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# Short- and Long-Term Effectiveness of Coral Disease Treatments

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Since 2014, stony coral tissue loss disease (SCTLD) has led to large-scale mortality of over 20 coral species throughout the Florida Reef Tract. In 2019, in-water disease intervention strategies were implemented to treat affected corals. Two treatment strategies were employed: (1) topical application of an amoxicillin paste directly to disease margins, and (2) application of a chlorinated epoxy to disease margins as well as an adjacent “disease break” trench. Effectiveness of treatments on 2,379 lesions from 725 corals representing five species was evaluated using mixed effects logistic regression models which demonstrated substantially greater effectiveness of amoxicillin compared to chlorine-treated lesions across all species up to 3 months post-treatment. As a result of the failed chlorinated epoxy treatments, any new lesions that appeared during subsequent monitoring events were treated with amoxicillin paste, and all corals were monitored and treated as needed approximately every 2 months for up to 24 months. The health status of 1664 amoxicillin-treated corals during each monitoring event was used to model the probability of a coral being uninfected over time. Models included species and geographic regions as variables. The appearance of new lesions (reinfection rates) varied by species, and offshore sites showed greater reinfection rates than inshore sites; however, all sites and species exhibited a decreased probability of reinfection with time since initial treatment. We conclude that topical amoxicillin treatments are highly effective at halting SCTLD lesions and that through initial and follow-up treatments as needed, colonies and reef sites will progress toward a lower prevalence of SCTLD.

**Keywords:** stony coral tissue loss disease, disease treatment, antibiotics, chlorinated epoxy, mixed effects logistic regression model

## INTRODUCTION

Coral diseases are found on reefs throughout the world and are one of the most rapid and prevalent sources of coral mortality, even among the myriad stressors that are driving the decline of reefs. The first report of coral disease was published in Squires (1965). Since then, diseases have been documented in over 100 coral species and over 50 different nations (Green and Bruckner, 2000; Bruckner, 2016). Even though disease is traditionally present in ecosystems, elevated prevalence and virulence has resulted in elevated “background” rates as well as increasingly common outbreaks that can affect coral cover and species diversity (Cróquer et al., 2005). Coral disease has led to substantial changes in reef communities, from localized declines in various species (Cróquer et al., 2005) to the widespread loss of over 90% of acroporid corals throughout the Caribbean (Aronson and Precht, 2001).

Many coral diseases have correlations with environmental stressors. For example, some diseases have peak prevalence during and following periods of thermal stress; these include white pox (Patterson et al., 2002), yellow band (Cervino et al., 2004; Cróquer and Weil, 2009), white plague (Cróquer and Weil, 2009), black band disease (Boyett et al., 2007; Lewis et al., 2017), and potentially white band disease (Randall and van Woessik, 2015). Water quality is also likely to play a role in overall disease prevalence; white pox has been tied to human fecal matter (Patterson et al., 2002), elevated nutrients have been shown to increase the prevalence and severity of dark spot disease (Thurber et al., 2014), and white plague outbreaks in the lower Florida Keys have correlated with periods of enriched nitrogen loading (Lapointe et al., 2019). Projections indicate that disease-conducive conditions are increasing rapidly and that disease is likely to be just as significant a driver of coral decline as bleaching (Maynard et al., 2015).

Despite this, successful identification of coral disease pathogens continues to be challenging (Richardson, 1998), and potential treatment options are rare. Some coral disease treatments have been conducted for experimental and diagnostic purposes. These have ranged across seven different coral diseases with varying levels of success (Figure 1). Treatment types can be classified as mechanical (e.g., removal of diseased tissue, shading, smothering, and creating trenches), chemical (antiseptics or antibiotics, sometimes included within a mechanical treatment), or biological (phage therapy). Most treatments to date have involved mechanical methods such as separating diseased tissue from healthy tissue or in some way covering the disease margin (Hudson, 2000; Muller and Van Woessik, 2009; Dalton et al., 2010; Williams, 2013; Miller et al., 2014; Aeby et al., 2015; Randall et al., 2018). Results using these mechanical treatments have ranged from ineffective to relatively successful. The use of antibiotics is common in treating human and animal diseases (including heavy prophylactic use in agriculture), but their usage, even experimentally, for coral diseases has been limited. Two treatments have been used diagnostically to determine the presence of a bacterial component in white band disease (Kline and Vollmer, 2011; Sweet et al., 2014), and a third was unsuccessfully used as a conservation effort for corals with black band disease (Gil-Agudelo et al., 2004). Biological control of bacterial pathogens in corals has also been tested using phage therapy, in which a viral phage is used to target the causative agent. On Red Sea corals affected with white plague, this has been shown to slow tissue loss (Atad et al., 2012), prevent transmission (Efrony et al., 2009), and halt disease when applied prophylactically or within early stages of infection (Efrony et al., 2007, 2009).

While not all historical coral disease treatments have resulted in disease cessation, the success of some demonstrates that field treatment of coral diseases, at least on targeted colonies, is possible. Though disease outbreaks are heavily managed for humans and agricultural biomass (both plant and animal), disease outbreaks within wildlife populations are generally left untouched. Though guidelines have been suggested for management at different stages of wildlife disease outbreaks

(Langwig et al., 2015), the relatively few instances of active management of an outbreak traditionally occur in cases in which:

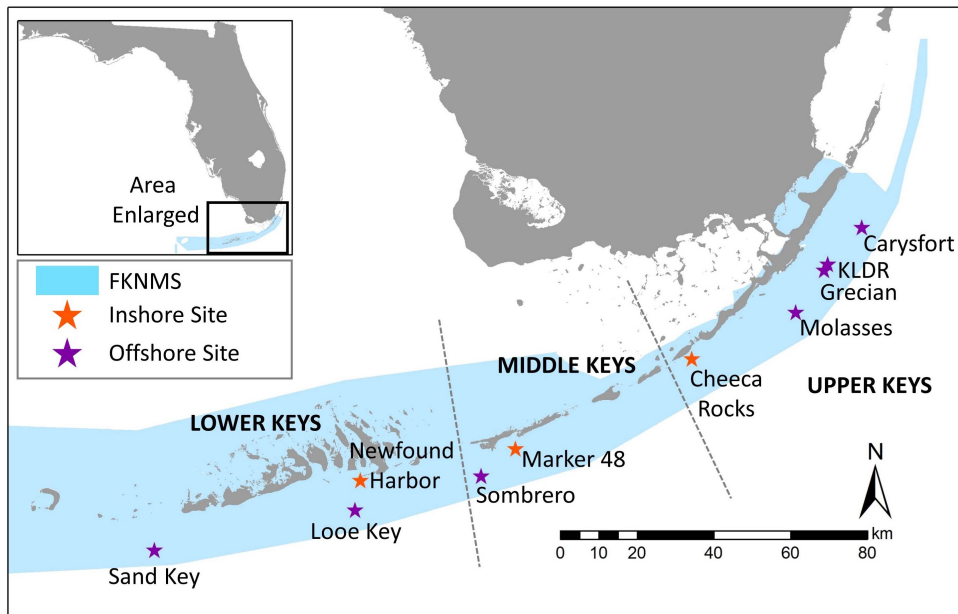
1. Human or agricultural health is threatened by the wildlife outbreak. Examples include baiting foxes with an antihelminthic to reduce risk of human infection (Tackmann et al., 2001), releasing sterile flies to control an outbreak of screwworms in Florida key deer to prevent transmission to animal stock (Skoda et al., 2018), and widespread wildlife vaccinations in Europe and North America to reduce and/or eliminate terrestrial rabies (Sterner et al., 2009; Mähl et al., 2014).
2. Ecosystem services (including harvest of a resource) are threatened. Examples of various treatment efforts include culling, chemical application, and biological control for pine beetles in American forests (Fettig et al., 2013).
3. Rare and highly managed species are at risk. Examples include California condors vaccinated against West Nile virus (Chang et al., 2007), Ethiopian and red wolves vaccinated against rabies (Harrenstien et al., 1997; Haydon et al., 2006), black-footed ferrets vaccinated against sylvatic plague (Abbott et al., 2012), arctic foxes treated with antiparasitic drugs for mange (Goltsman et al., 1996), and environmental disinfection and antifungal treatments on Mallorcan midwife toads (Bosch et al., 2015).

Stony coral tissue loss disease (SCTLD) has emerged as a major threat to Caribbean coral reefs. First documented near Miami, Florida in 2014 (Precht et al., 2016), it spread over the next 5 years throughout the majority of the Florida Reef Tract (Muller et al., 2020; Neely et al., 2021). Beginning in 2018, observations of SCTLD were documented in Mexico (Alvarez-Filip et al., 2019) and subsequently throughout other regions of the northern Caribbean (Weil et al., 2019). Work to identify the pathogen is ongoing, but transmission experiments show that infection is possible through physical contact as well as through sterile seawater (Aeby et al., 2019). Mortality rates are high, resulting in extensive loss of colonies (Precht et al., 2016) and localized near-extinction of highly susceptible species (Neely et al., 2021). Coral cover, species diversity, and colony density have all declined significantly in affected areas (Walton et al., 2018; Alvarez-Filip et al., 2019; Heres et al., 2021). Given the threat presented by SCTLD to Caribbean reefs, unprecedented actions have been taken to understand and mitigate this disease. In Florida, these include interagency steering committees, coral rescue activities, and scaled-up propagation and restoration plans. The response has also included novel and large-scale efforts to save infected colonies *in situ*.

