# Investigation of temperature as a driver of stony coral tissue loss disease dynamics

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Final Report

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## Management Summary

A better understanding of the role of temperature in influencing disease processes associated with stony coral tissue loss disease (SCTLD) has many practical management implications. First, it can inform managers about seasonal constraints in managing SCTLD and help predict times of year or locations on the reef where disease will be more or less of a problem. This can be useful for timing the outplanting of restoration corals, intervention activities, construction activities and other considerations. For example, it can inform managers involved in restoration efforts when outplanting of corals should proceed with minimal risk of disease transmission to new recruits. Outplanting could be timed to occur when SCTLD is less of a problem and intervention activities enhanced when SCTLD is more of a problem. It can also assist with determining the best time of year to conduct SCTLD surveillance, which would be especially important if the best times fall outside summer months when most regular monitoring efforts occur. This research will address these two current research priorities determined by managers: 1) What factors (genotypic, colony-specific, or environmental) drive resistance and/or resilience to SCTLD? 2) What conditions (environmental, ecological, anthropogenic, and/or in the coral populations) have allowed the outbreak to persist and spread?

This project continues our working collaboration and reporting to the Disease Advisory Committee that includes all the research groups and reef managers involved with work on the SCTLD outbreak. Understanding the effect of water temperature and thermal stress on SCTLD pathogenesis would complement the restoration efforts by the Florida Department of Environmental Protection (DEP), the Florida Fish and Wildlife Conservation Commission, NOAA Florida Keys National Marine Sanctuary, the National Park Service, the Association of Zoos and Aquariums as well as the various collaborating marine laboratories. The results from this project will be shared with the disease advisory committee (DAC), a collection of the government agencies mentioned above and researchers from universities investigating the SCTLD outbreak.

## Executive Summary

The role of temperature as a driver of stony coral tissue loss disease (SCTLD) has been unclear. A study that used spatial epidemiology models and analytical tools to examine the progression of SCTLD throughout Florida's Coral Reef, from 2014 to 2017 early in the outbreak did not indicate a significant role of temperature in the spread of SCTLD. The average monthly sea-surface temperature was not significantly related to the presence of the disease nor to the densities of colonies with the disease. Other studies show decreasing prevalence and severity of SCTLD in late summer months. Experimental studies of temperature effects on SCTLD have not been conducted, and these are necessary to examine the effects of temperature on disease progression under controlled conditions without co-occurring changes in other environmental variables. To do so, we tested the influence of temperature on rates of progression of stony coral tissue loss disease in laboratory aquaria on two coral species, *Montastraea cavernosa* and *Colpophyllia natans*. After collection, diseased corals were trimmed with a cleaned rock saw and each diseased lesion was cut into six fragments with relatively equal areas of living tissue and disease lesion. Each individual fragment was housed separately in aquaria with five liters of ocean seawater previously filtered through 0.22 µm-pore filters with an air bubbler for water motion. The aquaria were housed in larger, insulated water tables with recirculating fresh water that is pumped through an insulated sump with heaters and a chiller to maintain a constant water temperature in each aquarium. For adequate replication, 6 large water tables were used, with two water tables randomly assigned to each of 3 temperature levels (21, 26, 31 ºC). Photographs for each fragment were taken on day 0 and daily thereafter up to 28 days or until the fragment died. The total apparently healthy tissue was measured from these photographs using the image analysis software ImageJ. Cumulative percent healthy tissue and disease progression rates were calculated over time from these measurements. The two infected coral species demonstrated different responses to increasing temperature in our experimental aquaria. Across the three experiments, healthy tissue of infected *C. natans* declined more rapidly with increasing temperature. In contrast, *M. cavernosa* declined much less over the duration of the experiments overall and had no strong relationship with temperature. The very different responses of these two species are somewhat surprising and may be related to their different symbiont types (*Cladocopium* for *M. cavernosa* colonies and predominately *Breviolum* for the *C. natans* colonies) or other undetermined factors. These findings have management implications because they show that different diseased coral species respond differently to seawater temperature and there is no uniform relationship with temperature and disease progression rates among different coral species. This may have implications for managing reef activities such as construction, outplanting and treating diseased corals.

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Likewise, we would also like to thank Karen Neely and Michelle Dobler for their field assistance collecting corals near Ft. Lauderdale, FL.

Many thanks for our collaborators for this study, Julie Meyer at University of Florida and Carly Dennison and Andrew Baker at the University of Miami.

