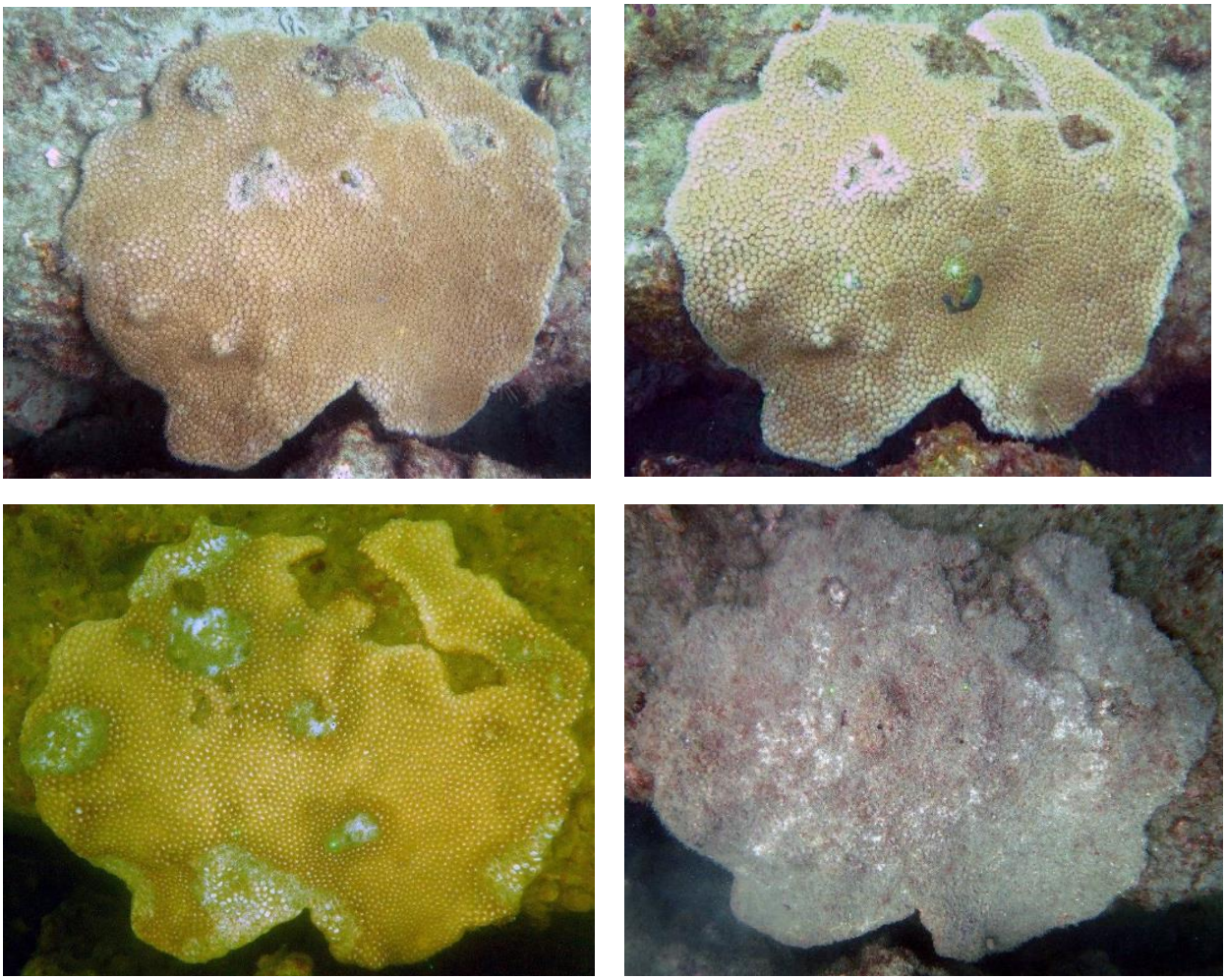
Fate tracking, molecular investigation, and amputation assessment of tissue loss disease on corals in the northern Florida Reef Tract



Final Summary Report

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1. BACKGROUND

Florida's coral reefs are currently experiencing a multi-year outbreak of a coral disease described as "tissue loss." While disease outbreaks are not unprecedented, this event is unique due to the presence of multiple symptoms and etiologies that have affected at least 21 species of coral across the Florida Reef Tract (FRT). The disease(s) are highly prevalent and are estimated to have resulted in the mortality of millions of corals across southeast Florida (SE FL), Biscayne National Park (BNP), and the Florida Keys. Hurricane Irma also recently impacted the entire FRT in September 2017, with subsequent freshwater discharge impacts particularly acute on coral reefs in Martin County. The work report here focuses on SE FL and is part of a larger effort to understand the impacts of disease and freshwater discharge on coral health in SE FL and leveraged funding from the Environmental Protection Agency (EPA) to FAU Harbor Branch.

