chlorine gas:

$$2H_2O = 4H^+ + O_2 + 4e^-$$

$$2Cl^- = Cl_2 + 2e^-$$

Which precipitates calcium and magnesium minerals from seawater:

$$OH^- + HCO_3^- + Ca^{+2} = CaCO_3 + H_2O$$

$$2OH^- + Mg^{+2} = Mg\,(OH)_2$$

The sum of the net reactions at both electrodes should be neutral regarding hydrogen ion production, and hence regarding CO_2 generation through acid–base equilibrium and carbonic acid hydrolysis:

$$2HCO_3^{-} = CO_3^{-2} + CO_2 + H_2O$$

Initially, it was assumed that the key factor to stimulate coral growth was the higher pH produced on the surface of the growing mineral accretion structure. Further work revealed that soft corals and organisms lacking limestone skeletons (e.g. sponges and tunicates) also seem to settle and grow at extraordinary rates on these surfaces (Goreau 2010). The surrounding environment also was positively impacted by the electrical field generated from the current. For example, Goreau (2012) reported that organisms, which broke off and fell to the ocean floor still gained the beneficial support of the system. There was also a much higher coral settlement and growth in the areas surrounding the structures (Goreau 2012).

Oysters filter large volumes of water daily, removing algae and sediments and aiding in cleaning up bodies of water (Coen *et al*., 2007, Beck *et al*., 2011). Oyster reefs play a vital role in the natural ecosystem of the Indian River Lagoon (IRL); however, their abundance has declined drastically over the past several decades (Garvis *et al*., 2015). Many oyster restoration efforts are currently underway in the IRL, but the majority of these involve the deployment of plastic oyster mats. Although these mats do reach the intended goals in the restoration, they can cause negative impacts as well. Plastic mats are far more brittle than steel mesh, and as a result, break down into smaller pieces that are released into the waterway. Once the plastic is in the ecosystem, it can be ingested by organisms throughout the food web and may eventually reach human consumers (Ogunola 2016).

A preferable alternative to the use of plastics for oyster restoration is the use of mineral accretion on steel mats. If steel mats break down, the elemental components are mainly iron and carbon, which are far less harmful to the ecosystem and may in fact enhance the growth of oysters and other calcareous organisms. Building off the above-mentioned work, this thesis will begin to address the use of cathodically protected steel mats in the IRL for oyster reef restoration.

2.0 Research Questions and Hypotheses

This thesis is part of a larger project to test the efficacy of using cathodically protected steel as an alternative to plastic in the IRL (Hunsucker *et al*., 2021). Specifically, the thesis will address the following questions and hypothesis through two experiments.

2.1 Research Questions

1. Will oysters grow faster on a cathodically protected steel substrate when compared to traditional plastic substrate?

2. Will the oyster survival rate be greater on a cathodically protected steel than plastic substrate?

3. How does the community composition on a cathodically protected steel mat compare to the community which develops on plastic oyster mat?

2.2 Hypotheses

Hypothesis 1:

Oysters will grow faster when cathodically protected steel is used as the substrate rather than traditional plastic substrate.

Hypothesis 2:

Survival rates of oysters will be greater on cathodically protected steel mesh when compared to a plastic mesh.

Hypothesis 3:

The biodiversity of sessile and mobile organisms will be greater on the steel mesh than that of the standard plastic mesh.

3.0 The Effect of Current on Oyster Growth & Survival

3.1 Introduction

Oysters filter large volumes of water daily, removing algae and sediments and aiding in cleaning up bodies of water (Coen *et al*., 2007, Beck *et al*., 2011). Oyster reefs play a vital role in the natural ecosystem of the Indian River Lagoon (IRL); however, their abundance has declined drastically over the past several decades (Garvis *et al*., 2015). Many oyster restoration efforts are currently underway in the IRL, but the majority of these involve the deployment of plastic oyster mats.

Although these efforts do reach the intended goals in the restoration, they can cause negative impacts as well. Overtime the plastic degrades and breaks down into smaller and smaller pieces that are released into the waterway. Once in the environment, plastic debris fragments into smaller particles: microplastics particles < 5 mm (NOAA, 2008) and nanoplastics particles < 10^{-4} mm (Gigault *et al.* 2018). In the marine environment, plastic goes through complex physicochemical and biological modifications like leaching chemicals into water or absorb pollutants from the water (Paul-Pont *et al.* 2018). Once the plastic is in the ecosystem, it can be ingested by organisms throughout the food web and may eventually reach human consumers (Ogunola 2016).

Bivalves are particularly vulnerable to contaminants in the estuarine and open coast environments they inhabit. As filter feeders, oysters consume at the lowest trophic level: phytoplankton and suspended material. This increases their potential for accumulation and biomagnifications of contaminants and subjects them to biological effects. The research of microplastics pollution in Mosquito Lagoon found that waters had an average of 21.4 ± 13.1 microplastic pieces per liter, and the mean number of microplastic pieces found in *Crassostrea virginica* was 16.5 microplastic pieces per adult oyster (Waite 2018).

Recently, many studies evaluated the effects of microplastics and nanoplastics on oysters. Microplastics in sizes greater than 100 μm are mostly rejected by oysters (Ward *et al.*, 2019). Nanoplastics affect gametogenesis of adult oysters (quantity and quality gametes) crating negative effects on the performance of offspring (Sussarellu *et al.*, 2016, Gonzalez-Fernandez *et al*., 2018). Further, nanoplastics can be injected by oyster larvae and cause significant decrease in fertilization and embryogenesis success, create numerous malformations in embryo-larval development (Cole and Galloway 2015, Tallec et al., 2018). The risk associated with nanomaterials is higher due to their reactivity and capacity to cross biological membranes. The toxic materials attached to nanoplastics can bioaccumulate on the cellular level (Gaspar *et al*., 2018)

A preferable alternative to the use of plastics for oyster restoration is the use of mineral accretion on steel mats. If steel mats break down, the elemental components are mainly iron and carbon, which are far less harmful to the ecosystem and may in fact enhance the growth of oysters and other calcareous organisms (Swain *et al*., 1994). This thesis is researching the possibility of the usage the cathodically protected steel mats in the IRL for oyster reef restoration.

This chapter was designed to test the hypotheses that oysters will grow faster when cathodically protected steel is used as the substrate rather than traditional plastic substrate and survival rates of oysters will be greater on cathodically protected steel mesh when compared to a plastic mesh.

3.1 Methodology

Oyster Box Experiment Design

Oyster growth and survival as a result of an applied mild electrical current on a steel mesh was tested using a box design as seen in Figure 3.1 (Hilbert *et al*., 1996)

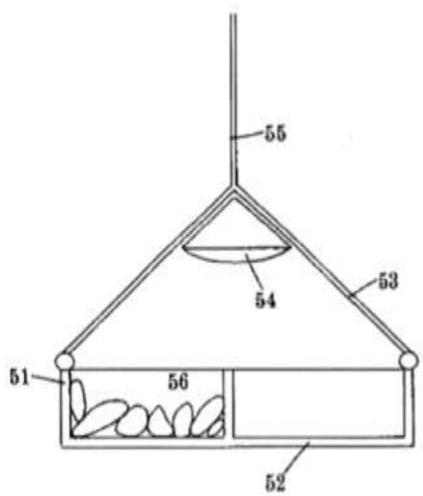


Figure 3.1. A sectional view of a rack utilized for cultivation of oyster, mussel, or other shellfish (56) where 51 and 52 is cathodic material, 53 and 55 are ropes, and 54 is light source. An anode (not shown) is suspended nearby (Hilbertz *et al*., 1996)

Figure 3.2. A set of steel boxes prior immersion (left) and deployed (right) at the site at the Port Canaveral, Florida.

Three replicate steel boxes were constructed for three treatments, for a total of nine boxes. Three additional plastic boxes were built to serve as controls. Construction began with the base. Each steel bottom was cut by hand from a large sheet to the size of 8 x 8 inch (20.32 x 20.32 cm) using steel cutting shears. After completing 9 individual bottoms the sides and top where cut out of a large sheet of plastic mesh and connected via zip ties. To ensure that the electrical field reached the oysters the sides and tops of the steel sets were plastic instead of steel. The 3 replicates for all the plastic boxes were cut out of a single piece of plastic mesh and folded to create a box and zip tied together in the same dimensions of the steel boxes. The same materials are used as in Chapter 4: 0.25" hole (0.64 cm) 18-gauge hot roll expanded flattened A36 steel mesh (onlimemetals.com) and aquaculture grade Aquatic Ecosystems, N0350, plastic mesh with ¾-inch (1.91 cm) square holes.

Once all the boxes were completed the steel sets were connected in a triangular position as seen in Figure 3.2. A metal bar from the same steel mesh was used to connect the 3 boxes together with nuts and bolts to ensure electrical current could flow through all the boxes from a single connection. Following the connection of 3 replicates to each other a wire was attached using 14 AWG marine grade wire to one of the boxes in the trio. The wire allowed electrical current to flow from the power source to the steel boxes. A nut and bolt held the wire to the steel mesh and epoxy covered the connection to insure a watertight connection. To complete a cathodic impressed current a mixed metal oxide (MMO) metal was cut into 3 individual sections for attachment to each treatment (Figure 3.2). Each anode had a wire attached to it via a nut and bolt was encased in epoxy at the top and bottom.

Three different treatments were tested on the steel boxes: a low current 60 mA (density 0.33 A/m^2) marked with a green wire, a medium current 150 mA (density 0.83 A/m^2) marked with an orange wire, and a high current 450 mA (density 2.50 A/m^2). For each treatment there was 3 replicates of steel boxes and 3 control plastic boxes. Three current levels were applied by one DC source using resistors to find the most beneficial setting for the oyster growth and survival rates (Figure 3.3). Connections are made with the replicates of each treatment in parallel from one DC source. The voltage was monitored and kept constant for each treatment throughout the experiment and measured using a voltmeter.

Two electrical wires were connected to the steel boxes with one for power and the other for electrical data collection. All electrical connections were secured with marine grade epoxy. The power source was connected to the boxes, along with a cathode and a mixed metal oxide (MMO) anode to complete the cathodic system. One anode was used for each set of steel boxes. The plastic boxes are the same size as the steel without any

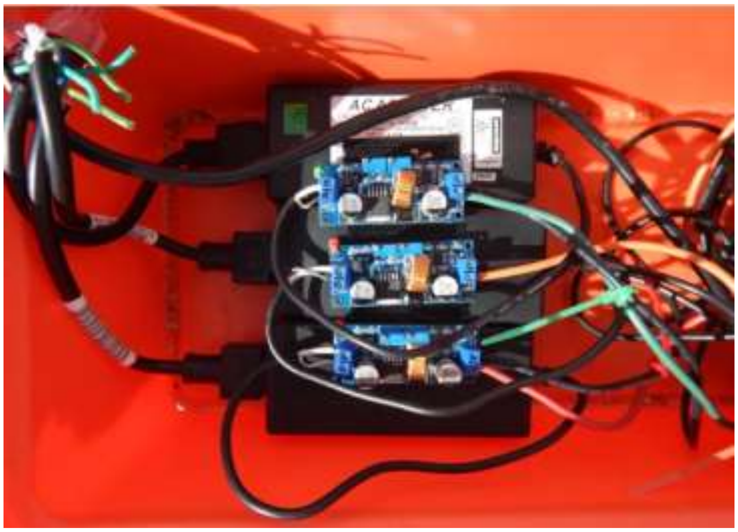


Figure 3.3. Power source setup for three different currents.

electrical wiring. The current and potential of the system were recorded each week using a clamp on ammeter and an Ag/AgCl reference electrode, respectively.

Oyster Collection & Growth

Under optimal conditions the oysters from the size 30 – 40 mm can grow up to 65 mm during May – October growing season (Doiron 2008), which should allow for results to be observed over the course of experiment. The testing was delayed until September due to the national lockdown as the result of COVID-19 and an August algae bloom (see green coloration of water in Figure 3.2). One hundred oysters were bought from

Figure 3.4. Oyster marked for easy measurement prior immersion.

The Indian River Oyster Company (IROC) located in New Smyrna Beach, Florida. All oysters were triploid which means they were sterile. Triploid oysters were chosen because they spend energy to grow larger in the summer months instead focusing on reproducing (Basics of Triploidy 2020). The size of the purchased oysters ranged from 1.0 - 1.7 inches (25.6 mm – 43.3 mm) and fit well in the range of 1-2 inches (25.4 mm - 50 mm) planned for the experiment.

The oyster shells were cleaned at the beginning of the experiment and groomed each week to remove the biofouling organisms settled on the oyster shell, such as: barnacles, bryozoans, tubeworms, etc. Oysters were marked the with a non-toxic paint pen for the identification during the experiment (Figure 3.4).

At the beginning of the experiment oysters were placed into a bucket of seawater for the conditioning. Conditioning allowed the acclimation of the oysters to the test site.

After the conditioning eight oysters were placed in each box. All boxes sets were deployed into water on September 11, 2020.

Data was collected on the growth of the oysters using measurements of length and weight. The measurement of length is chosen since the non-uniformly shape of oyster shell makes it hard to measure the width consistently (Figure 3.5**).** Weekly assessments of oyster length were done with digital calipers and the weight taken via a digital scale. Photos and notes were also taken weekly of each oyster. Figure 3.6 is showing images from a typical weekly assessment.

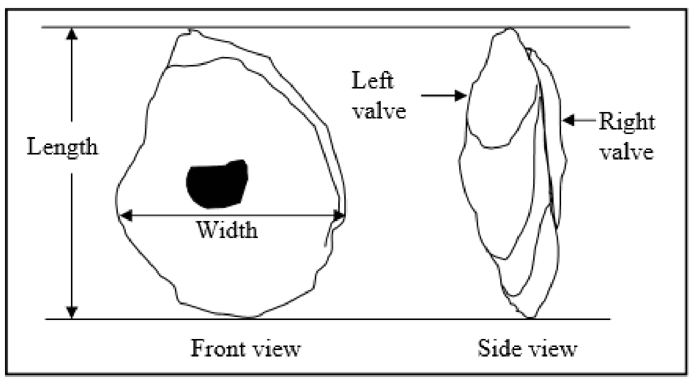


Figure 3.5. Illustration of the two valves of an oyster (Doiron 2008) to demonstrate how weekly oyster length measurements were taken.

Immersion Facility

During September, boxes with oysters were deployed at Florida Institute of Technology's Center for Corrosion and Biofouling Control (CCBC) research platform at in Port Canaveral (28°24'31.01"N, 80°37'39.54"W). This site has served as the location for preliminary testing and as well as part of a larger study which confirmed that the water

a) b) c) d) e) f)

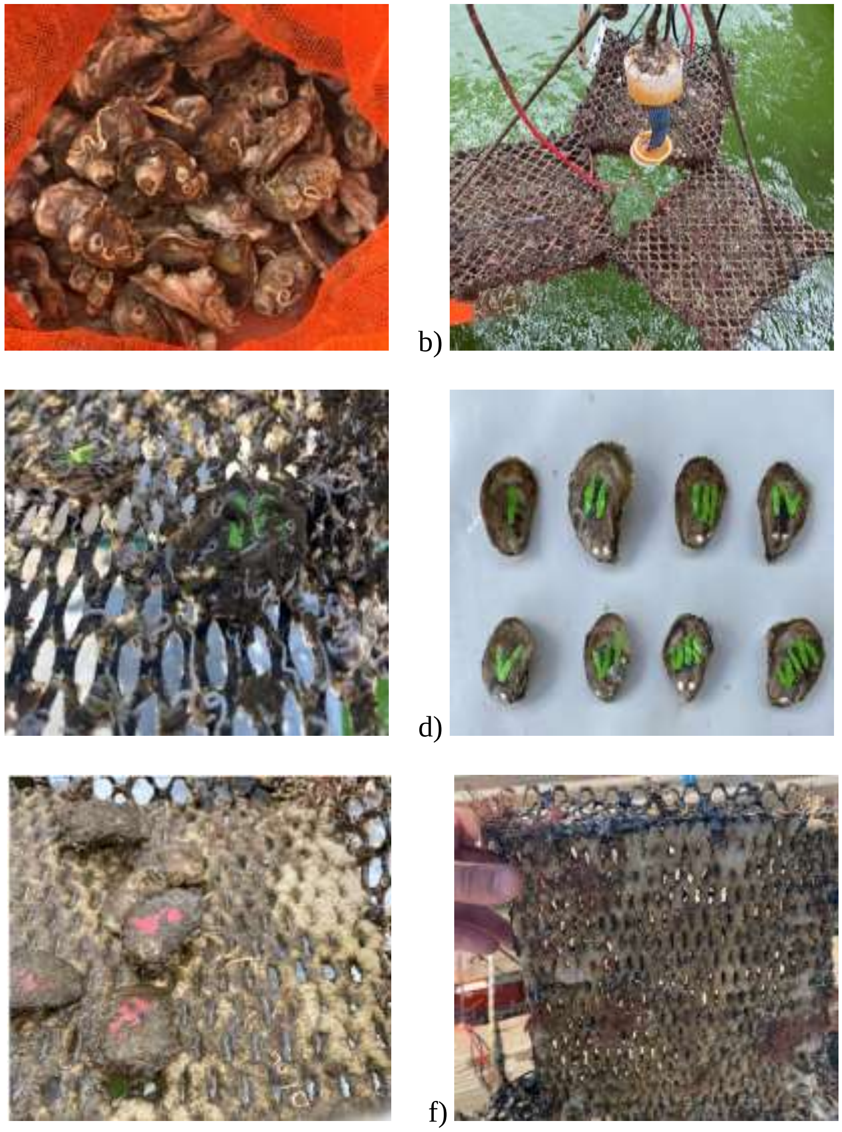


Figure 3.6. Images of purchased oysters and sets from a typical weekly assessment: a) initial stock, b) boxes pulled after immersion, c) uncleaned oysters, d) cleaned oysters before the immersion, e) mineral accretion on the steel bottom inside the box, f) mineral accretion outside the box.

quality is sufficient for oyster survival. The average salinity at this site is 34.4 ± 1.4 ppt, the average water temperature is 27.8 ± 1.0°C and the average pH is 8.2 ± 0.1 (as measured during an experiment in 2019). Port Canaveral is one of the test sites used for the experiments described in Chapter 4.

Statistics

Simple analysis of variance ANOVA test was used to identify variations between different electrical current treatments and plastic. If the significant difference was found in the test group, then the analysis was performed on sub-groups. Linear regression plots were used to visualize differences between the electrical treatments and plastic. The Data Analysis pack in MS Excel 2016 was used for data analysis.

3.3 Results

Current and Potential Measurements

Figure 3.7 shows the relationship between potential and current density at the three steel boxes sets. The low current boxes averaged the following measured values: current 63.11 ± 12.82 mA, potential -1.17 ± 0.18 V, and density 0.33 ± 0.03 A/m^2. The medium current boxes averaged the following measured values: current 133.89 ± 28.23 mA, potential -1.27 ± 0.17 V, and density 0.83 ± 0.05 A/m^2. The high current boxes averaged the following measured values: current 393.89 ± 105.45 mA, potential -1.45 ± 0.18 V, and density 2.32 ± 0.45 A/m^2.

Oyster Weight and Length Measurements

Oysters grow in different directions: some may grow more in length, some more in width, and others grow evenly in both directions. Data on the oyster growth

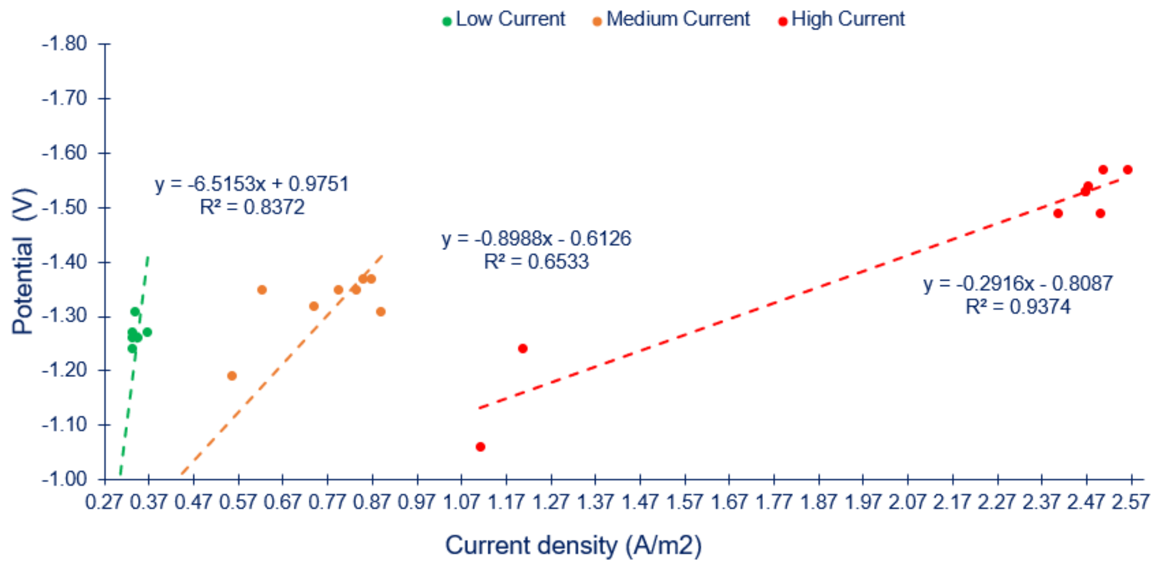


Figure 3.7. Potential versus current density recorded for steel boxes.

was collected using measurements of length and weight. Length was chosen due to the non-uniform shape of oyster shell which makes it hard to measure the width consistently.

