CORAL DISEASE INTERVENTION ACTION PLAN

Florida Department of Environmental Protection
Coral Reef Conservation Program

Coral Disease Intervention Action Plan

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Introduction

Florida's coral reefs are currently experiencing a multi-year disease-related mortality event that has resulted in massive die-offs in multiple coral species. Nearly half of Florida's species of stony coral, including federally-listed species (Endangered Species Act) and primary reef-building species, have high regional occurrences of tissue loss that usually result in whole colony mortality. First observed near Virginia Key in late 2014, the disease has since spread to the northernmost extent of the Florida Reef Tract (FRT) in Martin County as well as southward beyond the Middle Keys. The best available information indicates that the disease outbreak is continuing to spread southwest along the FRT.

The disease has been termed Stony Coral Tissue Loss Disease (SCTLD) to describe the impacted suite of species as well as the visual appearance of the disease lesions. The pathogen(s) has not been identified, though ongoing work is focusing on this effort. Initial experiments confirm that transmission can occur through physical contact or can be water-borne. Arresting of the disease via antibiotics suggests a bacterial component to the disease.

Management and stakeholder workshops have identified intervention action as a primary need in order to arrest disease progression on individual colonies, preserve colonies that would otherwise be lost, and potentially treat entire sites to try to maintain intact ecosystems with full coral species complements. Laboratory and field trials have provided guidance on treatment options that may have some effectiveness in treating lesions short-term. Ongoing research continues to look into alternatives and improvements to increase effectiveness.

This document:

1. Outlines four disease zones along the FRT, along with their location as of Fall 2018.
2. Outlines guiding principles for selection of sites and priority corals for intervention
3. Outlines the existing treatment options, including standard operating procedures (SOPs) as appendices.
4. Outlines the goals and methodologies to be undertaken in each of the zones, including selection of priority sites/corals, treatment recommendations, and monitoring.

These guidelines are based on the best available knowledge, but in this rapidly evolving event, should be considered a living document, adaptable to modification as new information is obtained.

Coral Disease Conditions

The status of reefs in Florida have been broadly categorized into four different SCTLD outbreak conditions: pre-invasion, invasion, epidemic, and endemic. Each category is indicative of a different time since potential exposure to the disease, disease prevalence, and the current coral community. As such, each reef category is subject to different intervention strategies and timelines. The outbreak conditions (fall 2018) are outlined as follows:

Condition	Duration of Exposure	Geography: Fall 2018 (Fig 1)	Disease Prevalence	Coral Community
Pre-Invasion	None	American Shoal through Marquesas	None (though background levels of other diseases may exist).	Normal, pre-disease coral communities.
Invasion	< 3 months	Looe Key through American Shoal	Low. Acute lesions visible only on early susceptible species.	Still has full suite of species, though early susceptible ones will be experiencing mortality.
Epidemic	3 months – 1 year	Middle Keys through Looe Key	High. Lesions acute as well as chronic.	Rapidly transitioning between pre-diseased community and one with lower abundances/absence of susceptible species.
Endemic	1-4 years	Martin County through Upper Keys	May be low, as susceptible species are rare. May be chronic on remaining susceptible species.	Few to no remaining susceptible species. Diminished coral cover and higher proportion of non-susceptible species.

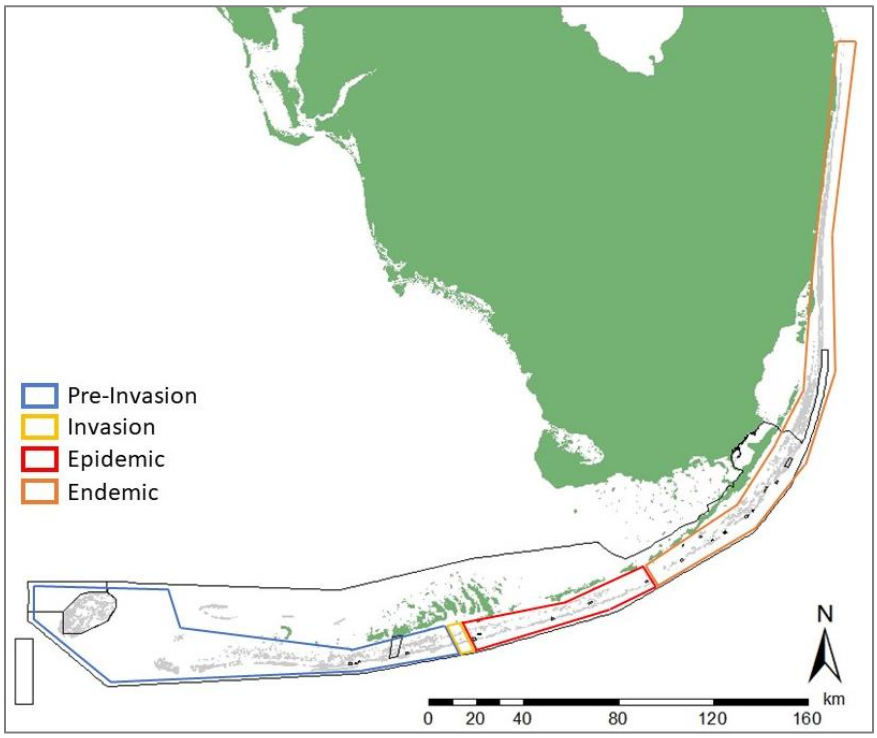


Fig 1. Location of disease zones in Fall 2018.

Guiding principles for determining priority reef sites and coral colonies

Within each region, intervention actions will need to be targeted at priority sites and/or priority colonies. Selection of sites/corals will be 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.

Available Intervention Efforts

The intervention efforts listed below have been proposed for immediate use as permitting allows. Topical applications of chlorine and amoxicillin have been conducted in laboratory and field experiments. Amputation and relocation are considered to pose limited additional risk to the environment, but have few data to support their effectiveness. All of these treatments are known/suspected to be limited in treating only the specific lesion on a single colony for only a limited amount of time. No long-term or colony-wide solutions are yet available. Ongoing research is examining alternatives, and this document will be updated to reflect the outcomes of those projects.

Topical antibiotics

Topical application of antibiotics has been shown to be effective on lesions in laboratory and field tests. To date, it is the most effective method for treating SCTLD lesions across all tested species (*Montastraea cavernosa*, *Orbicella faveolata*, *Diploria labyrinthiformes*, *Pseudodiploria strigosa*, *Meandrina meandrites*, *Dichocoenia stokesii*). Application can be done just on diseased edges, as part of a firebreak trench application, or as a combination of each. Success rates for all are similar (>90% after 4 weeks.

