12-6-2022

# Assessing Broad-Scale Stony Coral Tissue Loss Disease Intervention Activities in Southeast Florida, USA

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# Thesis of
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Submitted in Partial Fulfillment of the Requirements for the Degree of

## Master of Science
## Marine Science

Nova Southeastern University
Halmos College of Arts and Sciences

December 2022

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HALMOS COLLEGE OF ARTS AND SCIENCES

# ASSESSING BROAD-SCALE STONY CORAL TISSUE LOSS DISEASE INTERVENTION ACTIVITIES IN SOUTHEAST FLORIDA, USA

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December 2022

**Abstract:**

Reef-building corals are crucial to the long-term existence of Caribbean coral reef ecosystems, providing both direct and indirect, local and global, ecological, economic, and social benefits. Stony coral tissue loss disease (SCTLD) is endemic in southeast Florida first appearing in 2014 and present in 2022. Effective *in situ* disease intervention treatments using antibiotic paste stop disease progression ~ 90% of the time. Between May 2019 and April 2022, 1,037 corals, >85% of which were *Montastraea cavernosa,* were treated during disease intervention dives in southeast Florida. This study investigated intervention activities over three years in an effort to make them more efficient. Treatment density, calculated by dividing the number of corals treated by the distance covered in each dive, was significantly higher during the first year than subsequent years. Treatment density was significantly higher in the wet season each year compared to the dry season. Local Coastal Regions Haulover South and Haulover North had the highest treatment density of all regions throughout the project. High treatment density areas in the first year did not recur in subsequent years, suggesting intervention successfully decreased local disease incidence. Results indicate that disease intervention efforts in Southeast Florida should be prioritized during the wet season and peak disease months June, July, and September, and at known dense coral locations to optimize the number of corals treated. However, periodic effort should occur year-round. Disease intervention activities have provided optimistic results for the future of Florida's Coral Reef and are an effective tool for coral reef management.

**Keywords:** coral tissue loss disease, SCTLD, disease response, *Montastraea cavernosa, Orbicella faveolata*, amoxicillin, lesion treatment

**Acknowledgments:**

I would like to thank the Florida Department of Environmental Protection for funding the disease intervention teams activities conducted throughout this project under Award No. B96800. Without this support, our local reefs would have suffered even further damage from stony coral tissue loss disease.

I also want to thank my committee members, Dr. Brian Walker, Dr. Karen Neely, and Dr. Rosanna Milligan. Their assistance and support have been appreciated and valued. I would also like to thank past and present members of the GIS and Spatial Ecology Lab who have helped to collect and manage the study data.

I would like to express my sincere gratitude to my family and friends for supporting me throughout this chapter of my life. Their love and encouragement have been invaluable to me during my time at NSU.

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# 1.0 INTRODUCTION

## *1.1 Marine Diseases*

Marine diseases are ecologically and economically costly (Groner et al. 2016; Harvell et al. 2004), and are occurring at increasing rates while remaining less studied than their terrestrial counterparts (Glidden et al. 2022). Marine diseases benefit from residing in seawater, a relatively stable environment in regards to temperature and chemical composition allowing them to remain viable for much longer than most airborne diseases (Glidden et al. 2022; Harvell et al. 2004). Marine diseases create further challenges by being difficult to detect and often affecting species inaccessible to humans (Glidden et al. 2022). For example, one of the most ecologically destructive documented marine diseases was the 1983 disease outbreak in *Diadema antillarum* (Lessios 2016), which contributed to the cascading degradation of Caribbean coral reefs, tipping the balance towards a macroalgal-dominated system (Mumby et al. 2006; Bellwood et al. 2004). The agent of the disease was never identified.

The marine environment creates many hardships for researchers in determining disease agents, transmission rates, modes, and vectors, as well as containment methods following an outbreak (Glidden et al. 2022). Although numerous marine diseases have been acknowledged and some identified, few have led to the development of intervention methods. Intervention development often follows the declaration of a disease emergency, in which the disease causes direct threats to human health or the economy (Groner et al. 2016). Additionally, marine diseases can be characterized as emergencies if they disrupt ecosystem functioning which often occurs when the species impacted is foundational or an ecosystem engineer, such as sea urchins or reef-building corals (Groner et al. 2016). However, delays to recognize and diagnose diseases exacerbate the impacts to the environment as well as the ability to manage those impacts (Groner et al. 2016).

Many instances of disease intervention are non-traditional and involve preventive methods or adaptive management rather than direct treatment (Glidden et al. 2022). Intervention methods can be *in situ*, in which intervention occurs within the natural environment, or *ex situ*, in which intervention occurs outside the natural environment, often in a lab or aquaculture facility. *Ex situ* disease intervention has been heavily relied upon for large charismatic species, such as dolphins and turtles, in which rehabilitation is often used to assist in the recovery process prior to release, if possible (García-Párraga et al. 2014; Escobedo-Bonilla et al. 2022). Diseases causing high stock mortality in aquaculture, such as those in important fisheries species, have presented the need for extensive system-wide *ex situ* interventions where individuals can be monitored for extended periods, treated, and maintained within a controlled system (Mohapatra et al. 2013). This method is commonly used for corals living in aquaculture, or those removed from the reef, presenting with disease signs and symptoms (Mohapatra et al. 2013). However, *in situ* disease interventions have been limited for many marine diseases due to inaccessibility, the need for monitoring or retreatments, or simply the inability to detect diseased individuals (Groner et al. 2016).

### *1.2 Coral Diseases*

The first studied coral diseases occurred in the Caribbean in the 1970s and were informally termed 'plagues' as the disease-causing agent was unknown (Dustan 1977). Recently, diseases affecting corals have become more frequent and lethal likely due to an increase of environmental stressors, and many have shown a strong connection to widespread increases in coral bleaching associated with ocean warming and low water quality (Aeby et al. 2019; Hayes et al. 2022; Morais et al. 2022). The accumulation of stressors to corals and their symbiotic zooxanthellae and microbiome, such as increased temperature and turbidity, may increase coral colony susceptibility to pathogens, making them more likely to succumb to disease (Groner et al. 2016; Harvell et al. 2004). Coral disease pathogens, have been challenging to identify, as diseased corals can often display similar morphological signs of stress and recession when infected by different pathogens, either directly in their tissues or within their zooxanthellae (Gil-Agudelo et al. 2004; Sheridan et al. 2013). These signs include bleached coral tissue typically localized to the edge of disease lesions and tissue recession from natural or injured edges, leaving behind white dead coral skeleton. (Gil-Agudelo et al. 2004; Sheridan et al. 2013; Neely et al. 2021b).

Scleractinian coral diseases are often named for their appearance and vary in the types of corals they infect, such as bouldering or branching. For example, dark spot disease, identified by a dark pigmented area of tissue and slow lesion progression, has been shown to impact multiple bouldering species (Gil-Agudelo et al. 2004). Dark spot disease incidence has been related to water depth, temperature, and even silica, with disease incidence increasing when water temperatures exceeded 28°C (Gil-Agudelo et al. 2004; Aeby et al In review). Black band disease, identified by a dark microbial mat along the disease lesion front, was first identified in Belize in 1973, infecting bouldering species (Antonius 1973). Yellow band disease, identified by yellow concentric blotches spreading outward on bouldering corals, and was first reported in 1994 in the lower Florida Keys (Santavy and Peters 1997). White band disease is one of the most detrimental coral diseases in the Caribbean, however, it only impacts the Acroporidae family of branching corals and is identified by a distinct white band of dead tissue around coral branches (Gladfelter 1982). Various pathogens from white band disease have been identified from *in situ* and laboratory-infected individuals suggesting that multiple pathogens, alone or in concert, are responsible for infection (Gignoux-Wolfsohn and Vollmer 2015). It was also found that white band disease can spread through direct contact and via a corallivorous snail as a vector between corals (Gignoux-Wolfsohn and Vollmer 2015). White pox infects *Acropora palmata* and is identified by irregularly shaped white patches on the branching coral as the tissue dies and the skeleton becomes exposed (Patterson et al. 2002). Both white pox disease occurrence and severity of disease lesions were positively associated with the corals being bleached prior to disease incidence in St. John, United States Virgin Islands (Muller et al. 2008). White tissue loss diseases that affect bouldering corals, such as white plague, exhibit very similar signs and symptoms to stony coral tissue loss disease, including acute and subacute tissue loss (Cróquer, et al. 2021). White plagues were identified in multiple epizootic events and named with a type number for each event, however the pathology and etiology do not exhibit clear distinctions between the types (Cróquer, et al. 2021). Similar diseases causing tissue loss along the lesion, have been noted in corals and termed white syndromes (Aeby et al. 2019). White syndromes can be acute or chronic and may be comprised of different diseases, although lesions appear almost identical (Greene et al. 2020).

In general, it is crucial to understand the methods of coral disease transmission, along with detecting the putative agent, to develop optimum disease intervention. For example, in white plague type II, the initial detection of causative agents has not been reproducible after the initial identification suggesting changing disease agents or multiple factors influencing the disease characteristics and spread (Sheridan et al. 2013), which presents challenges for current and future disease agent identification. Disease intervention methods for stony corals have historically been limited and vary in their success. Previous studies have relied on various antibiotic tests to determine the acting disease agent. Sweet et al. (2014) found that *ex situ* ampicillin and paromomycin treatments completely arrested white band disease in *Acropora*, a genus of branching corals, while two other antibiotics had little to no effect on these disease lesions. Their findings helped identify up to three bacterial components found solely within white band diseased individuals (Sweet et al. 2014). Antibiotic treatments are effective in treating SCTLD lesions when used in water dosing in aquaria to arrest disease lesion progression on *Meandrina meandrites, Colpophyllia natans, Dendrogyra cylindrus*, and *Montastraea cavernosa* (Aeby et al. 2019; Miller et al. 2020).

*In situ* disease interventions for coral diseases have involved various treatment methods, typically focused on the lesion front. Methods tested on yellow band disease, included shading and aspiration of the lesion, which were unsuccessful, while the use of a trench, a channel cut into the coral tissue and skeleton, had a high short-term success of ~70% but the long-term success of only ~10% (Randall et al. 2018). Black band disease in the Looe Key National Marine Sanctuary in the late 1980s, was successfully treated by removal of the microbial mat followed by the application of clay to seal the lesion edge, halting the spread of the lesion (Hudson 2000; Teplitski and Ritchie 2009). Although effective, coral colonies were reinfected ~30% of the time (Hudson 2000). This method was highly time-consuming and exhibited the need for a wide-reaching intervention method that decreased the probability of disease re-emerging along the reef (Hudson 2000). Chlorinated epoxy (15 mL calcium hypochlorite to 50mL epoxy), applied at the lesion front, was 63% effective at stopping disease progression, demonstrating its potential for halting disease lesions of black band disease in Hawaii (Aeby et al. 2015). Phage therapy has been tested on corals with white plague in the Red Sea, and was successful when used in the early stages of infection (Efrony et al. 2009). Pro-biotic treatments for SCTLD have successfully been used *in situ*, providing promising results for improving the coral's microbiome health and therefore decreasing individual susceptibility to disease, and may be useful to enhancing coral health after initial disease treatment (Schul et al. 2022; Rosado et al. 2019). The varied success in treating stony coral diseases suggests coral disease intervention is underdeveloped and understudied, presenting the need for further disease-specific intervention method research.

*1.3 SCTLD and FCR*

Stony coral tissue loss disease (SCTLD) has been deemed the most destructive Caribbean coral disease to date because of its high disease prevalence (61%) (Precht et al. 2016), compared to previous white syndromes (<5%), and the decimation of coral populations in southeast Florida, in which some species were reduced to <3% of their initial densities (Precht et al. 2016; Alvarez-Filip et al. 2022; Neely et al. 2021a). SCTLD was first identified in 2014 in the Kristin Jacobs Coral Reef Ecosystem Conservation Area (Coral ECA), bounded by St. Lucie Inlet and the southernmost edge of Key Biscayne and extends eastward ~5.5 km from the coast. SCTLD has since spread throughout the entirety of Florida's Coral Reef (FCR) and much of the Caribbean (Kramer et al. 2019; Dobbelaere et al 2020; Walker et al. 2021a). The disease onset coincided with a massive sedimentation event caused by the 2013-2015 dredging in the Port of Miami (Cunning et al. 2019; Barnes et al. 2015) and following the 2014 thermal stress-driven coral bleaching event (Manzello 2015; Aeby et al. 2019; Precht et al. 2016). However, the causative agent of the disease remains unknown. Data support that the disease is associated with dysbiosis of the zooxanthellae living within the coral tissue, followed by a secondary bacterial infection affecting the coral tissue itself (Landsberg et al. 2020; Work et al. 2021), while other studies suggest it to be predominantly bacterial or influenced mainly by bacterial interactions (Iwanowicz et al. 2020; Rosales et al. 2022). The disease is often found within clusters among the reef in SEFL, suggesting a contagious mode of transmission (Muller et al. 2020). Dobbelaere et al. (2020) found that disease spread matched that of a neutrally buoyant particle, suggesting that it is water-borne and currents play an important role in the spread. However, additional environmental and ecological factors must be considered, such as genetic susceptibility, organism size and age, and the general location and depth, when making conclusions about disease and transmission characteristics (Aeby et al. 2019; Muller et al. 2008).

Florida's Coral Reef stretches 530 km from Port St. Lucie in the north to the Dry Tortugas in the southwest and consists of numerous susceptible reef-building coral species. SCTLD has been documented to infect over 24 of the estimated 45 species of hard corals found within FCR, making it wide-ranging and highly destructive to many of the reef-building species crucial to the preservation of the reef (Aeby et al. 2021; Walton et al. 2018; NOAA Stony Coral Tissue Loss Disease Case Definition 2018). Highly susceptible species of SCTLD, which include the meandroid species such as *Dendrogyra cylindrus, Dichocoenia stokesii, Meandrina meandrites, Colpophyllia natans*, and *Pseudodiploria spp*., have been significantly impacted in the past seven years since the disease was first identified (Walker et al. 2021a; Neely et al. 2021a; Walton et al. 2018, NOAA Stony Coral Tissue Loss Disease Case Definition 2018). SCTLD signs vary within and between coral colonies, with some showing fast, aggressive disease lesion progression, while others progress at much slower rates (Aeby et al. 2019). SCTLD lesions can occur in single or multiple focal areas on a colony and are typically defined by a stark white denuded skeleton, often preceded by bleached tissue in a circular or arced pattern, radiating outward (Aeby et al. 2021). However, the presence of a bleached region is not consistently observed, especially among corals with fast lesion progression (Aeby et al. 2019). SCTLD distinctly differs from previously studied white plagues and syndromes in that it often displays lytic necrosis of the coral tissue along the disease margin (Aeby et al. 2021). Overall, colony mortality varies by lesion morphology, the relative number of infected individuals nearby, coral species, and regional location (Aeby et al. 2021). SCTLD poses a more significant threat than previously identified and studied white plagues and syndromes because of its aggressive progression rate across individual corals and the wide range of susceptible species found within Caribbean reef locales (Aeby et al. 2019).

The intermediately susceptible species, as determined by the NOAA Stony Coral Tissue Loss Disease Care Definition (2018), *Montastraea cavernosa* and *Orbicella faveolata*, are the most significant bouldering species contributing to reef composition and structure in SEFL (Hayes et al. 2022; Walker et al. 2020). SCTLD has become endemic to SEFL due to its persistence since 2014 and consequent disease-driven declines in coral density and live tissue cover along the reef (Walker et al. 2021a; Walton et al. 2018; Jones et al. 2020). Hayes et al. (2022) found a 59% decrease in regional mean live tissue area following the disease outbreak in SEFL, and all species that exhibited significant declines in mean live tissue area were categorized as highly or intermediately susceptible by NOAA Stony Coral Tissue Loss Disease Case Definition (2018). The vast decimation of stony corals by SCTLD throughout FCR illustrates the need for effective, efficient, and practical disease intervention.

