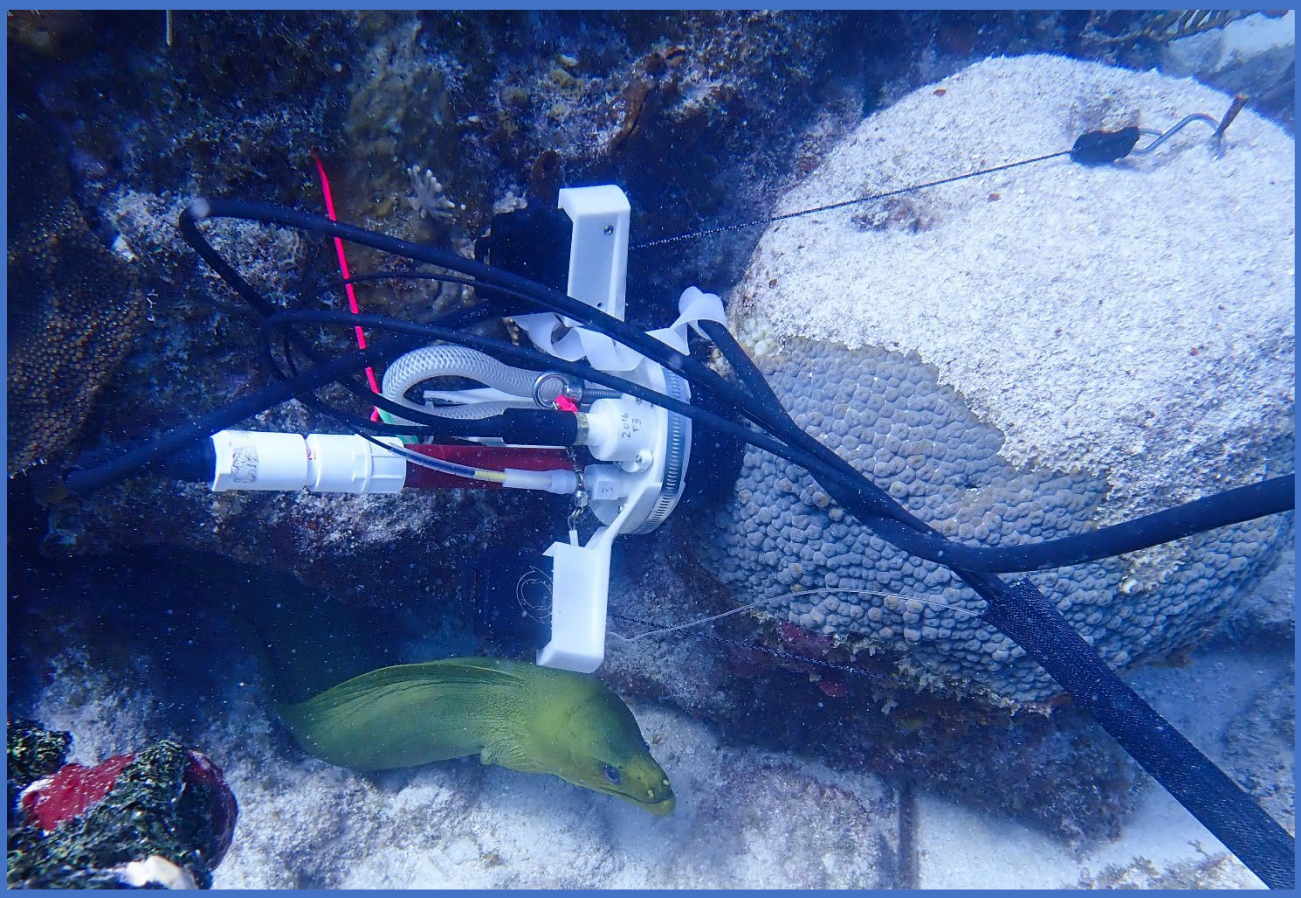
THE EFFECT OF DISEASE LESIONS AND AMOXICILLIN TREATMENT ON THE PHYSIOLOGY OF SCTLD-AFFECTED CORALS
FY 2021/2022 FINAL REPORT



Florida Department of Environmental Protection
Office of Resilience and Coastal Protection

THE EFFECT OF DISEASE LESIONS AND AMOXICILLIN TREATMENT ON THE PHYSIOLOGY OF SCTLD-AFFECTED CORALS FY 2021/2022 FINAL REPORT

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Background

Florida's Coral Reef is currently experiencing a multi-year disease-related mortality event that has resulted in massive die-offs in multiple coral species. Over 25 species of coral, including both Endangered Species Act-listed and the primary reef-building species, have displayed tissue loss lesions which often result in whole colony mortality. First observed near Virginia Key in late 2014, the disease has since spread throughout Florida's Coral Reef and parts of the Caribbean.

