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EFFECTS OF ARTIFICIAL SUBSTRATES ON THE RECRUITMENT OF JUVENILE FISHES IN THE NORTHWESTERN GULF OF MEXICO

A Thesis

by

STEFANY SALINAS

Submitted to the Graduate College of The University of Texas Rio Grande Valley In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2020

Major Subject: Ocean, Coastal and Earth Sciences

EFFECTS OF ARTIFICIAL SUBSTRATES ON THE RECRUITMENT OF JUVENILE FISHES IN THE NORTHWESTERN GULF OF MEXICO

A Thesis by STEFANY SALINAS

COMMITTEE MEMBERS

Dr. Carlos E. Cintra-Buenrostro Chair of Committee

> Dr. Daniele Provenzano Committee Member

Dr. Richard J. Kline Committee Member

May 2020

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ABSTRACT

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Most artificial reefs (ARs) in the Gulf of Mexico are considered intermediate to high vertical relief structures which have recruited large predatory species indicating lack of juvenile habitat. Small, inexpensive ARs, standard monitoring units for the recruitment of reef fish (SMURFs), were deployed at eight sites at -20m as habitat for juvenile fishes to understand and determine the effects of substrate type on juvenile fishes recruitment. Each site contained four SMURFs with four treatments: small and large rubble (~10 and ~20cm), oyster shells, and bare. Surveys yielded 39 juvenile species, including one of the most economically important species, *Lutjanus campechanus*, present across all replicates. Highest richness and diversity occurred in SMURFs containing oysters or small rubble while bare had the lowest. This study suggests deployment of low-relief structures with different substrate materials might affect recruitment of select species and provide further information useful to individuals designing ARs, aiding fisheries production.

DEDICATION

Dedicated to my mother and father, Alma and Juan Salinas, who taught me the value of education and for their endless love and encouragement. To my sister and all my friends without whom none of this would have been possible. Thank you all for your support.

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CHAPTER I

INTRODUCTION

According to the United Nations (2017), ~40% of the world's population resides within 100 km of a coast. However, Earth's shorelines are also ecological hotspots, regions hosting numerous threatened species (Myers et al., 2000). Coral reef ecosystems are estimated to harbor ~600,000 to > 9 million living species worldwide and are of critical economic importance; providing ecosystems that are beneficial to their inhabitants as well as to humans through fisheries, coastal protection, and tourism (Hoegh-Guldberg et al., 2007; Plaisance et al., 2011). Rapid human population growth and expansion accompanied by increased resource consumption has sparked vast concerns among scientists, government officials, and the public on fisheries. Mainly due to the fact that as population augments, urbanization and economic rise increases the amount of waste, pollution and chemical runoff, and the use of destructive fishing practices that can wreak havoc and lead to the depletion of species and habitat damage in many locations (Clausen & York, 2008). These effects have resulted in an increased deployment of artificial reefs (ARs) around the world to help buffer the effects of overfishing and habitat damage and destruction by providing additional habitat for marine life (Bohnsack et al., 1994; Clausen & York, op. cit.).

Artificial reefs are defined as man-made structures or materials that provide abiotic and/or biotic colonization substrates for marine organisms ranging from prokaryotes and

protozoa to barnacles, corals, sponges, clams, bryozoans, hydroids, etc. (Chang, 1985; Jardeweski & Almeida, 2006; Wiens et al., 2010). Fishermen have known for centuries that fishes congregate around sunken objects within hours of hitting the ocean floor, and evidence suggests that ARs and fish aggregating devices (FADs) have been used as early as 1789 to help populate coastal fisheries (Oren, 1968; Stone, 1982; Polovina, 1991). Primitive ARs consisted of mangrove tree branches, bamboo, tires, and concrete cubes that provided solid substrates that attracted species not usually found in soft-bottom sites (Polovina, op. cit.; Munro & Balgos, 1995; Deudero et al., 1999; Lukens & Selberg, 2004; Gratwicke & Speight, 2005; Jardeweski & Almeida, op. cit.). Hard substrates are initially colonized by bacterial biofilms providing a biotic substrate for films of periphyton, algae and sessile invertebrates that function as important "habitat-formers," and an important food source to grazing animals (Gratwicke & Speight, op. cit.). These habitat-formers or epibenthic organisms provide subsequent biotic substrates on ARs as forage items for crustaceans, polychaetes, molluscs, and fishes (Jessee et al., 1985). Increasing total hard surface area of a reef, elevates net productivity and hence boosts the number of fishes supported (Gratwicke & Speight, op. cit.).

In the US, the number of permitted AR projects to mitigate loss of habitat and increase fish populations has grown exponentially since the first documented ARs in the 1830s (Polovina, 1991; Bohnsack et al., 1994; Lukens & Selberg, 2004; Gratwicke & Speight, 2005). Most early ARs in the US consisted mainly of scrap materials (Buchanan, 1973; Parker et al., 1974; Smith & Mathews, 1979; Pybas, 1997; Lukens & Selberg, op. cit.) until in 1989 the Texas legislature recognized the importance of these efforts, and enacted the Artificial Reef Act directing Texas Parks and Wildlife Department (TPWD) to develop a state artificial plan, Texas Artificial Reef Plan (Bohnsack & Sutherland, 1985; Stephan et al., 1990).

Under the Texas Artificial Reef Plan, three AR programs were enacted the: Rigs-to-Reef, Ships-to-Reef, and Nearshore Reefing Program (Stephan et al., 1990). The Rigs-to-Reef Program involves sinking or tipping over decommissioned petroleum platforms constructed of corrosionresistant steel that meets the five major characteristics or standards for AR materials which are function, compatibility, durability, stability and availability giving rise to high profile ARs (Lukens & Selberg, 2004). Typically, oil rigs support a thriving reef ecosystem while in service (Stephan et al., op. cit.; Shively et al., 2003). The Ships-to-Reef Program intentionally sinks decommissioned boats for the same purpose; as of today, 12 liberty ships, four deck barges, two tugboats, one shrimp boat, and a ~30 m Navy dive barge have been sunk (Arnold, 1998; Froehlich & Kline, 2015). The Nearshore Reefing Program allows private citizens, organizations and corporations to deploy stable (material remaining in its original configuration on the site), durable (resistant to the chemical and physical forces of the marine environment such as steel or concrete), and complex (have lots of spaces or openings for marine life) AR materials under TPWD's guidance (Lukens & Selberg, op. cit.). Given the economic and ecological importance of Red Snapper, *Lutjanus campechanus* (Poey, 1860) in the Gulf of Mexico, most of these ARs have been deployed specifically targeting the recovery of this species (Syc & Szedlmayer, 2012; Powers et al., 2018). *Lutjanus campechanus* forms one of the largest fisheries in the region and numerous studies (e.g., Patterson III et al., 2001; Froehlich & Kline, op. cit.; Arney et al., 2017; Streich et al., 2017 Powers et al., op. cit.) have shown their presence, affinity, and high abundances for various types of ARs such as culverts, shipwrecks, oil platforms, concrete pyramids, etc. Most studies comparing natural to ARs have found similar community structure among the reefs but sometimes with fewer species on ARs (Carr & Hixon, 1997). Nevertheless,

