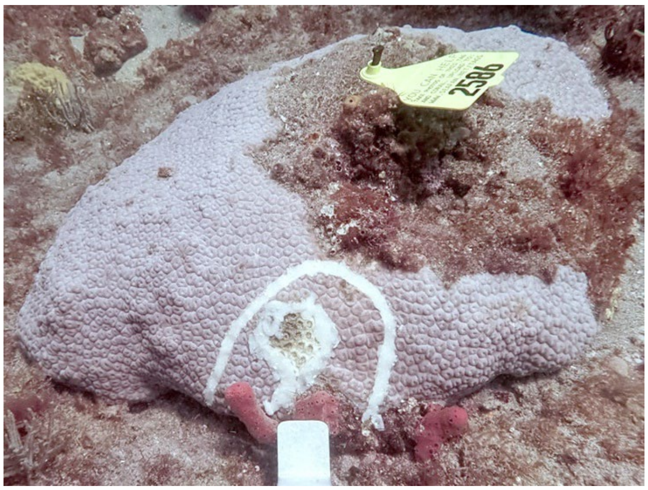
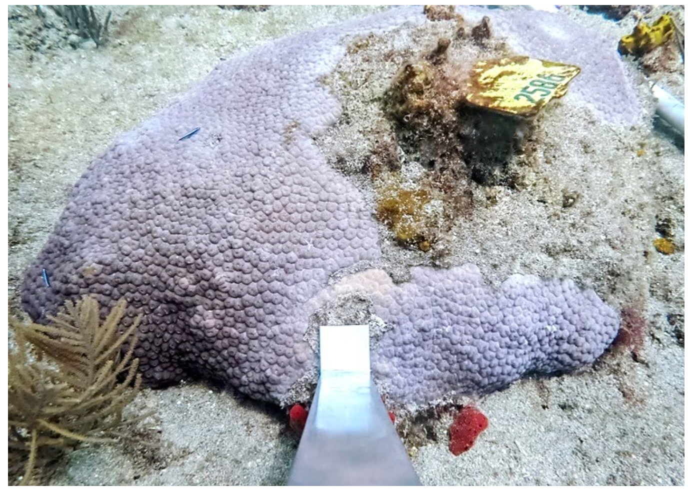
2022-2023 SE FL ECA Reef-building-coral Disease Intervention and Preparation for Restoration

Final Report

Florida Department of Environmental Protection
Coral Protection and Restoration Program

2022-2023 SE FL ECA Reef-building-coral Disease Intervention and Preparation for Restoration

Final Report

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List of Acronyms

FAU	Florida Atlantic University Harbor Branch Oceanographic Institute
DEP	Florida Department of Environmental Protection
ORCP	Office of Resilience and Coastal Protection
FWC	Florida Fish and Wildlife Conservation Commission
NSU	Nova Southeastern University
Coral ECA	Kristin Jacobs Coral Reef Ecosystem Conservation Area
FCR	Florida's Coral Reef
SCTLD	stony coral tissue loss disease
SE FL	Southeast Florida
NOAA	National Oceanic and Atmospheric Administration
NCRMP	National Coral Reef Monitoring Program
CIMAS	Cooperative Institute For Marine And Atmospheric Studies
GIS	Geographic Information System

Acknowledgements

Thank you to the Florida Department of Environmental Protection's Coral Protection and Restoration Program (DEP CPR) and NOAA CRCP for supporting these efforts. We thank the Florida Coral Disease Advisory Committee for the large number of volunteers assisting in the meeting and planning of coral disease efforts. We thank Lisa Gregg for assisting with permitting. Thanks to the DEP CPR staff including Kristi Kerrigan for contract and report-review coordination. Thanks to Miami-Dade Regulatory & Economic Resources for field assistance and boat time. Thank you to Katy Toth, Sasha Wheeler, Zach Graff, Allie Kozachuk, and Alex Wagner at the NSU GIS and Spatial Ecology lab.

Management Summary (300 words or less)

Coral diseases have caused enormous impacts to coral populations globally perpetuating the rapid need for large-scale coral reef restoration. SCTLD exemplifies that coral diseases are more devastating than ever before. However, implementing recent disease intervention techniques from the restoration toolbox reduces the necessity for costly and time-consuming post hoc restoration techniques.

Our work shows that SCTLD is still prevalent in the region, interventions are still needed, and these efforts are saving corals. This year, interventions helped maintain species diversity and ecosystem services within the Coral ECA and kept alive some of the largest and oldest animals in Florida. Monthly monitoring and treatments have reduced the loss of live tissue area and provided valuable information on the temporal and spatial variations of colonies with lesions. Corals not regularly treated with disease interventions have had drastic declines in live tissue area. Regular disease intervention is restoring colony health and saving the large priority corals, preempting post hoc restoration. For example, the current live tissue area of all previously treated large corals is 357.8 m^2. This is the equivalent to the area of 2 volleyball courts of live coral tissue.

Our reconnaissance for coral restoration sites identified two new large corals, a high-density coral site, and the largest known *Madracis aurotenra* site ever recorded. These are of high value to the State of Florida for restoration and eco-tourism.

The monthly monitoring data have been invaluable in linking environmental drivers to disease dynamics. Higher water temperatures, amounts of water following out of the inlets, and rainfall account for 56.4% of the temporal variability in the number of new lesions. Understanding these dynamics can help with water management strategies. Caution should be taken when choosing coral restoration sites as to avoid areas more prone to disease.

Executive Summary

SCTLD was first discovered in the Kristin Jacobs Coral Reef Ecosystem Conservation Area (Coral ECA) in 2014 and remains present in the region. As of June 15, 2023, a total of 1,451 colonies were treated by the coral disease interventions strike teams in the Coral ECA since 2018 (including the experimental sites, but not the large priority corals) totaling 972 meters of treatment. Between July 1, 2022 and May 31, 2023, 186 colonies were treated. 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 some are still succumbing to the disease.

Priority corals are still requiring monthly visits to ensure their survival. **This year had two months with the highest number of treatments and treated corals in the four years of this work.** Of the 107 monitored corals between July 1 and May 31, 2023, a total of 455 antibiotic ointment treatments were conducted on 55 colonies. One hundred and ten treatments failed, equating to 75.4% effectiveness on lesions. The number of new infections varied throughout the period. The months with the highest treatments were July, August, September, and January. September had the highest total treatment length.

Monthly visits should continue to maximize treatment success and capture new infection frequencies.

During this reporting period, NSU divers conducted a total of 151 recon and intervention dives over 35 dive days. **Three newly identified recon sites: the largest patch of *Madracis aurotenra* ever reported anywhere, and two rare large *Orbicella annularis* colonies, one in a patch of dense corals.**

This period NSU provided a lot of assistance to the Smithsonian Marine Station at Fort Pierce. NSU divers conducted a total of 44 dives over 10 days assisting with probiotics experimental treatments and monitoring.

NSU also assisted Dr. Joshua Voss' lab at FAU Harbor Branch Oceanographic Institute with investigating the impact of elevated nutrient levels on the spread and progression of SCTLD in *M. cavernosa*.

The large coral new treatment data collected herein and previously were summarized to evaluate statistical relationships to environmental predictors funded by DEP PO B9CAF9 and EPA award SF02D21722. They will also be used to evaluate the outcomes of the SCTLD Resistance Research Consortium (RRC) laboratory analyses funded by DEP B8A48D and the EPA grant. They are the primary dataset for many future research projects including hydrographic modeling and the continuation of the SCTLD RRC.

1. BACKGROUND

Florida's Coral Reef is currently experiencing a multi-year disease-related mortality event, that has resulted in massive die-offs in multiple coral species. Approximately 21 species of coral, including both Endangered Species Act-listed and the primary reef-building species, have displayed tissue loss lesions which often result in whole colony mortality. First observed near Virginia Key in late 2014, the disease has since spread to the northernmost extent of Florida's Coral Reef, and southwest through the Dry Tortugas in the Lower Florida Keys. The best available information indicates that the disease outbreak is continuing to spread west and throughout the Caribbean.

In 2022, DEP funded award C00BAE to continue Coral ECA coral disease intervention and restoration activities ongoing since 2018 (DEP PO# B2A150, B48140, B46AD7, B3C3AD, B558F2, B7B6F3, B96800) with the expectation of supporting existing collaborations and adapting to new methodologies to improve intervention success in the Coral ECA. These actions included maintaining the monitoring and continued treatment of 100 priority large corals, conducting broadscale strike team reconnaissance and disease interventions, further testing of permitted intervention techniques and materials, and the identification of unique colonies and sites (e.g. location of remaining highly susceptible species, remaining high coral density sites, restoration candidate sites). Information from these previously funded activities has strengthened local partnerships and provided data on treatment effectiveness, saved the largest large colonies from extreme tissue loss, and facilitated probiotics testing. It also provided data on tissue loss rates, survivor sites, new infection rates through time, and classifying large corals into categories based on infection rates setting up the study design for the stony coral tissue loss disease (SCTLD) Resistance Research Consortium.

This report summarizes the progress from our continued Coral ECA coral disease interventions through May 31, 2023, 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 is to perform disease interventions on the remaining reef-building coral species with active disease in the Coral 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 includes smothering diseased tissue with treatment, creating a disease-break to arrest disease progression, and covering the newly exposed skeleton with an antibiotic paste. The first objective was to apply these interventions to the 100 priority large corals as necessary to maintain their health and continue monthly monitoring. The second objective was to conduct broader-scale strike team disease intervention efforts in partnership with Smithsonian, Florida Atlantic University, DEP, Broward County, and Miami-Dade County to help save diseased colonies throughout the Coral ECA. A third objective was to further field test new permitted intervention techniques and materials (e.g. Smithsonian probiotics) including whole colony treatments as they are conceived, developed, and permitted.

The fourth objective of this project was to collect information to inform and aid in planning future Coral ECA restoration efforts. Future restoration will need to identify survivors for sexual reproduction, genetic analyses, and experimentation on stress hardening and disease resistance. Therefore, identified unique coral disease survivor sites to make restoration activities more efficient. This work is partially funded by a NOAA Coral Program grant (NA19NOS4820127).

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) is essential to avoid duplication, share lessons learned, and ensure alignment of needs.

3. METHODOLOGY

The antibiotic paste treatments were conducted under the State of Florida Special Activity License Permit SAL-21-2022-SRP and SAL-23-2022-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-22-2201-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 recording divers' movement along the reef track. Once a diseased coral was located, the time of treatment was recorded. Each coral was tagged and measured, photographed, and treated. GPS coordinates were loaded into ArcGIS and the locations that corresponded to each time recorded during treatment were copied into a GIS shapefile. All treated coral locations were supplied to FWC for inclusion in the Coral Disease Intervention Dashboard: https://arcg.is/0L1LWX.

3.2. Apply Interventions to 100 Large Corals (Task 3)

The largest known corals in the region were prioritized for monitoring and disease interventions. From September 2018 – June 2019, approximately 60 corals began being monitored and treated on a monthly basis. This increased to 90 colonies in July 2019, but it took several periods to establish all 90. As of October 2021, there were a total of 107 corals in the priority database. All priority corals were photographed monthly and visually assessed by a diver estimating the percentage of live tissue, diseased tissue, bleached tissue, recent mortality, and old mortality. If SCTLD was found, the lesion was treated with antibiotic paste. All margins were treated with the Ocean Alchemists antibiotic ointment CoreRx B2B with amoxicillin (1:8 ratio by weight). Photographs were taken of all areas before treatment at both the 0.5 m standard distance and wider scenes. The length of each treatment was estimated using a standardized scale in the photographs. Lesion treatments were determined failures if the active disease continued progressing past the treatment line. Treatment success was calculated as the reciprocal of the percentage of treatment failures. All prioritized 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 1).

