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One-Time Broadscale SCTLD Intervention Effectiveness on Montastraea cavernosa in an Endemic Zone

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HALMOS COLLEGE OF ARTS AND SCIENCES

One-time broadscale SCTLD intervention effectiveness on *Montastraea cavernosa* in an endemic zone.

By

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Abstract

Disease intervention using CoralCure with amoxicillin is effective at stopping stony coral tissue loss disease (SCTLD) lesion progression, however, it does not provide protection against reinfection. Prolonging colony survival may require regular visits to treat new lesions should they appear. Intervention activities in southeast Florida were allocated between regular treatment and monitoring of selected priority colonies and broadscale disease interventions. The latter involved strike team divers, who worked in small groups to cover large areas of reef treating all SCTLD lesions observed, tagging the colonies, and recording their locations via a floating GPS. Broadscale disease intervention efforts aimed to maximize the area covered and treat as many disease lesions as possible without the intent of returning to monitor treatment success. Between 2018 and 2023, over 1,800 colonies of 12 different species were treated at over 280 sites. We evaluated this strategy's effectiveness in preventing colony mortality by revisiting 178 *Montastraea cavernosa* colonies treated once after a year or more prior and recording their condition. 94% of all *M. cavernosa* colonies were still alive at the time of revisit. Categorizing colonies into elapsed timeframes since treatment yielded high proportions of survival: 100% of colonies treated 1-2 years prior, 97% treated 2-3 years prior, and 87% treated 3-4 years prior. The average percent decrease in live tissue coverage was 18%, 20%, and 30% after 1-2, 2-3, and 3-4 years, respectively. Compared to reported natural SCTLD senescence of about 30%, one-time broadscale interventions provide prolonged colony survival reducing the burden of post-hoc restoration.

Keywords: Amoxicillin, Colony Condition, Tissue Loss, In-situ, Southeast Florida

Acknowledgements

Thank you to the Florida Department of Environmental Protection's Office of Resilience and Coastal Protection (FDEP ORCP) and NOAA CRCP for funding and supporting these efforts. Thank you to Miami-Dade Regulatory & Economic Resources for all their assistance treating corals in the field and time spent on the water. My committee members: Dr. Brian Walker, Dr. Karen Neely, and Dr. Greta Aeby for all their valuable input. The NSU GIS and Spatial Ecology lab for treating corals during broadscale disease intervention dives and all their in-water assistance finding previously treated corals.

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Introduction

Coral tissue loss diseases were first described in the Caribbean in the 1970s (Richardson, 1998) and have been continuing to increase in prevalence and severity (Harvell et al., 2007). Among other environmental stressors, disease has shifted stony coral species assemblages (Hayes et al., 2022) and reduced genetic diversity (Weil & Rogers, 2011). Acroporid species that once dominated the reef crests and forereefs were essentially extirpated from white band and white-pox disease (Aronson & Precht, 2001; Patterson et al., 2002; Porter & Meier, 1992). White plague and black band disease continue to have devastating impacts on brain and star corals (Jones et al., 2012; Rutzler et al., 1983), however, the most impactful disease in affected parts of the Caribbean has been stony coral tissue loss disease (SCTLD).

SCTLD was first described off the southeast coast of Florida in 2014 near Miami, Florida (Jones et al., 2021; Precht et al., 2016). The initial outbreak of the disease occurred during the 2014-2016 summer bleaching events and a sedimentation event attributed to 2013-2015 dredging project in the Port of Miami. As of May 2024, the causative agent of SCTLD is unknown. It is hypothesized that the disease impacts the zooxanthellae within the polyps and is accompanied by a secondary bacterial infection affecting the lesion area (Landsberg et al., 2020; Work et al., 2021), or SCTLD could be mainly bacterial-based and include different bacterial interactions (Iwanowicz et al., 2020; Rosales et al., 2023). SCTLD is characterized by singular or multifocal lesions that appear at the base or throughout the colony. Disease lesions are accompanied by sloughing of necrotic tissue and, in some species, bleached tissue along the disease margin. Lesion progression rates vary by species due to differences in species susceptibility and often result in whole colony mortality.

Species are classified as highly-, intermediately-, and lowly-susceptible and share similar characteristics of lesion progression rates and start of infection during an outbreak between susceptibility groupings. Highly susceptible species display lesions first and total colony mortality can occur within one week to one to two months. Intermediately susceptible species display lesions about one month after highly susceptible species with smaller colonies experiencing total colony mortality after a couple months and larger colonies exhibiting waxing and waning of lesions and

new lesions occurring over years. Lowly susceptible species do not exhibit disease lesions and are presumed to be tolerant to SCTLD. Due to the differences in infection rate and lesion progression, highly susceptible species in southeast Florida were impacted the most by the onset of SCTLD with intermediately susceptible species displaying persistent SCTLD lesion infections over time. The epizootiology of SCTLD has three distinct phases that is started by a few infected colonies, expanded into a reef-wide outbreak, and continued to persist in the environment for years despite reduction in coral cover (Croquer et al., 2021). SCTLD disease incidence in southeast Florida peaked in 2016 and is still prevalent in the reef ecosystem, constituting southeast Florida an endemic zone (Croquer er al., 2021; Hayes et al., 2022; Toth et al., 2024; Walker et al., 2021).

The loss of corals due to SCTLD has perpetuated the rapid need for large-scale coral reef restoration in Florida. The majority of widely established coral restoration efforts have focused on reintroductions of individuals through direct transplantation, coral gardening, micro-fragmentation, asexual propagation, and larval enhancement (Boström-Einarsson et al., 2020). Motivations for conducting coral restoration studies range from experimental reasons to biodiversity enhancement (Bayraktarov et al., 2019; Boström-Einarsson et al., 2020; Moriarty et al., 2020). Conducting and assessing widely established coral restoration success and large-scale effectiveness is costly and time consuming due to high mortality rates, associated costs, and small spatial-temporal scales. (Bayraktarov et al., 2019; Boström-Einarsson et al., 2020; Moriarty et al., 2020). The above coral restoration efforts solely focus on replenishing the reef post-degradation utilizing new individuals (Moriarty et al., 2020). However, an emerging field within coral restoration is disease intervention.

Disease intervention aims to proactively treat infected colonies in efforts to stop coral mortality. This method utilizes preventative measures to reduce the loss of genetic diversity, biodiversity, natural colony density, and cover. Controlling disease and natural mortality from ever-increasing disease outbreaks has been an issue in the field of traditional restoration that has led to a decrease in the success of reintroduction of coral individuals back on the reef (Moriarty et al., 2020). Disease intervention helps alleviate the need for species reintroduction by aiding diseased colonies survival. Disease intervention on individual corals also appears to have an

indirect positive impact on untreated, infected corals offering a small benefit on a community scale (Forrester et al., 2022; Toth et al., 2024).

Historic literature on coral disease interventions is sparse. Treatment methods include disease lesion removal and other biological, chemical, and mechanical techniques (Neely et al., 2021). Most studies conducted on disease intervention have involved mechanically removing or separating diseased tissue from healthy tissue (Neely et al., 2021) utilizing various techniques resulting in mixed effectiveness (Miller et al., 2014; Muller & Van Woesik, 2009; Randell et al., 2018; Williams, 2013). The first published in situ disease intervention was conducted on mounding corals infected with black band disease (Hudson, 2000). In 1987, an aspirator device was created to remove and collect the microbial mat associated with black band disease and modeling clay was used to smother the disease lesion underneath in situ (Hudson, 2000). Other disease intervention methods conducted in situ include using phage therapy to slow tissue loss from white plague (Atad et al., 2012) and mechanical trenching and chlorinated epoxy to reduce tissue loss and stop lesion progression from black band disease (Aeby et al., 2015). In situ treatments, on colonies in the natural environment, allow for monitoring of colony health though various environmental changes and stressors, help preserve colonies along the reef tract, and any size coral can be treated. This method can be challenging due to the logistics of field operations, the inability to isolate colonies from diseased environments, and the difficulty of dosing whole colonies.

