FY 2018 IN-SITU DISEASE INTERVENTION

Florida Department of Environmental Protection
Coral Reef Conservation Program

FY 2018 In Situ Disease Intervention

Final Report

Prepared By:

Karen Neely, Ph.D.
and
Emily Hower

Nova Southeastern University
Halmos College of Natural Science and Oceanography
8000 N. Ocean Drive
Dania Beach, FL 33004-3078

January 31, 2019

Completed in Fulfillment of PO B3852C for
Florida Department of Environmental Protection
Coral Reef Conservation Program
1277 N.E. 79th Street Causeway
Miami, FL 33138

This report was prepared for the Florida Department of Environmental Protection, Florida Coastal Office by Nova Southeastern University. Funding was provided by the Florida Department of Environmental Protection. The views, statements, findings, conclusions and recommendations expressed herein are those of the authors and do not necessarily reflect the views of the State of Florida or any of its sub-agencies.

Work was conducted under permits FKNMS-2015-156-A3, FKNMS-2018-007-A1, and FKNMS manager's permit

Background:

Since 2014, a multi-year, multi-species disease outbreak has progressed geographically along the Florida Reef Tract from an origin near Virginia Key. Over half of the hard coral species on the reef are susceptible, and the disease often results in 100% infection rates and 100% subsequent mortality. Susceptible species include five of the seven ESA-listed Caribbean coral species and most of the reef-building species.

Beginning in December 2017, treatment options in laboratory settings were tested for effectiveness in halting advancing disease margins. Treatments included barriers such as trenching and/or application of clay or epoxy bands. They also included the addition of high chlorine concentrations or antibiotics to those barriers. Most treatments proved moderately to completely ineffective in the laboratory setting[1]. The most promising laboratory treatments, as well as successful work on chlorinated treatments on some Southeast Florida (SEFL) wild colonies[2], were conducted in the field in attempts to save wild colonies as well as to compare effectiveness of various treatments.

Project Design:

Disease treatments were applied to wild colonies between May 23, 2018 and October 14, 2018. Treatment applications were initially separated into four trials, but analyses combine these to present a more complete picture. The trials, as well as the number of colonies and treatments associated with each, are listed in Table 1.

Differences in objectives led to two different strategies in treatment applications among projects. The primary goal for treatment of the rare and declining *Dendrogyra cylindrus* (Projects 1, 2) was to prevent tissue loss using best practices from laboratory trials. Treatments were primarily antibiotic-based, though a few amputations were also attempted. Treatments were also repeated as new lesions appeared on already treated colonies. Treatments were conducted on 32

Project	Purpose/Comparisons	# colonies	Species	# lesions
Sombrero: DCYL	Rescue. Trench vs Margin, Amputations	3	DCYL only	32
Coffins/Critter: DCYL	Rescue. Patch vs Lesion, Trench vs. Margin	11	DCYL only	30
Sombrero/Coffins/ Critter: Non-DCYL	Test. Chlorine vs Amoxicillin. Comparison of bases	16	CNAT (7). DLAB (3). OFAV (2). MCAV (1). PSTR (1). SSID (1). MMEA (1).	53
Looe: Non-DCYL	Test (amoxicillin). Shea vs. Base 2b	7	DLAB (4). OFAV (1). PSTR (1). PCLI (1).	10
TOTAL		37		125

Table 1. Summary of field trials in the Middle and Lower Keys. Number of colonies, number of lesions, species, and comparisons from each trial are presented.

[1] Neely, KL. 2018. Ex-Situ Disease Treatment Trials. Final Summary Report for DEP. https://floridadep.gov/sites/default/files/Ex-Situ-Disease-Treatment-Trials.pdf

[2] Walker BK and A Brunelle. 2018. Southeast Florida large (>2 meter) diseased coral colony intervention summary report. Final Summary Report for DEP. https://floridadep.gov/sites/default/files/Large-Coral-Disease-Intervention-Summary-Report.pdf

disease lesions across 3 colonies at Sombrero Reef, and on 30 lesions across 11 colonies at Coffins Patch and Critter Ridge.

Treatments on other species were primarily conducted with the goal of comparing different treatment strategies. Colonies were treated only at one time point, and a variety of methods were employed. Primary comparisons at Sombrero, Coffins, and Critter Ridge (N=53 lesions) were between antibiotic- and chlorine-based treatments packed into a variety of delivery bases. Primary comparisons at Looe Key were between shea butter and CoreRx Base2b amoxicillin mixtures (N=10 lesions). In total, 125 lesions over 37 colonies representing 9 species were treated and monitored.

Treatments were opportunistic based on disease presence during the site visits; sample sizes vary accordingly. Monitoring design was set for weekly samples for the first month, bi-weekly sampling for a subsequent two months, and monthly sampling for an additional three months. Because of the opportunistic nature of treatments, as well as weather and logistical considerations, this varied slightly. However, for all lesions, monitoring was frequent in the weeks immediately following treatment, and less frequent later on. To date, monitoring of colonies has ranged from 8 to 30 weeks.

Test treatments included the following:

- Amoxicillin powder mixed with shea butter (1:16 by weight)
- Amoxicillin powder mixed with a specially-formulated silicone-based “CoreRx Base2b” (1:16 by weight)
- Amoxicillin powder mixed with Sargent Art Plastina modeling clay (1:16 by weight)
- Chlorine powder mixed with Splash Zone Epoxy (3:10 by volume)
- Chlorine powder mixed with Aves Apoxie (3:10 by volume)
- Chlorine powder mixed with Sargent Art Plastina modeling clay (3:10 by volume)
- Amputation of apparently healthy tissue for transplantation away from the infected colony
- Amputation of diseased tissue from nearby healthy tissue

Distribution of these treatments among lesions and species is outlined in Table 2.

	DCYL	CNAT	OFAV	DLAB	PSTR	MCAV	MMEA	SSID	PCLI	Total	
Amoxicillin + Shea	12	6	2	4		2	1	1	1	29	
Amoxicillin + Base	45	1	6	3	2					57	Total Amoxicillin
Amoxicillin + Clay							1			1	87
Chlorine + Zspar		7	4	2	1			1		15	
Chlorine + Aves		4	4	1	1					10	Total Chlorine
Chlorine + Clay		3	2	1	2					8	33
Amputation of healthy	3									3	Total Amputation
Amputation of diseased	2									2	5
Total	62	21	18	11	6	2	2	2	1	125	

Table 2. Summary of treatment types on lesions across species at all sites. A total of 87 amoxicillin treatments, 33 chlorine treatments, and 5 amputation treatments were applied.

Several variables appear to potentially affect treatment effectiveness, and current sample sizes do not allow for statistical analyses of each variable. To address potentially confounding variables, figures throughout this report are presented in two parts. Bar or area charts compare each primary variable of interest, but each is also paired with pie charts showing how colonies are distributed throughout the other variables in order to show similarities and differences. The primary variables of interest are:

1. Treatment type. Comparisons of amoxicillin and chlorine are presented, as are comparisons of application bases within each treatment type and the use of trenching and clay overlays on amoxicillin treatments
2. Coral species. Differential success of treatments is tied to species.

3. Site. Failure rates may vary among reef sites. From North to South, sites are: Coffins Patch/Critter Ridge, Sombrero, Looe (see Fig 12 for map)
4. Lesion size. Depending on the size of each lesion, treatments consisted of either direct topical application to the disease margin, a trench cut into presumably healthy tissue ~5 cm from the disease margin and packed with the treatment, or a combination of both. For the purposes of this report, "patches" are referred to as small lesions (<5 cm in diameter) on which the treatment was applied to cover the entire disease margin; "lesions" are >5 cm diameter diseased areas on which the treatment was applied to the margin of the diseased area and, in all chlorinated treatments and some amoxicillin treatments, also to a trench carved around the disease margin.