compared to open bottom areas, ARs have been found to host up to 35 times greater biomass and fishes density (Bohnsack & Sutherland, 1985).

The quantity and type of habitat structure has been shown to impact the distribution and abundance of fishes in response to different components of the habitat such as vertical relief, spatial heterogeneity and structural density that contribute to varying degrees of complexity that affect colonization, and therefore food availability and/or predation (Anderson et al., 1989; Svane & Petersen, 2001). Vertical relief refers to the height provided by the structure (Bohnsack & Sutherland, 1985). Several studies have shown that most high-relief structures such as ships and rigs generally provide habitat for larger, adult fishes resulting in high predation-mortality on juveniles recruited to these habitats (Bohnsack et al., 1994; West et al., 1994; Arnold, 1998). As a result, most juvenile fishes (age 0 and age 1), such as *L. campechanus*, will usually move to available small, low-relief $(1-3 \text{ m}^3)$ ARs (Syc & Szedlmayer, 2012). Numerous studies done on natural and ARs have evaluated influence of habitat structure and complexity on the community assembly of reef fishes, reporting that species diversity is positively related to habitat complexity (August, 1983; Gorham & Alevizon, 1989). Heterogeneity of the area can also strongly influence the abundance and distribution of fish assemblages especially in areas of low productivity of epibenthic organisms, and on the seafloor with low levels of physical heterogeneity such as sand and mud bottoms (Friedlander & Parrish, 1998; Svane & Petersen, op. cit.). The introduction of novel hard substrate such as ARs leads to colonization by settling larvae and spores belonging to a large number of epibenthic organisms thereby increasing the abundance of food for fish (Anderson et al., op. cit.; Svane & Petersen, op. cit.). Therefore, incrementing complexity and heterogeneity of the area is hypothesized to increase species diversity by providing more

protection per unit space, which as August (op. cit.) indicated is advantageous especially for juvenile fishes.

Predation is actually one of the major factors influencing population size and structure of ecological communities, as habitat structure may interfere with effects of predation by providing individuals with higher numbers of refuges where predators are unable to reach the prey or by diminishing encounters between interacting parties (Beukers & Jones, 1998). Several studies (e.g., Gratwicke & Speight, 2005; Kuffner et al., 2007; Alvarez-Filip et al., 2009) mentioned rugosity of a structure or substrate, a measure of small-scale variations of amplitude in the height of a surface, as an important and significant complexity variable positively affecting fish species abundance and richness, possibly due to the increased refuge from predators. Multiple linear regression models done by Friedlander & Parrish (1998) in Hanalei Bay, Hawaii indicated that a large part of the variability in species abundances, richness and diversity is explained by the size of crevice in and the rugosity of the substratum. They also recorded a strong linear relationship suggested that the volume of holes on the reef surface emphasized the importance of shelter for fish assemblages (Friedlander & Parrish, op. cit). Therefore, the type of the substrate or material utilized is expected to influence the species composition and diversity of fishes in an area (Luckhurst & Luckhurst, 1978). Results of these studies have practical applications in designing ARs as well as theoretical value in helping to explain the organization of reef fish assemblages as suggested by Friedlander & Parrish (op. cit.).

Several species indices are often utilized in ecology to accurately assess population and community dynamics of ARs, including: species abundance, richness, evenness and diversity (Gratwicke & Speight, 2005). According to Mason et al. (2005) these metrics are defined as: a) species abundance is simply a measure of the number of individuals per species, b) species

richness is the number of different species within a community, c) species evenness is a measure of how equally spread the abundances across the species in an area are, and d) species diversity takes into account both species richness and evenness, measuring the number and frequency of species in a community. Fish community assemblages are also commonly compared using Bray-Curtis similarity matrices with multidimensional scaling (MDS) to examine and visualize potential similarities among sites (Walker et al., 2002).

Objectives and Hypotheses

The objective of this study was to deploy small, inexpensive ARs, known as standard monitoring units for the recruitment of reef fish (SMURFs) (Ammann, 2004), as habitat for juvenile fishes along the Southern coast of the Gulf of Mexico in compliance with the TPWD's Nearshore Reefing Program to understand and determine the effects of substrate type on juvenile fishes recruitment, and assess fish assemblage differences among the varying treatments (small rubble $(\sim 10 \text{ cm})$, large rubble $(\sim 20 \text{ cm})$, oyster shells and bare), throughout SMURFs reset event, sampling dates, and for the month of July among three consecutive years. Additionally, identify an optimal low-relief AR substrate for the recruitment and survival of targeted species such as *L. campechanus* by comparing abundances and sizes.

Specific hypotheses to be tested included the following:

- 1. Fish richness, evenness, diversity, and abundance will differ among distinct types of substrates within SMURFs.
- 2. SMURFs with no substrate will harbor lower species richness, evenness, diversity and abundance given that fishes are typically found in more complex habitats.

3. SMURFs reset event will have an effect on fish assemblages as the disturbance might cause

some species to leave the units.

- 4. Observed fish assemblages will differ among samplings dates due to seasonal variations, spawning events and stochastic processes, but remain similar for the month of July among the three consecutive years.
- 5. *Lutjanus campechanus* will be the most abundant species found in all of the treatments.
- 6. Abundances of *L. campechanus* will be lowest at the bare treatment. Size range is expected to be low given the small area of the SMURF as larger individuals do not need cover.

