The role of algal symbionts in stony coral tissue loss disease (SCTLD): Comparison with cultured isolates from five different Symbiodiniaceae genera, and role of temperature in disease dynamics

The role of algal symbionts in stony coral tissue loss disease (SCTLD): Comparison with cultured isolates from five different Symbiodiniaceae genera, and role of temperature in disease dynamics

Final Report

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Management Summary (300 words or less)

We investigated the role of algal symbionts (Family Symbiodiniaceae) in stony coral tissue loss disease (SCTLD). In Task #1, we tested whether algal symbionts can show a disease response independently of their coral hosts by exposing isolated cultures of five different Symbiodiniaceae genera to SCTLD using a waterborne disease transmission assay. We found that cultures of the genera *Breviolum* and *Durusdinium* that were exposed to seawater from a tank containing corals with active SCTLD lesions exhibited a rapid (2-4 days) negative response, with a reduction in photochemical efficiency, reductions in cell motility, increased vacuolization, and eventual degradation (after 6-8 days). In contrast, control cultures of *Breviolum* and *Durusdinium* exposed to seawater from a tank containing healthy corals of the same species showed no response. Moreover, cultures of *Symbiodinium, Effrenium,* and *Fugacium* were also unaffected by disease exposure. These relative patterns of susceptibility match our previous experiments involving corals containing different algal symbionts, supporting the role of algal symbionts in stony coral tissue loss disease. Further transcriptomic analyses will investigate mechanisms associated with symbiont disruption. In Task #2, we collaborated with Valerie Paul and Julie Meyer to investigate rates of SCTLD lesion progression in diseased *Montastraea cavernosa* and *Colpophyllia natans* at different temperatures. In particular, we were tasked with characterizing the algal symbiont communities associated with experimental coral colonies to help interpret disease dynamics. We found that *M. cavernosa* colonies exclusively hosted *Cladocopium*, while *C. natans* predominantly hosted *Breviolum* (although five of nine colonies also contained background *Symbiodinium*). Overall, Symbiodiniaceae communities across the experimental colonies were stable with no significant variation between sample locations (i.e., at the lesion, 2cm from the lesion, 4cm from the lesion) and across time (even at 31ºC). We are now looking at intrageneric variation in a subset of these samples using ITS2 amplicon sequencing.

Executive Summary (max 1 page)

Since late summer 2014, Florida's Coral Reef has experienced unprecedented losses of brain and boulder corals due to stony coral tissue loss disease (SCTLD). These coral species appear to be unusually susceptible to this disease, but the factors underlying differential susceptibility (at both the species and colony level) are not well understood. However, studies have shown that algal symbionts (Family Symbiodiniaceae) are implicated in disease etiology (Landsberg et al., 2020) and that different algal symbionts can modulate susceptibility, both within and between species (Dennison et al., 2021). Here we tested the hypothesis that algal symbionts might be directly affected by a SCTLD pathogen(s) by exposing cultured symbionts from five Symbiodiniaceae genera (*Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, and *Fugacium*) to a 9-day controlled SCTLD exposure in the laboratory. Cultures were monitored daily using light microscopy and chlorophyll fluorescence, and a subset of cells from each culture were preserved daily for TEM, fluorescence microscopy, and transcriptomic analyses. We found that cultured algal symbionts can indeed be directly affected by SCLTD, and that different Symbiodiniaceae genera show patterns of differential susceptibility that match those documented in hospite. These findings indicate that algal symbionts, particularly certain taxa in the genus *Breviolum*, but also members of *Cladocopium* and *Durusdinium* (but not *Symbiodinium* or *Fugacium*) may be the direct targets of a SCTLD pathogen(s) and suggest that cultured symbionts may be informative model systems for understanding SCTLD, due to the relative ease of maintaining clonal lineages in the laboratory under standard conditions that can be easily replicated for comparative experiments across space and time.

In addition, this project also included our involvement in a collaborative experiment (led by PI Dr. Valerie Paul at the Smithsonian Marine Station) in which we used real-time PCR to identify and quantify Symbiodiniaceae communities in samples generated from a thermal experiment. We found that the colonies in the experiment were dominated by algal symbionts in the genera *Breviolum* and *Cladocopium* and we are in the process of analyzing a subset of these samples that will be analyzed using ITS2 amplicon sequencing (end June 2022), and investigating changes in symbiont community structure over time.

Acknowledgements

For Task #1 we thank Javier del Campo, Bradley Weiler, Anthony Bonacolta, and Alexandra Wen for help processing samples for fluorescence and TEM imaging. We also thank Vania Almeida at the Miller School of Medicine's TEM core. We also thank Mary Alice Coffroth and John Parkinson for sharing Symbiodiniaceae cultures and identifications. For Task #2 we thank Dr. Valerie Paul and her team at the Smithsonian Marine Station, in particular Jon Lefcheck and Kelly Pitts, for leading the experiment. For both tasks we thank Karen Neely and Michelle Dobler, who assisted with collections of diseased coral colonies. This project would not have been possible without the support from the Florida Department of Environmental Protection's Office of Resilience and Coastal Protection, the Florida Keys National Marine Sanctuary (FKNMS), and the Florida Fish and Wildlife Conservation Commission (FWC) who permitted the collection of corals. Finally, we would like to acknowledge the help of Victoria Barker, Samantha Cook, Kristi Kerrigan, Joanna Walczak, and Maurizio Martinelli for their assistance throughout this project.

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1. DESCRIPTION

1.1. Overview

Florida's Coral Reef is currently experiencing a multi-year disease-related mortality event that has resulted in massive die-offs in multiple coral species. Approximately 21 species of coral, including both Endangered Species Act-listed and the primary reef-building species, have displayed gross lesions which often result in whole colony mortality. First observed near Virginia Key in late 2014, stony coral tissue loss disease (SCTLD) is now endemic to all of Florida's Coral Reef, including the Dry Tortugas. SCTLD has also presented on corals in The Bahamas, Puerto Rico, and the US Virgin Islands, with the best available information indicating this outbreak is continuing to spread throughout the Caribbean (AGGRA 2021). Recent studies have found that SCTLD has reduced live tissue by more than 60% on some Florida reefs, however, reduction of live tissue is likely much greater than reported as juvenile corals are not captured in these surveys and a recent study found coral recruits and juveniles to be susceptible to SCTLD (Williamson et al., 2022, Walton et al., 2018). Nonetheless, several studies have noted significant changes to coral community composition throughout the Caribbean (Alvarez-Filip et al. 2019, Estrada-Saldivar et al. 2021, Heres et al. 2021).

