Project Title: Studies on environmental co-factors potentially influencing the disease dynamics of Florida's coral tissue loss diseases

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Disease dynamics through time

At our two field sites, Looe Key and Fort Lauderdale, we found differences in lesion appearance (Ft. Lauderdale: subacute often with a bleached band vs. Keys: acute tissue loss usually lacking a bleached band) and in mortality on tagged *M. cavernosa* colonies with tissue loss disease. Tagged *M. cavernosa* colonies at Looe Key had a >50% case mortality rate after 7 months but only 15% of the Fort Lauderdale colonies died after a year. There could be regional differences in environmental conditions, which are affecting pathogen-host dynamics, allowing the pathogen to have higher virulence at Looe Key. Alternatively, we did not begin monitoring the Ft. Lauderdale colonies until years after the event was first found in that region, and so it could be a natural progression in disease-host dynamics with pathogens evolving to a less virulent state and/or the most susceptible corals are already dead from the disease. Coral disease outbreaks are often more virulent at the start of an outbreak and then prevalence and virulence decrease through time (Brandt et al. 2012, Aeby et al. 2016). To determine whether differences in mortality among diseased *M. cavernosa* colonies at Looe Key vs. Fort Lauderdale are best explained by temporal (evolutionary) or regional (environmental) processes, we are continuing to monitor tagged colonies at both sites. We hypothesize that if regional differences are key, then *M. cavernosa* colonies will continue to display acute lesions at the Looe Key site. Conversely, if Looe Key colonies shift to subacute lesions, then evolutionary processes may be at work. In addition, if environmental factors differ at these two sites and if disease dynamics are affected by these potential differences, then this should be reflected in the prevalence and types of lesions present.

Our specific objectives are:

1) Compare disease prevalence, species affected and types of lesions present at Looe Key vs. Fort Lauderdale.
2) Compare mortality and lesion morphology among diseased *M. cavernosa* colonies at Looe Key vs. Fort Lauderdale sites through time.

Disease surveys in November 2019

Disease surveys using belt transects were completed at all three sites (FtLT, Tag site, Haslun's reef) in November 2019. The coral communities at all three sites were numerically dominated by *Siderastrea siderea*, and the 2nd most common coral was *M. cavernosa* (Table 1). The Tag Site in the Lower Keys and the Ft. Lauderdale Tag Site both had low coral cover (<3%) and high algal cover (>60%). Haslun's reef in the Lower Keys was in better condition with 28.6% coral cover and 28.6% algal cover (Table 1).

Table 1. Comparison of substrate characteristics, coral community structure, and disease prevalence at three sites surveyed in November 2019. Coral community data based on colony counts within belt transects at each site. Data show the percent of the coral community represented by each coral species. Coral and algal cover (%) based on point-intercept data at 50cm intervals.

Region	Fort Lauderdale	Keys	Keys
site	FtLT	tag site	Haslum's reef
tot area surveyed	60m^2	35m^2	55m^2
coral species			
Agaricia agaricites	3.88	0.00	0.00
Colpophyllia natans	0.00	0.00	0.00
Dichocoenia	0.43	3.85	0.00
Pseudodiploria clivosa	0.00	1.92	0.00
Diploria labyrinthiformis	0.00	0.00	0.46
Pseudodiploria strigosa	0.00	0.00	0.00
Eusmilia fastigiata	0.43	0.00	0.00
Meandrina meandrites	0.00	0.00	0.00
Montastraea cavernosa	**12.93**	**21.15**	**24.20**
Orbicella faveolata	1.72	3.85	0.00
Orbicella annularis	0.00	0.00	0.46
Porites astreoides	9.48	21.15	17.81
Siderastrea siderea	**61.64**	**36.54**	**41.55**
Siderastrea radians	1.72	0.00	0.00
Stephanocoenia intersepta	7.76	11.54	15.53
# corals/m^2	3.87	1.49	3.98
# coral species	9	7	6
coral cover (%)	2	2.9	28.6
algal cover (%)	60	65.7	28.6
overall disease prevalence (%)	1.62	8.97	5.38

We found disease in 5 of the coral species surveyed (*S. siderea, M. cavernosa, O. faveolata, S. intersepta, C. natans)*. Overall disease prevalence (proportion of corals surveyed with disease) was highest in the two Keys sites (9.0% and 5.4%) compared to the site off Ft. Lauderdale (1.6%). Disease prevalence also differed among coral species and regions (Fig. 1). Disease in *S.*

siderea was higher in the Keys sites as compared to the site in the Ft. Lauderdale region. Disease in *M. cavernosa* was high at tag site (Keys) and lower at FtLT (Ft. Lauderdale) and Haslun's reef (Keys). *C. natans* is an uncommon coral species at all three sites but disease was found on one colony at FtLT (Fig. 2). Disease prevalence for *C. natans* could not be calculated as the coral is so rare that no colonies were counted within the belt transects. SCTLD initially passed through the Ft. Lauderdale region years ago and so a newly infected *C. natans* (highly susceptible species to SCTLD) could suggest that the pathogen is still in the environment and that not all colonies that survived the event are resistant to the disease. Whether or not a colony becomes infected would depend on the infectious dose required by the pathogen to initiate infection and the dose that each specific colony received. Hence, healthy colonies on an infected reef could merely indicate lack of adequate exposure to the pathogen. However, the current infection in *C. natans* could be due to a different pathogen. Until we find the identity of the pathogen we cannot be sure of what the observed disease patterns indicate. It must also be noted that a single *O. faveolata* colony was found with lesions at Haslun's site (Keys) and at FtLT (Ft. Lauderdale). However, prevalence could not be calculated at Haslun's site as *O. faveolata* was so rare that it was not found within the belt transects.

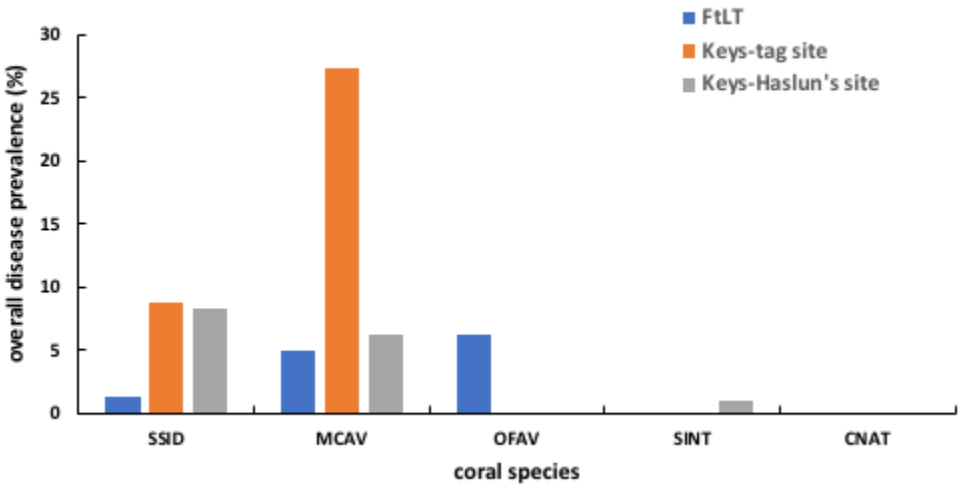


Fig. 1. Disease prevalence by species at three sites surveyed in November 2019. Two sites were located within the Lower Keys (tag site, Haslun's site) and one site at Ft. Lauderdale (FtLT).

Fig. 2. Diseased *C. natans* at FtLT surveyed in November 2019.

Types of lesions

For *S. siderea*, we documented six types of lesions (Table 2; Fig. 3). They were categorized according to type of discoloration (purple, bleached, none) and tissue loss (present or absent). *S. siderea* with purple discoloration (with or without tissue loss) was only found at the Keys sites and *S. siderea* with bleached lesions (with or without tissue loss) was found predominantly in Ft. Lauderdale with only a single colony found at Haslun's reef (Table 3). These lesion categories may not represent different diseases and could be different stages of the same disease, e.g. bleached patches could progress to bleached patches with tissue loss. To help understand the different lesions on *S. siderea* we are following tagged colonies with different types of lesions through time and collecting samples to examine the different lesions at the cellular level.