In order to have a meaningful comparison of oyster growth between different current treatments on the steel mats and plastic mats, it was important to make sure that the initial oyster stock had similar distribution.

The weight and length of the initial oyster stock was checked for a normal distribution with a 95% confidence level. The weight of initial oyster stock was not statistically different between testing samples (p-value > 0.05) (Table 3.1). The length of the initial oyster stock was different (p-value < 0.05) (Table 3.2). Although the analysis results show a difference in initial oyster lengths, no oysters were removed as outliers. The test groups were treated as comparable because the rate of growth is independent

Table 3.1. Initial weight (g) of the oyster stock.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Low current	24	111.34	4.64	1.53
Medium current	24	119.24	4.97	1.81
High current	24	119.54	4.98	1.51
Plastic	24	108.57	4.52	2.75

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.87	3	1.29	0.679	0.567	2.704
Within Groups	174.77	92	1.90			
Total	178.64	95				

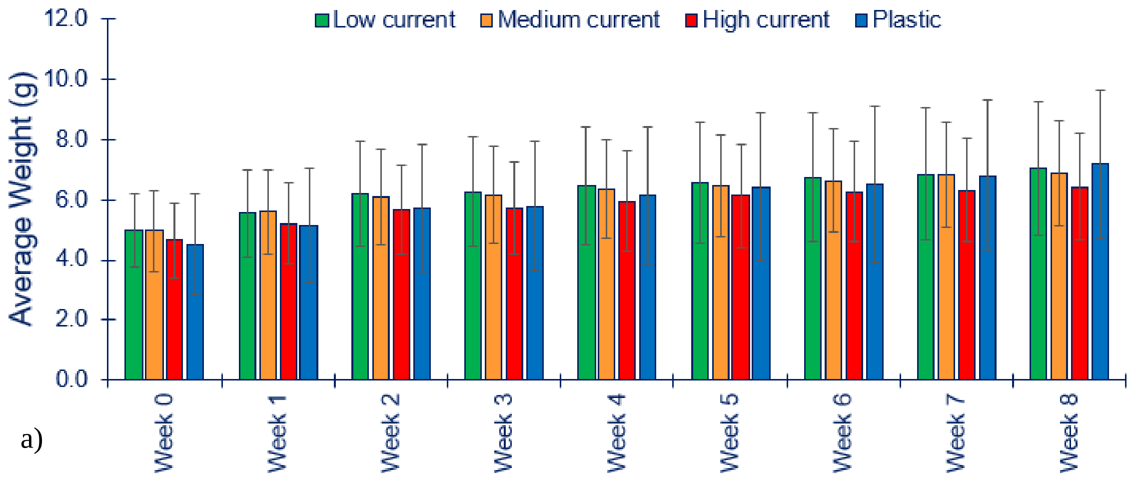
Table 3.2. Initial length (mm) of the oyster stock.

SUMMARY

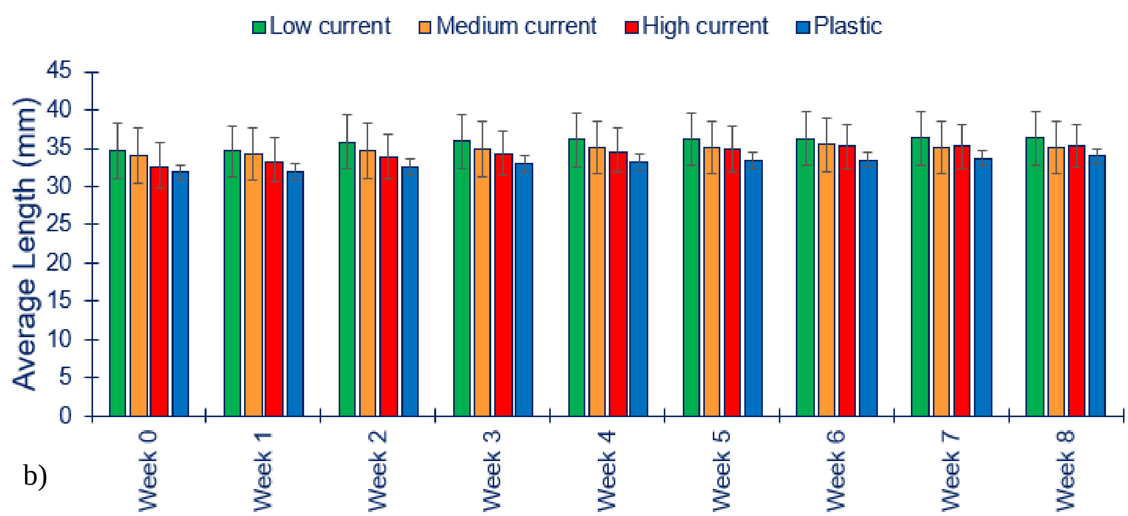
Groups	*Count*	*Sum*	*Average*	*Variance*
Low current	24	830.47	34.60	13.22
Medium current	24	816.85	34.04	13.61
High current	24	782.05	32.59	10.03
Plastic	24	763.99	31.83	7.80

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	117.51	3	39.17	3.509	0.018	2.704
Within Groups	1026.96	92	11.16			
Total	1144.47	95				



a)



b)

Figure 3.8. Average weekly measurements with standard deviation: a) weight, b) length.

from the overall size. Another reason to include all oysters in the analysis is not all oysters survived the experiment.

The average weight of oysters after eight weeks of immersion was 7.04 ± 1.76 g for oysters on the steel with the low current treatment, 6.87 ± 1.75 g for oysters on the steel with the medium current treatment, 6.43 ± 2.20 g for oysters on the steel with the high current treatment, and 7.18 ± 2.46 g for oysters on the plastic mats (Figure 3.8 a).

The average length of oysters after eight weeks of immersion was 36.34 ± 3.55 mm for oysters with the low current treatment, 35.18 ± 3.41 mm with the medium current treatment, 35.33 ± 2.74 mm with the high current treatment, and 33.99 ± 2.81 mm for oysters on the plastic mats (Figure 3.8 b).

The final weight and length of the oysters at the end of the experiment were checked for a normal distribution with a 95% confidence level. The final weight (Table 3.3) and length (Table 3.4) of oysters were not statistically different (p-value > 0.05).

Descriptive statistics for the final weight (a) and length (b) for all boxes at the end of the experiment is presented in the table 3.5. The oyster with the highest maximum weight was found in the first box (11.92 g) of the plastic set. The longest oyster was found in the third box (43.88 mm) of the low current boxes set.

Among the steel boxes sets, all oysters with the maximum weight (10.74 g, 11.86 g, 10.56 g) and length (40.24 mm, 42.69 mm, 43.88 mm) were found with the low current treatment in box one, two and three respectively.

The boxes with electrical treatments had series circuit connections. The oyster maximum weight in each electrical treatment was found in the second box in series: in

Table 3.3. Final weight (g) of the oyster stock.

SUMMARY

Groups	Count	Sum	Average	Variance
Low current	21	147.89	7.04	4.83
Medium current	18	123.57	6.87	3.07
High current	21	135.01	6.43	3.10
Plastic	18	129.17	7.18	6.06

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	6.39	3	2.13	0.502	0.682	2.728
Within Groups	313.88	74	4.24			
Total	320.27	77				

Table 3.4. Final length (mm) of the oyster stock.

SUMMARY

Groups	Count	Sum	Average	Variance
Low current	21	741.90	35.33	7.53
Medium current	18	633.20	35.18	11.62
High current	21	763.19	36.34	12.59
Plastic	18	611.79	33.99	7.92

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	53.93	3	17.98	1.811	0.152	2.728
Within Groups	734.40	74	9.92			
Total	788.33	77				

Table 3.5. Descriptive statistics for each test box after 8 weeks of immersion: a) weight (g), b) length (mm).

a)

Test Treatment	Count	Mean	St. Dev	Variance	Minimum	Maximum	Range
Low current box 1	6	7.80	1.80	3.23	6.24	10.74	4.50
Low current box 2	7	6.61	2.57	6.58	3.90	**11.86**	7.96
Low current box 3	8	6.85	2.27	5.14	3.77	10.56	6.79
Medium current box 1	7	6.48	2.50	6.24	2.91	9.01	6.10
Medium current box 2	5	7.41	1.38	1.91	5.75	**9.26**	3.51
Medium current box 3	6	6.86	0.96	0.93	5.17	7.79	2.62
High current box 1	6	7.23	1.71	2.93	4.80	9.04	4.24
High current box 2	7	6.46	1.98	3.92	3.56	**9.38**	5.82
High current box 3	8	5.80	1.55	2.41	2.70	7.77	5.07
Plastic box 1	6	6.87	2.74	7.50	4.65	**11.92**	7.27
Plastic box 2	5	7.24	1.99	3.96	4.19	9.67	5.48
Plastic box 3	7	7.39	2.85	8.12	4.16	11.47	7.31

b)

Test Treatment	Count	Mean	St. Dev	Variance	Minimum	Maximum	Range
Low current box 1	6	35.78	3.10	9.61	33.13	40.24	7.11
Low current box 2	7	35.45	3.59	12.87	31.90	42.69	10.79
Low current box 3	8	37.54	3.92	15.35	32.63	**43.88**	11.25
Medium current box 1	7	33.72	3.51	12.30	27.48	38.62	11.14
Medium current box 2	5	36.73	2.19	4.81	33.05	38.96	5.91
Medium current box 3	6	35.59	3.91	15.29	29.43	**41.24**	11.81
High current box 1	6	36.58	1.87	3.51	34.32	39.42	5.10
High current box 2	7	35.25	3.12	9.74	30.71	38.28	7.57
High current box 3	8	34.46	2.90	8.43	30.08	**40.18**	10.10
Plastic box 1	6	32.25	2.34	5.46	28.46	34.29	5.83
Plastic box 2	5	35.81	2.84	8.08	32.09	**39.82**	7.73
Plastic box 3	7	34.18	2.59	6.69	31.59	38.67	7.08

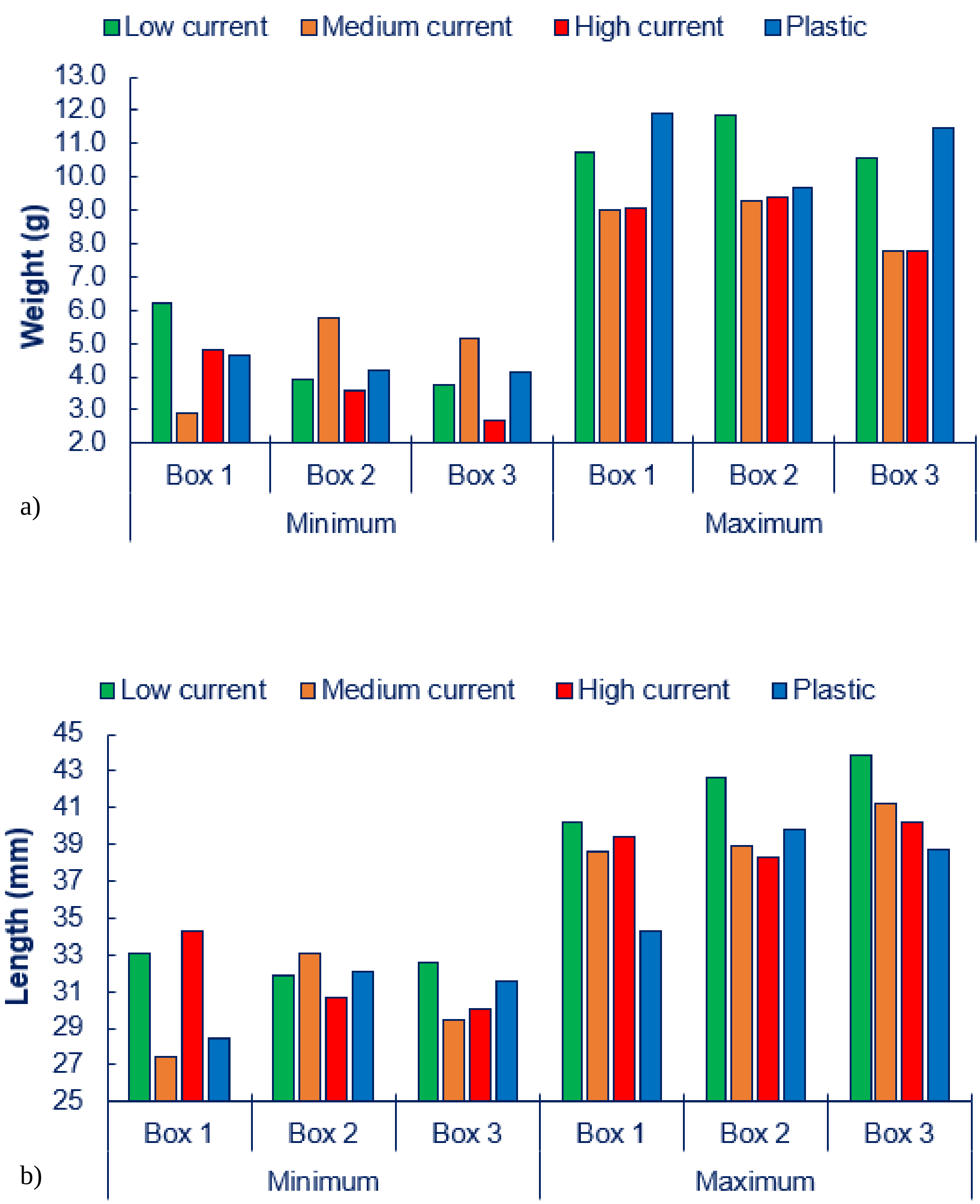


Figure 3.9. Minimum and maximum measurements for oysters in each box in all treatments: a) weight, b) length.

the second box (11.86 g) of the low current treatment set, in the second box (9.38 g) of the high current treatment set, and in the second box (9.26 g) of the medium current treatment set. The oysters with minimum weight were found in the third box (2.70 g) of the high current treatment set, in the first box (2.91 g) of the medium current treatment set, in the third box (3.77 g) of the low current treatment set (Figure 3.9 a).

The oyster maximum weight in each electrical treatment was found in the third box in series: in the third box (43.88 mm) of the low current treatment set, the third box (41.24 mm) of the medium current treatment set, in the third box (40.18 mm) of the high current treatment set. The oysters with the lowest minimum length were found in the first box (27.48 mm) of the medium current treatment set, in the first box (28.46 mm) of the plastic set, in the third box (30.08 mm) of the high current treatment set, and in the second box (31.90 mm) of the low current treatment set (Figure 3.9 b).

Figure 3.10 shows the growth rate of weight (a) and length (b) growth during the eight weeks of the immersion. The rates were calculated as the difference between each week value and initial value divided by initial value.

The growth weight rates did not have significant difference between all treatments and plastic (p value > 0.05). At the end of the experiment, the oyster highest weight growth rate (48%) was in the plastic set following by the low current treatment set (40%), the high current treatment set (36%), and the medium current treatment set (33%).

The growth length rates had significant differences between all treatments and plastic (p value = 0.004) (Table 3.6 a). The length of oysters was increased at significantly different rates between electrical treatments (p value = 0.002) (Table 3.6 b).

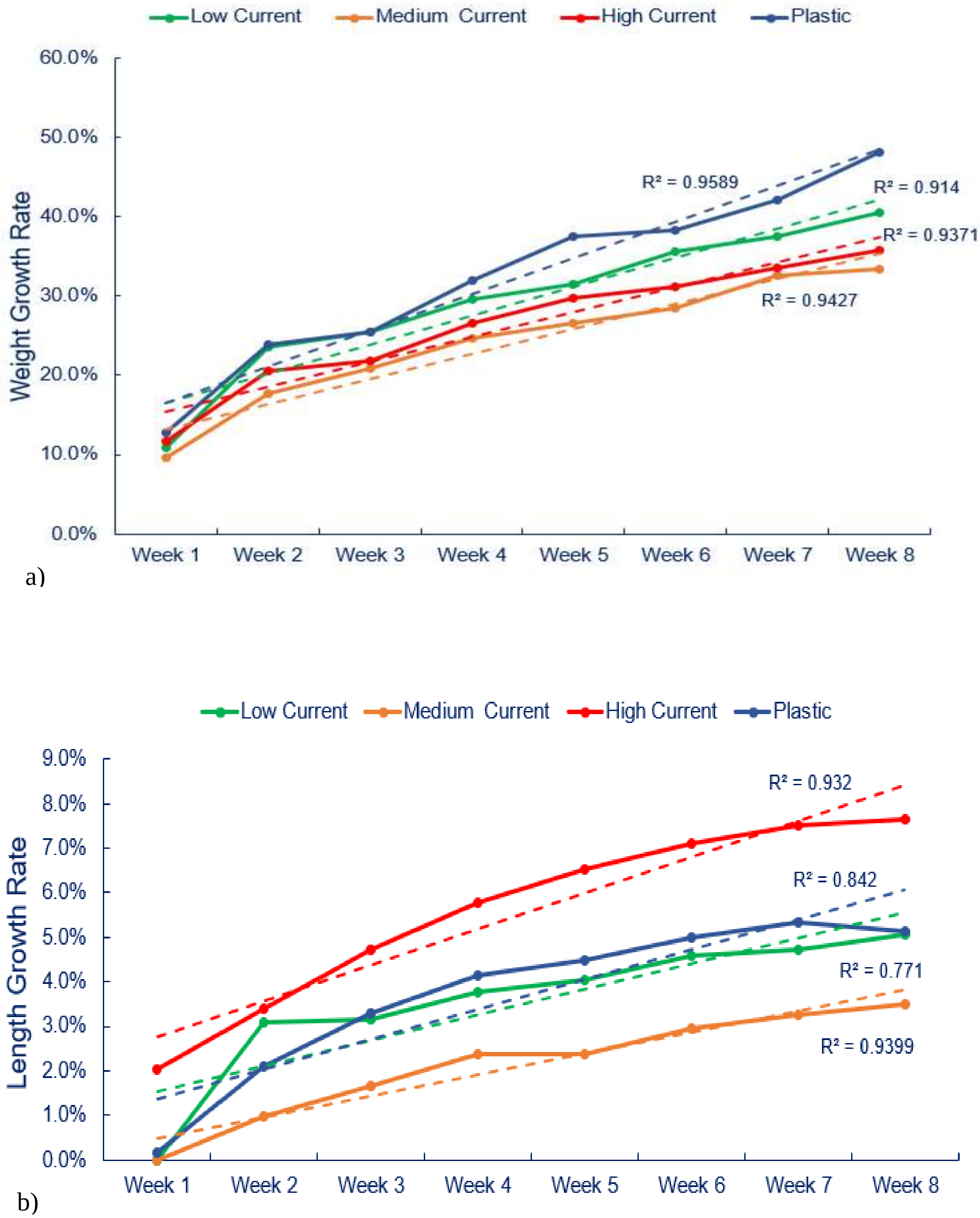


Figure 3.10. Observed oyster growth rates: a) weight, b) length.

Table 3.6. Oyster Length Growth Rates: a) all test treatments and plastic, b) all steel treatments.

a)

SUMMARY

Groups	Count	Sum	Average	Variance
Low current	8	0.28	0.04	0.00
Medium current	8	0.17	0.02	0.00
High current	8	0.45	0.06	0.00
Plastic	8	0.30	0.04	0.00

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.00	3	0.00	5.602	0.004	2.947
Within Groups	0.01	28	0.00			
Total	0.01	31				

b)

SUMMARY

Groups	Count	Sum	Average	Variance
Low current	8	0.283771	0.035471	0.000257
Medium current	8	0.171241	0.021405	0.000145
High current	8	0.446941	0.055868	0.00042

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.004804	2	0.002402	8.768468	0.001704	3.4668
Within Groups	0.005753	21	0.000274			
Total	0.010557	23				

At the end of the experiment, the highest growth rate based on length 7.7% was in the high current treatment set. The low current treatment and plastic set had a growth rate of 5.1%. The lowest final length growth rate was 3.5% in the medium current treatment set and plastic with 3.3%.

Oyster Survival Rate

Mortality counts were performed during each weekly assessment. The high current treatment and the low current treatment boxes both had 3 dead oysters in total during the experiment. The medium current treatment and plastic boxes both had 6 dead oysters in the total during the experiment.