Early observations of this disease noted differential disease susceptibility, both among and within affected species (e.g., Precht et al. 2016, Aeby et al. 2020), and our recent DEP-funded work investigated the roles that different algal symbionts (Family Symbiodiniaceae) play in disease susceptibility. By manipulating corals to host different algal symbionts and then exposing them to SCTLD, we found that corals which predominantly or exclusively associated with *Breviolum* were the most susceptible to SCTLD, followed by corals associated with *Cladocopium,* which are slightly more susceptible than those with *Durusdinium* (Dennison et al., 2021). Based on field observations (e.g., Precht et al., 2016, Walton et al., 2018, Aeby et al., 2019, Muller et al., 2020), all three of these genera are more susceptible than corals that associate with *Symbiodinium*, which do not appear to be susceptible to this disease. In fact, corals that exclusively associate with *Breviolum* are 2.5x more likely to present with SCTLD than those exclusively associated with *Durusdinium* (Dennison et al. 2021, in prep.). This relative risk model suggests that disease susceptibility increases as the proportion of *Breviolum* increases (Dennison et al. 2021). This suggests that either the disease affects the algal symbionts directly, or that corals that host these symbionts are affected in such a way that they become more susceptible (e.g., as a result of coral host immunosuppression by certain algal symbionts, Fuess et al. 2020). The former hypothesis is supported by a recent report which suggests that SCTLD may be the result of a virus affecting the algal symbionts (Work 2021, Aine Hawthorn DAC Presentation August 25, 2021). Moreover, damage to algal symbionts has also been observed in histological examinations of disease lesions (Landsberg et al. 2020).

Given the results of recent studies, assessing the role of algal symbionts in the susceptibility of corals to SCTLD remains a priority for ongoing disease research and, in the case of this report, directly comparing the response of different cultured Symbiodiniaceae to a SCTLD exposure independently of their coral hosts is an immediate goal. Understanding the role of algal symbionts in SCTLD disease dynamics,

and whether algal symbionts are the direct targets of the pathogen, are essential to understanding patterns of disease progression and transmission among different species. They may also be very helpful in understanding patterns of disease progression within colonies (Meiling et al., 2020, particularly species such as *Orbicella faveolata*, which routinely host diverse and patchily distributed symbiont communities within individual colonies, e.g., Kemp et al., 2014). Moreover, it will allow us to better understand the complex relationship between coral bleaching, algal symbiont community structure, and SCTLD, and help inform potential mitigation actions that may help reduce the spread of disease and/or help increase the success of restoration efforts, such as provisioning of sexual recruits with particular algal symbionts (Williamson et al. 2021) to increase both their thermal tolerance and their disease resistance.

1.2. Project Goals

This project tests two hypotheses aimed to expand our current knowledge of SCTLD, in particular regarding patterns of disease progression and transmission. In Task #1 we tested whether isolated cultures of Symbiodiniaceae exhibit a response to a disease challenge and whether different cultures (of five different Symbiodiniaceae genera) vary in their response, using a combination of physiological (e.g., chlorophyll fluorometry), microscopic (e.g., light, epifluorescence, TEM), and molecular techniques (e.g., quantitative PCR, transcriptomics) to characterize response. In Task #2, we used molecular techniques (i.e., qPCR and ITS2 amplicon sequencing) to identify and quantify algal symbionts in coral samples from a collaborative experiment with Dr. Valerie Paul (Smithsonian Marine Station) entitled "Investigation of temperature as a driver of stony coral tissue loss disease dynamics".

2. METHODS

2.1. Symbiont Disease Exposures (Task #1)

In early February, we followed up on our pilot experiment from November 2021 (reported in earlier interim reports) and conducted a 9-day disease exposure experiment, using two treatments (disease vs. control) and three replicates of cultures belonging to each of five different Symbiodiniaceae genera (*Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, and *Fugacium*), for a total of 2x3x5 = 30 culture flasks, each containing 40 mL of symbiont culture in f/2 media. To each "control" culture we added 5 mL of seawater (final concentration ~11%) from a 300 L tank containing healthy colonies of *Montastraea cavernosa*, *Pseudodiploria clivosa*, and *Pseudodiploria strigosa.* To each "disease" treatment culture we added 5 mL of seawater from a 300 L tank containing diseased colonies of the same three species. Seawater (10 um-filtered, UV-sterilized) from Bear Cut, Miami, FL was supplied to both the disease tank and the control tank at a rate of ~200mL per minute (turnover rate of water in tank ~24 h). After four days of incubation in these semi-recirculating set-ups, water from both the disease and control tank was then collected, filtered to 10 um (to remove coral cells, mucus and tissue debris), and used to inoculate each of the culture flasks. Following inoculation, cultures were monitored daily using light microscopy (using a VWR VistaVision Inverted

Microscope equipped with an iPhone 12 Mini lens adapter) and dark-adapted chlorophyll fluorometry (using an Imaging-Pulse Amplitude Modulated fluorometer, Walz, GmbH). A subset of cells from each culture was also fixed daily in 10% glutaraldehyde, stained with DAPI, and passed through a 0.8-um polycarbonate membrane filter for fluorescence microscopy analysis. Filters were analyzed in duplicate using Leica DM 2500 at 20x magnification. A total of 255 IPAM images and 279 DAPI-stained filters were generated using this sampling scheme.

2.2. Preservation for transmission electron microscopy (TEM)

Every other day, a subset of cells was preserved in glutaraldehyde fixative for TEM. Samples were centrifuged at 5,000 g for 10 minutes, the supernatant was removed, and 0.4 mL of 2% glutaraldehyde solution added. Samples were then stored at 4ºC until the conclusion of the experiment where they were then prioritized for analysis based on observations collected using light microscopy. Priority samples were then sectioned into 100 nm grids and stained with uranyl acetate and lead citrate. Grids were then imaged at the University of Miami's TEM core facility at 1400x, 5000x, and 8000x magnification.