Disease intervention conducted ex situ has included using phage therapy to prevent transmission of white plague (Efrony et al., 2007), ampicillian or paromomycin sulfate to treat white band (Kline & Vollmer, 2011; Sweet et al., 2014), and probiotics for treating SCTLD (Ushijima et al., 2023). Ex situ treatments, in onshore nursery systems, allow for continual monitoring and the control of external variables. However, coral collection, transportation, and tank maintenance are labor intensive and are only feasible with smaller colonies.

As SCTLD quickly spread along the southeast Florida reef tract, in situ disease intervention was established. In 2018, chlorinated (Chl) epoxy was permitted to treat SCTLD in southeast Florida based off success observed in treating black band disease (Aeby et al., 2015). Chlorinated epoxy consisted of two-part marine epoxy premixed with chlorine powder (Walker et

al., 2021). In 2019, amoxicillin was permitted for use on SCTLD-infected colonies in southeast Florida and became the main treatment type being more effective at halting disease lesion progression than Chl epoxy (Neely et al., 2021; Walker et al., 2021). With the overwhelming scope of this outbreak, two main disease intervention strategies in southeast Florida were developed to best use funding and allocate resources efficiently. Resources were assigned to priority coral monitoring and broadscale disease intervention. Priority coral monitoring entailed visiting the largest corals in the region monthly to document colony condition and treat disease lesions (Walker et al., 2023). These large corals have been deemed ecologically significant due to their size, age, and fecundity (Walker et al., 2023). Monthly monitoring is necessary to ensure disease treatment success and to stop the progression of any new lesions, as treatment does not prevent reinfection (Neely et al., 2021; Walker et al., 2021).

Broadscale disease intervention was established in response to the high disease prevalence and extensive coral mortality observed along the reef. The objective was to treat as many SCTLD-infected colonies along the reef tract without the intent to monitor treatment success (Toth et al., 2024). A one-time treatment was applied to infected coral colonies to halt lesion progression, assist in colony survival, and preserve tissue coverage.

Studies assessing treatment success of SCTLD disease intervention treatments typically had a monitoring range of one month to two years (Neely et al., 2020; Walker et al., 2021). These studies consisted of colonies treated multiple times on a single lesion or on new lesions that appeared throughout the study period (Neely et al., 2020, Walker et al., 2021). There is a lack of evidence on the long-term effectiveness of one-time antibiotic treatments on SCTLD-infected colonies. As the field of disease intervention continues to grow, it is vital to understand the impacts broadscale disease intervention has on long-term colony health.

This project aimed to evaluate the long-term effectiveness of a one-time antibiotic treatment, known as CoralCure, on *Montastraea cavernosa* colonies by revisiting a subset of corals previously treated one-time for SCTLD. Analyzing changes in colony condition at the time of treatment in comparison to their condition at the time of revisit provides a better understanding of how these colonies are faring in the long-term. Results from this study will be used to inform

management on this strategy's effectiveness and used to improve the allocation of resources used for disease intervention strategies.

Materials and Methods:

Broadscale Disease Intervention:

Broadscale disease interventions started in 2018 and were conducted in the southeast Florida Reef Tract spanning between Hillsboro Inlet, Pompano Beach, Florida to Biscayne National Park (Figure 1). Site selection, CoralCure materials, and broadscale disease intervention dive methods were equivalent to those outlined in Toth et al. (2024).

As of December 2023, a total of 1,805 colonies were treated for SCTLD along the Florida reef tract (Figure 2). The total number of treatments by species were 1,406 *Montastraea cavernosa*, 169 *Orbicella faveolata*, 60 *Pseudodiploria clivosa*, 58 *Pseudodiploria strigosa*, 44 *Colpophyllia natans*, 29 *Solenastrea bournoni*, 17 *Diploria labyrinthiformis*, 7 *Stephanocoenia intersepta*, 5 *Dichocoenia stokesii*, 4 *Mycetophyllia aliciae*, 3 *Siderastrea siderea*, 1 *Orbicella franksi*, 1 *Porites astreoides*, and 1 *Orbicella annularis*. Out of the 1,805 colonies, 1,680 (93.1%) were treated with CoralCure, 109 (6%) corals were treated with chlorinated epoxy, and 16 (0.9%) corals were treated with CoreRx B2B without antibiotics that were unsuccessful. A total of 1,030.32 meters of antibiotic paste treatments, 68.59 meters of chlorinated epoxy treatments, and 6.4 meters of CoreRx Base treatments were performed totaling 1,105.31 meters.

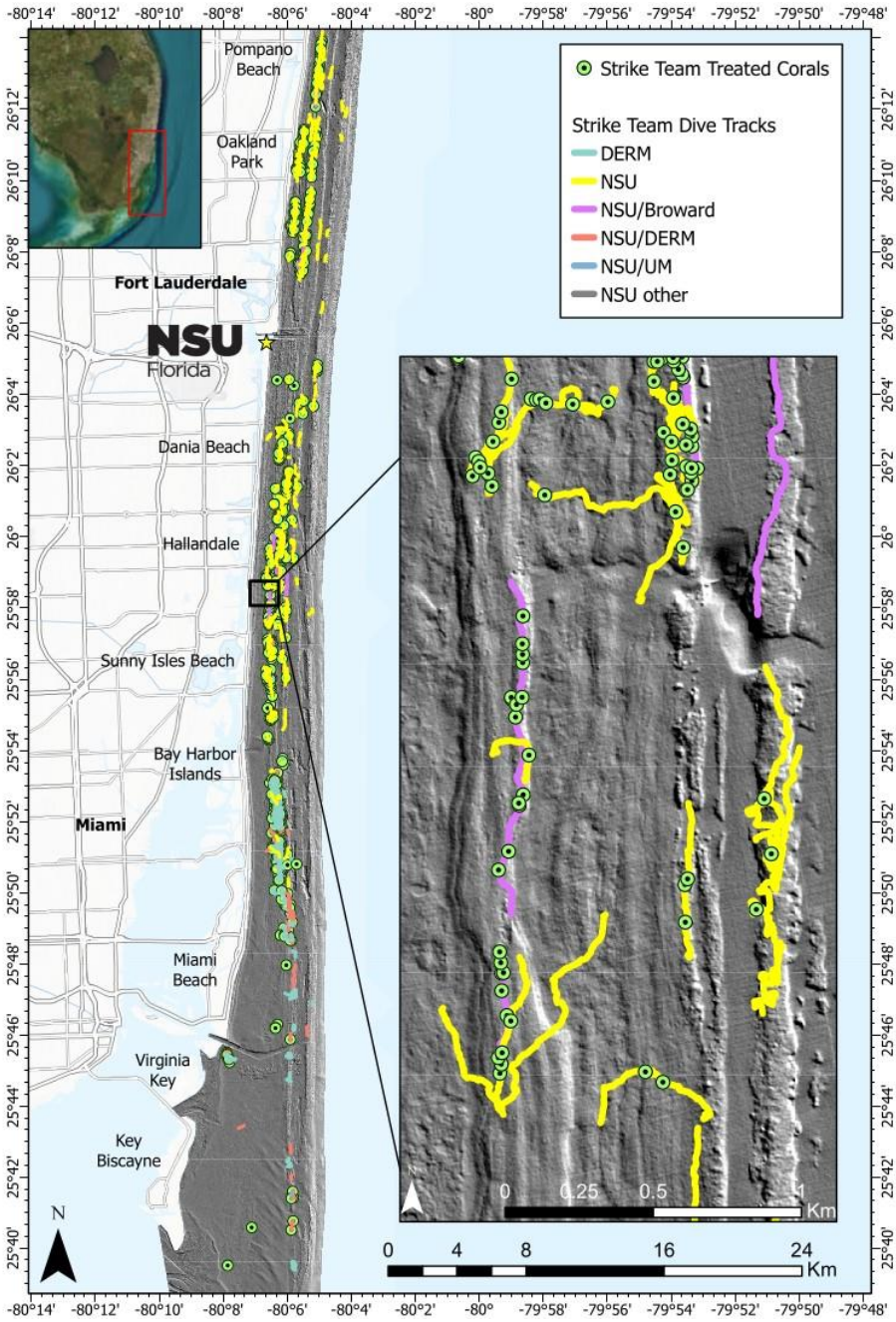

Figure 1. Map of strike team treated corals and dive tracks from January 2019 to December 2023. Close-up map frame showcases the dive tracks and treated corals occurring along areas of topographical relief indicative of hard bottom. Green points represent the locations of individual colonies treated for SCTLD. Strike team dive tracks are color-coordinated by the organization that completed that dive. NSU is abbreviated for Nova Southeastern University. DERM is Miami-Dade county's Department of Environmental Resources Management. Broward is Broward County. UM is University of Miami.

