

Investigating the Ongoing Coral Disease Outbreak in the Florida Keys: Collecting Corals to Diagnose the Etiological Agent(s) and Establishing Sentinel Sites to Monitor Transmission Rates and the Spatial Progression of the Disease.

> Florida Department of Environmental Protection Award

> > **Final Report**

FWC: FWRI File Code: F4364-18-18-F

William Sharp & Kerry Maxwell

Florida Fish & Wildlife Conservation Commission Fish & Wildlife Research Institute June 25, 2018 **<u>Project Title</u>**: Investigating the ongoing coral disease outbreak in the Florida Keys: collecting corals to diagnose the etiological agent(s) and establishing sentinel sites to monitor transmission rates and the spatial progression of the disease.

Principal Investigators: William C. Sharp and Kerry E. Maxwell

Florida Fish & Wildlife Conservation Commission Fish & Wildlife Research Institute 2796 Overseas Hwy., Suite 119 Marathon FL 33050

Project Period: 15 January 2018 – 30 June 2018

Reporting Period: 21 April 2018 – 15 June 2018

#### **Background:**

Disease is recognized as a major cause of the progressive decline in reef-building corals that has contributed to the general decline in coral reef ecosystems worldwide (Jackson et al. 2014; Hughes et al. 2017). The first reports of coral disease in the Florida Keys emerged in the 1970's and numerous diseases have been documented with increasing frequency (e.g., Porter et al., 2001). Presently, the Florida Reef Tract (FRT) is experiencing one of the most widespread and virulent disease outbreaks on record. This outbreak has resulted in the mortality of thousands of colonies of at least 20 species of scleractinian coral, including primary reef builders and species listed as Threatened under the Endangered Species Act. First reported near Key Biscayne in 2014 (Precht et al., 2016), this outbreak has progressed southward along the Florida Reef Tract, and by December 2017 had reached the vicinity of Coffins Patch Reef in the middle Florida Keys. The disease itself has been colloquially described as "white blotch" or "white plague", and affected colonies exhibit multiple symptoms and etiologies, suggesting this disease outbreak may be a consortium of several different diseases. However, at present there is limited capacity to rapidly and accurately diagnose these etiological agent(s), and its mode and rate of transmission are poorly understood. Consequently, our limited understanding of the disease outbreak has greatly hindered our ability to implement management efforts to control or prevent the spread of the disease(s).

#### **Project Goals and Objectives:**

The goal of this project was to improve our understanding of the causative pathogens responsible for the widespread coral mortality and improve understanding of its rates of transmission between coral colonies. The outcome of this project contributes to the on-going coral disease response effort that seeks to improve the understanding of this disease event and facilitate effective management actions to remediate its impacts. Ultimately, the results of this project will aid mitigation or prevention of future "white blotch" disease outbreaks.

The specific objectives of this project were:

- i) provide coral samples for an upcoming Florida State Wildlife Grants-funded project that will identify the causative pathogens responsible for this coral mortality.
- ii) establish and monitor "sentinel" coral colonies at locations off Marathon, FL in areas currently unaffected by the disease to monitor the outbreak's spatial progression along the reef tract and monitor disease transmission rates and evaluate the spatial epidemiology of the disease outbreak.

# **Methods**

#### **Objective 1: Collection of coral colonies to identify disease pathogens**

We had originally proposed to collect tissue from three coral species: *Montastraea cavernosa*, Orbicella faveolata, and Siderastrea siderea from five locations where white blotch disease was active and three locations where the disease was not active. At each of the diseased sites, we attempted to collect tissue samples from five diseased and three apparently healthy colonies. For diseased colonies, we collected samples from both the diseased and apparently unaffected areas of the colony, with the goal of collecting a total of 195 samples. At each of the inactive sites, we sought to collect tissue samples from three colonies of each of the target species, yielding 27 samples. We had initially proposed to also opportunistically collect samples from Dichocoenia stokesii, Meandrina meandrites, Pseudodiploria strigosa, and Colpophyllia natans if resources allowed. During the roving diver survey, it became clear that diseased colonies of two species that we intended to target opportunistically, C. natans and P. strigosa were common in the survey area. After conferring with Erinn Muller of Mote Marine Laboratory, Jan Landsburg of the FWC, the PI on the complementary State Wildlife Fund (SWG) award for which these samples are being collected, and Esther Peters of George Mason University, we decided to also directly target C. natans and P. strigosa. Given the prevalence of diseased colonies of these two species, we decided that including them with the three originally targeted species would not impede progress of the field effort. Including these species increased our targeted number of samples to 325 samples from diseased sites and 45 from non-diseased sites.

Sample Collections — Collection protocols followed NOAA's established protocols in the Coral Disease and Health Consortium's *Field Manual for Investigating Coral Disease Outbreaks* (Woodley *et al.*, 2008). At each collection site, a team of four to seven staff collected and processed coral tissue samples. Typically, four divers using SCUBA were responsible for the inwater collection of coral tissue. Cross-contamination minimization measures outlined under the QA/QC considerations detailed below were followed for all sampling activities. One diver recorded the disease status of the coral colony (*i.e.*, diseased or apparently healthy) before sampling, collected the prescribed biopsy samples from the colonies (see below), and took a representative photograph of the pre-and post- biopsy site. The second diver (the handler), organized the collection materials, verified the sample labeling (sample id, species, disease status, date, and site location), and measured to the nearest mm with calipers. The third diver on snorkel transported samples and paperwork between the collection site and the boat for further processing. Additional staff on the vessel received, labeled, processed, stored, and recorded the coral samples as described below.

*Tissue Biopsy* — For each sampled colony, we collected 25.4 mm circular cores (consisting of tissue and skeleton) using a stainless-steel corer/punch. For each apparently healthy colony from a diseased location or a healthy colony from a disease-free location, one histology tissue core and one molecular tissue slurry (obtained *via* a swab or syringe) was collected. For each diseased coral colony, one histology tissue core and one molecular tissue slurry was collected from unaffected tissue first, and then one histology core and one molecular tissue slurry was collected from the disease margin (tissue/exposed skeleton boundary). Each tissue core was placed in a pre-labeled Whirl Pack. Once aboard the vessel, the histology cores were preserved in a zinc-formalin solution (*e.g.*, Z-Fix® Anatech, Battle Creek, MI, USA) and stored in a cooler at ambient temperature (in the shade) during transportation to the lab. Tissue slurries were maintained on ice until they were frozen at FWC's South Florida Regional Laboratory, or if

more expedient, at Mote Marine Laboratory (MML) on Summerland Key. Three water samples were also collected at each site and transported to (MML) at the end of the collection day.

*QA/QC Considerations* — The above sampling activities followed the following protocols to ensure quality and integrity of the samples. During daily sampling activities, healthy corals were sampled before affected/ diseased corals. When sampling affected/diseased coral, unaffected tissues were sampled before disease margin tissues to ensure minimized contamination of samples. All sampling equipment was sterilized on land before use and placed in separate numbered collection bags for each coral colony. Each numbered collection bag (one for each sampled colony), contained a sterile corer, swabs, a pair of nitrile gloves, and pre-labeled Whirl Packs. To minimize cross contamination between colonies, each pair of nitrile gloves was discarded in a separate designated sealable bag after each colony is sampled. To minimize cross contamination between sites, all collection equipment was sterilized on the boat in a 5-10% sodium hypochlorite solution for 20 minutes.