2.3. Symbiodiniaceae identification and quantification (Tasks #1 and #2)

Genomic DNA was extracted using a modified organic extraction protocol (Baker & Cunning, 2016) and the associated Symbiodiniaceae genera in each colony/core was characterized using real-time PCR (qPCR) assays. TaqMan environmental master mix (Thermo Fisher Scientific) assays were used to amplify the actin gene in *Symbiodinium* (Winter, 2017), *Breviolum* (R. Cunning et al., 2015), *Cladocopium*, *Durusdinium* (Cunning & Baker, 2013), *Effrenium* and *Fugacium* (Meisterzheim et al, 2019). qPCR data were used to identify associated algal symbionts and their relative abundance using the StepOne package for R (Cunning 2018) adapted for these data. Additionally, for Task #1, Sanger sequencing of ITS2 rDNA was used to confirm qPCR results of the unialgal cultures.

2.4. ITS2 amplicon sequencing (Task #2)

To assess within-genus Symbiodiniaceae diversity and the relative abundance of different symbionts within samples, 25% of samples generated in Task #2 were chosen for ITS2 amplicon sequencing. Samples were prepared for Illumina MiSeq ITS2 amplicon sequencing following the Illumina 16S Metagenomic Sequencing Library Preparation (Illumina protocol, Part # 15044223 Rev. B) using ITS2 primers (Sym-Var 5.82S-forward: 5'-GAA TTG CAG AAC TCC GTG AAC C-3' and Sym-Var-reverse: 5'-CGG GTT CWC TTG TYT GAC TTC ATG C-3') in place of the 16S primers (Hume et al. 2019). Samples will be sequenced June 24-28, 2022 on the Illumina MiSeq platform with 2x300 bp paired-end read chemistry. Quality filtering, sequence assembly and taxonomic assignment will be performed using the dada2 pipeline (v1.12.1) for amplicon sequence variants (ASVs) and the SymPortal framework for symbiont taxa. ASVs will be filtered and analyzed using the dada2 package in R and then re-formatted for input into the R package phyloseq (v1.28.0) (McMurdie & Holmes 2013).

2.5. Transcriptomic samples

Each day, 1mL of media containing a subset of cells for each flask were centrifuged to produce a cell pellet, the supernatant was removed, and the pellet was flash-frozen in liquid nitrogen for future RNA analysis and then stored at -80ºC. In total, 280 samples were preserved for downstream transcriptomic analysis. Given the relatively small amount of biological material per sample, we first identified an RNA extraction kit that yielded usable amounts of RNA. After some trials the Direct-zol RNA Microprep Kit (Zymo) with an added bead beating step yielded RNA. Following extraction, all samples were quantified using Qubit 2.0 Fluorometer (Life Technology) to ensure high enough yields of RNA.

2.6. cDNA preparation and sequencing

192 samples of extracted mRNA will be prepared for sequencing using a CORALL mRNA-Seq (Lexogen). Samples will be sequenced at the University of Miami's, Miller School of Medicine, John P. Hussman Institute for Human Genomics sequencing core using an Illumina NovaSeq with 150 base paired end reads at a sequencing depth of ~20 million reads per sample for de novo assembly of symbiont transcriptomes.

De novo transcriptome assembly, raw reads will be passed through BBDuk (BBTools software suite) for raw read adapter and contaminant trimming and sickle for quality trimming. Reads that pass this QA/QC will then be screened for bacterial contaminants by aligning to the NCBI prokaryote database with STAR and prokaryote aligning reads are discarded. Resulting non-host and non-prokaryote reads are then assembled with transcriptome assembler TRINITY. Read data is then aligned back to the resultant TRINITY draft transcriptome to polish the assembly. The resulting transcriptome is then BLASTED to related species to confirm draft transcripts are not contaminants. Polished transcriptomes are then annotated using the TRINOTATE and InterProScan tools for functional annotation. Following de novo assembly standard differential expression will be explored.

2.7. Data Analysis

All data analysis was performed in Rstudio version 4.1.3. Linear regression and cubic polynomial regression assessed differences between symbionts and treatment. The emmeans package was used to assess pairwise differences in differential response. ImageJ and Cell Profiler pipelines were developed to analyze microscopy images.

3. RESULTS

3.1. Response of isolated cultures of different Symbiodiniaceae to SCTLD exposure

3.1.1. Chlorophyll fluorometry

Analysis of daily, dark-adapted, chlorophyll fluorometry data showed a significant decline ($P<0.01$, $R^2=0.8042$) in the photochemical efficiency of *Breviolum*, *Cladocopium*, and *Durusdinium* inoculate with seawater from the tank containing diseased corals, compared to identical cultures inoculated with seawater from the control tank containing healthy corals, with both *Breviolum and Durusdininum* declining the most rapidly in the days following inoculation (Figure 1a, 1b).

