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Strategy for Stony Coral Tissue Loss Disease Prevention and Response at Flower Garden Banks National Marine Sanctuary (Version 1)

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Cover photo: Large lobed star coral (*Orbicella annularis*) and symmetrical brain coral (*Pseudodiploria strigosa*) colonies at West Flower Garden Bank. Photo: G.P. Schmahl/NOAA

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Abstract

Coral reefs are an essential component of healthy and resilient marine environments, providing important ecosystem services such as tourism and fisheries. However, corals in the Atlantic and Caribbean region are currently experiencing an unprecedented epizootic. The newly described disease, termed stony coral tissue loss disease (SCTLD), is characterized by its large geographic range, extended duration, high rates of host mortality, and the substantial number of coral species affected. To date, SCTLD has not been observed in Flower Garden Banks National Marine Sanctuary (FGBNMS), located in the northwestern Gulf of Mexico, but the coral species present and high coral cover within the national marine sanctuary suggest this location may be susceptible to and strongly affected by the disease. Therefore, FGBNMS has established this plan to identify research needs and institute prevention, education, preparedness, early warning, response, and intervention strategies. Information and training materials outlined in this plan will be updated as new SCTLD information becomes available.

Key Words

coral, disease, Gulf of Mexico, management, monitoring, response

Introduction

Background

From the reefs of the Florida Keys to the Caribbean islands, coral reefs are fundamental to healthy and resilient ecosystems and provide critical services such as tourism and fisheries. However, coral reefs in the Atlantic and Caribbean are in the middle of an unprecedented coral disease outbreak characterized by swift spread, rapid tissue damage, and high mortality rates (Landsberg et al., 2020; Skrivanek & Wusinich-Mendez, 2020). Therefore, Flower Garden Banks National Marine Sanctuary (FGBNMS) has developed this prevention and response document to identify preemptive actions and have a response plan in place in the event the disease spreads to the reefs within the sanctuary, located in the northwestern Gulf of Mexico.

Stony coral tissue loss disease (SCTLD) was first observed in September 2014 off the coast of southeast Florida (Precht et al., 2016; Florida Coral Disease Response Research & Epidemiology Team, 2018). The disease has spread continuously since 2014, affecting the entirety of Florida's coral reef tract and a number of locations in the Caribbean, including Belize, the Dominican Republic, Jamaica, Puerto Rico, Saint Martin, and the U.S. Virgin Islands (Doyle & O'Sullivan, 2020; National Park Service, 2021) (Figure 1). The disease was undocumented prior to 2014, is thought to be waterborne and suspected to be caused by bacterial agents, and affects nearly half of the reef-building species of the Florida reef tract and one third of Caribbean species (Aeby et al., 2019; Muller et al., 2020).

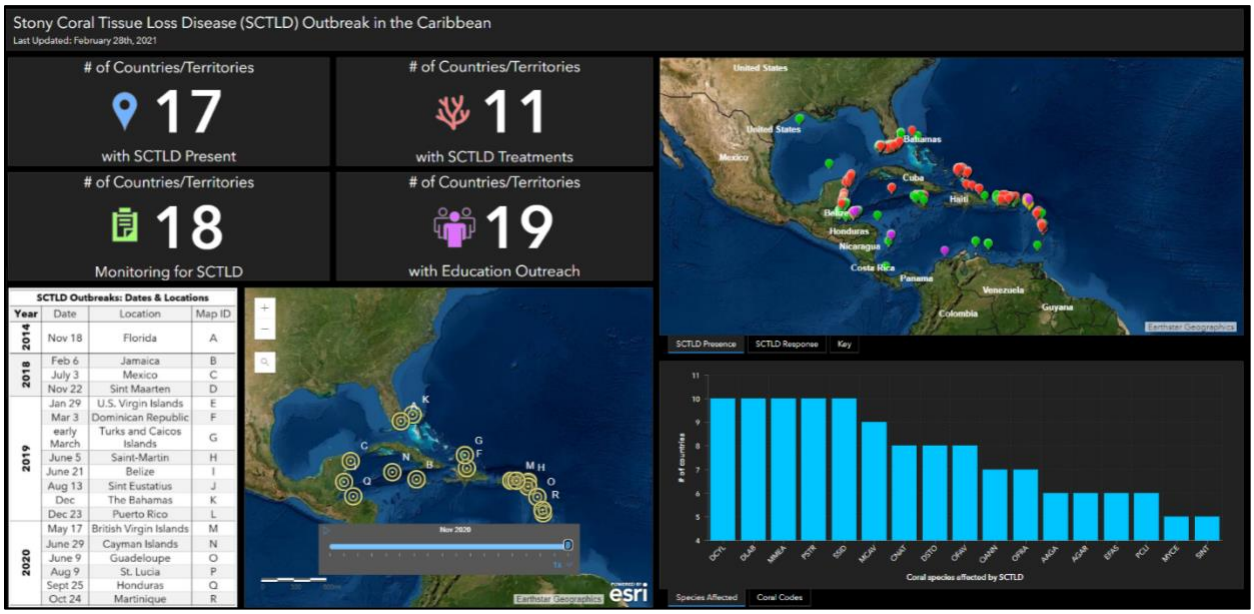

Figure 1. The Caribbean SCTLD Dashboard provides summary information on the status and spread of SCTLD. The dashboard was developed by the Atlantic and Gulf Rapid Reef Assessment (AGRRA) in collaboration with MPAConnect, the Gulf and Caribbean Fisheries Institute (GCFI), and NOAA. Image: AGRRA

For certain coral species, disease prevalence is high (66–100% of colonies at an affected site), though there are differences in lesion progression and rates of mortality among susceptible coral species and across locations (Sharp & Maxwell, 2018; Aeby et al., 2019, Meiling et al., 2021). SCTLD is identified by observation of rapid tissue loss resulting in the exposure of the white

coral skeleton (Figure 2). Tissue loss may begin as single or multiple lesions that appear within the live tissue area and radiate outward, eventually merging as tissue loss progresses. Tissue loss may also begin at the margin or base of the colony, or at the edge of a previously denuded area within the colony, advancing in a linear or crescent-shaped band as it progresses across the entire colony (Meiling et al., 2020). Once a coral is infected, mortality typically occurs within weeks to months without intervention. Intervention methods, such as the repeated use of the topical antibiotic amoxicillin, have shown nearly 95% success in halting or slowing lesion progression in both laboratory and field experiments (Neely et al., 2018a; Shilling et al., 2021); however, it remains uncertain whether bacteria is the primary causative agent or a secondary infection (Doyle & O'Sullivan, 2020).