*1.4 SCTLD Intervention*

The SCTLD impact has created an impetus for developing and studying novel intervention methods for efficacy in treating coral disease and practicality for real-world application. To conduct treatments on wild populations, it was necessary to develop an *in situ* tool where basic water attributes cannot be managed or maintained to specific levels, and water dosing is not feasible or applicable (Neely et al. 2020). However, *in situ* treatments presented more challenges, including the need for researchers to use SCUBA to apply treatments directly to corals in the marine environment. Novel *in situ* treatment types and methods began to be tested on corals showing evidence of SCTLD, and included the direct application of peroxide toothpaste, epoxy, chlorinated epoxy, and antibiotic paste to the disease lesion (Neely et al. 2021b; Walker and Brunelle 2018). The previous success of chlorinated epoxy to halt black band disease lesions (Aeby et al. 2015) demonstrated the potential for *in situ* SCTLD treatments, and therefore, began in May 2018 (Walker and Brunelle 2019). Concomitantly, amoxicillin (50 mg/mL) mixed with a proprietary mix of coral dental paste, a formulation used for drug delivery in dentistry, had success in stopping disease progression on SCTLD infected *Dendrogyra cylindrus* individuals (Miller et al. 2020; O'Neil et al. 2018). Ocean Alchemists LLC, run by a team of scientists, developed a silicone-based paste, CoreRx Base2b, to use with powdered amoxicillin, allowing for better lesion adhesion than dental paste, and delayed release over three days (Neely et al. 2021b; Walker et al. 2021b; Neely et al. 2020; Shilling et al. 2021). Amoxicillin paste, now referred to as CoralCure, outperformed chlorinated epoxy, in which treatments were successful 58.8 % of the time compared to 5.9 % of the time, on *M. cavernosa* in SEFL (Walker et al. 2021b). Additionally, CoralCure treated corals had a significant long-term lesion healing success on 95% of *M. cavernosa* lesions at 46 weeks after initial treatment in Broward County, FL (Shilling et al. 2021). Similarly, in the Florida Keys, 95% of corals treated with CoralCure, showed no signs of disease after two years, when monitored, and treated, if needed, every two months (Neely et al. 2021b).

SCTLD studies also compared the use of a 'firebreak', a trench cut into the coral skeleton and tissue to act as a disconnect between healthy and diseased tissue. The use of a 'firebreak' for coral disease treatment was first implemented by Aeby et al. (2015) for black band disease with the application of a band of marine epoxy adjacent to and at the disease lesion, acting as a second defense if the lesion were to spread past the initial extent. Walker et al. (2021b) found that treatment, at the disease lesion, combined with a ‘firebreak’ filled with CoralCure, placed about 5 cm from the disease lesion on the live tissue side, was the most effective *in situ* intervention method. On *Montastraea cavernosa*, the most frequently found diseased coral species during SCLTD intervention activities in SEFL, this method stopped disease  progression at a rate of 91.2 % with antibiotic paste and ‘firebreak’, 82.8 % with only antibiotic paste, 23.5 % with chlorinated epoxy and ‘firebreak’, and only 5.9 % with only chlorinated epoxy (Walker et al. 2021b). However, a ‘firebreak’ was not suitable for use on all diseased colonies, as it impacts a significant amount of tissue, uses valuable SCUBA dive time that could be used to treat more corals, and requires additional treatment materials. Divers were responsible for determining the need for a ‘firebreak’ based on the amount of size of the coral, live coral tissue area, and lesion morphology of each diseased individual. About 50% of corals treated with coral cure and a firebreak showed complete healing of the firebreak within 5-10 months and only 8% required retreatment (Walker et al. 2021b), providing promising results for disease intervention activities. Intervention practitioners recommend using amoxicillin alone without a ‘firebreak’ if intervention activity resources and time are constrained (Walker et al. 2021b; Shilling et al. 2021). CoralCure amoxicillin paste treatments without the use of a ‘firebreak’ were most effective on the star corals, *Montastraea cavernosa* (89%) and *Orbicella faveolata* (91%), and least effective on the brain corals *Diploria labyrinthiformes* (88%), *Psuedodiploria strigosa* (73%), and *Colpophyllia natans* (67%) (Neely et al. 2020). This disparity is likely due to variations in lesion and skeleton morphology impacting treatment adhesion (Neely et al. 2020).

Other SCTLD interventions in Southeast Florida include using probiotics to treat disease lesions and improve microbiome health (Paul et al. 2021). Probiotics are living microorganisms that benefit the host’s health, in this case, stony corals (Reshef et al. 2006). Scientists at the Smithsonian Marine Station investigated this relationship and began isolating microbial components from healthy coral individuals (Paul et al. 2021). McH1-7, isolated from healthy *M. cavernosa* colonies, has shown early success in fighting disease lesions and improving colony health to reduce individual susceptibility (Paul et al. 2021). At three research sites in Broward County, the probiotic strain *Pseudoaltermonas sp.*, McH1-7, is used to stop and prevent further SCTLD infections (Paul et al. 2021). An ongoing study will allow researchers to directly compare probiotic and antibiotic treatments within the same study site.

*1.5 Disease Response and Broad-Scale Interventions*

ECA interventions focused on four main tasks. First, interventions were used to attempt to save some of the region's largest colonies, identified using light detection and ranging (LIDAR) reef topography data and scouted via SCUBA, by monthly monitoring and applying treatments as necessary. These large corals were prioritized due to their old age, as suggested by their size and reproductive potential (Walker et al. 2021a). In preliminary research, corals monitored monthly, and treated if necessary, have roughly 50% greater live tissue cover than those visited biennially, since 2015 (Kozachuk 2022). The second task was to conduct broad scale, across the entire reefscape, interventions in which treatments were performed throughout the Coral ECA to attempt to reduce disease load on the reef without the intent of returning to monitor. This approach was determined due to the high short-term success rate (~90%) of the treatment methods, (CoralCure and firebreak) shown by Walker et al. (2021b). The third task was to conduct reconnaissance for restoration sites in which areas were classified based on their coral density and diversity (Walker et al. 2021a). Sites with abnormally high coral density and diversity, as determined by divers' observations and notes, were designated as sites of interest for future use. The last task was to field-test new intervention techniques and materials. This has been fulfilled by adding antibiotic treatments within the probiotic test sites in Broward County.

Due to resources and permitting, disease interventions in the Florida Keys were limited to specific areas defined by the Florida Keys National Marine Sanctuary (FKNMS) (Neely et al. 2021b). At the FKNMS sites, divers could be highly efficient in conducting treatments due to a large number of diseased colonies in close proximity along the reef. A recent study suggests that SCTLD has led to ~30% decline in coral density since 2014  in SEFL (Walton et al. 2018; Aeby et al. 2019; Simmons et al. 2022). In the FKNMS, protected reef sites exhibit less environmental and ecological stressors (Lirman et al. 2018) however, their high coral cover (Simmons et al. 2022), increases the likelihood of greater disease incidence. Conversely, the Coral ECA reefs have historically had lower species diversity, coral cover, and reduced coral populations than the Florida Keys (Walton et al. 2018). The endemicity of SCTLD to the Coral ECA has further reduced local coral populations and, in turn, declined reef health and functioning. As SCTLD becomes endemic to the Florida Keys region, intervention practitioners should utilize the information provided by the analyses and outcomes of this study to compare disease intervention patterns and management plans. If patterns seem consistent, practitioners should utilize the information from this study to amend disease intervention activities and management plans.

### *1.6 Project Overview*

This project valuated historical SCTLD interventions on coral reefs within the Coral ECA in SEFL to increase efficiencies in disease intervention activities. In this study, disease intervention activities were conducted by Nova Southeastern University's GIS and Spatial Ecology Lab and its partners at the Department of Regulatory and Economic Resources in the Environmental Resources Management Restoration and Enhancement Section based in Miami, Florida. Coral treatments were performed with the effective established method using CoralCure and a firebreak, if needed. The location and time of these treatments were used for spatial and temporal analysis to identify spatiotemporal patterns, local disease trends, and intervention densities. The outcomes of this project will be used to inform future intervention and disease management activities.

## 2.0 MAIN GOALS AND OBJECTIVES

### *2.1 Goals*

My thesis research goal was to evaluate spatiotemporal patterns of coral treatments and treatment density to provide information on historical disease interventions and recommendations for future disease event responses. Understanding spatiotemporal patterns of treatment density can prioritize future responses more effectively and increase the impact of intervention actions while reducing wasted effort. After this study, broad-scale intervention and data collection methods will be modified to better reflect reef sites visited throughout intervention activities.

### *2.2 Objectives*

The main objectives and questions to be addressed in this project are as follows:

1. Summarize and report disease intervention activities in SEFL to date
    a. How many disease intervention dives and coral treatments were conducted each month, season, year, and overall, throughout the project?

b. Which locations were visited during disease intervention activities? Were there apparent gaps in areas of disease intervention activities?

2. Statistically analyze data to determine if intervention was more efficient in certain locations or times

    a. Spatial analyses: Does regional and latitudinal variation exist within the treatment density and coral treatment data?

    b. Temporal analyses: Does monthly, seasonal, and yearly variation exist within the treatment density and coral treatment data?

    c. What data gaps need to be addressed in the future?

3. Make recommendations for future disease intervention activities

    a. Of the patterns identified throughout the data, which information is most beneficial to intervention practitioners designing projects? How can intervention resources be used most efficiently?

## 3.0 METHODS

### *3.1 Site Selection*

The study area was along FCR within the Coral ECA (Figure 1a). By 2017, SCTLD had spread through most of FCR, establishing an extensive area needing disease response (Lunz et al. 2017). With limited resources, initial disease response focused on areas of known high coral density and richness to increase efficiency and optimize the number of corals treated. The main goal was to treat as many diseased individuals and attempt to save as much live coral tissue as possible. Broad-scale disease interventions occurred in the Coral ECA between September 2018 and June 2022. All data were collected by the GIS and Spatial Ecology Lab and Department of Environmental Resources Management (DERM) members. Past and current members collected data from 2018 to 2022 and I began collecting data December of 2021.

Treatment sites were initially chosen using data from the Southeast Florida reef-wide post-hurricane Irma coral disease surveys (Walker 2018). The surveys collected sitewide information about coral species richness (number of species present), density (relative density), and cover (relative % live coral) in 2018. The 2018 surveys saw a significant decline of ~57.2% of coral cover on average across sites originally labeled as high density in 2015 surveys (Walker 2018). Additionally, the surveys collected information on the presence of SCTLD. Disease intervention activities in this study were prioritized at sites in which disease was recorded as present at the time of the post-hurricane Irma surveys, however, the presence of disease was unknown when visited for disease intervention.

National Coral Reef Monitoring Program (NCRMP) benthic and fish surveys were conducted in 2020 throughout SEFL. NOAA selected numerous NCRMP sites based on their depth, bottom habitat type, and fish assemblage, and then randomly assigned a portion for surveys. NCRMP surveyed site data were utilized to determine sites for disease intervention and sites were chosen if disease was marked as present in the area of the benthic survey or if numerous highly susceptible species were recorded at the site. Disease intervention efforts were focused on shallow habitats in the Broward-Miami ecoregion, bounded by Hillsboro Inlet, in the north, to Government Cut, in the south (Walker et al. 2021a) due to NCRMP sites in the shallow nearshore and inner reef regions recorded having disease more frequently than those in the middle and outer reef regions (Walker et al. 2021c). Additionally, Broward and Miami-Dade counties had the highest instances of NCRMP sites with disease out of the three counties surveyed, where disease was found at 41.9% and 51.6%, respectively, of all NCRMP sites within each county (Walker et al. 2021a). NSU efforts were explicitly focused on the area between Hillsboro Inlet and Biscayne National Park; however, data from all SEFL disease intervention teams within Broward and Miami-Dade counties were utilized within this study. The additional data collected during these studies were beneficial in determining areas of high coral density, abundant highly susceptible species, and relatively high disease prevalence (Walker et al. 2021a).

Additional intervention locations were haphazardly identified by selecting high topographic relief areas in the LIDAR bathymetry indicative of reef to fill spatial gaps between the prioritized sites. LIDAR inspection helped to ensure that divers spent time surveying reef habitats rather than other sandy or hardbottom areas. Prior to each dive day, approximately 4-6 sites were chosen from LIDAR, intentionally avoiding previously visited sites. GPS coordinates for each site were provided to the dive team, and divers were directed to enter the water at the site and swim in the direction of the current, to limit wasted effort while searching for diseased individuals. Typically, 2-4 sites were visited each dive day, but extra sites allowed teams to avoid other boaters and recreationalists that might risk the safety of divers. If divers came upon a large sand patch or did not find a diseased individual for an extended period, divers could decide to end the dive and move to another predetermined site.

Finally, intervention sites were added throughout the project when areas of high coral cover, disease prevalence, or species richness were located during other research activities or from public notification (Walker 2018). Spawning hubs, artificially created areas of threatened species placed in close-proximity to promote natural reproduction success, were visited for disease intervention to ensure the survival of important and highly susceptible reef-building species. However, the dives conducted, and corals treated at artificial sites were not included in this analysis because the corals did not occur naturally in such proximity to one another. Permanent coral reef monitoring stations and experimental research sites were avoided.

### *3.2 Intervention methods and materials*

Disease intervention teams required SCUBA divers capable of navigating, identifying, and treating corals underwater. Three divers would enter the water with a dive flag equipped with a GPS unit at a predetermined location, descend to the reef substrate and swim along the reef, following the underwater topography, until a diseased individual was located. Once identified, the coral was tagged with a Southeast Florida Action Network (SEAFAN) coral tag and treated underwater, allowing the individual to stay in its natural environment. Tags included information for citizen scientists and recreational divers to report information regarding the coral's health status or submit photographic updates. Broadscale, along the extent of the reef within the study area in SEFL, coral disease treatments were conducted in accordance with the previously determined effective treatment methods.

Disease intervention was conducted using CoralCure amoxicillin paste. The paste was made using an 8:1 ratio (400g container of Ocean Alchemists CoralCure Ointment Base2B Placebo Blend and 50g of powdered Amoxicillin. Once well mixed, it was placed into syringes for easy application underwater. Amoxicillin paste was applied directly to the disease lesion, pressing the paste into the coral skeleton, with a diver's finger. Firebreaks were used in conjunction with CoralCure when a coral had a fast disease lesion (extensive bright white skeleton (> 6cm) behind the disease lesion) and there was considerable live coral tissue remaining (> 0.5 $m^2$). If a firebreak disease trench was cut into the coral skeleton, amoxicillin paste was also pressed into the disease trench.

Divers were equipped with unique SEAFAN coral tags, hammer, nails, measuring device, underwater camera, datasheet, premade CoralCure amoxicillin paste in plastic syringes, and an underwater NEMO angle grinder. CoralCure amoxicillin paste, prepared in the lab the night before or the morning of planned dives, was refrigerated or chilled until use.

### *3.3 Data Collection*

The disease intervention team was equipped with underwater cameras and datasheets to document corals of interest, disease lesions, and coral treatments. Divers recorded the tag number of the coral, the coral species, length, width, and height, the approximate percent dead, diseased, and bleached tissue, the type of treatment used, and the length of treatment applied, in centimeters. Photographs documented the disease lesion prior to, and following, the treatment. The use of firebreaks was noted on the datasheet.

Using data collected and GPS location information, metrics were calculated to help with data analyses. Treatment density was calculated by dividing the number of corals treated by the dive distance, in meters. This allowed the data to be normalized because dives varied drastically in the distance covered. This metric was used for the bulk of analyses and to determine patterns within the data. Raw count coral treatments were used as a basis for comparison to treatment density. Average corals treated per dive were used to explain general spatial and temporal patterns.