As SCTLD has decimated Florida's reefs, in-water coral treatments were developed and implemented on ~3,500 priority corals from 2019-2021 (Walker and Pitts 2019; Neely 2020; Voss et al. 2020). Intervention involves the topical application of an amoxicillin paste to active SCTLD lesions. These applications are highly effective at halting lesion progression and preventing mortality of treated corals (Neely et al. 2020; Neely et al. 2021). However, concerns about unintended consequences of antibiotic treatments persist among management and regulatory authorities, and one identified research priority is to determine whether there are adverse effects of the antibiotic treatment.

SCTLD rapidly sweeps across the surface of a live coral, but mounting evidence suggests that it is the Symbiodiniaceae rather than the coral host that is affected by the disease. Histology has identified Symbiodiniaceae necrosis (Landsberg et al. 2020), loss of Symbiodiniaceae during paling and bleaching has correlated with slowed/halted disease progression (Meiling et al. 2020; Sharp et al. 2020), and susceptibility of species correlates roughly with dominant symbiont species (Dennison et al. 2021). An understanding of how SCTLD and any treatment options affects physiology of the corals should thus examine both the host response and the symbiont response.

Common physiological metrics of coral/symbiont health include 1) photosynthesis rate (P), 2) respiration rate (R), 3) P/R which compares photosynthesis and respiratory rates of the holobiont and is a metric of total metabolic health, and 4) calcification which measures whether a coral is growing. Such metrics have been used in the past to identify temperature and salinity levels where coral metabolism performs poorly (Dellisanti et al. 2020a,b), and also detect seasonal changes in metabolic performance (Szmant et al. 2019) even when changes in health cannot be detected by visual appearance.

Rapid in situ measurements of photosynthesis, respiration, and calcification can be conducted using the CISME instrument (Dellisanti et al. 2020b). Briefly, the instrument has a silicone-lined chamber head which lightly attaches to a ~5.5 cm diameter area of coral tissue for up to 30 minutes (Figure 1). No damage is done to the coral by this process (Dellisanti et al. 2020b). The head contains LED lights which are used for standardizing photosynthetic rates across samples. It also contains sensors for instantaneous collection of O_2, pH, and temperature which are used for the subsequent calculations of metabolic metrics. Finally, there is a water circulation pump which circulates the chamber water through an 18 mL loop; at the conclusion of the sampling period, the loop is removed, fixed, titrated for alkalinity, and used to calculate calcification rates.

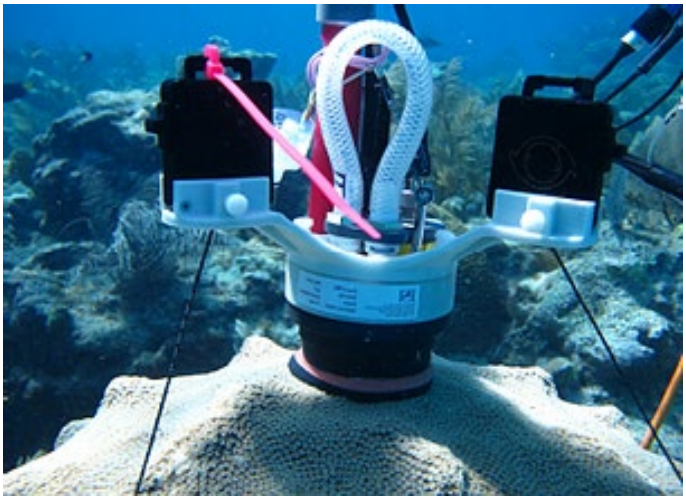

Figure 1. View of CISME chamber head attached to a live coral for metabolic measurements. Photo courtesy of cisme-instruments.com.

Through the use of this instrumentation, we examined metabolic health of the coral and symbionts of healthy, diseased, and disease-treated colonies over three time points to determine how SCTLD impacted the metabolism of the adjacent tissues and also how antibiotic treatments impacted the metabolic measures of adjacent tissues over immediate, short-term, and long-term time periods.

Permitting

Permitting to tag and test colonies using CISME instrumentation was authorized on March 3, 2022 under permit FKNMS-2021-247. Treatment work using amoxicillin paste was authorized under FKNMS-2020-077 (issued July 16, 2020).