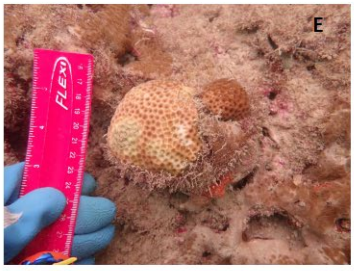
Table 2. Different lesion types found on colonies during surveys in November 2019. Data show the percent of each lesion type out of the total number of colonies recorded.

Coral species	Lesion type	Keys-tag site	Haslun's reef	Ft. Lauderdale FtLT
S. siderea	tot # colonies with lesions	5	23	7
	purple discoloration		21.7	
	purple discoloration + tissue loss	100	65.2	
	tissue loss		8.7	
	bleached patches			28.6
	bleached patches + tissue loss		4.3	57.1
	bleached stripe			14.3
M. cavernosa	tot # colonies with lesions	9	10	6
	bleached patches	33.3	10	100
	tissue loss	66.7	90	
O. faveolata	tot # colonies with lesions	0	2	1
	bleached patches			100
	tissue loss		100	
S. intercepta	tot # colonies with lesions	0	1	0
	dark discoloration		100	
C. natans	tot # colonies with lesions	0	0	1
	tissue loss			100

Lesions in *S. siderea*

Keys

Ft. Lauderdale



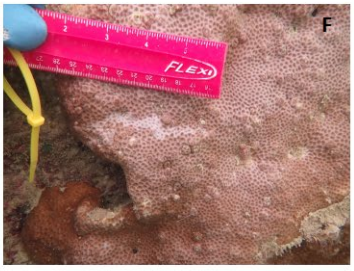


Fig. 3. Different lesion types encountered in *S. siderea* during disease surveys in November 2019. A) purple discoloration; B) purple discoloration with tissue loss; C) chronic tissue loss; D) bleached patches; E) bleached patches with tissue loss; F) bleached stripe

Disease in *M. cavernosa* was categorized as bleached patches or tissue loss (with or without bleached lesions) (Fig. 4). From our prior studies on tagged colonies, we found that the bleached borders along tissue loss lesions can wax and wane and that bleached areas on *M. cavernosa* don't always progress to tissue loss. At the Ft. Lauderdale site, disease prevalence was 5% for *M. cavernosa* and all were bleached patches. In contrast, prevalence was 27.3% at Tag site in the Keys of which 1/3 of the lesions were bleached patches and 2/3 were tissue loss. This is different from the types of lesions we found at this same site in 2018. In 2018 at Tag site, we found 19 diseased *M. cavernosa* and all were tissue loss lesions with no colonies with bleached patches. The other site in the Lower Keys (Haslun's reef) had much lower disease prevalence in *M. cavernosa* (6.2%) with the majority of lesions (90%) tissue loss.

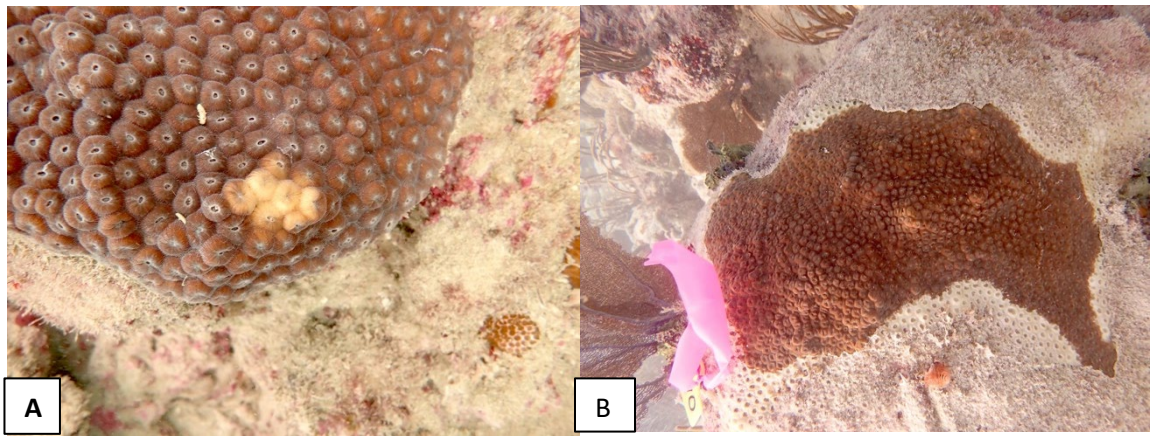


Fig. 4. Two types of lesions found in *M. cavernosa* during disease surveys in November 2019. A.) *M. cavernosa* with a bleached patch, B) *M. cavernosa* with tissue loss lesions bordered by healthy brown polyps.

Obj. 2. Compare mortality and lesion morphology among diseased M. cavernosa colonies at Looe Key vs. Fort Lauderdale sites through time.

Unfortunately, at our site at Looe Key (tag site), another research team treated some of the *M. cavernosa* colonies (n= 7) with antibiotics. This adds an additional variable to interpreting disease processes through time at this site. To counter this problem, we not only tagged additional colonies at our original site but established a 2nd site in the Lower Keys (Haslun's reef) that had *M. cavernosa* with subacute to acute lesions. Haslun's reef is located approximately 1.5 nmiles ENE of Looe Key. We are comparing disease dynamics at all three

sites: two in the Lower Keys and one in Ft. Lauderdale (Table 3). These three sites differ in region (Lower Keys vs. Ft. Lauderdale) and in timing of SCTLD emergence. SCTLD was observed on Ft. Lauderdale reefs by ~2015 whereas reports of SCTLD in the Lower Keys did not occur until 2018.

Table 3. Sites established for monitoring disease dynamics through time.

Date surveyed	Region	site	depth (m)	Latitude (N)	Longitude (E)
11.18.19	Lower Keys	Tag site	7.3	24.54599	81.404
11.19.19	Lower Keys	Haslun's reef	7	24.55234	81.43745
11.22.19	Ft. Lauderdale	FtLT	8.2	26.14858	80.09591

Ft. Lauderdale

At our Ft. Lauderdale site, (FtLT), we have 20 tagged *M. cavernosa* colonies that we have been monitoring since 2017, and as of June 2019, there are 14 that remain alive (case fatality=30%). For the current study, we are continuing to monitor the tagged *M. cavernosa* colonies at this site and all colonies were re-photographed in August and November 2019 and January, April, and July 2020.

Lower Keys

At our original permanent site, Tag site, we had three out of 19 remaining tagged *M. cavernosa* colonies that were still alive. Case fatality rate of tagged colonies between November 2018 and November 2019 was 84.2%. We mapped out and tagged an additional 36 *M. cavernosa* colonies. We photographed all 39 colonies (22 healthy and 17 diseased) and will follow their health state through time. At Haslun's reef, we tagged, mapped and photographed 20 *M. cavernosa* colonies (11 healthy and 9 diseased). All tagged colonies were re-photographed in January, March, May, and July 2020.

Results

Regional comparisons in lesion morphology, disease prevalence and colony mortality

Ft. Lauderdale (disease emerged in 2014-15)

At Ft. Lauderdale, most tagged colonies initially (2017) had subacute tissue loss lesions with a bleached border but by 2019 most lesions were bleached spots or edges (Fig. 5). Disease

prevalence on tagged colonies initially dropped through time, increased in Fall 2018 and then dropped again to lower endemic levels (Fig. 6). Colony survival was high with 65% of the colonies still alive after 3 years (Fig. 7).

Lower Keys- Tag site (Looe Key) (disease emerged in 2018)

At the Tag site, the pattern of change in lesion morphology and survival was similar to what is occurring on colonies at the Ft. Lauderdale site. In 2018, there were 17 tagged diseased *M. cavernosa* colonies with subacute to acute lesions, with or without bleached border. Disease prevalence remained high throughout the year (Fig. 6) and only 16% of the colonies survived after one year (Fig. 7). In contrast, at the same site in 2019-20, disease prevalence declined (Fig. 6), lesions changed to predominantly bleached polyps or subacute tissue loss with bleached border and survival was 78.6% between Nov 2019 and July 2020 (Fig. 5).

