Investigating the ongoing coral disease outbreak in the Florida Keys: continued SCTLD monitoring at middle and lower Florida Keys, experimental coral restoration of SCTLD-susceptible coral species, and assessing the prevalence of SCTLD on intermediate reef habitat

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Final Report

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Florida Fish & Wildlife Conservation Commission

Fish & Wildlife Research Institute

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Project Title

Investigating the ongoing coral disease outbreak in the Florida Keys: continued SCTLD monitoring at middle and lower Florida Keys, experimental coral restoration of SCTLD-susceptible coral species, and assessing the prevalence of SCTLD on intermediate reef habitat

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Background

Disease is recognized as a major cause of the progressive decline in reef-building corals that has contributed to the general decline in coral reef ecosystems worldwide. The first reports of coral disease in the Florida Keys emerged in the 1970's and have been documented with increasing frequency. Presently, the Florida Reef Tract is experiencing one of the most widespread and virulent disease outbreaks on record. This outbreak has resulted in the mortality of thousands of colonies of at least 20 species of scleractinian coral, including primary reef builders and species listed as Threatened under the Endangered Species Act. First reported near Key Biscayne in 2014, this outbreak, recently described as "stony-coral-tissue-loss disease" (SCTLD), has progressed southward along the Florida Reef Tract (FRT), and by December 2017, had reached the vicinity of Coffins Patch Reef in the middle Florida Keys. The Florida Fish and Wildlife Conservation Commission (FWC) began assessing its progression as it approached the reefs off Marathon in the Middle Keys. In January 2018 the FWC initiated a DEP-funded project that established four "sentinel sites" in the middle Florida Keys. These sites have been continuously monitored and the fate of approximately 1,400 individual coral colonies has been tracked to evaluate the small-scale spatial epidemiology of SCTLD (Sharp and Maxwell 2018). Additionally, FWC conducted another DEP-funded project that assessed the efficacy of colony-specific intervention efforts that treated SCTLD infected corals with antibiotics at three experimental plots in the lower Florida Keys (Sharp *et al.* 2019).

This project continues the work associated with both of those efforts. We have continued to monitor the middle Keys sentinel site locations to better understand the spatial and temporal dynamics of SCTLD in the area. We revisited the intervention sites in the lower Keys to assess the longer-term effectiveness of the intervention effort. Additionally, we initiated an experimental-scale restoration effort to translocate several species of SCTLD coral species into areas along the Keys reef tract in the endemic disease zone to better understand how outplanted coral colonies survive under the chronic persistence of SCTLD. Finally, this project includes an assessment of SCTLD prevalence in deep areas along the Keys reef tract where there has been minimal monitoring effort.

Project Goals and Objectives

There are four objectives to this project:

i) Continue collecting small-scale epidemiology information from previously established monitoring sites in the middle Florida Keys to better understand SCTLD dynamics;

ii) Conduct follow-up surveys at previously established intervention sites to assess longer-term effectiveness of this effort;

iii) Outplant coral colonies onto previously SCTLD-affected locations across the endemic zone and assess their survival;

iv) Conduct SCTLD surveys along the Intermediate Reef strata (10-25m) along the Florida Keys reef tract assess SCTLD prevalence in this habitat.

Task 1: Continue collecting small-scale epidemiology information at the existing sentinel sites in the middle Florida Keys to better understand longer-term spatio-temporal dynamics of SCTLD

During December 2017 and January 2018, the FWC established four sentinel sites off Marathon FL in the Middle Keys to assess the spatial epidemiology of SCTLD and assess species-specific disease progression rates (Figure 1). Supported by two previous DEP awards, these sites were monitored either monthly or bi-monthly through July 2019 (Sharp and Maxell 2018; Sharp *et al.* 2019). This effort documented the spatial and temporal epidemiology of SCTLD. From this effort we developed and refined a predictive model that indicated that disease transmission at scales < $100m^2$ was density and species-independent (Sharp *et al.* 2019). However, continued monitoring revealed possible seasonally related difference in disease progression, as well as differences in disease prevalence and progression rates between offshore and inshore locations. We found that from August 2018 to August 2019 SCTLD progression had slowed at the inshore sites but continued at offshore locations. The present project continued this monitoring effort to provide a longer-term assessment of SCTLD dynamics, information particularly relevant to a recommended activity summarized in the recently completed Action Plan drafted by the Restoration Trials Team (RTT), a multi-agency multi-disciplinary team assembled to plan and coordinate coral reef restoration activities (Coral Disease Workshop, Key Largo, FL July 10-13, 2018). That plan specifically prioritizes identifying when SCTLD-susceptible species can be restored on the FRT with acceptable survival rates.

Sentinel reef locations were monitored during the present award during September, October, and December 2019, and January and March 2020. Inclement weather during November 2019 and February 2020 limited the number of days available to safely conduct this project's field efforts. Given the minimal monthly changes in SCTLD progression we had recently encountered at the sentinel sites, we prioritized our field effort on the available field days toward our outplanting experiment detailed under 'Task 3'. The March 2020 monitoring effort was the last completed during this reporting period. The FWC suspended all field activities through April 2020 due to COVID-19-related health concerns. Limited field activities were resumed during May, and we choose to direct that effort towards the outplanting experiment.

The full timeline summarizing the proportion of coral colonies that have become infected with SCTLD, those that have died after becoming infected with SCTLD, and those that have died from unknown causes are summarized in Figure 2 and Figure 3. The SCTLD infection and related mortality rates plateaued during the summer of 2018, but SCTLD has remained

chronically active at all four sites and we have continued to document signs of active SCTLD (*i.e.*, colonies exhibiting tissue mortality) across the area, albeit at much lower incidence since early 2018 (Figure 4). Since 2018 we observed infected colonies that were no longer showing continued tissue mortality, the largest proportion of colonies that were infected prior to September 2019 exhibited no tissue mortality as of March 2020.

We have no immediate plans to continue our monitoring effort in the near term as FWC field activities remain curtailed due to COVID-19-related health concerns. However, as these sites are conveniently located near FWC's base of operations in Marathon, FL, it is our intent that when full-scale field activities resume, we will opportunistically evaluate them for the persistence of active SCTLD infection and update the Disease Advisory Committee's response teams when pertinent.

<u>Task 2</u>: Conduct follow-up surveys at previously established intervention sites to assess the potential longer-term effectiveness of this effort.

Supported by DEP funding, in the summer of 2018, the FWC initiated a project to test the effectiveness of colony-specific intervention methods discussed at the Coral Disease Workshop (Key Largo, FL July 10-13, 2018). In brief, these methods entailed applying either a disinfectant or antibiotic directly to SCTLD lesions on coral colonies (*see* Neely 2018). Using one of these methodologies -- shea butter impregnated with amoxicillin applied to SCTLD lesions and covered with modelling clay – we evaluated if this technique could alter the incidence of SCTLD within 100m^2 areas of reef habitat (Sharp *et al*. 2019).

In brief, we found that when SCTLD reached epidemic levels, treating the colonies using this method did reduce the overall loss of living coral tissue within those experimental plots relative to similar untreated plots used as controls, but required extensive manpower and effort that we deemed the effort unsustainable when SCTLD prevalence was epidemic.

The Coral Disease Workshop held in St. Petersburg, FL during August 6, 2019 identified as an action item the continued refinement of intervention techniques (either lesion or whole colony treatment). We completed treatment at intervention sites in the spring of 2019. However, as we had treated several hundred SCTLD-affected colonies during this effort, continued periodic monitoring of these sites would provide important information of the long-term effectiveness of this methodology. Accordingly, this project intended to conduct two follow-up surveys of those intervention sites, approximately six and 12 months after we completed the intervention effort described above. We conducted the first scheduled monitoring of the plots during September 2020. The second survey was scheduled for April but was not conducted due to COVID-19 related health concerns.

During September 2020 the three intervention plots were re-surveyed (Figure 5). Divers recorded the percentage of living tissue of each coral colony and if the colonies exhibited signs of SCTLD. Thirty-five colonies had died since the May 2019 survey. The incidence of living colonies exhibiting active SCTLD infection had decreased since May 2019. Twenty of the 960 (2%) colonies surveyed exhibited of active SCTLD during the September survey. Of the 1,016 surveyed during May, 194 (19%) of the living colonies showed signs of SCTLD. We identified 172 colonies during the September survey that we had been previously treated with antibiotics. Seventeen (~10%) had died. The proportion of living tissue of the remaining 155 living colonies had decreased from the May 2019 survey (Figure 6), and the overall the mean percentage of live tissue of the coral community had decreased at all three sites from the May to the September survey (Figure 7). These findings indicate that efforts to treat SCTLD-affected colonies with a

topically applied antibiotic will likely require regular re-treatments, at least while SCTLD remains present within the coral community being treated.

<u>Task 3</u>: Outplant coral colonies onto previously SCTLD-affected locations across the endemic zone of the FRT and assess their survival

As noted under 'Task 1', the RTT identified as a recommended activity an effort to determine experimentally whether fragments of susceptible species propagated in nurseries can be safely outplanted into SCTLD-affected areas of the FRT. An extensive portion of the FRT affected during the earlier stages of the epizootic now exhibits low disease prevalence, suggesting the possibility that resuming coral restoration activities should be considered.

