Can SCTLD-susceptible species be outplanted on Florida's Coral Reef with acceptable survival rates to warrant larger-scale coral restoration efforts to commence?

Restoration Team

September 2021

Executive Summary

A priority of Florida's Stony Coral Tissue Loss Disease (SCTLD) Response Restoration Team is to contribute to an understanding of whether SCTLD-susceptible coral species can be outplanted with acceptable survival rates to warrant large-scale coral restoration efforts using these foundational species. In advance of a project that directly tests this question, the Restoration Team compiled information from ongoing coral outplanting projects across Florida's Coral Reef. The following document includes findings on coral survivorship and, as applicable, disease prevalence on SCTLD-susceptible corals outplanted in 2018 and 2019 by five partner organizations: Mote Marine Laboratory, University of Miami, Coral Restoration Foundation, Florida Fish and Wildlife Conservation Commission, and The Nature Conservatory. To complement this document, the Restoration Team has developed a detailed spreadsheet to track ongoing outplanting efforts that include SCTLD-susceptible species. This spreadsheet can be made available to restoration partners upon request.

Based on the reports from partner organizations, SCTLD is not a major factor influencing outplant survival rates in Florida using SCTLD-susceptible species. However, differences in restoration efforts, such as location, species, coral density, colony size, time of outplanting, and genetic diversity prevent definitive conclusions. Predation, however, was routinely noted as a potential limitation to outplant survival and ultimately restoration success. Survivorship and disease prevalence of these restoration efforts, as well as others occurring with SCTLD-susceptible species, will continue to be tracked and this document will be updated as warranted.

Background

Most coral restoration along Florida's Coral Reef ("FCR"; also referred to as the Florida Reef Tract or FRT) to date has focused on *Acropora cervicornis* (staghorn coral) and *Acropora palmata* (elkhorn coral). Fortunately, these species do not appear to be susceptible to stony coral tissue loss disease (SCTLD) (Restoration Team, 2020). However, in recent years, several organizations have broadened their restoration efforts to include boulder and brain corals. While these coral species are important for Florida's coral community composition and are the main reef builders of FCR, they are also some of the most susceptible species to SCTLD. In light of these recent efforts, as well as the need to rebuild SCTLD-impacted species, the Restoration Team ("RT"; previously called the Restoration Trials Team) identified the need to better understand the successes and risks of using SCTLD-susceptible species in coral restoration efforts.

In April 2019, the RT created an Action Plan that identified priority restoration research questions to be addressed in the context of conducting restoration within the SCTLD outbreak. One of the questions identified as a priority was: "Can SCTLD-susceptible species be outplanted on the FRT with acceptable survival rates to warrant larger-scale coral restoration efforts to commence?" The RT Action Plan recommended that a cooperative, multi-organizational, multi-species coral outplanting effort using SCTLD-susceptible species be conducted along FCR in endemic, epidemic, invasion, and pre-invasion disease zones (see Table 1 below for descriptions of zones), at both nearshore and offshore reef habitats, to address this priority question. However, at the time of project implementation (May 2021), the endemic zone covered the entire reef tract from Martin County to the Marquesas with only the Dry Tortugas region being absent of SCTLD until summer 2021. As this project is in its early stages and will take several years to complete, the RT has compiled information of ongoing restoration activities that include SCTLD-susceptible species to elucidate the fate of boulder and brain corals outplanted in SCTLD-affected areas.

Disease Zones

Throughout this document, data is presented within a contextualized framework that describes the status of SCTLD within the outplanted sites. These are largely characterized as four different zones that describe stages of spatial and temporal progression of SCTLD throughout FCR (Table 1). The stage at which SCTLD is affecting the natural coral community within site locations at the time of outplanting, and thereafter will, in turn, affect the vulnerability of outplanted corals to SCTLD.

Table 1. The four stages of stony coral tissue loss disease invasion throughout Florida's Coral Reef (*sensu* Langwig et al. 2015)

Zone Name	Disease Characteristic
Vulnerable or Pre-Invasion	Disease absent
Invasion	Disease has just occurred
Epidemic	Moderate to high disease activity
Endemic	Disease at chronic low levels; been present for > 1 year

Objective

The primary goal of this document is to utilize field-based restoration efforts currently underway to help understand when and where it may be acceptable to continue coral restoration activities using historical outplant rates and practices. In effect, any field outplanting within FCR or directed experiment using susceptible coral species will have the opportunity to help address this question. This document summarizes the efforts and survival rates of susceptible coral species that have been outplanted on FCR within the SCTLD outbreak between 2018 and 2019. To note, these various restoration endeavors and their associated monitoring were not designed to be directly comparable to one another. Still, when pulled together, they provide a current, general understanding of the initial fate of SCTLD-susceptible boulder and brain corals outplanted within the SCTLD outbreak area.

Timeframe

This document concerns SCTLD-susceptible corals outplanted between 2018 and 2019 along FCR and covers any monitoring of those outplants through February 2021. This timeframe allowed for better comparability among the different efforts through evaluation across somewhat similar monitoring timeframes. This timeframe excludes outplanting activities and associated monitoring that may have been impacted by Hurricane Irma in 2017.

Outplant Species

Coral restoration efforts along FCR included six SCTLD-susceptible species: *Montastraea cavernosa* (Mcav), *Orbicella faveolata* (Ofav), *O. annularis* (Oann), *Pseudodiploria clivosa* (Pcli), *P. strigosa* (Pstr), and *Siderastrea siderea* (Ssid). Of these species, the two brain corals (Pcli and Pstr) are considered highly susceptible to SCTLD while the four boulder corals (Mcav, Ofav, Oann, and Ssid) are considered intermediately susceptible to SCTLD in Florida (SCTLD Case Definition, 2018). However, it should be noted that susceptibility may be temporally or spatially specific. For example, reports from outside Florida indicate that certain intermediately susceptible species, particularly Mcav, appear to be highly susceptible in other regions.

Partner Organizations

Five RT partner organizations contributed to the reports within this document: Mote Marine Laboratory (MML), Coral Restoration Foundation (CRF), University of Miami (UM), The Nature Conservancy (TNC), and Florida Fish and Wildlife Conservation Commission (FWC). In total, between 2018 and 2019, the five partners outplanted 6,242 SCTLD-susceptible corals. By species, partners outplanted: 2,931 Ofav, 619 Oann, 1,383 Mcav, 1,219 Pcli, 76 Pstr, and 10 Ssid. A summary of outplants can be found below as Table 2.

Table 2. Number and region of boulder and brain corals outplanted by Restoration Team partners Mote Marine Laboratory (MML), University of Miami (UM), Coral Restoration Foundation (CRF), The Nature Conservancy (TNC), and Florida Fish and Wildlife Conservation Commission (FWC) within 2018 and 2019. Outplanted species include *Orbicella faveolata* (Ofav), *O. annularis* (Oann), *Montastraea cavernosa* (Mcav), *Pseudodiploria clivosa* (Pcli), *P. strigosa* (Pstr), and *Siderastrea siderea* (Ssid). Wk: week; Mo: month, Yr: year

			# of Outplants							
Organization(s)	**Location**	**Disease Stage**	**OFAV**	**OANN**	**MCAV**	**PCLI**	**PSTR**	**SSID**	**Monitoring Frequency**	**Final Monitoring**
CRF	Upper Keys	Endemic	1250	619	--	--	--	--	1 mo; 1 yr	1 yr
FWRI/MML	Middle Keys	Endemic	120	--	120	120	--	--	1, 2, 6, 12 wk; 10 mo; 15 mo	15 mo
MML	Key West	Vulnerable/Invasion /Endemic	470	--	210	240	--	--	Monthly	1 yr
MML	Lower Keys	Invasion/Endemic	680	--	725	505	--	--	Monthly	1 yr
TNC/FWC/MML	Lower Keys	Endemic	117	--	120	119	--	--	Monthly	5 mo
TNC/FWC/MML	Middle Keys	Endemic	118	--	120	120	--	--	Monthly	5 mo
UM	S. Florida	Endemic	176	--	88	115	76	10	1 mo; 6 mo	6 mo

Summary of Outplant Survival

Partners reported survival rates ranging from 20 - 99% across different disease zones, locations, outplanting methodologies, species compositions, and monitoring methods and timeframes. These ranges depict low survival of Ssid in the endemic zone (20%) compared to Ofav, Mcav, and Pcli originally outplanted within the pre-invasion zone, but monitored as the sites became a part of the invasion zone (99%). Outplants occurring within the endemic zone (i.e., SCTLD had been present for at least a year within the region) showed an average 71.52% survival rate when including those with monitoring data at least 5 months post outplant. While the projects may not be directly comparable, the high survival rates of several SCTLD-susceptible species, reported across all outplanting efforts represented a pattern of general success when outplanting SCTLD-susceptible corals within the endemic zone. In general, disease was not observed as a major limiting factor associated with survival rates. However, most monitoring events were not focused on observing SCTLD effects, which requires high frequency monitoring. Interestingly, predation effects and dislodgement/missing corals were routinely identified as a threat to survival.

Below are summaries of the reports of outplanting efforts using SCTLD-susceptible corals between 2018 and 2019.

It should be noted that the RT is also collating more detailed information on ongoing SCTLD-susceptible coral outplanting activities across FCR. That information will be used to update this document, as necessary and appropriate. The detailed information can be provided to restoration partners upon request.

Mote Marine Laboratory (MML)

Between September and November 2018, MML outplanted a total of 1,930 colonies of Mcav, Ofav, and Pcli at nearshore, midchannel, and offshore sites near Big Pine/Summerland Key and Key West. These corals were outplanted for the explicit task of monitoring for the presence of disease and determining if there was genetic variation to susceptibility. Survival and disease prevalence rates reported here cover a twelve-month monitoring period. The full report from MML, including information on genotypic variability, can be found as Appendix 1.

The average survival rate across all sites at the twelve-month monitoring event was ~51%. Survival rates were highest at nearshore sites: 85.5% survival at the inshore site near Big Pine/Summerland Key and 99.6% survival at the inshore site near Key West. Early mortality (i.e., at the one-month monitoring event) was driven by dislodgement or poor adherence to the substrate, fish predation, or sedimentation as evidenced by missing plugs, obvious bite marks, and burial.

At the MML outplant sites, there was an approximate three-month lag between initial outplanting and the appearance of SCTLD on outplants. Qualitative observations suggest that disease prevalence of outplanted corals correlated to general disease prevalence within each site. Disease prevalence varied across time, species, and locations. Cumulative disease prevalence was highest at Big Pine/Summerland outplant sites as compared to Key West on all species initially, but by the one-year period the disease prevalence within the two regions was comparable. However, the midchannel sites had higher disease prevalence than either the nearshore or offshore sites. It should be noted, however, that Key West sites were not affected by SCTLD at the time of outplanting (i.e., within the vulnerable zone) and one site (nearshore) showed no disease signs throughout the entire one year of monitoring.

In addition to this disease transmission study, MML outplanted 450 susceptible corals in late 2019 for restoration purposes. These corals were dispersed among two recipient sites, one offshore (35 Ofav, 55 Mcav and 15 Pcli) and one nearshore site (115 Ofav, 95 Mcav and 135 Pcli) following best-practices developed during the

aforementioned transmission study. These practices include outplanting only to raised substrate (dead coral skeleton), and planting tightly clustered arrays of 10-20 coral.