# **Objective 2: Establishing and monitoring sentinel reefs**

## Marked Colony Sites

From late November through late December 2017, the FWC opportunistically identified and marked coral colonies to gather coarse disease transmission information (*i.e.*, within colony disease progression rates, and possible, inter- and intra-specific disease progression rates) at three near-shore patch reefs off Marathon FL: West Turtle Shoal (24°42'7.70"N; 80°57'47.63"W), East Washerwoman Shoal (24°39'52.42"N; 81° 4'26.94"W), and an unnamed patch reef (24°42'42.36"N; 80°56'47.40"W)(Figure 1; Appendix 3). We continued to monitor the marked coral colonies at approximately two-week intervals through the project period.

*Methodology* — During November and December 2017, Divers using SCUBA surveyed and marked coral colonies with 'cow ear' tags of the following species if present: *C. natans*, *D. labyrinthiformis*, *D. stokesii*, *M. cavernosa*, *M. meandrites*, *O. faveolata*, and *P. strigosa*. A central buoy was placed on the site, and a distance and bearing from this buoy to each marked coral was recorded to aid the diver's navigation of the site during routine monitoring. When each colony was initially marked, the proportion of older exposed skeleton that was not the result of the white blotch disease was recorded. We also measured each colony's length, width, and height to the nearest cm. At approximate two-week intervals, each marked coral head was examined for the presence of white blotch disease and if noted, the proportion of the colony affected was recorded. If disease was observed, a photo was taken of the colony.

## Sentinel Reef Monitoring

During January 2018, we established four additional reef locations off Marathon, southwestward down the FRT where the disease had not yet been reported to act as sentinel sites both to track the outbreak along the reef tract at > 1 km scale, and, when white blotch disease was observed, to map its spatial epidemiology at a finer scale (*i.e.*, within colony disease progression rates, and inter- and intra-specific disease transmission rates at a < 1m scale) This effort was designed to complement a similar effort by Mote Marine Laboratory (E. Muller, pers. com.)

*Methodology* — We selected two offshore bank reef locations and two near-shore patch reefs off Marathon, FL that were unimpacted by the disease and where boulder coral abundance was sufficient to track disease dynamics (Figure 1; Appendix 3). At each of those four reef locations, we established two replicate monitoring plots that contained colonies of several species of boulder corals that had been shown highly susceptible to the disease (*e.g., D. stokesii, M.* 

*meandrites*, *P. strigosa*, C. *natans*, *M. cavernosa*, *O. faveolata*). Monitoring plots were scaled based upon the number of coral heads present and ranged from  $25m^2$  to  $100m^2$ .

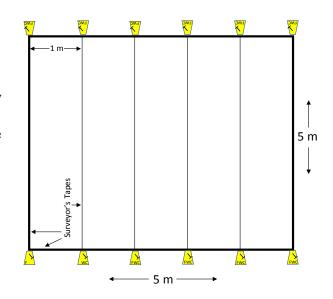
To establish each plot, two divers using SCUBA extended surveyor's tapes along the bottom at the appropriate distance (*e.g.*, 5 m for a  $25m^2$  plot or 10m for a  $100m^2$  plot) at 90° from one another. The divers swam diagonally the length of the hypotenuse of the tapes to ensure the two sides were square. Once the position of the tapes is confirmed, they installed nails at the plot corners. The remaining two sides of the plot were established using the same method. Divers installed marked tags every meter on the north and south sides of the plot. The tags facilitated the attachment of surveyor's tapes at 1-m intervals across the plot to orient divers within the plot and aided them in recording the relative position of each coral colony as detailed below (Figure 1).

Once the plots were established, they were surveyed using methods developed by the Florida Reef Resilience Program's Disturbance Response Monitoring for monitoring shallow coral reefs from the Florida Keys (*see* Lirman et al. 2010). Divers using SCUBA identified to species, measured, and mapped each coral colony > 10cm within the plot. Each coral colony was

measured (W x L x H to the nearest 0.1cm) and given an "x,y" coordinate (to the nearest 0.1m) that marked its relative position within the plot and to the other coral colonies. Each diver carried a 1m measuring pole to ensure that the location of each coral colony on the x and y axes within the plot was measured as accurately as possible. When each colony was initially marked, the proportion of older exposed skeleton that was not the result of the white blotch disease was recorded.

At approximate two-week intervals, divers surveyed the site for the presence of disease. Each marked coral head was examined for the presence of white blotch disease and other incidence of mortality. If mortality was noted, the proportion of the colony affected was recorded and a photo was taken of the colony.





#### Results

#### **Objective 1: Collection of coral colonies to identify disease pathogens**

On March 8, 2018 a training workshop was held at FWC's South Florida Regional Laboratory to exchange information on coral tissue collection methodologies. A list of attendees and their affiliations is summarized in Appendix 1. Lindsey Huebner and Kerry Maxwell of the FWC and Erinn Muller of Mote Marine Laboratory gave oral presentations on coral disease identification and sampling protocols. A smaller break-out group consisting of core field sampling personnel then participated in a hand-on demonstration of the field collection materials.

We have summarized all the following field activities for this project in Appendix 2. On March 18, 19, and 26, 2018 we conducted roving diver surveys at 21 reef locations in the middle Keys

to identify suitable reef locations for tissue collection where white blotch disease was active and the targeted coral species were present. The locations of the five selected sites are shown in Figure 2A and details summarized in Appendix 3.

Tissue collections at the disease-active sites began on April 9, 2018 and concluded on April 27, 2018. We had originally proposed to collect 195 core and tissue samples across three species: *M. cavernosa*, *O. faveolata*, and *S. siderea*. However, because we also collected samples from *C. natans* and *P. strigosa*, we ultimately collected 321 of the potential 325 samples each of tissue core and slurry samples (Table 1A). Four samples of *O. faveolata* were not collected at the West Turtle Shoal site as there were an insufficient number of disease colonies present at the sampling date.

In early May 2018, we conducted diver surveys west of Looe Key to identify reefs were white blotch disease was not evident. We selected three sites (Figure 2B; Appendix 3). Fifteen samples across the five targeted species were collected at each site, yielding a total of 45 samples (Table 1B). Field collections at these sites were completed on June 5, 2018.

All tissue core samples collected have been transported to FWRI's main laboratory in St. Petersburg Florida. All slurry samples have been transported to Mote Marine Laboratory on Summerland Key and stored in their -80° C freezer.

# **Objective 2: Establishing and monitoring sentinel reefs**

The monitoring of both the marked colony sites and the sentinel reef sites remains ongoing. Here, we report our activities through early June 2018. The QA/QC process on these datasets remains in progress. We present preliminary summary statistics that describe the relative speciesspecific susceptibility to the disease. We also calculated a preliminary coarse inter-specific disease progression rate for selected coral species. We note that these results were based on a relatively small sample size for several species. We will continue to refine this analysis as this work progresses.