During the first two years of the project, data were collected only on the number of diseased individuals but did not count healthy individuals, limiting the ability to determine disease prevalence and absence during these years. The methodology was altered in the third project year to include estimating disease prevalence and coral density for reef sites by counting healthy *Montastraea cavernosa* (MCAV) and *Orbicella faveolata* (OFAV) individuals. Disease prevalence values were calculated for each MCAV and OFAV count data by dividing the number of diseased individuals by the total number of individuals inspected throughout each dive (diseased + healthy), giving a percent diseased value for each species. Additionally, a coral density metric was calculated for each dive, MCAV and OFAV individually, using the number of corals counted (diseased + healthy) divided by the distance covered during the dive.

### *3.4 Quality Control*

Prior to conducting data analyses, data exploration was performed in R. The dataset compiling all disease intervention activities via GPS tracks of dives was cleaned in R using variable coercion to ensure variable type was correct, detection of inconsistencies, and correction of incorrect fields or values. Outliers were detected but not removed as they were checked to be accurate, provided valuable information to the study, and did not influence the outcomes of the analyses. Null or missing values were filled with "NA", and imputation was not used because values could not be created from available information. Once cleaned, the data were validated using the "validate" package (van der Loo and de Jonge 2020) to ensure that values made logical sense. Normality was tested for each variable using Shapiro-Wilkes tests and by reviewing the data distribution.

Similarly, the dataset for treated coral locations was cleaned using variable coercion and checked for outliers. All outliers were validated to the hardcopy datasheets and not removed as they provided accurate and valuable information to the study. Treated corals were removed with missing location information due to GPS failure during data collection. Once validated using the "validate" package (van der Loo and de Jonge 2020) to ensure values made logical sense, the data was imported into ArcGIS Pro for analysis. No analysis was conducted in R for this dataset.

Data was analyzed to determine if coral treatments were a function of dive distance. Various regression models were run and tested to determine the best fit model. The best model was chosen using the standard of the lowest Akaike Information Criterion (AIC) score. The outcome of these analyses will determine the applicability of further analyses.

The dataset for disease prevalence and absence, only collected in the last year of the project, was cleaned using variable coercion and checked for outliers. All outliers were validated to the hardcopy datasheets and not removed as they provided accurate and valuable information to the study. Dives in which the distance covered could not be calculated due to GPS failure during data collection were removed from coral density data analysis. However, the information collected during these dives was used when the distance covered was not a factor, such as the treated corals by species analyses and disease prevalence calculations, based on the number of diseased individuals and healthy individuals inspected throughout a dive. The data were validated using the "validate" package (van der Loo and de Jonge 2020) to ensure values made logical sense, and normality was tested for each variable using Shapiro-Wilkes tests and by reviewing the distribution of data values. Covariance of factors was tested using a Spearman's rank correlation test.

Once data were reviewed and tested for normality and covariance, the information was compiled to more easily determine the statistical test needed for analysis (Table 1).

**Table 1.** Project variables and descriptive information used for statistical analysis.

| Variable Name | Factor Type | Numeric Type | Normality | Variable Type | # of Values |
|---|---|---|---|---|---|
| **Coral Treatments** | Continuous | Integral | Not normal | Dependent | 1037 |
| **Treatment Density** | Continuous | Numerical | Not normal | Dependent | 333 |
| **Year** | Categorical | NA | NA | Independent | 3 |
| **Season** | Categorical | NA | NA | Independent | 2 |
| **Month** | Categorical | NA | NA | Independent | 12 |
| **Local Coastal Regions** | Categorical | NA | NA | Independent | 8 |
| **Latitude** | Continuous | Numerical | Not normal | Independent | 333 |
| **Prevalence** | Continuous | Numerical | Not normal | Dependent | 101 |
| **Coral Density** | Continuous | Numerical | Not normal | Dependent | 91 |

*3.5 Spatial Data Analyses*

A floating GPS unit time synced to a dive computer was used to attain each treated coral's location and the entire dive extent. The diver recorded the time at each treated coral so the location could be obtained from the GPS later. GPS point and line data collected from each dive were obtained using DNR Garmin (Version 6.1.0.6, Minnesota Department of Natural Resources 2011). Both line and point data were inputted to ArcGIS Pro (Version 2.9.3, Esri 2021). The line data were trimmed to ensure that only underwater survey time was included in the analysis. The GPS track dataset included the number of coral treatments conducted, dive track, dive time, dive track distance, day of dive, and the institution which conducted the survey and treatments. Dives conducted at spawning hubs and restoration sites were excluded from maps and data analyses. GPS dive track length and coral treatment data were used to calculate the total number of colonies treated over the linear distance covered (the number of corals treated by the dive distance). This metric was termed treatment density and is used for the bulk of the analysis. The treatment density metric is a measure of efficiency because it is the number of corals treated over the dive distance covered. Therefore, areas with higher treatment densities provided more treated corals per unit effort. The geometric center of each dive track was used to determine site classifications.

A layer depicting Local Coastal Regions (LCRs) was created to better understand spatial patterns of disease prevalence throughout the study area (Figure 1b). The inlet locations in SEFL were used to divide the study area into manageable, locally recognized regions. The midpoint between inlets was used to further define these regions as the area 'north of' and 'south of' each inlet. The eastern layer boundary was created using the Coral ECA outline. The LCRs were named according to the inlet and their location ("Port Everglades North" to designate the area between Port Everglades and the midpoint of Port Everglades and Hillsboro Inlet, Figure 1b).

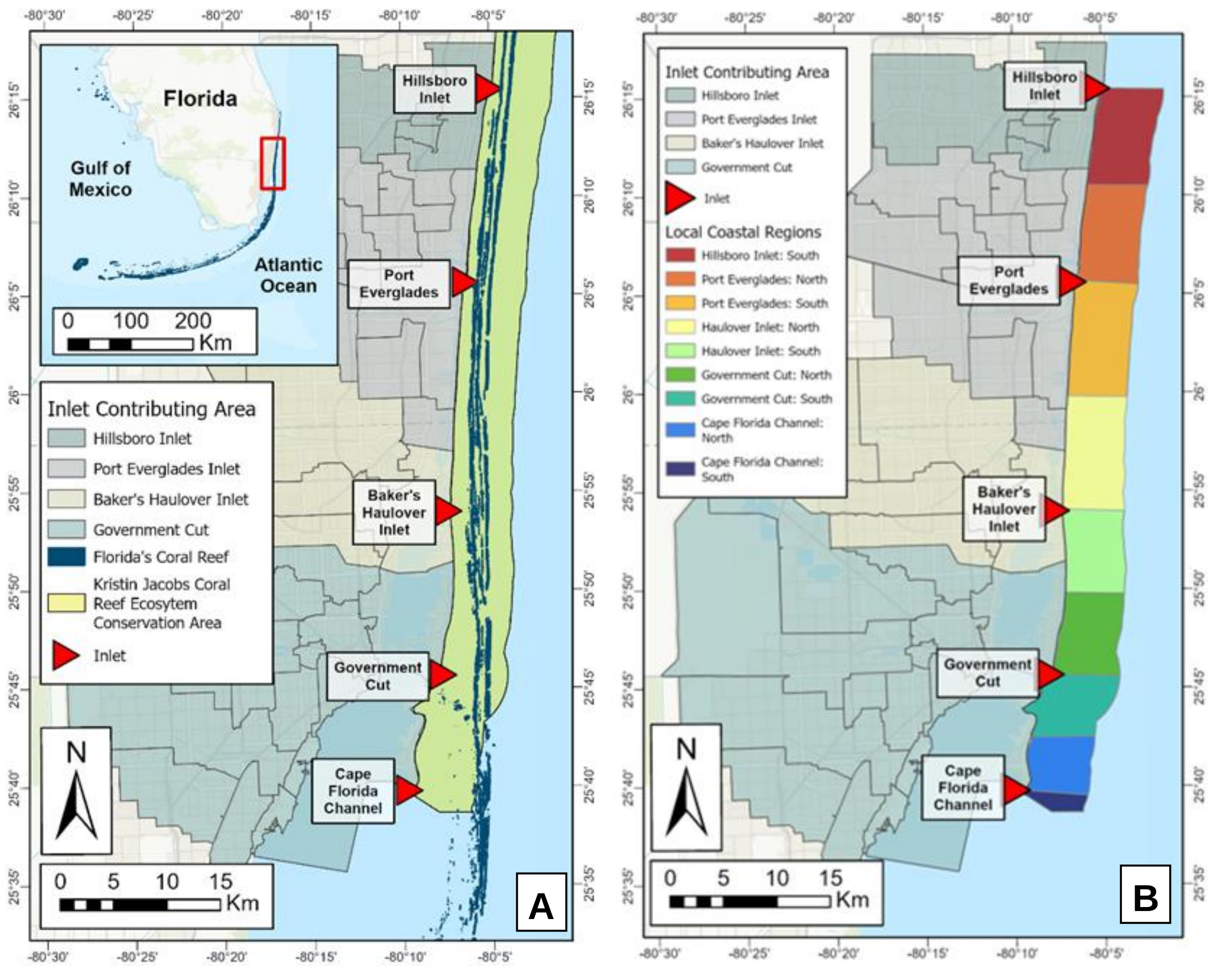


***Figure 1.*** *(a) Inlets and Inlet Contributing Areas in Southeast Florida. (b) Local Coastal Regions (LCRs) in Southeast Florida.*

Data analysis was conducted in ArcGIS Pro (Version 2.9.3, Esri Inc. 2021) and R (Version 4.1.0, R Core Team 2021). Prior to conducting further spatial analyses, it was necessary to conduct spatial autocorrelation tests on the treated coral location data as well as the dive track location data. The results of these analyses were used to determine the validity of conducting further statistical spatial analyses within the data. ArcGIS Pro Spatial Analyst tools Kernel Density estimation was used to determine spatial patterns within the data. Kernel Density estimation was completed using the location and value of the point features and calculates the density of these point features (Esri Inc. 2021). Kernel Density Estimation was performed for treatment density using the centralized dive track location and location of treated corals for the entire dataset and each project year individually. This allowed a comparison of treatments and treatment density patterns through time.

These outcomes were then compared to the epidemiological probability maps created by Muller et al. (2020).

Various maps were produced with previously validated datasets to show the extent and patterns within the data. These maps help to provide a visual basis for a better understanding of intervention efforts and treatment density throughout the study area. Maps were also used to depict project year differences, and variations over the entire timeframe of the project. Kernel Density estimation outputs were included in maps to visually represent the results found.

Treatment density values were not normally distributed for all LCRs except the Cape Florida Channel North region. A negative binomial GLM was used to compare treatment density between LCRs to one another using coral treatments with dive distance as a log-transformed offset factor to represent the values for treatment density. Treatment density was the dependent variable and were analyzed by inlet region, the independent variable. An ANOVA was completed for post-hoc testing and to obtain the chi-squared and p-value for the model. The residuals were plotted, and the "emmeans" package (Lenth et al. 2022) was used to understand the effect of each LCR on treatment density. The effect of coastal regions were directly compared using the "contrast" function and "pairwise" method (Spadafore et al. 2021).

Comparisons were conducted between latitude and treatment density. Treatment density was not normally distributed as determined by a Shapiro-Wilkes test and distribution plot of the variable (Table 1). Kendall's tau correlation tests were completed on treatment density and latitude due to the large n-value of 333.

Coral density analysis was limited to MCAV only as OFAV data were too scarce to identify patterns within the data. MCAV density analysis was conducted with a negative binomial Generalized Linear Model (GLM) using the number of individuals inspected with distance covered as the offset factor compared across all LCRs. MCAV coral density data distribution was compared to the Year C treatment density per dive distribution using a Kolmogorov–Smirnov test to determine if the datasets were drawn from different distributions. If each dataset was drawn from the same distribution, the datasets should not be further compared for effects. Once dataset distribution differences were confirmed, MCAV coral density metrics were compared to treatment density per dive using a negative binomial GLM to determine if coral density affected treatment density throughout SEFL. The negative binomial GLM was completed using the number of diseased and healthy MCAV individuals with dive distance as a logarithmic transformed offset factor and tested if MCAV density was a predictor of treatment density. Once completed, an $R^2$ value was calculated to determine the predictive power of the model, with a value over 0.4 suggests high predictive power and that the model fits the data well.

*3.6 Temporal Data Analyses*

Disease intervention dive tracks and coral treatment data were compiled to allow for temporal analyses. The data were categorized by each project year beginning in May and continuing through the following April. The project years were as follows: A: May 2019- April 2020, B: May 2020 - April 2021, C: May 2021 - April 2022. Seasons were categorized as wet from May to October and dry from November and April, as per Davis and Ogden (1994). The dry season is associated with cooler water and air temperatures, and the wet season with warmer water and air temperatures (Walton et al. 2018). Each dive track was labeled based on the month, season, and year it was conducted to allow for ease of analyses. Once categorized, each group was tested for normality using a Shapiro-Wilkes test and distribution plots of each variable. A compilation of descriptive information for each variable was used to determine the appropriate tests for significance (Table 1).

Comparisons were conducted between treatment density and months, seasons, and project years to detect temporal patterns within the data. Due to the non-normality of treatment density, negative binomial GLMs were used for analyses of treatment density and months, seasons, and project years, independently (Table 1). Negative binomial GLMs were conducted utilizing the coral treatment data with a logarithmic offset term of dive distance to represent the values of treatment density. An additional negative binomial GLM was run to explore the variation between seasons throughout each project year because of the drastic seasonal effect identified within the data.

All GLMs were followed by an ANOVA for post-hoc testing and to obtain the chi-squared and p-values. Where applicable, pairwise comparisons were completed using the “emmeans” package (Lenth et al. 2022), the “pairs” and “contrast” functions, and the “pairwise” method (Spadafore et al. 2021) to determine the significance between levels of factors.

## 4.0 RESULTS

### *4.1 Broadscale treatments*

Disease intervention teams conducted 333 dives completed across 120 days throughout the Coral ECA from May 2019 to April 2022 (Figure 2a). On average, each field day consisted of 2 dives covering an average distance of 723 meters on each dive. During the project period, 1,037 corals were treated (Figure 2b). Each diseased coral is considered one treatment and does not account for the number of treatments conducted on one coral. The average number of corals treated per dive was 2.81 corals. All treatments conducted within the study period used CoralCure amoxicillin paste and firebreak disease trenches, if needed. There are apparent data gaps within the dive track data and treated coral datasets due to safety concerns of diving near inlet mouths.

Data were analyzed to determine if coral treatments were a function of dive distance. A polynomial regression of coral treatments and dive distance determined that coral treatments are a function of dive distance ($p = 5.59 \times 10^{-5}$, Adjusted $R^2 = 0.05758$). However, the low adjusted $R^2$ value explains that only 5.8% of the variance in coral treatments were explained by dive distance.

The number of treatments conducted during the study period by species was 888 (86%) *M. cavernosa*, 76 (7%) *O. faveolata*, 27 (3%) *Pseudodiploria strigosa*, 19 (2%) *Pseudodiploria clivosa*, 15 (1.5%) *Colpophyllia natans*, 5 (<1%) *Solenastrea bournoni*, 4 (<1%) *Diploria labryinthiformis*, 2 (<1%) *Siderastrea siderea*, and 1 (<1%) *Orbicella annularias* suggesting there are some survivors of both the highly and intermediately susceptible species, assuming that all individuals in the region have been exposed to the disease (Figure 4, NOAA Stony Coral Tissue Loss Disease Case Definition, 2018).