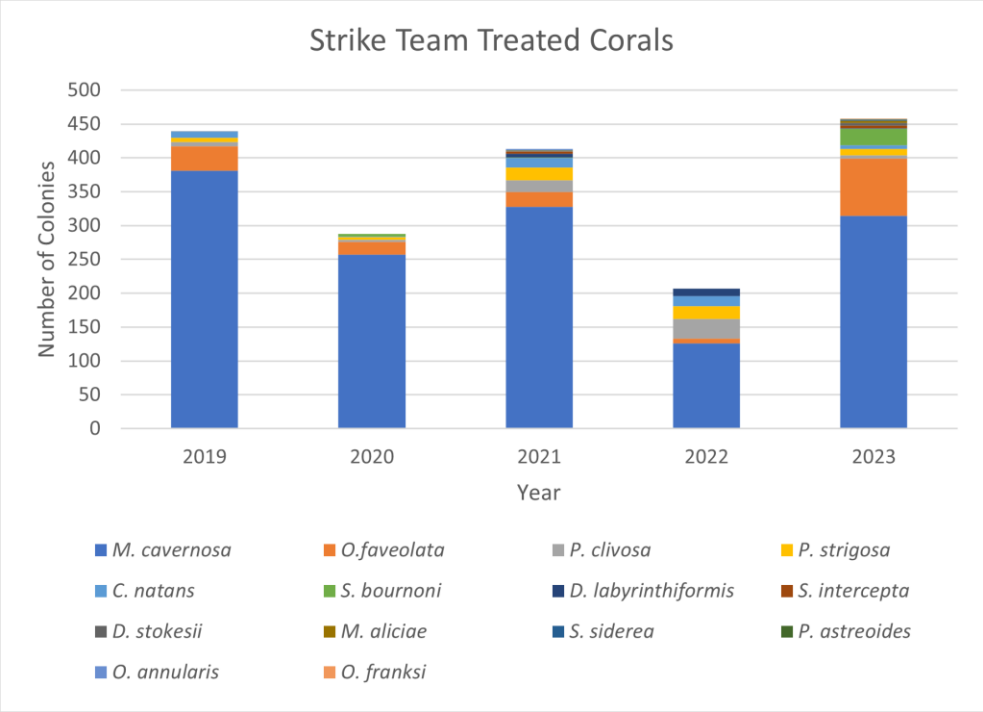

Figure 2. Total strike team efforts from January 2019 to December 2023 represented by the species treated.

Treatment Application:

Two disease intervention methods were used. One treatment method consisted of smothering the disease lesion only with CoralCure. The treatment type was applied where the colony exhibited recent morality and sloughing of live tissue. The amoxicillin paste was applied in a band anchored to the dead, exposed skeleton and over the adjacent live tissue along the colony's disease lesion. The band would cover the adjacent row of live, visually-infected polyps insuring contact with the remaining live colony.

The other disease intervention method utilized a disease break and smothering of the disease lesion. A disease break was applied about 5 cm above the disease margin into live tissue on the colony. The disease break was about 1 cm deep and created using a Nemo underwater angle grinder with a 11.4 cm masonry grinding disk (Figure 3). The disease break was then filled with CoralCure and the disease lesion was also treated as per the methods mentioned above. The purpose of the disease break was to isolate the remaining live colony from the active lesion.

Disease breaks were not always used when treating colonies with SCTLD. Disease breaks were applied when colonies exhibited extensive, rapid mortality characterized by large swathes of exposed skeleton that had not been colonized by algae or other biofouling species. Disease breaks were also applied when the treatment type was not sticking to the live tissue well. Due to polyp morphology, mucus production, and the extent of tissue breakdown of the polyps adjacent to the disease margin, the treatment type may not be able to fully adhere to live tissue reducing treatment application success.

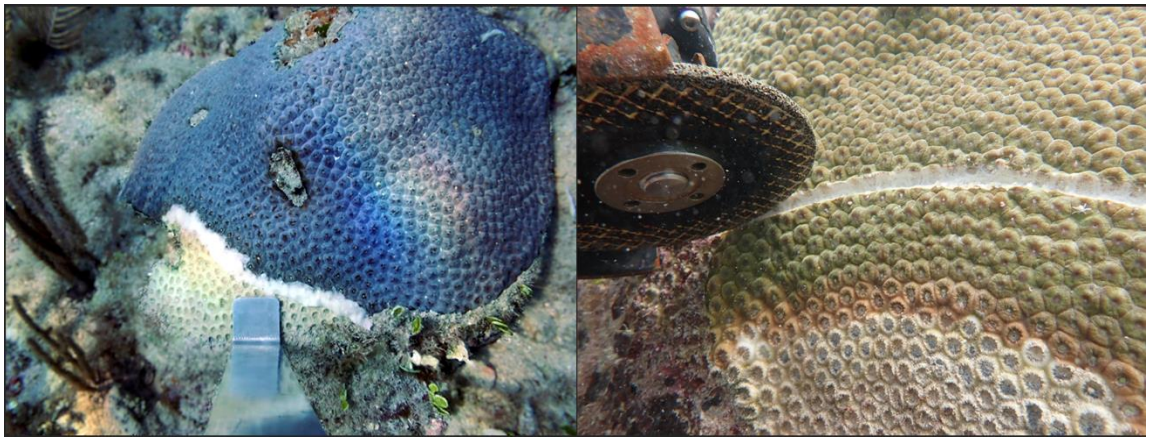

Figure 3. One treatment method included smothering of only the disease lesion (left). The other treatment method included implementing a disease break then applying the treatment type inside the disease break and along the disease lesion (right). The treatment type has not yet been applied (right).

Colony Revisits:

The locations of previous strike team corals treated with CoralCure were analyzed to determine the number of days since treatment. Corals that were previously treated with antibiotic paste at least 365 days prior were plotted in ArcGIS Pro to aid in the identification of high-density sites to target for revisits. High density sites allowed for the highest number of corals to be revisited during a single dive day. The number of days since treatment were analyzed to determine timeframe groupings for analysis. Revisits were planned to achieve a comparable sample size for each timeframe.

Revisit divers were equipped with an underwater navigation map to assist in finding old strike team corals (Figure 4). Underwater navigation maps contained compass headings and distance between colonies to allow divers to successfully swim from one colony to the next. Teams were mainly looking for SEAFAN tags attached to live or dead coral colonies in the area relative to the underwater map. SEAFAN tags were bright yellow when first attached to the colonies but were overgrown by algae during their duration underwater. The unnatural, bell-like shape of the SEAFAN tag was the main thing divers looked for (Figure 5). Once a tag was found, divers would verify the colony ID number and record various metrics on the coral's condition.