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# List of Acronyms

FSW: Filtered seawater
SCTLD: Stony coral tissue loss disease
USVI: United States Virgin Islands

## 1. DESCRIPTION

Florida's coral reefs are currently experiencing a multi-year disease-related mortality event that has resulted in massive die-offs in multiple coral species. Over 20 species of coral, including both Endangered Species Act-listed and the primary reef-building species, have displayed tissue loss lesions which often result in whole colony mortality. First observed near Virginia Key in late 2014, the disease has since spread to the northernmost extent of Florida's Coral Reef, and south to the Dry Tortugas. The best available information indicates that the disease outbreak is continuing to spread throughout the Caribbean (AGRRA 2021) with devastating consequences to the reefs of Florida and the Caribbean (Precht et al. 2016, Walton et al 2018, Alvarez-Filip et al. 2019, Estrada-Saldívar et al. 2021, Heres et al. 2021).

We have learned a lot about stony coral tissue loss disease (SCTLD) since it was first observed, but many fundamental questions remain including about the causes and environmental drivers of disease. We know that antibiotic treatment can stop disease lesions from progressing (Aeby et al. 2019, Neely et al. 2020, 2021, Walker et al. 2021) and that coinfections with the pathogen *Vibrio coralliilyticus* can cause lesions to progress more rapidly, indicating that bacteria are important in SCTLD etiology (Ushijima et al. 2020). There appear to be regional differences in disease dynamics between SE Florida and the Florida Keys that may be due to differences in environmental conditions on the reefs of these regions.

The role of temperature as a driver of SCTLD dynamics has been unclear. While spatial epidemiological studies suggest a limited role for temperature in the spread of SCTLD along Florida's Coral Reef (Muller et al. 2020), field observations indicate that SCTLD progression can slow or stop in the Florida Keys in late summer (Sharp et al. 2020), and it is not readily observed in late summer months when susceptible species of corals show signs of bleaching (K. Neely, E. Bartels, personal communication). Reduction in SCTLD progression during a thermal stress event was documented quantitatively by 3D photogrammetry in the USVI (Meiling et al. 2020). Damage to zooxanthellae is observed in histological examination of disease lesions (Landsberg et al. 2020), and the Baker laboratory at University of Miami has evidence that symbiont type is important in susceptibility of different coral species to SCTLD (Dennison et al. 2021). Thus, relationships between thermal stress leading to bleaching and SCTLD need to be better studied. It is fundamental to understanding SCTLD disease dynamics to examine the responses of SCTLD to temperature and to try to understand mechanisms explaining patterns of disease progression and transmission under thermal stress, especially as thermal stress to Florida's Coral Reef is expected to increase with climate change (Kuffner et al. 2015, Manzello 2015). A better understanding of the role that temperature may play in the etiology of SCTLD is needed for future management and mitigation of the disease.

Increased water temperature and temperature stress have been suggested to be contributing factors to the SCTLD outbreak. Reefs around Miami-Dade County during 2014-15 (the suspected starting period and location of the outbreak) experienced more days exceeding the bleaching threshold of 30.5°C compared to recent years (Walton et al. 2018). Warmer water temperatures can increase the growth and virulence of some coral pathogens (Ben-Haim et al. 2003, Bruno et al. 2007, Ward et al. 2007, Harvell et al. 2009), while thermal stress may affect the immune system of susceptible coral hosts (Palmer et al. 2011). It should be noted that not all coral disease outbreaks occur during periods of elevated water temperatures, for example, outbreaks of *Montipora* white syndrome in Hawaii occurred during the colder periods of 2010 and 2012 (Aeby et al. 2016). Additionally, at least one of these outbreaks was attributed to a unique strain of the coral pathogen *Vibrio coralliilyticus* that could maintain consistent levels of virulence at lower water temperatures, unlike other *V. coralliilyticus* strains that have reduced virulence during colder periods (Beurmann et al. 2018, Ushijima et al. 2014). Therefore, conclusions cannot be made about temperature and disease progression until experimentally investigated.

