EX-SITU DISEASE TREATMENT TRIALS

Florida Department of Environmental Protection
Coral Reef Conservation Program

Ex-Situ Disease Treatment Trials

Final Summary Report

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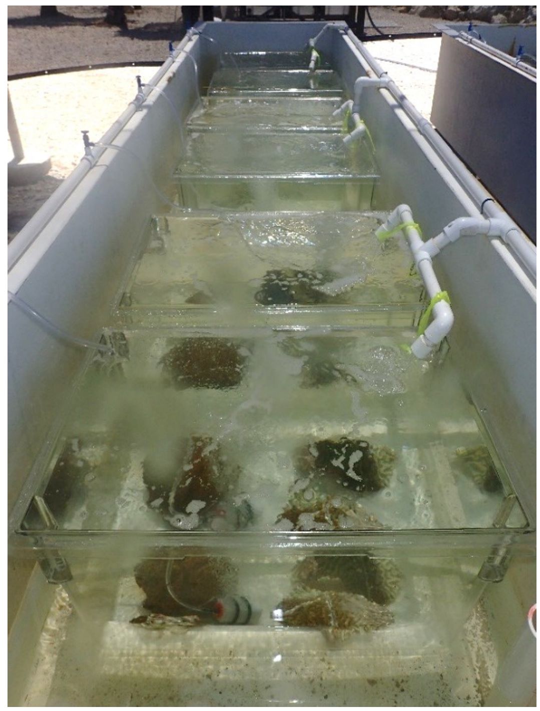
Experiment Overview:

Since 2014, the Florida Reef Tract has been experiencing a disease-related coral die-off that has affected numerous scleractinian species and been unprecedented in its geographic and temporal scope. As a potential management tool, treatment options are being explored to address infections at a colony level. Prior efforts at field treatments for other coral diseases have included aspiration/shading, barriers via epoxy bands or chiseled trenches, and barriers with the addition of chlorine powder; efforts in previous laboratory environments have included the use of antibiotics.

Beginning in December 2017, a series of treatment trials were conducted at Keys Marine Lab (Long Key) using sets of flow-through tanks supplied from a deep-water saline well. Experiments were conducted in an outdoor facility protected by shade-cloth from full ambient light. Colonies for testing were collected from reefs with high disease prevalence between Long Key and Marathon, administered treatments within 24 hours of collection, and observed for 5-14 days based on disease progression and mortality. Species tested were primarily: *Meandrina meandrites*, *Dichocoenia stokesii*, *Pseudodiploria strigosa*, and *Colpophyllia natans*. *M. meandrites* became more difficult to find as the disease progressed through the region, and so *Montastraea cavernosa* were also used in some trials. For each treatment, individuals from each species were treated and placed in multi-species tanks of the same treatment. Forty-gallon tanks were used for the first rounds of experiments and 22 gallon tanks for the later rounds (Fig 1). Three to four tanks were used as replicates for each treatment.

Fig 1. Experimental setup in treatment tanks.



Treatments, application methods, and dosages for trials conducted to date are outlined in Table 1. In addition to the treatment/delivery combinations outlined in Table 1, controls included colonies placed directly into tanks with no treatment, and also the application of non-treated barriers (either a trench or a band of the delivery vehicle applied directly to the coral tissue). Some of the more promising treatments in Table 1 were also paired with trenching in later trials. Trenching consisted of a 1 cm deep by 0.5 cm wide cut into the tissue with an angle grinder approximately 2 cm inward from the disease line.

	Amoxicillin	Amoxicillin + Kanamycin	Chlorine (73% Hypochlorite)	Marine Salt
Dental Paste	1:16 (weight)			
Z-Spar Epoxy		1:8 (weight)	3:10 (volume)	
Aves Epoxy	1:16 (weight)		3:10 (volume)	
Modeling Clay	1:16 (weight)	1:8 (weight)	3:10 (volume)	1:5 (weight)
Shea Butter	1:8 (weight)			
Vaseline	1:8 (weight)			
CoreRx Silicone Compound	1:16 (weight) 1:8 (weight)			

Table 1. Treatment/delivery combinations tested on diseased coral colonies.

Results:

Trials showed a range of successes depending on the treatment (e.g. antibiotic, chlorine), the delivery vehicle (e.g. epoxy, clay), the use of a trench to separate healthy from diseased tissue, and the species being treated (Table 2).

Overall trends show:

- Control treatment (nothing done) is ineffective (97% mortality)
- A barrier alone (either a trench, an epoxy/clay applied to the coral surface, or a combination of both) is ineffective (95% mortality)
- Chlorine treatment is ineffective (90% mortality)
- High salt concentrations within modeling clay may be effective (56% mortality, but more effective on *M. cavernosa*)
- Antibiotic applied to the surface is largely ineffective (79% mortality).
    - An exception was a combination of amoxicillin plus kanamycin mixed in Z-Spar epoxy to a ratio in a 1:8 ratio by weight (38% mortality).
- Antibiotic use combined with a trench is the most effective field-applicable method to date.
    - When mixed in a silicone base developed by CoreRx, mortality rate was 33%. The treatment was at least partially effective on all tested species.
- Full amputation of diseased tissue plus application of amoxicillin on cut edges was fully effective on all tested species.

