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Thesis of
Alysha Brunelle

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science
Marine Science

Nova Southeastern University
Halmos College of Arts and Sciences

August 2020

Approved:
Thesis Committee

Committee Chair: Brian Walker

Committee Member: Francisco Pagan

Committee Member: Bernhard Riegl

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NOVA SOUTHEASTERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

Prioritizing the largest, oldest corals for disease intervention in a coral disease-ravaged area: Southeast Florida Coral Reef Ecosystem Conservation Area.

By

Alysha Brunelle

Submitted to the Faculty of
Halmos College of Arts and Sciences
in partial fulfillment of the requirements for
the degree of Master of Science in Marine Science with a concentration in:

Coastal Zone Management
and
Marine Environmental Science

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August 28, 2020

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TABLE OF CONTENTS

LIST OF FIGURES……..……..…..i

LIST OF TABLES..…….……ii

ABSTRACT…….……..iii

1. INTRODUCTION…….…..1

2. METHODS……..5

3. RESULTS……9

a. Treatment Success……9

b. Untreatable Colonies……..19

c. Temporal Infection Patterns……...21

4. DISUSSION……...31

5. RECOMMENDATIONS……...36

LITERATURE CITED……..38

APPENDIX ……..…….46

LIST OF FIGURES

Figure 1. Map of the large priority monitoring corals.....7

Figure 2. Number of corals requiring treatments and retreatments in 2018 and 2019.....10

Figure 3……..

a. Graph of treatment type success by species including outlier colonies…….…11

b. Graph of treatment type success by species excluding outlier colonies……...….…12

Figure 1. Example of disease progression and treatment results of chlorinated epoxy on *Orbicella spp.,* LC-110……...12

Figure 5. The cumulative percent success of all treatments on all corals each treatment period…….14

Figure 6. The total number of treatments (grey) and failures (black) per monitoring period for all corals…….....14

Figure 7. Shade plot of total failure on each treated coral by monitoring period sorted by the maximum total number of failures…….....15

Figure 8. The cumulative total number of treatments (grey) and failures (black) per monitoring period for all corals…….....15

Figure 9. The cumulative percent success of all *Orbicella spp*. treatments on all corals each treatment period…….....16

Figure 10. The cumulative percent success of all *M. cavernosa* treatments on all corals each treatment period…….....17

Figure 11. The total number of treatments (grey) and failures (black) per monitoring period for *M. cavernosa*…….....17

Figure 12. The cumulative total number of treatments (grey) and failures (black) per monitoring period for *M. cavernosa*.....18

Figure 13. The cumulative percent success of antibiotic ointment treatments on all corals each treatment period…….19

Figure 14. Graph of total treatment success of all forty treated corals and excluding the seven outliers ……..…….20

Figure 15. Untreatable corals that showed blotchy, half-paling/half-diseased appearance.....20

Figure 16. Untreatable corals with heavy algae colonization…….....21

Figure 17. Corals that did not respond to treatment……..…….....21

Figure 18. The numbers of new treatments on all corals used as a proxy for new infections and the number of treated corals by treatment period..……23

Figure 19. Shade plot of total treatments on each treated coral by monitoring period……....…….28

Figure 20. Multidimensional scaling plot of Bray-Curtis similarities of total treatments per coral for each monitoring period…….……28

Figure 21. Shade plot of total treatments on each treated coral of all corals consistently monitored from September 2018-April 2020……32

Figure 22. Map of study site with priority large corals colored by category…….30

LIST OF TABLES

Table 1. Total treatment failure and success by species from April 2018 – April 2020….....13

Table 2. Cumulative success of amoxicillin ointment on all treated species from September 2018 to April 2020.....17

Table 3. The total number of corals assessed, total number of new treatments, and number of new treatments on newly assessed corals by monitoring period…….....23

Table 2. Corals needing new treatments between May 2019 and October 2019.....…….....24

Table 5. Corals needing new treatments between November 2019 and April 2020……..25

ABSTRACT

Coral diseases appear to be more devastating than ever before. When a virulent disease ravages a coral ecosystem, it can significantly change the population's demographics and cause local extinctions. Disease intervention response during such an event is impossible at a landscape scale, therefore priorities must be considered. Saving the largest, oldest colonies of reef-building species is a good choice due to their high fecundity and ecological function. Their size, as a proxy for age, is an indicator of their resistance to previous perturbations which may indicate higher fitness. Their size also provides habitat to many organisms and wave resistance in shallow water for shoreline protection. Saving these colonies is imperative to preserve present ecological functions and to prepare for future restoration. Condition assessments of ninety of the largest, healthiest-looking colonies in southeast Florida during the SCTLD were conducted recording live tissue area, colony size, and number of tissue isolates. Colonies were checked for disease and photographed monthly. If diseased, intervention was conducted which entailed covering diseased tissue margins with chlorinated epoxy and sometimes creating a trench in the skeleton between diseased and healthy tissue and filling it with chlorinated epoxy. Treatment success generally went down over time and varied by treatment method and coral species. Methods were most effective on *Orbicella spp.* (75%) and less effective on the limited number of treated *Montastraea cavernosa* (37%) colonies. Seven of the thirty-eight treated colonies did not respond favorably to intervention treatments. The number of new treatments varied monthly and was highest at the onset of rainy season and the warmest periods in late summer. After field-testing the effectiveness of antibiotic treatments, all treatments on the infected Large Corals were switched to antibiotic ointment in August 2019. This showed higher success for *Orbicella spp.* (88.3%) and much higher success for *Montastraea cavernosa* (73.7%) colonies. The low success of the chlorinated epoxy treatments lead to the termination of this method, continuing, disease treatments will be treated solely with antibiotic ointment. Monitoring and intervention efforts have shown these colonies continue to get disease periodically, which if not treated will lead to colony mortality. New infections could be due to environmental stressors (e.g. salinity, temperature, dissolved organic carbon) as well as adaptations and genetic variations between and within species. Observed infection rates may correspond to increases in certain water quality metrics therefore it is important to investigate temporal infections of the large corals with temporal changes in water quality as well as collect cores and tissue samples of our Large Coral inventory to identify the cause of infection rate differences between species and individuals.

Keywords: Stony Coral Tissue Loss Disease, chlorinated epoxy, treatment, antibiotic ointment, *Orbicella*, temporal, outbreak

1. Introduction

Coral reefs are among the most biologically rich and diverse ecosystems on Earth and yet are one of the most threatened. With a global distribution confined to the tropical latitudes due to specific ecological requirements such as warm, sunlit shallow water, coral reefs have evolved to be one of the most complex and productive natural resources (Guinotte, Buddemeier, & Kleypas, 2003). Corals with their endosymbiotic relationship with photosynthetic symbionts are most responsible for the production of calcium carbonate that form the base of the reefs creating essential habitat for an array of marine flora and fauna (Hightshoe, 2018; Schwarz et al., 2008; Sheppard, Davy, Pilling, & Graham, 2018) . Corals are unique in that they create their own ecosystems through time with geologic formations that affect larger scale biological and oceanographic zonation and processes. In many locations, they buffer oceanic currents and waves creating an ideal environment for seagrass beds and mangroves. The complex interaction between these ecosystems is critical for nutrient exchange and creates important spawning, nursery, and feeding grounds (Moberg & Folke, 1999). Millions of tourists are drawn to coral reefs for recreational activities such as snorkeling and SCUBA diving (Bellwood, Hughes, Folke, & Nyström, 2004; Costanza et al., 2014). Coral reefs also provide important protection to coastal populations by lessening the impact of strong storms, waves, and flooding to coastlines (Ferrario et al., 2014; Moberg & Folke, 1999). Without healthy and intact coral reefs, increased wave activity and violent storms could cause massive erosion and property damage to coastal communities (Ferrario et al., 2014). Storlazzi et al. (2019) reported the annual value of flood risk reduction provided by U.S. coral reefs as more than 18,000 lives and $1.805 billion in 2010 U.S. dollars

Coral reefs face many stressors that threaten their diversity, structure, function and resilience. Many reefs show widespread overexploitation and instability of reef fisheries resulting in a devastated reef habitat as well as diminished food security and economic development (Newton, Côté, Pilling, Jennings, & Dulvy, 2007). Impacts from costal development, crop cultivation, and farming cause significant runoff into coastal waters introducing the presence of excess sediments and nutrients that contribute to the decline of coral health (Burke, Reytar, Spalding, & Perry, 2011; Knowlton & Jackson, 2008) Local and regional stressors are compounded by global threats of ocean warming and increased acidification. The emission of carbon dioxide (CO2), a greenhouse gas, into the atmosphere as a result of burning fossil fuels causes heat to become trapped within

the atmosphere, warming sea surface waters. Simultaneously CO2 absorbs into the ocean increasing the ocean's acidity. Together, these two reactions result in drastic problems for the structure and function of coral reefs (Gattuso et al., 2015; Meinshausen et al., 2011; Solomon, Manning, Marquis, & Qin, 2007).

Coral reefs are sensitive to sea temperatures, with specific temperature tolerances. Maintaining a symbiotic relationship with their zooxanthellae symbionts is critical to the survival of zooxanthellate corals, which comprised the majority of shallow-water coral reefs (Baker, Glynn, & Riegl, 2008; Hoegh-Guldberg et al., 2007). These algae are responsible for photosynthesis inside the coral host and provide nutrients and energy transferring about 90% of its production to the coral (Sheppard et al., 2018). When water temperature exceeds the normal tolerances for an extended time, the coral expels its symbiont, a process called coral bleaching (Baker et al., 2008; Brown, 1997). Bleaching leaves the coral visually stark white, without the photosynthetic pigments of the zooxanthellae. As these algae are the main nutrient and energy source for the coral, their lack of presence leaves the bleached coral susceptible to death from starvation and disease from a weakened immune system. Since the 1980s, bleaching has been reported from almost every region that supports coral reefs, including Florida (Baker et al., 2008) as well as eventual colony recovery from bleaching events as water temperatures fluctuate and eventually return to normal (Schoepf, Stat, Falter, & McCulloch, 2015). With sea surface temperature continuously rising, mass bleaching events have increased in frequency and severity in the region (Baker et al., 2008; Eakin, Lough, & Heron, 2009; Hoegh-Guldberg et al., 2007; Hughes, Terry P. et al., 2018; Pandolfi, Connolly, Marshall, & Cohen, 2011). Since 1983 there have been three recorded major Global Coral Bleaching Events (GCBE), 1998, 2010, and 2014-2017 (Eakin, Lough, Heron, & Liu, 2018). As corals are capable of recovery if conditions were to improve, the continuation of warming temperatures severely affects the overall health, growth, and fecundity of these organisms (Hughes, Terence P. & Tanner, 2000; Pitts, Campbell, Figueiredo, & Fogarty, 2020; Richmond, 1997).

Along with mass bleaching events, prevalence of coral-mortality-inducing diseases have also been correlated with increased water temperature (Brandt & McManus, 2009; Bruno & Selig, 2007). Coral diseases can be due to biotic (e.g., bacteria) or abiotic (e.g., virus, radiation, toxicant) pathogens, or a combination of the two (Peters, 2015). Corals as well as disease pathogens are

sensitive to ocean temperature change (Rosenberg, Koren, Reshef, Efrony, & Zilber-Rosenberg, 2007). Warming temperatures impair the defense mechanisms of the corals while concomitantly increasing growth of disease pathogens (Boyett, Bourne, & Willis, 2007). Water quality parameters and environmental factors can also stress the coral allowing pathogens to thrive (Raymundo, Halford, Maypa, & Kerr, 2009). Increased water temperatures can lead to higher pathogen growth rates (Muller, Erinn M., Bartels, & Baums, 2018; Remily & Richardson, 2006) and increased virulence (Harvell et al., 2002; Kushmaro, Rosenberg, Fine, Haim, & Loya, 1998; Muller et al., 2018; Remily & Richardson, 2006; Toren, Landau, Kushmaro, Loya, & Rosenberg, 1998). Mass bleaching events can increase the risk of coral mortality from disease, whether due to higher disease susceptibility or increased pathogenic load and/or virulence and caused almost all previously resistant corals to become disease susceptible (Muller, E. M., Rogers, Spitzack, & van Woesik, 2007; Muller et al., 2018). Coral disease research is confounded by its microbiome, which includes a complex and dynamic community of bacteria, fungi, dinoflagellates, and algae making identification of pathogens extremely difficult (Hightshoe, 2018; Kline & Vollmer, 2011)

While some presence of disease in coral ecosystems is expected for a healthy reef ecosystem, it is apparent that the number and distribution of coral disease outbreaks are increasing in frequency and prevalence (Galloway, Bruckner, & Woodley, 2009; Sokolow, 2009). It is feared that the recent stony coral tissue loss disease (SCTLD), which was first reported in southeast Florida, is the worst yet (Alvarez-Filip, Estrada-Saldívar, Pérez-Cervantes, Molina-Hernández, & González-Barrios, 2019; Muller, Erinn M., Sartor, Alcaraz, & van Woesik, 2020; Precht, Gintert, Robbart, Fura, & Van Woesik, 2016)

Evidence of change in community structure and reef health along Florida's Coral Reef has been present since the early 1970s (Baker et al., 2008). This reef tract is of the most heavily impacted reef systems of anthropogenic impacts (Alvarez-Filip, Dulvy, Gill, Coˆté, & Watkinson, 2009; Carpenter et al., 2008; D'Antonio, Gilliam, & Walker, 2016), experiencing significant losses in stony coral cover and species abundance (Porter & Meier, 1992; Porter et al., 2001; Wheaton et al., 2001). Since the 1970s, reports of disease outbreaks along Florida's Coral Reef described tissue loss patterns that were later termed white plague; now known as one most virulent of coral diseases (Aeby, Greta et al., 2019; Aronson & Precht, 2001; Dustan & Halas, 1987; Richardson, Goldberg, Carlton, & Halas, 1998; Richardson, 1998). Large-scale outbreaks of black band disease, rapid

tissue loss disease, white pox, and white band disease have been responsible for massive reef-wide coral mortality (Harvell et al. 1999, Aronson and Precht 2001, Porter et al., 2001, Gardner et al. 2003, Harvell et al. 2004, Vollmer and Kline 2008).