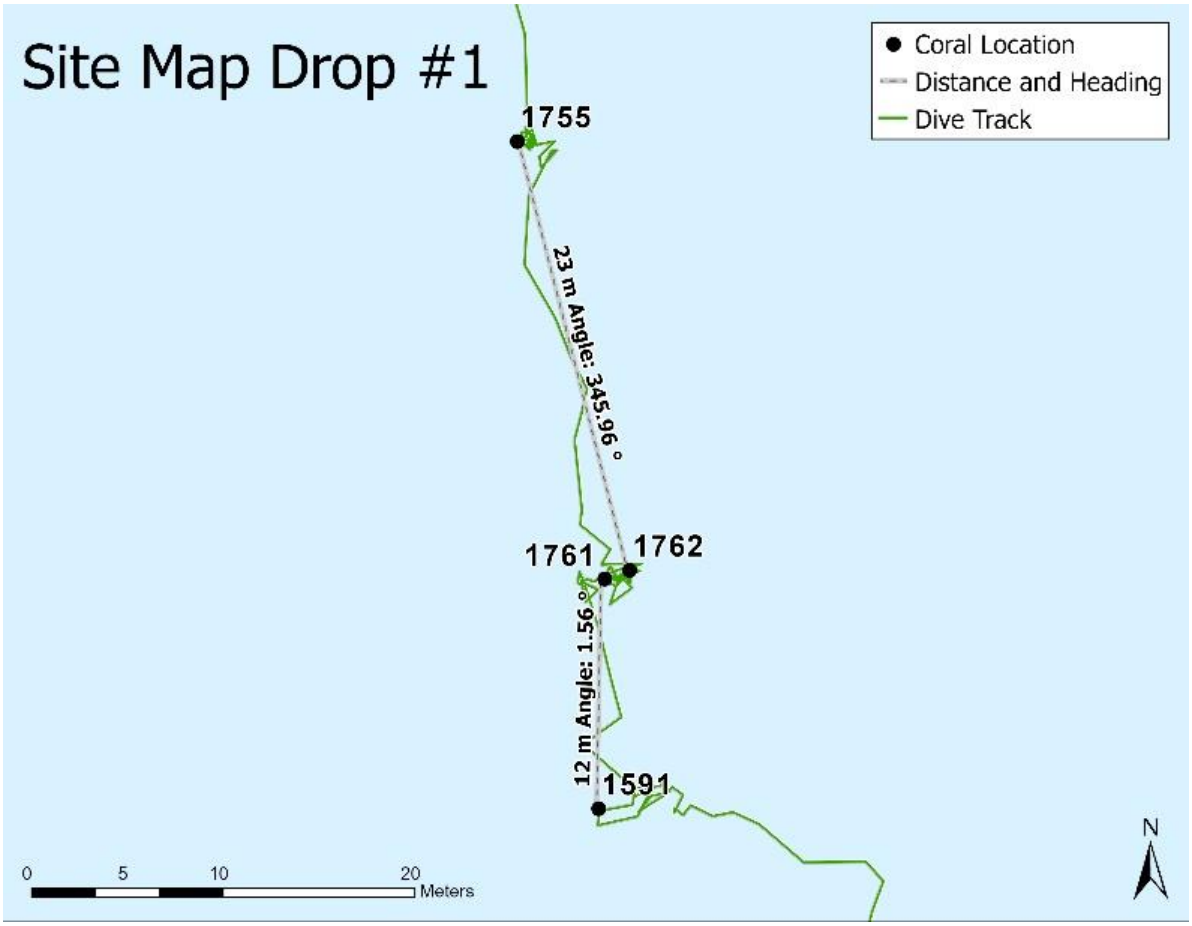

Figure 4. An example of an underwater navigation map that allowed divers to use calculated compass headings and distances between previously treated colonies.

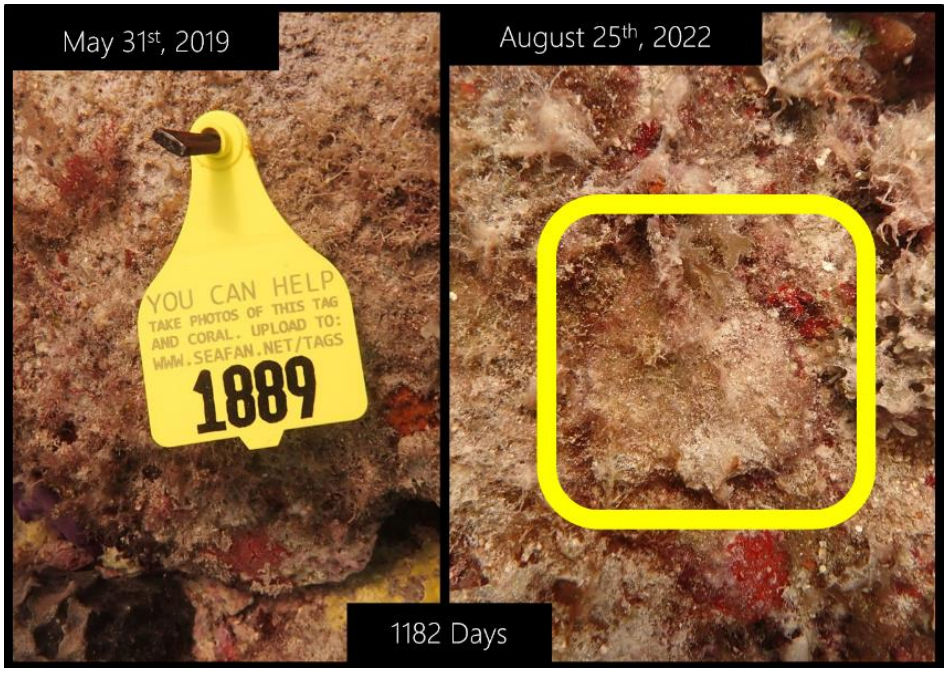

Figure 5. SEAFAN tag was attached at the time of treatment to a SCTLD-infected colony (left). SEAFAN tag from the same colony after 1182 days underwater (right). The tag color is no longer visible; however, the shape of the tag looks unnatural on the coral colony. Looking for the shape alone assisted divers during revisit dives.

Revisit dives recorded similar colony data as the original strike team dives. Nadir photographs of the colony and close-ups of disease margins were used to confirm percentage of total mortality and live tissue during the time of original treatment and at the time of revisit. For colonies that utilized a disease break, 1% was added to total mortality at the time of original treatment due to the loss of live tissue where the trench was created. Areas targeted for revisit typically contained colonies that were treated on the same day resulting in multiple corals having the exact same number of days between treatment and revisit. This led to the distribution of revisited colonies days post-treatment to cluster together with clear gaps between days lapsed (Figure 6). To get a broader picture of the metrics of the colonies throughout time, revisited corals were categorized into 1-2 years (n=55), 2-3 years (n=63), and 3-4 years (n=60) post-treatment and are referred to as year groupings.

Revisited colonies were categorized as alive or dead to account for colony condition. Percent change of live tissue was calculated (percent live tissue coverage at original time of treatment – percent live tissue coverage at time of revisit/ percent live tissue coverage at original

time of treatment * -100) (Furey, 2023). Percent change was used to standardize changes in live tissue coverage due to variations in colony size. Surface area (cm^2) of the coral colony was calculated using the surface area equation of an ellipsoid divided by two (Klamkin, 1971, 1976) (Figure 7). Live surface area (cm^2) was calculated using the surface area (cm^2) and the proportion of live tissue (Walton et al., 2018) (Figure 8).

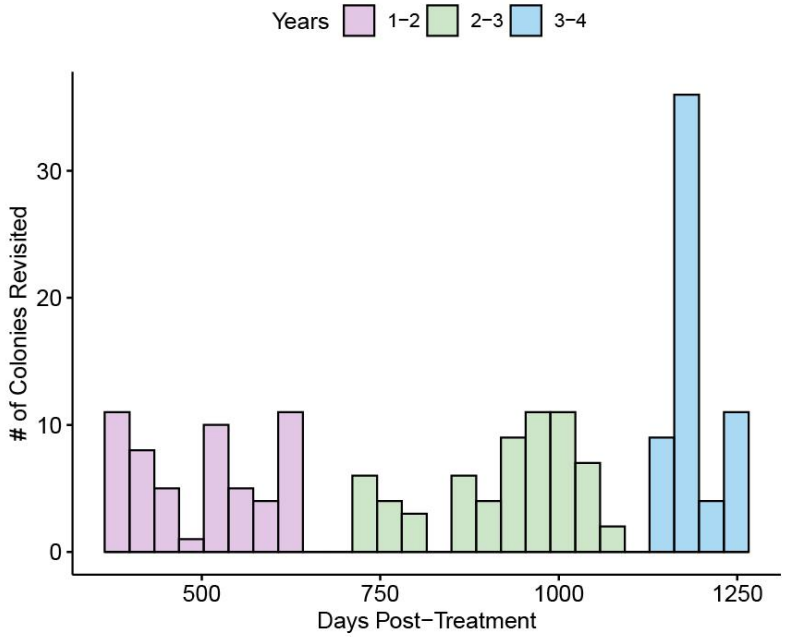

Figure 6. Distribution of days that have lapsed between initial treatment and revisits. Colonies in the same area were commonly treated and revisited on the same day leading to small groupings within the dataset. To gain a broader picture, corals were grouped into years post-treatment.

$$CSA = \frac{1}{2}\left[4\pi\left(\frac{(lw)^{1.6}+(lh)^{1.6}+(wh)^{1.6}}{3}\right)^{\frac{1}{1.6}}\right]$$

Figure 7: The coral surface area equation includes l = maximum colony length * ½, w = maximum colony width * ½, and h = maximum colony height. The surface area of an ellipsoid is multiplied by ½ to account for the shape of the *M. cavernosa* colony.

$$LTA = SA\left(\frac{\%LiveTissueCoverage}{100}\right)$$

Figure 8: Percentage of live tissue coverage is recorded during strike team dives. This is divided by 100 to change the percentage to a decimal. The decimal is multiplied by the surface area of that colony to find the live tissue area (cm^2).

