Initial assessments of a potentially novel coral disease affecting key reef building corals on Florida's Coral Reef

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Final Report

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Executive Summary (max 1 page)

A potential new coral disease, currently coined Fast Lesion Progression (FLP), has been observed affecting coral reefs across the Florida Keys. FLP manifests as large, quickly spreading lesions on *Orbicella faveolata* colonies, often accompanied by sloughy tissue at the lesion line. To investigate this potentially novel disease, we tagged and monitored 51 corals across three Florida Keys reef sites. We reexamined photos from 234 of the first *O. faveolata* colonies treated at six sites and found that 20.5% of presumed SCTLD-affected *O. faveolata* were instead FLP-style lesions, with offshore Upper Keys reefs having a higher percentage of historic FLP-affected colonies than other sites. 3% of colonies were affected with both SCTLD and FLP concurrently. We assessed photos of 40 amoxicillin-treated lesions from 2019-2020 and 2022-2023 and found treatment efficacy to be 45%, and an additional 23 from 2022-23 for which efficacy was 17.4%. Finally, we collected 220 samples, including TEM, histology, and microbiome samples, from FLP-affected corals as well as unaffected control corals for further analyses and comparison to previously studied coral diseases by collaborators.

FLP has been observed to be a relatively prevalent and highly virulent threat to *O. faveolata* colonies, which are a vital reef building species. We recommend the prioritization and funding of continued work on this topic to determine other aspects of the disease, such as seasonality, as well as potential treatments to stop the progression of lesions.

Acknowledgements

We thank Florida Department of Environmental Protection for funding this work. All work was conducted under Florida Keys National Marine Sanctuary permit FKNMS-2023-141-A1.

We would like to thank and acknowledge Sami Miller, Michelle Dobler, Hailey Vaughan, and Katy Toth who aided significantly in both field monitoring and data analyses.

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List of Acronyms

SCTLD: Stony coral tissue loss disease
FLP: Fast Lesion Progression
KLDR: Key Largo Dry Rocks
BTT: Blown through tissue
TEM: Transmission electron microscopy
FWC-FWRI: Florida Fish and Wildlife Conservation Commission- Fish and Wildlife Research Institute
EDTA: Ethylenediaminetetraacetic acid
UNCW: University of North Carolina Wilmington
sRNA: small RNA

1. Description

1.1 Fast Lesion Progression (FLP)

In 2022, Nova Southeastern University disease intervention team divers in the Florida Keys began noticing presumed Stony Coral Tissue Loss Disease, or SCTLD, lesions that did not respond to antibiotic treatments. These lesions manifested on *Orbicella faveolata* colonies and presented as both focal and multifocal. The lesions were typically linear, were observed progressing horizontally, vertically, or diagonally across colonies, and were sometimes accompanied by sloughy tissue along the lesion line (Figure 1). Alarmingly, these lesions progressed more rapidly than typical SCTLD lesions on *O. faveolata* colonies.

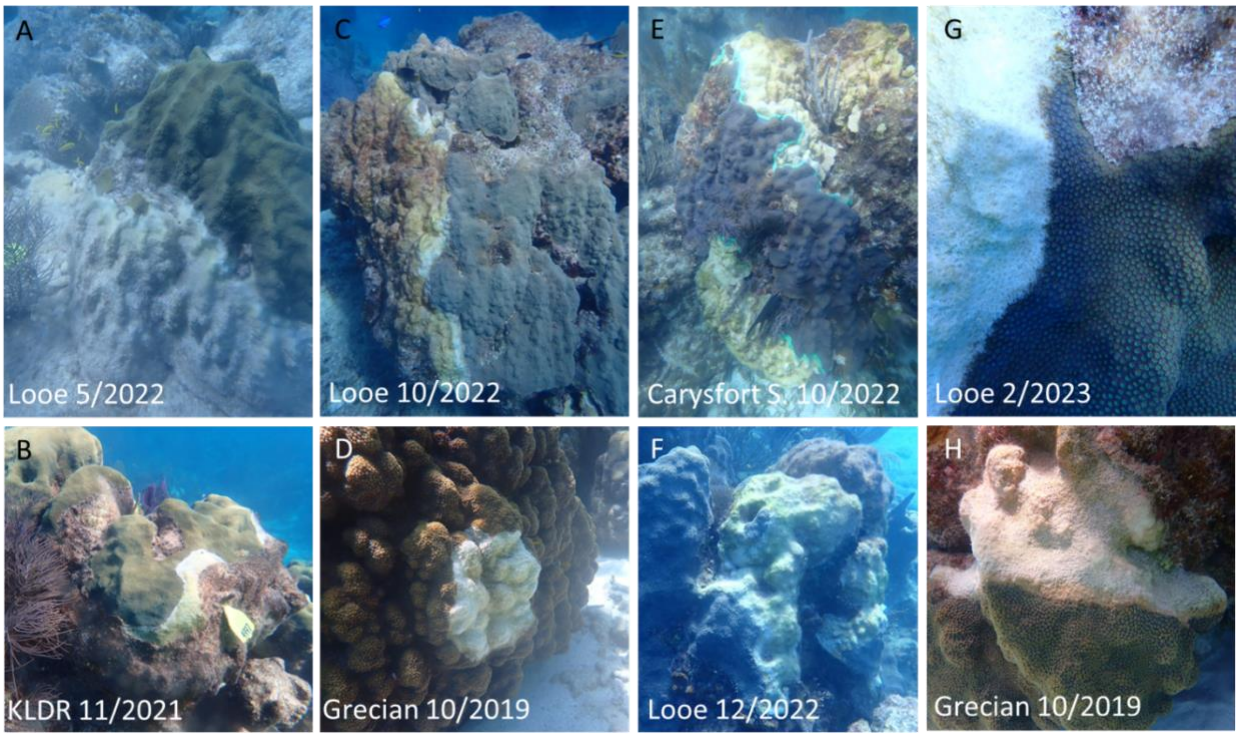

Figure 1: Appearance of Fast Lesion Progression (FLP) on *Orbicella faveolata* colonies. A&B: rapid lesion progression upwards, similar to white plague. C: Progression of lesion sideways across colony. D: Lesion radiating from a midpoint of the colony. E&F: Lesions progressing downwards from the top of the colony and, in image E, an example of multi-focal lesions (which have been treated with a green topical paste). G: Close-up of lesion. H: Close-up of lesion showing loose brown film seen on some lesions, particularly during times of calm water.