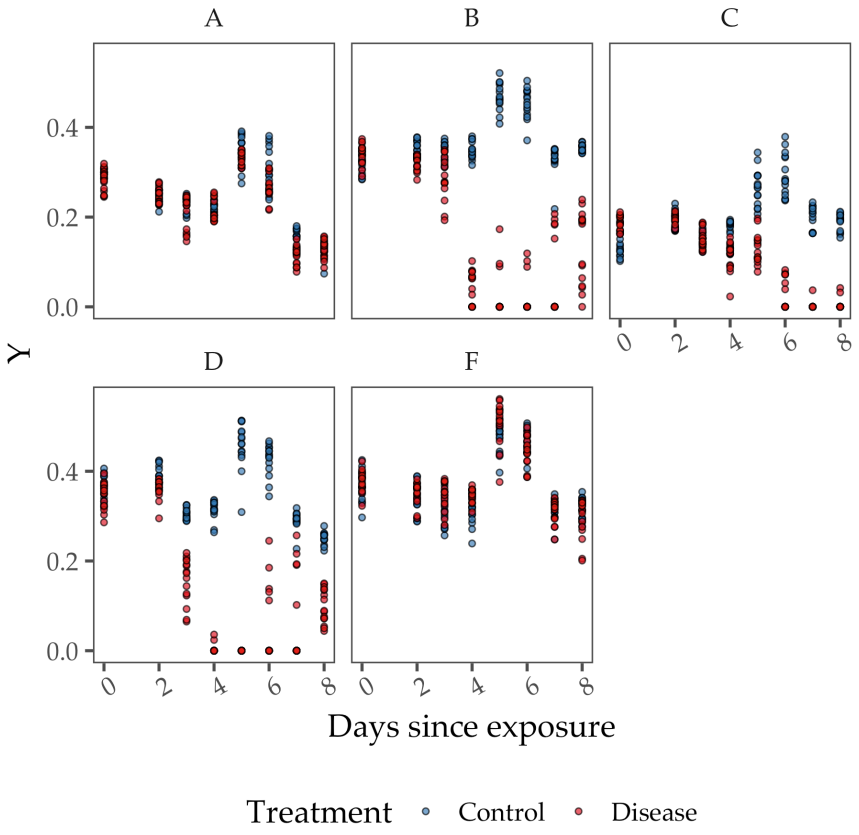

Figure 1a. Decline in photochemical efficiency of symbiont cultures.

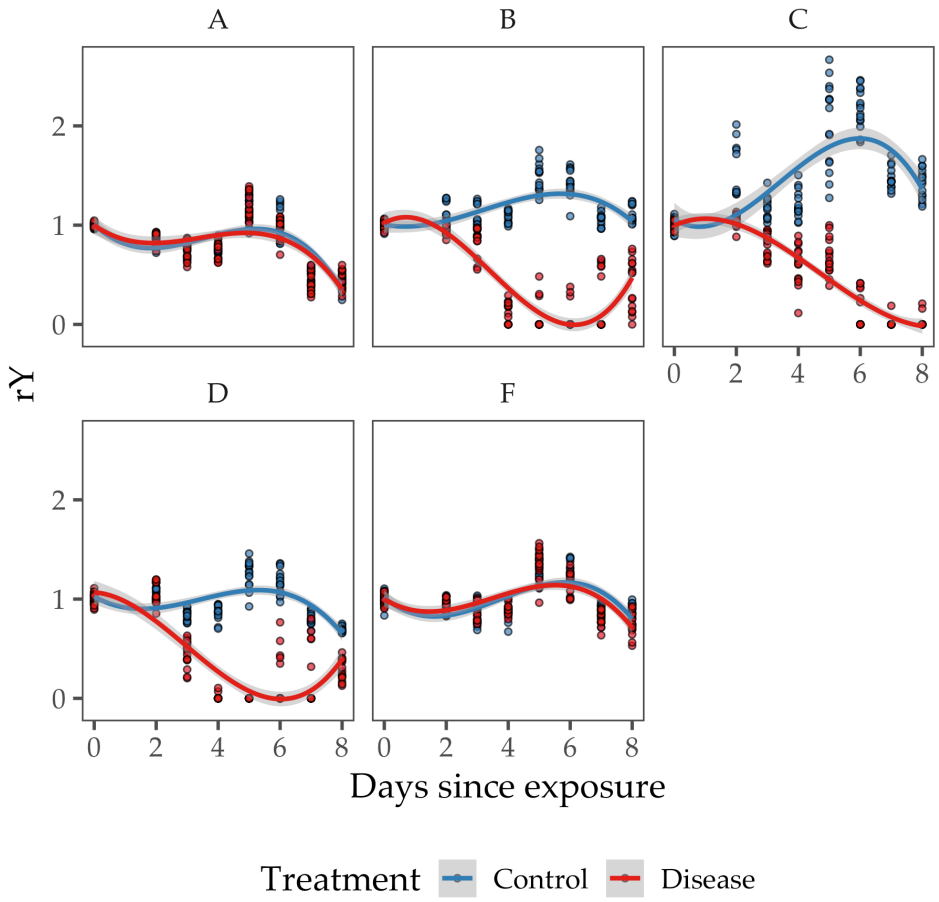

Figure 1b. Relative decline in photochemical efficiency of symbiont cultures exposed either to diseased or control water showing cubic polynomial best fit.

3.1.2. Cell Counts

Still images were extracted from videos taken daily using a Python script and cell counts were performed using an ImageJ pipeline to count the number of cells with symbiont diameters using the red color channel in the microscope focus area. Cubic polynomial regression on relative cell counts showed a significant decline in numbers of both *Breviolum* and *Durusdinium* following disease treatment (Figure 2, $P<0.01$, R^2: 0.46).