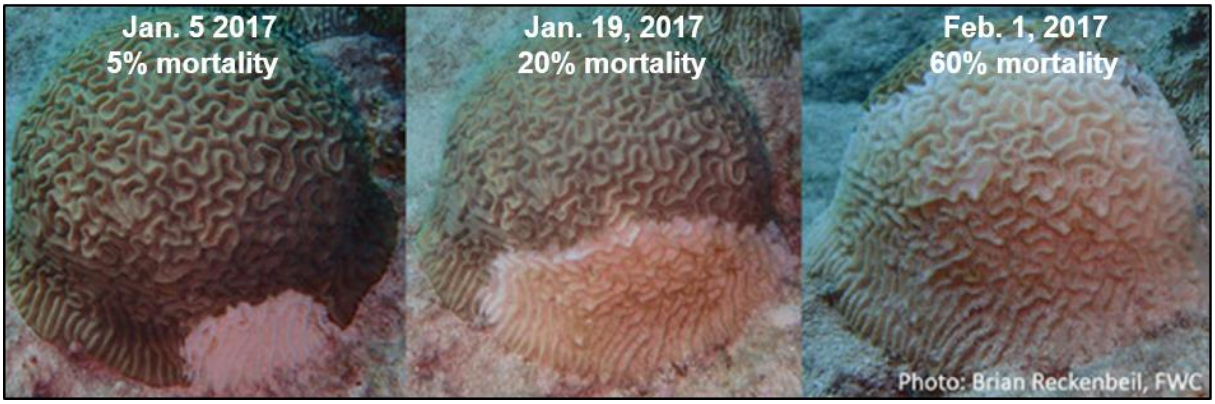

Figure 2. Rapid mortality progression in a brain coral colony (Pseudodiploria strigosa) on the Florida reef tract. Photos: Brian Reckenbiel/Florida Fish and Wildlife Conservation Commission

SCTLD affects many coral species, although some are more susceptible than others (AGRRA, 2019). Table 1 lists susceptible coral species and their level of susceptibility. Highly susceptible species are the first species affected during a SCTLD outbreak at a given site, with total mortality ranging from one week for smaller colonies to two months for larger colonies. The brain coral species *Pseudodiploria strigosa* and *Colpophyllia natans* are included in the highly susceptible species category. These species are present at FGBNMS and account for approximately 8% and 2% of coral cover in benthic long-term monitoring surveys, respectively (Johnston et al., 2020).

At a given site, tissue loss in intermediately susceptible species typically occurs approximately one month after the onset of SCTLD in highly susceptible species (Doyle & O'Sullivan, 2020). Smaller colonies that are intermediately susceptible experience complete mortality over several months, while larger colonies may show slowly progressing lesions that may result in total mortality over years. Star and starlet coral species are included in the intermediately susceptible species category (Table 1). These species are present at FGBNMS and account for the following percentage of total coral cover in benthic long-term monitoring surveys: *Orbicella franksi* (30%), *Orbicella faveolata* (3%), *Orbicella annularis* (1%), *Montastraea cavernosa* (3%), *Stephanocoenia intersepta* (0.6%), and *Siderastrea siderea* (0.5%) (Johnston et al., 2020).

Table 1. Susceptible coral species and degrees of susceptibility (adapted from AGRRA, 2019). Shaded boxes with an asterisk (*) indicate coral species found at FGBNMS above 40 m.

High Susceptibility	Intermediate Susceptibility	Presumed Susceptibility	Low/No Susceptibility
Colpophyllia natans (boulder brain coral)*	*Orbicella annularis* (lobed star coral)*	*Agaricia agaricites* (lettuce coral)*	*Porites astreoides* (mustard hill coral)*
Dendrogyra cylindrus (pillar coral)	*Orbicella faveolata* (mountainous star coral)*	*Agaricia* spp. (plate/saucer corals)*	*Porites porites* (finger coral)
Dichocoenia stokesii (elliptical star coral)	*Orbicella franksi* (boulder star coral)	*Madracis auretenra* (pencil coral)*	*Porites divaricata* (thin finger coral)
Diploria labyrinthiformis (grooved brain coral)	*Montastraea cavernosa* (great star coral)*	*Favia fragum* (golfball coral)	*Porites furcata* (branched finger coral)*
Eusmilia fastigiata (smooth flower coral)	*Solenastrea bournoni* (smooth star coral)	*Mussa angulosa* (spiny flower coral)*	*Acropora palmata* (elkhorn coral)
Meandrina meandrites (maze coral)	*Stephanocoenia intersepta* (blushing star coral)*	*Scolymia* spp. (disc coral)*	*Acropora cervicornis* (staghorn coral)
Pseudodiploria strigosa (symmetrical brain coral)*	*Siderastrea siderea* (starlet coral)*	*Isophyllia* spp. (sinuous cactus coral; rough star coral)	*Oculina* spp. (bush corals)*
Pseudodiploria clivosa (knobby brain coral)			*Cladocora arbuscula* (tube coral)

Information Gathering and Meetings with Experts

With the rapid spread of SCTLD throughout the entire Florida reef tract and reports of its spread in the wider Caribbean region beginning in 2018, FGBNMS staff began preparing for the potential spread of the disease to the sanctuary. The remote location and high coral cover (>50%) of FGBNMS would make disease response and treatment difficult (Johnston et al., 2020). Therefore, one of the first proactive actions taken by FGBNMS, beginning in the summer of 2019, was asking all scuba divers visiting the sanctuary to disinfect their dive gear. Chlorhexidine disinfectant, regularly used to decontaminate dive gear at the local Moody Gardens Aquarium in Galveston, Texas, was made available to divers aboard the sanctuary's R/V *Manta*. Large rinse tubs and chlorhexidine disinfectant were also provided to recreational divers visiting FGBNMS aboard M/V *Fling*, operated by Fling Charters, based in Freeport, Texas. Information was also sent to local dive shops and shared on FGBNMS social media outlets.

As SCTLD spread throughout the Caribbean region, FGBNMS staff gathered information and began researching the disease more strategically. Relevant response and action plans were reviewed, webinars were attended, and FGBNMS long-term monitoring data (reporting the absence of SCTLD at FGBNMS) was submitted to the Coral Monitoring Dashboard developed by the Atlantic and Gulf Rapid Reef Assessment (AGRRA) (Figure 1).

In March and April of 2021, FGBNMS staff organized information gathering virtual meetings with subject matter experts (in lieu of in-person meetings due to COVID-19 restrictions). FGBNMS staff met with colleagues within National Oceanic and Atmospheric Administration (NOAA) partner programs, Florida Sea Grant, Florida Fish and Wildlife Conservation Commission (FWC), the U.S. Environmental Protection Agency (EPA), AGRRA; academic

partners from the University of the Virgin Islands, Florida Atlantic University (FAU), University of North Carolina Wilmington (UNCW); and the private sector to learn from their experiences and discuss realistic response options for FGBNMS. This learning exchange included topics such as monitoring, response, sampling, training, treatment, and outreach.

The collaborative meetings assessed current methods used to respond to the disease and lessons learned from research partners in Florida and the U.S. Virgin Islands. Ultimately, the collective discussions resulting from the meetings led to the development of this response plan, which describes strategies for prevention and education, preparedness and early warning, response and intervention, and research needs for FGBNMS.

Prevention

Website and Education

Prevention is the first strategy described in this FGBNMS SCTLD plan, since, as of the writing of this plan, SCTLD has not been observed at FGBNMS. A new "Prevent Coral Disease" webpage was added to the FGBNMS website in 2019 in an effort to educate visitors about SCTLD. The primary purpose of the webpage is to provide a brief SCTLD overview with helpful links and detailed best practices for scuba divers, including dive gear decontamination protocols.

In addition to website updates, clear communication and educational products describing the disease will be utilized by FGBNMS staff. Lessons learned from Florida include cohesive messaging among researchers and with the public when communicating scientific terminology. Clear science communication includes appropriate terminology, limited use of acronyms and abbreviations, and avoiding the use of misleading language (Doyle and O'Sullivan 2020). Recommended coral disease language for use by resource managers is summarized in Figure 3.