While these lesions appeared similar to other white coral diseases such as SCTLD and white plague, there were key differences. These lesions progressed more rapidly than SCTLD is typically seen on *O. faveolata* colonies in the Florida Keys, leaving behind larger margins of bare skeleton. Additionally, the lesions were only observed on *O. faveolata* colonies, unlike SCTLD which is typically observed on numerous susceptible species at an affected site. Unlike white plague, these lesions did not always progress from the bottom of the colony upwards and were sometimes multifocal. These differences indicated that these lesions could possibly be

caused by a previously undiscovered coral disease, or a variation of a previously studied disease. Our team began to further investigate these lesions, and tentatively called the disease causing them Fast Lesion Progression, or FLP. We acknowledge that this is not a formally named disease, but it was necessary to come up with a temporary naming convention while completing initial assessments in the field.

1.2 Past Work

Observations of FLP were first described in Neely (2023). Preliminary investigations included a small number of assessments of FLP lesion antibiotic response, both in water and through past monitoring photos. Seven FLP lesions were fate-tracked in water for two months after being treated with the standard amoxicillin and Base2b topical paste. Five lesions continued past the treatment, later halting on their own. The remaining two lesions continued to be active, with one completely killing off an isolate of coral tissue, and one remaining active at the end of the two month period. Additionally, past monitoring photos of 23 corals from five different reefs were reassessed to determine amoxicillin treatment efficacy on FLP lesions. Of the 23 lesions reassessed, the amoxicillin treatment was only effective on three.

FLP lesions were tested for the presence of *Vibrio coralliilyticus*, a pathogen that is typically associated with coral diseases. We tested five FLP and five SCTLD lesions for presence of this pathogen using the testing kits developed in Ushijima et al. (2020). All five FLP lesions tested negative for *V. coralliilyticus*, while four of the five SCTLD lesions tested negative for the pathogen (Figure 2). The remaining lesion had an irregular result. Despite the irregular result, there was no indication that *V. coralliilyticus* was associated with FLP, and there were no differences in presence of the pathogen between FLP and SCTLD lesions.

Figure 2: FLP lesions, left, and SCTLD lesions, right, were tested for the presence of *Vibrio coralliilyticus*.

2. Methods

2.1 Task 1: Monitoring FLP-affected colonies through time

To investigate FLP lesions in-water, FLP-affected corals were monitored at three sites: the paired Carysfort South and Carysfort Main reefs, the Grecian/Key Largo Dry Rocks paired reefs, and Looe Key (Figure 3).

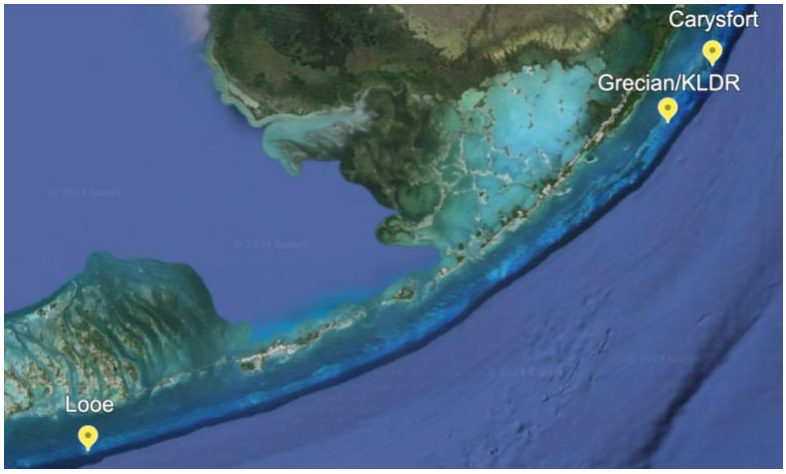

Figure 3: Map of FLP monitoring sites in the Florida Keys.

During these monitoring visits, *O. faveolata* corals with presumed FLP lesions were handled as follows:

- For FLP corals not previously in disease intervention strike team database:
  - Place N-tag at base of colony.
  - Record tag number, linear length, width, height, percent live cover, percent recent mortality, and location.
  - Place sets of two nails each, 10 centimeters apart from each other, along the active lesion line(s) (Figure 4). If a lesion line is longer than 50 centimeters, place multiple nail sets, each 20 centimeters apart (Figure 5).
  - Record lesion data including: lesion letter, lesion location, lesion bearing, and number of nail sets placed.
  - Take photos of the full coral colony with measuring device present in at least one. Take photos of all lesions, before and after nail placement.

- For FLP corals already in disease intervention strike team database:
  - Place orange tag adjacent to existing tag.
  - Record tag number, linear length, width, height, percent live cover, and percent recent mortality.
  - Place sets of two nails each, 10 centimeters apart from each other, along the active lesion line(s). If a lesion line is longer than 50 centimeters, place multiple nail sets, each 20 centimeters apart.
  - Record lesion data including: lesion letter, lesion location, lesion bearing, and number of nail sets placed.

- Take photos of the full coral colony with measuring device present in at least one. Take photos of all lesions, before and after nail placement.