Monitoring at month 1 showed an overall survival of 90% nearshore and 100% offshore, with all nearshore losses directly attributed to fish predation (complete coral removal from plug). Twelve-month monitoring has been conducted (nearshore) or is planned (offshore), with initial results showing 78% site survival (ranging 71.1% for Pcli, 75.7% for Ofav and 90.5% for Mcav). No disease lesions were recorded at these sites. Losses were primarily attributed to predation and unknown factors, which could represent mortality caused by disease that was not observed during the tissue loss process.

University of Miami (UM)

UM outplanted over 450 fragments of massive corals in 2018 and 2019 in the Key Biscayne area. These outplants included Mcav (88), Ofav (176), Pcli (115), Pstr (76), and Ssid (10). Outplants were generated by fragmenting colonies collected around the Miami area (including from the natural reef and from a sea wall). All fragments were held within in-water nurseries for approximately three months prior to outplanting onto the reef. Outplants were monitored for survival one month and six months after outplanting. The full UM report can be found as Appendix 2.

While there was high survivorship of fragments in the nurseries (>80%), there was highly variable survivorship post-outplanting to the natural reef. Survivorship did not change substantially between the two monitoring periods. At six months, survivorship for each species was: Mcav 31.8%, Ofav 76.6%, Pcli 60.9%, Pstr 31.6%, and Ssid 20.0%, with a total average of 44.18% overall survival. However, mortality appeared to be driven by fish predation and fragment dislodgement. No active disease was reported for any fragments in the nurseries or on outplants.

Coral Restoration Foundation (CRF)

CRF outplanted 1,168 Ofav and 629 Oann corals at Carysfort Reef in 2018 and 2019. Reported below are survival rates at approximately one-year post outplanting. It should be noted that monitoring was conducted according to putative genotype, and survival across genotypes was variable. The full CRF report, including one-month monitoring information, can be found as Appendix 3.

One-year post-outplanting, CRF found generally high, but variable, survival across genotypes of outplanted *Orbicella*. Across both species, eight of eleven genotypes (representing 501 Ofav clusters and 567 Oann clusters)

experienced ≥78% survival. The remaining three genotypes (representing 120 Ofav clusters and 60 Oann clusters) experienced 0-42% survival. Overall, 65.2% of the Ofav survived and 80.6% of Oann survived at 12 months after outplanting for a total average survival of 72.9%. High mortality occurred for many of the very first clusters CRF outplanted. Since then, CRF has improved outplanting techniques to decrease algae overgrowth and ultimately increase long-term survivorship. CRF did not report any active disease on their outplants and noted that while disease may be a likely cause, mortality could also have been driven by other factors such as unsuitable outplant location or predation.

Florida Fish and Wildlife Conservation Commission (FWC)

In November 2019, FWC established six outplant sites in the middle and upper keys: one offshore and one inshore site each at Pickles Reef, Tennessee Reef, and Delta Shoal. The experimental design included a total of 360 coral outplants, evenly distributed across three species: Mcav, Ofav, and Pcli. At each of the six sites, 20 colonies of each species were randomly outplanted in 4x15 arrays. Survival rates reported here are from the end of a 12-week followed by a 15-month monitoring period. The full report, including information on SCTLD incidence in the surrounding reef environment, is included as Appendix 4.

During the 12-week monitoring period, FWC reported that only nine outplants had died and four had gone missing, representing ~96% survival. All nine dead outplants were Pcli. FWC did not observe any clear incidence of SCTLD affecting the outplants. In fact, during the six-week post-outplant monitoring event, FWC marked 5 colonies that exhibited tissue loss that was possibly SCTD-related, but no evidence of disease was noted on these colonies at the end of the 12-week monitoring period. By the 15-month monitoring period there was still an overall survival rate of 76.4% with Mcav (80.8%) and Pcli (79.2%) showing higher survival rates compared with Ofav (69.2%).

As noted by other partners, fish predation is considered a likely driver of some tissue loss and/or mortality. During the one-week post-outplant monitoring event, 193 outplants (~55%) had evidence of fish predation. Through time, the proportion of colonies showing evidence of recent fish predation decreased progressively and few colonies showed evidence of predation at the conclusion of the 12-week monitoring period. Minimal subsequent predation effects were documented at the 15-month monitoring period.

The Nature Conservancy (TNC) w/ FWC & MML

TNC collaborated with FWC and MML on a project to determine the levels of maintenance required for massive coral outplants to overcome the competitive pressures of macroalgae and *Palythoa.* As part of this project,

researchers noted the condition of all coral outplants, including the presence of disease. While the project includes outplanting in 2016 and 2019, this summary only includes information from the 2019 cohort. The full report, including the fate of the 2016 cohort, can be found as Appendix 5.

In 2019, a total of 534 corals of three SCTLD-susceptible species were outplanted: 240 colonies of Mcav, 235 Ofav, and 239 Pcli. The 2019 outplant cohort was monitored monthly for five months. Due to the size of the outplants, cause of mortality (including from disease) was difficult to identify. While overall disease prevalence appeared low in the outplants, the highest prevalence was noted within two weeks of outplanting. The majority of mortality, estimated at approximately 20% (i.e., 80% survival), was from dislodgement/missing corals and finfish predation.

Conclusions

Efforts to outplant corals of susceptible species within the area affected by the SCTLD outbreak have shown minimal disease-associated mortality, especially within long-impacted endemic areas (i.e., South Florida and Upper Florida Keys). However, it is important to note that few studies conducted monitoring frequently enough to document SCTLD incidence appropriately (i.e., at least monthly) and data also suggest that 'unknown' mortality can contribute to substantial percentages of outplanted coral loss, which likely includes disease-associated mortality. The highest mortality rates directly attributed to disease included corals outplanted directly within the invasion zone (Lower Florida Keys and Key West) in late 2018, although disease prevalence only affected a total of ~10% of the fragments 12 months after outplanting. There was also notable variation in disease prevalence among sites, as well as among species and genotypes outplanted.

While these various outplanting and monitoring efforts were not designed to be directly comparable, the common relatively high survivorship of SCTLD-susceptible outplants, especially by well-established restoration practitioners within the endemic zone allows us to draw some tentative conclusions. It appears that, at the present scale and locations of outplanting, SCTLD has limited impact on susceptible coral outplant survivorship especially compared with other causes of mortality. No project reported an outbreak of SCTLD comparable to what has been observed in natural reef communities. It appears that outplanting within the endemic zone one- to two-years after the initial SCTLD outbreak, especially when following best practices, can have high success. It is recommended that these tentative conclusions be more thoroughly tested via a cooperative, multi-organizational, multi-species coral outplanting effort using SCTLD-susceptible species along FCR especially within the endemic zone, at appropriate habitats deemed high priority for restoration within the near future.

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APPENDIX 1

Mote Marine Laboratory Outplant Case Study: outplanting susceptible coral species within the stony coral tissue loss disease outbreak

Mote Marine Laboratory
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In August 2018, a case study was presented to the Restoration Trials Team (RTT) and subsequently approved, which aimed to quantify the following objectives:

Objectives

1) Quantify the impacts of stony coral tissue loss disease on outplanted microfragmented corals that were previously raised within a land-based nursery.
2) Identify whether any coral genotypes are resistant to the stony coral tissue loss disease.
3) Determine whether susceptibility of outplanted restoration corals changes through time.

In September 2018, after approval by the RTT, permission was provided by FWC and FKNMS allowing for the limited outplanting of *ex-situ* (land-based) corals. Permit conditions required that initially less than 2,000 corals be outplanted, that sites cover several of the habitats present throughout the region (inshore, midchannel, and offshore), and that additional monitoring be undertaken to assess for potential impacts due to the stony coral tissue loss disease (SCTLD) outbreak. Results from this initial monitoring were then used by the permitting agencies to make further decisions regarding potential restrictions placed on the release of corals from the *ex-situ* nursery.

Methods

A total of 1,930 massive corals were planted across six sites between September and November 2018, distributed along two cross-shelf transects that each included a nearshore, midchannel, and offshore site off of both the Lower Keys (Big Pine/Summerland Key) and off Key West (Table 1). Five clonal fragments were attached onto coral skeletons or reef substrate in arrays approximately 4-6" in diameter by inserting the post of each coral plug into a hole drilled in the dead substrate (Figure 1). A small amount of cement was used to attach the coral fragment and its associated ceramic plug flush to the surface of the reef. Once secured, the coral fragments grow over the ceramic plug, forming a new colony permanently affixed to the reef. These corals were outplanted for the explicit task of monitoring for the presence of disease and determining if there was genetic variation to susceptibility. All genotypes that were used to assess genetic variation (i.e., were present at more than one site) had been identified as unique genets using either microsatellite loci (*O. faveolata*, *M. cavernosa*) or 2bRAD sequencing (*P. clivosa*). Most genotypes were represented at all six sites. A small tag imprinted with a unique identifier was attached to the bare substrate directly adjacent to each array using a 1" masonry nail to permanently identify each array of coral fragments for monitoring. Sites were monitored for active disease approximately monthly for the first year post-outplanting, with data collection including documentation of survival/mortality, adherence to the reef, predation, and active disease lesions.

Table 1. Summary of ex-situ grown massive coral fragments outplanted for this project. A * denotes invasion zone at time of outplanting

Site	Species			Total Fragments Outplanted
	MCAV	OFAV	PCLI	
*Lower Keys - Nearshore	60	135	60	255
*Lower Keys - Mid Channel	75	150	90	315
*Lower Keys - Offshore	90	245	105	440
Key West - Nearshore	60	120	60	240
Key West - Mid Channel	75	150	75	300
Key West - Offshore	75	200	105	380
Total	**435**	**1000**	**495**	**1930**

Figure 1. Photos of outplanted *Pseudodiploria clivosa* (left) and *Orbicella faveolata* (right) at Lower Keys offshore site.

Results

Twelve months of monitoring was conducted for 1,930 massive corals outplanted across six sites distributed throughout the Lower Keys and Key West for the explicit task of monitoring for the presence of disease and determining if there was genetic variation to disease susceptibility. Disease was first noted in low levels on outplanted corals in month three in the Lower Keys (2% diseased fragments), and month four in Key West (<1% diseased fragments).

The presence of active disease lesions sharply increased through months six and eight of the study. While no new active disease was recorded at the 12-month timepoint, new mortality was noted at most sites (Figure 2). The cumulative total of active disease lesions to month 12 was highest in *M. cavernosa* fragments, with 13% (Lower Keys) and 15% (Key West) of coral outplanted, followed by *O. faveolata* (11% and 15% respectively), and *P. clivosa* (2% for both locations). Mid channel sites were the locations of highest disease prevalence averaging ~20% disease for all coral combined, and nearshore sites showed minimal disease activity (<1% of coral species combined). While not quantified, diver observations also suggest that disease rates were higher on wild corals than *ex-situ* raised and outplanted coral during this study.