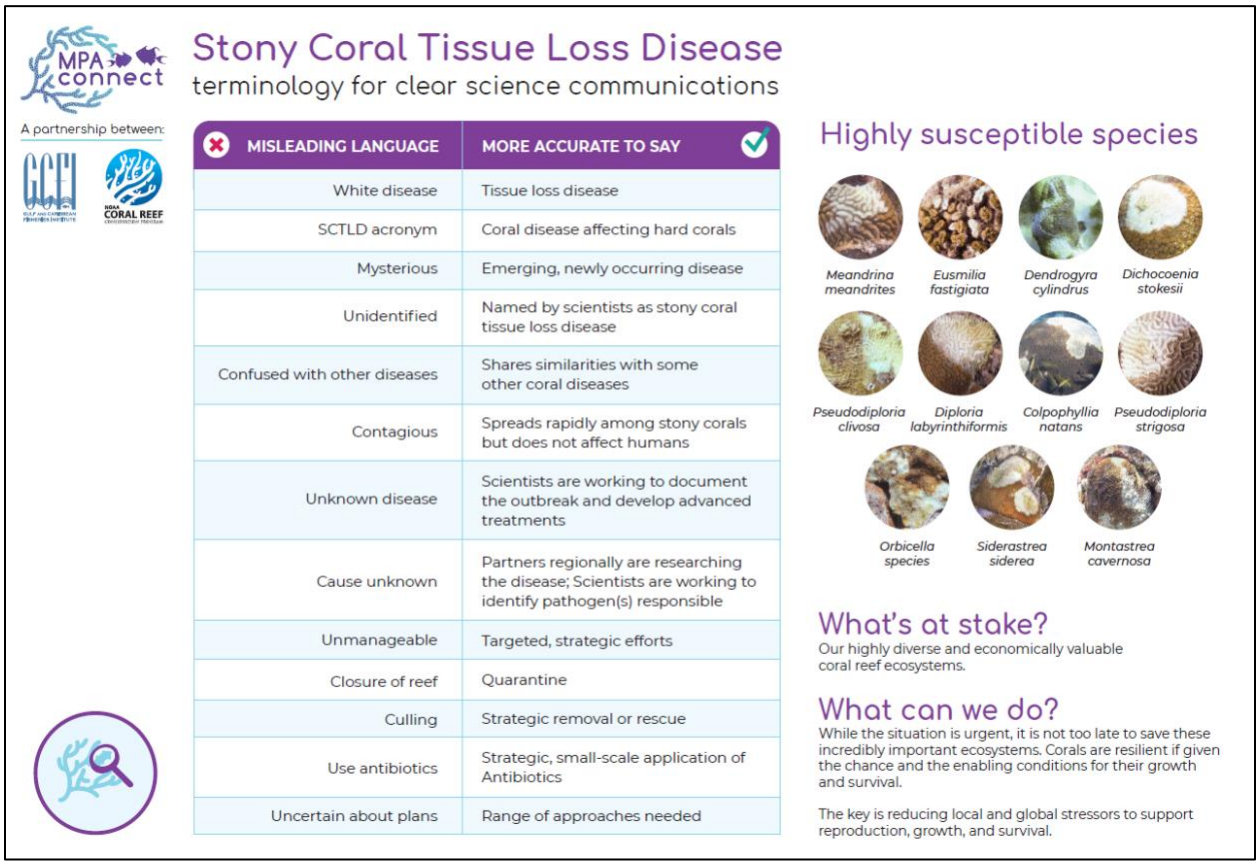

Figure 3. Terminology for clear coral disease science communications, including misleading vocabulary about SCTLD and alternatives for more accurate communications. Image: MPAConnect

Dive Gear Decontamination

Divers can reduce the likelihood of transferring SCTLD through proper buoyancy, avoiding touching marine organisms, and sanitizing equipment between dives and before and after each dive excursion, especially when travelling between countries or dive destinations.

Because many scuba divers visiting FGBNMS come from other dive destinations, in 2021, FGBNMS started requesting that all divers follow dive gear decontamination recommendations to help prevent SCTLD from reaching the sanctuary's otherwise healthy reefs. Because the disease may be caused by bacterial agents and transmitted to other corals through direct contact and water circulation, scuba gear may be a vector of disease spread between dive sites and/or locations. Neoprene gear, such as wetsuits, booties, and gloves, and the internal bladder of buoyancy compensation devices (BCDs) can harbor bacteria and other microorganisms by remaining damp and trapping water. Pathogens can also adhere to other dive and snorkel equipment. Therefore, just as hand washing is a common practice to prevent the spread of disease among humans, disinfecting dive gear is recommended as a precaution to prevent the accidental transmission of coral diseases between dive sites on the reef.

The following recommendations were developed by Florida Keys National Marine Sanctuary (FKNMS) as a result of working with many collaborating partners and dive professionals (Florida Keys National Marine Sanctuary, 2019). Decontamination methods will vary depending on equipment:

- Before entering the water for the first time, all dive gear must be decontaminated. As a best practice, gear should also be decontaminated between dives to prevent accidental transmission between dive sites.
- Non-sensitive equipment: After each dive, soak equipment (weight belts, tools, etc.) for 10 minutes in a 1% bleach solution (1/2 c bleach per 2 gal water). Rinse with fresh water, then air dry.
- Wetsuits, BCDs, masks, and fins: After each dive, soak these items for 10 minutes in one of the following: 0.5% RelyOn (four 5 g tablets per 1 gal water), 1% Virkon S (1.3 oz per 2 gal water), 6.6% Lysol (1 qt per 1 gal water), or an equal concentration of another quaternary ammonium disinfectant. Soak in fresh water for 10 minutes, then air dry.
- BCD internal bladders: Pour approximately 0.5 l disinfecting solution into the mouthpiece of the exhaust hose while depressing the exhaust button. Inflate BCD and gently rotate in all directions. Allow to sit for 10 minutes. Flush twice with fresh water.
- Regulators, computers, gauges, underwater cameras, and other sensitive scientific equipment**:** Soak for 20 minutes in a solution of warm water and antibacterial dish soap or OdoBan (5 oz per 1 gal water). Rinse in fresh water, then air dry. Additionally or alternately, thoroughly wipe with isopropyl alcohol.
- After diving, properly dispose of disinfectant solutions and rinse water in a sink, tub, or shower. The bleach wash solution should be allowed to break down in the sun for 24 hours before disposal. Never pour disinfecting solution into the ocean or a storm drain.

NOAA does not endorse, recommend, or favor any specific commercial product, process, or service or the use of any trade, firm, or corporation name, and this protocol is provided only to inform the public. Safety data sheets for chemicals and user 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, applying, and disposing of each product in a safe manner.

While the above protocols are encouraged, at a minimum, FGBNMS requests that all dive gear be decontaminated prior to entering sanctuary waters and gear be sanitized in a 1% bleach solution for 10 minutes between dives (Figure 4). Divers are reminded to maintain proper buoyancy and avoid touching marine organisms such as coral, not only to prevent disease transmission, but because several coral species at FGBNMS are listed as threatened under the Endangered Species Act.

To assist with gear decontamination, protocols will be shared with research divers prior to offshore field work on board R/V *Manta*, as well as M/V *Fling* dive operators. Updated posters with these minimum requirements (Figure 4) will also be printed and displayed on R/V *Manta*, and printed and shared with M/V *Fling* for display. FGBNMS also collaborated with Tennessee Aquarium to film a step-by-step dive gear decontamination video, which will be posted to the FGBNMS coral disease webpage. A detailed decontamination protocol is listed in Appendix A.