## Marked Colony Sites

These sites were established in late 2017. The West Turtle Shoal Site was established on 11/29/2017, followed by the Nearshore Patch site on 12/21/2017 and the Washerwoman Shoal site on 12/27/2017 (Figure 1; Appendix 3). In all, we marked 63 coral colonies encompassing 7 species (Table 2). Although it was our intent to establish these sites before disease was present to optimally track disease progression rates, one *M. meandrites* colony at the Nearshore Patch site was already exhibiting white blotch disease when the site was established. By January 10, 2018, the disease was present at all three sites. *M. meandrites*, initially exhibited the highest proportion of diseased colonies, and by April 5, 2018, 100% (15/15) of the colonies were diseased and one had already died (Table 2; Figure 3). By May 5, 2018, all species exhibited some degree of disease, with *M cavernosa* and *O. faveolata* being the last to exhibit tissue loss. Figure 4 shows the progression of disease through a *D. stokesii* colony over one month. By June 1, 2018, 32 of the 63 colonies either exhibited white blotch disease or had already died from the disease (Table 2).

## Sentinel Sites

Bi-monthly monitoring for the presence of disease at these four sites began January 10 - February 2, 2018. In all, we identified and monitored more than 1,350 coral colonies representing 23 species (Table 3).

The incidence of disease was low across all sites through February, and was noted in one *M*. *meandrites* at Grouper Reef, one *M*. *cavernosa* at East Washerwoman Shoal, and several *S*. *sideraea* at three sites (Figure 5; Figure 6). However, we note that the expression of disease in S. *sideraea* differs noticeably from the other species, and consequently the data remain unclear on the incidence of white blotch disease in this species.

By March the rate at which colonies began to exhibit white blotch disease increased, and among those species that were sufficiently abundant, in general the same species-specific pattern was observed as at our marked colony monitoring sites colonies. *M. meandrites* initially exhibited the highest prevalence, followed by *D. stokesii, C. natans,* and *P. strigosa,* followed by *O. faveolata, O. annularis,* and *M. cavernosa.* 

## Species-Specific Disease Progression Rates

We estimated interspecific disease progression rates for *M. meandrites*, *D. stokesii*, *C. natans*, and *P. strigosa*, *M. cavernosa*, *O. faveolata*, and *O. annularis* using the combined monitoring data from the both the Marked Colony sites and the Sentinel sites. Although the disease has affected many other species we are monitoring, the former four listed above are the only species with a sample size and time series sufficient to calculate a reasonably robust estimate. We have included *M. cavernosa* and *Orbicella* spp. as they are large, conspicuous species that become infected at a lower rate and have been purported to have much slower disease progression rates once becoming infected compared to those other four species. However, owing to these factors at present we only have a limited number of disease colonies to examine.

To calculate disease progression rates, we first estimated the surface area of each diseased colony. As the species of interest were massive growth forms, we assumed the geometric shape of each colony was generally hemispherical (*see* Naumann *et al.* 2009). Therefore, we calculated the surface area with the formula  $SA=2\pi r^2$ , where SA is the estimated surface area and r is the mean of the two radii derived from our two diameter measurements recorded for each colony. We then estimated the rate of disease progression for each colony by subtracting the difference between the maximum percentage of the colony with diseased tissue and the initial percentage of the colony with diseased tissue (*i.e.*, percent disease  $_{max}$  – percent diseased  $_{min}$ ), then dividing that value by the number of days between the corresponding monitoring dates. The surface area estimate was then divided by that value to yield an estimate of daily disease progression.

We evaluated mean disease progression rates of *M. meandrites, C. natans, P. strigosa* and *D. stokesii* by fitting a fixed one-factor GLM model to the data. The disease progression rates of *M. meandrites, C. natans,* and *P. strigosa* were significantly higher than that of *D. stokesii* (F = 33.4; df = 3; P < 0.01; log transformed data) Median disease progression rates of *M. meandrites, C. natans, P. strigosa* ranged from approximately 20-40 cm<sup>2</sup>/day, whereas the median value of *D. stokesii* was approximately 5 cm<sup>2</sup>/day (Figure 7). However, we note that *D. stokesii* colonies are typically smaller than the other three species (Figure 8). However, when the colonies of these four species that had completely died by the last monitoring date included herein, the number of days from when they were observed to be infected to the date the colonies were completely dead was comparable (F = 0.588; df = 3; P = 0.627; one-factor GLM, log transformed data) (Figure 9).

Our limited observations of *M. cavernosa and Orbicella* spp. suggest that they do have lower disease progression rates compared to *M. meandrites, C. natans, P. strigosa,* with median disease progression rates of approximately 10cm<sup>2</sup>/day (Figure 10). However, a larger sample size and longer time series is necessary to provide a more robust estimate.

#### **Ongoing Activities**

Monitoring data collected in late June will be added to the Marked Colony and Sentinel Site databases and subjected to QA/QC procedures, and exploratory analyses will continue to evaluate the spatial epidemiology of the disease outbreak. As funding allows, monitoring efforts will continue through 2018. We have also coordinated with Sarah Davies of Boston University to collect RNA samples from *S. siderea* to conduct transcriptomics work with this species.

## **Literature Cited**

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**Table 1.** Locations, sampling dates, and the number of tissue samples collected (both cores and slurry) for each of the five targeted coral species at (A) locations where white blotch disease was active and (B) locations where the disease was not evident.

Location/Dates	Species	No. Of	Samples
West Turtle Shoal		Targeted	Collected
	Montastraea cavernosa	13	13
	Orbicella faveolata	13	9
April 8-9, 2018	Siderastrea siderea	13	13
	Colpophyllia natans	13	13
	Pseudodiploria strigosa	13	13
Boot Key Patch			
	Montastraea cavernosa	13	13
	Orbicella faveolata	13	13
April 11,20, 2018	Siderastrea siderea	13	13
	Colpophyllia natans	13	13
	Pseudodiploria strigosa	13	13
Nearshore Patch			
	Montastraea cavernosa	13	13
	Orbicella faveolata	13	13
April 24-25, 2018	Siderastrea siderea	13	13
	Colpophyllia natans	13	13
	Pseudodiploria strigosa	13	13
Dustan 3			
	Montastraea cavernosa	13	13
	Orbicella faveolata	13	13
April 26-27, 2018	Siderastrea siderea	13	13
	Colpophyllia natans	13	13
	Pseudodiploria strigosa	13	13
East Turtle Shoal			
	Montastraea cavernosa	13	13
	Orbicella faveolata	13	13
April 25-26, 2018	Siderastrea siderea	13	13
	Colpophyllia natans	13	13
	Pseudodiploria strigosa	13	13

No. Of Samples Location Species Western Sambo 1 Montastraea cavernosa 3 3 Orbicella faveolata 3 May 8, 2018 Siderastrea siderea Colpophyllia natans 3 3 Pseudodiploria strigosa Western Sambo 2 3 Montastraea cavernosa Orbicella faveolata 3 May 8, 2018 3 Siderastrea siderea 3 Colpophyllia natans 3 Pseudodiploria strigosa Key West 3 Montastraea cavernosa 3 Orbicella faveolata June 5, 2018 3 Siderastrea siderea 3 Colpophyllia natans 3 Pseudodiploria strigosa

B

# A

**Table 2.** Summary of the number of coral colonies observed with white blotch disease at the three sites where colonies were marked and monitored at approximately two-week intervals.