Results are presented across time (weeks since treatment), with each time stamp showing the number of colonies in which the treatment was identified as:

1. Ineffective: lesion has progressed across/past the treatment line and is proceeding unimpeded across the colony. For treatments that include a firebreak treatment as well as a margin, failure indicates progression past both barriers.
2. Overtaken: the treatment has not failed, but the tissue directly on the protected side of the treatment is killed by an external lesion that developed elsewhere on the colony.
3. Effective: the tissue on the protected side of the barrier is still healthy at the time of monitoring.

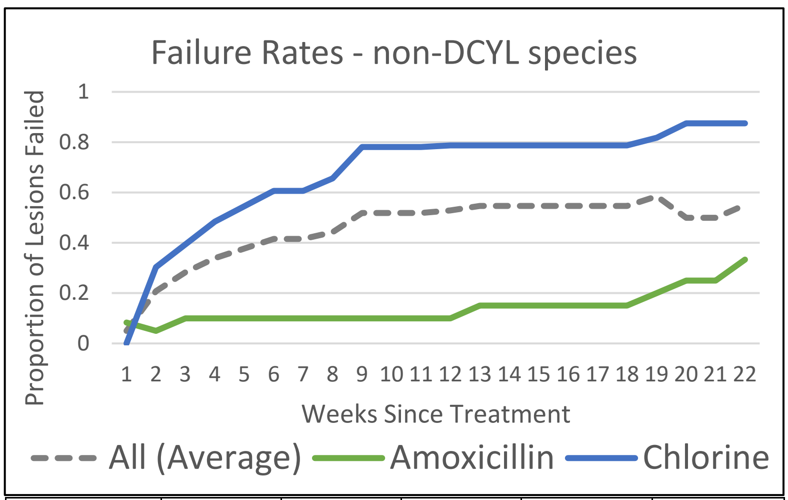
Because monitoring for all colonies was not uniform (e.g., some colonies were monitored at 4 weeks, and others at 5 weeks), the observation (ineffective, overtaken, effective) for each colony was extended across the subsequent weeks until the next observation was made. This inherently oversamples effectiveness. For example, if a treatment is deemed effective at week 4 and then ineffective during the next monitoring at week 8, the figures and analyses will show effectiveness of the treatment until week 8, regardless of the actual time after week 4 in which the treatment failed. Extrapolations were not made past the latest observation, so sample size of lesions decreases through time.

Results:

Chlorine vs. Epoxy

Overall, antibiotic treatments were more successful than chlorine treatments, both in lower overall failure rates and in length of time before failure. Figure 1 compares the failure rates over time of amoxicillin treatments and chlorine treatments on all non-*D. cylindrus* lesions. The figure omits *D. cylindrus* treatments because they were entirely amoxicillin based, with no chlorinated treatments to provide a comparison.

In acknowledgement that other factors may be confounding the failure rates of different treatments, all suspected variables are shown in Figure 2 for each treatment type. Note that antibiotic treatments were heavily biased towards *D. cylindrus* colonies and had a large proportion of treatments in the Coffins/Critter area. In contrast, chlorinated treatments were more heavily employed on *C. natans* colonies and were predominantly at Sombrero.



	Week 2	Week 4	Week 8	Week 12	Week 16
Amoxicillin	13%	15%	21%	16%	19%
Chlorine	30%	49%	66%	79%	79%

Figure 1: Proportional failure rates of amoxicillin and chlorine treatments in non-*D. cylindrus* species

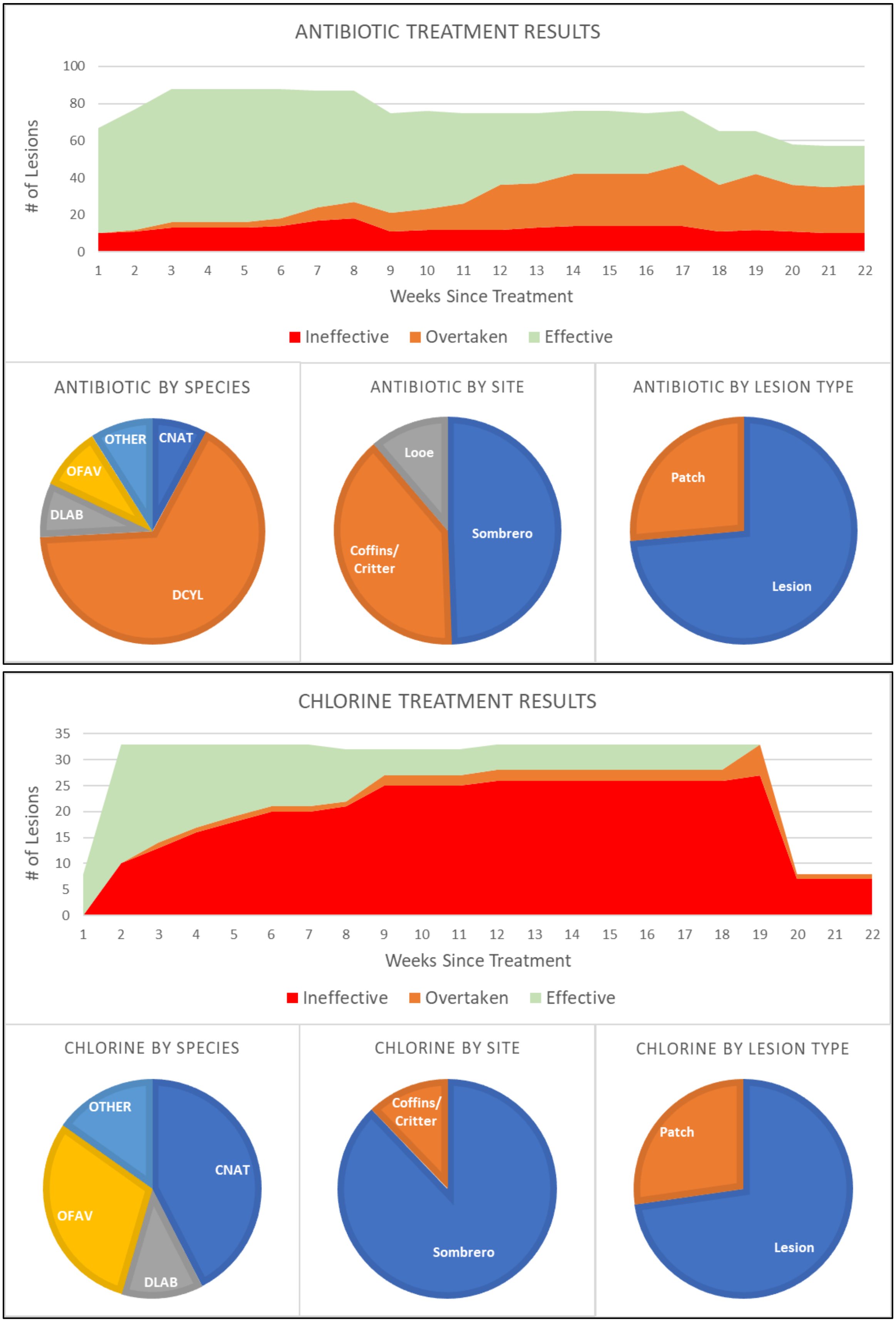


Figure 2: Comparison of ineffective/overtaken/effective rates on lesions in weeks following disease treatments. Comparisons are between all lesions treated with antibiotics and those treated with chlorine. Distributions of treatments among other variables (species, site, and patch/lesion) are shown for comparison.

These differences in the species treated (number of brain corals) and site distribution (proportion in endemic vs epidemic zones) between treatment types may have contributed to higher apparent failure rates in chlorine. However, when chlorine vs amoxicillin treatments are separated by species, results show that for all species in which both types of treatments were performed (N>5) (CNAT, OFAV, DLAB), failure rates are lower with amoxicillin treatments than with chlorinated ones (Fig 3).

