SE FL ECA Reef-building-coral Disease Intervention and Preparation for Restoration: Final Report

Florida Department of Environmental Protection
Office of Resilience and Coastal Protection

SE FL Reef-building-coral Response to Amoxicillin Intervention and Broader-scale Coral Disease Intervention

Final Report

Prepared By:

Brian K. Walker, Hunter Noren, Alysha Brunelle, and Samantha Buckley

Nova Southeastern University
Halmos College of Natural Science and Oceanography
8000 N. Ocean Drive
Dania Beach, FL 33004-3078

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List of Acronyms

FAU	Florida Atlantic University Harbor Branch Oceanographic Institute
DEP	Florida Department of Environmental Protection
FWC	Florida Fish and Wildlife Conservation Commission
NSU	Nova Southeastern University
SE FL ECA	Southeast Florida Coral Reef Ecosystem Conservation Area
FCR	Florida's Coral Reef

Acknowledgements

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Executive Summary

SCTLD was first discovered in the SE FL Coral ECA in 2014 and remains present in the region. It has had a devastating impact on the coral communities. This report describes the majority of the SCTLD disease intervention activities that have occurred north of Biscayne National Park and their outcomes thus far amidst the Coronavirus pandemic complications.

Broad-scale intervention strike teams treated 700 colonies totaling 341.6 m of treatments. The amount and species of corals treated in broad scale recon surveys indicates that there are still some rare survivors of the highly susceptible brain coral species in the area and they are still succumbing to the disease.

Topical interventions on coral disease lesions are highly effective in stopping the progression of disease lesions and saving large amounts of live tissue. Success varied between species, technique, and materials. Antibiotic paste was the most effective treatment for all species when applied both to the disease margin and to a disease-break (a trench separating diseased from healthy tissue). Margin treatments alone were 75-85% successful. The addition of a disease-break as a backup to margin treatment failure increased to 92%.

The majority of failed margins treatments occurred within the first nine days, indicating the optimum revisiting time is 10 - 14 days posttreatment. However, with a >80% success rate, one month is a practical re-visitation time for retreating failures and new lesions. The addition of a coral-break is recommended if re-visitation is not planned.

Amoxicillin treatment success indicates that the bacteria infecting the SE FL corals are not currently resistant to antibiotics from the outfalls and other sources. There were no measurable or observed impacts of the antibiotic treatments on the treated corals or surrounding organisms other than stopping the disease lesion progression. There were no apparent benefits provided to the individual coral colony.

Successful disease interventions have kept corals alive providing a unique opportunity to examine infection patterns with environmental correlates and categorize coral with differing infection patterns. New infections varied over time with total infections higher during June – October; the warmest, wettest time of year. New infections were not consistent between corals. Some were highly infected and unresponsive to treatments. Some exhibited high numbers of infections every month. Some exhibited low infections intermittently. Some only needed one treatment. And some were never infected.

Coral disease intervention saves corals, but reef-scape scale interventions are costly and time consuming. Interventions are the only effective stopgap tool while the larger causative agents are identified and remediated. Ideally, a treatment can be developed that bestows long-term comprehensive colony immunity with a single easy-to-administer application with no adverse environmental impacts. Monitoring priority colonies has saved many from extinction. A no-action alternative will lose large amounts of live tissue, promoting bioerosion, and let some of the oldest SE FL residents die.

1. BACKGROUND

Considering the ongoing global COVID-19 pandemic, the extreme effect of a pathogen without a vaccine is more apparent than ever. While the global population is reeling from the COVID-19 virus, the reefs of South Florida continue battling their own pandemic. First emerging on Miami-Dade reefs in 2014, the novel disease, Stony Coral Tissue Loss Disease (SCTLD), has rapidly spread across Florida's Coral Reef (FCR) and onward through parts of the western Caribbean (Precht et al. 2016; Alvarez-Filip et al. 2019). This disease affects up to 22 of the 45 species of scleractinian corals found on FCR (Meyer et al. 2019) including several important reef-building species and several classified as endangered on the International Union for Conservation of Nature (IUCN)'s Red List. This ongoing disease outbreak has resulted in widespread regional declines in both colony density and live tissue cover (Walton et al. 2018).

Evidence of change in community structure and reef health along Florida's Coral Reef has been present since the early 1970's (Baker et al., 2008). This reef system is heavily impacted by anthropogenic activities (Alvarez-Filip et al., 2009; Carpenter et al., 2008; D'Antonio et al., 2016), which has experienced significant losses in stony coral cover and species abundance (Porter & Meier, 1992; Porter et al., 2001; Wheaton et al., 2001). Since the 1970's, reports of disease outbreaks along Florida's Coral Reef described tissue loss patterns that were later termed white plague; now known as one of the most virulent of coral diseases (Aeby et al., 2019; Aronson & Precht, 2001; Dustan & Halas, 1987; Richardson et al., 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; Kline and Vollmer, 2008).

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 et al., 2009; Sokolow, 2009). Coral diseases arise from biotic (e.g., bacteria) or abiotic (e.g., virus, radiation, toxicant) sources, or a combination of the two (Bourne et al. 2009; Harvell et al. 2002; Peters, 2015). Corals as well as disease pathogens are sensitive to ocean temperature change (Brandt & McManus, 2009; Bruno & Selig, 2007; Rosenberg et al., 2007). Warming temperatures impair the defense mechanisms of the corals while concomitantly increasing growth of disease pathogens (Boyett et al., 2007). Water quality parameters and environmental factors can also stress the coral allowing pathogens to thrive (Raymundo et al., 2009). Increased water temperatures can lead to higher pathogen growth rates (Muller et al., 2018; Remily and Richardson, 2006) and increased virulence (Harvell et al., 2002; Kushmaro et al., 1998; Muller et al., 2018; Remily & Richardson, 2006; Toren et al., 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 et al., 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). White pox affecting Acropora palmata, for example, is caused by the bacterial pathogen Serratia marcescens (Patterson et al. 2002), while black band disease (BBD) affecting multiple scleractinian species is caused by a bacterial consortium (Cooney et al. 2002). Caribbean ciliate disease and brown band disease are caused by sessile and motile ciliates respectively (Randall et al. 2015). Aspergillosis, a common disease affecting Gorgonia ventalina is caused by the aspergillus fungus (Troeger et al. 2014). However, no putative pathogens have been identified for most coral diseases due to the diverse and transient populations of potential pathogens associated with corals and their microbiome and the difficulties of fulfilling Koch's postulates in (Pollock et al. 2011).

Historically, the Caribbean has been considered a disease "hotspot" with 66% of recorded disease events despite hosting only 8% of the world's coral reefs (Green and Bruckner 2000). However, coral disease in the Caribbean was considered seasonal, with higher prevalence during the summer months, and only impacting a limited number of species (Muller and van Woesik 2014). The SCTLD outbreak defies that supposition. It has been ongoing, occurring year-round, affecting half of the reef-building corals, and leading to significant declines in overall coral cover and colony density on the FCR (Precht et al. 2016; Walton et al. 2018). Southeast Florida total coral density has declined around 30% over a four-year period (Walton et al. 2018), with some sites decreasing to 57.2% of historic values (Walker 2018) and several species were quickly reduced to < 3% of their initial populations (Precht et al. 2016). The outbreak has been active for six years in Southeast Florida and is ongoing as of the date of this report.

SCTLD is highly virulent, able to spread both through direct contact and through the water column (Aeby et al. 2019). The Florida Keys National Marine Sanctuary (FKNMS), with the help of many experts, established a standard case definition that describes SCTLD as "Focal, multifocal, locally extensive to diffuse areas of acute to subacute tissue loss distributed basally, peripherally, or both. In some cases, tissues bordering areas of chronic tissue loss have indistinct bands (1–5 cm) of pallor progressing to normal pigmentation away from denuded skeleton." Disease lesions with bare skeleton extending >5cm from the margin are considered acute while sub-acute displays less than 5cm of white skeleton (Aeby et al. 2019). Disease presentation and lethality can vary greatly between and among species and regions (Aeby et al. 2019; Voss et al. 2019). 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 FCR within the Coral ECA (Gilliam et al., 2018). *Siderastrea siderea* and other brain corals are the next most susceptible species.

The pathogen(s) responsible for SCTLD are currently unknown, but the effective use of antibiotics halting the disease on multiple species implicates bacteria as a component of SCTLD lesions. O'Neill et al. (2018) found disease progression was halted on *Dendrogyra cylindrus* fragments solely treated with amoxycillin and a combination of

amoxicillin and kanamycin have halted disease progression on *Montastrea cavernosa* and *Meandrina meandrites* in aquaria (Aeby et al. 2019). Rosales et al. 2020 found elevated levels of *Rhodobacteriales* and *Rhizobiales* associated with SCTLD lesions; however, more work is needed to confirm their role in causing the disease.

In 2018, SCTLD intervention efforts began in southeast Florida to save individual corals and reduce the pathogen load in the environment. Initial interventions prioritized the largest corals. 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 and Bak, 1994). *Orbicella spp.* fertility and fecundity increase linearly with colony size (Sakai, 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. 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, therefore, we prioritized the largest, healthiest-looking corals for disease interventions with the expectation of adapting to new methodologies to improve intervention success in the SE FL Coral ECA so that the remaining coral population can be saved.

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 treatments had moderate success on *Orbicella spp*. (77%) but not for *Montastraea cavernosa* (40%) (Walker and Brunelle 2019). Concomitantly, an antibiotic paste was being developed by Ocean Alchemists for experimental use to increase intervention success. Based on the success of intervention trial using antibiotics (Neely et al. 2020; Walker and Pitts 2019), in August 2019, all margins were treated with the Ocean Alchemists antibiotic ointment CoreRx B2B with amoxicillin (1:8 ratio by weight). Antibacterial resistant genes have been found on southeast Florida reefs (Griffin et al 2020), putting into question that an antibiotic treatment may not be as effective as in the Florida Keys. Therefore, we tested the efficacy of the antibiotic paste and chlorinated

epoxy treatments on *M. cavernosa* to stop disease progression, prevent future infections, and the corals' ability to heal/regrow after treatment.

This report summarizes the results from our continued SE FL ECA coral disease interventions through April 2020, including the monitoring and continued treatment of the priority large corals, broadscale strike team reconnaissance and disease interventions, field activities for initial probiotics testing, and the identification of unique coral disease survivor sites.

2. PROJECT DESCRIPTION

One goal of this project was to perform disease interventions on the remaining reef-building coral species with active disease in the SE FL ECA. These activities are essential to saving the remaining corals in SE FL affected by disease that have the potential of recovering and building new reef structure. Coral disease intervention treatments included smothering diseased tissue with chlorinated epoxy and/or amoxicillin and, when necessary, creating a "fire break" to arrest disease progression and covering the newly exposed skeleton with the same treatment. This technique is hereafter referred to as a disease-break. The first objective for this goal was to conduct broader-scale strike team reconnaissance and disease intervention efforts in partnership with FAU, DEP, Broward County, and Miami-Dade County to help save diseased colonies throughout the SE FL ECA. The second objective was to apply these interventions to 90 priority large corals as necessary on a monthly basis to maintain their health. A third objective was to further field test new permitted intervention techniques and materials including whole colony treatments as they are conceived, developed, and permitted.

Another goal of this project was to collect information to inform and aid in planning future SE FL ECA restoration efforts. In order to conduct restoration in the future, it's important to identify and locate survivor colonies to be used for sexual reproduction, genetic analyses, and experimentation on stress hardening and disease resistance. Therefore, we aim to identify sites with dense populations of survivor colonies, large colonies, and locations of survivors of particularly impacted species to make restoration activities more efficient.

The findings of this project are being incorporated into the on-going coral disease response effort which seeks to improve understanding about the scale and severity of the Florida's Coral Reef coral disease outbreak, identify primary and secondary causes, identify management actions to remediate disease impacts, restore affected resources and, ultimately, prevent future outbreaks. As such, collaboration amongst partners and the Disease Advisory Committee (DAC) will ensure alignment of needs and avoid duplication.

3. METHODOLOGY

The antibiotic ointment and chlorinated epoxy treatments were conducted under the State of Florida Special Activity License Permit SAL-19-2022A-SRP which authorized the cutting of disease-breaks and the application of disease treatments containing amoxicillin

and chlorine. The probiotics work was permitted under the State of Florida Special Activity License Permit SAL-19-2201-SRP.

3.1. **Broad-scale Coral Disease Intervention Strike Team (Task 2)**

Southeast Florida coral disease intervention strike teams, consisting of personnel from NSU, Broward County, and Miami-Dade County, conducted disease intervention at various sites throughout both counties. Intervention sites were chosen based on previous information on the locations of diseased corals and high priority county sites. The NSU efforts discussed in this report targeted locations between Hillsboro Inlet and Biscayne National Park while avoiding known existing monitoring stations and experimental sites. At each location, divers towed a GPS buoy synced to a dive computer. Once a diseased coral was located, the time was taken from the dive computer to link to a point on the GPS track. Each coral was tagged and measured and treated. Detailed photographs were taken of the coral before and after treatment, as well as all treatments. At the end of the day the GPS coordinates were loaded into ArcGIS and the locations that corresponded to each time recorded during treatment were copied into a GIS file.

All strike team activities were supplied to FWC for inclusion in the Coral Disease Intervention Dashboard:
https://novasoutheastern.maps.arcgis.com/apps/opsdashboard/index.html#/55a759f02f3c486eb1d29a95f80fba0a

3.2. **Apply Interventions to 90 Large Corals (Task 3)**

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. 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 compilation of photos and data of the priority corals.

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 (2014), 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.

Starting in September 2019, all margins and disease-breaks (if necessary) were treated with the Ocean Alchemists antibiotic ointment CoreRx B2B with amoxicillin (1:8 ratio by weight). The length of each treatment was estimated using a standardized scale in the photographs.

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 Disease-break 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 disease-breaks were filled with treatment material. Disease-breaks ranged in length, width, and depth depending on coral morphology and hardness. A typical disease-break 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 Disease-break Treatment – the active disease margin and drilled disease-break was is covered/filled with chlorinated epoxy to isolate the disease.

Solo Margin Treatment – a treatment where the chlorinated epoxy or CoreRx B2B with amoxicillin was applied to the disease margin only.

Solo Disease-break Treatment – a trench was created about 5 cm from the disease margin and filled with chlorinated epoxy or CoreRx B2B with amoxicillin to isolate the disease. The active disease margin was is left untreated.

Most initial treatments were margin and disease-break. Solo margin and disease-breaks 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 disease-break. Solo disease-breaks were used when the disease was progressing rapidly, and the margin was too large to treat effectively and upon retreatments of previous disease-break failures (Figure 1).

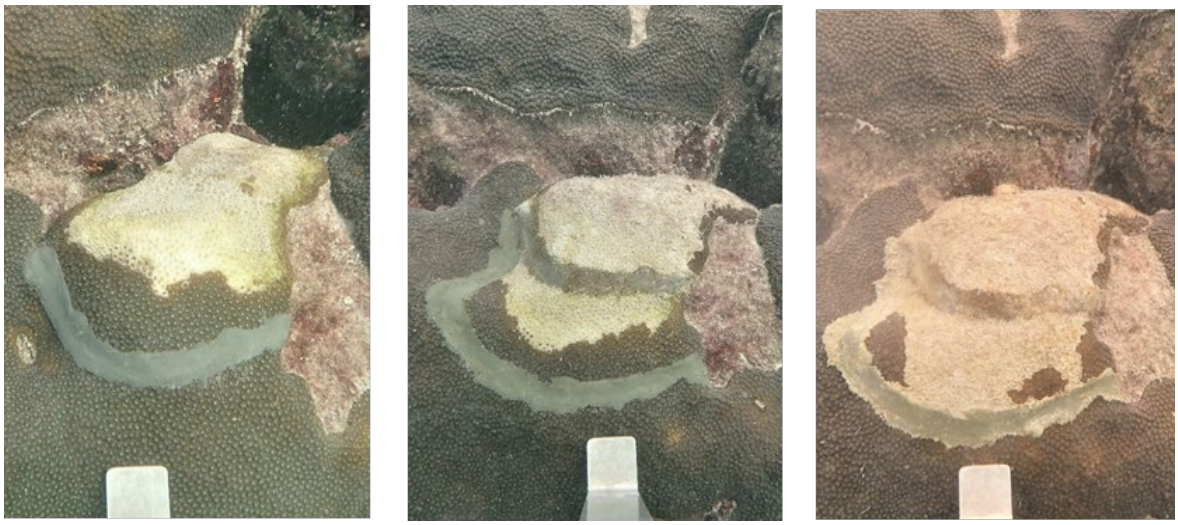


Figure 1. Example of disease progression and treatment results of chlorinated epoxy on *Orbicella spp.,* LC-110. Initial solo disease-break treatment (left) in January 2019. Failure of solo disease-break treatment with subsequent retreatment in February 2019 (middle). Success of solo disease-break retreatment in March 2019 (left).

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 and Pitts, 2019), no disease-breaks 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 disease-break treatment failed if the active disease margin progressed across the chlorinated epoxy filled disease-break to the other side. The margin and disease-break treatment failed if the active disease margin progressed past the disease-break. If the active disease progresses past the margin treatment portion of the combined treatment but not passed the disease-break, 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.
All treated colonies were tagged with a yellow tag with a unique number and instructions to photograph the coral and submit the photo to www.SEAFAN.net/tags (Figure 2).

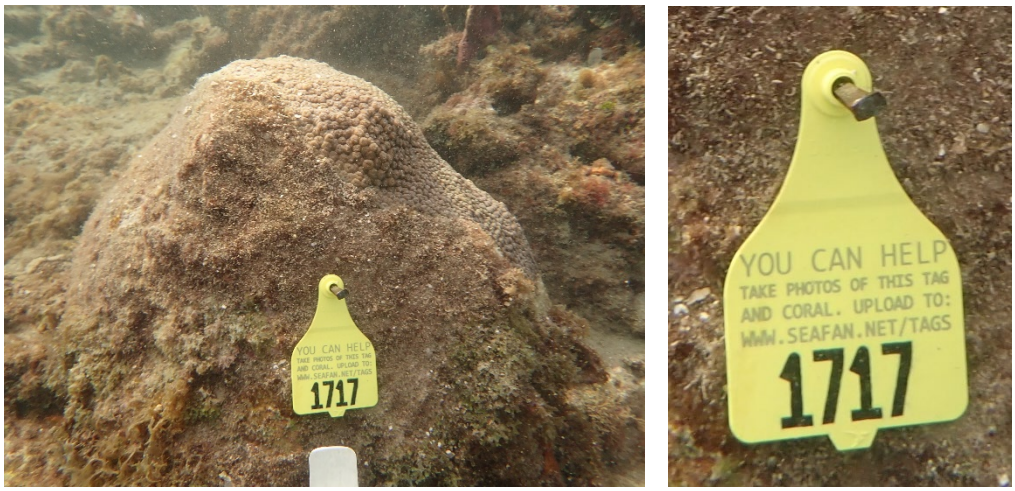


Figure 2. Example of coral tag placed on or next to each treated coral.

3.3. **Recon Sites (Task 4)**

Reconnaissance throughout Broward and Miami-Dade was conducted to identify corals and/or sites that respond better to treatments or that have resisted infection to-date and to identify unique coral disease survivor sites to make future restoration activities more efficient. Recon was guided by a desktop analysis of previous datasets that identify historic sites of high coral density and/or richness. Recon is also performed during strike team activities and other opportunistic times. Divers visit identified sites and haphazardly search the area towing a GPS buoy to find locations of visually noticeable high coral density or richness and if disease is present. If disease is found, these locations are treated and mapped. Large colonies of any species and smaller colonies of the species hardest by SCTLD (e.g. *M. meadrites*, *E. fastigiata*, *D. stoksii*, *C. natans*, *D. cylindrus, D. labyrinthiformis*) are mapped.

3.4. **Field test new permitted intervention techniques and materials (Task 5)**

3.4.1. Antibiotic paste versus Chlorinated Epoxy on Montastrea cavernosa

All experimental treatment sites are located in northern Miami-Dade County (Figure 3). Study Sites were established on April 17, 2019 near Golden Beach (GB1), April 29, 2019, near Surfside (SS1), and May 7, 2019, near Surfside (SS2). All visibly infected *M. cavernosa* colonies were tagged, measured, photographed, and mapped from a central GPS location by distance and heading. A floating GPS was used to obtain positions for all treated colonies.

Treatments were determined for each site by evaluating colony location, size, number of lesions, and the percentage of diseased versus healthy tissue to keep the treatments relatively equivalent between treatment types and avoid any bias in the analyses from these factors. Initially, 18 corals were treated with chlorinated epoxy and 22 corals with antibiotic paste. On May 6, 2019 at GB1, 23 lesions were treated on 10 colonies with

chlorinated epoxy and 36 lesions on 14 colonies with antibiotic paste. At SS1, eight lesions were treated on five colonies with chlorinated epoxy and five lesions on four colonies with antibiotic paste. On May 8, 2019 at SS2, three lesions were treated on three colonies with chlorinated epoxy and 11 lesions on four colonies with antibiotic paste. No colonies had mixed treatments at the onset of the study. Upon subsequent visits, newly diseased corals and treatments were added as they were found.

Two materials were used in this comparison. We used an antibiotic paste delivered via a silicone-based 36hr extended-release paste as one treatment and chlorinated epoxy as the other. The antibiotic paste was mixture of amoxycillin trihydrate from PhytoTechnology Laboratories and, a silicone-based paste labeled Base 2b (originally from CoreRX Pharma, now Ocean Alchemists). The Base 2b and amoxycillin were mixed by a weight ratio of 8:1 (Base 2b: amoxicillin). The resulting antibiotic paste was thoroughly mixed then spread in an approximate two-centimeter-thick layer for at least 15 minutes to allow any of the ethanol-based preservative in the Base 2B to evaporate. The antibiotic paste was then transferred to 60ml catheter tip syringes with the tips cut to facilitate application. These syringes were then kept on ice until application. The chlorinated epoxy treatment was ZSPAR A-788 Splash Zone™ two-part marine epoxy where Part A was premixed with the chlorine powder (Poolife™ Turboshock©) at a ratio of 15g of chlorine to approximately 50 mL of Part A epoxy. Equal epoxy parts (Part A with CL and Part B) were kept separate and then mixed underwater before application.

Treatment application consisted of methods outlined in Aeby et al (2015). The disease margin was smothered with treatment material covering all visibly infected polyps. Then a disease-break about 5 cm from the visibly diseased margin was created using a Nemo underwater angle grinder (AG-22-5Li-50) with a Diablo 4 ½ inch masonry grinding disk. This disease-break was then filled with treatment material.