Location				Diseased/Dead																						
West Turtle Shoal	Species	N Tagged 11/29/2017	12/2	7/18	1/1	0/18	1/2	2/18	2/5	/18	2/1	5/18	3/1	/18	3/1	8/18	4/5	/18	4/1	9/18	5/7	//18	5/1	8/18	6/1	/18
			Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead
	D. stokesii	7	0	0	0	0	0	0	0	0	1	0	3	0	5	0	5	1	3	3	1	4	1	5	1	6
	M. cavernosa	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
	M. meandrites	5	5	0	4	1	3	2	2	3	2	3	1	4	1	4	1	4	1	4	0	5	0	5	0	5
	P. clivosa	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
	P. strigosa	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	1	0	1	0	1
Near Shore Patch	Species	N Tagged 12/21/2017	12/2	7/18	1/1	0/18	1/2	2/18	2/5/18 2/16/18 3/1/18		1/18 3/18/18		4/5/18		4/1	9/18	5/7	/18	5/13	8/18	6/1	/18				
			Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead
	C. natans	5	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	2	0	2	0
	D. labyrinthiformis	1	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	0	1
	D. stokesii	5	0	0	0	0	1	0	3	0	2	0	3	0	3	0	3	1	4	1	2	3	1	4	1	4
	M. meandrites	5*	2	0	2	0	2	0	4	1	3	2	1	4	1	4	1	4	1	4	1	4	1	4	1	4
	P. strigosa	5	0	0	0	0	0	0	0	0	2	0	2	0	1	1	1	1	1	1	2	1	3	1	3	1
East Washerwoman Shoal	Species	N Tagged 12/27/2017	12/2	7/18	1/1	0/18	1/2	2/18	2/5	/18	18 2/16/18 3/2/18		/18	3/1	6/18	8 4/5/18		4/5/18 4/19/18		8 5/7/18		5/1	8/18	6/1	/18	
Silvai		12/2//2017	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead	Dis	Dead
	C. natans	6	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	2	0	3	0	4	1
	D. labyrinthiformis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	D. stokesii	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	4	0	3	1	3	2	1	4
	M. cavernosa	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M. meandrites	5	0	0	1	0	2	0	3	0	1	2	2	3	2	3	1	4	0	5	0	5	0	5	0	5
	O. faveolata	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
	P. strigosa	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	3	0
Total		63	7	0	8	1	9	2	14	4	13	7	15	11	17	12	18	15	18	18	17	24	21	27	19	32

\*One *M. meadrites* diseased when site established on 12/21/2018

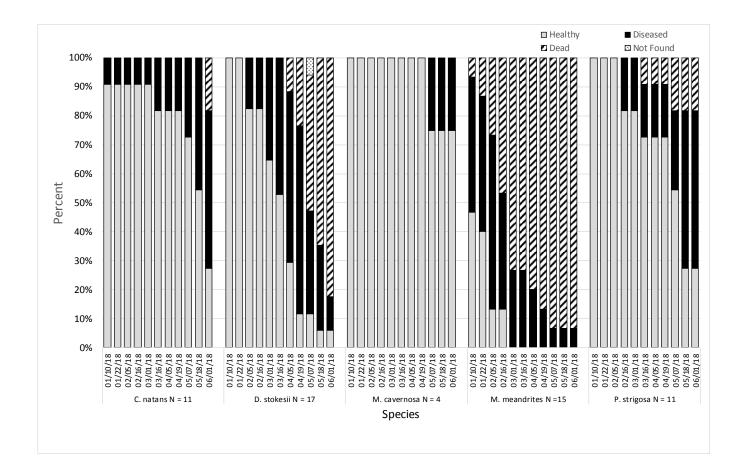
**Table 3.** The number of coral colonies by species that were identified and have been monitored bi-weekly at the four sentinel sites. Asterisk denotes species suspected of exhibiting white blotch disease.

Species	Boot Key Patch Reef	East Washerwoman Shoal	Grouper Reef	Sombrero Reef
Acropora cervicornis	0	0	2	0
Colpophyllia natans *	76	19	4	0
Dichocoenia labyrinthiformis*	5	1	2	2
Dichocoenia stokesii*	11	9	63	8
Eusmilia fastigiata*	6	11	1	0
Mycetes aliciae	1	0	0	0
Montastraea cavernosa*	29	37	16	25
Meandrina jacksoni*	0	0	1	0
Meandrina meandrites*	3	3	6	12
Mycetophyllia spp. *	4	1	0	0
Orbicella annularis *	0	25	0	0
Oculina diffusa	1	0	0	0
Orbicella faveolata*	9	8	7	6
Porites astreoides*	10	33	13	13
Pseudodiploria clivosa*	1	1	1	0
Porites porites*	1	8	2	3
Pseudodiploria strigosa*	19	0	22	6
Solenastrea bournoni*	6	0	4	0
Scolymia spp.	1	0	0	0
Stephanocoenia intersepta*	81	101	65	10
Siderastrea radians	2	0	0	0
Siderastrea siderea*	163	156	137	68
Undaria agaricites*	0	6	2	5
Total	429	419	348	158

**Figure 1.** (A) Maps showing (A) locations of the marked coral sites, sentinel monitoring sites, and the tissue collection sites where white blotch disease was active, and (B) tissue collection sites where disease was not evident.



**Figure 3.** Time series of the percentage of healthy, diseased, and dead coral colonies pooled across the three marked colony sites beginning January 10, 2018. Not shown are the coral species represented by two or fewer colonies. Data for these species is presented in Table 2.



**Figure 4.** Time series of photographs taken during regular monitoring at a site with marked coral colonies showing the progression of disease through a *Dichocoenia stokesii* colony.



**Figure 5.** Time series of the percentage of selected healthy, diseased, and dead coral colonies for selected species at each of the four sentinel sites.