In 2014, a white-plague-like coral disease outbreak was first reported in high levels near Virginia Key, Florida later termed SCTLD (Precht et al., 2016) which has brought upon catastrophic coral loss in SE FL (Precht et al., 2016; Walker, 2018; Walton, Hayes, & Gilliam, 2018). This outbreak coincided with the hottest water temperatures on record to that point, a regional large-scale bleaching events, and a dredging operation that created large amounts of turbidity and sedimentation (Aeby et al., 2019; Barnes et al., 2015; Cunning, Silverstein, Barnes, & Baker, 2019; Manzello, 2015; Walton et al., 2018). It is likely that the combination of the mass bleaching event and increased turbidity and sedimentation stressed the stony corals, allowing for the pathogen to invade the colonies (Miller et al., 2016). Most tissue loss diseases are spatially and temporally limited and their effects tend to dissipate relatively quickly (Aeby, Greta S. et al., 2016; Brandt, Smith, Correa, & Vega-Thurber, 2013; Williams & Miller, 2005), however, SCTLD continued to spread southward over the course of at least five years after the initial stressors subsided (Muller et al., 2020). Some impacted species on affected reefs have been reduced to <3% of their initial population densities (Precht et al., 2016) with severe regional declines reported in coral densities and live tissue (Aeby et al. 2019; Walton et al., 2018; Walker 2018). Meandroid colonies (*Dendrogyra cylindrus*, *Dichocoenia stokesii, Eusmilia fastigiata* and other Meandroid spp.) are the most susceptible to SCTLD (Aeby et al., 2019). Between 2015 and 2018, both *M. meandrities and D. stokesii* each lost 70% of live tissue area across the northern third of Florida's ECA (Gilliam, D.S., Hayes, N.K., Ruzicka, R., and Colella, M, 2018). *Siderastrea siderea* and other brain corals are the next most susceptible species. Additional data from Walton et al. (2018) estimated that regionally as much as 30% of all coral colony density and 6770% of live tissue area was lost regionally.

When a virulent disease ravages a coral ecosystem, it can significantly change the populations demographics and cause local extinctions. Disease intervention response during such an event is virtually impossible at a landscape scale, therefore priorities must be considered. Saving the largest, oldest colonies of reef-building species is a good choice due to their high fecundity and ecological functions. Due to the greater surface area of polyps able to release more gametes on

large colonies compared to small colonies, it can be inferred that large colonies would have higher potential fertilization (Rinkevich & Loya, 1987; Van Veghel & Bak, 1994). *Orbicella* fertility and fecundity increase linearly with colony size (Kazuhiko, 1998). These massive colonies grow about 1 cm per year in SE FL, therefore, colony size can be used as a proxy for age, the largest colonies being the oldest in a population. One large colony (>2 m) was cored and dated to be over 320 years old (Helmle and Dodge, per obs). Their age makes them some of the oldest living residents in south Florida and demonstrates that they have persisted through a multitude of natural and anthropogenic impacts of the region. Many of the large colonies have, since discovery, remained alive and untouched by numerous bleaching and disease events, indicating exceptional resistance to major stress events. Increased reproduction of these species is extremely important for the health and restoration of massive coral species currently declining along Florida's Coral Reef.

To this end, I conducted coral disease interventions with the expectation of adapting to new methodologies to improve intervention success in the SE FL ECA. These actions include but were not limited to the monitoring and continued treatment of the priority large corals, broadscale strike team reconnaissance and disease interventions, further testing of permitted intervention techniques and materials, identification of unique coral disease survivor sites, as well as testing coral histology and testing micro fragmentation restoration techniques on select large coral skeletons. It is important that actions are taken to curtail this disease quickly so that the remaining coral population can stabilize, and recovery and restoration efforts can begin. There should be continued focus on the remaining corals because they are apparently resistant to the disease and perhaps better acclimated to the stressful conditions over recent years. This information will provide us with data on which treatment option to use to save the remaining corals in broader-scale disease intervention efforts and will inform future restoration actions.

2. Methods

Previous studies used high resolution Light Detection and Ranging (LIDAR) bathymetry (<4 m) and NOAA's Office of Coast Survey Hydrographic Division bathymetry (1 m) and aerial photography (1 ft) to identify the location of large corals on the relatively flat nearshore habitats in southeast Florida (Walker & Klug, 2014); Walker et al. *In prep*). At the onset of disease intervention only few large corals were selected to receive intervention treatments. As funding increased, more corals were prioritized for intervention treatments. From September 2018 – June

2019, approximately 60 corals were monitored and treated. Priority corals were increased to 90 colonies in July 2019, but it took several periods to establish all 90.

In May 2018, the large coral database was sorted by live tissue area estimates, estimated percent mortality, and colony size. This resulted in 50 corals with either more than four square meters of live tissue remaining and colonies with <10% mortality (Figure 1). In July 2019, the next colonies in the list were visited to establish 40 more monitored corals. Many of these corals had lost significant tissue or died; therefore, we had to broaden our criteria to include more corals. This meant revisiting some smaller diameter corals (1 - 2 m) that were originally excluded due to their smaller size, but still had a substantial amount of live tissue remaining. We also included several new corals found during other activities or reports. See Appendix A for a table of the priority corals and a summary of their treatments from April 2018 – October 31, 2019.

High resolution photographs and video were collected of each coral as a permanent record of its condition. Photographs were taken from above and at each cardinal direction of the compass: north (0°), east (90°), south (180°), and west (270°). Distance of photos from the colony depended on water clarity, but the objective was to capture the entire coral in the image. In cases where the coral was too large, or the visibility was poor, multiple pictures of the coral were taken at a closer distance to photo document the entire structure. Coral condition was visually estimated using methods and personnel of Walker and Klug (2015), where a diver floated above the colony and estimate the percentage of live tissue, diseased tissue, bleached tissue, recent mortality, and old mortality. The presence of paling and the number of tissue isolates were also recorded. Each colony was initially measured using a stiff meter stick to estimate the maximum length, maximum width perpendicular to the max length axis, and height from the seafloor. Two divers scanned the colony for potential diseased areas then conferred. If tissue loss was found, it was scrutinized to determine the possible cause based on visual cues. If it was thought to be SCTLD, then a decision on treatment was made based on how much live coral tissue would be saved and the present condition of that tissue. Small isolates were usually not treated. Photographs were taken of all areas before treatment at both the 0.5 m standard distance and wider scenes.

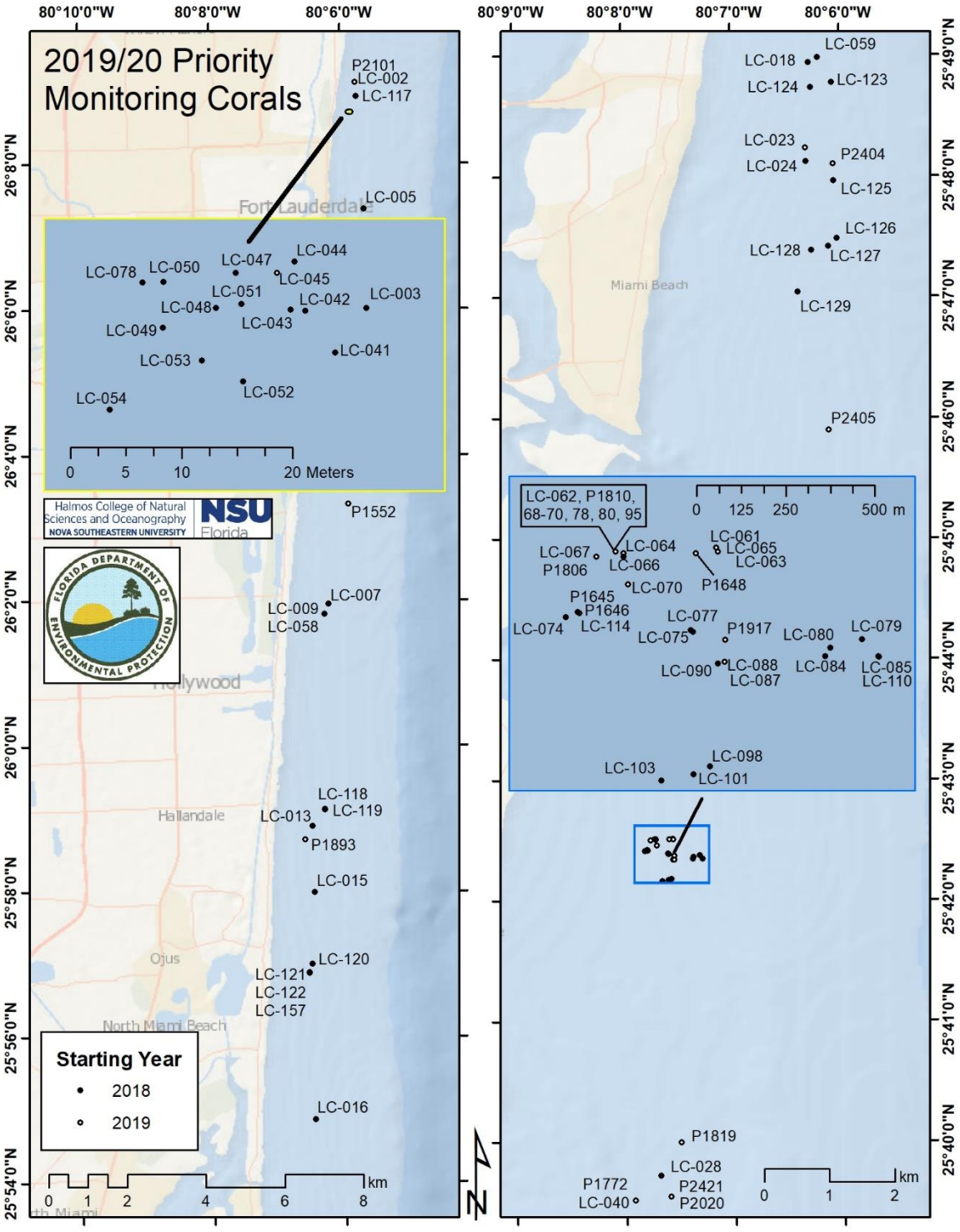


Figure 3. Map of the large priority monitoring corals.

SCTLD lesions typically present as an area of tissue loss exposing the bare skeleton. This is often, but not always, associated with paling or bleached polyps near the lesion. As the disease progresses over time, lesions radiate outward from the initial onset. Once a colony shows signs of infection, the disease often spreads rapidly, leading to whole colony mortality in weeks to months. Aeby et al. (2015) reported the successful in situ use of a disease intervention to cease black band disease (which has a similar radiating disease presentation) in Hawaii using a mixture of marine epoxy and chlorine powder. Considering the immediate need for disease intervention, we used the successful techniques in Aeby et al. (2015) to start saving corals while others conducted laboratory trials on many other materials.

Chlorinated epoxy was created using the same ingredients (ZSPAR A-788 Splash Zone epoxy & Poolife™ TurboShock© powder), recipe, and application methodology as described in Aeby et al. (2015). The chlorinated epoxy was pushed onto the disease margin covering 1-2 cm of live tissue and 1-2 cm onto the recently dead skeleton across the entire diseased portion. In many cases, a Firebreak was also created by using a Nemo V2 underwater angle grinder and hammer and chisel to cut a trench and isolate the progressing margin from apparently healthy tissue. The firebreaks were filled with treatment material. Firebreaks ranged in length, width, and depth depending on coral morphology and hardness. A typical firebreak was one to two centimeters wide and deep. The disease area was first scored with chisel about five centimeters away from the margin, and then a trench was created along the scored tissue. Scalable photographs were taken of all treatment areas before and after treatments and monthly thereafter.

Treatments were categorized into the following types:

> *Margin and Firebreak Treatment* – the active disease margin and drilled firebreak was is covered/filled with chlorinated epoxy to isolate the disease.
>
> *Solo Margin Treatment* – a treatment where the chlorinated epoxy was applied to the disease margin only.
>
> *Solo Firebreak Treatment* – a trench was created about 5 cm from the disease margin and filled with chlorinated epoxy to isolate the disease. The active disease margin was is left untreated.

Most initial treatments were margin and firebreak. Solo margin and firebreaks were created opportunistically based on special cases. Solo margin treatments were used in cases where the disease did not appear to be progressing rapidly or where there was not a lot of tissue to allow for an effective firebreak. Solo firebreaks were used when the disease was progressing rapidly, and the margin was too large to treat effectively and upon retreatments of previous firebreak failures.

Starting in August 2019, all margins were treated with the Ocean Alchemists antibiotic ointment CoreRx B2B with amoxicillin (1:8 ratio by weight) based on the increased success of this treatment material (Walker et al *In prep*), no firebreaks were used this portion of the study.

Treatment success was based on if the entire treatment stopped the disease in the photographs. The solo margin treatment failed if the active disease continued progressing past the treatment line. The solo firebreak treatment failed if the active disease margin progressed across the chlorinated epoxy filled firebreak to the other side (Figure 4). The margin and firebreak treatment failed if the active disease margin progressed past the firebreak. If the active disease progresses past the margin treatment portion of the combined treatment but not passed the firebreak, this was not considered a treatment failure.

Initial treatment monitoring was dictated by the State of Florida Special Activities License. Colonies were monitored 2-3 days (or as soon as possible thereafter) after the first treatment and revisited and photographed every two weeks through June 2018. In July 2018, monthly monitoring began for all colonies.

3. Results

a. Treatment Success

In total, 90 corals were monitored monthly (Figure 4a). Forty-six (51%) needed treatment, leaving the other 44 assessed corals not infected during the monitoring. Twenty-one (45.6%) of the treated colonies needed additional treatments over multiple monitoring periods. Eleven colonies only required treatment one time. Monitoring of some colonies stopped as they were almost or completely dead and it was no longer effective to spend time on them. Figure 2 shows the proportion of treatments on corals in the monitoring database from the start of the project in 2018 to present and the proportion of treatments on the thirty-three corals added to the monitoring database in 2019.