N=38), and can best be situationally determined by an analysis of available resources. Ongoing research continues to refine the practice. See Appendix I for current suggested protocol.

Topical chlorinated epoxy

Topical chlorinated epoxy has shown moderate success on treatment of lesions on wild colonies of *Montastraea cavernosa* and *Orbicella faveolata* (>95% after 6 weeks with firebreak. N=86) in the southeast Florida region. However, this success has not been replicated in the Florida Keys (<50% after 4 weeks with firebreak. N=25) on *O. faveolata* or three other species. At this point, we recommend that non-experimental use of this treatment be considered in relation to the region and species being treated, and followed up with frequent monitoring to re-treat in case of failure. See Appendix II for protocol.

Amputation of affected regions of diseased corals

Amputation of affected regions to prevent further spread through connected tissue can be considered, but has had minimal testing. The procedure may remove the immediate threat of disease spread, but may also result in new pathways for pathogen entry and re-establishment of disease in treated corals. Very limited field testing (on two regions of a single *Dendrogyra cylindrus* colony) showed success in that SCTLD did not appear at the cut sites, but these data should not be assumed to expand across other species or individuals. See Appendix III for protocol.

Relocation of healthy regions of diseased corals

Relocation of healthy tissue away from spreading disease margins may also be considered as a treatment effort. There have been limited field data to address this; three regions from a single *Dendrogyra cylindrus* colony were removed and replanted, and lesions appeared on all relocated fragments. However, these data should not be assumed to apply to other species or individuals, and may be considered as an option for further experimentation. See Appendix IV for protocol.

Monitoring Protocol

Treated sites and individual corals will require monitoring to determine effectiveness at the lesion, colony, and site level to assess whether intervention goals are being met.

At the lesion level, treatments should be monitored for failure, as determined by unabated progression of the disease margin past the treatment line. In some cases (such as the highest priority corals), it is prudent to monitor every treated lesion as well as the remainder of the colony in order to treat new lesions or re-treat existing ones as necessary. During initial treatments, enacting protocols to allow for re-finding of treated colonies and lesions will ease subsequent monitoring efforts. These protocols may be situationally dependent, but can include precise GPS points, distance/bearing data from other colonies or permanent fixtures, photographs compiled into contact sheets, tags affixed to the colony, etc.

If any scientific data are being collected (e.g. treatment success rates), specific records should be collected for each visited lesion. Data can be recorded using underwater data sheets and/or photographs. Photographs of the whole colony and of each treated lesion are highly recommended as they provide more effective time-series visuals and may require less underwater time (note that compiling and analyzing these on land does require effort). Time series photos provide evidence of treatment effectiveness after initial treatment and help to identify the temporal and spatial scope of any potential conferred resistance in the region of treatment.

At a site level, regular monitoring of non-priority corals may be a burden that exceeds capacity or takes away from other primary goals. In this case, a subset of lesions and/or colonies may be selected for monitoring. Sub-samples could be defined by pre-selecting a set of treated corals or lesions to be revisited. This subset could be focused on high-priority corals to ensure they get re-assessed for potential follow up treatment, or could focus on a random sample or a selection of species or treatment types. If this selected sub-set is to be followed through time, steps should be taken during treatment to make finding and assessing these colonies and lesions at subsequent intervals as easy as possible (e.g. GPS points, distance/bearings, photographs, tags, etc). Alternately, in an area with a high number of lesions that are visible during a roving swim, a datasheet that records treatment success on the first subset of lesions found (e.g. 10 lesions, or 10% of treated lesions) could be used. Note that while some treatment options (relocation, trenching) are relatively easy to see, others such as topical antibiotic paste on non-trenched margins are not.

If corals are being treated in attempts to alter the site-wide disease prevalence through time, a metric for success should address corals at the site level. Several alternatives, including random transects, fixed transects, plot mapping, or roving diver surveys, may be appropriate. The most rapid of these is a roving diver survey which has the benefit of finding susceptible species not generally present on transects. However, roving diver surveys are not standardized and can not be used for statistical analysis. The method can, however, be used in conjunction with scouting/searching for priority corals or initial lesions, and is recommended for rough estimates of prevalence. See Appendix V for Roving Diver Survey protocol.

Other Concurrent Considerations

Reduction of Enabling Environmental Conditions

While no link has yet been established between environmental conditions and the spread or severity of SCTLD, general knowledge of other disease outbreaks suggests that stressed organisms are more susceptible to disease.

Any treatments proposed within this document are applied at a lesion level. No colony-wide or ongoing protection is conferred to the rest of the colony. Treatments should always be considered within an ecosystem-management framework. Any efforts to mitigate stressful factors are likely to help mitigate disease. Factors to consider are water quality, water temperature, fishing, and boating/visitor impacts.

Decontamination of Diver Gear

While no direct evidence of diver transmission of SCTLD between colonies or sites has been found, precautionary principle suggests undertaking efforts to minimize any potential risk of transmission. Diver decontamination protocols should be followed to the extent that circumstances allow (Appendix VI).

Human Health Concerns

While no evidence of negative impacts of SCTLD on human health has been documented, certain treatment options may carry risks. Use of chlorine should be conducted with care for human tissues as well as equipment. Application of antibiotics may carry risks of aggravating antibiotic allergies in humans and/or setting up antibiotic-resistant genes within the surrounding environment, overall reef community, or those administering treatment. All treatments involving amputation, relocation, and trenching of corals involve sharp and potentially high-velocity tools. In all endeavors, risk to those providing treatments as well as others in the area should be considered and planned for.

Regulatory considerations

Existing field treatments have included chlorinated epoxy, topical antibiotic application, amputation, and relocation in limited application within the Florida Keys National Marine Sanctuary (FKNMS). Chlorinated epoxy has also been permitted within state waters in southeast Florida. Any additional use of treatments will require consideration and permitting by the relevant management authorities (FKNMS, National Park Service, State of Florida).

Funding and capacity have to date been provided by most of the government, academic, and non-profit agencies in the south Florida region. Numerous personnel within each of these agencies have had experience with many of the treatment and monitoring protocols. High priority action items can be ranked to expedite funding, collaboration, and regulatory approvals.