1.1. Project Goals & Objectives

The purpose of this project is to supplement ongoing efforts to understand the impacts of tissue loss disease and freshwater discharge on corals in SE FL's northern FRT. This project was designed to improve understanding of the current spatial extent of the disease outbreak, prevalence, species affected, timeline of disease progression within colonies, likelihood of mortality due to disease among species, and the physiological responses of

corals to disease. The outcomes of this project will contribute to on-going and future coral disease response efforts which seek to improve understanding about the severity and impacts of the Florida Reef Tract coral disease outbreak, identify management actions to remediate disease impacts, and, ultimately, prevent or mitigate the effects of future outbreaks. The project was designed with input from agency representatives and Martin County stakeholders to improve adaptive management regarding coral susceptibility to disease and impacts from infection. Finally, to expand our understanding of *M. cavernosa* population connectivity in SE FL, additional sampling effort was focused in southern Palm Beach and Broward counties.

2. METHODOLOGY

This project expanded and extended the monitoring efforts completed during long-term monitoring in the St. Lucie Inlet Preserve State Park by Dr. Voss's team at Florida Atlantic University's Harbor Branch Oceanographic Institute (HBOI) and recent post Irma surveys completed by Drs. Voss and Walker in 2017. In 2017 targeted sites were surveyed using both disease response monitoring (DRM) and roving diver protocols (see Walker 2018). This project combined repeated surveys, imaging, and coral sampling to address the objectives listed above. Table 1 below summarizes the operational activities at each of the project sites.

Table 1. Operational Activities at Each Project Site

Site Name	County	Activity	Dates
SLR North	Martin	Samples & Surveys	3/19/18, 6/12/18
SLR Central (SEFL01)	Martin	Samples & Surveys	11/19/17, 3/19/18, 6/12/18
SLR South (SEFL02)	Martin	Samples & Surveys	11/19/17, 3/19/18, 6/12/18
SLR Ledge	Martin	Samples & Surveys	3/19/18, 6/12/18
SEFL03	Martin	Surveys	11/19/17
SEFL04	Palm Beach	Surveys	12/8/17, 5/30/18
SEFL05	Palm Beach	Surveys	12/8/17, 6/1/18
SEFL06	Palm Beach	Surveys	12/8/17, 6/1/18
SEFL07	Palm Beach	Surveys	12/7/17
SEFL08	Palm Beach	Surveys	12/7/17, 4/19/18
SEFL09	Palm Beach	Surveys	12/7/17, 4/19/18
SEFL10	Palm Beach	Surveys	12/7/17
SEFL11	Palm Beach	Surveys	12/7/17
SEFL12	Palm Beach	Surveys	12/7/17
SEFL13	Palm Beach	Surveys	12/12/17
SEFL14	Palm Beach	Surveys	12/12/17
SEFL15	Palm Beach	Surveys	12/12/17
SEFL16	Palm Beach	Samples & Surveys	1/10/18, 5/11/18, 6/7/18
SEFL17	Palm Beach	Surveys	12/12/17
SEFL18	Palm Beach	Surveys	1/10/18
SEFL19	Palm Beach	Surveys	1/10/18, 5/11/18
SEFL20	Palm Beach	Surveys	1/10/18
SEFL21	Palm Beach	Surveys	1/10/18
SEFL22	Palm Beach	Surveys	1/10/18
BC1	Broward	Sample Collection	6/19/18
BC2	Broward	Sample Collection	6/19/18
BC3	Broward	Sample Collection	6/19/18

2.1. Roving Diver Surveys

High resolution video imaging was the intended method to conduct surveys at the target sites to determine disease prevalence and coral community demography. However, two initial efforts in this regard were unsuccessful due to poor visibility at all sites. Therefore, roving diver surveys were also conducted at each site (see Figure 1) to record disease prevalence, species impacted, and disease incidence across sites. For 20 minutes, investigators swam around the site where previous DRM transects occurred and collected data. For coral disease, the rover counted every coral species greater than 10 centimeters in diameter. These corals were tallied as either diseased or not diseased. Any coral disease was noted by general descriptors (e.g. Dark spot, White plague). Paling, partial bleaching and bleaching were also noted utilizing the following codes to indicate the severity of discoloration. Bleaching or paling directly associated with a disease (next to a margin of recent mortality) was not recorded as paling/bleaching, but this was difficult to distinguish in many cases of diffuse bleaching without decaying tissue. Any discoloration of coral tissue was considered pale (P). Patches of fully bleached or white tissue were considered partially bleached (PB), and totally white tissue with no visible zooxanthellae was considered bleached (BL). Diver propulsion vehicles were particularly useful for maintaining position and effectively conducting surveys (and later sampling) at sites such as Jupiter and Palm Beach Breakers where currents were up to two knots.