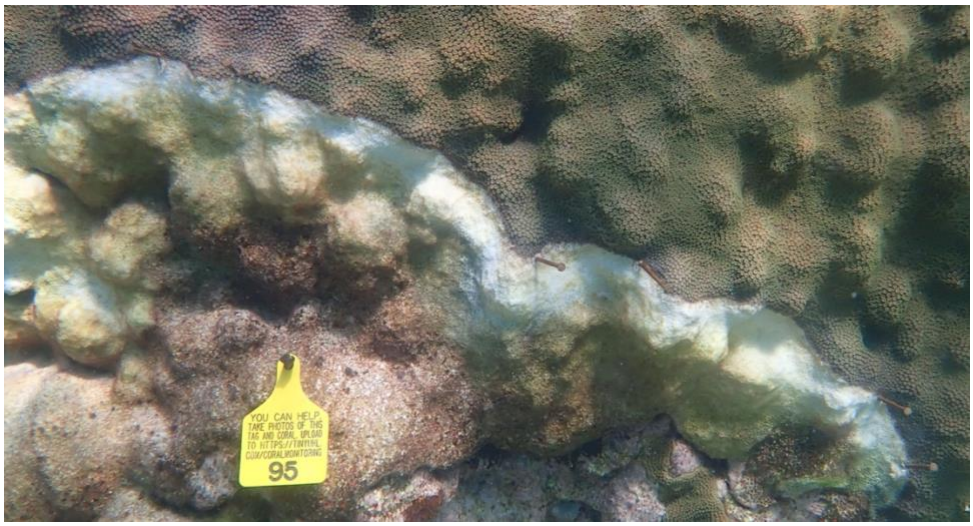

Figure 4: FLP lesion with three sets of nails placed along the lesion line.

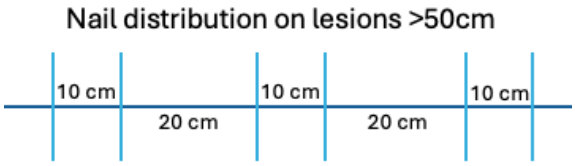

Figure 5: Schematic describing the correct space distribution of nail sets on FLP lesions longer than 50 centimeters.

The three sites were re-visited at approximately two month intervals. During these monitoring visits, any new FLP-affected corals were handled using the protocol above, and all previously established FLP corals were assessed as follows:

- Record coral data including: tag number, percent live cover, and percent recent mortality.
- Measure lesion growth of previously nailed lesions using measurement tool (Figure 6).
- Record status of each lesion: Active, no active disease (NAD), blown through remaining tissue in path (BTT), or halted on own (HALT).
- Remove nails and replace at new lesion line if lesion is active, tag any new lesions, and record lesion-level data.
- Take photos of the full coral colony with measuring device present in at least one. Take photos of all lesions, before and after nail placement.

Figure 6: Lesion growth being measured from nail sets placed at previous monitoring visit to current lesion line.

Monitoring began under an amendment to Sanctuary permit FKNMS-2020-077.We began sampling FLP-affected corals under Sanctuary permit FKNMS-2023-141-A1 which was issued January 26, 2024.

2.2 Task 2: Assessing past FLP presence and amoxicillin effectiveness

We used imagery from previous disease intervention monitoring events to assess whether FLP had been present since 2019, as well as to assess past efficacy of amoxicillin treatments on FLP lesions. Photos of the first 40 *O. faveolata* colonies treated for assumed SCTLD lesions by any treatment method at six monitoring sites were reexamined to determine if corals have been affected with FLP in years prior. The sites included four offshore sites, Carysfort, Molasses, Sombrero, and Looe, and two inshore sites, Cheeca Rocks and Marker 48 (MK48) (Figure 7).

Figure 7: Map of monitoring sites from which past monitoring photos were examined for

presence of FLP and efficacy of amoxicillin treatments on presumed FLP lesions. Offshore sites are indicated by a yellow marker, and inshore sites are indicated by red markers.

For each of the first 40 *O. faveolata* colonies treated at each site, the lesion photographs from the first visitation were visually examined, and the coral was identified as SCTLD-affected, FLP-affected, or both, if lesions caused by both diseases were present. Additionally, monitoring photos of *O. faveolata* FLP-style lesions treated with a standard treatment mixture of amoxicillin and Base2b topical paste at each of the six sites were re-assessed for treatment efficacy based on photos from the subsequent monitoring event. Monitoring photos from 2019-2020 and from 2022-2023 were reassessed to determine whether efficacy had changed with time, with an original goal of 50 lesions reassessed from both time periods. Amoxicillin treatment efficacy was determined by comparing the initial monitoring photos with the subsequent monitoring follow-up photos, taken no more than three months after the initial monitoring. Effective treatment was defined as the lesion halting at the treatment line, while ineffective treatment was defined as the lesion continuing past the treatment line. The proportion of FLP lesion treatments that were affected across sites and time periods were compared using X^2 tests, followed by post-hoc Fisher Exact tests.

2.3 Task 3: Sampling of FLP-affected colonies for histology, TEM, and microbiome

Samples were taken from *O. faveolata* colonies at three monitoring sites to be sent to partner facilities for histology, TEM, and microbiome analyses (Figure 8).