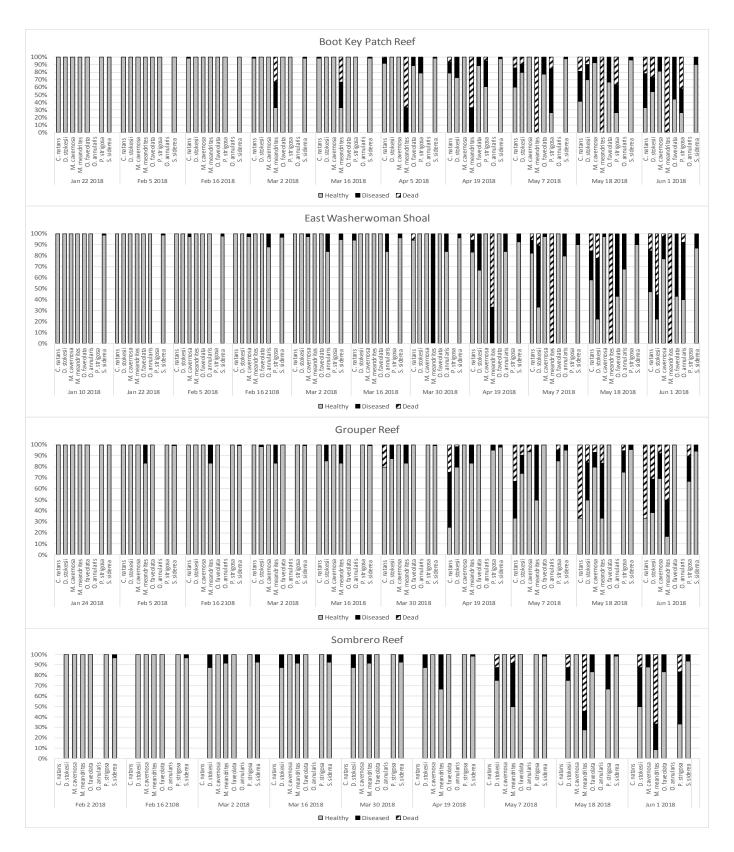
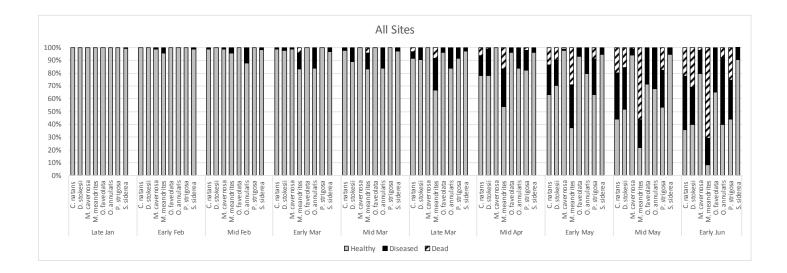
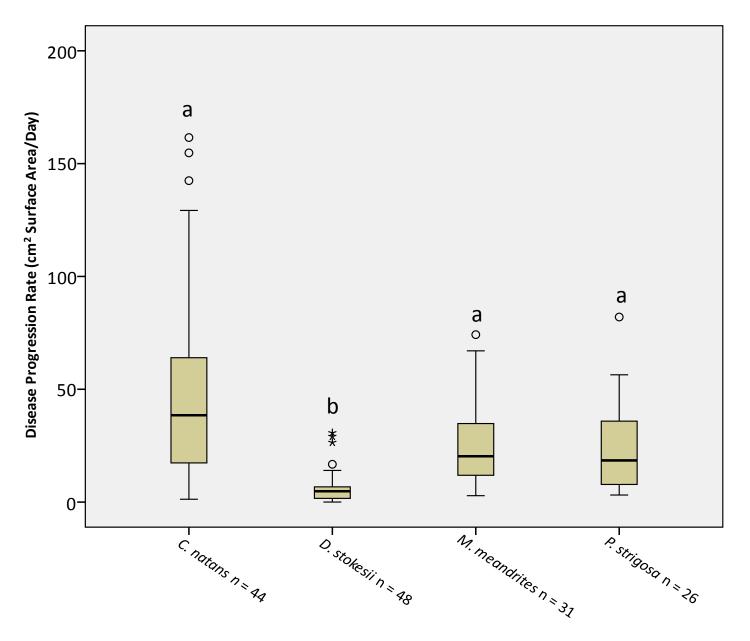


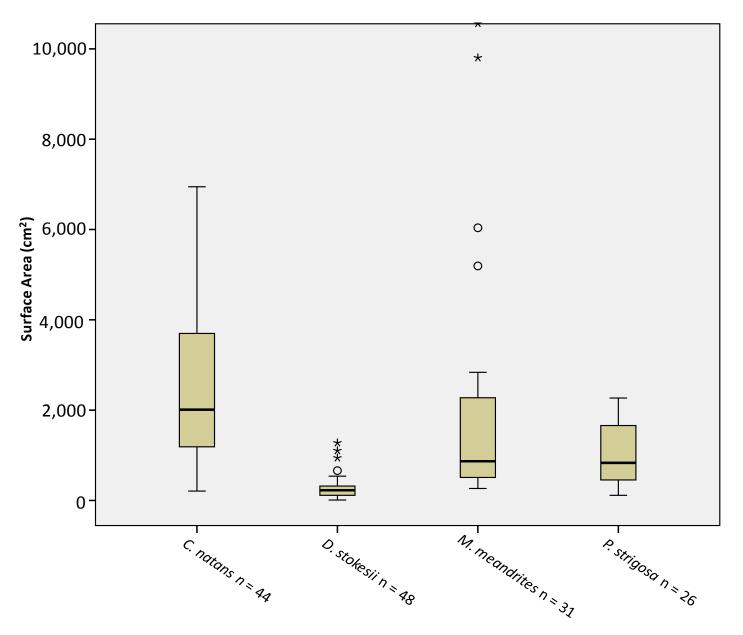
Figure 6. Time series of the percentage of healthy, diseased, and dead coral colonies pooled across the four sentinel sites.



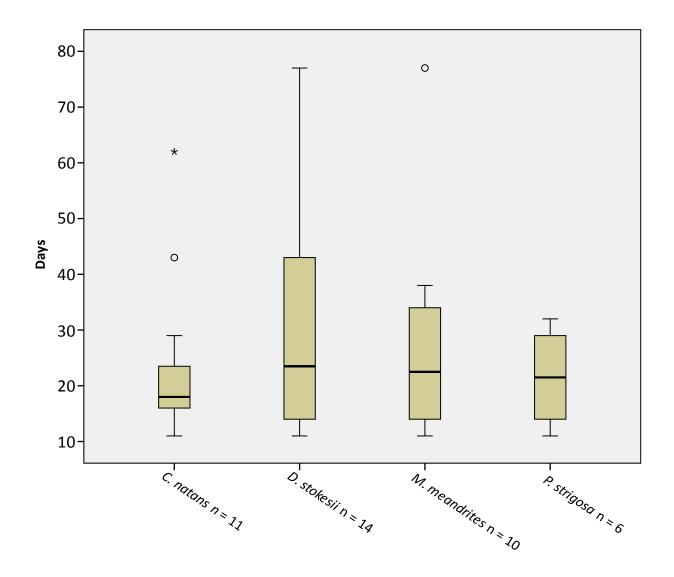
**Figure 7.** Boxplots summarizing white blotch disease progression rates of. *M. meandrites, D. stokesii, C. natans,* and *P. strigosa* from the marked colony and sentinel monitoring sites. Boxes represent the interquartile range; the solid line bisecting the box represents the median. Observations >1.5 times the interquartile range is represented by the open circles. Observations >3 times the interquartile range is represented by asterisks. Some outlying observations have been omitted from the figure to enhance the detail of the figure. Letters above each box denote homogenous subsets identified by Tukey's HSD post-hoc test following a one-factor General Linear Model analysis.