Roving Diver Code Legend:
UK = Unknown
DS = Dark Spot
BB = Black Band
RB = Red Band
YB = Yellow Band
WB = White Blotch
WP = White Plague
WS = White Syndrome
P = Paling
PB = Partially Bleached
BL = Bleached
STLD = Scleractinian Tissue Loss Disease**
**Not noted in the original Roving Diver Survey Data Sheets since this convention was not yet adopted. Early notes on the datat sheets list these observations as “White Blotch”

2.2. QA/QC

All site data were entered into Excel where QA/QC and data summaries were performed. Once entered, data were reviewed to ensure consistency with data sheets. During the summary table creation, the data were once again reviewed for consistency between teams especially for coral species and disease identifications. In some cases, site pictures were reviewed to help this QA/QC process.

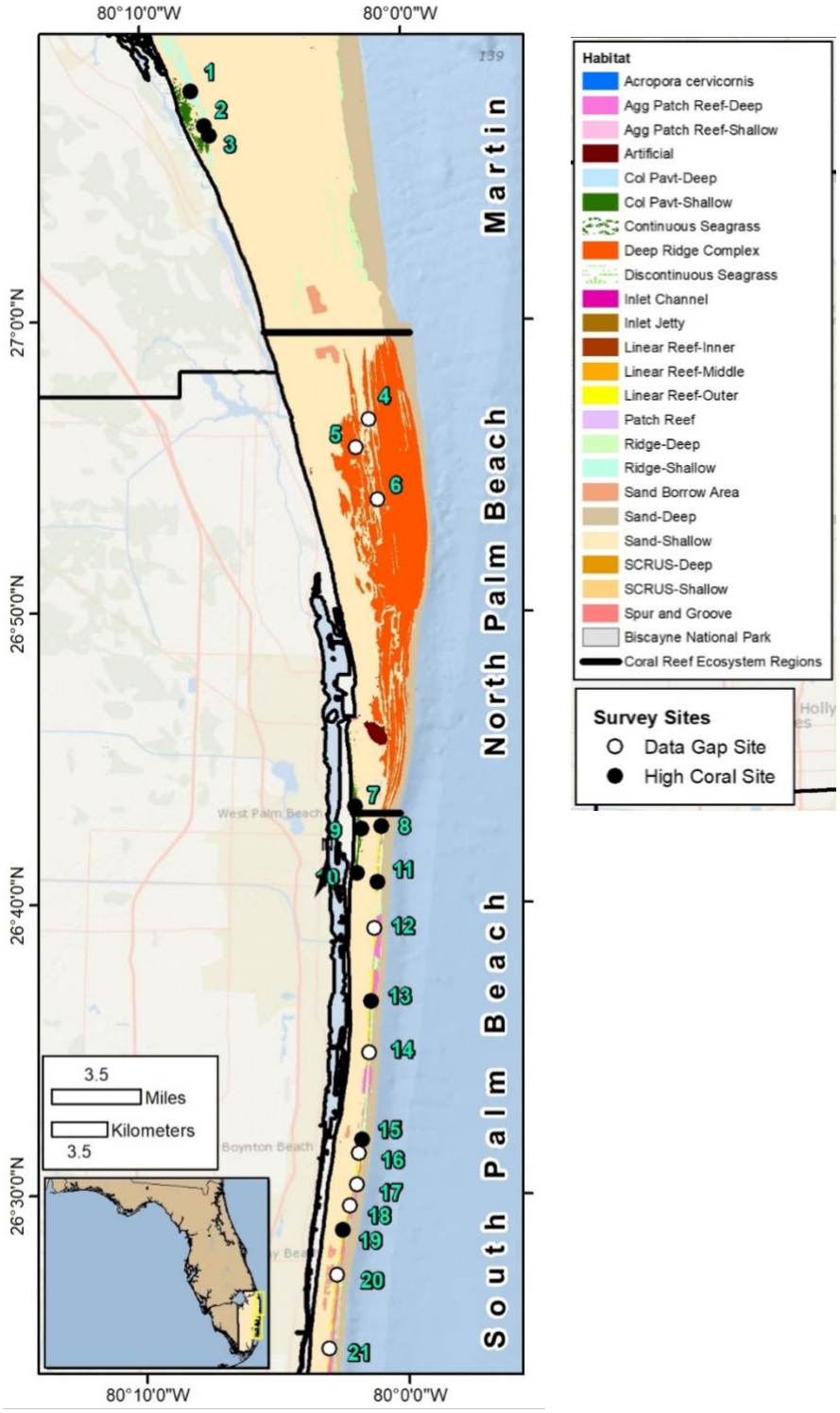


Figure 1. Southeast Florida disease survey site map. White sites were chosen to fill data gaps in the SE FL Irma response project and black sites were previously surveyed sites with high coral values (Walker, 2018). Sites reassessed in this project included sites 1, 2, 3, 4, 5, 6, 8, 10, 16, 19, 20, 21, and 22 (not pictured but just south of 21).