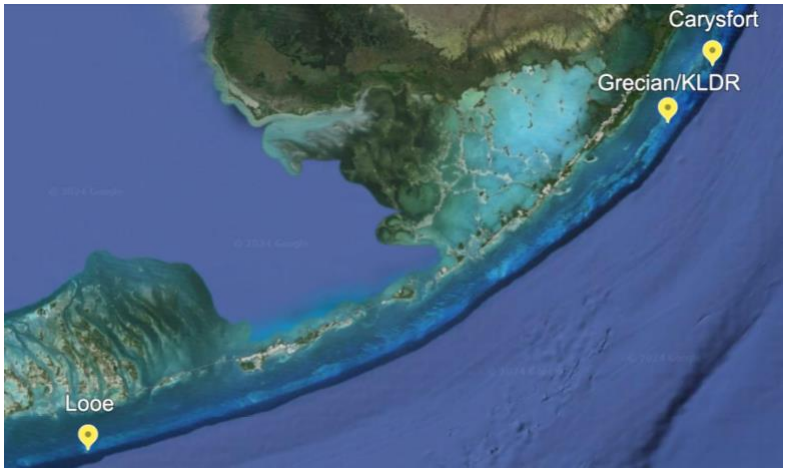

Figure 8: Map displaying FLP sample collection sites in the Florida Keys.

At each of the three sites, 2.5 centimeter cores and 1.5 centimeter cores were taken for histology and TEM analyses, respectively (Table 1). Cores were taken using a hollow punch that is hammered into the coral tissue, producing a core containing both coral tissue and skeleton. Syringe samples were taken for microbiome analysis and were collected by scraping the coral tissue with the syringe tip and gathering the mucus and tissue released. All samples were collected from healthy corals unaffected by FLP, and from two different areas of FLP-affected corals. On diseased colonies, samples were taken directly from the active lesion line, as well as from an area of the coral without active lesions.

	Apparently Healthy	Diseased: Unaffected	Diseased: Lesion
Histology	3 colonies 1 core each Size: 2.5 cm	5 colonies 1 core each Size: 2.5 cm	5 colonies 1 core each Size: 2.5 cm
TEM	3 colonies 2 cores each Size: 1.5 cm	5 colonies 1 core each Size: 1.5 cm	5 colonies 2 cores each Size: 1.5 cm
Microbiome	10 colonies 1 syringe each	10 colonies 1 syringe each	10 colonies 2 syringes each

Total Samples	# per Site	Total
2.5 cm cores	13	39
1.5 cm cores	21	63
Syringe samples	40	120

Table 1: Sampling schematic describing the samples needed for histology, TEM, and microbiome analysis.

Samples were brought back to the boat and immediately processed. Histology samples were fixed with Z-Fix for histological analysis and delivered to FWC-FWRI Lab at St. Petersburg, FL on February 8, 2024. All fixed samples were archived, and FWRI's accession numbers were given to each sample alongside the numbers already assigned in the field collection. Prior to processing samples into histological slides, the external surface area was examined with a dissecting microscope, especially focusing on presence or absence of the mesenterial filaments' protrusion from the surface tissue. Photomicrographs were taken for all samples at low and high magnifications. Subsequently, all samples began the decalcification process, which was initiated on May 1, 2024, using 10% EDTA solution.

TEM samples were fixed using a recipe originally from Thierry Work to standardize fixation across experiments for comparative analyses. Samples were fixed in a combination of 2.5% glutaraldehyde and 2% paraformaldehyde in Instant Ocean (pH 8, 35ppt) and kept at 4°C. Coral tissue was then cut into 1 mm^3 chunks. Samples were rinsed three times for 15 minutes each with 0.35 M sucrose in a 0.1 M sodium cacodylate buffer solution. Samples were post-fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for two hours at room temperature. Samples were rinsed twice for 15 minutes each with 0.1 M sodium cacodylate buffer. Samples were then dehydrated with a series of ethanol solutions (50, 70, 95, 100, 100%) for 15 minutes each. Samples were then added to a 1:1 mixture of Spurr's resin with 100% ethanol for one hour. Samples were then embedded in 100% Spurr's resin overnight. Fresh 100% Spurr's resin was added to samples and samples were put into a 70 °C vacuum oven overnight. All samples collected have been embedded into resin. For the next phase (FY 24-25), samples will be sectioned with a diamond knife and placed onto a 0.25% formvar coated copper grid. Sections will be stained with UranyLess for five minutes, rinsed with ultrapure water, stained with lead citrate for five minutes, rinsed with ultrapure water, and then allowed to dry overnight. Sections will then be imaged using a FEI Tecnai Spirit Bio Twin TEM at UNCW's Richard Dillaman Bioimaging Facility.

Microbiome samples of disease lesions from *Orbicella faveolata* corals exhibiting Fast Lesion Progression (FLP) were received for microbiome characterization. The bacterial community was successfully characterized through 16S rRNA gene libraries (V4 region) for 77 FLP samples.

3. Results

3.1 Task 1: Monitoring FLP-affected colonies through time

FLP corals were tagged and/or monitored at three sites across 22 field days (Table 2).

New corals tagged	Upper Keys Monitoring	Looe Monitoring
9/19/2023		
9/21/23		
11/22/2023		
12/1/2023		
1/11/2024	1/11/2024	
1/16/2024		
1/19/2024	1/19/2024	
1/28/2024	1/28/2024	
1/30/2024	1/30/2024	
1/31/2024		1/31/2024
2/1/2024		
2/3/2024		
		2/16/2024
2/17/2024		
		2/23/2024
		2/25/2024
4/5/2024	4/5/2024	
4/6/2024		4/6/2024
		5/8/2024
		5/9/2024
		5/13/2024
5/21/2024	5/21/2024	

Table 2: FLP field work dates. Upper Keys monitoring consisted of both Carysfort and Grecian/KLDR.

A total of 85 FLP lesion measurements were obtained from 51 corals across three sites since the commencement of this project (Table 3). 17 FLP colonies were fatetracked at Carysfort, including 10 new corals and 7 previously monitored for SCTLD, 14 at Grecian/KLDR, including 7 new corals and 7 previously monitored corals, and 20 FLP-affected corals at Looe, including 7 new corals and 13 previously monitored corals. Not all fate-tracked FLP corals had lesions that were measurable.