Corals were initially treated on May 6 and 8, 2019 and revisited weekly until June 5 (May 15, May 21, May 29, June 5), after which they were revisited on June 19, June 27, July 10, July 30, August 13, and October 10, 2019 and January 31 and April 21, 2020. During treatment and monitoring, high resolution photographs and videos were taken to record the total coral condition, each treatment, and new infections. These consisted of whole-colony nadir photographs and individual treatments taken perpendicularly to the colony center at a fixed distance away with a standard measuring scale in the image. We used these photographs to visually assess colony health, disease progression, and treatment effectiveness. Healing was assessed during the final two monitoring visits (January 31 and April 21, 2020) by measuring the length of fully healed tissue along the disease-breaks. In order to be considered fully healed, tissue had to be connected over the disease-break.

After the initial ineffectiveness of chlorinated epoxy was evident, the antibiotic paste was applied to the disease margin without additional disease-breaks on all corals during monitoring.

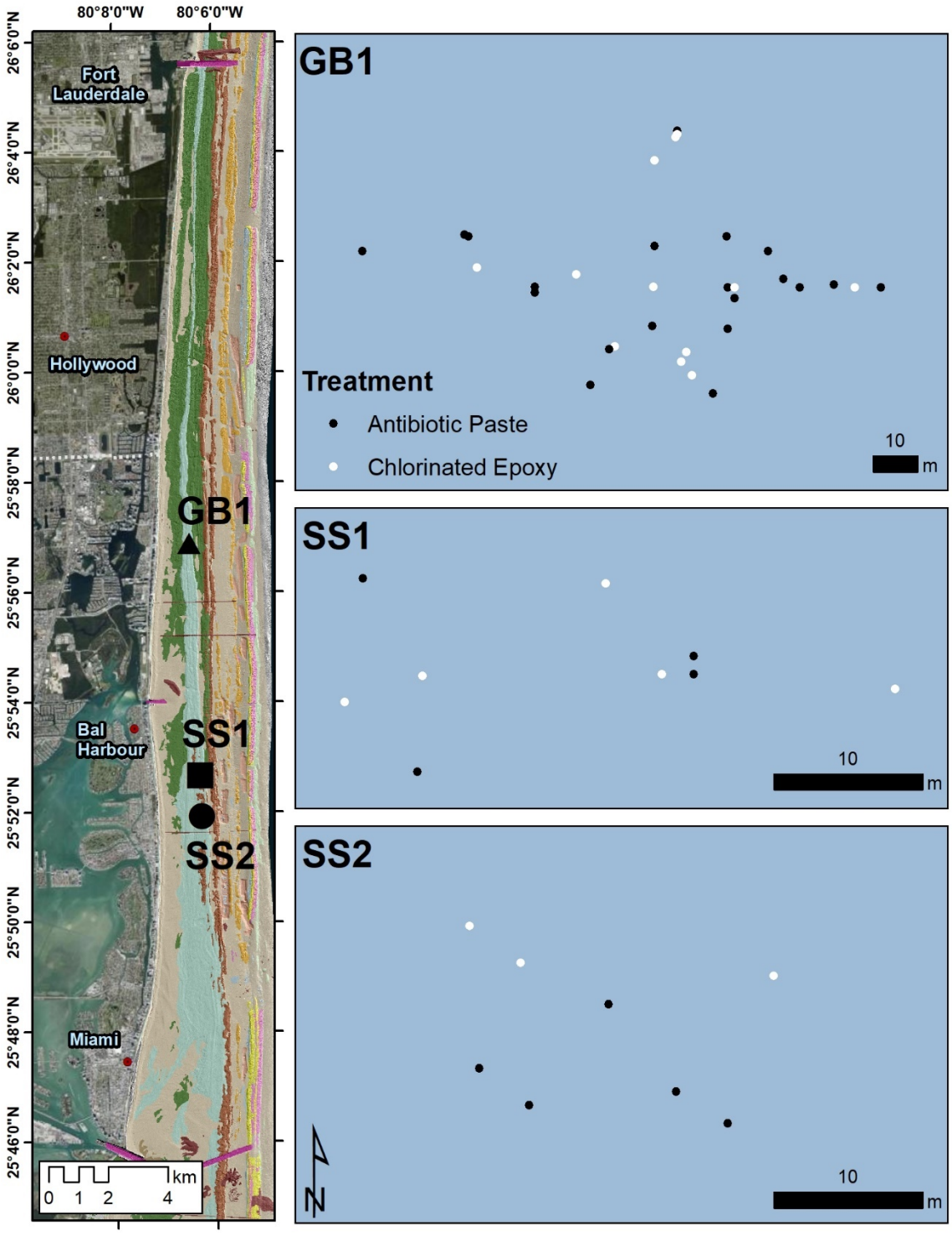


Figure 3. Map of the *M. cavernosa* corals at the three northern Miami-Dade County treatment sites. Black dots and gray dots represent corals treated with antibiotic paste or chlorinated epoxy respectively.

3.4.2. Probiotics on Montastrea cavernosa

The Smithsonian Marine Station at Fort Pierce is developing several probiotics and several treatment methods to be tested on *M. cavernosa*. We set up two field experiment sites for testing and monitoring the corals' responses.

An experimental site, Broward Site 1 (BS1), was set up on September 26, 2019 (Figure 4). Nineteen corals were tagged and photographed that looked diseased. The site was revisited fourteen days later (10/10/19) and photos were retaken. On this date, six additional diseased corals were tagged to incorporate into the experimental site. Photographs were compared to assess the rate of disease progression and estimate the length of disease margin. Six of the originally tagged corals were excluded due to insufficient disease characteristics (bleaching or burying associated mortality rather than disease). The remaining nineteen corals were randomly selected and treated with probiotics or a control procedure without the probiotics.

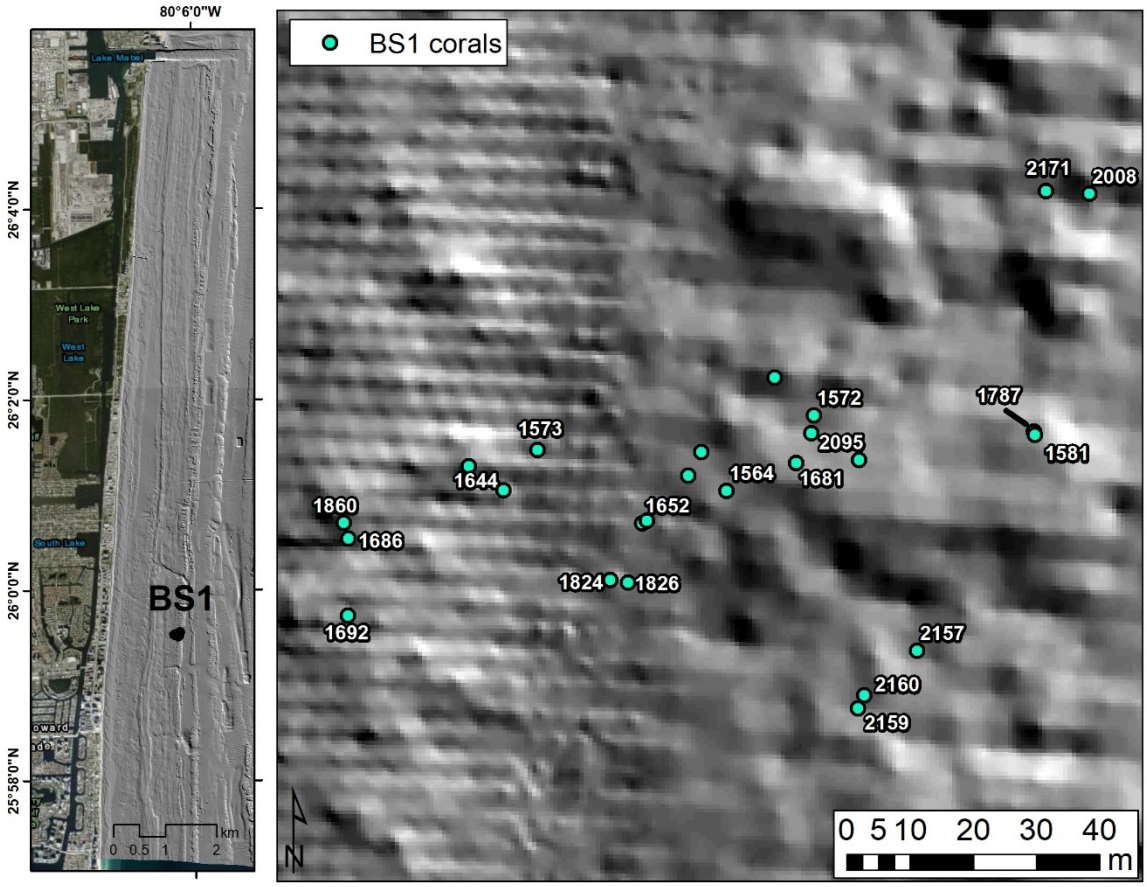


Figure 4. Map of probiotics site BS1 corals established September 26, 2019.

A second experimental site location (BS2) was identified on March 16, 2020 and thirteen diseased *Montastrea cavernosa* were tagged, photographed and mapped (Figure 5). The site was revisited on March 23, 2020 and an additional eight diseased colonies were added to the site. BS2 treatments were delayed due to the coronavirus pandemic. This site was revisited August 19, 2020 to evaluate disease progression and then treated with whole-colony probiotic treatments soon after.

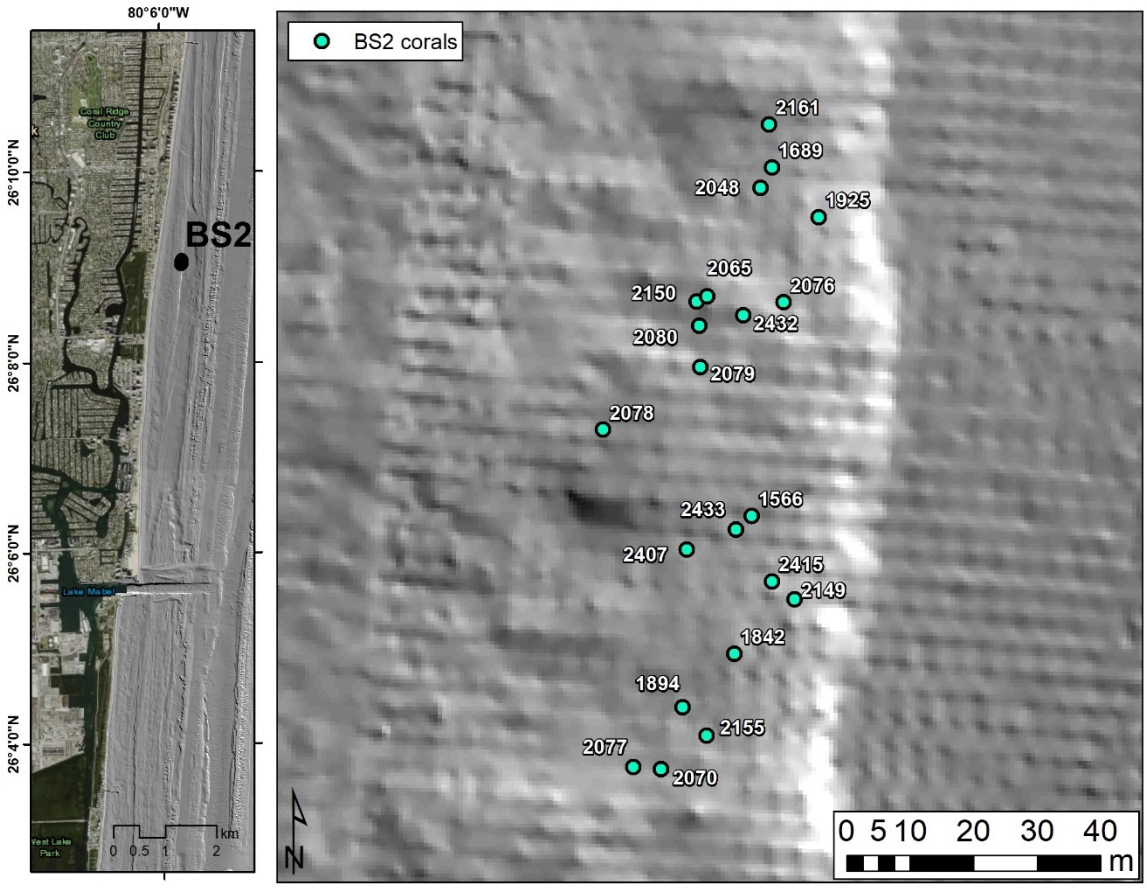


Figure 5. Map of probiotics site BS2 corals established March 23, 2020.

4. RESULTS

4.1. Broad-scale Coral Disease Intervention Strike Team (Task 2)

As of August 12, 2020, a total of 700 colonies were treated by the coral disease interventions strike teams in the SE FL ECA (including the treatment comparison sites and probiotic site, but not large corals) (Figure 6). The total number of treatments by species were 624 *M. cavernosa*, 44 *O. faveolata*, 8 *Colpophyllia natans*, 10 *Pseudodiploria strigosa*, 9 *Pseudodiploria clivosa*, 4 *Solenastrea bournoni* and 1 *Siderastrea siderea* (Figure 7).

Out of the 700 colonies, 529 were treated with antibiotic ointment (463 *M. cavernosa*, 39 *O. faveolata*, 6 *C. natans,* 8 *P. strigosa*, 8 *P. clivosa,* 4 *Solenastrea bournoni* and 1 *S. siderea*); 109 corals were treated with chlorinated epoxy (102 *M. cavernosa*, 4 *O. faveolata*, 2 *P. strigosa*, and 1 *C. natans*); and 16 treated with CoreRx B2B without antibiotics (that were not successful) (Figure 8).

A total of 266.61 meters (26,661 cm) of antibiotic ointment treatments, 68.59 meters (6,859 cm) chlorinated epoxy treatments, and 6.4 meters (640 cm) of CoreRx Base treatments were performed totaling 341.60 meters (34,160 cm). The average treatment length per coral was 58.49 cm which varied by species: *M. cavernosa* = 58.03 cm, *O. faveolata* = 68.14 cm, *P. strigosa* = 46.00, *P. clivosa* = 53.75, *C. natans* = 51.86, *S. bournoni* = 51.67, and *S. siderea* = 100.00cm.

The average treated colony length was 63.4 cm and height was 34.6 cm. The maximum colony length was 200 cm and height was 140 cm both of which were *Orbicella* colonies. GPS locations were obtained for all the treated corals however, as of August 12th, 2020, only 2 previous corals have been opportunistically retreated during strike team operations and no treatment success rates have been calculated. Coral tags have instructions for the general public to photograph and upload photos to a website. If this happens, we will be able to compare our previous photos and measure treatment success. These tags get covered with biota in a couple of months and the instructions are no longer visible without scraping them with a knife.

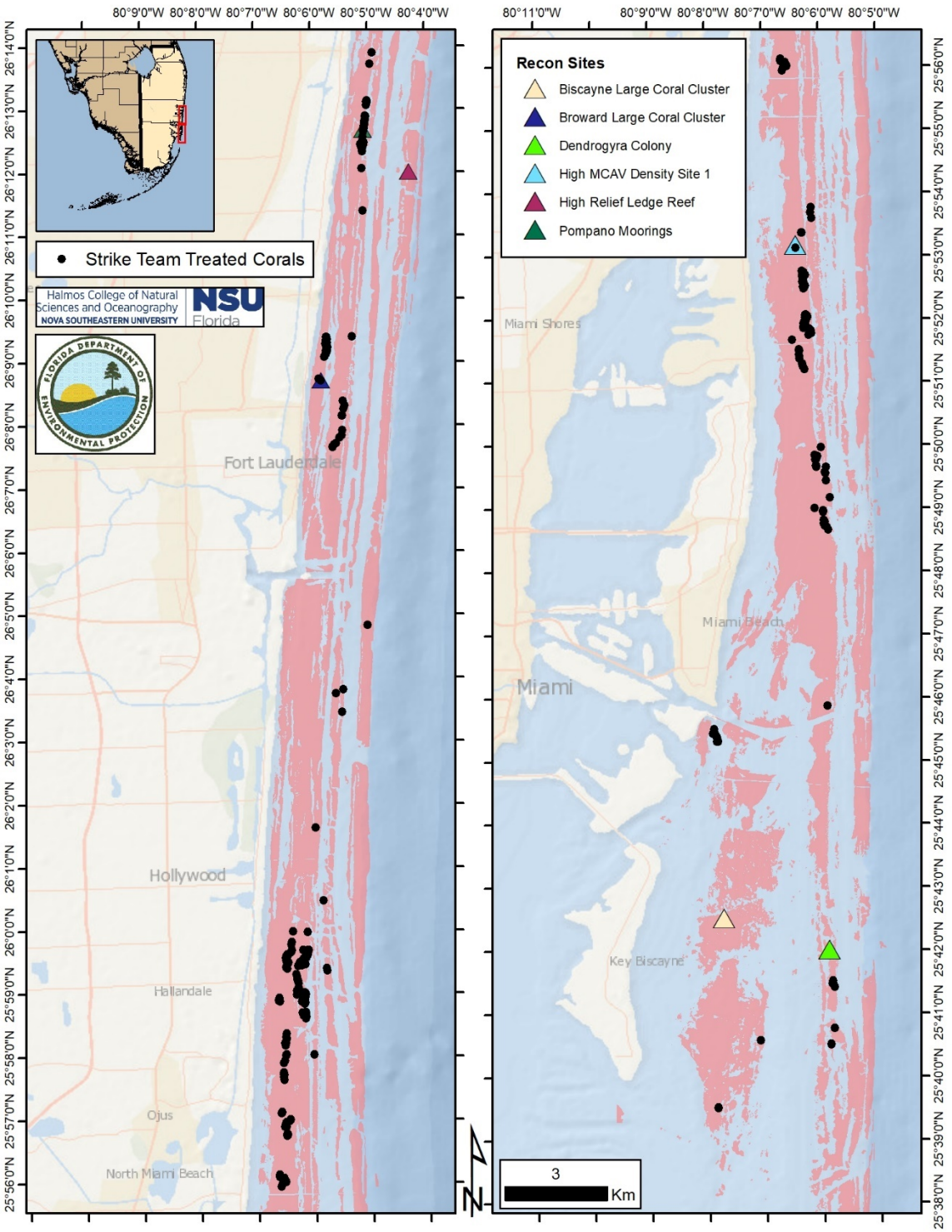


Figure 6. The location of the 700 strike team treated corals and six recon sites for restoration as of August 12th, 2020.

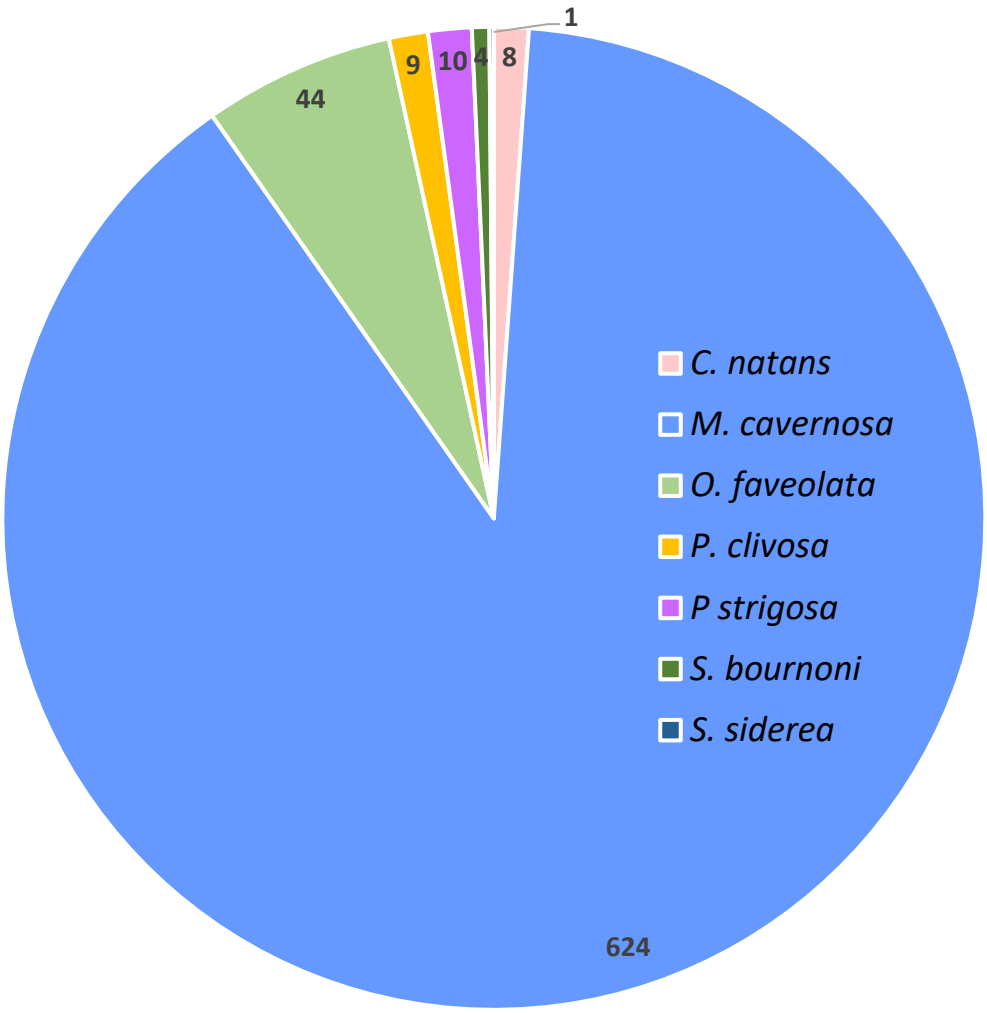


Figure 7. The number of colonies by species treated by the coral disease interventions strike teams throughout southern Broward and northern Miami-Dade by species as of August 12th, 2020.

