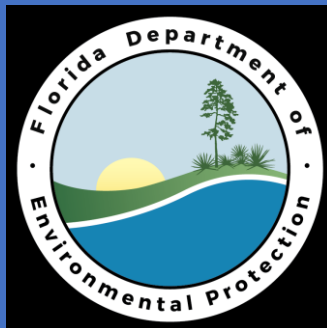


# SURVEYING THE FLORIDA KEYS SOUTHERN DISEASE BOUNDARY



Florida Department of Environmental Protection  
Coral Reef Conservation Program



# Surveying the Florida Keys Southern Coral Disease Boundary

Final Summary Report

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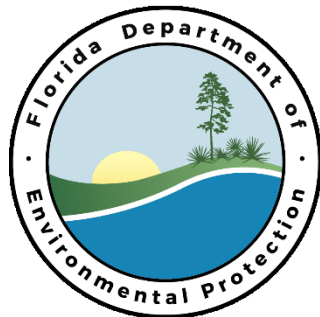
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## Executive Summary:

Since 2014, multi-year, multi-species disease outbreak has progressed geographically along the Florida Reef Tract from an origin near Virginia Key. From fall 2017 to spring 2018, 134 reef sites were surveyed to identify the geographic boundary of the disease as well as provide information on rates of progression, cross-reef prevalence differences, and differences in responses of impacted species. Roving diver surveys were conducted during two reef-tract wide assessments in fall 2017 and spring 2018, and roving diver and belt transect surveys were conducted in a targeted effort near the southern disease boundary in winter 2017-18 between Long Key and Marathon.

Surveys confirmed prevalence of the disease on 15 coral species, including the primary reef builders and five ESA-listed species. Disease was conspicuously absent on some other species, including the *Acropora* and *Porites* spp. Disease hotspots and geographic differences in disease prevalence showed that tissue loss begins on different species at different times. Broadly, the brain corals (particularly *Meandrina meandrites*) are the first to show tissue loss. Boulder coral infections generally appear shortly thereafter, with *Montastraea cavernosa* in particular often a later species to exhibit tissue loss.

During Fall 2017, the disease boundary was determined to be north of Long Key. By winter 2017-18, it was off Marathon, and by Spring 2018, it was observed at Looe Key off of Big Pine. The rate of disease boundary progression is estimated at between 8 and 22 km/month, with the prediction that the whole of the Florida Reef Tract (excluding Dry Tortugas) will be infected between June 2018-January 2019.

No differences in progression rates or infection susceptibilities were found based on reef zone (mid-channel patch reefs, offshore patch reefs, fore reefs). However, the progression was found to be not entirely linear. In several instances, signs of infection were present “downstream” of apparently healthy sites. These infected sites were sometimes up to 10 km southwest of the nearest known disease site.

Anecdotal observations include observations of probable inter- and intra-specific transmission by touch as well as corals showing at least short-term resilience in heavily infected areas.

Roving diver surveys proved to be an effective and efficient way to collect large amounts of site data on susceptible species. Belt transects were valuable for assessing common species (particularly *Siderastrea siderea*). *Meandrina meandrites* is identified as a primary “early warning” species, generally showing signs of disease ahead of all other common species. The progression of disease signs through the various species, as well as the varying speeds at which they progress to full mortality, are important to consider in developing early warning systems or potential treatment options to reduce pathogen load or save susceptible colonies. The widespread extent of the disease suggests a heavy pathogen load on the Florida Reef Tract, and consideration of minimizing or preventing the water-borne spread of this by anthropogenic means to other regions is recommended.

## Introduction:

Since 2014, the Florida Reef Tract has been experiencing a coral die-off that has affected numerous scleractinian species and been unprecedented in its geographic and temporal scope. First appearing near Virginia Key, it progressed rapidly northward to the northern boundaries of the reef tract and also showed a slower but steady progression southward into the Florida Keys National Marine Sanctuary.

The “white syndrome” die-off presents as tissue loss radiating from disease lesions. Within most species, a stark line between apparently healthy tissue and denuded skeleton progresses rapidly across the colony, sometimes from multiple lesions. Within *Montastrea cavernosa*, a bleached area of tissue lies between dead skeleton and apparently healthy tissue. And in *Siderastrea siderea*, multiple irregularly shaped lesions across the colony eventually coalesce. In almost all affected species, tissue loss leads to full colony mortality.

Beginning in fall 2017, an effort was undertaken to determine the location of the southern disease boundary. In addition to identifying the geographic extent of infection, surveys also examined differences in impacted species, habitat-related susceptibility, and rates of progression across the reef tract.

## Methods:

### Sites:

Sites were first assessed following Hurricane Irma between September 2017 and April 2018. Initial site visits (September 23, 2017 – October 17, 2017) were opportunistically conducted either off of Rainbow Reef Dive Shop boats during post-storm assessments (3 sites), or as part of a collaborative National Oceanographic and Atmospheric Administration (NOAA) post-hurricane assessment cruise (55 sites). These sites were located from Key Largo to Key West and were selected to focus primarily on “high-value” tourist sites, long-term monitoring sites, and Florida Reef Resilience Program (FRRP) Disturbance Response Monitoring (DRM) sites with high coral cover.

Based on the results from the September-October 2017 surveys, a second survey effort was conducted between November 21, 2017 and January 21, 2018. Some of these (8 sites) were surveyed opportunistically by Florida Fish and Wildlife Conservation Commission’s Restoration Ecology team as researchers were on site for other projects. The majority (28 sites) were specifically

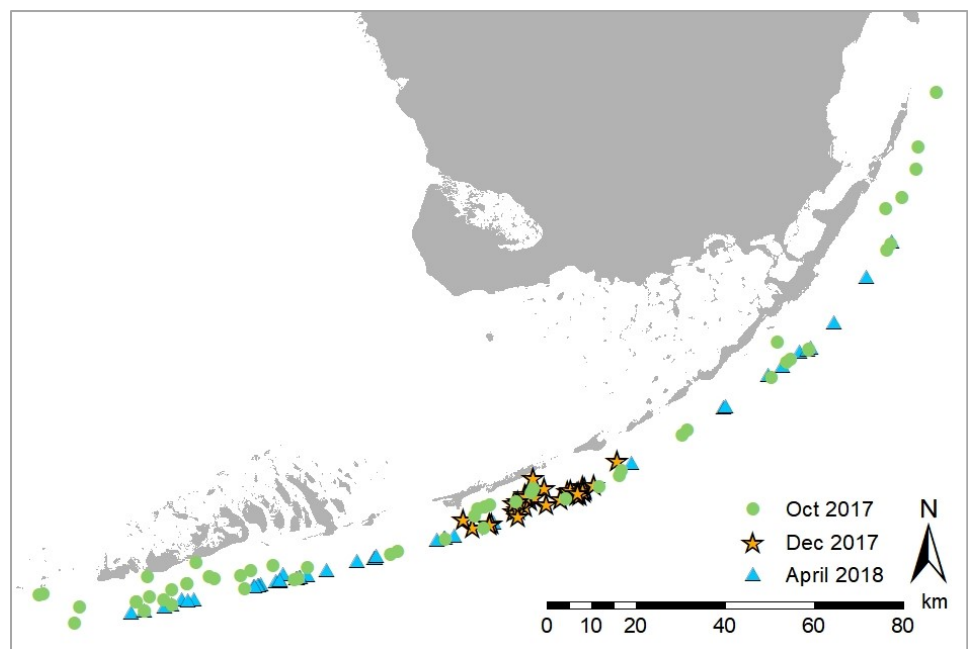


Fig 1. Location of all survey sites, distinguished by project/date.

directed towards disease assessments within a projected disease zone (between Long Key and Grassy Key).

These targeted sites were initially selected using a stratified random design to select 30 primary sites positioned on inshore patch reefs, mid-channel patch reefs, offshore patch reefs, and forereef habitats. However, after two days of sampling, 7 of these sites were found to be sand/grass, and others had inadequate densities of corals to conclude whether disease was present. In order to maximize field efforts (visiting only sites with corals) and probability of disease detection (targeting sites with high coral densities), a more strategic approach was applied. Past FRRP survey records were sorted to identify sites within the disease margin zone that recorded high coral cover as well as the presence of suspected “disease indicator” species like *Meandrina meandrites* and *Dichocoenia stokesii*. These targeted sites comprised the remainder of the November to January assessments.