Experimental studies of temperature effects on SCTLD have not been conducted, and these are necessary to examine the effects of temperature on disease progression and transmission under controlled conditions without co-occurring changes in other environmental variables. This will clarify relationships between SCTLD and thermal stress and investigate mechanisms. This is especially important as Florida's Coral Reef is under increasing threat from warming temperatures and bleaching already occurs during many years in summer months (Manzello et al. 2007, Manzello 2015). Enhanced understanding will facilitate predictions and better management of SCTLD and its treatment during summer months and could be applied to treatment of SCTLD when it occurs in aquarium settings. Although controlling water temperature in the field is not practical, understanding its effect on SCTLD is (1) essential for the land-based nurseries housing corals for restoration purposes that can control growth conditions and (2) will assist managers in making informed decisions on selecting coral restoration timing and locations and disease monitoring efforts.

The objective of this work is to investigate rates of lesion progression in diseased corals at different temperatures. A range of temperatures (21-31 ºC) was tested in 5 ºC increments, which includes temperatures near bleaching thresholds, to determine if SCTLD progression slows at higher temperatures as we hypothesize based on preliminary data and published studies (Meiling et al. 2020). Target coral species include *Montastraea cavernosa* and *Colpophyllia natans* because diseased corals of these species can still be readily collected. Given that temperature tolerance of corals in different locations differ, we will try to source corals from different locations including the Florida Keys as well as Southeast Florida. Corals will be tested for the presence or absence of *Vibrio coralliilyticus*, a bacterium that causes tissue loss and increases the virulence of SCTLD, and the amounts of the VcpA protease will be quantified at different temperatures. Higher amounts of that protease toxin could explain higher disease virulence. Changes in the Symbiodiniaceae communities will be determined by

collaborators Carly Dennison and Andrew Baker at the University of Miami. Corals will be preserved for histology to determine changes to the lesions at different temperatures and to confirm whether the changes on a cellular and tissue level attributed to SCTLD are consistent with those seen in diseased corals in the field.

## 2. METHODS

The study focused on measuring SCTLD progression with two coral species across water temperatures ranging from 21 to 31 °C, which correspond to high and low seasonal temperatures observed in SE Florida and the Florida Keys (NOAA National Centers for Environmental Information). We conducted temperature treatments that have the greatest potential effect of slowing or speeding up the rates of disease progression relative to an early summer temperature of ~27 ºC, which is well below temperatures causing bleaching in Florida (Manzello et al. 2007, Manzello 2015). Diseased corals were collected from sites in the Florida Keys under collection permit FKNMS-2019-160-A1 to Valerie Paul. Coral fragments were the maximum size allowed under the permit (20 cm diameter) to ensure they could be cut into at least 6 pieces for the experiments. Attempts were made to source corals from different locations based on availability of diseased colonies, assuming these represent distinct genotypes. However, only *M. cavernosa* corals were collected in two locations, Ft. Lauderdale and Florida Keys reefs near Marathon because we did not find *Colpophyllia natans* colonies on Ft. Lauderdale reefs.

Diseased corals were trimmed using a cleaned rock saw and each diseased lesion was cut into six fragments with relatively equal areas of living tissue and disease lesion. Each individual fragment was housed separately in aquaria with five liters of ocean seawater previously filtered through 0.22 µm-pore filters (FSW). Each coral was placed upon elevated plastic grating (1.5 cm x 1.5 cm spacing) with an air bubbler for aeration and water motion. The aquaria were housed in larger, insulated water tables with recirculating fresh water that is pumped through an insulated sump with heaters and a chiller to maintain a constant water temperature in each aquarium. For adequate replication, 6 large water tables were used, with two water tables randomly assigned for each of 3 temperatures levels. The experiment followed a split-plot design, where each of the 10 source corals (“genotypes”) constitutes the within-plot treatment, and the 3 temperature levels the between-plot treatment. Each genotype was cut into 6 approximately equal sized pieces, and one representative of each genotype was placed in each of the 6 water tables for a total $n = 60$. To prevent temperature shock, all corals were initially housed at the same temperature they were collected at in the field, then the temperature was slowly adjusted warmer or colder (1°C/day) to their corresponding experimental temperature. Water quality was maintained through partial water changes three times per week. Photographs of all fragments were taken at day 0 and 1 and every other day thereafter. An appropriate scale bar was included in each photograph, and distance to fragments, angle of camera, white balance and lighting were controlled to ensure comparable images are taken. The total apparently healthy tissue was measured from these photographs using the image analysis software Image J (https://imagej.nih.gov/ij/). Cumulative percent disease and disease progression rates

were calculated over time from these measurements. Corals were monitored for a maximum of 28 days. Assuming a modest ~10% difference in final percent cover, a preliminary power analysis suggested that our ability to detect a significant temperature effect could be achieved with as little as two-thirds of the proposed replication, suggesting 20 replicates per treatment is more than adequate to observe even a mild temperature effect (if present).

