FLORIDA KEYS CORAL DISEASE STRIKE TEAMS: FY 2018/2019 FINAL REPORT

Florida Department of Environmental Protection
Coral Reef Conservation Program

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FY 2018/2019 FINAL REPORT

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Background

Since 2014, a multi-year, multi-species disease outbreak has progressed geographically along the Florida Reef Tract from an origin near Virginia Key. Termed Stony Coral Tissue Loss Disease (SCTLD), it affects over half of the stony coral species on the reef and usually results in 60-100% infection rates and 100% subsequent mortality. Susceptible species include five of the seven ESA-listed Caribbean coral species and most of the reef-building species.

A response priority has been active in-water intervention to treat diseased corals. As such, a collaborative coral disease response strike team was established between Nova Southeastern University (NSU) and FORCE BLUE, a nonprofit organization composed of former elite combat divers who are retrained and deployed on missions of conservation. The strike team consisted of FORCE BLUE divers who were contracted for 50 days of dive support, and NSU divers contracted separately for 40 days of oversight and collaborative efforts with FORCE BLUE plus an additional 30 days of associated in-water coral treatment and data collections. All FORCE BLUE operations were ultimately completed in collaboration with NSU. From December 2018 to June 2019, the NSU/FORCE BLUE strike team conducted extensive treatments on corals at locations designated by the ECT (Executive Coordination Team) as high priority sites. Laboratory and field trials conducted since December 2017 (Neely and Hower 2019; Neely 2018a) provided disease treatment options that might prove effective on wild diseased corals, and these were employed by the in-water teams. This document reports on work conducted through June 19, 2019.

Permitting and Logistics

Permitting to conduct diseased coral treatments using antibiotic pastes and chlorinated epoxies, including grinding a trench to create a disease firebreak, was federally authorized on November 30, 2018 under permit FKNMS-2018-141. The permit approved activity within upper Keys Sanctuary Preservation Areas (SPAs). Revisions to the permit on January 28, 2019 and March 26, 2019 incorporated additional sites by authorizing treatment at all locations from Looe Key SPA north. Permission to apply antibiotics was separately authorized by the FDA's Office of Minor Use and Minor Species.

Logistics were coordinated by both NSU and FORCE BLUE personnel. For the 50 collaborative in-water days, FORCE BLUE arranged dive charters with upper Keys (Captain Slate's) and middle and lower Keys (Captain Hook's) outfitters. Nova Southeastern University personnel coordinated acquisition of supplies, organized training activities, conducted early scouting and information gathering on sites, coordinated daily dive plans, and managed work flow, data entry and analyses, photo management, map making, and overall coordination with other agencies. NSU also coordinated boat logistics for days without a FORCE BLUE presence, including all follow-up monitoring and most retreatment activities.

Training

Training for FORCE BLUE personnel occurred over one classroom day and one field day and focused on the following activities:

- Overview of reef importance at a global, regional, and local scale
- Coral physiology
- Coral identification, with priority focus

Fig 1. FORCE BLUE personnel are trained on tools and treatment materials, and on how to identify priority corals.

on the target species most likely to be encountered for treatment
- Identification of disease and other coral stressors
- Identification of priority corals using the guiding principles laid out in the Disease Intervention Action Plan (Neely 2018b)
- Types of intervention treatments
- Hands-on practice for preparing and applying interventions

Training materials were provided to FORCE BLUE personnel, who trained new strike team members as they cycled through. The training materials were also provided to other practitioners in SEFL and modified for training sessions for reef managers from Mexico and the US Virgin Islands who visited to learn the techniques.

Protocols and Quality Assurance

During the NSU/FORCE BLUE strike team work, two teams of divers, each consisting of 1 NSU diver and 1-2 FORCE BLUE divers, identified and treated priority corals. Each of the two teams was responsible for one type of treatment (detailed protocols for each treatment are in Appendices I and II):

- Amoxicillin mixed with CoreRx paste in a 1:8 by weight ratio. The compound was applied directly to the disease margin using a syringe and fingertip (Figure 2)
- Powdered chlorine mixed with Splash Zone epoxy in a 3:10 by volume ratio. The compound was applied directly to the disease margin and also to a trench approximately 5 cm from the disease margin. The trench was created using an underwater angle grinder and was 1-2 cm deep and ~1 cm wide (Figure 3)

Teams followed standard operating procedures, which included:

- Selecting priority corals as outlined in the Disease Intervention Action Plan (Appendix III)
- Taking photos of the coral and the lesions
- Taking diameter and height measurements of the coral
- Affixing a numbered tag with citizen science instructions to dead coral skeleton or adjacent substrate
- Applying the treatment
- Taking photos of treated lesions
- Getting distance/bearings from other tagged corals or fixed points in order to build a map for subsequent monitoring

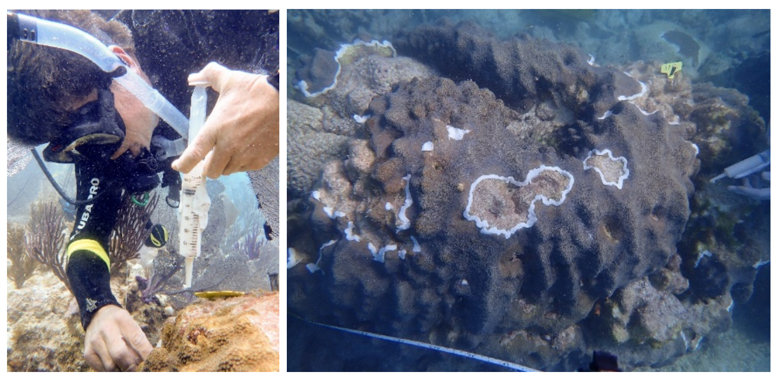

Fig 2. Application and appearance of treatment using amoxicillin paste on disease margins.