A final reef-wide assessment was conducted in April 2018 (Apr 17- May 10), primarily during a Nova Southeastern University/Florida Aquarium *Dendrogyra cylindrus* assessment cruise. All sites were centered on points which contained known *D. cylindrus* colonies, and thus were confined mostly to the forereef. In total, 48 sites between Carysfort Reef (Key Largo) and Sand Key (Key West) were surveyed during the spring assessment.

In total, 141 sites were assessed. Of these, 134 yielded reef-related habitat data suitable for disease assessment (Fig 1. Appendix I). Seventy of these were located between Tennessee Reef and Looe Key, which were estimated as the southern boundaries in August 2017 and April 2018 respectively.

#### Assessments:

The primary mode of disease assessment was roving diver surveys. During each survey event, one or more divers surveyed the area by tallying coral colonies larger than 10 cm. If multiple divers were surveying, care was taken to not overlap survey areas. Divers on all surveys excluded Acroporids, Milleporids, *Siderastrea siderea*, and *Porites astreoides* from their tallies in order to focus on species that either show susceptibility (unlike Acroporids, Milleporids, and *P. astreoides*), or were not so common as to overwhelm the census (like *S. siderea*). During most surveys, the minimum survey time was 20 minutes. During the April 2018 surveys, survey times could be as short as 5 minutes as they were being conducted opportunistically with another project. Roving divers tallied colonies by species into one of four categories: 1) recently dead in a manner suggesting disease-related mortality, 2) active white disease, 3) symptoms of concern (paling or bleaching spots), and 4) healthy.

During the November to January disease-specific surveys (28 sites between Tennessee and Sombrero), 10x1m belt transects were also conducted following the FRRP DRM methodology. At each site, two divers deployed non-overlapping transects and surveyed the status of all hard corals greater than 4cm in diameter within the transect. Proportions of healthy versus diseased *S. siderea* and *P. astreoides* at each site were derived from these transects.

## Results/Discussion:

### Species susceptibility:

Across all surveys, 24 hard coral species were recorded. Of these, nine were completely or almost completely asymptomatic: *Porites astreoides*, *Porites porites*, *Madracis decactis*, *Madracis mirabilis*, *Mussa angulosa*, *Scolymia cubensis*, *Oculina* spp., *Stephanocoenia michilini*, and *Agaricia agaricites*. The invasive *Tubastraea coccinea* was also observed (but not tallied) in an area of high disease and displayed no visible signs of susceptibility. These species had disease proportions lower than 0.006, which are less than four times those of any other species (Table 1). For some of these (*M. mirabilis*, *M. angulosa*, *S. cubensis*, and *Oculina* spp), sample sizes are small, but for others, a large number of colonies were recorded. These proportions are not indicative of reef-tract or even regional values as they are calculated from a variety of site selection methodologies. However, the noted absence of disease on the nine largely asymptomatic species, particularly as compared to disease observations on other species, strongly suggests a list of species that are not susceptible.

Susceptibility and infection ratios of species were further determined by focusing on patterns within areas of active disease. These areas were identified by calculating a site disease index: the proportion of recently dead and diseased colonies divided by the total number of recorded colonies for the eight most susceptible species: *Meandrina meandrites*, *Dichocoenia stokesii*, *Colpophyllia natans*, *Pseudodiploria strigosa*, *Diploria labyrinthiformes*, *Orbicella* spp, *Solanaestera bournoni*, and *Montastrea cavernosa*. The eleven sites with a disease index greater than 0.3 (30% of index colonies infected) were used to look at proportional infection rates at disease “hotspots.” Seventeen species were documented at these sites, and the proportion of symptomatic colonies ranged from greater than 80% (*Meandrina meandrites*) to 0% (*Eusmyllia fastigiata*, *Mycetophyllia lamarckiana*, *Madracis decactis*, *Porites porites*, *Porites astreoides*) (Fig 2).

One plausible explanation for these species-specific differences in visible infections is the length of time each species has been displaying signs of disease. While

| Species                          | N    | Proportion Affected |
|----------------------------------|------|---------------------|
| <i>Porites astreoides</i>        | 139  | 0                   |
| <i>Porites porites</i>           | 81   | 0                   |
| <i>Madracis decactis</i>         | 20   | 0                   |
| <i>Madracis mirabilis</i>        | 4    | 0                   |
| <i>Mussa angulosa</i>            | 4    | 0                   |
| <i>Scolymia cubensis</i>         | 1    | 0                   |
| <i>Oculina</i> spp.              | 1    | 0                   |
| <i>Stephanocoenia michilini</i>  | 943  | 0.004               |
| <i>Agaricia agaricites</i>       | 178  | 0.006               |
| <i>Eusmilia fastigiata</i>       | 80   | 0.025               |
| <i>Pseudodiploria clivosa</i>    | 182  | 0.033               |
| <i>Orbicella annularis</i>       | 234  | 0.038               |
| <i>Orbicella faveolata</i>       | 1263 | 0.041               |
| <i>Montastrea cavernosa</i>      | 3236 | 0.046               |
| <i>Solenastrea bournoni</i>      | 232  | 0.047               |
| <i>Colpophyllia natans</i>       | 1621 | 0.057               |
| <i>Orbicella franksii</i>        | 439  | 0.062               |
| <i>Siderastrea siderea</i>       | 899  | 0.071               |
| <i>Mycetophyllia</i> spp         | 33   | 0.091               |
| <i>Dichocoenia stokesii</i>      | 887  | 0.100               |
| <i>Diploria labyrinthiformis</i> | 461  | 0.108               |
| <i>Pseudodiploria strigosa</i>   | 947  | 0.126               |
| <i>Meandrina meandrites</i>      | 387  | 0.233               |
| <i>Dendrogyra cylindrus</i>      | 49   | 0.286               |

Table 1. Proportion of each recorded coral species affected by disease across all 134 sites. Proportions are determined by tallies of colonies showing active disease or 100% recent mortality divided by total number of observed colonies within each species. Recent mortality is identified as bright white skeleton; as algal colonization makes these indistinguishable from old mortality within weeks, the proportions represent a snapshot of disease at a given time rather than a cumulative impact. Tallies were conducted by roving diver survey for all species except *Porites astreoides* and *Siderastrea siderea*, which were determined via 1x10 meter belt transects during the December surveys. Species shaded in gray show little to no susceptibility to the disease.

individual sites/colonies were not fate-tracked in these surveys, species with higher disease proportions at “hotspots” are also the species in which infections are first observed in downstream (the most newly infected) sites. For example, at three survey sites around Coffins Patch (Marathon), the site disease index decreased in a southwesterly direction. At the most downstream site, the disease index was 0.02, and only *M. meandrina* was infected. A site 1 km to the northeast had a disease index of 0.06, four infected species, and some colony mortality of *M. meandrina*. At the northeastern most site, which had a disease index of 0.11, five species were infected, and colony mortality of both *M. meandrina* and *D. stokesii* had occurred (Fig 3). These geographic patterns suggest that signs of disease do not appear concurrently in all species, and that proportional differences in disease as well as mortality rates are driven by the susceptibility to infection and/or the early display of disease signs.

#### Observations of transmission:

Within high density sites (Long Key Bridge Rubble and inshore patch reefs), corals frequently have physical contact with conspecifics as well as individuals of other species. Anecdotal observations note many instances in which the disease appears to transmit from an infected individual into an individual of the same or different species at the contact margin (Fig 4). However, examples of colonies in physical contact with both inter- and intra-specific diseased/dying colonies that are not diseased or do not display signs of disease at the region of contact also abound (Fig 5).

Fig 2a. Average proportion of each species infected at eleven “hotspot” sites. Sites all had overall colony infection rates greater than 0.3 of susceptible species. Error bars are +/- one standard deviation. Species names on x-axis are derived from first letter of genus and first three letters of species (e.g., MMEA is *Meandrina meandrites*).

Fig 2b. Location of hotspot sites used in analysis. High disease prevalence sites were concentrated off Key Largo in Fall 2017 and off of Long Key and Marathon in Winter 2017-18 and Spring 2018.