2.3. Coral Fate Tracking and Imaging

Corals at St. Lucie Reef including *Montastraea cavernosa* and *Pseudodiploria clivosa* have been tracked since 2010 and were monitored throughout this project (until complete mortality occurred). Each coral was photographed using a Canon G16 camera in a Fantasea housing using underwater green laser arrays scaled at 15-centimeter spacing. The camera was oriented perpendicular to the colony at a linear distance suitable for capturing the entire colony with no zoom. If abnormalities were observed, more detailed close-up images of disease lines or bleached tissue were also collected. The objectives of this project also included tracking infected coral colonies in Palm Beach and later Broward counties based on the observed disease prevalence in 2017. However, no active diseases were observed in Palm Beach County during the course of the study. When active diseases were observed, we revisited the colonies for repeated imaging approximately every two months as weather and current conditions allowed. In addition, qualitative notes on the appearance of the lesions as well as the number of active lesions were recorded. If the colony had experienced complete mortality or if the colony had been removed from the reef, presumably due to strong wave events including those during Hurricane Irma, these were noted in our observations.

2.4. Coral and disease sample collection

Coral Gene Expression Samples
To quantify the impacts of tissue loss disease on coral physiology, naturally infected *Montastraea cavernosa* and *Pseudodiploria clivosa*. were sampled. For infected individuals of each species, 2.5-centimeter diameter core samples were collected at the disease margin and on the most distal unaffected area of the colony. Baseline non-infected transcriptomes have already been generated and analyzed on *M. cavernosa* at St. Lucie and Palm Beach. The samples were collected and preserved for future research. The optimized pipeline developed with recent FL Sea Grant funding will be used, including Tag-Seq transcriptomic analyses with Illumina HiSeq and DESeq2 to quantify differential gene expression.

Pathogen and Histological Samples
When possible, subsamples of disease and distal samples were collected for histological analyses. These samples returned to the boat and fixed in z-fix and, when possible, Trump's fixative per the instructions provide by Florida Fish and Wildlife Conservation Commission (FWC; see appendix 1). Sampling supplies and preservatives for histological samples were provided on May 25, 2018 by FWC (Jan Landsberg and Yasu Kiryu).

Contamination control
Operational considerations for minimizing cross contamination or pathogen spread were used in this study. All sampling equipment were sterilized on land before use (using chemical or heat and pressure sterilization techniques as appropriate) and placed in separate numbered sterile collection bags for each sample target. To minimize cross contamination between colonies, each pair of nitrile gloves were used and discarded in a

separate designated sealable bag after each colony was sampled. To minimize cross contamination between sites, all collection equipment were sterilized on the boat in a 5-10% sodium hypochlorite (bleach) solution for 20 minutes, while traveling between sampling sites. Dive gear and wetsuits were sterilized using a 10% Odoban solution between operations.

Photogrammetry of Affected Corals
Before and after coral sampling, laser-scaled digital images were collected using custom cameras systems with Canon G16 cameras and Fanatasea underwater housings. For each sampled colony 90° overhead planer photographs, 45° colony profiles, and close ups of disease margins were included.

Coral Population Genetics Samples
For *M. cavernosa*, approximately five square centimeter fragments were collected using a hammer and chisel from the tissue of apparently healthy (non-diseased) colonies in Boynton Beach (SEFL16) and Pompano Beach (BC1, BC2, BC3). Each sample was placed into an individual numbered bag for transport to the boat, photographed, and preserved in molecular grade ethanol (EtOH). Laser-scaled photographs of each colony were taken before and after collection for each colony. These samples have been extracted using established protocols in the Voss laboratory and will be analyzed using nine previously developed microsatellites.

2.5. Intervention Strategies
Sufficient numbers of tissue loss affected colonies were observed in St. Lucie Inlet State Park on March 19, 2018 to have carried out intervention efforts. However, intervention under the existing state park sampling permit was not permitted. In a fortuitous change for SE FL's corals, no active lesions were observed on any corals during survey activities from March to June at any of the Palm Beach County sites. However, with no active lesions observed, we were unable to trial intervention efforts. As a result, we proposed a change in the scope of work to include expanded sampling for coral population genetics and connectivity as described above.

3. RESULTS

3.1. Disease Observations and Sampling
The following quick look reports summarize our disease observations during the course of this project and provide preliminary statistical analyses regarding the relative abundance of coral species at each site where roving diver surveys were conducted

On March 19, 2018 members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted four sampling dives at St. Lucie State Park (SLR). *Montastraea cavernosa* and *Pseudodiploria clivosa* colonies that have been monitored over the past seven years at SLR were revisited, photographed, and small tissue biopsies were taken for gene expression analysis. Additionally, broader disease sampling occurred on untracked colonies. The individuals displaying physical signs of disease were sampled at two locations, adjacent to the disease margin (< 1cm) and on visibly healthy tissue away

from the disease margin (> 5cm), using a hammer and chisel. Across the four sites, nine diseased colonies were sampled at North, Central, and South sites, of those colonies sampled, only one previously tracked colony was sampled for disease at the North site. A total of 16 samples were collected from 11 different colonies, 10 *Psuedodiploria clivosa* colonies and 1 *Solenastrea bournoni* colony. Not all samples had enough visibly healthy tissue for a distal sample. All samples were stored in ethanol, placed on ice, and transferred to a -80 ºC freezer to await molecular analyses. No diseased colonies were found at the Ledge site; however, multiple bleached *Montastraea cavernosa* colonies were observed, two of which were previously tracked colonies (Mcav3 and Mcav4). *Madracis decactis*, *Siderastrea radians*, *Siderastrea siderea*, and *Isophyllia sinuosa* colonies were observed without disease. Of the previously tracked colonies 1 *Psuedodiploria clivosa* was infected, two *Pseudodiploria clivosa* were deceased and one *Montastraea cavernosa* colony was deceased.