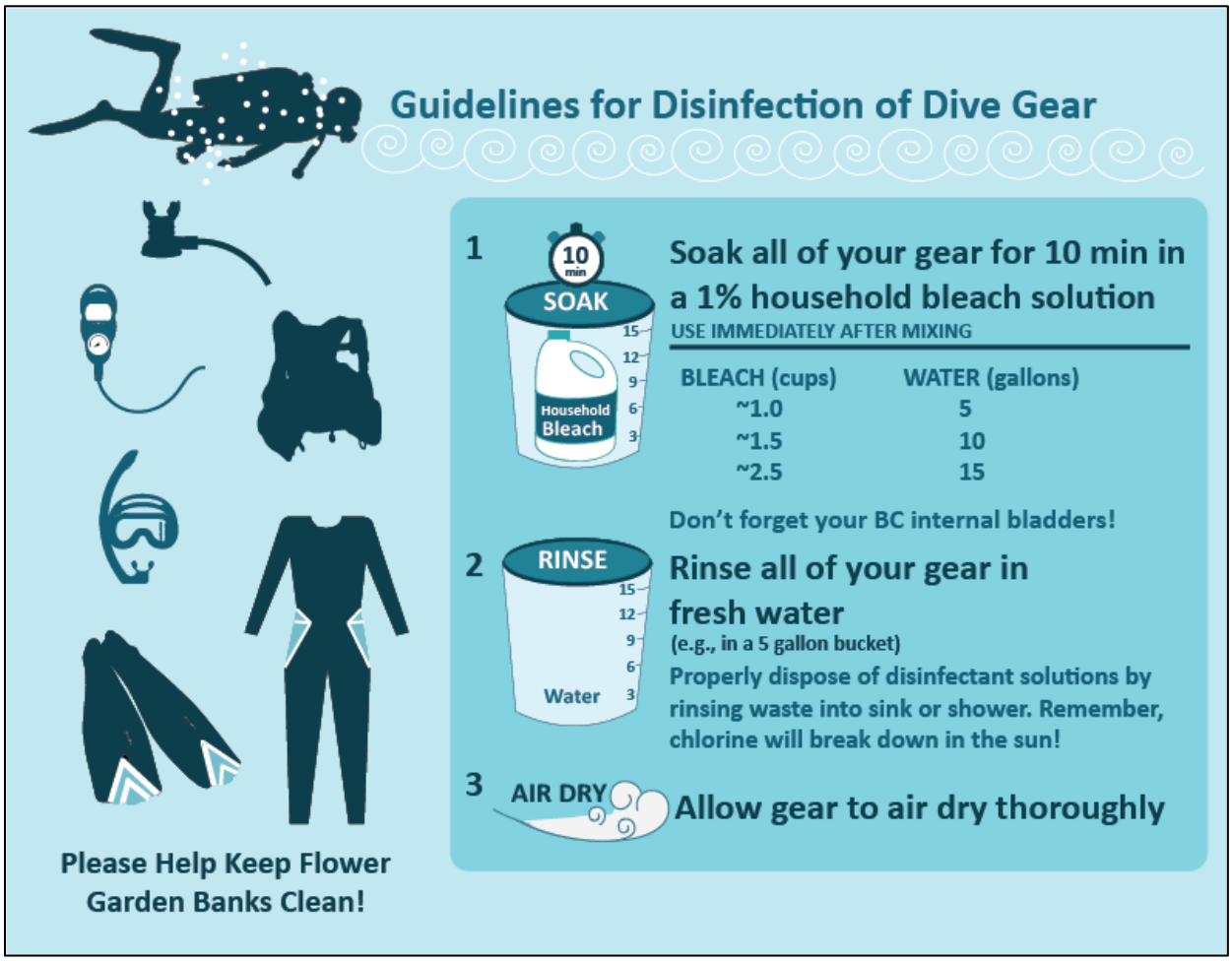

Figure 4. Dive gear disinfection protocol for FGBNMS. Image: NOAA

Ballast Water

Ballast water discharge from ships has been suggested as a potential vector of disease spread. In September 2019, at the request of NOAA, the U.S. Coast Guard (USCG) issued a Marine Safety Information Bulletin (OES-MSIB: 07-19) warning mariners of SCTLD, highlighting ballast water management regulations, and providing voluntary best management ballast water exchange

practices to diminish the risk of spreading the disease via ballast water (U.S. Coast Guard [USCG], 2019). Notably, FGBNMS is located in close proximity to a major shipping fairway leading to the Port of Houston, which is one of the busiest ports in the United States (Figure 5). Boundaries from three banks, including Horseshoe Bank, Geyer Bank, and Elvers Bank, overlap with the shipping fairway. It should be noted that the banks with the highest density of susceptible coral species are East Flower Garden Bank (EFGB) and West Flower Garden Bank (WFGB) (Johnston et al., 2020). Susceptible coral species also inhabit Stetson Bank, Sonnier Bank, Bright Bank, and McGrail Bank, but in lower densities.

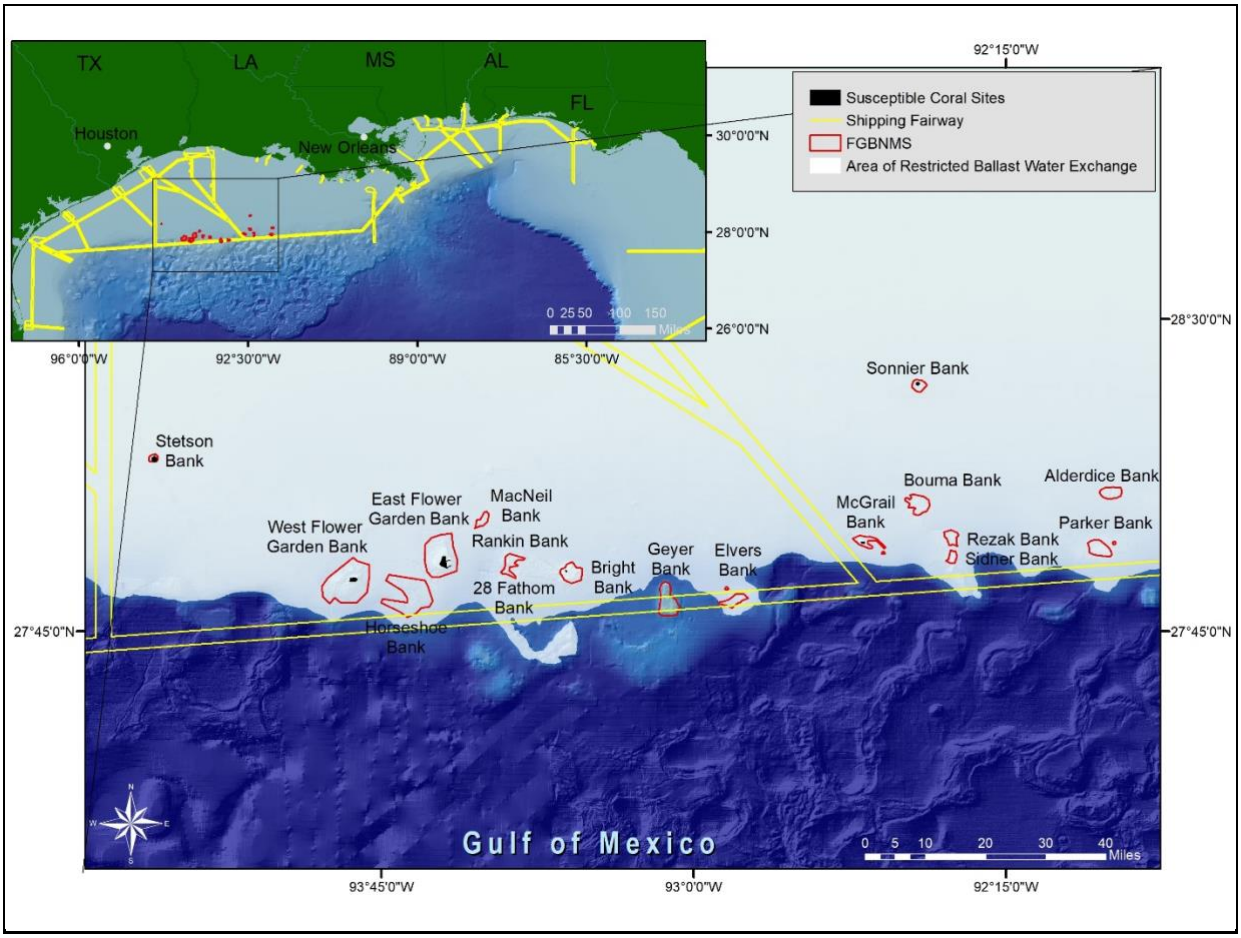

Figure 5. Locations of shipping fairways near FGBNMS sanctuary boundaries outlined around banks in the Gulf of Mexico. The area of restricted ballast water exchange is the entire white area shoreward of the continental shelf break. Susceptible coral sites on the banks are in black. Image: NOAA