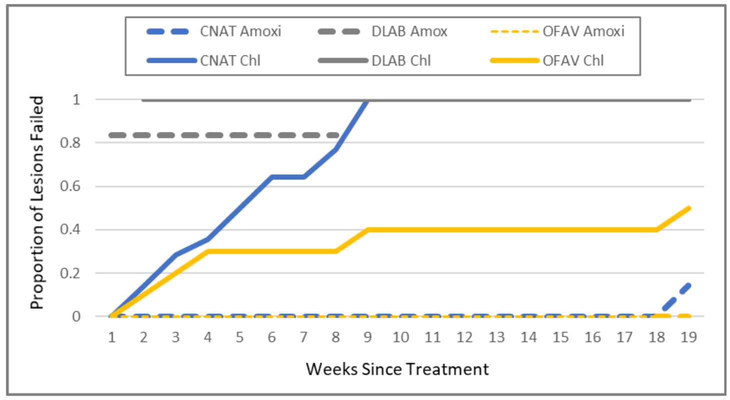


Figure 3: Failure rates over time for amoxicillin (dash) and chlorine (line) treatments. Failure rates vary by species, but each species (CNAT, DLAB, OFAV) has fewer amoxicillin failures than chlorinated ones.

Chlorine Bases: Aves Apoxie vs. Modeling Clay vs. Splash Zone Epoxy

Chlorine was mixed into three different bases for experimental comparisons of effectiveness: Aves Apoxie, modeling clay, and Splash Zone Epoxy. Failure rates of all three chlorinated bases were high, exceeding 40% by week 4 and 75-80% by week 9 (Fig 4). Any differences in product effectiveness is likely masked by the overall failure rates. As such, none of these applications are recommended for successful interventions.

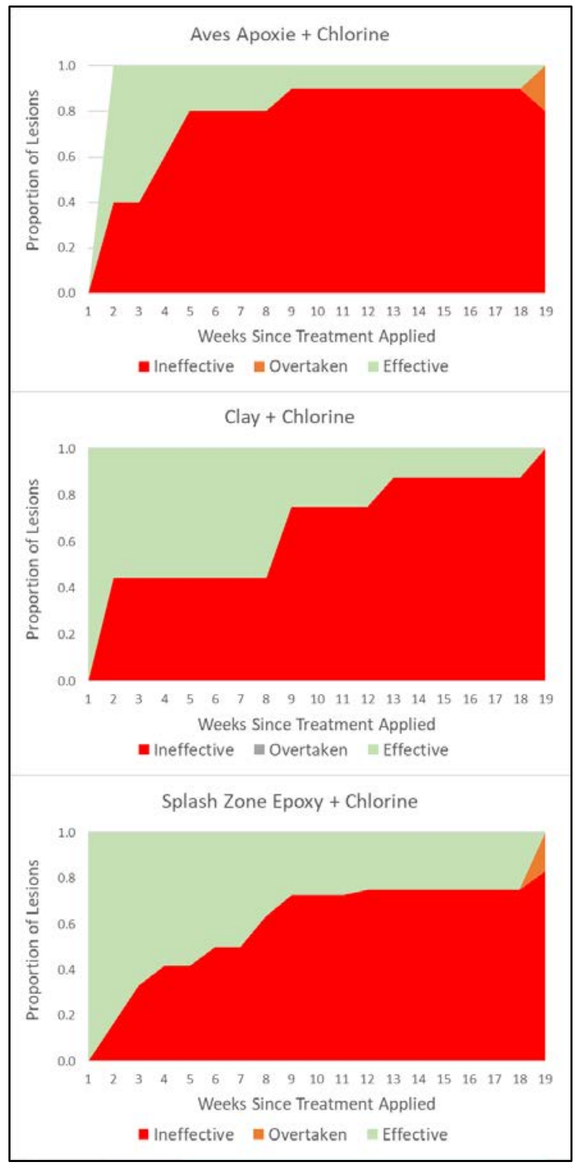


Figure 4. Proportional failure rates of chlorine mixed with three different bases. Failure rates are similar for all base types.

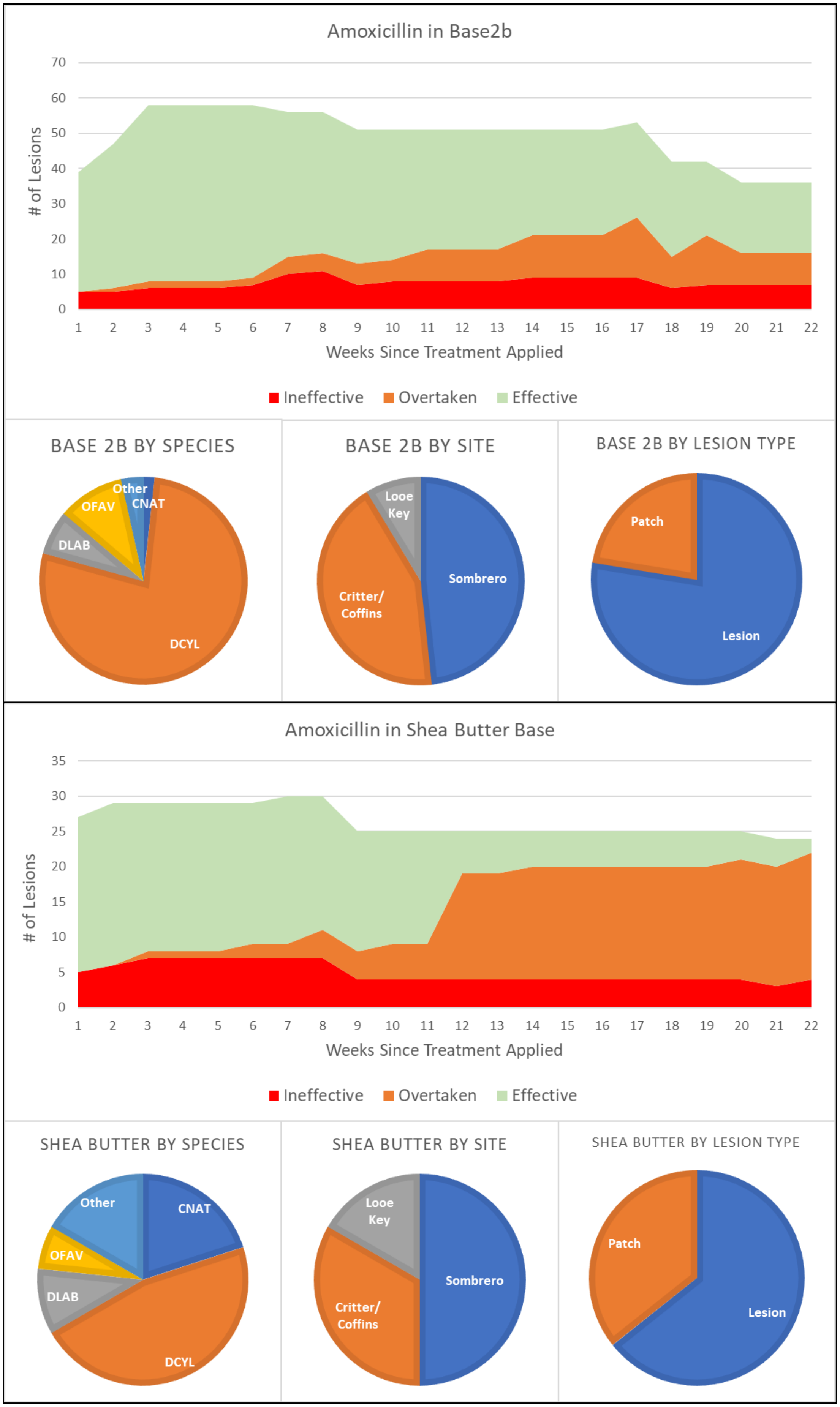


Figure 5. Comparison of the number of ineffective/overtaken/ effective lesion treatments over time between amoxicillin mixed with CoreRx Base2b formula, and amoxicillin mixed with shea butter. Distributions of treatments among other variables (species, site, and patch/lesion type) are shown for comparison.

Amoxicillin Bases: CoreRx Base2b vs. Shea Butter

Corals treated with CoreRx Base2b and with shea butter had similar distributions among sites and also between lesions and patches (Fig 5). Base2b treatments were more common on *D. cylindrus*, while shea butter included more *C. natans* treatments.