By utilizing and modifying methodologies from the literature and expanding aquarium practices of antibiotic dosing to develop a topical application (Miller et al., 2018), two disease intervention procedures were implemented on Florida Keys reefs in an attempt to arrest active SCTLD lesions. We compared the effectiveness of chlorinated epoxy treatments and antibiotic paste treatments on halting disease lesions, and assessed the long-term infection rates of treated colonies.

		Black Band	Yellow Band	White Band	White Plague	Dark Spot	Aus. White Syndrome	Growth Anomalies
Mechanical	Transplantation			1				
	Removal						2	3
	Shading		4		5			
	Aspiration	6	4					
	Smother			1				
	Trench		4					
Chemical	Trench + Chlorine	7						
	Antibiotics			8,9		10		
Biological	Phage Therapy				11, 12, 13			

**FIGURE 1 |** Effectiveness of treatments on corals afflicted with seven diseases, as reviewed from the literature. Effectiveness is color coded as follows: green (effective), yellow (mixed results), red (ineffective). References: 1. Miller et al. (2014); 2. Dalton et al. (2010); 3. Williams (2013); 4. Randall et al. (2018); 5. Muller and Van Woesik (2009); 6. Hudson (2000); 7. Aeby et al. (2015); 8. Sweet et al. (2014); 9. Kline and Vollmer (2011); 10. Gil-Agudelo et al. (2004); 11. Efrony et al. (2007); 12. Efrony et al. (2009); 13. Atad et al. (2012).



**FIGURE 2 |** Location of coral disease treatment sites within the Florida Keys National Marine Sanctuary (FKNMS). Regions are defined as lower, middle, and upper Keys. Colors represent inshore (orange) and offshore (purple) sites.

## MATERIALS AND METHODS

### Sites and Treatments

Ten sites throughout the Florida Keys National Marine Sanctuary (FKNMS) were selected for treatment (Figure 2). Sites ranged in depth from 4 to 12 m. Most were located in no-take marine reserves; exceptions were Marker 48 as well as approximately

half of the corals at Cheeca Rocks, which straddled the boundary of a no-take area. Initial treatments occurred between January 2019 and April 2020 (Table 1). As SCTLD spread through the FKNMS in a north to south gradient as well as an offshore to inshore gradient, reefs were not all experiencing the same level of disease during initial treatments; for example, offshore sites in the upper Keys were first treated several years after the arrival

**TABLE 1** | Dates and number of corals (N) treated with amoxicillin paste and chlorinated epoxy treatments during the initial visits to each site.

Site	Treatment dates	N amoxicillin	N chlorine	# diseased corals per 100 m <sup>2</sup>
Upper Keys	January 2019	23	14	0.04
Sombrero	February 2019	61	46	0.35
Looe Key	February to April 2019	329	303	1.03
Sand Key	October 2019	23	0	N/A
Cheeca Rocks	April 2020	241	0	1.03
Marker 48	April 2020	120	0	3.04
Newfound Harbor	April 2019	160	0	1.75

of SCTLD while some inshore and southern sites were treated within a year of first appearance. Disease status during treatment of offshore sites in the upper Keys was similar, and so corals from Carysfort South, Grecian, Key Largo Dry Rocks, and Molasses are clumped for analysis. No pre-treatment prevalence data were taken, but considering that most to all of the diseased corals were treated, we used a GIS to estimate the area searched at each site and divided by the number of corals treated in order to estimate the density of diseased corals in an area (Table 1). Not all corals at Sand Key were treated and so no estimate is provided for that location.

Habitat characteristics surrounding each treatment site were analyzed in a GIS using the Unified Florida Coral Reef Tract Map v1.2 (Florida Fish and Wildlife Research Institute, 2014). For each treatment site, a 4km buffer was overlaid on the habitat map's Level 1 classification scheme to determine the percentages of the surrounding radial area that were comprised of reef, pavement/unconsolidated substrate, seagrass, land, and offshore habitat.

At each treatment site, divers searched the reef to identify corals for treatment using the "priority coral guiding principles" outlined in the Coral Disease Intervention Action Plan (Neely, 2018). Treated corals were generally large, had a large amount of remaining live tissue, and were not overwhelmed with SCTLD lesions. At sites treated between January and April 2019, each coral was haphazardly assigned one of two treatments: chlorinated epoxy or amoxicillin paste. As a result of high failure rates on chlorine-treated corals, all sites treated after April 2019 used only amoxicillin paste. In total, 1320 corals were treated during initial site visits and an additional 865 were treated during subsequent monitoring events through October 2020 (total of 2185).

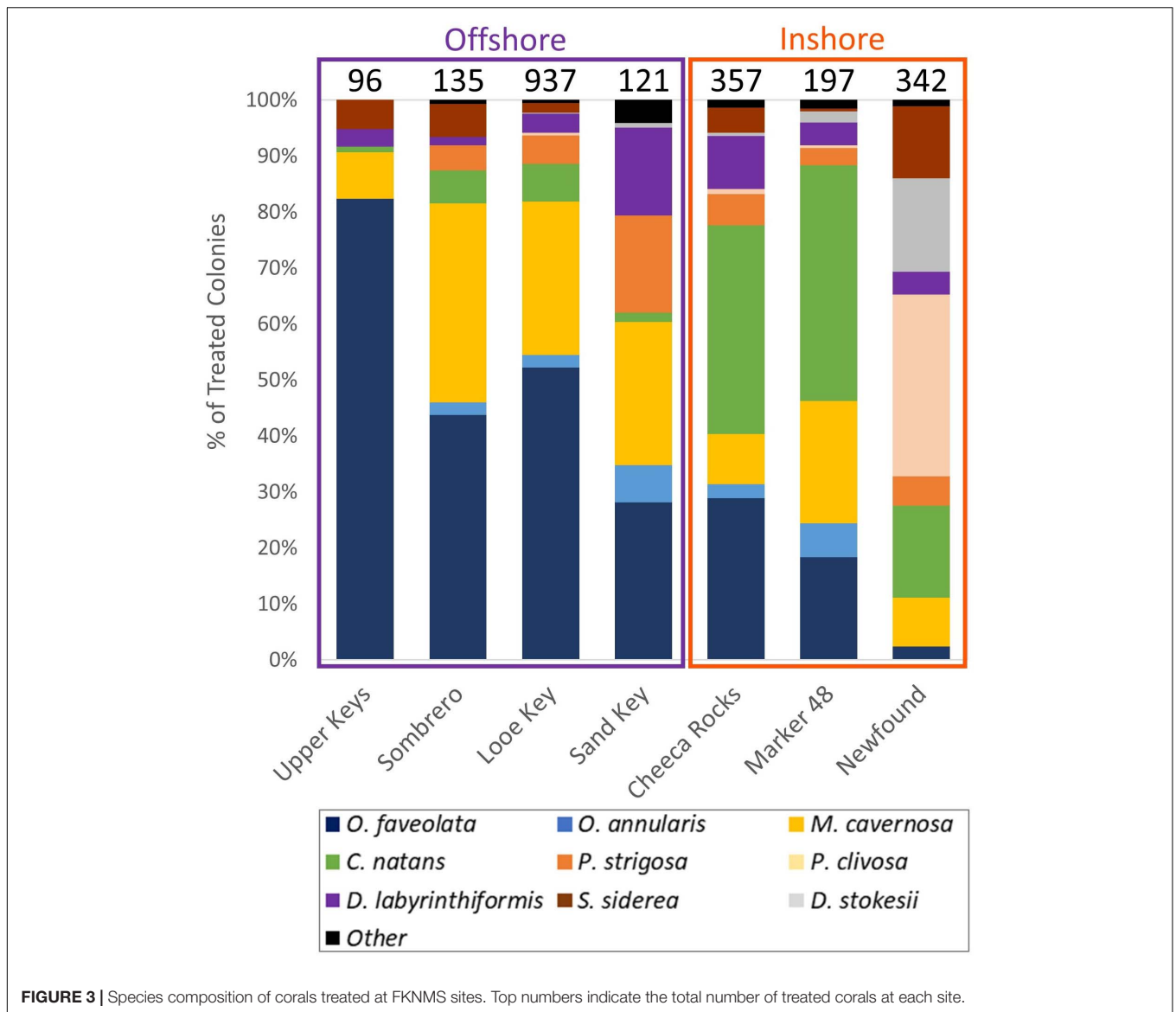
Because many corals and species had already perished at long-diseased sites, the abundance and diversity of corals that could be treated at each site varied (Figure 3). The density of treatable corals was notably lower at the Upper Keys and Sombrero sites (0.04 and 0.35 corals per m<sup>2</sup>) compared to offshore Looe Key and the three inshore sites (1.03 to 3.04 corals per m<sup>2</sup>). Across all sites, the three most commonly treated species were *Orbicella faveolata* (37%), *Montastraea cavernosa* (21%), and *Colpophyllia natans* (16%). *O. faveolata* and *M. cavernosa* dominated offshore reef treatments, while inshore reef treatments were dominated by the brain corals *C. natans* and *Pseudodiploria clivosa* (at the Newfound Harbor site). The average maximum diameter of treated colonies was 115 cm ( $\pm$  84 SD).