In November 2020, we initiated an experimental outplanting of SCTLD susceptible coral species along the reef tract in the upper and middle Keys. We selected three locations: Pickles Reef region in the upper Keys, and Tennessee Reef and Delta Shoal region in the middle Keys. Because our sentinel site monitoring indicated the potential for differences in SCTLD prevalence between offshore and inshore habitats (Sharp *et al.* 2019), we selected an offshore bank reef site and an inshore patch reef site at each of the three locations (Figure 8). Figure 9 presents a conceptual diagram of one outplant site. At each of the six sites, we outplanted 20 colonies of each of three SCTLD-susceptible coral species: *M. cavernosa*, *Pseudodiploria clivosa*, and *O. faveolata*. Half of the colonies of each species were sourced from FWC's *in situ* coral nursery, which was in the endemic zone but isolated from reef habitat and has been minimally affected by SCTLD. The other half was sourced from Mote Marine Laboratory's (MML) land-based coral nursery and had not been exposed to SCTLD (E. Muller, personal communication).

Colonies were outplanted randomly (species × source) by securing each to bare reef substrate using underwater epoxy (Figure 10). The monitoring methodology followed the procedures detailed in the RTT Action Plan. In brief, before the corals were outplanted, we determined the baseline prevalence of SCTLD at each restoration site and at an adjacent site using a roving diver survey. The survey recorded the number of SCTLD-susceptible coral colonies, their size (cm diameter), and health status (healthy or diseased). Following outplanting, the outplanted coral colonies and the natural coral community at the restoration site and at the adjacent site were scheduled to be monitored at one week, two weeks, four weeks, 12 weeks, three months, and six months post-outplanting for changes in SCTLD prevalence.

Outplant sites were established during November 2019, and the sites were surveyed as scheduled at one-week and two-weeks post-outplanting. The next survey was scheduled to occur during late December 2019, but inclement weather postponed this effort until early January 2020, approximately 6 weeks after the outplanting. The sites were again monitored during February, approximately 12-weeks post outplant. The FWC suspended all field activities during March 2020 due to COVD-19-related health concerns. After consultation with the DEP, it was agreed that the FWC would survey the outplant sites one more time before the end of the project. When minimal field activities resumed during May 2020, the sites were surveyed for the last time as per that agreement.

By May 2020, the survival rate of the outplanted colonies was ~91%. Thirty-one colonies had died (8.6%), and three (1%) were not found. None of the dead colonies had previously shown signs of SCTLD. During December 2019, we identified five colonies that exhibited some tissue loss that we suspected was SCTLD-related. However, when the sites were revisited during February, there was no evidence of disease on these colonies. During February, two additional colonies exhibited signs we believed were SCTLD. But again, no sign of disease was observed

on these or any of the colonies during May. Those seven colonies that had been earlier suspected of showing signs of SCTD were alive.

The roving diver surveys of the natural coral communities were conducted through the February monitoring period. These natural communities exhibited a low incidence of colonies showing signs of SCTLD (Figure 11). At 12 weeks post-outplant, 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 did not observe any coral colonies that showed clear signs of SCTLD on the outplanted colonies, they were subjected to intense predation consistent with parrotfish corallivory after outplanting (Figure 12). Of the 359 colonies found during the 1-week post-outplant survey, 193 (54%) exhibited bite marks (Figure 13). However, the proportion of colonies of each species with evidence of bite scars decreased progressively each time the sites were re-visited, and by 12-weeks post outplant, only 10 (2.88%) of the 347 colonies surveyed bore evidence of bite scars (Figure 14). Additionally, the percent of live tissue per surviving colony, which had decreased across our sites immediately after outplanting due to predation, began to increase (Figure 15). Colonies that experienced predation during the 1-week post-outplant were no more likely to die by 12-weeks post-outplant than colonies that did not experience predation ($\chi^2_{1,356} = 2.313$, $p = 0.1283$; Chi-Squared).

<u>Task 4</u>: Conduct SCTLD surveys along the intermediate reef strata (10-25m) along the Florida Keys reef tract assess SCTLD prevalence in this habitat.

Most of the *in-situ* SCTLD research and reconnaissance surveys has occurred along the shallow habitats of the offshore bank reef and inshore patch reef and hard-bottom habitats. Lesser effort has been directed at evaluating the prevalence of SCTLD along the deeper intermediate reef habitat, typified by low relief spur-and-groove reef structure whose coral community includes several SCTLD-susceptible species. During the summer of 2019, we observed what appeared to be a higher incidence of SCTLD-affected colonies in this deeper habitat compared to the adjacent shallower bank reef habitat. Although this habitat is deeper than where most restoration efforts occur, the presence of SCTLD in this habitat could increase the risk of its transmission along the shallower habitats should restoration efforts commence. Therefore, understanding the risk of SCTLD-affected coral communities in these deeper habitats is vital to evaluating the risk of restoring shallower reef habitats.

This Task included a survey of SCTLD prevalence at a minimum of 30 sites in the reef habitat at depths ranging from 10-25m. This survey uses a rapid assessment approach outlined in the RTT Action Plan. This approach entails using a 30-minute roving diver survey to record the number of SCTLD-susceptible coral colonies, their size (cm diameter), and health status (healthy, diseased, or recently dead).

We completed 29 of the planned 30 surveys. We conducted six surveys at Carysfort Reef, four at Alligator Reef, and two near Pickles Reef, three at Tennessee Reef, four on reefs off Marathon, four near Looe Key and six within Western Sambos Ecological Reserve. Survey depths ranges from approximately 10-25m. The surveys were conducted from late October 2019 through early February 2020, a wider timeframe than originally envisioned, and the interpretation of our results are made with this consideration.

SCTLD infected coral colonies were observed across the surveyed regions. Carysfort Reef had the lowest proportion of SCTLD-infected diseased colonies (0.66%) and Western Sambos ER region the highest (6.14%) (Figure 16). Perhaps not surprisingly, the two survey regions in the lower Keys showed the most evidence of recent SCTLD infections, as evidenced by the

proportion of colonies that had recently died. Overall, the coral species that had the highest proportion exhibiting signs of SCTLD were *D. stokesii* (10.53%), *C. natans* (10.26%), and *M. cavernosa* (9.47%). Again, the majority of the actively diseased or recently dead individuals for these species were observed in the Lower Keys at the intermediate reefs near Looe Key and Western Sambo (Table 1).

Literature Cited

Sharp W.C., Maxwell, K.E. 2018. Investigating the ongoing coral disease outbreak in the Florida Keys: Collecting corals to diagnose the etiological agent(s) and establishing sentinel sites to monitor transmission rates and the spatial progression of the disease. Final Report to the Florida Department of Environmental Protection.

Sharp W.C. Maxwell, K.E. and Hunt JH. 2019. Investigating the ongoing coral disease outbreak in the Florida Keys: Evaluating its small-scale epidemiology and mitigation techniques. Final Report. Florida Department of Environmental Protection Award. Pp. 1-34.

Sharp W.C. Shea, C., Hunt, J.H., Maxwell, K.E. Muller, E. *In review*. Evaluating the small-scale epidemiology of Stony Coral Tissue Loss Disease in the middle Florida Keys.

Table 1. The number and percentage of coral colonies observed with SCTLD and the total number of colonies found (healthy and actively diseased) in the Intermediate Reef habitat of the Upper, Middle and Lower Keys by species. A blank cell indicates no colonies, healthy or diseased, were found in that region.

	Upper Keys		Middle Keys		Lower Keys		Total	
Species	No. of Colonies with SCTLD	Percent of Colonies with SCTLD	No. of Colonies with SCTLD	Percent of Colonies with SCTLD	No. of Colonies with SCTLD	Percent of Colonies with SCTLD	No. of Colonies with SCTLD	Percent of Colonies with SCTLD
Diploria labyrinthiformis	6	0.0%	11	0.0%	4	0.0%	21	0.0%
Mycetophyllia spp.	9	0.0%	7	0.0%	20	0.0%	36	0.0%
Mussa angulosa	1	0.0%			11	0.0%	12	0.0%
Madracis decactis	13	0.0%			12	0.0%	25	0.0%
Porites astreoides	218	0.0%	247	0.0%	314	0.0%	779	0.0%
Porites divaricata	7	0.0%			2	0.0%	9	0.0%
Porites porites	54	0.0%	16	0.0%	20	0.0%	90	0.0%
Siderastrea radians	11	0.0%	10	0.0%			21	0.0%
Stephanocoenia intersepta	591	0.1%	424	0.6%	684	0.2%	1699	0.9%
Agaricia lamarcki	48	1.0%	14	0.0%	41	0.0%	103	1.0%
Agaricia agaricites	585	0.1%	165	0.5%	266	0.4%	1016	1.0%
Meandrina meandrites	21	0.0%	4	0.0%	19	2.3%	44	2.3%
Pseudodiploria strigosa	9	0.0%	11	0.0%	11	3.2%	31	3.2%
Siderastrea siderea	833	0.5%	1278	2.7%	1263	2.2%	3374	5.4%
Orbicella annularis	2	0.0%	10	0.0%	6	5.6%	18	5.6%
Eusmilia fastigiata	18	3.1%	1	0.0%	13	3.1%	32	6.3%
Orbicella franksi	14	0.0%	13	6.9%	2	0.0%	29	6.9%
Solenastrea bournoni	4	0.0%	7	7.7%	2	0.0%	13	7.7%
Orbicella faveolata	101	1.1%	58	1.7%	177	5.0%	363	7.7%
Montastraea cavernosa	140	0.2%	98	1.8%	364	7.5%	602	9.5%
Colpophyllia natans	5	0.0%	5	0.0%	29	10.3%	39	10.3%
Dichocoenia stokesi	1	0.0%	7	0.0%	11	10.5%	19	10.5%

Figure 1. Map showing locations of SCTLD sentinel monitoring sites.