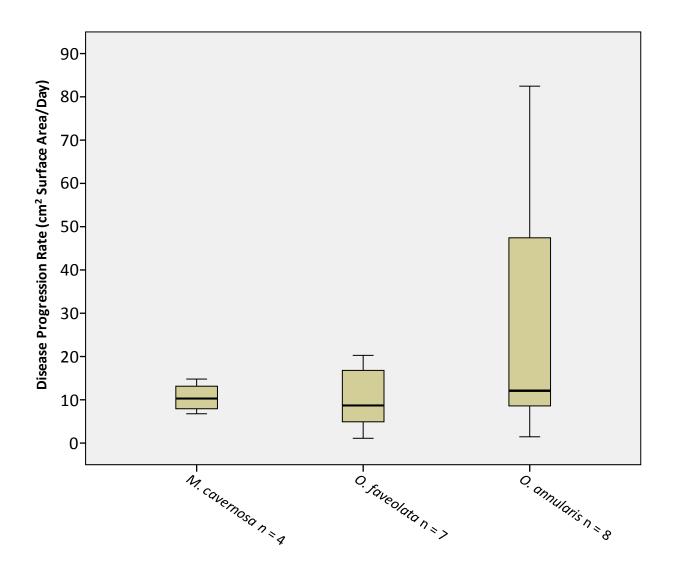
**Figure 8.** Boxplots summarizing the surface area of. *M. meandrites, D. stokesii, C. natans,* and *P. strigosa.* from the marked colony and sentinel monitoring sites. Boxes represent the interquartile range; the solid line bisecting the box represents the median. Observations >1.5 times the interquartile range is represented by the open circles. Observations >3 times the interquartile range is represented by asterisks. Some outlying observations have been omitted to enhance the detail of the figure.



**Figure 9.** Boxplots summarizing the number of days from the monitoring date that *M. meandrites, D. stokesii, C. natans,* and *P. strigosa.* were first observed to be diseased until the date the colonies had completely died. Boxes represent the interquartile range; the solid line bisecting the box represents the median. Observations >1.5 times the interquartile range is represented by the open circles. Observations >3 times the interquartile range is represented by asterisks.



**Figure 10.** Boxplots summarizing the white blotch disease progression rates *M. cavernosa*, *O. faveolata*, and *O. annularis* from the marked colony and sentinel monitoring sites. Boxes represent the interquartile range; the solid line bisecting the box represents the median. Some outlying observations have been omitted to enhance the detail of the figure.



Appendix 1: Workshop Attendees

Attendee	Affiliation
John Hunt	Florida Fish & Wildlife Conservation Commission
William Sharp	Florida Fish & Wildlife Conservation Commission
Kerry Maxwell	Florida Fish & Wildlife Conservation Commission
Brian Reckenbeil	Florida Fish & Wildlife Conservation Commission
Elliot Hart	Florida Fish & Wildlife Conservation Commission
Mike Bollinger	Florida Fish & Wildlife Conservation Commission
Krissy Fisher	Florida Fish & Wildlife Conservation Commission
Ananda Ellis	Florida Fish & Wildlife Conservation Commission
Jennifer Stein	Florida Fish & Wildlife Conservation Commission
Lindsay Huebner	Florida Fish & Wildlife Conservation Commission
Stephanie Schopmeyer	Florida Fish & Wildlife Conservation Commission
Yasu Kiryu	Florida Fish & Wildlife Conservation Commission
Jan Landsberg (remote)	Florida Fish & Wildlife Conservation Commission
Erinn Muller	Mote Marine Laboratory
Abigail Clark	Mote Marine Laboratory
Cindy Lewis	Keys Marine Laboratory
Josh Farmer	Keys Marine Laboratory
Karen Bohnsack	Florida Department of Environmental Protection
Kristi Kerrigan	Florida Department of Environmental Protection
Murphy MacDonald	Nova Southeastern University
Karen Neely	Nova Southeastern University