To determine whether changes in Symbiodiniaceae occurred in response to our temperature treatments, particularly at the higher temperatures, we removed small tissue biopsies (typically a few square millimeters) using a razor blade to use standard protocols to extract and purify DNA from these biopsies (Baker & Cunning 2016). Samples were shipped to Andrew Baker's laboratory at the University of Miami who have established quantitative PCR (qPCR) methods that are used routinely (Cunning and Baker 2013) to identify and quantify the relative abundance of algal symbiont communities (to the level of Symbiodiniaceae genus). Since colonies can contain multiple algal symbionts and the relative abundance of symbionts can vary over the colony surface, we took multiple (2-3) biopsies from the initial experimental fragments adjacent to the disease lesion and at standard distances of 2 and 4 cm away from the lesion and can relate them to a photograph taken of each fragment. Based on experience of the Baker laboratory, we did not expect Symbiodiniaceae communities to change very much in our aquaria in these short-term assays due to standard holding conditions, which mimic the temperature and light environment in the field; however, they may change in response to temperature treatments, especially as we near bleaching conditions. We will be able to compare the communities immediately after collection and at the start of the experiment with any changes that occur during the experiments to determine how temperature affects the symbiont communities.

All corals were tested after collection to detect a toxic zinc-metalloprotease produced by *Vibrio coralliilyticus*, VcpA. *V. coralliilyticus* may cause coinfections that make SCTLD lesions progress much more rapidly, and testing for VcpA is used to indicate the presence of this bacterial toxin (Ushijima et al. 2020). After collection, diseased corals were tested using the VcpA *RapidTest* cassette (mAbDx, Inc), an improved and simplified version of a 2-site immunoassay for VcpA. Samples of corals at the beginning and end of temperature treatments were also sent to Julie Meyer's laboratory at the University of Florida for microbiome analyses.

For data analysis, because the initial area of coral fragments varied over several orders of magnitude (0.795-39.067 $cm^2$), we expressed the response as the proportional change in healthy tissue by dividing the area of each individual at each time point by its initial area. A single excessively large individual of *C. natans* was removed from the analysis (58.454 $cm^2$).

To analyze proportional change in healthy tissue (0-100% of initial area), we fit generalized linear mixed effects models including both fixed and random effects using the *lme4* package (Bates et al., 2015). We modeled each species (*C. natans* and *M.*

*cavernosa*) separately but pooled across all sets of experiments for each species. For both models, we included the following fixed effects: a temperature treatment-by-day interaction, to test whether the effect of increasing temperature increased coral mortality through time; experiment, to test for differences in the timing and source populations for each experiment; and initial size, to account for differences in the amount of beginning tissue. We further fit a varying-intercept fixed-slope random effect of water table, as well as a random effect of individual, allowing both the intercept and slope to vary by day (accounting for the fact that some individuals declined more rapidly through time than others).

We initially fit each model to a binomial distribution to account for the fact that change is bound between 0-1, but to account for potential overdispersion, we additionally fit each model to a negative binomial distribution and compared the two models using Akaike Information Criteria (AIC). In the end, the binomial distribution was heavily favored for models of change in both *C. natans* ($\Delta$AIC = 419.5) and *M. cavernosa* ($\Delta$AIC = 1762.7). Finally, we assessed model assumptions of normality of errors and homogeneity of variance using the *DHARMa* package (Hartig, 2022). We examined the model output using Type II ANOVA as implemented in the *car* package (Fox & Weisberg, 2018). We held an experiment wide $\alpha = 0.05$ for assessing significance. We assessed the total $R^2$ explained by each model using the *piecewiseSEM* package (Lefcheck, 2016). All analyses were run in R version 4.2.0 (R Core Team, 2022).

## 3. RESULTS

All corals of both species (*Colpophyllia natans* and *Montastraea cavernosa*) for all experiments tested negative for toxic zinc-metalloprotease produced by *V. coralliilyticus*, VcpA, using the VcpA *RapidTest* cassette (mAbDx, Inc), an improved and simplified version of a 2-site immunoassay for VcpA. This means that temperature effects on *V. coralliilyticus*, which may cause coinfections that make SCTLD lesions progress more rapidly (Ushijima et al. 2020), were not driving the patterns we observed for relationships between temperature and rates of SCTLD progression.