Lower Keys – Haslun's reef (disease emerged in 2019)

At Haslun's reef, all lesions were chronic to subacute tissue loss, with or without a bleached border (Fig. 5). Prevalence remained high throughout the study (Fig. 6) and colony survival between Nov 2019 and July 2020 was 12.5 % (Fig. 7).

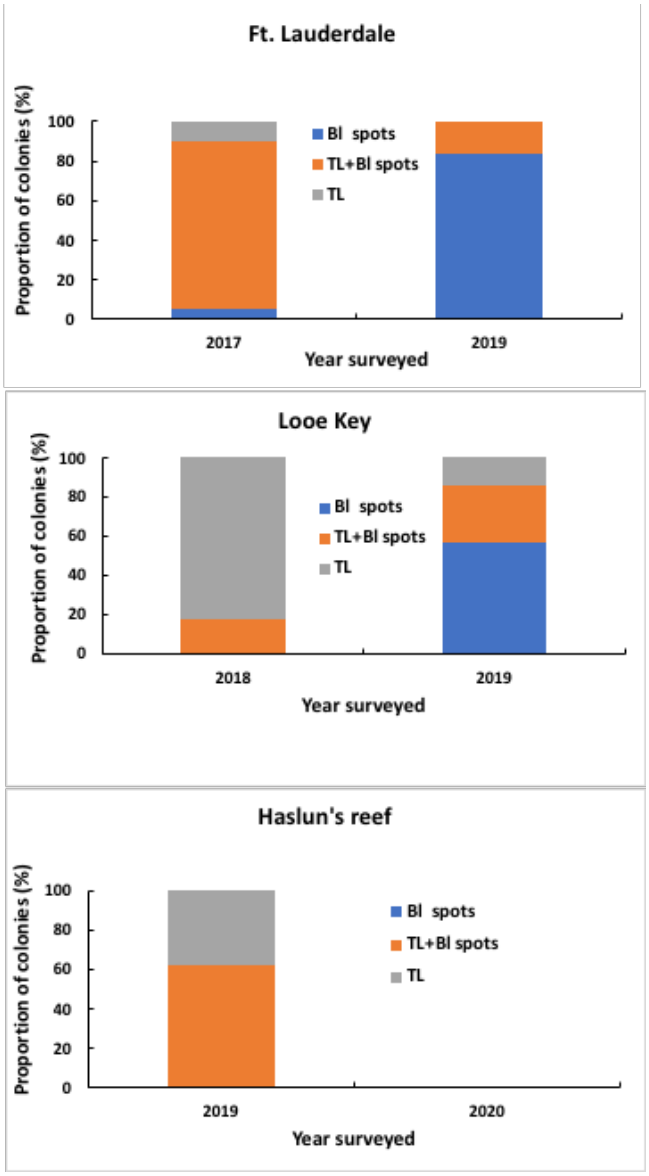


Fig. 5. Differences in lesion morphologies through time at three sites where SCTLD emerged at different times. Estimated times of disease emergence is 2014-15 in Ft. Lauderdale, 2018 at Tag Site (Looe Key) and 2019 at Haslun's reef.

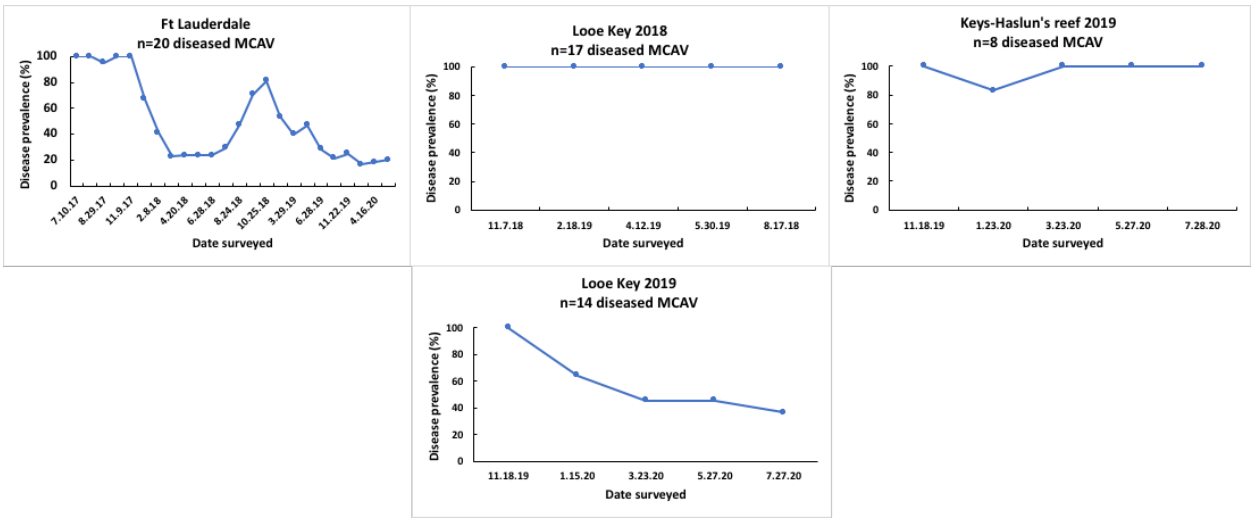


Fig. 6. Changes in disease prevalence through time at three sites where SCTLD emerged at different times. Estimated times of disease emergence is 2014-15 in Ft. Lauderdale, 2018 at Tag Site (Looe Key) and 2019 at Haslun's reef.

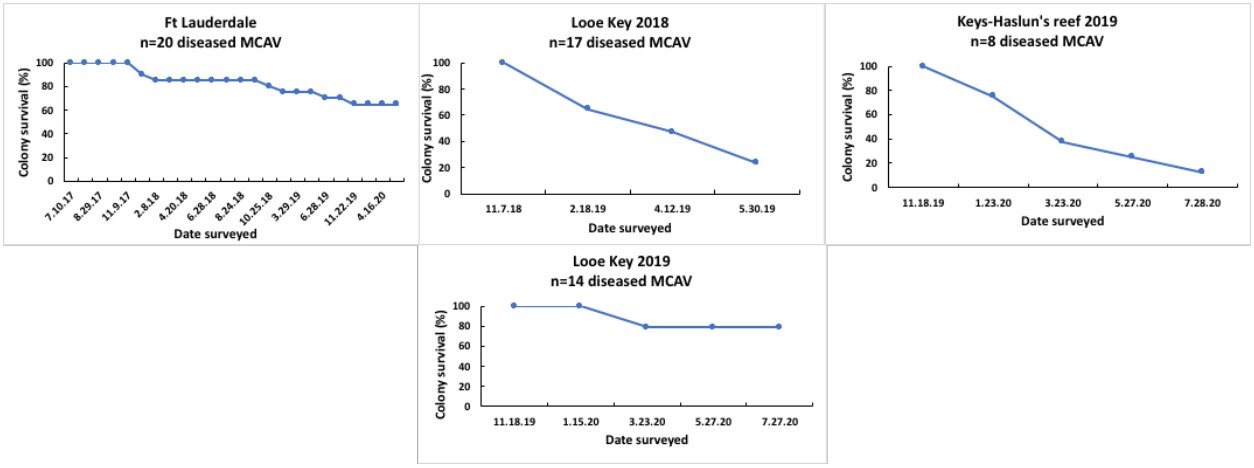


Fig. 7. Differences in colony mortality through time at three sites where SCTLD emerged at different times. Estimated times of disease emergence is 2014-15 in Ft. Lauderdale, 2018 at Tag Site (Looe Key) and 2019 at Haslun's reef.