Recommendations:

- Further trial work is necessary to improve efficacy of treatments. Current "best practices" survival rates are still too low to be effectively applied in the field, and longer-term reinfection rates are unknown.
- Future ex situ trials should focus on:
    - Easier and more adhesive methods of application. The silicone base that shows the most promising results is very difficult to apply and manage underwater. CoreRx has agreed to continue working with this material to improve it

Table 2: Trial results

Category	Treatment	Spp	# Effective	# Ineffective	% Effective
Control	Control (No treatment)	DSTO	1	8	11%
		CNAT	0	9	0%
		PSTR	0	9	0%
		MMEA	0	6	0%
Barrier	Barrier (Clay)	DSTO	0	3	0%
		CNAT	0	3	0%
		PSTR	0	3	0%
		MMEA	2	0	100%
	Barrier (Z-Spar)	DSTO	1	2	33%
		CNAT	0	3	0%
		PSTR	0	3	0%
		MMEA	0	1	0%
		MCAV	0	2	0%
	Trench Only	DSTO	0	3	0%
		PSTR	0	3	0%
		CNAT	0	3	0%
		MMEA	0	1	0%
	Trench + Z-Spar	DSTO	0	3	0%
		PSTR	0	3	0%
		CNAT	0	3	0%
		MMEA	0	3	0%
Chlorine	Chlorine Z-Spar	DSTO	1	2	33%
		CNAT	0	3	0%
		PSTR	0	3	0%
	Trench + Chlorine Z-Spar	DSTO	1	2	33%
		PSTR	0	3	0%
		CNAT	0	3	0%
		MMEA	0	2	0%
	Chlorine Modeling Clay	DSTO	0	5	0%
		CNAT	0	3	0%
		PSTR	1	3	25%
		MMEA	0	4	0%
	Chlorine Aves Epoxy	DSTO	0	3	0%
		CNAT	0	3	0%
		PSTR	1	2	33%
		MMEA	1	2	33%
Salt	Trench + Clay + Salt	DSTO	1	3	25%
		PSTR	1	3	25%
		CNAT	1	3	25%
		MCAV	4	0	100%
Antibiotics	Amoxicillin Dental Paste	DSTO	0	2	0%
		CNAT	0	3	0%
		PSTR	1	2	33%
		MMEA	0	3	0%
	Amoxicillin Modeling Clay	DSTO	1	1	50%
		PSTR	0	1	0%
		MMEA	0	1	0%
	Amoxicillin & Kanamycin Modeling Clay	DSTO	0	3	0%
		CNAT	0	3	0%
		PSTR	1	2	33%
		MMEA	0	3	0%
	Amoxicillin Aves Epoxy	DSTO	1	2	33%
		CNAT	0	3	0%
		PSTR	0	3	0%
		MMEA	1	2	33%
	Amoxicillin & Kanamycin Z-Spar	DSTO	3	0	100%
		CNAT	1	1	50%
		PSTR	1	2	33%
Antibiotics + Trench	Trench + Amoxicillin Z-Spar	DSTO	0	3	0%
		PSTR	1	2	33%
		CNAT	0	3	0%
		MMEA	1	1	50%
	Trench + Amoxicillin Shea Butter	DSTO	0	4	0%
		PSTR	3	1	75%
		CNAT	0	5	0%
		MMEA	4	2	67%
	Trench + Amoxicillin Vaseline	DSTO	1	1	50%
		PSTR	1	1	50%
		CNAT	0	2	0%
	Trench + Amoxicillin Silicone Base	DSTO	2	0	100%
		PSTR	1	2	33%
		CNAT	3	0	100%
	Trench + Double Dose Amoxicillin Silicone Base	DSTO	2	1	67%
		PSTR	1	1	50%
		CNAT	1	1	50%
Disease Removal	Aggressive Lab Treatment (Amputation + Lugols + Amoxicillin Dental Paste)	DCYL	~30	0	100%
		DSTO	7	0	100%
		PSTR	2	0	100%
		CNAT	1	0	100%

    - Testing of other antibiotics or combinations of antibiotics to increase effectiveness
    - Manipulating dosages to maximize effectiveness while minimizing unnecessary transmission to the environment
    - Testing the necessity and effectiveness of reapplication following an ineffective initial treatment or reinfection
    - Resistance to reinfection after successful treatment
- Future in situ trials should focus on:
    - Using the most promising option (to date: silicone base with amoxicillin) to experimentally treat field colonies paired with untreated controls
    - Using monitoring to determine effectiveness of:
        - The slowing/halting of disease
        - The short- and long-term resistance to reinfection at the treatment site
        - The short- and long-term resistance to disease throughout the treated colony
    - Adapting laboratory methodologies for efficient field-based practices
        - Determining effective, efficient, and financially feasible ways to trench, manage treatment materials, and monitor success over short- and long-term time scales.