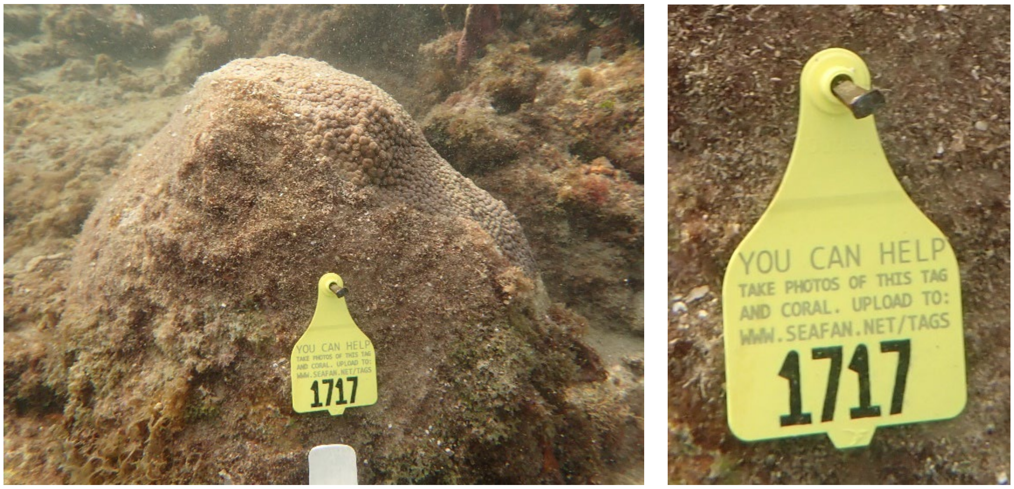

Figure 1. Example of coral tag placed on or next to each treated coral.

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

Reconnaissance throughout Broward and Miami-Dade was conducted by coral disease response strike teams 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. Recon was performed during strike team disease interventions and other opportunistic times. Divers visited sites and haphazardly searched the area towing a GPS buoy to find locations of visually noticeable high coral density or richness and to identify if disease is present. If disease was found, these locations were treated and mapped. Large colonies of any species and smaller colonies of the species hit hardest by SCTLD (e.g. *M. meandrites*, *E. fastigiata*, *D. stoksii*, *C. natans*, *D. cylindrus, D. labyrinthiformis*) were mapped.

3.4. **Field Test New Permitted Intervention Techniques and Materials (Task 5)**

3.4.1. Probiotics on Montastraea cavernosa

The Smithsonian Marine Station at Fort Pierce is developing several probiotics and several treatment methods to be tested on *M. cavernosa*. NSU has previously set up 3 experimental sites in the middle of Broward County: BS1 (no longer used), BS2 (Figure 2 and Figure 3), BS3 (Figure 4). During this period, NSU assisted the Smithsonian Marine Station at Fort Pierce with probiotics treatments, setting up new experiments, and the collection of monitoring data. The experiment involved examining the efficacy of probiotics through two different delivery vehicles: 1) probiotic paste and 2) probiotics injected into a bagged coral. The second one was a comparison between probiotics and antibiotic paste (amoxycillin in Base2B). During our disease intervention and recon activities, we located a new site with 8 diseased *Montastrea cavernosa* which became BS4 (Broward Site #4) (Figure 5). We also collected 3-D photographs at each site several times to track tissue loss over time.

3.4.2. *Nutrient enrichment on* Montastraea cavernosa

The Voss lab at FAU Harbor Branch investigated the impact of elevated nutrient levels on SCTLD spread, progression, and impact on changes in microbial communities in *M. cavernosa* found on nearshore reefs in Southeast Florida. NSU helped to re-run a field experiment site for FAU by contributing a boat, captain, and a diver.

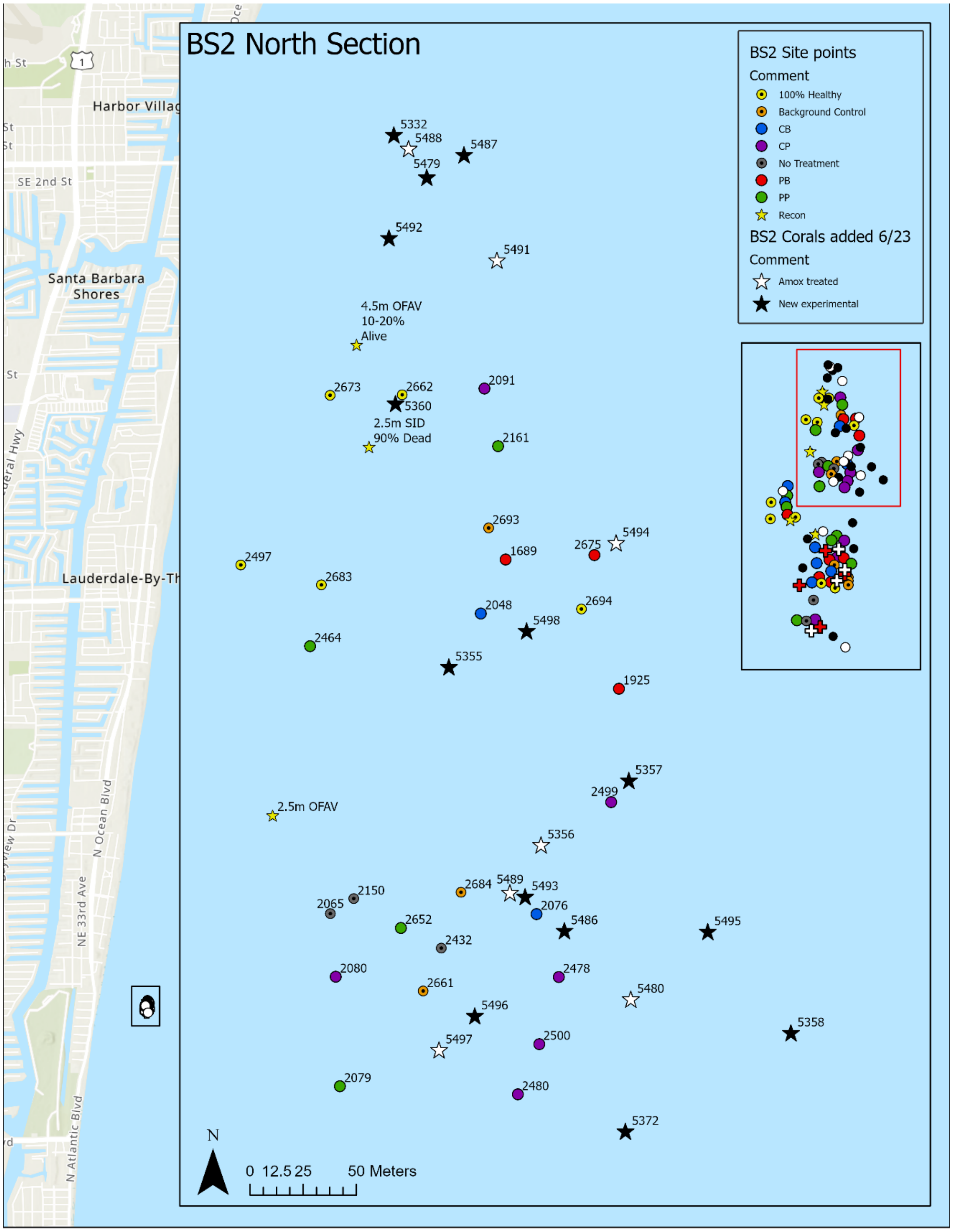

Figure 2. Map of the northern corals at BS2 symbolized by treatment.

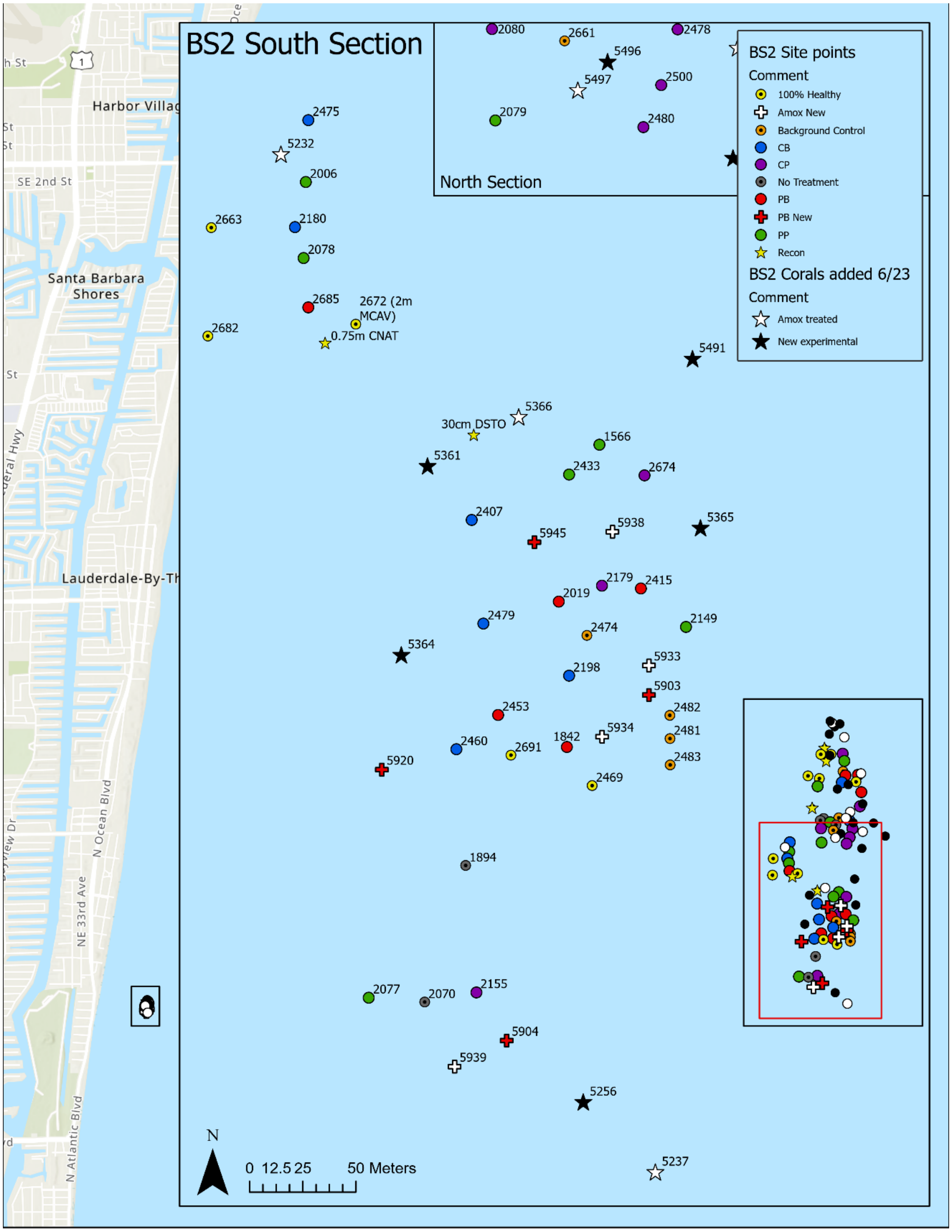

Figure 3. Map of the southern corals at BS2 symbolized by treatment.