Each selected coral was tagged and mapped for future identification. Corals were identified to species, and 1" masonry nails were placed at each active lesion. Photographs of the colony and each lesion were taken before and after treatments.

Chlorinated epoxy was mixed following methodology formerly used on black band disease on Pacific reefs (Aeby et al., 2015). Before entering the water, chlorine powder (78% calcium hypochlorite) was folded by hand into Part A of SplashZone two-part epoxy in a 3:10 by volume ratio. Once stationed at a selected coral, divers would hand-mix the chlorinated Part A with the non-chlorinated Part B and apply the mixture directly to all active disease margins. Additionally, an underwater angle-grinder was used to create a trenched disease break approximately 5 cm from each active disease lesion. Disease breaks were approximately 1 cm wide and 1 cm deep. Each disease break was also packed with the chlorinated epoxy mixture.

The amoxicillin paste was created by hand-mixing amoxicillin trihydrate (98.1% purity, sourced from Phytotechnology Laboratories) into a specially formulated silicone-based paste termed Base2b. The proprietary base (CoreRx/Ocean Alchemists) includes polymers to mimic coral mucus consistency and releases amoxicillin over a 3-day time period. The Base2b and amoxicillin were hand-mixed no more than 36 h before application to the corals and packed into 60cc catheter-tip syringes. At the corals selected for treatment, divers would syringe the product onto active disease lesions and then press it into the bare skeleton for adhesion. The product made a band approximately 1 cm in width, with about half overlaying live tissue and the other half anchoring to the skeleton.

Colonies were revisited over the course of up to 710 days for monitoring and treatment of new lesions as needed. The goal for monitoring was for each coral to be revisited at 1 month, and then every 2 months thereafter. Actual monitoring varied slightly, particularly at Looe Key which was initially split into two regions for monitoring every other month. During each monitoring event, photographs were taken of the whole colony and of each current and previously treated disease lesion. Photographs from each monitoring event were used to assess whether the treatments had been effective (defined as the active lesion halting at the treatment line) or ineffective (defined as the lesion continuing past the treatment line across the colony) (Figure 4). For lesions with chlorinated epoxy applications, ineffective treatments were defined as those that had passed both the margin treatment and the disease break.



**FIGURE 3 |** Species composition of corals treated at FKNMS sites. Top numbers indicate the total number of treated corals at each site.

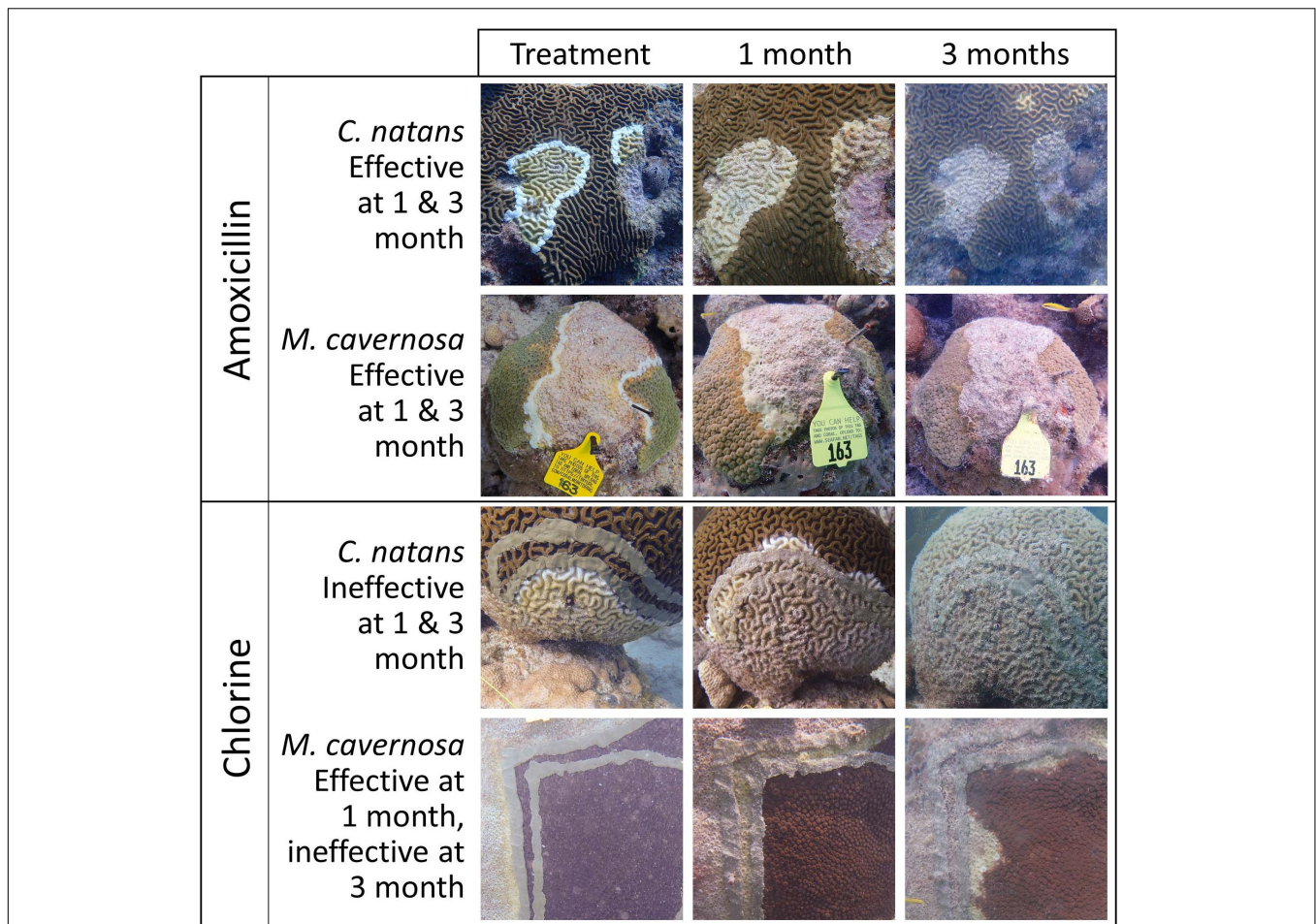
Corals that had active disease lesions during subsequent monitoring events received additional treatments on new lesions as needed. However, there were some instances where this did not occur. In the upper Keys, Sombrero, and some parts of Looe Key, colonies monitored 1 month after the initial treatment did not receive additional treatments at that time. Additionally, a small number of corals across all sites displayed active disease during monitoring, but no longer qualified as priority corals and were not treated again. By April 2019, it was clear that chlorine treatments were largely unsuccessful, and follow-up treatments were done with amoxicillin paste regardless of whether the coral was initially treated with amoxicillin or chlorine. The exception was 38 corals at Looe Key that were initially treated with chlorinated epoxy and were re-treated with chlorinated epoxy on active disease areas during the 2-month monitoring; these were all treated during subsequent monitoring events with amoxicillin if needed. During each monitoring event, every coral

was categorized as either (1) dead, (2) active lesions – untreated, (3) treated, or (4) no active disease (NAD).

## Analyses

### Lesion-Level Effectiveness

The effectiveness of amoxicillin and chlorine treatments at halting disease lesion progression for 3 months post-treatment was evaluated using a mixed effects logistic regression analysis. The binary response variable represented the effectiveness (1) or ineffectiveness (0) of treatment on 2,379 individual lesions present on 725 colonies from five species (*Colpophyllia natans*, *Pseudodiploria strigosa*, *Diploria labyrinthiformis*, *Montastraea cavernosa*, and *Orbicella faveolata*). Treated lesions were monitored periodically (**Supplementary Figure 1A**) during the initial 140-day time frame in the upper Keys (Carysfort South, Key Largo Dry Rocks, Grecian Rocks), middle Keys (Sombrero Reef), and lower Keys (Looe Key). If a lesion required



**FIGURE 4 |** Time series photos showing effective amoxicillin treatments and ineffective chlorinated treatments on two coral species: *Colpophyllia natans* (CNAT) and *Montastraea cavernosa* (MCAV). Disease lesions were defined as ineffective only once they had crossed both the margin and disease break chlorinated epoxy treatments.

retreatment, it was considered ineffective for that and future monitoring events.

We fit a candidate set of eight logistic regression models, each of which included a different combination of the predictors and two-way interaction terms (treatment type; region; species; number of days since treatment; number of days since treatment × treatment type, region × treatment type, and species × treatment type). To account for non-independence of repeated observations of lesions on the same colonies, we included a random intercept associated with unique corals (Gelman and Hill, 2007). We ranked the plausibility of each candidate model using Akaike’s Information Criterion [AIC; Akaike (1973)] with a small-sample bias adjustment [AICc; Hurvich and Tsai (1989)]. To quantify the relative support of each candidate model, we calculated Akaike weights (*w*) that range from zero to one, with the best-approximating model having the highest weight (Burnham and Anderson, 2002). The ratio of Akaike weights for two candidate models can be used to assess the degree of evidence for one model over another; for example, a model with an Akaike weight of 0.9 is 10 times more likely to be

the best-approximating model compared to a model with an Akaike weight of 0.09. We based all inferences on the best-approximating model i.e., the model with the lowest AICc score. Following model-fitting and model selection, we assessed goodness-of-fit for each model in the candidate set using a residual-based, simulation approach implemented in the R package “DHARMA” (Hartig, 2019). Additionally, we assessed the performance for each model by calculating an area under the receiving operator characteristic curve (AUC) statistic. AUC values > 0.5 indicate that a model predicts a categorical outcome, on average, better than random chance alone. All statistical analyses were conducted in R v.4.0.3 (R Development Core Team, 2013) using the packages “glmmTMB” [model fitting: logistic regression with logit link function; Brooks et al. (2017)] and “ROCR” [predictive performance; Sing et al. (2005)].