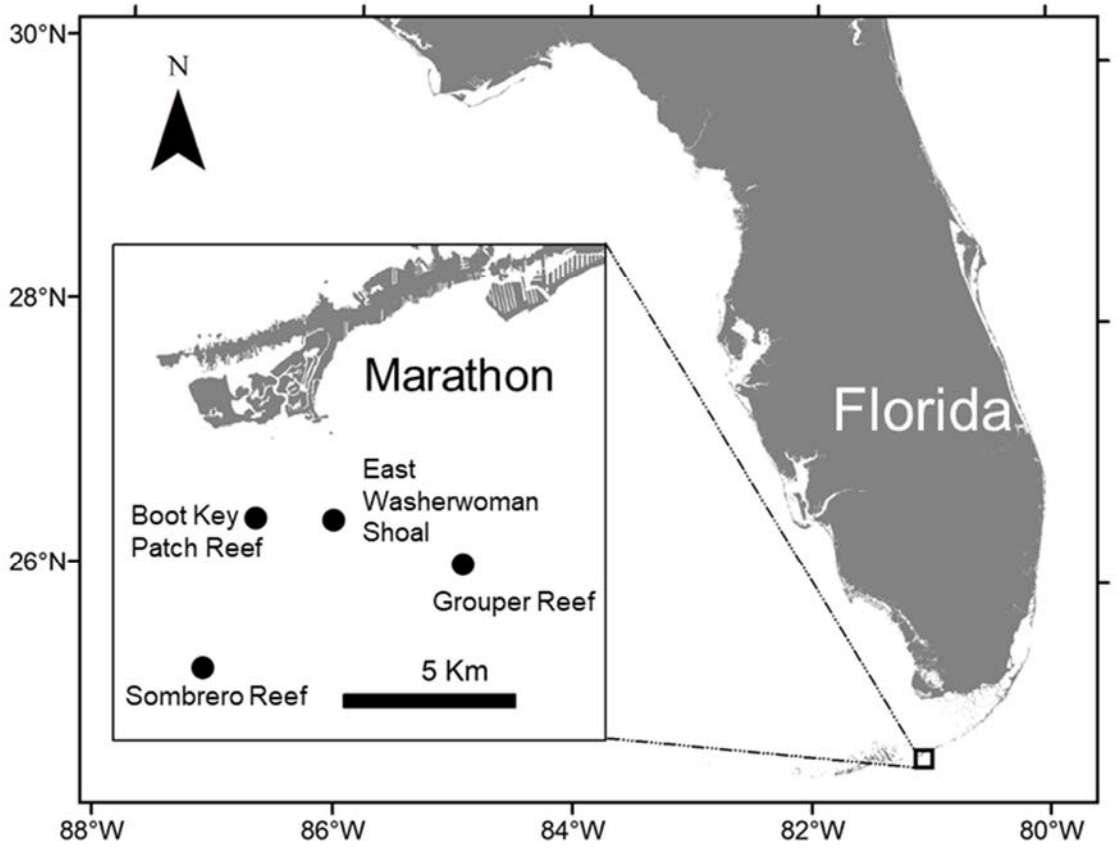

Figure 2. Time series summarizing the status of coral colonies at (A) Boot Key Patch Reef and (B) East Washerwoman Shoal.

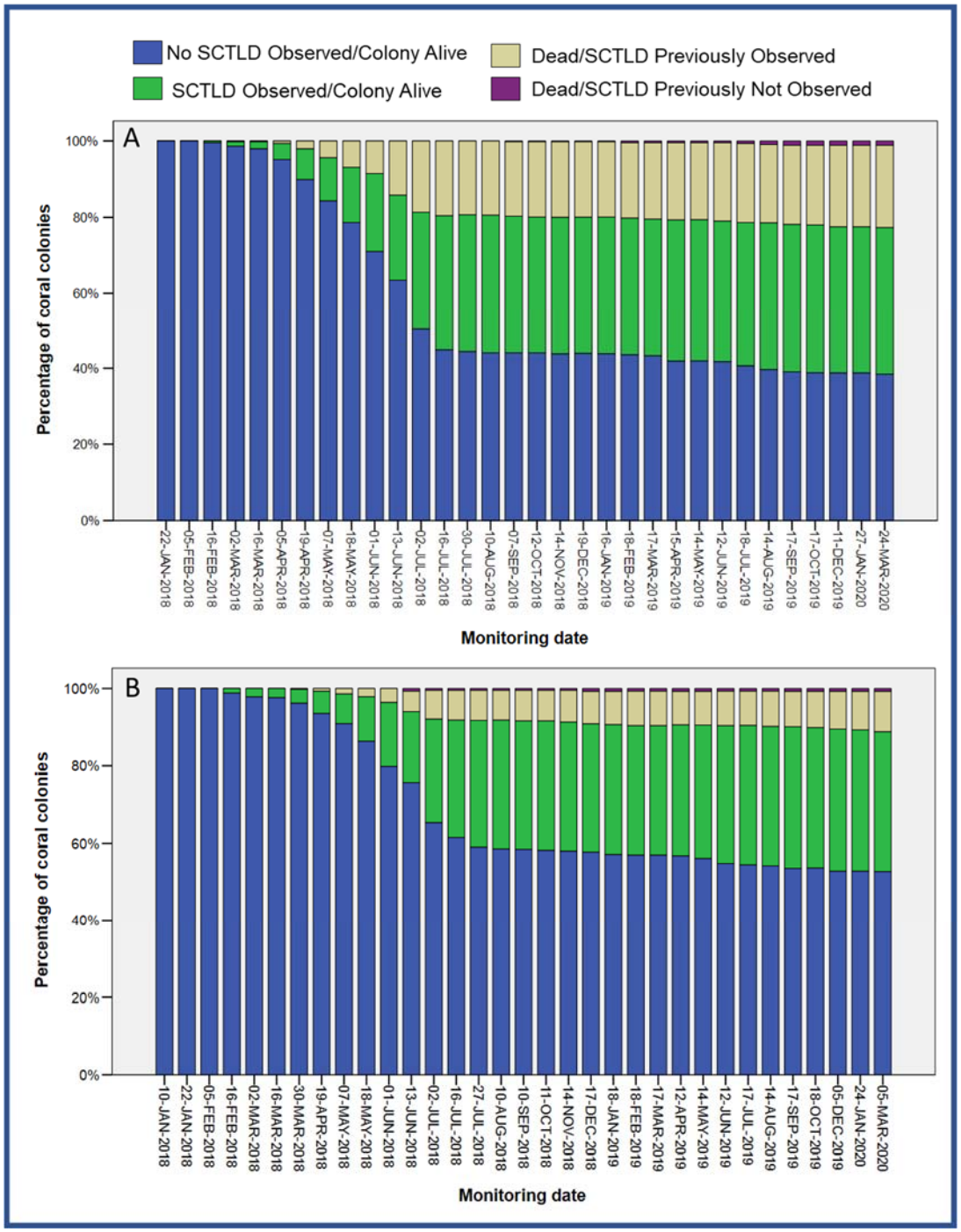

Figure 3. Time series summarizing the status of coral colonies at (A) Grouper Reef and (B) Sombrero Reef.