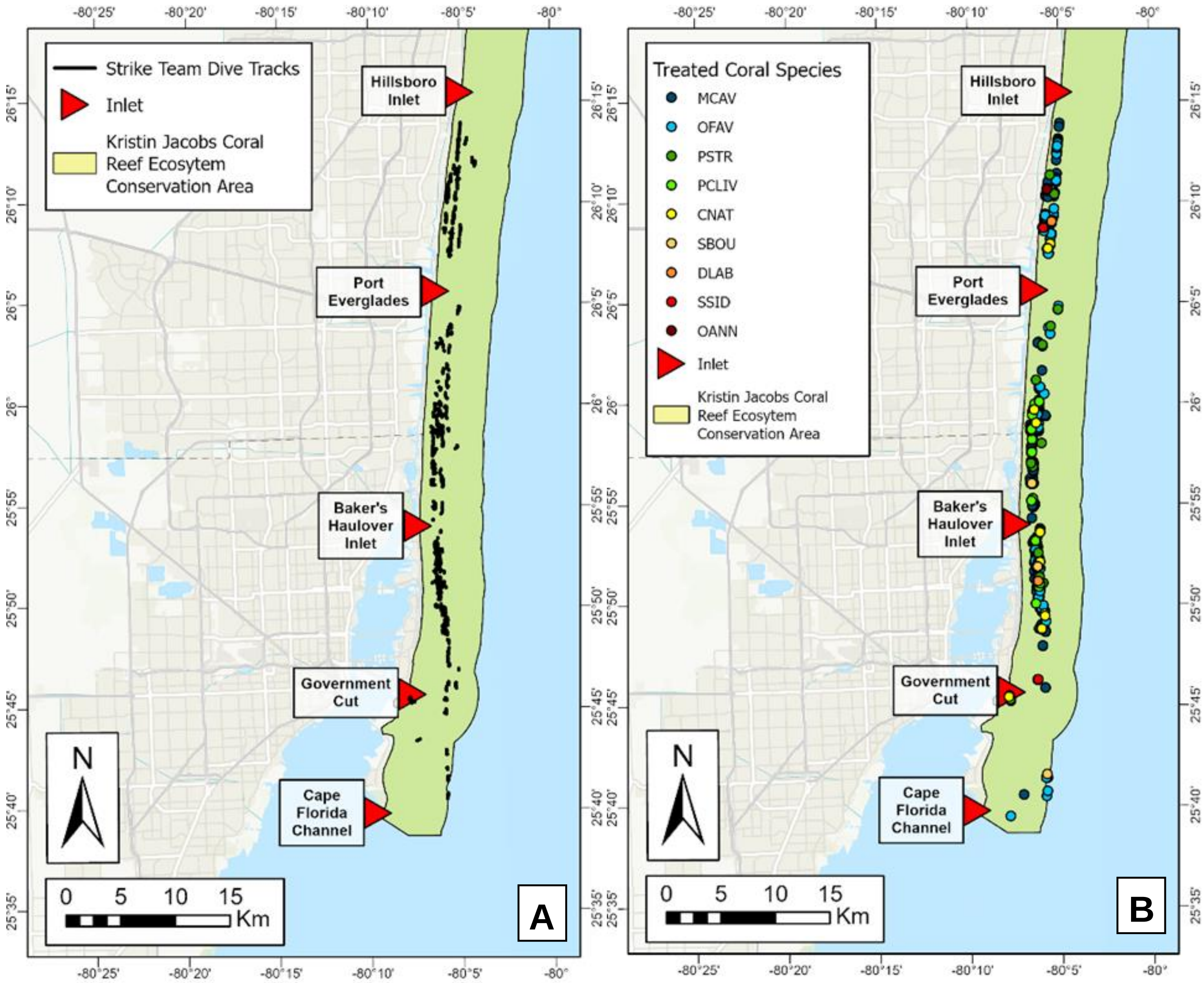


***Figure 2.*** *(a) Strike team disease intervention dive tracks. (b) Strike team disease intervention treated corals by species.*

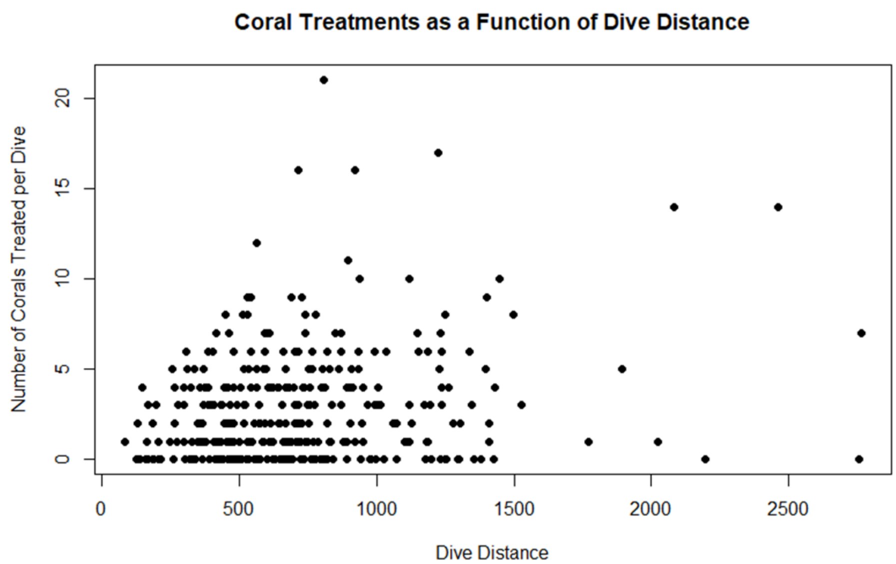


***Figure 3***. *Coral treatments are a function of dive distance (3rd degree Polynomial Regression: p = 5.59 x 10-5, Adjusted* $R^2$ *= 0.05758). Coral treatments per dive plotted against the dive distance covered during the dive (meters).*

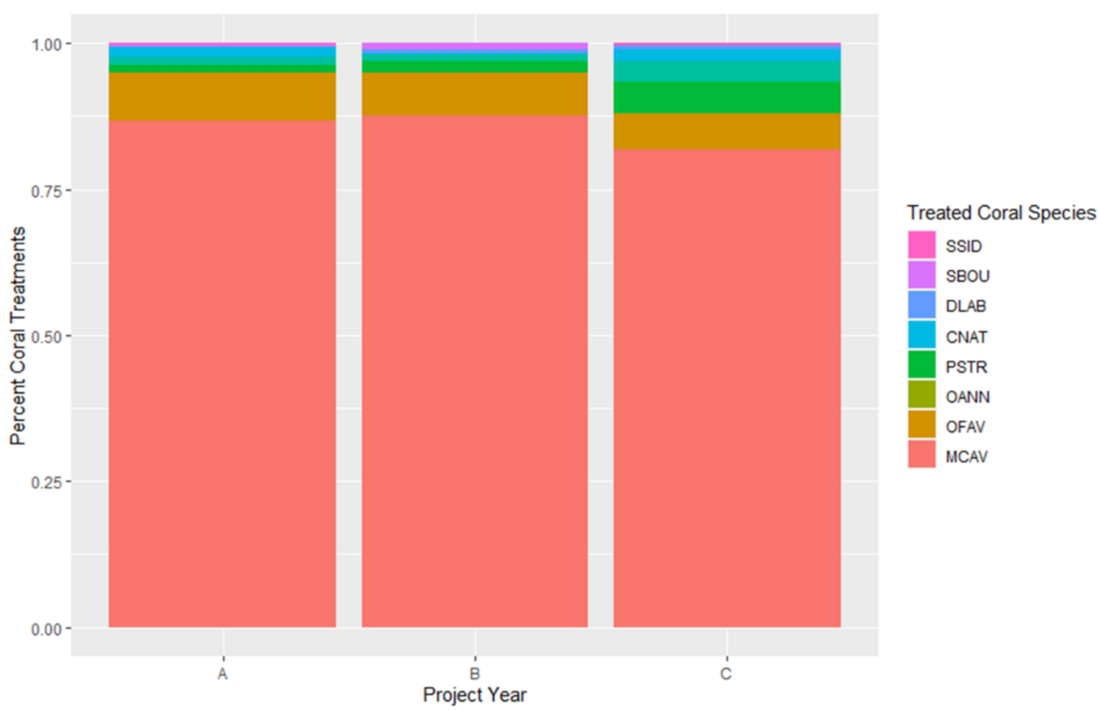


***Figure 4.*** *Species proportions of treated corals for each project year. A: May 2019 - April 2020, B: May 2020 – April 2021, C: May 2021 – April 2022. Treated Coral Species ID Codes: SSID: Sidastrea siderea, SBOU: Solenastrea bournoni, DLAB: Diploria labyrinthformis, CNAT: Colpophyllia natans, PSTR: Pseudodiploria strigosa, OANN: Orbicella annularis, OFAV: Orbicella faveolata, MCAV: Montastraea cavernosa.*

*4.2 Spatial Analyses*

*4.2.1 Number of Dives and Coral Treatments*

Spatial autocorrelation tests were run in ArcGIS Pro to determine if the data was statistically different from a random distribution. Geometrical mean dive track locations and treatment density were spatially autocorrelated and the data distribution was more clustered than random spatial processes (Moran's Index = 0.997269, z= 38.743816, p=0.00). Geometrical mean dive track locations and coral treatments were also spatially autocorrelated and the data distribution was more clustered than random spatial processes (Moran's Index = 1.00157, z= 59.43677, p=0.00). Therefore, Kernel Density estimations were used to visualize patterns and spatial analyses were limited to the latitude and LCR analyses conducted in R. LCRs will be used heavily throughout the results and discussion. The regions are within the Coral ECA, from north to south, as follows: HiS: Hillsboro South, PEN: Port Everglades North, PES: Port Everglades South, HaN: Haulover North, HaS: Haulover South, GCN: Government Cut North, GCS: Government Cut South, CFCN: Cape Florida Channel North.

Total number of dives and coral treatments varied by LCR (Figure 5). The number of dives was lowest in Cape Florida Channel North (8) and highest in Haulover South (107). The number of treatments followed a similar pattern with the highest in Haulover South (333) and lowest in Cape Florida Channel North (11).

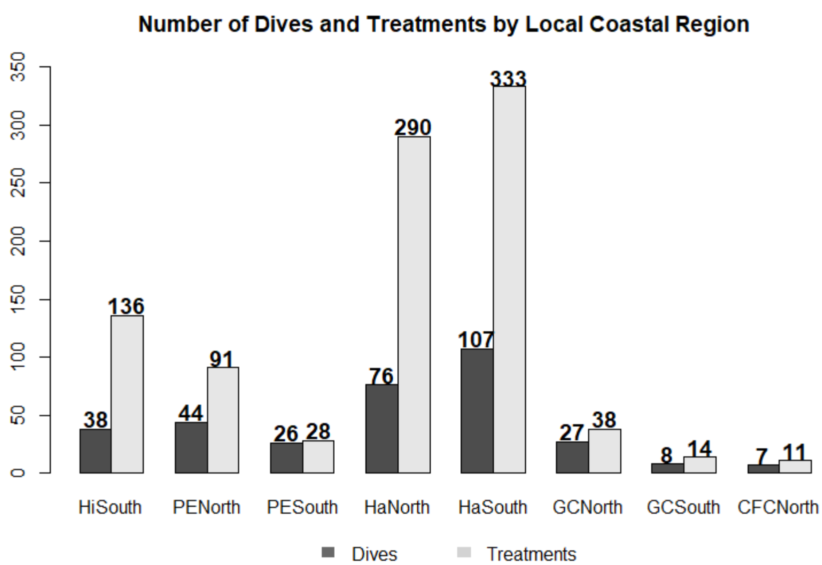


***Figure 5.*** *Number of dives and coral treatments over the course of the project for each LCR.*

### *4.2.2 Overall Spatial Patterns and Analyses (All years combined)*

Treated coral locations spatially varied with the most treated corals occurring near Bakers Haulover Inlet and Hollywood, FL, south of Port Everglades (Figure 6a). Another region of medium-high coral treatments was in Hillsboro South. These locations were dependent on dive effort, which was not randomly distributed along the coast. However, more dive effort does not lead to a comparable increase in coral treatments.

The Kernel Density estimation for treatment density per dive exhibited a large area of high kernel density in Haulover South and medium values in Hillsboro South (Figure 6b). This map indicates that overall, divers treated more corals per meter in the Haulover South region than any other LCR. Haulover North was also relatively efficient with more corals treated per meter than most LCRs but distinctly lower than the Haulover South region.

Treatment density per dive was not significantly correlated to latitude ($z= -0.18352$, $p= 0.8544$, Figure 7), however, the treatment density negative binomial GLM showed that LCRs were a significant predictor of treatment density (LR $\chi^2= 47.249$, df=7, $p=4.992 \times 10^{-8}$, Figure 8). The Haulover North region was a significant predictor of treatment density per dive ($z= 2.169$, $p=0.0301$).

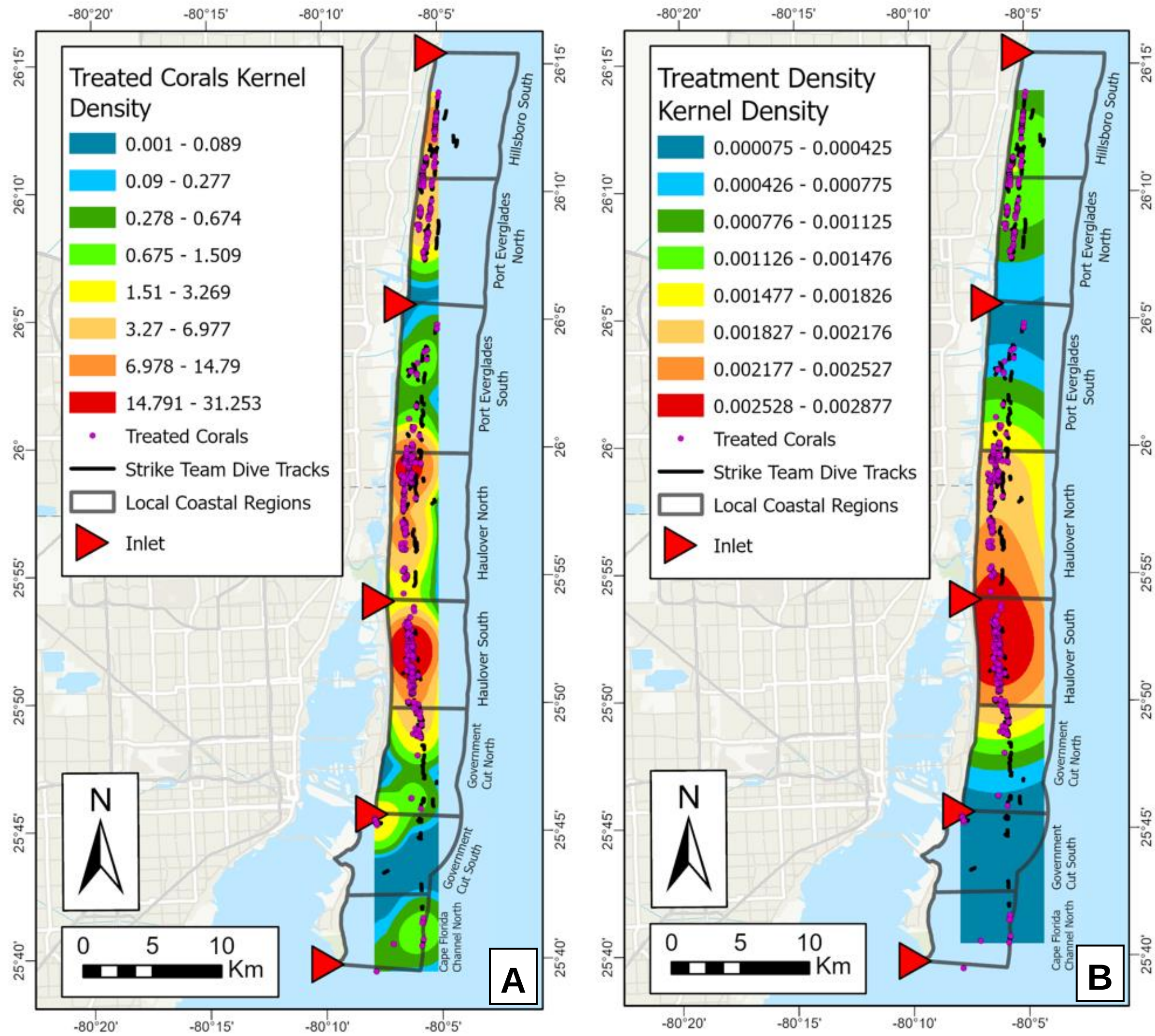


***Figure 6.*** *(a) Treated corals Kernel Density estimation for the entire project May 2019 to April 2022. (b) Treatment density Kernel Density Estimation density for dive tracks for the entire project from May 2019 to April 2022.*