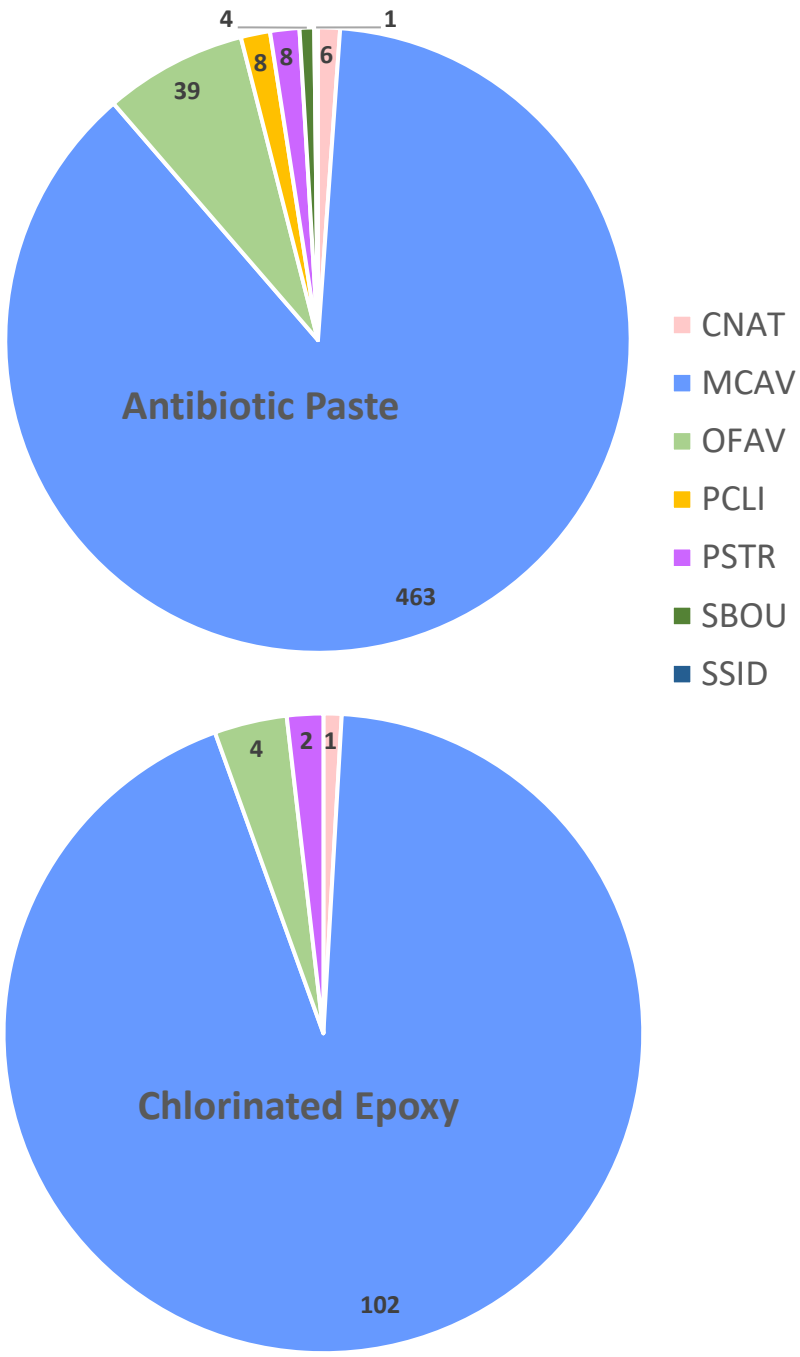


Figure 8. The number of colonies by species treated with antibiotic ointment (top) and chlorinated epoxy (bottom) by the coral disease interventions strike teams throughout southern Broward and northern Miami-Dade by species as of August 12th, 2020.

4.2. **Apply Interventions to 90 Large Corals (Task 3)**

It was a challenge finding the thirty additional priority corals. First, we revisited the remaining corals on the original priority list from 2018 that didn't make the original cut for priority monitoring. 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 1 < 2 meter diameter corals that were originally excluded due to their smaller size, but still had a substantial amount of live tissue remaining. We also included several corals found during strike team dives. Thus, we surveyed 80 corals in July, 83 in August, 87 in September, and 90 in October (Figure 9). See Appendix A for a table of the priority corals and a summary of their treatments from April 2018 – April 20, 2020.

a. Treatment Success

Not all monitored corals required treatments (Figure 8). 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 10 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.

Success varied drastically by species and treatment method (Figure 11). Disease progression was halted on 83% of the *Orbicella spp.* solo margin chlorinated epoxy treatments, 48.9% of the solo disease-breaks, and 34.5% when both a disease-break and direct margin treatment to the active disease margin was applied. Contrastingly, *Montastrea cavernosa* showed to only respond to the disease treatment 47% of the time when a chlorinated epoxy-filled disease-break as well as margin treatment were both applied to the individual. Results showed that *M. cavernosa* species did not respond positively to either solo margin treatment or solo disease-break treatments.

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 12). 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.

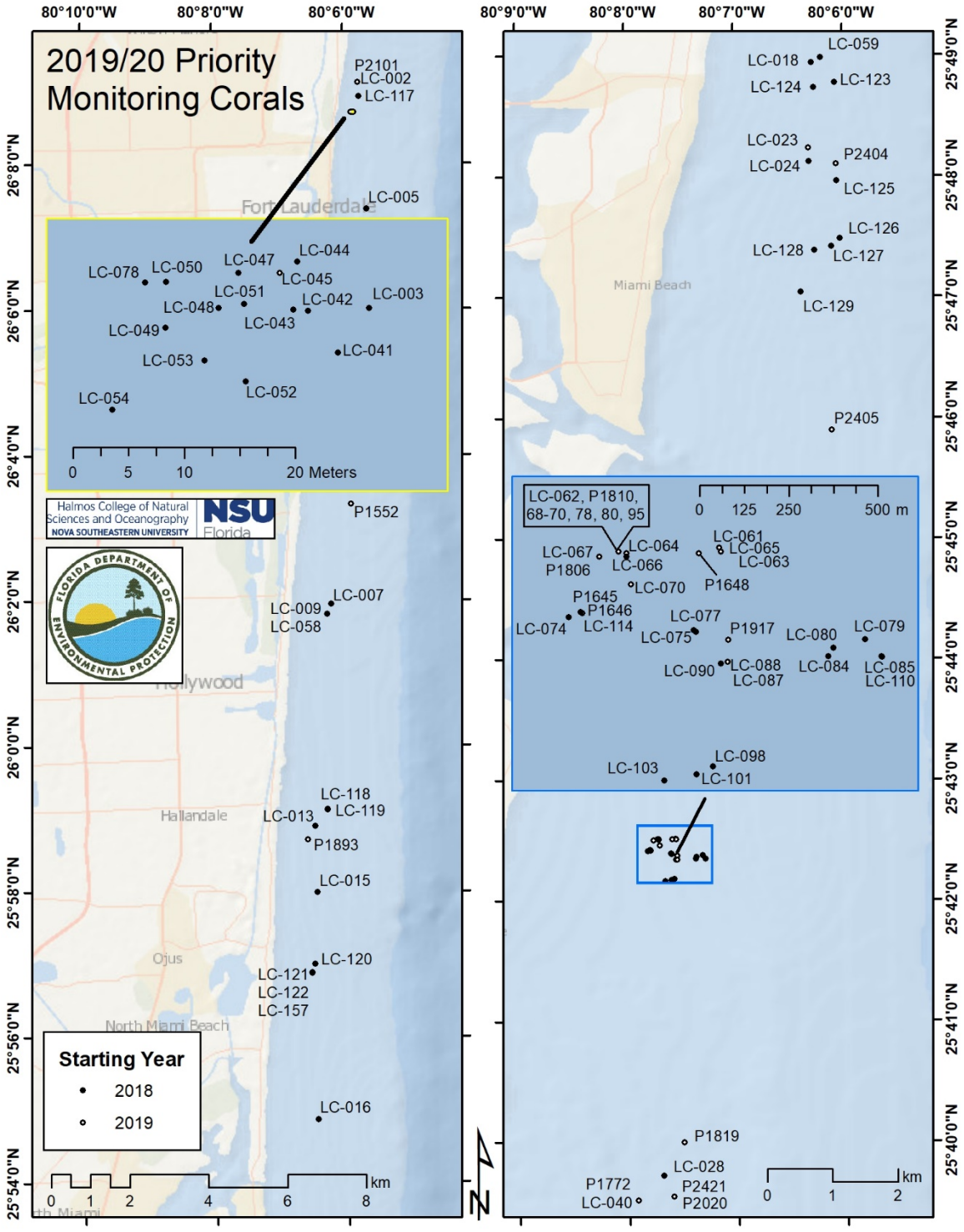


Figure 9. Map of the large priority monitoring corals.

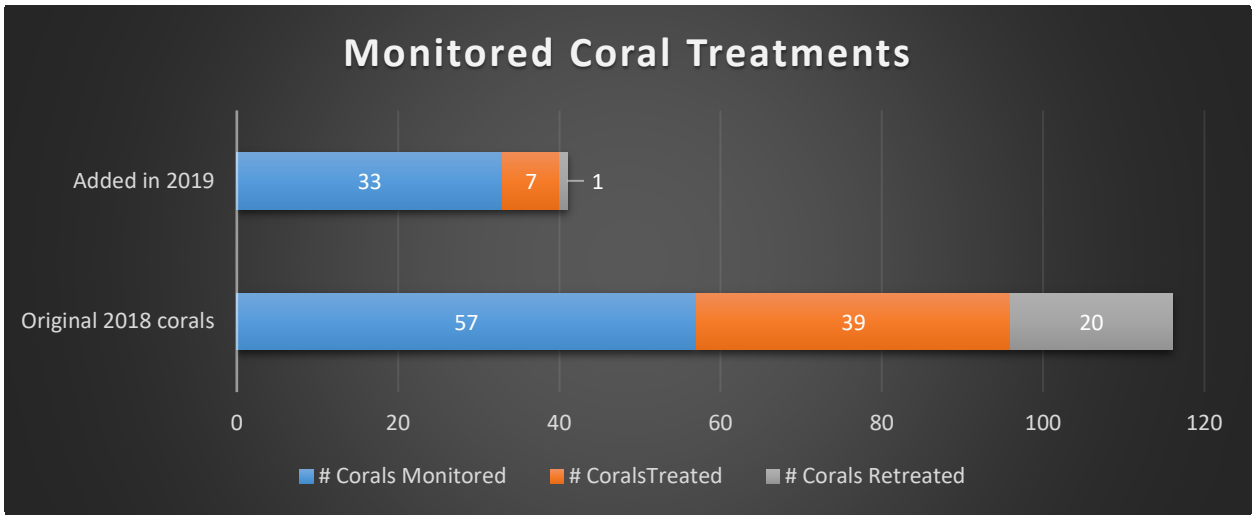


Figure 10. Number of corals requiring treatments and retreatments out of the total monitored corals in 2018 and 2019.

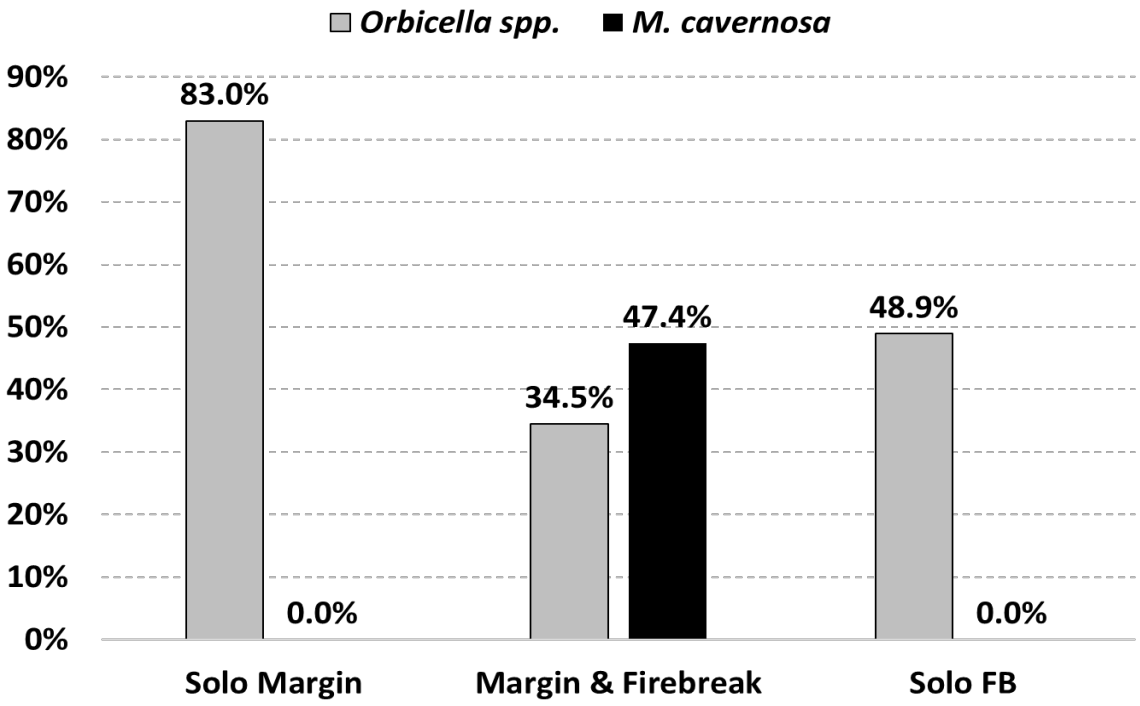


Figure 11. Graph of treatment type success by species. Solo margin treatments on *Orbicella spp*. were most successful.

Cumulative treatment success varied between months (Figure 12). 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 13). 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 14). 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 15). Twenty-three corals (44%) of the total treated corals never failed after the initial treatment.

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%

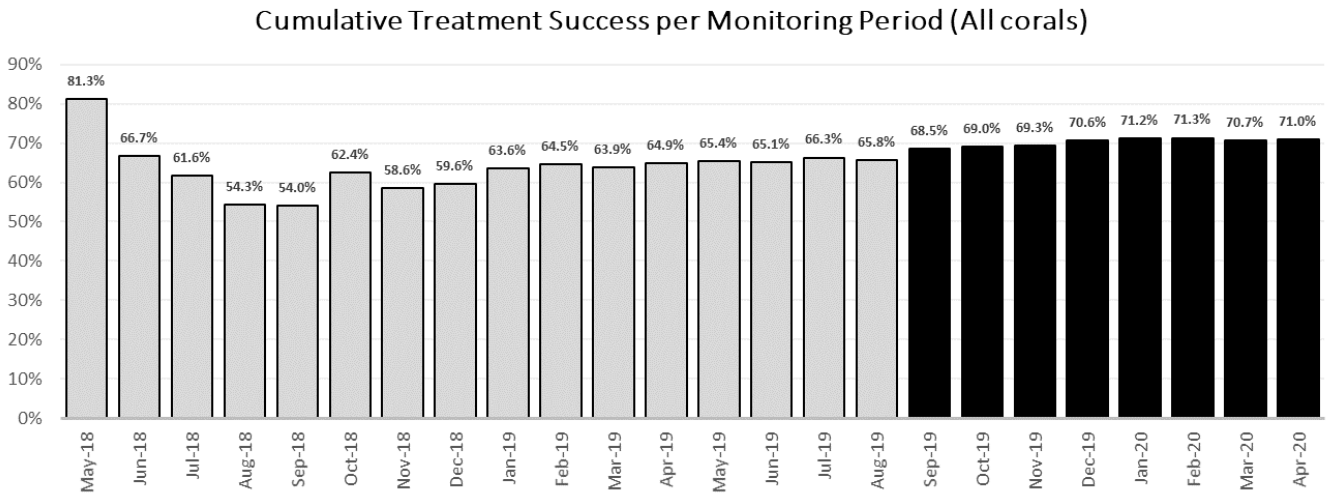


Figure 12. The cumulative percent success of all treatments on all corals each treatment period. Grey bars indicate chlorinated epoxy treatments. Black bars indicate antibiotic ointment treatments.

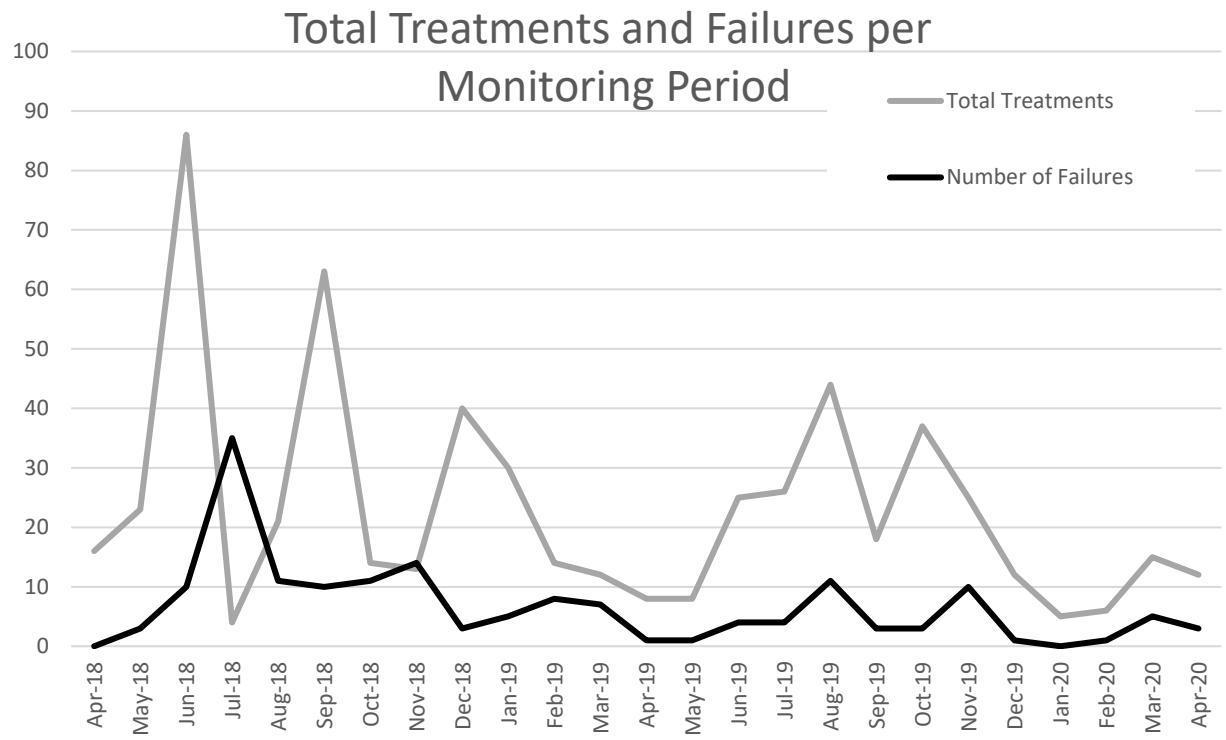


Figure 13. The total number of treatments (grey) and failures (black) per monitoring period for all corals.

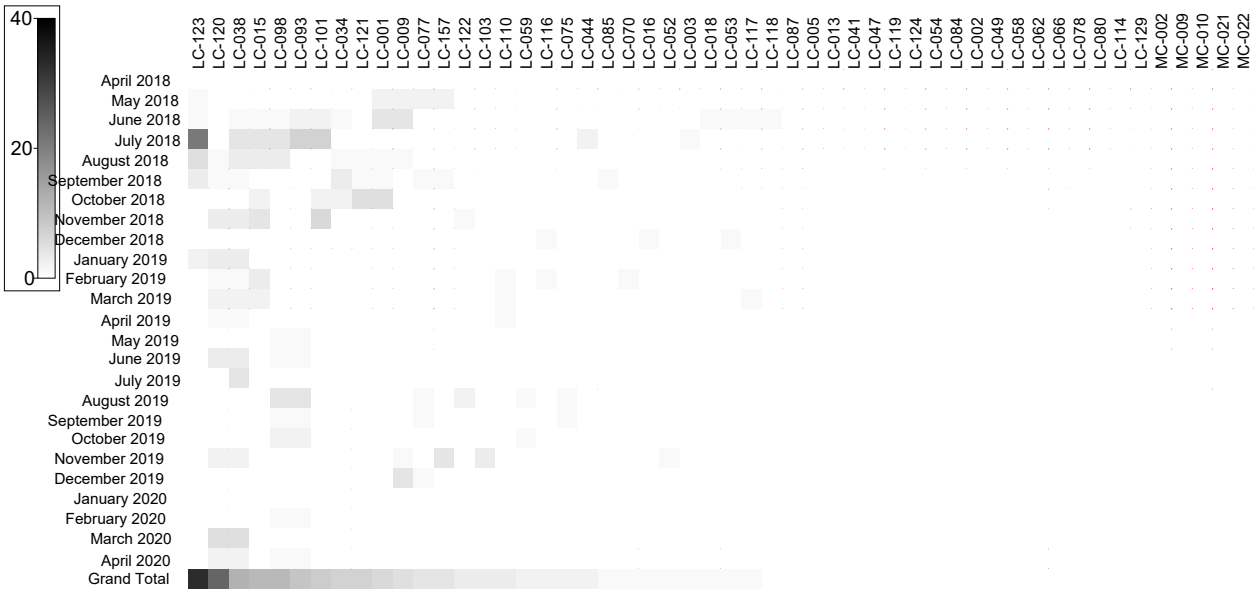


Figure 14. Shade plot of total failure on each treated coral by monitoring period sorted by the maximum total number of failures.