Statistical Analyses:

All statistical analyses were conducted in RStudio version 4.2.1 (2022-06-23). Descriptive statistics in the library package "dplyr" and "ggpubr" were used to better visualize the dataset based on the percentage of all colonies still alive at the time of revisit, treatment methods by year groupings, percentage of live colonies by year groupings, and percent change in live tissue coverage. The datasets were not normally distributed (Shapiro-Wilk $p < 0.05$) and non-parametric methods were used. Fisher's exact test was performed using the RStudio library package "vcd" to test significance between colony condition and years post-treatment. Kruskal-Wallis one-way analysis of variance tests were performed using the RStudio library package "ggpubr" to test significance in the surface area (m^2), starting live tissue area (m^2), remaining live tissue area (m^2), and percent change in live tissue coverage between the years post-treatment groups. A Wilcoxon ranked sum test was performed to compare the change in live tissue coverage between treatment of the disease lesion versus treatment of the disease lesion and disease break.

Results:

In total, 79 dives and a total of 34 hours of dive time over 13 dive days yielded 178 revisited *Montastraea cavernosa* colonies. Colonies were revisited between 387 days to 1,287 days post-treatment, with some clustering of similar days lapsed between treatment and revisit (Figure 9).

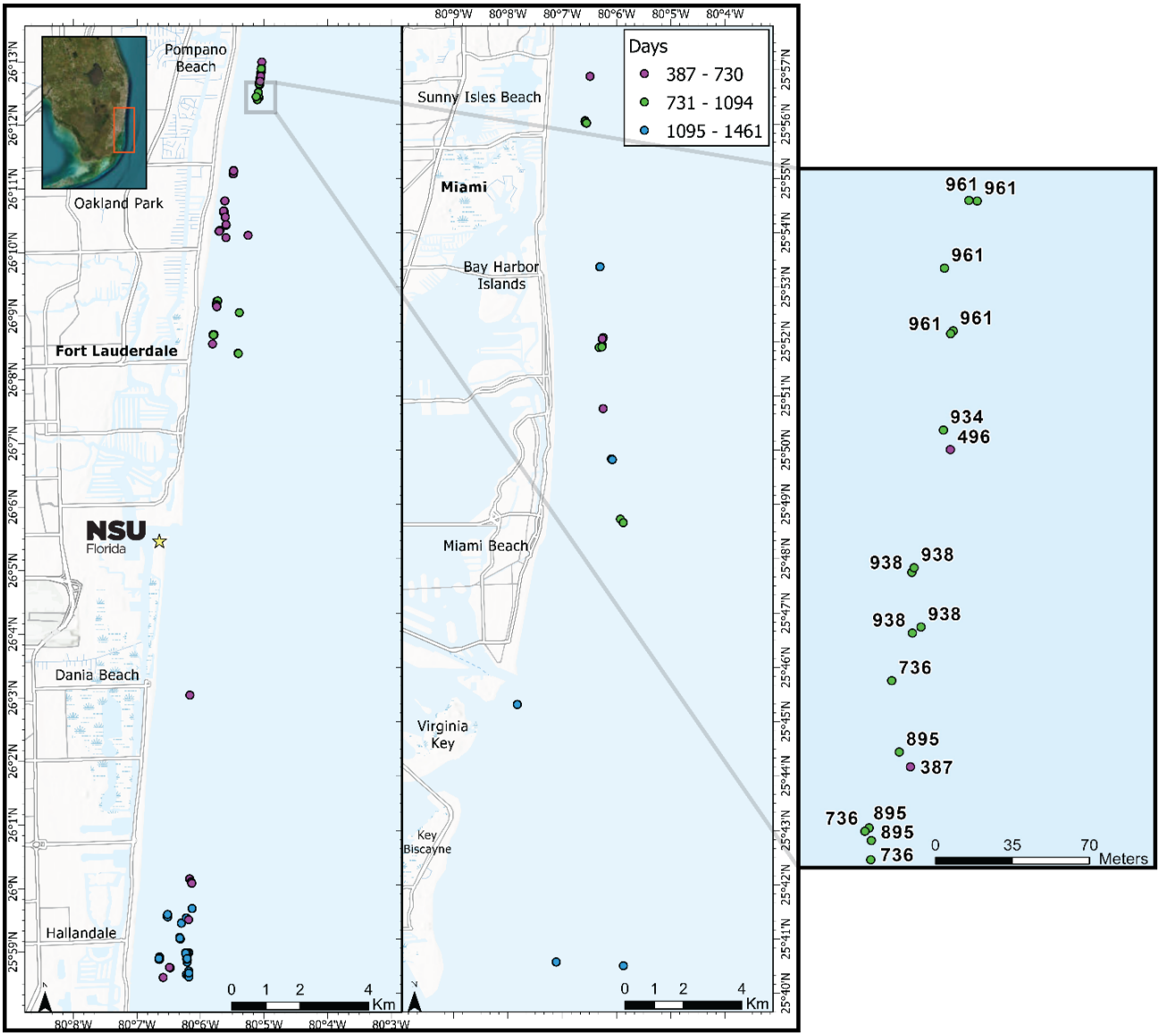

Figure 9. Revisited corals color coded by the days between treatment and revisit. Colonies in the same region were often treated and revisited on the same day leading to clusters of the same number of days for groups of corals in the dataset. The example on the right-most map pane showcases groupings of corals with the same days lapsed between treatment and revisit.

In total, 94% (168) of all *M. cavernosa* colonies were still alive at the time of revisit. All corals treated <799 days prior were alive. Total colony mortality occurred in one colony 799 days post-treatment, one colony 934 days post-treatment, one colony 1,146 days post-treatment, three colonies 1,168 days, one colony 1,175 days post-treatment, and three colonies 1,244 days post-treatment. Significantly more colonies died in the years 3-4 post-treatment group (Fisher's Exact; p=0.005) (Figure 10). Of the colonies that died, nine were antibiotic paste lesion only treatments and one colony was treated with antibiotic paste lesion and a disease break treatment.

SCTLD disease prevalence of revisited colonies was 2%, with four colonies having active lesions. No revisited colonies prior to 1,042 days post-treatment exhibited active disease lesions. Colonies that did display an active SCTLD disease lesion were 1,042, 1,201, and 1,244 days post-treatment.

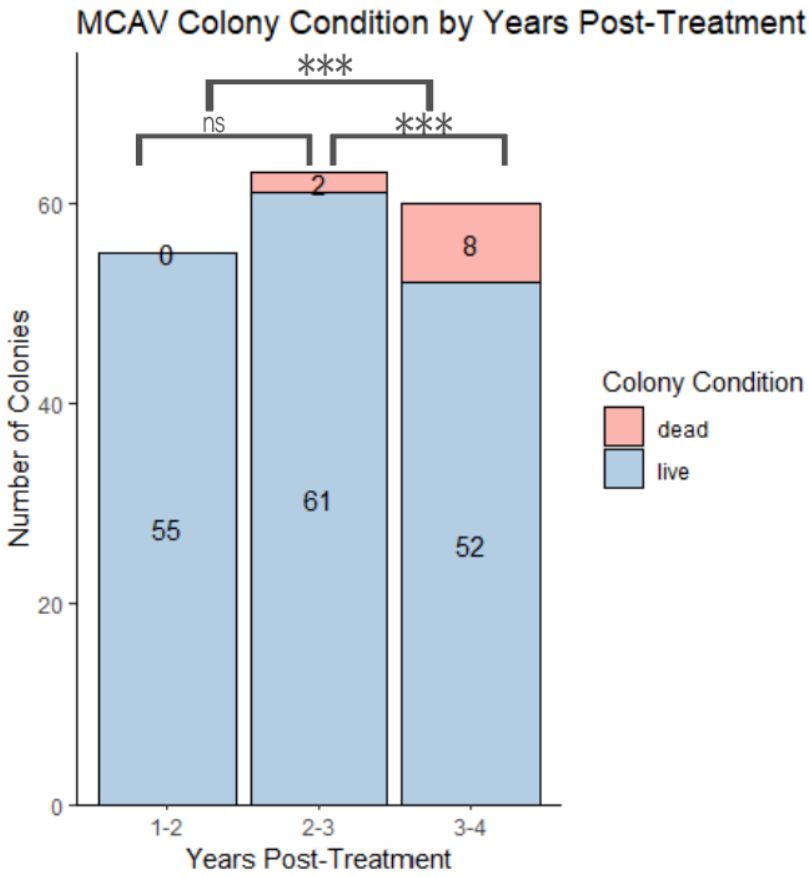

Figure 10. Significantly more corals died in years 3-4 post-treatment. Sample size is represented by the number displayed on each section of the bar graph. The three asterisks above the brackets represent a significant difference between the years post-treatment groups. The bracket labeled with "ns" is an abbreviation of "no significance".