Failure rates in the first eight weeks were similar though slightly higher for for shea butter treatments compared to Base2b treatments. However, of note is the rapid increase in treatments that are overtaken by external lesions on the shea butter treatments after 11 weeks, which is not observed in the Base2b treated lesions (Fig 5). One hypothesis is that the amoxicillin is generally effective at treating the lesions to which it is applied, regardless of the base it is incorporated into. However, shea butter may limit delivery to the region directly under the application, while the Base2b, which is specially formulated to facilitate easy transfer of the antibiotic into coral tissue, may allow for wider distribution of product that halts the development, spread, or encroachment of lesions near the application site. Some further evidence of this may be indicated by patterns of treated lesions halting as they approach amoxicillin barriers, and by encroaching lesions halting some distance (usually a few cm) from the application (Fig 6).

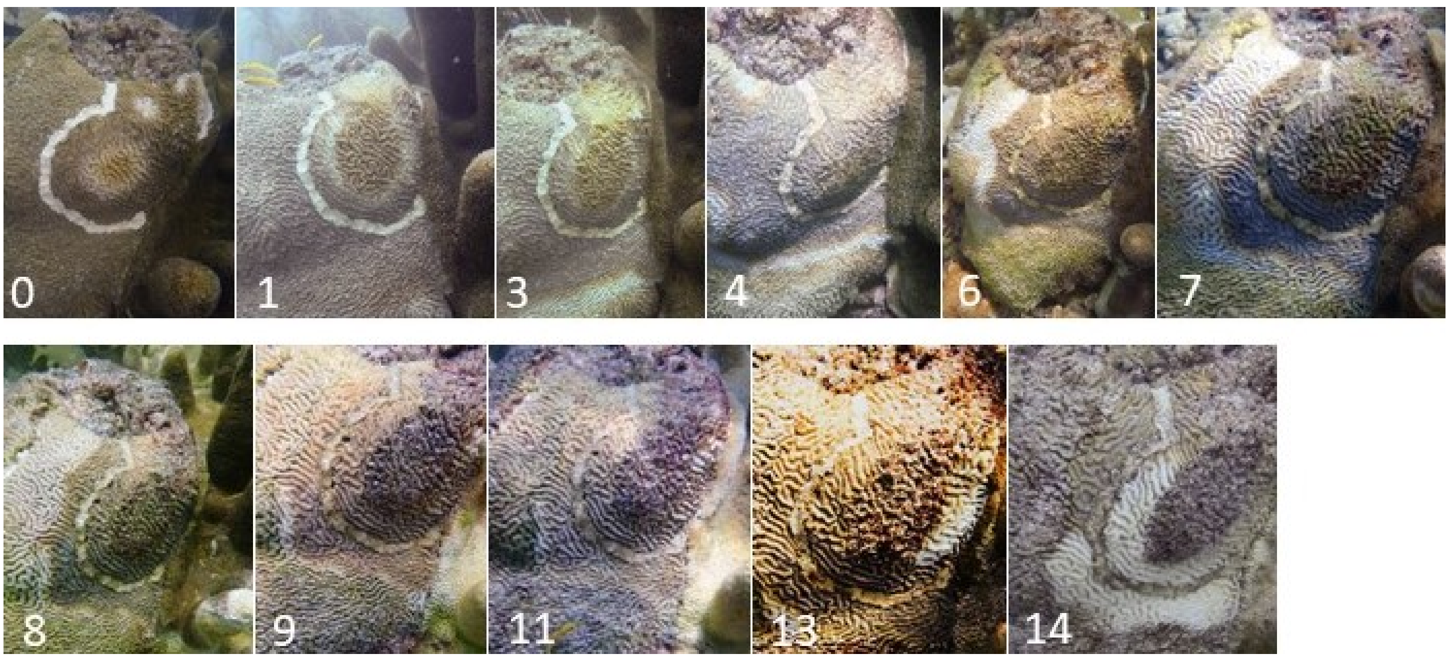


Figure 6. Progression of disease around an antibiotic treatment line. Numbers represent weeks after treatment. Advancement of the initial lesion halted a few centimeters from the treatment line. External lesions approached the treatment line but ceased nearby, leaving a characteristic living tissue "halo" around the treatment. However, in weeks 13-14, renewed infection led to mortality of the previously protected tissue.

Amoxicillin: Covering with Clay

Colonies in which clay was applied over the amoxicillin mixture had lower failure rates than those without clay (Fig 7). However, factors that may confound this perceived effectiveness include:

- Coral species: Lesions without clay were predominantly DCYL, while those with clay were a more evenly distributed mix of species.
- Types of lesions: treatments without clay were predominantly lesions, while treatments with clay were predominantly patches, which are generally more effective.
- Site: Lesions without clay included all of the Looe Key treatments, which were generally less effective
- Application base: Lesions without clay were predominantly treated with a Base2b mixture, while those with clay were predominantly shea butter.

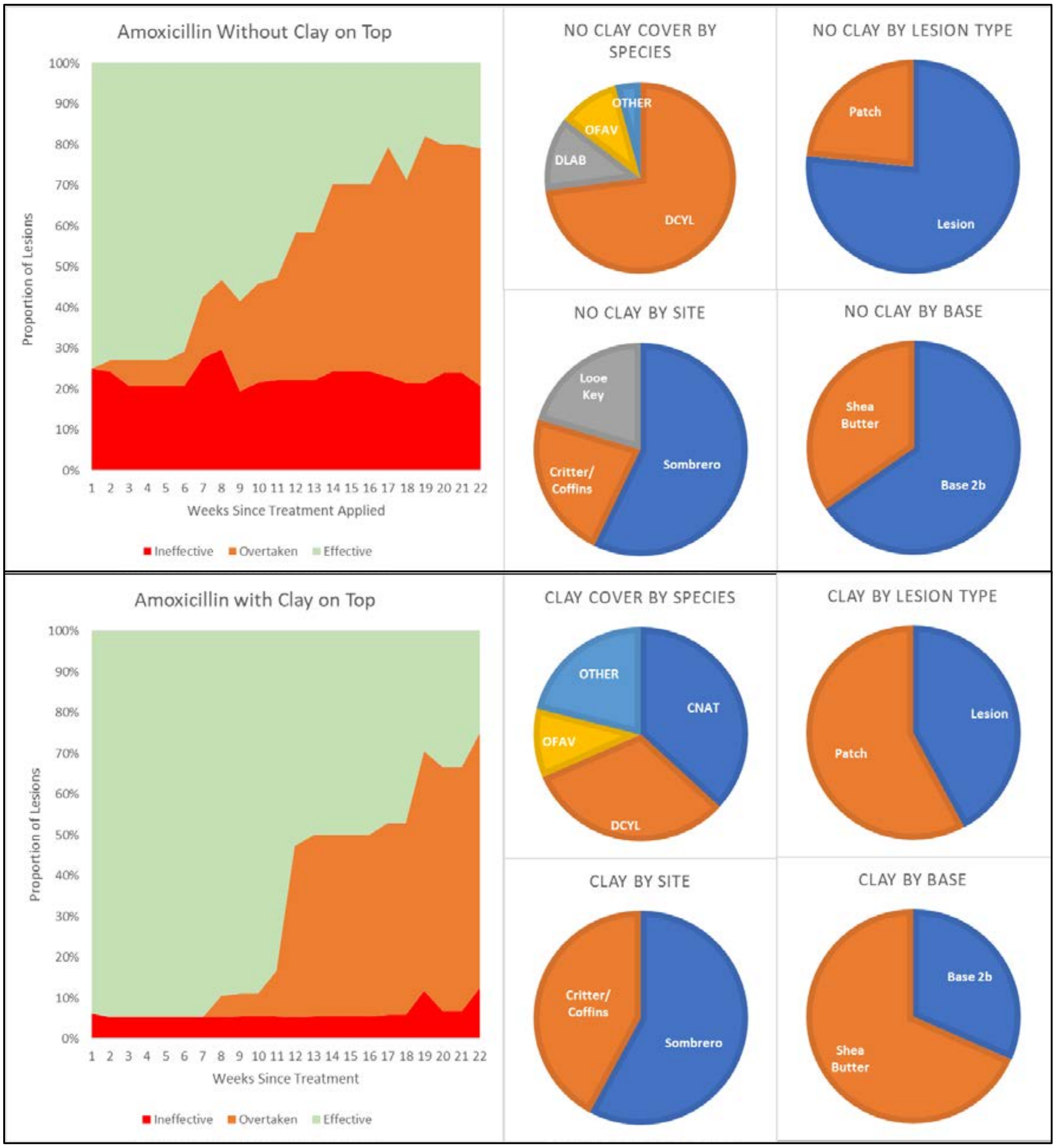


Figure 7. Comparison of failure rates between amoxicillin treatments without clay (N varies between 24-48) and those covered with clay (N varies between 15-19). Comparisons of treatments among other variables are presented as pie charts.