Protocols and Quality Assurance

Measurements of coral holobiont metabolism were conducted from late April– early June 2022 in order to minimize other variables (Szmant et al. 2019) that may impact the metrics of interest such as temperature variability and rapid changes in Symbiodiniaceae counts. During the initial sampling period, a total of 30 corals (14 *Orbicella faveolata* and 16 *Montastraea cavernosa*) were selected at Looe Key, within the Florida Keys National Marine Sanctuary (FKNMS). While the initial proposal was to sample at a previously untreated site, extensive scouting in March/April 2022 did not identify any non-treated sites within the FKNMS with enough SCTLD-affected corals of the two target species to conduct the experiment. In conversations with FKNMS and DEP managers, the options of 1) utilizing sites within the Dry Tortugas region, 2) utilizing a variety of sites to reach the needed sample size, and 3) utilizing a site where previous treatments had been conducted were considered; the managers selected option 3 as the preferred choice. Scouting at Looe Key identified the colonies for treatment with the requirement that no selected colony would have received antibiotic treatment within the previous 3.5 months. The bases of the selected colonies were all between 18-27’ in depth and were spread across approximately 0.5 linear kilometers of the spur and groove reef (Figure 2).

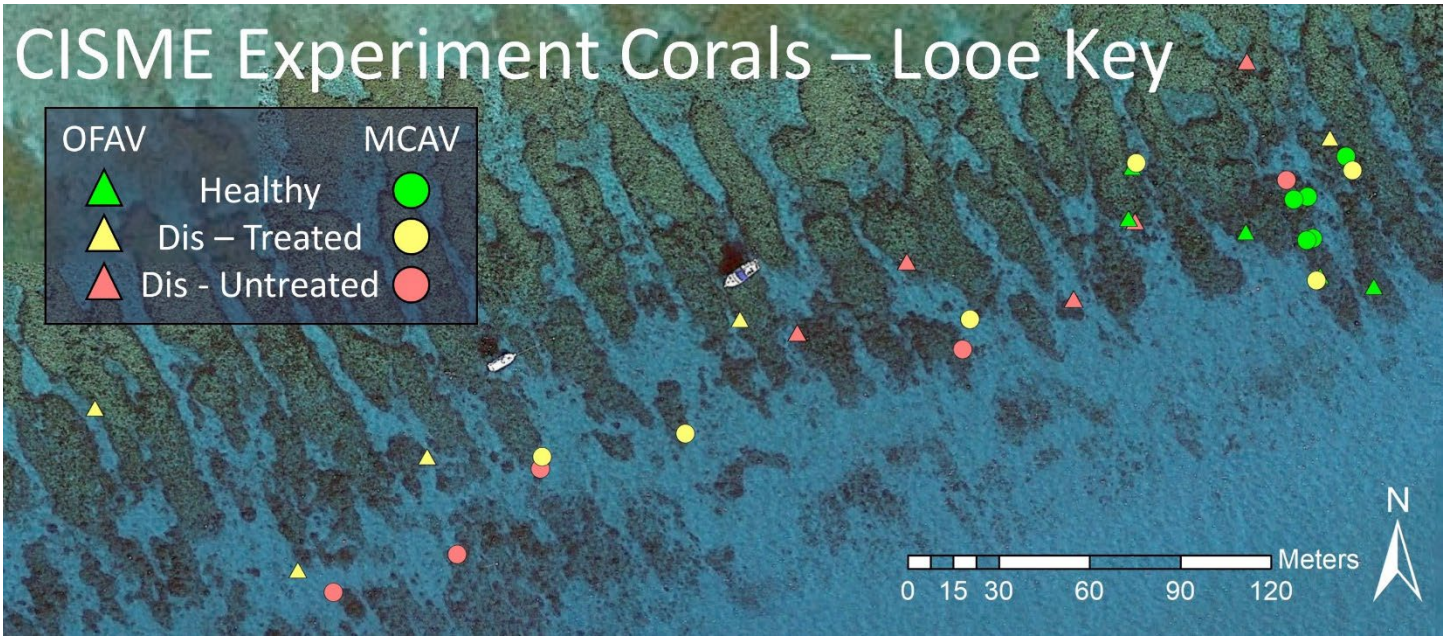

Figure 2. Spatial arrangement of selected coral colonies within Looe Key, Florida Keys National Marine Sanctuary.