Priority intervention efforts for each condition

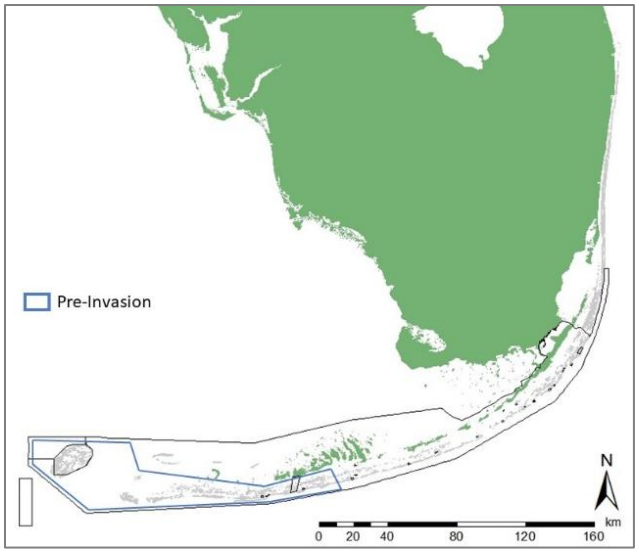
Pre-Invasion Zone

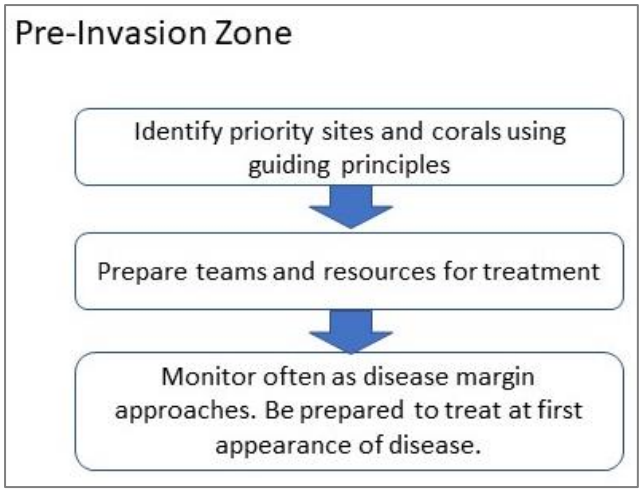
General Considerations

By definition, disease has not yet reached the pre-invasion zone. These intact communities do not presently require active treatment. However, monitoring is essential for knowing when a pre-invasion site and/or priority coral first exhibits signs of the disease and may become an immediate target for treatment.

Goals for this zone may include:

- Identifying priority sites and corals.
- Monitoring priority sites and corals as the disease line approaches.
- Considering any preventative measures such as mitigating enabling conditions.





Selection of Sites/Corals for Treatment

Within this zone, decisions and responses may be more measured as treatment actions are not immediate. Priority sites and corals can be scouted and selected using the guiding principles, and the most recent information can be used to establish the protocols and actions necessary to most effectively begin treatment if/when SCTLD appears at a site or coral.

Types of Treatment

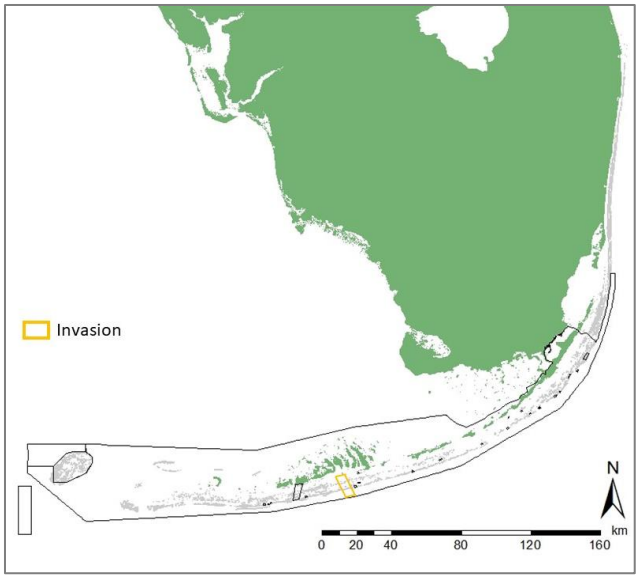
Best practices should be considered and implemented at each site as treatments become necessary. If any preventative measures, including potentially mitigating exacerbating factors, are possible, they should be implemented before disease appears.

Monitoring

If specific priority sites/corals have been selected, regular monitoring as the disease front approaches is recommended in order to spot the first signs. As the disease margin approaches the site, weekly monitoring is recommended if possible, with thorough surveys throughout the site focusing on early susceptible species.

Invasion Zone

General Considerations



Within the invasion zone, prevalence of disease is low and likely appearing only on early susceptible species. While existing evidence suggests that disease is likely to move through a region regardless of prevalence or presence of untreated neighbors, this zone potentially represents a chance to alter disease progression at a site level. This success is likely to be increased if disease lesions are treated early and often.

Sites within this zone may vary in coral diversity and density, but due to the early onset of the disease, most if not all lesions should be located and treated. High-intensity surveys to find diseased colonies, as well as aggressive and frequent treatment of all lesions is recommended.

Goals for this zone may include:

- Identification of priority sites.
- Treatment of all disease lesions to maintain the intact coral community structure.
- Treatment of all disease lesions to attempt to minimize rates of reappearance of disease or development of new lesions on other colonies at the site.

Selection of Sites/Corals for Treatment

The nature of and goals of the invasion zone suggest treatment of ALL infected corals and lesions up to a point where resources or efforts cannot maintain such a regime. At that point, the site should be treated as within the epidemic zone, with efforts shifting to targeted priority corals (see next section).

The invasion zone is likely to contain a range of sites for consideration, with a variety of habitats, coral community representation, and patterns of human use. Site types to consider for prioritization are:

- Iconic high-rugosity spur-and groove sites. These generally have a moderate to high species diversity and density and are potentially highly visited by divers and snorkelers due to the presence of mooring balls. Many of the largest, most iconic corals on the FRT are located at these sites, and potential citizen engagement is highest at these locations.
- Mid-channel or inshore patch reefs. These are generally the highest diversity and highest coral-cover sites on the FRT. They likely contribute substantially to coral reproduction, and potentially provide the highest number of colonies treatable within set time, logistical, and financial resource constraints.
- Other offshore areas of low-relief spur and groove or hardbottom can also harbor large amounts of corals. Though colony density has not been shown to impact disease prevalence, it is possible

that treatments in these areas could be more effective because rates of disease reappearance or new lesion development might be lower.

- Very small and isolated patch reefs. Treatment might be easiest and most effective at these small and isolated scales as the entire patch could be regularly surveyed and treated. Isolation might decrease susceptibility for high rates of disease occurrence.