The dead oysters were found during different weeks. Figure 3.11 shows the mortality counts and survival rates during the eight weeks immersion period. At the end of the experiment the high current treatment and the low current treatment boxes had 90% survival rate. The medium current treatment and plastic boxes had 75% survival rate.

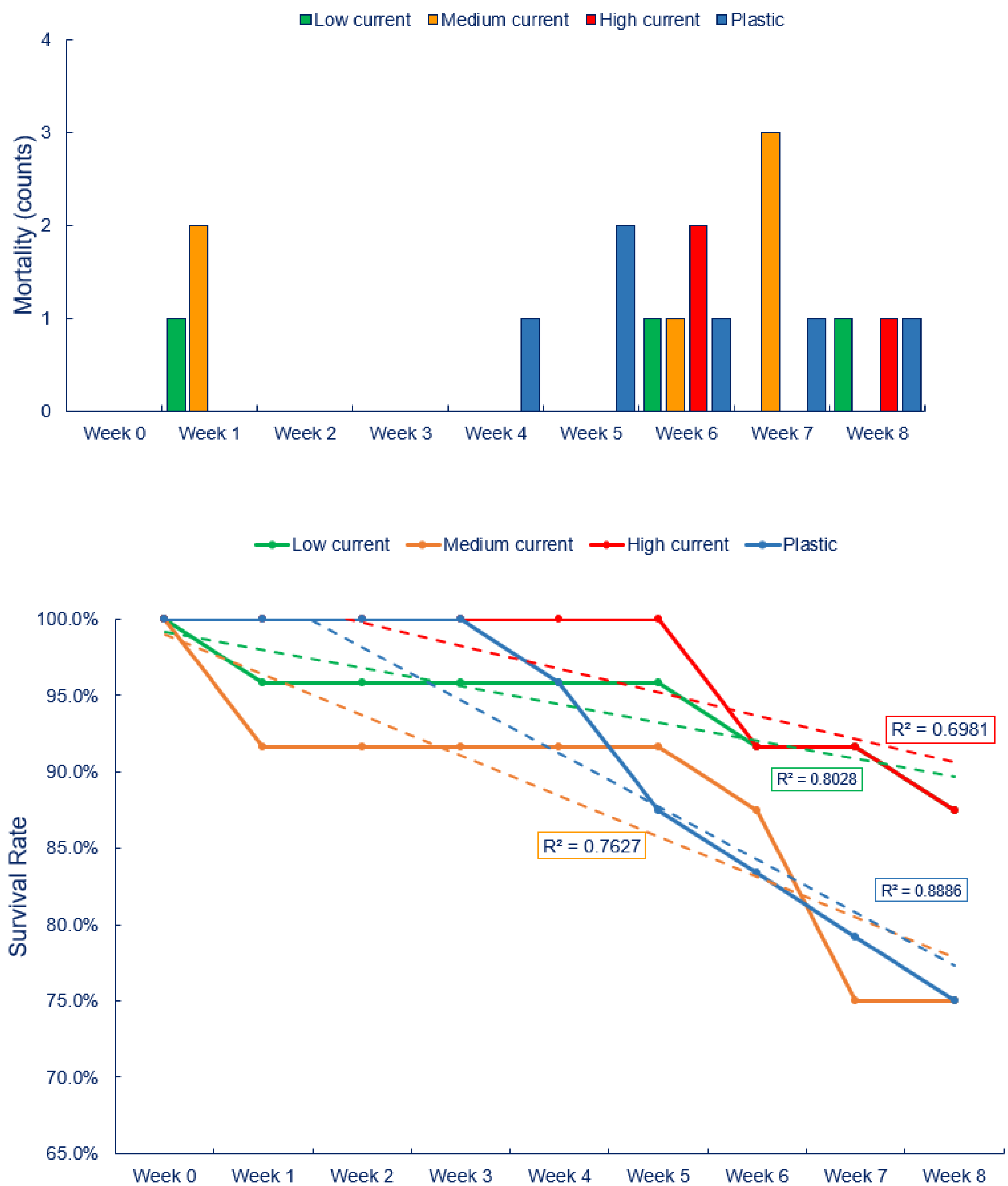


Figure 3.11. Mortality counts (upper) and survival rates (lower) for all test samples.

3.4 Discussion

To determine the efficacy of using a cathodically protected steel for oyster restoration and to evaluate oyster growth and survival, a steel mesh was compared to the traditional plastic mesh mats. This chapter was designed to test the hypotheses that oysters will grow faster when cathodically protected steel is used as the substrate rather than traditional plastic substrate and survival rates of oysters will be greater on cathodically protected steel mesh when compared to a plastic mesh.

During the eight weeks immersion period, the measurements of weight and length of oysters were performed weekly. There are three different current treatments applied to the steel boxes: a high current 450 mA (2.50 A/m^2), a medium current 150 (0.83 A/m^2), and a low current 60 mA (0.33 A/m^2). The potentials were typically more negative than -1.2 V. The measured current densities had the following values: current 63.11 ± 12.82 mA, potential -1.17 ± 0.18 V, and density 0.33 ± 0.03 A/m^2 for the low current treatment, current 133.89 ± 28.23 mA, potential -1.27 ± 0.17 V, and density 0.83 ± 0.05 A/m^2 for the medium current treatment, and current 393.89 ± 105.45 mA, potential -1.45 ± 0.18 V, and density 2.32 ± 0.45 A/m^2 for a high current treatment. These current densities were in the suggested range between 0.1 and 30.0 A/m^2 of cathode surface area from the previous studies (Hilbertz & Goreau 1996).

The results of weight measurements did not support the hypothesis that oyster will grow faster on the electrical stimulated steel mat than on plastic mat, but the results were comparable. The *ANOVA* statistical analysis for the oyster weight showed the final weight of oysters was not statistically different between all four test samples (*p*-value

>0.05) (Table 3.3). The oyster with the highest maximum weight (11.92 g) was found at the plastic set. Among the steel boxes sets, all oysters with the maximum weight (10.74 g, 11.86 g, 10.56 g) were found with the low current treatment boxes set (Figure 3.9 a). The rate of weight growth was not statistically different either (*p*-value >0.05). At the end of the experiment, the highest oyster weight growth rate (48%) was in the plastic boxes set following by the low current treatment boxes set (40%), the high current treatment boxes set (36%), and the medium current treatment boxes set (33%) (Figure 3.10 a). A previous study of low-voltage electrical stimulation on the early growth stage of pearl oyster *P. maxima* (Jameson) had the similar result. The weight of oysters after a three-month immersion period was not significantly different among electrical and non – electrical settings (Karissa *et al*., 2012).

The results of length measurements were partially supported the hypothesis that oyster will grow faster on the electrical stimulated steel mat than on plastic mat. After eight-week immersion, the oyster survival rates were 90% for the high and low currents and 75% for the medium current and plastic boxes.

The *ANOVA* statistical analysis for the oyster length shows the final length of oysters was not statistically different between all four test samples (*p*-value >0.05) (Table 3.4). The average length of oysters after eight weeks of immersion was 36.34 ± 3.55 mm for oysters with the low current treatment, 35.18 ± 3.41 mm with the medium current treatment, 35.33 ± 2.74 mm with the high current treatment, and 33.99 ± 2.81 mm for oysters on the plastic mats (Figure 3.8 a). On the other hand, the oyster length growth rate was statistically different (*p*-value < 0.05). The oyster highest length growth rate of 7.7%

was in the high current treatment set. The low current treatment and plastic set had the rate of 5.1%. The lowest length growth rate was 3.5% in the medium current treatment set (Figure 3.10 b). Other studies investigating electrical stimulation on oysters also found significantly higher growth rates based on the length measurements for *Crassostrea virginica* oysters (Berger *et al*., 2012) and *P. maxima* (Jameson) oysters (Karissa *et al*., 2012). A yearlong experiment in New York Bay with *Crassostrea virginica* oysters found they grew 9.30 times faster under the high power and 5.82 times faster under the medium power (Shorr *et al*., 2012)

More significant results between steel and plastic boxes may be achieved with a longer immersion time. For example, Berger *et al*., 2012 and Karissa *et al*., 2012 looked at the impact of electrically stimulated steel for three months of immersion, and Shorr *et al*., 2012) deployed experiments for twelve months. Also, the deployment of the experiment in the middle of September did not cover the typical May - October growing season for oysters (Doiron 2008). It is possible higher growth rates and increased weights may have been observed should this experiment have been conducted during the growing season.

4.0 Biodiversity Associated with Cathodically Protected Steel Mats

4.1 Introduction

The Indian River Lagoon (IRL), an estuary along Florida's east coast, is one of the most biologically diverse ecosystems in North America (Kjerfve 1986). However, ecosystem health has declined over the past few decades which has resulted in algal blooms, detrimental muck accumulation, seagrass decline, and fish kills. Human activities have increased nitrogen loads, total suspended solids, and nutrient concentrations in the lagoon which have created a major eutrophication problem resulting in algal blooms (Anderson *et. al.*, 2008). The decline in seagrass cover is the consequence of the increase in turbidity and phytoplankton abundance that blocks sunlight from reaching the benthos (Fronseca *et. al.*, 1998, Wall *et. al.*, 2008). Harmful algal blooms (HABs) are producing toxins that negatively impact marine mammals in the IRL, particularly bottlenose dolphins (*Tursiops truncatus*) which frequently experience mass mortality events (Fire *et. al.*, 2020).

Oyster reefs play a vital role in the natural ecosystem of the IRL; however, their abundance has declined drastically over the past several decades. For example, it has been reported that oyster reef cover has declined by 24% in Mosquito Lagoon and 40% in Canaveral National Seashore region (Garvis *et. al.*, 2015). One of the causes of recent large-scale algal blooms may be linked to the diminishing oyster population.

The eastern oyster, *Crassostrea virginica,* is often used for mitigation of eutrophication in coastal waters (Beseres *et. al.*, 2013, Kellogg *et. al.*, 2014). The ecosystem services provided by the eastern oyster, *Crassostrea virginica*, are important for the health of the estuary (Garvis et al. 2015; Dame 2012). The restoration of a solid biodiverse oyster reef is important for improving the water quality, increasing the richness of fish species and creating a strong trophic network (Harding and Mann 1999). Oysters and organisms associated with the oyster reef have water filtration capacities which act as a control on phytoplankton abundance that could help with the eutrophication issue in the IRL (Forrest *et al*., 2009**).**

Oysters filter large volumes of water daily by removing algae and sediments, and aid in cleaning up bodies of water (Coen *et. al.*, 2007, Beck *et. al.*, 2011). Oysters consume nitrogen contained in phytoplankton and reduce turbidity and concentrations of particulate organic nitrogen (PON) in the water column (Grizzle *et. al.*, 2008; Dame, 2012, Chambers *et al*., 2018). Nitrogen is assimilated into oyster shells and tissue, buried into the sediments in feces and pseudo-feces, or returned to the atmosphere as N_2 gas (zu Ermgassen *et. al.*, 2016). The restoration of oyster population can help with the abundance of muck in the IRL that releases nitrogen and phosphorous into the water column (Fox *et al.*, 2018).

Oyster reefs provide habitat to other benthic filter feeding organisms like sponges, mussels, barnacles, bryozoans, and tunicates (sea squirts). These organisms also filter suspended particles and algae from the water and can improve the health of the IRL. One oyster can filter up to 50 gallons of water, and tunicates filter up to 23 gallons of water per day (Volety 2015, Weaver *et. al.*, 2018). The filtering capacity of the tunicates can help reduce toxins and pathogens (Burge *et. al.*, 2016). It is reported that use of Biorock technology for coral reef restoration had significant recruitment rates for filter feeding organisms such as tunicates and calcareous tube-dwelling polychaetas (Goreau 2012).

Oyster reefs provide another important ecosystem service by creating habitat, refuge and feeding grounds for juvenile fish and invertebrates (zu Ermgassen *et al*., 2016). High recruitment of juvenile fishes is often reported for the restored oyster reefs (Harding and Mann 1999, Bourdreaux *et al*., 2006). During the restoration efforts on Palace Bar Reef, Piankatank River, Virginia, the following fishes were recorded around the restored oyster reefs: striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*), weakfish (*Cynoscion regalis*), skilletfish (*Gobiesox strumosus*), naked goby (*Family: Gobiidae*), etc. These fish used oyster reefs as both feeding and nursery grounds (Harding and Mann 1999).

Previous work, which has used the low-voltage electrical fields for coral and oyster restoration is thought to also provide organisms with essential elements for growth, reproduction and resistance to environmental stress (Goreau 2012).

This chapter was designed to test the hypothesis that the biodiversity of sessile and mobile organisms will be greater on the cathodically protected steel mesh than that of the standard plastic mesh.

4.2 Methodology

To determine the efficacy of using a cathodically protected steel for oyster restoration and evaluate associated biodiversity, a steel mesh was compared to the traditional plastic mesh mats. The steel mats were constructed from 0.25" hole (0.64 cm)

18-gauge hot roll expanded flattened A36 steel mesh (onlimemetals.com). Each steel mat was cut into 18 x 18 inches (45.7 x 45.7 cm) replicates and affixed with 36 dead and dried oyster shells accordingly to the previous research on restoration with oyster mats (Barber *et al*., 2010, Walters 2013). Oyster shells were attached to steel mats using stainless steel screws, washers, and nuts. Marine grade wire was attached to all steel samples, which allowed for a connection to a power source. Plastic mats were constructed from aquaculture grade Aquatic Ecosystems, N0350, plastic mesh with ¾-inch (1.91 cm) square holes in the same dimensions, and with the same shell count, using UV resistant zip ties to attach the oyster shells. Nine steel and nine plastic mats were constructed. Mats were mounted on six PVC frames. Each frame was holding 3 samples. PVC frames were submerged from the floating docks at the test locations (Figure 4.1).

Before the deployment at the testing sites, all steel and plastic mats were immersed for about 24 hours at FIT's Port Canaveral immersion facility. The steel mats were connected via the wire to two deep cycle batteries driving a potential (-1.3V, 6A) for the pre-chalking. This prevented the steel from rusting overnight and allowed for the elimination of the battery source at the more remote test locations.

All mats were rinsed after pre-chalking. Two PVC frames with the replicate steel and control plastic mats were submerged at each test site: Port Canaveral (28°24'31.01"N, 80°37'39.54"W), Grant (27°55'47.53"N, 80°31'28.97"W), and Melbourne Beach (27°59'51.3" N, 80°31' 33.9" W) (Figure 4.2). At each location, steel mats were connected via marine grade wires to a solar panel (12V, 50W) to continue mineral a)

b)

c)

Figure 4.1 a) PVC frame with steel mats before immersion, b) PVC frame with plastic mats before immersion, c) the floating dock located along the IRL, where all mats were immersed.

accretion. The titanium anode was attached to a 1-Ohm resistor, and each steel mesh attached to a bus bar connected to a solar panel with a 12 DC V battery to complete the current flow.

Figure 4.2 A map depicting the three locations utilized for experiments.

The selection of the test sites was based on previous projects that demonstrated the development of mineral accretion. Each of these locations have a variety of environmental factors such as salinity, temperature, pH, water clarity and have history of oyster settlement.

Mineral Accretion

Hilbertz (1970) developed a method to stimulate the mineral accretion on cathodically protected steel using a low-voltage electrical current. For the evaluation of mineral accretion, the four sacrificial coupons 2 x 2 inches (5.1 x 5.1 cm) were cut, pre-weighed, and attached to each of the steel mats. One of the coupons was removed after the pre-chalking and the remaining coupons were removed at one, two, and three months of immersion (Figure 4.3). Coupons were dried and weighed. The initial weight and weight upon removal were taken via a digital scale. The mineral accretion chemical composition was analyzed using Energy Dispersive X-Ray Analysis (EDAX), which is an x-ray technique used to identify the elemental composition of materials (Figure A1). Photographs were made using a scanning electron microscope (SEM) (Figure A2). The thickness of the chalking was measured using an Elcometer 456 coating thickness gauge. The average thickness was calculated using ten random points on each coupon.

a) b) c)

Figure 4.3. Examples of the sacrificial coupons after a) month one, b) month two, c) month three.

Data Collection

The test samples were submerged for about five months (21 weeks) at the three locations. During week six all mats were moved to a secure location in preparation for Hurricane Dorian (September 2019). The hurricane sites provided immersion and protection from the storm. Mats were returned to Port Canaveral and Melbourne Beach locations on week seven. The Grant test location had issues during week seven and were not returned until week nine.

Weekly assessments were performed at each of the sites. The current and potential of the system were recorded using a clamp on ammeter and an Ag/AgCl reference electrode, respectively. ONSET HOBO data loggers were used to record water quality parameters: temperature, salinity, and pH. The growth and diversity of benthic organisms, interactions of mobile organisms with the mats were visually assessed with field notes and photographs. Each mat was analyzed on both surfaces to record total coverage of sessile benthic organisms using ASTM D 6990 (2005). Benthic organisms were placed into functional groups following the same standard, and the percent coverage for each group was recorded weekly.

After each month of immersion, videos were taken of the mats. This was done by placing a Go Pro Session affixed to a pole and positioning it to view the mats from the side to observe fish and other mobile species which may be living or associating within/near the mats. Ten-minute videos were taken of both steel and plastic mats to help identify the mobile organisms.

The note records, photographs and videos were utilized to create an associated communities list of organisms which settled on the steel and plastic mats or were found to interact with the steel mats.

Statistics

Simple analysis of variance ANOVA test was used to identify variations between mesh types and locations. If the significant difference was found in the test group, then the analysis was performed on sub-groups like all steel mesh mats or plastic mats at all locations. Linear regression plots were used to visualize differences between the steel and plastic, as well as among the sites. The Data Analysis pack in MS Excel 2016 was used for data analysis. The significance of differences in the chalking thickness and coupon weight based on immersion time and location was evaluated using a two-way analysis of variance (MATLAB R2020) (Hunsucker *et al*, 2021).

4.3 Results

Environmental Conditions

Each site had slightly different water quality conditions (Figure 4.4, 4.5). Over the five months period of immersion, Port Canaveral had an average salinity of 34.3 ± 1.3 ppt, with an average temperature of 25.9 ± 2.9 ºC and pH 8.3 ± 0.1. The Melbourne Beach site had an average salinity of 20.1 ± 4.1 ppt, with an average temperature of 26.7 ± 4.3 ºC and pH 8.5 ±0.4. The Grant site had an average salinity of 19.9 ± 5.7 ppt, with an average temperature of 26.5 ± 4.8 ºC and pH 8.3 ± 0.3.

The test sites had different average salinities: Melbourne Beach and Grant around 20 ppt and Port Canaveral around 34 ppt. All the test sites had a close fit into the optimal salinity for oyster growth that is in the range 10-30 ppt (Walters *et al*., 2001).

There were fluctuations in pH values at each site (Figure 4.4). The Port Canaveral and Grant test sites had very similar average pH 8.3 ± 0.3 and 8.3 ± 0.1 respectively. At

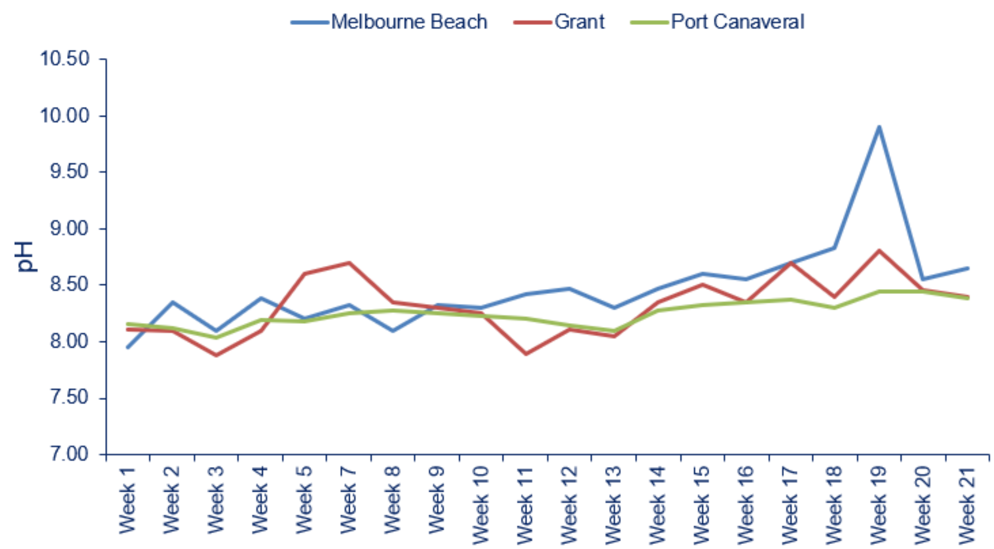


Figure 4.4. pH for the 21-week immersion period at each site (Note: Week 6 excluded since assessment was not performed due to Hurricane Dorian).

Melbourne Beach, pH had average 8.5 ± 0.4 with a large spike in the value (9.9) on the week 19.

The water temperature did not vary between the three locations and was in 22.4 ºC – 30.4 ºC range that fit perfectly into optimal range of 20 ºC to 32.5 ºC for the oyster growth and reproduction (Banks *et al*., 2007).