Goals for coral selection included five healthy corals from each species, five diseased corals from each species that remained untreated until the conclusion of the experiment, and five diseased corals from each species that were treated using the standard amoxicillin paste. The goal of 15 corals of each species was supplemented with additional corals; for *M. cavernosa*, this resulted in a sample size of 16, while in *O. faveolata* two of the selected corals could not be tested due to skeletal bumps near the disease lesions which prevented a seal on the instrumentation, resulting in a sample size of 14 for that species. Of the diseased corals, selection of those to be treated versus left untreated was randomized. All selected corals were tagged, measured, mapped, and photographed for future identification.

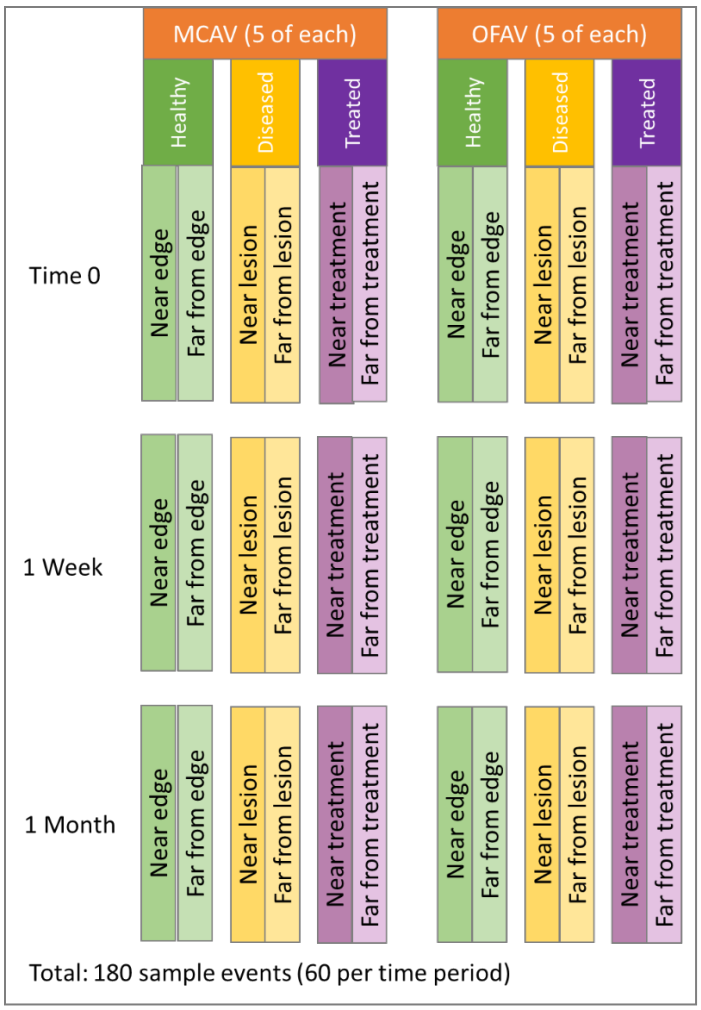

Figure 3. Sample design for experiment. Metabolic measurements were taken at two locations on healthy corals, at two locations on diseased corals, and at two locations on antibiotic-treated corals across three time points following disease treatment. Measurements occurred on two coral species: *Montastraea cavernosa* (MCAV) and *Orbicella faveolata* (OFAV).

On healthy corals, one sampling run was conducted within 1 cm of the corals' edge, and another was conducted inward (ideally 30+ cm) from any tissue margins. On diseased corals, one sampling run was conducted on tissue directly adjacent to the SCTLD lesion (the outside of the sensor head aligned with the live tissue margin), and one was conducted on visually healthy tissue ideally 30+ cm from the lesion. On the SCTLD-treated colonies, the standard amoxicillin paste was applied to any active SCTLD lesions, and the sampling run conducted within 1 hour of application on tissue directly adjacent to the treatment (the outside of the sensor head aligned with the treatment/live tissue margin). To standardize dosage, lesions were at least 10 cm in length, and the sensor head was placed such that at least 2 cm of treated lesion extended past each side of the measurement area. An additional sampling run was conducted on healthy tissue ideally 30+ cm from the treatment location. See Figure 3 for sample design. At two subsequent intervals, the same colonies were revisited and resampled. On healthy and treated colonies, subsequent samples were at the same locations on the colony as the initial samples as indicated by two nails placed around the sensor head after the initial measurement. On diseased colonies, lesions continued to progress, and so, as possible, sampling occurred adjacent to the disease margin at the time

of each sample. Subsequent sample runs occurred at 7-10 days and 30-35 days after the initial treatment and sampling runs (Appendix I).