Surface area (m^2), starting live tissue area (m^2), and remaining live tissue area (m^2) differed between year grouping (Figures 11 and 12). Corals 1-2 years post-treatment had a significantly higher mean surface area on the colony (100.99 ± 9.80 m^2) ($p < 0.0001$), mean starting live tissue area (50.39 ± 6.36 m^2) ($p=0.0001$), and mean remaining live tissue area (941.75 ± 5.6 m^2) ($p < 0.0001$) than corals treated 2-3 years and 3-4 years post-treatment. There were no significant

differences for mean surface area, mean starting live tissue area, and mean remaining live tissue area between corals treated 2-3 and 3-4 years ago (Table 1).

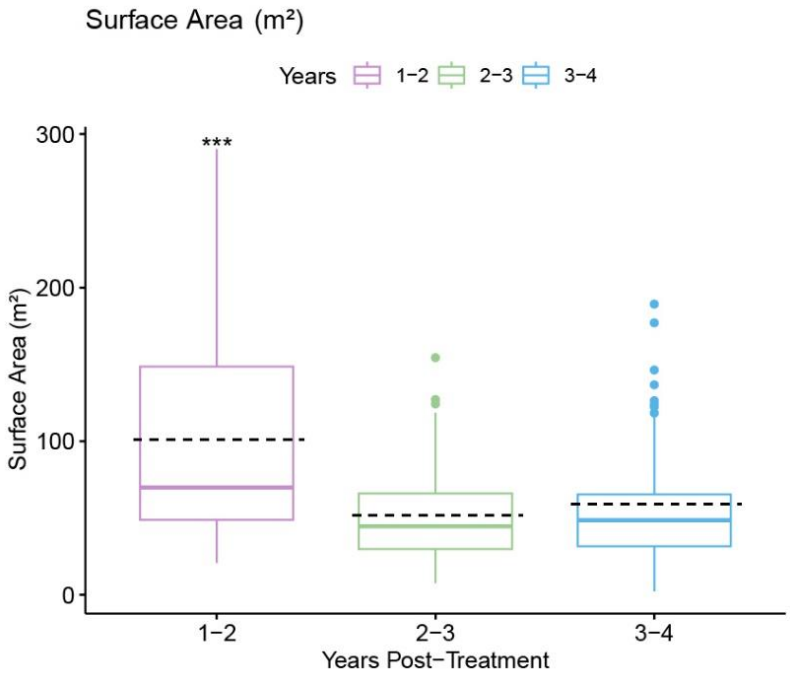

Figure 11. Mean surface area (m^2) was significantly higher for corals in 1-2 years post-treatment grouping. Dashed line in the boxplots represented the mean surface area (m^2) for each years post-treatment grouping.

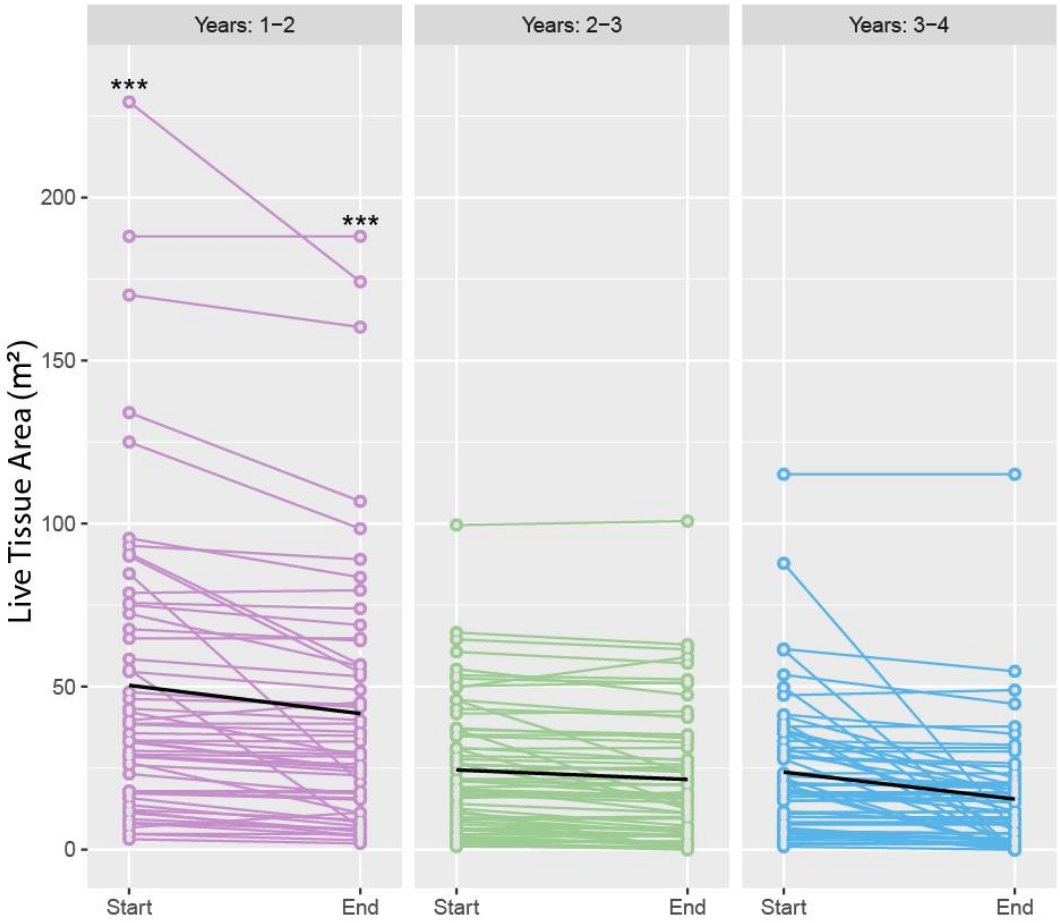

Figure 12. Starting live tissue area (m^2) and remaining live tissue area (m^2) for revisited colonies in their year groupings. Each colony is plotted by the starting and remaining live tissue area (m^2) connected by a line to showcase how each colony changed from the time of treatment to revisit. Solid black line represents the mean starting and remaining live tissue area (m^2) for each year post-treatment grouping. The three asterisks represent the starting and ending live tissue coverage was significantly higher in 1-2 years post-treatment than 2-3 years and 3-4 years post-treatment.

Table 1. Mean (±SE) for surface area (cm2), remaining live tissue area (cm2), and remaining percent live tissue coverage for revisited colonies in their year groupings. Bolded numbers are significantly different from other years post-treatment groupings.

Years Post-Treatment	Surface Area (m^2)	Starting Live Tissue Area (m^2)	Ending Live Tissue Area (m^2)
1-2	**100.99 ± 9.80**	**50.40 ± 6.36**	**41.75 ± 5.60**
2-3	51.3 ± 3.90	24.46 ± 2.55	21.54 ± 2.57
3-4	58.92 ± 3.44	23.80 ± 2.74	15.51 ± 2.38

There was no significant difference in mean percent change in live tissue between year groupings (Figure 13). Corals treated 1-2 years prior lost a mean percent change of 18.43% (±3.25% SE), corals treated 2-3 years lost 20.19% (±3.50% SE) and corals treated 3-4 years prior lost 29.67% (±4.89% SE).