Following model fitting, we implemented *post hoc* contrasts (Tukey-adjusted pairwise comparisons with a 95% familywise confidence level) in R using the “emmeans” package (Lenth, 2018) to provide a more detailed assessment of differences in treatment effectiveness among species and regions.

## Colony Health Status

The health status of amoxicillin-treated corals was evaluated using mixed effects logistic regression to estimate the probability of a coral within the treatment regime being observed with no active disease (NAD) during monitoring. A total of 1,664 coral colonies representing eight species (*C. natans*, *D. labyrinthiformis*, *D. stokesii*, *M. cavernosa*, *O. annularis*, *O. faveolata*, *P. strigosa*, and *S. siderea*) were visited a total of 9956 times ranging from 8 to 710 days after initial treatment (**Supplementary Figure 1B**). The binary response variable represented the absence (1) or presence (0) of disease. Here, the absence of disease was defined as instances where a colony previously afflicted by SCTLN no longer exhibited any evidence of active disease. Any colonies with active lesions or mortality were considered diseased. We fit a candidate set of eight mixed effects logistic regression models, each of which included a different combination of the predictor variables (region; habitat; species; number of days since treatment; and all two-way interactions between region, habitat, and species). As with the 3-month lesion treatment analysis, we accounted for non-independence of repeated observations on the same colonies by including a random intercept associated with unique corals. We again ranked the plausibility of each candidate model using AICc and Akaike weights. Lastly, we based all inferences on the model with the lowest AICc score and assessed goodness of fit and predictive performance as described above for the 3-month lesion treatment effectiveness model.

Following model fitting, *post hoc* contrasts (Tukey-adjusted pairwise comparisons with a 95% familywise confidence level) were again implemented in R using the “emmeans” package to provide a more detailed assessment of differences in long-term disease status among species, regions, and habitats.

## RESULTS

### Lesion-Level Effectiveness

The best-approximating mixed effects logistic regression model assessing the probability of successful lesion treatment included number of days since treatment, treatment type, region, species, number of days since treatment  $\times$  treatment type, region  $\times$  treatment type, and species  $\times$  treatment type. Akaike weights indicated that this model was 10.1 times (0.91/0.09) more plausible than the next-best approximating model, which was similar but excluded the region  $\times$  treatment type interaction term. There was no support for the remaining six candidate models (**Supplementary Table 1**). The goodness of fit assessments based on scaled residuals indicated that all candidate logistic regression models provided an adequate fit to the data. Lastly, the AUC statistics for the eight candidate models ranged from 0.96 to 0.97, indicating they were all capable of predicting the observed data well.

Across all species and regions, the probability of amoxicillin treatment effectiveness was high. At 109 days, predicted effectiveness exceeded 95% among all tested species at Looe and the upper Keys. Effectiveness was slightly lower for *D. labyrinthiformis* and *O. faveolata* at Sombrero, but still

exceeded 75% (**Figure 5**). Across regions, amoxicillin treatments responded similarly, with the exception of corals at Sombrero, which did not respond to treatment as effectively as corals at Looe (**Tables 2, 3**). This regional pairwise comparison is significant ( $p < 0.0001$ ), but the magnitude of differences was minor as effectiveness was still high at both sites. Based on the best approximating model across all regions and times within the 3-month analyses, all five species responded equally well to amoxicillin (**Table 4**).

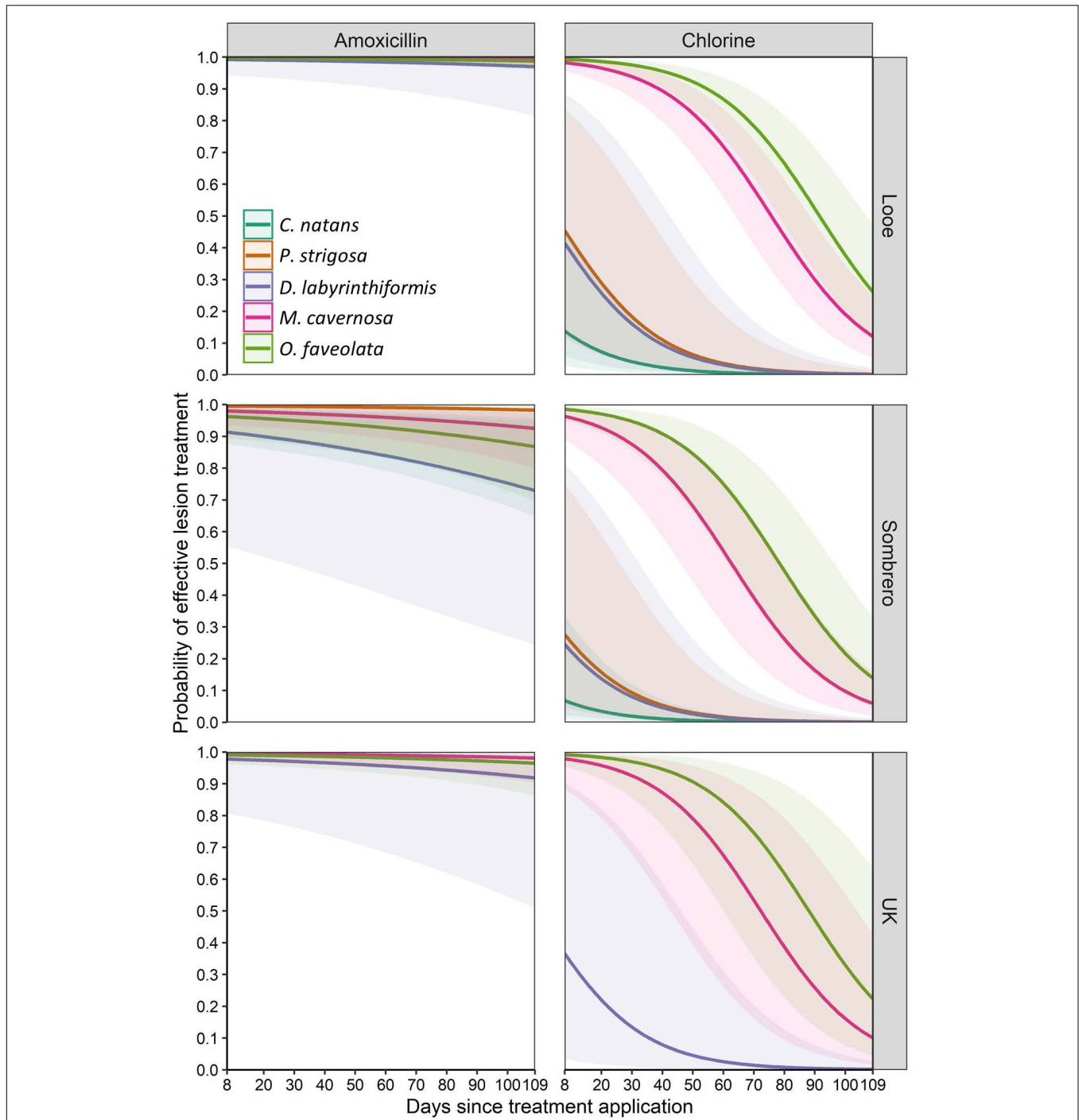
In contrast to amoxicillin treatments, chlorinated epoxy treatments were ineffective (**Figure 5**). This chlorinate epoxy effectiveness was equally poor across all regions, and was significantly less effective than amoxicillin treatments across all regions ( $p < 0.001$ ) (**Tables 2, 3**). Among species, the brain corals *C. natans*, *D. labyrinthiformis*, and *P. strigosa* had lower effectiveness rates than the boulder corals *M. cavernosa* and *O. faveolata* (**Tables 2, 4**). However, these summaries are across all times after treatment; while *M. cavernosa* and *O. faveolata* treatments did not fail as early as those of the brain corals, their rate of effectiveness 3 months after treatment was less than 20%.

### Colony Health Status

To assess the probability of a coral having no active disease (NAD) at up to 24 months after initial treatment and necessary touchups, eight mixed effects logistic regression models were considered for goodness of fit. The best fit model contained the following parameters: number of days since treatment, habitat (inshore or offshore), region (upper Keys, middle Keys, or lower Keys), species, a habitat  $\times$  species interaction term, and a region  $\times$  species interaction term. Akaike weights ( $w$ ) indicated that this model was 6.9, 8.6, and 11.5 times more plausible than the second, third, and fourth best-approximating models, respectively, and there was very little support for the remaining four candidate models (**Supplementary Table 2**). The goodness of fit assessments based on scaled residuals indicated that all candidate logistic regression models provided an adequate fit to the data. Lastly, the AUC statistics for the eight candidate models ranged from 0.86 to 0.87, indicating they were capable of predicting the observed data reasonably well.