Disease in S. sidereal

For the current Florida disease outbreak, the disease signs vary among affected coral species with differences in rate of tissue loss, lesion morphology and occurrence. One coral species, *S. siderea*, displays unusual lesions with multi-focal bleached spots, spots of purple discoloration or discolored spots (bleached or purple) with tissue loss. So, the question remains as to whether this species is affected by the same pathogen(s) as the other coral species affected by SCTLD but disease signs vary or if this coral has a different disease altogether. Through manipulative studies, we have shown that *S. siderea* with lesions are transmissible, but lesions do not respond to antibiotics in the same manner as other coral species with SCTLD-type lesions (*Meandrina meandrina, M. cavernosa, Colpophyllia natans, Orbicella faveolata*) that have been tested. This suggests that the etiology of the disease affecting *S. siderea* differs from the other species affected in the SCTLD outbreak. We also noted that during our preliminary transmission studies, there were differences in transmission success dependent upon the lesion morphology. Fragments presenting with bleaching with tissue loss transmitted more readily than fragments having other lesion types. However, our sample sizes were too small to adequately evaluate this. Some coral species respond to any irritation with a pigmentation response and so those lesions do not usually indicate disease. For example, compromised tissues of *Porites* spp. in the Indo-Pacific express red fluorescent proteins which produce a pink lesion whether from disease or other natural processes such as algal abrasion, competition, etc. (Palmer et al. 2009). During coral reef surveys, where you examine the health of the reef, it is important to be able to distinguish between disease and non-disease lesions. To resolve interpretation of different lesions in *S. siderea*, we will examine different lesion types at the cellular level and test for differences in transmission success in aquaria studies.

Our specific objectives are:

3) Follow lesion progression on diseased *S. siderea* with different lesion types
4) Conduct histopathological studies on different lesion types in *S. siderea* to compare them at the cellular level.

***S. siderea* lesions through time**

As part of our objective to examine different lesion types in *S. siderea*, we tagged 8 *S. siderea* (6 healthy and 2 diseased) at our Ft. Lauderdale site in 2018 and are following them through time. Colonies were re-photographed in January, April, and July 2020. In November 2019, at one of our sites in the Keys (Haslun's reef) we tagged an additional 18 *S. siderea* colonies, with different lesions, along our permanent transect and will follow colonies through time. All tagged colonies were re-photographed in January, March, May, and July 2020.

Histopathology of *S. siderea* lesions

Samples from 13 diseased *S. siderea* were collected in the Keys and 3 diseased *S. siderea* from the Ft. Lauderdale site. They were fixed and preserved in 20% Z-fix-seawater (i.e. 10% formalin; equivalent to 4% formaldehyde) and transported to the FWRI-FWC labs where they were processed.

Results

Obj. 3. *S. siderea* lesions through time

Ft. Lauderdale – FtLT

Seven tagged colonies with multi-focal bleached lesions were followed between Nov 2019 and July 2020. During those seven months only one colony lost tissue (-13.2%). One colony showed no change in status and the remaining five colonies showed signs of re-pigmentation and healing (Fig. 8).

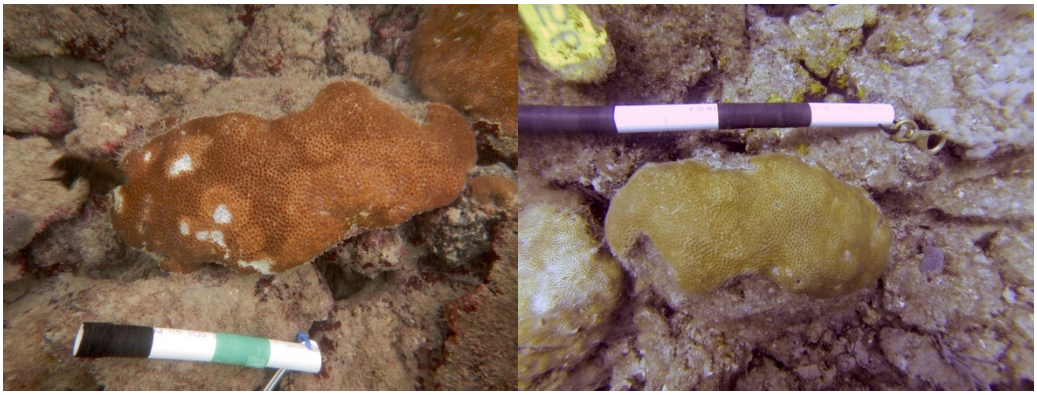


Fig. 8. Example of *S. siderea* healing from multi-focal bleached lesions. Photo on left is from Nov 2019 and photo on the right is from July 2020.

Keys – Haslun's reef

A total of 18 colonies with disease lesions were followed between Nov 2019 and July 2020. Eight colonies (44.4%) presented with purple discoloration with chronic tissue loss, 6 colonies (33.3%) had purple discoloration, 2 colonies had a purple linear discoloration (11.1%) and a single colony each have tissue loss with no discoloration and bleached lesion with tissue loss. Overall, all colonies, except one, showed progressive tissue loss with an average of 68.4% loss (6.6%/month) (Fig. 9). Of the two lesion types with sufficient sample sizes, purple discoloration with tissue loss (n=8) and purple discoloration (n=6), average tissue loss was 9.9% per month and 7.4% per month respectively. Case fatality rate was 11.1%.

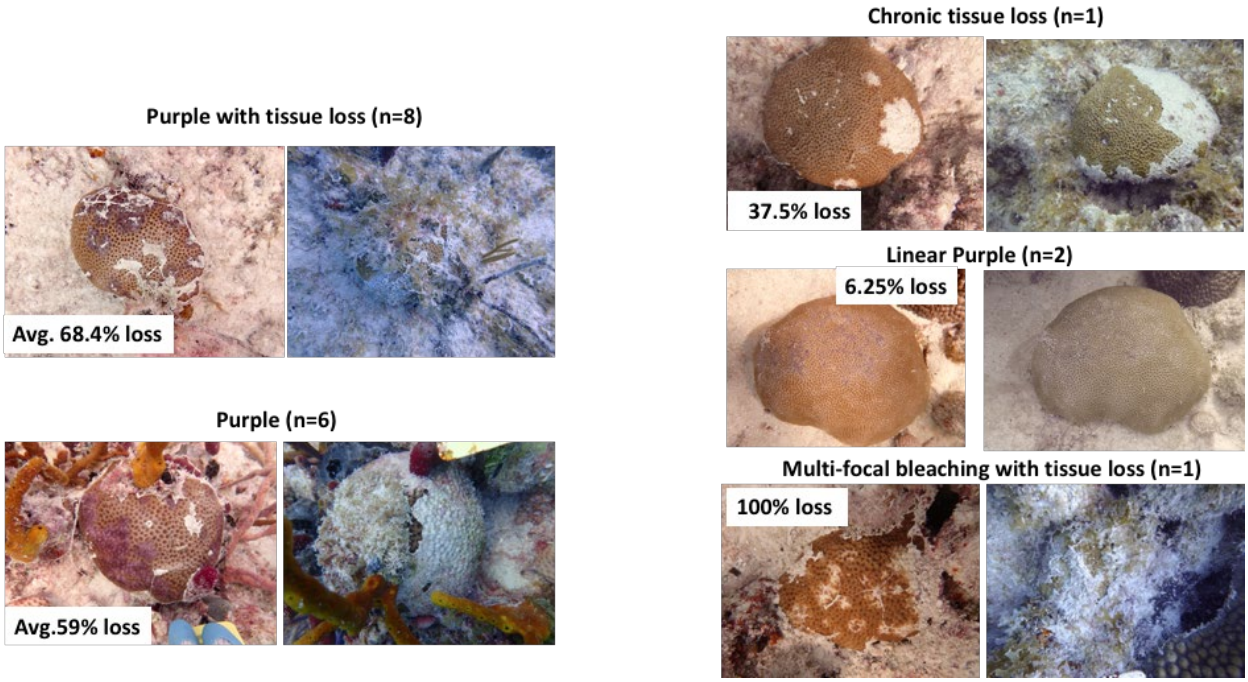


Fig. 9. Disease progression on *S. siderea* with different lesion types. Pictures on left side of each pair are from Nov 2019 and on the right side the same colonies from July 2020. Average tissue loss is total loss after 8 months.