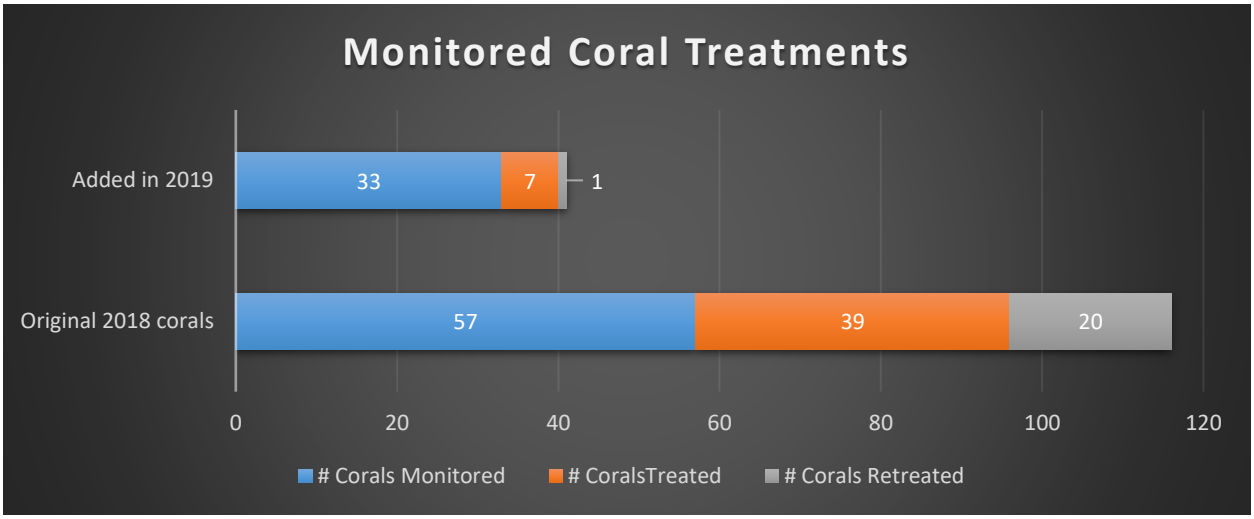
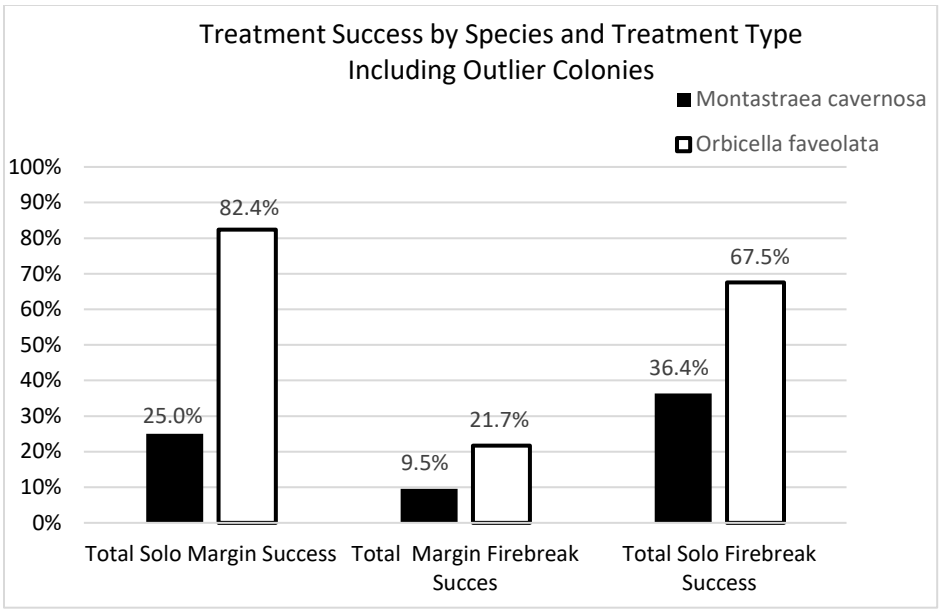


Figure 4. Number of corals requiring treatments and retreatments out of the total monitored corals in 2018 and 2019.

Success varied drastically by species and treatment method (Figure 3). Analysis of success of treatments on all treated corals in the large coral database, showed disease progression was halted on 82% (154/187) of the *Orbicella spp.* solo margin chlorinated epoxy treatments (Figure 3a). When calculating the success of treatments that include the use of a firebreak (eg. solo firebreak or margin firebreak treatments) the number of failures must be considered in relation to the number of firebreaks that were "tested," meaning the active disease margin must reach the firebreak and halt progression at the firebreak to be considered successful. Therefore, to calculate the success of solo firebreak and margin firebreak treatments, the number of failed treatments must be divided by the number of "tested" treatments, rather than the total number of firebreak treatments created. *Orbicella spp.* were 67.5% (47/60) successful at halting disease with the use of solo firebreak treatments, followed by 36.4% (19/36) successful when both a firebreak and direct margin treatment to the active disease margin was applied. Contrastingly, *Montastrea cavernosa* showed to have much less success when compared to *Orbicella spp.* The highest success of *Montastrea cavernosa* was 36% (4/11) when disease lesions were treated with a solo firebreak filled with chlorinated epoxy. Results showed that *M. cavernosa* species responded positively solo margin treatments 25% (1/4) of the time. It was found that the least successful treatment type on

Montastrea cavernosa was the combined treatment of a chlorinated epoxy-filled firebreak as well as margin applied to the individual which was found successful 9.5% (2/21) of the time.

Figure 3b shows the treatment success by species and treatment type excluding outlier colonies (*see b. Untreatable Colonies)*. The seven outlier colonies excluded prove drastically change the treatment success. *Orbicella spp.* show increases in success across all treatment types with 87% (145/149) success when applying chlorinated epoxy directly to the active disease margin only. Disease was halted on 80% of solo firebreak treatments (12/15), and 63% (12/19) of combined margin firebreak treatments. Similarly, *M. cavernosa* also showed increased success when excluding the outlier corals from the results, however like Figure 3a, still prove to me much less successful when compared to *Orbicella spp.* The use of a margin treatment alone on *M. cavernosa* was successful 50% of the time (1/2), followed by 40% (2/5) success with the use of a solo firebreak treatment. The least effective treatment type for *M. cavernosa* species was the combined margin firebreak treatment which was successful 16.7% (1/6) of the time. It is clear that when excluding the outlier corals, success for both species increases, and the number of treatments decrease.



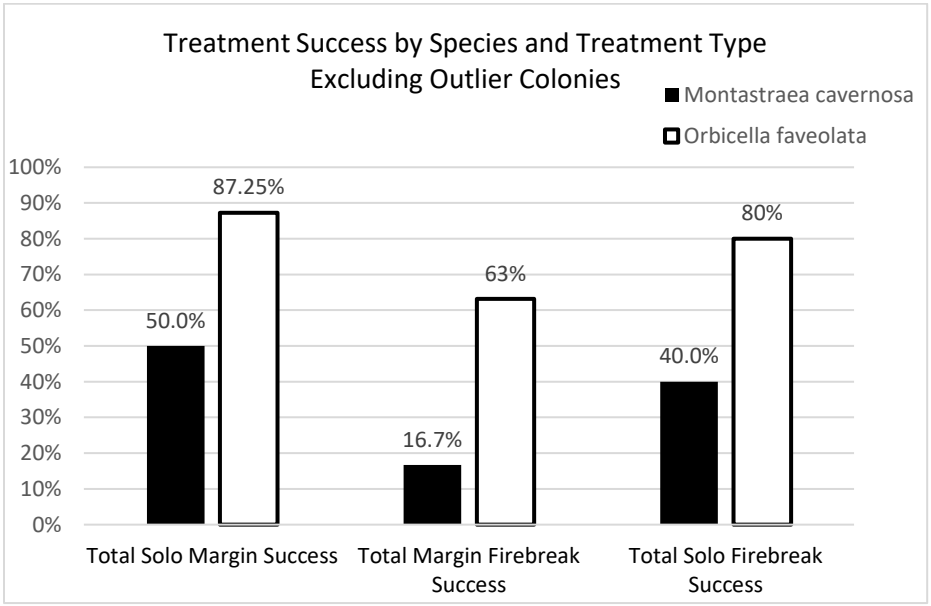


Figure 5) a. Graph of treatment type success by species including outlier colonies (above). **b.** Graph of treatment type success by species excluding outlier colonies (below).

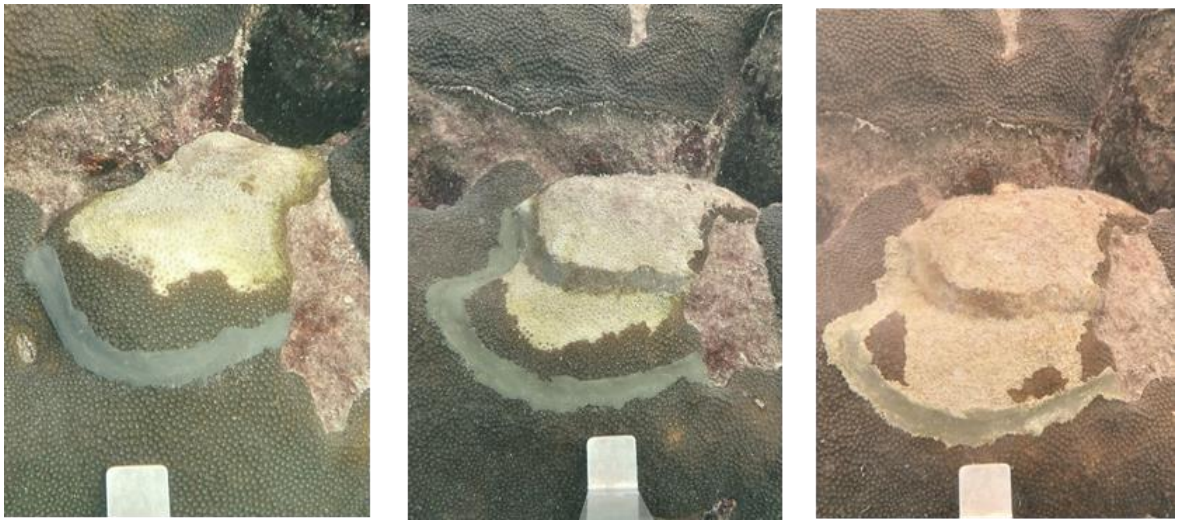


Figure 6. Example of disease progression and treatment results of chlorinated epoxy on *Orbicella spp.,* LC-110. Initial solo firebreak treatment (left) in January 2019. Failure of solo firebreak treatment with subsequent retreatment in February 2019 (middle). Success of solo firebreak retreatment in March 2019 (left).

Treatment success for all corals varied through time. Both chlorinated epoxy and amoxicillin ointment treatments combined, had a success of 71.2% (166/577), but these were very different between species (Table 1), between treatment type, and through time (Figure 5). The success for treating *Orbicella spp.* and *Siderastrea siderea* using chlorinated epoxy was high (75.5% and 80% respectively). Contrastingly, chlorinated epoxy success for *Montastraea cavernosa* was low (37.5%). Eighty-eight percent of treatments were on *Orbicella spp.,* thus the total success was mostly reflective of this species. However, the poor outcomes of *M. cavernosa* treatments did affect the total success values. In August 2019, all treatments were switched to amoxicillin powder mixed in CoreRX B2B, thus all success from September 2019 onward was on antibiotic ointment treatments.

Cumulative treatment success varied between months (Figure 5). Initial treatment success was relatively high (81%) in May 2018, however it dropped substantially to 54.3% by August. The initially high success may have been the low number of early treatments to date (16) or because the treatments had not had enough time to fail between assessments. There appears to have been a one to two-month lag between increases in treatments and increases in treatment failures (Figure 6). July 2018 had the highest number of failures (35). These almost exclusively came from three corals LC-123 (21), LC-093 (7), and LC-038 (4) (Figure 7). Since November 2018, the number of

treatment failures declined even with periodic increases in higher number of treatments. This is evident in the cumulative treatments versus failures by monitoring period where the total number of treatments has a much steeper slope than the cumulative total number of failures (Figure 8). Twenty-three corals (44%) of the total treated corals never failed after the initial treatment (Figure 7).

Table 1. Total treatment failure and success by species from April 2018 – April 2020.

APRIL 2018-APRIL 2020	MONTASTRAEA CAVERNOSA	ORBICELLA SPP.	PSUEDODIPLORIA STRIGOSA	SIDERASTREA SIDEREA	ALL SPECIES
TOTAL TREATMENTS	56	507	1	13	577
TOTAL FAILURES	57.14%	26.23%	0.00%	7.69%	28.77%
TOTAL SUCCESS	42.86%	73.77%	100.00%	92.31%	71.23%

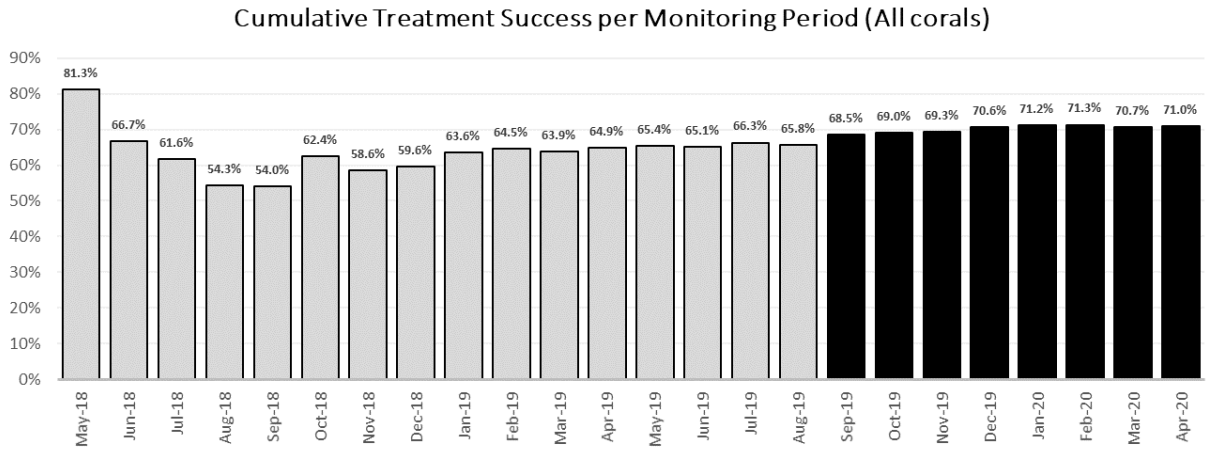


Figure 7. The cumulative percent success of all treatments on all corals each treatment period. Grey bars indicate chlorinated epoxy treatments. Black bars indicate antibiotic ointment treatments.