On April 19, 2018 members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted 25-minute roving diver surveys at Breakers Reef, off West Palm Beach, recording colony size and the presence or absence of disease. No visibly diseased colonies were observed. A total of 306 colonies across two sites were recorded, of those, only two were observed as bleached. Of the total colonies observed, 46% were *Montastraea cavernosa* and 33% were *Porites astreoides.* The remaining 20% of coral cover consisted of *Agaricia agaricites, Dichocoenia stokesi, Madracis decactis, Madracis formosa, Meandrina meandrites, Orbicella faveolata, Pseudodiploria strigosa, Siderastrea radians, Siderastrea siderea, Solenastrea bournoni,* and *Stephanocoenia intersepta* comprised the remaining species. The two bleached colonies were both *Montastraea cavernosa*.

On May 11, 2018 members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted 25-minute roving diver surveys at Boynton Beach, recording observed colonies and the presence or absence of disease. Three surveys were conducted and no visibly diseased colonies were observed. A total of 151 colonies were observed across three sites, of those, only one was observed as paling. Of the total colonies observed, 50% were *Montastraea cavernosa*, 13% were *Porites astreoides,* and 9% were *Siderastrea siderea.* The remaining 28% of coral colonies consisted of *Agaricia agaricites*, *Agaricia lamarcki*, *Eusmilia fastigiata, Madracis decactis, Madracis formosa*, *Orbicella annularis*, *Orbicella faveolata*, *Solenastrea bournoni*, and *Stephenocoenia intersepta*.

On May 30 and June 1, 2018, members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted three, 25-minute roving diver surveys at Jupiter Ledge, recording observed colonies and the presence or absence of disease. Due to unfavorable conditions, the surveys were split between two days. If high enough disease prevalence was found, those colonies were to be tagged and sampled for future molecular work, however no visibly diseased colonies were observed. A total of 142 colonies were observed across three sites, and no active lesions was observed. Of the total colonies observed, 68% were *Montastraea cavernosa*, 11% were *Agaricia agaricites,* and 9% were *Porites astreoides.* The remaining 12% of colonies consisted of *Dichocoenia stokesi, Helioseris cucullata,*

Oculina diffusa, Orbicella annularis, Siderastrea radians, Siderastrea siderea, Solenastrea bournoni, and *Stephanocoenia intersepta*.

On June 7, 2018 members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted 2 sampling dives off Boynton Beach. *Montastraea cavernosa* colonies were sampled for population genetics and connectivity analyses. Small tissue biopsies were taken from *Montastraea cavernosa* colonies using hammer and chisel. 33 samples were collected throughout two dives. All samples were preserved in ethanol, placed on ice, and transferred to a -80 ºC freezer to await molecular analyses. Only visibly healthy, non-diseased colonies were selected for sampling, however, overall disease prevalence was low.

On June 12, 2018 members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted four sampling dives at St. Lucie State Park. *Montastraea cavernosa* and *Psuedodiploria clivosa* colonies that have been monitored over the past seven years at SLR were revisited and photographed. Small tissue biopsies were taken for gene expression and histological analyses on tracked and untracked colonies that exhibited disease. The individuals displaying physical signs of disease were sampled at two locations, adjacent to the disease margin (<1cm) and on visibly healthy tissue away from the disease margin (> 5cm) using hammer and chisel. Across four sites, five diseased colonies were sampled at Central and Ledge. Of those, only one previously tracked colony was sampled for disease at the North site. A total of 10 samples were collected from five different colonies. Two *Montastraea cavernosa* colonies, two *Pseudodiploria clivosa* colonies, and one *Isophyllia sinuosa* colony. To our knowledge, this is the first recorded occurrence of tissue loss disease on *I. sinuosa.* All colonies had enough visibly healthy tissue to take an adjacent and distal sample. All samples were preserved in ethanol, placed on ice, and transferred to a -80 ºC freezer to await molecular analyses. Of the previously tracked colonies, 25 were located, 10 were found apparently healthy, 6 were found dead, 4 were found diseased, and the remaining 5 were found with considerable recent mortality.

On June 19, 2018, members of the Voss Lab (FAU Harbor Branch) and Jeff Beal (FWC) conducted three sampling dives off Pompano Beach. *Montastraea cavernosa* colonies were sampled for population genetics and connectivity analyses. Small tissue biopsies were taken from *Montastraea cavernosa* colonies using hammer and chisel. Thirty samples were collected throughout two dives. All samples were preserved in ethanol, placed on ice, and transferred to a -80 ºC freezer to await molecular analyses. There was substantial healthy *M.* cavernosa prevalence that only visibly healthy colonies were sampled.