Comparing the nearshore, mid-channel and offshore sites over the 12-month period, it is evident that coral survival was highest in nearshore sites throughout the study period, with 90% of outplanted coral surviving. Survival in mid-channel

and offshore sites declined rapidly from month six (Figure 3), with 38-41% survival respectively at 12-months post-outplant. This high intensity monitoring has further generated data on site suitability and informed outplanting methodology and habitat selection for the outplanting of boulder corals across the shelf in the lower Florida Reef Tract. Offshore and Mid-channel sites remain locations of lowest coral survival, with mortality related to sedimentation, algal overgrowth or other unknown causes, which could include disease-related mortality that was not observed. This study indicates that in the Lower Keys near-shore sites remain the strongest candidates for outplanting of boulder corals for restoration purposes regardless of SCTLD activity (Figure 3).

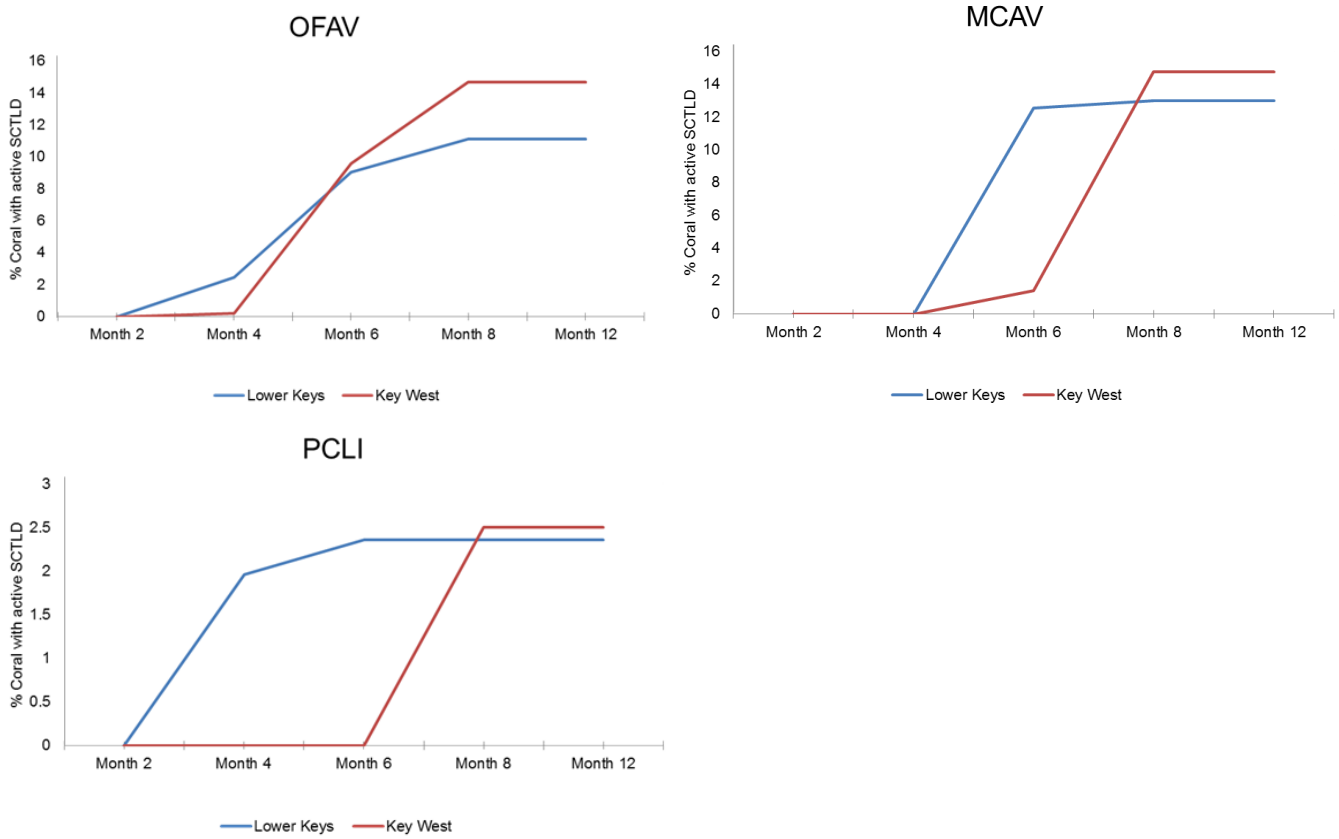

Figure 2: Active cumulative disease presence on three boulder coral species (*Orbicella faveolata* (OFAV), *Montastraea cavernosa* (MCAV) and *Pseudodiploria clivosa* (PCLI)) as a percentage of total outplants during twelve-month monitoring of boulder corals outplanted for disease transmission studies in Key West and the Lower Keys.

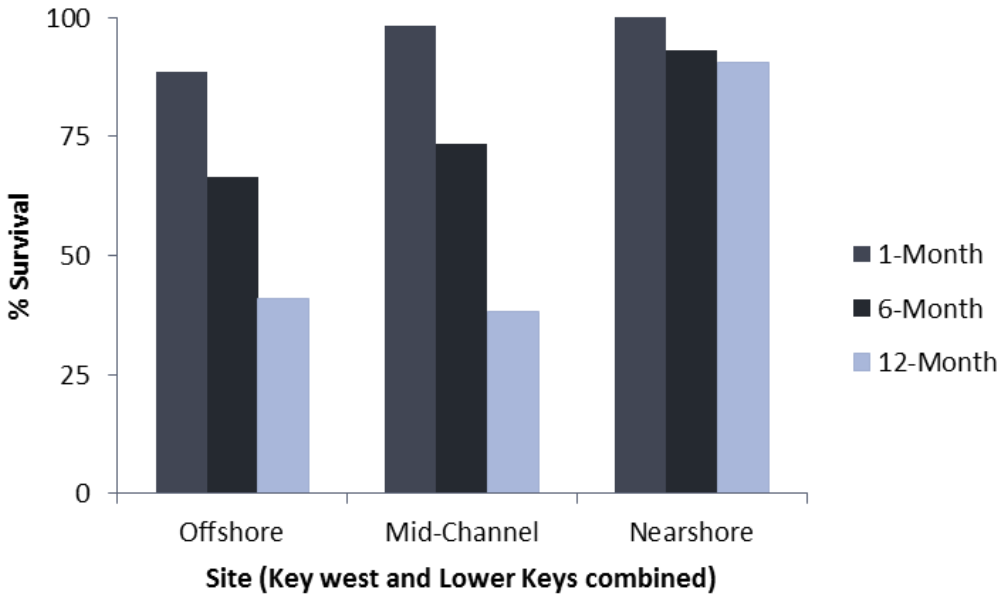

Figure 3: Comparison of massive coral survival for 12-month disease transmission study conducted at two replicate offshore, mid-channel and nearshore sites in the Lower Keys and Key West.

Genotypic susceptibility

Disease prevalence ranged from 0 to 24% within individual genotypes (Figure 4), which appeared largely driven by the outplant site. For example, genotype F27 (*O. faveolata*), which was outplanted at all six sites, showed little disease-related mortality at offshore and nearshore sites but had 93% and 33% disease prevalence at the midchannel sites. High disease prevalence at only certain sites also occurred for several other genotypes, suggesting that site specific effects have significant influence on disease prevalence of outplants. Additionally, disease prevalence may need to reach a particular threshold before genotypic susceptibility can be quantified within the field. One genotype, D12 (*P. clivosa*), was outplanted at every site and showed no disease-related mortality after 6 months, suggesting this genotype may be more resistant to stony coral tissue loss disease compared with others. However, follow-up monitoring, paired with laboratory trials, may be needed to provide more confident conclusions on genotypic susceptibility.

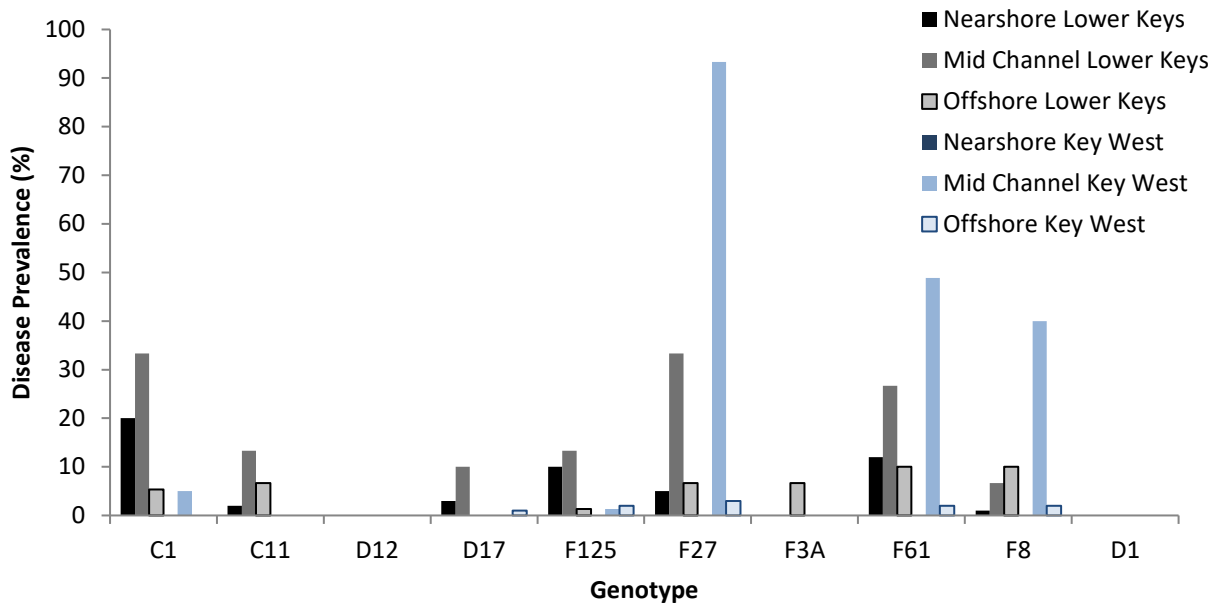

Figure 4. Prevalence of stony coral tissue loss disease of outplanted genotypes within each site. Only the genotypes outplanted at two or more locations are included. Genotypes with 'C' represent *Montastraea cavernosa*, 'D' represent *Pseudodiploria clivosa*, and 'F' represent *Orbicella faveolata*.

Conclusions

Our study suggests that there may be a time lag of at least 3 months post outplant before disease activity is observed on outplanted fragments from the Mote's *ex-situ* nursery, even within the invasion zone. Qualitative observations also suggest that disease prevalence of the outplanted corals correlates to general disease prevalence within each site. Additionally, although the Key West sites were initially within the pre-invasion zone at the time of outplanting, disease was present at the Key West sites within 4 months post outplanting (i.e., within the invasion zone). Finally, variation in genotypic susceptibility was observed, but was generally overwhelmed by site specific patterns. Continued monitoring of long-term survival of genotypes within sites that experience high disease prevalence will help confirm genotypic resistance to stony coral tissue loss disease.

APPENDIX 2

Outplanting of microfragmented massive corals in Miami-Dade County

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The University of Miami's Reef Restoration Program has outplanted > 450 small (<4 cm in diameter) fragments of massive corals from 2018-2019 (Table 1). These corals were sourced from colonies collected from reefs in the area around Key Biscayne and Miami Beach as well as corals collected from a seawall at Fisher Island, Miami. After fragmentation, these corals were grown at our in-water nursery in Key Biscayne for at least 3 months prior to outplanting. Corals were outplanted onto 3 reefs in the Key Biscayne area using ceramic plugs and cement pucks as platforms (Fig. 1). Coral survivorship was monitored at 1 and 6 months.