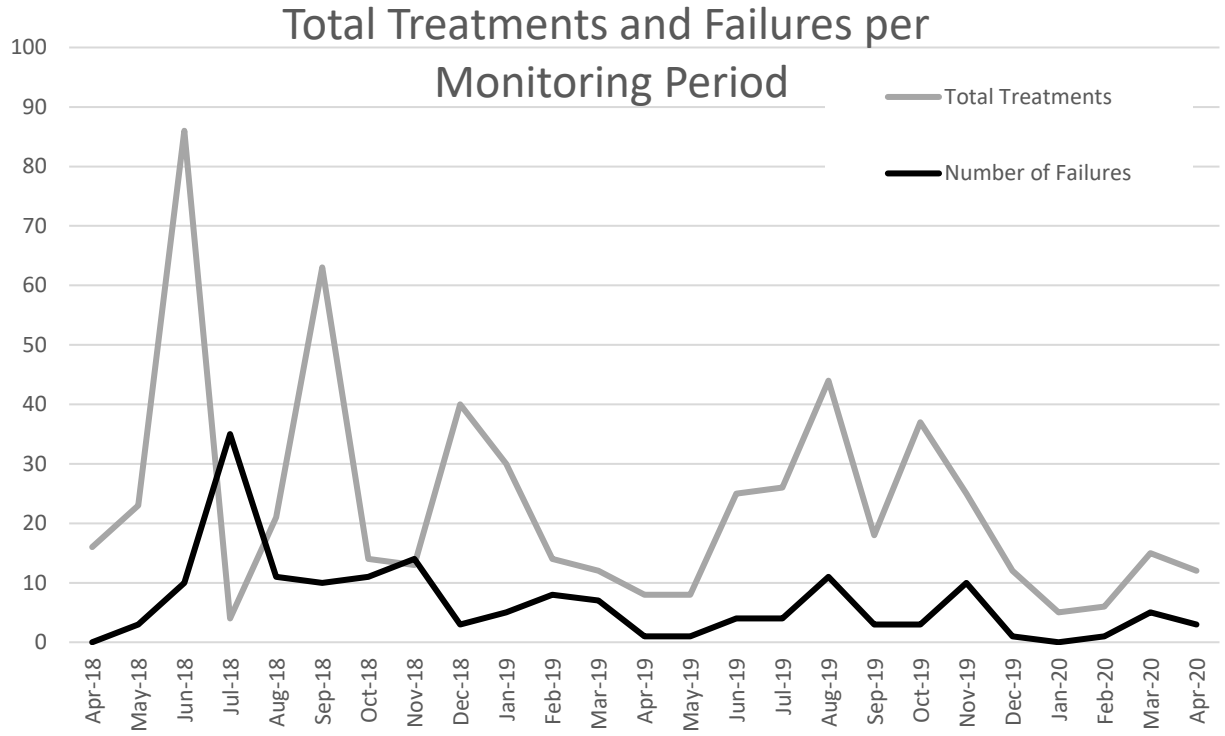


Figure 8. The total number of treatments (grey) and failures (black) per monitoring period for all corals.

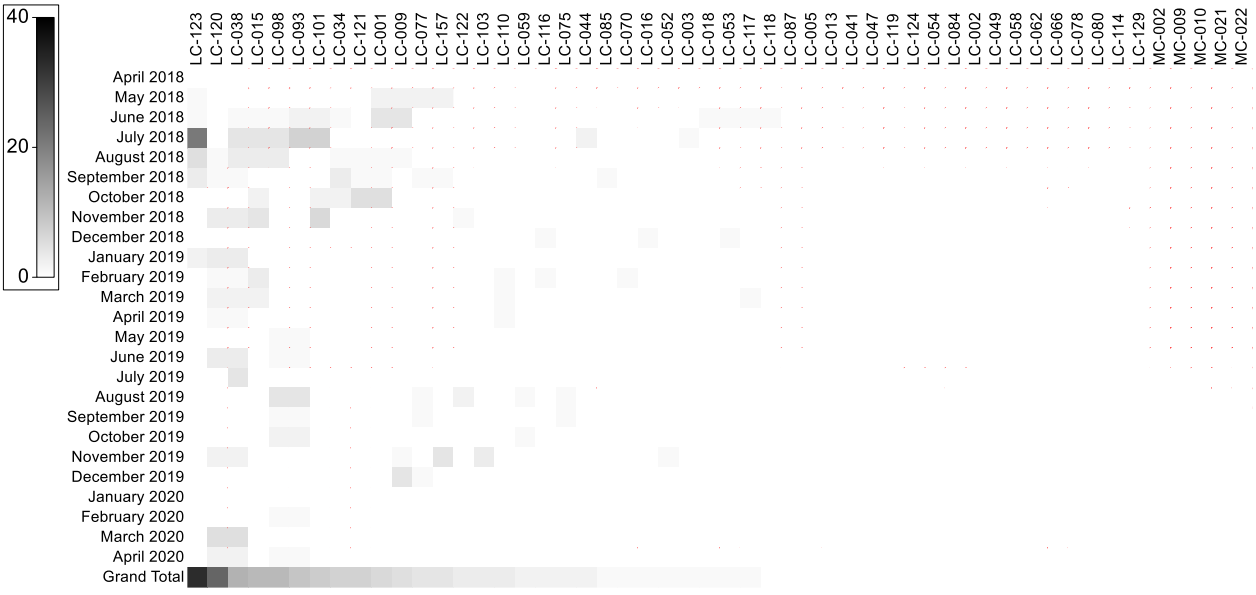


Figure 9. Shade plot of total failure on each treated coral by monitoring period sorted by the maximum total number of failures.

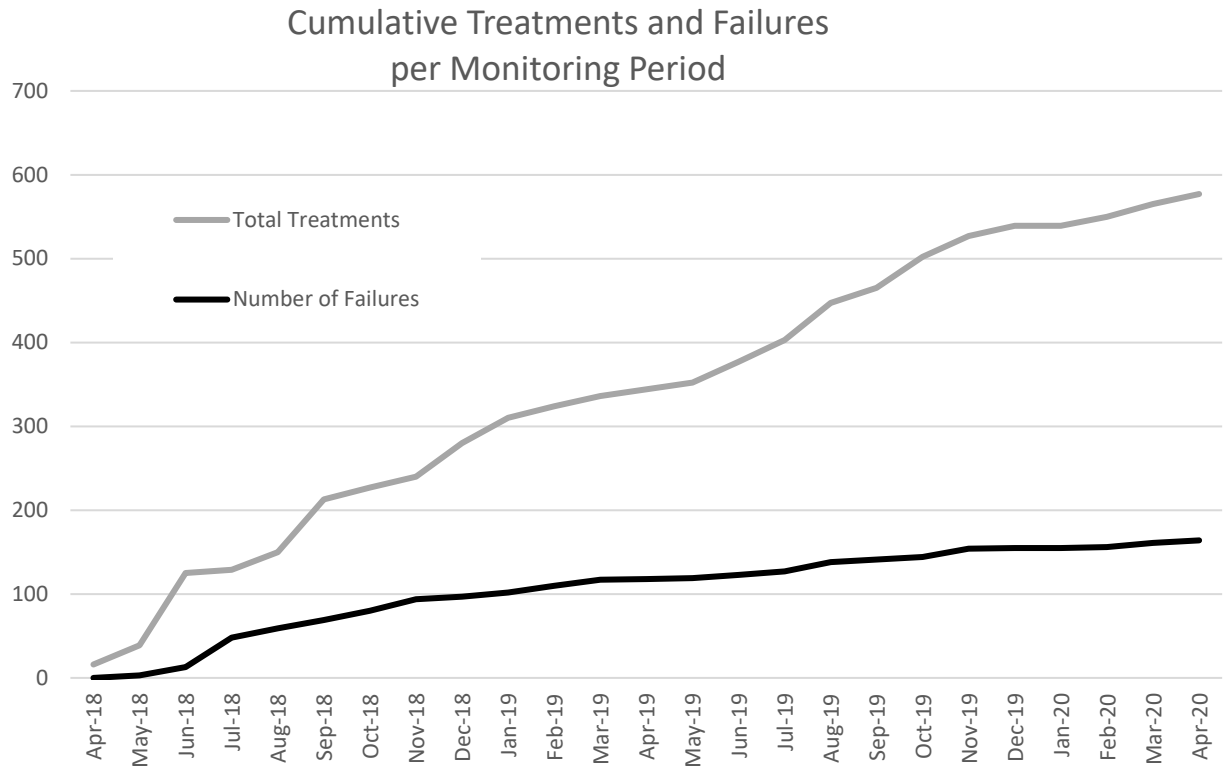


Figure 10. The cumulative total number of treatments (grey) and failures (black) per monitoring period for all corals.

The patterns of total cumulative success were mostly driven by *Orbicella spp.* which comprised most of the monitored corals (77%), hence Figure 9**Error! Reference source not found.**. looks similar to Figure 5**Error! Reference source not found.**. However, the *M. cavernosa* success was notably different (Figure 10). Like the *Orbicella spp.*, they initially seemed successful but failed over time (Figure 10**Error! Reference source not found.**). In July 2018, there had been 25 treatments on *M. cavernosa* and only 6 failures (Figure 11). Although no additional treatments were needed until November, the failures continued to rise leading to a 40% success in October 2018. This was evident in the steep slope in failures in the cumulative data during a flat slope for treatments (Figure 12). Since November 2018, chlorinated epoxy treatment success on *M. cavernosa* has been a dismal 2% (3/16). The poor success of chlorinated epoxy on *M. cavernosa* led to an experiment comparing the two treatment types and the recommendation to switch all treatments to the Ocean Alchemist (CoreRX B2B) coral disease antibiotic ointment (Walker & Pitts, 2019).

Between August 2019 and April 2020, 174 antibiotic ointment treatments were conducted on 25 corals including four species (Table 2). Since the beginning of antibiotic ointment treatments (August 2019 - April 2020), 22 treatments had failed. This equates to 87.4% success rate for those treatments. Success varied by species where *Orbicella spp.* treatments were 88.3% successful (128/145) and *M. cavernosa* treatments were 73.7% successful (14/19). The cumulative success of amoxicillin treatments per monitoring period was higher than chlorinated epoxy and consistent thus far (Figure 13**Error! Reference source not found.**).

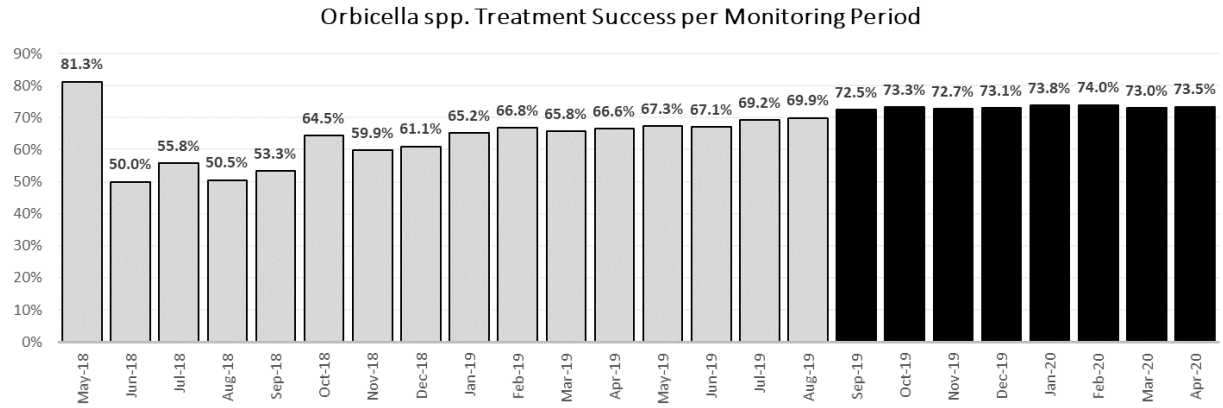


Figure 11. The cumulative percent success of all Orbicella spp. treatments on all corals each treatment period. Grey bars indicate chlorinated epoxy treatments. Black bars indicate antibiotic ointment treatments.

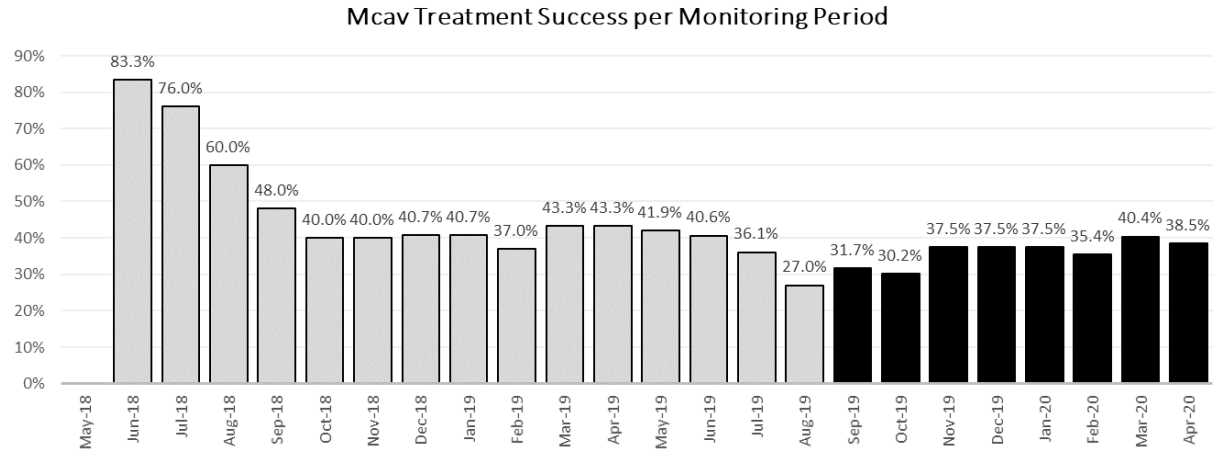


Figure 12. The cumulative percent success of all M. cavernosa treatments on all corals each treatment period. Grey bars indicate chlorinated epoxy treatments. Black bars indicate antibiotic ointment treatments.

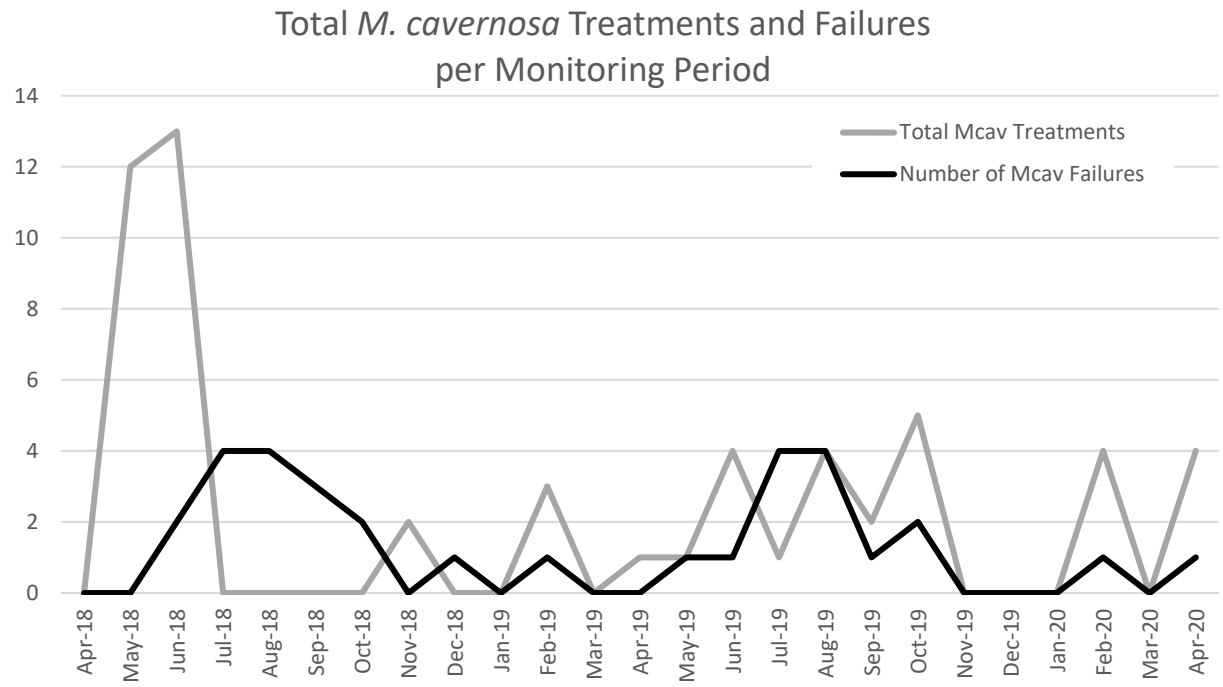


Figure 13. The total number of treatments (grey) and failures (black) per monitoring period for M. cavernosa.