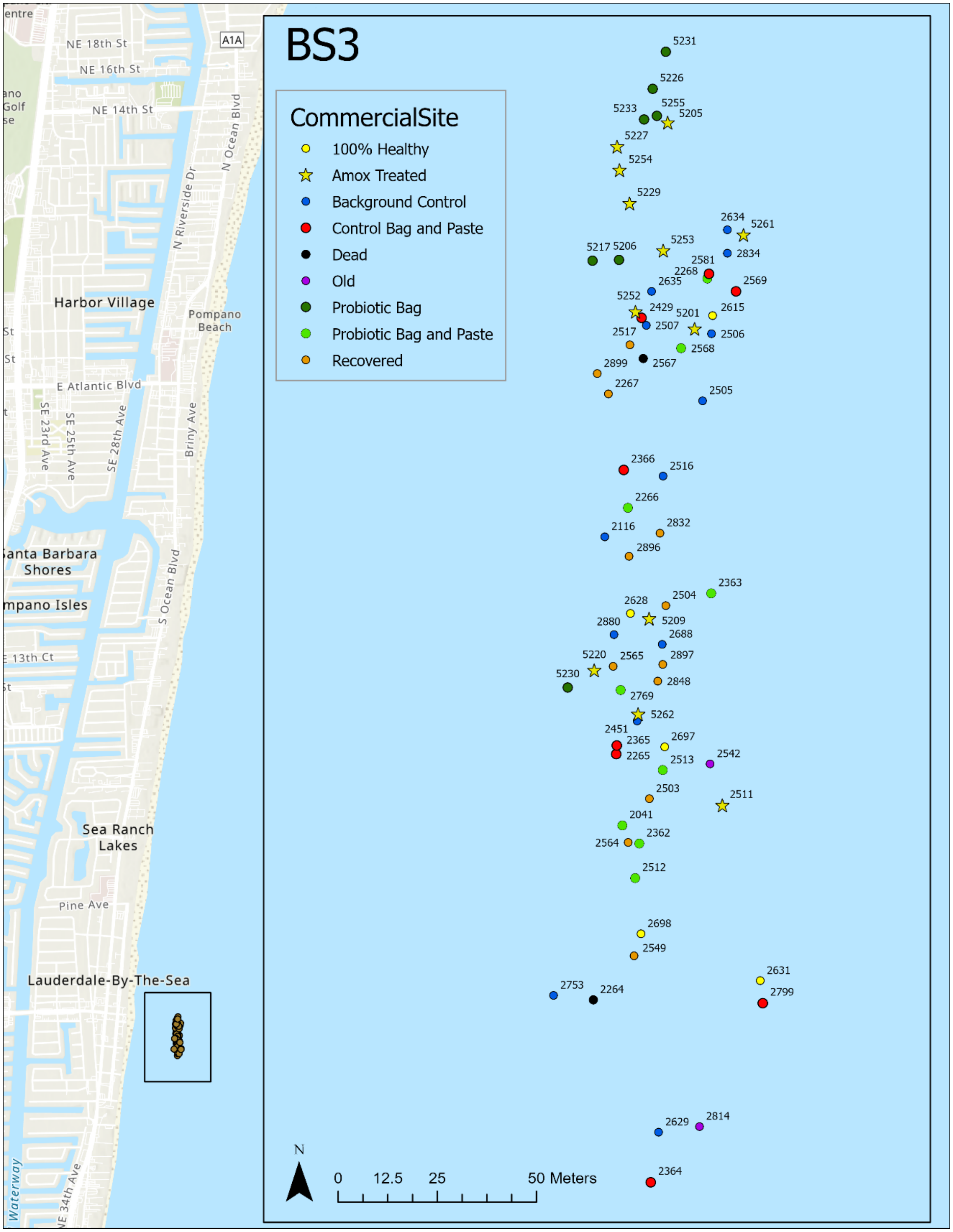

Figure 4. Map of probiotics site BS3 treatment corals established July 23, 2021.

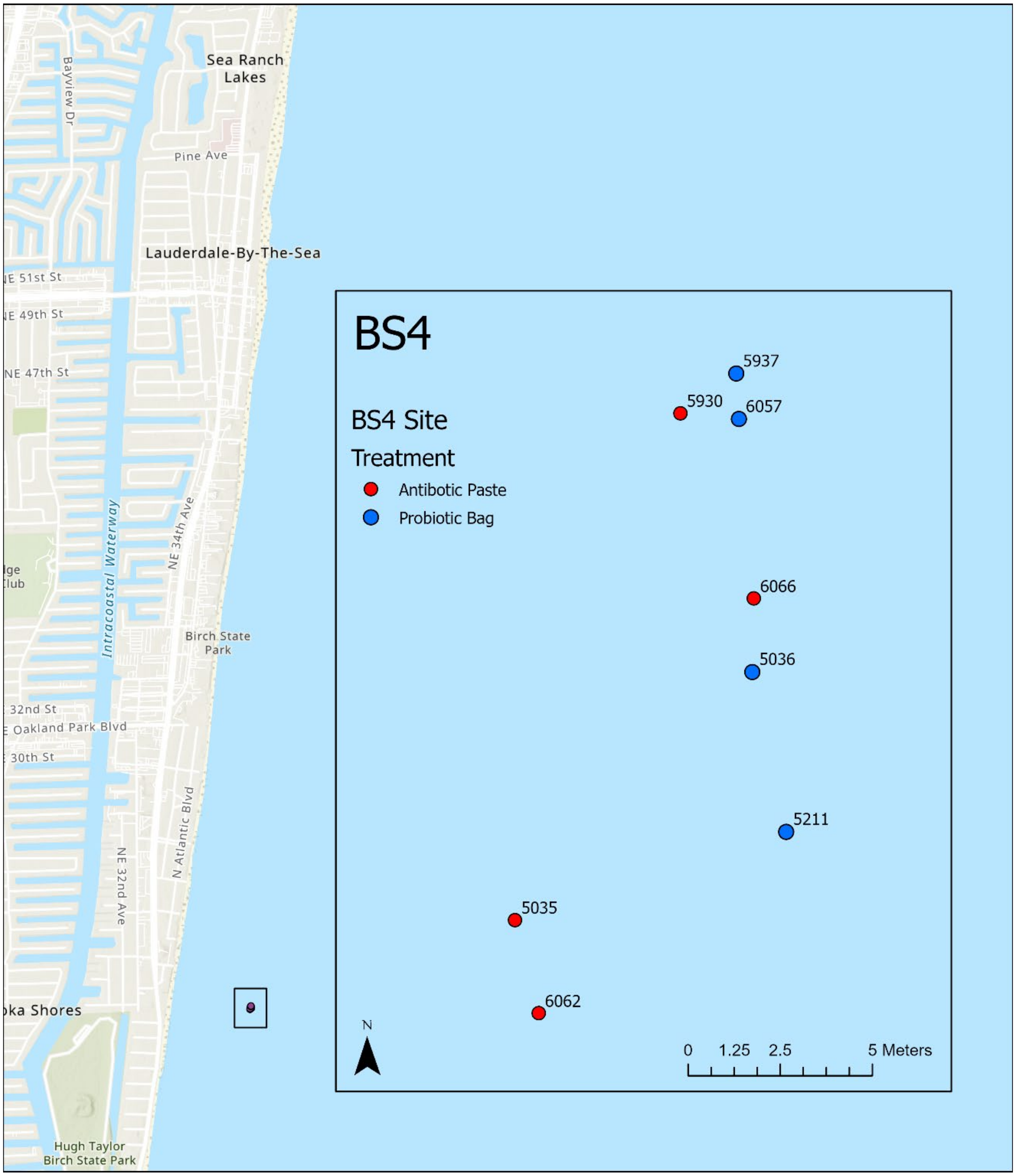

Figure 5. Map of probiotics site BS4 treatment corals.

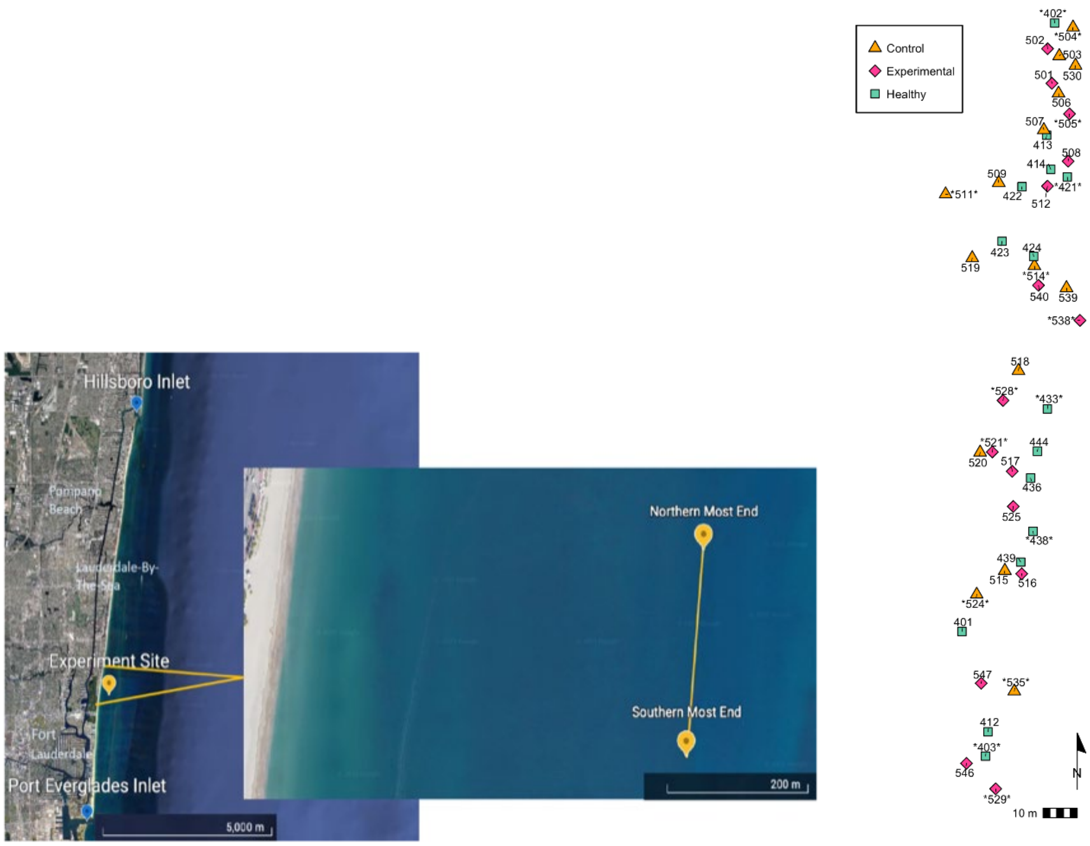

Figure 6. Map of the FAU nutrient site established September 17, 2021 and revisited March 5, 2023. Numbered shapes indicate individual corals by treatment type.

3.5. **3-D and MSS testing and evaluation (Task 6)**

Structure from motion is a technique recently developed to create relatively accurate 3-dimensional models on computers by processing many 2-dimensional images around an object. In 2020 and 2021, we collected and processed images for every priority colony using Agisoft as part of our activities. The models are impressive and offer a glimpse at the coral without being underwater. The present monthly large coral monitoring cover estimates vary between observers up to 10%. Regardless of how much calibrating of diver estimates, this variation remains inherent in the data collection. This inhibits our ability to confidently measure live tissue cover changes below 10%. If employed properly, 3-D modeling methods promise to provide much more precise tissue estimates with much less variation between surveys. The main disadvantage to the 3-D modeling is current processing times are slow and cumbersome. But new techniques have been published that allow for the models to be automatedly quantified to determine estimates of live coral tissue area that are much more precise than diver estimates (Pierce et al., 2021).

Pictures for 3D modeling have been added into large coral monitoring days to begin testing this protocol while continuing visual estimations. A subset of corals was selected each monitoring day for 3D modeling pictures with different corals selected each month. This protocol includes laying four cardinal direction tiles around the base of the coral, along with a meter stick for scale, and taking photos using a TG5 camera on the “continuous low” setting. These images were uploaded into Agisoft for the creation of the 3D models. The Agisoft software first aligns the photos by detecting points to match images to each other. Next, using the batch process tool, the software creates the dense point cloud, mesh, and texture to create the full 3D model (*Figure 7*).