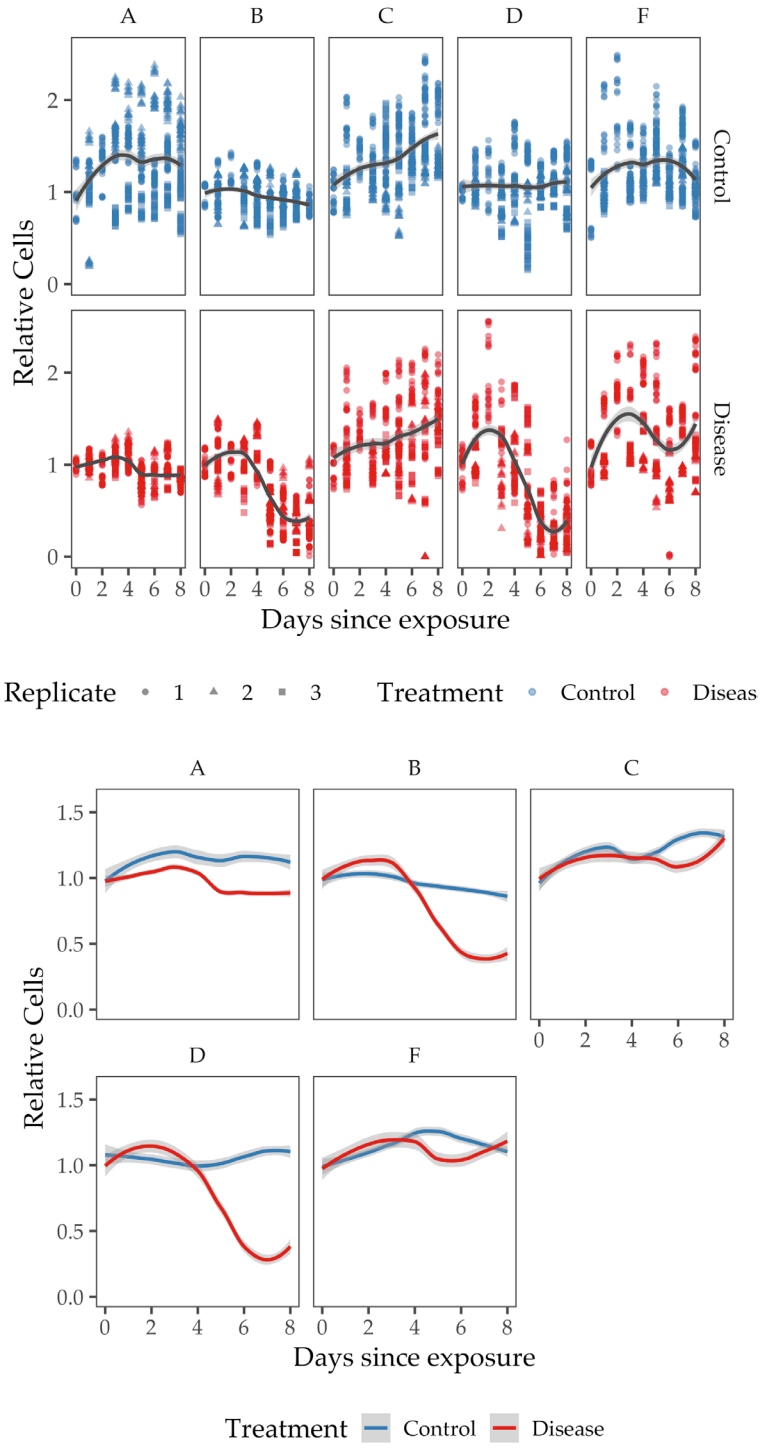

Figure 2. Upper panel: Relative cell counts for each treatment and symbiont type. Lower panel: Overlaid fitted cubic polynomial for relative number of cells per viewing area.

3.1.3. Epifluorescence microscopy

Fluorescence data analysis is still ongoing, but all filters have been imaged in duplicate for chlorophyll and positive nuclei with DAPI staining using a Leica DM 2500 microscope at 20x magnification. Cell profiler was used to count the number of nuclei, chlorophyll cells, and percentage of cells that overlapped with nuclei. Preliminary analysis shows a decrease in the number of symbiont cells in disease treatments, but analysis of percent positive nuclei and number of nuclei is still ongoing (Figure 3, $P<0.01$, R^2: 0.42).

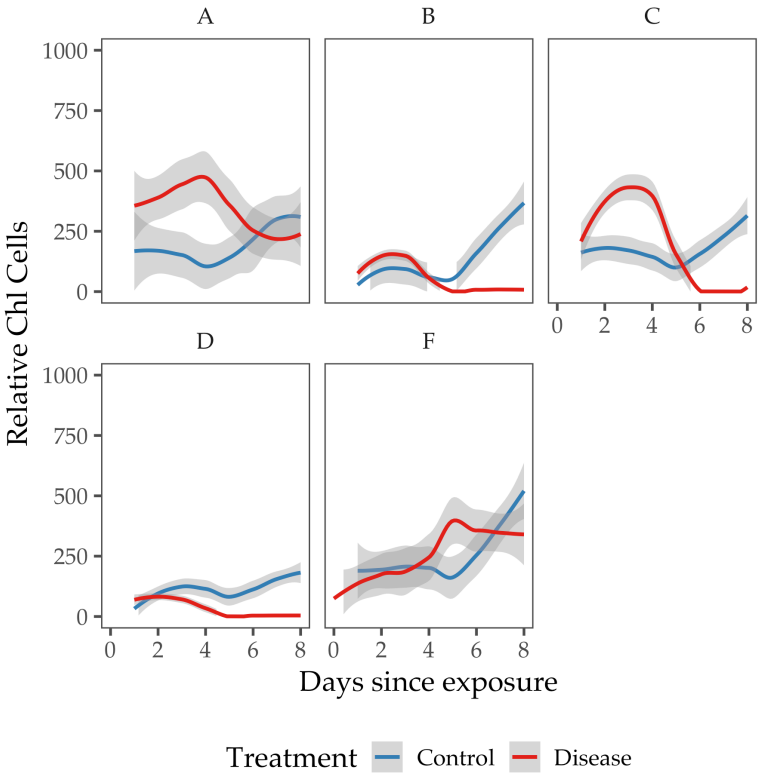

Figure 3. Analysis of chlorophyll-positive cells using fixed filter samples.

3.1.4. TEM analyses

All samples prepared for TEM analysis have been imaged. All TEM images are available at: https://miami.box.com/s/tvalyvu7i699sk0l7tqh45ceejbq1cgs. Expert analysis is needed to confirm whether anisometric viral like particles (AVLPs) are present, but *Breviolum* and *Effrenium* cultures appear to show increased abundances of electron-dense material in the disease treatment prior to cellular degradation (beginning 2-4 days after disease exposure). We observed loss of cellular structure in *Breviolum*, *Effrenium*, and *Durusdinium* following disease exposure, including a loss of chloroplast structure, degraded nuclei, degraded pyrenoids, increased cellular vacuolization, and larger accumulation bodies (Figure 4). However, further analysis and interpretation is pending from collaborator Esther Peters at George Mason University.