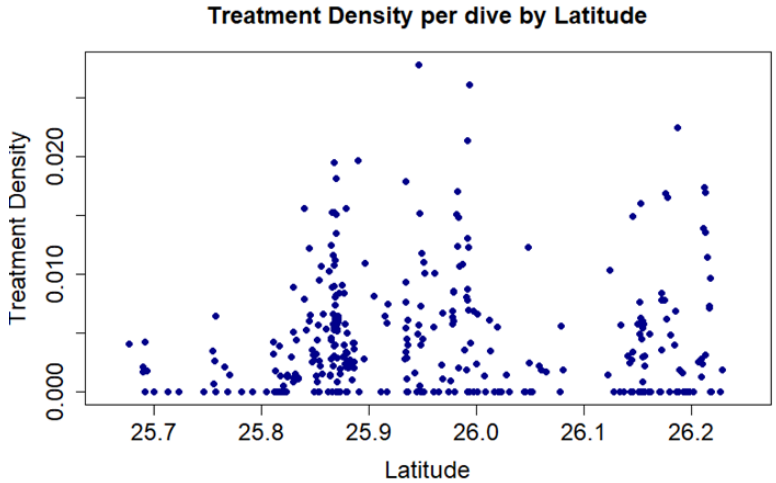


*__Figure 7.__ Treatment density per dive by latitude over the entire project from May 2019 to April 2022.*

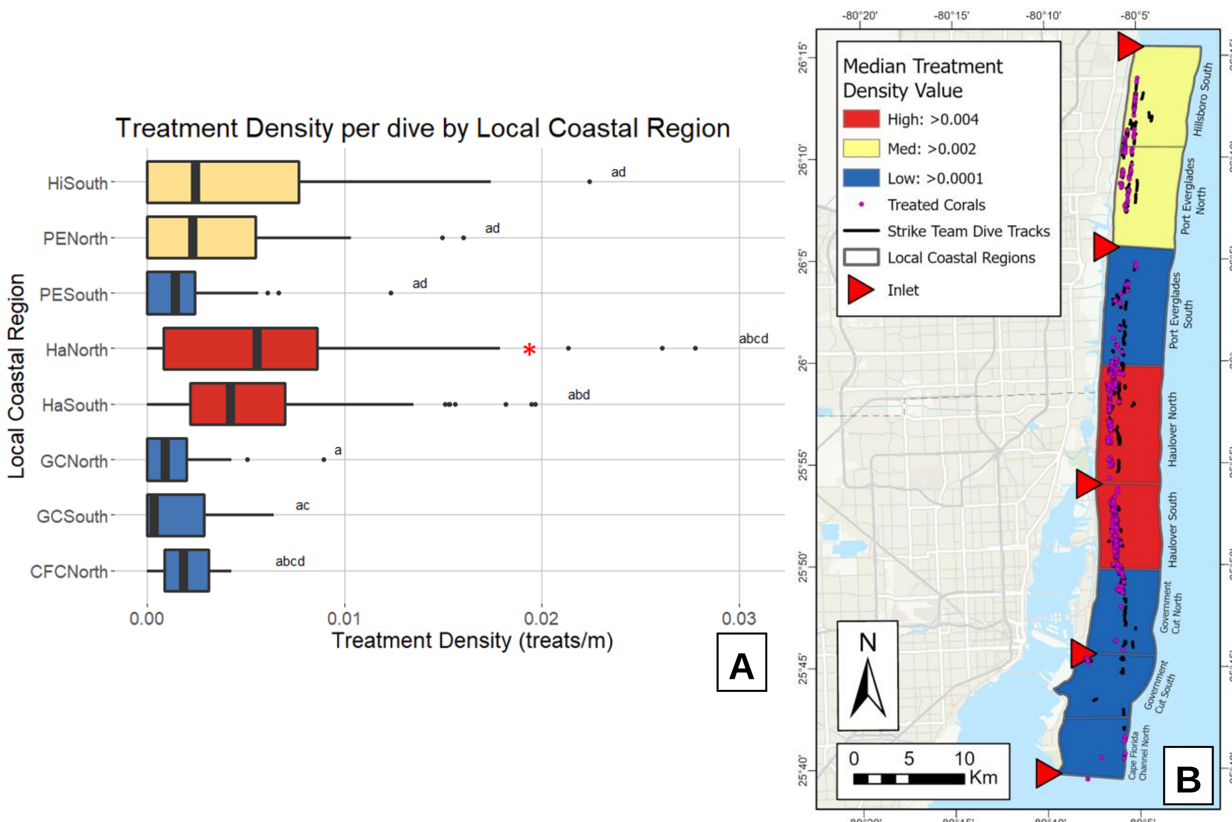


*__Figure 8.__ (a) Treatment density per dive by LCRs over the entire project. Boxes represent the interquartile interval, and the dark line represents the median of the data. Error bars represent the upper and lower quartiles of the data. Points to the right represent outliers of the data. Letters right of boxes represent statistical significance. Red asterisks represent levels that had significant effects on the model. (b) Median treatment density per dive by Local Coastal Region shown through gradient, high values shown in red, moderate in yellow, and low values shown in blue.*

*4.3 Yearly Analysis*

Number of dives and coral treatments varied by project year (Figure 9). Year A (May 2019 – April 2020) had the fewest number of dives (99) but most coral treatments (402). Similar numbers of coral treatments (262 and 277, respectively) were conducted in Year B (May 2020 – April 2021) and Year C (May 2021 – April 2022), but 32 more dives were conducted in Year C.

A negative binomial GLM showed that project year was a significant predictor of treatment density per dive (LR $\chi^2$=21.863, df=2, p=1.789 x $10^{-5}$, respectively; Figure 10). Additionally, the model showed treatment density varied significantly by project year. Treatment density was significantly higher in Project Year A than in Project Years B and C (p=0.0102 and p<0.0001, respectively; Figure 10).

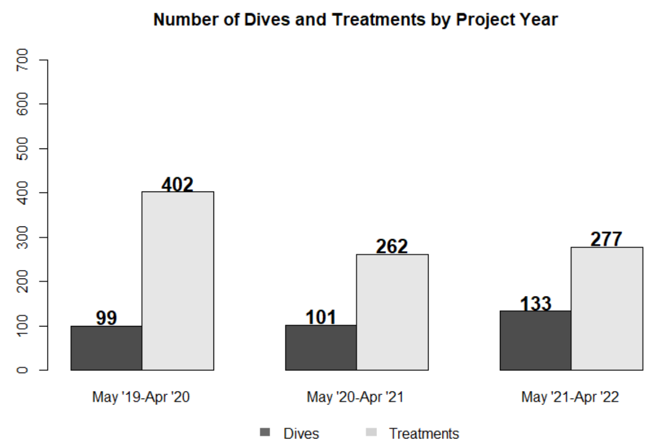


***Figure 9.*** *Yearly number of dives and coral treatments over the course of the project.*

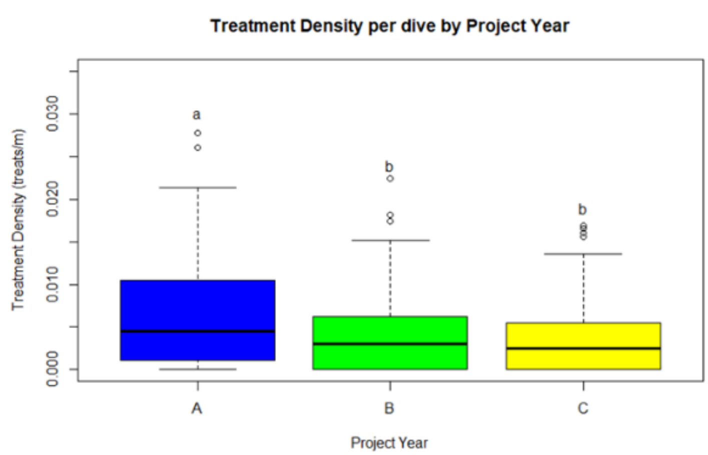


***Figure 10.*** *Treatment density per dive by project year over the course of the project. Boxes represent the interquartile interval, and the dark line represents the median of the data. Error bars represent the upper and lower quartiles of the data. Letters above the boxes represent significance. A: May 2019 - April 2020, B: May 2020 – April 2021, C: May 2021 – April 2022.*

### *4.3.1 Yearly Spatial Patterns*

Yearly Kernel Density estimations of treated coral locations showed differences in the locations of treated corals. Project Year A showed the highest density of treatments in Port Everglades South, Haulover North and Hillsboro South (Figure 11a). Project Year B and C showed the highest density of treatments in Haulover South. near Haulover South (Figure 11a).

Yearly Kernel Density estimations of treatment density per dive showed drastic variations in density patterns through time (Figure 11b). Project Year A had the most treated corals per meter in Haulover North and Port Everglades South. Project Year B did not show much of a spatial pattern at all between dives, despite each region having varying dive effort. Project Year C showed higher treatment density in Haulover South and Port Everglades South. Interestingly lots of Year C dives in Hillsboro South yielded low treatment densities unlike Year A.

A negative binomial GLM was conducted using local coastal regions, project years, and coral treatments with dive distance as a logarithmic transformed offset factor. Local Coastal Region and project year were identified as significant predictors of treatment density per dive (LR $\chi^2$= 54.266, df= 7, p= 2.083 x $10^{-9}$ and LR $\chi^2$= 22.422, df= 2, p= 1.352 x $10^{-5}$, respectively, Figure 12). Haulover South was the only region with significant effects across all three years (p <0.0001, p= 0.001, p=0.0162). Haulover North, Hillsboro South, and Port Everglades North had significant effects in Year A (p<0.0001, p<0.0001, and p=0.0225, respectively). Government Cut North, Haulover North, and Hillsboro South had significant effects in Year B (p=0.0012, p=0.0162, and p=0.0162, respectively). Government Cut North had significant effects in Year C (p= 0.0001).

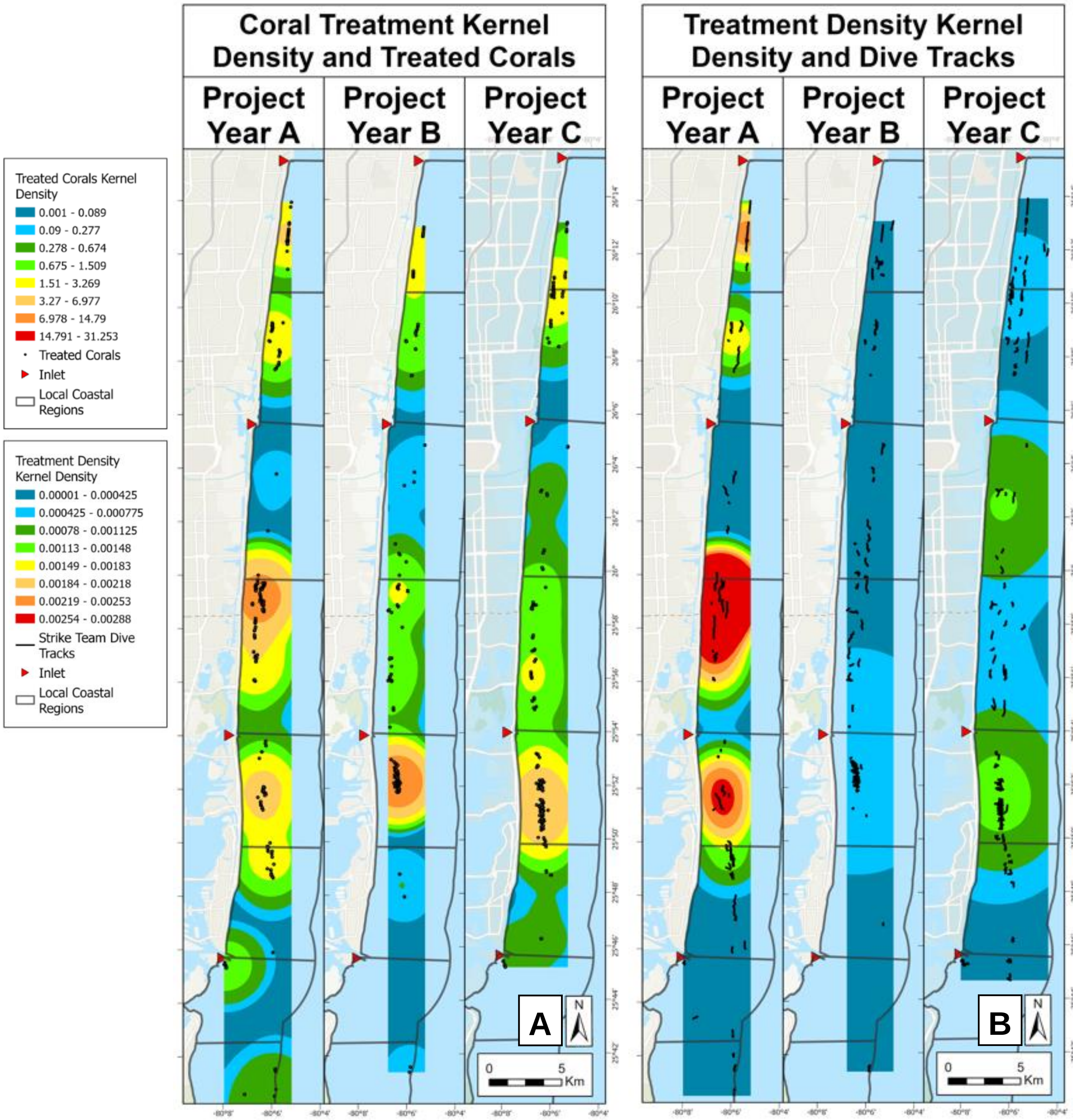


***Figure 11.*** *(a) Treated Corals Kernel Density Estimation run independently for each project year. (b) Treatment density per dive Kernel Density Estimation run independently for each project year. A: May 2019 – April 2020, B: May 2020 – April 2021, C: May 2021 – April 2022.*