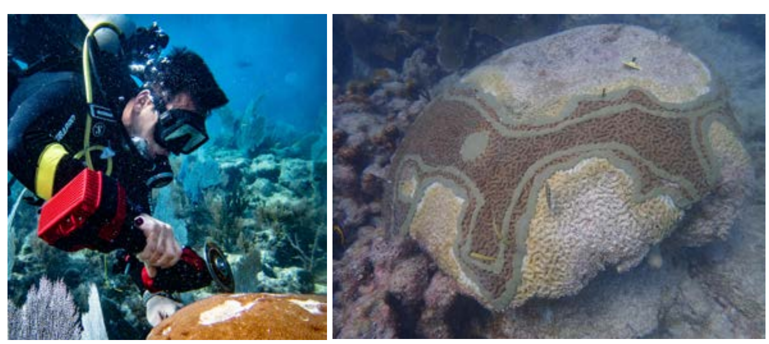

Fig 3. Firebreaking a coral for application of chlorinated epoxy and appearance of a treated coral showing treatments on the margins as well as the firebreaks.

Depending on the site and circumstance, an additional NSU diver would sometimes scout for nearby priority corals, take photo/video, and during the initial days or when new team members were brought in, supervise and approve treatments.

A Quality Assurance (QA) plan was developed to lay out the site selection process, work plan, and monitoring guidelines (Neely 2019). Site selection was guided by the management and ECT teams which initially prioritized upper and middle Keys Sanctuary

Preservation Areas (SPAs). SPAs were selected because of their high stakeholder use, visibility and ease of access (all have mooring buoys), and potentially easier mapping and monitoring conditions. After the selected SPAs were treated or found to have no treatable corals, permitting and site prioritization shifted to Looe Key and Newfound Harbor SPAs in the upper part of the lower Keys.

The proposed monitoring plan was to check at least 10 treated colonies or 10% of treated colonies (whichever was greater) at each treatment site. Monitoring was set for one-month post-treatment, and retreatment of colonies with failed or new lesions was to be incorporated. Monitoring protocols were to take photos of the whole colony, each visible treatment area (epoxy line, nails), and any active disease areas. If further treatment was conducted, the number of lesions treated was recorded and separated into "retreatments" (fixing a break in a previously treated lesion) or "new treatments" (treating a new lesion). With only a few early exceptions, all follow-up treatments were conducted with amoxicillin, regardless of the original treatment type. Photos from pre-treatment, post-treatment, and all monitoring events (including citizen science reports) were compiled into time-series documents that track each colony and lesion through time (see example in Appendix IV). These time-series photos were used to quantify failure rates by assessing the change in the disease margin between each treatment/monitoring period. Each lesion was classified as either "ineffective" (disease progressed past the treatment and proceeded unimpeded across the tissue) or "effective" (disease progression halted at or before the treatment line). Interobserver discrepancies were accounted for by each of three NSU team members conducting full photo assessments of all monitored corals. These differences in reported treatment ineffectiveness are represented as error bars in figures.

Work Accomplished

As of June 22, 2019, a total of 51 collaborative NSU/FORCE BLUE days and 29 additional NSU field days had been conducted. Further NSU work continued through the end of the fiscal year (June 30).

During FORCE BLUE collaborative days, a total of 1191 colonies were treated; 32% were treated with chlorinated epoxy and 68% with amoxicillin (Table 1). As most colonies had multiple lesions, this work also represents the treatment of 5687 lesions; 16% were chlorine, 84% were amoxicillin. This discrepancy in sample size between treatments is due to a) more rapid and efficient application of the amoxicillin treatment, b) chlorinated treatments encompassing multiple lesions via a single firebreak surrounding an infected area, and c) supply problems that necessitated only amoxicillin treatments during the days at the Newfound Harbor site.

Location	Total #	Amoxicillin	Chlorine	# Days at Site
Molasses Reef	19	10	9	4
Crocker Reef	4	3	1	1
Carysfort South	37	23	14	2
Key Largo Dry Rocks	4	2	2	0
Grecian Rocks	1	1	0	0
Sombrero Reef	107	61	46	4
Looe Key	739	431	308	32
Newfound Harbor	280	280	0	6
Total:	**1191**	**811**	**380**	**51**

Table 1. Number of total corals treated by FORCE BLUE/NSU collaborative teams, number treated with amoxicillin paste, number treated with chlorinated epoxy, and days of work conducted at each treatment site.