Parameter estimates from the best-approximating model indicated a positive relationship between the date after initial treatment and the probability of NAD (**Table 5** and **Figure 6**). The most pronounced variable affecting this probability was habitat (inshore vs. offshore). After 24 months, the proportion of colonies with NAD exceeded 95% for most species at inshore sites. In contrast, several species had NAD rates of less than 80% at offshore sites. Of the eight compared species (*C. natans*, *D. labyrinthiformis*, *D. stokesii*, *M. cavernosa*, *O. annularis*, *O. faveolata*, *P. strigosa*, and *S. siderea*), all but *D. stokesii* and *O. annularis* had significantly higher NAD values at inshore sites than offshore sites (**Table 6**). Some species-specific variations in NAD rates were apparent within different geographies, with more differences occurring at offshore sites (**Table 7**). *Orbicella faveolata* performed more poorly (lower NAD values) than *C. natans* (offshore middle Keys, offshore upper Keys, inshore upper Keys), *D. labyrinthiformis* (offshore





**FIGURE 5 |** Mean predicted probability (solid lines) of effective lesion treatment for three months after application. Amoxicillin and chlorinated epoxy effectiveness levels are shown for five species across three sites. Shaded areas represent 95% confidence intervals.

lower Keys), *P. strigosa* (inshore upper Keys), and *M. cavernosa* (offshore middle Keys). *S. siderea* also performed more poorly than *P. strigosa* (offshore lower Keys) and *M. cavernosa* (offshore lower Keys and offshore upper Keys). *Colpophyllia natans* performed more poorly than *M. cavernosa* in the offshore middle Keys.

## DISCUSSION

Clear differences between treatment types were apparent in assessing effectiveness on SCTLD lesions. Chlorinated epoxy treatments on SCTLD lesions were highly ineffective, with corals of all species at all sites exhibiting high rates of failure

**TABLE 2 |** Parameter estimates affecting the probability of halted disease progression up to 3 months after treatment.

	Parameter	Estimate	SE	LCL	UCL	p-value
<b>Fixed effects</b>						
	Intercept	6.458	0.960	4.576	8.340	<0.0001
Treatment	Chlorine	-7.814	1.345	-10.450	-5.178	<0.0001
Time	Days	-0.013	0.005	-0.024	-0.003	0.011
Region	Sombrero	-2.478	0.494	-3.446	-1.509	<0.0001
	Upper Keys (UK)	-1.049	0.675	-2.371	0.273	0.120
Species	<i>D. labyrinthiformis</i> (DLAB)	-1.521	1.253	-3.977	0.936	0.225
	<i>M. cavernosa</i> (MCAV)	-0.007	0.907	-1.785	1.771	0.994
	<i>O. faveolata</i> (OFAV)	-0.634	0.873	-2.345	1.076	0.467
	<i>P. strigosa</i> (PSTR)	1.510	1.683	-1.788	4.808	0.370
Treatment × Time	Chlorine × Days	-0.046	0.008	-0.061	-0.031	<0.0001
Treatment × Region	Chlorine × Sombrero	1.698	0.575	0.570	2.826	0.003
	Chlorine × UK	0.842	1.042	-1.200	2.884	0.419
Treatment × Species	Chlorine × DLAB	3.009	1.889	-0.693	6.711	0.111
	Chlorine × MCAV	5.875	1.321	3.286	8.464	<0.0001
	Chlorine × OFAV	7.444	1.353	4.793	10.095	<0.0001
	Chlorine × PSTR	0.139	2.052	-3.883	4.161	0.946
<b>Random effects</b>						
	Intercept (Coral ID)	2.813				

Parameter estimates, standard errors (SE), lower and upper 95% confidence limits (LCL and UCL), and p-values are from the best-approximating mixed effects logistic regression model. All estimates are on the log-odds scale, and random effects are reported as standard deviations. The default parameters for comparison were: Treatment – Amoxicillin, Days – 0, Region – Looe Key, Species – *Colpophyllia natans* (CNAT).

**TABLE 3 |** Significant differences (p-values) in modeled treatment effectiveness among treatment types (amoxicillin and chlorine) and regions (Looe: lower Keys, Sombrero: middle Keys, and UK: upper Keys).

		Amoxicillin			Chlorine		
		Looe	Sombrero	UK	Looe	Sombrero	UK
<b>Amoxicillin</b>	<b>Looe</b>		<0.0001	0.629	<0.0001	<0.0001	<0.0001
	<b>Sombrero</b>	<0.0001		0.421	<0.0001	<0.0001	<0.0001
	<b>UK</b>	0.629	0.421		<0.0001	<0.0001	<0.0001
<b>Chlorine</b>	<b>Looe</b>	<0.0001	<0.0001	<0.0001		0.626	1.000
	<b>Sombrero</b>	<0.0001	<0.0001	<0.0001	0.626		0.990
	<b>UK</b>	<0.0001	<0.0001	<0.0001	1.000	0.990	

Tests are Tukey-adjusted pairwise comparisons. All amoxicillin treatments were more successful than all pairwise chlorinated treatments. Chlorinated treatments all performed equally poorly. Among amoxicillin treatments, Looe treatments were slightly but significantly more effective than those at Sombrero.

within the first 3 months. Effectiveness rates decreased with time and varied by species, largely based on the species-specific rate of lesion progression. On brain corals (*P. strigosa*, *C. natans*, and *D. labyrinthiformis*), lesions progressed quickly across the colonies, and by the first monitoring period, lesions had usually progressed past both the margin treatment and the disease break treatment, constituting a fully ineffective lesion treatment. In contrast, on species with slower lesion progression (*O. faveolata* and *M. cavernosa*) lesions had generally only crossed the chlorinated disease margin treatment 1 month after treatment; during later observations, the continually progressing lesion had also crossed the disease break and was scored as ineffective (Figure 4). Thus, the seemingly high rates of chlorine treatment effectiveness on some species immediately following treatment were not representative of treatments that halted disease progression.

In contrast, amoxicillin treatments were highly effective, quickly halting lesion progression on all species at all sites. There were no differences in amoxicillin effectiveness on treated lesions among species, indicating this methodology is suitable and effective for the suite of SCTLD-affected species. Though sample sizes for other coral species were too small for inclusion in the logistic models, a total of 16 species have been treated with the amoxicillin paste with similar success rates. In comparing treatments with placebos, Neely et al. (2020) confirmed amoxicillin rather than the application paste as the active ingredient. As a result of these comparative studies, chlorinated epoxy treatments were discontinued in Florida and are not recommended for SCTLD-affected corals.

Tracking of long-term colony health confirmed that while SCTLD lesions can be halted by amoxicillin treatments, additional distinct lesions may subsequently appear on treated

**TABLE 4 |** Significant differences (*p-values*) in modeled treatment effectiveness among species under two different treatment types (amoxicillin and chlorinated epoxy).

Amoxicillin treatments					
	CNAT	DLAB	PSTR	MCAV	OFAV
CNAT		0.744	0.898	1.000	0.950
DLAB	0.744		0.411	0.572	0.895
PSTR	0.898	0.411		0.854	0.603
MCAV	1.000	0.572	0.854		0.651
OFAV	0.950	0.895	0.603	0.651	
Chlorinated epoxy treatments					
	CNAT	DLAB	PSTR	MCAV	OFAV
CNAT		0.838	0.639	<0.0001	<0.0001
DLAB	0.838		1.000	0.007	<0.001
PSTR	0.639	1.000		<0.001	<0.001
MCAV	<0.0001	0.007	<0.001		0.227
OFAV	<0.0001	<0.001	<0.001	0.227	

Tests are Tukey-adjusted pairwise comparisons. There was no difference among modeled success rates of amoxicillin treatments among species. Among chlorinated epoxy treatments, *Montastraea cavernosa* and *Orbicella faveolata* performed significantly better, but these results were summed over the 3-month period and do not reflect results toward the end of that time period.

colonies. These lesions may be the result of systemic infection or of reinfection from the environment. Much of the histological and microbiome studies on SCTLD-affected corals remains inconclusive as to whether SCTLD is systemic within a colony or localized to the visible infection site. For example, gastrodermal necrosis was found in 87% of visually diseased histological samples as well as in 11% of apparently healthy areas of tissue (Landsberg et al., 2020). And microbial communities on apparently healthy tissue of affected colonies are sometimes similar to those of unaffected colonies while other times more similar to those at active lesions (Meyer et al., 2019; Rosales et al., 2020; Thome et al., 2021). It has also been suggested that the bacteria seen in microbiome studies are purely opportunistic, capitalizing on other underlying health issues (Landsberg et al., 2020). Whether new lesions are caused by independent infection events or an underlying systemic condition has important implications for long-term colony health and disease management and certainly warrants future research.

Though new lesions could appear on previously treated colonies, corals that received regular monitoring and lesion touch-ups as needed progressively exhibited improved health (no active lesions) over time. Possible mechanisms by which initial and follow-up treatments as necessary could contribute to this include:

1. Reducing the potentially systemic infection within colonies, sometimes through multiple treatments, resulting in decreased lesions development.
2. Reducing opportunistic harmful bacteria on a colony that lead to rapid tissue loss, even if bacteria are not the underlying cause of SCTLD.