Since the test site at Port Canaveral is located about 4 km from the Atlantic Ocean, there was little fluctuation in the water quality. The test sites at Melbourne Beach and Grant are both located in the IRL and are more susceptible to environmental changes with alter the water conditions (e.g. increased precipitation or evaporation). Grant is located near a freshwater tributary that may have played a role in fluctuation in the salinity especially during rainy days.

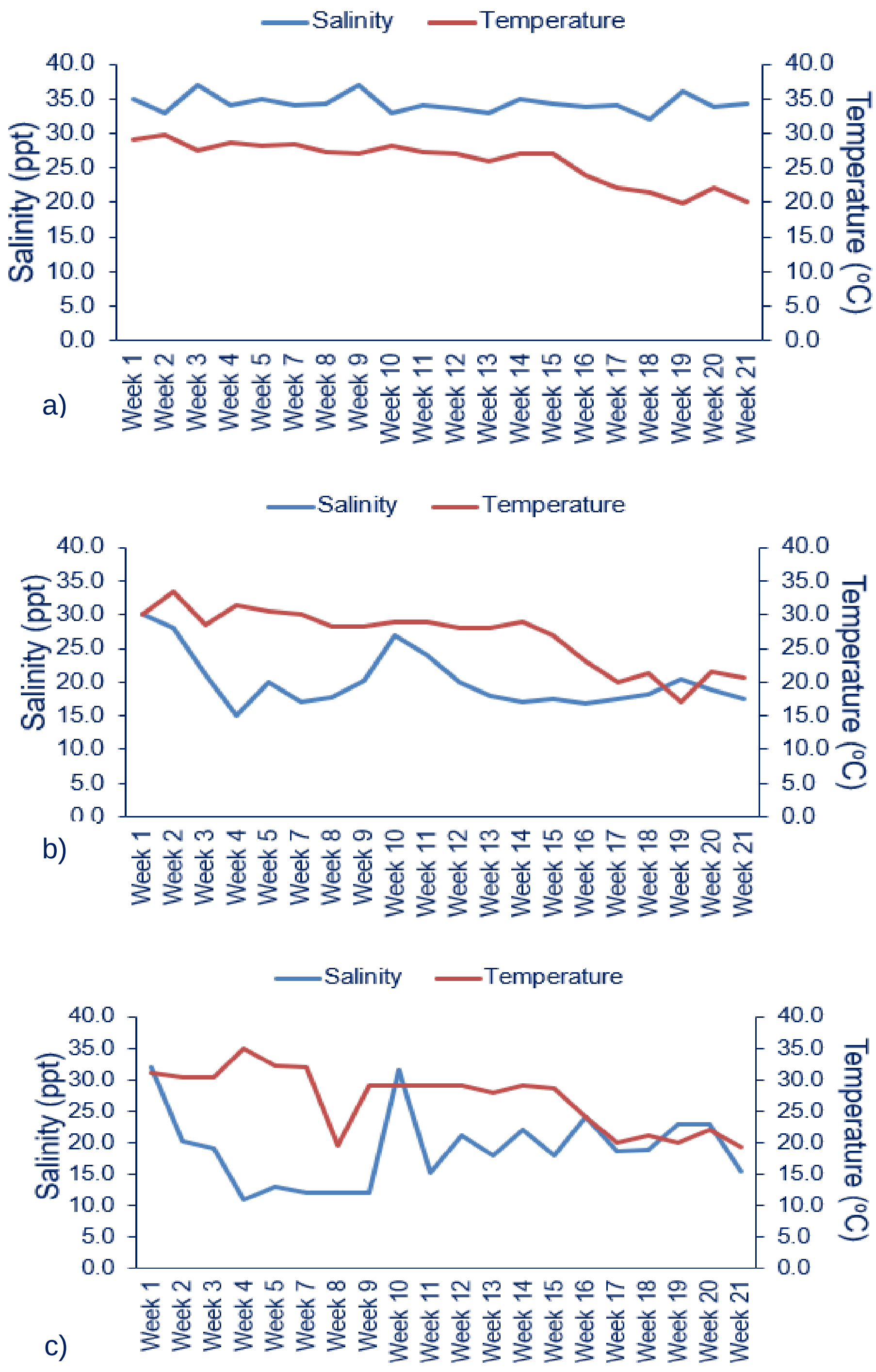


Figure 4.5. Salinity and temperature for the 21-week immersion period at each site: a) Port Canaveral, B) Melbourne Beach, c) Grant. (Note: Week 6 excluded since assessment was not performed due to Hurricane Dorian).

Current and Potential Measurements

The potential and current were measured weekly during daylight hours when the solar panel was providing power. The measured current ranged between 0.20 and 3 A during the immersion period. The potentials were typically more negative than -1.0 V which demonstrated the degree to which the steel was being driven cathodically. Average current density over the course of the experiment was 1.91 ± 1.32 mA/cm^2 at Melbourne Beach, 2.09 ± 1.25 mA/cm^2 at Grant, and 1.89 ± 1.12 mA/cm^2 at Port Canaveral. Figure 4.6 shows the relationship between potential and current density at the three test sites.

Mineral Accretion

The thickness and weight of the mineral accretion were measured on the steel coupons for pre-chalking and after one, two, and three months of immersion (Figure 4.7). The thickness of accretion layer after pre-chalking was around 200 μm and coupon weight was on the average 0.67 g. The steady increase in thickness and weight were recorded at Port Canaveral and Melbourne Beach. At Port Canaveral a final thickness of the chalk was 981 ± 255 μm and weight 6.3 ± 1.3 g after two-month immersion. Due to heavy biofouling, the measurement was not completed after the third month. The Melbourne Beach test site had a chalking thickness of 789 ± 289 μm and weight 4.4 ± 1.0 g after three-month of immersion. The data from Grant was mixed due to changes in

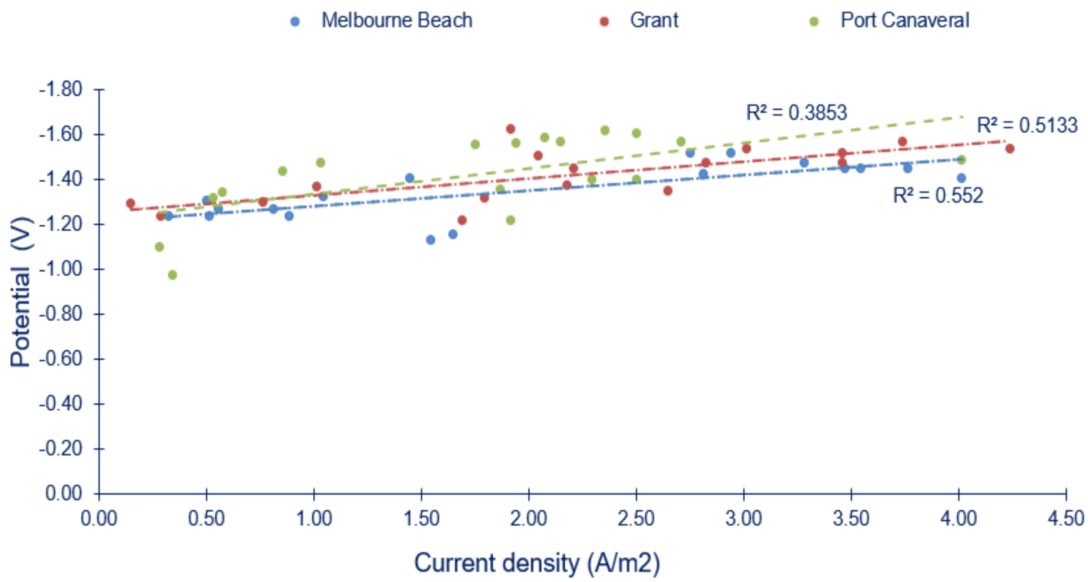


Figure 4.6. Potential versus current density recorded for each of the three test sites over the five-month immersion. Data is not present for week 6 due to Hurricane Dorian.

the water quality caused by Hurricane Dorian. At Grant a final thickness was 890 ± 306 μm with a slight decrease after the second month and weight was 3.5 ± 1.2 g. The EDAX analysis showed the pre-chalking stage was dominated by magnesium carbonate. Month one, month two, and month three of accretion also had a heavy abundance of magnesium carbonate within the chalk. However, this was not uniform across all coupons, as some were calcium carbonate dominated: three coupons at Port Canaveral, two coupons at Melbourne Beach and one coupon at Grant (Table A1).

Figure A1 shows the EDAX analysis report for the coupon at Port Canaveral. There are significant differences in weight and thickness based on length of immersion ($p< 0.05$) and among the test sites (Hunsucker *et al*, 2021).

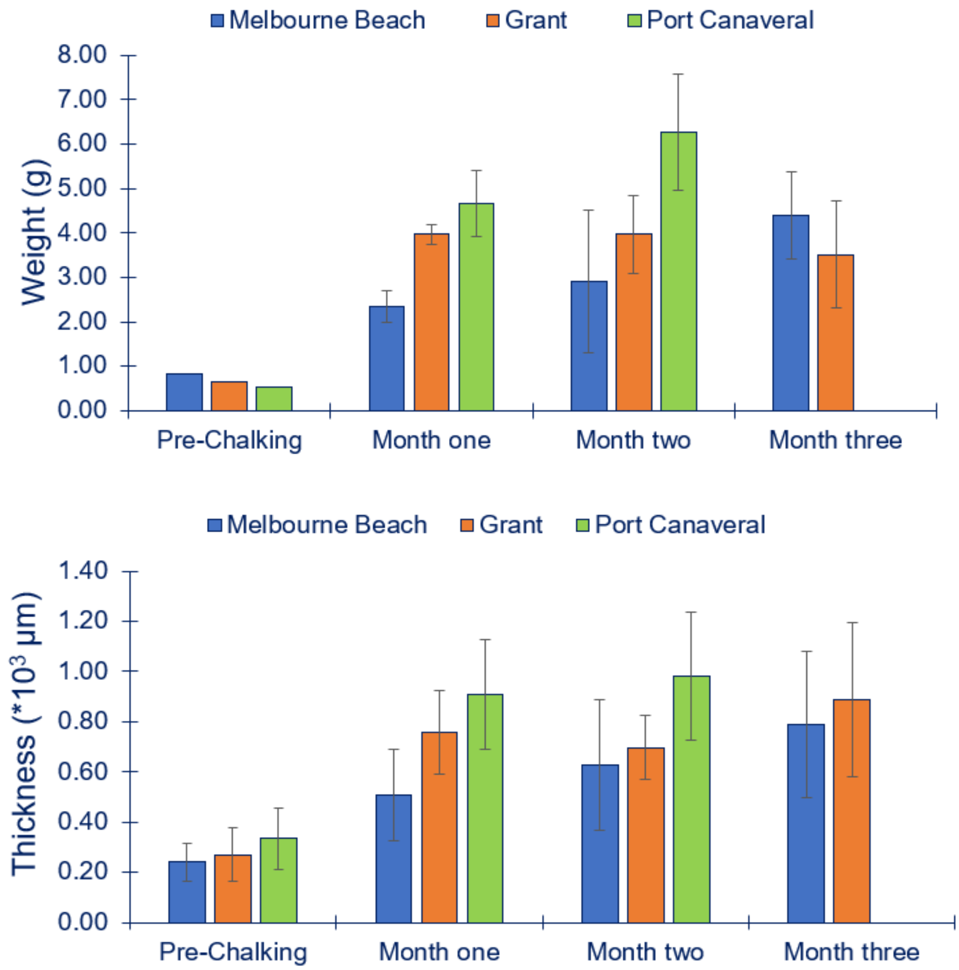


Figure 4.7. Mineral accretion average weight (top) and thickness (bottom) with standard deviation for steel coupons at the three test locations. (Note: the measurements were not performed at Port Canaveral after the third month due to heavy biofouling).

Oyster Growth

The three test sites had varying water quality and environmental conditions which affected the oyster recruitment. Oyster growth was recorded at all locations after one month. The oyster counts were similar between steel and plastic mats at Port Canaveral. The higher number of oysters were on plastic mats at Grant and no oysters on plastic mats at Melbourne Beach (Figure 4.8a-b). The second month counts were affected by

Hurricane Dorian since the mats were moved to a secure location from the test sites. Starting at week 9 the oyster growth increased at all test sites. Photographs of oysters for month three, four and five for all locations are available in Appendix B. The oyster spat was also found growing directly on the steel mats not just the oyster shells.

The oyster counts were different between the steel and plastic mats, and among the sites. The Port Canaveral test site had the highest salinity around 34 ppt which is out of the preferred salinity range for eastern oysters (10-30 ppt) and heavy competition from other benthic organisms. As the result, it had the lowest out of the three test sites. Oyster counts on the steel mats were within the range of 1-15 oysters (Figure 4.8 a). The plastic mats had oyster recruitment greater than the steel with the range of 7 – 14 (Figure 4.8 b).

Melbourne Beach was the ideal location for oyster studies. The salinity was in the perfect range as well non-turbid waters. It had a healthy population of oysters in the area. The oyster counts on the steel mats were within the range of 10-90 oysters (Figure 4.8 a). The plastic mats on the other hand did not do as well as the steel having a range of 0 -30 oysters (Figure 4.8 b).

The Grant site turbid water also created a weak environment for the oysters to inhabit. The oyster counts on the steel mats were within the range of 0 – 23 oysters that was greater than Port Canaveral, yet lower than at Melbourne Beach (Figure 4.8 a).

The plastic mats had the oyster counts within the range of 0 – 25 oysters which is lower than the steel mats recruitment rate (Figure 4.8 b).

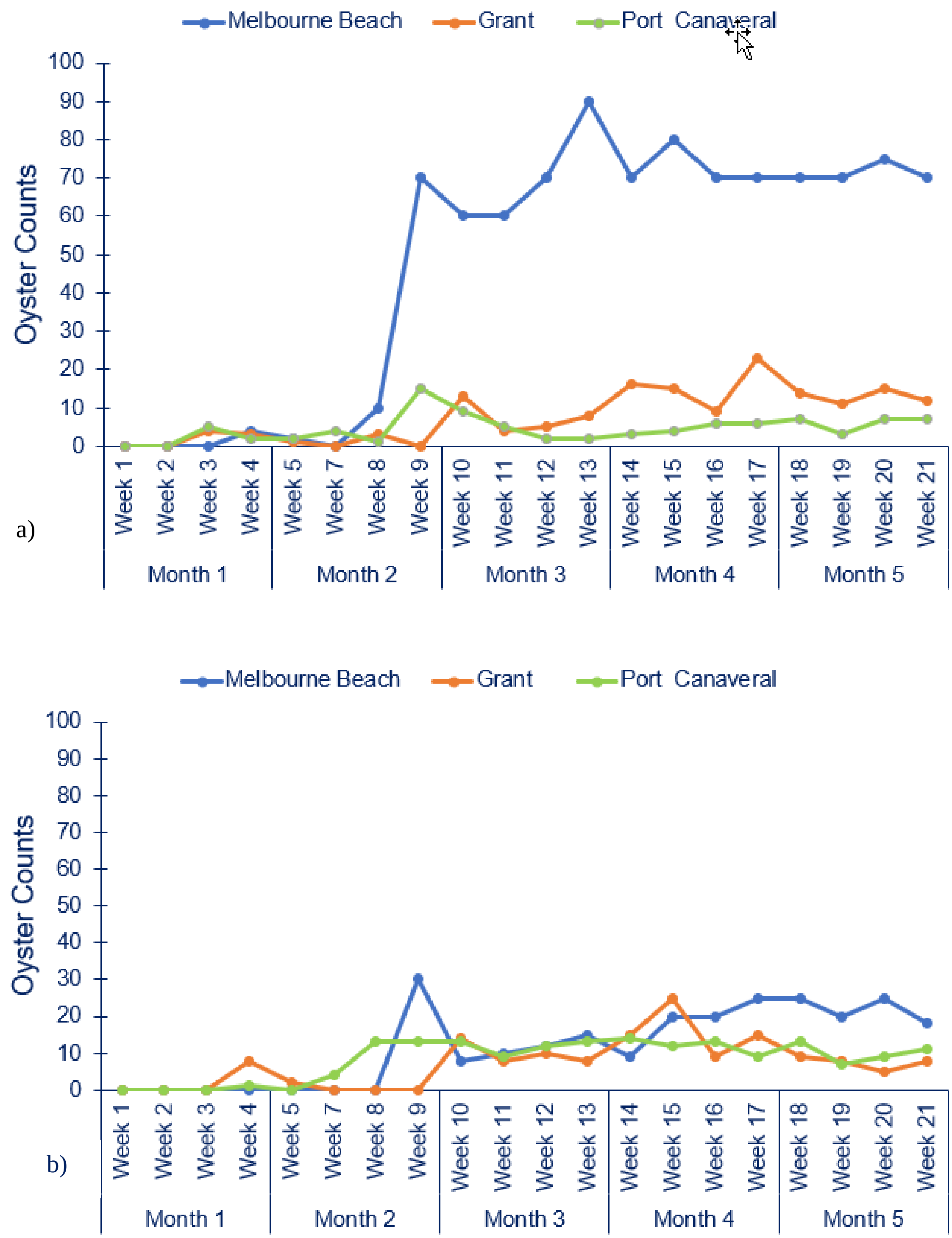


Figure 4.8. Oyster counts for steel mats (a) and plastic mats (b) over the 21 weeks sampling period. Counts include oyster present on all three mat replicates, including the oyster shells and mat material. (Note: Week 6 assessment was not performed due to Hurricane Dorian).

The biodiversity of sessile organisms

Over the course of the five-month experiment, sessile organisms were observed at the three test sites. Only organisms directly attached to the mats are recorded. The list of settled sessile organisms for biofouling community is shown in Table 4.1.

Table 4.1. The sessile organisms observed on mats at the three test sites (adopted from Center for Corrosion and Biofouling Control Florida Institute of Technology, Melbourne FL).

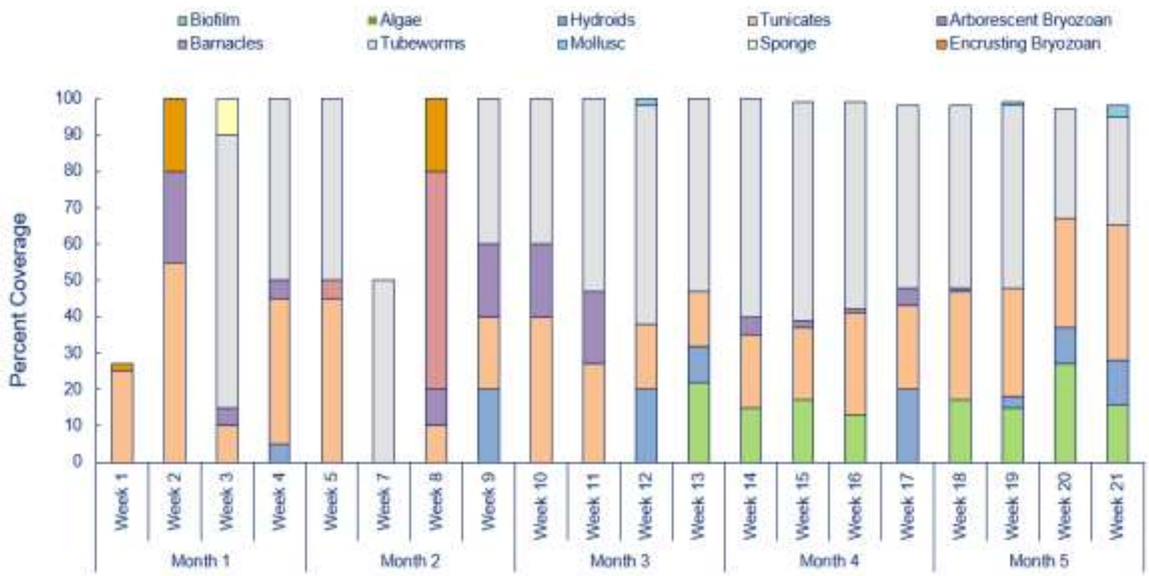
Common Name	Definition
Biofilm	Diatoms, initial algae germination, and low form algae, bacterial growth.
Algae	Fully established alga types and larger forms, e.g. *Ulva* sp., *Enteromorpha* sp. and *Ectocarpus* sp.
Hydroid	Low form, highly branching organisms.
Tunicates	Soft animals that may be solitary or colonial; solitary types may reach several centimeters in height and colonial forms tend to form a think cover over the surface.
Arborescent Bryozoan	Colonial animals forming a turf like mat rarely exceeding 3 cm in length; they may be mistaken for plants.
Barnacle	A hard shelled crustacean that cements itself permanently to a substrate, and is difficult to remove; the outer shell is generally whitish in color and shaped like a truncated cone. The barnacles in this area may grow to 2 cm in height and 2 cm in width at the base; the most abundant species at the FIT test site is Balanus eburneus.
Calcareous tubeworm	Tubeworms that form a hard calcareous exoskeleton which becomes cemented to the substrate; the individuals rarely exceed 2 cm in length and may show some coiling .
Moluscs	Animals with two hard shells, hinged along one edge; typical examples are oysters and mussels. Note: oysters are counted separately.
Sponges	Soft animals with sponge like texture forming thin surface cover or thicker accumulations, often brightly colored.
Encrusting Bryozoan	Colonial animals forming an encrusting layer over the surface; these layers are generally 1-2 mm thick and have rough texture.
Sea anemone	Soft animals, with a solitary tube-like body and a ring of stinging tentacles around the oral opening.