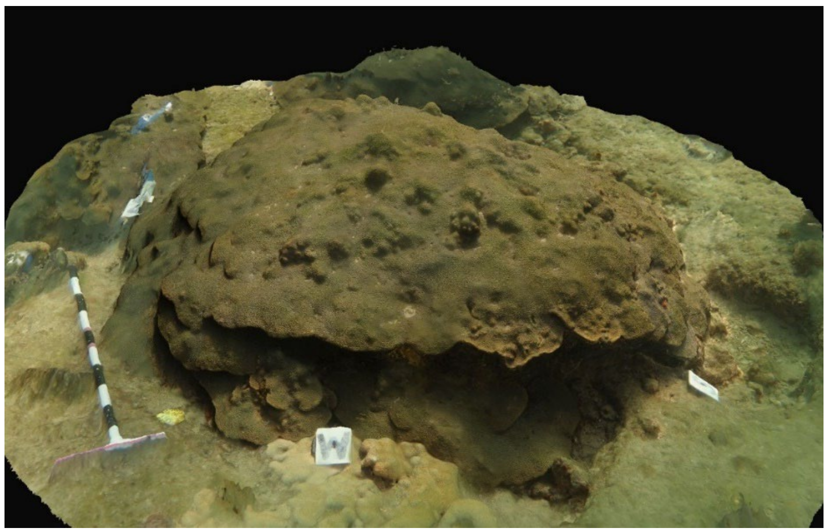

Figure 7. 3-D model of large coral (LC-1745).

For this task we developed a protocol for the incorporation of multi-view semantic segmentation machine learning techniques to automate live tissue area quantification of our large corals. Pictures used for the 3D models were uploaded to CoralNet to create sparse labels for the images. The class categories chosen include “*Orbicella faveolata*”, “Cliona”, “dead coral”, “substrate”, “fish”, “water”, “target”, “other” (i.e. gorgonian, sponge, or palythoa), or “unknown”. These annotated patches will be extracted and used to train a Convolutional Neural Network (CNN) in Python which can then serve as an automatic annotator to provide sparse labels for images. Once >0.01% of pixels are labeled, the sparse labels can then be converted to dense labels using a fast multilevel semantic segmentation (Fast-MSS). Dense labels can be used to train a fully convolutional network (FCN) which will be able to perform pixel-level classification on unannotated images. Once successfully trained, the FCN will be all that is needed to annotate images without any other of previous training steps.

A classified 3D model will then be created alongside the original reconstructed 3D models. Using the “change paths” tool in Agisoft, the original photos will be swapped with the dense labels. A classified textured mesh will be created using the “build texture” tool which allows the shape of the original 3D model but with the added set of dense labels. The “Colorize Vertices” and “Colorize Dense Cloud” tools can then be used to create classified shaded mesh and dense point clouds which can be exported with their classifications to be used in spatial analysis software.

4. RESULTS

All broad-scale strike team activities have been reported to FWC's coral disease intervention dashboard (https://arcg.is/0L1LWX).

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

As of June 7th, 2023, a total of 1,451 colonies have been treated by the coral disease interventions strike teams in the Coral ECA since 2018 (including the experimental sites, but not the large priority corals in section 4.2) (Figure 8). The total number of treatments by species were 1,174 *M. cavernosa*, 101 *O. faveolata*, 1 *O. annularis*, 40 *Colpophyllia natans*, 40 *Pseudodiploria strigosa*, 55 *Pseudodiploria clivosa*, 16 *Diploria labyrinthiformis*, 7 *Solenastrea bournoni,* 2 *Siderastrea siderea,* 3 *Stephanocoenia intersepta*, 1 *Dichocoenia stokesii* and 1 *Mycetophyllia aliciae*.

Out of the 1,451 colonies, 1,326 (91.4%) were treated with antibiotic ointment (1,059 *M. cavernosa*, 96 *O. faveolata*, 1 *O. annularis*, 38 *Colpophyllia natans*, 48 *Pseudodiploria strigosa*, 54 *Pseudodiploria clivosa*, 16 *Diploria labyrinthiformis*, 7 *Solenastrea bournoni* 2 *Siderastrea siderea,* 3 *Stephanocoenia intersepta*, 1 *Dichocoenia stokesii* and 1 *Mycetophyllia aliciae*); 109 (7.5%) corals were treated with chlorinated epoxy (102 *M. cavernosa*, 4 *O. faveolata*, 2 *P. strigosa*, and 1 *C. natans*); and 16 (1.1%) corals were treated with CoreRx B2B without antibiotics that were not successful (13 *M. cavernosa,* 1 *O. faveolata,* 1 *P. clivosa,* and 1 *C. natans*).

A total of 897.1 meters of antibiotic paste treatments, 68.59 meters chlorinated epoxy treatments, and 6.4 meters of CoreRx Base treatments were performed totaling 972.08 meters. That's 2.6 times the height of the Empire State Building. The average treatment length per coral was 67.98 cm which varied by species: *M. cavernosa* = 69.62 cm, *O. faveolata* = 85.3 cm, *O. annularis* = 149.00 cm, *P. strigosa* = 57.18 cm, *P. clivosa* = 47.38 cm, *C. natans* = 47.38 cm, *D. labyrinthiformis*= 45.6 cm, *S. bournoni* = 41.00 cm, *S. siderea* = 68.50 cm, *S. intersepta* = 21.00 cm, *D. stokesi* = *20.00* cm, and *M. aliciae* = 30.00 cm.

Between July 1, 2022 and June 7th, 2023, a total of 186 colonies were treated with antibiotic paste by the coral disease interventions strike teams in the Coral ECA (not including the large priority corals (Section 4.2) and probiotics (Section 4.4)) (Figure 9). The total number of treatments by species were 151 *M. cavernosa*, 22 *O. faveolata*, 4 *Colpophyllia natans*, 3 *Pseudodiploria strigosa*, 3 *Pseudodiploria clivosa*, 1 *Diploria labyrinthiformis*, and 2 *Solenastrea bournoni.* These totaled 77.21 meters of antibiotic ointment treatments were performed. The average treatment length per coral was 44.37 cm which varied by species: *M. cavernosa* = 42.86 cm, *O. faveolata* = 58.71 cm, *P. strigosa* = 34.33 cm, *P. clivosa* = 29.00 cm, *C. natans* = 49.25 cm, *D. labyrinthiformis* = 12.00 cm, and *S. bournoni* = 30.00 cm.

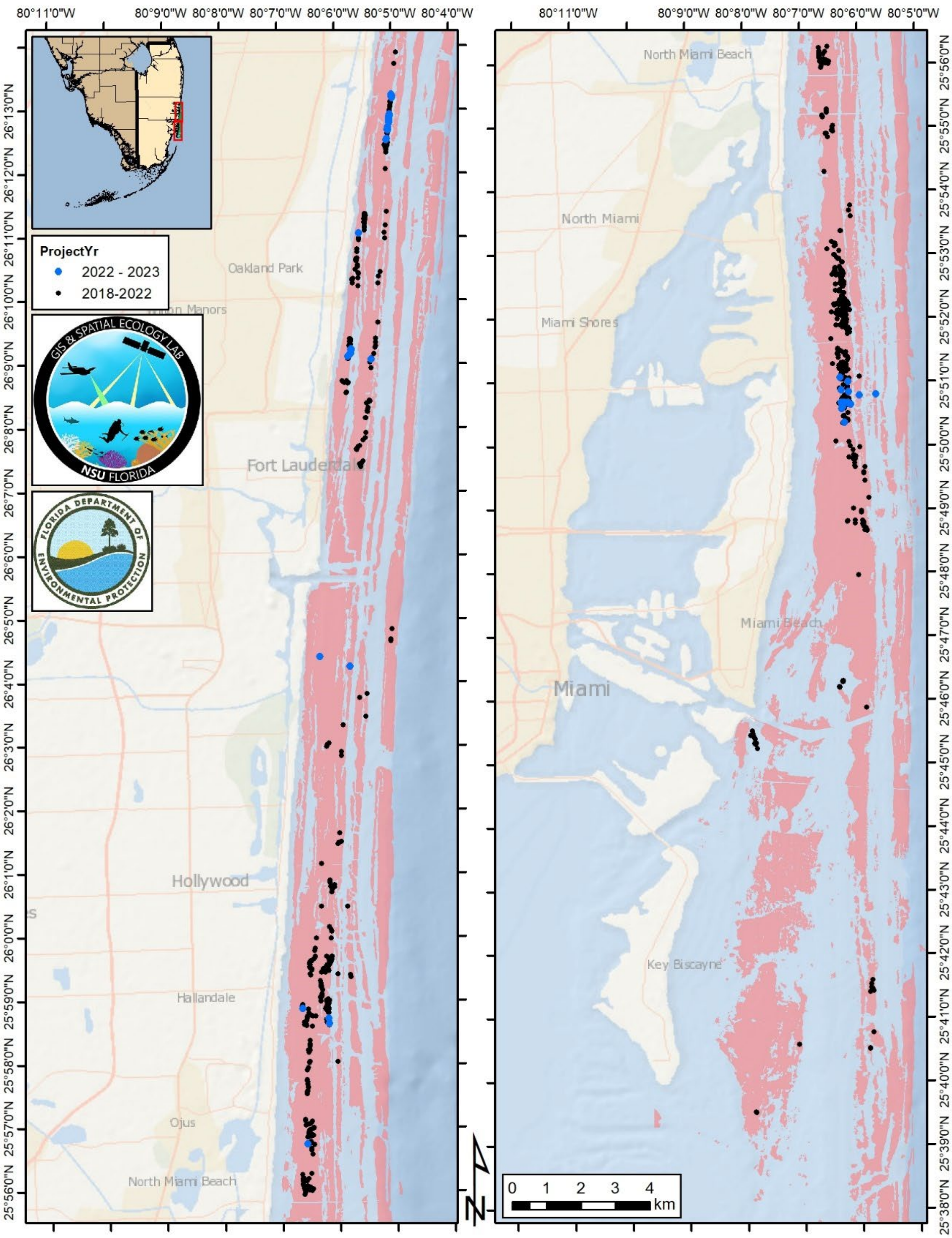

Figure 8. The location of the 1,451 strike team treated corals as of June 13, 2023. The blue dots are those 186 colonies treated during the current project period.

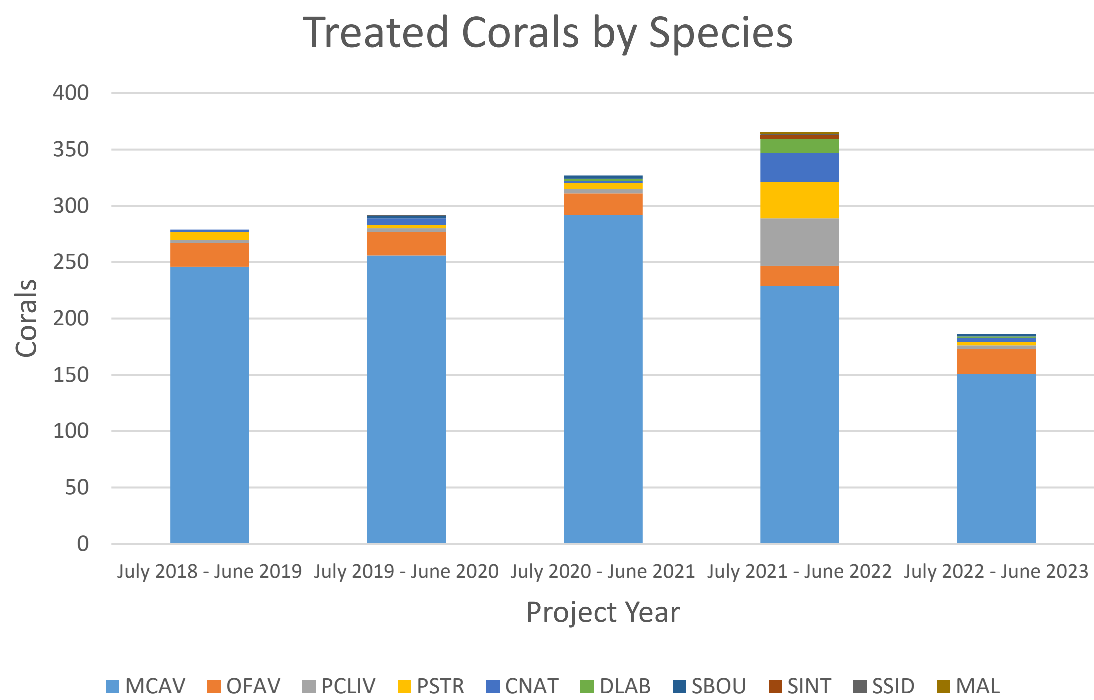

Figure 9. The number of strike team treated corals by species by project year.