3. Reducing the pathogen load of either disease-inducing or opportunistic bacteria across the site as a whole through multiple visitations and treatments, thus minimizing new infections and lesions across all colonies.

The mechanism behind treatments leading to healthy colonies is recommended as a topic for further research. In particular, why some colonies do not show signs of SCTLD after a single treatment while others continually exhibit new lesions is relevant for questions of resilience and assisted reproduction. The impact of treatments in reducing pathogen load and protecting the surrounding non-infected colonies is also unknown and of importance for effective SCTLD management.

For the purposes of terminology, we will refer to the appearance of a new distinct lesion as a “reinfection,” though as acknowledged above, alternate hypotheses suggest it may be a result of systemic influences. The colony-level assessment models show that the probability of reinfection (i.e., the inverse of the probability of NAD) was not the same across all species. In particular, *O. faveolata* and *S. siderea* were more likely than other species to develop new lesions, particularly at offshore sites. One possible explanation is that the particularly large size of the *O. faveolata* colonies in relation to the other species provides more surface area for new infection events. However, *S. siderea* were comparable in size to other species, and also showed high reinfection rates. We suggest that somehow these species are physiologically more inclined toward reinfection. Considering that *O. faveolata* is the most important reef builder remaining on offshore Florida Keys reefs, and that *S. siderea* is the most abundant species on Florida Keys reefs, determining why these species do not perform as well as others following treatment is an important research question.

Almost all treated species showed significantly better long-term response rates at inshore reefs compared to offshore reefs. We here propose four hypotheses for this, which are recommended avenues of future research.

1. Bleaching at inshore reefs halted disease progression annually. During the summers of 2019 and 2020, severe paling and bleaching was seen on most individuals at the inshore sites, but not on the offshore sites. This paling/bleaching coincided with an almost complete cessation of active disease. This correlation between zooxanthellae loss and SCTLD lesions has also been observed at other Florida sites (Sharp et al., 2020) and in the United States Virgin Islands (Meiling et al., 2020). These inshore bleaching events may have reduced or eliminated the reef pathogen load, either superseding or working in collaboration with our intervention efforts, to rapidly diminish the possibility of subsequent new infections. Meiling et al. (2020) suggest that disease treatment efforts could be made more efficient by working during and immediately following such bleaching events; we suggest that year-round intervention even in areas likely to experience regular bleaching is important in order to keep colonies alive through times when disease is more likely to be abundant.

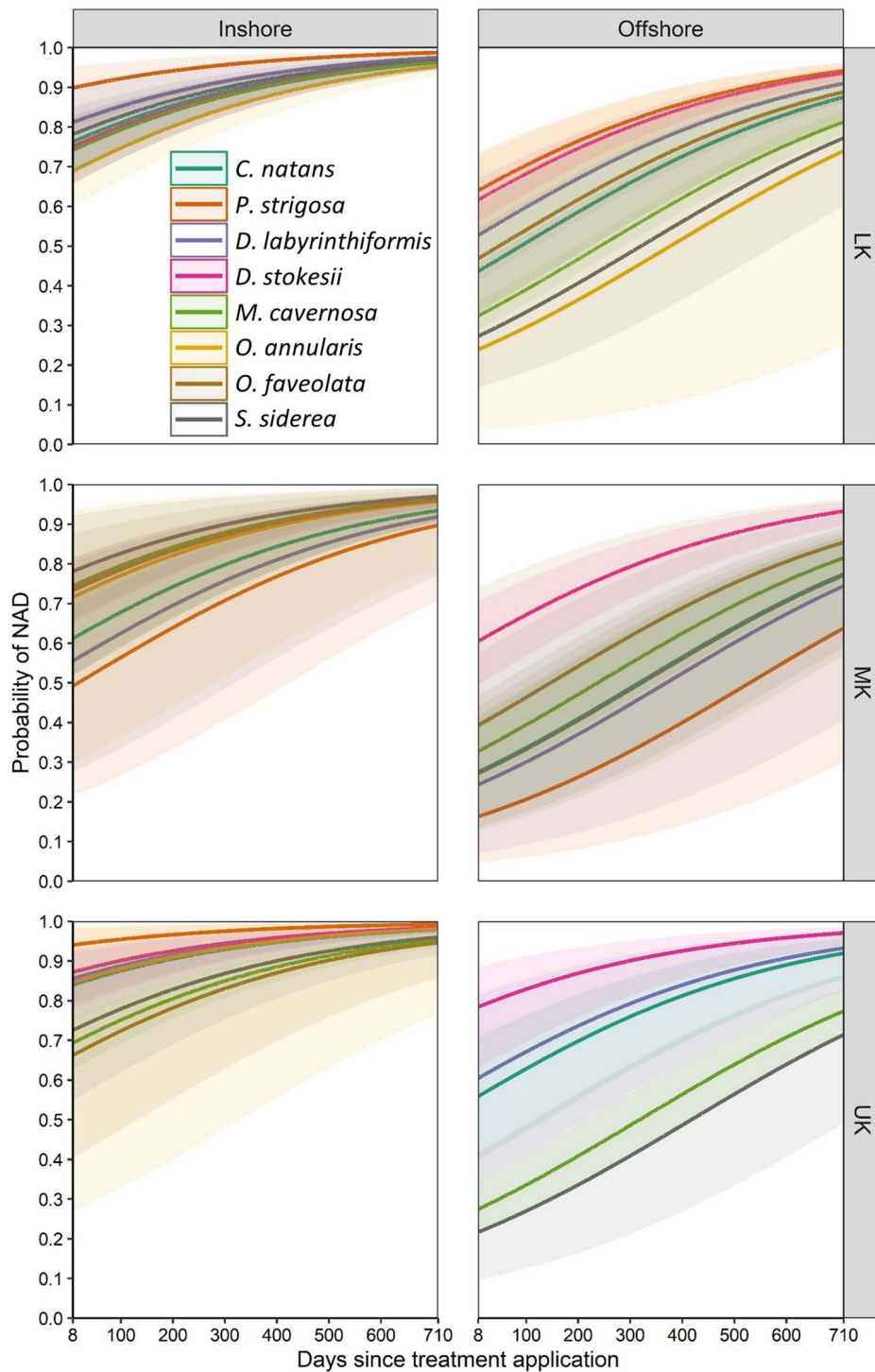
**TABLE 5 |** Parameter estimates affecting the probability of a colony within the monitoring and touch-up regime being without disease up to 24 months after initial treatment.

	Parameter	Estimate	SE	LCL	UCL	p-value
<b>Fixed effects</b>						
	Intercept	1.143	0.210	0.732	1.554	<0.0001
	Days	0.003	0.000	0.002	0.004	<0.0001
Habitat	Offshore	-1.423	0.286	-1.983	-0.863	<0.0001
Species	<i>D. labyrinthiformis</i> (DLAB)	0.295	0.462	-0.609	1.200	0.52
	<i>D. stokesii</i> (DSTO)	-0.372	0.285	-0.932	0.187	0.19
	<i>M. cavernosa</i> (MCAV)	-0.066	0.307	-0.667	0.535	0.83
	<i>O. annularis</i> (OANN)	0.149	0.960	-1.734	2.031	0.88
	<i>O. faveolata</i> (OFAV)	-0.114	0.289	-0.680	0.451	0.69
	<i>P. strigosa</i> (PSTR)	1.012	0.462	0.106	1.918	0.03
	<i>S. siderea</i> (SSID)	0.107	0.308	-0.496	0.710	0.73
Region	Middle Keys (MK)	-0.713	0.270	-1.243	-0.182	0.01
	Upper Keys (UK)	0.492	0.265	-0.028	1.011	0.06
Habitat × Species	Offshore × DLAB	0.068	0.538	-0.986	1.123	0.90
	Offshore × DSTO	-0.530	1.142	-2.767	1.708	0.64
	Offshore × MCAV	0.796	0.368	0.076	1.517	0.03
	Offshore × OANN	-0.019	0.938	-1.858	1.820	0.98
	Offshore × OFAV	-0.365	0.344	-1.039	0.310	0.29
	Offshore × PSTR	-0.180	0.542	-1.243	0.883	0.74
	Offshore × SSID	-0.832	0.522	-1.856	0.192	0.11
Region × Species	MK × DLAB	-0.529	0.761	-2.021	0.963	0.49
	MK × DSTO	0.841	0.963	-1.047	2.728	0.38
	MK × MCAV	0.664	0.357	-0.036	1.364	0.06
	MK × OANN	0.403	0.850	-1.262	2.069	0.63
	MK × OFAV	0.728	0.334	0.074	1.382	0.03
	MK × PSTR	-1.499	0.752	-2.972	-0.025	0.05
	MK × SSID	0.710	0.648	-0.560	1.980	0.27
	UK × DLAB	-0.175	0.577	-1.305	0.955	0.76
	UK × DSTO	0.418	1.419	-2.363	3.199	0.77
	UK × MCAV	0.323	0.463	-0.585	1.232	0.49
	UK × OANN	-1.134	1.116	-3.321	1.052	0.31
	UK × OFAV	-0.730	0.320	-1.357	-0.102	0.02
	UK × PSTR	0.083	0.767	-1.420	1.587	0.91
UK × SSID	-0.795	0.504	-1.783	0.194	0.12	
<b>Random effects</b>						
	Intercept (Coral ID)	1.024				

Parameter estimates, standard errors (SE), and lower and upper 95% confidence limits (LCL and UCL) are from the best-approximating mixed effects logistic regression model. All estimates are on the log-odds scale, and random effects are reported as standard deviations. The default parameters for comparison were: Days = 0, Habitat = Inshore, Region = lower Keys, and Species = *C. natans* (CNAT).