Two CISME instruments were used to conduct sample runs. Briefly, CISME instrumentation consists of an electronics housing unit attached to an incubation head. The head is a silicone seal which gently seals around a 5.6cm diameter section of coral, creating a chamber where pH and oxygen concentrations can be measured. Elastic cords attached to surrounding substrate secure the instrument. A sample tube is also affixed to the chamber for calcification measurements via titration. Instrument control and real-time readouts are available through an underwater affixed tablet. Full specifications are available at https://www.cisme-instruments.com/the-instrument.

Barring technical problems, approximately 10 corals could be assessed within a day using both instruments. Sample runs were conducted at least 3 hours after sunrise to 3 hours before sunset. We sampled non-diseased colonies first, then moved onto diseased colonies, with one CISME dedicated to non-diseased areas and the other dedicated to diseased areas. Once a sensor touched a diseased area of a colony, it was not used on a non-diseased area. Between sample runs, pumps were run continually and were flushed by wafting surrounding saltwater into the head. After each field day, the CISME units were soaked, with the pump running, in fresh water for at least 30 minutes. The sensor seal was then placed in ozonated water for at least 20 additional minutes for further decontamination. All decontamination protocol were discussed with the developers of the instrumentation to ensure no damage to sensors, and also with FKNMS permitting and research personnel to ensure suitability.

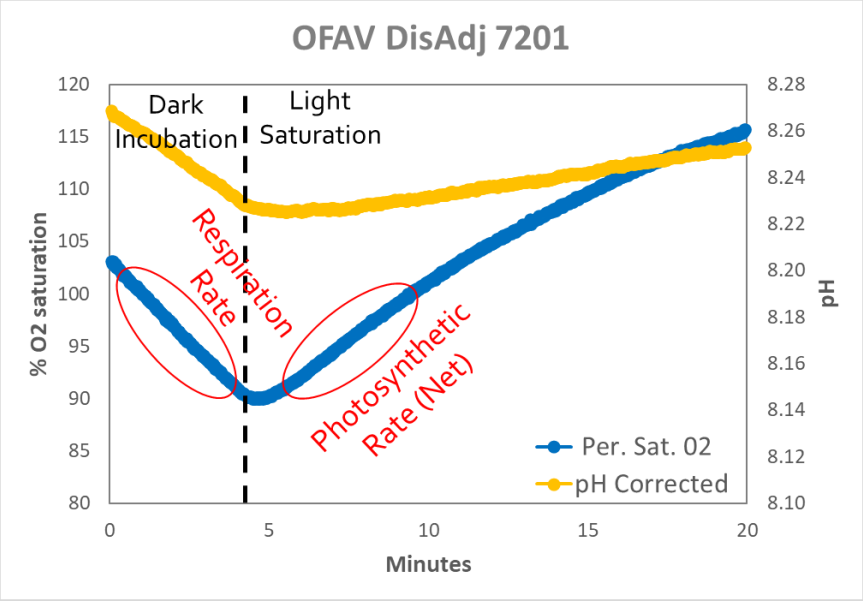

Figure 4. An example sampling run showing the pattern of O_2 saturation and pH through the 4 minute dark incubation and the 16 minute light incubation. Respiration and net photosynthetic rates are calculated from the slopes created by ΔO_2 during each incubation period.

During each sampling, the sensor head was placed on the selected area of tissue. Elastic cords were attached to surrounding rocks, coral lips, or other secure points to stabilize the instrument. The integrity of the seal was tested by looking for light leaks, and the light was turned off for one minute before the run was started to allow the coral to dark acclimate. The sensor head measured respiration during an initial 4 minutes of dark incubation, followed by 16 minutes of light incubation at 450 µEins/m^2/s. Respiration rates (R) were calculated from the

ΔO_2 during dark respiration. Net photosynthesis was calculated from the ΔO_2 during light incubation (from 2-4 minutes after the light turned on) (Figure 4). Gross photosynthesis was then calculated by adding the respiration rate to the net photosynthesis rate. Photosynthesis to respiration ratios (P/R) were calculated by dividing gross photosynthesis by respiration rates. The sample tubes were removed after the light incubation period and fixed at the end of the day in mercuric chloride. These samples will be processed via titration for calculation of calcification rates by staff at UNC Wilmington. Briefly, sea water alkalinity will be measured using an open-cell, potentiometric titration. The seawater sample will be acidified to a pH of approximately 3.0 in a two-stage hydrochloric acid titration. The final titration will be done in 0.05 cm^3 increments, recording dispensed volume, e.m.f, and temperature and calculated using a non-linear least-squares approach. The full operating procedures are outlined in SOP3b of the Guide to Best Practices for Ocean CO_2 Measurements (Dickson et al. 2007).