In total, 16 SPAs and one buoyed non-SPA (Crocker Reef) were visited for treatment (Figure 4). At seven of these sites (six in the upper Keys and one in the middle Keys), no live corals of the susceptible species were found. At two inshore SPAs, susceptible species were found, but without active disease. These sites appeared to have had extensive disease in the past as most colonies had patchy older mortality indicative of the infection pattern. It is hypothesized that these inshore sites exhibited a similar phenomenon as other nearshore patches near Marathon in which disease halted entirely in

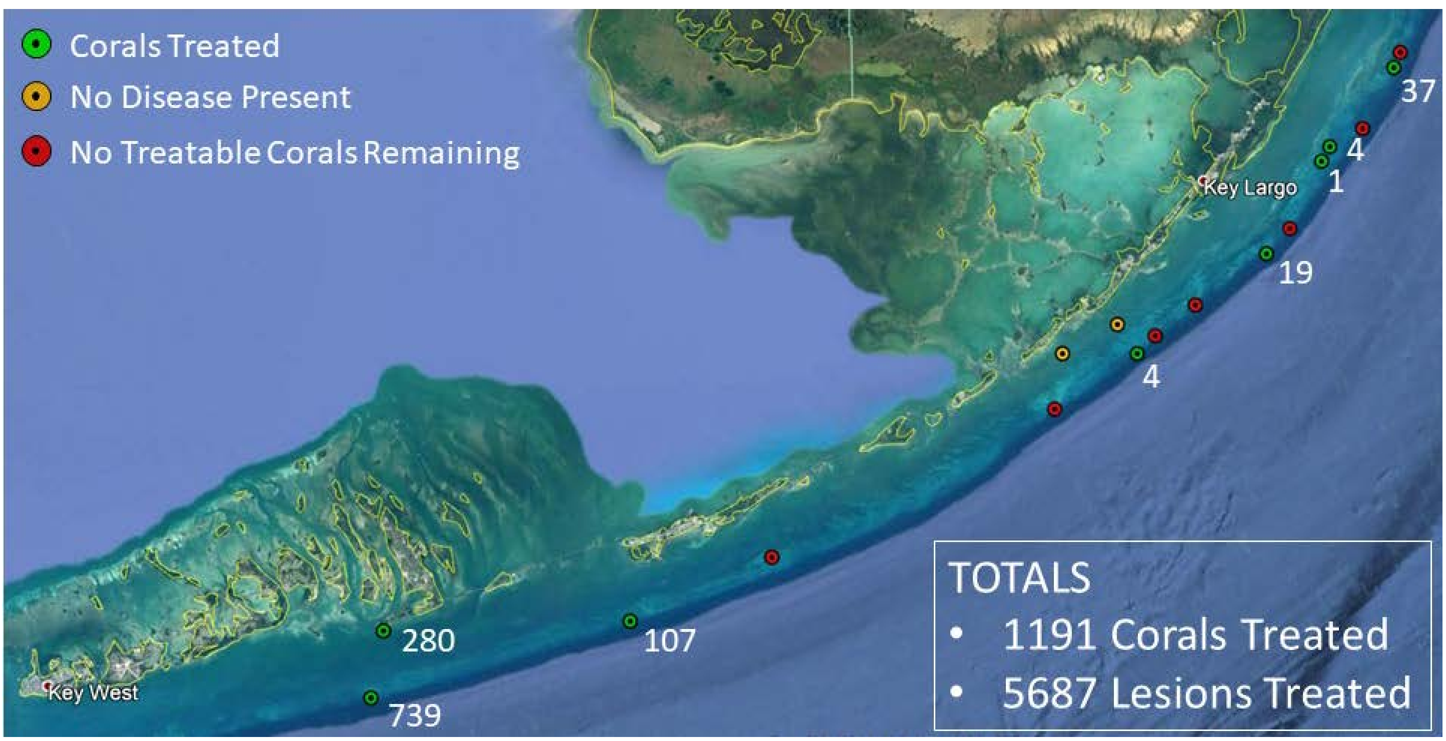

Fig 4. Map showing all visited sites. Sites where no corals were treated are indicated in red (no treatable colonies remaining) and orange (susceptible species present, but no active disease). Sites where colonies were treated are green and numbers indicate the number of colonies treated at each site.

summer 2018. Overall, upper Keys sites had few treatable corals, and thus the majority of treatments were at lower Keys sites where diseased corals were abundant and work proceeded with high efficiency.

At sites that did have active disease, all priority corals were treated. Over 60% of the total treated corals were *Montastraea cavernosa* or *Orbicella* spp. *Orbicella faveolata* dominated upper Keys treatments. Species diversity increased at sites further south, and treatments on the recently infected inshore Newfound Harbor site were dominated by brain corals and *Dichocoenia stokesii* (Figure 5).

Monitoring has been conducted over 22 field days and has substantially exceeded minimum monitoring standards. One-month monitoring was conducted at all sites except Crocker (4 colonies). However, prioritization towards utilizing FORCE BLUE for initial treatment work substantially delayed retreatments at some sites (Table 2) and made follow-up monitoring less consistent than desired. Monitoring and retreatment was primarily conducted by the NSU team and continues to be the ongoing priority. Monitoring was also conducted in a limited manner by citizen scientists.