Site	Total *O. faveolata* FLP colonies	Total Lesion Measurements	Active Measurements	BTT Measurements	HALT Measurements
Carysfort	17	25	72%	12%	16%
Grecian/KLDR	14	25	36%	12%	52%
Looe	20	35	20%	20%	60%

Table 3: Total number of FLP-affected *O. faveolata* colonies monitored at each of the three monitoring sites, as well as the number of lesion measurements collected. Of the total lesion measurements, the proportion of active, blown through tissue (BTT), and halted (HALT) lesion measurements taken from each site is also displayed.

Of the 85 lesion measurements gathered across all three sites, 38 (44.7%) halted between visits. Some of these halted lesions had 0 centimeters of lesion growth between monitoring visits, indicating that lesions most likely halted shortly after the monitoring visit. An additional seven lesions partially halted, or continued progressing in a non-linear direction, resulting in lesions that could not be accurately measured to calculate lesion progression rates.

For the remaining, active, normally-progressing lesions, progression rates were calculated using the formula:

$$\frac{\text{lesion growth (cm)}}{\text{number of days between monitoring visits}}$$

We also calculated lesion progression rates for halted and BTT lesions. Lesion progression rates were found to be as high as 1 cm/day (Figure 9). For active lesions, the average FLP lesion progression rates for the three sites ranged from 0.42-0.54 cm/day (Table 4). There was no significant difference in active lesion progression rates among sites (One-Way ANOVA; $p = 0.46$)

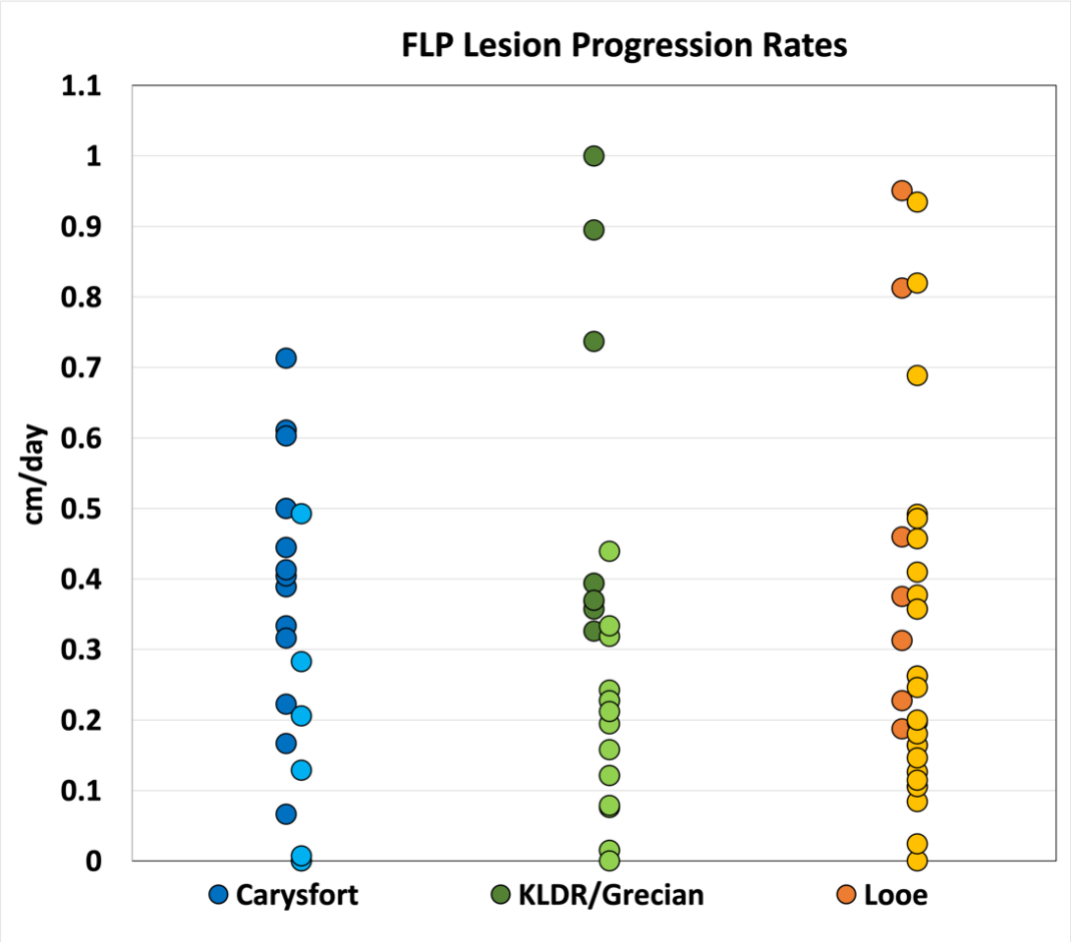

Figure 9: Lesion progression rates of FLP lesions at the three monitoring sites. Darker colored cirlces indicate lesion progression rates from active lesions, while lighter circles of the same color indicate lesion progression rates from blown through tissue (BTT) and halted lesions.

Site	Average FLP Lesion Progression Rate
Carysfort	0.42 cm/day
Grecian/KLDR	0.54 cm/day
Looe	0.48 cm/day

Table 4: Average lesion progression rate of active lesions at three sites.