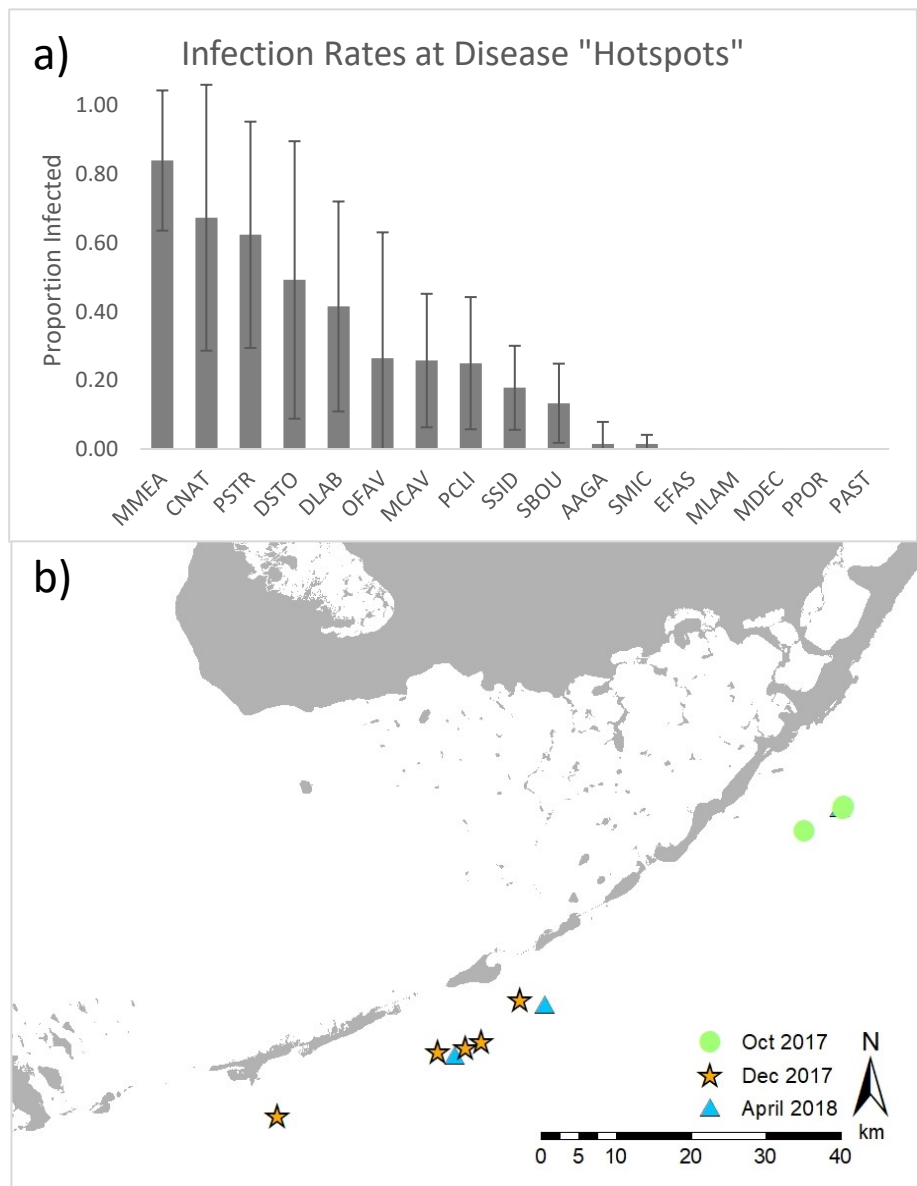
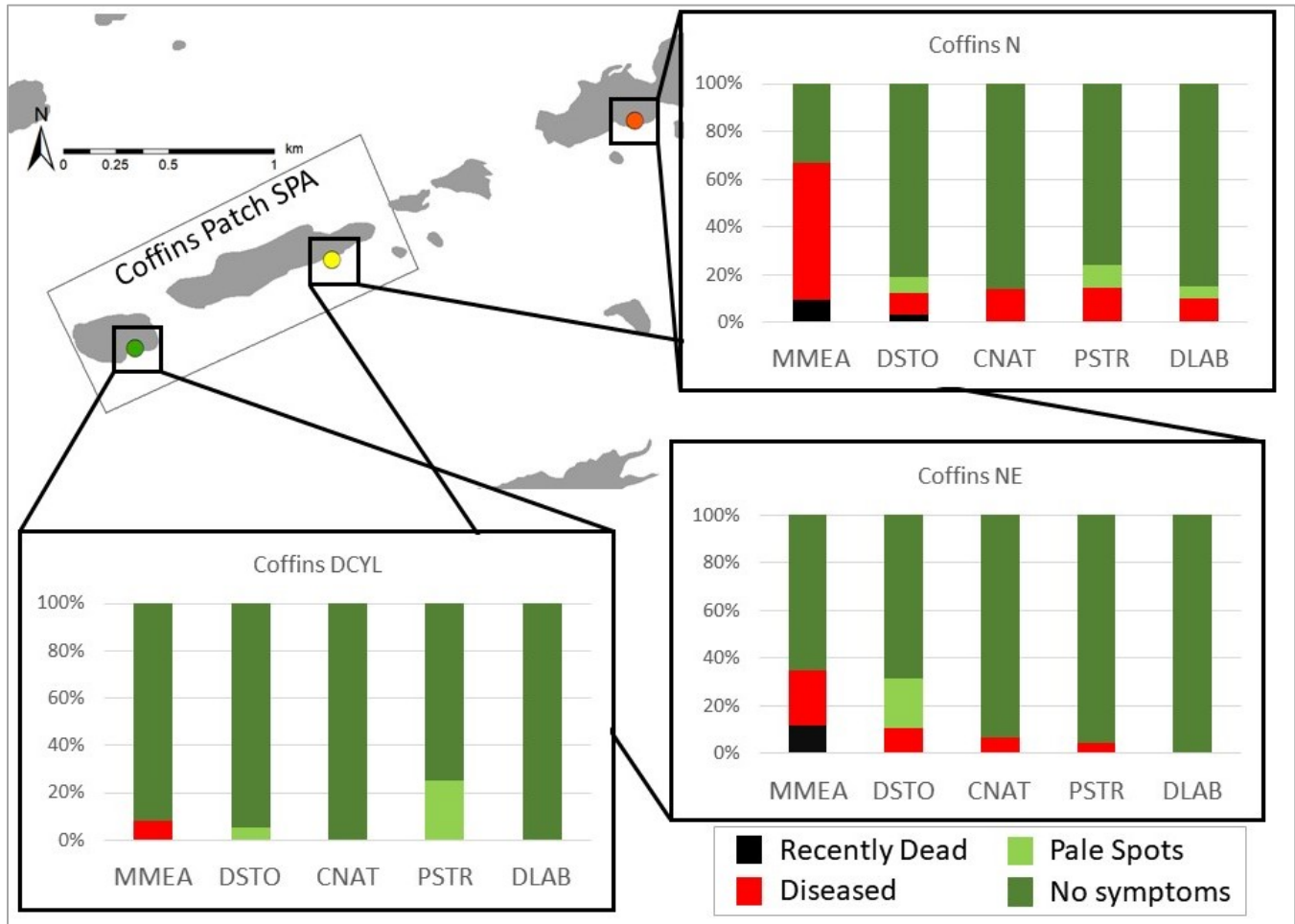


Fig 3. Prevalence of disease on five early indicator species on three reef patches within and near Coffins Patch (Marathon). Assuming that the disease infects from northeast to southwest, signs of infection appear first on *M. meandrites*, then on other species. Mortality follows, with species showing the first signs of infection also showing the first signs of complete mortality.



Observations of corals becoming infected at the point of contact with another diseased colony at both the inter- and intra-specific level suggest that transmission is heightened by physical contact. However, the large number of colonies infected that are not in contact with other hard corals, the transmission between reefs, and the “jumps” in infection between areas that sometimes pass over intermediate reefs suggests more widespread transmission mechanisms. Laboratory transmission experiments (Val Paul, pers comm) show infection of healthy corals by diseased ones even through sterile seawater.

That some colonies appear non-diseased when surrounded by or even touching diseased colonies of the same or different species may be of interest for determining disease resistance. As these surveys represent only snapshots of each site, they can not determine whether disease signs for these exposed but apparently healthy colonies would soon appear or whether some long-term resistance was being observed. However, the presence of some healthy individuals even at highly-diseased sites suggests at least a short-term resistance within species. Fate tracking of individual colonies throughout the infection period is advised to further explore this topic.



Fig 4 (below). Probable transmission via contact between intraspecific colonies (left: two *Pseudodiploria strigosa*) and interspecific colonies (right: recently dead *Meandrina meandrites* colony and new signs on *Dichosoenia stokesii*).