CHAPTER II

METHODS

Study Site

The study was conducted from July 2017 to July 2019 at the South Padre Island-1047 Reef previously known as the Port Mansfield Nearshore Reef (PS-1047, thus hereafter), located 11.3 km east of Port Mansfield, TX (26°31'32.10" N and 97° 9'12.91" W, Figure 1). This region is composed of 4,922 concrete culverts (1 m by 3 m) ranging in density (1-190 culverts), and a sunken tugboat at a depth of 20 m deployed by TPWD and the Coastal Conservation Association of Texas (Shively et al., 2003; Froehlich & Kline, 2015). The bottom is flat, and the sediment is characterized generally by soft sand and mud. The site also includes four naturally occurring reefs composed of hard clay and sandstone varying in length from 5 to 200 m (Froehlich & Kline, op. cit.).

Artificial Reef Construction and Deployment

Each of the 32 SMURFs was constructed as follows: two 108 x 36 cm plastic netting with grid sizes of 4.5 x 4.5 cm were laid down perpendicular one on top of the other, a concrete base (36 x 36 cm) was then placed at the center for the purpose of weighing the unit down, the remaining net was then molded into the base and the edges were bound with nylon cable ties

producing a crater-like structure with an opening at the top (Figure 2). The purpose of the plastic mesh was to help reduce predation but provide juvenile fishes access and prevent the non-fixed substrates from washing out. Thus, no tests were performed on potential effects of plastic mesh, which was consistent among all treatments (detailed below). The SMURFs were deployed at eight different locations around the perimeter of the South Padre Island-1047 Reef (Figure 1). All sites were randomly selected based on images produced by Humminbird 1198s SI side scan sonar (455 kHz, Johnson Outdoors Marine Electronics, Inc., Eufaula, Alabama) of the PS-1047 reef site to ensure a minimum distance of 160 m from any existing structure, and from one another to reduce possible movements of individuals among SMURFs (Zeller et al., 2003; Topping & Szedlmayer, 2011). At each location, four units along with three 18.9 L (5-gal.) buckets filled with three different types of substrates by volume, rubble diameter of either ~10 or \sim 20 cm, and \sim 10 cm oyster shells were deployed. A 1.8 m fence post and two cinder blocks were also deployed at each site to serve as the center point to facilitate diver orientation given the characteristic poor visibility conditions in this area of the Gulf of Mexico.

Figure 1. South Padre Island-1047 Reef map of the eight sites (red dots) used in this study located off the South Padre Island coastline, 11.3 km east of Port Mansfield, TX, USA. Modified from Google Earth (2018).

Figure 2. Standard monitoring unit for the recruitment of juvenile fish (SMURF) surrounded with plastic netting (bare treatment) at -20 m deep. Photo courtesy of Kirsten Lara.

The experimental set up was based on a randomized complete block design with four distinct treatments: the two non-overlapping categories of rubble diameter $(\sim 10 \text{ and } \sim 20 \text{ cm})$, oyster shell and a bare substrate treatment as a control consisted of only the cement block with plastic netting (Figure 2). At each site, the 10 m circumference circle was divided into four quadrants to account for any variation so that the observed differences were due to true differences among treatments. The treatments were then assigned at random to a quadrant and allocated within a 360° circumference with the use of a diving compass. The four treatments were set up randomly on June 2017 at the edge of 10 m circumference circles (blocks) in barren areas around the South Padre Island-1047 Reef (Figure 3).

Figure 3. Diagram of experimental setup at each site containing a **(A)** bare, **(B)** large rubble, **(C)** oyster shell and **(D)** small rubble treatment, as well as a **(E)** center point. Treatments were randomly allocated at each site (see text for details).

Sampling Regime

Sites were originally planned to be surveyed quarterly; however, due to unfavorable conditions this was not possible. Sampling was conducted from July 2017 to July 2019, resulting in a total of nine surveys. Field research was suspended after the first survey (July 2017) due to Hurricane Harvey, which crossed the Caribbean Sea, and into the Gulf of Mexico on August 2017 where it intensified into a category 4 strength bringing heavy rainfall (van Oldenborgh et al., 2017), and unfavorable diving conditions to the study sites. The study site was visited approximately one month and again three months after the storm to determine the condition of the SMURFs, which were found to be intact; however, visibility was too low to conduct

appropriate visual surveys. Surveys resumed June 2018 as weather conditions allowed to account for known recruitment cycles of commercially important species such as *L. campechanus* (June-August) (Alexander, 2015; Glenn et al., 2017). By the third survey (July 2018), the concrete base of most SMURFs had sunk below the plane of the ocean floor. After being surveyed, sunken SMURFs were pulled to the surface, cleaned and reset. SMURFs whose base did not sink were cleaned in the same manner during the reset process to avoid confounding factors. A five-week period was allotted before surveys resumed. Five more surveys, weather, vessel and divers' availability permitting, were completed in 2018; and one more in July 2019 to allow for a comparison of this month among years. Because of these adjustments, inherent modifications to data analyses were needed (detailed below).

Surveys were conducted by SCUBA using visual and video documentation, where two divers entered the water. The first diver (surveyor) was equipped with a slate, pencil and a data sheet to conduct a visual SCUBA fish survey following the Bohnsack & Bannerot (1986) stationary sampling method with some modifications. The second diver carried a GoPRO HD Hero 3+ camera to conduct the video documentation and reels to attach to the center post and conduct a 10 m sweep to locate each SMURF at each site. Upon encountering each SMURF, both divers recorded adult and juveniles surrounding the unit coming from opposite positions and rotating clockwise as they approached the SMURF. The survey lasted around two to three minutes depending on the time needed to count all fishes present. The surveyor would then look into the SMURF through the opening at the top to record hidden fishes and remove excess algal growth that impairs visibility within the unit. All fishes observed were included in the analyses. Video documentation was then analyzed to confirm fish counts by SCUBA surveys. Additionally, *L. campechanus* approximate age and size (total length: TL) were recorded from

the videos using the mesh surrounding the SMURF as a scale. Age/size life stages were assigned based upon data from Gallaway et al. (2009) as follows: (a) settlement stage: age 0 (26-30 days old, < 20 mm TL), (b) post-settlement stage: age 0 (30-66 days old, 20-50 mm TL), (c) postrecruit stage: age $0-1$ ($> 67-365$ days old, 50-200 mm TL), and (d) adult stage: age 2 (2 years old, > 200 mm TL).