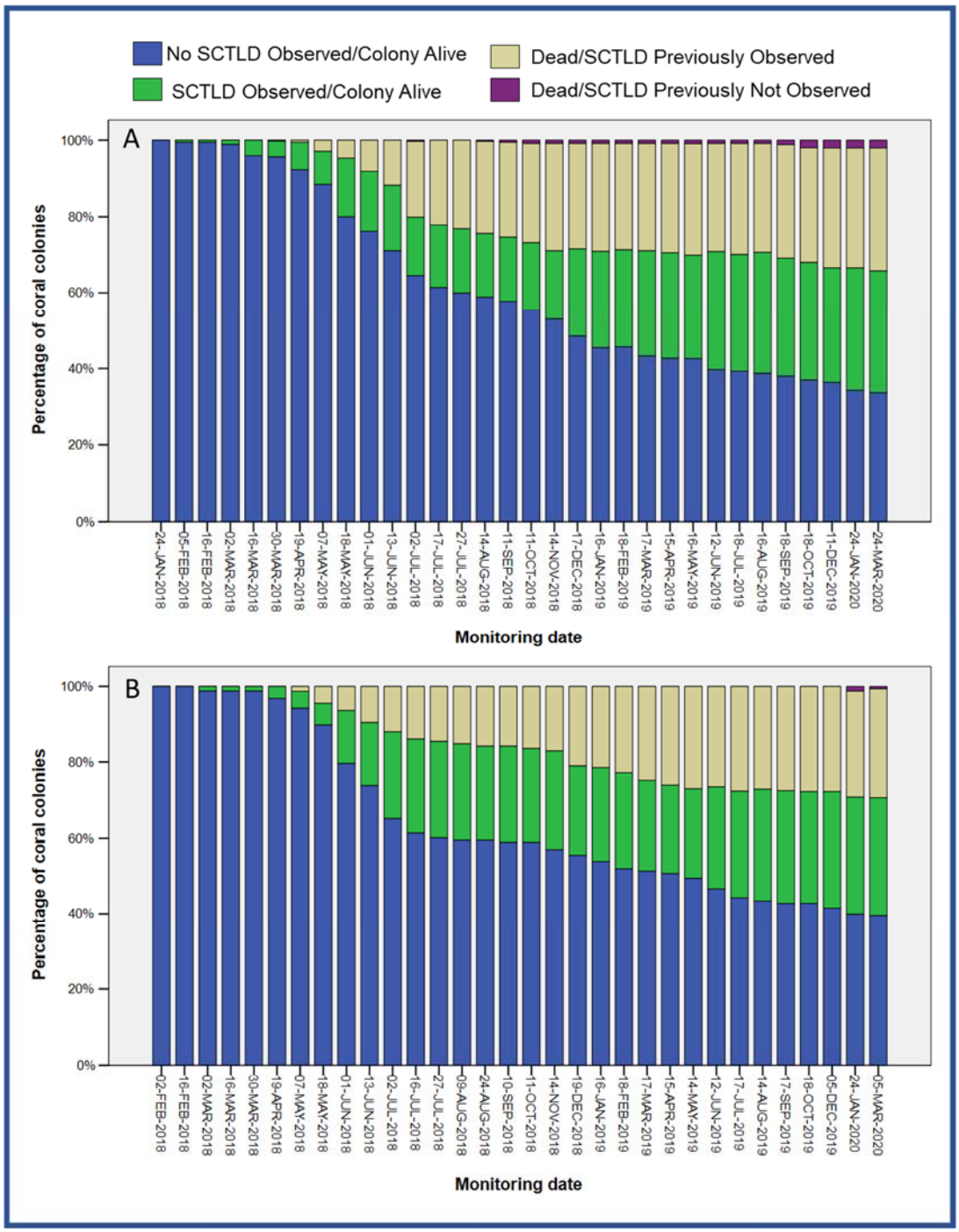

Figure 4. Summary of the disease status of the coral communities at the four sentinel sites during March 2020. “SCTLD Active” denotes colonies that showed signs of SCTLD infection and had exhibited tissue mortality during the September 2019 – March 2020 survey period. Colonies denoted as “SCTLD inactive” are those that had shown signs of SCTLD infection prior to September 2019 but exhibited no tissue mortality.

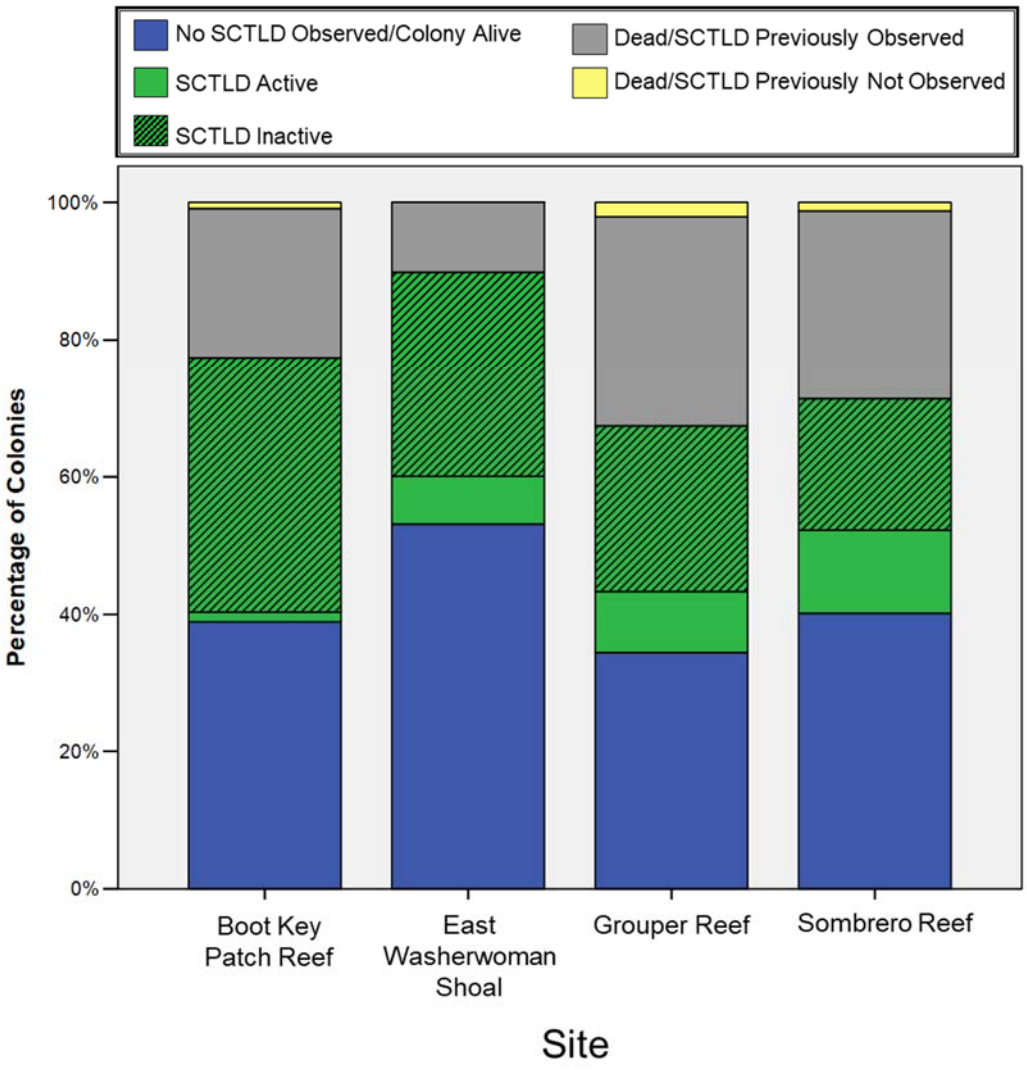

Figure 5. Map of the middle and lower Florida Keys showing locations of FWC's sentinel monitoring sites and MML's sentinel sites where FWC's intervention trials were conducted from August 2018 through May 2019. The FWC resurveyed the MML sites during September 2019.