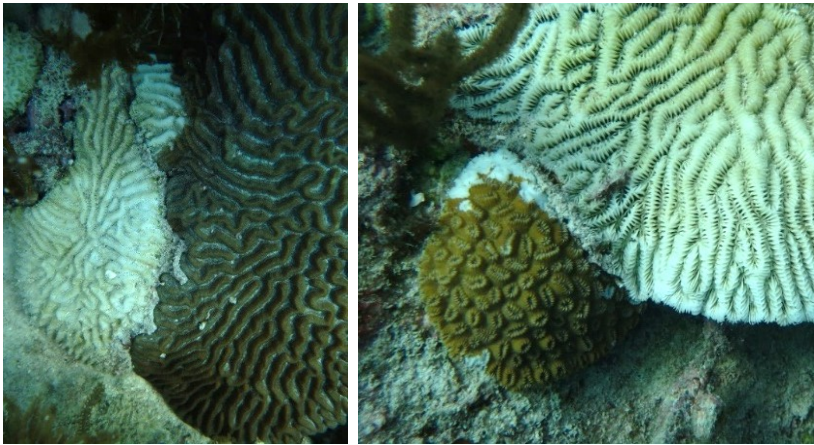


Fig 5 (below). Colonies in which contact may not be causing disease transmission. Left: Infected *Orbicella faveolata* adjacent to diseased/dead *Colpophyllia natans* and *Diploria labyrinthiformes*. Lesions on the *O. faveolata* are not adjacent to the infected colonies. Right: nearly dead *Colpophyllia natans* and adjacent asymptomatic *Pseudodiploria strigosa*.



January 2018, infected colonies were documented off of the south end of Marathon. By April 2018, infected colonies were documented at Looe Key (Big Pine Key) (Fig 6). Using these southernmost observations over the three survey periods (Oct 2017, Dec 2017, and April 2017), the rate of boundary movement down the reef tract is estimated at between 8-22 kilometers per month. Between the December 2017 and April 2018 surveys, it crossed any hydrographic barrier that the Seven Mile Bridge flow may have caused, leaving few if any natural barriers until past Key West. At this rate, the entirety of the Florida Reef Tract (not including Dry Tortugas) is expected to be symptomatic between June and August 2018.

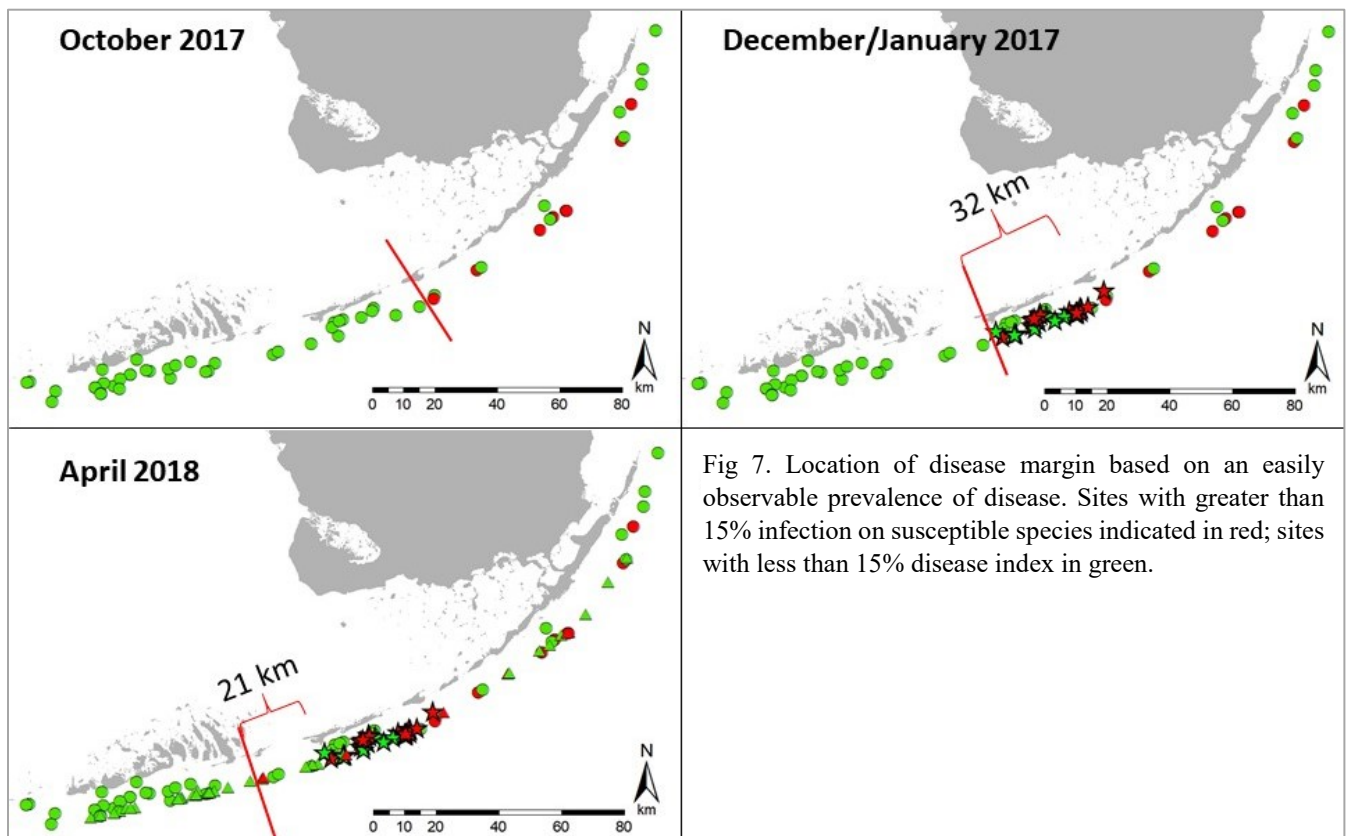
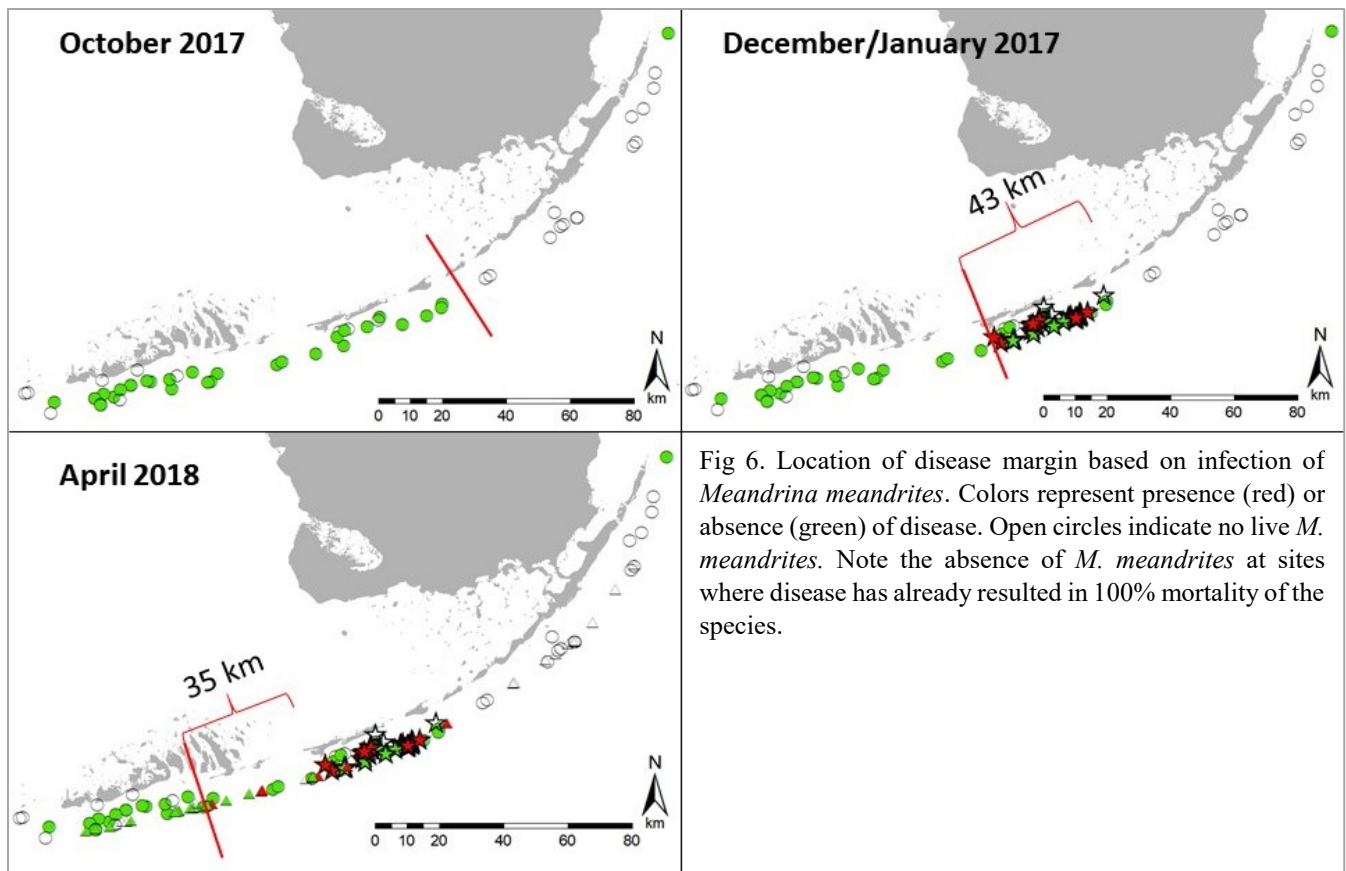
Progression can also be documented by using the total proportion of susceptible colonies, a measure that is more indicative of the point at which the “general public” would take notice of the disease. Widespread coral mortality and disease rates at a level noticeable to divers or other members of the community lag behind biological infection disease margins. Though some early indicators (*M. meandrina* and *D. stokesii*) are rapidly infected, they are relatively small, uncommon, and quickly undergo 100% mortality. Infection at a reef level is more visible when the brain and boulder corals become heavily infected. This is not a