Table 1. Survivorship of corals outplanted in the Key Biscayne area 1 and 6 months after outplanting.

Species (sample size)	% Survivorship (1 month)	% Survivorship (6 months)
OFAV (176)	76.7	76.7
MCAV (88)	38.6	31.8
PCLI (115)	65.2	60.9
PSTR (76)	32.9	31.6
SSID (10)	20.0	20.0

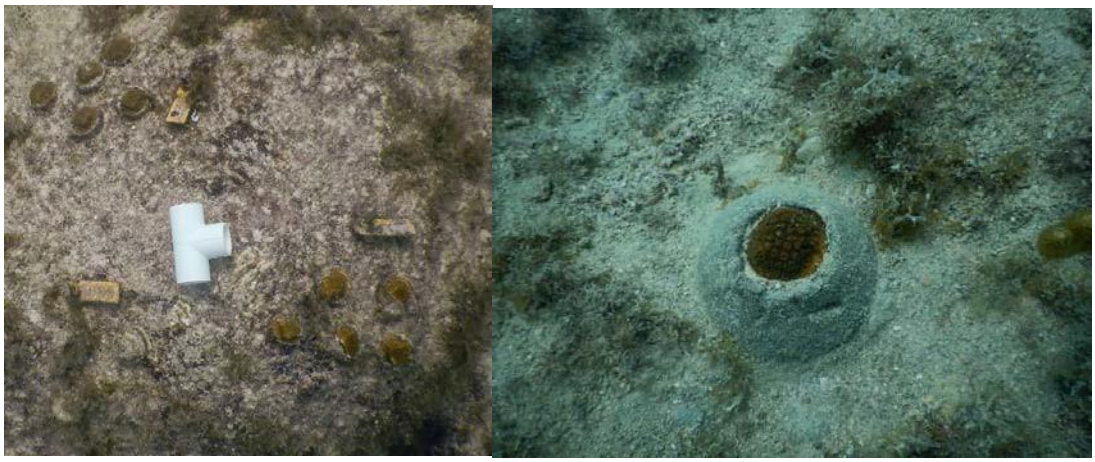

Fig. 1. Images of the small corals outplanted in this study using ceramic plugs (left image) and cement pucks (right image).

Result Highlights:

1. Only presumably healthy parent colonies collected from the endemic zone with no signs of disease were used in this study
2. The corals collected had high survivorship during the nursery phase (> 80% survivorship for all species combined) and showed no signs of disease at the nursery
3. Once outplanted, survivorship was highly variable among the species used, ranging from 20% for *Siderastrea siderea* to 77% for *Orbicella faveolata* during the first month

4. The large majority of the mortality was observed during the first month, with very limited increases in mortality recorded between 1 and 6 months
5. The main cause of mortality for these small corals was fish predation. Parrotfish and butterflyfish were observed actively feeding on the new outplants immediately after deployment
6. Evidence of fragment dislodgement (likely by fish) and fish bites were the main source of tissue loss in this study. On average, 35% of outplanted fragments were physically removed by fish during the first month.
7. Another potential source of disturbance for these corals was sediment smothering
8. No signs of disease were recorded at 1 and 6 months for any of the outplanted small massive corals
9. Corals glued onto ceramic plugs had higher rates of removal by fish compared to corals outplanted using cement

Reference

Koval, G., N. Rivas, M. D'Alessandro, D. Hesley, R. Santos, and D. Lirman. 2020. Fish predation hinders the success of coral restoration efforts using fragmented massive corals. PeerJ 8:e9978 https://doi.org/10.7717/peerj.9978.

APPENDIX 3

2018-2019 CRF Boulder Coral Update

Coral Restoration Foundation
Dan Burdeno, Restoration Program Coordinator
Jessica Levy, Restoration Program Manager
Amelia Moura, Science Program Manager

Objective 1: Outplant *Orbicella spp.* coral plugs onto Carysfort Reef, utilizing a NEMO drill and two-part underwater epoxy.

Objective 2: Test the viability and efficacy of a 3D boulder coral outplanting structure. Note: Several materials to be tested for best use and are still in early development.

Objective 3: Determine the 1-year survival of CRF *Orbicella spp.* outplants at Carysfort Reef planted between 2018 and 2019. Continue with 1-month monitoring for any new outplants.

Methods:

Coral Restoration Foundation outplanted monogenetic clusters of individuals placed close together onto either existing dead boulder heads or directly on the reef. Monogenetic clusters consisted of between 1 and 22 individual plugs, spaced approximately 1 cm apart. The target substrate was cleared of algae and subsequently drilled with a Nemo hydraulic drill. This action created holes in the substrate and individual coral plugs were inserted within the holes. A two-part marine epoxy was used to adhere the plugs to the newly cleared substrate.

Corals were monitored 1-month and 1-year after outplanting (Figure 1). All outplant data presented is from corals outplanted to Carysfort Reef. One-month monitoring data was collected and is represented below. Percent tissue mortality was determined from partial mortality data. Most often it is undeterminable as to what caused the mortality, although common known factors include predation, sedimentation, disease, and bleaching in the summer. If any signs of active disease are noted the coral outplant is removed from the water immediately.

BOULDER CORAL MONITORING

Surveyor:
Date:
Site/Mooring #:
GPS Coordinates:
Water Temp:

Overall Tagged Plot							Remaining Live Fragments					
Tag #	Species	Genotype	# Alive	# Dead (NEW)	# Dead (OLD)	# Missing	% Partial Mortality (NEW)	% Partial Mortality (OLD)	Disease?	Bleaching?	Predation?	Comments

Figure 1. CRF Boulder coral in-water monitoring sheet.

Results:

CRF has documented high survival rates associated within outplanting boulder corals at the one month monitoring event. Since the two initial outplant events in 2018, there was a uniform 100% survival 1-month following outplanting for each genotype and equal or less partial mortality in 2019 (Table 1 & Figure 2) compared to 2018 outplants. Genotypes were chosen based solely on available stock. CRF has not yet seen signs of active stony coral tissue loss disease margins on its outplanted corals during monitoring events.

Table 1: Percent survival of CRF Orbicella spp. outplants at Carysfort Reef after 1 month

Orbicella faveolata				***Orbicella annularis***			
Genotype	**# Clusters**	**# Corals**	**% Survival**	**Genotype**	**# Clusters**	**# Corals**	**% Survival**
Ofav5	6	67	100	Oann8	7	65	100
Ofav8	1	22	100	Oann26	6	65	100
Ofav9	4	33	100				
Ofav11	7	80	100				
Ofav12	3	45	100				
Ofav14	10	109	100				
Ofav16	4	33	100				
Ofav22	6	69	100				
Ofav25	9	94	99				

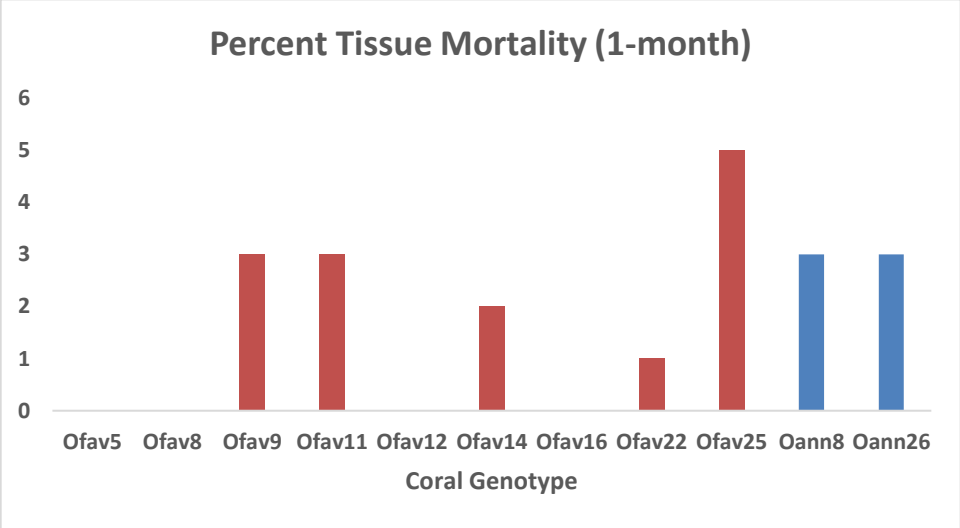

Figure 2: 1-month percent tissue mortality for Orbicella faveolata and Orbicella annularis, averaged across all clusters for each genotype.

The 1-year survival and tissue mortality (Table 2 & Figure 3) data shows a clear difference in performance among putative genotypes. CRF plans to investigate this genotype response further, potentially avoiding genotypes that have limited survival. As expected, there was further mortality from 1-month to 1-year, but CRF documented high survival (>75%) for most genotypes. Ofav9, Ofav12, and Oann22 had less than 50% survival rates, which were also reflected in the percent tissue mortality. Ofav9 showed 100% mortality. Cluster numbers and quantities thus far have been small, so it remains to be seen whether this full mortality was genotype driven or due to other factors such as bad cluster location, disease, or predation. Of the 123 clusters monitored at the 1-year time point, 61 of them exhibited fusion across plugs within the clusters. While there have been no signs of active disease on outplants, several genotypes exhibited extreme mortality after one year which could have been caused by disease that was not directly observed. It should be noted that the genotypes which showed high mortality rates were planted within a similar cohort (time + location) whereas other outplanted cohorts did not show similar rates of loss.

Table 2: Percent survival of CRF Orbicella spp. outplants at Carysfort Reef after 1 year

Orbicella faveolata				*Orbicella annularis*			
Genotype	**# Clusters**	**# Corals**	**% Survival**	**Genotype**	**# Clusters**	**# Corals**	**% Survival**
Ofav1	32	318	99	Oann1	18	184	97
Ofav5	6	67	82	Oann8	13	125	88
Ofav9	6	60	0	Oann17	19	192	78
Ofav12	6	60	17	Oann22	6	60	42
Ofav22	6	60	98	Oann26	6	66	98
Ofav25	5	56	95				

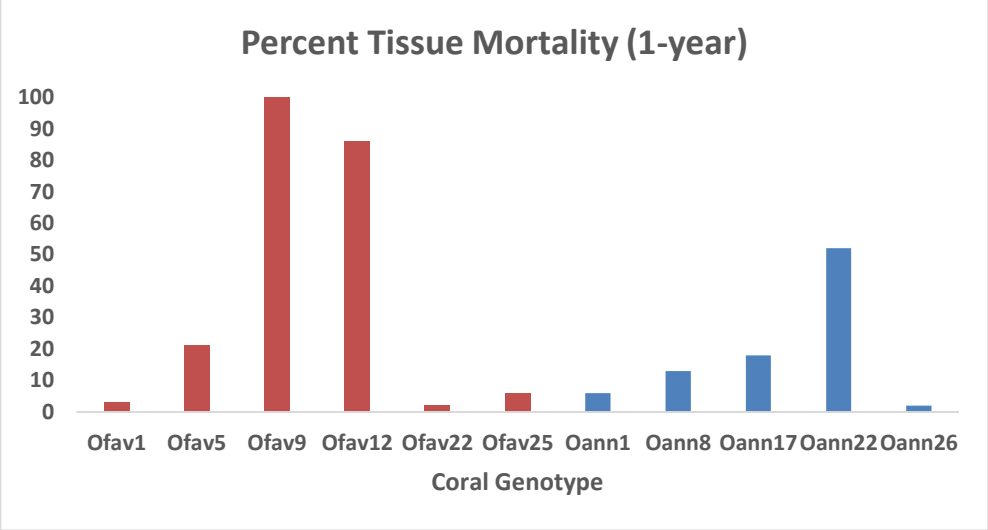

Figure 3: 1-year percent tissue mortality for Orbicella faveolata and Orbicella annularis, averaged across all clusters for each genotype.