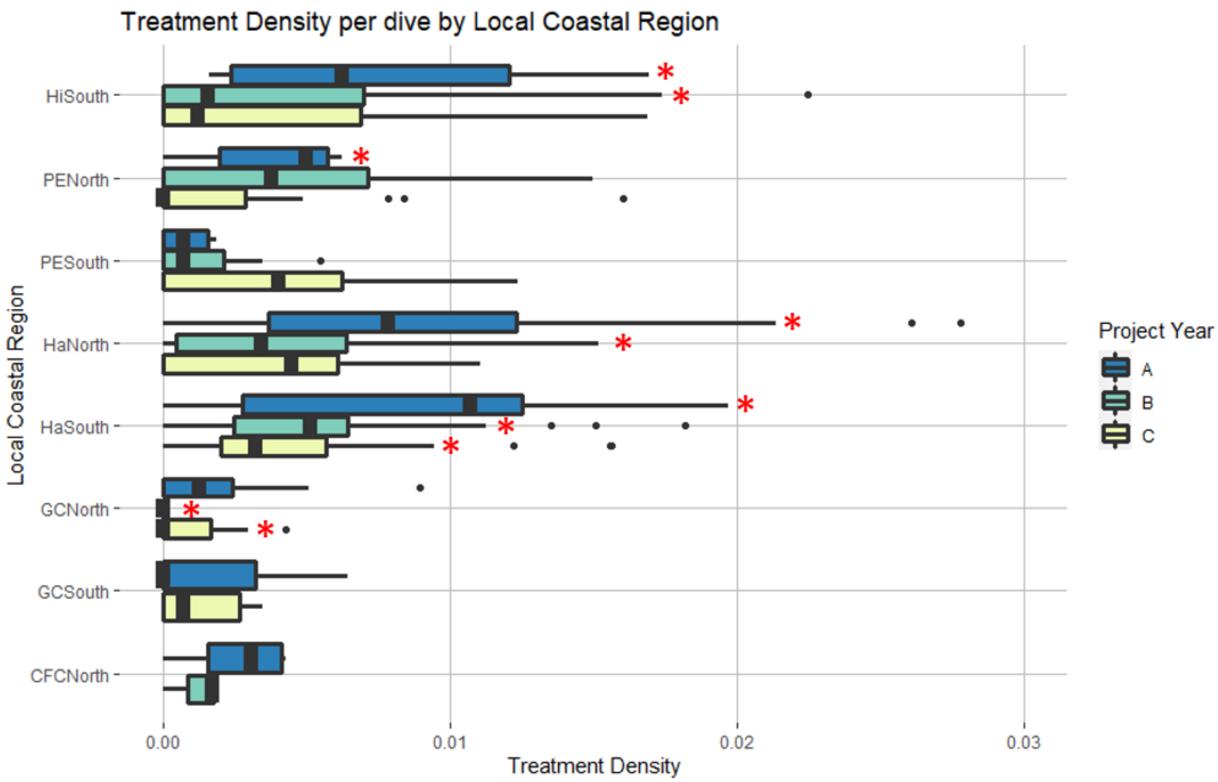


***Figure 12***. *Local Coastal Region and project year were identified as significant predictors of treatment density per dive. Red asterisks represent levels that had significant effects on the model. Haulover South was the only region with significant effects across all three years. A: May 2019 – April 2020, B: May 2020 – April 2021, C: May 2021 – April 2022.*

*4.4 Seasonal Analysis*

Seasonal dives and coral treatments varied over the project duration (Figure 13). The wet season had a higher number of coral treatments (673) than the dry season (268). However, only 1.6 times more dives occurred in the wet season (206) compared to the dry season (127). Treatment density significantly differed by season. A negative binomial GLM showed that season was a significant predictor for treatment density per dive (LR $\chi^2$=13.893, df=1, p=1.935 x $10^{-4}$; Figure 14). Treatment density per dive was significantly higher in the wet season than in the dry season (p= 0.0001). Season explained the variance in treatment density and had the same relative effect each year of the project. A negative binomial GLM showed that season and project year were significant predictors of treatment density ($\chi^2$=19.662, df=1, p=9.240 x $10^{-6}$ and $\chi^2$=26.243, df=2, p=2.002 x $10^{-6}$, respectively, Figure 15). The wet season was significantly higher than the dry season each year (A: p=0.0002, B: p=0.0002, C: p=0.0002, Figure 15).

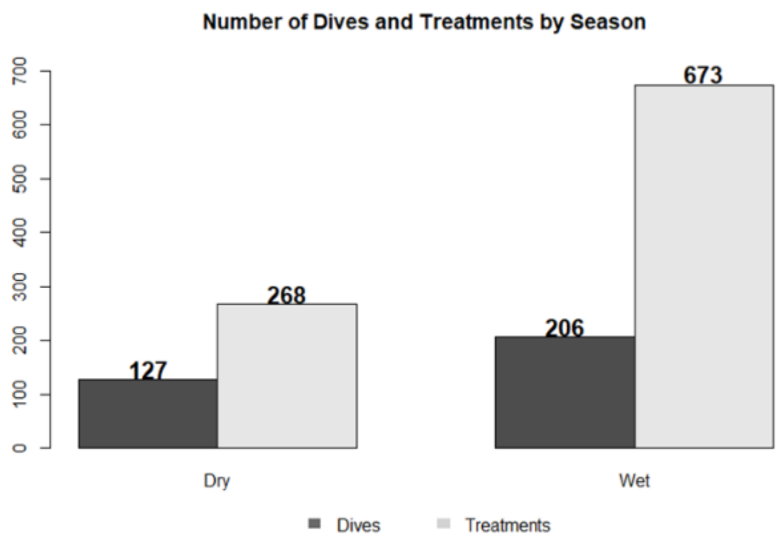


***Figure 13.*** *Total seasonal number of dives and coral treatments over the course of the project.*

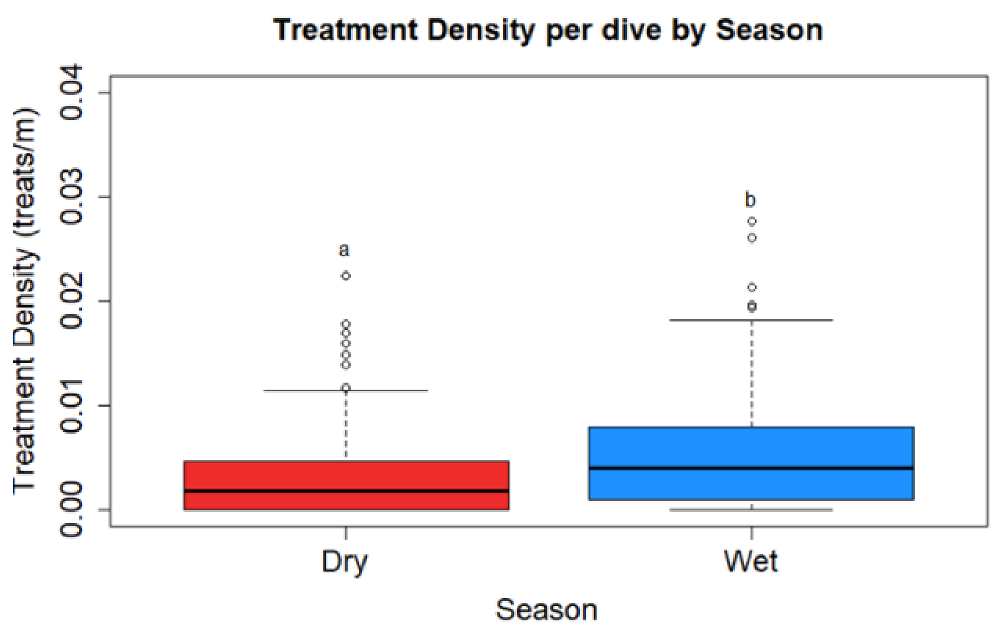


***Figure 14.*** *Treatment density per dive by season over the course of the project. Boxes represent the interquartile interval, and the dark line represents the median of the data. Error bars represent the upper and lower quartiles of the data. Points to the right represent outliers of the data. Letters above the boxes represent significance.*

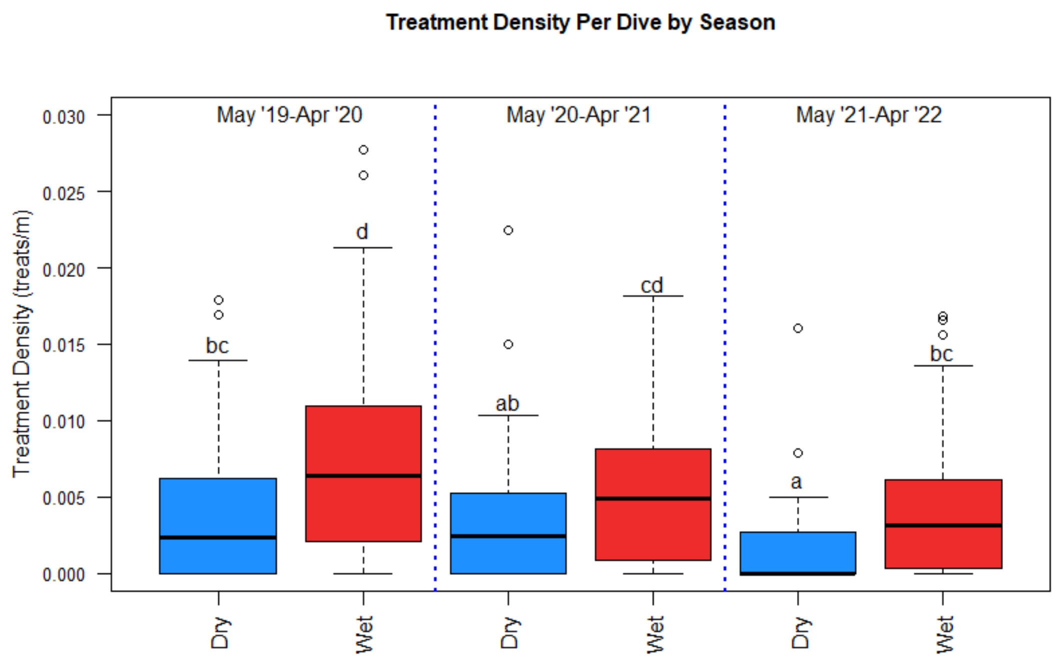


***Figure 15.*** *Seasonal treatment density per dive throughout each project year. Boxes represent the interquartile interval, and the dark line represents the median of the data. Error bars represent the upper and lower quartiles of the data. Points above represent outliers of the data. Letters above boxes represent statistical significance.*

Seasonal differences in treatment density by local coastal regions were also significant. A negative binomial GLM was conducted using local coastal regions, season, and coral treatments with dive distance as a logarithmic transformed offset factor. Local Coastal Region and Season were identified as significant predictors of treatment density per dive (LR $\chi^2$ = 44.279, df = 7, p = 1.887 x $10^{-7}$ and LR $\chi^2$ = 11.271, df = 1, p = 7.872 x $10^{-4}$, respectively, Figure 16). Hillsboro South and Haulover North were the only regions with significant effects across both seasons, wet and dry (p <0.0001, p=0.0421, and p= 0.0187, p<0.0001, respectively). Haulover South and Port Everglades North had significant effects in the wet season (p<0.0001 and p=0.0421). Government Cut North, Government Cut South, and Port Everglades South had significant effects in the dry season (p=0.0020, p=0.0421, and p=0.0187).

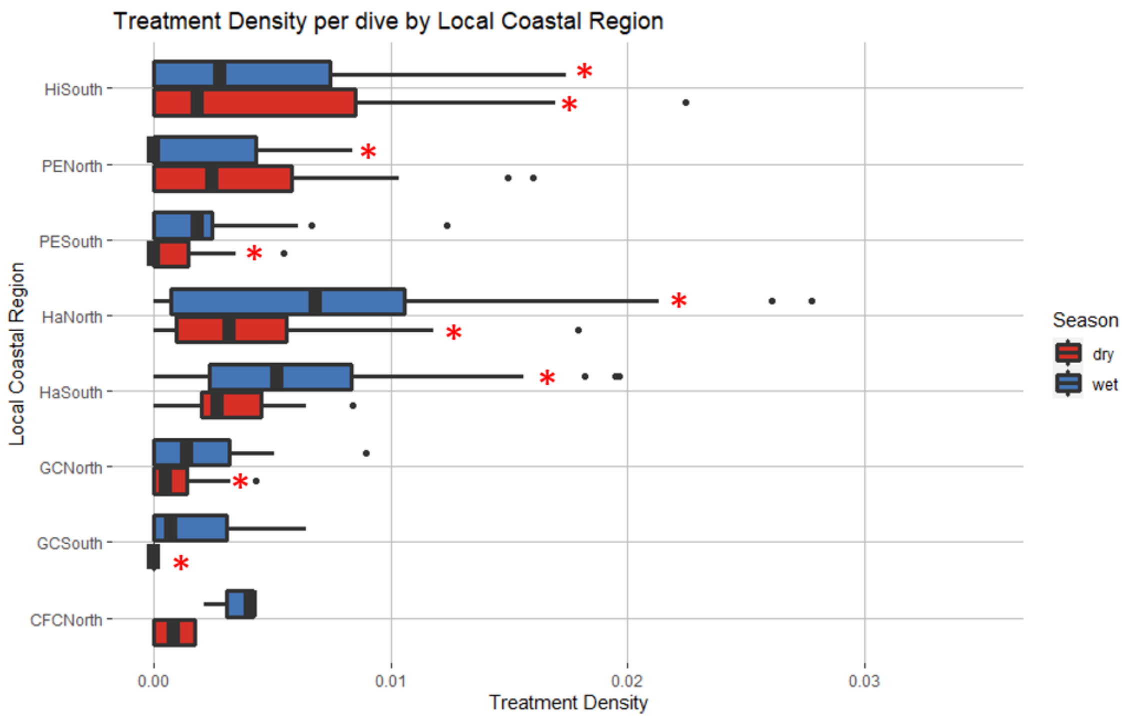


***Figure 16****.Local Coastal Region and Season were identified as significant predictors of treatment density. Red asterisks represent levels that had significant effects on the model. Hillsboro South and Haulover North were the only regions with significant effects across both seasons. (Wet: May to October, Dry: November to April).*

*4.5 Monthly Analysis*

The number of dives and coral treatments varied by month over the course of the project (Figure 17). July and June had the most coral treatments, with 153 and 126, respectively. The fewest number of treatments occurred during November and December with 21 and 13, respectively. April and March had the highest number of dives with 41 and 40, respectively. However, May, June, July, and September all had values between 36 and 39 dives. The fewest dives occurred in November, December, and January, with nine dives each. Month was a significant predictor of treatment density per dive as shown by a negative binomial GLM (LR $\chi^2$=47.262, df=11, p=1.932 x $10^{-6}$). April, October, and November had significant effects (z=-4.067, p=4.75 x $10^{-5}$; z=-2.072, p=0.03830; z=-2.755, p=0.00587, respectively). Although the highest total number of dives were in April, treatment density was significantly lower than that of January, February, March, May, June, July, August, and September (p=0.0028, p=0.0341, p=0.0382, p=0.0001, p =0.0023, p <0.0001, p=0.0005, p=0.0001, respectively; Figure 18). Monthly treatment density per dive over each project year could not be statistically tested due to low sampling in certain months (n<5).