2. Presence of more biodiverse and intact coral communities at inshore reefs confers resilience. The inshore reefs of the Florida Keys, including those treated here, have higher coral cover, larger average colony size, more evenly distributed population structures, greater species diversity, and higher coral growth rates than their offshore counterparts (Lirman and Fong, 2007; Ruzicka et al., 2013; Manzello et al., 2015; Vega-Rodriguez et al., 2015). Such biodiversity may have an impact on community disease susceptibility [see review in Rohr et al. (2020)]. Arguments for high biodiversity helping to protect individuals within an ecosystem include higher densities of non-susceptible hosts, lower numbers of susceptible hosts,

and potential consumption of pathogens. In contrast, for a disease such as SCTLD which affects a large number of species, increased diversity and coral density may instead provide more hosts and worsen disease. Epidemiological models of SCTLD have indicated that more diverse sites may have higher SCTLD prevalence (Muller et al., 2020), while prevalence is independent of colony density (Sharp et al., 2020). Devastating SCTLD impacts on other regions with more intact reefs further suggest that this hypothesis of diverse and intact reef communities reducing SCTLD impact or improving treatment success is incorrect, but this may warrant future research.



**FIGURE 6 |** Mean predicted probability (solid lines) of corals exhibiting no active disease (NAD) for eight species across two geographic variables: inshore/offshore and upper/middle/lower Keys (UK/MK/LK). Shaded areas represent 95% confidence intervals. Model predictions represent corals initially treated with amoxicillin and retreated as necessary during subsequent monitoring events.

3. Presence of more resistant and resilient coral colonies at inshore reefs results in lower reinfection rates. Historic losses of non-Acroporid corals on offshore reefs

are generally attributed to early (1989–1998) bleaching events (Sommerfield et al., 2008), which did not cause the same levels of mortality on inshore reefs. One

**TABLE 6 |** Tukey-adjusted pairwise comparisons (*p-values*) of “no active disease” (NAD) rates between inshore and offshore sites for eight coral species using the best approximating model.

Species	estimate	SE	df	t. ratio	p.value
CNAT	1.423	0.286	8920	4.981	<0.0001
DLAB	1.355	0.457	8920	2.965	0.003
DSTO	1.953	1.106	8920	1.766	0.078
MCAV	0.627	0.232	8920	2.698	0.007
OANN	1.442	0.894	8920	1.612	0.107
OFAV	1.788	0.195	8920	9.19	<0.0001
PSTR	1.603	0.462	8920	3.473	0.001
SSID	2.255	0.439	8920	5.137	<0.0001

For six species (*Colpophyllia natans*, *Diploria labyrinthiformis*, *Montastraea cavernosa*, *Orbicella faveolata*, *Pseudodiploria strigosa*, and *Siderastrea siderea*) amoxicillin-treated corals at inshore sites were more likely to be NAD than those at offshore sites.

hypothesis is that these inshore corals are stress-hardened as a result of higher temperature fluctuations, higher turbidity, and generally poorer water quality (Lirman and Fong, 2007), making individual colonies more resilient to bleaching-related mortality and perhaps to disease. Such resilience could present within the SCTLD treatment regime as an enhanced ability to resist reinfection events and/or to fight off a systemic infection with the help of treatment more effectively than offshore corals.

- Isolation of inshore reefs may reduce reinfection potential. The reef tract off the Florida Keys consists of a largely continuous forereef punctuated by high-relief spur and groove formations. Though not densely populated with corals, this forereef presents a relatively continuous habitat for susceptible species that may act as continual reservoirs of SCTLD, capable of continually causing forereef reinfections even if pathogens are managed locally. In contrast, inshore sites are localized patch reefs separated from other coral habitat by sand or seagrass (Figure 7). Given sufficient time and the dynamic water

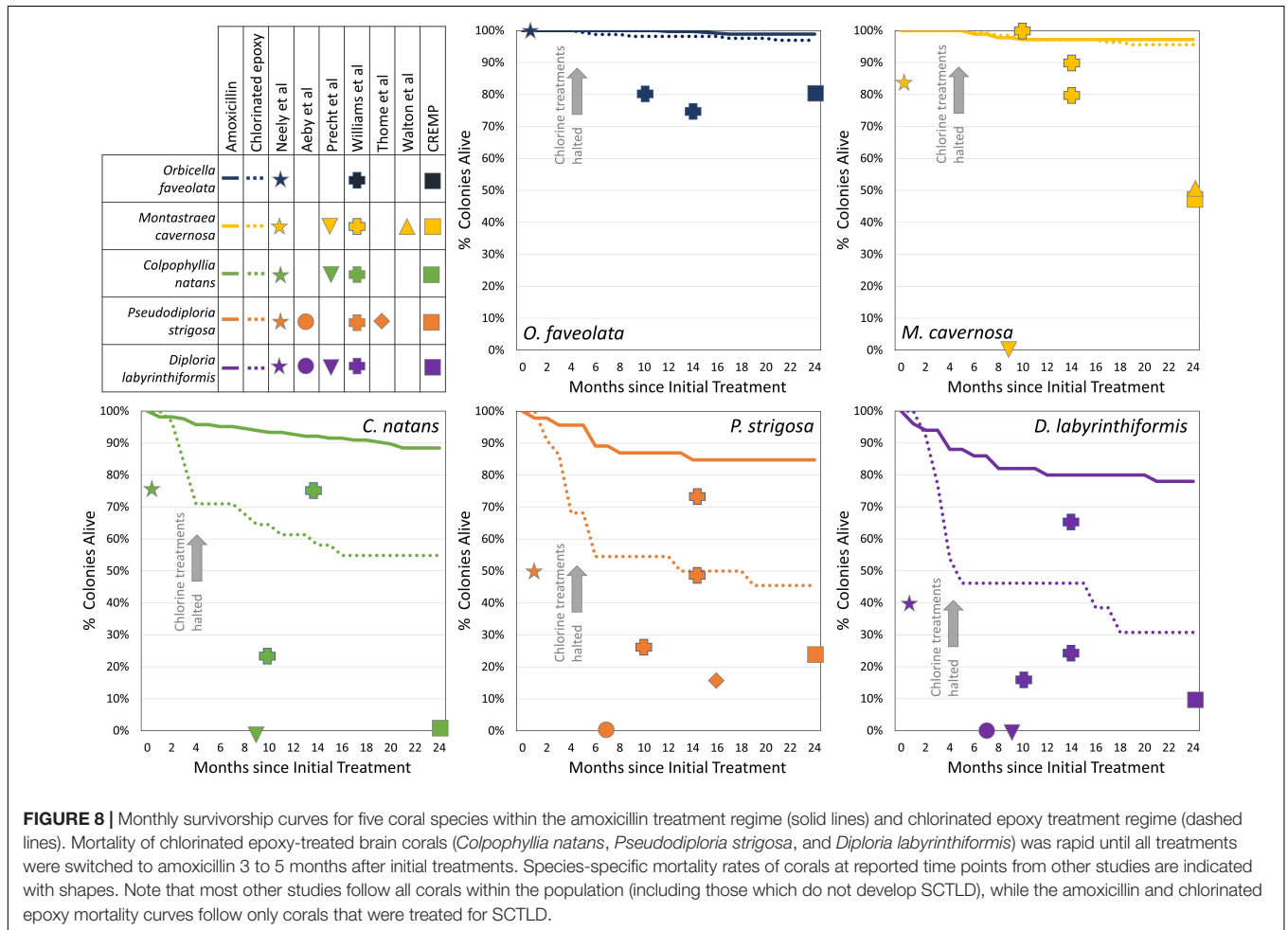
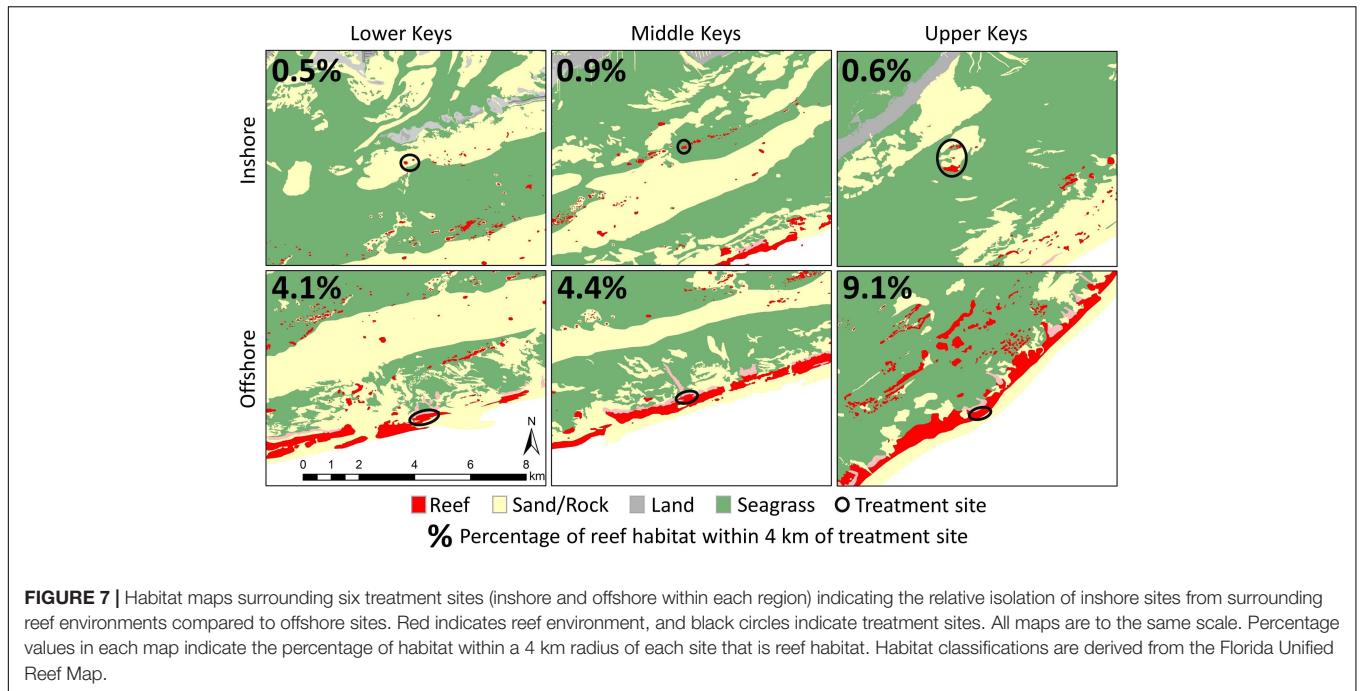
movements of the region, all sites are likely to receive pathogen loads that result in infections. Those initial infections are likely to result in localized community spread within sites (Williams et al., 2021). During our initial treatment periods, more diseased colonies per unit area were present on inshore reefs than on offshore reefs. This would have suggested a higher localized pathogen load which would be expected to result in higher rates of reinfection at inshore reefs; however, the opposite pattern was observed. We suggest that localized intervention efforts were effective at reducing or even eliminating community spread within reefs, and that any subsequent reinfections on treated reefs resulted from transmission from surrounding untreated areas. On average, 6.5% ( $\pm 3.2$  SD) of the habitat within 4 km of the offshore treatment sites was coral reef. In contrast, only 0.7% ( $\pm 0.2$  SD) of the habitat within 4 km of the inshore treatment sites consisted of reef. We speculate that while both offshore and inshore reefs benefited from reduction of local pathogen loads as a result of treatment, the lower reinfection rates of inshore reefs were the result of their isolation and hence their decreased probability of reinfection from surrounding areas.