4.2. Apply Interventions to 100 Large Corals (Task 3)

a. Treatment Success

Figure 10 illustrates the locations of all 107 priority corals. Not all monitored corals required treatments this period; 55 corals (51.4%) were treated, and 52 corals (48.6%) did not show active lesions (Figure 11 and Table 1). The proportion of colonies requiring treatment were fairly similar between species, with 41 out of the 84 (51.2%) monitored *Orbicella* spp., and 6 of the 14 *M. cavernosa* (42.9%) requiring treatment.

Over the project period (June 2022 to May 2023), a total of 455 antibiotic ointment treatments were conducted on 55 large colonies. 110 treatments failed, equating to 75.4% effectiveness on lesions.

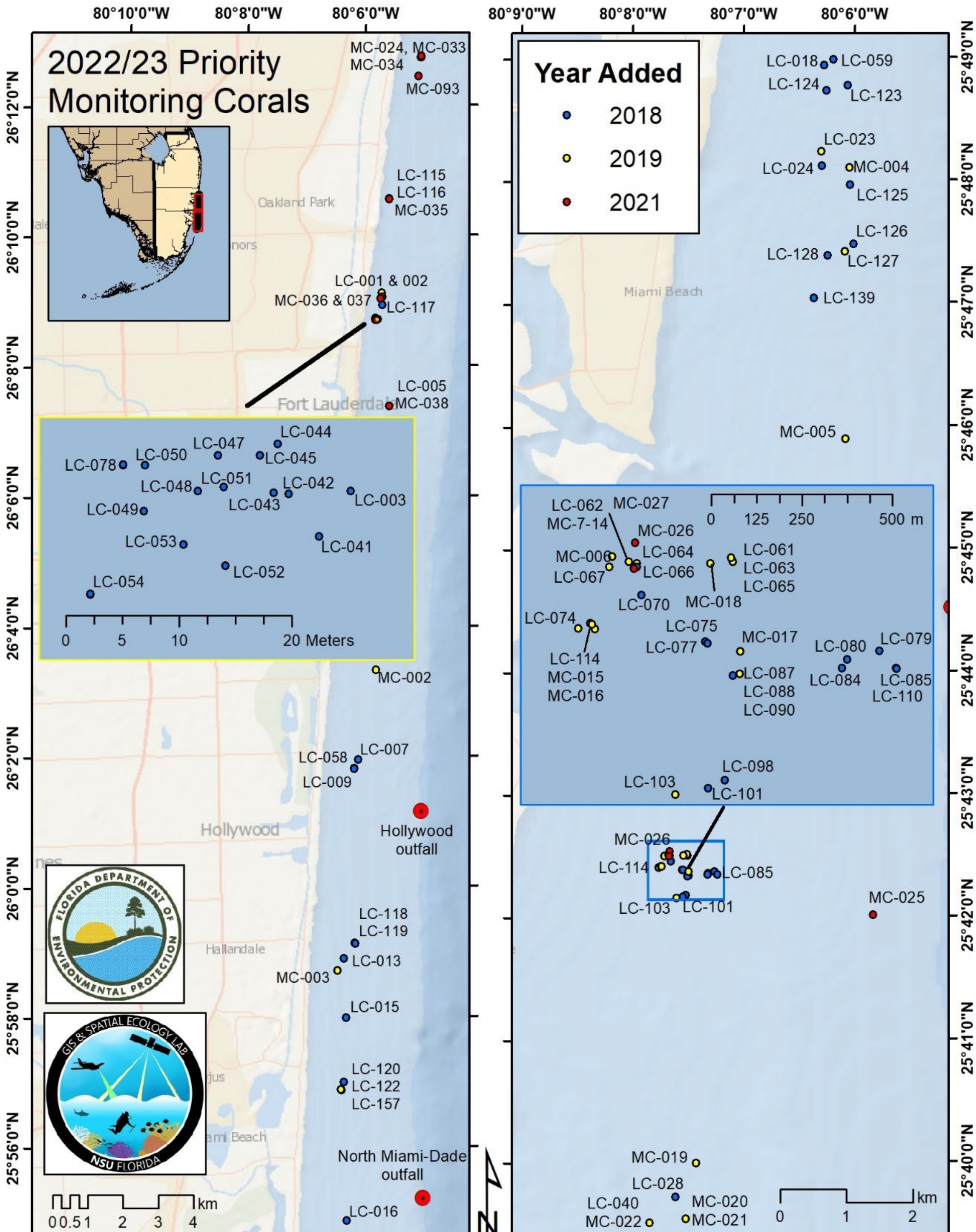

Figure 10. Map of the large priority monitoring corals colored by the year they were added to the monitoring.

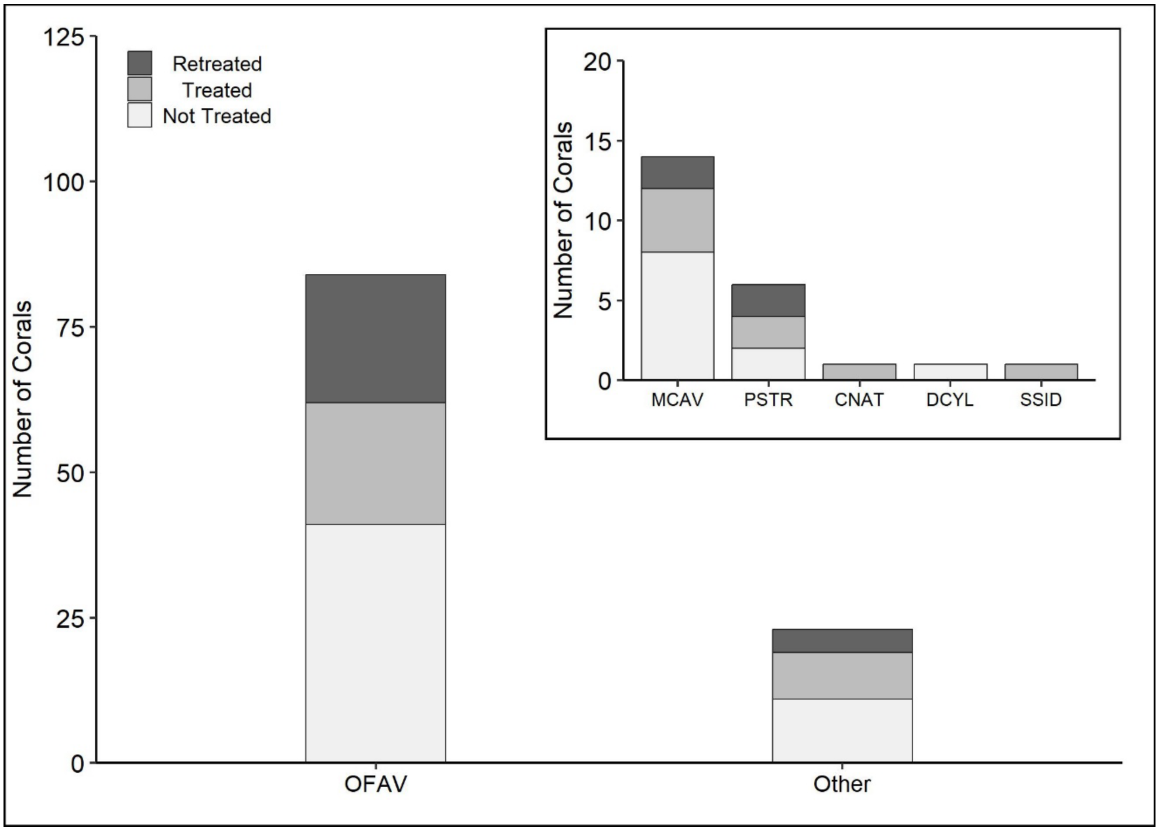

Figure 11. Total number of corals monitored and Not Treated (no disease), Treated (once), and Retreated for each species from July 2022 to May 2023. "Other" has been enlarged to show the number of treatments for the less abundant species.

Table 1. Total antibiotic ointment treatments by species from July 2022 – May 2023.

	MCAV	OFAV	PSTR	ALL SPECIES
Total Colonies	14	84	6	107
Total Treated Colonies	6	43	4	55
% Treated	42.9%	51.2%	66.7%	51.4%

a. Temporal Infection Patterns

All disease lesions were treated at each monitoring period, 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 12 summarizes the number of new treatments required (black) and number of treated corals (grey) per monitoring period for all corals during the project period. The number of new infections and corals requiring treatment varied through time with relatively high numbers of treatments and colonies treated, especially in July 2022 and September 2022. Figure 13 summarizes the amount of treatment required in each period. The most treatments on the most corals of any time prior were performed in the summer of 2022 (Figure 14), indicating the last year's temperature stress increased SCTLD in the ECA.

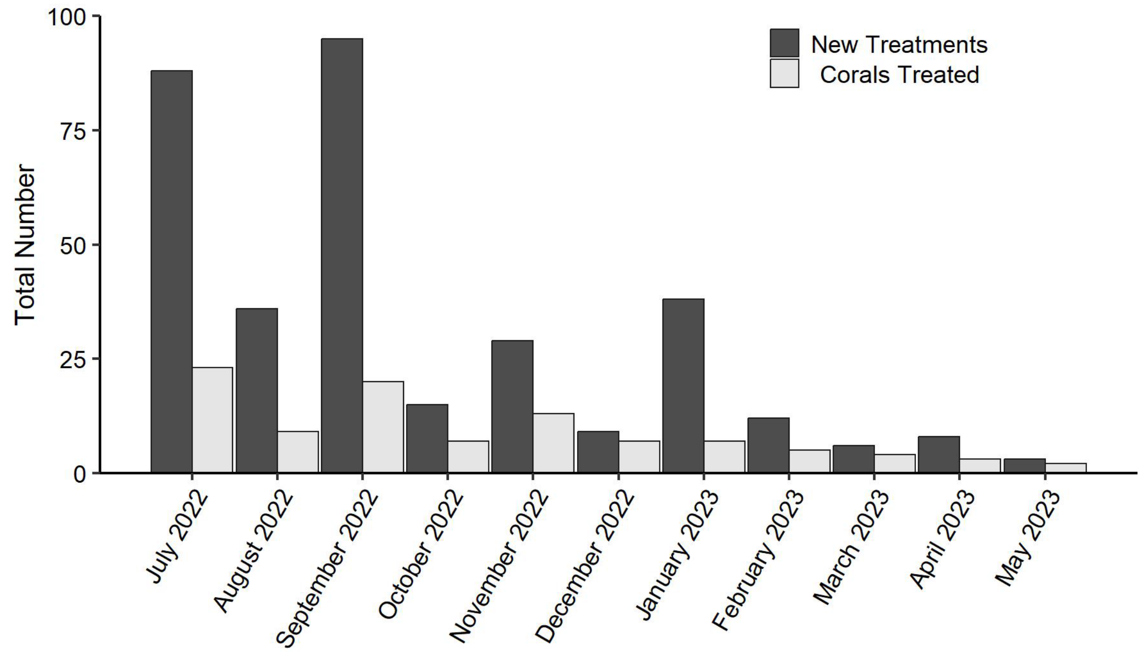

Figure 12. The number of new treatments (black) on all corals used as a proxy for new infections and the number of treated corals (light gray) by treatment period.