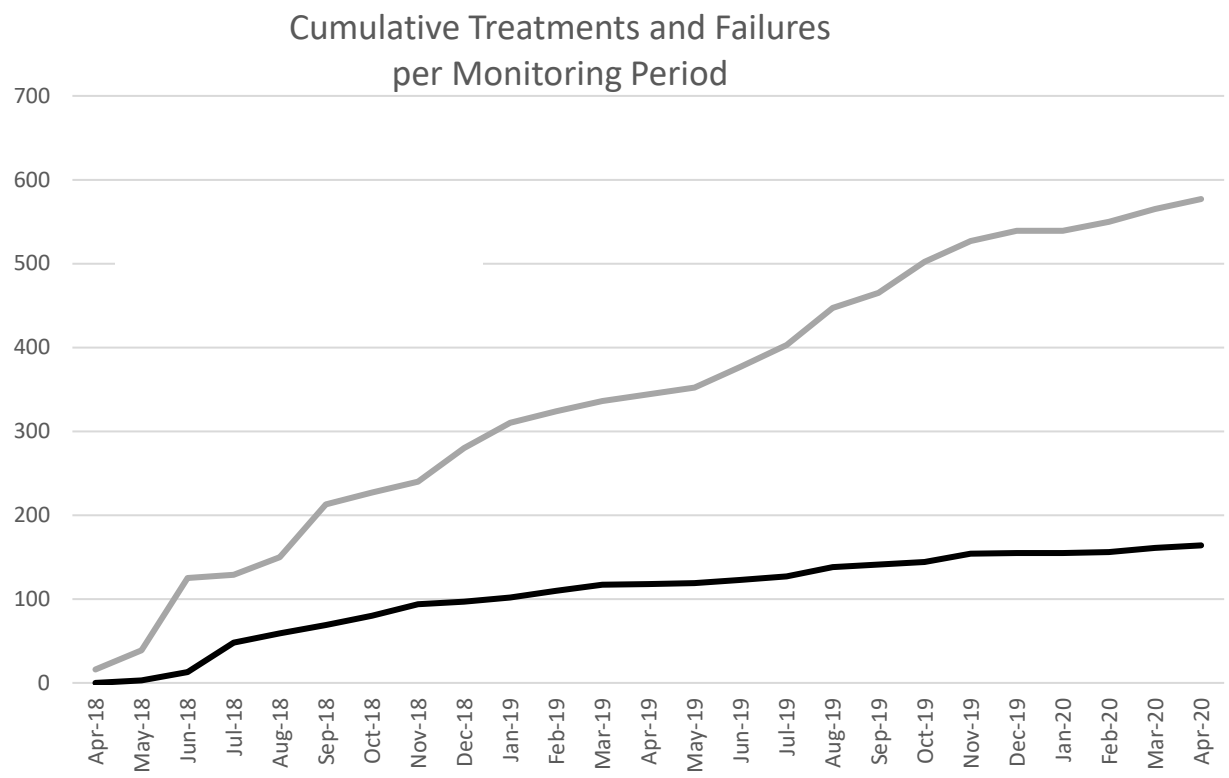


Figure 15. The cumulative total number of treatments (grey) and failures (black) per monitoring period for all corals.

The patterns of total cumulative success were mostly driven by *Orbicella spp.* which comprised most of the monitored corals (77%), hence Figure 14 looks similar to Figure 10. However, the *M. cavernosa* success was notably different (Figure 17). Like the *Orbicella spp.*, they initially seemed successful but failed over time (Figure 18). In July 2018, there had been 25 treatments on *M. cavernosa* and only 6 failures. 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 19). 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 and 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 20).

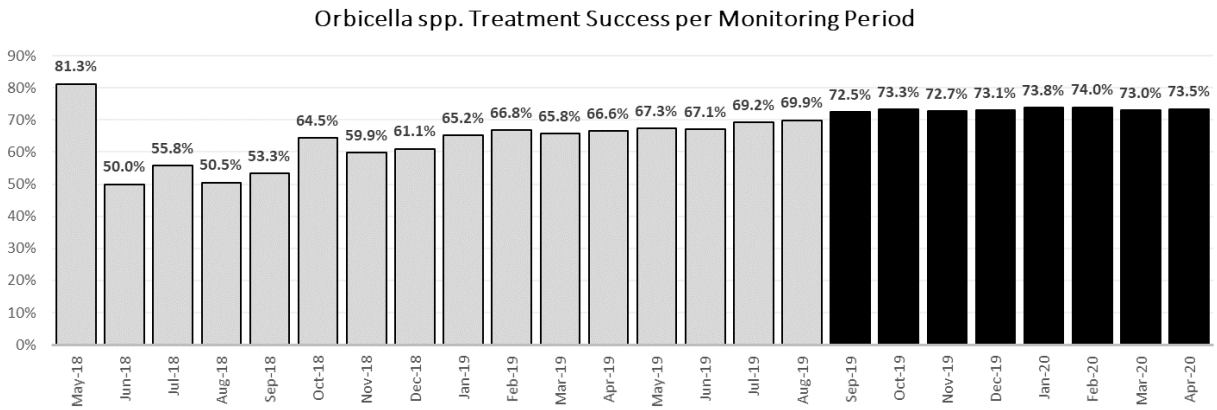


Figure 16. The cumulative percent success of all Orbicella spp. treatments on all corals each treatment period. Grey bars indicate chlorinated epoxy treatments. Black bars indicate antibiotic ointment treatments.

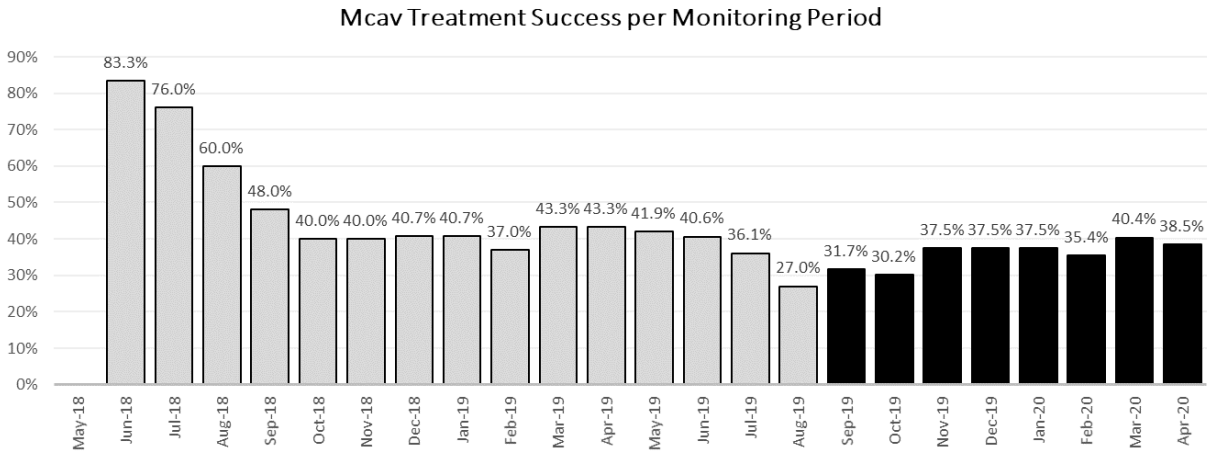


Figure 17. The cumulative percent success of all M. cavernosa treatments on all corals each treatment period. Grey bars indicate chlorinated epoxy treatments. Black bars indicate antibiotic ointment treatments.

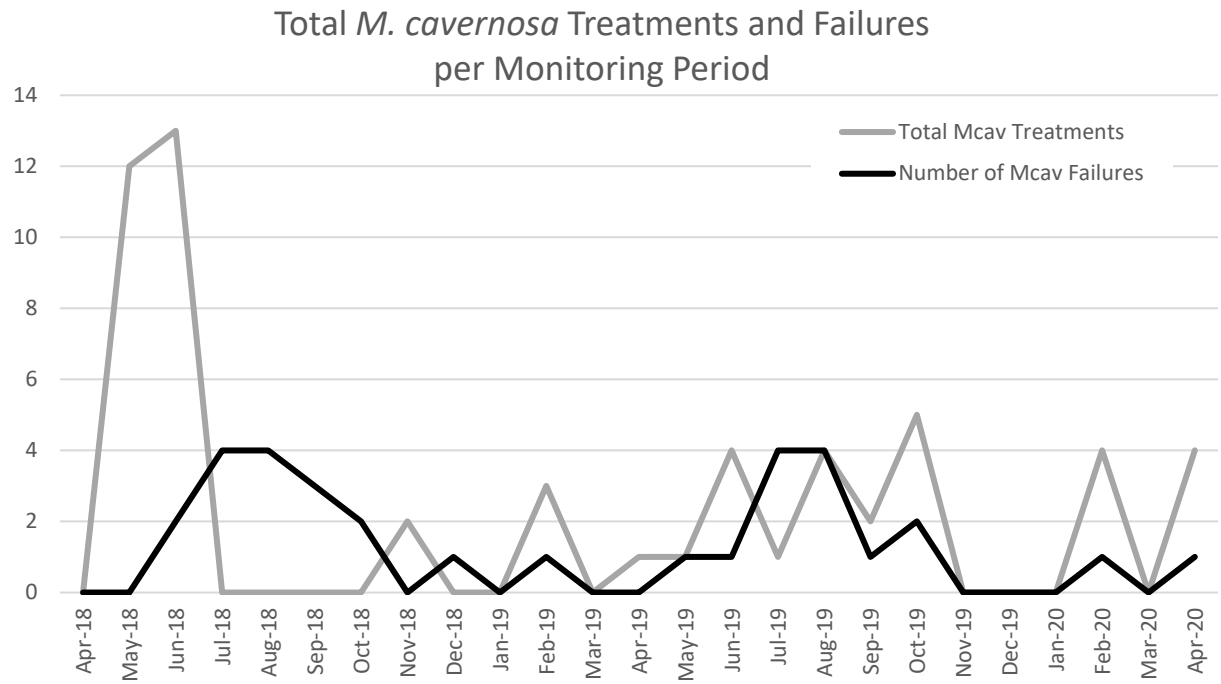


Figure 18. The total number of treatments (grey) and failures (black) per monitoring period for M. cavernosa.

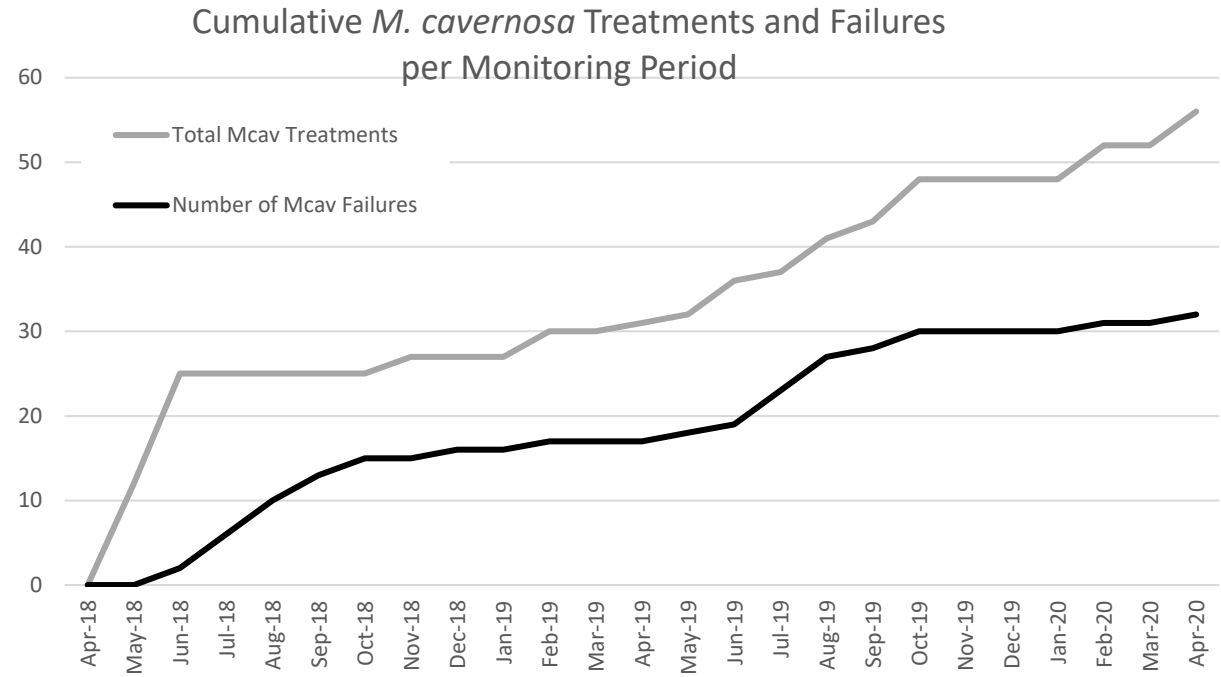


Figure 19. The cumulative total number of treatments (grey) and failures (black) per monitoring period for M. cavernosa.

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%

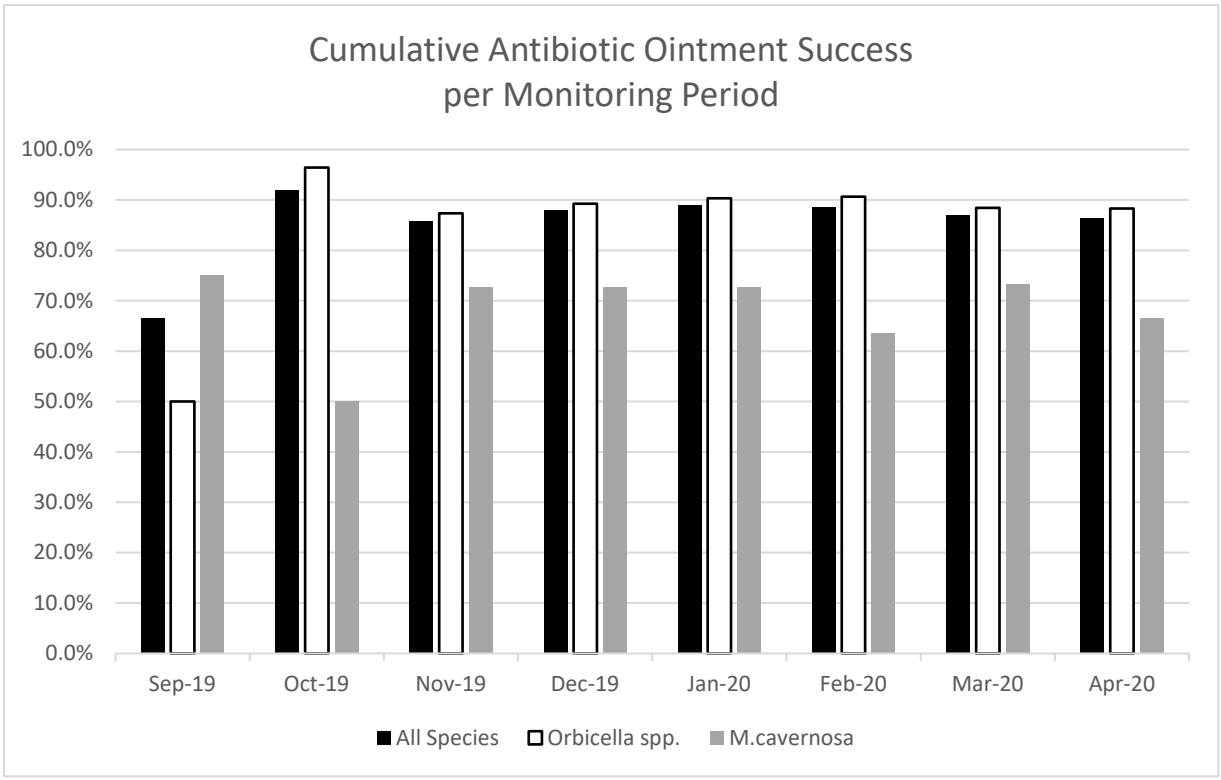


Figure 20. The cumulative percent success of antibiotic ointment treatments on all corals (black), *Orbicella spp*. (white), and *M. cavernosa* (grey) each treatment period.

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 21), usually followed by heavy algal growth (Figure 20). Three colonies seemingly did not respond to treatment and required excessive work: LC-034, LC-120, LC-123 (Figure 23). If we remove these seven outlier colonies, treatment success improved substantially to 80.5% (Figure 24).

Figure 21. 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.

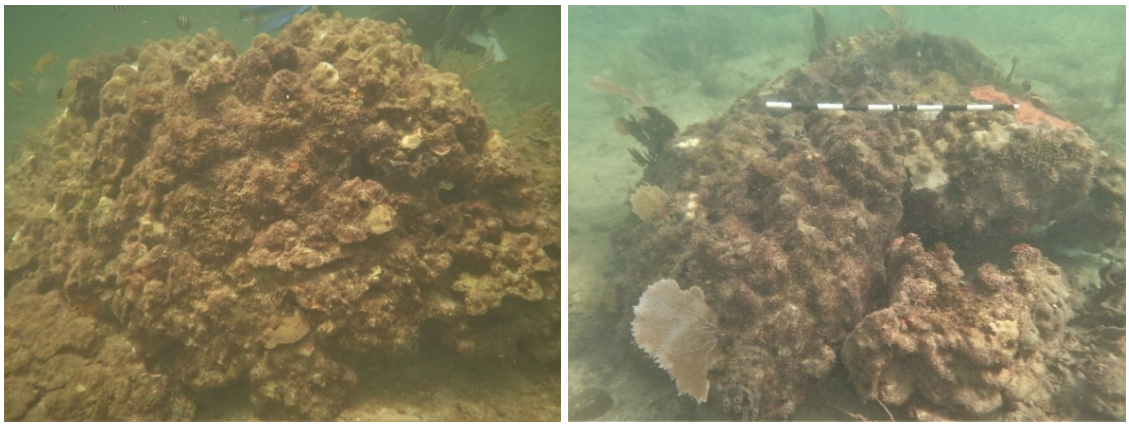


Figure 22. Untreatable corals with heavy algae colonization. Left: LC-001 & right: LC-092.

Figure 23. Corals that did not respond to treatment. Left: LC-034, Middle: LC-120, & Right: LC-123.

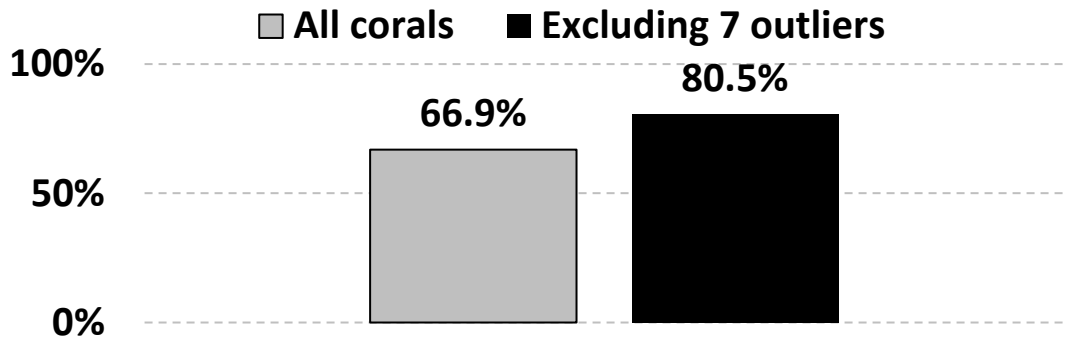


Figure 24. Graph of total treatment success of all forty treated corals (grey) and excluding the 7 outliers (black).

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 15 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 25 and Table 3). Between April and June 2018, the number of corals visited was low 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 in multiple monitoring periods. LC-120 required treatments in five out of the six periods. LC-157 required treatments in four out of the six periods while LC-118, LC-103, and LC-059 required treatments in 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).

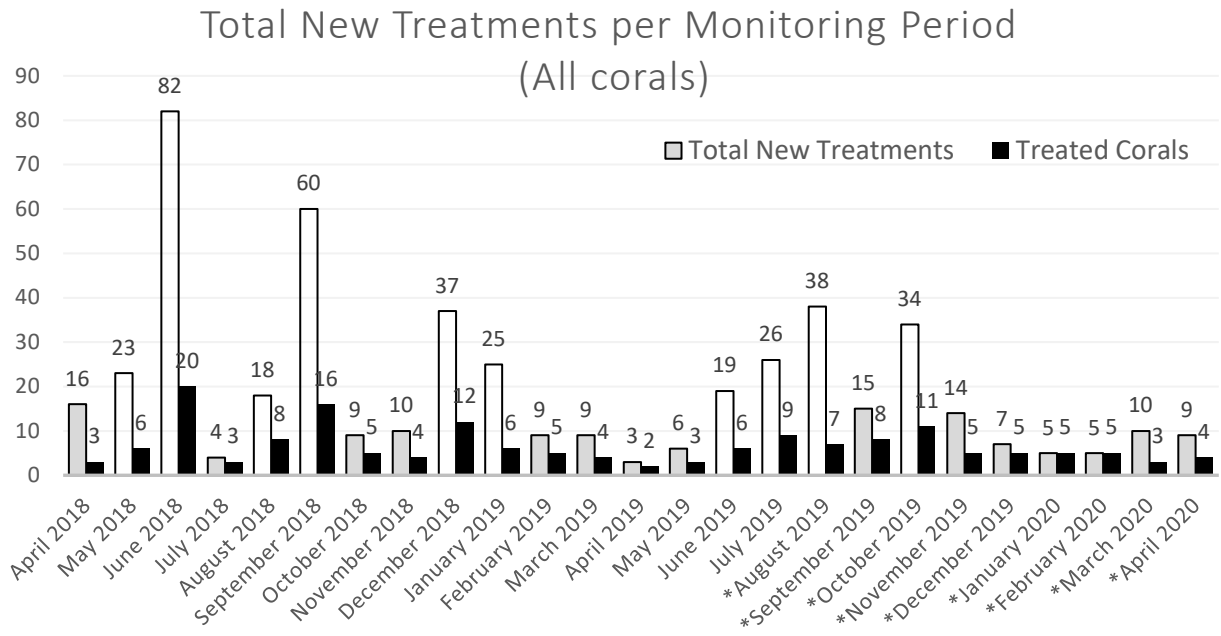


Figure 25. The numbers of new treatments on all corals used as a proxy for new infections (grey) and the number of treated corals (black) by treatment period. *Indicates antibiotic ointment treatments.

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			

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.

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			

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 26). 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).

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 27). 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.

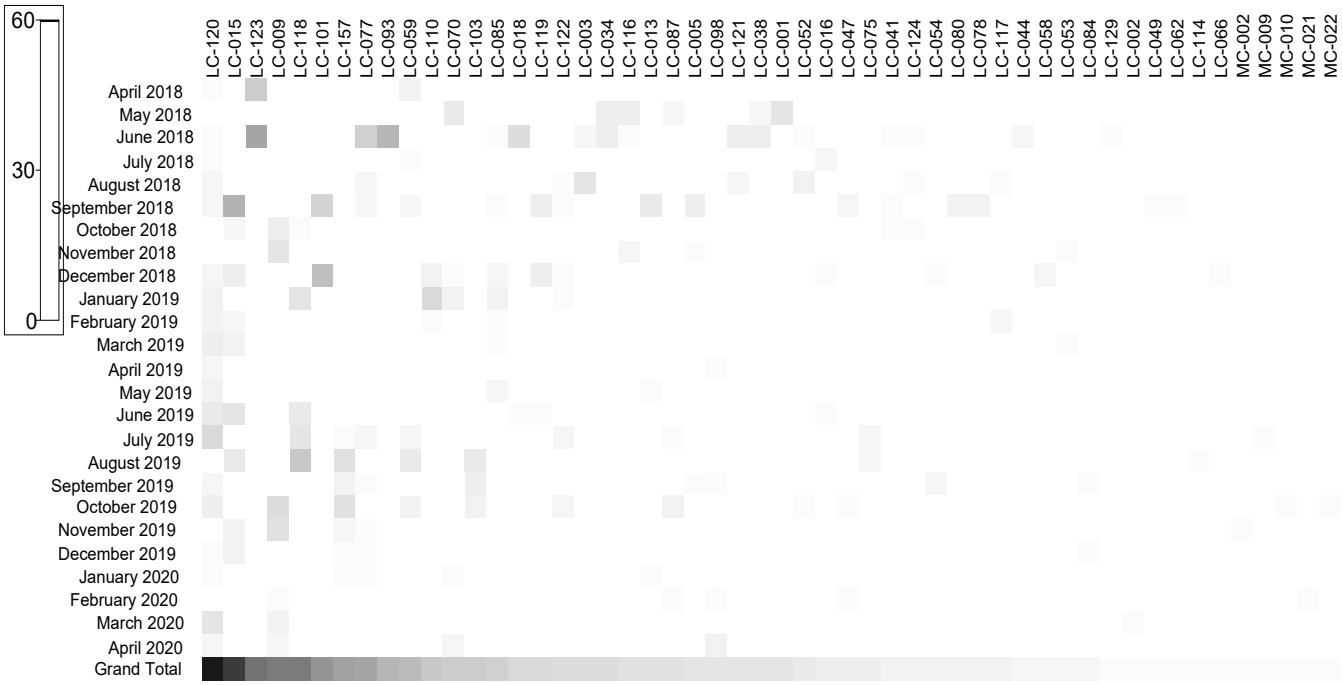


Figure 26. Shade plot of total treatments on each treated coral (column) by monitoring period (row) sorted from left to right by the maximum total number of treatments.