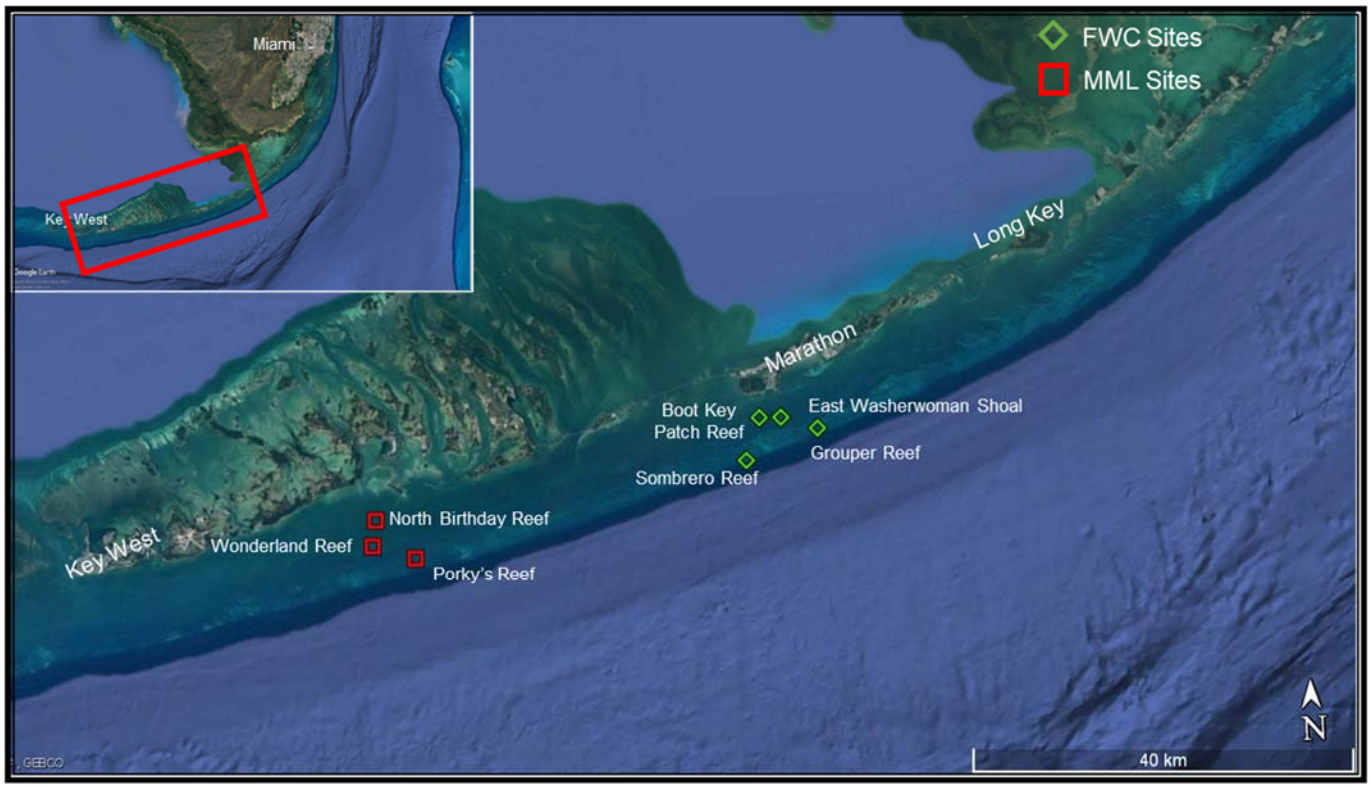

Figure 6. Mean (±1SE) Percent living tissue of colonies during May 2019 and September 2019 that had been treated with antibiotics at some point during the intervention effort conducted from August 2018 through May 2019.

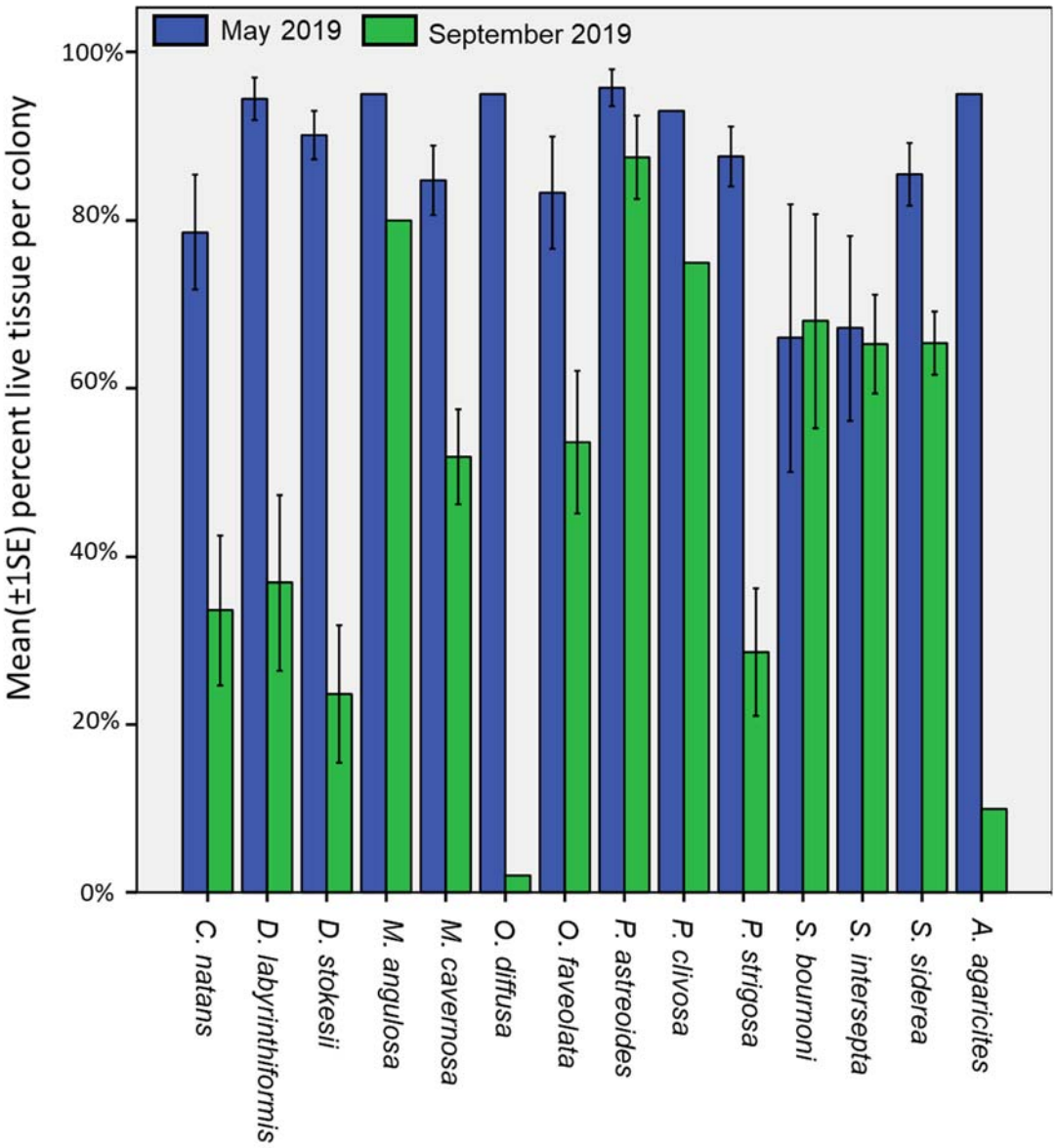

Figure 7. Time series of the mean (±1 SE) percentage of living tissue per colony at the three FWC intervention reefs in the lower Keys. From August 2018 through May 2019 sites were surveyed for colonies exhibiting signs of SCTLD. SCTLD lesions on infected colonies were treated by applying an antibiotic at regular monitoring intervals. Sites were revisited during September 2019, six months after the effort had concluded to assess the coral community. (A) Porky's Reef, (B) Wonderland Reef, and (C) North Birthday Reef.

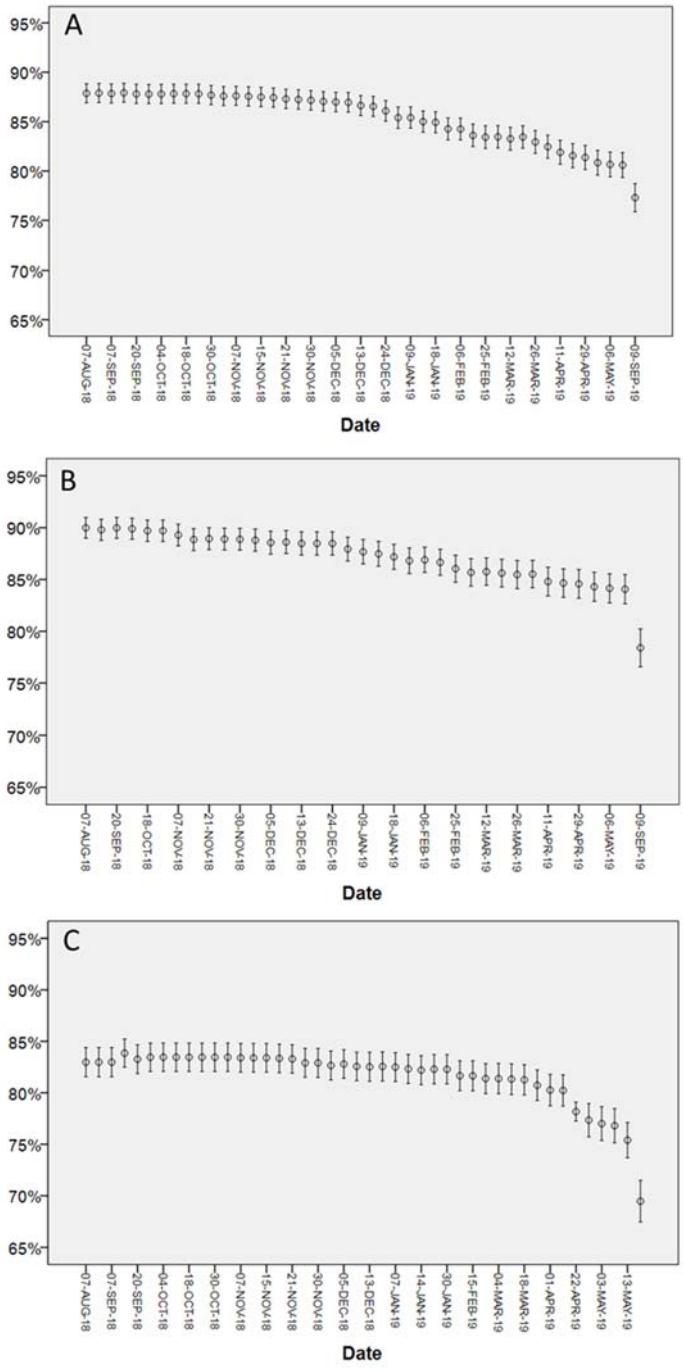

Figure 8. Map of the coral outplant locations spanning the upper and middle Florida Keys.