### Boundary Determination:

Boundary determination analyses were conducted using two metrics: a biological concept (site of first observable infection) and an anthropocentric social/economic one (site where prevalence is easily observable).

The biological disease boundary is here defined as the most downstream site where signs of disease indicative of this outbreak are observed. As an early indicator species, *Meandrina meandrites* is the primary candidate for this determination. Geographic extent of disease in other species was also examined, but in no instances did other species correctly identify infection further downstream than *M. meandrina* colonies. In two cases, use of other species gave a “false positive,” as a single colony with “background” white plague indicated infection tens of kilometers ahead of all other indications of the disease boundary.

Using this method, the following boundaries were identified. In October 2017, no diseased *M. meandrites* colonies were recorded south of Tennessee Reef (Long Key). By



biological boundary (the outbreak is already well established at this point), but more of an economic and social one. Setting the disease index at 0.15 (15% of susceptible colonies showing signs of infection) to assume a noticeable ratio by the dive community, the margin has moved at between 7-10 km month (Fig 7), which if continued at the same rate will affect tourist-visited reefs off Key West between September 2018 and January 2019.

#### Onshore to Offshore Gradient:

Reports from summer 2017 suggested that transmission may be proceeding faster along the outer reef tract than among inshore sites. To test this, the December 2017 disease surveys were designed to sample habitats from nearshore to offshore reef environments as determined by benthic habitat maps. Sites surveyed within the disease zone included offshore patch reefs (N=4), forereef (N=17), and mid-channel patch reefs (N=5). An ANOVA was used to compare the proportions of infected species (all eight indicator species - DSTO, MMEA, CNAT, PSTR, SBOU, DLAB, MCAV, *Orbicella* spp - as well as the total proportion of infected colonies among indicator species) among these cross-reef habitats. For no species or proportional total was habitat zone a significant indicator of prevalence. This suggests that, at least within the Middle Keys, the disease is moving across all zones at an equal rate.

or proportional total was habitat zone a significant indicator of prevalence. This suggests that, at least within the Middle Keys, the disease is moving across all zones at an equal rate.

#### Disease “Jumping”

Though generally moving in a steady rate and direction, the progression of the disease margin was not always strictly linear. In two cases, actively diseased reefs were observed further southwest than apparently disease-free reefs.

- Disease “hotspots” were observed at Long Key Bridge Rubble and Coffins Patch in December 2017, but not at sites between the two (Fig 8). The distance between observed infected reefs was 9.6 km. While there may have been other intermediate reefs that were infected and not surveyed, the presence of non-infected reefs between two heavily diseased areas suggests that the movement is not

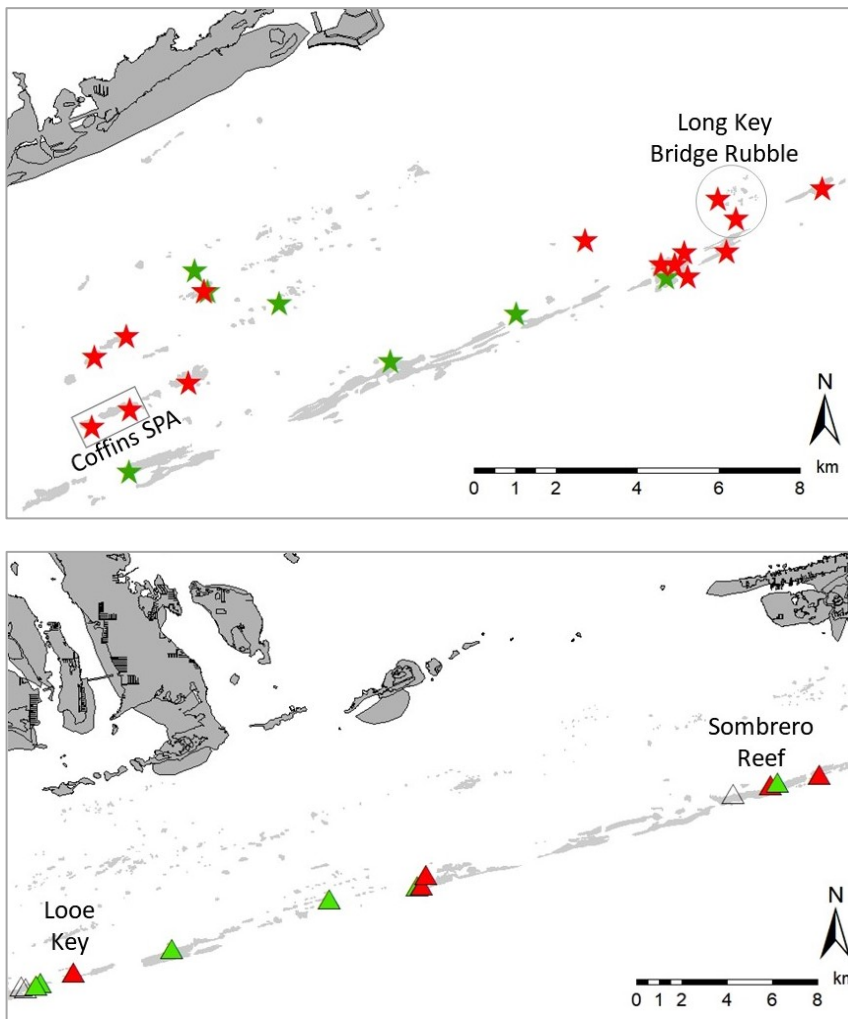


Fig 8. Top: Regions of uninfected *Meandrina meandrites* (green) between infected sites (red) near Marathon. Bottom: Sites with uninfected *M. meandrites* (green) interspersed among infected regions between Looe Key and Sombrero Reef.

uniform but may “jump” at these scales.

- Disease was observed on *M. meandrites* at Looe Key in April 2018. Surveys within the same two weeks confirmed disease at Sombrero (Marathon), but at less than 50% of the survey sites in between. Five sites remained clear of disease, even on the most early susceptible species (Fig 8).

## Recommendations:

### Boundary surveys

Roving diver surveys proved to be an effective means of determining disease presence and severity. The method is rapid, can be conducted by a single diver, is applicable in areas of varying coral density, and is comparable between sites regardless of time spent or area covered because of the proportional nature of the survey.