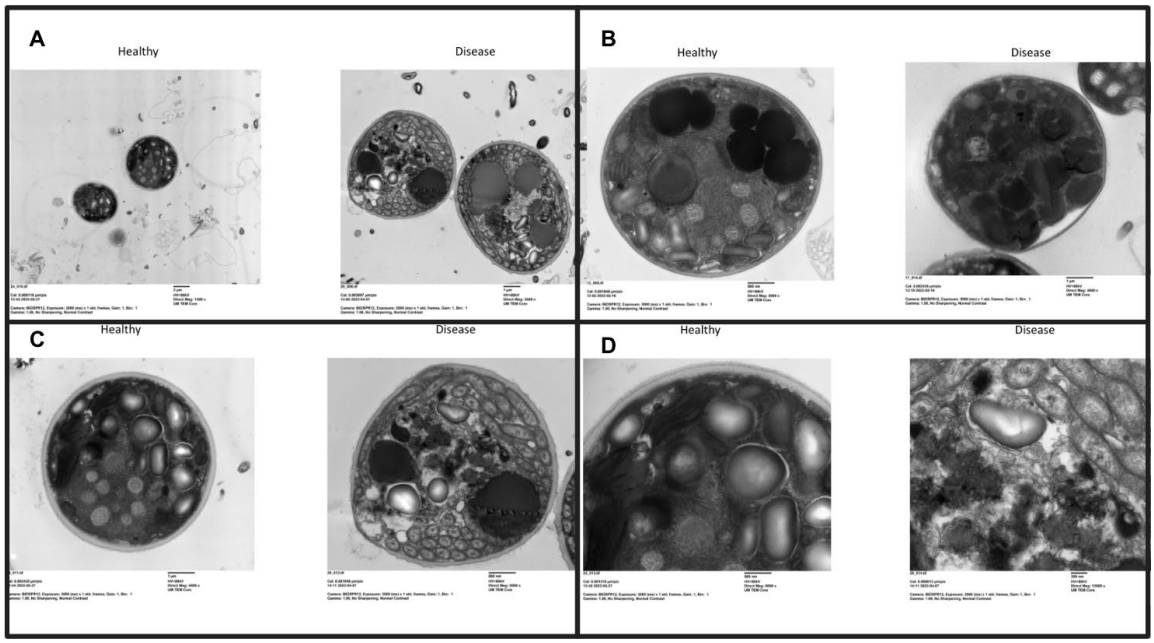

Figure 4. TEM slides of *Breviolum* cultures from both control and disease treatments. A) and B) are *Breviolum* cells 2 days after exposure at 1200x and 2500x magnification respectively. C) and D) are *Durusdinium* cells 4 days after exposure at 5000x and 8000x magnification respectively.

3.1.5. RNA Sequencing

RNA analysis will help inform the molecular mechanisms by which symbionts are affected by exposure to the SCTLD pathogen(s). A total of 192 samples across all treatments, symbionts, and timepoints are being prepared for sequencing with CORALL mRNA-Seq (Lexogen) and we are currently ~50% complete in this pipeline. Samples will be sequenced at the University of Miami Sequencing Core using an Illumina NovaSeq with 150 base paired end reads at a sequencing depth of ~20 million reads per sample for de novo assembly of symbiont transcriptomes. The de novo assembly allows for a more targeted approach for symbionts in culture, since transcriptomes are only available at the genus level for many of the Symbiodiniaceae.

3.1.6. qPCR and Sanger sequencing for symbiont identity

qPCR analysis of the actin gene for each symbiont genera has identified *Symbiodinium*, *Breviolum*, *Durusdinium*, and *Fugacium* to the genus level, but DNA from all symbiont samples used in the experiment have been extracted and will be sent for Sanger sequencing in order to identify the cultures at the ITS2 (species) level. It is our understanding that the *Cladocopium* culture originally obtained from Mary Alice Coffroth may actually be *Effrenium voratum*, based on independent analyses of the same culture source (John Parkinson, personal communication).

3.2. Temperature effects on SCTLD progression: Role of algal symbionts

3.2.1. Identification and quantification of Symbiodiniaceae

Small tissue biopsies from the corals in each of the three thermal experiments were collected by Dr. Valerie Paul and the Smithsonian Marine Station team. Samples were flash frozen in liquid nitrogen, and sent to us for symbiont identification and quantification. From these three experiments, 341 samples were generated (194 *Colpophyllia natans* samples and 237 *Montastraea cavernosa*). Samples were extracted using an organic DNA extraction protocol and analyzed using real-time PCR (qPCR). In total, 11 samples did not pass QA/QC and are being re-extracted. Generally speaking, *C. natans* were dominated by *Breviolum* while *M. cavernosa* exclusively hosted *Cladocopium* (Figures 5-8). Interestingly, two *C. natans* genotypes (26 and 27) from the second thermal trial and all three genotypes (33, 34, 35) from the third thermal trial contained trace amounts of *Symbiodinium* (ranging from 0.02% to 1.5%). Further discussions and analysis are ongoing between the Smithsonian team and RSMAS team to interpret these joint findings.