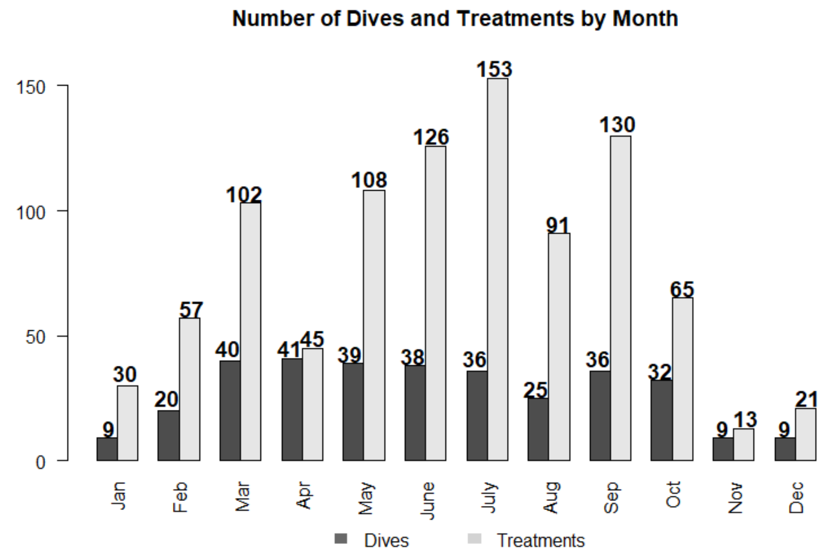


***Figure 17.*** *Monthly number of dives and coral treatments over the course of the project.*

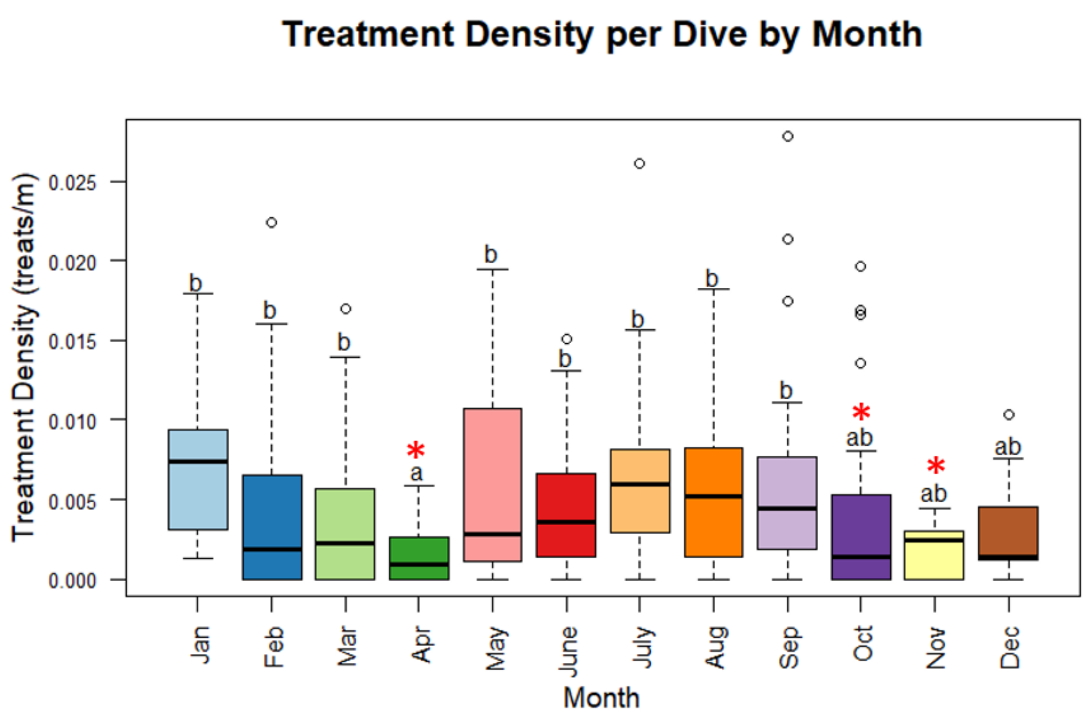


***Figure 18.*** *Treatment density per dive by month over the course of the project. Boxes represent the interquartile interval, and the dark line represents the median of the data. Error bars represent the upper and lower quartiles of the data. Points to the right represent outliers of the data. Letters above the boxes represent significance. Red asterisks represent levels that had significant effects on the model.*

### *4.6. Montastraea cavernosa Disease Prevalence and Coral Density*

#### *4.6.1 Disease Prevalence using Data from May '21 to April '22*

Disease prevalence was only measured in the last year of the Project (May '21 - April '22). Disease prevalence was calculated by dividing the number of diseased individuals by the number of total individuals (healthy and diseased) seen throughout the dive. The values were then multiplied by 100 to produce a percentage. The significant regional pattern identified within the treatment density per dive data suggested regional analysis be conducted on disease prevalence. A negative binomial GLM conducted using the diseased individual counts by local coastal regions with total individual counts as a logarithmic transformed offset factor showed that disease prevalence did not vary significantly by local coastal region (LR $\chi2 = 9.3718$, df = 6, p = 0.1537, Figure 19).

Seasonal analysis was conducted on disease prevalence data collected during the last year of the project (May 2021 to April 2022). A negative binomial GLM conducted using the diseased individual counts by season with total individual counts as a logarithmic transformed offset factor showed that disease prevalence did not vary significantly by season (LR $\chi2 = 1.672$, df = 1, p = 0.196, Figure 20).

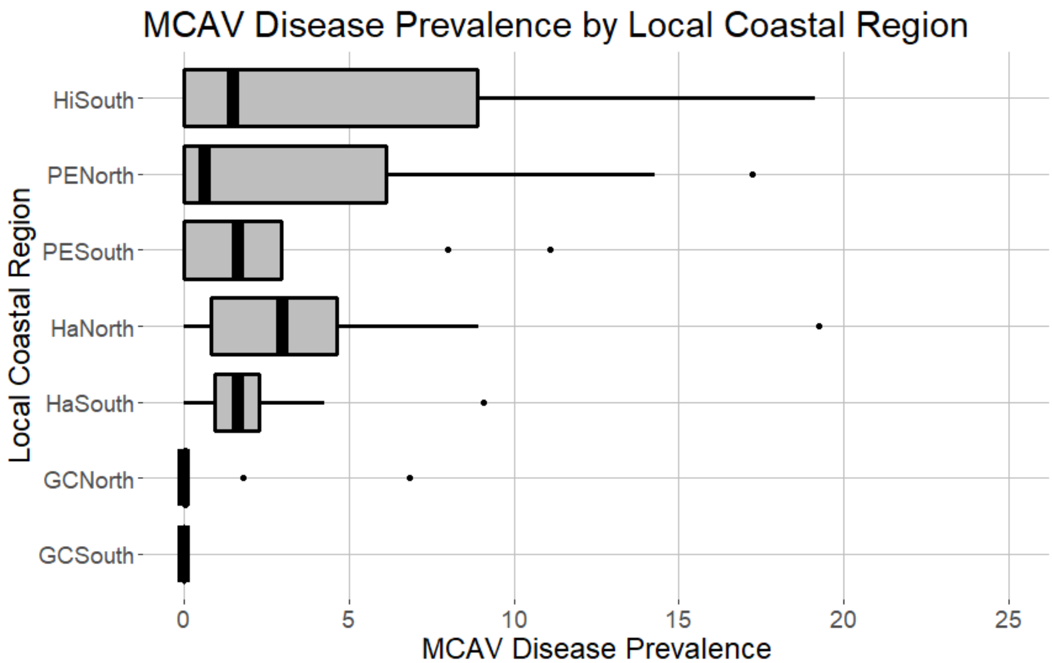


***Figure 19.*** *Montastrea cavernosa (MCAV) disease prevalence varied by Local Coastal Regions during the last year of the project (May '21 to Apr '22). Boxes represent the interquartile interval, and the dark line represents the median of the data. Error bars represent the upper and lower quartiles of the data. Points to the right of the boxes represent outliers of the data.*

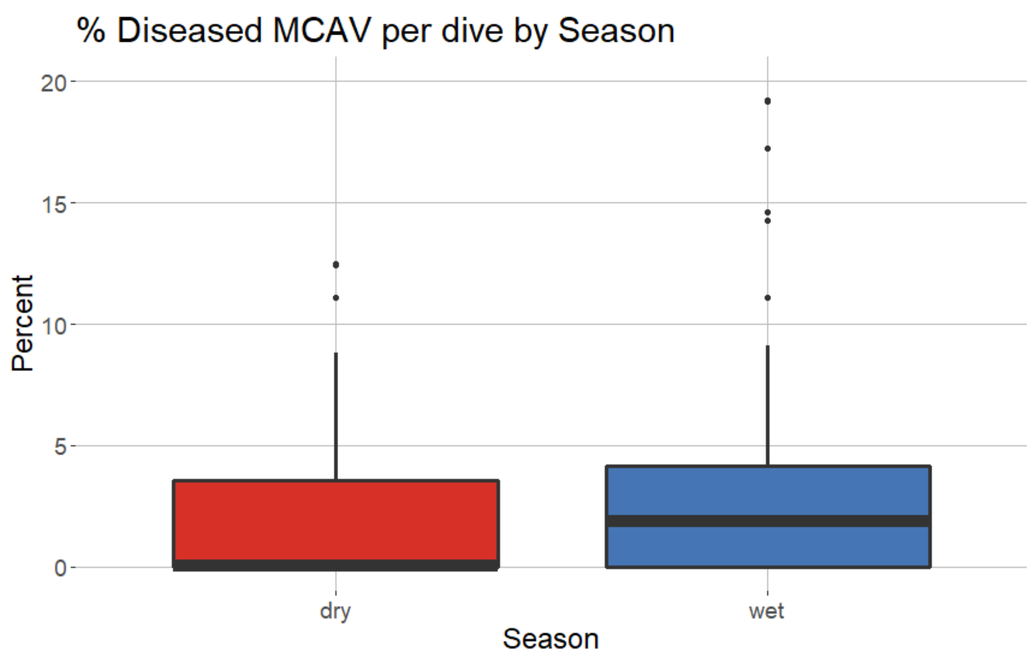


***Figure 20.*** *Montastraea cavernosa (MCAV) disease prevalence by season, wet and dry, during the last year of the project (May '21 to April '22). Boxes represent the interquartile interval, and the dark line represents the median of the data. Error bars represent the upper and lower quartiles of the data. Points above the boxes represent outliers of the data.*

*4.6.2 Coral Density using Data from May '21 to April '22*

*Montastraea cavernosa* (MCAV) coral density (total number of MCAV individuals, healthy and diseased, divided by distance covered) was significant when tested against the LCRs using a negative binomial GLM (LR $\chi^2$= 14.039, df = 6, p = 0.0292, Figure 21). Haulover South had significant effects (t.ratio=3.366, p=0.0081). Government Cut: North had significantly lower MCAV coral density than Baker's Haulover Inlet: South (t.ratio=-3.045, p= 0.0471).

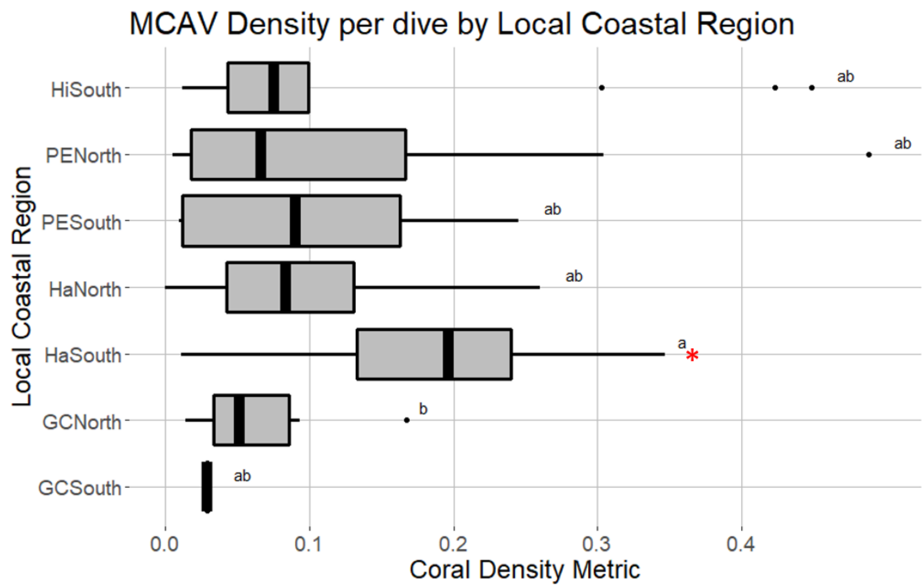


***Figure 21***. *Montastraea caverosa (MCAV) density (diseased + healthy individuals/distance covered (m)) per dive by LCRs over the last year of the project (May '21 to Apr '22). Boxes represent the interquartile interval, and the dark line represents the median of the data. Error bars represent the upper and lower quartiles of the data. Points to the right represent outliers of the data. Letters right of boxes represent statistical significance. Red asterisks represent levels that had significant effects on the model.*

*4.6.3 Treatment Density and MCAV Density*

To determine the relationship between treatment density per dive and MCAV density per dive, a Kolmogorov- Smirnov test was done to compare the distributions of treatment density and coral density. A Kolmogorov-Smirnov test showed that MCAV coral density and treatment density were not drawn from the same distribution (D= 0.86617, p< 2.2 x $10^{-16}$, Figure 22). Additionally, a negative binomial GLM using diseased individual counts and total individual counts, and the distance covered as a logarithmic transformed offset factor, allowing for comparison of treatment density, diseased individuals divided by distance covered, and MCAV density, total individuals divided by distance covered to be compared. The negative binomial GLM showed that MCAV coral density was a significant predictor of treatment density per dive (z=2.36, p=0.0183, Figure 23). However, the model does not fit the data well and has low predictive power as shown by the calculated McFadden's $R^2$ value (0.046).

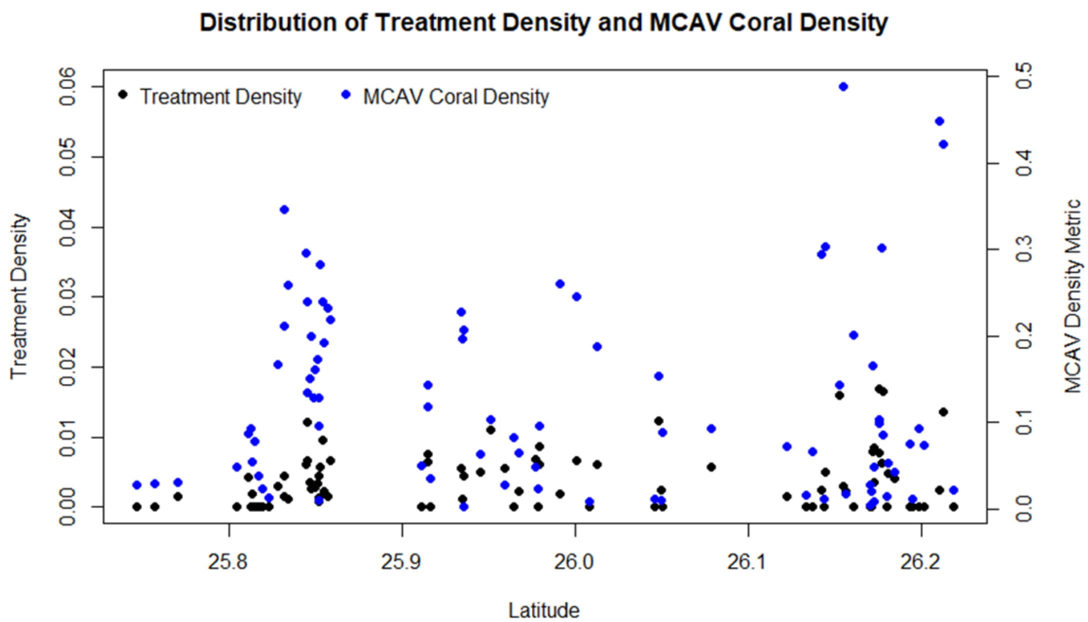


***Figure 22.*** *Treatment density per dive and Montastraea cavernosa (MCAV) density per dive during Year C (May '21 to April '22).*