We compared photosynthesis, respiration, and P / R metrics across the treatment groups (healthy corals edge, healthy corals non-edge, diseased corals adjacent to the lesion, diseased corals far from the lesion, treated corals near the treatment line, treated corals far from the treatment line), and across the three time periods (within 1 hour of treatment, ~1 week after treatment, ~1 month after treatment) using a 2-way ANOVA with Holm-Sidak pairwise comparisons.

Results

Respiration rates, photosynthesis rates, and P/R rates were calculated for sample runs. Respiration and photosynthetic rates were both notably higher for *O. faveolata* colonies (Respiration median: 1020 nmol / cm^2 / h. Photosynthesis median: 1629 nmol / cm^2 / h) than for *M. cavernosa* colonies (Respiration median: 475 nmol / cm^2 / h. Photosynthesis median: 1026 nmol / cm^2 / h) and thus the two species are presented independently.

The three physiological metrics were compared across treatments (healthy middle, healthy edge, treated colony lesion, treated colony away from lesion, non-treated colony lesion, non-treated colony away from lesion) and across the three time periods using a 2-Way ANOVA.

For *O. faveolata*, values for respiration and photosynthesis were significantly different between all three time periods, with rates of both significantly decreasing during each subsequent time period (Figure 5). These decreases were independent of the treatment type. The P / R ratios were not significantly different among time periods, nor were there any treatment effects (see Appendix II for ANOVA tables). P/R values were greater than 1 for all treatment types and time periods.