Next Steps:

CRF has continued outplanting Ofav and Oann in 2020, with one cohort planted to Cheeca Rocks on 4/17/20. 305 *Orbicella faveolata* plugs, across 4 genotypes, were outplanted in 14 separate clusters, consisting of about 20 plugs each. CRF has plans to begin experimenting with a new outplant methodology for *Orbicella spp*. This technique is still in early development. It would involve outplanting a "discrete cluster unit", a fabricated 3D structure (half sphere) that coral plugs can be inserted into. This would allow for elevation off the reef substrate as well as eliminate any need for drilling with the NEMO tool. CRF will be testing multiple materials for use in making this structure including ceramic, natural limestone, and a potential cement additive. These structures are currently planned to hold 20 plugs, but varying sizes might be tested as well. This is still in early development prototypes are currently being tested.

APPENDIX 4

Florida Fish & Wildlife Conservation Commission Coral Outplant Case Study: outplanting SCTLD-susceptible coral species in the middle Florida Keys

Florida Fish & Wildlife Conservation Commission
Florida Fish & Wildlife Research Institute

Introduction

During August 2018, the Restoration Trials Team (RTT) identified as a recommended activity the outplanting of SCTLD-susceptible coral species along the Florida Reef Tract (FRT) at an experimental scale to assess the suitability of undertaking larger scale coral restoration efforts. In response to this RTT directive, we sought and were subsequently granted funding support from the Florida Department of Environmental Regulation to conduct experimental outplanting of SCTLD susceptible coral species at previously SCTLD-susceptible coral species at previously SCTLD-affected locations across the endemic zone of the FRT. The objectives of our study were:

1) Quantify the SCTLD infection rates on three species of SCTLD-susceptible coral species among three locations in the upper and middle Florida Keys
2) Compare SCTLD infection rates between inshore and offshore reef strata
3) Compare SCTLD infection rates between *in situ* and *ex situ*-nursery sourced coral colonies
4) Compare SCTLD infection rates among coral colonies of known genet
4) Monitor SCTLD prevalence of the surrounding coral community at the outplant sites

Methods

During November 2019 we established outplanting sites at three locations: Pickles Reef in the upper Florida Keys, and Tennessee Reef and Delta Shoal in the middle Keys. At each of these locations we selected one outplanting site on offshore bank reef habitat and one on an inshore patch reef. Prior to outplanting, the natural coral community on the site was surveyed to assess SCTLD prevalence. In addition, a roving diver survey was conducted to assess disease prevalence at a nearby site. We recorded the number of SCTLD-susceptible coral colonies, their size (cm diameter), and health status (healthy or diseased).

Only presumably healthy corals (robust coloration, no sign of disease, no open wounds on the surface) were selected for outplanting. Each of the selected colonies (3-4cm diameter) was epoxied to the top of a 4 cm diameter circular cement base plate several months before outplanting. To facilitate anchoring to the substrate, a nylon pin was installed on the underside of the base plate; a similar shaped concrete plug protruded from the Mote corals for the same purpose. We selected slightly elevated areas for attachment to the reef and avoided low areas where sediment could pool. Immediately prior to outplanting, the substrate directly under the baseplate was cleared of algae and sediment. A hole was drilled to accommodate the nylon bolts and concrete plugs and an underwater epoxy was pressed into the hole and under the baseplate where it attached to the substrate.

At each of the six sites, we outplanted 20 colonies each of the SCTLD-susceptible coral species *Montastraea cavernosa*, *Pseudodiploria clivosa*, and *Orbicella faveolata* (Figure 1). Half of the colonies of each species were sourced from FWC's *in situ* coral nursery, and the other half were sourced from Mote Marine Laboratory's (MML) land-based coral nursery. Colonies were outplanted randomly (species × source) 1 m apart within a 4 × 15 colony array. This design used a total of 360 colonies.

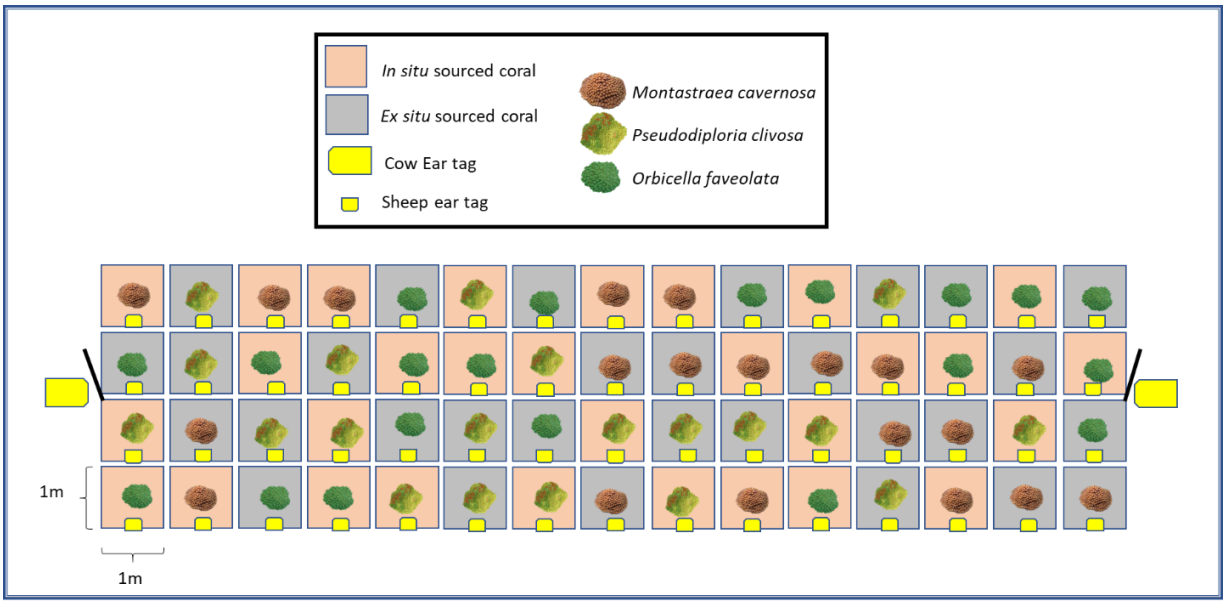

Figure 1. Conceptual diagram of a proposed coral outplant site. Colonies are arrayed randomly by species, genotype, and nursery source (*i.e.*, sourced from FWC's *in situ* nursery and MML's *ex situ* nursery).

Following the recommendation from the RTT Action Plan, our monitoring protocol included an evaluation of the natural coral community within and around the restoration site and at an adjacent location over an area judged to be sufficient to evaluate changes in disease dynamics related to outplanting activities. The Action Plan notes that because SCTLD is waterborne, a true control for this effort is unlikely, but it may be possible that control sites separated from outplant sites are at less risk of experiencing an increase in the prevalence of SCTLD than the coral community at the outplant site. Therefore, we choose to include this monitoring effort into this project.

Accordingly, immediately preceding outplanting, the natural coral community at each outplanting site and an adjacent site was assessed for SCTLD using a roving diver survey. Because of differences in natural coral cover between offshore and inshore sites, roving diver surveys were 10 minutes at the offshore sites and were 20 minutes at the inshore sites. Control sites were separated by 100-150 m from outplant sites, except at the Tennessee inshore site (37m), which was a comparatively small patch reef. After baseline data was collected on the outplants and the natural coral community, the outplanted colonies and the natural coral communities were monitored 1 week, 2 weeks, 1 month, 12 weeks, and 24 weeks post-outplanting for changes in SCTLD prevalence. Inclement weather postponed the 1-month monitoring to 6 weeks post-outplanting. We also completed the 3-month post-outplant assessment in March 2020. However, after learning that SCTLD was first observed on coral colonies associated with an experimental outplanting conducted by MML at 4-months post-outplant (E. Muller, MML, personal communication), we attempted to monitor our sites during April 2020, but the FWC suspended field activities before this could be completed. Due to Covid-19 related limitations in our ability to conduct field work, roving diver surveys were not conducted after the 12-week monitoring. We monitored outplanted colonies in May, August, and September 2020 and in February 2021. Here, we summarize the results of this project through 15 months.

Results

Prevalence of SCTLD among the experimental coral colonies

At the 12-week monitoring period, the survival rate of the outplanted colonies was ~96%. Nine colonies had died (2.5%), and 4 (1.1%) were missing. All the dead colonies were *P. clivosa*, and 7 of those colonies were from the Pickles Reef offshore location. At the 15-month monitoring period, the survival rate of the outplanted colonies was 76.4%. Seventy-seven colonies had died (21.4%) and 8 (2.2%) were missing. One outplanted colony at the Delta Shoal offshore location was observed with disease during the 10-month monitoring period (Figure 2) and then was observed as dead 15 months after outplanting, but no other clear incidences of SCTLD were observed during any of the other monitoring periods. However, there have been colonies that have died for unknown reasons (Figure 3), including approximately 10 colonies that appeared healthy 10 months after outplanting but were dead 15 months after outplanting, so it is possible that SCTLD caused their death.

Figure 2. Observation of SCTLD in *Orbicella faveolata* at Delta Shoal offshore location in September 2020**.**

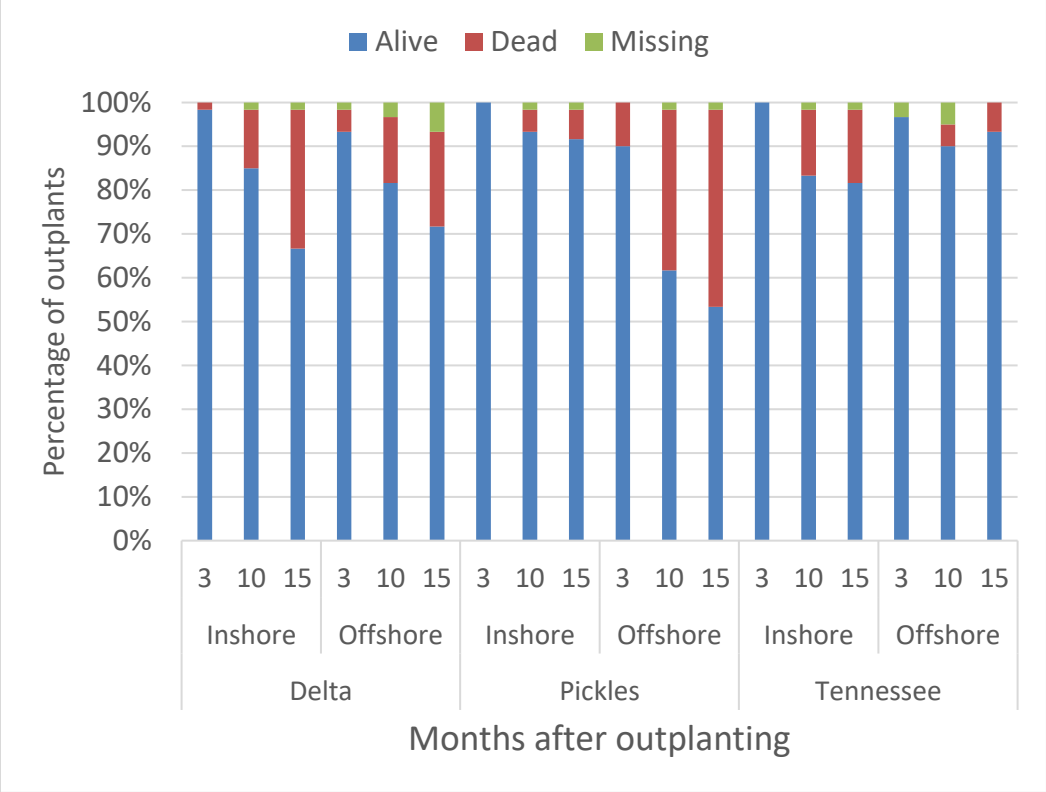

Figure 3. The percentage of outplants that were alive, dead, and missing at inshore and offshore locations at Delta Shoal, Pickles Reef, and Tennessee Reef during monitoring 3, 10, and 15 months after outplanting.