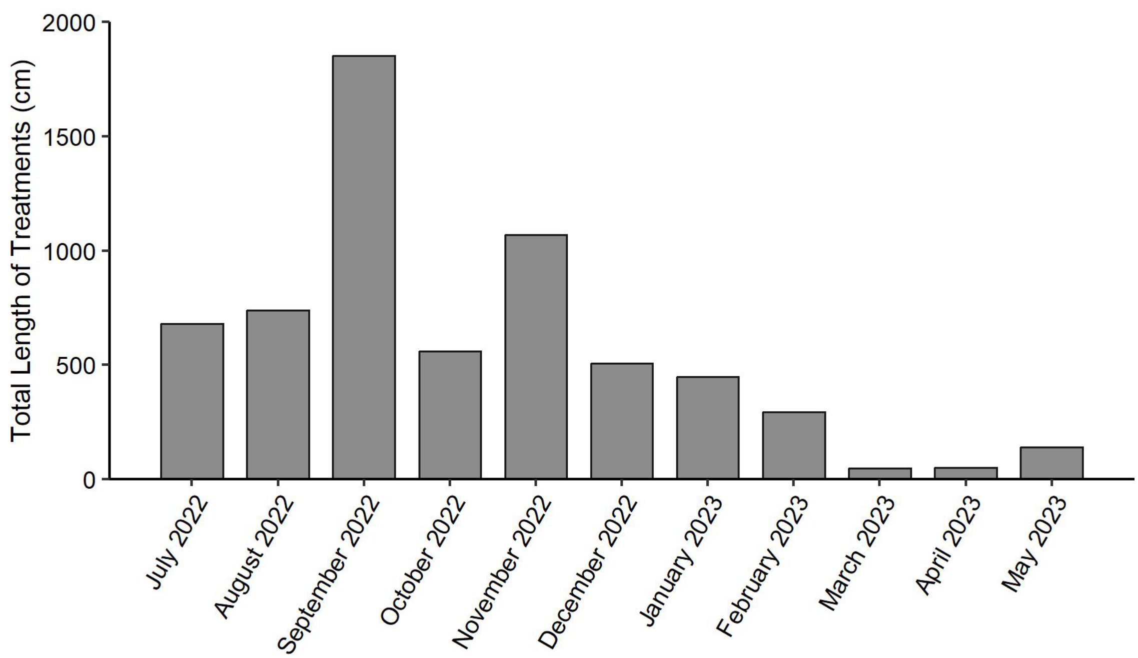

Figure 13**.** The total treatment length (cm) on all corals by monitoring period. Shaded area indicates antibiotic ointment treatments.

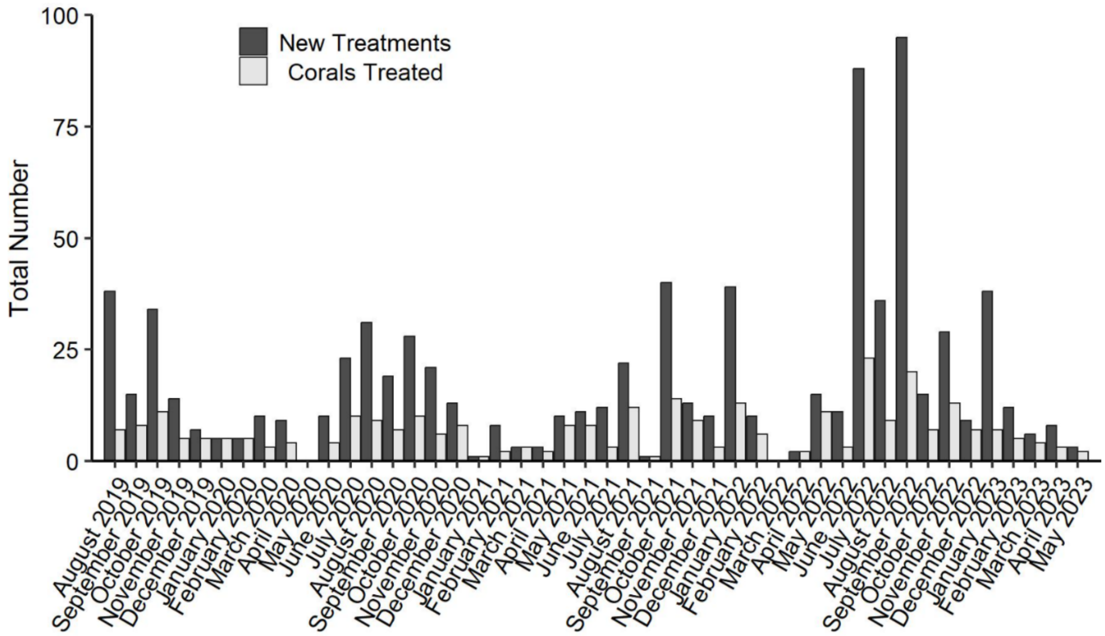

Figure 14. The number of new treatments (black) on all corals used as a proxy for new infections and the number of treated corals (light gray) by treatment period since 2019.

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

During this reporting period we located three new sites of interest (Table 2). Previously, while conducting NCRMP fish counts in south Palm Beach County our divers located an extraordinarily large patch of *Madracis spp.*. We revisited this site on October 27th to document it further and to collect skeletal samples to identify the species (Figure 15). Indications are that this may be the largest uniform patch of *Madracis* that has been described anywhere. We are in the process of verifying this and documenting it further.

The second site of interest was a large colony of *Orbicella faveolata* that was discovered during a strike team disease intervention dive (Figure 16). Our divers located a single large colony measuring approximately 3.5m tall and 3m across with extremely high live tissue coverage (85-90% live). This coral is noteworthy due to its large size, and growth morphology as well as its high tissue cover (especially since it has persisted in the SCTLD endemic zone). This coral was given the tag #5322 and will be entered into our large coral database and monitored periodically.

The final site of interest, located in North Broward, is a large *Orbicella faveolata* colony over 2.5m across with very high tissue cover (Figure 17). It was tagged with #5385. In addition, there were several medium/large colonies in close proximity with slightly less live tissue coverage. The persistence of so many large *Orbicella faveolata* colonies indicates suitable conditions for their growth and may be a potential site for our restoration efforts. We plan on returning to this colony to collect accurate measurements and live tissue estimates as well as health assessments for the smaller satellite colonies. Table 3 and Figure 18 contain information on all recon sites identified to date.

Table 2. Recon sites identified during this reporting period.

Site name	County	Coordinates	Description
Large *Madracis* patch	West Palm Beach	N 26.489237, W 80.037500	Large patch of *Madracis aurotenra*
Large *O. faveolata* #7 (Tag3522)	Dade	N 25.933700, W 80.10921	Very healthy and large Ofav. Just north of Haulover, Approx 3.5m tall and 3m across 85-90%live
Large *O. faveolata* #8 (Tag5385)	Broward	N 26.208512, W 80.085002	Large very healthy Ofav with several other large Ofav nearby (slightly smaller and less healthy). Possible site for restoration.

Figure 15. Large, previously undocumented *Madracis aurotenra* patch.

Figure 16. *Orbicella faveolata* #5322. Note the height and high tissue coverage.

Figure 17. *Orbicella faveolata* #5385, a good candidate for monitoring due to its size and high coral cover. On right: several of the *Orbicella faveolata* satellite colonies located close to 5385. Their presence indicates potential for restoration activities.

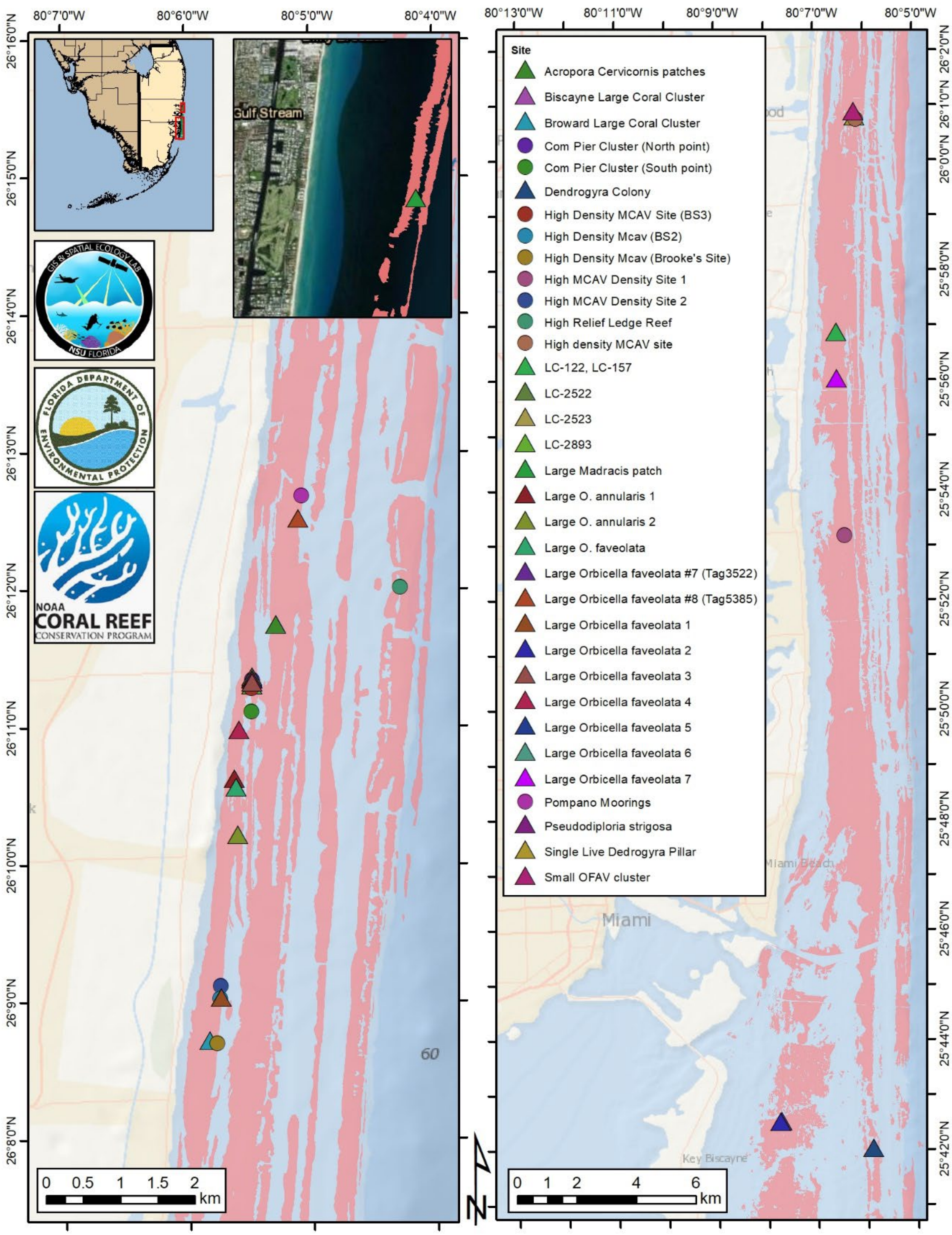

Figure 18. The location of all identified recon sites to date.

Table 3. Preliminary information on all of the recon sites of interest identified to-date.