3.2 Task 2: Assessing past FLP presence and amoxicillin effectiveness

Photos from the first 40 *O. faveolata* colonies treated for presumed SCTLD from six sites were assessed to determine if they were affected by FLP or SCTLD. Because not all sites were first treated at the same time, and because *O. faveolata* are more common at some sites than others, the years for the first 40 colonies treated is not consistent across sites. For example, both Looe and Sombrero were first treated in 2019 when SCTLD was relatively new to the sites, thus all 40 of the first *O. faveolata* colonies' assessments are from 2019. In contrast, the Upper Keys sites

Carysfort and Molasses had already suffered substantial losses to SCTLD when treatments began, and so first appearances of disease on *O. faveolata* colonies across multiple years were required. By expanding the range of years available for assessment, we were able to reexamine 40 *O. faveolata* colonies from all sites except Molasses, at which only 34 *O. faveolata* colonies qualified for assessment.

FLP was found to be present at all six monitoring sites during first visitations (Figure 10). Proportions of FLP affected corals ranged from 5-41.8% between sites, with Molasses having the highest proportion. Additionally, four out of the six sites were found to have corals affected with both FLP and SCTLD concurrently.

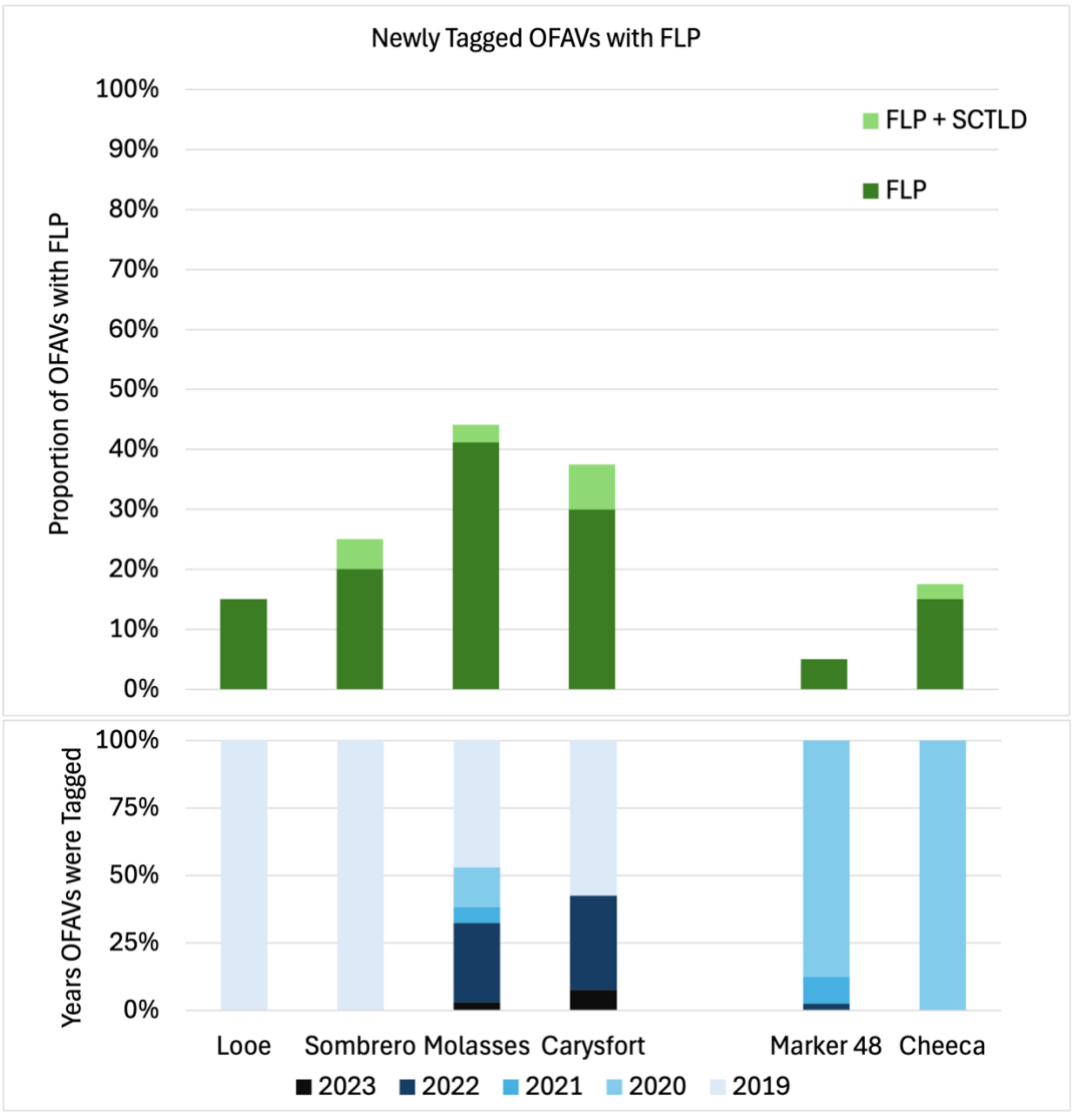

Figure 10: The top graph displays the percentages of the first 40 *O. faveolata* colonies tagged at each site affected with FLP. The bottom graph displays the percentages of *O. faveolata* colonies reexamined from various years ranging from 2019-2023. Sites on the left are offshore reefs, and sites to the right are inshore reefs. Both groups are arranged from West to East.

To determine the effectiveness of amoxicillin and Base2B topical paste on FLP lesions we attempted to assess 50 of the first FLP lesions treated with amoxicillin during two time periods, 2019-2020 and 2022-2023, at six sites. For this task, only *O. faveolata* colonies that had been initially treated with amoxicillin were reexamined, leading to the discrepancy among total FLP-affected *O. faveolata* colonies between tasks. Photos from all 955 *O. faveolata* colonies treated with amoxicillin in 2019-2020 and 2022-2023 were reexamined (Table 5), however there were not 50 available treated FLP lesions per time period for any of the six sites.