The two infected coral species demonstrated different responses to increasing temperature in our experimental mesocosms. Across the two experiments, healthy tissue of *C. natans* declined more rapidly with increasing temperature: by the end of the experiment, this coral species had declined by -69.9 ± 10.3% (mean % change ± 1 standard error) in the 21°C treatment; by -83.8 ± 8.8% in the 26°C treatment; and by -93.3 ± 6.7% in the 31°C treatment (Figure 1). In contrast, *M. cavernosa* declined much less over the duration of the experiments and had a parabolic relationship with temperature: healthy tissue for this species declined by -20.0 ± 5.4% in the 21°C treatment; by -39.6 ± 8.2% in the 26°C treatment; and declined by a more modest -29.9 ± 7.7% in the 31°C treatment (Figure 1).

The trajectory of individual fragments varied widely (Figure 2). In the first set of experiments, declines were, on average, more drastic for *C. natans* than the second

experiment (-90.7 vs. -64.1%), whereas loss of healthy tissue in *M. cavernosa* was higher in the second experiment than in the first or third (-52.4 vs. -22.6 and -25.7%, respectively), keeping in mind that, unlike *C. natans*, individuals of *M. cavernosa* were from different source populations for the different experiments. A higher proportion of *C. natans* survived until the end of the second experiment (5 out of 16 replicates) than in the first (4 out of 32 replicates), and again, the reverse was true for *M. cavernosa* (32 out of 36 replicates remaining in the first experiment and 26 out of 34 in the third experiment vs. 6 of 16 in the second experiment). On average, a fragment of *C. natans* lasted 24.5 days until losing all tissue to disease in the 21°C treatment versus just 9.6 days in the 31°C treatment. In contrast, *M. cavernosa* lasted 28.6 days on average at 21°C and 27.3 days at 31°C, but only 24.5 days at 26°C.