Statistical Analyses

Species abundance, richness, evenness and diversity were estimated using the Shannon-Wiener Diversity and Pielou's Indices generated by the diversity routine in PRIMER-E (v7). Data were tested for normality using Kolmogorov-Smirnov test and for homoscedasticity using Levene's test to meet parametric analyses assumptions (Sokal & Rohlf, 2012). A square root transformation was applied to data that failed the normality test which did not offset the distribution. Nonetheless, species indices were analyzed independently using a one-way analysis of variance (ANOVA) followed by Tukey post hoc test to identify where any difference(s) among substrate treatments lie with untransformed data because ANOVAs are robust to violations of normality (Underwood, 1997).

For further analyses (nonparametric tests), a square root transformation was applied to all the data to increase the influence of less abundant species and stabilize abundant species, and a dummy variable was added to include samples with no species observed (Verdiell-Cubedo et al., 2012; Clarke et al., 2014). Bray-Curtis similarity index was constructed from the transformed data and a non-metric multidimensional scaling (NMDS) using 100 iterations of bootstrap averaging with 95% confidence ellipses were applied to this matrix to visualize differences in reef community assemblages among dates and treatments. The default in PRIMER-E (v7) is 50

iterations, but according to Clarke et al. (op. cit.) and Clarke & Gorley (2015) doubling that number ensures a near-optimal solution. It also mentions that if the same (lowest) stress value is obtained over 50% of the time, which was the case, it is very unlikely to be improved by further iterations. Furthermore, three independent two-way analysis of similarity (ANOSIM) were performed on the Bray-Curtis similarity matrixes to compare reef community assemblages from surveys done before, during and after resetting the SMURFs, surveys done within the year of 2018; and the surveys done in the month of July across the years with an *a priori* null hypothesis of no differences among dates or treatments. The ANOSIM generates an R statistic that quantifies the extent of segregation between groups and a p value indicating the significance of the difference observed. The R statistic ranges from zero to one, where the closer the value to one indicates groups are similar and the closer to zero indicates groups are different (Clarke & Warwick, 2001). Similarity percentages test (SIMPER) was also applied to determine which species drove dissimilarities among treatments and dates (Clarke & Warwick, op. cit.; Clarke et al., op. cit.).

Lutjanus campechanus abundances and size (life stage) comparisons among treatments and dates were evaluated separately using one-way ANOVAs. If significant differences occurred, Tukey post hoc test results were performed. All statistical analyses were performed at an α level of 0.05 using the IBM SPSS® statistical package (v25.0) and the PRIMER-E (v7) (Clarke & Warwick, 2001; Clarke et al., 2014; Clarke & Gorley, 2015).

CHAPTER III

RESULTS

A total of 5,238 individual fishes consisting of 39 juvenile reef species across 23 families were surveyed across all sites (Table 1). Out of the 23 families, those from family Lutjanidae dominated (48.85%) followed by fishes from Serranidae (16.51%), Sciaenidae (12.49%), and Haemulidae (11.76%), contributing to almost 90% of the taxa. The dominant species in each of these families were *L. campechanus* (45.67%), *Diplectrum bivittatum* (6.05%), *Pareques umbrosus* (12.49%), and *Haemulon aurolineatum* (10.35%), respectively (Table 1). Out of the 39 species, nine were only observed three times or less. These rare species included *Myripristis jacobus, Ocyurus chrysurus, Cantherhines pullus, Stegastes partitus, Synodus foetens, Anisotremus virginicus, Holocentrus adscensionis, Brotula barbata*, and *Rachycentron canadum* (Table 1).

Table 1. Fish collection data from SMURFs sampled from July 2017 to July 2019 from eight different stations, nine surveys. Fishes are presented by family and were ordered alphabetically. Valid family and species names from World Register of Marine Species [WORMS] (2019). Number of fish observed (n) across the different treatments: B = bare or no substrate, O = oyster shells (~10 cm), S = small rubble (~10 cm), L = large rubble (~ 20 cm). Total number of fish (N) and percentage (%).

Community Indices

The average species richness $(\pm S E)$ was significantly different across the different substrate treatments (ANOVA: $F_{3,281} = 10.014$, $p < 0.001$). Species richness increased from bare (3.54 ± 0.2) , to large (4.46 ± 0.2) , to small (4.96 ± 0.2) , and to oyster (4.99 ± 0.2) . On two occasions SMURFs with bare substrate and once SMURF with oyster shells substrate could not be found thereby affecting sample size. Tukey post hoc analysis revealed that the mean increase from the bare to oyster substrate (1.45, 95% Confidence Intervals (CI) [0.66, 2.22]) was statistically significant ($p = 0.001$), as well as the increase from bare to small (1.42, 95% CI [0.64, 2.19], $p = 0.001$), and even from bare to large (0.92, 95% CI [0.14, 1.69], $p = 0.013$, Figure 4A). One-way ANOVA of average fish abundance showed significant differences among substrates ($F_{3,281} = 5.680$, $p < 0.001$, Figure 4B). Species abundance increased from bare (14.69) \pm 1.1), to oyster (17.82 \pm 1.1), to large (18.61 \pm 1.2), to small (22.29 \pm 1.7). Tukey post hoc analysis revealed that the mean increase from the bare to small rubble substrate (7.60, 95% CI $[2.81, 12.40]$) was the only significantly different treatment ($p = 0.001$; Figure 4B). No significant differences in species evenness ($F_{3,266} = 0.944$, $p = 0.420$, Figure 4C) were found across substrate treatments; however, highest species evenness was observed at the SMURFs with the oyster treatment (0.75 \pm 0.02) and the lowest at the SMURFs containing small rubble (0.71 ± 0.02) . The change in degrees of freedom from 281 to 266 was due to the omission of samples that did not yield an evenness value as a result of species richness values equating to zero or one. Average species diversity also showed significant differences among treatments $(F_{3,281} = 6.606, p < 0.001,$ Figure 4D). There was a significant mean increase from bare to small rubble (0.23, 95% CI [0.04, 0.43], $p = 0.012$), and even a greater increase to the oyster treatment $(0.33, 95\% \text{ CI } [0.13, 0.52], p = 0.001).$

Figure 4. Average \pm SE (bars) for **(A)** species richness (*S*), **(B)** abundance (*N*, number of fish), **(C)** species evenness (*J'*), and **(D)** species diversity (*H'*) of all fishes observed at the SMURFs across the different substrate treatments: B = bare (n = 70), O = oyster (~10 cm, n = 71), S = small rubble (~10 cm, n = 72), L = large rubble (~20 cm, n = 72); * represents significant differences at $p < 0.05$.