In short, the variability amongst these factors makes it difficult to determine whether this perceived difference in effectiveness with clay coverings is real, or a result of the confounding factors. Further experimentation is advised.

Amoxicillin: Trenching

Treatments without a trench exhibited a slightly higher failure rate than those with a trench (Fig 8). Confounding variables that differ between the two types of treatment include site (no trenching treatments were conducted at Looe Key) and lesion type (treatments with trenches did not include any patches). These differences suggest that trenches might be more effective, but warrant further experimentation.

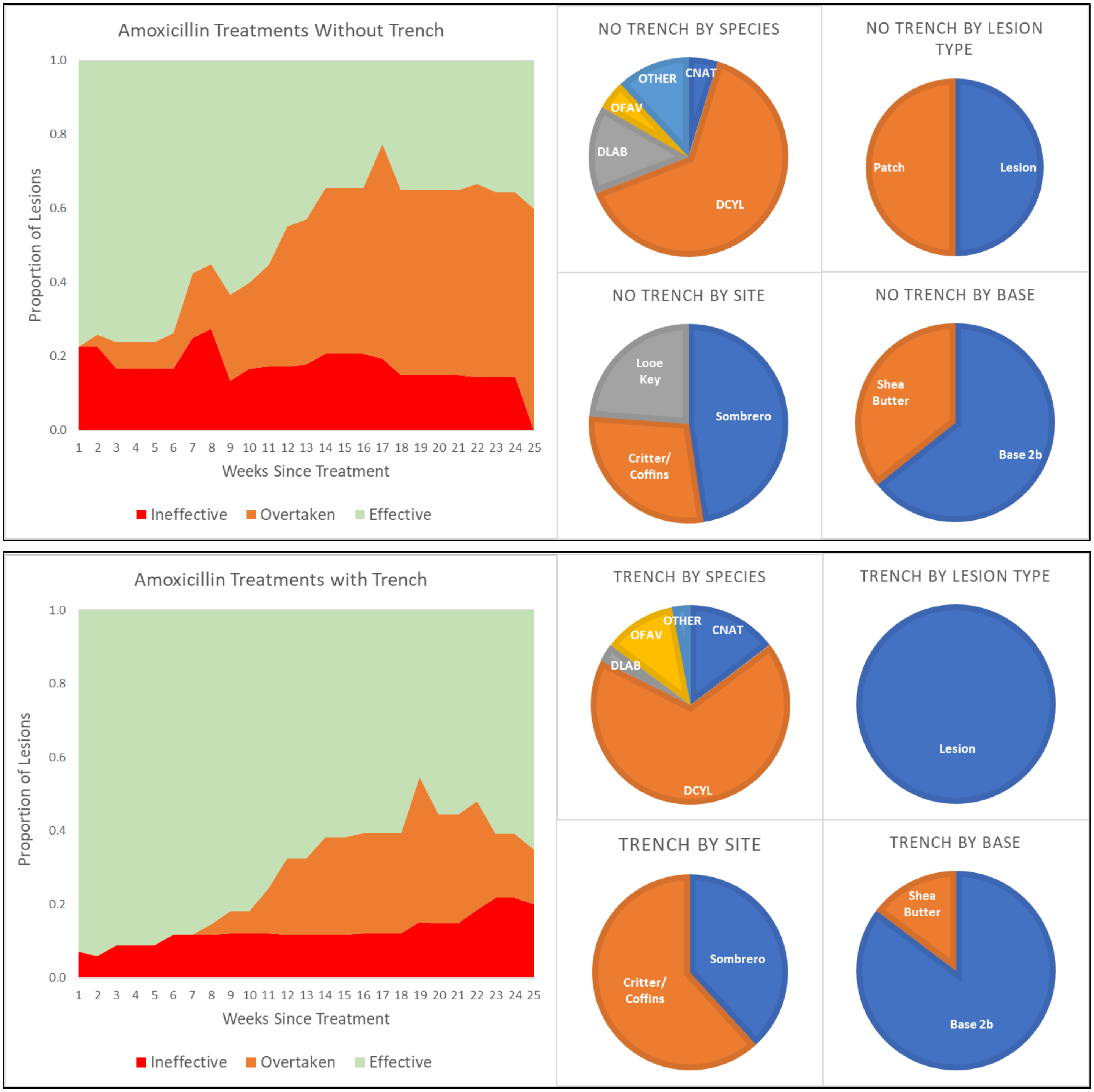


Figure 8. Comparison of failure rates between amoxicillin treatments within trenches (N varies between 25-35) and amoxicillin treatments not within a trench (N varies between 20-42). Comparisons of treatments among other variables are presented as pie charts.

Amputations

Two diseased *D. cylindrus* pillars were amputated and transported ashore for bleaching and disposal. The tissue at the base of the amputations remained healthy for 11 weeks until new lesions elsewhere on the colony overtook the area. Based on these two instances, we hypothesize that removal of diseased lesions can be an effective method for halting a progressing lesion.

Three healthy *D. cylindrus* pillars were removed from a heavily diseased colony and transplanted ~30 meters away. These transplants had poor survival, with two of the three diseased and dead within two weeks. The third exhibited disease after five weeks and was treated with amoxicillin. It showed initial recovery, but was also dead within 19 weeks. We hypothesize that amputation from a diseased colony is only likely to be effective if the tissue is already uninfected, which may be difficult to ascertain, and also unlikely to become immediately reinfected.

Lesions vs Patches

Small (< 5 cm) lesions that are fully covered exhibit lower failure rates than larger lesions that require more extensive treatments. Species compositions, site, and treatment type were similar across treatments on these two lesion types. However, after two weeks, failure rates on lesions were nearly 25%, but only 7% on patches. Longer-term failure rates were similarly higher on lesions after four months (44%) than on patches (19%) (Table 3, Figure 9).

	Week 2	Week 4	Week 8	Week 12	Week 16
Patch	7%	9%	9%	16%	19%
Lesion	24%	29%	42%	42%	44%

Table 3: Comparison of failure rates between all patches and all lesions over time.

It is unclear why these differences might occur, but we hypothesize that infection is either less established in small lesions and thus responds more to treatment, or that smaller treatments are more likely to create complete barriers to prevent spread, unlike larger lesion treatments which may have "weak points" at which disease can slip past and continue infecting protected tissue.

Species:

A total of nine species were treated in these field trials. Four of these (CNAT, DCYL, DLAB, and OFAV) had over ten lesions treated and are suitable for comparison. Failure rates were markedly different between species. DLAB colonies had over 75% failure within two weeks. CNAT treatments failed more slowly, but neared 50% within 6 weeks. DCYL and OFAV treatments responded with lower failure rates, not exceeding 25% even after 18 weeks (Fig 10).

These results are slightly confounded by location and treatment type (Fig 11). DCYL treatments were exclusively amoxicillin, which likely contributed to this lower failure rate compared to other species. Treated DLAB lesions were primarily at Looe Key, which may respond more poorly than other sites due to its epidemic disease status. However, we note that DLAB will generally be found only in epidemic zones, and thus these results exemplify what might be typical of *in situ* treatment of this species.