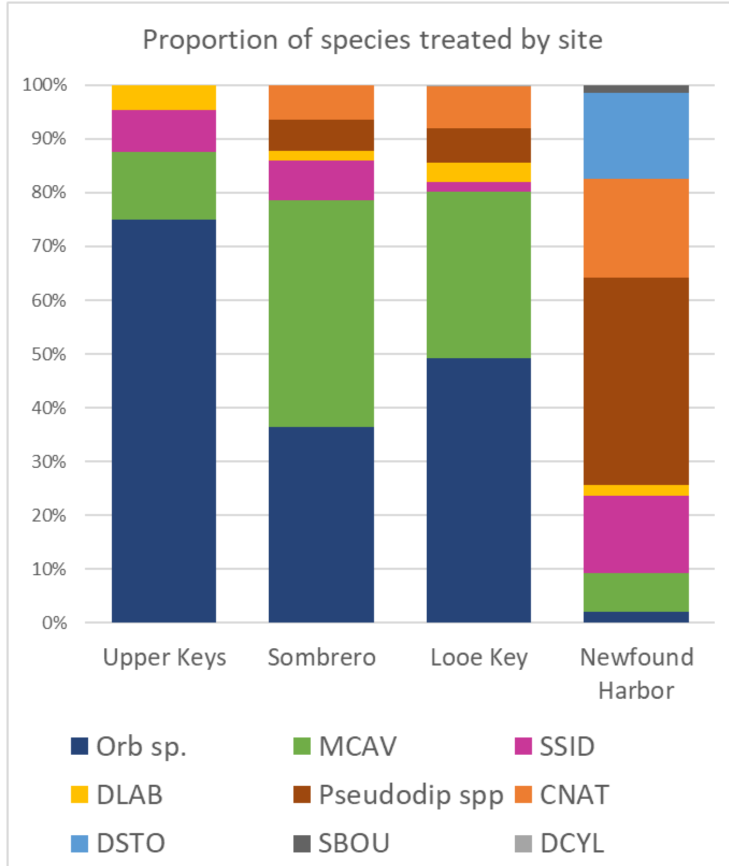

Fig 5. Proportion of treated colonies by species in each region. Labels in the legend represent four-letter species codes. Upper Keys reefs were dominated by *Orbicella* spp. Other species were more present in middle and lower Keys reefs.

Citizen Science

Each treated coral was tagged with an identifying number and instructions directing citizens to an FWC-developed database (www.seafan.net/tags) to upload photos (Figure 6). NSU staff combined these photos, along with the formal monitoring data, into photo time series showing lesion fate over time. Approximately 150 flyers distributed in dive shops, marinas, and local businesses, as well as numerous social media posts, alerted citizens to this project. Over the subsequent five months, a total of 19 usable citizen science reports were submitted. These represent < 3% of the total analyzed observations. Though a valuable way of engaging the community and raising awareness, current citizen science efforts are not enough to replace organized monitoring trips.

	1 month	2 month	3 month	4 month
Newfound Harbor	M & R			
Looe	M & R	M & R	M & R	
Sombrero	M		M & R	
Crocker				
Molasses	M			M & R
Grecian/KLDR	M			M & R
Carysfort	M			M & R

Table 2. Monitoring and retreatment regimes for treated sites. M= monitoring. R = retreatment. One-month monitoring occurred at all sites. Retreatment was delayed at most sites due to logistics.

Fig 6. Identification tags attached to all treated corals. Information for citizen science photo submission directs users to an FWC-created submission form and photo database.

Results

Monitoring results from the first three months show notable differences in effectiveness between chlorinated epoxy and amoxicillin treatments. Failure of epoxy-treated firebreaks increases linearly from 25% (month 1) to 72% (month 3). Failure of amoxicillin-treated lesions remains at 11-12% during months 1 and 2 and increases to 27% in month 3 (Figure 7). Sample size decreases with later months as newer treatments have not yet reached those monitoring thresholds. Additionally, it is worth noting that 88% of chlorine-treated lesions were found and monitored, but that only 59% of amoxicillin treatments were; the disparity is accounted for by the difficulty in re-finding effective amoxicillin treatments, which leave no mark and appear as old mortality. It is thus likely that the failure rates of amoxicillin treatments are significantly lower than represented by this data.