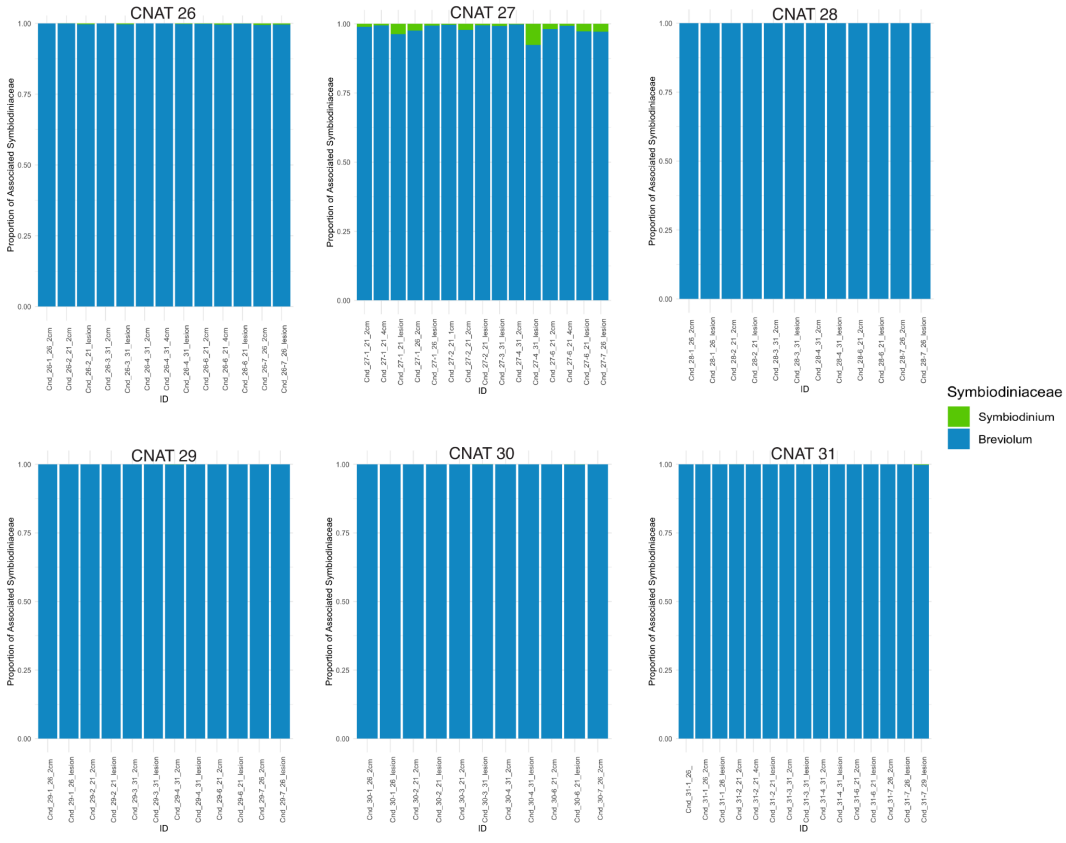

Figure 5. Symbiodiniaceae community structure of *C. natans* in thermal trial 2 conducted in November 2021. Colonies 28, 29, 30, and 31 are exclusively hosting algal symbionts in the genera *Breviolum* while colonies 26 and 27 predominantly host *Breviolum* and contain background amounts of *Symbiodinium* (<2%)

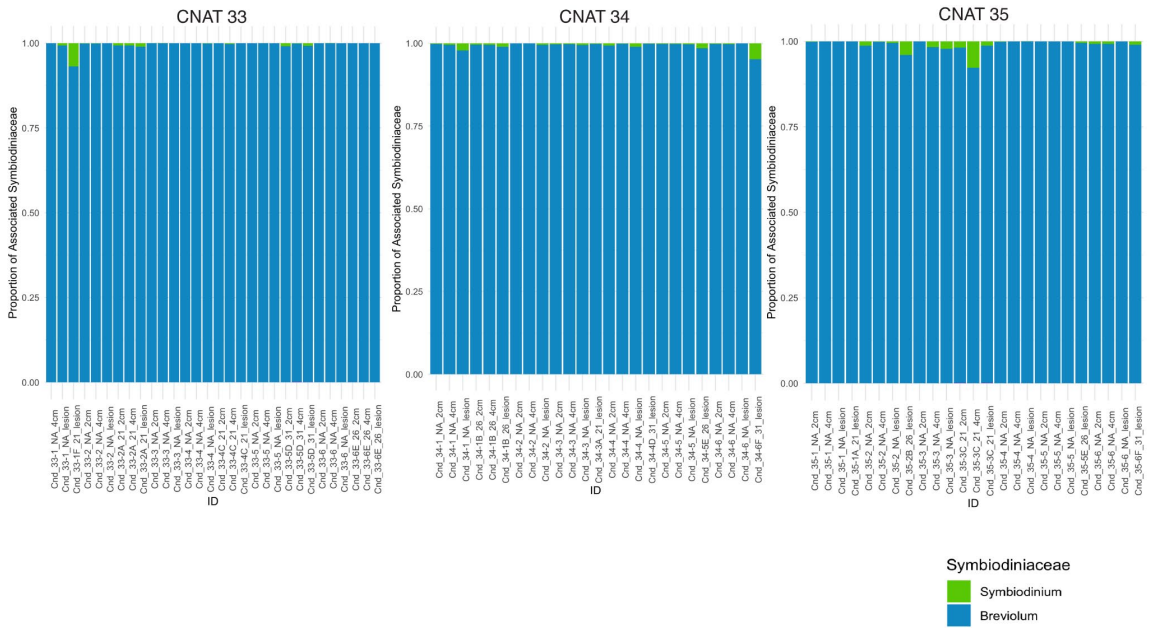

Figure 6. Symbiodiniaceae community structure of *C. natans* in thermal trial 3 conducted in January 2022. All colonies are predominantly hosting *Breviolum* with background amounts of *Durusdinium* (<2%).

Figure 7. Symbiodiniaceae community structure of *M. cavernosa* in thermal trial 1 conducted in September 2021. All colonies exclusively associate with *Cladocopium.*