CNAT and DCYL colonies exemplified very high rates of "Overtaking" by external lesions. As visually confirmed at many sites, diseased DCYL and CNAT diseased colonies are generally characterized by a high number of lesions. Treatments of lesions on these species may be moderately successful, but protected tissues are very often overtaken by external lesions. Within the scope of this study, OFAV stands out as a species that responds well to treatments. After 4 weeks, only three of ten chlorinated lesions had failed, and even up to 18 weeks, none of the amoxicillin treatments had failed (N=8 to eight weeks, N=6 to eighteen weeks).

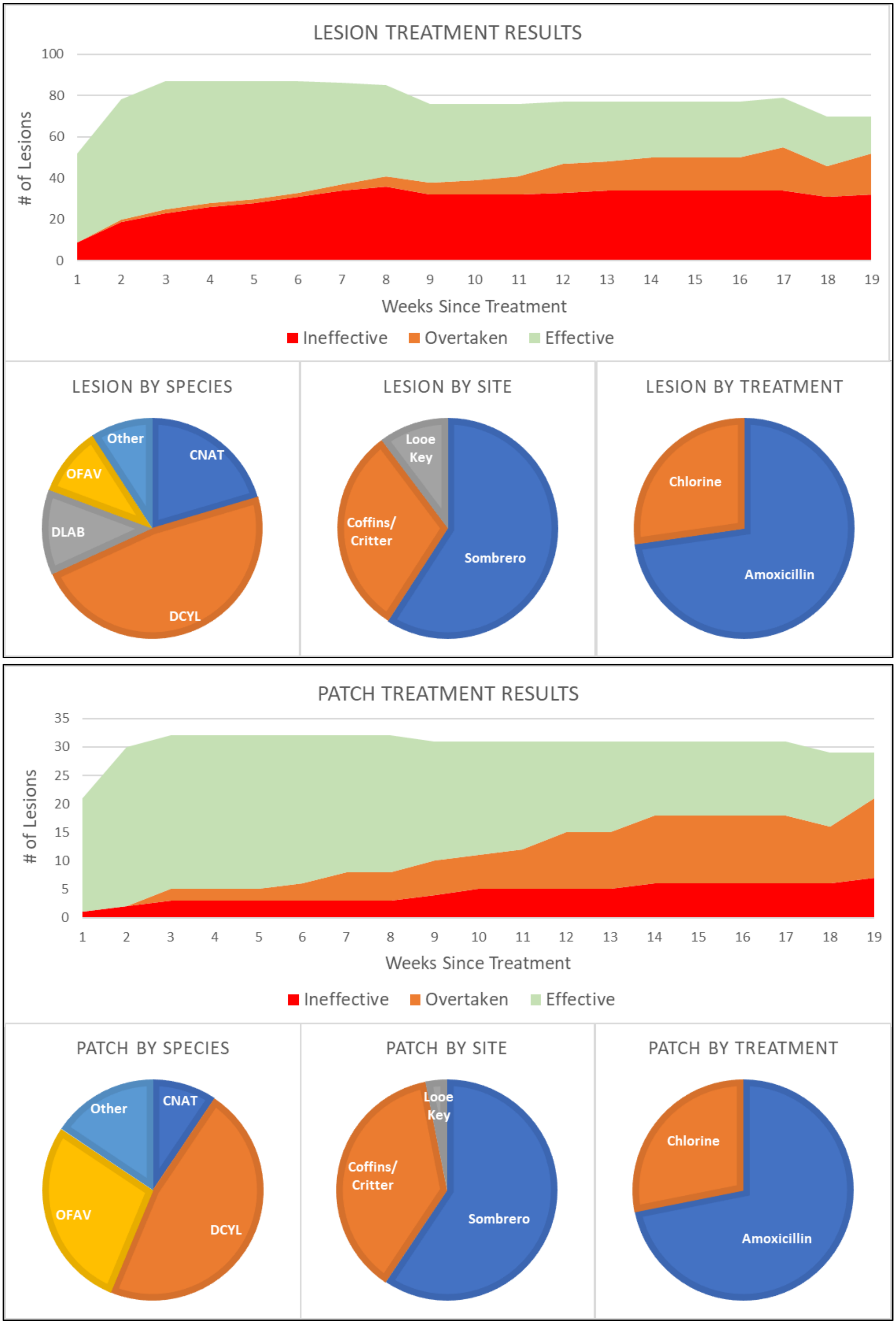


Figure 9. Comparison of failure rates between treatments on lesions (long linear disease margins) and patches (< 5 cm spots). Distribution of patch/lesion treatments are shown across other potentially confounding variables within the pie charts, but are overall similar.

Appearance of new lesions on OFAV colonies does occur, but at a slower rate. Lesion progression rate is also slower, which allows for new lesions (or failed treatments) to potentially be treated before overtaking a large portion of a colony.

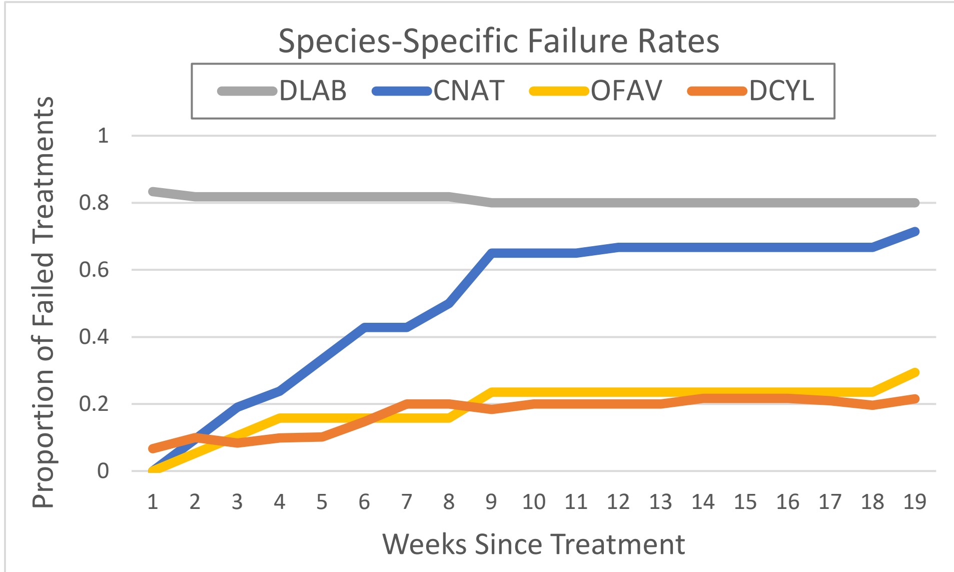
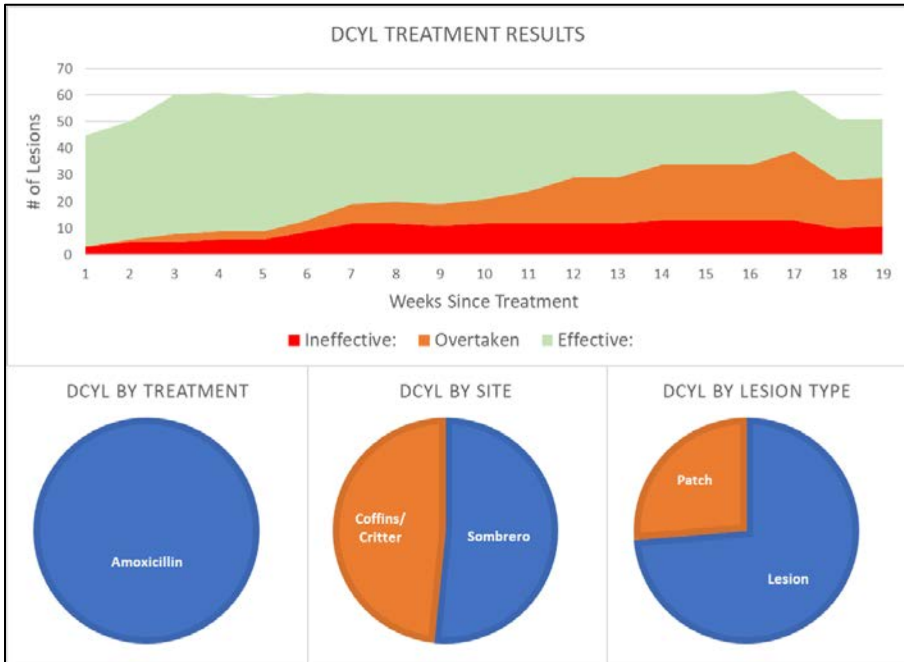