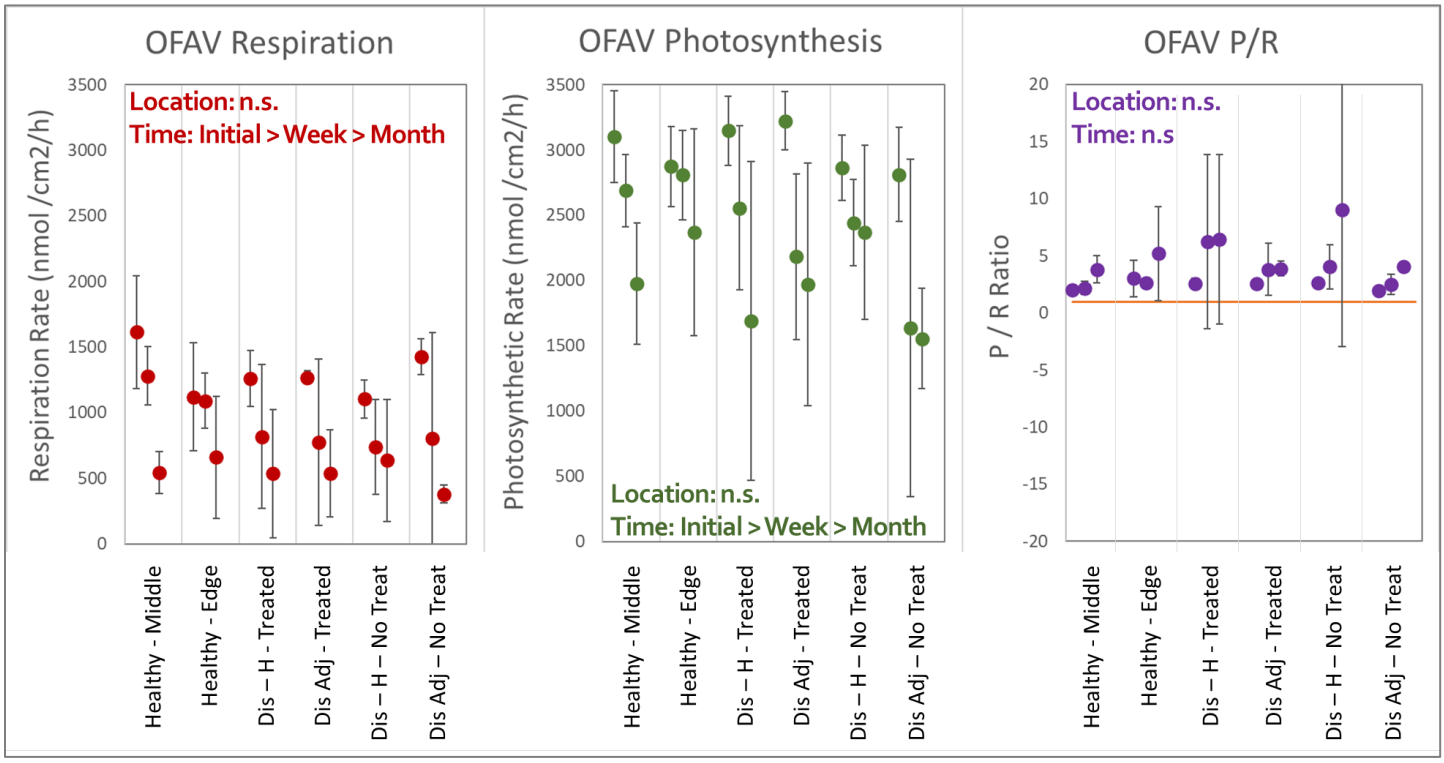

Figure 5. Respiration rates, photosynthesis rates, and P/R ratios for CISME runs on *Orbicella faveolata* colonies. The three values within each treatment type represent (from left to right), the initial sample run, the sample run at one week, and the sample run at one month. All values represent the mean, with error bars showing standard deviation. Significance is labeled on each graph and derived from 2-Way ANOVA tables in Appendix II (n.s. = not significant, > 0.05).

For *M. cavernosa*, treatment had no significant impact on respiration rates, but the one month respiration rates were significantly lower than those at the initial and one-week runs (Figure 6). Photosynthetic rates were similarly higher during the initial runs than during the one month runs. Though the effect of treatment was significant for *M. cavernosa* photosynthesis ($p = 0.014$), no pair-wise comparisons were significant. The trend was for healthy coral respiration rates to be the highest (average 1646 nmol / cm^2 / h and 1670 nmol / cm^2 / h for healthy middle and edge respectively), healthy tissues of SCTLD-affected corals to be lower (1394 nmol / cm^2 / h and 1443 nmol / cm^2 / h for treated and non-treated healthy regions), and disease-adjacent tissues to be least productive (1249 nmol / cm^2 / h and 1273 nmol / cm^2 / h for treated and non-treated lesion-adjacent runs). P/R values were not significantly different based on treatment or time period.