Obj. 4. S. siderea histopathology

A total of 16 *Siderastrea siderea* samples were collected and preserved in 1-part Z-Fix (zinc formalin; Z-Fix concentrate [18.5% formaldehyde; Anatech Ltd. Battle Creek, MI]) mixed with 4 parts 0.2 µm–filtered natural seawater (or 35 salinity artificial seawater) for histology. These 16 samples comprised diseased specimens exhibiting diverse lesions from Florida (8 from Looe Key [LK; 24.54599°, –81.404°], 5 from Haslun's Reef [HR; 24.55234° –81.43745°], and 3 from Ft Lauderdale, [FtLT, 26.148585°, –80.095915°]).

Post-fixed samples were shipped to the FWC/FWRI lab at St. Petersburg, FL for processing with routine paraffin embedded histological specimens. Briefly, each specimen was macroscopically photographed using a digital camera fitted with a macro lens (Nikon, Tokyo). Samples, especially those exhibiting lesions, were further observed using low power magnification with a dissecting microscope attached to an Olympus DP71 digital camera (Tokyo), and photomicrographs were taken.

For ease of orientation and maintaining coral integrity, samples were enrobed with 1.5% agarose to hold tissues (under a heat vacuum oven [60°C] at 22 mm Hg [Jones and Calabresi 2007]) and associated surface biota in place after the skeleton was removed following decalcification processing with 10% ethylenediaminetetraacetic acid ($Na_2 \cdot 2H_2O$; MW = 372.1) solution. Decalcified tissues were organized for sectioning orientation at both radial (cross, parallel to the polyp mouth) and sagittal (longitudinal, perpendicular to the polyp mouth) angles.

Routine paraffin embedded histologic sections were cut at 4 µm, stained with Mayer's hematoxylin and eosin (H&E) and thionin stains (Luna 1968). Tissues were also embedded with glycol methacrylate plastic resin (JB-4; Electron Microscopy Sciences, Hatfield, PA) with arbitrary angle, sectioned at 4.0 µm, and stained with Weigert's iron hematoxylin and eosin (H&E), thionin, and periodic acid–Schiff–metanil yellow (PAS-MY; Quintero-Hunter et al. 1991). Slides were examined with an Olympus BX51 light microscope equipped with an Olympus DP71 digital camera (Olympus Inc., Tokyo).

Siderastrea siderea colonies at the different locations sampled for histology appeared to exhibit either single lesion types or could present with multiple lesion types that were focal or multifocal. Diseased *Siderastrea siderea* colonies at the three locations in Florida were observed in the field to exhibit (1) multifocal bleached spots (bleaching discoloration, BD), (2) spots of purple discoloration (PD) (i.e., varying degrees of dark to light purple discoloration, or combinations of lesion types), (3) bleached spots with tissue loss (BD/TL), or (4) purple discoloration spots with tissue loss (PD/TL) (Fig. 3).

The gross and enlarged gross appearance of the PD and BD lesions on colonies in the field is compared to macroscopic observations of the biopsy samples post-fixed in the lab (Fig. 10, Table 4). The apparent gradation of some lesions in the field that grossly showed PD is visually compared following sample fixation and when macroscopically viewed under artificial light conditions in the lab (Fig. 10). In some cases, PD changed in degree of intensity, faded, or disappeared with time (or under fixed conditions).

PD determined grossly in the field was separated macroscopically by the visual appearance of PD along the septa (septal PD) contrasted against the color of oral lesions. Grossly, oral lesions could appear grey-purple (Fig. 10A, D) or white-beige (Fig. 10B, E), but macroscopically were pink-purple (Fig. 10G) or white beige, respectively (Fig. 10H). The purple discoloration and the varying degrees of intensity were a reflection of the development and extent of the lesion and the expansion of the lesion in individual polyps. Histologically, the oral lesions were seen to have liquefactive necrosis with sloughing of necrotic tissue into the oral cavity (Fig. 11).

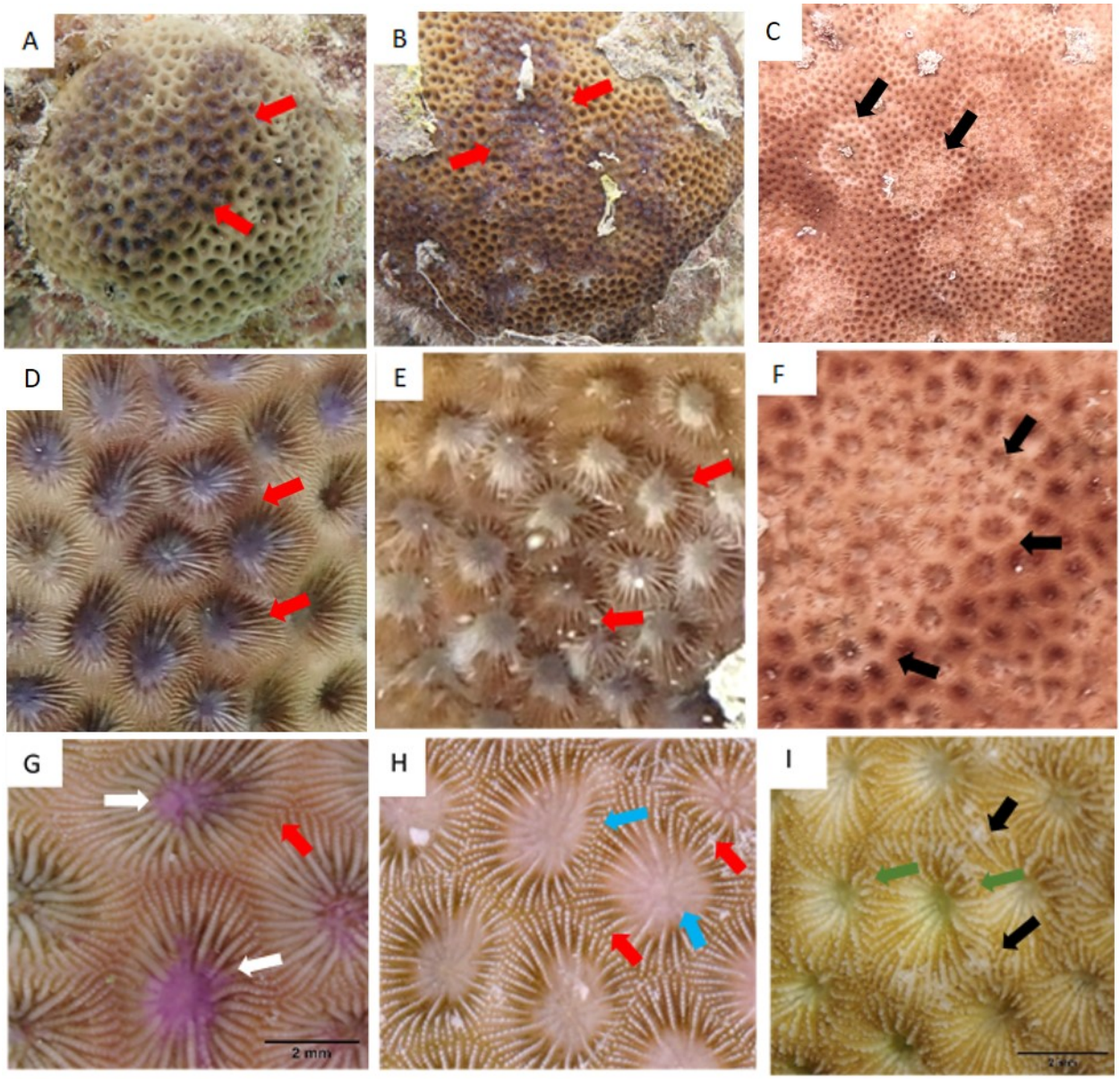


Fig. 10. Gross (A-C) and enlarged gross (D-F) observations of purple discoloration (PD) (A-B, D-E) and bleaching discoloration (BD) (C, F) in colonies compared to macroscopic views (G-I) of post fixed samples showing dark coloration of septal ridges (red arrows) contrasted against grey-purple (white arrows; D, G) or white-beige oral lesions (blue arrows; E, H), or gross bleaching on septal ridges (black arrows) contrasted against white-green endolith oral lesions (green arrows; F, I). A, D, G = specimen #7 from LK; B, E, H specimen # 1 from LK, and C, F, I, specimen #16 from FtLT.

Table 4. Comparison of gross field descriptions of lesion types with macroscopic observations of core samples post fix.