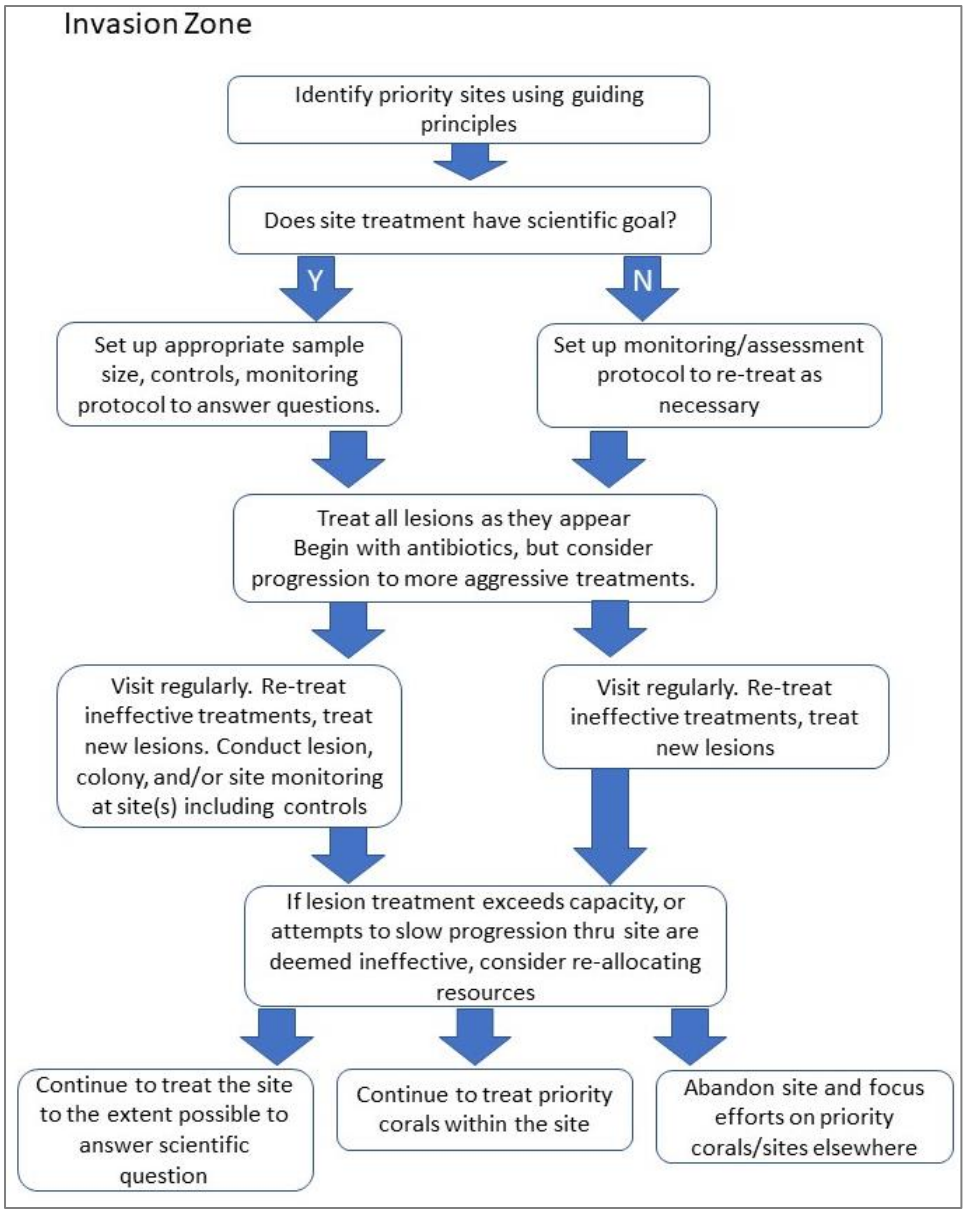
The guiding principles for site selection laid out earlier in this document provide further suggested considerations for determining priority sites.

Types of Treatment

Topical antibiotic application (Appendix II) is the most effective and well-tested treatment to date. Treatment on individual colonies and at small plots using this method has been previously permitted by FKNMS. This is the primary recommended treatment option at this time.

Though mostly effective on individual lesions, existing antibiotic treatment options do not prevent development of new lesions. Colonies that have been treated should be re-visited and monitored for effectiveness and re-treatment if necessary.

As the goal in this zone is to eliminate all disease lesions, treatments may need to become more aggressive than those applied in other regions. If lesions cannot be treated with topical antibiotics, amputation of the lesion may be warranted.



Monitoring

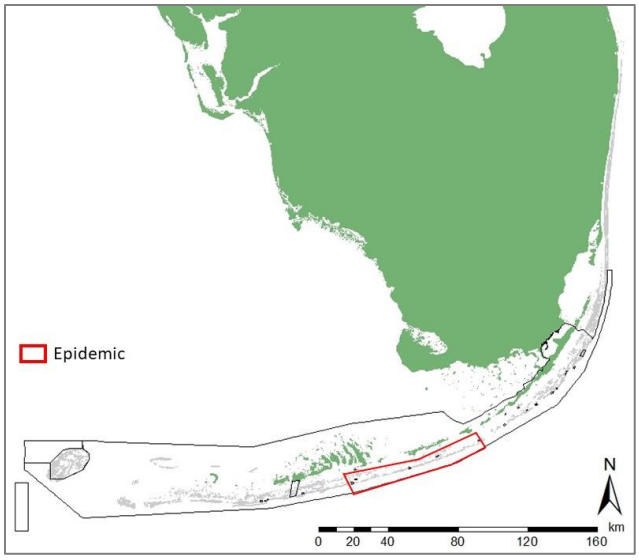
Treatment of individual lesions does not provide colony-wide or long-term effectiveness to a coral. Treated colonies should be revisited frequently (weekly if possible) to re-treat any failed treatments and to identify and treat any new lesions.

Re-treatments will likely need to continue long-term, though there are no data to suggest how the disease will progress through such a heavily treated area. If the site shows increasingly worsening disease occurrence, particularly if prevalence rate increases are similar to those at untreated sites, treatment at a site level may be determined to be ineffective. At this point, treatments should switch either to alternative sites or to priority corals within the existing site, depending on the value of the site as identified by the guiding principles.

Epidemic Zone

General Considerations

Within the epidemic zone, disease prevalence rates are high and new lesions develop quickly. Early susceptible species can undergo rapid mortality with acute, quickly-moving disease margins. Comparisons of high vs low-density sites, as well as nearest neighbor analyses suggest that reduction of the "pathogen load" through treatment of individual colonies is unlikely to influence rates of disease occurrence or severity of the outbreak at the site. However, targeted application on specific corals may help preserve those individuals through the outbreak.



Large colonies of highly susceptible species are likely to still be present in this zone and thus represent an opportunity to maintain those populations and the reef-building capacity they provide. The potentially large number of remaining corals provides the ability to prioritize individuals based on desired characteristics. Goals for treatment in this region include:

- Prioritization of high value coral colonies.
- Prevention of localized (reef) extinction of a variety of susceptible species.
- Prioritization to maximize colony-saving treatment efforts across desired criteria.