Due to the proximity of FGBNMS to the shipping fairway, FGBNMS staff held discussions with EPA Region 6 (South Central) colleagues and corresponded with regional USCG officials in April and May of 2021. While FGBNMS is located outside of the area regulated by U.S. ballast water requirements, USCG, EPA, and the International Maritime Organization have all incorporated a concept of ballast water exchange as a management technique in certain instances (i.e., exchanging ballast taken on in port with water from the mid-ocean based on the principle that organisms taken on in port will not survive in the mid-ocean and vice versa). Also, the locations of these ballast water exchanges are required to be reported to the USCG for vessels bound for the United States. The 2019 Marine Safety Information Bulletin (USCG, 2019) provides

additional details of mid-ocean exchange requirements and considerations with respect to SCTLD.

Sanctuary regulations prohibit discharging within the sanctuary; however, if best management practices associated with the discharge of ballast water are not followed, FGBNMS reefs may be at risk. In order to avoid potential disease spread via ballast water, additional regulations may be needed for this area. FGBNMS will continue to be in close contact with regional USCG and EPA colleagues, and NOAA has identified the need to further examine links between ballast discharge and disease spread, as well as the effectiveness of ballast water treatment methods (Skrivanek & Wusinich-Mendez, 2020). Additional information needed on this topic includes:

- Research on ballast uptakes/discharges in the FGBNMS area,
- Determining which vessels frequenting the northwestern Gulf of Mexico have old ballast treatment systems,
- Tracking vessels in the northwestern Gulf of Mexico that have traveled from high-risk areas known to have SCTLD, and
- Assessing the use of sodium percarbonate to disinfect bilges of small vessels visiting FGBNMS.

Preparedness and Early Warning

Training

Accurate identification of SCTLD can be difficult, as lesions on stony corals may be mistaken for white plague disease. In some cases, a bleached margin adjacent to denuded skeleton can represent early signs of SCTLD, while a band or blotch of pale yellow tissue on *Orbicella* spp. colonies may be indicative of yellow band disease. Furthermore, divers must be able to differentiate signs of predation and other causes of tissue loss from disease. To ensure accurate diagnosis of SCTLD, all FGBNMS divers will participate in disease identification training prior to the 2021 field season, with annual refresher training before every field season, as needed. Training will consist of watching the September 2020 MPAConnect webinar titled "Identifying Stony Coral Tissue Loss Disease." In addition, staff will study disease identification cards (Appendix B) and review the "How do you recognize and describe stony coral tissue loss disease lesions?" presentation created by FKNMS research coordinator Andy Bruckner in 2018. Furthermore, these training materials will be made available to dive operators on M/V *Fling*. Training materials will be updated as new presentations and updated SCTLD information become available. At this time, key criteria for identifying SCTLD include:

- Distinct patterns of spread among susceptible species, with early susceptible species (e.g., brain corals) showing the first signs of disease and other species (e.g., star corals) subsequently showing signs of disease,
- Total loss of affected tissue or sloughing away of tissue,
- Absence of a distinct dark band at the margin of denuded tissue,
- Appearance of new lesions on the colony surface that are completely surrounded by living tissue,
- High prevalence of disease among early susceptible coral species shortly after initial emergence, and
- Rapid mortality among susceptible coral species.

In addition to training for FGBNMS divers and M/V *Fling* dive operators, disease identification cards will be laminated and made available to divers for reference underwater. In an attempt to have as many informed eyes in the water as possible, FGBNMS will display the "Identification of Stony Coral Tissue Loss Disease on Gulf of Mexico Reefs" poster created by MPAConnect (Figure 6) in the FGBNMS office and on R/V *Manta*, and will provide Fling Charters with posters to display on M/V *Fling*. The summary poster aims to guide FGBNMS divers on the detection and correct identification of the disease (Figure 6). For early warning and detection, divers visiting FGBNMS will be asked to pay specific attention to FGBNMS highly susceptible brain coral species, *Pseudodiploria strigosa* and *Colpophyllia natans*.