Site	N	Gross lesion			Macro/microscopic lesion post fix				
		BD	PD	TL	Septal BD	Oral lesion (PD)	Septal PD	Oral lesion (no PD)	TL
LK	8	0	5	8	2	2	6	4	8
HR	5	0	2	5	1	2	3	1	5
FtLT	3	2	2	1	2	1	2	2	3

BD = bleaching discoloration, PD = purple discoloration, TL = tissue loss
LK = Looe Key, HR = Haslun's Reef, FtLT = Fort Lauderdale

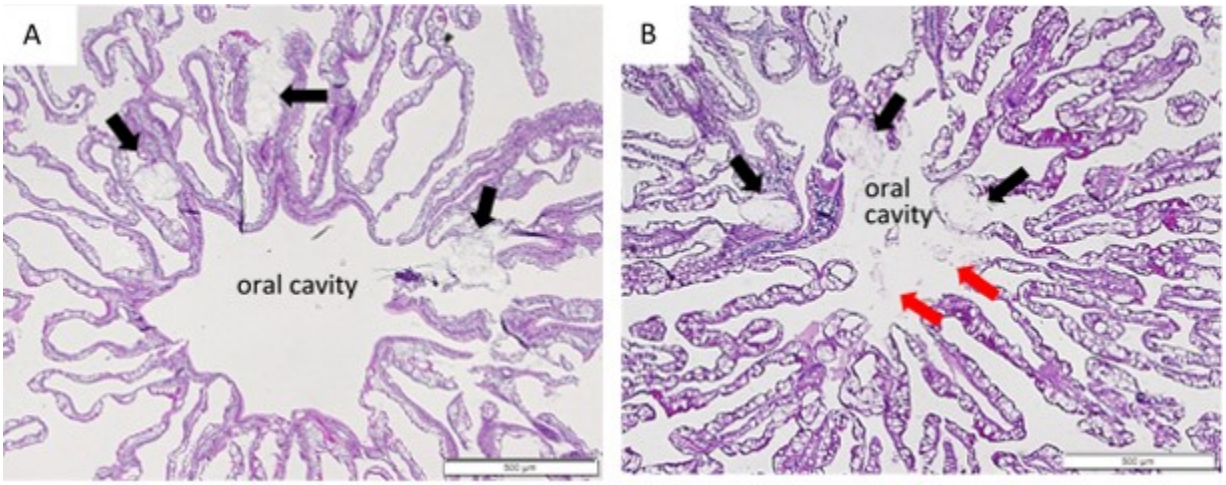


Fig. 11. Radial histologic sections (H&E) across the oral cavity of specimen #8 (A) from LK compared to specimen #13 (B) from FtLT showing similar liquefactive necrosis lesions (black arrows) and sloughing of necrotic tissue into the oral cavity (red arrows).

Although initially observed only in two specimens at FtLT, BD was also observed macroscopically in two specimens from LK and 1 specimen from HR. There was no apparent difference between bleaching at the sites, rather BD could be a progressive stage in the development of oral lesions (Fig. 11).or bleaching along the septal ridge (Figs. 10 C, F, I; 12, 13), both of which can lead to TL (Figs. 11-13). Bleaching (loss of zooxanthellae) was histologically confirmed at all three sites (62.5–80%; Figs. 12, 13).

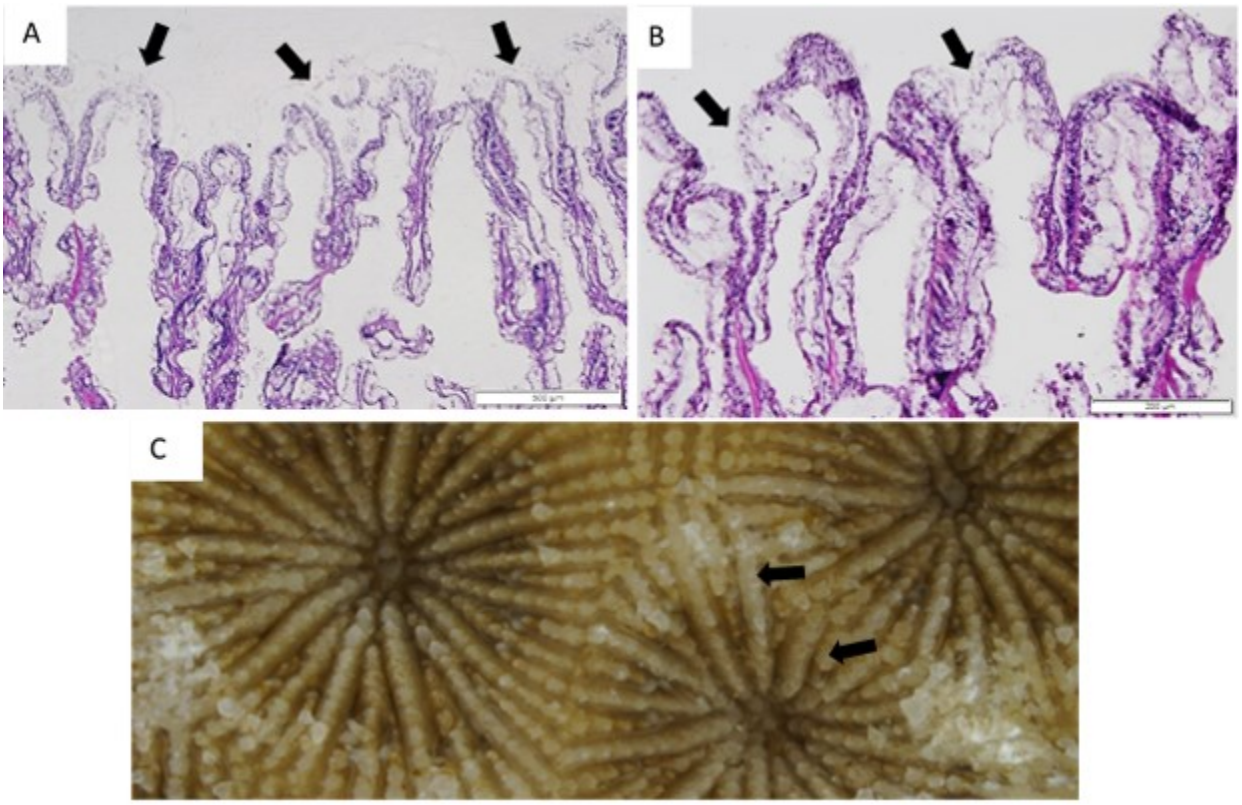


Fig. 12. Sagittal histologic sections (H&E) along the septa of specimen #8 (A) from LK compared to specimen #15 (B) from FtLT showing surface bleaching and early TL lesions compared to the macroscopic appearance of the corallite septa with bleaching and TL in specimen #16 from FtLT (C, black arrows).

Additional histological observations were noted for descriptive purposes (Table 5). It is unknown from this preliminary investigation to what extent any of these were significant in the appearance or response to lesions. All samples examined histologically exhibited TL, with early lesions

showing liquefactive necrosis (LN) (n =13) characteristic of SCTLD found at the gastrodermis at both basal and surface body wall throughout three sites (Fig. 11; 62.5–100%). However coagulative necrosis (Fig. 14A; N = 10) in advanced TL was commonly found at the grossly observable lesion border, especially at LK (87.5%) with less prevalence at HR (40%) and FtLT (33.3%). This supports the progression of disease observed in field that LK site was sub-acute compared to HR and FtLT sites were more likely acute.