Selection of Corals for Treatment

Selection of high value coral colonies relies on both site and colony selection. A balance between ecological, management, and feasibility criteria should be established to maximize available resources. Trade-offs will likely have to be made between treating large numbers of lower-value corals at a few sites versus smaller numbers of higher-value corals across many sites.

The guiding principles outlined at the beginning of this document can help with site selection. Habitats to consider include:

- High-relief, iconic reef sites. Sites generally have some of the largest corals on the FRT, and are frequented by stakeholders. Citizen awareness, engagement, and benefit to human use could be high in these sites.
- Mid-channel or inshore patch reefs contain the highest diversity and coral cover in the region as well as many large corals. Ecologically, these reefs probably contribute the most in reproductive potential. Because of their density, they also represent areas in which the most corals could be treated and monitored with the least resources.
- Other areas of low-relief spur and groove can also contain many corals, though at much lower densities. Though colony density has not been shown to impact disease prevalence, it is possible that treatments in these areas could be more effective because rates of disease reappearance or new lesion development might be lower.

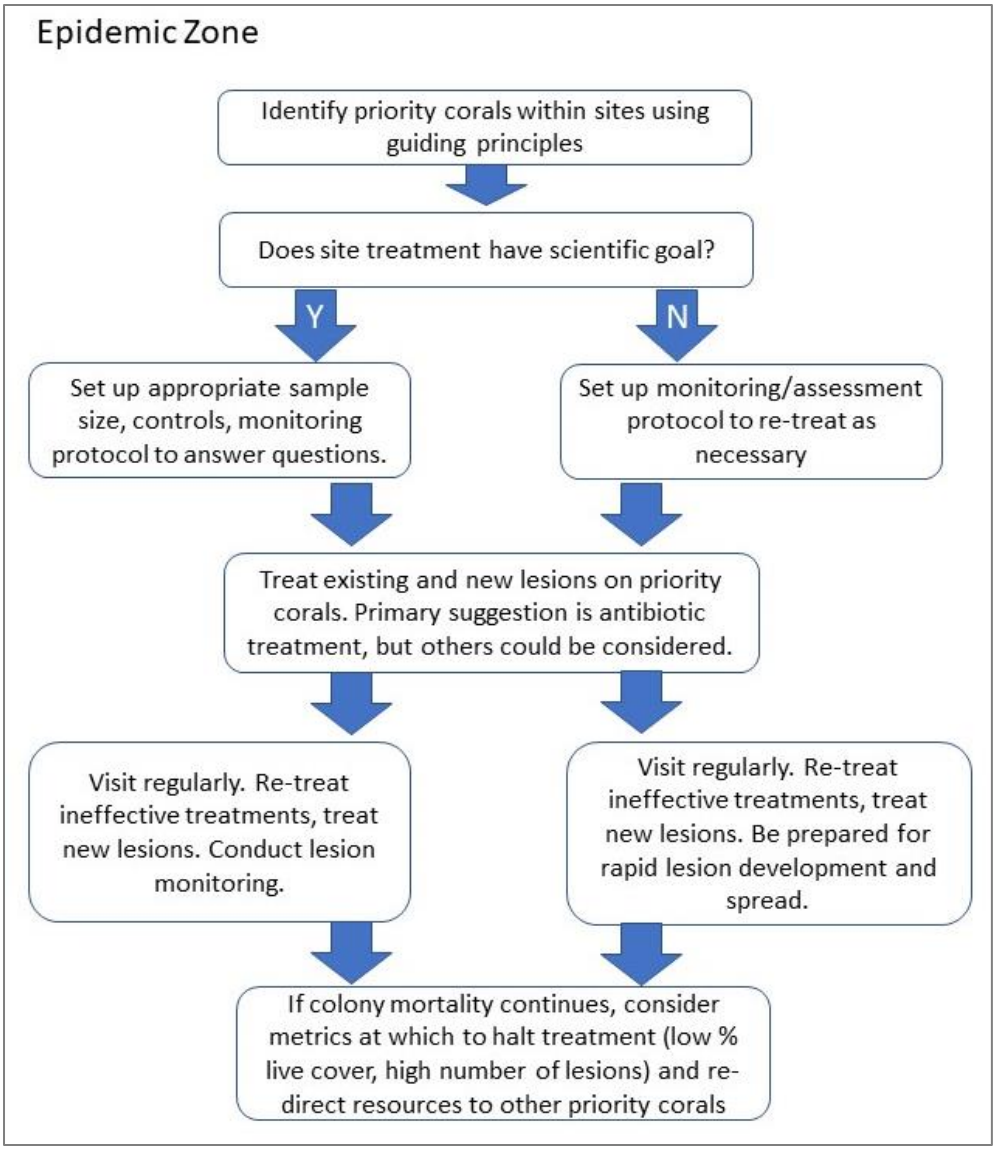
Within sites, the additional set of guiding principles for coral colony selection will help prioritize corals and promote the treatment goals. These goals may vary by site: at highly visited sites, these may lean towards protection of stakeholder-identified corals or common guided dive pathways. At less-visited but ecologically important sites, goals may be primarily preserving colonies for reproductive potential or species diversity.

Types of Treatment

Chlorinated epoxy has been found to be ineffective on corals within this region. This treatment is not recommended.

Topical antibiotic application (Appendix II) has been conducted in this region and found to be the most effective treatment on individual lesions. Permitting on individual colonies and at small plots using this method has been previously authorized. This is the primary recommended treatment option at this time.

Experimental amputation of diseased tissue regions, and relocation of healthy tissue regions, may also be considered for priority corals in this region.



Monitoring

Reappearance of disease and/or development of new lesions is very high in this region. Treatment of individual lesions does not provide colony-wide or long-term effectiveness to a coral. Treated colonies should be revisited frequently (weekly if possible) to re-treat any failed

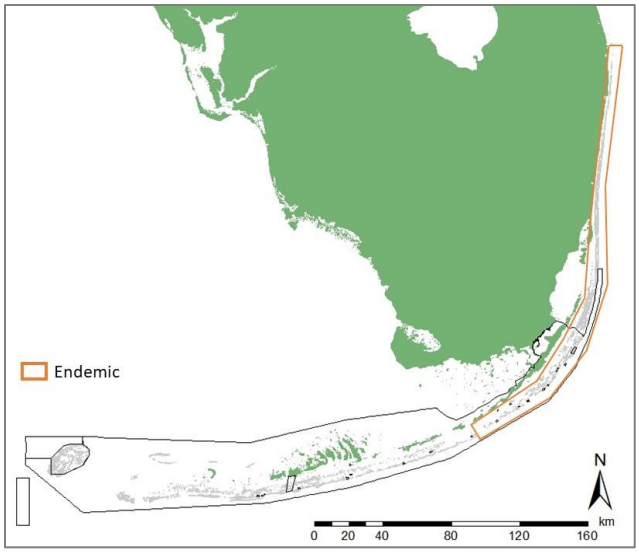
treatments and particularly to identify and treat any new lesions.