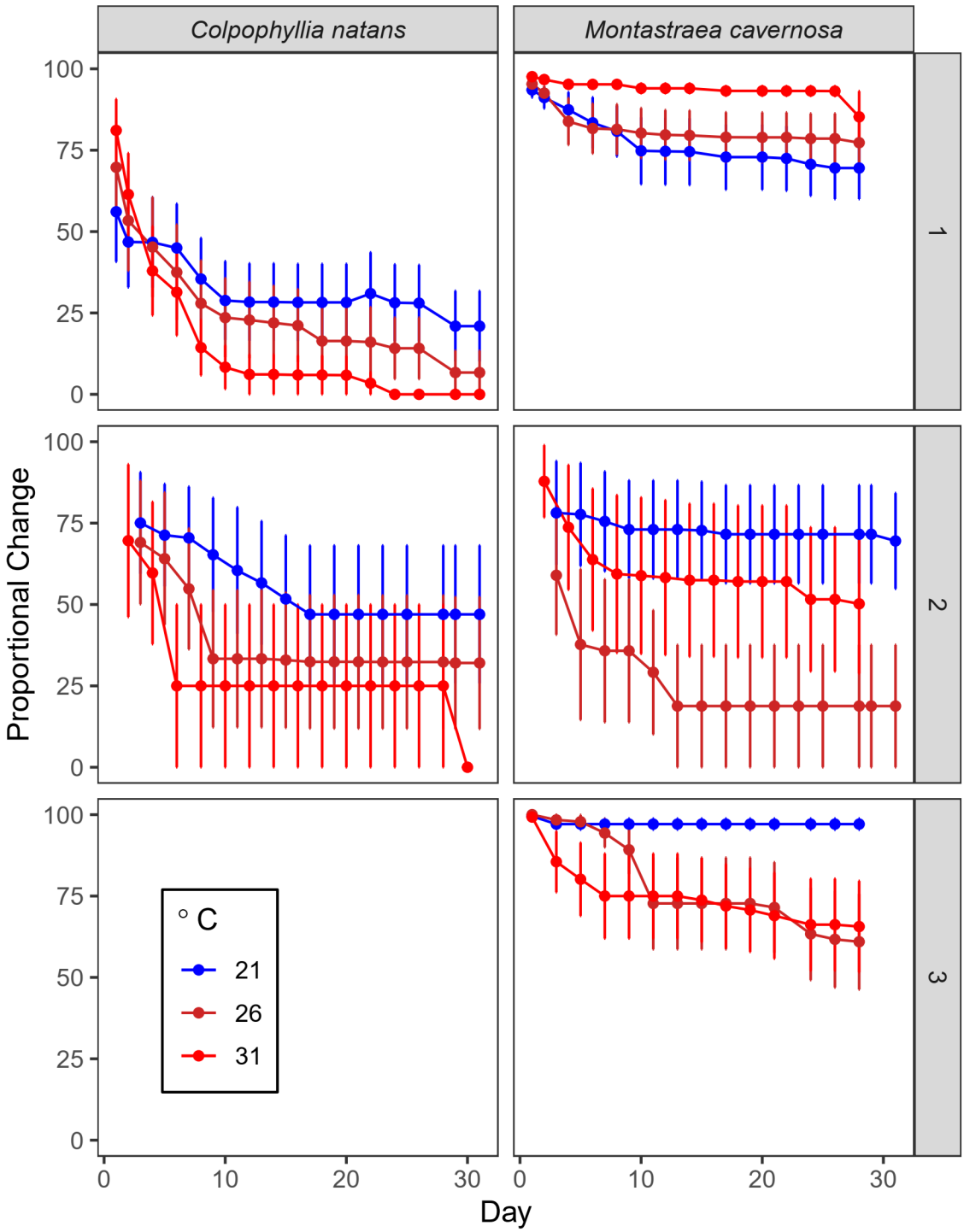


***Figure 1.*** *Change in the proportion of healthy tissue over time for the two species of diseased corals (columns), and for the different sets of experiments (rows). Values are averages ± 1 standard error of the mean.*

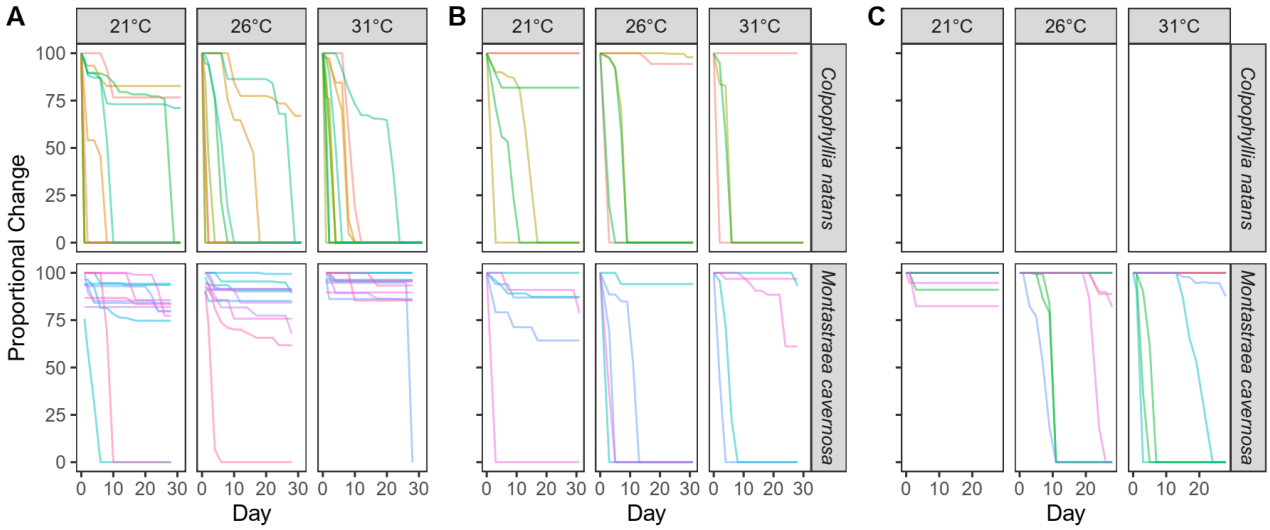


***Figure 2.*** *Trajectories of individual fragments of* Colpophyllia natans *and* Montastraea cavernosa *in* ***A)*** *the first experiment,* ***B)*** *the second experiment, and* ***C)*** *the third experiment, involving M. cavernosa only.*

Our statistical models revealed a strong interaction between the temperature treatments and time for *C. natans* ($P < 0.001$; Table 1). Examination of the linearized coefficients revealed that loss in tissue accelerated by 12.3% with each additional day over the course of the two experiments, and the decline was steepest for the 31°C treatment and shallowest for the 21°C treatment (Figure 3A). Additionally, initial fragment size was significant ($P < 0.001$) and for each additional $cm^2$, the individual was 51.5% more likely to survive. The response of *C. natans* did not differ among the two experiments ($P = 0.060$). In total, the model explained $R^2 = 74.2\%$ of the total deviance in the proportion of healthy tissue lost through time based on the fixed effects only, with an additional 23.8% of variance explained by the random effects of individual and block.