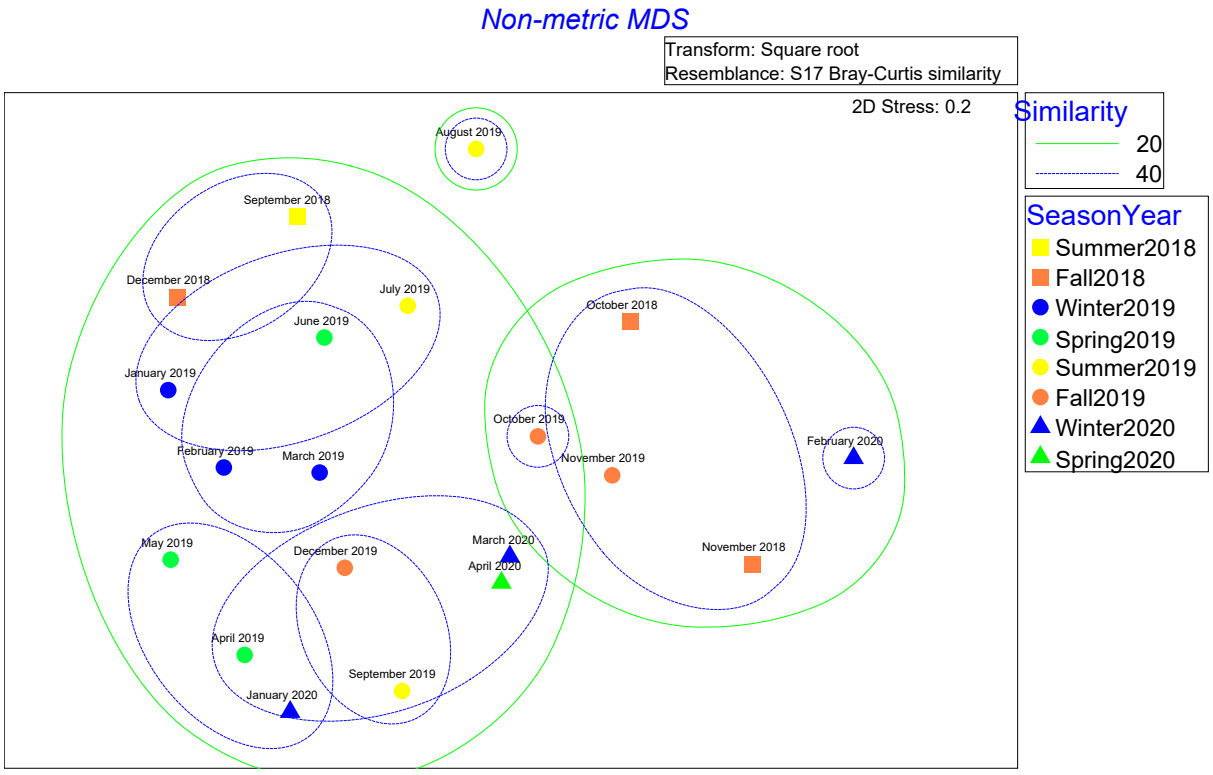


Figure 27. Multidimensional scaling plot of Bray-Curtis similarities of total treatments per coral for each monitoring period.

4.3. **Recon Sites (Task 4)**

During our strike team and NCRMP survey activities we searched for areas of high coral density and diversity. Recon efforts are ongoing, funded by NOAA Coral Program Award NA19NOS4820127 and DEP B7B6F3. As of August 12, 2020, six sites were identified during recon to consider for restoration activities. Table 6 contains the data associated with these sites and Figure 6 illustrates their locations.

We identified several sites of interest that meet these criteria including a reef with a previously unknown colony of *Dendrogyra cylindrus* in Miami-Dade County (Figure 28). As well as several reefs with high densities of *Montastrea cavernosa*. One site observed while drifting on safety stop after a NCRMP fish survey appeared to have high relief and rugosity. However, at a depth of ~20 m and limited visibility, individual colonies were not identified. We plan to revisit to investigate further. During our strike team activities, we also found a site with both high coral density and diversity along the Pompano mooring buoys, despite high traffic, a large number of colonies were healthy and of significant size.

We plan on continuing our reconnaissance over the next couple months while conducting coral intervention work along the nearshore reef in Broward and Miami-Dade Counties. We will also use our remaining NCRMP fish and benthic sites in Broward, West Palm and Martin Counties to scout for additional sites of interest/importance.

Table 6. Preliminary information on six sites identified for restoration activities.

Nr	Site name	County	Coordinates	Description
1	*Dendrogyra* Colony	Miami-Dade	N 25.700211 W 80.098248	One partly live *Dendrogyra cylindrus* colony on a shallow reef in South Miami-Dade
2	Pompano Moorings	Broward	N 26.211332 W 80.084531	Nice relief, high coral cover and diversity
3	High Relief Ledge Reef	Broward	N 26.200196 W 80.071329	High rugostiy deep reef, appeared to have coral colonies, lots of large fish
4	Broward Large Coral Cluster	Broward	N 26.145267 W 80.097367	Cluster of large OFAV
5	Biscayne Large Coral Cluster	Miami-Dade	N 25.708467 W 80.12895	Cluster of large OFAV
6	High MCAV Density Site 1	Miami-Dade	N 25.886240 W 80.106852	High density of MCAV on a patch reef, when visited not enough disease to make a site but need to revisit

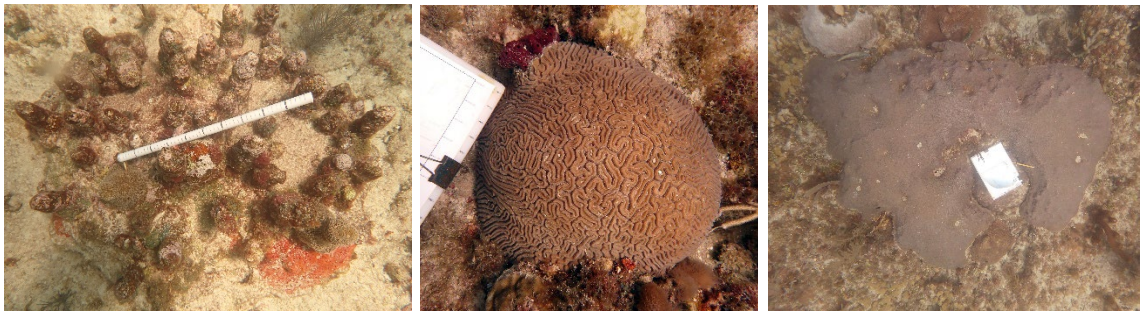


Figure 28. Examples of new corals found during recon dives.

4.4. **Field test new permitted intervention techniques and materials (Task 5)**

4.4.1. Antibiotic paste versus Chlorinated Epoxy on Montastrea cavernosa

Treatment comparison corals were treated on May 6 and 8, 2019 and revisited weekly until June 5 (May 15, May 21, May 29, and June 5) after which they were revisited on June 19, June 27, July 10, July 30, August 13, October 10, 2019 and January 31 and April 20, 2020 (Figure 3). Treatments before June 30, 2019 were conducted under DEP PO B46AD7.

In total by April 2020, 118 lesions were treated on 32 colonies with antibiotic paste, 37 lesions on 20 colonies with chlorinated epoxy and 70 lesions on 19 (of the 20) corals originally treated with chlorinated epoxy and subsequently treated with antibiotic paste after the initial failure of the chlorinated epoxy. Table 7 shows the number of corals and lesions treated each visit. Treatment success differences were clear within one week, where antibiotic paste treatment success was 76% and chlorinated epoxy was 11%. Due to the clear failure of epoxy treatments observed one-week post-treatment, chlorinated epoxy treatments were abandoned, and all new treatments used the antibiotic paste. We refer to these as mixed treatments. This was done to test the antibiotic paste treatment on corals that the other treatment had failed. Antibiotic paste success on corals where chlorinated epoxy failed would eliminate any possibility of a treatment distribution bias in the study design. However, this resulted in a much greater overall number of antibiotic paste treatments.

Table 7. The total number of treated corals and disease lesions by date. Blue shading are data associated with initial chlorinated epoxy treatments. Green shading are from subsequent antibiotic paste treatments after initial treatments failed.

		Antibiotic Paste				Chlorinated Epoxy		Mixed Treatment			
Date	New Treated Corals	Treated Corals	Re-Treatments	New Lesions: Original corals	New Lesions: On new corals	Treated Corals		Re-Treatments		New Lesions	
6-May-19	24	14		36		10				23	
8-May-19	20	10		18		10				14	
15-May-19											
21-May-19	3	3			4						
29-May-19	2	6	1	5	3		9		7		3
5-Jun-19		4	4	5			12		11		6
19-Jun-19	1	2	2		3		9		10		4
27-Jun-19											
10-Jul-19		3		5			7		8		3
30-Jul-19	1	2		2	7		5		4		1
13-Aug-19		3	2	1			6		5		1
10-Oct-19	1	6	2	3	1		1		1		
31-Jan-20		2		3			2				4
21-Apr-20		3	2	9			2				2
Totals	52	58	13	105		20	53	0	46	37	24

The number of new treatments varied through time (Figure 29). The two weeks post initial treatment did not require any new margin treatments. During the third week, we applied four treatments to three corals. The following week, we applied eleven treatments to eight corals. Thus, one month after treatments, 15 new lesions occurred on eleven corals. Treatments spiked in July, with four colonies requiring ten treatments, but declined through October. Treatments spiked in April 2020 with eleven lesions on four corals. These results somewhat correspond to the priority corals new infections (Figure 25).

Two corals, 1891 and 1932 accounted for 44% of the new infections with 1891 acquiring 10 new infections and 1932 acquiring five. Interestingly, 1891 was originally treated with antibiotic paste and 1932 was treated with chlorinated epoxy. There were no obvious differences in re-infection patterns over time for corals that received the two different treatments.

Eight additional corals were treated after site establishment: three were added on May 21; two on May 29; and one each on July 19, July 30, and October 10, 2019. Six of the eight corals were added during May and early June 2019, which corresponded closely to the peak of new infections at these sites during the monitoring.

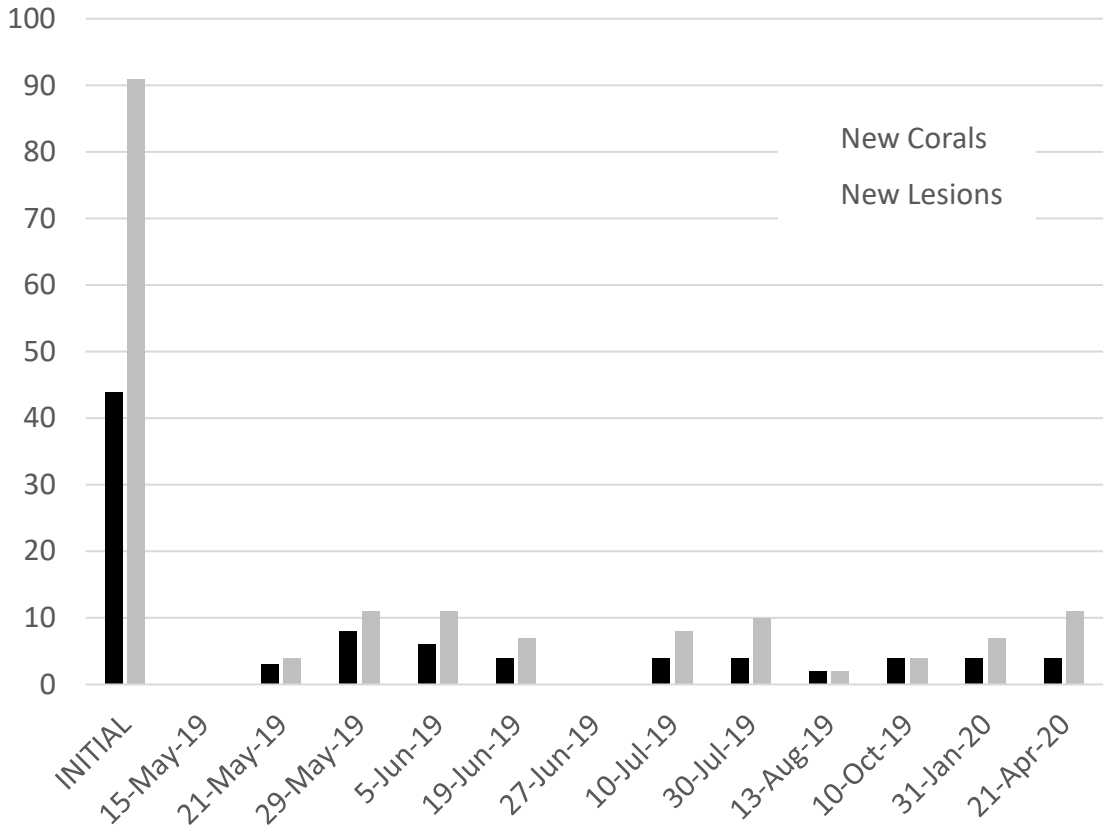


Figure 29. Number of new treatments, colonies with new treatments, and new colonies per monitoring period.

Table 8 shows the 324 lesion treatments by types and date. There were 114 total antibiotic margin treatments; 105 originals and 9 retreatments where the original treatment was unsuccessful. There were 37 total disease-breaks and 10 total margin treatments of disease-break failures; eight were disease break retreatments and 2 were retreatments of the retreatments. There were 37 chlorinated epoxy margin treatments and 35 disease-breaks at the onset. No further chlorinated epoxy treatments were performed and all failures were treated with antibiotic paste (mixed treatment). The mixed treatments included 26 total antibiotic margins, 4 total disease-break treatments, and 61 total disease-break failure retreatments. The 26 mixed margin treatments were 24 treatments of antibiotic paste on a new margin not associated with the original chlorinated epoxy treatments and 2 retreatments of failures. There were 4 new antibiotic treatments on the mixed treatment corals that included disease-break margins. The 56 disease-break failure treatments were antibiotic paste margin treatments of chlorinated epoxy disease-break failures. Five of these required retreatments with more antibiotic paste.

Table 8. Lesion treatments (T) and re-treatments (R) by treatment type and date. *Retreatments for Chlorinated epoxy disease breaks were done with Antibiotic paste. Blue shading are data associated with initial chlorinated epoxy treatments. Green shading are from subsequent antibiotic paste treatments after initial treatments failed.

	Antibiotic Paste						Chlorinated Epoxy				Mixed Treatment				
	Margin		Disease-Break		DB Failure Re-treatment		Margin		Disease-Break		DB Failure Re-treatment				Treatments
Date	T	R	T	R	T	R	T	R	T	R	T	R			by Date
6-May-19	36		26				23		22						**107**
8-May-19	18		10				14		13						**55**
15-May-19															**0**
21-May-19	4														**4**
29-May-19	8				2			3					8		**21**
5-Jun-19	5	2			3	2		6					15	1	**34**
19-Jun-19	3	2						4		1			12	2	**24**
27-Jun-19													0		**0**
10-Jul-19	5							3					8	1	**17**
30-Jul-19	9							1		1			5	1	**17**
13-Aug-19	1	1			3			1					7		**13**
10-Oct-19	4	2											1		**7**
31-Jan-20	3		1					4		4					**12**
21-Apr-20	9	2						2							**13**
Treatments	**105**	**9**	**37**	**0**	**8**	**2**	**37**	**24**	**0**	**2**	**35**	**4**	**0**	**0**	**0**	**56**	**0**	**5**	**324**
Total	**114**		**37**		**10**		**37**		**26**		**35**		**4**		**0**		**61**		

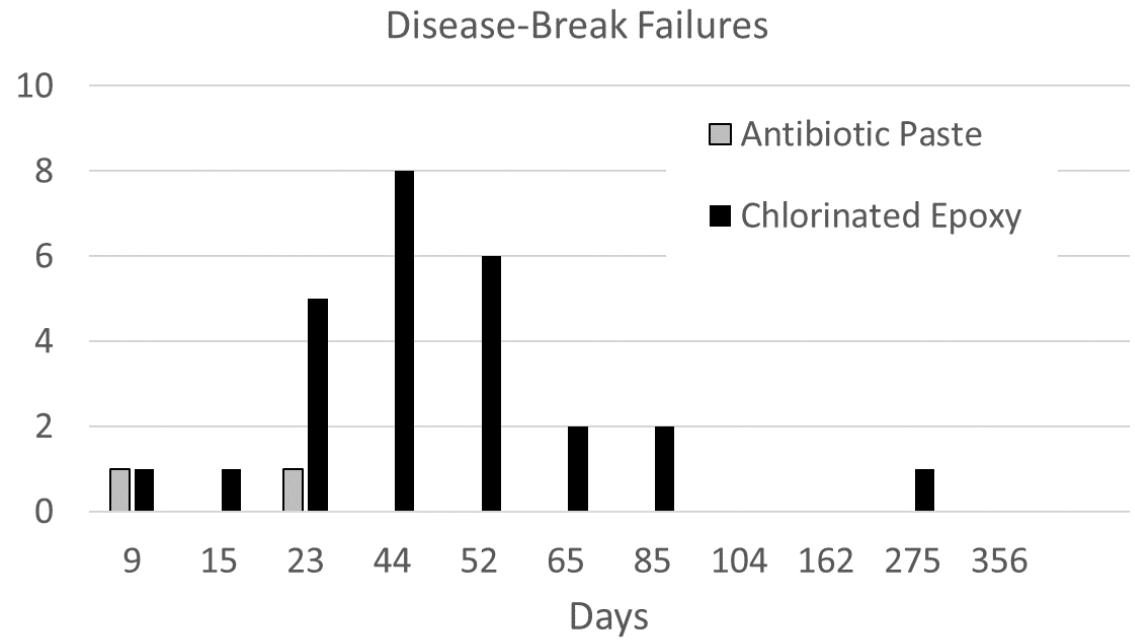
Table 9 shows the 103 treatment failures by treatment types and date. There were 29 antibiotic paste margin treatment failures, 3 disease-break failures, and 2 disease-break retreatment failures. There were 34 margin and 25 disease-break chlorinated epoxy failures. There were 3 margin and 7 disease-break retreatment antibiotic paste treatment failures on the mixed treatment corals.

Success rate varied between treatment application and material. Lesions treated with antibiotic paste on the disease margin and disease-break combination had the highest rate of success (91.9%) whereas those with chlorinated epoxy had the lowest (28.6%). When looking at just the disease margin application only, the antibiotic paste stopped disease progression in 74.6% of cases, whereas chlorinated epoxy margin treatments only achieved a success rate of 8.1% (Table 9). On the mixed treatments, the antibiotic paste margin treatments were 88.5% successful on failed chlorinated epoxy disease-break failures. Combining all of these antibiotic margin treatments gives a total success of 79.4%. Hence, the addition of a disease-break increased the success of "margin only" treatments by 12.5%, indicating that treatments combining margins and disease-breaks are the most effective strategy.

Table 9. Lesion treatment failures by treatment type and date. Blue shading are data associated with initial chlorinated epoxy treatments. Green shading are from subsequent antibiotic paste treatments after initial treatments failed.

	Antibiotic Paste			Chlorinated Epoxy			Mixed Treatment			
Date	Margin	Disease-Break	DB Failure Re-Treatment	Margin		Disease-Break		DB Failure Re-Treatment		Failures by date
6-May-19										**0**
8-May-19										**0**
15-May-19	13	1		33		1				**48**
21-May-19	1					1				**2**
29-May-19	2	1		1		5				**9**
5-Jun-19	3		2			8			2	**15**
19-Jun-19	3				2	6			2	**13**
27-Jun-19	2					1				**3**
10-Jul-19						2			1	**3**
30-Jul-19					1				1	**2**
13-Aug-19	1	1								**2**
10-Oct-19	2					1			1	**4**
31-Jan-20										**0**
21-Apr-20	2									**2**
Total Failures	**29**	**3**	**2**	**34**	**3**	**25**	**0**	**0**	**7**	**103**
Total Treatments	**114**	**37**	**10**	**37**	**26**	**35**	**4**	**0**	**61**	
Failure Rate	25.44%	8.11%	20.00%	91.89%	11.54%	71.43%	0.00%	N/A	11.48%	
Success Rate	**74.56%**	**91.89%**	**80.00%**	**8.11%**	**88.46%**	**28.57%**		**N/A**	**88.52%**	

Treatment failure timing varied between treatment method (Figure 30). Most margin treatment failures occurred within 9 days of initial treatment whereas most coral-break failures occurred within 52 days. Four margin treatments occurred between 15 and 44 days post-treatment and five disease-break failures occurred between 65 and 275 days.