Site	Total *O. faveolata* colonies	FLP-affected *O. faveolata* colonies	Usable FLP Lesions
Carysfort	82	27	21
Cheeca	216	27	11
Marker 48	92	2	0
Looe	470	13	11
Molasses	33	9	6
Sombrero	62	7	14

Table 5: Total number of amoxicillin-treated *O. faveolata* colonies assessed, the number of FLP-affected colonies, and the number of FLP lesions assessed for each site.

FLP lesion assessment was limited by a variety of factors such as the small number of FLP-affected *O. faveolata* at some sites, the lack of FLP-lesion monitoring photos within the three month post-treatment time period, and some environmental factors such as poor visibility which led to uncertainty in lesion efficacy assessment. Furthermore, in 2022, our team implemented a new protocol where instead of monitoring and documenting every previously tagged coral at a site, only actively diseased colonies were documented. This change in protocol led to a large number of corals being excluded due to monitoring photos being longer than three months apart when FLP was no longer active on a coral.

In the case of Marker 48, past monitoring photos of 92 *O. faveolata* colonies that were treated with amoxicillin were reexamined for this project. Of the 92 possible *O. faveolata* colonies, only two were found to be afflicted with FLP. This is in line with earlier findings, where only 5% of the first 40 *O. faveolata* colonies at the site had FLP-style lesions. Of the two potential FLP lesions found at Marker 48, both were unable to be assessed as their follow-up photos did not meet the standards put in place for this task.

We assessed 63 lesions (40 from 2019-2020 and 23 from 2022-2023) that were treated with the standard amoxicillin and Base2B topical paste to determine treatment efficacy on FLP lesions (Table 6).

Site	2019-2020 Total	2019-2020 Effective %	2022-2023 Total	2022-2023 Effective %
Carysfort	10	40%	11	0%
Cheeca	6	33.3%	5	60%
Looe	6	50%	5	20%
Molasses	6	33.3%	0	N/A
Sombrero	12	58.3%	2	0%

Table 6: The number of FLP lesions treated with amoxicillin by site and time period, and the percentage of FLP lesions for which treatment was effective.

Of the 63 amoxicillin-treated FLP lesions, treatments were effective on 35%. Effectiveness of amoxicillin treatments did not vary across sites during the 2019-2020 time period (X^2; p = 0.6) (Figure 11). It did vary by site during the 2022-2023 time period (X^2; p = 0.03), with post-hoc tests identifying the 60% efficacy of treatments on FLP lesions at Cheeca Rocks to be significantly greater than the 0% efficacy rate at Carysfort (Fisher Exact; p = 0.02). There were no other significant differences in efficacy among sites in 2022-2023. At most sites, there was no difference in efficacy between the 2019-2020 lesion treatments and the 2022-2023 lesion treatments. The exception was Carysfort Reef where 2019-2020 treatments were effective 40% of the time, but 2022-2023 treatments were completely ineffective (Fisher Exact; p = 0.04).

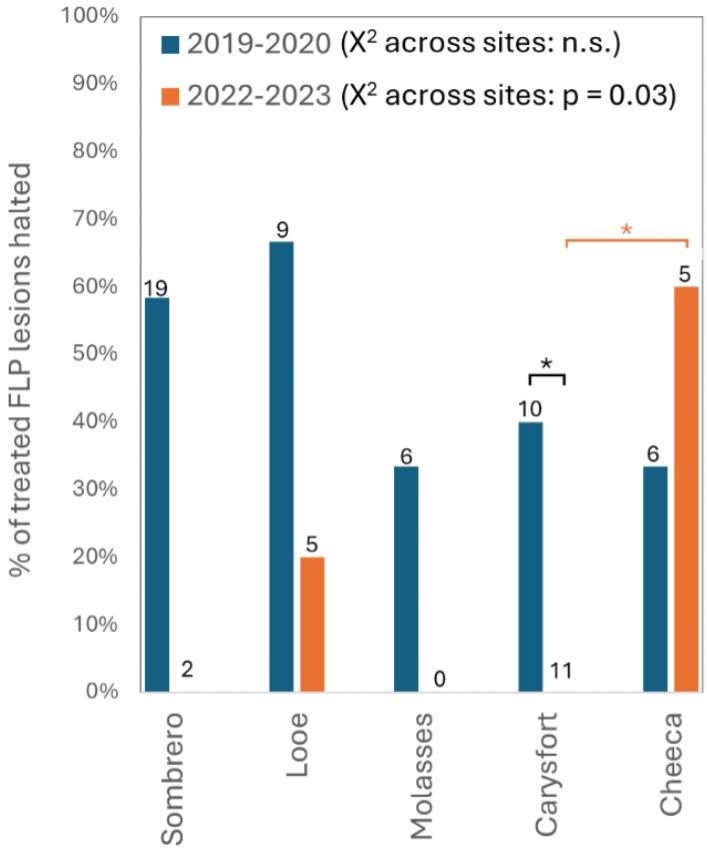

Figure 11: The percentage of FLP lesion treatments that were effective across reef sites and time

periods. Numbers over the bars represent the number of lesions assessed. * indicates significant differences.

3.3 Task 3: Sampling of FLP-affected colonies for histology, TEM, and microbiome

Project partners are continuing their histology, TEM, and microbiome analyses. The decalcification process for the histology samples is still ongoing. During TEM analysis, researchers found that *O. faveolata* tissue is darker near the basal body wall when compared to the surface body wall. This can be difficult to observe prior to decalcification. More observations will be recorded once processing is completed and imaging begins. For the microbiome analyses, there were 625 - 112,101 sequencing reads per sample after quality filtering (average = 11,059 reads per sample). Raw sequencing reads are publicly available in NCBI under BioProject PRJNA1120359 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1120359). A total of 5,522 microbial taxa were detected from these 77 samples. Analysis of the microbiome libraries will continue in the next fiscal year.