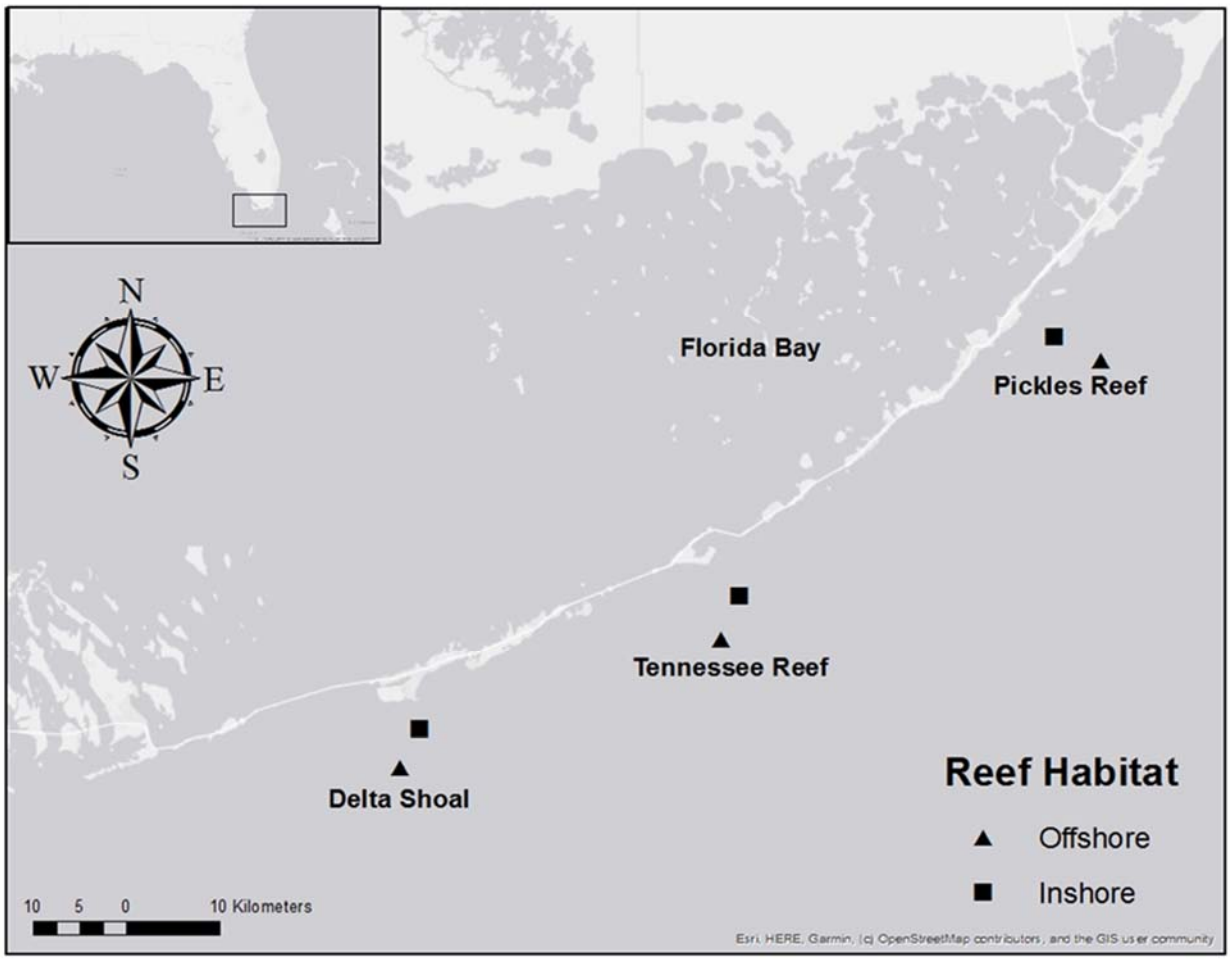

Figure 9. Conceptual diagram of the outplant study's experimental design. Sites were established at an inshore patch reef and on an offshore bank reef location at three locations: Pickles Reef, Tennessee Reef, and Delta Shoal.

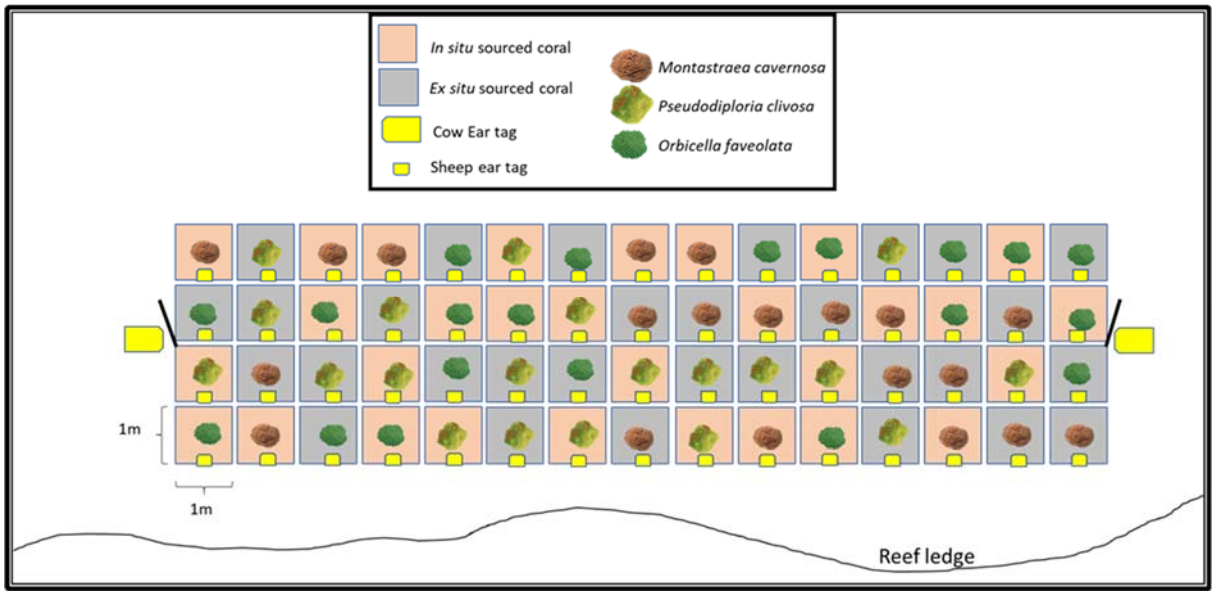

Figure 10. Photograph of an FWC diver adhering a coral colony to the substrate at part of the experimental outplanting effort.

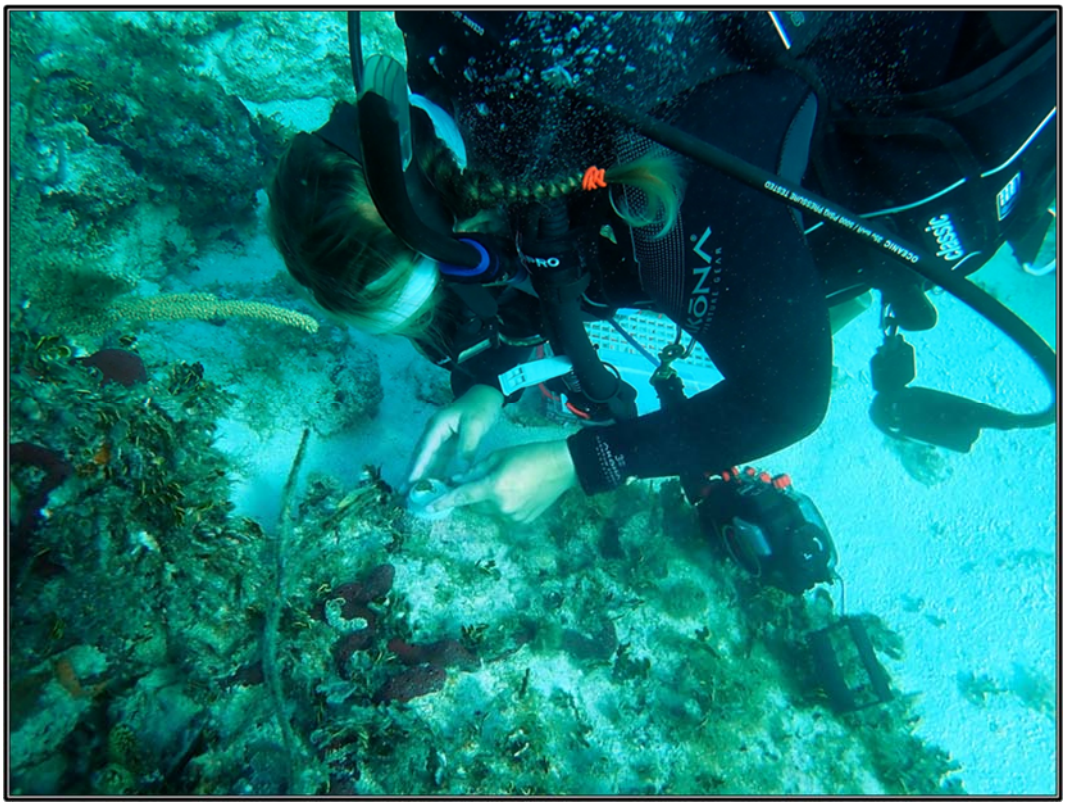

Figure 11. Time series of SCTLD incidence between the natural coral communities at coral outplanting sites and control sites observed by 30-minute roving diver surveys through 1two weeks post outplanting. Coral colonies not exhibiting SCTLD were categorized as "Uninfected", colonies with clear SCTLD infections were defined as "Infected". Colonies defined as "Recent Whole Colony Death" were those exhibiting signs of rapid tissue loss resulting in whole colony death that was consistent with SCTLD infection. Numerals on the bars represent the number of coral colonies.