Prevalence of SCTLD in the natural coral community

The roving diver surveys detected SCTLD-infected colonies both in the immediate vicinity of the outplant sites and at nearby control sites (Figure 4). The mean (1±sd) incidence of disease observed pooled across the outplant sites was 0.5 (±0.001%) and 1.4 (±0.01%) at the control sites. Although we recognize this is a limited time series of information, it does underline a potential challenge in assessing the cause-and-effect dynamic between coral outplanting and SCTLD-prevalence *via* this effort, particularly within the endemic zone if SCTLD persists chronically at low infection rates.

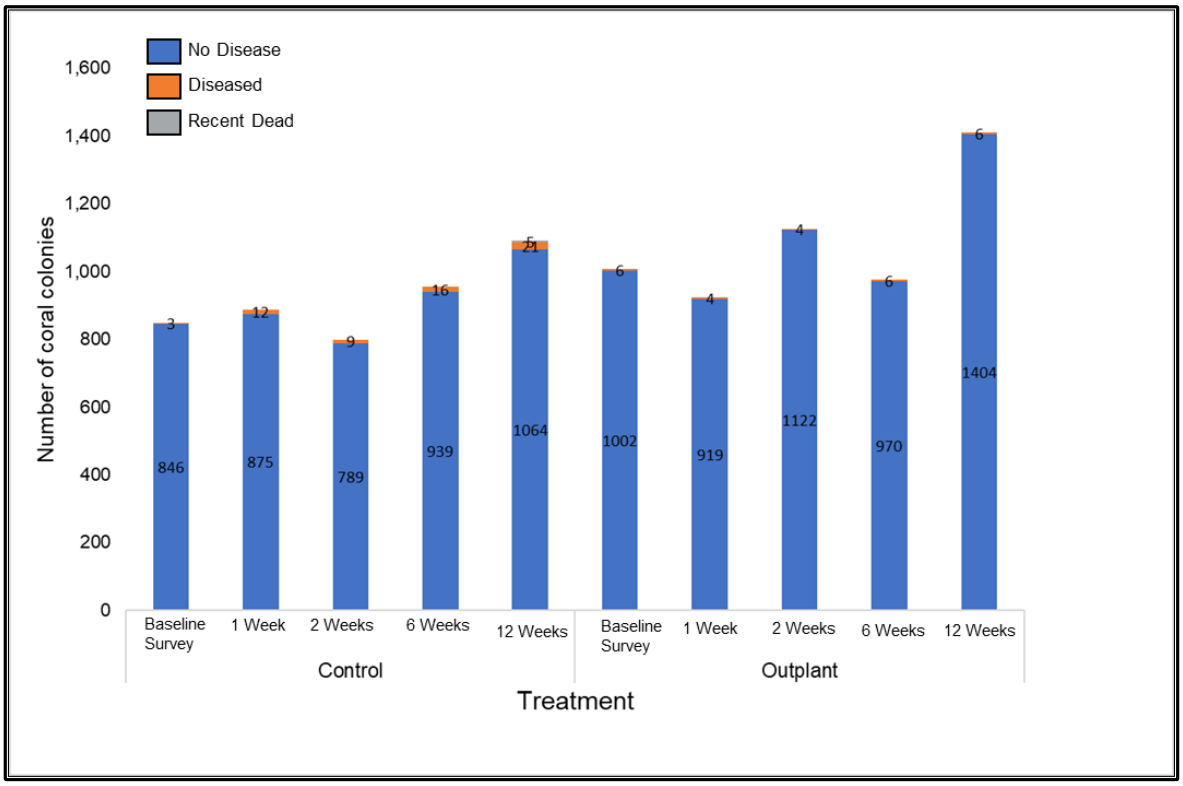

Figure 4. Time series of SCTLD incidence between the natural coral communities at coral outplanting sites and control sites observed by 30-minute roving diver surveys through 12 weeks post outplanting. Control sites were areas of reef similar to the outplant site but assumed to be independent. Coral colonies not exhibiting SCTLD were categorized as "No Disease", colonies with clear SCTLD infections were defined as "Diseased". Colonies defined as "Recent Dead" were those exhibiting signs of rapid tissue loss resulting in whole colony death that was consistent with SCTLD infection.

Finfish Corallivory

Although we have observed only one incident of SCTLD on our outplanted colonies, we did observe intense predation consistent with parrotfish corallivory at all the outplant sites during the 1-week post outplant monitoring effort. During that monitoring period 193 of the 359 colonies monitored (54%) had evidence of finfish predation. However, the proportion of colonies that showed evidence of recent finfish predation decreased progressively each monitoring period and few colonies showed evidence of predation by the 12-week monitoring period. We found that fragments produced by the *in situ* nursery (Figure 5A) had significantly lower predation compared to fragments produced in the *ex situ* nursery (Figure 5B). In addition, predation during the first week varied by reef location (Pickles, Tennessee, and Delta) and reef type (offshore vs nearshore). Higher predation was observed at the offshore sites at both Delta and Tennessee and at the nearshore sites at Pickles (Figure 6). Predation during this first week and origin source did not impact the survivorship of these corals at 12-week post-outplanting. These results indicate that early predation may not lead to increased mortality in boulder coral outplants.

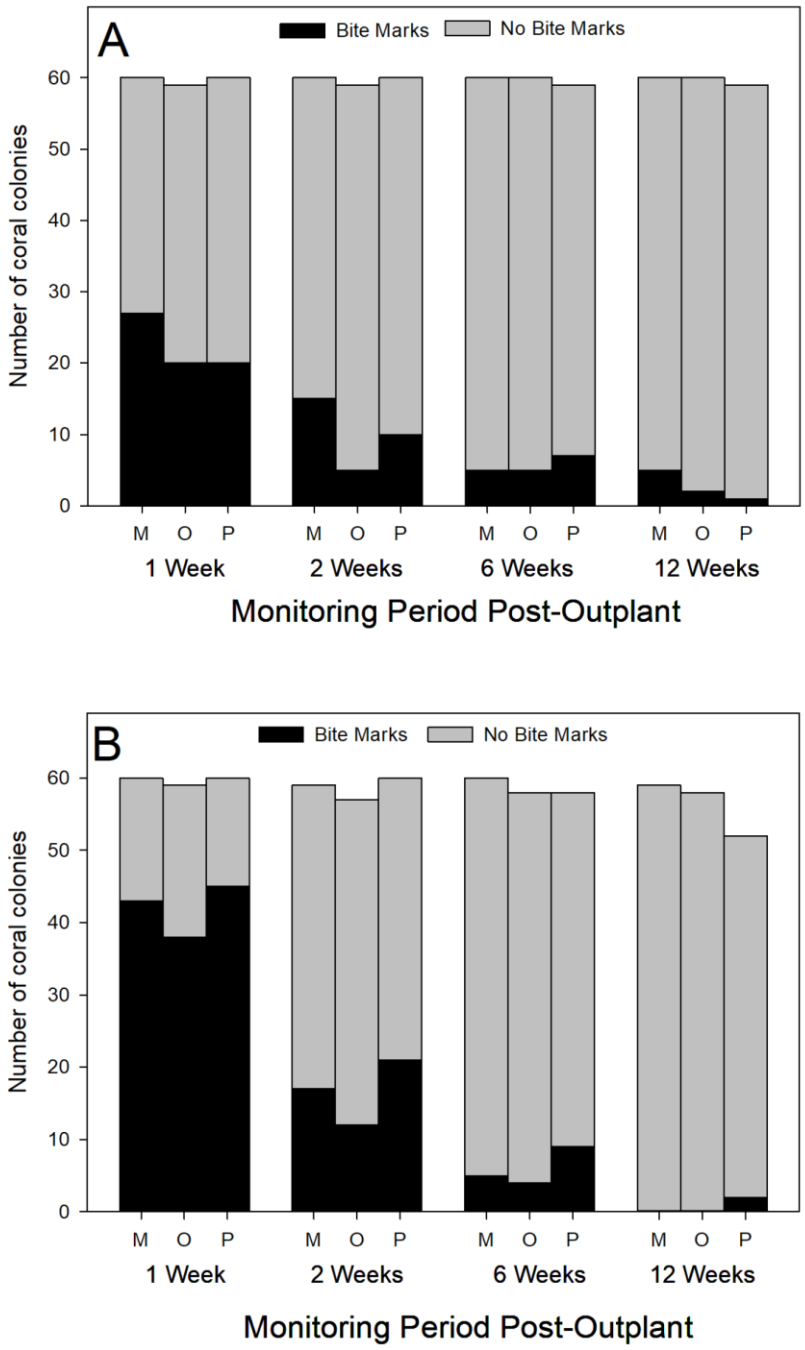

Figure 5. The number of coral colonies with finfish predation for each monitoring period by species (M – *Montastraea cavernosa*, O – *Orbicella faveolata*, P – *Pseudodiploria clivosa*) for in situ- (A) and ex situ- (B) propagated colonies. Colonies that died or were not relocated are excluded.