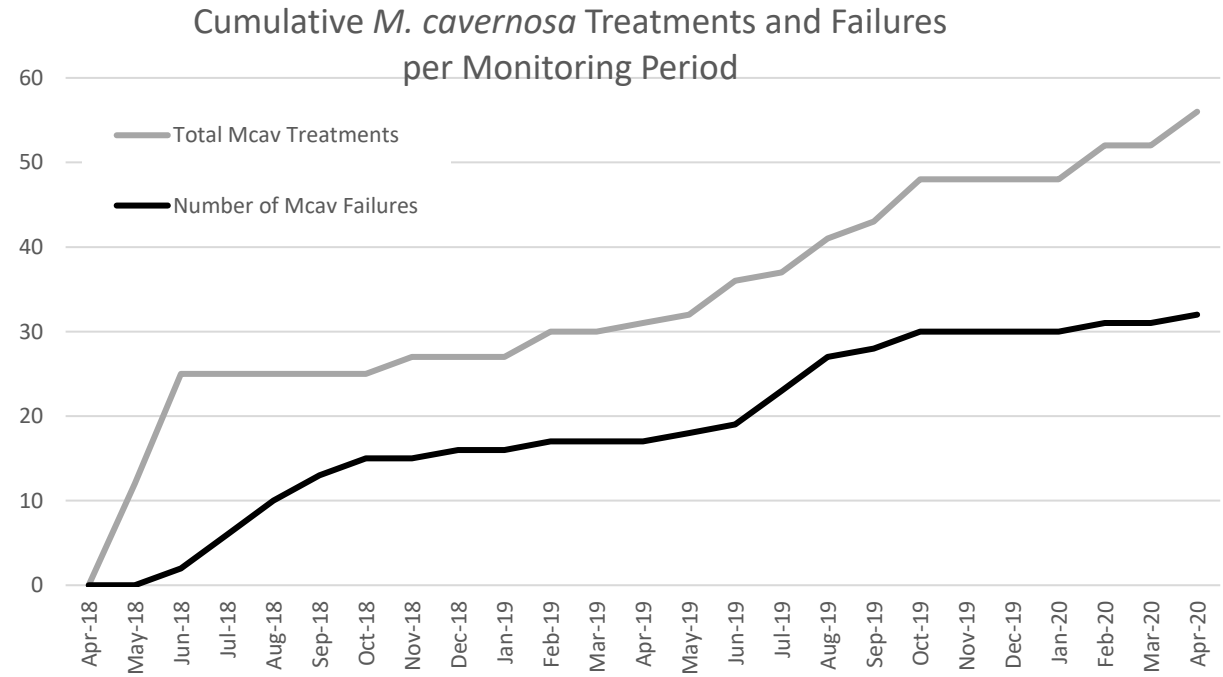


Figure 14. The cumulative total number of treatments (grey) and failures (black) per monitoring period for M. cavernosa.

Table 2. Cumulative success of amoxicillin ointment on all treated species from September 2018 to April 2020.

MONITORING PERIOD	ALL SPECIES	ORBICELLA SPP.	M.CAVERNOSA	S. SIDERAEA	P. STRIGOSA
SEP-19	66.7%	50.0%	75.0%		
OCT-19	91.9%	96.4%	50.0%		
NOV-19	85.9%	87.4%	72.7%		100%
DEC-19	87.9%	89.3%	72.7%		100%
JAN-20	89.0%	90.3%	72.7%		100%
FEB-20	88.7%	90.6%	63.6%	100%	100%
MAR-20	87.1%	88.5%	73.3%	100%	100%
APR-20	86.4%	88.3%	66.7%	100%	100%

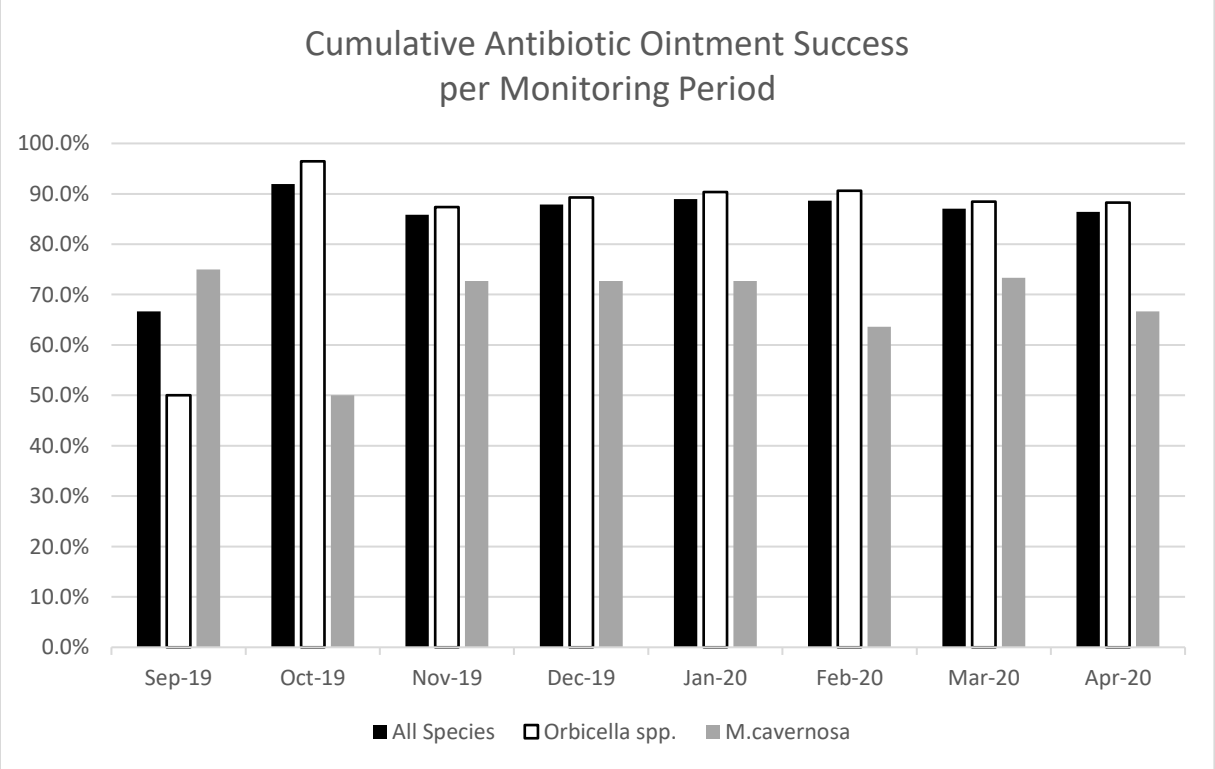


Figure 15. The cumulative percent success of antibiotic ointment treatments on all corals (black), Orbicella spp. (white), and M. cavernosa (grey) each treatment period.

b. Untreatable Colonies

Four colonies were untreatable: LC-001, LC-014, LC-092 & LC-093. These were colonies that showed blotchy, half-paling/half- diseased appearance (Figure 15), usually followed by heavy algal growth (Figure 16). Three colonies seemingly did not respond to treatment and required excessive work: LC-034, LC-120, LC-123 (Figure 17). If we remove these seven outlier colonies, overall treatment success improved substantially to 80.5% (Figure 14) as well as by success by species and treatment type (Figure 3).

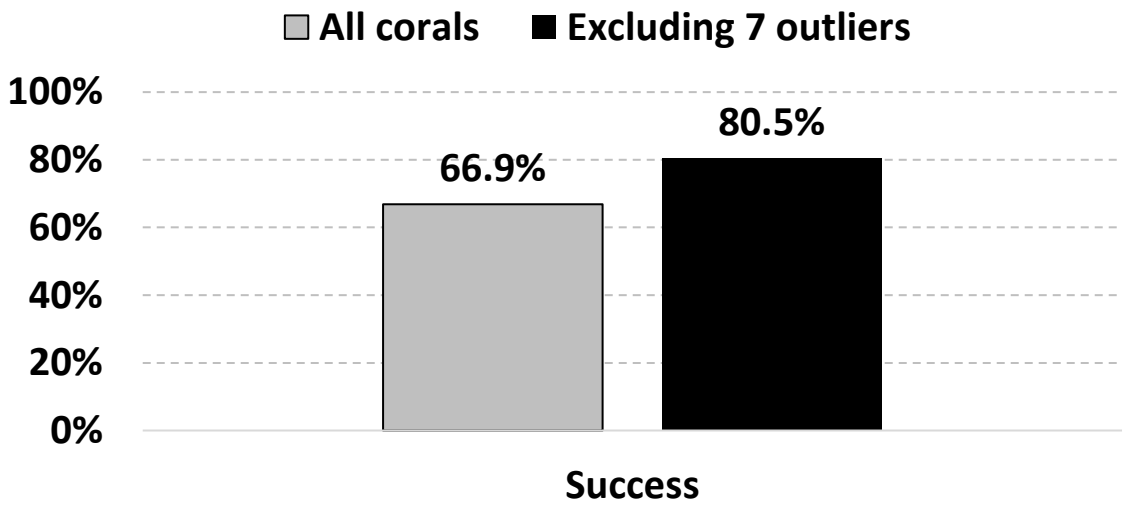


Figure 16. Graph of total treatment success of all forty treated corals (grey) and excluding the 7 outliers (black).

Figure 17. Untreatable corals that showed blotchy, half-paling/half-diseased appearance. Top left: LC-001, top right: LC-0014, bottom left: LC-092 & bottom right: LC-093.

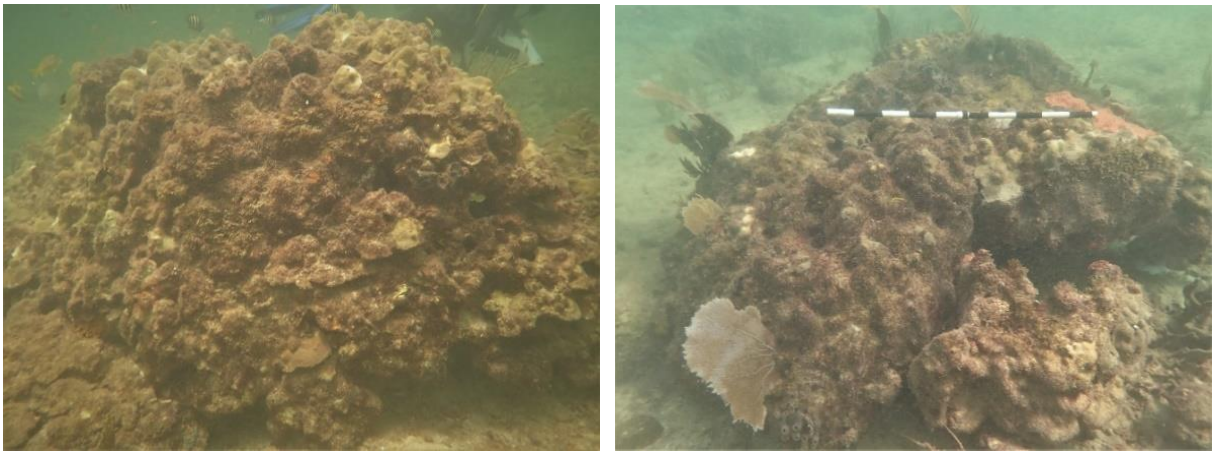


Figure 18. Untreatable corals with heavy algae colonization. left: LC-001 & right: LC-092.

Figure 19. Corals that did not respond to treatment. Left: LC-034, Middle: LC-120, & Right: LC-123.

c. Temporal Infection Patterns

At each monitoring period, all disease lesions were treated, thus the total number of new treatments indicates the amount of new disease found on the monitored corals over time after their initial visit. Figure 18 summarizes the number of new treatments required (grey) and number of treated corals (black) per monitoring period for all corals since April 2018. The number of new infections and corals requiring treatment varied through time. At the beginning of the project (April and May 2018) the number of new margins per period was affected by the addition of new corals that needed treatment (Figure 2b and Table 3). Between April and June 2018, the number of corals visited was low (Figure 2b) but increasing and the addition of new corals contributed to a substantial increase in the number of new treatments .The increase in August 2018 was not due to the addition of new corals, however the high number of treatments in September 2018 was because thirty-nine new corals were added and treated for the first time. The number of newly added corals did not affect the number of new margin treatments after September 2018 (Table 3), indicating variable amounts of infections over time.

In October and November 2018 only five and four corals respectively needed treatment and far fewer treatments (<10) were required than in December 2018 (37) and January 2019 (25). February and March required fewer treatments (9) and the number of treated corals dropped through April 2019. In June 2019, the number of treatments tripled and remained high in July 2019 (25). Interestingly only two corals treated in June 2019 (LC-118 and LC-120) required treatment again in July 2019. Six of the nine treated in July 2019 were not showing disease in June. In August

2019, the number of treatments spiked to thirty-eight, but this was on seven corals. Three of the seven corals required treatment in July and August, meaning there were five additional corals in August requiring treatment. Three of seven corals (different than the three in July and August) required treatment in August and September 2019.

In total, twenty-six corals required 138 new treatments from May through October 2019 (Table 4). Seventy-six percent of the treatments (106/138) were conducted on just seven corals. Six of these seven corals required treatments multiple monitoring periods. LC-120 required treatments five out of the six periods. LC-157 required treatments four out of the six periods while LC-118, LC-103, and LC-059 required treatments three out of six periods. LC-009 required treatment only in October 2019, however this one period required a high number of treatments with 6% of the total treatments (8/138).