Appendix 2: Log of all field activities associated with the project

						Coring				
Date	Vessel	# Staff	Personel	Task (plots, tags, tissue sampling)	Site	Site Code	Disease Present	# of dives	# corals sampled	Latitude/longitude
1/22/2018	3 25 Whaler		Reckenbeil, Binstein, Kneely	Monitor Tagged Corals	Nearshore Patch	couc	no	1	0	24.711950°, -80.945340°
	3 25 Whaler 3 25 Whaler	3	Reckenbeil, Binstein, Kneely Reckenbeil, Binstein, Kneely	Monitor Tagged Corals Roving Diver	West Turtle Shoal Washerwoman		no no	1	0	24.702140°, -80.963230° 24.664260°, -81.073850°
1/22/2018	3 Twin Vee	4	Maxwell, Stein, Bollinger, Hart	Plot Set Up	Boot Key		no	2	0	24.664900°, -81.096330°
	3 Twin Vee 3 Twin Vee	5	Maxwell, Stein, Bollinger, Hart, Reckenbeil Maxwell, Stein, Bollinger, Hart, Reckenbeil	Roving Diver Roving Diver	West of Trop Rocks1 West of Trop Rocks2		no no	1	0	24.65587°, -81.01691° 24.65479°, -81.02050°
1/23/2018	8 Twin Vee	5	Maxwell, Stein, Bollinger, Hart, Reckenbeil	Roving Diver	South Ledge		no	1	0	24.65744°, -81.02035°
	3 Twin Vee 3 Twin Vee	5	Maxwell, Stein, Bollinger, Hart, Reckenbeil Maxwell, Stein, Bollinger, Hart, Reckenbeil	Roving Diver Roving Diver	Near Delta Fraggle Rock		no no	1	0	24.63990°, -81.06328°
1/23/2018	8 Twin Vee	5	Maxwell, Stein, Bollinger, Hart, Reckenbell	Roving Diver	Pop Rocks		no	1	0	24.64776°, -81.05517°
	3 21 RE Parker 3 Twin Vee	4	Maxwell, Bollinger, Hart, Reckenbeil Maxwell, Bollinger, Hart, Stein, Binstein	Plot Set Up Plot Set Up	Grouper Reef Sombrero		no no	3	0	24.65257°, -81.03652° 24.625390°, -81.111550°
	3 25 Whaler	3	Maxwell, Stein, Reckenbeil	Plot Monitoring	Boot Key		no	1	0	24.664900°, -81.096330°
2/5/2018		3	Maxwell, Stein, Reckenbeil	Tag and Plot Monitoring	Washerwoman		yes	1	0	24.664260°, -81.073850°
	3 25 Whaler 3 25 Whaler	3	Maxwell, Stein, Reckenbeil Maxwell, Stein, Reckenbeil	Plot Monitoring Plot Monitoring	Grouper Reef West Turtle Shoal		maybe yes	1	0	24.65257°, -81.03652° 24.702140°, -80.963230°
2/5/2018	25 Whaler	3	Maxwell, Stein, Reckenbeil	Plot Monitoring	Nearshore Patch		yes	1	0	24.711950°, -80.945340°
	3 25 Whaler 3 25 Whaler	3	Maxwell, Bollinger, Reckenbeil Maxwell, Bollinger, Reckenbeil	Plot Monitoring Tag and Plot Monitoring	Boot Key Washerwoman		no yes	1	0	24.664900°, -81.096330° 24.664260°, -81.073850°
2/16/2018	25 Whaler	3	Maxwell, Bollinger, Reckenbeil	Plot Monitoring	Sombrero		no	1	0	24.625390°, -81.111550°
	25 Whaler 25 Whaler	3	Maxwell, Bollinger, Reckenbeil Maxwell, Bollinger, Reckenbeil	Plot Monitoring Monitor Tagged Corals	Grouper Reef Nearshore Patch		maybe yes	1	0	24.65257°, -81.03652° 24.711950°, -80.945340°
	3 25 Whaler	3	Maxwell, Bollinger, Reckenbeil	Monitor Tagged Corals	West Turtle Shoal		yes	1	0	24.702140°, -80.963230°
	3 25 Whaler	3	Maxwell, Reckenbeil, Fisher	Monitor Tagged Corals	West Turtle Shoal		yes	1	0	24.702140°, -80.963230°
	3 25 Whaler 3 25 Whaler	3	Maxwell, Reckenbell, Fisher Maxwell, Reckenbell, Fisher	Monitor Tagged Corals Plot Monitoring	Nearshore Patch Boot Key		yes yes	1	0	24.711950°, -80.945340° 24.664900°, -81.096330°
3/2/2018	25 Whaler		Maxwell, Reckenbeil, Fisher	Plot Monitoring	Sombrero		no	1	0	24.625390°, -81.111550°
	25 Whaler 25 Whaler		Maxwell, Reckenbell, Fisher Maxwell, Reckenbell, Fisher	Plot Monitoring Tag and Plot Monitoring	Grouper Reef Washerwoman		yes yes	1	0	24.65257°, -81.03652° 24.664260°, -81.073850°
3/16/2018			Maxwell, Reckenbell, Fisher, Stein	Plot Monitoring	Sombrero		no	1	0	24.625390°, -81.111550°
	25 Whaler 25 Whaler	4	Maxwell, Reckenbeil, Fisher, Stein	Plot Monitoring	Grouper Reef		yes	1	0	24.65257°, -81.03652°
	3 25 Whater 3 25 Whater	4	Maxwell, Reckenbell, Fisher, Stein Maxwell, Reckenbell, Fisher, Stein	Tag and Plot Monitoring Plot Monitoring	Washerwoman Nearshore Patch		yes yes	1	0	24.664260°, -81.073850° 24.711950°, -80.945340°
3/18/2018	3 25 Whaler	4	Maxwell, Reckenbeil, Fisher, Stein	Roving Diver	Nearshore Patch		yes	1	0	24.711950°, -80.945340°
	3 25 Whaler 3 25 Whaler		Maxwell, Reckenbell, Fisher, Stein Maxwell, Reckenbell, Fisher, Stein	Roving Diver Roving Diver	Nearshore Patch Ledge West Turtle Shoal		yes yes	1	0	24.710879°, -80.946985° 24.702140°, -80.963230°
	25 Whaler		Maxwell, Reckenbeil, Fisher, Stein	Roving Diver	Coffins Patch		yes	1	0	; 24.684615°, -80.965603°
	25 Whaler 25 Whaler		Maxwell, Reckenbeil, Fisher, Stein	Roving Diver	Shallow Ledge		yes	1	0	24.657442°, -81.020349° 24.65257°, -81.03652°
3/18/2018		4	Maxwell, Reckenbeil, Fisher, Stein Maxwell, Reckenbeil, Fisher, Stein	Roving Diver Roving Diver	Grouper Reef Dustan 3		yes yes	1	0	24.685319°, -81.042770°
3/19/2018		3	Hart, Reckenbeil, Stein	Roving Diver	Hawks 11		yes	1	0	24.652072°, -81.122572°
	3 Twin Vee 3 Twin Vee	3	Hart, Reckenbeil, Stein Hart, Reckenbeil, Stein	Roving Diver Roving Diver	Hawks 2.1 Washerwoman West		yes yes	1	0	24.663830°, -81.098120° 24.664021°, -81.076905°
3/19/2018	8 Twin Vee		Hart, Reckenbeil, Stein	Roving Diver	Washerwoman East		yes	1	0	24.666945°, -81.067402°
	3 Twin Vee 3 Twin Vee	3	Hart, Reckenbeil, Stein Hart, Reckenbeil, Stein	Roving Diver Roving Diver	Dustan 4 Dustan 3		yes yes	1	0	24.691929°, -81.026386° 24.685319°, -81.042770°
3/26/2018		3	Maxwell, Reckenbeil, Stein	Roving Diver	Tenn Patch		yes	1	0	24.782934°, -80.763299°
	25 Whaler	3	Maxwell, Reckenbeil, Stein	Roving Diver	Tenn Patch Investigate 2		yes	1	0	24.789032°, -80.751268°
3/26/2018 3/26/2018	3 25 Whaler 3 25 Whaler	3	Maxwell, Reckenbeil, Stein Maxwell, Reckenbeil, Stein	Roving Diver Roving Diver	Tenn Patch Investigate 1 LKBR 6 (ABC, EFG)		yes yes	1	0	24.778528°, -80.749371° 24.731560°, -80.827611°
3/26/2018	3 25 Whaler	3	Maxwell, Reckenbeil, Stein	Roving Diver	Long Key Ledge		yes	1	0	24.719284°, -80.841645°
	3 25 Whaler 3 25 Whaler	3	Maxwell, Reckenbeil, Stein Maxwell, Reckenbeil, Stein	Roving Diver Roving Diver	11 Foot Mound EE Turtle		yes yes	1	0	24.725792°, -80.856476° 24.732820°, -80.912300°
3/26/2018	25 Whaler	3	Maxwell, Reckenbeil, Stein	Roving Diver	Volcano		yes	1	0	24.725045°, -80.918835°
	3 25 Whaler 3 25 Whaler	3	Maxwell, Reckenbeil, Stein Maxwell, Reckenbeil, Bollinger	Roving Diver Plot Monitoring	CRF P9 Washerwoman		yes yes	1	0	24.720117°, -80.928733° 24.664260°, -81.073850°
	25 Whaler	3	Maxwell, Reckenbell, Bollinger	Plot Monitoring	Grouper Reef		yes	1	0	24.65257°, -81.03652°
3/30/2018		3	Maxwell, Reckenbeil, Bollinger	Plot Monitoring	Sombrero		yes	1	0	24.625390°, -81.111550°
	3 25 Whaler 3 25 Whaler	2	Maxwell, Fisher Maxwell, Fisher	Monitor Tagged Corals Monitor Tagged Corals	Nearshore Patch West Turtle Shoal		yes yes	1	0	24.711950°, -80.945340° 24.702140°, -80.963230°
4/5/2018	3 25 Whaler	2	Maxwell, Fisher	Tag and Plot Monitoring	Washerwoman		yes	1	0	24.664260°, -81.073850°
	3 25 Whaler 3 25 Parker	2	Maxwell, Fisher Reckenbeil, Maxwell, Bollinger, Spadaro, Barbera, Fisher, Murfy	Plot Monitoring Core Corals	Boot Key West Turtle Shoal	Δ	yes yes	1	0	24.664900°, -81.096330° 24.701680°, -80.96407°
4/10/2018	8 25 Parker	7	Reckenbeil, Maxwell, Bollinger, Sinstein, Barbera, Fisher, Murfy	Core Corals	West Turtle Shoal	A	yes	3	16	24.701680°, -80.96407°
	25 Parker 25 Whaler	6	Reckenbeil, Maxwell, Bollinger, Binstein, Fisher, Stein	Core Corals	Boot Key	В	yes	3	26	24.663830°, -81.098080° 24.711950°, -80.945340°
4/19/2018		4	Reckenbeil, Bollinger, Maxwell, Fisher Reckenbeil, Bollinger, Maxwell, Fisher	Monitor Tagged Corals Monitor Tagged Corals	Nearshore Patch West Turtle Shoal		yes yes	1	0	24.702140°, -80.963230°
4/19/2018	3 25 Whaler		Reckenbeil, Bollinger, Maxwell, Fisher	Plot Monitoring	Sombrero		yes	1	0	24.625390°, -81.111550°
	3 25 Whaler 3 25 Whaler		Reckenbeil, Bollinger, Maxwell, Fisher Reckenbeil, Bollinger, Maxwell, Fisher	Tag and Plot Monitoring Plot Monitoring	Washerwoman Boot Key		yes yes	1	0	24.664260°, -81.073850° 24.664900°, -81.096330°
4/20/2018	3 25 Parker		Hart, Reckenbeil, Bollinger, Fisher, Maxwell, Barbera, Stein	Core Corals	Boot Key	В	yes	1	14	24.663830°, -81.098080°
	3 25 Parker 3 25 Parker	7	Hart, Reckenbeil, Bollinger, Fisher, Maxwell, Barbera, Stein Hart, Reckenbeil, Bollinger, Fisher, Maxwell, Berkebile, Stein	Core Corals Core Corals	Nearshore Patch Nearshore Patch	C C	yes yes	3	25 15	24.711210°, -80.947210° 24.711210°, -80.947210°
	3 25 Parker	7	Hart, Reckenbeil, Bollinger, Fisher, Maxwell, Berkebile, Stein	Core Corals	East East Turtle Patch	D	yes	3	32	24.73290°, -80.912270°
	25 Parker	7	Hart, Reckenbeil, Bollinger, Fisher, Maxwell, Berkebile, Stephan Lindsay	Core Corals	East East Turtle Patch	D	yes	1	8	24.73290°, -80.912270°
	3 25 Parker 3 25 Parker	6	Hart, Reckenbeil, Bollinger, Fisher, Maxwell, Berkebile, Stephan Lindsay Burkebile, Reckenbeil, Ellis, Fisher, Stein, Maxwell	Core Corals Core Corals	Dustan 3 Dustan 3	E	yes yes	2	16 24	24.685690°, -81.04186° 24.685690°, -81.04186°
5/7/2018	3 25 Whaler		Reckenbeil, Hart, Stein, Fisher	Plot Monitoring	Sombrero		yes	1	0	24.625390°, -81.111550°
	3 25 Whaler 3 25 Whaler		Reckenbeil, Hart, Stein, Fisher Reckenbeil, Hart	Plot Monitoring Monitor Tagged Corals	Grouper Reef Nearshore Patch		yes yes	1	0	24.65257°, -81.03652° 24.711950°, -80.945340°
5/7/2018	25 Whaler	2	Reckenbeil, Hart	Monitor Tagged Corals	West Turtle Shoal		yes	1	0	24.702140°, -80.963230°
	25 Whaler 25 Whaler		Reckenbeil, Hart, Stein, Fisher Reckenbeil, Hart, Stein, Fisher	Tag and Plot Monitoring Plot Monitoring	Washerwoman Boot Key		yes ves	1	0	24.664260°, -81.073850° 24.664900°, -81.096330°
	3 25 Whater 3 21 RE Parker	4	Reckenbeil, Hart, Stein, Fisher Reckenbeil, Hart, Ellis, Fisher	Core Corals	Western Sambo Patch 2 (F)	F	yes no	1	15	24.520120°, -81.726720°
5/8/2018	3 21 RE Parker	4	Reckenbeil, Hart, Ellis, Fisher	Core Corals	Western Sambo Patch 1 (G)	G	no	1	15	24.501560°, -81.744870°
	3 21 RE Parker 3 21 RE Parker		Reckenbeil, Binstein, Bollinger, Fisher Reckenbeil, Binstein, Bollinger, Fisher	Plot Monitoring Tag and Plot Monitoring	Sombrero Washerwoman		yes yes	1	0	24.625390°, -81.111550° 24.664260°, -81.073850°
5/18/2018	3 21 RE Parker	4	Reckenbeil, Binstein, Bollinger, Fisher	Plot Monitoring	Boot Key		yes	1	0	24.664900°, -81.096330°
	21 Fish Parker		Maxwell, Hart	Plot Monitoring Monitor Tagged Corals	Grouper Reef Nearshore Patch		yes	1	0	24.65257°, -81.03652° 24.711950°, -80.945340°
	8 21 Fish Parker 8 21 Fish Parker		Maxwell, Hart Maxwell, Hart	Monitor Tagged Corals Monitor Tagged Corals	West Turtle Shoal		yes yes	1	0	24.711950°, -80.945340° 24.702140°, -80.963230°
6/1/2018	3 21 Fish Parker	2	Reckenbeil, Hart	Plot Monitoring	Grouper Reef		yes	1	0	24.65257°, -81.03652°
	3 21 Fish Parker	2	Reckenbeil, Hart Reckenbeil, Hart	Monitor Tagged Corals Monitor Tagged Corals	Nearshore Patch West Turtle Shoal		yes yes	1	0	24.711950°, -80.945340° 24.702140°, -80.963230°
	21 Fish Parker									
6/1/2018 6/1/2018	21 Fish Parker 21 RE Parker	3	Bollinger, Maxwell, Fisher	Plot Monitoring	Sombrero		yes	1	0	24.625390°, -81.111550°
6/1/2018 6/1/2018 6/1/2018								1	0	