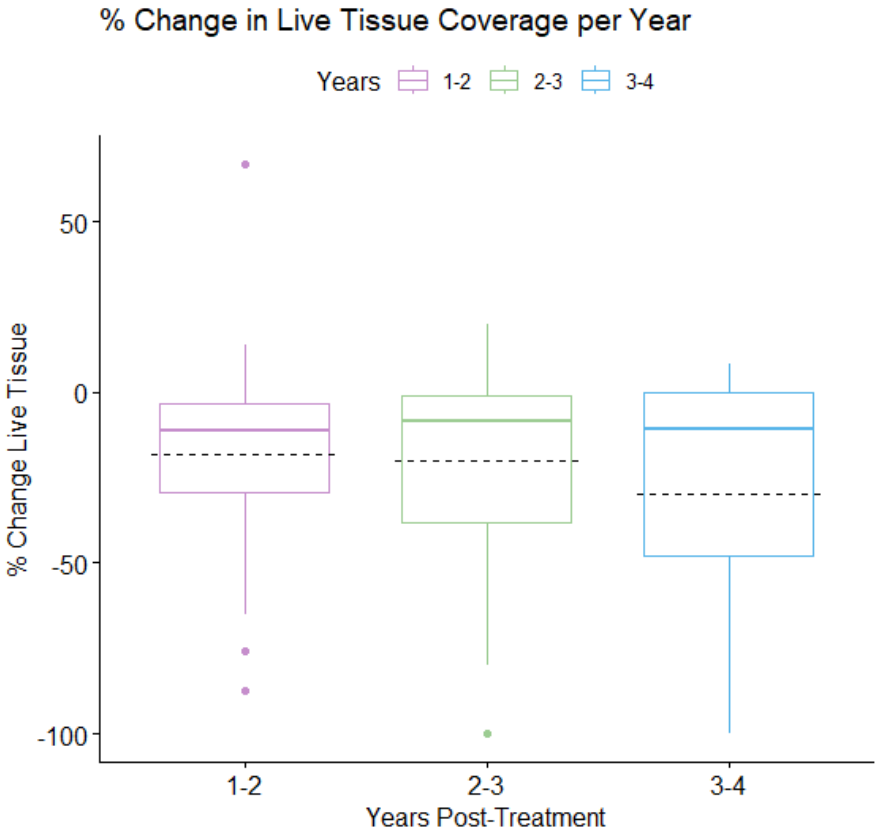

Figure 13. Percent change in live tissue coverage for each year grouping. Dashed lines are the mean percent change in live tissue coverage.

The mean percent loss in live tissue for revisited colonies treated only along the disease lesion was 23.04% (±5.38% SE) for 1-2 years post-treatment (n=28), 30.60% (±8.37% SE) for 2-3 years (n=14), and 26.76% (±5.15% SE) for 3-4 years (n=58). The mean percent loss in live tissue for lesion and disease-break treatment corals revisited was 13.66% (± 3.42% SE) for 1-2 years post-treatment (n=27), 17.21% (±3.77% SE) for 2-3 years (n=49), and 45.61% (±7.51% SE) for 3-4 years (n=2). Due to uneven sample size of the treatment method in each year grouping, significance was not tested (Figure 14). For colonies treated less than 2 years prior, the treatment

method utilizing a disease break (n=27) had significantly lower percent change in live tissue cover than colonies treated only along the disease lesion (n=28) (p-value=0.017) (Figure 13).

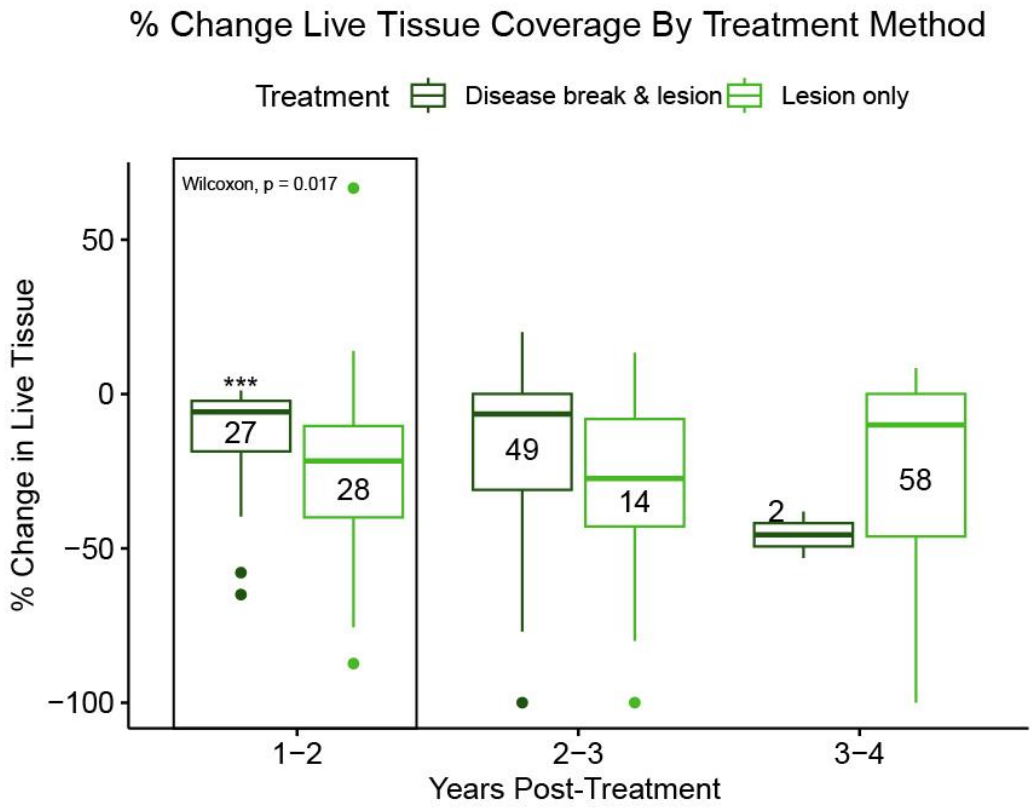

Figure 14. Percent change in live tissue coverage for each year grouping by treatment type: Disease break and lesion treatment and lesion only treatment. Wilcoxon ranked sum test was only performed for 1-2 years post-treatment. Sample size is represented by the number in each individual box plot.

Discussion:

Broadscale strike team SCTLD interventions were highly effective in keeping *M. cavernosa* corals alive after just a one-time treatment in southeast Florida with 100% still alive after 2 years and 87% still alive 3-4 years post-treatment. Due to the nature of the disease response, this study did not incorporate untreated controls, however several other co-located studies monitored untreated *M. cavernosa* corals during the same timeframe. In 2017, Aeby et al. (2019) found that 15% (3/20) of untreated colonies died after seven months and 60% quiesced after one year. Between 2018-2021, Aeby et al. (2021) found that 17.4% (4/23) of colonies died. Between

2019 and 2020, Shilling et al. (2021) found that 20% (2/10) of untreated colonies died and 40% quiesced after 46 weeks. 94% of the broadscale treatment corals were alive with a disease prevalence of 2%. This equates to a 98% SCTLD quiescence, far exceeding natural quiescence rates in other studies (40-60%).

Reduced live tissue coverage between years post-treatment groupings showcases the continual decline in colony health. The 1-2 years post-treatment group lost an average of 18.43% of tissue coverage. In comparison, Aeby et al. (2019) found that *M. cavernosa* colonies with subacute lesions in Fort Lauderdale lost an average of 34% tissue after 1 year of monitoring. The duration of the Aeby et al. (2019) study is comparable to 1-2 years post-treatment group. However, these studies were monitored during different timeframes. Regardless, 34% average tissue lost is still higher than our 3-4 years post-treatment of 29.67%. This comparison is indicative of treatments helping reduce the percentage of live tissue loss to SCTLD lesions. While most of these corals are still alive, they continue to lose their live tissue coverage. Besides disease, there are other environmental drivers contributing to coral decline such as high temperature, water turbidity, and water quality (Jones & Gilliam, 2024).