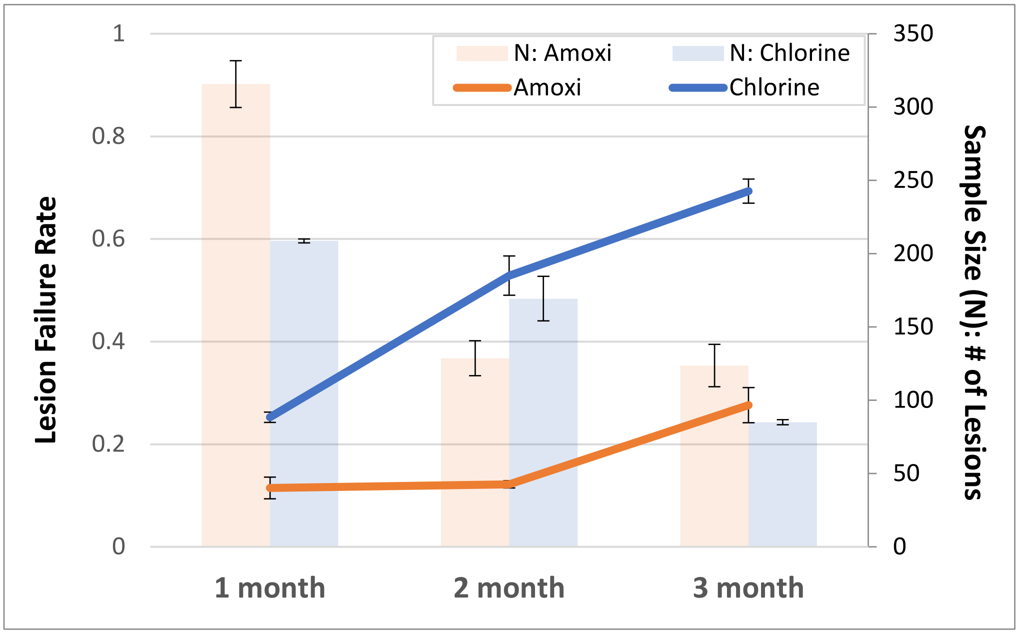

Fig 7. Lesion failure rates at one, two, and three-month intervals summarized across all sites and species. Chlorinated treatments failed at higher rates than amoxicillin treatments across all time periods. Chlorinated treatment failure rates increased linearly across three months while amoxicillin rates remained static for the first two months with a moderate increase in the third month. Sample sizes (N) for all treatments are shown as bars. Sample sizes decrease as time goes on, but will increase as more recent treatments are monitored. Error bars indicate interobserver differences in proportions and lesion counts based on photo timeseries analyses.

The continued rise of chlorinated failure rates with time is attributable to the continued progression of disease across the colony and past the firebreak. Failure rates of the chlorinated treatment applied on the margin application are 70% in the first month and 84% in the second month. In species with rapid progression rates, the disease margin usually also crosses the firebreak by month 1, but in all species, unstopped disease margins continue to spread until they pass the firebreak in subsequent months.

Failure rates can be separated by species. Across five of the most treated species (*Colpophyllia natans*, *Diploria labyrinthiformis*, *Pseudodiploria strigosa*, *Montastraea cavernosa*, and *Orbicella faveolata*), chlorinated epoxy failure rates were higher than amoxicillin failure rates for all species across all time periods (1, 2, and 3 month). Failure rates for chlorine treatments on the brain corals (*C. natans*, *D. labyrinthiformis*, and *P. strigosa*) all exceeded 60% by month 1, 80% by month 2, and reached 100% by month 3 (Figure 8). Failure rates were lower and slower-progressing on *M. cavernosa* and *O. faveolata.* On these species, disease margins progressed more slowly, but still crossed chlorinated firebreaks on 40% of corals by month 2, and 76% (*M. cavernosa*) and 51% (*O. faveolata*) by month 3.

Analyses to date have compared failure rates across the upper Keys sites, Sombrero, and most of Looe Key. Across all sites, failure rates of chlorine are higher than those of amoxicillin. However, there is no clear regional or geographic pattern of differing failure rates across sites (Figure 9). Additional monitoring data at Looe and other sites (3+ months) will help inform longer term trends. However, the former prediction that treatments may be less effective in epidemic areas than in endemic ones is not supported by these monitoring data.