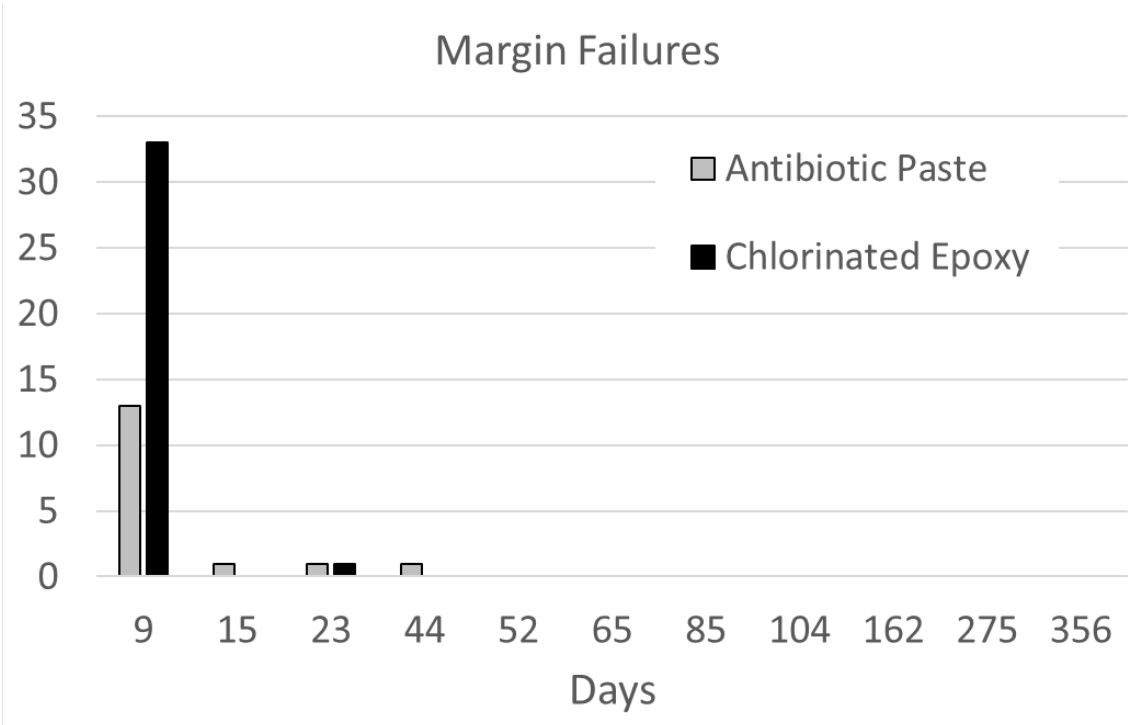


Figure 30. Timing of margin (top) and coral-break (bottom) failures during the study. Most margins failed within 9 days whereas most coral-breaks failed within 52 days.

Tissue regrowth over disease-breaks treated with antibiotic paste was more rapid and more frequent than regrowth over epoxy-filled disease-breaks (Figure 31). Considering the healing of the original disease-breaks (cut during the initial site setup May 6 and 8, 2019), 90.5% of antibiotic paste-filled disease-breaks exhibited some healing compared to just 15% healing among disease-breaks filled with chlorinated epoxy. By the final healing assessment, 50.7% of the antibiotic paste disease-breaks were fully healed compared to just 1.5% of the chlorinated epoxy-filled disease-breaks (Figure 32). In the approximately two months between healing assessments, we saw a 5.7% increase in healed antibiotic paste disease-breaks and a 1.4% decrease in healed chlorinated epoxy disease-breaks.

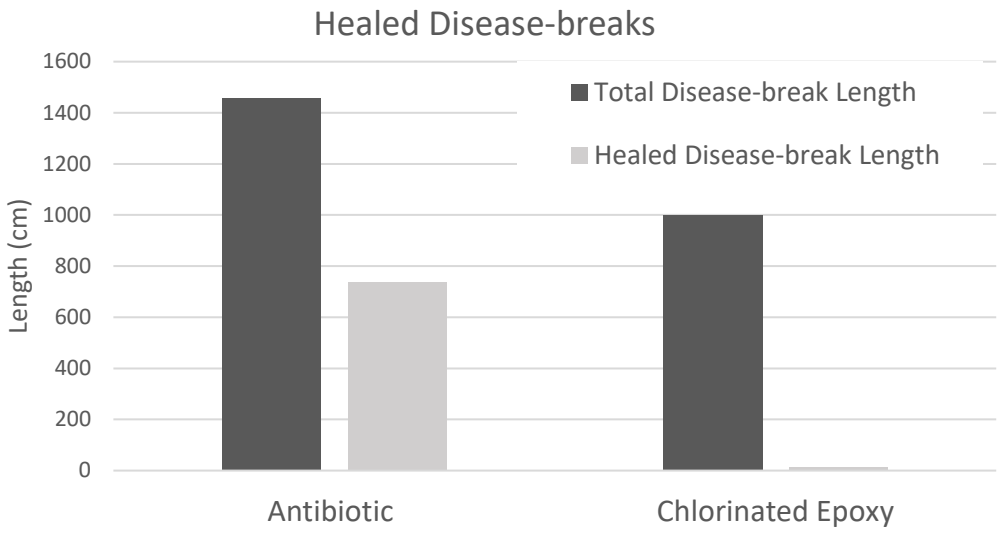


Figure 31. Comparison of fully healed disease-breaks between the antibiotic (50.7%) and the chlorinated epoxy (1.5%) treatments.

Thirty percent (8/24) of the antibiotic corals had new infections, whereas forty-seven percent of the epoxy corals had new infections. The new infections on epoxy corals were treated with antibiotic ointment. There were no obvious differences in infection patterns through time on corals receiving different treatment types.

There were no obvious spatial patterns of new infections during the monitoring (Appendix B).

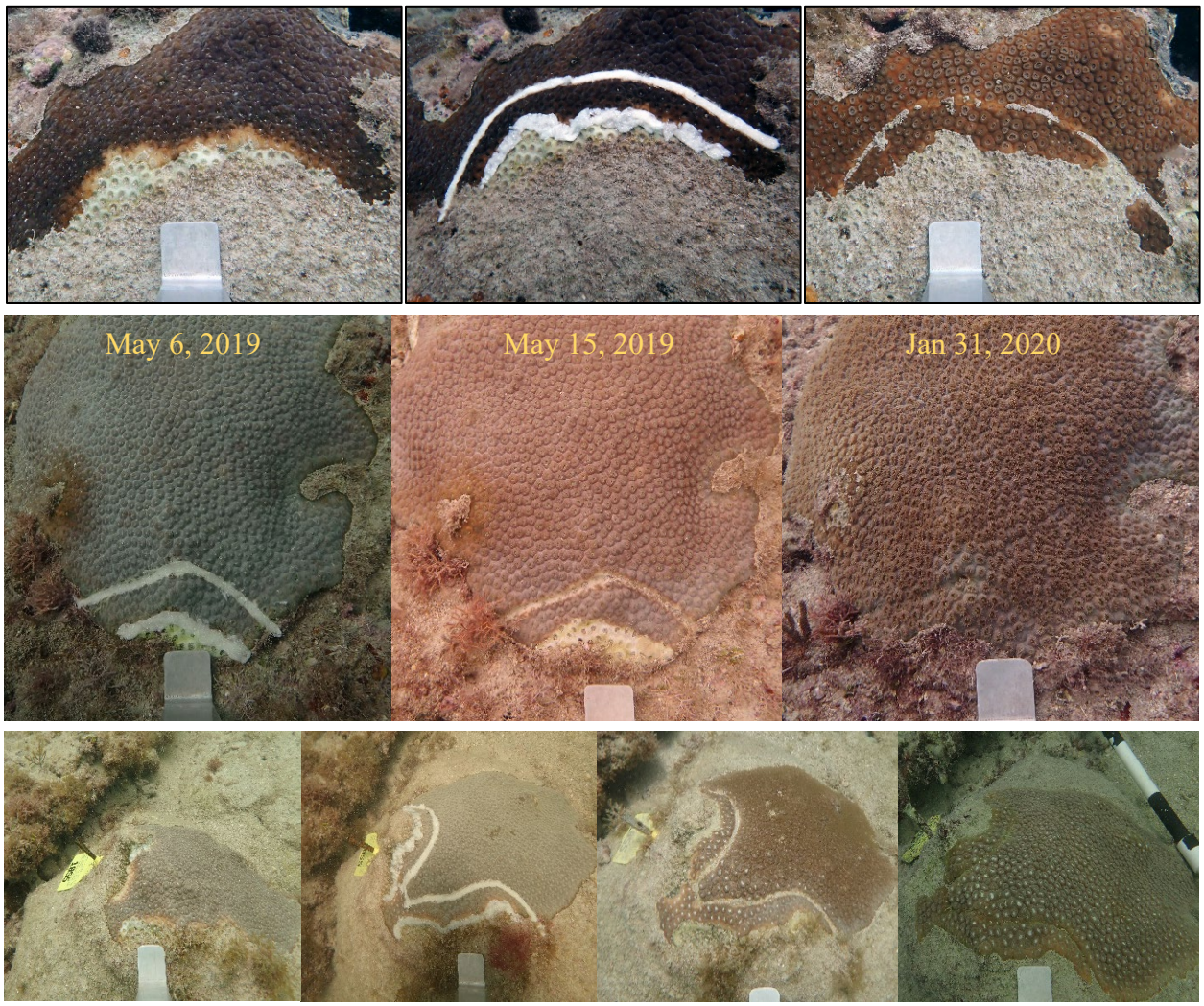


Figure 32. Examples of disease-break success and healing. Top: the lesion (left) was treated on May 6, 2019 (middle), and by June 5, 2019 displayed significant tissue growth over the disease-break (right). Note that the disease-break also contained the spread of the disease where the margin treatment was not effective. Middle: Colony #1507 showing initial margin and disease-break treatments, post-treatment bare skeleton and subsequent tissue recovery. Bottom: Colony 1855 showing initial disease and margin and disease-break treatments on May 19, 2019 (far left and left center), post treatment bare skeleton on May 21, 2019 (right center) and subsequent tissue recovery on Oct 9, 2019.

4.4.2. Probiotics on Montastrea cavernosa

NSU assisted the Smithsonian Marine Station at Fort Pierce with probiotics treatments at site BS1 on January 16, 2020. Divers bagged corals, released a probiotic slurry into the bag, and incubated them for about two hours (Figure 33). Please contact Valerie Paul for more information on those experiments.

Figure 33. Diver treating a coral with probiotics at BS1.

5. DISCUSSION

This report provides updates to the previous reports on the strike team activities and *M. cavernosa* treatment comparison by Walker and Pitts (2019) and the 2018-2019 large coral treatments and monitoring by Walker and Brunelle (2019).

5.1. **Broad-scale Coral Disease Intervention Strike Team (Task 2)**

The amount and species of corals treated in broad scale recon surveys indicates that there are still some rare survivors of the highly susceptible brain coral species in the area and they are still succumbing to the disease. Anecdotally, SCTLD prevalence is low, but remains high in *M. cavernosa*, *O. faveolata*, and to a lesser extent *P. clivosa*.

As of August 12, 2020, a total of 700 colonies were treated by the coral disease interventions strike teams in the SE FL ECA (including the treatment comparison sites and probiotic site, but not large corals) totaling 341.60 m of treatment; 75.5% with antibiotic paste. The total number of treatments by species were 624 *M. cavernosa*, 44 *O. faveolata*, 8 *Colpophyllia natans*, 10 *Pseudodiploria strigosa*, 9 *P. clivosa*, 4 *Solenastrea bournoni* and 1 *Siderastrea siderea*.

5.2. **Apply Interventions to 90 Large Corals (Task 3)**

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. The large coral treatments had 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 disease-break during the observation period. Neeley et al. (2020) 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 recently developed 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 (Neely 2020; Neely et al., 2020). Using the same mixture, the *M. cavernosa* treatment comparison study (Section 4.4.1) yielded 91.9% success of treated *M. cavernosa* lesions using a margin and disease-break treatment versus 28.6% success with chlorinated epoxy. The large coral 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 on large corals 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. Our antibiotic paste versus chlorinated epoxy comparisons found that most chlorinated epoxy disease-breaks on *M. cavernosa* failed between 23 and 52 days after initial treatment. There was a similar lag on the *M. cavernosa* chlorinated epoxy disease-break 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 and Bythell, 2005; Ritchie, 2006).

Intraspecific colony differences may have contributed as well. Individuals within 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 and Van Oppen, 2006; Glynn et al., 2001; Rowan, 2004; Tchernov et al., 2004). Therefore, having a combination of zooxanthellae may provide many ecological advantages in different niches to cope with stress (Baker, 2001; Berkelmans and Van Oppen, 2006), such as disease pathogens. It is possible that the SE FL corals have different clades which may affect disease intervention success.

Coral mucus production could also affect treatment success. Coral mucus provides protection from UV desiccation and increased sediment loading (Brown and 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 and Bythell, 2005; Ducklow and Mitchell, 1979; Meikle et al., 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.

SCTLD 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 et al., 2008), likely playing a role in the pathology (Aeby 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 and Bythell, 2012; Work and 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 et al., 2009; Ward et al., 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, no temporal patterns of groups of specific corals getting infected in certain seasons or years were found. 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.

New infections were not consistent between corals. 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, 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. Seventy-eight percent of the treatments from June through October 2019 were conducted on just seven corals whereas fourteen corals (58%) only required treatments one monitoring period and twelve corals only required one treatment. 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.

There were no spatial patterns to new infections, indicating that differences in individual corals may be causing differing infection rates. 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 et al., 2004; Mydlarz et al., 2010; Ritchie, 2006; Shore-Maggio et al., 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 and Santavy, 2006; Page and Willis, 2008; Willis et al., 2004). Additionally, common fish corallivores like butterflyfish and wrasses can act as vectors and can actively transmit disease among relatively close coral colonies (Aeby and 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 et al., 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. However, it is certain that without disease interventions, many of these corals would have been lost. By monitoring the conditions of the large live corals along the reef monthly, I have been able to treat lesions promptly in the earliest stages of infection and to track the onset of new lesions on large corals to identify temporal and individual coral infections patterns and rule out spatial effects.

5.3. **Field test new permitted intervention techniques and materials (Task 5)**

5.3.1. Antibiotic paste versus Chlorinated Epoxy on Montastrea cavernosa

Our comparison study demonstrates that antibiotic paste (amoxycillin in Base 2b at a 1:8 ratio by weight) is a highly effective treatment for SCTLD on *M. cavernosa* when applied both to the disease margin and to a disease-break cut into healthy tissue. Chlorinated epoxy proved far less effective at halting disease progression and appeared to interfere with tissue healing over the disease-break. These results are consistent with previous reports on the ineffectiveness of chlorinated epoxy on *M. cavernosa* margins (Section 4.2; Walker and Brunelle 2019).

As discussed in section 5.2, there are numerous possible reasons for this ineffectiveness including colony morphology, tissue thickness, and increased mucous production in *M. cavernosa*. Differences in binding time may also contribute to the treatment's success. Unlike the chlorinated epoxy that binds the chlorine inside during its short hardening time (~2 hrs) rendering it ineffective, the delivery vehicle for the amoxycillin was

formulated to deliver a high dose over a 36hr period (Neely et al. 2020). It is unknown how much antibiotic is leaching out over time or if that rate is linear, however it appears to be a lethal dose to the coral cauterizing the tissues it contacts. A longer exposure time of chlorine may prove effective as well, however there are currently no proven treatment methods demonstrating this as chlorine is difficult to work with in a time release medium and reacts/dissipates quickly when exposed to seawater.

Antibiotic paste is effective at halting SCTLD lesions, however it poses a risk that it may promote antibiotic resistance in corals or other organisms and have unintending environmental impacts. There are many environmental concerns about releasing antibiotics into the ocean, however it has been done for many years in southeast Florida through the effluent of ocean outfalls. South Florida houses multiple wastewater ocean outfalls that expel over 510 million gallons per day of treated effluent (Koopman et al. 2006). The Miami-Dade Central Wastewater Treatment Plant located on Virginia Key, releases ~143 million gallons of wastewater daily (Koopman et al. 2006). This effluent is partially treated but still contains nutrients and chemicals such as hormones and antibiotics (Englehardt et al. 2001). Multiple antibiotic resistance genes, including ampC (which bestows amoxicillin resistance), were found year-round in samples taken from the water column and in the sediments around outfall pipes (Griffin et al. 2020). The ecological impact of this to the reef system is unknown, however the scale of what is released into the ocean by the outfalls dwarfs the amounts released in disease interventions.

The treatment success with amoxicillin in SE FL indicates that the bacteria infecting the corals may not have antibiotic resistance from the outfalls and other sources.
During this study, there were no measurable or observed impacts of the antibiotic treatments on the treated corals or surrounding organisms other than stopping the disease lesion progression (e.g. no fish or invertebrate grazing on material, no obvious new maladies in surrounding organisms, no new blooms or mortality). To minimize the risk of developing antibiotic resistance, we used a highly concentrated dose which killed both the pathogen and the underlying coral tissue. This dose is far less likely to foster/generate antibiotic resistance than multiple lower doses designed to boost the coral's immune response (Roberts et al. 2008). Furthermore, treating with a single high dose is more efficient and cost-effective. However, revisiting corals the day after initial treatments revealed the antibiotic paste sloughed off many treatments. It was noted that adhesion was diminished when treating the bleached tissue band proceeding the dead skeleton since the antibiotic paste does not adhere to bleached tissue as well as to bare skeleton. It also did not adhere well to parts of the disease break due to the wide smooth skeletal areas between corallites. This resulted in the treatments laying in the surrounding sediments and reef algal matrix releasing the remaining antibiotics away from the intended target. There were no observed adverse effects or impacts from this occurrence, however it is not ideal.

There were no apparent benefits provided to the individual coral colony from the antibiotic paste treatment apart from lesion cessation. The re-infection rates were similar

to each treatment group, thus we conclude that the antibiotic paste treatment did not prevent subsequent infections.

The timing of treatment failures provides useful information on when to revisit initial treatments to check for success and retreat. The majority of failed margins treatments occurred within the first nine days (Figure 28). This indicates the optimum revisiting time is around 10 - 14 days posttreatment for antibiotic paste margin treatments. This is considerably sooner than the one-month visitation (Section 4.2 of this report; Neely et al. 2020), however, with an 80-90% treatment success rate, one month is a practical re-visitation time for retreating failures and new lesions. The antibiotic paste failures were likely due to incomplete application of the antibiotic paste at the disease margin. Neely et al. (2020) found reduced success when paste was not fully contacted with tissue. It is important to cover all visibly diseased tissue where possible but recognize there will be some level of failure that may be due to the application itself.

The addition of a disease-break as a backup to margin treatment failure increased treatment success by 12.5%. When disease-breaks are used, re-visitation to check treatment failure may not be necessary. Since 50.7% of the disease-breaks healed during our study, we recommend using disease-breaks on one-time treatments where re-visitation is not planned. However, the increased success must be weighed against the increased treatment application time, materials, coral stress, and healing rates to determine if it is worth doing.

Disease-breaks filled with chlorinated epoxy were far less likely to yield tissue regrowth (almost none), likely due to the permanent presence of the epoxy. Although almost all antibiotic paste disease-breaks were bare the day after initial treatments, they were still successful in stopping the disease after the margin failed. This indicates that bare disease-breaks might be sufficient so long as tissue healing is not faster than disease progression. Bare coral-breaks would reduce the amount of antibiotics used at a specific location and would provide a cost benefit from using less paste. Neely (pers. comm.) found bare coral-breaks were not successful in a limited tank study, but this method has not been field-tested. Ocean Alchemist has recently changed the formulation of the paste to adhere better.

Almost all chlorinated epoxy margin treatments failed along with many of the associated disease-breaks. The timing between the initial treatments and disease-break failures provides information on disease progression rates. Most disease-breaks failed between 23 and 52 days while peaking at 44 days (Figure 28). On average the disease-breaks were created 5 cm away from the margins. This indicates an average disease progression rate of about 1.1 (±0.96 – 2.2) mm per day. Although not directly comparable because we did not measure area of tissue loss, our disease rate estimates seem much lower than tissue area loss reported by Aeby et al. (2019). They estimated an average daily tissue area loss of 0.82 ± 0.28 cm^2 day^{-1} of Fort Lauderdale coral colonies with the highest average percent colony loss (November–December 2017).

In summary, our research shows that the antibiotic paste is effective on stopping disease lesions on *O. faveolata* and *M. cavernosa*. Conducting this at a reef-scape scale (1-10 km²) is very costly and time consuming, especially since the treated corals may get re-infected again later. These endeavors are only effective as a stopgap measure while the larger causative agents are identified and remediated. Coral disease interventions can save corals, but at a high cost. Ideally, a treatment can be developed that bestows long-term comprehensive colony immunity with a single easy-to-administer application. One candidate is probiotic dosing; dosing the colony with healthy probiotics has been shown to halt disease progression in aquaria (O'Neil et al., 2018; Aeby et al. 2019) and may strengthen coral immunity to subsequent pathogen exposure. Two major drawbacks to using probiotics are the difficulty in application and the cost of dosing. Initial application required corals to be tented for two hours while being dosed with antibiotics. This is much longer than ~5 minutes of lesion treatments. Furthermore, dosing multiple times would increase costs per coral.

Phage therapy has been shown to control infection of multiple coral diseases (Efrony et al. 2007). Phages also possess the ability to go dormant during lulls in infection bestowing the colonies with long-term immunity. However, phages are host-specific so the pathogenic bacteria must first be identified (Atad et al. 2012) and the impact risk of using phages in the field is unknown.

We encourage others to continue testing new treatments and addressing other factors affecting coral health while we strive to keep the remaining reef-building corals alive until SCTLD has subsided or a treatment effective at a seascape scale (10 – 100 km²) is found. Ideally these are fast one-time highly effective treatments with materials that have a low risk of adverse impacts to the corals or other reef organisms that would heal quickly.

6. RECOMMENDATIONS

Continue monthly monitoring and treatment of large priority corals – Monitoring these colonies has saved many from extinction. Treating them monthly has facilitated the classification of corals based on differing infection rates.

Investigate temporal infections of the large corals with temporal changes in water quality, temperature, and other available environmental data – 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.

Continue broad-scale strike team efforts – Conducting strike team efforts to reduce the active disease prevalence and save the genetic diversity remaining on the reef.

Continue measuring comparison site infections and healing – It is important to continue monitoring the treated corals to monitor the tissue healing and new infection rates.

Continue use of antibiotic ointment CoreRx B2B and amoxicillin (1:8 weight ratio) – Perform margin treatment and disease-break interventions using antibiotic paste. This includes the large *O. faveoata*.

Limit or eliminate present epoxy treatments – Epoxy treatments are only comparatively successful on *O. faveolata* margins. The material is expensive, and treatments are time consuming and wasteful.

Do not use chlorinated epoxy on **M. cavernosa** – This study showed dismal success of this approach.