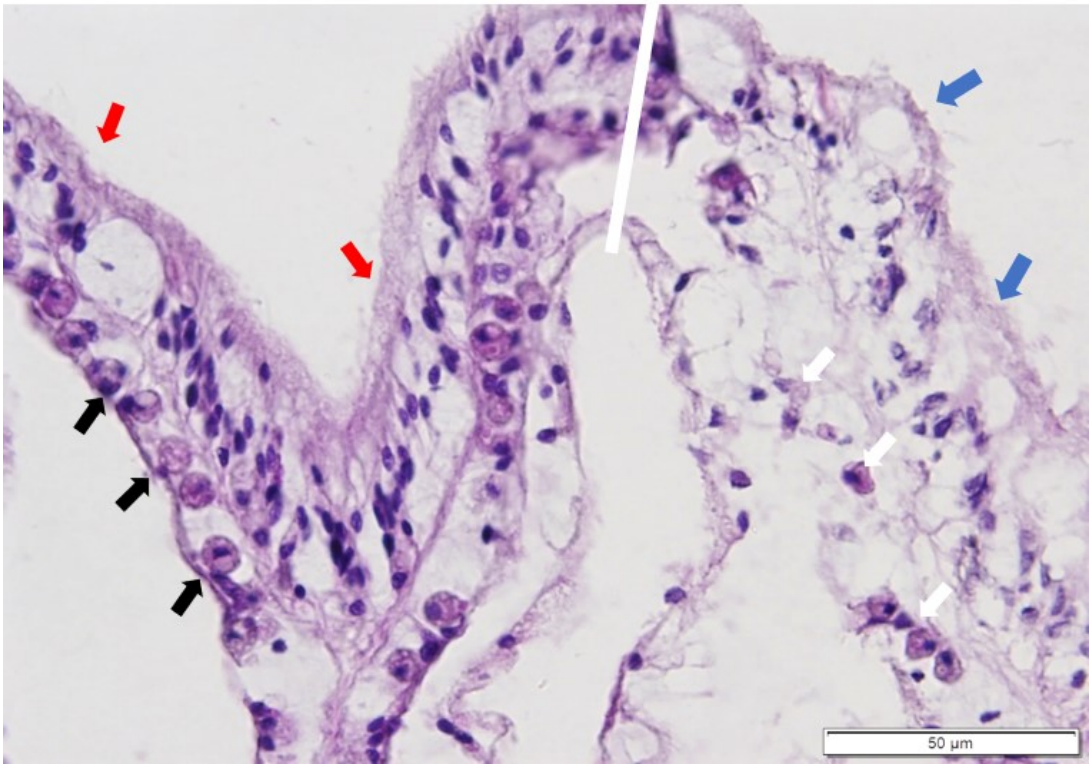


Fig. 13. Sagittal histologic section (H&E) along the septa of *S. siderea* specimen #16 from FtLT showing border (white line) between surface bleaching (blue arrows) with reduced density of, loss of, or *in situ* necrosis of zooxanthellae with abnormal appearance (white arrows), and apparently healthy tissue (red arrows) with zooxanthellae (black arrows). Note abnormal appearance of remnant zooxanthellae (white arrows) and general loss of cytoplasm or increased mucus (white space) in lesioned area.

Sponges, some of which were degraded (along with spicules and sometimes associated with eosinophilic granulocytes) were detected in the skeletal tissue especially at the aboral region, and found at LK (37.5%) and HR (80%). Only one sample (#11, HR) had a clionid (a boring sponge with zooxanthellae) near the septal surface and in the basal area of the skeletal tissue. Coral-acid rich protein (CARP) granules in the calicodermis were generally present in all specimens examined (characteristically present in *S. siderea*), and multifocally, prominently aggregated

CARPs (Fig. 14B) were found in all the sites (60–66.6%). Possibly, this aggregation reaction is a host response to some of the endolithic organisms.

Zooxanthellae with intracytoplasmic PAS-positive materials (starch) were seen at the LK site (Fig. 14D; 62.5%) but were not found at HR (0%) and only one at FtLT (Fig. 14C; 33.3%). However, this may be due to low sample size at the latter site. Note that the starch collar around the pyrenoid was stained PAS-positive in zooxanthellae at all three sites (Fig. 14C, D). Possibly the difference in starch storage reflects the nutritional health status of the zooxanthellae. Histologically, mucus was abundant (and stained brown to thick purple with thionin, not shown) on the oral side compared to the aboral side. Presumptively, the brown color staining with thionin may be indicative of degraded mucus along with debris in the dead tissues.

Table 5. Comparison of histologic features at three locations. Examples of LN are shown in Fig. 11, BD in Figs. 12, 13 and the other features in Fig. 14. CARPs = coral acid-rich proteins, zx = zooxanthellae

Site	Liquefactive necrosis (LN) N (%)	Coagulative necrosis (CN) N (%)	CARPs aggregates N (%)	Zx with PAS + cytoplasm (starch granules) N (%)	BD N (%)
LK	5/8 (62.5)	7/8 (87.5)	5/8 (62.5)	5/8 (62.5)	5/8 (62.5)
HR	5/5 (100)	2/5 (40)	3/5 (60)	0/5 (0)	4/5 (80)
FtLT	3/3 (100)	1/3 (33.3)	2/3 (66.6)	1/3 (33.3)	2/3 (66.6)

LK = Looe Key, HR = Haslun's Reef, FtLT = Fort Lauderdale

In conclusion, PD and BD was associated with progressive TL in *S. siderea*. Field observations and histopathological results generally matched well. However, it was not possible to determine the cause of the PD. PD was noted to be lost over time during histological processing and was retained in formalin fixative and EDTA solution but was dissolved in alcohol and solvent. This may possibly suggest that PD is associated with hydrophobic chemicals. Grossly, purple

pigments appeared to adhere to the oral disc area and they were apparently located deeper in the polyp more towards the aboral tissue.