The low mortality and high quiescence in treated corals could be influenced by temporal changes in disease dynamics. Toth et al. (2024) found that strike team treated corals and treatment densities per dive decreased between May 2019 and April 2022, possibly due to treatments reducing overall prevalence. Several studies have shown that SCTLD is affected by seasonal changes where prevalence drops in the dry, cooler months (Aeby et al., 2019; Walker et al., 2021; Walker et al., 2022). However, treatment density has not declined uniformly across disease intervention strategies in southeast Florida. For example, there has been no indication of reduced prevalence on the largest corals monitored monthly and treated as needed which had the highest number of treatments and treated corals in four years in the summer of 2022 (Walker et al., 2023).

Utilizing a disease break and lesion treatment, in comparison to a lesion only treatment, on *M. cavernosa* infected with SCTLD resulted in an increase in the mean amount of tissue saved in 1-2 years post-treatment. Walker et al. (2021) found that implementing a disease break and treating both the disease break and disease margin was the most effective at stopping lesion progression

(92%) in comparison to only treating the disease lesion (82.8%). The reduction in mean percentage of tissue loss further supports that utilizing a disease break is the more effective method to abate SCTLD lesions. Implementing a second line of defense does cause polyp mortality where the trench is applied. However, colonies treated with a disease break exhibited various stages of healing at the time of the revisit. Some colonies exhibited full regrowth of live tissue along the trench, partial growth of tissue connecting across the trench, and no regrowth at all. 100% mortality of the section isolated by the disease break was also observed. In these cases, live tissue on the other side of the disease break remained unaffected by disease suggesting that the isolation prevented the disease margin from spreading to the rest of colony. Since all colonies treated with a disease break were filled with CoralCure, it is unclear whether the mechanical aspect of a trench, additional antibiotics, or a combination of the two is the reason for higher lesion quiescence rates. Utilizing a disease break does increase the time it takes to treat a single colony and requires more CoralCure to be applied but is the most effective option for one-time treatments on *M. cavernosa* colonies that will not be monitored for treatment failure.

Broadscale disease intervention actively stopped disease progression and preserved large amounts of living tissue. Utilizing the average live tissue decline of 34% and total mortality rate of 15% of all untreated *M. cavernosa* colonies in Fort Lauderdale (Aeby et al., 2019), the amount of live tissue saved on revisited broadscale disease intervention colonies can be calculated. The declining rate of 34% was applied to the starting live tissue area (cm^2) of revisited colonies to represent how much the colony would have declined if the SCTLD lesions were not treated during broadscale disease intervention. The average live tissue decline of 34% was comprised of a range of +1% for healed colonies to -100% for complete mortality (Aeby et al., 2019). The total mortality rate of 15% was randomly distributed throughout the revisited corals to represent colonies that would have experienced total mortality due to SCTLD lesion progression. The average size of colonies and average live tissue coverage was calculated using the surface area and starting live tissue coverage (cm^2) of revisited colonies. The sum of hypothetical remaining live tissue on revisited colonies was compared to the actual sum of remaining live tissue area (cm^2) resulting in 1,395 (m^2) of live tissue saved. This is the equivalent to 20 colonies of average size with 100% live tissue coverage or 50 colonies of average size and average live tissue coverage (cm^2).

If the live tissue was not saved by broadscale disease intervention and instead relied on microfragmentation and outplanting to replenish live tissue area (cm^2) of *M. cavernosa* colonies, it would require 33,217 *M. cavernosa* microfragments of average size (Page et al., 2018). Utilizing the average of microfragments produced per year by Mote Marine Laboratory and Aquarium from 2015-2018, it would take nine years to produce the amount of microfragments to replace the 1,395 m^2 saved from revisited broadscale disease intervention (Page et al., 2018). When accounting for the 40% success rate of outplanted *M. cavernosa* microfragments in 6 m of water in the Florida Keys, it would require 46,504 *M. cavernosa* microfragments to be outplanted and take 13 years to produce (Page et al., 2018). Combating coral disease and reef degradation is a complex issue and will require a multifaceted toolbox of restoration techniques to give degraded reef areas a better chance of survival. However, due to low survival of outplants and slow growth rates of bouldering species, increasing live tissue area (cm^2) on a reef utilizing microfragment outplants will take decades to account for the live tissue area that is currently being lost due to ongoing environmental stressors. Whereas, utilizing disease intervention, especially on these highly and intermediately susceptible bouldering species, we can save live tissue that would otherwise take decades to grow, effectively cutting out the costs and time associated with rearing corals, tank maintenance, and outplanting.

Concerns have been raised about the impact antibiotic treatment has on the molecular responses of corals and the potential increase of antibiotic resistance among surrounding bacterial communities. Antibiotics can enter the environment through agricultural runoff, wastewater, pharmaceutical manufacturing, and the treatment of animals (U.S. Department of Health and Human Services, 2019). Where antibiotics are released, it is more likely that resistant bacteria will follow similar routes of dispersal and could have impacts on humans and other organisms in the area (Berglund, 2015). While the molecular mechanisms of coral immunity are not widely understood, Studivan et al. (2024) found treatment of corals with amoxicillin 'normalize' the transcriptional pathways associated with the response to SCTLD and of algal symbiont pathways. SCTLD-treated corals were statistically indistinguishable from healthy, uninfected SCTLD corals and significantly different from SCTLD-diseased *M. cavernosa*. Studivan et al. (2024) also found that antibiotic intervention was successful in promoting recovery from SCTLD and could provide additional benefits to immune processes. However, the long-term impact antibiotic treatment has

on the coral transcription and the potential increase of antibiotic resistance is unknown. Rosales et al. (2023) generated 16 metagenome-assembled genomes from corals infected with SCTLD and found antibiotic resistance genes present across all samples. These corals were not treated with antibiotics, thus antibiotic resistance cannot be attributed to SCTLD treatment, however the presence of such genes may have future implications on SCTLD treatment efficacy. The potential for antibiotic resistance bacteria to arise from SCTLD treatments is currently unknown and future studies should be conducted to shine light on possible environmental consequences of utilizing antibiotic treatments on diseased corals in the field.

Southeast Florida is considered an endemic zone, with SCTLD persisting in the environment for over a decade. SCTLD is still prevalent along the reef tract. In 2023, broadscale disease intervention efforts treated over 450 colonies, which was the highest number of treated corals since the start of the project in 2018. Most of these treatments were conducted during the summer months of May to September, indicative of the seasonal variability of SCTLD presence in the southeast Florida area (Walker et al., 2021; Walker et al., 2022). Broadscale disease intervention treatment density and number of SCTLD treatments on large *Orbicella faveolata* colonies were much higher in the summer months (Walker et al., 2022).

Future studies should assess the impact lesion morphology has on the long-term effectiveness of one-time treatments. Aeby et al. (2021) found that the starting lesion morphology affected colony mortality, with bleached lesions progressing slower than subacute to acute lesions. Understanding how the lesion morphology impacts treatment success will allow broadscale disease intervention efforts to better target colonies that would benefit the most from treatment. Accounting for lesion morphology could also provide insight on rates of tissue decline and colony mortality post-treatment. Designating untreated, SCTLD-infected colonies and monitoring their condition throughout multiple years would allow for a direct comparison of tissue loss rates and colony mortality. This study did not designate controls due to the nature of this broadscale disease intervention strategy. When SCTLD emerged, it had quick and devastating impacts on the coral coverage and species diversity, so divers did not leave any infected colonies untreated. Future studies should also look at the impact that spatial variations have on colony condition post-treatment. Toth et al. (2024) found coastal regions categorized by proximity to inlets in southeast

Florida were significant predictors of coral disease treatment density. The spatial relation of treated corals to inlets in southeast Florida may influence the colony's ability to survive post-treatment.

Determining the long-term effectiveness of one-time CoralCure treatments on SCTLD disease lesions provides valuable information to stakeholders on the benefits of utilizing this disease intervention method. The comparatively lower cost of treating corals in situ, the large amounts of tissue being preserved, and the overall high survival rate of treated colonies showcases the benefit of including disease intervention in the restoration toolbox. As SCTLD continues to spread throughout the Caribbean, the results from this study can provide guidance on proactive measures to help preserve live tissue cover and species diversity on newly infected reefs.

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