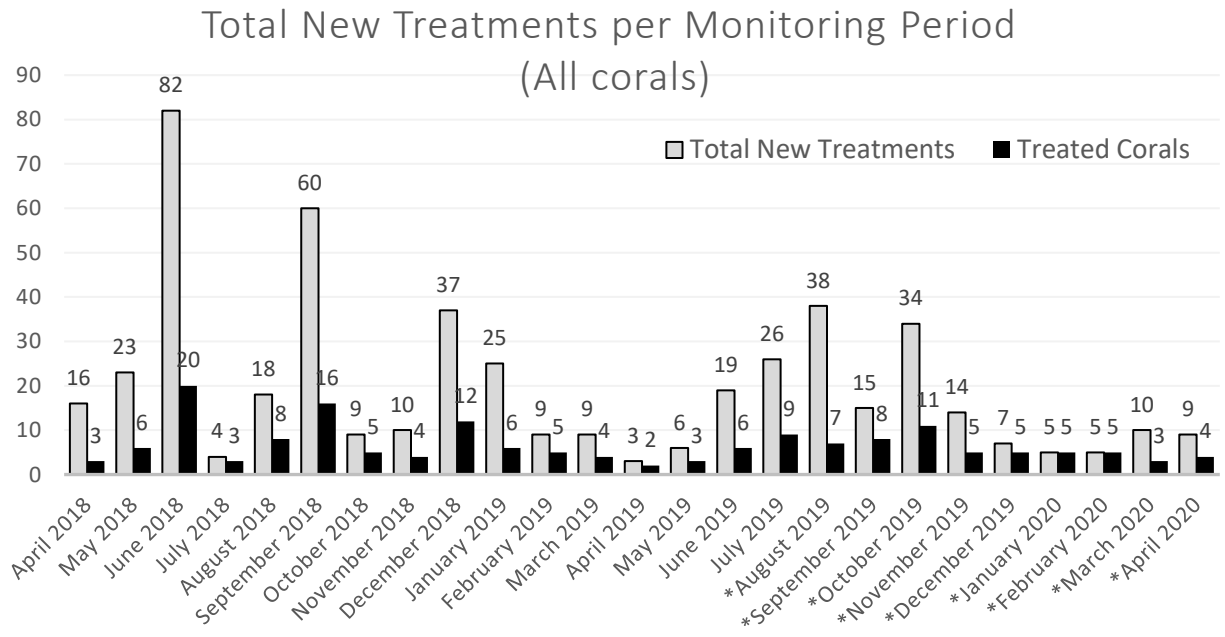


Figure 20. The numbers of new treatments on all corals used as a proxy for new infections (grey) and the number of treated corals (black) by treatment period. *Indicates antibiotic ointment treatments.

Table 3. The total number of corals assessed, total number of new treatments, and number of new treatments on newly assessed corals by monitoring period.

MONITORING PERIOD	# OF CORALS	# OF NEW MARGIN TREATMENTS	# OF MARGIN TREATMENTS ON NEW CORALS	% MARGIN TREATMENTS FROM NEW CORALS
APR- 2018	4	16	1	6.3%
MAY- 2018	11	23	21	91.3%
JUN- 2018	24	82	29	35.4%
JUL- 2018	23	4	2	50.0%
AUG- 2018	25	18	0	0.0%
SEPT- 2018	57	60	48	80.0%
OCT- 2018	53	9	0	0.0%
NOV- 2018	59	10	0	0.0%
DEC- 2018	57	37	0	0.0%
JAN- 2019	59	25	0	0.0%
FEB- 2019	60	9	0	0.0%
MAR- 2019	60	9	0	0.0%
APR- 2019	60	3	0	0.0%
MAY- 2019	60	6	0	0.0%
JUN- 2019	60	19	0	0.0%
JUL- 2019	80	26	2	7.7%
AUG- 2019	83	38	0	0.0%
SEPT- 2019	86	15	0	0.0%
OCT- 2019	90	34	0	0.0%
NOV- 2019	90	10	0	0.0%
DEC- 2019	90	11	0	0.0%
JAN- 2020	90	5	0	0.0%
FEB- 2020	90	5	0	0.0%
MAR- 2020	90	10	0	0.0%
APR- 2020	90	9	0	0.0%

Table 4. Corals needing new treatments between May 2019 and October 2019 with the * signifying months treated with antibiotic ointment.

CORAL ID	MAY 2019	JUN 2019	JUL 2019	AUG* 2019	SEPT* 2019	OCT* 2019	SUM	% NEW TREATMENTS OF TOTAL	NUMBER OF MONTHS REQUIRING NEW TREATMENTS	% MONTHS REQUIRING TREATMENT OF TOTAL MONTHS
LC-005	0	0	0	0	1	0	1	0.7%	1	2.3%
LC-009	0	0	0	0	0	8	8	5.8%	1	2.3%
LC-013	1	0	0	0	0	0	1	0.7%	1	2.3%
LC-015	0	6	0	5	0	0	11	8.0%	2	4.7%
LC-016	0	1	0	0	0	0	1	0.7%	1	2.3%
LC-018	0	1	0	0	0	0	1	0.7%	1	2.3%
LC-047	0	0	0	0	0	1	1	0.7%	1	2.3%
LC-052	0	0	0	0	0	1	1	0.7%	1	2.3%
LC-054	0	0	0	0	2	0	2	1.4%	1	2.3%
LC-059	0	0	2	5	0	3	10	7.2%	3	7.0%
LC-075	0	0	2	2	0	0	4	2.9%	2	4.7%
LC-077	0	0	2	0	1	0	3	2.2%	2	4.7%
LC-084	0	0	0	0	0	0	1	0.7%	1	2.3%
LC-085	2	0	0		0	0	2	1.4%	1	2.3%
LC-087	0	0	1	0	0	3	4	2.9%	2	4.7%
LC-098	0	0	0	0	1	0	1	0.7%	1	2.3%
LC-103	0	0	0	5	4	3	12	8.7%	3	7.0%
LC-114	0	0	0	1	0	0	1	0.7%	1	2.3%
LC-118	0	5	6	13	0	0	24	17.4%	3	7.0%
LC-119	0	1	0	0	0	0	1	0.7%	1	2.3%
LC-120	3	5	9	0	2	4	23	16.7%	5	11.6%
LC-122	0	0	2	0	0	2	4	2.9%	2	4.7%
LC-157	0	0	1	7	3	7	18	13.0%	4	9.3%
MC-009	0	0	1	0	0	0	1	0.7%	1	2.3%
MC-010	0	0	0	0	0	1	1	0.7%	1	2.3%
MC-022	0	0	0		0	1	1	0.7%	1	2.3%
SUM	6	19	26	38	14	34	138		43	
COUNT	3	6	9	7	7	11	26			

Table 5. Corals needing new treatments between November 2019 and April 2020 with the * signifying months treated with antibiotic ointment.

CORAL ID	NOV* 2019	DEC* 2019	JAN* 2020	FEB* 2020	MAR* 2020	APR* 2020	SUM	%NEW TREATMENTS OF TOTAL	NUMBER OF MONTHS REQUIRING NEW TREATMENTS	% MONTHS REQUIRING TREATMENT OF TOTAL MONTHS
LC-002	0	0	0	0	1	0	1	2.0%	1	3.7%
LC-009	7	0	0	1	3	2	13	26.0%	4	14.8%
LC-013	0	0	1	0	0		1	2.0%	1	3.7%
LC-015	3	3	0	0	0	0	6	12.0%	2	7.4%
LC-047	0	0	0	1	0	0	1	2.0%	1	3.7%
LC-070	0	0	1	0	0	2	3	6.0%	2	7.4%
LC-077	1	1	1	0	0	0	3	6.0%	3	11.1%
LC-084	0	1	0	0	0	0	1	2.0%	1	3.7%
LC-087	0	0	0	1	0	0	1	2.0%	1	3.7%
LC-098	0	0	0	1	0	3	4	8.0%	2	7.4%
LC-120	0	1	1	0	6	2	10	20.0%	4	14.8%
LC-157	2	1	1	0	0	0	4	8.0%	3	11.1%
MC-002	1	0	0	0	0	0	1	2.0%	1	3.7%
MC-021	0	0	0	1	0	0	1	2.0%	1	3.7%
SUM	14	7	5	5	10	9	50		27	
COUNT	5	5	5	5	3	4	14			

From November 2019 through April 2020, fourteen corals required treatments. Seven corals (50%) required treatment in only one monitoring period. Two corals (LC-009 & LC-120) required treatment four out of six periods, two corals (LC-157 & LC-077) required treatments during three periods, and three corals (LC-015, LC-070, & LC-098) (21%) required treatments during two periods.

Comparing the treated corals between May 2019 through October 2019 to November 2019 through April 2020 showed substantial differences. In total, from November 2019 through April 2020, fourteen corals required 50 treatments (Table 5); over 50% less than May through October 2019

in both the number of corals requiring treatment as well as the number of overall treatments created.

Ten corals, one-third of all treated corals, required treatments in both time periods. LC-120 required treatments nine of the twelve monitoring periods with a total of 33 treatments equaling about 18% of all treatments across one year. LC-157 required treatment seven out of twelve months of treatments and a total of 22 treatments throughout one year.

A shade plot of the number of treatments per coral over time did not show any obvious infection patterns across all of the priority corals (Figure 19). Six corals required 45% of the treatments over the total length of the project (25 months). Some of these, like LC-120 and LC-015 required a low number of treatments nearly every monitoring period while others required a very high number of treatments during more discrete time periods (e.g. LC-123, LC-118).

Fifty one large corals that have been monitored through the entirety of the project (September 2018- April 2020) were sorted out of the shade plot from Figure 19 and were then used to identify and categorize groups of corals according to frequency and severity of infections trough time (Figure 21). Categories were broken up into corals displayed high numbers of infections every month (5), corals needed few monthly treatments (9), corals that exhibited low infections intermittently (11), corals that only needed one treatment (8) and corals that were never infected (18).

From September 2018 through April 2020, a set of fifty-one corals were monitored each period. A multivariate analysis of Bray-Curtis similarities of the number of treatments on each treated coral for each monitoring period in this subset of corals did not show any significant clustering when categorizing the monitoring periods by season and year (Figure 22). An analysis of similarity with season and year as factors did not yield any significant comparisons, indicating that there were no sets of similarly infected coral during different times of the year in any annual pattern. In other words, the corals requiring treatments in one season were not the same set of corals the next season or the next year in the same season.

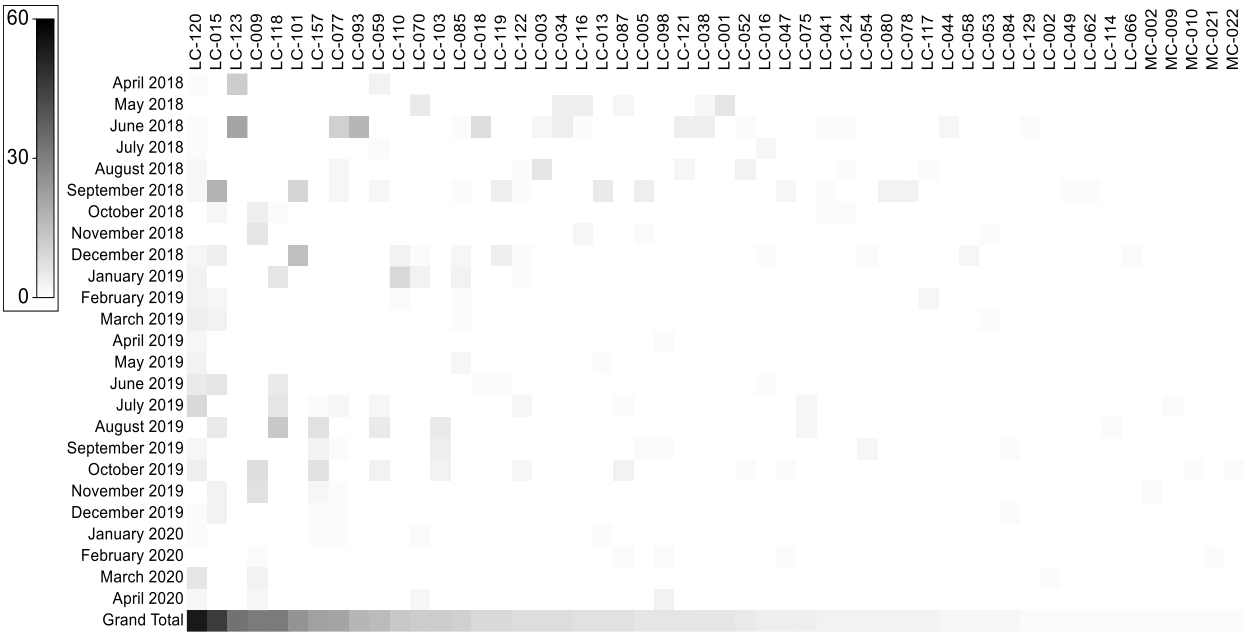


Figure 21. Shade plot of total treatments on each treated coral (column) by monitoring period (row) sorted from left to right by the maximum total number of treatments.

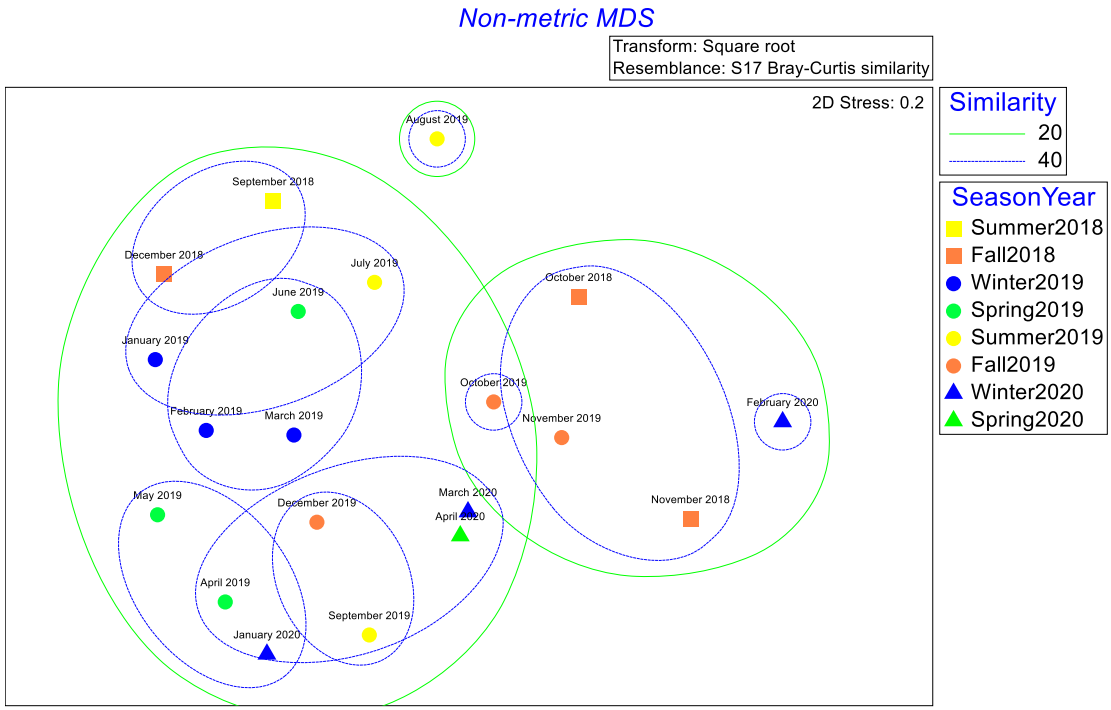


Figure 22. Multidimensional scaling plot of Bray-Curtis similarities of total treatments per coral for each monitoring period.