Mature forms of biofouling are recorded as percentage cover (Figures 4.9, 4.11, and 4.12) using the same color coding as on the table. Figure 4.10 shows biofouling communities on steel and plastic mats at each site after five months immersion. Photographs after one, two, three- and four-month immersions are available in Appendix

C. Each site location was geographically unique which created differences among the test sites. The benthic community varied between test sites.

There was a different dominant sessile organism that prevailed over the rest of biofouling community at each site. The most abundant biofouling organism at Port Canaveral were calcareous tubeworms with a high abundance of colonial tunicates (Figure 4.9). Organisms also present at this location included: hydroids, arborescent bryozoan, barnacles, algae, biofilm, sponges, mussels, encrusting bryozoan, sea anemone. It was difficult to look for oysters on either the steel or plastic mats due to the heavy amount of biofouling present at this location. Out of the three test sites the Port had the least number of oysters throughout the experiment possible due to the competition with tubeworms and tunicates. The Melbourne Beach test site was dominated by barnacles for most of the experiment and switched over to an encrusting bryozoan dominated community towards the end of the experiment (Figure 4.11). Out of the three test sites Melbourne Beach was also the most productive in oyster growth.

The Grant test site was dominated by barnacles throughout the course of the experiment (Figure 4.12) . Organisms also present at this location included: hydroids, tunicates, tubeworms, algae, biofilm, sponges, encrusting bryozoan. The oyster count was higher than at Port but still less than at Melbourne Beach. The amount of biofouling was much less than that of the Port, which made it easy to count oysters each week.



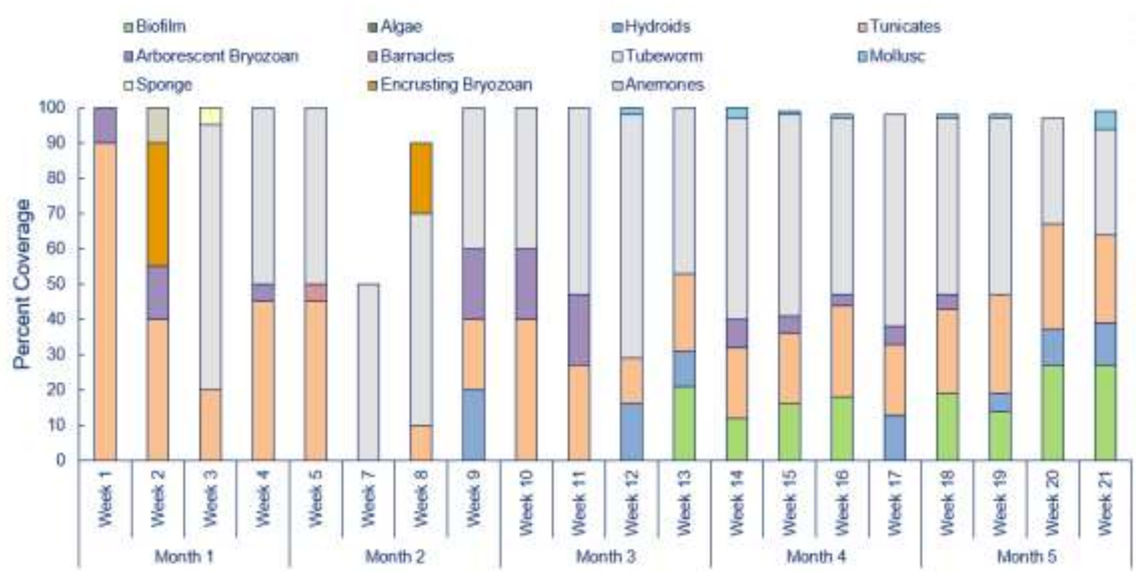


Figure 4.9. Percent cover of benthic organisms attached to steel mats (top) and plastic mats (bottom) at Port Canaveral over a 21-week immersion period (Note: Week 6 assessment was not performed due to Hurricane Dorian).

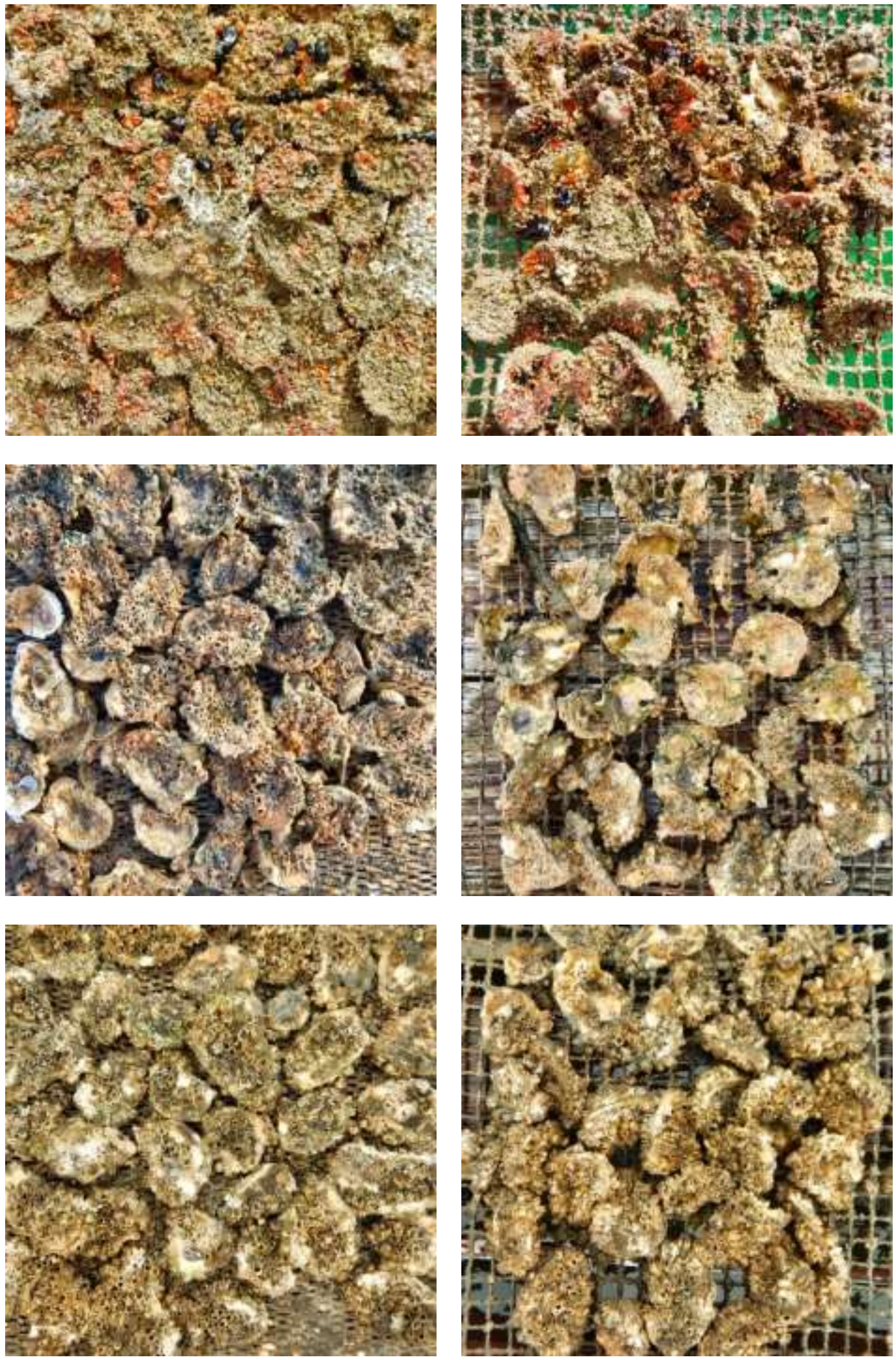
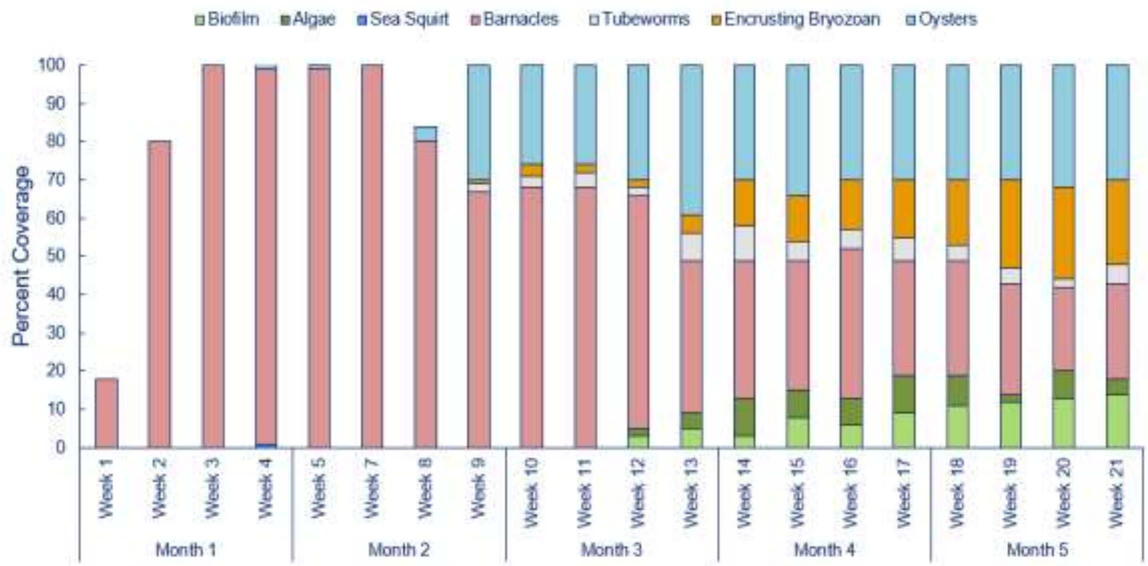


Figure 4.10. Oyster mats after month five immersion. Steel (left) and plastic (right) mats: a) Port Canaveral, b) Melbourne Beach, c) Grant.



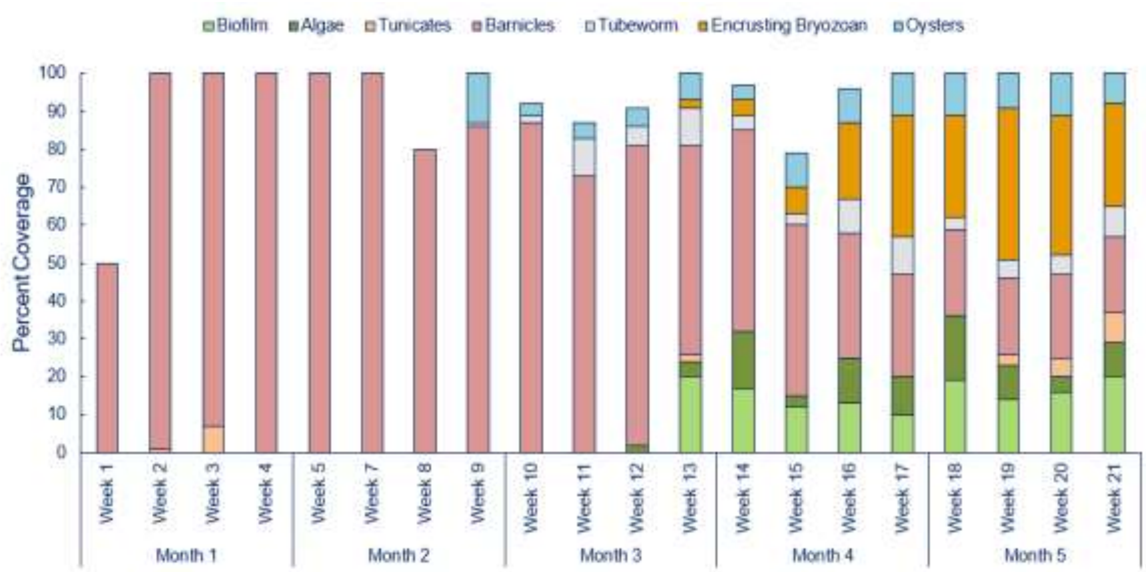
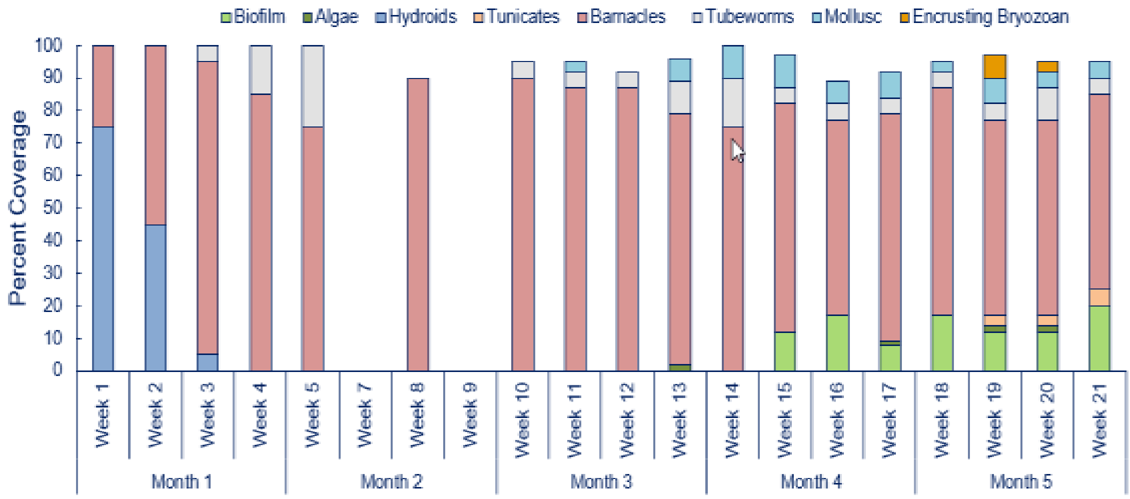


Figure 4.11. Percent cover of benthic organisms attached to steel mats (top) and plastic mats (bottom) at Melbourne Beach over a 21-week immersion period (Note: Week 6 assessment was not performed due to Hurricane Dorian).



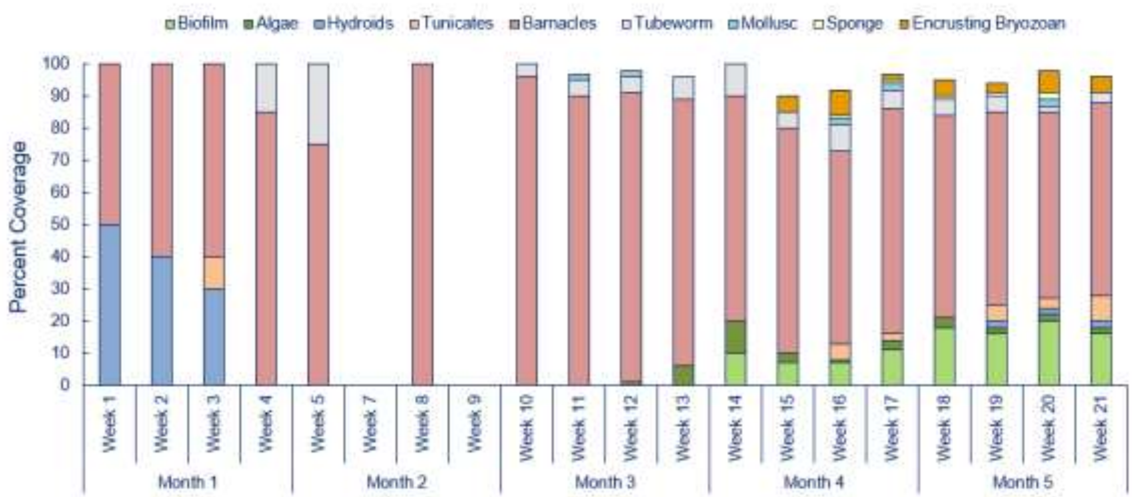


Figure 4.12. Percent cover of benthic organisms attached to steel mats (top) and plastic mats (bottom) at Grant over a 21-week immersion period (Note: Week 6 assessment was not performed due to Hurricane Dorian and Week 9 was not performed due to repairs).

The electrical stimulated mineral accretion created a suitable substrate for the oyster and other hard and soft benthic organisms' growth. Some of them were found to settle directly on the front and back of steel mats (Figure 4.13).

Figure 4.13. Photographs of oyster settlement directly on the steel mats: front of the mat (left), close -up (center) and back of the mat (right).

Species Richness

At the Port Canaveral test site, the average species richness was in favor of the steel mats towards the end of the experiment. The average richness was at 4 ± 0.5 on the steel mats and 3 ± 0.7 on the plastic mats after the first month of the immersion. After five months of immersion, the average richness increased to 5 ± 0.5 on the steel mats and 4 ± 0.4 on the plastic mats (Figure 4.14 a).

At the Melbourne Beach test site, the average species richness for the steel mats was the lowest of the three locations at 1 ± 0.5 and the plastic mats had 2 ± 0.6 after the first month of the immersion. Over the five-months immersion the average species richness increased to 5 on the steel mats and to 6 ± 0.5 (Figure 4.14 b).

At the Grant test site, the average species richness was 2 ± 0.4 for the steel and plastic mats after the first month of the immersion. After the five-month immersion, the

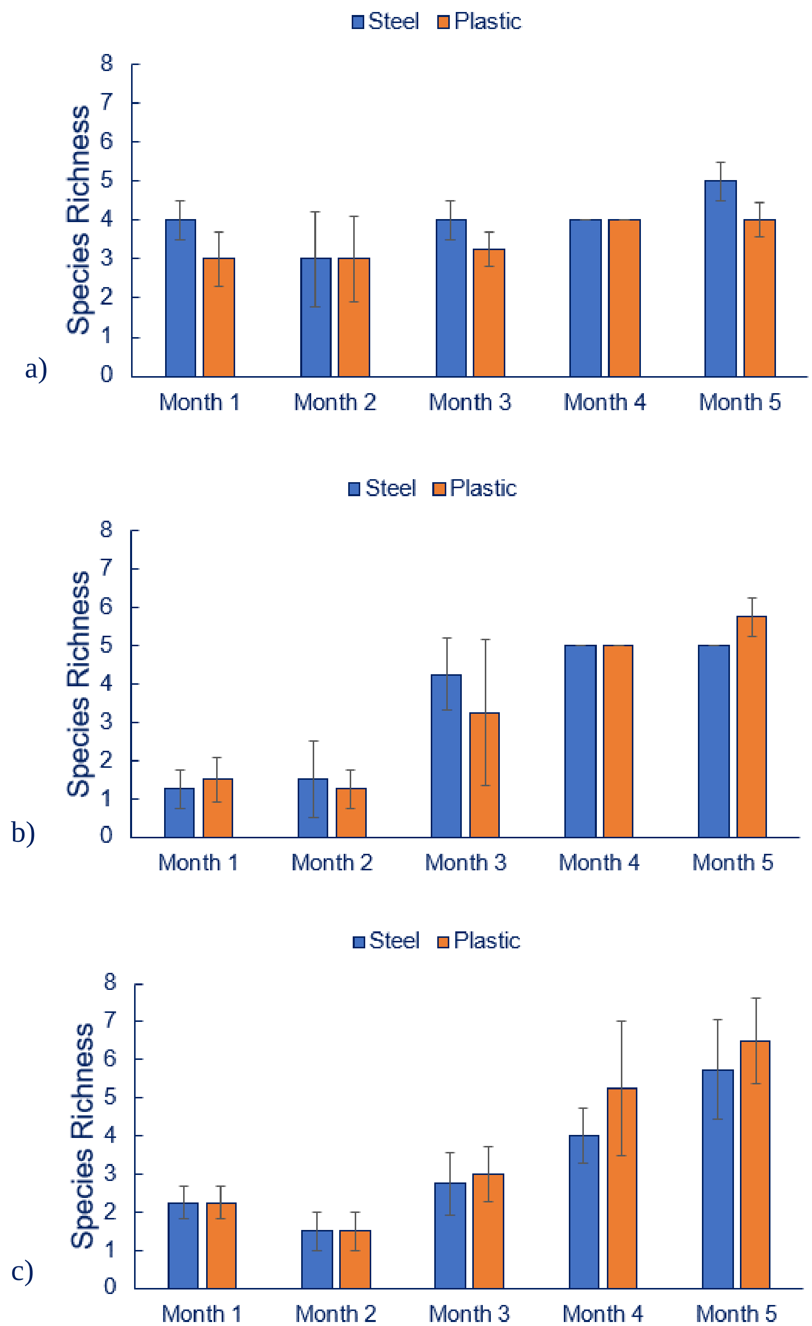


Figure 4.14. Average species richness of benthic organisms with standard deviation: a) Port, b) Melbourne Beach, c) Grant.

species richness increased greatly to 6 ± 1.3 for the steel mats and to 7 ± 1.1 for the plastic mats (Figure 4.14 c).