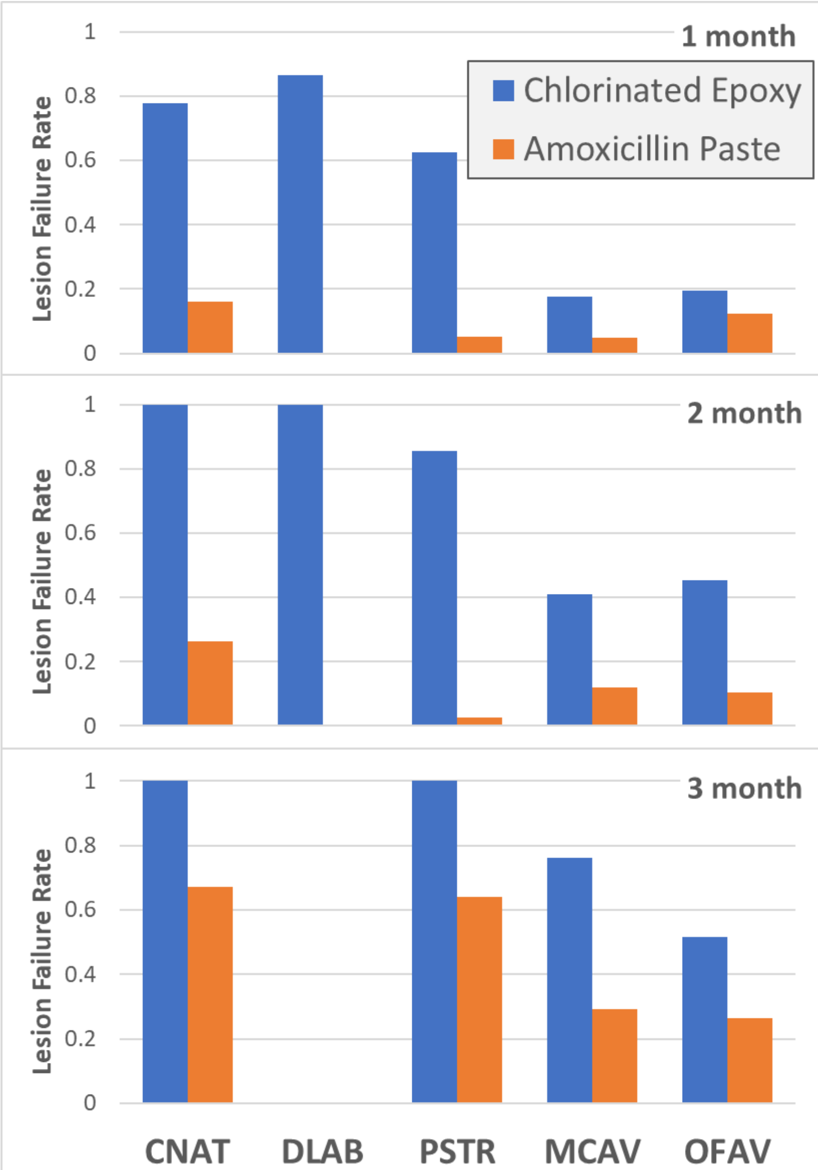

Fig 8. Species-specific failure rates for chlorinated epoxy and amoxicillin paste treatments. For all species across all months, chlorinated epoxy failed more frequently than amoxicillin paste. Letter codes at the bottom represent species (CNAT= *C. natans*. DLAB= *D. labyrinthiformis*. PSTR= *P. strigosa*. MCAV= *M. cavernosa*. OFAV= *O. faveolata*). Failure rates of chlorine were particularly rapid on the brain corals (CNAT, DLAB, PSTR). Amoxicillin treatment failure rates were below 25% during months one and two for all species. At three months, failure rates of amoxicillin treatments rose, perhaps indicating the tissue life of the antibiotic. Longer-term monitoring and increased sample sizes as more treatments age into the three-month monitoring period may help better inform this.

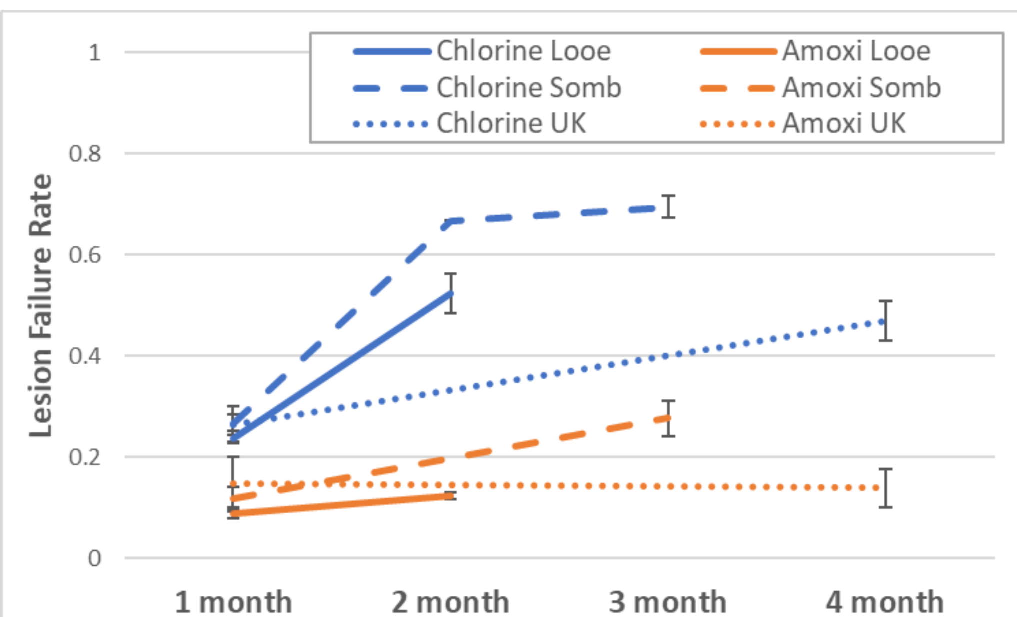

Fig 9. Failure rates of chlorine and amoxicillin treatments over time separated by upper Keys (UK) sites, a middle Keys site (Somb), and a lower Keys site (Looe). At all sites, chlorine treatments fail more frequently than amoxicillin treatments. Differences along a geographic gradient are not as apparent. Subsequent monitoring data may help resolve these patterns.

Failure rates on these corals are similar to those in preliminary field trials as well as laboratory trials (Table 3). Concerns that laboratory conditions might poorly replicate field conditions in terms of disease treatment responses may be unwarranted.

	Chlorinated Epoxy	Amoxicillin Paste
Laboratory Trials	90%	22%
Initial Field Trials	75%	19%
Strike Team Field Trials	85%	28%

Table 3. Comparisons of failure rates of two treatments on diseased Florida Keys corals. Results are summed across all species. For field trials, failure rates from the 3-month monitoring are presented.

Retreatments of lesions have occurred at all original treatment sites except Crocker (Table 4). Retreatments occur when either a) a prior treatment has failed or b) a new lesion has appeared. Retreatment is only conducted if the coral is still considered a priority coral; this precludes colonies in which the coral died, the remaining tissue amount is small, or the number and distribution of lesions make retreatment too difficult. As such, at this stage of analysis, retreatment numbers are representative of, but not equivalent to, the proportion of previously treated colonies in which signs of disease are present during monitoring.

	# Treated		1 month	2 month	3 month	4 month
Newfound Harbor	212	% Monitored	95%	N/A	N/A	N/A
		% Retreated	44%	N/A	N/A	N/A
Looe	401*	% Monitored	49%	58%	8%	N/A
		% Retreated	0%	54%	55%	N/A
Sombrero	107	% Monitored	89%	N/A	93%	N/A
		% Retreated	0%	N/A	69%	N/A
Carysfort South	37	% Monitored	100%	N/A	N/A	100%
		% Retreated	0%	N/A	N/A	46%
Molasses	16	% Monitored	100%	N/A	N/A	100%
		% Retreated	0%	N/A	N/A	81%
Grecian/ KLDR	5	% Monitored	100%	N/A	N/A	100%
		% Retreated	0%	N/A	N/A	40%

Table 4. Proportion of colonies monitored and of monitored colonies retreated during each post-treatment site visit. Except for Newfound Harbor, no retreatments were conducted during month 1 monitoring. *Only the first 401 of the total 695 colonies at Looe are included as analyses are ongoing. The retreatment values highlight the necessity of continued treatments to maintain priority coral survival.