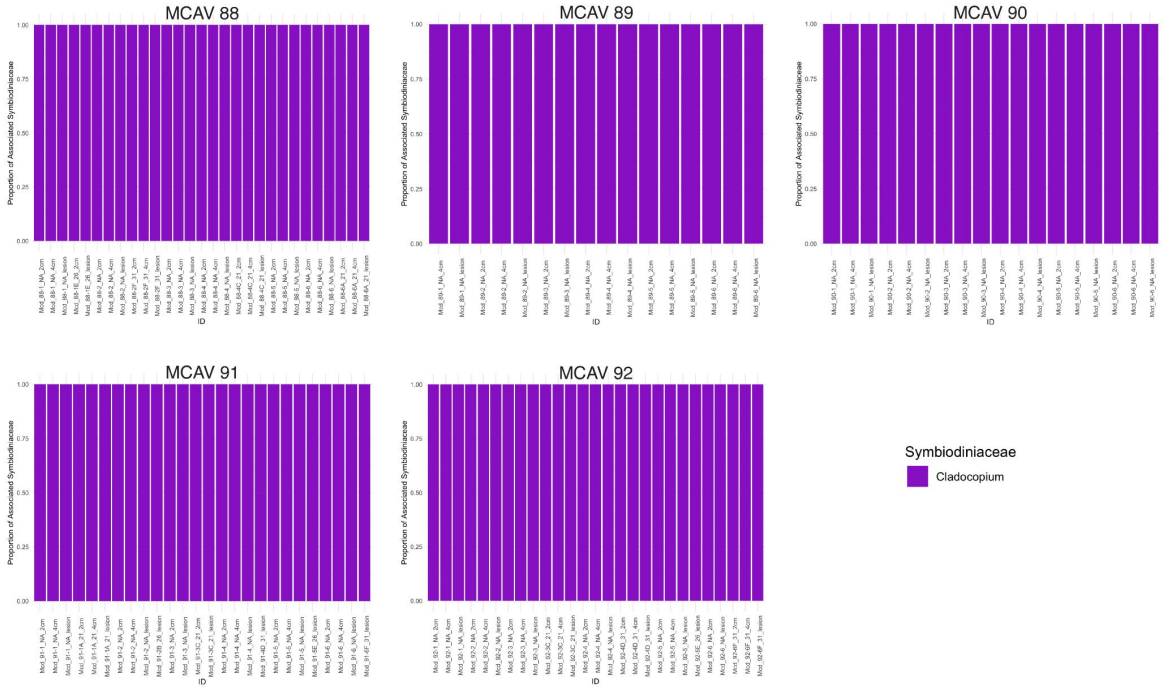

Figure 8. Symbiodiniaceae community structure of *M. cavernosa* in thermal trial 3 conducted in January 2022. All colonies exclusively host *Cladocopium*.

4. DISCUSSION

Results of this study, and the pilot studies that led up to it (reported in previous interim reports), confirm that some Symbiodiniaceae cultures are sensitive to exposure to SCTLD and elicit a response. Using physiological (chlorophyll fluorometry), microscopic (light, epifluorescence, TEM), and molecular (qPCR, Sanger sequencing, transcriptomics) techniques, we conclude that members of some Symbiodiniaceae genera are susceptible to disease following exposure to seawater surrounding corals affected by stony coral tissue loss disease (SCTLD), with some genera being susceptible and others showing no effect of exposure. Although we cannot distinguish a clear difference between the susceptibility of *Breviolum* and *Durusdinium* in culture, as we did in our laboratory studies of symbionts *in hospite*, the physiological responses are similar and continue to support the hypothesis that hosting certain algal symbionts (in particular the genus *Breviolum*) is a key risk factor in this coral disease. Indeed, the overall congruence between these findings and our earlier work characterizing the disease response of corals hosting different symbionts, strongly suggest that algal symbionts may be the actual targets of the SCTLD pathogen(s). We will be further investigating this by analyzing patterns of gene expression using samples preserved in this study.

During this study, unialgal strains of *Breviolum* and *Durusdinium* showed increased susceptibility to disease-exposed water compared to controls, and compared to

disease-exposed cultures of *Symbiodinium*, *Cladocopium*/*Effrenium* (see Section 3.1.6), and *Fugacium*. Here, *Breviolum* and *Durusdinium* cease photosynthetic activity within days of exposure to a ~10% concentration of disease-exposed seawater in f/2 culture media. Additionally, disease-exposed *Breviolum* and *Durusdinium* cultures showed a reduction in the number of cells over the course of this experiment due to cell lysis, degradation, and overall cell death. Although it is not quantified in this study, during light microscopy sessions we also noted impairment of cell motility in affected cultures, with rapid declines in cell motility between days 1 and 2 in *Breviolum* cultures and between days 3 and 5 in *Durusdinium* cultures, suggesting that exposure to disease impairs cellular function quite rapidly. After this time, motility stopped completely.

In addition to physiological changes measured using chlorophyll fluorescence and light microscopy, TEM analysis showed a marked degradation of cell structure, with increased vacuolization, loss of chloroplasts, and degraded nuclei in *Breviolum* and *Durusdinium* exposed to disease-containing water compared to non-disease-containing water. RNA sequencing analysis will further elucidate potential molecular pathways involved in symbiont exposure to disease.

Importantly, the seawater used for both the disease and healthy treatments of this study was collected from disease baths that were also used for a contemporaneous laboratory study in which both juvenile and adult coral colonies exhibited SCTLD-like lesions, although the results of the associated histology are still pending to confirm this diagnosis.

Although the findings of this study do not suggest a clear difference between the susceptibility of *Breviolum* and *Durusdinium*, this may be due to the high concentration of disease water (~11%) used in this study and the relatively high cell density of symbionts in our cultures. While this approach elicited a disease response, which was a goal of the project, it may also limit our ability to detect subtle differences in disease susceptibility. Additionally, differences in symbiont density in the cultures used in this study may also have limited our ability to distinguish hierarchies in susceptibility. Despite these promising results, much of the data we collected are still being explored. In particular, we are developing a way to automate the collection of data from our light microscopy videos (i.e., motility, cell density, etc.), visualize changes in epi-fluorescence, and complete our transcriptomic analyses. Nevertheless, this study suggests that algal symbiont cultures may be useful model systems for understanding SCLTD, due to the relative ease of maintaining clonal lineages in the laboratory under standard conditions that can be easily replicated for comparative experiments across space and time. This approach can greatly simplify experiments by removing complex holobiont responses and/or corals (sometimes unknown) prior experience of SCTLD and/or uncontrolled environmental history. Our results indicate that algal symbionts are sensitive to disease independently of their coral host, and may help explain previous histological findings (e.g., Landsberg et al. 2020). Moreover, if SCTLD is a disease affecting single-celled algal symbionts, this may also explain why the disease can take effect so rapidly. Following rapid impairment of photosynthesis, symbionts exhibit vacuolization before degrading in situ, resulting in damage to the coral gastrodermal cell layer and leading to the rapid appearance and spread of gross lesions on the coral surface. Our future work will take advantage of this new model system by fractionationating disease-exposed seawater to attempt to further identify the size class of the pathogen, with the ultimate

goal of isolating and potentially culturing it in order to identify mechanisms of stony coral tissue loss disease and find ways to slow its spread.

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