4. Discussion

4.1 Task 1: Monitoring FLP-affected colonies through time

Active FLP lesion progression rates averaged 0.48 cm/day across all three sites, with some lesions progressing as quickly as 1.0 cm/day. This progression rate is alarming, as it indicates that FLP could kill a centuries old coral in just a few months. Some lesions slowed or halted partially or completely between monitoring events, identifying progression rates to be highly variable and not consistent across time. Of the 85 lesions measurements gathered, 44.7% halted on their own. This behavior is different from SCTLD lesions, which are not typically observed to halt lesion progression without application of treatment. More frequent monitoring (perhaps every two weeks) is necessary to better understand the variability in these rates, including potential maximum rates as well as potential environmental variables affecting progression rates, and to better understand the halting behavior of FLP lesions.

Through strike team intervention work, 65 FLP affected corals were documented across Carysfort, Grecian/KLDR, and Looe between July 2023 and May 2024. Some of these FLP-affected corals are included in the FLP monitoring, while others are not,due to them not being good contenders for monitoring. Only 15.4% of corals with active FLP lesions were observed to have active FLP lesions at subsequent monitoring visits, suggesting that FLP either consumes all available coral tissue, or halts on its own. Only one coral was affected by FLP across three consecutive monitoring visits. Additionally, two corals were observed to be affected with FLP at the first monitoring visit, observed without active disease at the second, then found to be re-affected with FLP at the third monitoring visit, indicating that corals that halt may become reinfected at a later point.

By utilizing our team's intervention tags, as well as the data gathered during our monitoring events, we were able to investigate FLP on various reefs throughout the Florida Keys. We have found FLP lesions on corals from Key Largo to Key West. In the past year, we have documented FLP on up to 7% of tagged *O. faveolata* colonies at Carysfort reef, up to 5% at KLDR/Grecian,

and up to 4% at Sand Key (Figure 12). A decrease in incidence of FLP correlated with the 2023 bleaching event, but resumed as corals regained their color.

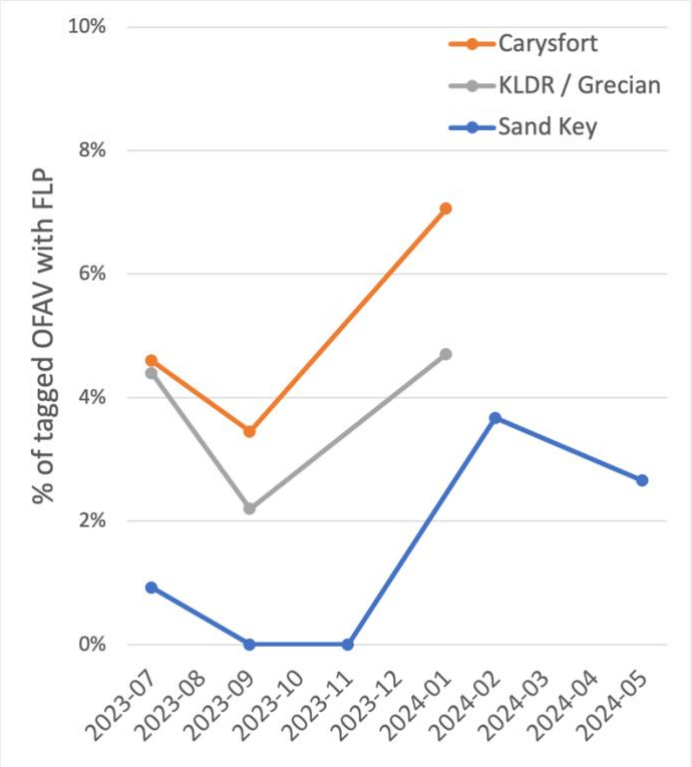

Figure 12: Proportions of tagged *O. faveolata* colonies affected by FLP at three monitoring sites.

4.2 Task 2: Assessing past FLP presence and amoxicillin effectiveness

FLP was found in a proportion of the first tagged *O. faveolata* colonies at all six sites reexamined, revealing that it has been affecting Florida Keys reefs since at least 2019. Unfortunately, it seems that FLP lesions do not respond to standard SCTLD lesion treatments. In comparison to the 91% (Neely et al, 2020) and 93% (Neely et al, 2021) efficacy rate of the same amoxicillin treatment of SCTLD lesions on *O. faveolata* colonies in the Florida Keys, the average efficacy rate on FLP lesions was 34.9%. It is possible that the examples of effective treatment on FLP lesion were explainable through natural halting, but further monitoring would be required to confirm this.

4.3 Task 3: Sampling of FLP-affected colonies for histology, TEM, and microbiome

The results of the analyses conducted by our partners is still ongoing. We hope these analyses will aid in determining whether FLP is truly a novel coral disease. Regular collaborative meetings and a workshop are planned for the next fiscal year to facilitate these analyses and synthesis.

4.4 Future Work

While this project has provided initial assessments of a potentially novel coral disease, further work is required to fully understand the potential threat FLP poses to reefs. FLP poses a relatively prevalent and highly virulent threat to *O. faveolata* colonies, which are a vital reef building species. We recommend the prioritization and funding of continued work on this topic to determine:

1. What the specific temporal patterns of lesion halting are, whether they are consistent across colonies and reefs, and whether there are environmental correlates like seasonality or bleaching events.
2. What the prevalence of FLP-affected colonies is on affected reefs, potentially through the use of already tagged *O. faveolata* colonies with known health histories.
3. Whether there are treatment options for FLP-affected corals. These could include firebreaking, chlorinated epoxy smothering, or natural products already being tested on black band disease.
4. Continued processing of collected samples and collaborative synthesis to compare those samples to other known coral diseases.

5. References

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