The proportion of monitored colonies with new treatments was 53%. At sites that received both amoxicillin and chlorine treatments, chlorine-treated colonies always had a higher prevalence of new lesions for treatment (Table 5). At Sombrero, these proportions differed by only 1%, but at other sites ranged from 18%-32%.

The prevalence of new lesions requiring retreatments further confirms that treatment effects are limited to the treated lesion(s) and do not provide much if any resistance to the rest of the colony. Revisitation for retreatments is an important component of maintaining these priority corals. Current efforts to retreat and further monitor will hopefully answer two important questions: 1) are some individual colonies more prone to new lesions than others, 2) can continued retreatment minimize subsequent development of additional lesions.

	Treatment	1 month	2 month	3 month	4 month
Newfound Harbor	Amoxi	45%			
Looe	Amoxi		70%		
	Chlorine		93%		
Sombrero	Amoxi			77%	
	Chlorine			78%	
Carysfort South	Amoxi				41%
	Chlorine				73%
Molasses	Amoxi				71%
	Chlorine				89%

Table 5. Proportion of monitored colonies in which new lesions were found and retreated, separated by initial treatment type (amoxicillin or chlorine). Some monitored colonies with new lesions did not receive new treatments because they were not deemed treatable or were dead/too diseased to save; those colonies are not reflected in these percentages. At all sites, amoxicillin-treated corals had a lower proportion of colonies retreated than chlorine-treated ones.

Recommendations

- Continue monitoring and re-treatment (including new lesions) of treated colonies at two-month intervals. Use the ongoing monitoring data to determine whether some corals are more highly prone to reinfection or margin failure. This can help inform intervention decisions about retreatment priorities.
- Abandon current chlorinated epoxy treatments in the Florida Keys because of high inefficiency and high failure rates.
- Field trial results closely mirror laboratory trial results. Initial presumptions should be that results from laboratory are relevant for measures of field performance.
- When selecting priority sites, differences in effectiveness within different regions do not seem to be of concern and should not be a consideration. However, it is relevant to consider work efficiency by selecting areas with a high number of treatable corals. Sites of more recent infection will likely contain corals with smaller lesions and greater live tissue, which offers opportunities for saving more colonies and tissue with less time and expense.
- Continue searching for new disease treatments that may be effective at the colony level.

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APPENDIX I: Protocol for Topical Antibiotic Treatment

1. Create an amoxicillin mixture utilizing powdered amoxicillin and either a specially developed base (created by CoreRx), or basic shea butter. Take appropriate precautions for working with chemicals/pharmaceuticals; risks are unknown. Rubber gloves for touch and hood/masks for inhalation during mixing should be considered.
2. Mix powdered amoxicillin into the base in a 1:8 by weight ratio. 5 g amoxicillin + 40 g base will pack a single syringe for smaller jobs. For larger batches, 400g of base and 50g of amoxicillin will fill ~7 syringes. If using shea butter, it helps to heat it in a warm water bath to make it softer and easier to mix. A small spatula or sturdy rod can be used for mixing.
3. Pack the mixture into a 60 cc syringe. A catheter (tapered) syringe can be helpful as it can be cut higher up if application is difficult. If using shea butter, cooling it before applying to corals (e.g. on ice en route to the site) creates a firmer compound.
4. Pack a goody bag with compound-filled syringes and modeling clay. Syringes are positively buoyant; modeling clay is negatively buoyant. Be careful to secure and close your bag. Sticking clay onto the syringes keeps the materials handy and also makes all items negatively buoyant.
5. Identify treatable lesions and use the syringe to cover the lesion and the immediately surrounding area (~0.5-1 cm) with the compound. It adheres better to skeleton than to tissue, and will require manipulation with fingers to smear along the lesion. Small pieces may detach during application, but can generally be caught and remolded into to the application. Modeling clay can be applied over the top of the treated margin if the paste does not adhere.

APPENDIX II: Protocol for Chlorinated Epoxy Treatment

1. Prepare a chlorinated epoxy mixture utilizing powdered chlorine and 2-part epoxy (the standard is Splash Zone).
    a. The mix ratio of chlorine to Part A of the epoxy is 3:10 by volume. Bundles of 15 mL of chlorine folded into 50 cc of Part A make for a manageable size in field applications. This can be scaled up by using a 3-cup hard sandwich container filled with Part A mixed with 4 x 15 cc vials of chlorine powder.
    b. Use protective measures such as gloves for protection. The epoxy is very messy and sticky, so work somewhere that can get dirty, or lay down protective coverings. Consider any tools you use for this (spatula, spoons, containers, etc.) ruined for any other future non-epoxy use.
        i. ***IMPORTANT NOTE:*** *dry rubber gloves will adhere strongly to the epoxy. Keep a bucket of water near the mix station. Soak hands before handling the epoxy and regularly throughout if they start to get dry.*
    c. Using hands/and or spoon, add and mix chlorine to Part A in 3:10 by volume ratio.
    d. Take an equivalent volume of Part B and place in a different container.