Appendix 3: Location details of tissue collection sites, sentinel sites, and tagged sites

**Tissue Collection Sites** 

Sites- White Blotch Disease Present	Dates Sampled	Lat	Long
West Turtle Shoal	4/9-10/2018	24.70168	80.96407
Boot Key Patch	4/11, 20/2018	24.66383	81.09808
Nearshore Patch	4/24/2018	24.71121	80.94721
Dustan 3	4/26, 4/27/2018	24.68544	81.04391
East-East Turtle Patch	4/25, 4/26/2018	24.73290	80.91227
Sites- White Blotch Disease Absent			
Western Sambo 1	5/8/2018	24.50156	81.74487
Western Sambo 2	5/8/2018	24.52012	81.72672
Key West	6/5/2018	24.51244	81.78568

# Tagged Sites

					# Corals
Site	Date Set-Up	Lat	Long	Name in GPS	Tagged
West Turtle Shoal	11/29/2017	24.70214	80.96323	CD17-WTURT	18
Nearshore Patch	12/21/2017	24.71195	80.94534	CD17-NSP	21
Washerwoman	12/27/2017	24.66429	81.07405	RUMAR-21	24

# Sentinel Sites

Site	Date Set-Up	Lat	Long	Plot	Name in GPS	Tag
Washerwoman	1/15/2018	24.66426	81.07385	1	CD17-WASH1	A1
Washerwoman	1/15/2018	24.66417	81.07378	2	CD17-WASH2	A2
Boot Key Patch	1/22/2018	24.66489	81.09616	1	CD17-BOOT1	B1
Boot Key Patch	1/22/2018	24.6649	81.09633	2	CD17-BOOT2	B2
Grouper Reef	1/24/2018	24.65257	81.03652	1	CD17-GROU1	C3
Grouper Reef	1/24/2018	24.65259	81.03615	2	CD17-GROU2	C4
Sombrero	2/2/2018	24.62536	81.11140	1	CD17SOMBD1	D1
Sombrero	2/2/2018	24.62539	81.11155	2	CD17SOMBD2	D2