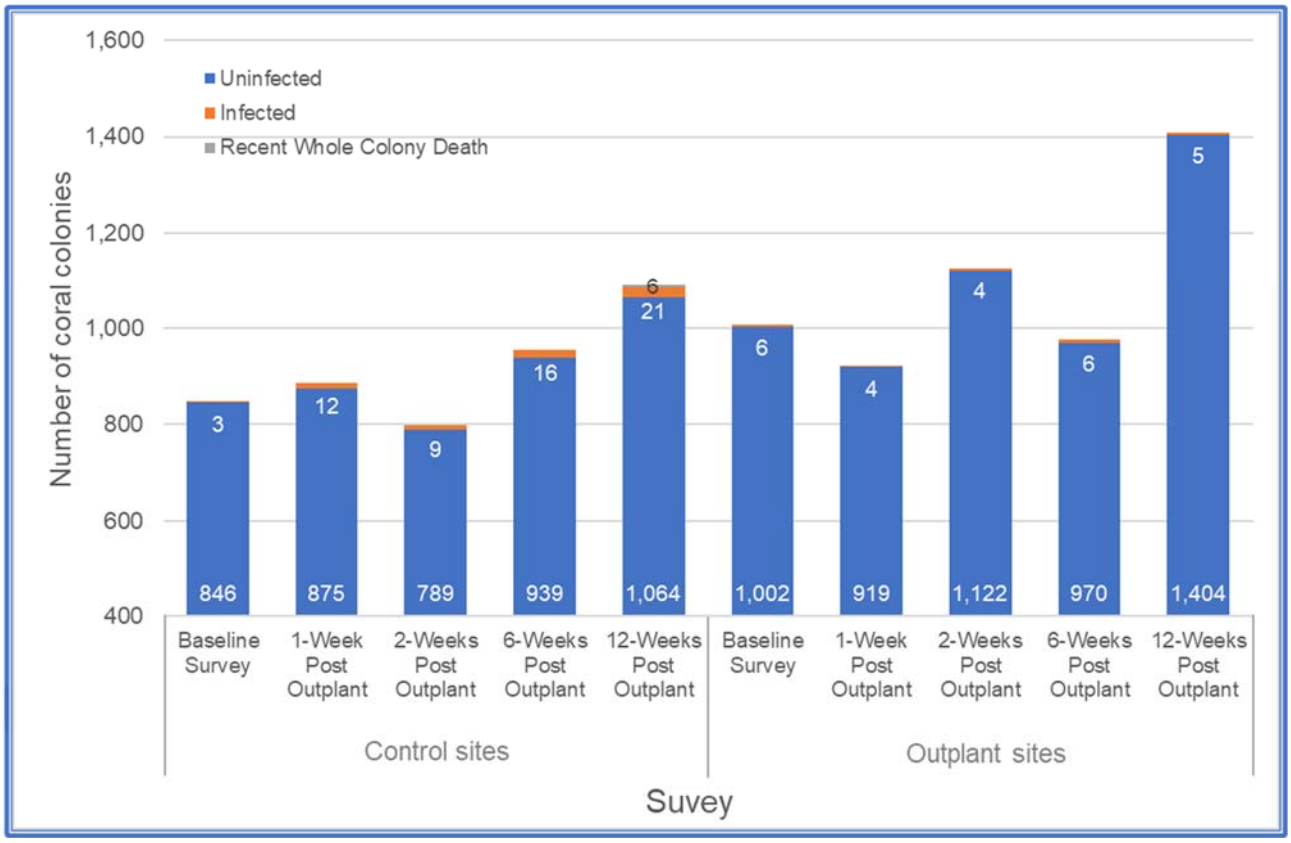

Figure 12. Photographs taken of individual coral colonies taken immediately after outplanting (A, C, E) and one-week post-outplanting (B, D, F). (A, B), *O. faveolata*; (C, D), *M. cavernosa*; *P. strigosa* (E, F).

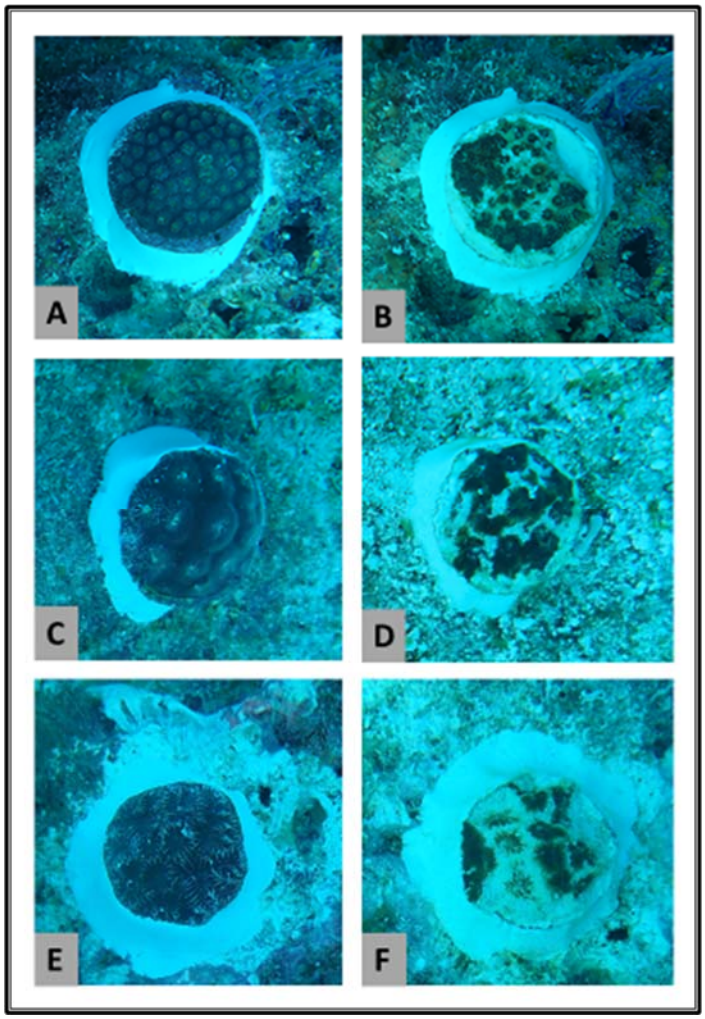

Figure 13. The number of coral colonies with finfish predation scars observed on inshore and offshore sites at each location one-week post-outplanting for *in situ*- (A) and *ex situ*-propagated (B) coral colonies.