Figure 21. Shade plot of total treatments on each treated coral of all corals consistently monitored from September 2018-April 2020 (column) by monitoring period (row) sorted from left to right by the maximum total number of treatments. Corals are categorized by color denoting frequency and severity of infections.

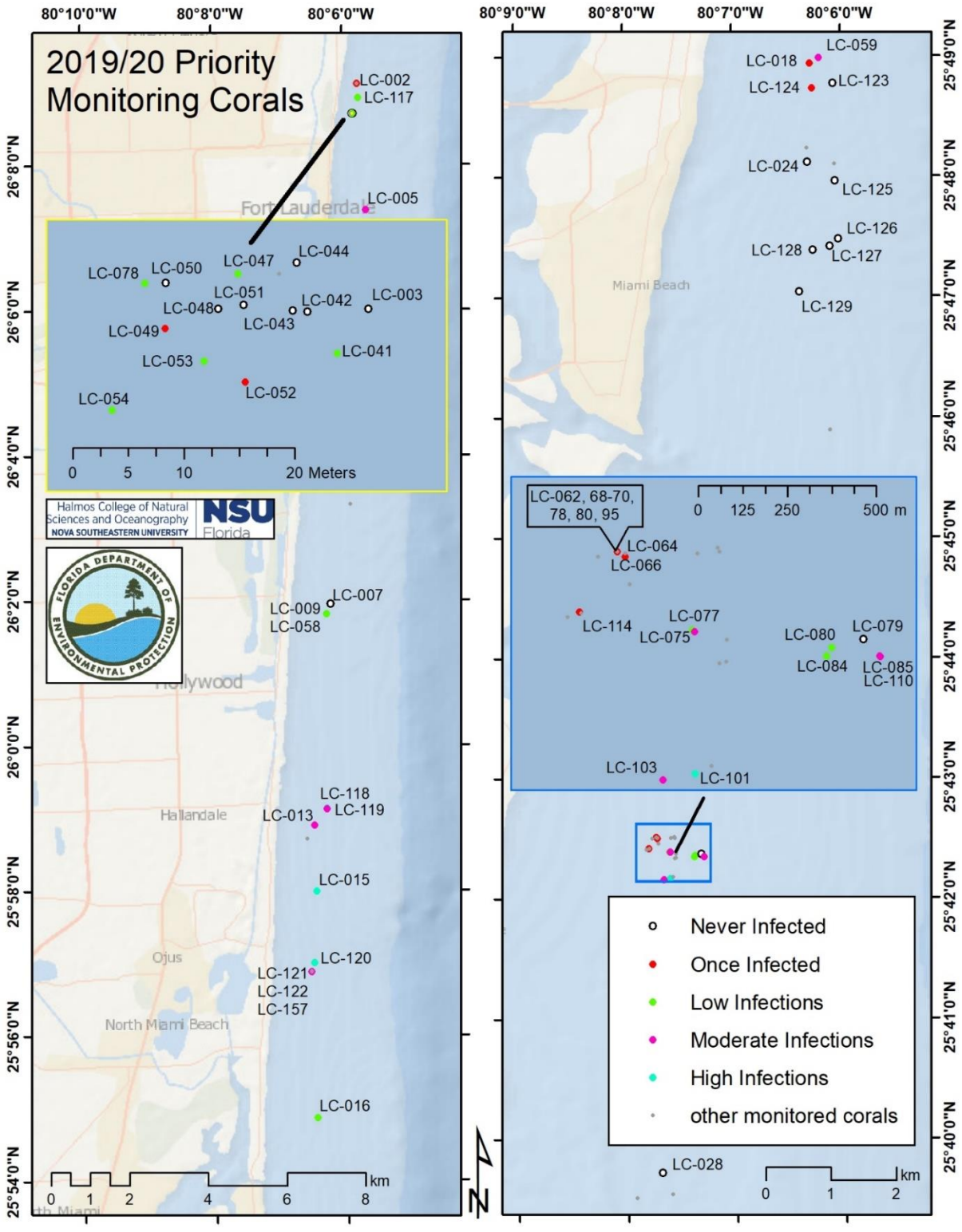


Figure 22. Map of study site with priority large corals colored by category (*see Figure 21*) according to frequency and severity of infection.

4. Discussion

Topical interventions on coral disease lesions are a useful tool in stopping the progression of disease lesions with a high rate of success and saving large amounts of live tissue. We found an overall intervention success of 71% with 74% for *Orbicella spp.* and 43% for *Montastrea cavernosa*. Other researchers obtained mixed results when applying chlorinated epoxy. Aeby et al. (2015) reported 63% success on five out of eight *M. capitate* colonies. One colony had no tissue loss beyond the active margin treatment and four (50%) additional colonies showing no tissue loss beyond the firebreak during the observation period. Neeley et al. (2019) showed low success using chlorinated epoxy with an 85% failure rate across all species, however, following a similar pattern, they had 49% success on *Orbicella spp.* and 24% on *M. cavernosa* across three months.

The recent development of a high concentration antibiotic ointment has resulted in the highest success at lesion treatments where 91% of antibiotic ointment lesion treatments on all species across one year were successful with *Orbicella spp.* having 91% success and *M. cavernosa* 89% (Neely, K., 2020; Neely, Karen L., Macaulay, Hower, & Dobler, 2020). Using the same mixture in a comparison study, Walker et al. (*In prep*) found 71% success of treated lesions on diseased *M. cavernosa* colonies after a single treatment versus 20% success with chlorinated epoxy. The antibiotic ointment success increased to 89.2% upon subsequent treatments. My intervention antibiotic ointment treatments increased total success to 85.4%, with 85.1% success on *Orbicella spp.* and 68.4% success on *M. cavernosa.* The transition to antibiotic ointment in August 2019 resulted in success increasing by 14.4% in *Orbicella spp.* and by 10.5% in *M. cavernosa*, further supporting that antibiotic ointment is a substantially more effective treatment than chlorinated epoxy.

Our data provided a cumulative treatment success because tracking every lesion on every coral individually was not feasible. Disease progression rates complicated success calculations because the time lag between treatment and failure was often longer than the monthly monitoring period. Walker et al. (*In prep*) found that most chlorinated epoxy firebreaks on *M. cavernosa* failed between 23 and 52 days after initial treatment. There was a similar lag on my *M. cavernosa* chlorinated epoxy firebreak treatments (Figure 11), therefore calculating monthly success was not possible and determining temporal success differences was challenging. Nevertheless, *Orbicella spp.* treatment success increased through time before the switch to antibiotic ointment, as evident

in the slope of the cumulative total treatments line versus the cumulative total failures (Figure 8). This was not evident in *M. cavernosa* where the treatment and failure lines are extremely similar except for a slight separation after the switch to antibiotic ointment (Figure 11).

Differences in success between *Orbicella* and *M. cavernosa* were likely due to physical and physiological differences. The margin failures were likely a result of the treatment not getting deep enough into the diseased tissue to completely smother the entire active disease lesions' margin and stop its progression. Both intervention materials, antibiotic ointment and chlorinated epoxy, adhered better to *Orbicella* than *M. cavernosa*. *Orbicella* has a comparatively smaller polyp size and thinner tissue than *M. cavernosa* which could account for the higher success of interventions on *Orbicella*. Morphological differences between species including polyp and corralite size can affect disease infection rates (Brown & Bythell, 2005; Ritchie, 2006).

Not only between species, but also individuals with species have genetic variations and adaptations for less optimal water conditions that coincide with increased disease prevalence such as polyp retraction, and lowered photosynthetic rates (Lirman & Manzello, 2009; Sofonia & Anthony, 2008). Buddemeier et al. (2004) introduced the concept of coral "ecospecies" to describe the idea that a single coral or species can be functionally different as a result of type of zooxanthellae it is associated with attributing to a clear adaptive significance. While most coral colonies are known to associate with a single zooxanthellae type, evidence has shown that there are coral species that can associate with several types of zooxanthellae simultaneously (Baker, 2003; Berkelmans & Van Oppen, 2006). Studies of coral colonies on the Great Barrier Reef, Australia have shown that while dominated by one type of zooxanthellae, a second type was also found present but in much lower quantities (Ulstrup & Van Oppen, 2003). There has been evidence to support individuals within the same species containing certain clades of zooxanthellae are more resistant to stress than those with other clades (Berkelmans & Van Oppen, 2006; Glynn, Maté, Baker, & Calderón, 2001; Rowan, 2004; Tchernov et al., 2004) Therefore, having a combination of zooxanthellae associated with a host may provide many ecological advantages in different niches to cope with stress (Baker, 2001; Berkelmans & Van Oppen, 2006), such as disease pathogens thus affecting our disease intervention success.

Coral mucus production could also affect treatment success. Coral mucus provides protection from UV, desiccation and increased sediment loading (Brown & Bythell, 2005) and is proposed to

enhance resistance by numerous mechanisms, including providing a physical barrier between the coral and the environment (Ritchie, 2006). Though little is known about the protective properties of mucus in disease resistance, it is understood that there is extensive variation in mucus composition and production both within and between species (Brown & Bythell, 2005; Ducklow & Mitchell, 1979; Meikle, Richards, & Yellowlees, 1988). Therefore, differences between species and within individuals' protective mucous layer could account for differences in topical treatment success. Alternatively, Aeby et al. (2019) speculated that there may be multiple pathogens involved in *M. cavernosa* lesions that contribute to differences in species mortality. Therefore, investigating the histology of tissue among our inventory of large corals would be a critical next step in providing valuable information about infection and intervention of large coral colonies.

The Stony Coral Tissue Loss Disease pathogen(s) remains unknown at the time of this publication. Several studies show distinct changes in the microbiome of disease lesions with the pathogen family *Vibrionacae,* which is well known from the coral bleaching pathogen *Vibrio coralliilyticus* (Sussman, Willis, Victor, & Bourne, 2008), likely playing a role in the pathology (Aeby, Greta Smith et al., 2020). In addition, other potential causes include ciliates, viruses, parasites, helminths, as well as cellular apoptosis have been linked with disease causation (Aeby et al., 2020; Sussman et al., 2008; Sweet & Bythell, 2012; Work & Aeby, 2011). Regardless of whether bacteria are the causative agent or a secondary infection taking advantage of the host's weakened immune system, antibiotics have been effective in stopping disease lesions (Aeby et al., 2020; Neely et al., 2020) Walker et al. *In prep*).

The antibiotic ointment treatments used a high concentration of antibiotic in the specialized CoreRx base 2B designed to release the antibiotic over a 72-hour period. An effective dosing of antibiotics to stop lesions in topical applications has not been determined, nor has the dosage release rate of CoreRx base 2B. The result of the topical application shows a cauterization of live tissue along the applied area, effectively killing everything underneath. This leaves questions remaining as to how the antibiotic ointment is specifically working. Is it a lethal dose of antibiotic that, when combined with the CoreRX base 2B, essentially kills everything it touches? Is the antibiotic working to increase the corals ability to fight the infection? Does the type of antibiotic matter? Would another topical cauterization material be as effective? Neely et al. (2020) have found that reducing the concentration of antibiotic decreases its effectiveness in stopping lesions

indicating that the high concentration of antibiotics in the base is factor in the treatment's effectiveness. More work needs to be done to understand the underlying mechanisms of the antibiotic ointment effectiveness. It is unknown what effect the release of antibiotics are having in the environment, so a topical application without antibiotics or other longer term environmental effects is highly preferred; however, as of this publication the antibiotic ointment is the most effective disease intervention material tested.

In addition to testing the efficacy of applications methods and materials, the high success of our disease interventions facilitated investigations into spatial and temporal patterns of new infection and identifying corals with different infection patterns. If not intervened, these corals would die or lose significant live tissue and not be available to investigate such aspects, making disease intervention a critical tool in the investigation of coral disease studies and an arrow in the quiver of reef managers to reduce disease prevalence and maintain coral cover.

New infections varied over time throughout this study indicating that the disease is still present in southeast Florida and environmental conditions may be affecting disease prevalence. In total, twenty-four corals required 132 new treatments during the summer and fall in 2019 (June through October 2019) compared to 52 treatments on 11 corals in the winter and spring of 2019 (January through May 2019) and 36 treatments on 13 corals in the winter and spring of 2020 (Dec 2019 through April 2020). This suggests a seasonal influence on new infections where the highest were found in the warmest, wettest time of year and new infections lessoned in the coolest, driest times. Haapkylä et al. (2011) reported seasonal increases in atramentous necrosis outbreaks in Great Barrier Reef where disease prevalence was negatively correlated with salinity, but positively correlated with high nutrient levels in the water column. Haapkylä et al. (2011) suggested that high rainfall and associated run off could be facilitating seasonal disease outbreaks. Other studies have shown coral diseases such as black band, aspergillosis, dark spots and white plague to have higher disease prevalence in warmer temperature months (Bruno et al., 2007; Haapkylä et al., 2011; Sato, Bourne, & Willis, 2009; Ward, Kim, & Harvell, 2007). In addition, Aeby et al. (2015) noted an increase of prevalence and rate of tissue loss in corals infected with black band disease in Hawaii during the warm water months. However, I did not find temporal patterns of groups of specific corals getting infected in certain seasons or years. The corals requiring treatments in one season were not the same set of corals the next season or the next year in the same season.

The disease interventions kept most of the corals alive providing data on their propensity for new lesions and their response to treatment. New infections were not consistent between corals (Figures 19 & 21) and there were no apparent spatial patterns to these differing infections (Figure 22) , which indicates that differences in individual corals may be affecting infection rates. This information can provide a baseline of expected SCTLD impacts on large colony populations.

During this study, which occurred after the initial wave of disease hit the region, 12.1% (7/58) of corals were unresponsive to disease intervention techniques (Figure 16 &17), 9.8% (5/58) of the colonies had high numbers of infections every month, 17.6% (9/58) required a few monthly infections, 21.6% (11/58) had low infections intermittently, 15.7% (8/58) only needed one treatment, and 35.3% (17/58) never become infected. Thus, without disease interventions, one might expect to have up to 65% of the colonies infected by SCTLD and lose a substantial amount of live tissue after six years of active disease in an endemic zone.