In contrast to *C. natans*, *M. cavernosa* exhibited no main or interactive effect of temperature treatment (Table 2). Nor did initial size significantly affect the progression of the coral disease in this species ($P = 0.79$), although we note that fragments of this species had a 25% smaller range in initial area than *C. natans*. Interestingly, experiment—a proxy for source population, as the first experiment retrieved corals from Ft. Lauderdale, and the second and third from the Florida Keys—was a highly significant predictor ($P < 0.001$, Table 2). After accounting for expected declines through time (Figure 1), individuals of *M. cavernosa* from Ft. Lauderdale were expected to generally fare well irrespective of temperature, while those from the Keys experienced more severe declines on the order of 75-84% healthy tissue remaining, on average, at the end of experiments two and three (Fig. 3B). Notably, the variance in tissue remaining was much greater in the second and third experiments, suggesting highly variable responses in individuals from this population compared to the first experiment (Fig. 3B). However, we note that the total deviance explained by the fixed effects only was $R^2 = 4.6\%$, with a

further 93.2% explained by the random effects. Thus, the progression of this disease is overall less severe and less predictable for *M. cavernosa* than for *C. natans.*

***Table 1.*** *ANOVA results for a model of change in healthy tissue of* Colpophyllia natans *across both experiments.*

| Predictor | $\chi^2$ | d.f. | *P*-value |
|---|---|---|---|
| Temperature | 0.903 | 1 | 0.342 |
| Day | 7.332 | 1 | 0.007 |
| Initial area ($cm^2$) | 39.339 | 1 | <0.001 |
| Experiment | 3.533 | 1 | 0.060 |
| Temperature × Day | 21.844 | 1 | <0.001 |

***Table 2.*** *ANOVA results for a model of change in healthy tissue of* Montastraea cavernosa *across all experiments.*

| Predictor | $\chi^2$ | d.f. | *P*-value |
|---|---|---|---|
| Temperature | 1.326 | 1 | 0.250 |
| Day | 22.342 | 1 | <0.001 |
| Initial area ($cm^2$) | 0.071 | 1 | 0.790 |
| Experiment | 23.795 | 2 | <0.001 |
| Temperature × Day | 0.747 | 1 | 0.388 |