SMURF Treatments Sampled Throughout Reset Event

The NMDS ordination comparing fish assemblages before, during and after the SMURF reset event revealed clustering of samples with a stress value of 0.03 indicating fish assemblages present among these dates were different; however, overlapping clusters were evident among substrate treatments indicating fish assemblages were quite similar, stress value of 0.16 (Figure 6A and B). The two-way crossed ANOSIM revealed no significant differences in fish assemblages among substrate treatments ($R = 0.037$, $p = 0.077$). However, significant differences among reset event dates ($p = 0.0001$) were apparent, a low R value ($R = 0.336$) indicated date did not have a great effect on the fish assemblages overall. Although no significant differences are present among reset event dates as per the ANOSIM, the visualization separation (Figure 6B) was further investigated with the pairwise tests indicating that before (June 2nd) and after (August $25th$) the reset event showed somewhat different fish assemblages (R = 0.485, p = 0.0001). The SIMPER analysis also indicated the greatest dissimilarity between these two dates (68.91%) and identified the top three species that were most responsible for this distinction: *D. bivittatum* (20.06%), *L. campechanus* (17.97%), and *P. umbrosus* (14.90%). The bare and small rubble treatment also displayed the greatest dissimilarity (59.80%) among treatments sampled throughout the reset event. The species responsible for this difference were *L. campechanus* (23.88%), *D. bivittatum* (16.31%), and *Centropristis philadelphica* (8.95%). The bare differed from the large rubble treatment 59.25% and from the oyster shells treatment 57.82%. The top two species contributing the most to these dissimilarities were *L. campechanus* and *D. bivittatum.*

Figure 5. Nonmetric multidimensional scaling (NMDS) ordination plot from 100 bootstrap averaging iterations, and 95% confidence ellipses of species composition observed on SMURFs **(A)** before (June 2), during (July 15), and after (Aug 25) SMURF reset event based on substrate treatment (see Figure 4) and **(B)** dates sampled. The two-dimensional (2D) stress value

represents the precision of the NMDS plot. The closer two points are to each other, the more similar their species composition.

SMURF Treatments Sampled in 2018

The ordination through the NMDS revealed groupings of samples according to the type of substrate treatment, and the date sampled on fish assemblages for the year of 2018 yielding stress values of 0.14 and 0.06, respectively (Figure 5A and B). The two-way crossed ANOSIM test based on Bray-Curtis similarity matrix revealed no significant differences in fish assemblages among substrate ($R = 0.023$, $p = 0.103$). However, significant differences among dates ($p = 0.0001$) were evident, but a low R value ($R = 0.313$) indicating that dates did not have a great effect on the fish assemblages overall. The SIMPER analysis identified which substrate treatment and dates differed the most and identified the species that caused those discrepancies. Although no significant differences were indicated by ANOSIM values, the bare and small rubble treatment displayed the greatest dissimilarity (55.39%) among treatments sampled within the year 2018. The species that contributed the most to this difference were *L. campechanus* (21.20%), *Haemulon aurolineatum* (9.46%), and *P. umbrosus* (9.15%). The bare treatment differed from the oyster shell treatment 54.96% and from the large rubble treatment 54.03%. The greatest dissimilarity among dates occurred between June $2nd$ and September 9th (71.55%) with *D. bivittatum, L. campechanus,* and *P. umbrosus* contributing the most to this difference (17.93%, 16.17%, and 12.81%, respectively).

Figure 6. Nonmetric multidimensional scaling (NMDS) ordination plot of species composition observed on SMURFs in the year of 2018 based on **(A)** substrate treatment (see Figure 4) and **(B)** dates sampled. Two-dimensional (2D) stress value, space among points, bootstrap averaging iterations and outlined ovals, as in Figure 5.

SMURF Treatments Sampled in July Over Three Consecutive Years

The NMDS ordination comparing fish assemblages in July across the three years sampled revealed distinct fish assemblages with no overlaps across the years, but among substrate it showed overlapping groupings meaning no differences among substrate treatments, except for bare (Figure 7A and B). Two-way crossed ANOSIM confirmed separations among fish assemblages throughout the years for the month of July ($R = 0.537$, $p = 0.0001$) and that no differences in fish assemblages were evident among substrate treatments ($R = 0.033$, $p = 0.101$). Pairwise tests showed fish assemblages in the year 2017 and 2019 differed most significantly (R $= 0.753$, p = 0.0001), 2017 and 2018 followed (R = 0.666, p = 0.0001). Even though fish assemblages between 2018 and 2019 were significantly different ($p = 0.0001$), the R value was low (R= 0.212) indicating that the factor (year) had a small effect on the fish assemblages. The SIMPER analysis identified the species that typified each group and those that distinguished between factor levels. The average dissimilarity between 2017 and 2018 was 75.55% with *L. campechanus* contributing most to the dissimilarity (15.93%) followed by *Lutjanus synagris* (11.79%), and *C. philadelphica* (11.54%). The average dissimilarity between 2017 and 2019 was 76.54% with greatest dissimilarity contribution from *P. umbrosus* (19.13%), followed by *L. campechanus* (16.20%), and *L. synagris* (12.57%). The average dissimilarity between 2018 and 2019 was 58.42% as a result of *P. umbrosus* (18.70%), *L. campechanus* (18.10%), and *C. philadelphica* (14.12%).

Figure 7. Nonmetric multidimensional scaling (NMDS) ordination plot of species composition observed on SMURFs during **(A)** July across all years sampled based on substrate treatment (see Figure 4) and **(B)** dates sampled. Two-dimensional (2D) stress value, space among points, bootstrap averaging iterations and outlined ovals, as in Figure 5.

Lutjanus campechanus **Abundances and Size Comparisons**

A total of 2,392 *L. campechanus* were observed throughout the SMURFs (Table 1). *Lutjanus campechanus* abundances did not significantly differ among the substrate treatments (ANOVA: $F_{3,281} = 1.788$, $p = 0.2$, Figure 8); however, the highest abundance was observed at the SMURFs with the small rubble treatment (9.57 \pm 0.87) followed by the large rubble treatment (8.90 ± 0.94). The SMURFs containing the bare treatment yielded the lowest abundances of *L. campechanus* (6.84 ± 0.76).