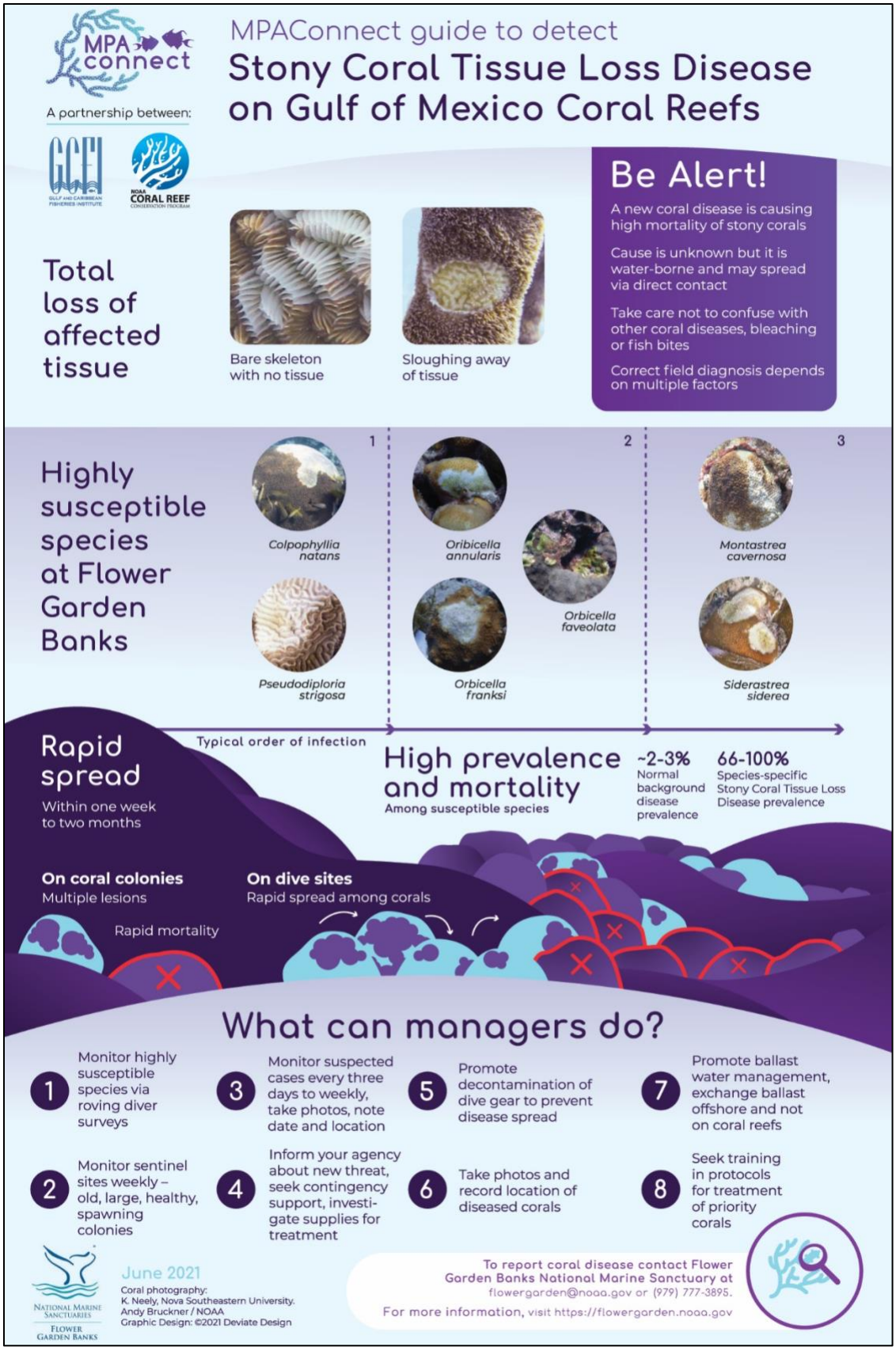

Figure 6. MPAConnect guide poster to detect stony coral tissue loss disease. Image: MPAConnect

Annual coral reef monitoring, in partnership with the Bureau of Ocean Energy Management, has occurred at FGBNMS since 1989 and utilizes random benthic photo transects as well as repetitive photographic monitoring stations to document coral cover and overall reef health (Johnston et al., 2020). While FGBNMS divers conduct regular monitoring and research cruises, it is impossible to monitor all areas of the coral reef; therefore, additional observations from recreational divers will be critical for early detection of SCTLD, should it arrive at FGBNMS. Recreational divers visiting the sanctuary are encouraged to take pictures to help document the location of suspected colonies that may be infected and email or text images with detailed location information to flowergarden@noaa.gov or (979) 777-3895.

Additional needs associated with this topic include:

- Funds for FGBNMS volunteer divers to participate in monthly M/V *Fling* dive trips to inspect the reef for signs of disease,
- Ensuring other research vessels visiting the FGBNMS region adhere to set protocols, and
- Development of a FGBNMS online reporting tool so divers can easily submit photos of suspected diseased colonies from their mobile phone.

Supplies for Response and Intervention

Should SCTLD spread to the coral reefs of FGBNMS, experts from Florida and the U.S. Virgin Islands recommend that it is critical to have supplies and funding available for immediate response. Therefore, before the 2021 field season begins, FGBNMS staff will order supplies (e.g., powdered amoxicillin, Ocean Alchemists LLC/CoreRx Base2B, syringes, hammers, chisels, waterproof paper, tags, etc.) for intervention methods.

Additional needs associated with this topic include:

- Staff training for treatment methods, such as antibiotic treatment application, and
- Establishment of emergency funds for SCTLD response, including additional cruises, volunteer divers, etc.

Alternative Treatment Investigations

An alternative treatment under investigation is the development of probiotics to stop SCTLD progression. However, the development of probiotic strains are location and potentially species specific. To contribute to this area of research, FGBNMS will collaborate with probiotic and marine pathogen research expert Dr. Blake Ushijima at UNCW. A priority for FGBNMS will be to collect mucus samples from highly susceptible and intermediately susceptible coral species in FGBNMS. These samples will be provided to Dr. Ushijima for FGBNMS probiotic screening and the development of FGBNMS-specific probiotic strains as a future alternative treatment option. Samples from FGBNMS will contribute to the evolving work on coral probiotics as a potential treatment option for SCTLD, as well as add to the understanding of how probiotics can benefit their coral hosts.

Response and Intervention

Monitoring

As mentioned previously, FGBNMS manages an established long-term coral reef monitoring program that began in 1989 (Johnston et al., 2020). The program focuses on benthic and fish community observations, as well as measurement of water quality parameters within the sanctuary. Monitoring has traditionally occurred within one-hectare study sites at EFGB and WFGB. Beginning in 2021, FGBNMS will incorporate a reef-wide stratified random sampling design for benthic and fish surveys, as well as continued monitoring within fixed study sites. FGBNMS also partners with NOAA's National Coral Reef Monitoring Program, which conducts reef-wide monitoring every two years at FGBNMS (NOAA Coral Reef Conservation Program, 2020). It should be noted that regular monitoring also occurs at Stetson Bank. Bright Bank, Sonnier Bank, and McGrail Bank all have monitoring plans in development. However, due to the depth of Bright and McGrail banks and low coral cover of susceptible species at Stetson and Sonnier banks, EFGB and WFGB are the priority locations considered in this plan.

Early Disease Identification

Despite the well-established monitoring program already in place at EFGB and WFGB, if SCTLD is identified at FGBNMS, a multi-level monitoring methodology will be needed based on the stage of the disease and management needs of FGBNMS. Based on recommendations from experts located in Florida and the U.S. Virgin Islands, the location where SCTLD is first identified will be the priority response area, with quick and aggressive treatment needed to minimize spread.

If identified, monitoring will begin immediately with diver propulsion vehicle (DPV) and/or roving diver surveys used to determine the extent of the disease outbreak and characterize the general reef area for vulnerability. DPV surveys will be performed to map the extent of disease. Latitude and longitude, along with estimates by divers of the percent of coral affected per location, will be marked during DPV surveys to delineate the edges of the affected area. Once the affected area is defined, roving diver surveys within the area will be conducted to define the prevalence of disease among susceptible species (Appendix C). DPV and roving diver surveys from the priority response area would be conducted every two weeks to one-month to map disease spread if weather allows.

Disease Progression

After SCTLD has been identified and the disease area delineated, disease progression will be monitored using time-series photography. If multiple corals are infected, large colonies providing substantial reef structure and reproductive capacity will be selected for monitoring. Coral colonies of interest will be marked by permanent pins with numbered tags on the reef, and a compass heading and distance to each tag will be taken from a centralized mooring buoy, subsurface buoy, or GPS location if a mooring is not nearby. Each colony, upon initial marking, will be measured for length, width, and height (cm). The proportional colony mortality will be estimated along with size of lesion(s). If lesion(s) are active, the repetitive photostation method described in Johnston et al. (2020) will be used for time-series analysis at approximately two-week to one-month intervals.

Intervention and Treatment

Once SCTLD has been documented, quick and aggressive treatment to minimize spread is advised by subject matter experts. FGBNMS will utilize two treatment strategies: culling and antibiotic treatment.

Culling

Culling is the removal of an entire colony from the reef to promptly reduce the pathogen load in a particular area and prevent disease spread to other reef locations (Meiling et al., 2020). Culling should only be utilized for small colonies (<30 cm in diameter) exhibiting clear signs of presumed mortality. Culling protocol is below:

- Remove colony with hammer and chisel,
- Place diseased colony immediately in sealed plastic bag,
- Preserve samples if required or euthanize in 20% bleach and water solution, and
- Transport to shore for either sample processing or disposal.