Re-treatments will likely need to continue throughout the epidemic time period, and potentially well into the endemic phase. Dedication to re-treatment may need to be balanced with the value accorded to each individual coral. Factors to consider when deciding whether to repetitively re-treat as the outbreak worsens may include:

- Value of the coral as identified by the coral selection guiding principles.
- Effectiveness of treatment (does each treatment extend the life of the colony substantially, or for only short time periods).
- Reappearance of the disease or new lesion development rate (do only a small number of new lesions emerge with each visitation, or are the number and severity growing exponentially?).

Monitoring efforts should also identify any pockets in the region where disease is not present.

Endemic Zone



General Considerations

Within the endemic zone, few if any highly susceptible species will remain. Surviving susceptible species may show no signs of disease and thus be considered either resistant or not yet infected, or may show more chronic lesions with slow progression.

Treatment options rest primarily on these colonies with chronic disease. These remaining live corals may be of particular importance due to their now rare status in the region, which increases their importance for genetic diversity, reproductive potential, and contributions as live coral habitat. Goals for treatment in this region include:

- Preservation of any remaining individuals from highly susceptible species
- Preservation of genetic diversity and prevention of regional extinction of affected species
- Identifying differences in disease progression or treatment options between the endemic and other zones

Selection of Corals for Treatment

The density of corals remaining for potential treatment is likely to be lowest in this zone and restricted to the less-susceptible species. However, the amount of the reef tract within this area is larger than any other zone, providing lots of opportunities for treatment. The set of guiding principles can help prioritize corals for treatment, but local resources and needs can also be taken into consideration.

Types of treatment

Chlorinated epoxy treatment of colonies of *M. cavernosa* and *O. faveolata* within the endemic zone have shown some success. This methodology has been previously permitted within Florida state waters as well as FKNMS. Its use in the endemic zone may be considered as a feasible option following protocols outlined in Appendix I.

Topical antibiotic application (Appendix II) has not been permitted in SE Florida. This, however, remains the most effective treatment in side-by-side comparisons within the epidemic zone and should be considered.

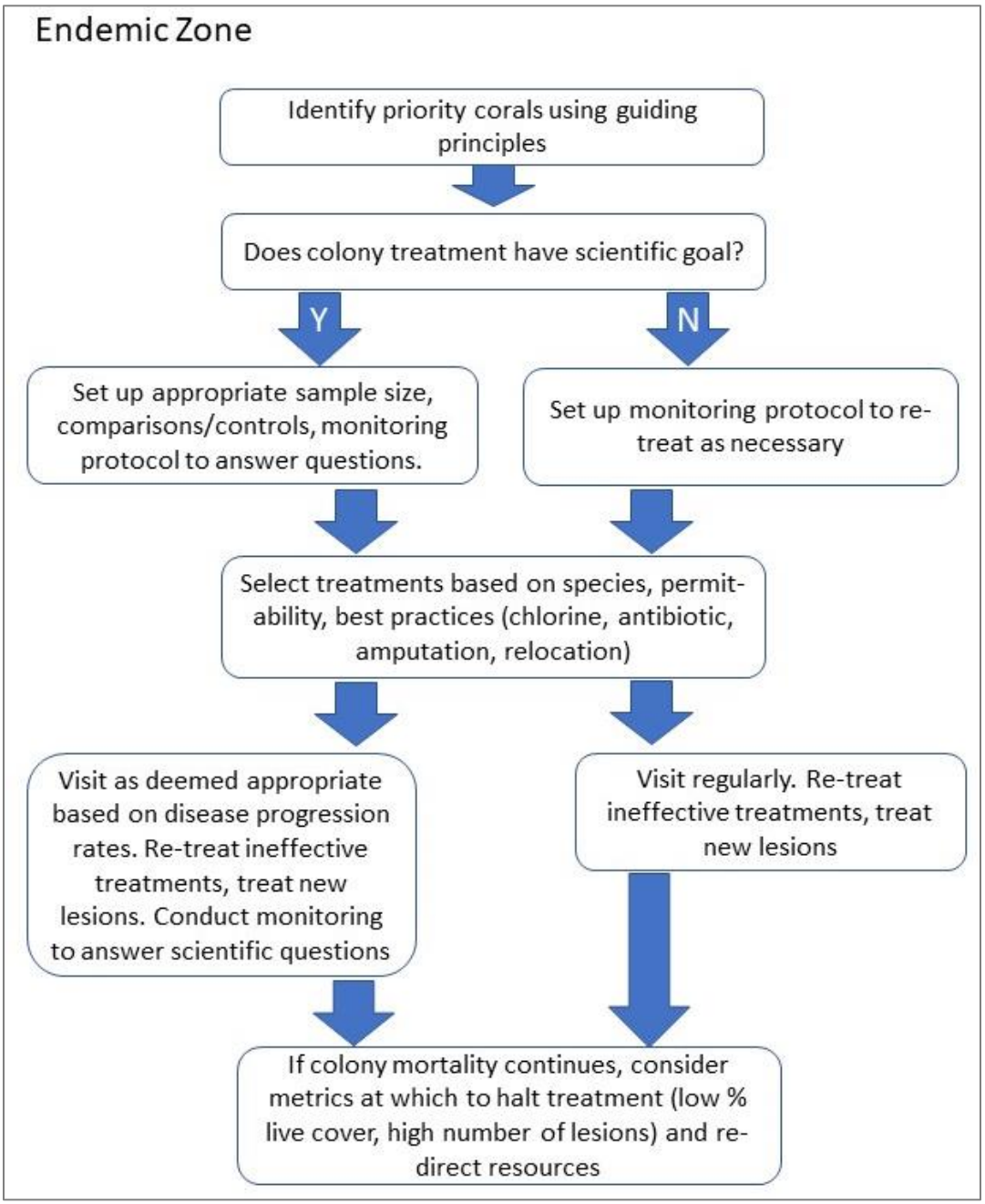
Amputation of diseased tissue regions and relocation of healthy tissue regions may also be considered in this region.

Monitoring

Monitoring within this region may be complicated by the highly dispersed nature of the remaining (and thus treated) corals. While considering logistics, monitoring should ideally be across species and across

treatments, with potentially more attention being given to newer protocols (amputation, relocation) and also to chlorinated epoxy applied to previously untested species in the region. Corals in this zone may also be prone to reappearance of disease, and so treatments should include a plan to revisit and re-treat.