Continue testing new treatments to improve treatment success in treatments without antibiotics.

Test a bare disease-break on field corals – Amoxicillin disease-breaks were 86.7% successful in this study even though much of the material did not stay in the disease-break overnight. It is possible a bare disease-break would be just as effective. A bare disease-break would allow tissue to regrow quickly after the disease has passed. The risk is that the disease progression is slower than tissue regrowth. In which case it may not be effective in halting the disease.

Revisit strike team treated corals other than **M. cavernosa** ***and*** **O. faveolata** ***to gauge amoxicillin success on other species in SE FL.***

Use caution when revisiting corals treated with CoreRx Base 2 and amoxicillin as they can still appear diseased and the diver may retreat without knowing, especially with frequent visiting (weekly).

Continue ongoing efforts to determine the disease agent/etiology and investigate how to prevent its spread and/or treat corals to resist the disease – DEP CRCP and FWC are conducting workshops and phone calls to coordinate many coral and disease experts with managers. These efforts should continue.

Conduct restoration efforts to aid 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. We 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 outplanted strategically to help regrow tissue on recently dead large colonies.

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APPENDIX A. PRIORITY LARGE CORAL TREATMENT SUMMARY TABLE (APRIL 2018 – APRIL 20, 2020).

Coral ID	Count of Assessment Date	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 Disease-breaks Created	Sum of Margin Treatment Disease-break Retreatment	Sum of Margin Treatment Disease-break Treatment Length (cm)	Sum of # Margin Treatment Disease-breaks Tested	Sum of Margin Treatment Disease-break Treatment	Sum of Solo Disease-breaks Created	Sum of Solo Disease-break Retreatment	Sum of Solo Disease-break Treatment Length (cm)	Sum of # Solo Disease-breaks Tested	Sum of Solo Disease-break Treatment Failure
LC-001	5			9/23/2015		3		9.5		3	1		19		1	2		24.7		2
LC-002	20	1602	75		1	1		3.882												
LC-003	24	1703	20	6/25/2018		6		49.78		1	2		55.1	2						
LC-004B	10		90																	
LC-005	20	2105	50	9/14/2018	1	3		197.4								3		33.06		
LC-007	20	1797	45																	
LC-009	20	1839	70	9/11/2018	6	31	5	423.7	5											
LC-013	20	1813	34	9/11/2018	1	6		94.24								1		41.8	1	
LC-014	1				1															
LC-015	20	1815	85	9/11/2018	7	26		354.16		5						20	5	406.6	12	6
LC-016	22	1796	18	7/16/2018		4	1	36.1		1										
LC-018	25	1848	20	6/18/2018		2		24.7		1	7		324.9	9						
LC-023	9	1723	65																	
LC-024	20	1724	55																	
LC-028	19	1768	50																	
LC-034	17		90	3/1/2018		2		30.4		1	6	1	359.1	6	6					
LC-038	10		85	3/20/2018							6	1	285.76	14	12					
LC-040	8	2040	40																	
LC-041	23	1741	23	6/25/2018		2		62.7			1		39.9	1						
LC-042	20	1742	5																	
LC-043	20	1743	2																	
LC-044	24	1744	5	6/18/2018							2	2	182.4	3	2					

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LC-045	11	1745	1																	
LC-046	2		85																	
LC-047	20	1747	7	9/14/2018	2	4		19												
LC-048	20	1778	5																	
LC-049	20	1749	10	6/18/2018		1		5.7												
LC-050	20	1750	10																	
LC-051	20	1751	2																	
LC-052	24	1752	9	6/25/2018	2	3	1	58.6	1		2		98.8	1						
LC-053	20	1753	13	11/15/2018		1	1	74.1		1	1		53.2							
LC-054	20	1754	30	12/3/2018	1	3		129.7												
LC-055	10	1655	20																	
LC-056	8	1656	20																	
LC-058	20	1858	10	12/3/2018		2		26.6												
LC-059	27	1659	55	3/16/2018	2	13	2	652.6	1	1	3		104.5	1						
LC-061	9	1651	30																	
LC-062	20	1922	65	9/10/2018		1		11.4												
LC-063	10	1653	50																	
LC-064	10	1879	70																	
LC-065	9	1565	10																	
LC-066	19	1816	11	12/4/2018		1		5.7												
LC-067	8	1567	40																	
LC-070	27	1570	82	5/7/2018	2	12	1	210.14		1										

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LC-074	10	1574	70																	
LC-075	20	1775	80		2	4	2	133	1	1										
LC-077	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	1778	10	9/12/2018		3		22.8												
LC-079	20	1699	50																	
LC-080	20	1660	20	9/10/2018		2		36.1			1		68.4							
LC-084	18	1684	75		2	2		19												
LC-085	24	2085	72	12/19/2017		8		172.9			2	1	123.5	3	1	1		49.4	1	
LC-087	18	1687	76	11/12/2015	2	5		68.4			2		53.2	1						
LC-088	10	1688	45																	
LC-090	20	1690	30																	
LC-092	10			3/1/2018																
LC-093	6			6/4/2018		6				4	11				5					
LC-098	20	1698	65	4/15/2019	5	5	9	294.5	5							1	5	609.6	2	6
LC-101	20	1801	45	9/10/2018		25		248.9		8										
LC-103	20	1803	65		4	12	3	191.9	3											
LC-110	20	1810	60	12/4/2018		11		77.9								2	5	203.3	7	3
LC-114	20	1814	31		1	1		7.6												
LC-115	19	2115	40																	
LC-116	29		78	3/21/2018		1		19		1	5	1	419.9	8		1	2	152	1	1
LC-117	20		35	7/16/2019		3	1	57		1										
LC-118	20	1918	55	9/11/2018	1	25		387.6								6		336.3	1	

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LC-119	20	1919	81	10/25/2018		7		102.6								2		24.7		
LC-120	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		85	5/10/2018							1		38	1	1	5	1	131.1	6	6
LC-121A	10		10	6/1/2018												1		22.8	1	
LC-121B	10		5	6/1/2018												1		19	1	
LC-122	27	1822	41	5/8/2018	1	6	1	114			2	1	159.6	1	3					
LC-123	28	1923	90	4/5/2018		4		117.8		4	23	2	1436.4	25	25	6		190	6	4
LC-124	25	1924	50	3/4/2018		2		91			1		26.6	1						
LC-125	19	1825	65																	
LC-126	20	1726	30																	
LC-127	20	2127	50																	
LC-128	20	2128	65																	
LC-129	24	2129	65	6/4/2018							1		45.6							
LC-157	10	1557	55	6/4/2018	6	22	4	315.4	4											
MC-001	10	2101	10					0												
MC-002	10	1552	26	11/12/2019	1	1		7.6												
MC-003	10	1893	2																	
MC-004	9	2404	10																	
MC-005	9	2405	45																	
MC-006	10	1806	15																	
MC-007	9	1868	30																	
MC-008	9	1869	50																	

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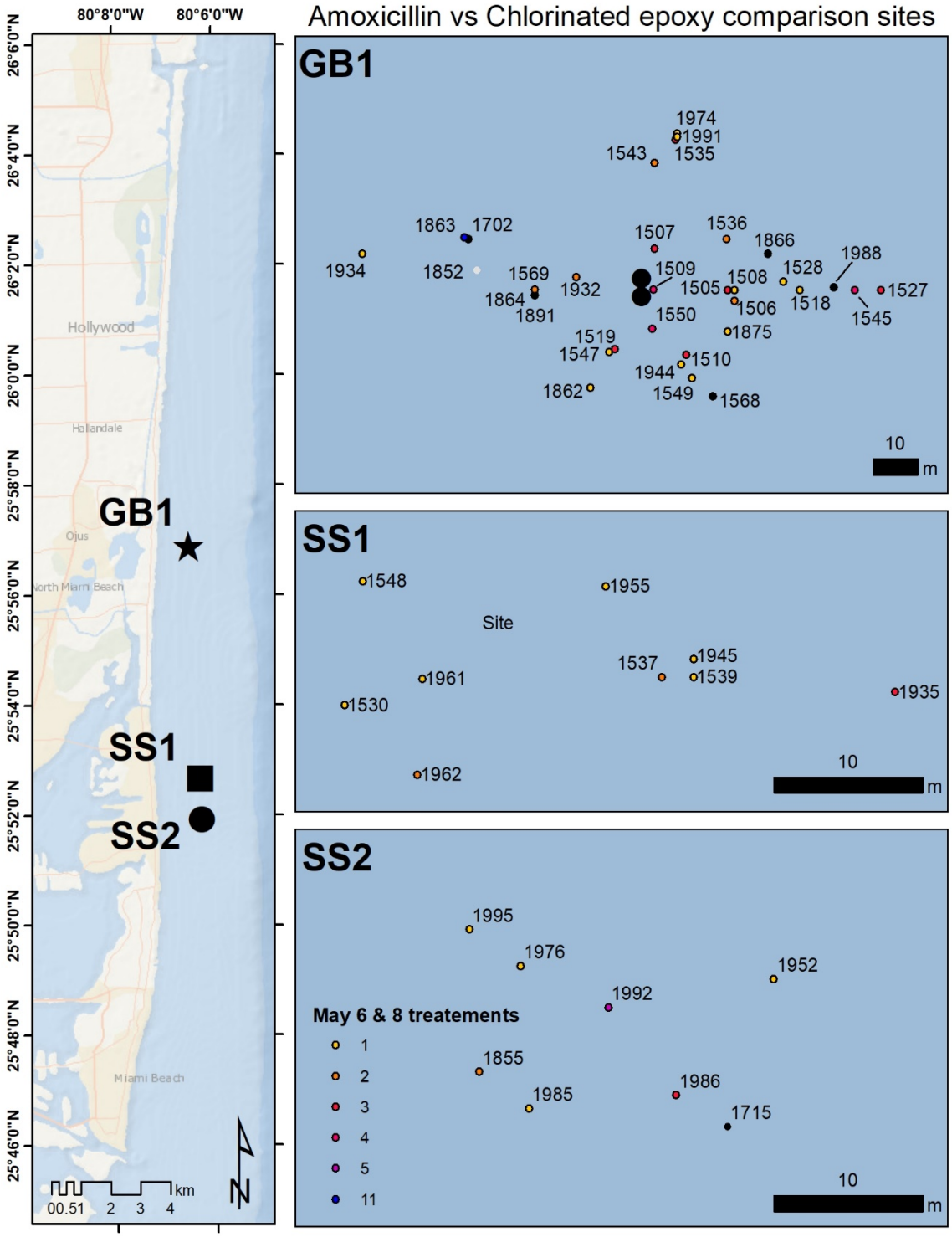
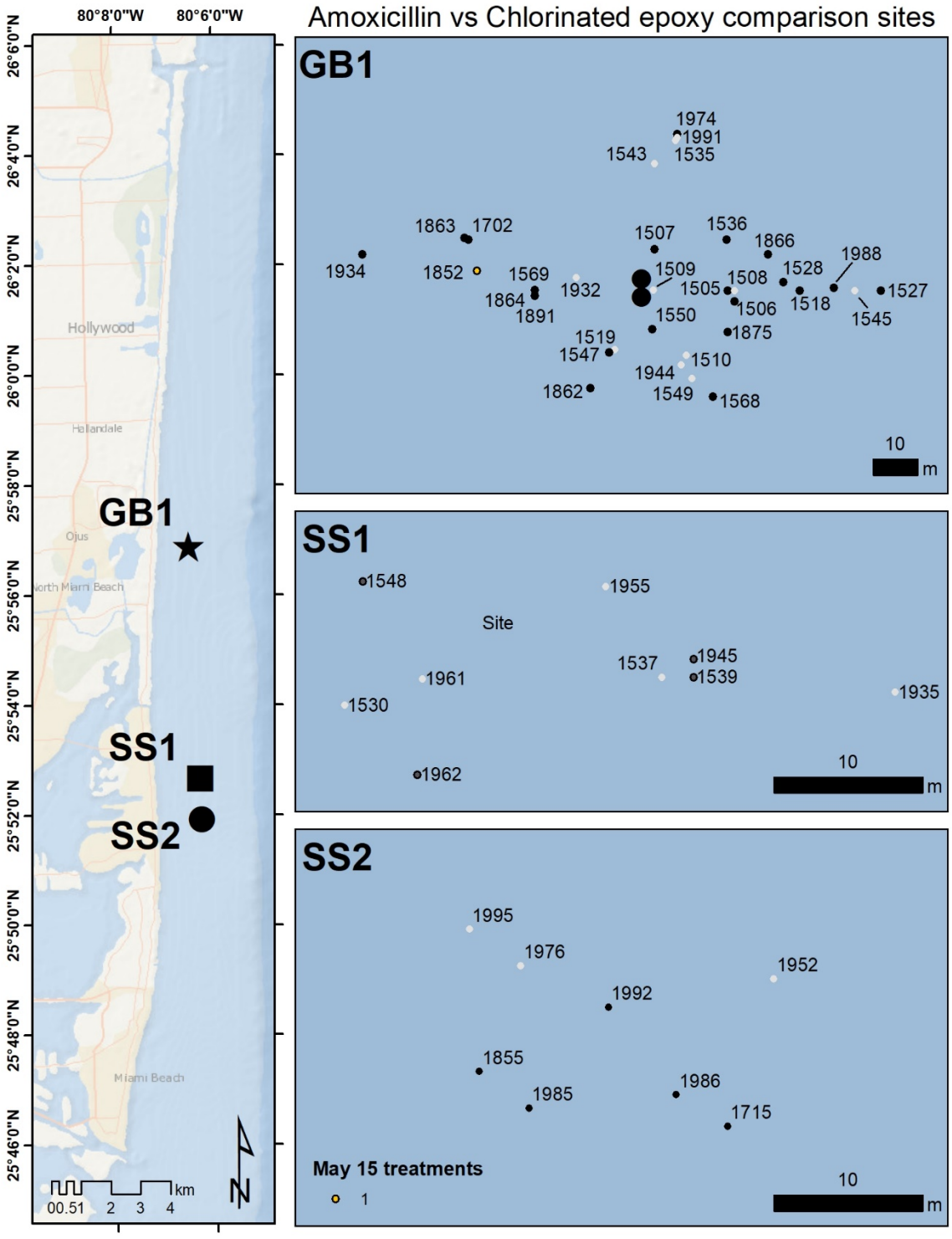
Coral ID	Count of Assessment Date	Tag #	% Total Mortality	Initial Infection	Number of Antibiotic Ointment Treatments	Sum of New Margin Treatment	Sum of Margin Retreatment	Sum of Margin Treatment Length (cm)	Sum of Amox Margin Treatment Failure	Sum of Epoxy Margin Treatment Failure	Sum of Margin Treatment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

APPENDIX B. A SERIES OF MAPS OF THE *M. CAVERNOSA* TREATMENT COMPARISON SITES SHOWING THE SPATIAL DISTRIBUTIONS OF NEW INFECTIONS EACH MONITORING PERIOD.