Antibiotic Treatment

Infected coral colonies larger than 30 cm and with 50% or more living tissue will receive antibiotic treatment, with priority given to Endangered Species Act listed corals, highly susceptible species, colonies located within shallower diving depths (to facilitate treatment application), and/or large colonies with high reproductive capacity (Figure 7). Experts suggest resources should be focused on large *Orbicella* spp. colonies, as they are important reef builders and respond well to treatment (Neely, 2018a). It should be noted that colonies with fewer than five lesions are more treatable than those with more than five lesions (Doyle & O'Sullivan, 2020). Antibiotic treatment protocol (Neely, 2018b) is below:

- Mix amoxicillin trihydrate powder into CoreRx Base2B (manufactured by Ocean Alchemists, LLC) in a glass beaker in a 1:8 ratio, by weight, just prior to application on the same day it is to be used. The total amount to prepare depends on the number of coral colonies to be treated that day;
- Once mixed, pack the mixture into 30 cc or 60 cc syringes;
- Place antibiotic syringes and rubber gloves in a dive bag; and
- Once on the reef location, press the antibiotic mixture from the syringe to cover the lesion and the area adjacent to the lesion. Use gloved fingers to press the antibiotic mixture to the colony to guarantee that it sticks to the lesion.

It is important to note that some people are allergic to amoxicillin, a beta-lactam class of antibiotic. Divers helping with intervention treatments should verify that they are not allergic to this type of antibiotic prior to the start of field work.

If not already marked, treated coral colonies will be marked by permanent pins with numbered tags on the reef, and a compass heading and distance to each tag will be taken from a centralized mooring buoy, subsurface buoy, or GPS location if a mooring is not close by. Each colony, upon initial marking, will be measured for length, width, and height (cm). Proportional colony mortality will be estimated, and the repetitive photostation method described in Johnston et al. (2020) will be followed for time-series analysis at approximately two-week to one-month intervals. During these intervals, treated colonies will be reexamined and antibiotic treatment

will be repeated if colonies show signs of reinfection or new lesions. If the disease were to spread and become endemic, reexamination of treated colonies will be reduced to every two months.

Figure 7. Diver applies antibiotic paste to diseased coral in Florida. Photo: Joshua Voss/FAU

On reef locations where SCTLD is present and intervention treatments are being applied, benthic photo transects to quantify percent coral cover and the extent of the disease will be conducted at periodic intervals if time allows. Surveys will follow benthic photo transect methodology in Johnston et al. (2020). In addition, should SCTLD spread to the one-hectare long-term monitoring study sites at EFGB and/or WFGB, treatment interventions will take priority within the study sites in an attempt to save valuable colonies from the 30-year monitoring dataset.

The intervention strategies described above are currently the most effective methods known, but will be updated and adapted if more efficient treatment methods are discovered.

Additional needs associated with this topic include:

- Funding for seasonal or part-time highly trained divers to assist with monitoring and intervention,
- Funding for DPV training for all divers,
- Funding for additional DPVs and batteries,

- Funding for regular access to vessels and supplies for disease documentation and intervention, and
- Collection of samples for research partners studying SCTLD (histology, proteomics, transcriptomics, microbial analysis, etc.).

Management

In the event one bank within FGBNMS becomes infected with SCTLD and another does not, FGBNMS may attempt to contain the disease by taking emergency action to close the bank to all activity, including recreational diving, fishing, and boating. Preparation for such an action will include drafting emergency closure language.

Additional actions may include removing certain moorings to limit activity, such as diving and recreational vessel traffic, in an attempt to reduce spread to other areas on the same bank if surrounding areas are affected by disease.

Research

The healthy FGBNMS reef ecosystem provides an opportunity to investigate several research topics that can contribute to the growing body of knowledge on disease treatment and restoration.

FGBNMS plans to partner with coral reef and molecular ecology expert Dr. Joshua Voss from FAU to experimentally assess the susceptibility of corals from FGBNMS to SCTLD through *ex situ* trials. Additionally, genomic differences between FGBNMS corals and corals in Southeast Florida and the Florida Keys will be compared to determine the potential for FGBNMS corals or larvae to serve as source material for coral restoration efforts in FKNMS and the Southeast Florida Ecosystem Conservation Area (SEFL ECA). This work includes two complementary approaches to understand the potential risks SCTLD poses to FGBNMS and potential restoration options for FKNMS and the SEFL ECA.

Research objectives include:

- *Ex situ* experimental tests to determine SCTLD susceptibility among four coral species from FGBNMS:
    - Ten coral colony fragments (10–20 cm diameter each) of susceptible species (*Montastraea cavernosa*, *Orbicella faveolata, Pseudodiploria strigosa,* and *Colpophyllia natans*) will be collected from FGBNMS and sampled (~1 cm^2) for genome-wide genotyping.
    - Coral fragments will be sent to FAU and exposed to SCTLD by either 1) inoculating them with a tissue slurry from a natural SCTLD infection collected in Southeast Florida or 2) exposing them to direct contact with a fragment with an active SCTLD lesion.
    - Colonies will be monitored daily for 10 days and photographed to determine lesion activity and coral tissue loss.
- 2b-RAD sequencing to identify and characterize genetic variation in coral populations and individuals in FGBNMS and FKNMS/SEFL ECA:
    - These data will support targeted coral genetic rescue and stock enhancement efforts and provide insight on overall genomic diversity among corals in FGBNMS. *Pseudodiploria strigosa* and *Colpophyllia natans* samples will be sequenced to add to ongoing work by Dr. Voss on *Montastraea cavernosa*, *Orbicella faveolata,* and *Stephanocoenia intersepta* using a 2b-RAD approach to identify genomic diversity based on single nucleotide polymorphisms throughout the species' genomes.
    - Small tissue samples (~1 cm^2) will be collected from ~50 individuals for each species and preserved. This approach could also be opportunistically applied to other coral species to inform restoration strategies and provide insight into population connectivity.
- Sample preservation and archiving for future analyses of microbiomes, histology, algal symbionts, transcriptomes, etc., should resistant and susceptible individuals be identified.

Once funding is established, the outcomes of this research will contribute to ongoing and future coral disease response efforts, improve understanding about the severity and impacts of the SCTLD outbreak, identify management actions to remediate disease impacts, and, ultimately, prevent or mitigate the effects of future outbreaks. This project will also provide key information for coral population enhancement efforts to ensure that restoration efforts maintain community and population level diversity.

In addition to applied research, FGBNMS will be partnering with Moody Gardens Aquarium, located in Galveston, Texas, to house representative samples of healthy FGBNMS coral species. Moody Gardens Aquarium is one of numerous facilities working to preserve genetic diversity and provide broodstock for future restoration efforts by collecting healthy coral colonies ahead of the disease front and caring for them in land-based, Association of Zoos and Aquariums-accredited facilities across the United States (Figure 8). Moody Gardens Aquarium has the capacity and facilities to house susceptible FGBNMS coral species, which will promote education and outreach on SCTLD and FGBNMS, as well as potentially contribute to reef diversity in the future. This collaboration will build upon the Aquarium-Sanctuary Partnership program already in place.

Figure 8. Coral monitoring dashboard providing information about corals rescued from Florida's coral reef and housing facilities as of June 2021. Image: FWC

Conclusions

To date, SCTLD has not been observed in FGBNMS, but the species of coral present and the relatively high colony densities suggest these important reefs may be susceptible to SCTLD. This strategy for disease prevention and response outlines a course of action to avert disease spread to FGBNMS and creates a response framework should the need arise. This plan highlights ongoing preparation actions by FGBNMS and the response actions most likely to prevent or mitigate widespread impacts to reef corals.