3.2. Fate Tracking

Using photogrammetry to track disease progression was successful for the few colonies observed with active lesions during this study. By combining images collected during this project and historical images from a previous Florida Sea Grant funded study the effective duration of fate tracking was longer for some colonies. We have compiled the

images and historical records for individual colonies at St. Lucie Reef. Of the tracked corals at each of the St. Lucie Reef sites, 33%, 73%, 70%, and 58% of the colonies have died since September 2017 at the North, Central, Ledge, and South sites, respectively. Below are examples of images from tracked colonies demonstrating resilience and survival in some colonies (e.g. PCLI4, PCLI6 at North site) while total colony mortality was observed in others (e.g. MCAV6, MCAV8 Ledge). The catastrophic losses of the primary scleractinian corals at these sites is likely to have significant impacts on the overall community structure and ecosystem services of these reefs. Only the Northern site, lying closest to St. Lucie Inlet, had relatively lower disease prevalence, though 1/3 of the track corals perished. St. Lucie North may benefit from a lack of *M. cavernosa* within the site acting as additional targets (i.e. reduced density dependent disease prevalence), or perhaps frequent freshwater discharges from the inlet are unfavorable for any pathogenic agents.

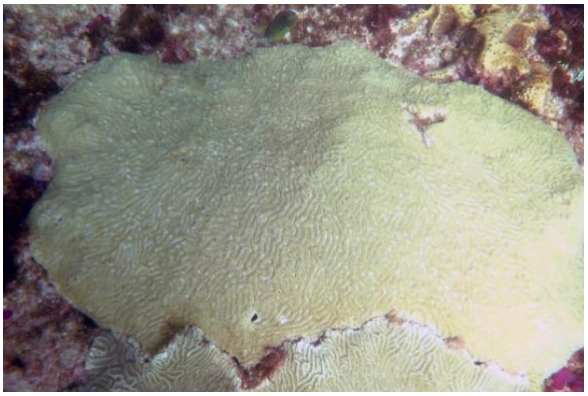
***P. clivosa* PCLI4, St. Lucie Reef North**

6.30.17

3.19.18

6.12.18

P. clivosa PCLI6, St. Lucie Reef North



6.30.17

3.19.18

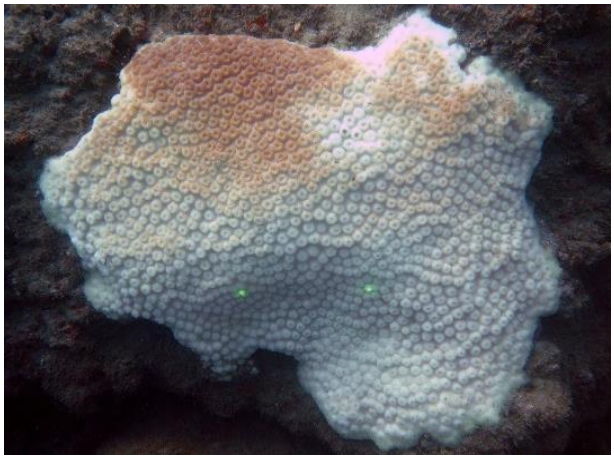
6.12.18

M. cavernosa MCAV3, St. Lucie Reef Ledge

6.30.17

8.10.17

10.9.17



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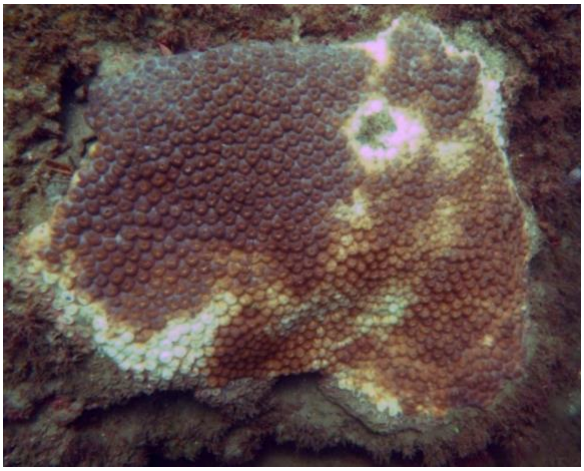
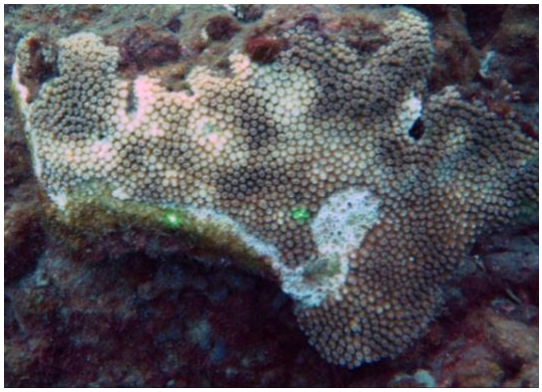