2. Apply the mixture to the diseased colony
    a. Identify lesions for treatment and use angle grinder to create a firebreak ~ 5 cm from each lesion. Firebreak should be ~1 cm wide and 1-2cm deep.
    b. Mix Part A and Part B underwater in a 1:1 ratio. It will eventually become a dark olive green putty consistency.
    c. Smush well-mixed epoxy onto the lesion, spreading into regions that might be infected but not yet dead (sometimes a few polyps in from the lesion)
    d. Pack the firebreak with the chlorinated epoxy mixture as well.

APPENDIX III: Guiding principles for determining priority reef sites and coral colonies

Within each region, intervention actions are targeted towards priority sites and/or priority colonies. Selection of sites/corals are determined by the goals of the region and the management/regulatory bodies, but the following guiding principles are suggested for consideration in selection.

Guiding principles for determining priority reef sites

Ecological:

- Coral diversity: a diverse community may provide more opportunity to protect an intact ecosystem and preserve reproductive capacity of many species with less effort.

- Coral density: high colony density may be representative of a more intact ecosystem with greater habitat, reproductive potential, and ecosystem services. However, such sites *may* be more prone to ongoing infections.

- Coral composition: sites that contain a high number of desired colonies of particular species may be prioritized. For example, sites with ESA-listed species and/or structure-building species might be valued over reefs containing mostly “weedy” or non-susceptible species.

- Demographic structure: Sites with large, reproductively active, structure-producing coral heads contribute disproportionately to habitat and propagation. These sites are usually high relief spur-and-groove structures or substantial patch reefs.

- Isolation: Sites isolated by sand/non-reef structure *may* be less susceptible to ongoing or high prevalence rates from water-borne pathogens. Discrete sites are easier to scout/search, and may be able to be treated more effectively.

Regulatory:

- Within an MPA: In addition to housing many of the ecological features listed above, SPAs and Ecological Reserves *may* potentially mitigate stressors caused by fishing pressure or other activities, and thus may respond more positively to treatment.

- Within a recreational area (near mooring balls): Treating corals within a heavily utilized recreational area may increase project visibility. It may also allow for some involvement by stakeholders such as dive shops that visit the area frequently and could provide feedback. In contrast, any potential concerns about human safety during or after treatments may warrant additional consideration in these regions.

Treatability:

- Coral density: high density sites may allow for more corals to be located, treated, and monitored in a smaller amount of time. However, such sites will require more effort to search for the full suite of infected corals and early lesions.

- Size of site: If all lesions within a discrete site are to be treated, site size is important. There are currently no projects to suggest what the ideal size is, but project considerations such as potential visitation and treatment rate (based on number of people, experience, and time of year), availability of supplies, and ability to permit should all be considered in site selection.

- Number of sites: The suggestions for size of site should also be considered in determining how many sites will be targeted at one time. Additionally, determining whether the treatment process is experimental will affect whether appropriate controls also need to be considered.

- Location of sites: In addition to ecological considerations, logistical considerations may determine site location. Distance from shore, distance from dock/boat ramp, ability to moor/anchor, and general visibility at the site may all be considerations in selecting treatment sites. Co-occurrence with other natural or cultural resource management and protection efforts may help maximize returns.

Guiding principles for determining priority coral colonies

Ecological:

- Structure builder: Some susceptible species contribute substantially to reef-building and the associated ecosystem services that provides (*Orbicella* spp., *Montastraea cavernosa*, *Colpophyllia natans*). These species may be prioritized over others that are not primary structure builders.

- Size: Larger colonies are likely to have greater reproductive capacity and provide more habitat. Corals larger than 2 meters may be prioritized for these features.

- Relative size: Colonies that are large for their species are likely to be older and thus more resilient to long-term environmental conditions. They also likely contribute more substantially to reproduction within their species. Corals in the top 5% of size for their species may be prioritized.

- Localized reproductive capacity: A coral surrounded (in the same general reef area) by other live colonies of the same species may have greater reproductive capacity because fertilization rates are likely to be greater.

Regulatory:

- Iconic coral: Corals identified by stakeholders as important for historical, educational, or economic reasons. This could include colonies popular at dive sites.

- Within an MPA: Corals within zones of extra protection may be living under better environmental conditions.

- Within a recreational area (within FKNMS – on a reef with mooring balls): Corals near mooring balls likely have more visitors who utilize the resource. This could provide additional awareness of treatment action and potentially greater involvement through citizen engagement.

- An ESA-listed species.

Treatability:

- Portion of colony unaffected: Treatment is likely to be more effective if the majority of the coral survives as a result. A recommended guideline is if greater than 75% of colony is still alive.

- Number of active SCTLD lesions: Each lesion requires initial treatment as well as follow-up. A greater number of lesions may also signify poorer overall health of a colony and thus a higher chance of new lesions developing. Colonies with fewer than 5 lesions are more treatable than those with more.

- Monitoring efficiency: Colonies in proximity to other treated corals, sites, or other ongoing projects will ease subsequent monitoring and re-treatment events.

- Suitability for treatment: Certain colonies may be disqualified for treatment for external reasons. For example, certain treatments (e.g. removal) may not be practicable if the coral is attached to a cultural resource. Individual sites and projects should consider these additional factors.

APPENDIX IV: Example time series showing photos of treated lesions on a colony over time