The weekly species richness was checked for a normal distribution with a 95% confidence level. There are no significant differences between the steel and the plastic mats and among test sites (p-value > 0.05) (Table 4.2).

Diversity (the Shannon Index)

The typical values for Shannon index are generally between 1.5 and 3.5 in most ecological studies, and the index is rarely greater than 4. The Shannon index increases as both the richness and the evenness of the community increase (Magurran 2004).

The average diversity at the Port Canaveral test site was 0.78 ± 0.3 for the steel mats and 0.77 ± 0.34 after the first month of immersion. After the five-month immersion period, the average diversity on the steel mats changed to 1.2 ± 0.15 and to 1.29 ± 0.12 (Figure 4.15 a).

After the one-month immersion at the Melbourne Beach test site, the average diversity for the steel mats was 0.04 ± 0.07 and 0.08 ± 0.1 on plastic. After the five-month period the average diversity raised to 1.57 ± 0.03 for the steel mats and 1.66 ± 0.08 for the plastic mats (Figure 4.15 b).

The average diversity at the Grant test site was 0.52 ± 0.12 for the steel mats and 0.66 ± 0.16 for the plastic mats after the one-month test period. At the end of the experiment, it increased to 1.1 ± 0.19 for the steel mats and 1.2 ± 0.1 for the plastic mats (Figure 4.15 c).

Table 4.2. Weekly Average Species richness: a) all steel and plastic mats at all test locations, b) weekly plastic mats at all test locations, c) weekly steel mats at all test locations.

a)

SUMMARY

Groups	Count	Sum	Average	Variance
Steel Port	20	74	3.70	0.75
Plastic Port	20	67	3.35	0.66
Steel Melbourne	20	68	3.40	3.31
Plastic Melbourne	20	67	3.35	4.13
Steel Grant	18	62	3.44	2.97
Plastic Grant	18	71	3.94	4.76

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	5.43	5	1.087	0.399	0.849	2.297
Within Groups	299.49	110	2.723			
Total	304.92	115				

b)

SUMMARY

Groups	Count	Sum	Average	Variance
Port Steel	20	74	3.70	0.75
Melbourne Steel	20	68	3.40	3.31
Grant Steel	18	62	3.44	2.97

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.04	2	0.52	0.224	0.800	3.165
Within Groups	127.44	55	2.32			
Total	128.48	57				

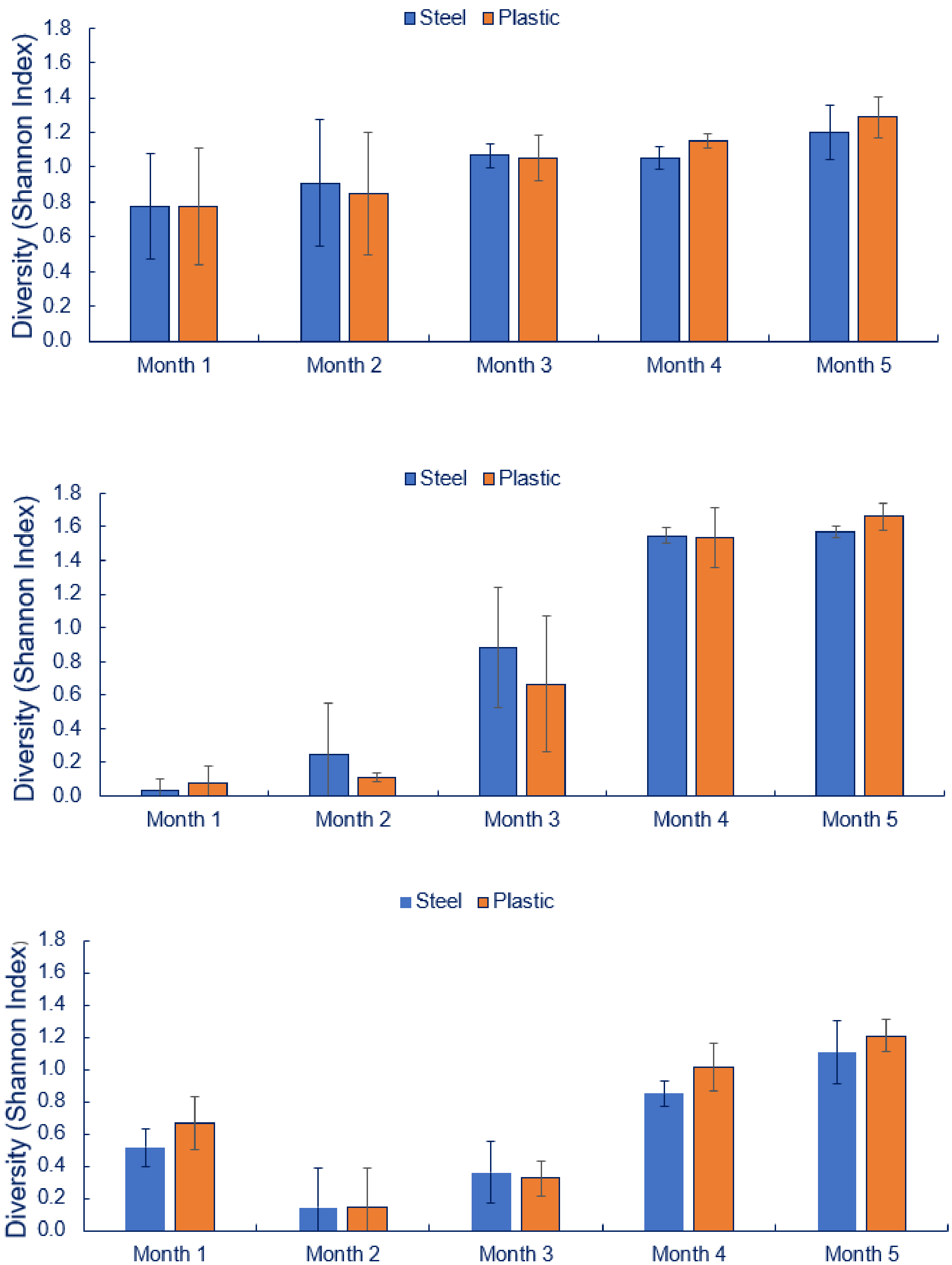
c)

SUMMARY

Groups	Count	Sum	Average	Variance
Port Plastic	20	67	3.35	0.66
Melbourne Plastic	20	67	3.35	4.13
Grant Plastic	18	71	3.94	4.76

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4.39	2	2.19	0.701	0.500	3.165
Within Groups	172.04	55	3.13			
Total	176.43	57				



a)

b)

c)

Figure 4.15. Average diversity of benthic organisms with standard deviation: a) Port, b) Melbourne Beach, c) Grant.

The diversity (Shannon Index) values were checked for a normal distribution with a 95% confidence level for 21 weeks immersion period. Table 4.3 (a) shows the *ANOVA* statistical analysis for the steel and plastic mats at all three test sites. There were statistical differences for the average diversity for the steel and plastic mats, and among the test sites (p-value = 0.011). Table 4.3 (b) shows the *ANOVA* statistical analysis for the diversity values between plastic mats at the test sites. The diversity was not statistically different (p-value = 0.052). The diversity was statistically different between sites on the steel mats with p-value of 0.013 (Table 4.3 c).

Figure 4.16 shows the linear regressions for the average diversity between the steel and plastic mats, and among the test sites.

The biodiversity mobile organisms.

Mobile organisms were recorded and photographed at each of the locations. Examples of some of these organisms found on the steel mesh are shown in Figure 4.17. Go Pro videos were collected for assessment to identify fish or other mobile organisms which may interact with the mats. Examples of some fishes found on the video are shown in Figure 4.18. The comprehensive list of organisms which live on or near the steel mats can be found in Table 4.4.

Overall all locations had juvenile schools of fish present at each assessment. The main organisms that were identifiable through the videos were mostly fish and one crab. The location at Port Canaveral had the best results in capturing footage that could be used to ID some organisms. Species that were identifiable are as follows: Atlantic

Table 4.3. Diversity (the Shannon Index): a) all steel and plastic mats at all test locations, b) weekly plastic mats at all test locations, c) weekly steel mats at all test locations.

a)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Port Steel	20	20.01	1.00	0.08
Melbourne Steel	20	13.18	0.66	0.38
Grant Steel	20	11.89	0.59	0.16
Port Plastic	20	20.44	1.02	0.09
Melbourne Plastic	20	13.76	0.69	0.46
Grant Plastic	20	13.41	0.67	0.20

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.53	5	0.71	3.108	0.011	2.294
Within Groups	25.89	114	0.23			
Total	29.42	119				

b)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Port Plastic	20	20.44158	1.022079	0.094885
Melbourne Plastic	20	13.7584	0.68792	0.461648
Grant Plastic	20	13.40931	0.670466	0.197255

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.570659	2	0.78533	3.125533	0.051523	3.158843
Within Groups	14.32197	57	0.251263			
Total	15.89263	59				

c)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Port Steel	20	20.01	1.00	0.08
Melbourne Steel	20	13.18	0.66	0.38
Grant Steel	20	11.89	0.59	0.16

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.91	2	0.95	4.6935	0.0130	3.1588
Within Groups	11.57	57	0.20			
Total	13.47	59				

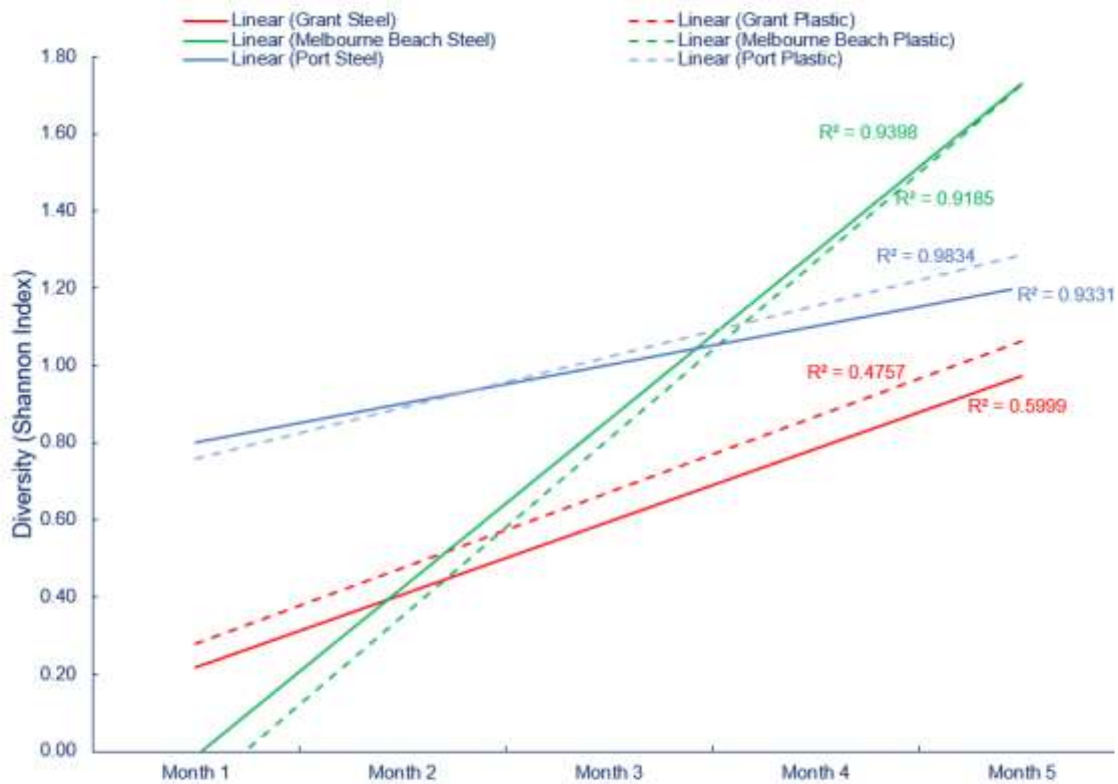


Figure 4.16. Linear regression for diversity for each mat type and location.

Spadefish (*Chaetodipterus faber*), Grey Snapper (*Lutjanus griseus*), and Sheepshead (*Archosargus probatocephalus*) (Figure 4.18). Grant had poor water quality regarding turbidity and made gathering video very difficult. At Melbourne Beach, a Blue Crab (*Callinectes sapidus*) was seen interacting with the steel mats, as well as many juvenile schools of fish. When the mats were removed from the water, mobile organisms were still attached and easily identifiable. Species that were identifiable this way are as follows: Grass Shrimp (*Palaemonetes pugio*), Green Porcelain Crab (*Petrolisthes armatus*), Florida Stone Crab *(Menippe mercenaria*), Lined Seahorse (*Hippocampus erectus*), and juvenile Spiny Lobster (*Panulirus argus*).

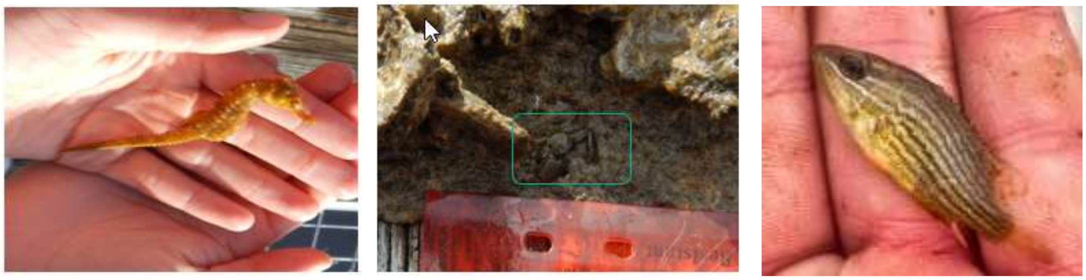


Figure 4.17. A Slender seahorse (*Hippocampus reidi*) (left) and a Green porcelian crab (*Petrolisthes armatus*) (center) and Green sunfish (*Lepomis cyanellus*) (right) were recorded on the steel mats.

Figure 4.18. Common fish which were found interact with the mats: Sheepshead (*Archosargus probatocephalus*), Grey Snapper (*Lutjanus griseus*), Atlantic Spadefish (*Chaetodipterus faber*) and Atlantic Tripletail (*Lobotes surinamensis*).

Table 4.4. Mobile organisms that were identified living on the mats or found to interact with the steel mats in the water column.

Common Name	**Scientific Name**
Amphipods	Order Gammaridea
Asian Green Mussel	*Perna viridis*
Atlantic Spadefish	*Chaetodipterus faber*
Atlantic Tripletail	*Lobotes surinamensis*
Blue Crab	*Callinectes sapidus*
Dusky Pipefish	*Syngnathus floridae*
Florida Blenny	*Chasmodes saburrae*
Florida Stone Crab	*Menippe mercenaria*
Grass Shrimp	*Palaemonetes pugio*
Green porcelain crab	*Petrolisthes armatus*
Grey Snapper	*Lutjanus griseus*
Green Sunfish	*Lepomis cyanellus*
Hermit crab	*Pagurus sp.*
Isopod	*Sphaeroma* sp.
Juvenile spiny lobster	*Panulirus argus*
Lined Seahorse	*Hippocampus erectus*
Scorched mussel	*Brachidontes exustus*
Sheepshead	*Archosargus probatocephalus*
Shore crab	*Pachygropsus* sp.
Skilletfish	*Gobiesox strumosus*
Slender Seahorse	*Hippocampus reidi*
Snail	Family Conidae

4.4 Discussion

To determine the efficacy of using a cathodically protected steel for oyster restoration and to evaluate the associated biodiversity, a steel mesh was compared to traditional plastic mesh mats. During the five-month immersion period, mineral accretion, oyster growth, benthic sessile organisms' community and mobile organisms interacting with the mats were observed weekly. The three test sites had varying water quality and environmental conditions. Port Canaveral is the northern most test site and has the most consistent water quality over time. Grant, the southernmost test site is in an area with freshwater discharge from the river into the IRL, which creates fluctuations in the water quality especially with heavy rain. The large amount of nutrients and suspended sediment entering this location can cause poor water quality. Melbourne Beach is located between Port Canaveral and Grant. It has a freshwater discharge in the vicinity but the fresh water at Melbourne Beach does not fluctuate the water quality as much as at Grant. Melbourne Beach is a unique location since it is next to an environmentally protected area rich in wildlife and has less anthropogenic disturbances.

Marine calcareous organisms grow their shells and skeletons using minerals dissolved in seawater. Coral and oysters live in colonies and form reefs, which are cemented together by skeletons of calcium carbonate ($CaCO_3$) produced by extracellular biomineralization. Biomineral accretion depends on the saturation of seawater with required ions, pH, and the special chemical conditions inside cells (Furla *et al.,* 2000). The electrical stimulated mineral accretion provides supersaturation of calcium and carbonate ions in the vicinity of the cathode, increased efficiency of cation uptake and transport due to the availability of electrons, increased metabolic efficiency since free electrons are available for Adenosine triphosphate (ATP) biochemical energy production (Hilbertz and Goreau 1996).

Different amounts of mineral accretion were recorded at each test site which corresponded to the associated water quality conditions. During the pre-chalking phase, the cathode was overcharged and as the result the mineral accretion layers was compounded with brucite (($Mg(OH)_2$) (Figure A.1). The same results are reported in the previous studies (Goreau 2012). During the experiment, on several of the coupons the mineral accretion composition was replaced by aragonite ($CaCO_3$) formation for some of the coupons. The salinity and pH are also affecting the production of mineral accretion. The Port Canaveral test site had the highest salinity around 34 ppt and mineral accretion on the sacrificial coupon had the highest weight 6.3 ± 1.3 g and thickness 981 ± 255 μm after two-month immersion between three locations. The electrical stimulated chalking created a suitable substrate for the oysters and other hard and soft benthic organisms to grow not only on the dead oyster shells but also directly on the steel mats. The future research of this finding can provide an interesting result since majority of oyster reef restoration efforts are using the dead oyster shells (Barber *et al*., 2010, Walters 2013, Garvis *et. al.*, 2015).

This chapter focused on the research questions and hypotheses which compared how the community composition on a cathodically protected steel mat to the community which develops on plastic mat. Experiments with oysters show that, under electrical stimulation, oysters grow faster and have higher survival rates (Berger *et al*., 2012, -

Karissa *et al*., 2012, Shorr *et al*., 2012) and organisms lacking limestone skeletons (e.g. sponges and tunicates) also seem to settle and grow at extraordinary rates on these surfaces (Goreau2010). Over the course of five-month immersion period, oyster growth and benthic communities were observed at all the test sites. The overall recruitment, species richness and diversity on the steel and plastic mats were different at each location depending on environmental and ecological conditions. The oyster counts and biodiversity of sessile and mobile organisms demonstrated the effectiveness of the steel mats at promoting the growth at the rate that was equivalent to plastic mats.

Melbourne Beach was the ideal location for oyster studies. The salinity was in the perfect range for oyster growth and this site had non-turbid waters. This location also had a healthy population of oysters in the area which was able to seed the mats. The oyster count on the steel mats was significantly higher compared to plastic. At the end of the experiment, there were 70 individual oysters on the steel and 18 on the plastic mats. The oysters were one of the dominant organisms and occupied more space than at the other sites (Figure 4.11). The oysters at the Port Canaveral test site were subjected to heavy biofouling competition from tunicates and tubeworms. Oysters need enough space to open and close to filter in and out water for the feeding and growth. The competition increased the stress on oyster recruitment which led to the lowest oyster counts of the three locations, but numbers were similar between the steel and plastic: 7 and 11 respectively at the end of month five. The Grant test site had lower competition from biofouling community but still had low overall oyster recruitment. Oyster counts were 12 individuals on the steel mats and 8 individuals on plastic.

Previous studies showed that filter feeding organisms like tunicates, tubeworms, barnacles, bryozoans, sponges had significant competition with oysters for food resource and the oxygen supply (Su *et al.*, 2007). Despite the competition for resources, oysters and other filter feeders provide the valuable water-filtering ecosystem service which act as a control on phytoplankton abundance that could help with eutrophication issue in the IRL (Forrest *et al*., 2009**).** One oyster can filter up to 50 gallons of water removing particles with the 4- to 100-µm when tunicates filter up to 23 gallons of water per day removing particles much smaller (Volety 2015, Galimany *et al*., 2017, Weaver *et. al.*, 2018). The filtering capacity of the tunicates can help reduce toxins and pathogens (Burge *et. al.*, 2016). It is reported that use of Biorock technology for coral reef restoration had significant recruitment rates for filter feeding organisms such as tunicates and calcareous tube-dwelling polychaetas (Goreau 2012).