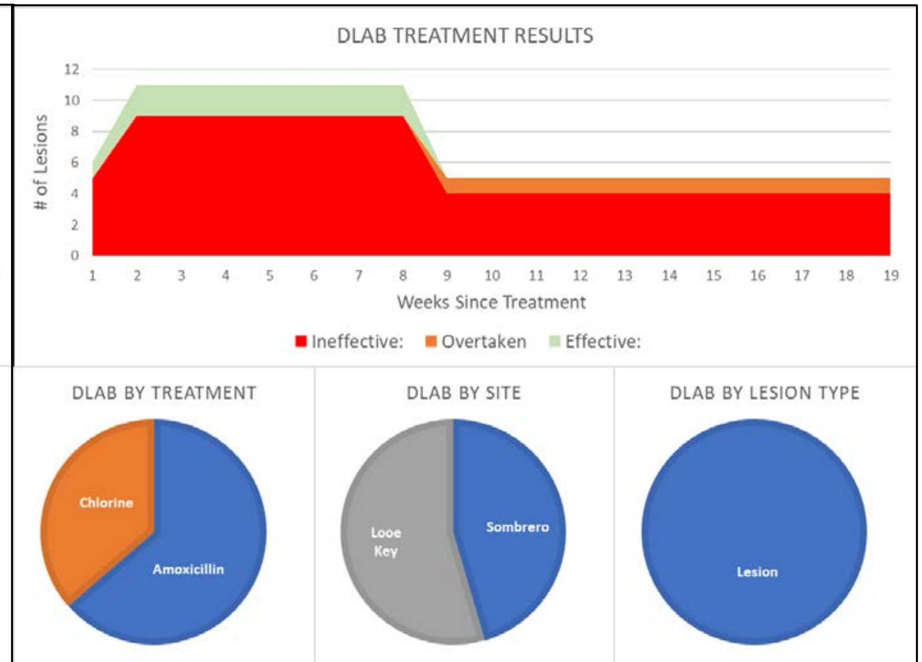
Figure 10. Comparison of lesion treatment failure rates among four frequently treated species. Sample sizes and confounding variables are shown in Fig 12.

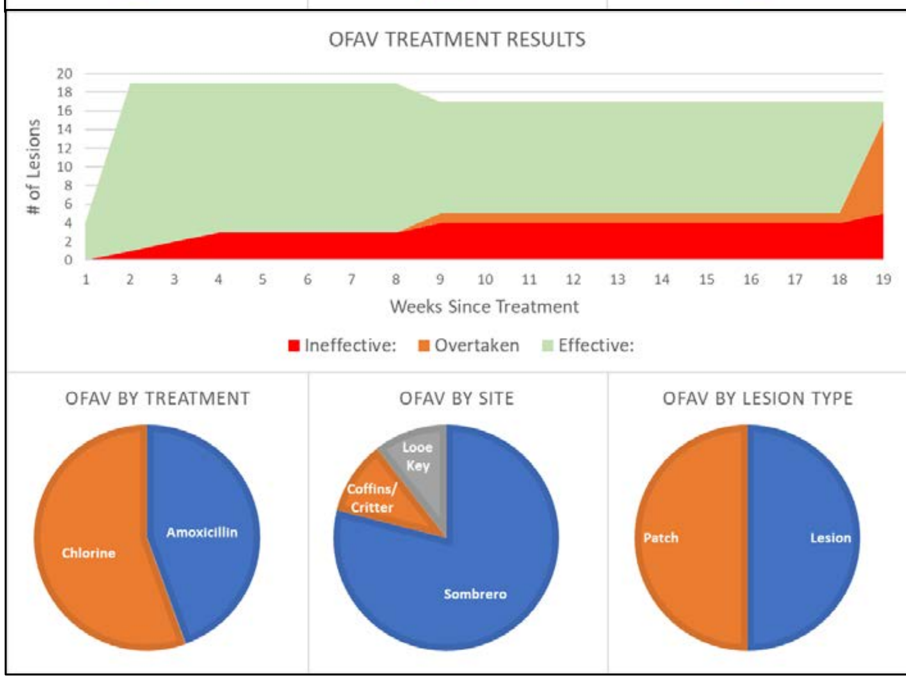
Site:

Responses to treatments varied among the three treatment locations (Fig 12). These results are complicated by large variations in species treated and types of treatment, but nevertheless the pattern should be considered. Coffins/Critter Ridge treatments (primarily DCYL) were the most successful, with failure rates of less than 20% up to 22 weeks. This was largely driven by low failure rates of amoxicillin treatments on DCYL at Coffins Patch. Treatments on a single SSID, OFAV, and MCAV within the region were also largely successful. Treatments on a single CNAT at Coffins (the only brain coral treated in the area) were unsuccessful. At Sombrero Reef, which was experiencing an epidemic during treatment, treatments were overall less successful, with failure rates exceeding 40% by week 9. Further south, within the early epidemic zone of Looe Key, treatments were solely amoxicillin-based, but were also focused primarily on DLAB. Failure rates at this site, possibly compounded by the heavy influence of poor DLAB response to treatment, were at 60% only 1 week after treatment.

Figure 11 (next page). Number of treated lesions documented as ineffective, overtaken, and effective during each monitoring period, split among the four most treated species. Distributions of treatments among treatment type, site, and lesion type for each species are presented as pie charts.


DCYL TREATMENT RESULTS
# of Lesions
0 10 20 30 40 50 60 70
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Weeks Since Treatment
Ineffective: Overtaken Effective:
DCYL BY TREATMENT
Amoxicillin
DCYL BY SITE
Coffins/ Critter
Sombrero
DCYL BY LESION TYPE
Patch
Lesion


DLAB TREATMENT RESULTS
# of Lesions
0 2 4 6 8 10 12
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Weeks Since Treatment
Ineffective: Overtaken Effective:
DLAB BY TREATMENT
Chlorine
Amoxicillin
DLAB BY SITE
Looe Key
Sombrero
DLAB BY LESION TYPE
Lesion


OFAV TREATMENT RESULTS
# of Lesions
0 2 4 6 8 10 12 14 16 18 20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Weeks Since Treatment
Ineffective: Overtaken Effective:
OFAV BY TREATMENT
Chlorine
Amoxicillin
OFAV BY SITE
Looe Key
Coffins/ Critter
Sombrero
OFAV BY LESION TYPE
Patch
Lesion