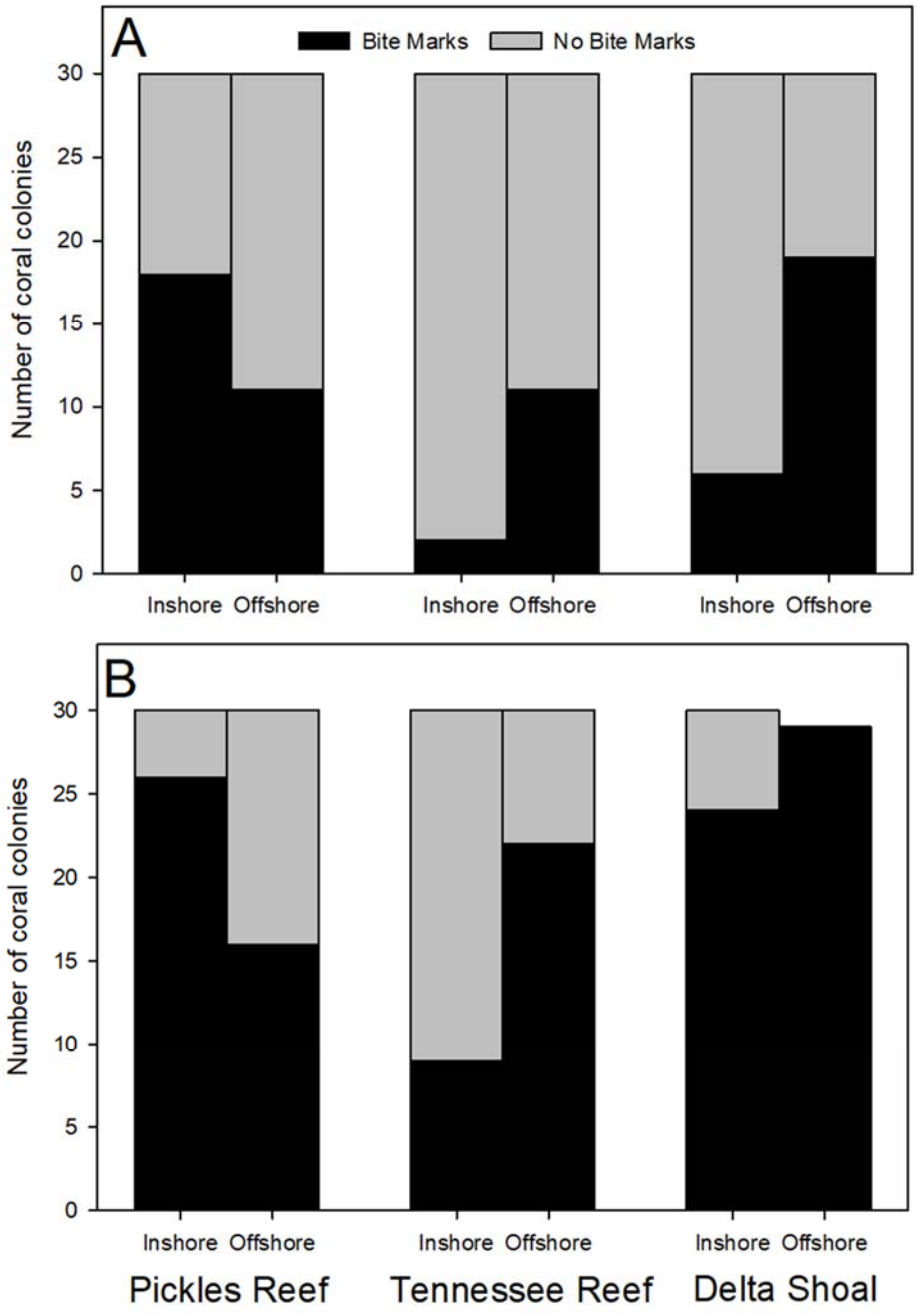

Figure 14. The number of coral colonies with finfish predation for each monitoring period by species (M – *M. cavernosa*, O – *O. faveolata*, P – *P. clivosa*) for *in situ*- (A) and *ex situ*- (B) propagated colonies. Colonies that died or were not relocated are excluded.

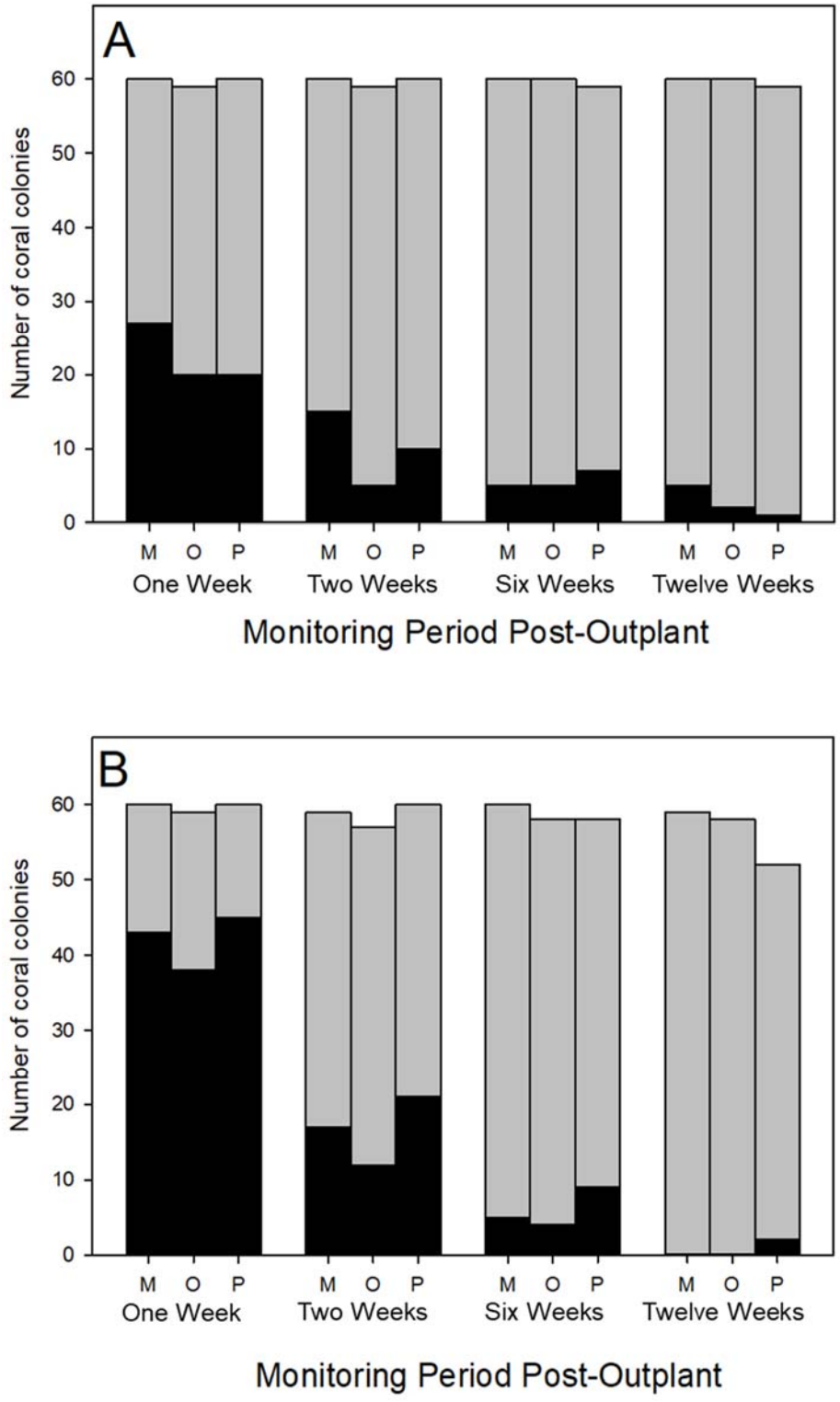

Figure 15. Mean (±1SE) percentage of live tissue per colony during each monitoring period for *ex situ*- and *in situ*-propagated colonies.

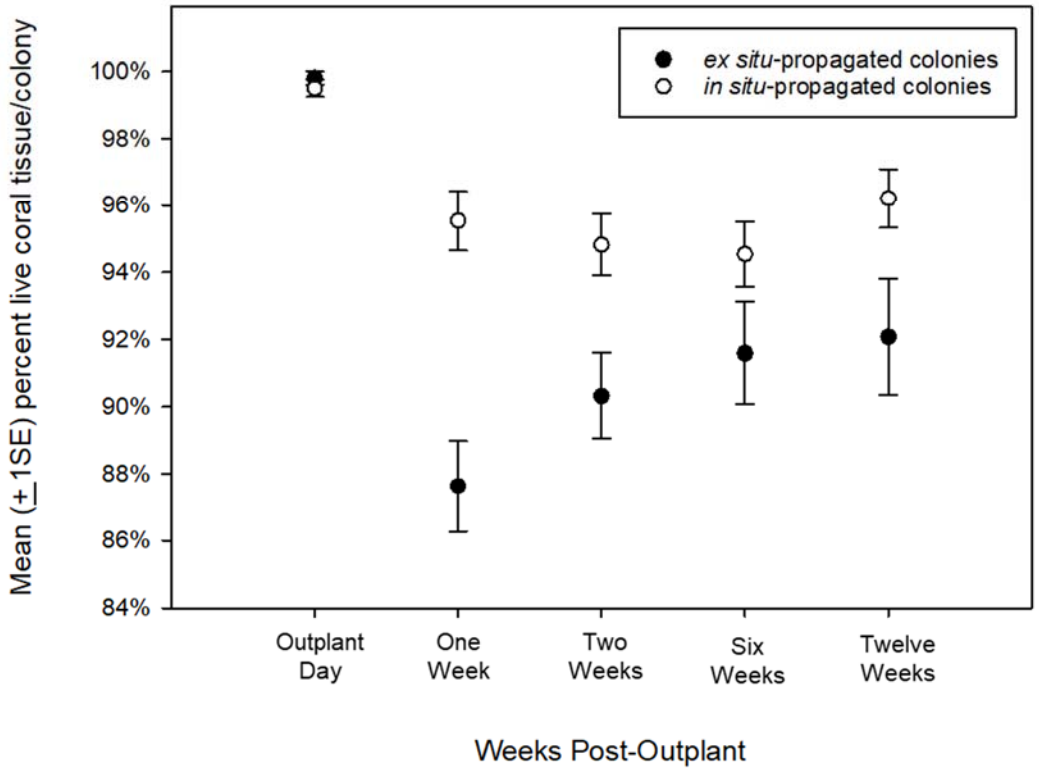

Figure 16. The percentage of coral colonies observed during roving disease surveys within the Intermediate Reef zone along the Florida Reef Tract. Coral colonies not exhibiting SCTLD were categorized as "Uninfected", colonies with clear SCTLD infections were defined as "Infected". Colonies defined as "Recent Whole Colony Death" were those exhibiting signs of rapid tissue loss resulting in whole colony death that was consistent with SCTLD infection. Numerals on the bars represent the number of coral colonies.