For the quantification of biodiversity ecologists use two separate components: number of species present (species richness) and their relative abundances (evenness). Species richness and evenness can be combined into a single indicator, and in ecology the Shannon Index is commonly used (Magurran 2004). The species richness and Shannon Index (diversity) were calculated for all three test sites.

At the Port Canaveral test site, the average species richness was in favor of the steel mats towards the end of the experiment and had a value of 5 on the steel with plastic value of 4. Melbourne Beach (steel – 5, plastic – 6) and Grant (steel – 6, plastic – 7) had the higher species richness on the plastic than steel mats. The number of species in a local assemblage is an intuitive index of community structure since some species can be overlooked (Gotelli and Chao, 2013). Overall after five months, the species richness on the steel mats are comparable to the plastic. The statistical analysis did not find a significant difference.

The good diversity of benthic organisms on the oyster mats is important in improving the water quality and increasing the richness of fish species (Harding and Mann 1999). During the five-month experiment, diversity of benthic sessile organisms was increased at all sites, but the rates and performance over time were different between locations.

The videos displayed an abundant increase of mobile organisms associated with the oyster shells on the steel mats. Ecologically important species such as spotted sea trout, blue crabs, stone crabs, and shrimp were observed during the experiment. This presence of fish and other mobile organisms relates to other studies that found restored oyster reefs act as important habitats for fish and invertebrates (Harding and Mann 1999, Bourdreaux *et al*., 2006). Other studies have also found the electrical stimulated mineral accretion technology provide an increase in the fish after 8 months of the deployment, with individual numbers ranging from 92 to 142 and a species diversity ranging from 6 to 14 (Hilbertz 1979)**.** The observations made around the steel mats are important, as juvenile fish and invertebrates use oyster reefs as habitat, refuge and feeding grounds (zu Ermgassen *et al*., 2016).

The hypothesis that the biodiversity of sessile and mobile organisms will be greater on the cathodically protected steel mesh than that of the standard plastic mesh was not confirmed with the experiment. Even though, there was not significant difference between steel and plastic results they are comparable in oyster recruitment, biodiversity of sessile and mobile organisms. The steel mesh mats can be used as an environmentally friendly alternative material for oyster reef restoration.

5.0 Conclusion and Recommendation for Future Work

Overall, the research supported the idea that the steel mesh mats can be used as an environmentally friendly alternative material for oyster reef restoration. The steel and plastic mats had comparable results in oyster recruitment, biodiversity of sessile and mobile organisms. The mineral accretion on electrical stimulated steel mats created a suitable substrate for the oysters and other hard and soft benthic organisms to grow not only on the dead oyster shells but also directly on the steel mats. More significant results between steel and plastic mats and boxes may be achieved with a longer immersion time (Shorr *et al*., 2012).

Many oyster restoration projects in Florida are done through citizen science projects. The steel mesh is easy to work with and a solar panel can be used as a power source. This technology is not only beneficial to oyster restoration but also aquaculture industry that use a lot plastic for production (Arthur *et. al*., 2008).

Further research is needed to optimize the current and voltage requirements for the best stimulated oyster growth. While this was attempted in a field setting, laboratory experiments would allow more stable environment for the fine tuning that later can be repeated in field experiments. Previous research (Goreau 2012) showed that electrically stimulated steel created a beneficial environment around the electrical and magnetic fields. In this thesis, the steel mesh and plastic mesh were in close proximity, which may have influenced comparisons in environmental benefits.

In a laboratory setting, the steel mesh and plastic mesh can be separated in the different tanks for comparison the results without interfering.

The future investigation of oyster larvae settlement directly on the electrically stimulated steel mats may provide the useful results in oyster restoration and aquaculture production. These can be conducted in laboratory settings, which will eliminate the presence of biofouling organisms and competition for space with the oyster larvae. Additionally, the orientation of steel substrates can be altered to achieve greater oyster settlement. The previous research has shown that orientation (vertical vs horizontal) can influence the settlement of oysters (Jonson 2017).

The replication of the oyster boxes experiment (Chapter 3) in the laboratory will allow more precise measurements of weight, length and electrical parameters. It can be done at any time and does not depend on May - October oysters' growing season (Doiron 2008). The design of boxes should use only steel mesh for walls and bottom and be opened at the top. The separate tanks for each current treatment and control plastic mesh will help with independent results.

The restoration of coral reefs with electrically stimulated steel technology is successfully used for shore protection and recovery of the severely eroded beaches (Goreau and Prong 2017). The hypothesis that electrical fields produced by this method may repel sharks (Goreau 2012) is an interesting topic for the future research. Positive results may address the safety of restored shorelines.

References

Anderson, D. M., Burkholder, J. M., Cochlan, W. P., Glibert, P. M., Gobler, C. J., Heil, C. A.,Vargo, G. A. 2008. *Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the united states.* doi: https://doi-org.portal.lib.fit.edu/10.1016/j.hal.2008.08.017

Arthur, C, Baker J. and Bamford H. eds. (2008). Proceedings of the International Workshop on the Occurrence, Effects and Fate of Microplastic Debris". University of Washington, Tacoma.

ASTM D6990. 2005. Standard practice for evaluating biofouling resistance and physical performance of marine coating systems. West Conshohocken, PA: ASTM International.

Banks, P., Berrigan, M., Choudhury, A., Craig, L., Diaz, D., Kern, F., Wikfors, G. 2007. Status review of the eastern oyster (*Crassostrea virginica*). NOAA Technical Memo NMFS F/SPO-88, Northeast Regional Office. Report to the National Marine Fisheries Service.

Bakti, L.A.A., Virgota, A., Damayanti, L.P.A., Radiman, T.H.U., Retnowulan, A., Hernawati, Sabil, A. and Robbe, D. 2012. Biorock Reef Restoration in Gili Trawangan, North Lombok, Indonesia. In: Goreau, T.J. and Trench, R.K., Eds., Innovative Methods of Marine Ecosystem Restoration, CRC Press, Boca Raton, 59-80.

Barber, A., Walters, L., & Birch, A. 2010. Potential for restoring biodiversity of macro-flora and macrofauna on oyster reefs in Mosquito Lagoon, Florida. Florida Scientist. 73(1):47-62. Retrieved from http://www.jstor.org/stable/24321598.

Basics of Triploidy. 2020. Retrieved from http://www.4cshellfish.com/about-triploidy/index.php.

Baxter, D., Britton J. 2007. Offshore cathodic protection 101. https://stoprust.com/technical-library-items/cp-101/

Beck, Michael W., Robert D. Brumbaugh, Laura Airoldi, Alvar Carranza, Loren D. Coen, Christine Crawford, Omar Defeo, Graham J. Edgar, Boze Hancock, Matthew C. Kay, Hunter S. Lenihan, Mark W. Luckenbach, Caitlyn L. Toropova, Guofan Zhang, and Ximing Guo. 2011. "Oyster reefs at risk and recommendations for conservation, restoration, and management." *BioScience*, vol. 61, no. 2, 2011.

Berger, N., Haseltine, M., Boehm, J., Goreau, T.J. 2012, Increased oyster growth and survival using Biorock Technology, In Innovative Technologies for Marine Ecosystem Restoration, Ed TJ Goreau and RK Trench, CRC Press.

Beseres Pollack, J., Yoskowitz, D., Kim, H. C., Montagna, P. A. 2013. Role and Value of Nitrogen Regulation Provided by Oysters (Crassostrea virginica) in the Mission-Aransas Estuary, Texas, USA. PLoS ONE, 8(6). https://doi.org/10.1371/journal.pone.0065314

Borell, E., Romatzki, S., Ferse, S. 2009. Differential physiological responses of two congeneric scleracrtinian corals to mineral accretion and an electrical field. J. Exp. Mar. Biol. Ecol.10.

Bourdreaux, M. L., Stiner, J. L., & Walters, L. J. (2006). Biodiversity of sessile and motile macrofauna on intertidal oyster reefs in mosquito lagoon, Florida. *Journal of Shellfish Research, 25*(3), 1079-1089. doi:10.2983/0730-8000(2006)25[1079:BOSAMM]2.0.CO;2

Burge, Colleen A., Collin J. Closek, Carolyn S. Friedman, Maya L. Groner, Cody M. Jenkins, Amanda Shore-Maggio, and Jennifer E. Welsh. 2016."The Use of Filter-feeders to Manage Disease in a Changing World." *Integrative and Comparative Biology*, vol. 56, no. 4, 2016, pp. 573–587.

Chambers, L., Gaspar, S., Pilato, C. Steinmuller, H., McCarthy, K., Sacks, P., Walters, L. 2018. How well do restored intertidal ouster reefs support key biogeochemical properties in a costal lagoon? Estuaries and Coasts.(2018) 41: 784-799. doi 10.1007/s12237-017-0311-5.

Coen, L.D., R.D. Brumbaugh, D. Bushek, R. Grizzle, M.W. Luckenbach, M.H. Posey, S.P. Powers, and S.G. Tolley. 2007. Ecosystem services related to oyster restoration. *Marine Ecology Progress Series* 341: 303–307.

Cole, M., Galloway, T. S. (2015). Ingestion of Nanoplastics and Microplastics by Pacific Oyster Larvae. Environ. Sci. Technol. 2015, 49, 14625−14632.

Dame, Richard F. 2012. "Ecology of Marine Bivalves: An Ecosystem Approach." CRC Press LLC, London.

Doiron, S. 2008. The Reference Manual for Oyster Aquaculturists. Agriculture and Aquaculture. 2008.

The Editors of Encyclopedia Britannica. “Electrolysis.” *Encyclopedia Britannica*, Encyclopedia Britannica, Inc., 6 Feb. 2020, www.britannica.com/science/electrolysis.

Fire, S., Browning, J., Noke Durden, W., Stolen, M. 2020. Comparison of during-bloom and inter-bloom brevetoxin and saxitoxin concentrations in Indian River Lagoon bottlenose dolphins, 2002–2011. Aquatic Toxicology, Volume 218, January 2020, 105371. ISSN 0166-445X, https://doi.org/10.1016/j.aquatox.2019.105371.

Fonseca, M.S., Kenworthy, J., Thayer, G.W. (1998). Guidelines for the conservation and restoration of seagrasses in the United States and adjacent waters. *NOAA Coastal Ocean Program Decision Analysis Series, 12,* NOAA Coastal Office, Silver Spring, MD; 222.

Forrest, B.M., Keeley, N.B., Hopkins, G.A., Webb, S.C., & Clement, D.M. 2009. Bivalve aquaculture in estuaries: review and synthesis of oyster cultivation effects. Aquaculture. 298:1-15. https://doi.org/10.1016/j.aquaculture. 2009.09.032.

Fox, Austen L., John H. Trefrey. “Environmental Dredging to Remove Fine-Grained, Organic-Rich Sediments and Reduce Inputs of Nitrogen and Phosphorus to a Subtropical Estuary.” *Marine Technology Society Journal*, vol. 52, no. 4, 2018, pp. 42 – 57.

Furla, Paula., Galgani, Isabelle, Durand, Isabelle, Allemand, Denis. 2000. Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis**.** The Journal of Experimental Biology 203, 3445–3457 (2000).

Galimany, E., Lunt, J., Freeman, C.J., Reed, S., Segura-García, I., & Paul, V.J. 2017. Feeding behavior of eastern oysters *Crassostrea virginica* and hard clams *Mercenaria mercenaria* in shallow estuaries. Mar Ecol Prog Ser. 567:125-37. https://doi.org/10.3354/meps12050.

Garvis, Stephanie K., Paul E. Sacks, and Linda J. Walters. "Formation, Movement, and Restoration of Dead Intertidal Oyster Reefs in Canaveral National Seashore and Mosquito Lagoon, Florida." *Journal of Shellfish Research*, vol. 34, no. 2, 2015, pp. 251– 58.

Gaspar, T., Chi, R., Parrow, M., Ringwood, A. (2018). Cellular Bioreactivity of Micro- and Nano-Plastic Particles in Oysters. Front. Mar. Sci. 5:345. doi: 10.3389/fmars.2018.00345.

Gigault, J., et al. (2018). Current opinion: what is a nanoplastic? Environ. Polllut. 230, 1030–1034. doi: 10.1016/j.envpol.2018.01.024.

Gonzalez-Fernandez, C., et al. (2018). Cellular responses of Pacific oyster (Crassostrea gigas) gametes exposed in vitro to polystyrene nanoparticles. Chemosphere 208, 764-772. https://doi.org/10.1016/j.chemosphere.2018.06.039.

Goreau, T. J., Gervino, J.M., Pollina, R. 2004. Increased zooxanthellae numbers and miotic index in electrically stimulated corals. Symbiosis 37, 107-120.

Gigault, J., et al. (2018). Current opinion: what is a nanoplastic? Environ. Polllut. 230, 1030–1034. doi: 10.1016/j.envpol.2018.01.024.

Goreau, T. J., Hilbertz, W. 2005. Marine ecosystem restoration: cost and benefits for coral reefs. World Resource Review, 17: 375-409.

Goreau, T.J. & Hilbertz, W. 2008. Bottom-up community-based coral reef and fisheries restoration in Indonesia, Panama, and Palau. In: Handbook of Regenerative Landscape Design. France, R.L. (Ed.). CRC Press: 143-150

Goreau, T. J. 2012. Marine electrolysis for building materials and environmental restoration. In V. Linkov (Ed.), Electrolysis, InTech Publishing, Rijeka, Croatia.

Goreau, T.J. and Hilbertz, W. 2012. Reef Restoration Using Seawater Electrolysis in Jamaica. In: Goreau, T.J. and Trench, R.K., Eds., Innovative Methods of Marine Ecosystem Restoration, CRC Press, Boca Raton, 36-46.

Goreau, T.J., Hilbertz, W., Hakeem, A., Sarkisian, T., Gutzeit, F., Spenhoff, A. 2012. Restoring Reefs to Grow Back Beaches and Protect Coasts from, Erosion and Global Sea-Level rise. In: Goreau, T.J. and Trench, R.K., Eds., Innovative Methods of Marine Ecosystem Restoration, CRC Press, Boca Raton, 11-35.

Goreau, T. J., Prong, P. 2017. Biorock Electric Reefs Grow back severely Eroded Beaches in Months. J. Mar. Sci. Eng. 5, 48; doi:10.3390/jmse5040048.

Gotelli, N. J., Chao, A. 2013. Measuring and Estimating Species Richness, Species Diversity, and Biotic Similarity from Sampling Data. In: Levin S.A. (ed.) Encyclopedia of Biodiversity, second edition, Volume 5, pp. 195211. Waltham, MA: Academic Press.

Grizzle, R.E., Greene, J.K., Coen, L.D., 2008. Seston removal by natural and constructed intertidal eastern oyster *(Crassostrea virginica)* reefs: a comparison with previous laboratory studies, and the value of in situ methods. Estuaries Coasts 31, 1208-1220 .Lestari (Eds.). Proceedings of the COREMAP Symposium on Coral Reef Management in the Coral Triangle. Invited Keynote Talk, World Ocean Congress, Manado, Sulawesi, Indonesia.

Harding JM, Mann R. 1999. Fish species in relation to restored oyster reefs, Piankatank River, Virgina. Bull Mar Sci 65(1): 289-300.

Hilbertz, W. 1975. Toward self-growing structures. *Industrialization Forum* 6:53-56.

Hilbertz, W. 1979. Electrodeposition of minerals in sea water: Experiments and applications. IEEE Journal on Oceanic Engineering 4:1–19.

Hilbertz, W. 1992. Solar-generated building material from seawater as a sink for carbon. *Ambio* 21: 126-129.

Hilbertz, W., Goreau, T. J. 1996. Method of enhancing the growth of aquatic organisms, and structures created thereby. Patent 5,543,034. United States Patent Office.

Hunsucker, K., Melnikov, A., Gilligan, M., Gardner, H., Erdogan, C., Weaver, R., Swain, G. 2021. Cathodically Protected Steel as an Alternative to Plastic for Oyster Restoration Mats. Ecological Engineering (in revision).

Johnson, K. 2017. Laboratory settlement of the eastern oyster *Crassostrea Virginia* influenced by substratum concavity, orientation, and tertiary arrangement. *Journal of Shellfish Research,* Vol. 36 No. 2. 315-324.

Karissa, P.T., Sukardi, Priyono, S.B., Mamangkey, G.F. and Taylor, J.J.U. 2012. Utilization of low-voltage electricity to stimulate cultivation of pearl oysters *Pinctada Maxima* (Jameson). Innovative Methods of Marine Ecosystem Restoration, CRC Press, Boca Raton, 131 – 139.

Kellogg, M. Lisa, Smyth, Ashley R., Luckenbach, Mark W., Carmichael, Ruth H., Brown, Bonnie L., Cornwell, Jeffrey C., Piehler, Michael F., Owens, Michael S. Dalrymple, D. Joseph, Higgins, Colleen B.2014. Use of oysters to mitigate eutrophication in coastal waters. Estuarine, Costal and Shelf Science 151 (2014) 156-168. http://dx.doi.org/10.1016/j.ecss.2014.09.025

Kjerfve, B. 1986. Comparative Oceanography of Coastal Lagoons. Estuary Variability. 63-81. https://doi.org/10.1016/B978-0-12-761890- 6.50009-5

Koster JW. 2017. Electrolysis, halogen oxidizing agents and reef restoration. Master's Thesis. UC Santa Cruz.

Magurran, A. E. 2004. Measuring Biological Diversity. Blackwell.

Ogunola, O., Palanisami, T. 2016. Microplastics in the Marine Environment: Current Status, Assessment Methodologies, Impacts and Solutions. Journal of Pollution Effects & Control. 04. 10.4172/2375-4397.1000161.

National Oceanic and Atmospheric Administration (NOAA). (2008). Proceedings of the International Research Workshop on the Occurrence, effects, and fate of Microplastic Marine debris eds C Arthur, J. Baker, and H. Bamford. Technical Memorandum NOS-OR&R-30 (Tacoma, WA University of Washington Tacoma).

Paul-Pont, I., et al.(2018). Constraints and Priorities for Conducting Experimental Exposures of Marine Organisms to Microplastics. Front. Mar. Sci. 5:252. doi: 10.3389/fmars.2018.00252.

Shorr, J., Cervino, J., Lin, C., Weeks, R., and Goreau, T. J. 2012. Electrical stimulation increases oyster growth and survival om restoration projects. Innovative Methods of Marine Ecosystem Restoration, CRC Press, Boca Raton, 151 – 159.

Stomberg, S. M., Lundalv, T., Goreau, T. J. 2010. Suitability of mineral accretion as a rehabilitation method for cold-water coral reefs. J. Exp. Mar. Biol. Ecol. 395: 53-161.

Su, Z., Yan, Y., & Huang, L. 2007. Effect of Fouling on Feeding, Oxygen Consumption and Waste Excretion of Pearl Oyster *Pinctada martensii* in Daya Bay Cultivation. Marine Science Bulletin, Vol.9 No2 Oct. 2007.

Sussarellu, R., Suquet, M., Thomas, Y., et al. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. *Proc Natl Acad Sci U S A*. 2016;113(9):2430–2435. doi:10.1073/pnas.1519019113.

Swain G.W., Muller E., Polly D. 1994. A Cathodic Protection System for the Living Seas-Epcot Center. Materials Performance. 33 (10): 21.

Volety, A.K. 2015. Southwest Florida Shelf Coastal Marine Ecosystem-Habitat: Oyster Reefs, Florida Gulf Coast, University. Southwest Florida Shelf Coastal Marine Ecosystem Habitat: Oyster Reefs, MARES, 67-79.

Wall, C.C., Peterson, B.J., & Gobler, C.J. 2008. Facilitation of seagrass *Zostera marina* productivity by suspension-feeding bivalves. Mar Ecol Prog Ser. 357:165-74. https://doi.org/10.3354/meps07289.

Walters, L.J., Roman, A., Stiner, J., & Weeks, D. 2001. Water Resource Management Plan, Canaveral National Seashore. Titusville, FL: National Park Service, Canaveral National Seashore. p. 224.

Walters, L. 2013. "How to Make an Oyster Restoration Mat" (2013). CEELAB Outreach Materials. https://stars.library.ucf.edu/ceelaboutreach/1

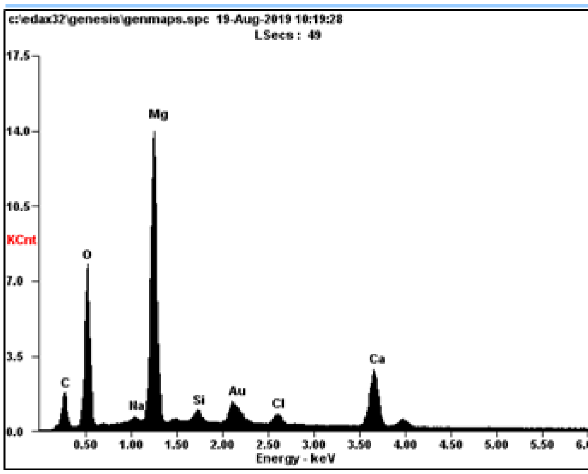
Weaver, Robert J., and Kelli Hunsucker. 2018. “The Living Dock: A Study of Benthic Recruitment to Oyster Substrates Affixed to a Dock in the Indian River Lagoon.” Marine Technology Society Journal, vol. 52, no. 4, 2018, pp. 7–18.

zu Ermgassen, P., B. Hancock, B. DeAngelis, J. Greene, E. Schuster, M. Spalding, and R. Brumbaugh. 2016. *Setting objectives for oyster habitat restoration using ecosystem services: A manager’s guide*. The Nature Conservancy, Arlington, VA, 2016.