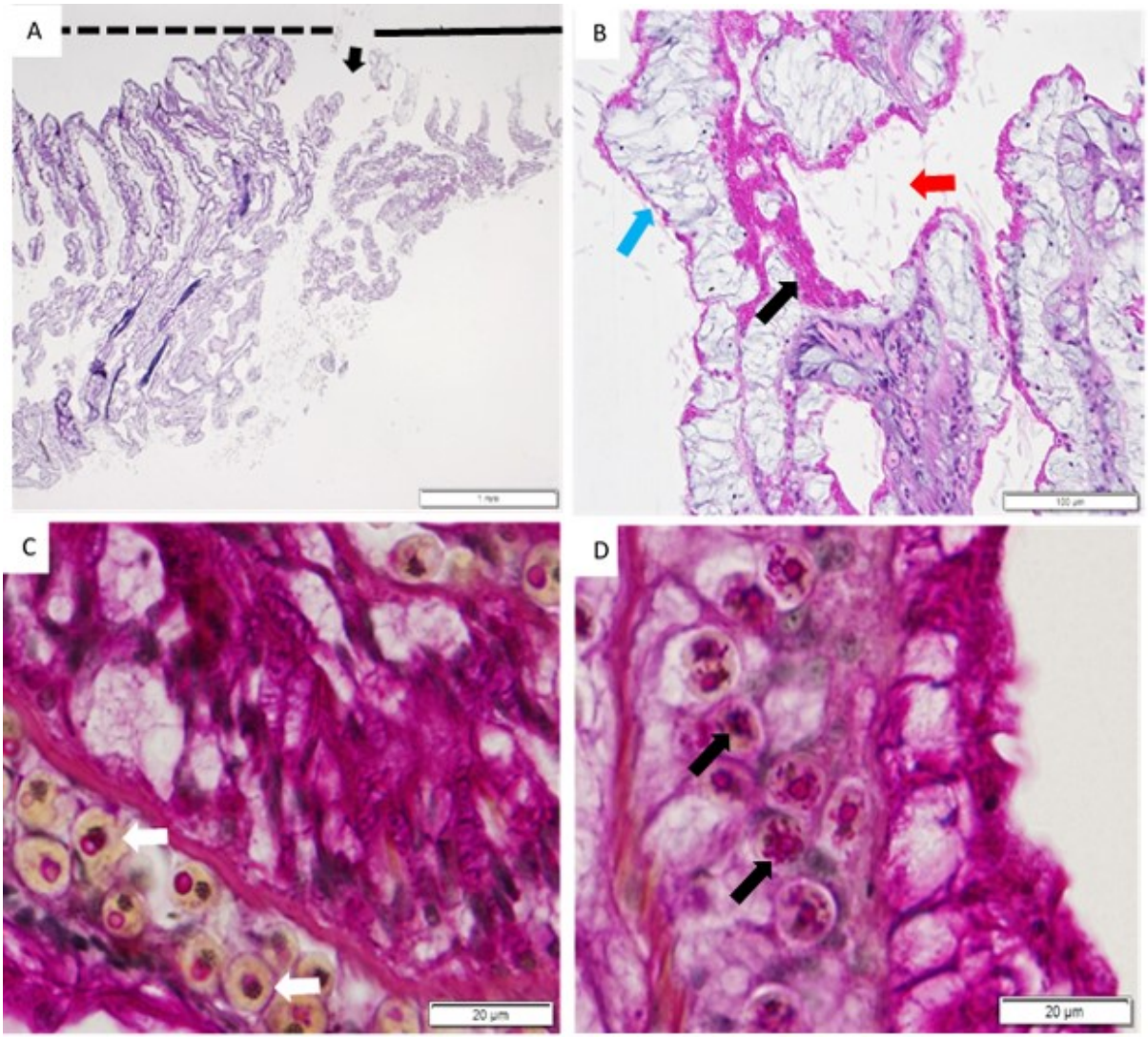


Fig. 14. Histologic sections of *S. siderea* (A-B, H&E; C-D, PAS) showing various histopathological features. A. Sagittal section showing TL border (black arrow) with area of coagulative necrosis (solid line) and apparently healthy tissue (dotted line) (#2, LK). B. Deeper mesenterial filament area showing prominent coral-acid rich protein (CARP) granules (black arrow) in the calicodermal layer, possibly reacting to nearby endoliths (red arrow) in the skeleton. Compare with apparent (subjective) lower density of CARPs (blue arrow) on the other side of the mesentery with less endoliths (#2, LK). C. Deeper gastrodermal layer showing zooxanthellae with clear cytoplasm (brown yellow color, white arrows) (#15, FtLT). D. Deeper gastrodermal layer showing zooxanthellae filled with PAS-positive (pink-red) starch granules (black arrows) (#1, LK).

Summary

Obj. 1. Compare disease prevalence, species affected and types of lesions present at Looe Key vs. Fort Lauderdale.

- Disease surveys at the Lower Keys sites (Tag site, Haslun's reef) and Ft. Lauderdale (FtLT) were conducted in November 2019
- Coral communities at all three sites were numerically dominated by *Siderastrea sidera* and *Montastraea cavernosa*
- The Tag Site in the Keys and the Ft. Lauderdale site both had low coral cover (<3%) and high algal cover (>60%).
- Haslum's reef in the Keys was in better condition with 28.6% coral cover and 28.6% algal cover.
- Overall disease prevalence was 1.62% at the Ft. Lauderdale site, and at sites in the Lower Keys it was 9% and 5.4% at Tag site and Haslun's reef, respectively.
- Overall disease prevalence in *S. siderea* was higher at the Keys sites (8.8%, 8.3%) compared to the Ft. Lauderdale site (1.2%)
- Overall disease prevalence in *M. cavernosa* was higher at the Tag site in the Keys (27.3%) compared to Haslun's reef in the Keys (6.2%) or Ft. Lauderdale site (5%)

Obj. 2. Compare mortality and lesion morphology among diseased *M. cavernosa* colonies at Looe Key vs. Fort Lauderdale sites through time.

- 29 prior tagged *M. cavernosa* colonies are being followed through time at FtLT in Ft. Lauderdale
- 3 originally tagged *M. cavernosa* remain alive at our original site in Looe Key (Tag site)
- 36 additional *M. cavernosa* colonies were mapped and tagged at Tag site
- A total of 39 tagged colonies at Tag site have been followed through time
- A new site in the Lower Keys was set up (Haslun's reef) and 20 *M. cavernosa* colonies were mapped and tagged
- Colonies at sites in both regions (Lower Keys & Ft. Lauderdale) were re-photographed in January, March, May and July, 2020

Regional comparisons in lesion morphology, disease prevalence and colony mortality

Ft. Lauderdale (disease emerged in 2014-15)

- Tagged *M. cavernosa* colonies initially (2017) had subacute tissue loss lesions with a bleached border but by 2019 most lesions were bleached spots or edges
- Disease prevalence on tagged colonies dropped through time and remain at lower endemic levels although there were some fluctuations
- Colony survival was high with 65% of the colonies alive after 3 years
- Of the 22 healthy colonies at the start of the study, 6 became infected, all with bleached spots. Disease incidence=4.5%.

Lower Keys- Tag site (Looe Key) (disease emerged in 2018)

- In 2018, tagged *M. cavernosa* colonies had subacute to acute lesions, with or without bleached border, but by 2019 lesions changed to predominantly bleached polyps or subacute tissue loss with bleached border
- Disease prevalence remained 100% throughout the first year (2018-19) but declined to 36.4% on the reef during the second year (2019-20). Note that only 3 colonies from the first year survived so change in prevalence not directly comparable to Ft. Lauderdale colonies.
- Only 16% of the colonies survived after the first year (2018-19) but survival was 78.6% during the 2nd year (Nov 2019 -July 2020).
- Of the 24 healthy colonies at the start of the study, 9 became infected, 7 with bleached spots and 2 with tissue loss. Disease incidence=37.5%.

Lower Keys – Haslun's reef (disease emerged in 2019)

- All tagged *M. cavernosa* colonies had chronic to subacute tissue loss lesions, with or without a bleached border.
- Prevalence remained high (80-100%) throughout the study.
- Colony survival between Nov 2019 and July 2020 was 12.5%.
- Of the 12 healthy colonies at the start of the study, 6 became infected, 5 with tissue loss lesions bordered by bleaching and 1 as a bleach spot. Disease incidence=50%.

Obj. 3. *S. siderea* lesions through time

Ft. Lauderdale – FtLT

- *S. siderea* colonies presented with multi-focal bleached lesions
- Between Nov 2019 and July 2020 there was 100% survival and 6 out of 7 colonies showed signs of re-pigmentation and healing
- The lesion of one colony progressed onto tissue loss

Keys – Haslun's reef

- *S. siderea* colonies presented with 5 types of lesions

- 8 colonies (44.4%) presented with purple discoloration with chronic tissue loss, 6 colonies (33.3%) had purple discoloration, 2 colonies had a purple linear discoloration (11.1%) and a single colony, each, had tissue loss with no discoloration and bleached lesions with tissue loss.
- All colonies, except one, showed progressive tissue loss with an average of 68.4% loss after 8 months (6.6%/month)
- Case fatality rate was 11.1%
- Rate of tissue loss was 9.9% per month for colonies with lesions showing purple discoloration (n=8)
- Rate of tissue loss was 7.4% for colonies with lesions showing purple discoloration (n=6)

Obj. 4. Conduct histopathological studies on different lesion types in *S. siderea* to compare them at the cellular level.

- 13 diseased *S. siderea* from the Keys and 3 diseased *S. siderea* from Ft. Lauderdale were collected and sent to FWRI-FWC histology labs for processing
- 8 *S. siderea* colonies are being followed through time at our Ft. Lauderdale site
- In the Keys (Haslum's reef) we tagged an additional 18 *S. siderea* colonies with lesions along our permanent transect and will follow colonies through time.

***S. siderea* histopathology**

- Oral lesions typically seen first with SCTLD in *S. siderea*
- Oral lesions radiate out on the septa to the outer edge of the corallite
- Oral lesions are grossly grey-purple (pink-purple macroscopically) or white-beige (in field/macroscopically)
- Histologically oral lesions manifest as liquefactive necrosis (LN) of basal body wall septa with sloughing of necrotic tissue into the oral cavity
- All samples (n = 16) had tissue loss (confirmed macroscopically/histologically with LN [n =13] and/or coagulative necrosis [n =10])
- Purple discoloration (PD) determined in the field was separated macroscopically by septal PD (contrast with color of oral lesion) and oral lesions (with or without PD)
- Bleaching can be associated with early oral lesions and on the septa
- Oral lesions (PD/no PD) and BD can lead to tissue loss (SCTLD)
- BD observed grossly at FtLT (n = 2), and detected histologically in LK (N = 2) and HR (n =1).
- PD observed grossly on septal ridges, contrasted against grey-purple or white-beige oral lesions
- PD cannot be characterized histologically and is lost over time during processing

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