Transect surveys, while statistically stronger, are extremely limited in documenting any but the most common species. Disease proportions derived from transects are overwhelmed by *Siderastrea siderea*, which are not good indicators of disease prevalence, particularly in the early stages of infection. For example, at the survey site with the highest susceptible species disease index (70% of colonies from indicator species infected), transects documented disease on only 6 to 7% of corals. While this represents a biological truism for the proportion of corals infected, it is unlikely to identify the site as diseased and makes differences between sites difficult to discern.

Thus, while the roving diver method’s limitations include replicability or statistical application, it is recommended as an assessment tool for a quick determination of infection and between-site comparisons of disease prevalence.

Determination of the disease margin boundary is confounded by its continual movement, varying susceptibilities between species, and evidence of non-linear progression at the scale of up to 10 kilometers. As such, the following guidelines are recommended:

- Single observations of disease on one colony, particularly on species that are not early indicators, can lead to false positives. Observations on *M. meandrina* can be highly indicative of the presence of disease, especially if observed on more than a single colony. Disease presence on other species should be viewed with caution unless present on multiple colonies, multiple species, or multiple sites in the region.
- Disease presence on *M. meandrina* appears to be a good indication of site infection and indicates future outbreaks on other species. Presence on 15% of colonies of vulnerable species can also be tracked as a progression likely to be detected by the public.
- An absence of diseased corals is not always indicative of a healthy site. If only proportions are compared, sites in which the disease has already passed through look similar to healthy ones because all susceptible colonies are dead. A lack of, or severe decrease in the number of susceptible-species colonies may indicate a disease-ravaged site even if no disease is presently observed.

### Recommendations for Fixed Sites

Based on the location of diseased colonies and the rate of progression, any fixed sites aiming to assess a community before it is infected should be established before June 2018 and be located as far south as possible. These fate-tracked fixed sites could confirm the order of species infections and also determine whether there are any “warning signs” of disease on individual colonies. Throughout the disease surveys, colonies with what were recorded as “symptoms of concern” were noted; these symptoms included paleness around margins (particularly *P. strigosa*) or blotchiness (particularly *D. stokesii*) (Fig 9). Fate-tracking such colonies would determine whether these symptoms develop into full tissue lesions or provide early indications of regions within the colony that will exhibit (or be resistant to) tissue loss.



Fig 9. Unusual appearance of susceptible species seen within or near disease margin zones. Fate-tracking of individual colonies may show whether these symptoms are early signs of disease that will soon lead to tissue loss and colony mortality.

### Proposed Management Actions

Surveys clearly show a rapidly progressing, highly fatal disease front that has already infected the majority of the Florida Reef Tract and will likely infect the rest within upcoming months. Transmission appears to be possible via water currents and is unlikely to be contained. At this point, no highly effective or large-scale treatments are available.

Ongoing treatment trials should continue in the hopes of finding a treatment that can be applied to individual colonies or perhaps small sites. It may be possible that early treatment on early signs of disease, coupled with continual monitoring and treatment, could confer a level of protection to a site by reducing the localized pathogen load. Ideas for treatment at greater scales should also be sourced; inclusion of medical professionals specializing in large-scale outbreaks and broad-scale treatments should be considered.

Additionally, a regional approach should be considered to advise, protect, and consider early intervention at other sites. Within the continental United States, the Dry Tortugas, Florida Gulf Coast, and Flower Garden Banks may be naturally isolated from the disease but may be highly susceptible to receiving it through anthropogenic means. Other Caribbean countries and territories may similarly be more vulnerable if human-related transmission is not considered and addressed. Creating an early warning system for reporting, response, and treatment at even a localized level may help protect these other regions.

### Acknowledgements:

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Appendix I: Site list for disease surveys. Gray sites at the bottom were non-reef habitats. Yellow sites were the hotspot sites used for the species-specific prevalence data shown in Figure 2

| <u>Site</u>         | <u>Date</u> | <u>Timing</u> | <u>Lat</u> | <u>Lon</u> | <u>Depth (ft)</u> | <u>Project</u> | <u>Disease Index</u> |
|---------------------|-------------|---------------|------------|------------|-------------------|----------------|----------------------|
| Snapper Ledge       | 9/23/2017   | Oct 2017      | 24.9816    | -80.4219   | 30                | Rainbow Reef   | 0.33                 |
| Molasses Wench Hole | 9/23/2017   | Oct 2017      | 25.0092    | -80.3745   | 30                | Rainbow Reef   | 0.38                 |
| French B14          | 9/23/2017   | Oct 2017      | 25.0333    | -80.3478   | 42                | Rainbow Reef   | 0.24                 |
| Irma 44             | 10/17/2017  | Oct 2017      | 24.5122    | -81.9298   | 15                | Irma Cruise    | 0.00                 |
| Irma 36             | 10/17/2018  | Oct 2017      | 24.5139    | -81.9225   | 17                | Irma Cruise    | 0.00                 |
| Irma 89             | 10/17/2017  | Oct 2017      | 24.4545    | -81.8589   | 14                | Irma Cruise    | 0.00                 |
| Irma 52             | 10/17/2017  | Oct 2017      | 24.4865    | -81.8486   | 21                | Irma Cruise    | 0.00                 |
| Irma 25             | 10/18/2017  | Oct 2017      | 24.4978    | -81.7333   | 19                | Irma Cruise    | 0.31                 |
| Irma 35             | 10/17/2017  | Oct 2017      | 24.4978    | -81.7333   | 22                | Irma Cruise    | 0.00                 |
| Irma 88             | 10/17/2017  | Oct 2017      | 24.4796    | -81.7176   | 25                | Irma Cruise    | 0.00                 |
| Irma 99             | 10/17/2017  | Oct 2017      | 24.4780    | -81.7171   | 34                | Irma Cruise    | 0.00                 |
| Irma 50             | 10/16/2017  | Oct 2017      | 24.5486    | -81.7124   | 13                | Irma Cruise    | 0.00                 |
| Irma 31             | 10/16/2017  | Oct 2017      | 24.5083    | -81.7083   | 20                | Irma Cruise    | 0.00                 |
| Irma 109            | 10/16/2017  | Oct 2017      | 24.5014    | -81.6791   | 26                | Irma Cruise    | 0.00                 |
| Irma 87             | 10/17/2017  | Oct 2017      | 24.4917    | -81.6636   | 12                | Irma Cruise    | 0.00                 |
| Irma 70             | 10/16/2017  | Oct 2017      | 24.5209    | -81.6621   | 27                | Irma Cruise    | 0.00                 |
| Irma 45             | 10/16/2017  | Oct 2017      | 24.5347    | -81.6323   | 16                | Irma Cruise    | 0.00                 |
| Irma 51             | 10/16/2017  | Oct 2017      | 24.5348    | -81.6321   | 17                | Irma Cruise    | 0.00                 |
| Irma 104            | 10/16/2017  | Oct 2017      | 24.5768    | -81.6134   | 12                | Irma Cruise    | 0.00                 |
| Irma 75             | 10/16/2017  | Oct 2017      | 24.5475    | -81.5866   | 22                | Irma Cruise    | 0.00                 |
| Irma 105            | 10/16/2017  | Oct 2017      | 24.5449    | -81.5765   | 28                | Irma Cruise    | 0.00                 |
| Irma 46             | 10/15/2017  | Oct 2017      | 24.5500    | -81.5237   | 29                | Irma Cruise    | 0.00                 |
| American Shoal      | 10/15/2017  | Oct 2017      | 24.5233    | -81.5161   | 21                | Irma Cruise    | 0.00                 |
| Irma 69             | 10/16/2017  | Oct 2017      | 24.5603    | -81.5021   | 23                | Irma Cruise    | 0.00                 |
| Irma 106            | 10/15/2017  | Oct 2017      | 24.5703    | -81.4577   | 30                | Irma Cruise    | 0.00                 |
| Irma 97             | 10/15/2017  | Oct 2017      | 24.5421    | -81.4153   | 43                | Irma Cruise    | 0.00                 |
| Irma 86             | 10/15/2017  | Oct 2017      | 24.5452    | -81.4071   | 26                | Irma Cruise    | 0.00                 |
| Irma 32             | 10/15/2017  | Oct 2017      | 24.5669    | -81.3886   | 26                | Irma Cruise    | 0.02                 |
| Irma 61             | 10/15/2017  | Oct 2017      | 24.5926    | -81.2208   | 44                | Irma Cruise    | 0.00                 |
| Irma 64             | 10/15/2017  | Oct 2017      | 24.6004    | -81.2063   | 38                | Irma Cruise    | 0.00                 |
| Irma 96             | 10/16/2017  | Oct 2017      | 24.6231    | -81.1105   | 16                | Irma Cruise    | 0.07                 |
| Irma 68             | 10/14/2017  | Oct 2017      | 24.6715    | -81.0506   | 21                | Irma Cruise    | 0.00                 |
| Irma 47             | 10/14/2017  | Oct 2017      | 24.6857    | -81.0450   | 14                | Irma Cruise    | 0.02                 |
| Irma 115            | 10/16/2017  | Oct 2017      | 24.6467    | -81.0322   | 65                | Irma Cruise    | 0.00                 |
| Irma 74             | 10/14/2017  | Oct 2017      | 24.6895    | -81.0302   | 16                | Irma Cruise    | 0.00                 |
| Irma 53             | 10/14/2017  | Oct 2017      | 24.6929    | -81.0210   | 20                | Irma Cruise    | 0.00                 |
| Irma 73             | 10/14/2017  | Oct 2017      | 24.6993    | -80.9669   | 24                | Irma Cruise    | 0.00                 |
| Irma 67             | 10/14/2017  | Oct 2017      | 24.7176    | -80.9357   | 24                | Irma Cruise    | 0.01                 |
| Irma 38             | 10/14/2017  | Oct 2017      | 24.7258    | -80.9315   | 23                | Irma Cruise    | 0.00                 |