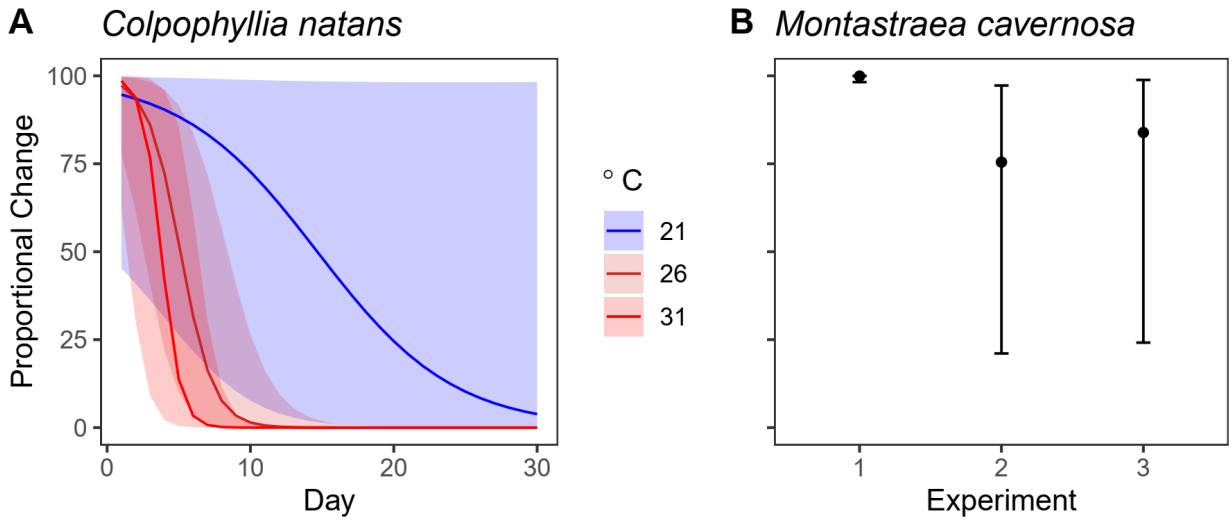


***Figure 3. A)*** *Predicted fits ± 95% confidence intervals from a generalized linear model of the proportional change in healthy tissue of* C. natans *as a function of both time and temperature treatments.* ***B)*** *Predicted means ± 95% confidence intervals from a second generalized linear model of the proportional change in healthy tissue of* M. cavernosa *as a function of experiment (source population).*

## 4. DISCUSSION

Rather than showing a similar response to temperature for the two species of corals, *Montastraea cavernosa* and *Colpophyllia natans*, the results of this study demonstrate a striking difference in the disease progression rates for these two common coral species on Florida's Coral Reef in response to different temperatures. Diseased *C. natans* corals had a strong negative response to increasing temperature, and amount of live tissue on *C. natans* fragments declined more rapidly with increasing temperature. In contrast, *M. cavernosa* declined much less over the duration of the experiments overall and did not show a strong temperature effect in terms of tissue loss during the experiment. The different rates of disease progression match the overall susceptibility of the two coral species, with *C. natans* listed as highly susceptible to disease and *M. cavernosa* listed as medium susceptibility to SCTLD (https://floridadep.gov/rcp/coral/documents/stony-coral-tissue-loss-disease-sctld-case-definition).

Little was known about the temperature responses of different corals to SCTLD. No experimental studies had been previously conducted where temperature effects were studied under controlled conditions. Conducting these experiments did have challenges. Once the corals were cut into small fragments, some of them, especially *C. natans* fragments, died relatively quickly. In some cases, fragments died during the acclimation period when the corals were slowly adjusting to their assigned water temperatures at a rate of 1 ºC per day and never made it into the experiment. This meant that there were not always the same number of fragments in each water table at each temperature. Fortunately, we still had an adequate sample size for all experiments and the results of statistical analyses were quite clear. We also observed a pattern that has been observed for diseased *M. cavernosa* corals in past studies that the disease on some of these fragments stops progressing. This also happens on the reef with *M. cavernosa* and is probably related to interactions between host, pathogen(s), and environmental conditions, which we do not understand for SCTLD.

The very different responses of these two species are somewhat surprising and may be related to their different symbiont types (*Cladocopium* for *M. cavernosa* colonies and predominately *Breviolum* for the *C. natans* colonies), their microbiome, previous exposure to the disease, or other undetermined factors. Some studies have suggested that thermal stress, which causes loss of symbionts and bleaching, reduces SCTLD progression in diseased colonies (Meiling et al. 2020). We did not reach temperatures that normally cause bleaching for Florida corals, which can regularly experience temperatures of 32 ºC in summer months (Muller et al. 2020). Understanding the role of thermal stress on corals with SCTLD could be the subject of future investigations.

It is important to note that all individuals used in our experiments were already infected with stony coral tissue loss disease. Therefore, we cannot disentangle the independent roles of disease and temperature on coral survival. However, uninfected corals have been

maintained over this range of temperatures in our aquaria as well as by other organizations, so we are confident that the differences we witnessed were in fact due to the interactions of the disease with our experimental treatments. Further, it does not reflect the susceptibility of corals to the infection under different temperature regimes. Further experiments could be useful in determining how susceptible uninfected corals are to the disease under different temperature regimes.

These findings have management implications because they show that different diseased coral species respond differently to seawater temperature and there is no uniform relationship with temperature and disease progression rates among different coral species. This is important information and may have implications for managing reef activities such as construction, outplanting, and treating of diseased corals. It suggests that some activities such as construction and outplanting of some species, especially with more susceptible species such as *C. natans,* should be limited during warmer times of the year. Treatment of diseased corals could be enhanced in the summer months. In contrast, outplanting of *M. cavernosa*, which has a lower response to temperature when corals are diseased, could proceed. Looking at natural patterns of disease emergence and subsidence among different coral species on the reef under seasonal changes in temperature would be important to expand the findings of this study to more species of corals in their natural environment. This could be complemented with additional aquarium studies under controlled conditions with certain abundant species of corals to continue to explore the differences in responses of additional species of corals to SCTLD.

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