Amoxicillin vs Chlorinated epoxy comparison sites
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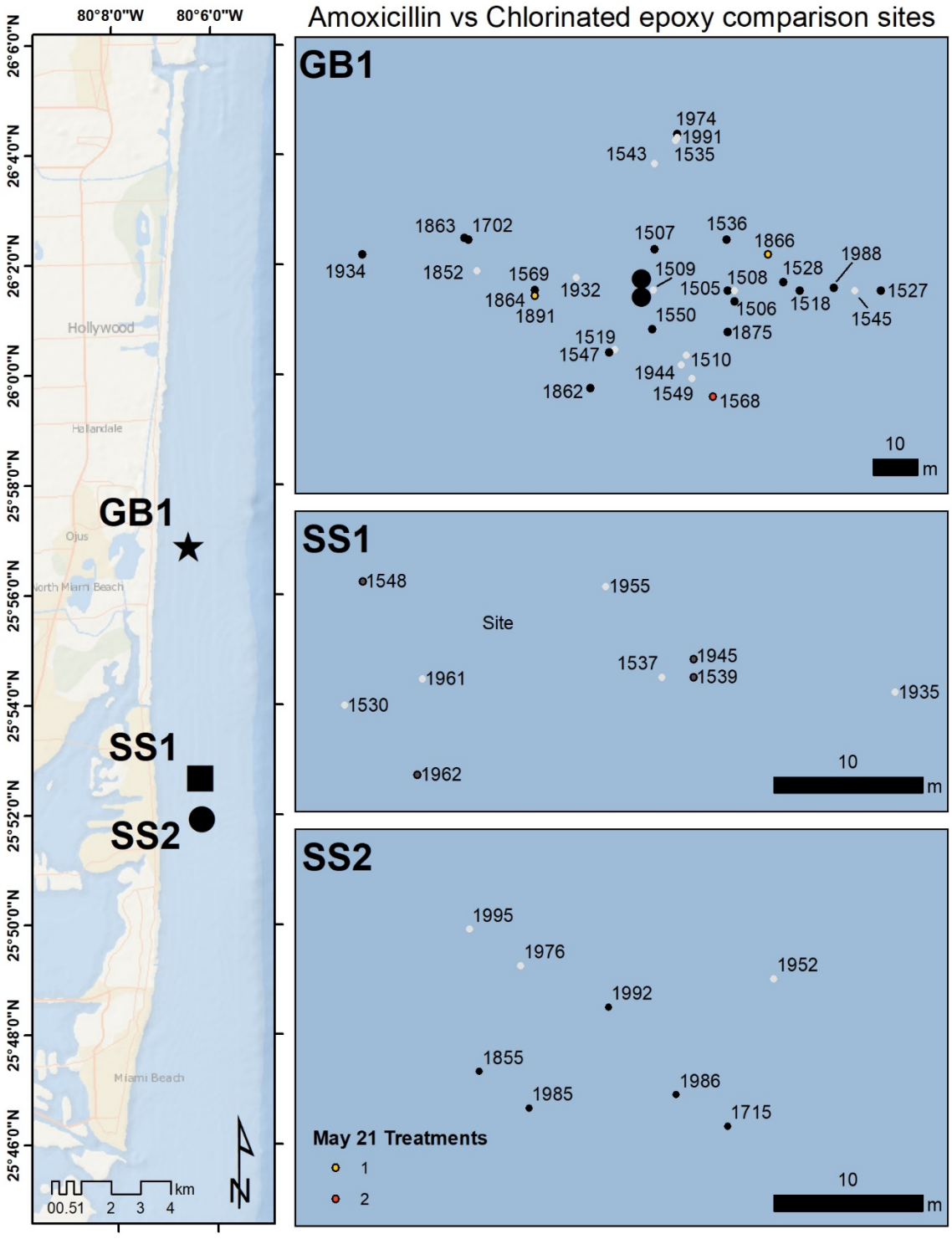
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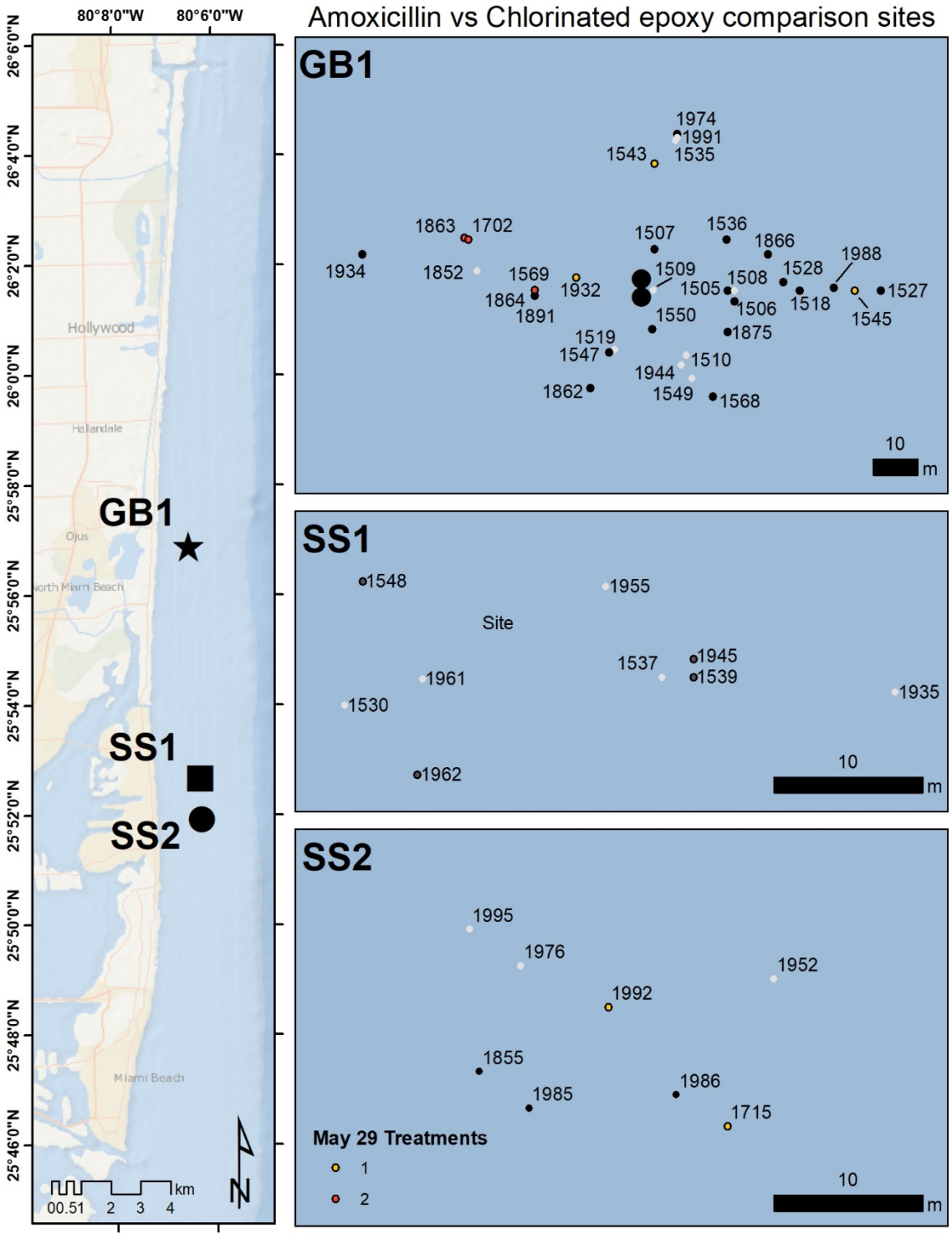
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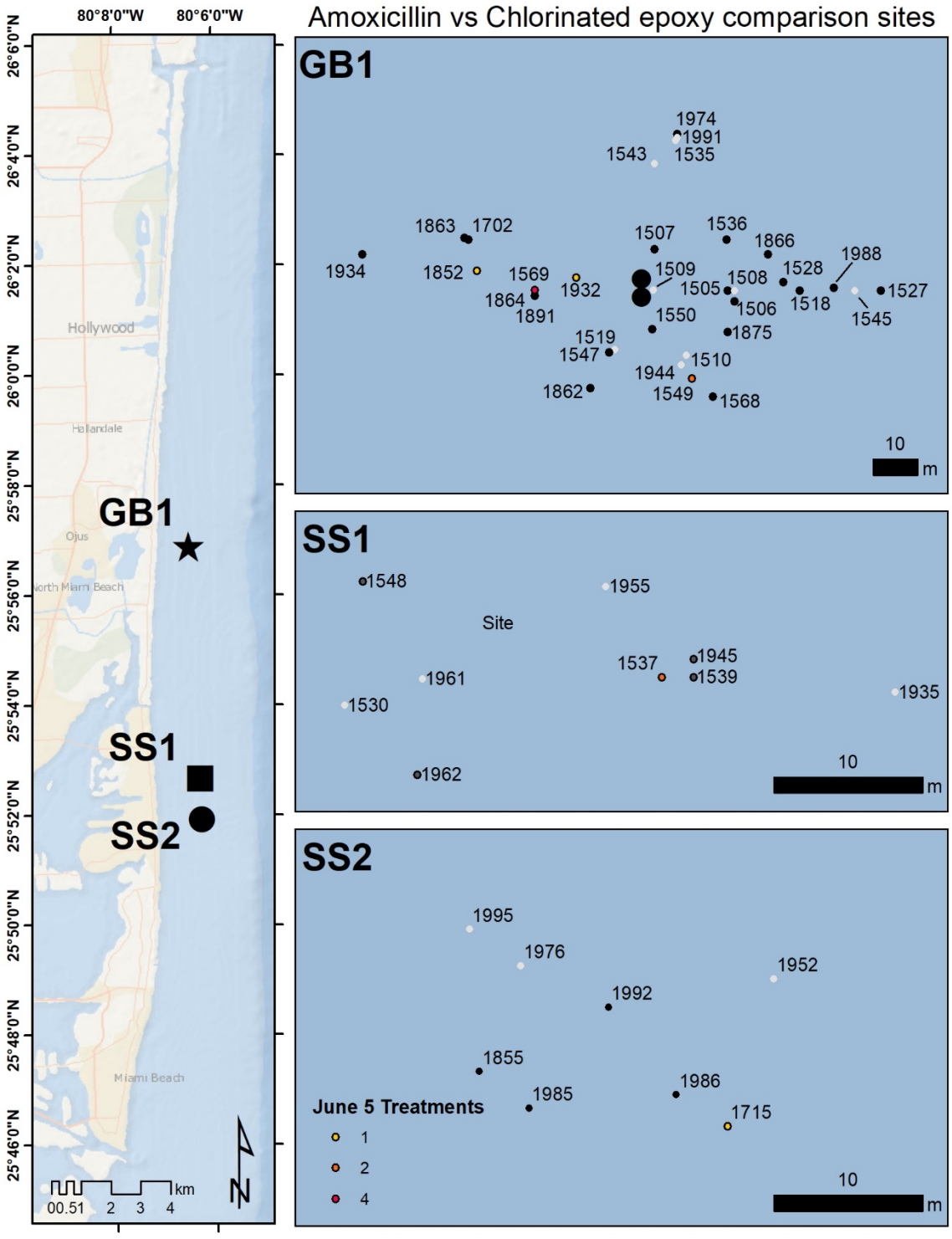
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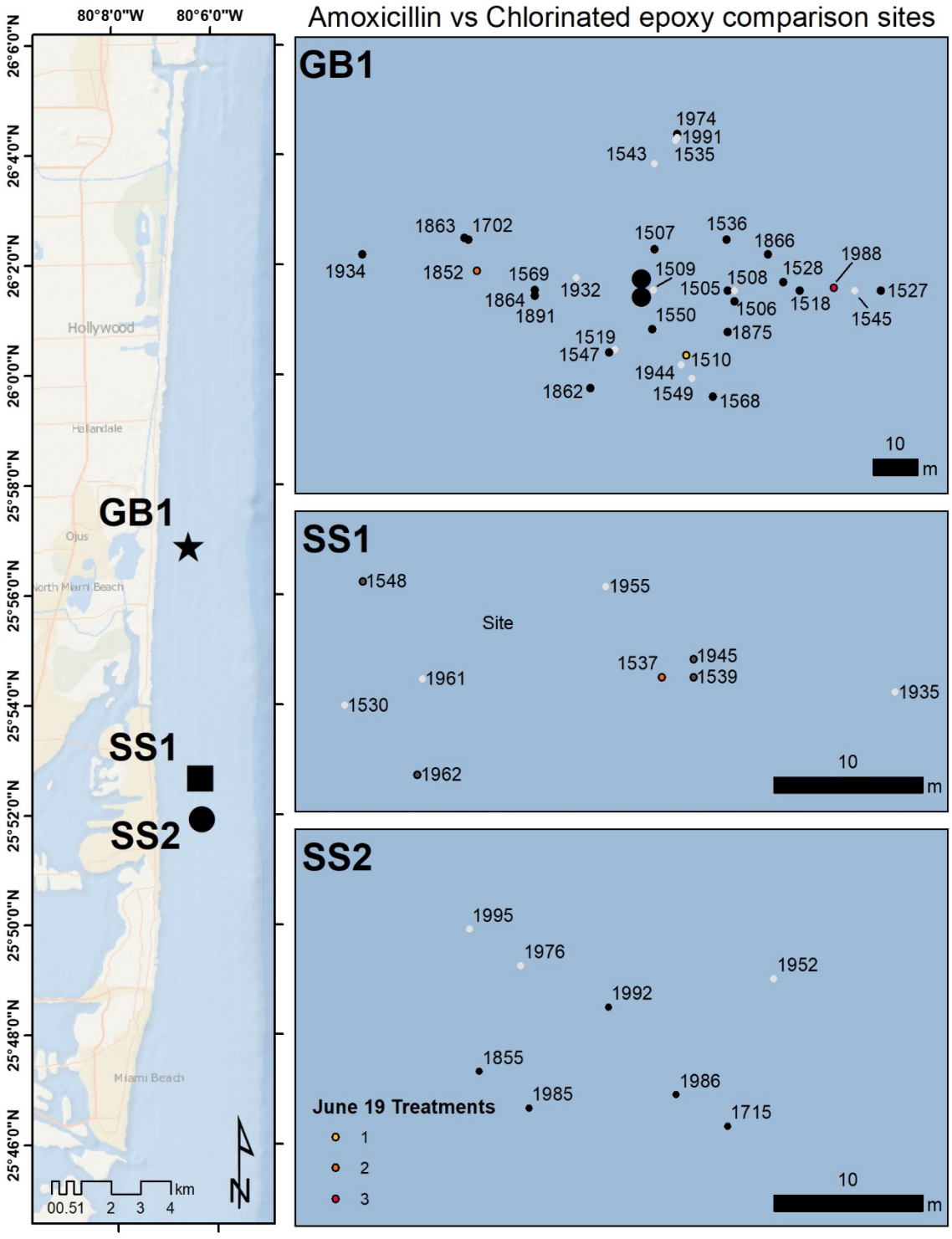
Amoxicillin vs Chlorinated epoxy comparison sites
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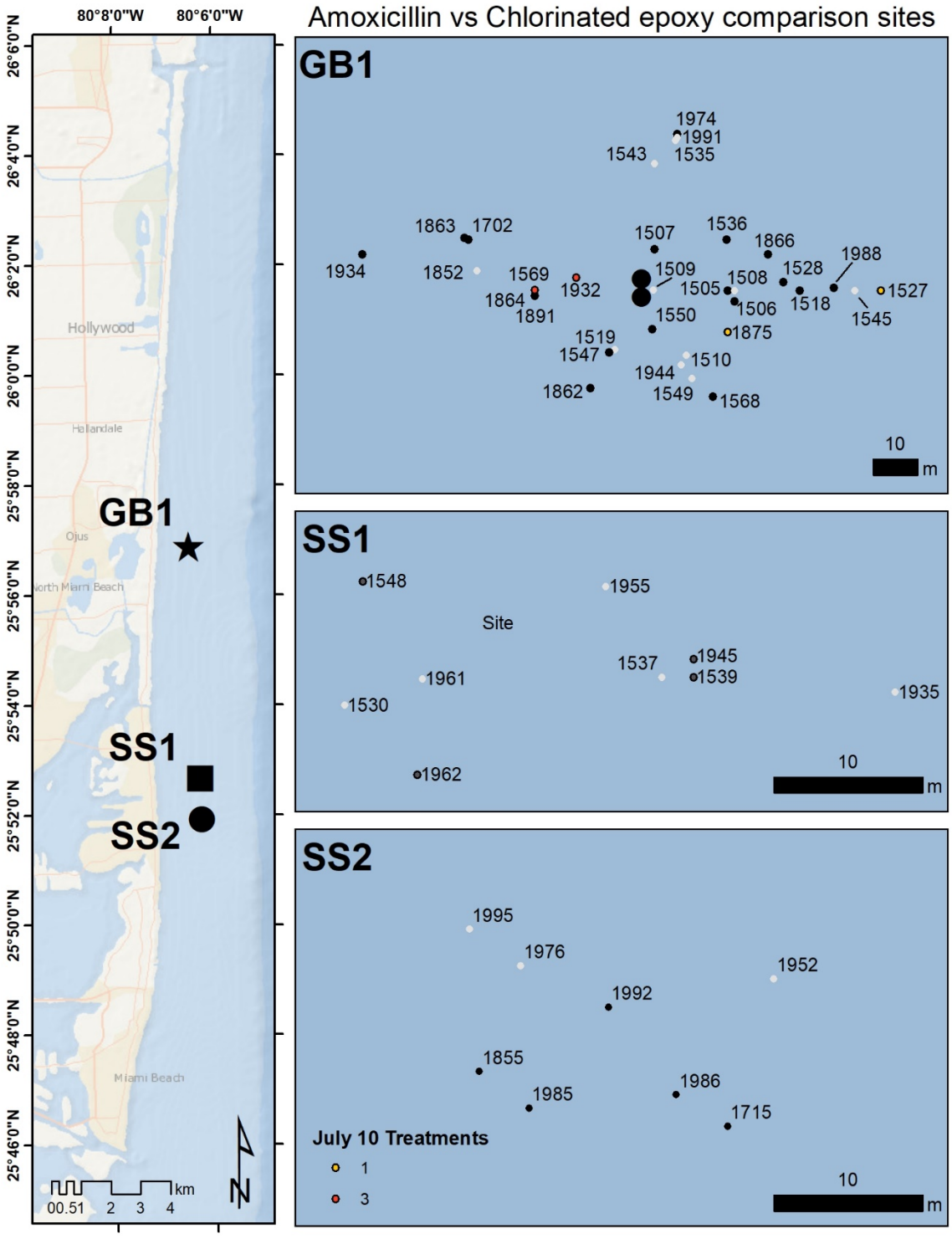
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25°58'0"N
25°56'0"N
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25°52'0"N
25°50'0"N
25°48'0"N
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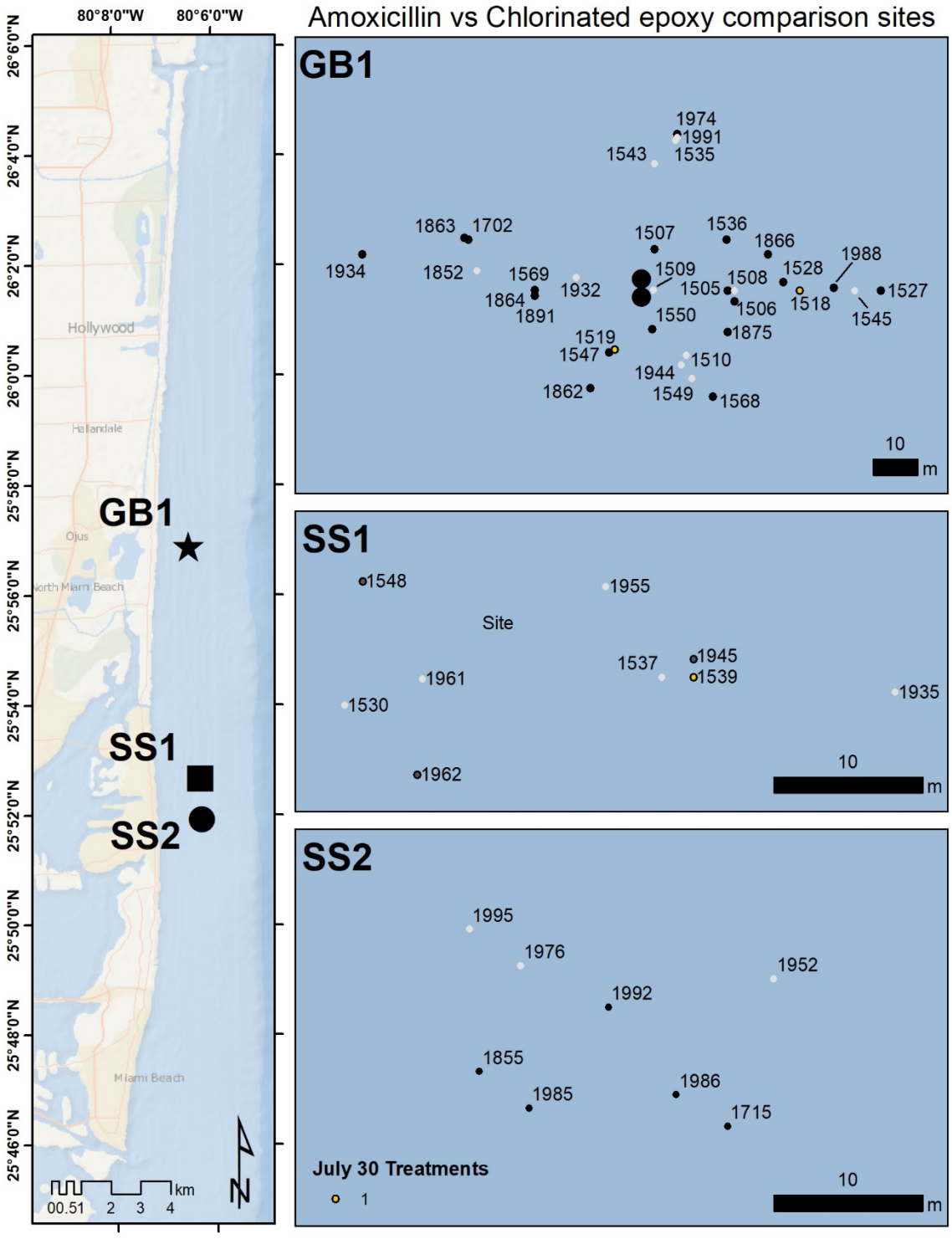
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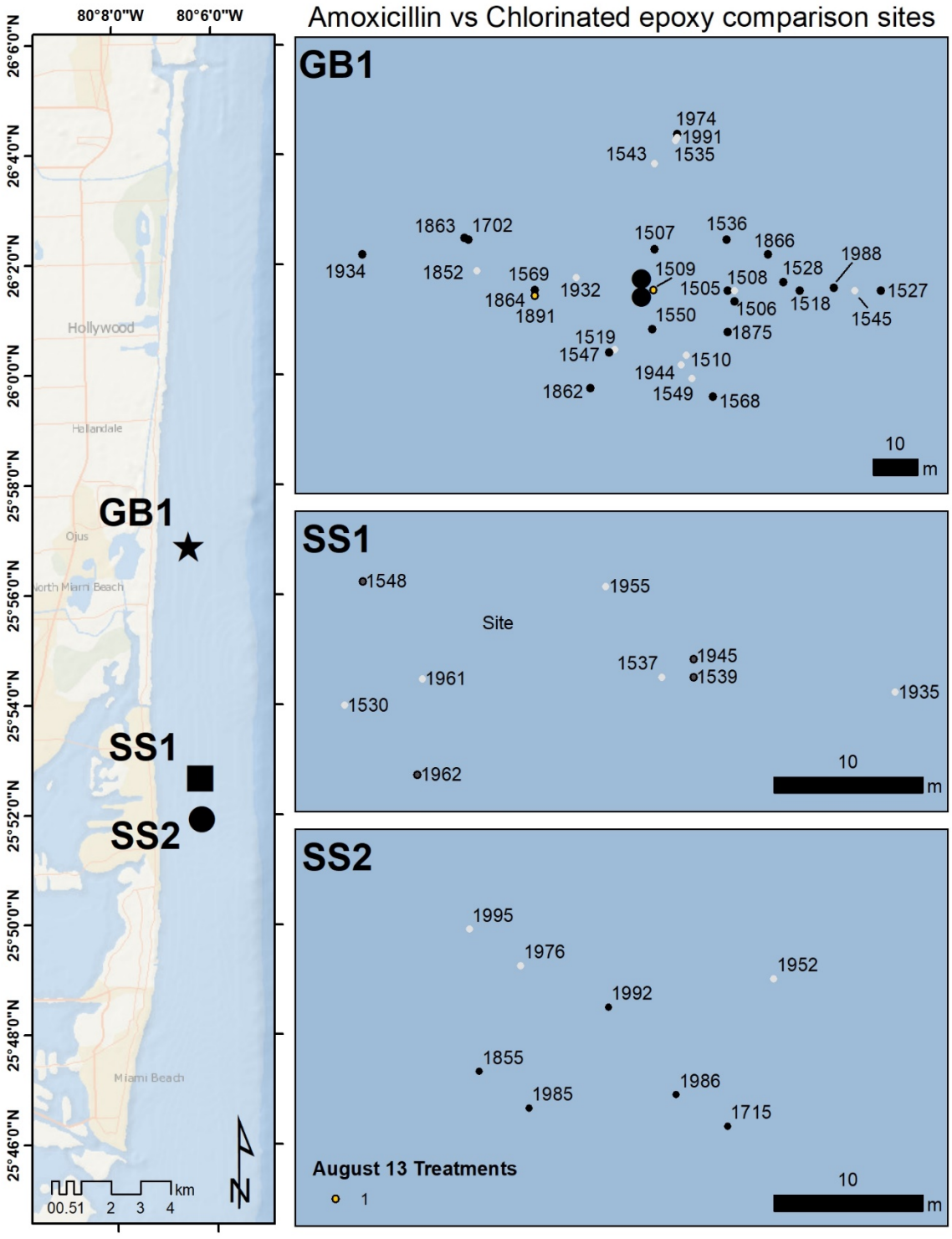
Amoxicillin vs Chlorinated epoxy comparison sites
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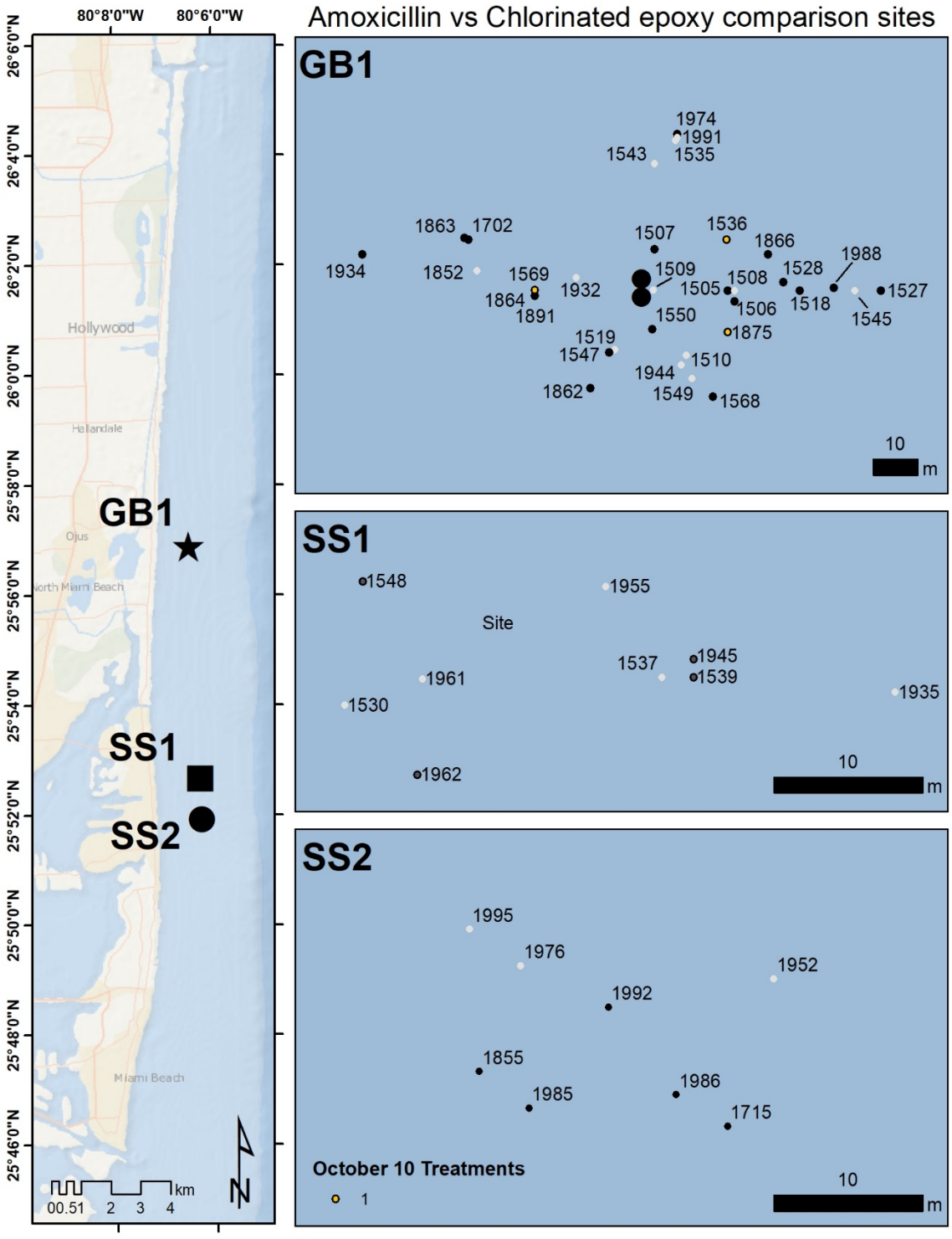
Amoxicillin vs Chlorinated epoxy comparison sites
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