Figure 8. Average *Lutjanus campechanus* abundances $(N,$ number of fish) \pm SE (bars) observed at the SMURFs across the different substrate treatments: $B = bare$, $O = o$ yster (~10 cm), $S =$ small rubble $(\sim 10 \text{ cm})$, and L = large rubble $(\sim 20 \text{ cm})$. No significant differences were found.

The average *L. campechanus* abundances showed significant differences across the different sampling dates (ANOVA: $F_{8,276} = 4.262$, p < 0.001, Figure 9). The change in degrees of freedom from 281 to 276 was due to a change in number of parameters from four substrate treatments to the nine different sampling dates. Tukey post hoc analysis revealed that the average *L. campechanus* abundance decreased significantly from July 2017 ($n = 32$, 11.03 \pm 1.78) to July 2018 (n = 32, 4.97 \pm 1.14) by a mean difference of 6.06 (95 % CI [0.55, 11.57], p = 0.019) and to July 2019 (n = 32, 5.38 \pm 0.92) by 5.65 (95% CI [0.14, 11.17], p = 0.039). A significant *L*. *campechanus* abundance increase was evident from July 2018 to November 8th of the same year $(n = 31, 12.23 \pm 1.27)$ by a mean difference of 7.26 (95% CI [1.70, 12.81], p = 0.002, Figure 9), and an abundance decrease from November $8th$ to July 2019 (6.85, 95% CI [1.29, 12.41], p = 0.004).

Figure 9. Average *Lutjanus campechanus* abundance $(N,$ number of fish) \pm SE (bars) observed at the SMURFs across the different sampling dates; * represents significant differences at p *˂* 0.05.

Total lengths of *L. campechanus* ranged from approximately 15 mm up to 300 mm. No significant differences among life stages could be discerned across substrate treatments $(ANOVAs: F_{3,281} = 2.139, p = 0.095, F_{3,281} = 0.582, p = 0.628, F_{3,281} = 0.669, p = 0.572, F_{3,281} =$ 0.860, p = 0.462, respectively, Figure 10). *Lutjanus campechanus* < 20 mm abundances increased from large (0.15 \pm 0.09), to bare (0.17 \pm 0.09), to small rubble (0.21 \pm 0.12), and to oyster (0.69 \pm 0.31). The highest abundance for individuals between 20-50 mm TL was observed at the SMURFs with the large rubble treatment (1.51 \pm 0.50). Individuals between 50100 mm and > 200 mm had the highest abundances in the small rubble treatment (6.67 \pm 0.75, 1.19 ± 0.50 , respectively).

Figure 10. Average *Lutjanus campechanus* abundances (*N*, number of fish) by age/size life stages, settlement stage: 26-30 days old (< 20 mm total length [TL]), post-settlement stage: 30- 66 days old (20-50 mm TL), post-recruit stage: $> 67-365$ days old (50-200 mm TL), and adult stage: 2 years old (> 200 mm TL), observed at the SMURFs across the different substrate treatments as defined in Figure 8. No significant differences were found.

Individuals < 20 mm TL showed significant differences across the sampling dates (ANOVA: $F_{8,276} = 3.639$, $p = 0.001$) as they were only seen from July through the beginning of September of 2018 (Figure 11). The highest abundances were found on August $25th$ (1.16 \pm 0.64). Individuals from 20-50 mm TL also showed significant differences across the sampling dates (F_{8,276} = 15,069, p = 0,001). July 2017 (6.66 \pm 1.57) and August 2018 (4.09 \pm 0.97) displayed the highest mean abundances that differed from the rest of the dates but not between them (Figure 11). Individuals 50-200 mm TL were found across all sampling dates and differed among them ($F_{8,276} = 13.726$, p = 0.001). November 8th of 2018 yielded the highest mean abundance of this size range (11.32 \pm 1.10) followed by June 2nd of the same year (9.97 \pm 1.60) (Figure 11). No significant differences were found across sampling dates for individuals > 200 mm TL even though *L. campechanus* were not observed on three dates ($F_{8,276} = 2.090$, $p = 0.067$; Figure 11).

Figure 11. Average *Lutjanus campechanus* abundances (*N*, number of fish) by age/size life stages, as defined in Figure 10, observed at all the SMURF treatments across the different sampling dates. Non-matching letters represent significant differences at p < 0.05.

CHAPTER IV

DISCUSSION

Substrate Treatment Species Differences

As expected, the largest differences in recruitment and species composition occurred between SMURFs with a type of substrate and those with no substrate (bare). Even though species richness, abundance and diversity did not differ significantly among the different types of substrates, the oyster shell treatment yielded higher species richness and diversity and the small rubble treatment yielded the highest species abundance. Various studies such as Luckhurst & Luckhurst (1978) suggest that reefs with a variety of hole sizes offer shelter to numerous body shapes and sizes, increasing species richness which would be the case for the oyster shell and small rubble treatment when compared to the large rubble. Regardless of the fact that all the SMURFs were surrounded by a plastic mesh, the way the large rubble would lay on top of each other did not offer much protection for small, cryptic species such as those from the family Blenniidae (*Ophioblennius macclurei, Parablennius marmoreus*) and Gobiidae (*Gobiosoma robustum*). These species were most abundant in the oyster shell and small rubble treatment (Table 1) and most divers described seeing them peeking out from within small crevices in between the substrate. In fact, most were often missed by survey divers but were fortunately caught on video. Even *L. campechanus* < 20 mm also had the highest abundance in the oyster shell and small rubble treatments (Figure 10) further confirming the idea that smaller fish

preferred these types of substrates due to the variety of hole sizes. This concurs with studies done by Gratwicke & Speight (2005) which constructed several ARs in the British Virgin Islands to investigate the effects of habitat complexity on fish assemblages and found out that hard substrate and refuge crevices were key factors influencing species richness, and that increasing the number of small reef holes increased fish abundance on reefs. Other species such as *Opsanus beta, A. virginicus, H. aurolineatum, Holocentrus adscensionis, L. griseus, L. synagris, Lactophrys trigonus, Stegastes variabilis, C. philadelphica, Rypticus maculatus,* and *Serranus subligarius*, were mostly seen as juveniles, and as a result were shorter in height and most commonly observed in the oyster and small rubble treatment as well. Those who were vertically longer (height) such as the *P. umbrosus,* which have a long sailfin like dorsal fin as juveniles, were found across all treatments but most abundantly in the large rubble treatment given the crevices were much larger and accommodated to their anatomy.