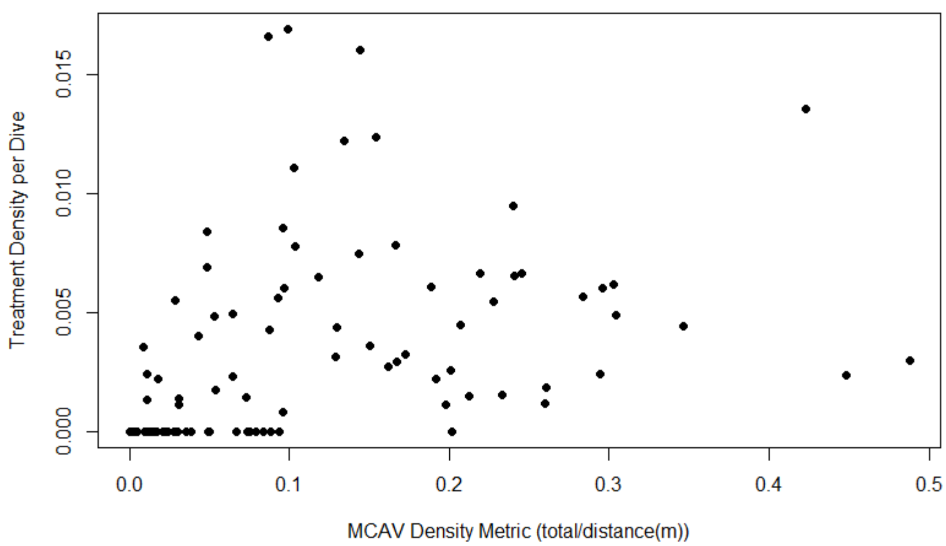


***Figure 23.*** *Treatment density per dive compared with Montastraea cavernosa density per dive during the last project year (May '21 to April '22).*

## 5.0 DISCUSSION

Reef-building corals are crucial to the long-term existence of Caribbean coral reef ecosystems, which provide direct and indirect, local and global, ecological, economic, and social benefits (Lirman et al. 2018; Moberg and Folke 1999). SCTLD is now endemic in the Coral ECA due to the disease being present since 2014, as it continues to kill reef building corals, and the data herein show high annual number of colonies needing SCTLD treatments. Disease interventions using CoralCure are highly effective at stopping lesions and less costly than post hoc restoration, therefore, broadscale disease intervention activities in the Coral ECA should progress as long as SCTLD persists. These disease interventions require extensive labor and field work because divers must find and identify colonies presenting with lesions on the reef and treat them individually (Neely et al. 2021b, Walker et al. 2021c). This study provides data analyses to inform future efficiencies in those operations. Broadscale intervention treatment density exhibited distinct seasonal and spatial patterns throughout each year, suggesting future activities should prioritize certain areas and times of the year over others. Currently, SCTLD is spreading unabated throughout many Caribbean and Mesoamerican locales threatening the future of Caribbean coral reefs (Kramer et al. 2019; Lee Hing et al. 2022; Walker et al. 2021c). The information provided herein assist in planning disease intervention response in other locales.

*In situ* disease intervention efficiency ultimately depends on the proximity of disease corals from each other. High density coral reefs naïve to a new disease, in the context of the highest susceptible individuals having yet to be removed by disease-related mortality, may result in a high number of disease corals in close proximity, making treatment dives highly efficient (high density of treated corals per search area). However, once the disease moves through the highly susceptible species, it leaves the less susceptible species behind (Sharp et al. 2020). This leads to disease being more spatially and temporally irregular, making disease intervention efforts less efficient and more costly (Walker et al. 2021c). A prime example is SCTLD in the Coral ECA and Florida Keys, where the Coral ECA disease exposure was three years prior to the lower Keys, resulting in the disease being endemic to the Coral ECA region but not to the Keys, at the time of disease interventions in this study. Between January 2019 and August 2022, 3,765 corals were treated in a survey area of ~720,500 $m^2$ within the Florida Keys National Marine Sanctuary under the guidance of Dr. Karen Neely. However, much of the dive effort was dedicated to monitoring and revisiting previously diseased corals. Conversely, during disease intervention activities included in this study ~1,100 colonies have been treated at over 280 sites covering ~400,000 $m^2$ in the Coral ECA. Additionally, divers in the FKNMS would typically treat, on average, 25-50 corals per dive, while divers would only conduct 2-5 treatments per dive, on average, in the Coral ECA. The likely cause of this disparity is due to differences in the time since disease exposure, the prevalence of highly susceptible coral species, and varying host densities (Sharp et al. 2020). These factors greatly affect treatment density and, therefore, efficiency by decreasing the distance needed to travel and search between diseased individuals. The additional three years of disease in the endemic Coral ECA have reduced the density of susceptible colonies, shifted population demographics, and reduced live tissue cover by 59% at long-term monitoring sites (Hayes et al. 2022).

This study found that a larger proportion of disease intervention effort should be prioritized during the wet season (and possibly after heavy rainfalls or large managed water releases). Climatology indicates that Florida has two seasons (Davis and Ogden 1994), the dry season (Nov-Apr) and the wet season (May-Oct). Walker et al. (2022) have reported seasonal variations of SCTLD in large Coral ECA *Orbicella faveolata* colonies. This study found that treatment density per dive and number of treatments per dive were significantly higher in the hotter wet season each year. Treatment density per dive was lowest in April, at the end of the dry season, and highest in January, March, and throughout May-September, mainly during the wet season. However, the high number of total treatments conducted during June, July, and September suggest the increased need of disease intervention during the wet season. However, a proportion of disease intervention activity should still be conducted in the dry season since disease remains year-round and corals can die in a matter of weeks (Walker et al. 2021b, Neely et al. 2021b). However, this might be more efficient by conducting many spot checks over broader areas instead of long local dives. Despite having the highest number of dives being conducted, April had the lowest treatment density throughout the project. Disease intervention efforts should be minimized in April in southeast Florida.

High coral density areas should be identified and revisited proportionally more often. Higher coral disease prevalence can be associated with higher host density (Bruno et al. 2007; Hayes et al. 2022; Walton et al. 2018). As the reef becomes sparser due to coral mortality, it may be more efficient to visit areas of high coral density because sites with high coral densities allow more colonies to be assessed over a dive. Interventions at high-density sites may increase the likelihood of successful natural reproduction by attempting to save corals already located nearby one another. This is especially important at restoration sites where corals are being planted at densities higher than the natural surrounding areas, such as spawning hubs. Spawning hubs are areas where corals of targeted restoration species are brought in close spatial proximity to increase local densities with the hopes of increasing fertilization success and natural reef recovery (Gilliam et al. 2021). It is unknown how this activity affects disease dynamics; however, since disease prevalence is often related to host density, disease interventions are necessary to attempt to reduce local disease loads and save the live tissue of relocated corals at these sites.

Regular disease intervention dives should be conducted proportionally throughout the Local Coastal Regions. Haulover North and Haulover South had the highest treatment densities and thus should receive the highest proportion of effort. No region, however, should be excluded from periodic disease intervention dives. Coastal ecosystems continue to be threatened directly and indirectly. Human activities have exacerbated the decline in water quality through coastal urbanization and poor management strategies and have led to an increase in on-reef nutrient levels, intensifying risks to coral health (Walker et al. 2021c; Whitall et al. 2019). The majority of coral reefs in Southeast Florida (SEFL) occur within three miles of the coast (Walker 2012), making them highly influenced by coastal activities such as beach restoration (Jordan 2010), dredging and shipping (Walker 2012), runoff from land, and discharge from inlets and outflow pipes (Wear and Thurber 2015). Additionally, anthropogenically induced water quality decline has been linked to increased coral disease prevalence (Bruno et al. 2003). Coastal urbanization and water management influence the number of coral disease lesions and disease corals in the Coral ECA by the amount of water flowing out of the inlets (Walker et al. 2021c). Walker et al (2021c) reported temperature stress, inlet flow, and total rainfall explained 56.4% of the temporal variation in the total number of SCTLD infections over time. Treatment density per dive was not correlated with latitude, indicating that disease differences were not dimply due to latitudinal variation. Disease intervention treatment density was highest in the Baker’s Haulover North, Baker’s Haulover South, and Hillsboro South regions. Muller et al. (2020) conducted a study modelling the spatial epidemiological probability of the spread of SCTLD and found peaks near Baker’s Haulover Inlet and Hillsboro Inlet in 2016. However, the study only estimated values through 2017. This study found the highest treatment densities in Haulover North, Haulover South and Hillsboro South during the wet season. The association of higher disease near certain inlets may be an indicator of the relationship of coral disease to inlet flows found in Walker et al. (2021c).

Coral treatments and treatment densities decreased throughout the project where Year A had higher values of coral treatments with fewer dives than Years B and C and more dives were needed in Year C to match the number of treatments in Year B. This indicates that disease prevalence decreased during the project. However, it is unknown if this is a result of disease interventions reducing disease, a decline in the conditions that cause disease, or the mortality of susceptible colonies.

Broadscale intervention stops disease lesions which may decrease local pathogen load and the likelihood of transmission to nearby colonies. Treatment density decreased dramatically after the initial year of intervention activity as shown by the increased effort required to treat the same number of corals. The initial high treatment values may have been because the disease had spread unabated for approximately four years prior to intervention. Returning to previous locations of higher treatment density did not yield the same treatment density or number of treatments. This is evident in the annual kernel density estimations where high areas in Year A dissipated throughout the broadscale interventions in Years B and C.

Alternatively, natural processes could lead to decreases in disease prevalence. Firstly, the source of disease remains unknown and therefore undetectable. Disease abundance or virulence might fluctuate through space and time with varying environmental factors. Without knowing the agent of disease, we can only speculate about disease exposures. Second, the mortality of susceptible individuals affects prevalence (Walker in prep). The associated modeling study utilizes a four-year database to show significant decreases in disease prevalence estimates following the mortality of susceptible large Coral ECA colonies (Walker in prep). The death of colonies could leave the less susceptible individuals remaining, resulting in less disease prevalence. However, while controlling for susceptibility by keeping all colonies alive, Walker et al (2022) found no indication of reduced disease incidence in the large coral population (107 individuals) by year over the same period. This dataset was dominated by *Orbicella faveolata* but may be indicative of *Montastraea cavernosa* as well.

Lastly, utilizing other available datasets to inform site selection of activities is highly recommended when possible. SCTLD is still widely present and active in the Coral ECA, but it is recorded more frequently at sites in shallow nearshore habitats (Walker et al. 2021a). Regional National Coral Reef Monitoring Program data from 2020 and monthly large coral monitoring indicated that the majority of SCTLD incidence in the ECA occurred in shallow, nearshore habitats with few records of disease in middle and outer reef habitats (Walker et al. 2021a). This led to most strike team disease interventions occurring in shallow habitats (<10m depth). Without this information, broadscale intervention efficiencies would have been much lower as dives would have been executed along all three reef lines of SEFL to depths of up to ~30m.

## 6.0 RECOMMENDATIONS

This project found that dive effort increased in order to treat the same number of corals each year. By implementing the recommendations herein, it is expected that fewer dives will be needed to find and treat a comparable number of corals. Based on this study, the NSU GIS and Spatial Ecology Lab and its disease intervention partners should plan to conduct ~100 disease intervention dives per year, proportioned to the aforementioned categories in the discussion above. In addition, I have provided other recommendations below.

### *6.1 Potential Data Collection Modifications*

Data collection methods should be modified to allow researchers to better understand disease patterns and drivers with Florida's Coral Reef. In addition to the metrics previously recorded, categories could be added to represent dive conditions. A metric of relative visibility; recorded by estimated distance or categorized (little to none, moderate, and very good) will allow researchers to associate the data to dive conditions to understand any possible effects. Additionally, data collected for each coral should be labeled with the number of lesions and relative lesion progression rate, fast or slow. The addition of this information will allow better connections between disease prevalence and possible drivers of disease.

New areas of high coral density should continue to be located through random disease intervention activities, as time allows. Information from other projects, such as Large Coral Monitoring and NCRMP, should be used to increase the chances of divers finding high-density reef sites and sites with an increased prevalence of highly susceptible species. By systematically identifying, labeling, and mapping reef sites by relative density (low, medium, high), previous visitation status (never, old: >2 years, recent: <2 years), and species breakdown (target (mainly MCAV and OFAV) vs non-target (other spp.)), teams can make better informed decisions when choosing dive locations. This information can be highly beneficial in future strike team disease intervention activities as disease dynamics change or for developing other research activities needing to target reefs with specific characteristics. Additionally, previously identified areas of low coral or treatment density should be visited periodically to ensure that a resurgence of disease prevalence has not occurred. Revisitation of previously visited sites will ensure that the disease prevalence in the area is monitored, and local disease loads can be regulated.

Communication with local divers and dive shops could help to better understand disease at certain locations. Local dive shops often frequent a set of named, popular dive sites along the reef and therefore have a better ability to detect disease outbreaks as they occur and notify researchers. In addition to gaining valuable information from locals, researchers should continue to collaborate and extend any helpful information to locating high-density reefs or areas heavily impacted by the disease.

Prioritizing intervention dives during the wet season months while still conducting some dives during the dry season months, as disease is recorded at some level throughout the entire year, is a recommended practice. However, as discussed earlier, the reef composition and structure vary greatly in the Florida Keys compared the reef within the Coral ECA. This presents some added challenges when constructing recommendations likely to be beneficial to the Florida Keys regions.

With a myriad of information from locals, stakeholders, and researchers, a database should be created to identify sites with high coral density and other reef characteristics. Within this database, information about site relative historical disease prevalence, species richness, and relative density of highly and moderately susceptible species will be stored and able to be used for future disease intervention or other research studies. This database can then be updated with site visit information, including the presence of highly susceptible species or corals exhibiting disease lesions. This database will then be used to systematically determine sites in GIS, allowing divers to monitor a greater diversity of reefscapes and cover a greater span within the Coral ECA.

*6.2 Recommendations for Disease Interventions in SCTLD non-endemic regions*

Since 2017, SCTLD has spread throughout the Caribbean and is presently seen as far south as Grenada (Kramer et al. 2019). SCLTD has yet to be confirmed in numerous southern Caribbean locales including Bonaire, Aruba, and Barbados, and Curacao (Kramer et al. 2019), but white syndrome-like lesions have been appearing among reefs in these regions. It is crucial to clearly identify SCTLD and differentiate it from other white tissue loss diseases seen throughout the Caribbean region since 1975 (Cróquer et al. 2021). Once SCTLD has been confirmed, it is important to deploy disease intervention teams to assess the impact and conduct treatments as soon as possible after the outbreak is recognized. Additionally, prior to the disease being realized, locations likely to be impacted by the disease should prepare management and action plans to allow for swift response. Create or utilize existing outreach programs to educate locals and visitors on the signs and symptoms of SCTLD, bridging the gap between scientists and recreationalists, and allowing for a more consistent flow of information.

The findings in this study can help to educate and guide future disease intervention activities to increase intervention efficiency once SCTLD becomes spatially and temporally irregular. If possible, action plans should continue to be created for locales where SCTLD has yet to be identified or areas beginning to experience the disease outbreak. Action plans have been made by NOAA and various governing bodies, and implemented in the Mexican Caribbean, Puerto Rico, and the U.S. Virgin Islands, and can provide valuable advice and guidance for locales newly impacted with SCLTD. Once the disease is present, effective and adaptive management is necessary to limit the impacts of SCTLD on coral reefs. Management teams should consult researchers, local stakeholders, and intervention practitioners to best develop action plans in the wake of SCTLD. The increasing incidence of coral disease outbreaks due to climate warming (Harvell et al. 2004) establishes the urgency for disease response plan creation and management. Maynard et al. (2009) suggest creating a plan with three components: an early warning system, assessment and monitoring, and communication. Beeden et al. (2012) add to this response plan by incorporating prioritization of management investments and adaptive management, allowing for the development of new techniques and conserving resources where applicable.

By designing disease intervention activities more efficiently, resources can be saved and used elsewhere. Continued research is necessary to fully identify the disease-causing agent and specific stressors leading to the development of disease lesions to develop better treatment methods specific to stony coral tissue loss disease. The activities completed under the strike teams throughout Southeast Florida have saved significant amounts of coral tissue and provided optimistic results for the health of current and future Caribbean coral reefs.

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