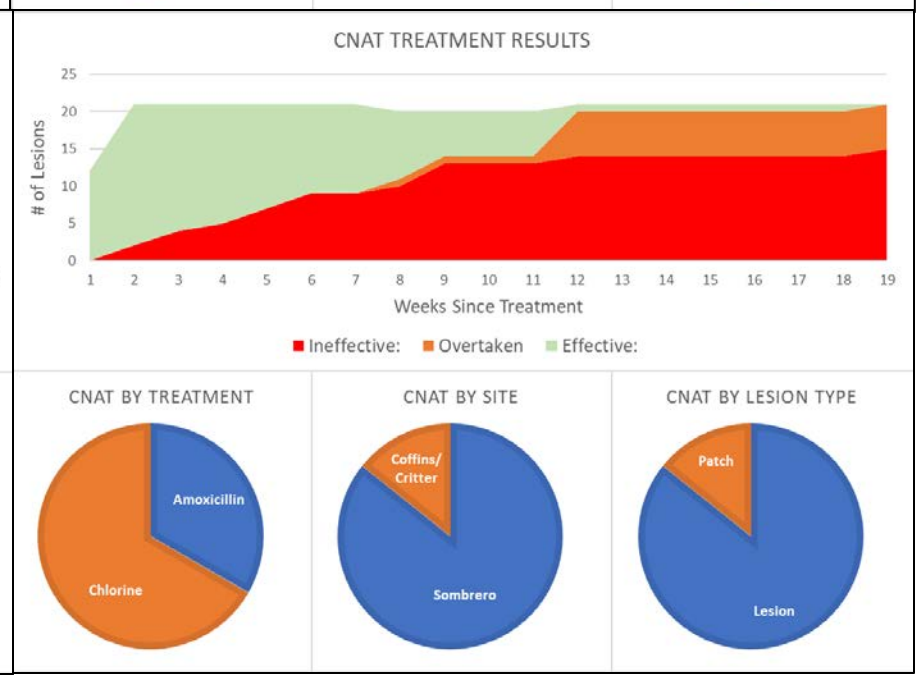
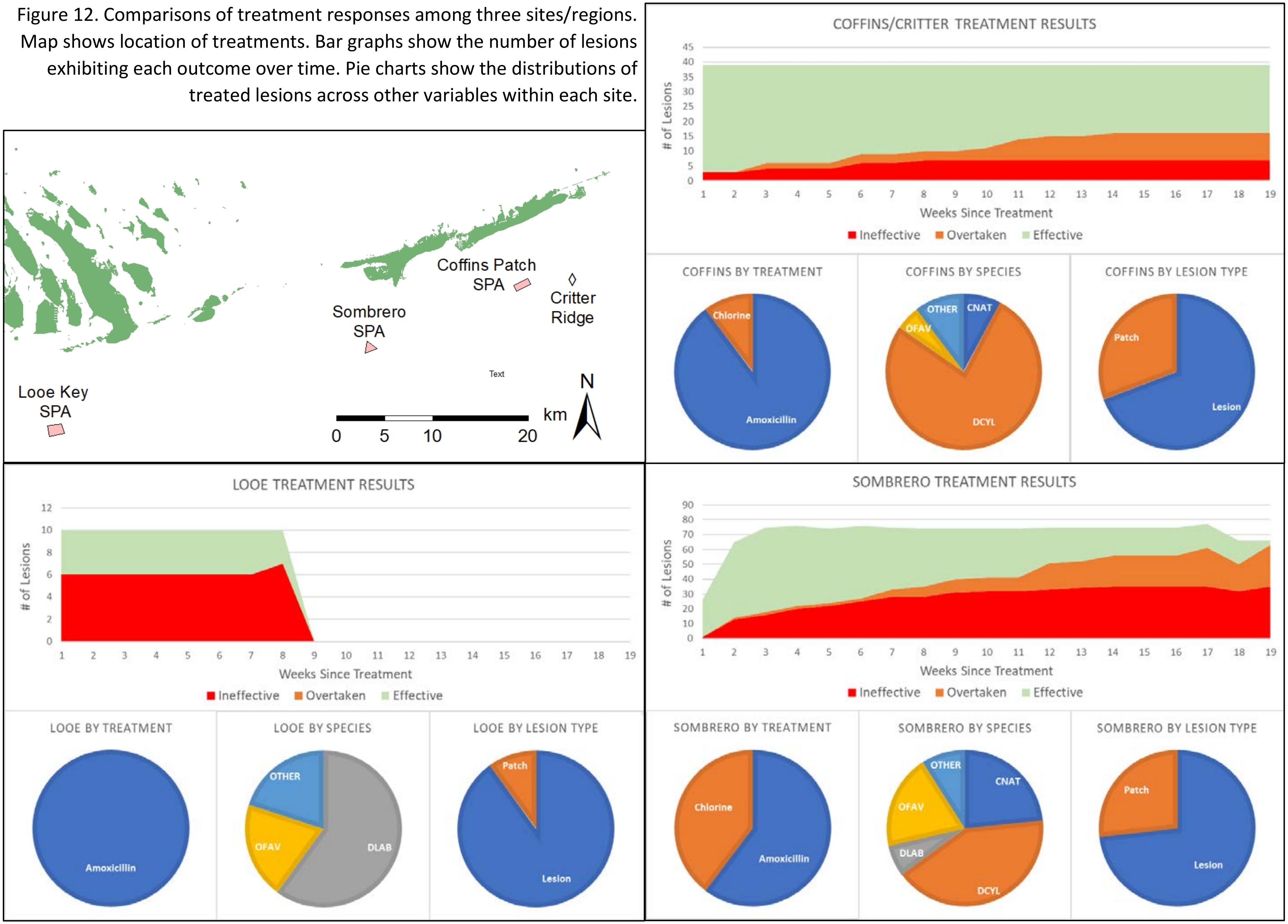

CNAT TREATMENT RESULTS
# of Lesions
0 5 10 15 20 25
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Weeks Since Treatment
Ineffective: Overtaken Effective:
CNAT BY TREATMENT
Amoxicillin
Chlorine
CNAT BY SITE
Coffins/ Critter
Sombrero
CNAT BY LESION TYPE
Patch
Lesion

Figure 12. Comparisons of treatment responses among three sites/regions. Map shows location of treatments. Bar graphs show the number of lesions exhibiting each outcome over time. Pie charts show the distributions of treated lesions across other variables within each site.



Conclusions:

- Amoxicillin treatments are much more effective than chlorinated treatments. This effectiveness is seen in summary analyses, but also when treatments among species (CNAT, OFAV, DLAB) are compared.
- Within chlorine treatments, the three tested bases (Aves Apoxie, modeling clay, and Splash Zone Epoxy) all had similar high failure rates.
- Within amoxicillin applications, the CoreRx Base2b and the shea butter both appear to be similarly effective at the onset, but subsequent encroachment of external lesions is much lower on Base2b-treated corals. This may be a result of better dissemination of the product to adjacent tissue.
- Results suggest that trenching and clay application over the treatment may contribute to lower failure rates, but these should be further tested to confirm.
- Very limited amputation work on DCYL suggests that removal of diseased tissue may help prevent transmission to adjacent healthy tissue. It also found transplantation of healthy tissue away from a diseased colony to be ineffective, though whether this is a result of new infections or already-existing infection within the transplanted pieces is unknown.
- Small lesions respond better to treatment than large linear ones.
- Response to treatments is species-specific. Brain corals (particularly DLAB) respond poorly, while OFAV responds well.
- Location of treatments within the disease zones (endemic, epidemic) may influence success rates, with colonies in epidemic/outbreak areas exhibiting lower treatment success than endemic zones. This may be due to the species that remain to be treated (OFAVs in the endemic zone vs poorly responding DLABs in the epidemic zone), resiliency of any susceptible individuals that are still alive within the endemic zone, lower pathogen load in the endemic zone, or other factors.
- Even the most effective treatments are acting only at the lesion level. New lesions continue to erupt and overtake treatments, leading to ongoing tissue loss as well as necessary frequent revisitation to apply additional treatments.
- Schedules for retreatment and monitoring vary based on species and perhaps disease status. Epidemic zones and highly susceptible species require more frequent monitoring to determine effectiveness, and more frequent retreatments to catch new lesions or repair broken treatment lines.

Suggestions for future work:

- Efforts were confounded by numerous (and initially unexpected) variables that could be teased out with additional work. In particular, addressing whether species like OFAV that respond well to treatment would also respond well in epidemic or invasion zones would prioritize these corals before they experience significant tissue loss, and while lesions are still small might better respond to treatments.
- Only one MCAV was treated as part of this work. Targeting this species in zones where it exists in treatable form and numbers would help identify effectiveness on this priority species.
- Further comparisons of amoxicillin treatments with and without trenching or the application of clay to cover the treatments would elucidate the effectiveness of these additional measures.
- Reef-scale intervention may be considered on small patch reefs by treating all lesions within an isolated region to determine whether the elimination of lesions in such an environment could have reef-scale consequences.
- Treatments are all occurring at a lesion level, but infections are occurring at colony and reef scales. Treatments of this type are capable of targeting only a minute fraction of diseased corals and, even if effective, will need to be repeated for potentially years until the outbreak concludes. A high priority research topic should be to address intervention at the colony or reef level, and management questions relating to this should be considered well in advance. Potential options may include antibiotic dosing by bagging or medicated feed, and could include probiotic application or phage therapy.