The SCTLD epizootic outbreak is unprecedented in its large geographic range, extended duration, high rates of coral mortality, and the substantial number of coral species affected, but lessons learned from Florida are being shared with researchers and managers in other locations with the hope of minimizing the outbreak's impact. While this disease is seriously threatening the health of coral reef ecosystems in the Atlantic, Gulf of Mexico, and Caribbean region, corals may be resilient with the help of intervention and treatment, particularly if other pressures are reduced to maintain continued growth and reproduction. While the situation is serious, everyone can take steps to help reduce pressures on coral reefs:

- Wear protective clothing or reef-safe sunscreen when recreating;
- Reduce marine debris by properly disposing of trash;
- Avoid touching corals when diving;
- Adhere to diving, mooring, and fishing guidelines;
- Decontaminate dive gear; and
- Report any suspected signs of SCTLD.

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Glossary of Acronyms

AGRRA – Atlantic and Gulf Rapid Reef Assessment

BCD – buoyancy compensation device

DPV – Diver propulsion vehicle

EFGB – East Flower Garden Bank

EPA – Environmental Protection Agency

FAU – Florida Atlantic University

FGBNMS – Flower Garden Banks National Marine Sanctuary

FKNMS – Florida Keys National Marine Sanctuary

FWC – Florida Fish and Wildlife Conservation Commission

GCFI – Gulf and Caribbean Fisheries Institute

NOAA – National Oceanic and Atmospheric Administration

SCTLD – stony coral tissue loss disease

SEFL ECA – Southeast Florida Ecosystem Conservation Area

UNCW – University of North Carolina Wilmington

USCG – United States Coast Guard

WFGB – West Flower Garden Bank

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APPENDIX A:
Detailed Decontamination Protocol

The following detailed decontamination protocol outlines general guidelines for gear disinfection as well as gear-specific guidelines (Florida Keys National Marine Sanctuary, 2019).

Reduce Disease Spread by Gear Decontamination

Neoprene gear, such as wetsuits, booties, and gloves, and the internal bladder of BCDs can harbor and support proliferation of pathogenic bacteria and other microorganisms by remaining damp and trapping water. Pathogens can also adhere to other dive and snorkel gear, especially when the diver directly contacts the bottom and touches corals and other benthic organisms. Pathogens on dive gear may survive for extended periods and can be transferred among reefs on subsequent dives, and, potentially, transmitted to reefs internationally, unless dive gear is disinfected.

Dive and snorkel gear can contribute to the overall transmission of pathogenic bacteria among reefs. Just like handwashing is a common practice to prevent the spread of disease among humans, disinfecting gear and following other best practices is recommended to prevent the accidental transmission of coral disease between reefs. Divers and snorkelers can reduce their likelihood of encountering and transferring pathogenic bacteria by maintaining proper buoyancy and avoiding contact with marine organisms. As a precautionary approach, divers and snorkelers can further minimize transmission of pathogens by sanitizing equipment between dives and before and after each dive excursion, especially when travelling between countries or between infected and uninfected locations.

Ammonium-based disinfectants and chlorine bleach are effective antiseptics that minimize the spread of disease-causing pathogens from infected to uninfected reefs and corals. However, proper use and technique are necessary to properly sanitize gear and avoid damaging sensitive equipment. Freshwater washing alone will not eliminate pathogens. Detailed disinfection guidelines are listed below (Neely, 2018b; Doyle & O'Sullivan, 2020).

General Guidelines for Disinfection

- 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/or 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.
- To minimize spillage on dive platforms and possible ocean contamination, quaternary ammonium solutions should only be used to decontaminate dive gear when on shore. Sanitization between dives should involve a bleach solution kept in a sealed container to

disinfect equipment that makes contact with corals (transect tapes, gloves, tools), and other gear should be washed in freshwater containing an antibacterial soap.

- 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

Tools, collection bags, sampling gear, transect tapes, clipboards, underwater slates, weight belts, and other equipment that comes in contact with the bottom should be decontaminated using diluted chlorine bleach. Bleach is extremely corrosive to metals and should not be used to decontaminate regulators or neoprene wetsuits, 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 (1/2 c bleach per 2 gal water, prepared in a 5-gal bucket with a lid).
- Rinse with fresh water, air dry.

Wetsuits, BCDs, masks, and fins should be decontaminated using quaternary ammonium disinfectants such as Virkon S1, RelyOn1, and Lysol1 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: 0.5% RelyOn (four 5-g tablets per 1 gal water), 1% Virkon S (1.3 oz per 2 gal water), 6.6% Lysol (1 qt per 1 gal 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 should be given to decontaminating wetsuits and the internal bladders of BCDs because of their ability to trap water that can house transmissible pathogens. Pour approximately ½ liter of solution into the mouthpiece of the BCD's exhaust hose while depressing the exhaust button, inflate the BCD, 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, then immediately dump the solution into a container for proper disposal on land. Flush the BCD two times with fresh water.

Regulators, computers, gauges, underwater cameras, and other sensitive scientific equipment should be decontaminated using fresh water with antibacterial dish soap or an isopropyl alcohol wipe and dried.

- Prepare a solution of warm water and antibacterial dish soap or OdoBan1 (5 oz per 1 gal water). 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 isopropyl alcohol. Be sure to wipe any small areas where water might accumulate.

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 for chemicals and user 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 disposal of each product in a safe manner. Familiarization with the safety data sheets for chemical products and manufacturers' product care and use standards will help to ensure appropriate use of these materials and safeguard human health.

Figure A. Dive gear decontamination poster developed by MPAConnect. Image: MPAConnect

APPENDIX B:
Training Materials

A selection of webinars and presentations hosted by AGRRA and MPAConnect can be accessed at https://www.agrra.org/webinars/. Webinars that will be specifically utilized for FGBNMS training are listed below:

- Identifying stony coral tissue loss disease (September 20, 2020) http://gcfi.adobeconnect.com/pqc7j15sbrw4/?OWASP_CSRFTOKEN=4d04cd13e3452f0a58f4000188387e9a38e42c3798878aa41f0188b63cf04945

- How to recognize and describe stony coral tissue loss disease lesions (September 15, 2020) https://www.agrra.org/wp-content/uploads/2020/09/SCTLD-ID-sep-2020-Bruckner.pdf

Downloadable disease identification cards are available on the FKNMS website: https://floridakeys.noaa.gov/coral-disease/disease.html

Figure B. Disease identification cards aid in determining if susceptible corals are affected by SCTLD. Image: NOAA

APPENDIX C:
Roving Diver Protocol

Roving diver protocol (adapted from Neely, 2018b; Doyle & O'Sullivan, 2020):

1. Swim around the reef site (no greater than 50 m from the recorded coordinates) for at least 10 minutes or longer for a more complete sample size.

2. On the data sheet (next page), record the following metadata:

    a) 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)
    f) Initial depth of survey
    g) Habitat surveyed and any additional notes

3. Record the species code of stony coral species observed during the survey. Exclude milleporids, acroporids, and *Porites astreoides*. Focus on colonies greater than 4 cm in diameter. For each species, tally the number of colonies exhibiting each of the following conditions:

    a) Newly dead colonies (bright white skeleton, polyp structure intact). Colonies with obvious other causes of mortality (breakage, toppling) should be excluded.
    b) Actively diseased colonies. Colonies with any level of SCTLD should be included here.
    c) Undiseased colonies with signs of concern (i.e., colonies that do NOT have any active mortality due to SCTLD, but are showing unusual pale spots or focal bleaching).
    d) Healthy colonies. No active disease or unusual signs.

4. Photos can be taken of unusual or interesting disease sightings.

Roving Diver Data Sheet

Name:		Site:	Time Start:	
Date:		Lat:	Time End:	
Depth:		Long:	Notes:	
Species Code	Tally colonies with recent mortality	Tally actively diseased colonies	Tally undiseased colonies w/ symptoms of concern (pale spots or bleaching)	Tally undiseased colonies

AMERICA'S UNDERWATER TREASURES