|                       |            |          |         |          |    |                |      |
|-----------------------|------------|----------|---------|----------|----|----------------|------|
| Irma 16               | 10/12/2017 | Oct 2017 | 24.7064 | -80.8680 | 45 | Irma Cruise    | 0.14 |
| Irma 4                | 10/12/2017 | Oct 2017 | 24.7308 | -80.7988 | 68 | Irma Cruise    | 0.14 |
| Irma 95               | 10/12/2017 | Oct 2017 | 24.7527 | -80.7578 | 43 | Irma Cruise    | 0.26 |
| Irma 43               | 10/12/2017 | Oct 2017 | 24.7634 | -80.7552 | 33 | Irma Cruise    | 0.13 |
| Irma 119              | 10/11/2017 | Oct 2017 | 24.8357 | -80.6326 | 60 | Irma Cruise    | 0.17 |
| Irma 94               | 10/11/2017 | Oct 2017 | 24.8452 | -80.6209 | 38 | Irma Cruise    | 0.00 |
| Irma 93               | 10/11/2017 | Oct 2017 | 24.9519 | -80.4513 | 54 | Irma Cruise    | 0.25 |
| Irma 65               | 10/11/2017 | Oct 2017 | 25.0219 | -80.4390 | 8  | Irma Cruise    | 0.00 |
| Irma 24               | 10/18/2017 | Oct 2017 | 24.9822 | -80.4216 | 25 | Irma Cruise    | 0.00 |
| Irma 23               | 10/10/2017 | Oct 2017 | 24.9889 | -80.4137 | 17 | Irma Cruise    | 1.00 |
| Irma 81               | 10/18/2017 | Oct 2017 | 25.0088 | -80.3765 | 12 | Irma Cruise    | 0.21 |
| Irma 92               | 10/10/2017 | Oct 2017 | 25.0072 | -80.3756 | 45 | Irma Cruise    | 0.50 |
| Irma 71               | 10/9/2017  | Oct 2017 | 25.2931 | -80.2209 | 14 | Irma Cruise    | 0.09 |
| Irma 108              | 10/9/2017  | Oct 2017 | 25.2097 | -80.2182 | 17 | Irma Cruise    | 0.25 |
| Irma 91               | 10/10/2017 | Oct 2017 | 25.2208 | -80.2099 | 54 | Irma Cruise    | 0.03 |
| Irma 103              | 10/9/2017  | Oct 2017 | 25.3162 | -80.1880 | 12 | Irma Cruise    | 0.18 |
| Irma 102              | 10/9/2017  | Oct 2017 | 25.3732 | -80.1603 | 9  | Irma Cruise    | 0.00 |
| Irma 57               | 10/9/2017  | Oct 2017 | 25.4183 | -80.1554 | 11 | Irma Cruise    | 0.00 |
| Irma 63               | 10/9/2017  | Oct 2017 | 25.5284 | -80.1187 | 25 | Irma Cruise    | 0.00 |
| Pop Rocks - FWC       | 1/23/2018  | Dec 2017 | 24.6478 | -81.0552 | 24 | FWC            | 0.30 |
| W of TR2 - FWC        | 1/23/2018  | Dec 2017 | 24.6548 | -81.0205 | 18 | FWC            | 0.00 |
| South Ledge - FWC     | 1/23/2018  | Dec 2017 | 24.6574 | -81.0203 | 17 | FWC            | 0.26 |
| W of TR1 - FWC        | 1/23/2018  | Dec 2017 | 24.6559 | -81.0169 | 18 | FWC            | 0.00 |
| Nearshore Patch - FWC | 12/15/2017 | Dec 2017 | 24.7119 | -80.9461 | 26 | FWC            | 0.02 |
| 11' Mound - FWC       | 1/9/2018   | Dec 2017 | 24.7231 | -80.8620 | 21 | FWC            | 0.49 |
| Tennessee Patch - FWC | 1/9/2018   | Dec 2017 | 24.7832 | -80.7632 | 12 | FWC            | 0.39 |
| Washerwoman           | 1/22/2018  | Dec 2017 | 24.6643 | -81.0739 | 24 | Disease Margin | 0.01 |
| Coffins DCYL          | 12/15/2017 | Dec 2017 | 24.6820 | -80.9710 | 15 | Disease Margin | 0.02 |
| Inshore Coffins       | 12/15/2017 | Dec 2017 | 24.6974 | -80.9704 | 19 | Disease Margin | 0.13 |
| W Turtle Shoal        | 1/22/2018  | Dec 2017 | 24.7019 | -80.9633 | 29 | Disease Margin | 0.15 |
| Coffins Offshore      | 12/15/2017 | Dec 2017 | 24.6720 | -80.9628 | 35 | Disease Margin | 0.01 |
| Coffins NE            | 12/15/2017 | Dec 2017 | 24.6857 | -80.9626 | 15 | Disease Margin | 0.06 |
| Coffins North         | 12/15/2017 | Dec 2017 | 24.6917 | -80.9497 | 17 | Disease Margin | 0.11 |
| Irma 9                | 11/21/2017 | Dec 2017 | 24.7164 | -80.9483 | 26 | Disease Margin | 0.00 |
| Nearshore Patch - KN  | 1/22/2018  | Dec 2017 | 24.7119 | -80.9454 | 26 | Disease Margin | 0.10 |
| Irma 7                | 11/21/2017 | Dec 2017 | 24.7091 | -80.9296 | 26 | Disease Margin | 0.00 |
| L2152                 | 11/30/2017 | Dec 2017 | 24.6965 | -80.9050 | 25 | Disease Margin | 0.01 |
| K2255                 | 11/30/2017 | Dec 2017 | 24.7069 | -80.8771 | 23 | Disease Margin | 0.00 |
| LKL1                  | 12/7/2017  | Dec 2017 | 24.7178 | -80.8452 | 26 | Disease Margin | 0.05 |
| F2067                 | 12/7/2017  | Dec 2017 | 24.7148 | -80.8441 | 50 | Disease Margin | 0.03 |
| LKL2                  | 12/7/2017  | Dec 2017 | 24.7178 | -80.8421 | 32 | Disease Margin | 0.14 |
| K1114                 | 12/7/2017  | Dec 2017 | 24.7204 | -80.8400 | 25 | Disease Margin | 0.14 |
| LKL offshore          | 12/14/2017 | Dec 2017 | 24.7150 | -80.8393 | 60 | Disease Margin | 0.01 |