Balistes capriscus, also with a deep highly compressed body shape, was most commonly found in the small rubble treatment followed by the large rubble (Table 1). Visualization graph (Figure 12) of average *B. capriscus* abundances by size based on fork length (FL) similar to those of *L. campechanus* revealed that older, larger individuals > 151 mm FL were most abundant in the small and large rubble treatment; whereas, the younger, shorter in height individuals were those found in the bare and oyster treatment, except for one instance where three *B. capriscus* < 50 mm were found at a SMURF with large rubble (Figure 12). This instance occurred in July 2019 which falls during its known spawning season (June-August) (Lee et al., 2018) suggesting these individuals had just left the pelagic zone which is uncommon at this size. Earlier studies indicate that most juvenile *B. capriscus* spend four to seven months in the pelagic

zone before recruiting to benthic artificial reefs (Bortone et al., 1977; Wells & Rooker, 2004; Hoffmayer et al., 2005).

Figure 12. Average *Balistes capriscus* abundances (*N*, number of fish) by size (fork length [FL]) observed at the SMURFs across the different substrate treatments as defined in Figure 8.

Species from the family Pomacanthidae (*Holacanthus bermudensis* and *Pomacanthus paru*) were found across all substrate treatments but were most abundant in either the small or large rubble treatment (Table 1), possibly showing preference to the type of substrate rather than size of the crevices. *Chaetodon sedentarius*, sister taxon to Pomacanthidae (Bellwood et al., 2004) (juveniles < 50 mm), were also found more abundant in the large rubble treatment.

Overall, the fish assemblages captured in SMURFs consisted mainly of juveniles compared to those usually found in intermediate to high relief structures indicating that either predation protection, structural complexity, and/or sites lacking any structure in a 160 m radius is a driving factor for these species to settle on these SMURFs. Nevertheless, *Scorpaena plumieri* species was composed mainly of adults (1 individual considered juvenile) found across all substrate treatments (Table 1). *Scorpaena plumieri* belong to the family Scorpaenidae and are widely known for their effective camouflage capabilities and their venomous spines (Humann, 1994). These are known as ambush predators as they lie most of the time motionless on the seafloor waiting to surprise potential prey (Lieske & Myers, 1994). Although considered piscivorous, feeding mainly on other fish larvae and juveniles, *S. plumieri* are also known to prey on crustaceans (Heck & Weinstein, 1989). As previously mentioned, predators play an important role in structuring ecological communities and have consistently shown negative effects of predators on abundance, species richness and recruitment (Beukers & Jones 1998; Webster, 2002). For example, Caley (1993) periodically removed predatory species on small, ARs and found that species richness and total abundance were generally greater than those in which predators were not removed. Carr & Hixon (1985) also removed piscivore species from isolated coral patch reefs and found that *Chromis cyanea* (Poey, 1860) and *Halichoeres pictus* (Poey, 1860) were significantly greater on reefs where resident predators had been removed. Given that

the presence of *S. plumieri* might have affected abundance for the rest of the species, a visualization graph was constructed comparing averaged abundances of *S. plumieri* against averaged abundances of the remaining species among substrate treatments (Figure 13). *Scorpaena plumieri* were most abundantly found at SMURFs with the oyster shell substrate and the least at the small and large rubble (Figure 13A). Abundance of the remaining species was highest for the small and large rubble treatments and the least at the bare treatment and oyster treatment suggesting *S. plumieri* could possibly be a potential cause for these differences (Figure 13B), but this hypothesis requires further examination.

Figure 13. Average abundances (*N*, number of fish) of **(A)** *Scorpaena plumieri* and **(B)** all other fish species observed at the SMURFs across the different substrate treatments as defined in Figure 8.

Differences among July Over Three Consecutive Years

No significant month to month variations regarding fish assemblages in the year of 2018 were recorded, regardless of the SMURF reset event, seasonal patterns due to water temperature, or spawning events that as per Gallaway et al. (2009) could influence and affect species composition. The only significant differences regarding fish assemblages occurred across the various years of the month of July, most likely due to the large-scale climate event, Hurricane Harvey, that happened not too long after the first survey (July 2017). From July 2017 to July 2018, *L. campechanus* decreased from an average abundance of 11.03 to 4.97, having the greatest mean difference between these two years (Figure 9). *Lutjanus synagris* also decreased between the years from 3.19 to 0.09 average abundance, whereas *Centropristis philadelphica* increased from 0.03 to 3.03. Both Lutjanidae species, *L. campechanus* and *L. synagris* have very similar spawning periods from April to September and peaking during June to August (Luckhurst et al., 2000; Gallaway et al., op. cit.; Alexander, 2015). *Centropristis philadelphica,* on the other hand, spawns from January to April showing preference to colder water temperatures (Herrema et al., 1985). A drop in water temperature did occur from July 2017 $(25.5^{\circ}C)$ to July 2018 (23.4 $^{\circ}C$) but perhaps not a sufficient amount to explain why both Lutjanidae species decreased and *C. philadelphica* increased in abundance. It is more reasonable to suggest Hurricane Harvey could have caused Lutjanidae species to disperse/move from the unit and/or to recruitment variations, spawning success and changes in water currents. Something similar happened to Watterson et al. (1998) in their mark and recapture study on *L. campechanus.* A few months after the start of their study the eye of Hurricane Opal passed 40 km within the AR sites located 20-32 km south of Mobile Bay, Alabama and fish demonstrated greater movement during the hurricane covering distances over 100 to 200 km. Hurricane Opal

affected not only the movement of *L. campechanus* but the site fidelity of the fish which is generally high even to ARs (Strelcheck et al., 2007; Froehlich et al., 2018). An even greater difference in mean abundance was recorded between July 2017 and July 2019 when *P. umbrosus* counts increased from 0.16 to 6.81, *L. campechanus* decreased by 5.66 mean difference (Figure 9), and *L. synagris* numbers decreased to zero. During this window of time, the spawning success of *Pareques umbrosus* could have increased which are known for their high spawning frequency and high relative fecundity (Holt & Riley, 1999).