6.12.18

M. cavernosa MCAV6, St. Lucie Reef Ledge

6.30.17



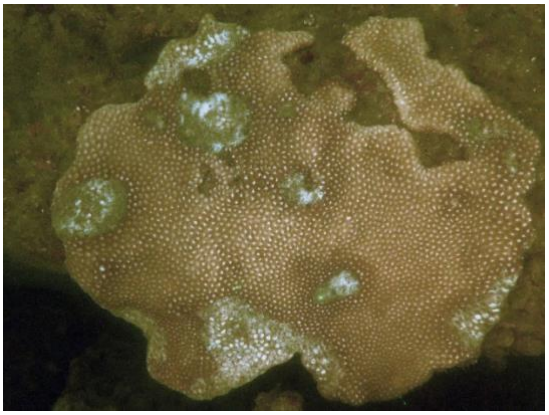
8.10.17

10.8.17

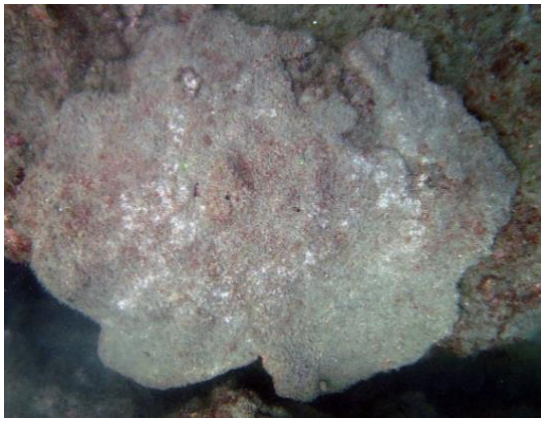
***M. cavernosa* MCAV8, St. Lucie Reef Ledge**

6.30.17

8.10.17



10.9.17



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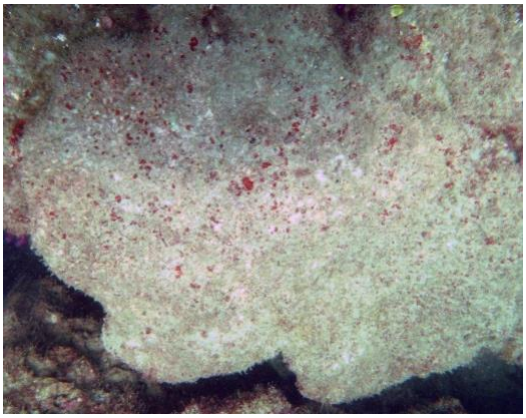
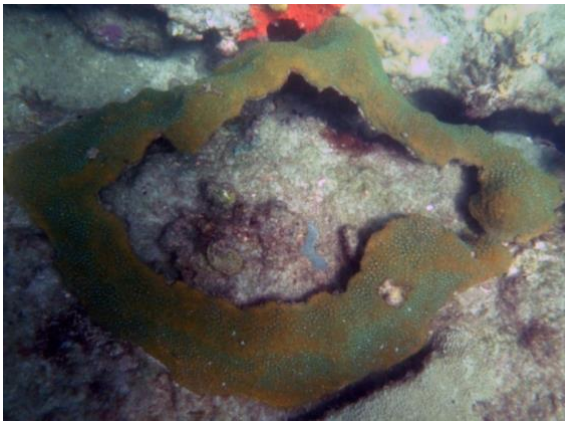


6.12.18

M. cavernosa MCAV2, St. Lucie Reef Central



6.30.17

8.10.17

3.19.18

6.12.18

***P. clivosa* PCLI803, St. Lucie Reef Central**

3.19.18

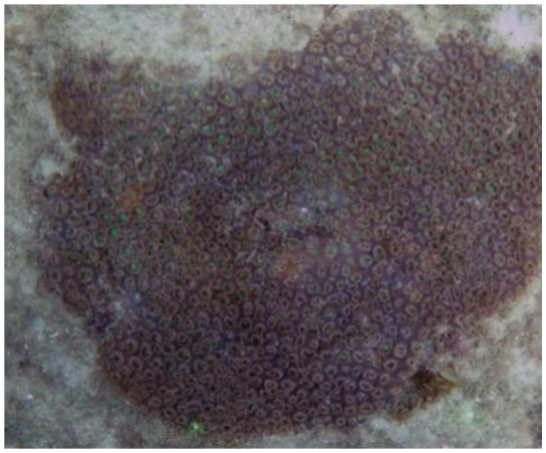
6.12.18

***M. cavernosa* MCAV4, St. Lucie Reef South**

6.30.17

10.8.17

3.19.18



6.12.18

P. clivosa PCLI4, St. Lucie Reef South

6.30.17

10.9.17

3.19.18

3.3. Coral and Disease Sample Summary

As a result of relatively infrequent disease observations following March 19, 2018, only 26 tissue loss affected coral samples were collected during this project, the vast majority of which were *P. clivosa* (n=19). Of the 26 disease samples, 10 were subsampled for standard histological analyses and 7 for transmission electron microscopy. Since no disease was observe in Palm Beach County during this project, all of these samples were collected at St. Lucie Reef. In southern Palm Beach and Broward County, 64 additional *M. cavernosa* colonies were sampled to assess coral population connectivity across the region. Diseased samples will be analyzed using a Tag-Seq transcriptomic analyses with Illumina HiSeq and DESeq2 to quantify differential gene expression.