|                           |            |            |         |          |    |                |      |
|---------------------------|------------|------------|---------|----------|----|----------------|------|
| K1115 - artificial        | 11/30/2017 | Dec 2017   | 24.7323 | -80.8326 | 25 | Disease Margin | 0.22 |
| LK Deep Offshore          | 12/14/2017 | Dec 2017   | 24.7206 | -80.8307 | 50 | Disease Margin | 0.09 |
| Ari's pillar (artificial) | 12/7/2017  | Dec 2017   | 24.7278 | -80.8287 | 19 | Disease Margin | 0.71 |
| Q2158                     | 11/30/2017 | Dec 2017   | 24.7346 | -80.8096 | 34 | Disease Margin | 0.32 |
| Sand Key                  | 5/10/2018  | April 2018 | 24.4521 | -81.8769 | 22 | DCYL Cruise    | 0.04 |
| Eastern Dry Rocks 2       | 5/10/2018  | April 2018 | 24.4598 | -81.8458 | 20 | DCYL Cruise    | 0.00 |
| Eastern Dry Rocks 1       | 5/10/2018  | April 2018 | 24.4592 | -81.8441 | 18 | DCYL Cruise    | 0.00 |
| 9' Stake                  | 5/10/2018  | April 2018 | 24.4719 | -81.7648 | 17 | DCYL Cruise    | 0.00 |
| Marker 32                 | 4/20/2018  | April 2018 | 24.4738 | -81.7441 | 26 | DCYL Cruise    | 0.00 |
| W Sambo                   | 4/20/2018  | April 2018 | 24.4793 | -81.7174 | 22 | DCYL Cruise    | 0.00 |
| Sambo                     | 5/10/2018  | April 2018 | 24.4876 | -81.6898 | 29 | DCYL Cruise    | 0.00 |
| Middle Sambo              | 4/20/2018  | April 2018 | 24.4867 | -81.6774 | 26 | DCYL Cruise    | 0.00 |
| FRRP 1620                 | 4/20/2018  | April 2018 | 24.4919 | -81.6621 | 13 | DCYL Cruise    | 0.00 |
| Lonny's Pillars           | 4/20/2018  | April 2018 | 24.5007 | -81.6424 | 28 | DCYL Cruise    | 0.00 |
| Pelican Shoal 2           | 4/20/2018  | April 2018 | 24.5003 | -81.6296 | 13 | DCYL Cruise    | 0.00 |
| Pelican Shoal 1           | 4/20/2018  | April 2018 | 24.5020 | -81.6185 | 23 | DCYL Cruise    | 0.00 |
| W of Looe 4               | 4/20/2018  | April 2018 | 24.5281 | -81.4966 | 24 | DCYL Cruise    | 0.00 |
| W of Looe 5               | 4/20/2018  | April 2018 | 24.5281 | -81.4948 | 28 | DCYL Cruise    | 0.00 |
| W of Looe 3               | 4/20/2018  | April 2018 | 24.5300 | -81.4896 | 23 | DCYL Cruise    | 0.00 |
| W of Looe 2               | 4/20/2018  | April 2018 | 24.5307 | -81.4863 | 22 | DCYL Cruise    | 0.00 |
| Acer 21                   | 4/19/2018  | April 2018 | 24.5317 | -81.4829 | 20 | DCYL Cruise    | 0.00 |
| Mote SWG                  | 4/19/2018  | April 2018 | 24.5385 | -81.4529 | 23 | DCYL Cruise    | 0.05 |
| Acer 11                   | 4/19/2018  | April 2018 | 24.5387 | -81.4438 | 34 | DCYL Cruise    | 0.00 |
| W of Looe 1               | 4/19/2018  | April 2018 | 24.5400 | -81.4429 | 26 | DCYL Cruise    | 0.00 |
| Acer 24                   | 4/19/2018  | April 2018 | 24.5528 | -81.4373 | 22 | DCYL Cruise    | 0.00 |
| Looe 2                    | 4/19/2018  | April 2018 | 24.5453 | -81.4103 | 18 | DCYL Cruise    | 0.00 |
| Looe 3                    | 4/19/2018  | April 2018 | 24.5447 | -81.4086 | 26 | DCYL Cruise    | 0.00 |
| Looe Buoy 9               | 4/19/2018  | April 2018 | 24.5458 | -81.4044 | 18 | DCYL Cruise    | 0.00 |
| Looe Buoy 3               | 4/19/2018  | April 2018 | 24.5468 | -81.4027 | 14 | DCYL Cruise    | 0.00 |
| E of Looe 1               | 4/19/2018  | April 2018 | 24.5511 | -81.3894 | 25 | DCYL Cruise    | 0.06 |
| E of Looe 2               | 4/19/2018  | April 2018 | 24.5603 | -81.3504 | 27 | DCYL Cruise    | 0.00 |
| BH1                       | 4/19/2018  | April 2018 | 24.5800 | -81.2878 | 30 | DCYL Cruise    | 0.00 |
| BH3                       | 4/19/2018  | April 2018 | 24.5851 | -81.2529 | 28 | DCYL Cruise    | 0.00 |
| BH4                       | 4/19/2018  | April 2018 | 24.5856 | -81.2511 | 29 | DCYL Cruise    | 0.14 |
| BH5                       | 4/19/2018  | April 2018 | 24.5899 | -81.2492 | 29 | DCYL Cruise    | 0.15 |
| Stag Acres                | 4/17/2018  | April 2018 | 24.6220 | -81.1270 | 24 | DCYL Cruise    | 0.00 |
| Sombrero SPA 1            | 4/17/2018  | April 2018 | 24.6255 | -81.1122 | 11 | DCYL Cruise    | 0.19 |
| Sombrero SPA 2            | 4/17/2018  | April 2018 | 24.6263 | -81.1094 | 22 | DCYL Cruise    | 0.00 |
| Sombrero Out              | 4/17/2018  | April 2018 | 24.6297 | -81.0930 | 32 | DCYL Cruise    | 0.05 |
| Parallel                  | 4/17/2018  | April 2018 | 24.6559 | -81.0122 | 26 | DCYL Cruise    | 0.25 |
| Long Key Ledge 2          | 4/17/2018  | April 2018 | 24.7178 | -80.8421 | 32 | DCYL Cruise    | 0.30 |
| Stag Party East           | 4/18/2018  | April 2018 | 24.7776 | -80.7335 | 25 | DCYL Cruise    | 0.42 |
| Crocker 3                 | 4/18/2018  | April 2018 | 24.8891 | -80.5480 | 42 | DCYL Cruise    | 0.15 |



|                           |            |            |         |          |    |                |      |
|---------------------------|------------|------------|---------|----------|----|----------------|------|
| Crocker 4                 | 4/18/2018  | April 2018 | 24.8927 | -80.5449 | 35 | DCYL Cruise    | 0.14 |
| Conch 3                   | 4/18/2018  | April 2018 | 24.9564 | -80.4574 | 19 | DCYL Cruise    | 0.00 |
| Pickles 10                | 4/18/2018  | April 2018 | 24.9731 | -80.4286 | 37 | DCYL Cruise    | 0.00 |
| Molasses 1                | 4/18/2018  | April 2018 | 25.0018 | -80.3950 | 26 | DCYL Cruise    | 0.00 |
| Molasses Buoy 19          | 4/18/2018  | April 2018 | 25.0075 | -80.3787 | 24 | DCYL Cruise    | 0.33 |
| Molasses 7                | 4/18/2018  | April 2018 | 25.0107 | -80.3729 | 17 | DCYL Cruise    | 1.00 |
| French 4                  | 4/18/2018  | April 2018 | 25.0622 | -80.3249 | 34 | DCYL Cruise    | 0.00 |
| Elbow 5                   | 4/18/2018  | April 2018 | 25.1546 | -80.2600 | 36 | DCYL Cruise    | 0.00 |
| Carysfort 8               | 4/18/2018  | April 2018 | 25.2256 | -80.2076 | 25 | DCYL Cruise    | 0.00 |
| Irma 54 - sand/grass      | 11/21/2017 | Dec 2017   | 24.7047 | -80.9392 | 20 | Disease Margin |      |
| Irma 12 - grass           | 11/21/2017 | Dec 2017   | 24.7498 | -80.9323 | 14 | Disease Margin |      |
| Irma 28 - sand            | 11/21/2017 | Dec 2017   | 24.7285 | -80.9104 | 32 | Disease Margin |      |
| K2111 - grass             | 12/7/2017  | Dec 2017   | 24.7250 | -80.8566 | 15 | Disease Margin |      |
| LK Maybe offshore - sand  | 12/14/2017 | Dec 2017   | 24.7221 | -80.8333 |    | Disease Margin |      |
| LK direct offshore - sand | 12/14/2017 | Dec 2017   | 24.7244 | -80.8281 |    | Disease Margin |      |
| Irma 8 - sand/grass       | 11/21/2017 | Dec 2017   | 24.7128 | -80.0921 | 32 | Disease Margin |      |