Nr	Site name	County	Coordinates	Description
1	Dendrogyra Colony (MC-025)	Dade	N 25.700211, W 80.098248	Located a live Dcyl 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	Dade	N 25.708467, W 80.12895	Cluster of large OFAV
6	High MCAV Density Site 1	Dade	N 25.88624, W 80.106852	High density of MCAV on a patch reef.
7	High MCAV Density Site 2 (NCRMP site 3204)	Broward	N 26.151909, W 80.095806	Near BS2 High Density of large MCAV and some CNAT, observed some SCTLD
8	LC-122, LC-157	Broward	N 25.94788, W 80.10913	LCM on 11/24/2020 noted many diseased MCAV and Ofav
9	Commercial Pier Cluster (South point)	Broward	N 26.185208, W 80.09145	High density of MCAV
10	Commercial Pier Cluster (North point)	Broward	N 26.189059, W 80.091318	High density of MCAV
11	LC-2893	Broward	N 26.188383, W 80.091359	Large OFAV Colony
12	LC-2523	Broward	N 26.189161, W 80.091371	Large OFAV Colony
13	LC-2522	Broward	N 26.189415, W 80.091368	Large OFAV Colony
14	Pseudodiploria strigosa	Broward	N 26.189059, W 80.091318	Large PSTG Colony
15	High density Mcav (Brooke's Site)	Broward	N 26.144958, W 80.096331	High density of MCAV
16	High Density Mcav (BS2)	Broward	N 26.150397, W 80.09594	SSI Probiotic Site (High density of MCAV)
17	Acropora Cervicornis Multiple patches	Broward	N 26.195693, W 80.088124	Area with extensive A. cervicornis thickets
18	Single Live Dedrogyra Pillar	Broward	N 26.013177, W 80.102561	Old Dendrogyra colony skeleton with single live pillar
19	High Density MCAV Site (BS3)	Broward	N 26.188045, W 80.091429	High density MCAV site along 1st reef
20	Large O.Annularis #1 (5915)	Broward	N 26.177027, W 80.09383	Large OANN colony 91%old dead but over 5.8m across
21	Large O.Annularis #2	Broward	N 26.170234, W 80.093439	Large OANN colony #2 over 6m across, high living tissue coverage
22	Large O. Faveolata (5029)	Broward	N 26.175988, W 80.093569	Large OFAV colony 3.6m x3.15m
23	High density MCAV site (also many Ofav)	Broward	N 26.012524, W 80.10229	High rugosity reef with very high density MCAV (also many Ofav)
24	Small OFAV cluster (3 colonies close, many nearby, near above site)	Broward	N 26.01474, W 80.102898	Unusually high density of OFAV
25	Large *Orbicella faveolata* #1	Broward	N 26.150438, W 80.095793	Large *Orbicella faveolata* >90% live and healthy
26	Large Orbicella faveolata #2	Dade	N 25.70833, W 80.12949	>1.5m *Orbicella faveolata* 60% live
27	Large Orbicella faveolata #3	Broward	N 26.188699, W 80.091328	~4m *Orbicella faveolata* 85%live and healthy

28	Large Orbicella faveolata #4	Broward	N 26.182942, W 80.093148	>2m *Orbicella faveolata* >85%live and healthy
29	Large Orbicella faveolata #5	Broward	GPS shutoff	~3m *Orbicella faveolata* >90% live and healthy
30	Large Orbicella faveolata #6	Broward	GPS shutoff	*Orbicella faveolata* >1.5m, healthy
31	Large Madracis patch	West Palm Beach	N 26.489237, W 80.037500	Large patch of Madracis
32	Large Orbicella faveolata #7 (Tag3522)	Dade	N 25.933700, W 80.10921	Very healthy and lg Ofav. Just north of Haulover, Approx 3.5m tall and 3m across. 85-90% live
33	Large Orbicella faveolata #8 (Tag5385)	Broward	N 26.208512, W 80.085002	Large very healthy Ofav with several other large Ofav nearby (slightly smaller and less healthy). Possible site for restoration

4.4. Field Test New Permitted Intervention Techniques and Materials (Task 5)

4.4.1. Probiotics on Montastraea cavernosa

During this period, NSU assisted the Smithsonian Marine Station at Fort Pierce with probiotics treatments, setting up new experiments, and the collection of monitoring data. The experiment involved examining the efficacy of probiotics through two different delivery vehicles: 1) probiotic paste and 2) probiotics injected into a bagged coral. The second one was a comparison between probiotics and antibiotic paste (amoxycillin in Base2B). During our disease intervention and recon activities, we located a new site with 8 diseased *Montastrea cavernosa* which became BS4 (Broward Site #4). We also collected 3-D photographs at each site several times to track tissue loss over time.

On June 9, 2022, we took 3-D photographs of all previously tagged corals at BS3 as well as tagging and treating newly diseased colonies with probiotic bags. On June 23, 2022, we set up a new site named Broward Site 4 (BS4) which consisted of 8 diseased *Montastrea cavernosa*. Four of these corals were randomly selected and treated with antibiotic paste. On July 22, 2022, we treated the remaining four corals at BS4 with probiotics bags (the delay in the probiotics treatment was to allow for the antibiotics to fully dissipate so as not to influence the probiotics results). Additionally, we also took 3-D photographs of all corals at BS3 (both from prior probiotics study and the Pro- vs Antibiotic study).

Finally, we revisited the BS2 site and started a new trial in the Pro- vs Anti-biotic study by treating four newly diseased colonies with antibiotic paste. We returned to BS2 one week later on June 29 and treated four additional newly diseased corals with probiotics bags. While these colonies were being treated 3-D photographs were taken of all corals at BS2. Finally, 3-D photographs were taken of all corals at BS4. On August 24, 2022, we took 3-D pictures of all corals at BS2, BS3 and BS4 (both old and new treatments).

On October 20, 2022, we again took 3-D pictures of all corals at BS2, BS3 and BS4. On December 19, 2022, we took 3-D pictures of the corals at BS2, BS3 and BS4 in the Pro- vs Antibiotic project. On February 15, 2023, we attended a zoom meeting between the Walker and Paul lab to discuss previous progress and future directions. On March 14, 2023, we took 3-D pictures of all corals at BS2, BS3 and BS4. Colonies that had no live tissue were noted and excluded. On March 16, 2023, we attended a second meeting between the Walker and Paul lab to go over the current status of BS2, BS3 and BS4 as well as to discuss setting up a new research site or possibly expanding upon one of the existing sites.

On June 5, 2023, we visited BS2 and BS3 to assess active disease at sites to determine the possibility of starting another experimental round comparing probiotic vs antibiotic vs control. During this swim through we counted over 20 newly diseased colonies at BS2. On June 7, 2023, we revisited BS2 to tag and map all non-tagged diseased colonies. NSU divers located and tagged 29 newly diseased colonies. 3-D pictures and GPS location were also collected. Then NSU divers treated 10 randomly chosen corals with antibiotic paste. NSU divers will return together with Smithsonian personnel to treat ~10 colonies with probiotic bags and the remaining corals will serve as untreated controls.

4.4.2. Nutrient enrichment on Montastraea cavernosa

On March 5, 2023, NSU assisted FAU with investigating the impact of elevated nutrient levels on the spread and progression of SCTLD in *M. cavernosa* by captaining their team to the sight. Please contact Joshua Voss and Ashley Carreiro for more information on those experimental outcomes.

4.5. **3-D and MSS testing and evaluation (Task 6)**

We have constructed recent 3-D models of 38 of the large corals in preparation for comparison to the annotated models. At the AMLC conference in St. Kitts several lab members attended a workshop led by Dr. Art Gleason called “Introduction to Underwater Photomosaics for Coral Reef Science and Restoration Monitoring” which provided helpful tips to increase efficiencies of the 3-D modeling process. While our previous models were created from on average about 800 photos (500 – 1000 range), we learned models of similar quality can be produced much faster with less than 100 photos (Figure 19). Making this change has decreased the time underwater divers collect the images by 5-7 minutes and the model processing time by 8-10 hours. With 107 corals on the list, this adds up to saving near 12 hours underwater and up to a 1000 hours of computer processing time. These savings will make it much more feasible to incorporate much more precise live tissue estimates from 3-D modeling as a metric.

We also began using the Agisoft coded targets to increase model accuracy and allow easy scaling of the model. These are partially visible in Figure 19. With these targets, it is also possible to add more with assigned depths and coordinates for more precise modeling estimations. The current main challenges in creating clear and consistent 3-D models include imaging in low visibility conditions and making sure the cameras are white balanced correctly to get clear colors and textures. We cannot control local conditions so if the visibility is poor upon visitation, we cannot capture images for that time period.

Currently 634 photos (4,520 annotated points) have been annotated using the CoralNet online resource. Throughout this stage, we tested the efficiency of using 5 annotation points on 500 photos versus 50 annotation points on roughly 100 photos (Figure 20, top). The automated annotation classifier appeared to have more accurate predictions in a shorter time with more annotation points per photo, however we are still adding more confirmed annotations. Once roughly 10,000 patches are created, our next step will be to extract patches for different class categories (Figure 20, bottom) to begin the training process of the Convolutional Neural Network (CNN). The CNN will be used to add additional sparse labels to our images that can then be passed to a fast multilevel semantic segmentation (Fast-MSS). We will be following the recommendations of Pierce et al., (2021) for the rest of our workflow (Figure 21), as well as using the provided python codes for the CNN and Fast-MSS.

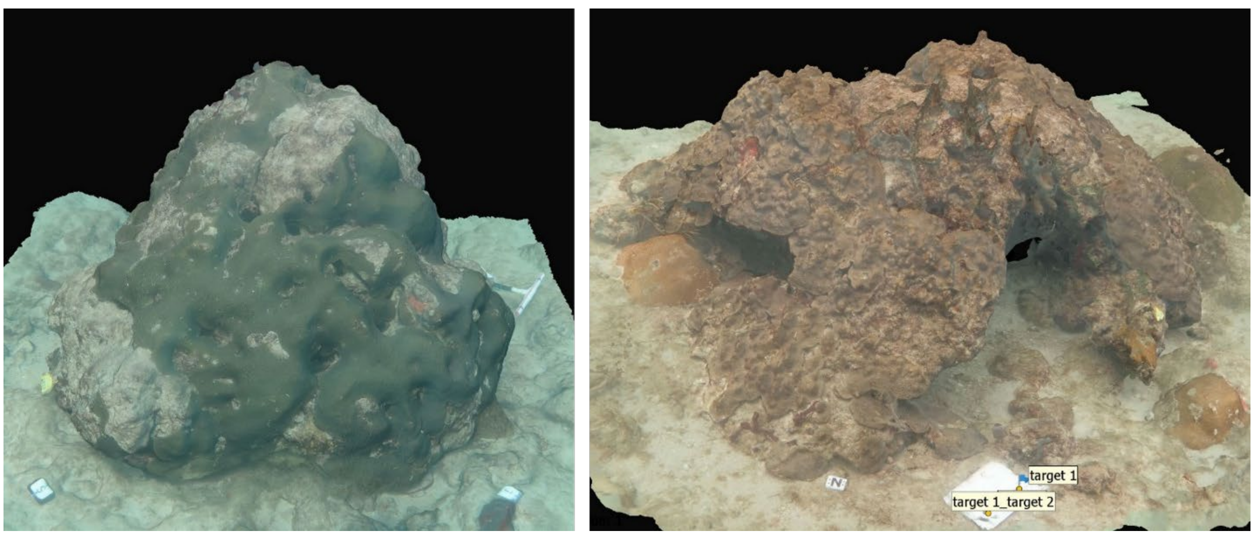

Figure 19. Illustration of 3-D model using original methodology with 830 photos (left) and figure of a 3-D model using updated protocol with 82 photos and Agisoft targets for scale (right).