Efforts should also identify any pockets where susceptible species are still part of an intact coral community or where natural recovery is taking place.



APPENDIX I: Protocol for Chlorinated Epoxy Treatment

1. Prepare a chlorinated epoxy mixture utilizing powdered chlorine and 2-part epoxy (the standard is Splash Zone).
    a. 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
    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. Take a small handful (50mL is about racquetball size) of Part A and smear onto a piece of parchment paper. Pour 15 mL of chlorine power onto the Part A and mix together.
    d. You can use a spatula to mix this on the paper, or hand mix using wet gloves. Hands are easier, but spatula is a little less messy. Mix as thoroughly as possible.
    e. Fold up the four corners of the parchment paper to seal the mixture inside
    f. Take an equivalent size (racquetball) of Part B, place it on a separate piece of parchment paper, and fold it up.
    g. Place both parts into a Zip-Lock bag or Tupperware containers for transport and application.

2. Apply the mixture to the diseased colony
    a. Pack a goody bag with rubber gloves and bag(s) of epoxy (equal numbers of parts A and B). The epoxy doesn't mix as well or stick after lengthy exposure to the seawater, so try to only take down what you think you might need.
    b. Open parchment packets and begin mixing Part A and Part B in a 1:1 ratio. It will eventually become a dark olive green putty consistency. If you err a little on ratios, use a little extra black (Part B).
    c. Smush onto the lesion, spreading into regions that might be infected but not yet dead (sometimes a few polyps in from the lesion)
    d. Use an angle grinder or hammer/chisel to carve a firebreak into the colony about 5 cm above the disease line. Pack the trench with the chlorinated epoxy mixture as well.

Product	Weight	Price	Notes	Weblink
Powdered Chlorine	5 pounds	$38.45	1 lb bag may be preferred	https://www.amazon.com/POOLIFE-22403-Poolife-Turboshock-Pounds/dp/B00JCYFNV6/ref=sr_1_4?ie=UTF8&qid=1538480202&sr=8-4&keywords=poolife+turboshock
Splash Zone Epoxy	1 quart	$75.45		https://www.amazon.com/Pettit-Paint-Splash-Zone-Quart/dp/B0032FXM9Q/ref=sr_1_1?ie=UTF8&qid=1538480340&sr=8-1&keywords=splash+zone+epoxy
Parchment Paper	205 feet	$11.89	Waxed paper does NOT work	https://www.amazon.com/PaperChef-Natural-Non-Stick-Culinary-Parchment/dp/B000E7D45W/ref=sr_1_1_sspa?s=kitchen&ie=UTF8&qid=1538480449&sr=1-1-spons&keywords=parchment+paper&psc=1

APPENDIX II: Protocol for Topical Antibiotic Treatment

1. Create an amoxicillin mixture utilizing powdered amoxicillin and either a specially developed silicone 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 shea butter is a manageable amount. Weigh into a glass beaker or other small container. If using shea butter, it helps to heat it up 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 30 or 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 rubber gloves, antibiotic syringes, and modeling clay. Syringes are positively buoyant; modeling clay is negatively buoyant. Be careful of how you secure and close your bag.
5. Select your lesion and use the syringe to cover the lesion and the immediately surrounding area (~0.5-1 cm) with the compound. It adheres better to the skeleton than to the tissue, and may ultimately require some manipulation with your fingers to apply it. Small pieces may detach during application, but can generally be caught and remolded into to the application.
6. Alternative or additional intervention can be accomplished by creating and treating a firebreak about 5 cm away from the disease margin. An underwater angle grinder provides a rapid and clean trench, but this can also be accomplished with a hammer/chisel. Use the syringe to squeeze the amoxicillin mixture into the resulting trench, and then cover it with modeling clay. The clay helps hold the mixture in place, as it tends to wash out of the trenches easier than off of the margins.

Product	Weight	Price	Notes	Weblink
Antibiotic (Amoxicillin)	25g	$55.95	5 grams treats about 10 lesions	https://phytotechlab.com/amoxicillin.html
Shea butter	2.2 lb	$16.99	We've used 2 different brands before. This isn't one of them, but it's bulk	https://www.amazon.com/Naissance-Organic-Shea-Butter-2-2/dp/B00MY7O91S/ref=sr_1_14_sspa?s=arts-crafts&ie=UTF8&qid=1532007707&sr=1-14-spons&keywords=shea+butter&psc=1
Catheter Syringe	10 syringes	$10.99		https://www.amazon.com/Catheter-Syringe-Syringes-Care-Touch/dp/B01M1R392V/ref=sr_1_1_sspa?ie=UTF8&qid=1537552151&sr=8-1-spons&keywords=catheter+syringe&psc=1
Modeling clay	5 lb	$13.91		https://www.amazon.com/dp/B00FR7TTBM/ref=asc_df_B00FR7TTBM5364043/?tag=hyprod-20&creative=394997&creativeASIN=B00FR7TTBM&linkCode=df0&hvadid=216501935499&hvpos=1o3&hvnetw=g&hvrand=14404616702206994139&hvpone=&hvptwo=&hvqmt=&hvdev=c&hvdvcmdl=&hvlocint=&hvlocphy=9011849&hvtargid=pla-350998893738

APPENDIX III: Protocol for Amputation of Diseased Tissue Regions

1. Removal of a diseased tissue region may be considered if:
    - The diseased area is physically accessible for easy removal
    - The rest of the colony is unaffected or can be treated with other methods
2. Removal can be accomplished using a hammer and chisel, but is likely to be more time effective with an angle grinder.
3. It is recommended to chisel/cut at least 10 cm away from the disease margin to ensure that any potentially diseased tissue forward of the observed margin is also removed.
4. While there is no evidence that disturbing the lesion line might disperse pathogens more readily into the environment, precautionary principle suggests minimizing that disturbance and considering disposal of the diseased fragment. Options may include:
    - Placing the fragment into a plastic bag to return to the surface for bleaching/disposal on land (preferred)
    - Carefully moving the fragment away from the reef for burial in sand.