4. PRELIMINARY CONCLUSIONS

This study demonstrated that tissue loss disease incidence and prevalence may be highly variable over space and time on coral reefs in SE FL. For example, tissue loss was observed at these sites from November 2017 to January 2018 at >20% prevalence in multiple locations. However, disease was not observed during surveys at Palm Beach County sites from March through June of 2018. . In contrast, scleractinian tissue loss disease and bleaching were observed continually throughout the project period among corals at St. Lucie Reef. Perhaps these observations are indicative of the timing and progression of this disease. For example, a potential pathogen may have moved through coral populations in Palm Beach County sites earlier and then subsided during this project. Contemporaneously, it appears the pathogen was reaching St. Lucie Reef and beginning to spread, resulting in relatively higher incidence. While coral populations in Palm Beach County sites appear to maintain some diversity in the coral communities after disease, the relative loss of corals, and coral cover, at St. Lucie Reefs is greater based on preliminary analyses of the roving diver surveys.

Previously we hypothesized that St. Lucie Reef may have been buffered from tissue loss impacts by 1) relative distance from other infected coral communities, and/or 2) stress hardened coral colonies resistant to disease. However, the observation of high disease prevalence and up to 73% losses of coral colonies counter these hopeful hypotheses. The losses at St. Lucie Reef cannot be attributed to disease impacts alone. During the time of these losses impacts from Hurricane Irma and subsequent discharges from the St. Lucie Estuary were also critical drivers that contributed to a severe multiple stressors scenario. The temporal confounding of these events makes interpretation of the proximal causes of coral loss difficult.

During the project period of performance, Dr. Voss has reported the findings of this research and overall information about the status of the outbreak at one SEFCRI meeting, three Florida coral disease working group calls, and one South Atlantic Fisheries Management Council meeting. Dr. Voss has also participated in the Florida Disease Advisory Committee convened by FWC and FDEP to address the coral disease outbreak by developing research priorities, intervention strategies, mitigation efforts, and outreach possibilities. FAU Harbor Branch master's student Ian Combs presented a poster on the project at the 2018 Benthic Ecology Meeting in Corpus Cristi, TX. In addition, several public lectures have been given highlighting this research including the Harbor Branch

Ocean Science Lecture Series, an invited lecture at Nova Southeastern University, and one student lecture at Harbor Branch.

5. RECOMMENDATIONS

Recommendation 1*: Ongoing efforts to identify tissue loss disease agent(s) should be coupled with efforts to identify the etiological mechanisms driving pathogenicity.* Coordinated efforts to share both environmental and experimental samples among multiple researchers aid these complementary goals and can be facilitated by the DAC email communications and calls. We will be using EPA support to investigate the transcriptomes of affected corals and potentially identify signatures of physiological responses of the corals when affected by disease. Ideally the same samples will also be assessed using histological and bacteriological methods.

Recommendation 2*: Because the prevalence of many coral diseases are known to correlate positively with temperature, high frequency monitoring at key sites during periods of thermal stress should be a priority.* Given the speed and severity of tissue loss disease, more frequent monitoring is needed to understand the impacts on Florida's coral communities and to direct any potential mitigation efforts (see below).

Recommendation 3*: Invest in disease mitigation strategies and testing to reduce losses of key ecosystem components.* In the northern SEFCRI region *M. cavernosa* and *P. clivosa* represent the majority of coral cover and are typically the largest coral colonies in the community. With typically flattened morphologies, amputation of affected areas is relatively easy (as compared to massive bouldering corals) and should be tested as a mitigation strategy. Euthanizing and sterilizing affected coral colonies should also be considered to reduce spread.

Recommendation 4*: Advance coral conservation initiatives with support from Magnuson-Stevens Act and implement actions/regulations for the Southeast Florida Coral Reef Ecosystem Conservation Area.* The threat posed to Florida's coral reefs by the tissue loss disease are severe. Any additional efforts to reduced stressors or known impacts to coral reef communities should be implemented to enhance the likelihood of coral resilience and recovery.

Recommendation 5: *To support effective management for coral reef populations and communities in Florida, additional information on population connectivity and source-sink dynamics is needed.* After severe disturbance events like the tissue loss disease outbreak, allocated effort/ resources to particular regions should be based on predicted coral recruitment and recovery. Likewise, effective coral restoration strategies will require knowledge of genetic stocks among various coral populations.