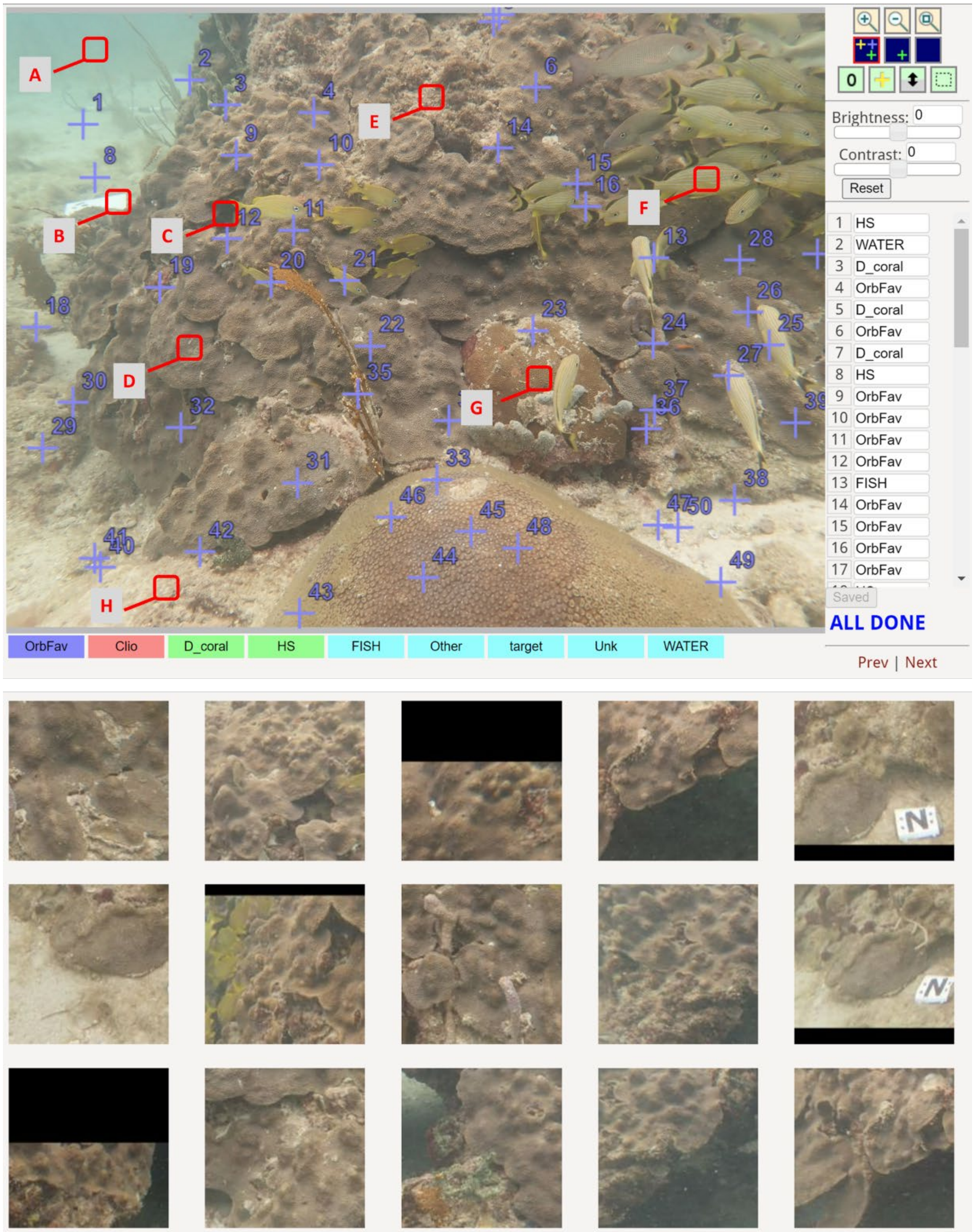

Figure 20. Screenshot of the annotation process using the CoralNet online program. Example annotations are shown with the labeled boxes: A: water, B: target, C: unknown, D: *Orbicella faveolata*, E: dead coral, F: fish, G: other, H: hard substrate (top). Examples of annotated patches that will be used to train the CNN (bottom).

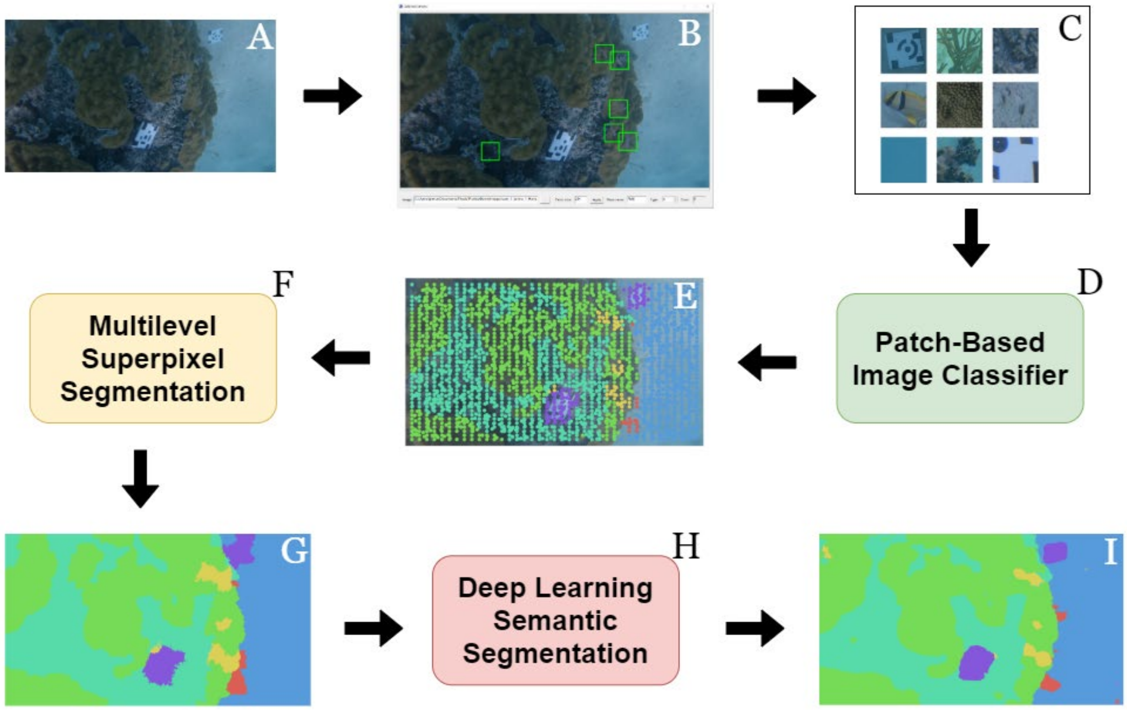

Figure 21. Workflow recommendations from Pierce et al., 2021 for obtaining dense labels that can then be used to classify our 3D models. We are currently developing steps D and E.

5. DISCUSSION

Coral diseases have caused enormous impacts to coral populations globally perpetuating the rapid need for large-scale coral reef restoration. SCTLD exemplifies that coral diseases are more devastating than ever before. When a virulent disease like SCTLD ravages a coral ecosystem, it significantly alters the population's demographics and causes local extinctions. However, recent disease intervention techniques are over 75% effective at stopping lesions. Implementing these in the restoration toolbox reduces the necessity for costly and time-consuming post hoc restoration techniques (e.g. microfragmentation, coral husbandry, and outplanting).

Our work shows that SCTLD is still prevalent in the region, that interventions are still needed, and that these efforts are worthwhile. This past year, interventions helped maintain species diversity and ecosystem services within the Coral ECA and kept alive some of the largest and oldest animals in Florida. Although disease interventions are very effective at stopping disease lesions, they do not provide protection against reinfection. This requires regular visits to ensure colony survival. Since disease intervention response during such an event is virtually impossible on a landscape scale, priorities must be considered. Intervention activities are usually allocated between prioritizing saving certain colonies by regular monitoring and treating large areas without the intent to monitor. The latter involves a team of divers, known as a strike team, covering large areas of reef, treating coral lesions that they encounter, tagging treated colonies, and recording their locations. The purpose of the strike team is to maximize the area covered to treat as many disease lesions as possible without the intent of returning to monitor treatment success. By treating more of the remaining corals less susceptible to SCTLD, more live coral tissue and genetic diversity are saved. This allows post hoc restoration to be focused on the species more susceptible to SCTLD that are now functionally extinct.

Prioritizing the largest, oldest colonies of reef-building species is imperative to preserve the high fecundity, ecological function, and genetic diversity of the most resilient corals. Large corals have a higher reproductive potential thereby increasing the natural ability to replenish the reef as well as harness the potential for assisted reproduction through gamete collection. Their size also preserves ecological functions such as existing reef structures and habitats. As a proxy for age, coral size is an indicator of their resistance to previous perturbations which may indicate higher fitness.

Monthly monitoring and treatments have reduced the loss of live tissue area and provided valuable information on the temporal and spatial variations of colonies with lesions. Corals not regularly treated with disease interventions have had drastic declines in live tissue area. Regular disease intervention is restoring colony health and saving the large priority corals from SCTLD and is an efficient method to save live tissue area preempting slower, more costly post hoc restoration efforts.

By stopping disease lesions, *in situ* disease interventions can save a higher magnitude of live tissue in comparison to the amount that can be generated from years of microfragmentation. Our intervention activities have saved species and size classes that would take decades to achieve through asexual restoration at a fraction of the cost that equivalent restoration would require to achieve similar coral cover. Considering the Coral ECA large coral monitoring alone, the current live tissue area of all previously treated large corals is 3,578,091 cm^2 (357.8 m^2 or 3,851 ft^2). This is the equivalent to the area of 2 volleyball courts of live coral tissue. If 36 outplant plugs covered one sq ft with 100% survival and no disease, that equates to 138,636 coral restoration fragment plugs. Granted, we can't assume all tissue would've been lost without treatment or that the treatments are 100% effective or what will be lost if we stop monitoring those corals today, but it gives a rough estimate of the restoration effort required to get a similar amount of tissue. There is also the considerable advantage that the saved corals are already reproductive, and the largest ones have the most reproductive potential. Disease interventions have the added benefit of reducing disease loads as well. But the use of antibiotics poses an unknown risk in the environment.

Our reconnaissance for coral restoration sites identified two new large *Orbicella* and one massive *Madracis* patch. One *Orbicella* was in an area of high coral density. These locations are good targets for periodic strike team activities to recon for lesion outbreaks and perform interventions when necessary. These locations can also be useful in collecting hardened colonies that may be more resistant to the disease.

These new corals/sites are of high value to the State of Florida. These colonies can be used as genetic stock for gamete collections for assisted reproduction. They can also be monitored and treat for disease to assist in understanding disease dynamics and coral resistance to disease.

Spatiotemporal disease patterns occur on portions of Florida's Coral Reef, where SCTLD is endemic. Frequent monitoring and successful disease interventions have kept diseased corals alive, revealing variable lesion outbreak patterns where some colonies frequently or occasionally exhibit new lesions, while others appear resistant to SCTLD and have never been observed with lesions. The monthly monitoring data have been invaluable in linking environmental drivers to disease dynamics (Walker et al., 2021). Higher water temperatures, amounts of water following out of the inlets, and rainfall account for 56.4% of the temporal variability in the number of new lesions. Understanding these dynamics can help identify the disease and optimum times to treat. Caution should be taken when choosing coral restoration sites as to avoid areas more prone to disease.

6. RECOMMENDATIONS

Continue monthly monitoring and treatment of large priority corals – Monitoring these colonies has saved many from extinction. Monthly monitoring and treatment have facilitated the classification of corals based on differing infection rates which has informed the design of the SCTLD Resistance Research Consortium.

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

Continue use of antibiotic ointment CoreRx B2B and amoxicillin (1:8 weight ratio) – Perform margin treatment and appropriate disease-break interventions using antibiotic paste on SCTLD lesions.

Continue opportunistic recon for potential new monitoring colonies and restoration sites.

Continue public outreach for SEAFAN and other citizen reporting mechanisms to educate the public on reporting diseased corals.

Analyze previous data collections to understand pre-SCTLD coral densities and richness.

Avoid areas prone to increased coral disease when choosing restoration sites.

7. CITATIONS

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