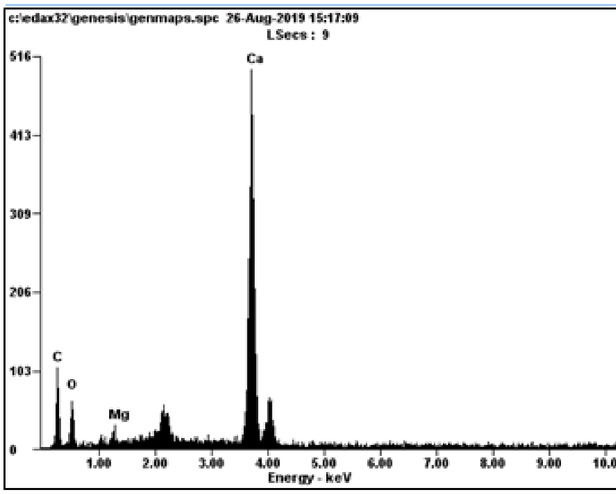
Appendix A

Table A1. Mineral Accretion Chemical Compassion.

		Port Canaveral	Melbourne Beach	Grant
Pre-chalking Wt%		*MgK*	*MgK*	*MgK*
		28.69	35.25	22.1
		CaK	*CaK*	*CaK*
		14.53	11.91	6.46
Month One Wt%	Grey Coupon	*MgK*	*MgK*	*MgK*
		0.59	3.14	9.76
		CaK	*CaK*	*CaK*
		65.58	16.99	7.48
	Orange Coupon	*MgK*	*MgK*	*MgK*
		1.26	4.96	13.91
		CaK	*CaK*	*CaK*
		73.58	21.01	8.45
	Purple Coupon	*MgK*	*MgK*	*MgK*
		1.68	41.28	14.18
		CaK	*CaK*	*CaK*
		64.87	7.39	7.61
Month Two Wt%	Grey Coupon	*MgK*	*MgK*	*MgK*
		25.54	36.96	9.63
		CaK	*CaK*	*CaK*
		2.16	5.95	6.42
	Orange Coupon	*MgK*	*MgK*	*MgK*
		14.07	11.69	11.85
		CaK	*CaK*	*CaK*
		3.09	4.65	3.33
	Purple Coupon	*MgK*	*MgK*	*MgK*
		5	18.51	1.1
		CaK	*CaK*	*CaK*
		0	5.41	15.43
Month Three Wt%	Grey Coupon	*MgK*	*MgK*	*MgK*
		4.03	17.42	6.1
		CaK	*CaK*	*CaK*
		0	2.94	0
	Orange Coupon	*MgK*	*MgK*	*MgK*
		8.51	6.3	5.32
		CaK	*CaK*	*CaK*
		3.57	0	1.08
	Purple Coupon	*MgK*	*MgK*	*MgK*
		13.39	7.7	4.2
		CaK	*CaK*	*CaK*
		4.1	1.92	0.68



Element	Wt%	At%
CK	14.08	24.83
OK	29.22	38.68
NaK	00.74	00.68
MgK	28.69	24.99
SiK	01.31	00.99
AuM	09.55	01.03
ClK	01.88	01.12
CaK	14.53	07.68
Matrix	Correction	ZAF



Element	Wt%	At%
CK	11.76	26.43
OK	13.40	22.62
MgK	01.26	01.39
CaK	73.58	49.56
Matrix	Correction	ZAF

Figure A1. The EDAX report for chemical composition of mineral accretion on the steel coupon at Port Canaveral: the pre-chalking (top) and after month one immersion (bottom).

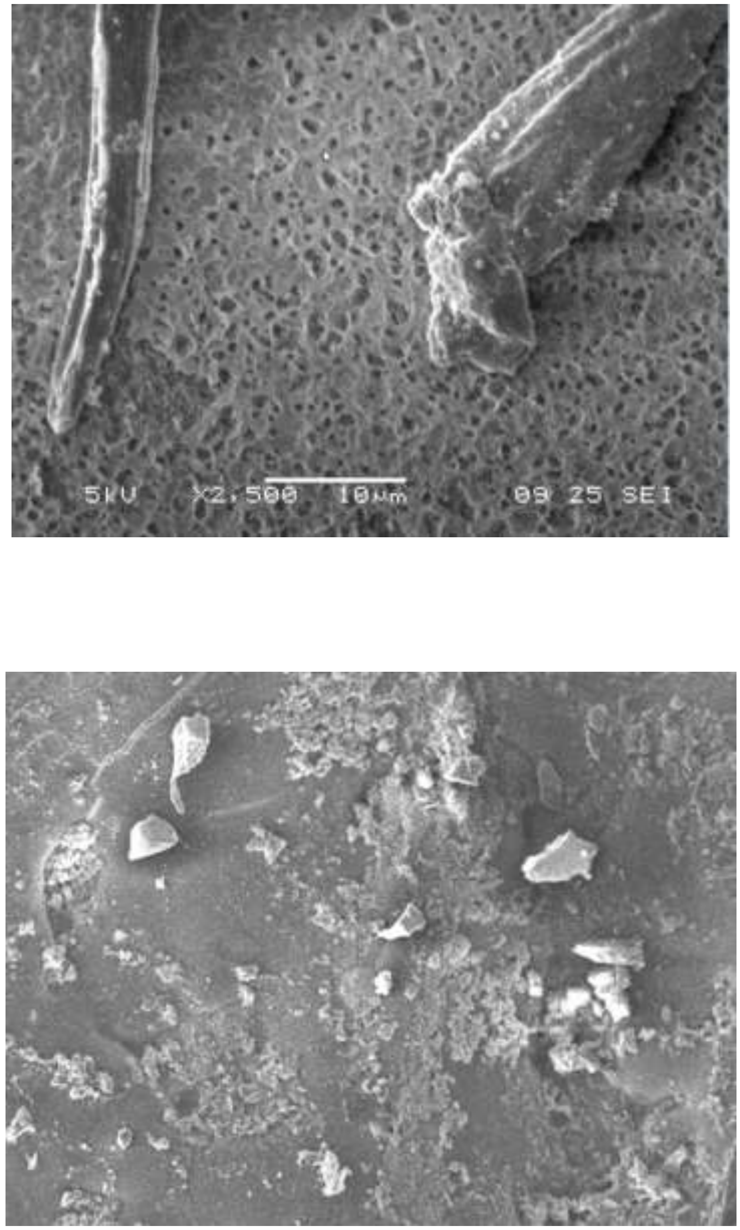


Figure A2. SEM images of the mineral accretion on steel coupons.

Appendix B

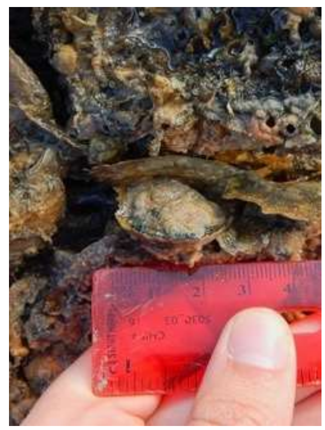
Grant Month 3

Grant Month 4

Grant Month 5

Melbourne Month 3

Melbourne Month 4



Melbourne Month 5

Port Month 3

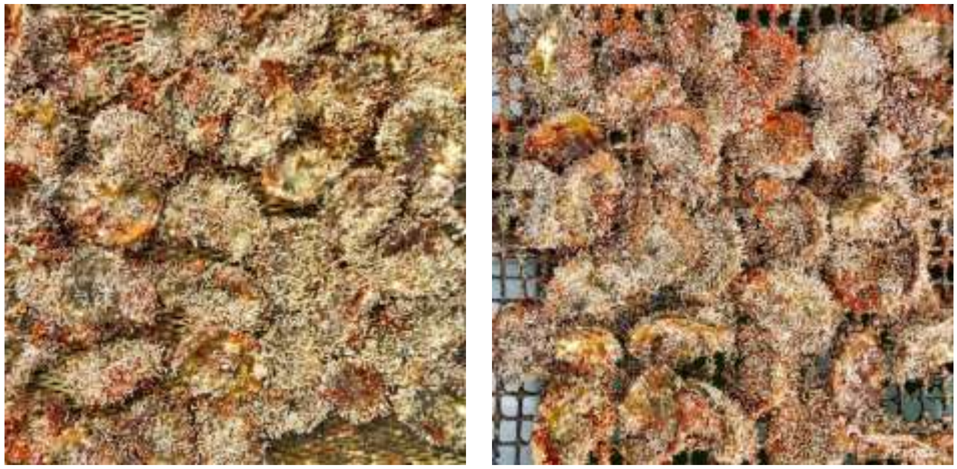
Port Month 4

Port Month 5

Figure B1. Photographs of oysters after month three, four and five of the immersion at the test locations.

Appendix C

a)



b)

c)

Figure C1. Oyster mats after month one immersion. Steel (left) and plastic (right) mats: a) Port Canaveral, b) Melbourne Beach, c) Grant.

a)

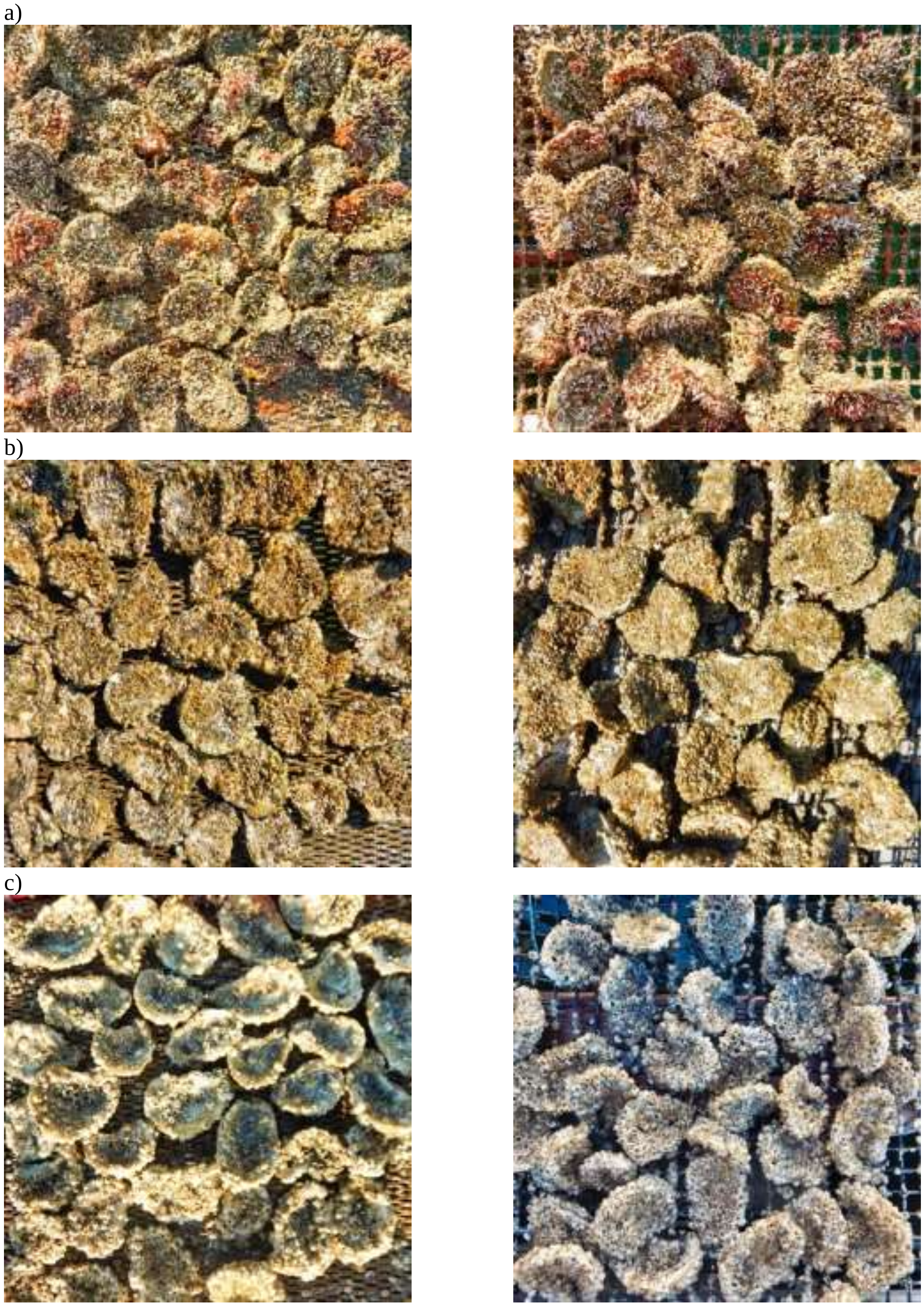


Figure C2. Oyster mats after month two immersion. Steel (left) and plastic (right) mats: a) Port Canaveral, b) Melbourne Beach, c) Grant.

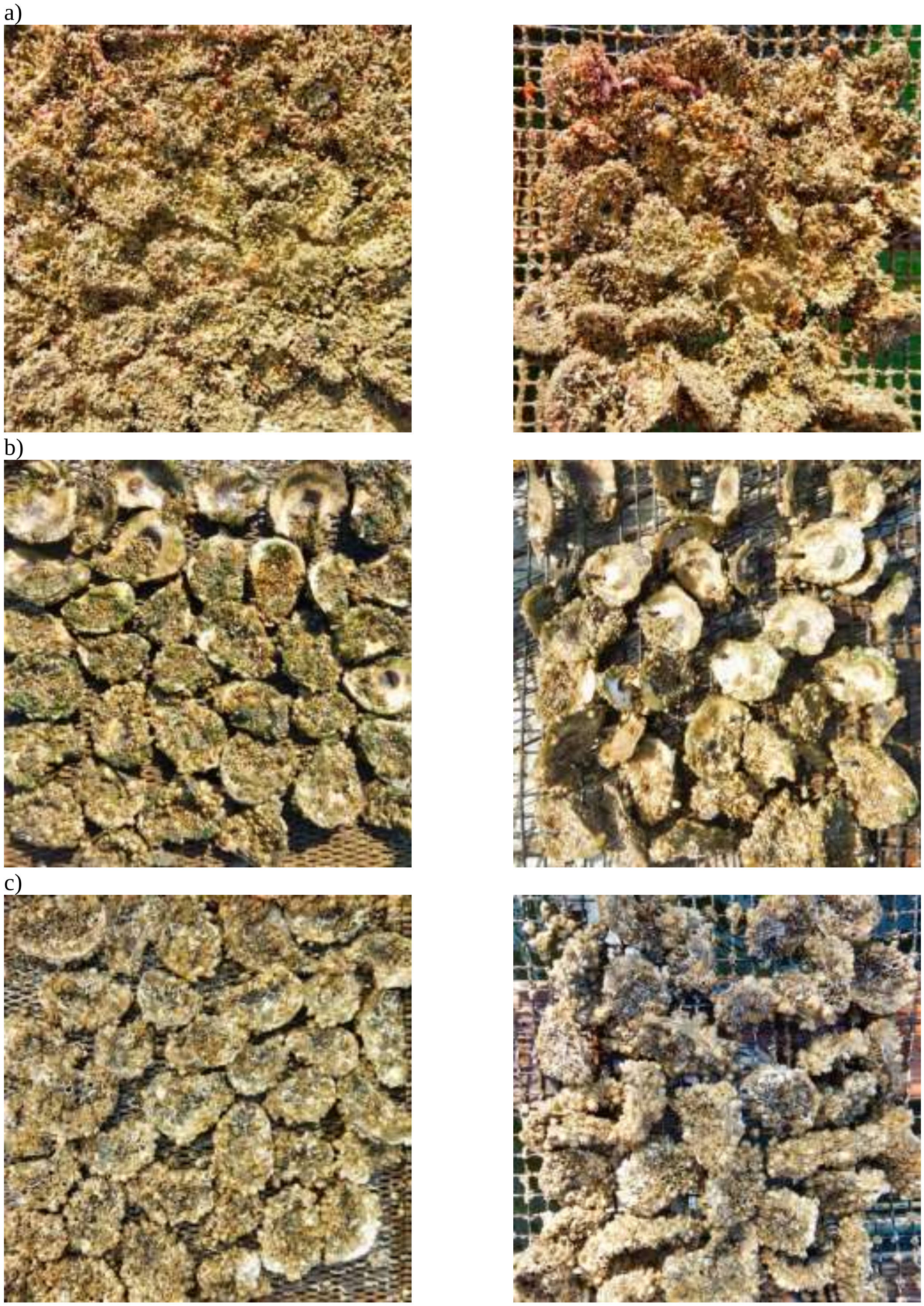
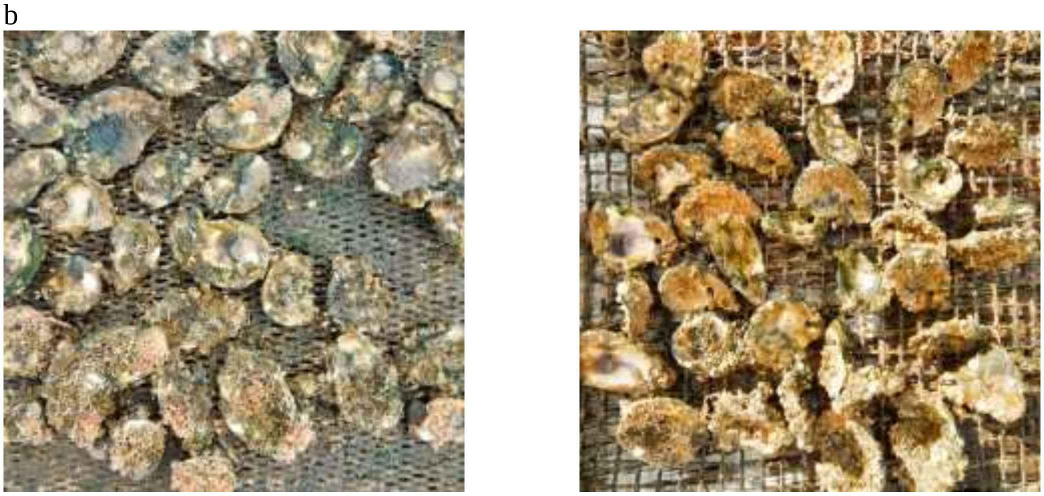


Figure C3. Oyster mats after month three immersion. Steel (left) and plastic (right) mats: a) Port Canaveral, b) Melbourne Beach, c) Grant.

a

b



c

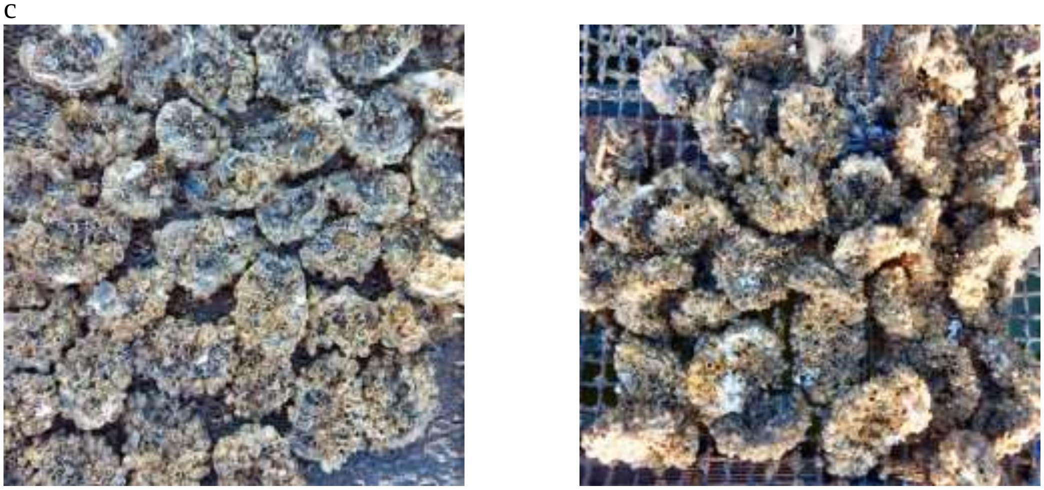


Figure C4. Oyster mats after month four immersion. Steel (left) and plastic (right) mats: a) Port Canaveral, b) Melbourne Beach, c) Grant.