Corals have a suite of defense mechanisms to protect themselves from potential pathogens, including the production, release, and biochemical properties of mucus, mucus-associated bacterial communities, phagocytic cells that can engulf and destroy micro-organisms, and antimicrobial chemical defenses that vary among families, genera, and species and at the level of the individual colony (Aeby et al., 2019; Bourne et al., 2009; Gochfeld & Aeby, 2008; Mullen, Peters, & Harvell, 2004; Mydlarz, McGinty, & Harvell, 2010; Ritchie, 2006; Shore-Maggio, Runyon, Ushijima, Aeby, & Callahan, 2015). These differences might allow individuals to have an advantage over others in resisting or overcoming invasion by pathogens.

Along with the previously mentioned broad range of species-level traits that could potentially influence aspects of disease intervention success, other factors such as ecological and reproductive characteristics can also play a role in disease susceptibility and resistance between species. For example, ecological studies have reported higher prevalence of white syndrome coral disease in areas of greater coral cover suggests that coral species living at higher local abundances can be more susceptible to disease (Aeby, Greta S. & Santavy, 2006; Page & Willis, 2008; Willis, Page, & Dinsdale, 2004). Additionally, common fish corallivores like butterflyfish and wrasses can act as vectors and can actively transmit disease among relatively close coral colonies (Aeby & Santavy, 2006).

Species morphology and reproductive strategy is another important characteristic that is related to energy allocation to physiological processes such as growth and colony defense (Díaz & Madin, 2011). When compared to massive corals, branching corals invest more energy in growth and allocate less energy to maintenance and potentially disease resistance (Buss & Jackson, 1979; Palmer, Mydlarz, & Willis, 2008). Broadcast spawning species recover faster after bleaching events in the Indo-Pacific and in the Arabian Gulf (Glynn et al., 2008) and are potentially more resilient to certain stressors.

Although currently unknown, understanding the possible causes of the differing infection rates between individual corals is critical to future disease intervention work. As previously mentioned, individual colonies can have specific physiologies, zooxanthellae symbionts, and completely different genetic makeups all of which can individually, or in any combination greatly affect how the colony reacts to an invasive disease pathogen (Baker, Glynn, & Riegl, 2008; Hoegh-Guldberg et al., 2007). If we were to identify the relationships between these characteristics in the large coral population, we would likely be able to identify disease infection patterns along the reef. In order to test these different characteristics, there needs to be a diverse coral population to sample.

With the large coral population in this study, we were able to identify the frequency and severity of the diseased individuals within the populations (Figure 19). Sequentially, we were then able to identify and categorize groups of corals according to frequency and severity of infections trough time (Figure 21). With this information, samples can then be collected from corals from each of the categorizations to then compare and identify differences between genetics, tissues, pathogens, etc. that could be linked to disease dynamics within the large coral population.

With this project successfully treating and, in some cases, completely stopping death by disease progression, I have been able to effectively preserve the largest, oldest, most ecologically valuable and potentially most reproductive individuals for future studies which if left untreated, would likely have died over the last two years proving these colonies to be critical to the future of disease dynamics research.

5. Recommendations

Monitoring and intervention efforts have shown these colonies continue to get disease periodically, which if not treated will lead to colony mortality. Moving forward, the large corals inventory

should continue to be visited monthly to monitor their condition and, if disease outbreaks occur, be targeted for disease intervention efforts. The low success of the chlorinated epoxy treatments lead to the termination of this method, continuing, disease treatments will be treated solely with antibiotic ointment.

New infections could be due to environmental stressors (e.g. salinity, temperature, dissolved organic carbon). Observed infection rates may correspond to increases in certain water quality (WQ) metrics obtained by the WQ monitoring project, therefore it is important to investigate temporal infections of the large corals with temporal changes in water quality.

Lastly, the health and resilience of the large coral population could be essential to restoration efforts aiding in coral population recovery. Once the disease has passed and prevalence is low again, coral restoration efforts should be conducted to improve the probabilities of reproductive success and regain coral diversity and density in the system. I recommend collecting gametes from sites with multiple large corals, fertilizing them, and rearing them in a land-based nursery to save the genetic diversity of these resistant colonies. These corals should be grown out for several years and then out planted strategically to help regrow tissue on recently dead large colonies.

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Appendix A- Large Coral Appendix

Coral ID	Count of Assessment Date	Count of Monitoring Period	Tag #	% Total Mortality	Initial Infection	# Antibiotic Ointment Treatments	Sum of New Margin Treatment	Sum of Margin Retreatment	Sum of Margin Treatment Length (cm)	Sum of Amox Margin Treatment Failure	Sum of Epoxy Margin Treatment Failure	Sum of Margin Treatment Firebreaks Created	Sum of Margin Treatment Firebreak Retreatment	Sum of Margin Treatment Firebreak Treatment Length (cm)	Sum of # Margin Treatment Firebreaks Tested	Sum of Margin Treatment Firebreak Treatment Failure	Sum of Solo Firebreaks Created	Sum of Solo Firebreak Retreatment	Sum of Solo Firebreak Treatment Length (cm)	Sum of # Solo Firebreaks Tested	Sum of Solo Firebreak Treatment Failure
LC-001	5	5			9/23/2015		3		9.5		3	1		19		1	2		24.7		2
LC-002	20	20	1602	75		1	1		3.882												
LC-003	24	24	1703	20	6/25/2018		6		49.78		1	2		55.1	2						
LC-004B	10	10		90																	
LC-005	20	20	2105	50	9/14/2018	1	3		197.4								3		33.06		
LC-007	20	20	1797	45																	
LC-009	20	20	1839	70	9/11/2018	6	31	5	423.7	5											
LC-013	20	20	1813	34	9/11/2018	1	6		94.24								1		41.8	1	
LC-014		1				1															
LC-015	20	20	1815	85	9/11/2018	7	26		354.16		5						20	5	406.6	12	6
LC-016	22	22	1796	18	7/16/2018		4	1	36.1		1										
LC-018	25	25	1848	20	6/18/2018		2		24.7		1	7		324.9	9						
LC-023	9	9	1723	65																	
LC-024	20	20	1724	55																	
LC-028	19	19	1768	50																	
LC-034	17	17		90	3/1/2018		2		30.4		1	6	1	359.1	6	6					
LC-038	10	10		85	3/20/2018							6	1	285.76	14	12					
LC-040	8	8	2040	40																	

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Coral ID	Count of Assessment Date	Count of Monitoring Period	Tag #	% Total Mortality	Initial Infection	Number of Antibiotic Ointment Treatments	Sum of New Margin Treatment	Sum of Margin Retreatment	Sum of Margin Treatment Length (cm)	Sum of Amox Margin Treatment Failure	Sum of Epoxy Margin Treatment Failure	Sum of Margin Treatment Firebreaks Created	Sum of Margin Treatment Firebreak Retreatment	Sum of Margin Treatment Firebreak Treatment Length	Sum of # Margin Treatment Firebreaks Tested	Sum of Margin Treatment Firebreak Treatment Failure	Sum of Solo Firebreaks Created	Sum of Solo Firebreak Retreatment	Sum of Solo Firebreak Treatment Length (cm)	Sum of # Solo Firebreaks Tested	Sum of Solo Firebreak Treatment Failure
LC-045	11	11	1745	1																	
LC-046	2	2		85																	
LC-047	20	20	1747	7	9/14/2018	2	4		19												
LC-048	20	20	1778	5																	
LC-049	20	20	1749	10	6/18/2018		1		5.7												
LC-050	20	20	1750	10																	
LC-051	20	20	1751	2																	
LC-052	24	24	1752	9	6/25/2018	2	3	1	58.6	1		2		98.8	1						
LC-053	20	20	1753	13	11/15/2018		1	1	74.1		1	1		53.2							
LC-054	20	20	1754	30	12/3/2018	1	3		129.7												
LC-055	10	10	1655	20																	
LC-056	8	8	1656	20																	
LC-058	20	20	1858	10	12/3/2018		2		26.6												
LC-059	27	27	1659	55	3/16/2018	2	13	2	652.6	1	1	3		104.5	1						
LC-061	9	9	1651	30																	
LC-062	20	20	1922	65	9/10/2018		1		11.4												
LC-063	10	10	1653	50																	
LC-064	10	10	1879	70																	
LC-065	9	9	1565	10																	
LC-066	19	19	1816	11	12/4/2018		1		5.7												
LC-067	8	8	1567	40																	
LC-070	27	27	1570	82	5/7/2018	2	12	1	210.14		1										

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Coral ID	Count of Assessment Date	Count of Monitoring Period	Tag #	% Total Mortality	Initial Infection	Number of Antibiotic Ointment	Sum of New Margin Treatment	Sum of Margin Retreatment	Sum of Margin Treatment Length	Sum of Amox Margin Treatment	Sum of Epoxy Margin Treatment	Sum of Margin Treatment	Sum of Margin Treatment Firebreak	Sum of Margin Treatment Firebreak	Sum of # Margin Treatment	Sum of Margin Treatment Firebreak	Sum of Solo Firebreaks Created	Sum of Solo Firebreak	Sum of Solo Firebreak Treatment	Sum of # Solo Firebreaks Tested	Sum of Solo Firebreak Treatment
LC-074	10	10	1574	70																	
LC-075	20	20	1775	80		2	4	2	133	1	1										
LC-077	25	25	1777	61	11/1/2015	4	10	2	131.1	1	2	3	1	68.4	1	1	8		264.1	5	
LC-078	21	21	1778	10	9/12/2018		3		22.8												
LC-079	20	20	1699	50																	
LC-080	20	20	1660	20	9/10/2018		2		36.1			1		68.4							
LC-084	18	18	1684	75		2	2		19												
LC-085	24	24	2085	72	12/19/2017		8		172.9			2	1	123.5	3	1	1		49.4	1	
LC-087	18	18	1687	76	11/12/2015	2	5		68.4			2		53.2	1						
LC-088	10	10	1688	45																	
LC-090	20	20	1690	30																	
LC-092	10	10			3/1/2018																
LC-093	6	6			6/4/2018		6				4	11				5					
LC-098	20	20	1698	65	4/15/2019	5	5	9	294.5	5							1	5	609.6	2	6
LC-101	20	20	1801	45	9/10/2018		25		248.9		8										
LC-103	20	20	1803	65		4	12	3	191.9	3											
LC-110	20	20	1810	60	12/4/2018		11		77.9								2	5	203.3	7	3
LC-114	20	20	1814	31		1	1		7.6												
LC-115	19	19	2115	40																	
LC-116	29	29		78	3/21/2018		1		19		1	5	1	419.9	8		1	2	152	1	1
LC-117	20	20		35	7/16/2019		3	1	57		1										
LC-118	20	20	1918	55	9/11/2018	1	25		387.6								6		336.3	1	

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Coral ID	Count of Assessment Date	Count of Monitoring Period	Tag #	% Total Mortality	Initial Infection	Number of Antibiotic Ointment Treatments	Sum of New Margin Treatment	Sum of Margin Retreatment	Sum of Margin Treatment Length (cm)	Sum of Amox Margin Treatment Failure	Sum of Epoxy Margin Treatment Failure	Sum of Margin Treatment Firebreaks Created	Sum of Margin Treatment Firebreak Retreatment	Sum of Margin Treatment Firebreak Treatment Length (cm)	Sum of # Margin Treatment Firebreaks Tested	Sum of Margin Treatment Firebreak Treatment Failure	Sum of Solo Firebreaks Created	Sum of Solo Firebreak Retreatment	Sum of Solo Firebreak Treatment Length (cm)	Sum of # Solo Firebreaks Tested	Sum of Solo Firebreak Treatment Failure
LC-119	20	20	1919	81	10/25/2018		7		102.6								2		24.7		
LC-120	28	28	1920	86	4/19/2018	7	40	10	647.6	6	6	6	11	918.46	14	8	8	2	361	9	4
LC-121	17	17		85	5/10/2018							1		38	1	1	5	1	131.1	6	6
LC-121A	10	10		10	6/1/2018												1		22.8	1	
LC-121B	10	10		5	6/1/2018												1		19	1	
LC-122	27	27	1822	41	5/8/2018	1	6	1	114			2	1	159.6	1	3					
LC-123	28	28	1923	90	4/5/2018		4		117.8		4	23	2	1436.4	25	25	6		190	6	4
LC-124	25	25	1924	50	3/4/2018		2		91			1		26.6	1						
LC-125	19	19	1825	65																	
LC-126	20	20	1726	30																	
LC-127	20	20	2127	50																	
LC-128	20	20	2128	65																	
LC-129	24	24	2129	65	6/4/2018							1		45.6							
LC-157	10	10	1557	55	6/4/2018	6	22	4	315.4	4											
MC-001	10	10	2101	10					0												
MC-002	10	10	1552	26	11/12/2019	1	1		7.6												
MC-003	10	10	1893	2																	
MC-004	9	9	2404	10																	
MC-005	9	9	2405	45																	
MC-006	10	10	1806	15																	
MC-007	9	9	1868	30																	
MC-008	9	9	1869	50																	

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Coral ID	Count of Assessment Date	Count of Monitoring Period	Tag #	% Total Mortality	Initial Infection	Number of Antibiotic Ointment Treatments	Sum of New Margin Treatment	Sum of Margin Retreatment	Sum of Margin Treatment Length (cm)	Sum of Amox Margin Treatment Failure	Sum of Epoxy Margin Treatment Failure	Sum of Margin Treatment Firebreaks Created	Sum of Margin Treatment Firebreak Retreatment	Sum of Margin Treatment Firebreak Treatment Length	Sum of # Margin Treatment Firebreaks Tested	Sum of Margin Treatment Firebreak Treatment Failure	Sum of Solo Firebreaks Created	Sum of Solo Firebreak Retreatment	Sum of Solo Firebreak Treatment Length (cm)	Sum of # Solo Firebreaks Tested	Sum of Solo Firebreak Treatment Failure
MC-009	10	10	1870	25	7/22/2019		1		22.8												
MC-010	9	9	1700	60	10/17/2019	1	1		76												
MC-011	9	9	1880	35																	
MC-013	9	9	1878	30																	
MC-014	9	9	1895	30																	
MC-015	10	10	1645	10																	
MC-016	10	10	1646	5																	
MC-017	10	10	1917	50																	
MC-018	10	10	1648	45																	
MC-019	10	10	1819	50																	
MC-020	9	9	2020	10																	
MC-021	9	9	2421	11	2/14/2020	1	1		7.6												
MC-022	9	9	1722	15	10/19/2019	1	1		38												