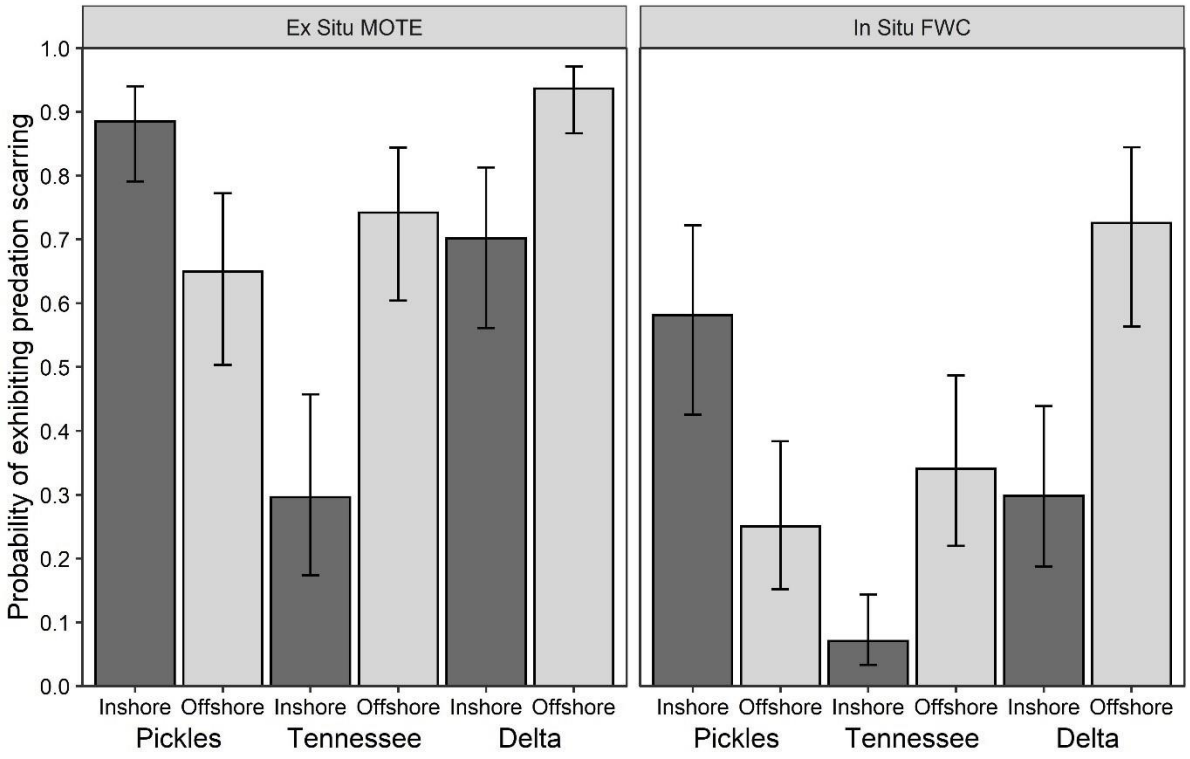

Figure 6. Predicted probabilities of a coral colony exhibiting finfish predation scars 1-week post-outplanting on inshore and offshore sites for in situ- and ex situ-propagated coral colonies. Error bars represent 95% confidence intervals.

APPENDIX 5

Site-specific multi-species coral reef restoration pilot project

The Nature Conservancy, Mote Marine Laboratory, Fish and Wildlife Research Institute

Caitlin Lustic, The Nature Conservancy
Erich Bartels, Mote Marine Laboratory
Kerry Maxwell and Brian Reckenbeil, Fish and Wildlife Research Institute

The study described here was not designed to provide data on the progression of disease, but rather to better understand the amount of maintenance massive coral outplants need in order to overcome the competitive pressures of macroalgae and *Palythoa caribaeorum*. However, monitoring included monthly assessments on the condition of each coral and taking photographs. As stony coral tissue loss disease (SCTLD) moved through the region, those methods proved useful in detecting disease on the outplanted corals. The first set of outplanting occurred in 2016 before the first observations of SCTLD in the Lower Keys subregion. SCTLD was first officially reported at Looe Key in April 2018 but, as described in the text below, we had observed outplants with a tissue loss disease prior to that at the site.

Objectives

This project was completed in two phases. The first two objectives applied to the first phase of the project in which 15-25 cm diameter corals were outplanted to one site in the Lower Keys. All four objectives applied to the second phase in which ~4 cm diameter corals were outplanted to 4 sites total within the Middle Keys (n=2 sites) and Lower Keys (n=2 sites). Importantly, the outplant sites of the project were within the vulnerable "pre-invasion" zone during the first phase of the project (2016) and within the endemic zone during the second phase of the project (2019). The final objective was to specifically document the impacts of SCTLD on the outplanted coral.

1) Can we successfully outplant non-Acroporid coral species in an effort to move towards multi-species habitat restoration?
2) What can we do to improve coral survivorship? Does removal of competitors significantly influence the survivorship and health of outplanted corals?
    a. Is *P. caribaeorum* detrimental to newly outplanted corals? Is coral survivorship increased with the initial removal of *P. caribaeorum*? Is it further increased with maintenance of that removal?
    b. Is algae detrimental to newly outplanted corals? Is survivorship increased with the initial removal of algae? Is it further increased with maintenance of that removal?
3) Are there size-specific differences in the survival rates between non-acroporid coral colonies outplanted at 15-25 cm diameter and those outplanted at ~4 cm diameter?
    a. Among ~4 cm diameter outplants, is there a difference in survival between in situ nursery-grown coral colonies and ex situ nursery-grown coral colonies?
4) How do these dynamics change following a dramatic disturbance, such as Hurricane Irma and/or the ongoing coral disease outbreak?

Methods

Phase 1 (2016). A total of 180 colonies representing three species (*Montastraea cavernosa*, *Orbicella faveolata*, and *Acropora cervicornis*) were outplanted to a site between Looe Key and American Shoal on March 17-18, 2016 (Table 1). The massive corals, for which the genotypes were unknown, were sourced from the Florida Keys National Marine Sanctuary dockside rescue nursery, whereas all of the *A. cervicornis* colonies, which were all from a single genotype, came from Mote's offshore nursery near Looe Key. All massive corals were between 15-25 cm in diameter with recently cut

edges on all sides, and the *A. cervicornis* fragments were 10-25 cm total linear extension (TLE). The massives were attached to the reef using a mix of Portland cement and plaster of paris, and the *A. cervicornis* were outplanted using masonry nails and nylon cable ties. Because of the original intent of this study, corals were divided randomly into 4 treatment categories that received different levels of exposure to competitors or cleaning regimes. The metrics collected through monthly monitoring included: survivorship, percent live tissue, growth, and condition. During every monthly monitoring event, corals were assessed in-water by a diver for survivorship, percent live tissue, and condition (including disease, bleaching, predation, burial, and anything else unusual that the diver noticed). Additionally, a photograph was taken using a tripod frame attached to a 50 cm x 50 cm quadrat to allow for growth measurements using ImageJ. Quarterly, three measurements were taken in the field for each coral (maximum width, perpendicular width, and height) in addition to the other monitoring data.

Phase 2 (2019). A total of 954 colonies representing four species (*M. cavernosa*, *O. faveolata*, *Pseudodiploria clivosa* and *A. cervicornis*) were outplanted in May and June 2019 to each of four sites: an *in situ* nursery-grown massive coral site in the Lower Keys and the Middle Keys and an *ex situ* nursery-grown massive coral site in the Lower Keys and Middle Keys (Table 1). SCTLD had been observed at both the Middle Keys and Lower Keys sites at least a year before the corals were ouplanted (i.e., considered within the endemic zone). For the *in situ* nursery-grown massive corals, the original intent was to utilize *in situ* grown fragments sourced from wild collected "corals of opportunity" that had been collected after Hurricane Irma, moved to Mote's *in situ* nursery, and prepared months in advance for this experiment. However, because disease impacted the *in situ* nursery-grown fragments shortly before the experimental outplanting was to be conducted, the *in situ* massive colonies were again sourced from the FKNMS dockside rescue nursery. FKNMS rescue nursery collections targeted three parent colonies per species, producing equal numbers of replicates of three putative genotypes for each species. All massive fragments were approximately 4 cm in diameter and were mounted on pre-cast cement plugs. *Ex situ* nursery-grown corals were sourced from Mote Marine Laboratory. All massive colonies were outplanted on their plugs by using a nail to create a hole in the reef and securing the plug with epoxy (Figure 1). The *A. cervicornis* colonies for all four sites were sourced from *in situ* nurseries because we did not have an *ex situ* nursery source for *A. cervicornis*. Equal numbers of the same three Lower Keys *A. cervicornis* genotypes were outplanted to all four sites, but the Middle Keys sites outplanted colonies grown in the FWC Middle Keys nursery and the Lower Keys sites outplanted colonies grown in Mote's offshore nursery near Looe Key. All *A. cervicornis* colonies were outplanted using masonry nails and nylon cable ties. Outplants from each species and genotype were randomly assigned one of four treatment categories that received different levels of exposure to competitors or cleaning regimes. Data collected during monitoring was the same as data collected for the Phase 1 outplants.

Table 1. Summary of corals outplanted for the multi-species coral reef restoration pilot project.

Phase	Site	Date outplanted	Species and nursery origin				Total outplanted
			A. cervicornis	*M. cavernosa*	*O. faveolata*	*P. clivosa*	
1	Lower Keys	March 2016	60 (in situ)	60 (in situ)	60 (in situ)	0	180
2	Lower Keys	May 2019	60 (in situ)	60 (in situ)	60 (in situ)	60 (in situ)	240
2	Middle Keys	May 2019	60 (in situ)	60 (in situ)	60 (in situ)	60 (in situ)	240
2	Lower Keys	June 2019	60 (in situ)	60 (ex situ)	57 (ex situ)	59 (ex situ)	236
2	Middle Keys	June 2019	60 (in situ)	60 (ex situ)	58 (ex situ)	60 (ex situ)	238

Figure 1. Examples of *in situ* nursery sourced massive corals outplanted as part of Phase 2.

Results

Phase 1 (2016). Six months post-outplanting, coral outplants had 100% survivorship and relatively little partial mortality. After six months (September 2016), we began to see corals with what appeared to be tissue loss disease. Over the next 18 months, and prior to April 2018 when SCTLD was officially reported at Looe Key, 14 colonies were observed with disease (note: photos were used to identify disease observations where there was no tissue loss including colonies with pale spots and/or dark spots and these disease observations were removed, Figure 2). Of those 14 colonies, two were dead within a month after disease was first observed (Figure 3) and four others were dead within three months after disease was observed. Another five were either dead or missing after Hurricane Irma, so the disease progression could not be followed.

Figure 2. These two colonies exhibited signs of tissue loss and were dead within one month of the observations.

Hurricane Irma impacted the Florida Keys in September 2017 and approximately half of the remaining live colonies were either lost or killed during the storm, so we were unable to continue to fate-track some of the colonies that had shown signs of tissue loss disease. SCTLD was officially reported at Looe Key in April 2018 (Neely, 2018, DEP report). We observed incidences of SCTLD on ten of the 46 remaining outplants between September 2018 and March 2019, and nine of those were dead within one month of the observation.

Figure 3. Two corals that experienced apparent SCTLD infection and died within a month of the observation.