APPENDIX IV: Protocol for Relocation of Healthy Tissue Regions

1. Relocation of healthy fragments from a diseased colony may be considered if:
    - A non-diseased, easily-removable area can be separated with a large (10+ cm) margin away from any lesions
    - A suitable relocation area is available within 100' from the parent colony.
2. Cut the fragment as far away from any lesion or potential lesion (pale or suspicious areas) as possible (minimum 10 cm). An angle grinder will make this easier, but hammer/chisel can be used.
3. When choosing a relocation site, use the following considerations:
    - Should be within 100' of the parent colony (this will help minimize potential disease spread)
    - Should have few if any surrounding susceptible species so that if the fragment is diseased (but not showing signs), it isn't potentially spreading disease to other corals
    - Should be in conditions similar to those of the parent colony (consider depth, light, water flow)
    - Should be an area where long-term survival is possible (consider burial/toppling/abrasion)
4. Affix colony to substrate using a strong epoxy (Splash Zone, Aves Apoxie, All-Fix, or others previously used in coral outplanting processes)

APPENDIX V: Roving diver survey protocol

A diver will conduct a census swim of the site, focusing on species that are primarily impacted SCTLD. Multiple divers can conduct the survey at one site, but should work in opposite directions or parallel to each other to avoid duplicating data. Data should be entered separately.

1. Swim around the site (no greater than 50 m from the recorded coordinates) for at least 10 minutes.
2. On the datasheet (next page), record the following metadata:
    a. Observer Name
    b. Date
    c. Site Name
    d. Latitude and Longitude in Decimal Degrees
    e. Time start and Time end of roving diver swim (10 minutes minimum, but longer is fine)
3. Record the species code of stony coral species seen on the swim. Exclude Milleporids, Acroporids, *Siderastrea siderea* (SSID), and *Porites astreoides* (PAST). Focus on colonies greater than 4 cm. For each species, tally the number of colonies exhibiting each of the following conditions:
    a. Recently dead colonies (white skeleton, polyp structure intact) presumed dead due to SCTLD. Colonies with obvious other causes of mortality (breakage, toppling) should be excluded.
    b. Actively diseased colonies. Colonies with any level of SCTLD disease should be included here.
    c. Undiseased colonies with symptoms of concern. Colonies that do NOT have any active mortality due to SCTLD, but are showing unusual pale spots or focal bleaching. Colonies with dark spot disease should also be included here. In meta-analyses, these colonies will be lumped in with “non-diseased” colonies.
    d. Healthy colonies. No active disease or unusual signs.
4. Photos can be taken of unusual or interesting disease sightings, but are not required.

NAME:	SITE:	TIME START:
DATE:	LATITUDE:	TIME END:
Exclude Milleporids, SSID, and PAST	LONGITUDE:	

Species Code	Tally colonies w/ recent mortality (likely due to disease)	Tally actively diseased colonies	Tally undiseased colonies w/ symptoms of concern (pale spots or focal bleaching)	Tally undiseased colonies

APPENDIX VI: Diver decontamination protocol

Ammonium-based disinfectants and chlorine bleach are effective antiseptics that can minimize the spread of disease-causing pathogens from infected reefs and corals to uninfected sites. However, proper use and technique are necessary to not only properly sanitize gear but avoid harming equipment. Freshwater washing alone may not eliminate pathogens.

General Guidelines for Disinfection

- All divers should decontaminate dive gear at the end of the day.
- Divers should inspect all dive gear and equipment carefully and remove any debris such as seagrass, algae and sediment following each dive.
- Divers should sanitize all gear between dives at sites with a high prevalence of disease, especially if subsequently moving to an uninfected site and if coming into close contact with diseased corals or the bottom. The preferred option is to dive the "cleanest" site first and move to the "dirtiest site last.
- Gear should be decontaminated, between dive sites separated by large distances >10 km), and in sensitive areas.
- Gear should be decontaminated when travelling between countries.
- Properly dispose of disinfectant solutions and rinse water in a sink, tub or shower. Never pour into the ocean or a storm drain. Quaternary ammonium wastewaters should not be drained through septic systems because of the potential for system upset and subsequent leakage into groundwater.

Gear-Specific Guidelines for Disinfection

1. <u>Tools, collection bags, sampling gear, transect tapes, clipboards, underwater slates, weight belts and other equipment that comes in contact with the bottom</u> should be decontaminated using diluted chlorine bleach. Bleach is extremely corrosive to metals and should not be used to decontaminate regulators or neoprene wet suits as it can compromise the integrity of polymers such as neoprene and silicone rubber components in regulators. Bleach should never be mixed with ammonia-based solutions. Bleach rapidly degrades and must be used immediately after mixing; it should be changed daily.
    - After each dive, soak non-sensitive equipment and tools for 10 minutes in a 10% bleach solution (1 qt bleach per 2 gallons water prepared in a 5-gallon bucket with a lid).
    - Rinse with fresh water, air dry.
2. <u>Wet suits, Buoyancy Compensation Devices (BCD's), mask and fins</u> should be decontaminated using quaternary ammonium disinfectants such as Virkon S[1], RelyOn[1] and Lysol[1] All Purpose Cleaner. These are broad spectrum disinfectants and are effective for treating bacteria, viruses, fungi, larval mollusks and other microorganisms.
    - After each dive, soak dive gear for 10 minutes in one of the following: a 0.5% solution of RelyOn (four 5 g. tablet/gallon of water), 1% Virkon S (1.3 oz./2 gallons of water), Lysol (1 qt. per gallon; 6.6% Lysol in water), or an equal concentration of another quaternary ammonium disinfectant.
    - Remove from disinfecting solution, soak in fresh water for 10 minutes, and allow to air dry.

- Particular attention needs to focus on decontaminating wetsuits and the internal bladders of BCs because of their ability to trap water that can house transmissible pathogens. Pour approximately ½ liter into the mouthpiece of the BC's exhaust hose while depressing the exhaust button, inflate the BC, and gently rotate the BCD in all directions to ensure the solution has reached all of the internal parts. Allow the BCD to sit for 10 minutes. After 10 minutes, immediately dump the solution into a container for proper disposal on land and flush the BCD two times with fresh water.

3. Regulators, computers, gauges, underwater cameras and other sensitive scientific equipment should be decontaminated using fresh water with anti-bacterial dish soap or an isopropyl alcohol wipe and let dry.
    - Prepare a solution of warm water and anti-bacterial dish soap or OdoBan[1] (5 oz/gal). After each dive, soak regulators and other sensitive equipment for 20 minutes, rinse in fresh water and allow to dry.
    - Additionally/alternatively, equipment can be wiped down with an isopropyl alcohol wipe. Be sure to wipe any small areas where water might accumulate.

[1] This protocol does not endorse, recommend, or favor any specific commercial product, process, or service, or the use of any trade, firm or corporation name and is provided only to inform the public. Safety data sheets (SDS) for chemicals and user's manuals for equipment developed by product manufacturers provide critical information on the physical properties, reactivity, potential health hazards, storage, disposal, and appropriate first aid procedures for handling, application, and disposing of each product in a safe manner. Familiarization with the SDS for chemical products, and manufacturer's product care and use standards, will help to ensure appropriate use of these materials and safeguard human health.