These hypotheses, or any combination of them, may render corals on inshore reefs less susceptible to reinfection and/or more responsive to treatment. Experimentation would be necessary to identify the relevant variables, and such research is recommended as it carries consequential management and biological implications. However, even without knowing the causal factors, we have identified that high-diversity, high-cover, isolated reefs that are susceptible to regular bleaching respond better to amoxicillin treatment than low-cover reefs surrounded by reef habitat that do not bleach. In the Florida Keys, these reefs further represent the highest potential for reproductive capacity based on proximity of conspecifics and abundance of SCTLD-susceptible species. Further, colony density at these sites results in much higher treatment and monitoring efficacy

**TABLE 7 |** Tukey-adjusted pairwise comparisons (*p-values*) of “no active disease” (NAD) rates among amoxicillin-treated colonies of eight coral species by region.

	CNAT	DLAB	PSTR	DSTO	MCAV	OANN	OFAV
DLAB							
PSTR							
DSTO							
MCAV	O-MK (0.01)						
OANN							
OFAV	O-MK (0.005) O-UK (0.024) I-UK (0.005)	O-LK (0.014)	I-UK (0.031)		I-UK (0.050)		
SSID			O-LK (0.019)		O-LK (0.014) O-UK (0.001)		

Regions are Offshore (O-) and Inshore (I-) as well as upper Keys (UK), middle Keys (MK), and lower Keys (LK). *Orbicella faveolata* had lower probability of being NAD than six other species/habitat combinations. *Siderastrea siderea* had a lower probability than three other species/habitat combinations. And *Colpophyllia natans* had a lower probability than one other species/habitat combination.



compared to offshore sites that require considerable search time to find remaining live colonies. We recommend that intervention resources be focused primarily on these areas of high coral cover, high species diversity, better response to treatment, and high work efficiency.

Two years after initial treatments, mortality of SCTLD-affected colonies treated under the amoxicillin regimens was only 5%. One limitation of this study is the lack of non-treated controls; the decision to treat all SCTLD-affected corals was based on observations and studies throughout Florida and now through the Caribbean identifying extremely high mortality rates. Comparisons with non-treated corals monitored through these other studies highlight the value of such work (Figure 8).

1. Neely et al. (2020) tested controls and placebo treatments alongside amoxicillin treatments in the Florida Keys and identified control/placebo 1-month mortality rates of 60% on *D. labyrinthiformis*, 25% on *C. natans*, 50% on *P. strigosa*, 17% on *M. cavernosa*, and 0% on *O. faveolata*.
2. In work by Precht et al. (2016) on tagged SCTLD-affected colonies in Southeast Florida, mortality of SCTLD-afflicted colonies after 9 months was 100% across four species (9 *Meandrina meandrites*, 10 *D. stokesii*, 1 *C. natans*, 4 *P. strigosa*, 8 *M. cavernosa*, 1 *P. clivosa*, and 2 *Solenastrea bournoni*).
3. Thome et al. (2021) tagged 96 *P. strigosa* colonies on an SCTLD-affected reef in Mexico; within 306 days, mortality was greater than 84%, with nearly half of the surviving colonies actively diseased at that point.
4. Aeby et al. (2019) tagged 13 actively diseased colonies in the Florida Keys (5 *P. strigosa*, 5 *D. labyrinthiformis*, 2 *D. stokesii*, and 1 *Meandrina jacksonii*); within 7 months, 100% of these were dead.
5. Williams et al. (2021) followed colonies at offshore and mid-channel Florida Keys reefs through 14 months after SCTLD onset, and nearshore colonies through 10 months after SCTLD onset; mortality rates were 50–71% on *D. stokesii*, 33–83% on *D. labyrinthiformis*, 1–20% on *M. cavernosa*, 0–25% on *O. faveolata*, 25–77% on *C. natans*, and 27–75% on *P. strigosa*.
6. Though not tracking individual colonies, Walton et al. (2018) documented declines in density of 90% in *D. stokesii*, 95% in *M. meandrites*, 50% in *M. cavernosa*, and 32% in *S. siderea* over 2 years through the SCTLD outbreak.
7. Fixed survey sites in the upper Florida Keys documented substantial losses in colonies between pre-SCTLD years (average of 2014 – 2016) and post-SCTLD (2018): 18% of *Orbicella* colonies, 52% of *M. cavernosa*, 78% of *P. strigosa*, 84% of *D. stokesii*, 91% of *D. labyrinthiformis*, and 100% of *C. natans* and *M. meandrites* (CREMP, unpublished data).

These known rates of exceptionally high mortality as a result of SCTLD infection highlight the importance of in-water treatment if *in situ* colonies are to be saved. These colonies represent centuries of growth highlighting a track record of hardiness against stressors. The habitat, reproductive output, and ecosystem services they provide are essential for reefs, even those

undergoing restoration efforts. The effectiveness of amoxicillin treatments in halting SCTLD lesions and the long-term reduction in diseased colonies at sites that are regularly monitored and treated as needed identifies the use of this in-water intervention on SCTLD-affected sites as a viable method for saving corals and coral diversity in the presence of this unprecedented disease.

## CONCLUSION

Corals affected by SCTLD were treated using two experimental methods to halt disease lesions: chlorinated epoxy and an amoxicillin paste. Logistic regression models from post-treatment monitoring data identified amoxicillin treatments as effective across all species; in contrast, chlorinated epoxy treatments failed across all species, with failure rates more rapid on species with faster lesion progression rates. Corals that were initially treated and then revisited approximately every 2 months for treatments on new lesions as needed showed long-term improvements in health, with nearly 95% of treated corals exhibiting no signs of disease after 2 years. Species-specific comparisons in long-term health identified some species (particularly *O. faveolata*) as more susceptible to reinfections than others. Geographic comparisons showed that treated corals on inshore reefs had better long-term prognoses than those on offshore reefs. Hypotheses as to why inshore reefs respond better include: summer inshore bleaching events may reduce pathogen load, isolation from other reefs may limit reinfections, and stress-hardening or benefits of biodiversity may enhance colony resilience. The short- and long-term effectiveness of amoxicillin treatments provides an effective tool for preventing mortality of corals affected by SCTLD.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

KN secured funding, conceived the data collection design, and wrote the manuscript with assistance from CS. CS conceived the model design and conducted statistical assessments. KN, KM, EH, and MD conducted data collection and prepared data for analysis. KN and CS made the tables and figures. All authors edited and approved the manuscript.

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information here: <https://forceblueteam.org/coral-disease-intervention/>).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.675349/full#supplementary-material>

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