Lutjanus campechanus **Abundances and Size Comparisons**

In the present study, *L. campechanus* abundance did not vary significantly among substrate treatments despite length sizes as these were found across all treatments. This coincides with the notion that *L. campechanus* quickly settle over any hard substrates displaying an attraction to low-relief habitat that provides adequate shelter and protection (Watterson et al., 1998; Wells & Cowan Jr, 2007; Gallaway et al., 2009). The small and large rubble treatments reported the highest abundance values and as expected, the bare treatment yielded the least which provided little protection compared to the others (Figure 8). Treatments separated by age/size did provide some insight as to how *L. campechanus* utilized the various treatments throughout its life cycle. Individuals < 20 mm TL appeared in higher abundances at the oyster shell treatment and were only seen throughout the months of July through September coinciding with peak spawning months of *L. campechanus* (June-August) (Alexander, 2015; Glenn et al., 2017). Various studies have demonstrated relic-shell habitat such as oyster shells offer primary nursery habitat for new settlers (juveniles) due to the smaller hole sizes provided by these shells (Lingo & Szedlmayer, 2006; Gallaway et al., op. cit.). Highest abundance of *L. campechanus* of

increased size/age (> 20-300 mm TL) settled at the small and large rubble treatment given that crevices were much larger in size. The most abundant individuals throughout the treatments and sampling dates were those age 0-1 indicating that these SMURFs were essential habitat at this stage in their lives. Most individuals sized $2 > 200$ mm TL, better characterized as subadults since none were greater than 300 mm TL, were found roaming around the SMURFs rather than entering and exiting the structure despite that the plastic netting was open at the top. These subadults had the lowest abundances at the oyster shell treatment coinciding with the concept that as fish size increases the fish require and seek structured habitat with larger hole size (Szedlmayer & Lee, 2004). Moreover, these subadults might have been foraging for food from their actual permanent residence given that studies have reported adults from age 2-7 to feed on smaller fishes, crabs, and shrimps from the surrounding areas of their habitat (Gallaway et al., op. cit.). In this study, divers reported the presence of shrimps, crabs, sea urchins and other organisms in or around the SMURFs across all substrates which was not the case in the barren ocean floor. Given that age 2 *L. campechanus,* at least smaller than 300 mm TL, did not show significant differences among treatments nor dates (Figure 10 and 11), suggests their presence was more stochastic. Perhaps these individuals were traveling from nearby sites, such as the culverts or natural patch reefs present at the South Padre Island-1047 Reef, in search of food as there might have been excessive competition with older individuals as well as added pressures from predatory species at those sites.

CHAPTER V

CONCLUSIONS

In summary, all treatments recruited reef associated species. Moreover, fish assemblage captured in SMURFs consisted mainly of juveniles compared to those usually found in intermediate to high relief structures indicating that either predation protection, structural complexity, and/or sites lacking any structure in a 160 m radius is a driving factor for these species to settle on these SMURFs. Even though not statistically significant, species richness and diversity was highest at SMURFs with the oyster shell substrate followed by the small and large rubble. In terms of fish abundance, the small rubble substrate yielded the highest value. As expected, SMURFs with no substrate (bare treatment) had the lowest values indicating that plastic netting was not optimal to recruit and provide suitable habitat for most juvenile species. SMURFs reset event did not have an effect on fish assemblages regardless of the disturbance. Seasonal fish assemblage variations were not seen across 2018 dates. However, reef fish assemblages were different among the summers and this was attributed to Hurricane Harvey and perhaps variations in recruitment and spawning success in subsequent years as well as changes in water currents. *Lutjanus campechanus* was the most abundant species across all treatments and as expected lowest at the bare treatment. Most *L. campechanus* individuals were between 50- 200 mm TL suggesting SMURFs were more suitable for smaller individuals to thrive. This species was also observed across all treatments but depending on their current life stage

generally preferred the oyster shell substrate as they began to settle from the water column, and as they grew older, they preferred small or large rubble substrate. These findings suggest that oyster shells and small rubble substrate could be the most effective types of substrate to deploy especially during the peak spawning months of *L. campechanus* to provide essential habitat for these individuals to settle and use as protection as they grow before moving to much larger reefs. If these substrates are deployed using SMURFs, it would be necessary to include some mechanism to avoid SMURFs from sinking. Perhaps adding friction pilings which rely on the friction between the sediment and the pole, like those used for construction when the ground is soft, could prevent SMURFs from sinking below the plane of the seafloor (Hansbo, 1984).

Predators such as *S. plumieri* were observed at SMURFs in some cases even inside the SMURFs, lying on top of the substrate. A study monitoring the effects of caging effects on fish recruitment at One Tree Island in the Great Barrier Reef indicated that plots with completely closed off cages, rather than partial cages or open plots, always harbored more recruits (Doherty & Sale, 1985). Completely closing the plastic netting on SMURFs could have added protection to juveniles from *S. plumieri* or other piscivorous species. However, Arney et al. (2017) used completely enclosed SMURFs and reported similar results. Additionally, assessing the effects the invertebrate community present including crabs, sea urchins, octopuses (which were occasionally found inside or under the SMURFs), sponges or even algal growth have on the recruitment of fishes could provide more insights.

Nevertheless, finding commercially important species as juveniles at sites lacking significant structure suggests that fisheries management may benefit from producing ARs with small, nontoxic, and inexpensive substrates distant from larger structures where juveniles can recruit and grow, and thus enhance desired fish stocks. This project may also help increase

abundance and potentially contribute to establishment and maintenance of recreational fisheries and diving activities. Moreover, monitoring information regarding the performance of the project overall and its constituent features will be highly useful to individuals designing current and future AR projects with similar goals.

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BIOGRAPHICAL SKETCH

Stefany Salinas was born in Brownsville, Texas. After receiving her high school diploma in the year 2012 from James Pace High School, Stefany entered the University of Texas at Brownsville in the Fall of 2012. She was a NOAA-ECSC (National Oceanic and Atmospheric Administration- Environmental Cooperative Science Center) scholar and worked as a laboratory assistant helping other graduate students. She received a Bachelor of Science in Environmental Sciences on Spring 2016 from what later became the University of Texas Rio Grande Valley, Brownsville, Texas. During the following years, she was employed as a research assistant at the same institution and successfully completed her Master of Science in Ocean, Coastal and Earth Sciences on May 2020. Her permanent mailing address is 1465 Calle Espacio, Brownsville, Texas 78520.