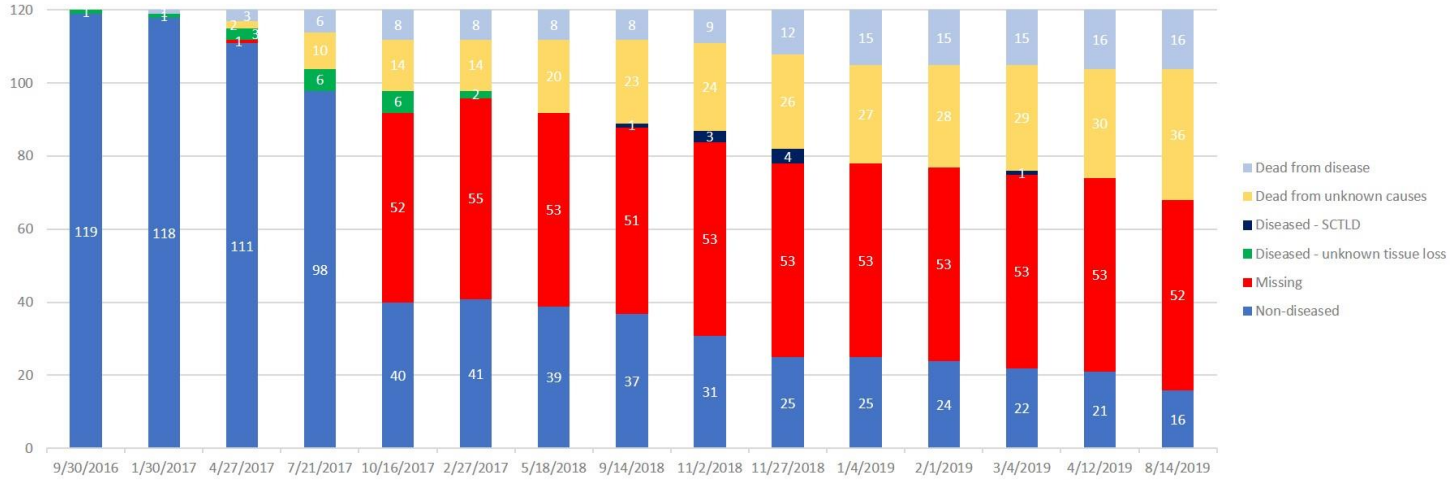

Figure 4. Monitoring results of colony condition over the period of the grant. Note that the time periods of reporting are not consistent; they were chosen to highlight the major changes over time.

Phase 2 (2019). The corals outplanted as part of Phase 2 were monitored monthly for five months at each site. Disease on the smaller massive colonies was challenging to identify, so many of the observations of tissue loss were noted as possible disease or possible fish predation (Figure 5). Disease observations were rare during the five months of monitoring, but the highest number of disease observations for *in situ* nursery-grown massive colonies (Figure 6 A and B) occurred 2 weeks after outplanting. This was also the monitoring period during which the most predation was observed, so it is possible that some of the disease observations were actually predation injuries. Disease observations in *ex situ* nursery-grown massive colonies were also infrequent (Figure 7 A and B) and the percentage of colonies with signs of disease was relatively constant through time.

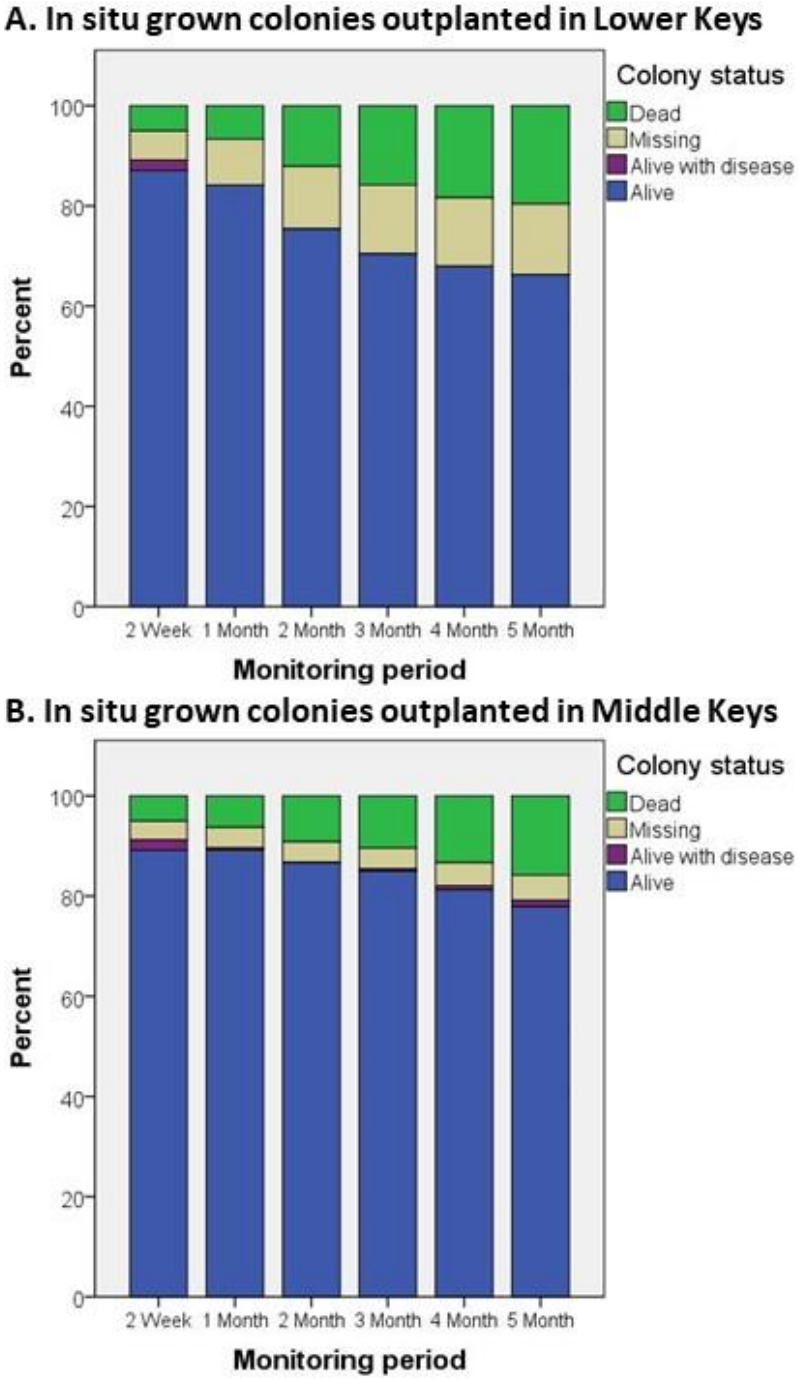

Figure 6. The percentage of *in situ* nursery-grown colonies during each sampling period that were dead, missing, alive and observed with active disease, and alive with no active disease during the first five months of the study.

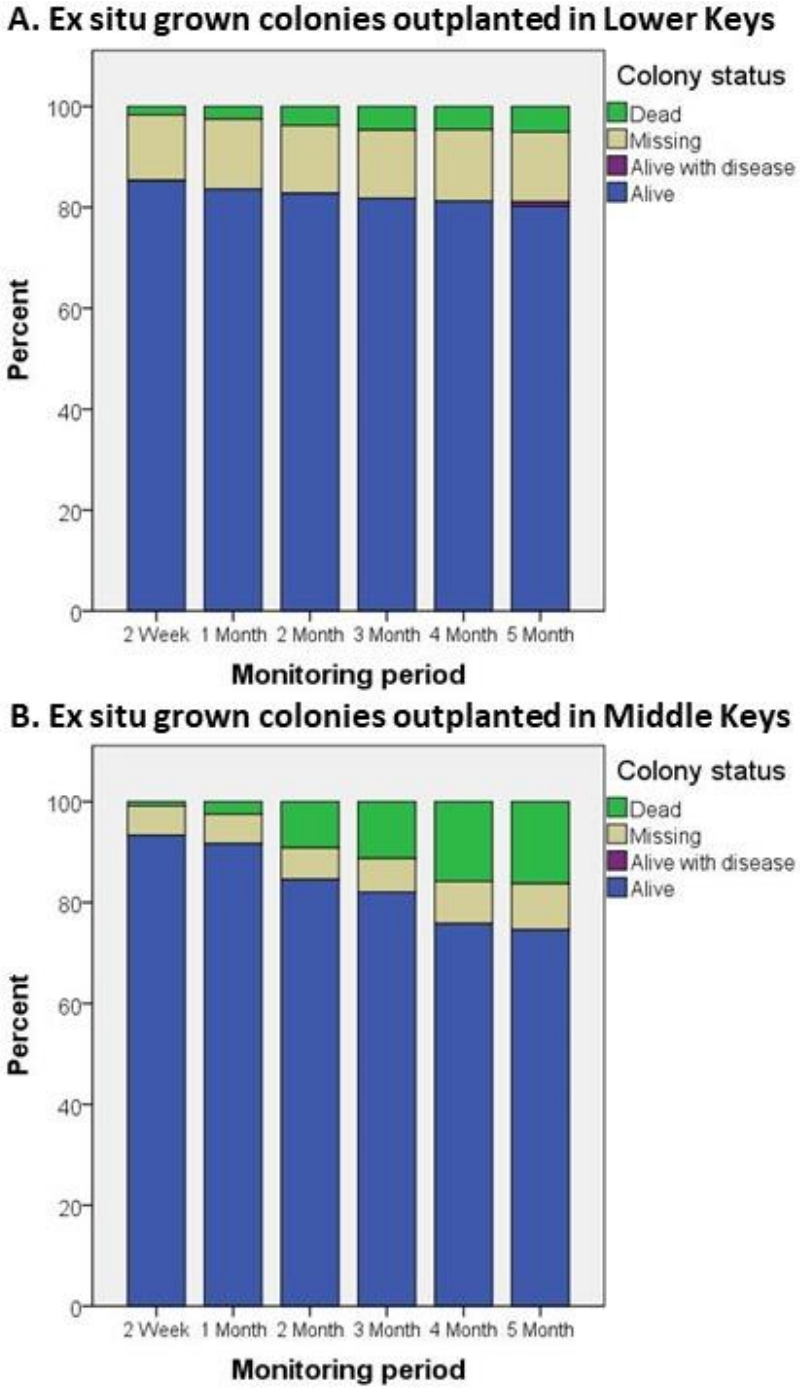

Figure 7. The percentage of *ex situ* nursery-grown colonies during each sampling period that were dead, missing, alive and observed with active disease, and alive with no active disease during the first five months of the study.

Conclusions

The phase 1 part of the study demonstrated that *in situ* sourced massive colonies outplanted at 15-25 cm diameter size are susceptible to SCTLD. However, many of the disease observations from Phase 1 of the study occurred prior to the arrival of SCTLD to the Lower Keys and some of these early observations presented similarly to SCTLD. It is unknown what disease was observed on the site prior to the arrival of SCTLD to the Lower Keys. During phase 2 of the project, the cause of many observations of tissue loss was unclear because it was challenging to differentiate disease from predation on small massive colonies, especially when predators were absent. However, 7 out of 8 *in situ*-grown massive colonies with

possible disease observations during the first 2 weeks died within a month of the disease observation. Smith et al. (2021) demonstrates that predation occurring at the time of outplanting on coral outplants similar in size to the corals outplanted in Phase 2 of this study was unlikely to result in mortality 12 weeks after outplanting. Since the outplants with questionable predation in our study died within a month of the possible disease observation, Smith's study (2021) suggests that the possible disease observations were indeed disease. Regardless of these limited observations of disease, this phase 2 study showed overall that disease was not a significant cause of mortality for either *in situ* or *ex situ* sourced massive corals outplanted at two different regions within the previously SCTLD-affected endemic zone of the FRT.

References

Smith, K. M., Pharo, D. M., Shea, C. P., Reckenbeil, B. A., Maxwell, K. E., & Sharp, C. (2021). Recovery from finfish predation on newly outplanted boulder coral colonies on three reefs in the Florida Keys. *Bulletin of Marine Science*, *97*(2), 337-350.