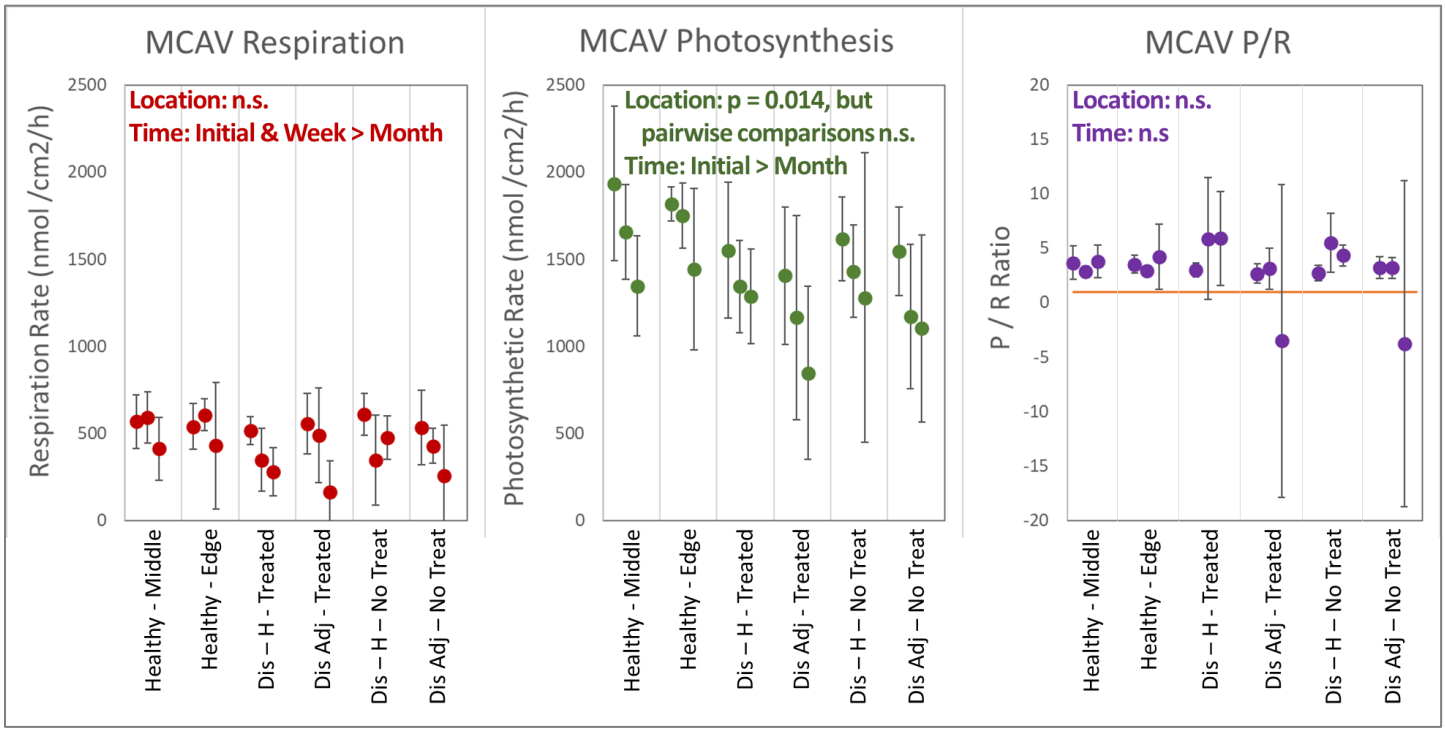

Figure 6. Respiration rates, photosynthesis rates, and P/R ratios for CISME runs on *Montastraea cavernosa* colonies. The three values within each treatment type represent (from left to right), the initial sample run, the sample run at one week, and the sample run at one month. All values represent the mean, with error bars showing standard deviation. Significance is labeled on each graph and derived from 2-Way ANOVA tables in Appendix II (n.s. = not significant, > 0.05).

Two of the P/R averages at one month are below 0 and warrant explanation. Respiration values for many of the *M. cavernosa* colonies were extremely low, and for two of these (one treated disease-adjacent run and one disease-treated run), the ΔO_2 values read slightly positive, making the P/R ratio strongly negative. We will further examine these two anomalies to determine whether they should be omitted or recalculated in an alternate way.

Summary and Recommendations

The primary takeaway from these experiments is that neither SCTLD nor SCTLD amoxicillin treatments appear to be impacting the physiological metrics of the tissues adjacent to (1-6 cm away from) lesions or amoxicillin paste applications. Respiration, photosynthesis, and P/R rates of these disease and treatment-adjacent tissues

are not distinguishable from those of healthy tissues elsewhere on the colony nor from healthy colonies. This pattern was true across three time points. For disease treatments, this suggests there are neither immediate, short-term, nor long-term impacts of antibiotic treatments on the metabolism of these tissues. From a management perspective, apprehensions about antibiotics negatively impacting these metrics do not appear to be of concern.

We were interested to find how much lower the respiration and photosynthetic rates of *M. cavernosa* were compared to *O. faveolata*, and are interested in exploring possible reasons for this in the future. Calcification rates, which will be measured in the future from the titration tubes collected during this experiment, may yield further information on this. We also find the temporal differences in physiological metrics to be of interest, and we plan to look into possible mechanisms for the declines in measured respiration and photosynthetic rates.

The use of in situ methods for examining coral physiology with the CISME instrumentation proved to be an efficient way of assessing metrics like photosynthesis and respiration rates without causing damage to the corals. This non-invasive methodology also allows for repeated measurements on the same coral to track changes through time – a feat not possible through sample collection. We see many opportunities for other experiments that could help answer important questions about coral health using this or similar technology.

Acknowledgements

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Appendix I: Metadata for CISME experiment corals

Tag #	Species	Status	Length (cm)	Width (cm)	Height (cm)	% live	Depth (feet)	Date of S1	Date of S2	Date of s3
55	MCAV	Healthy	41	54	46	90	24	5/5/2022	5/16/2022	6/8/2022
57	MCAV	Healthy	25	20	50	98	23	5/8/2022	5/16/2022	6/1/2022
43	MCAV	Healthy	39	25	34	95	22	5/5/2022	5/16/2022	6/8/2022
45	MCAV	Healthy	63	35	41	95	22	5/5/2022	5/16/2022	6/1/2022
38	MCAV	Healthy	33	38	40	98	21	5/8/2022	5/16/2022	6/1/2022
7117	MCAV	SCTLD - Not Treated	72	58	50	60	27	5/11/2022	5/20/2022	6/10/2022
609	MCAV	SCTLD - Not Treated	52	40	38	50	27	5/11/2022	5/19/2022	6/10/2022
567	MCAV	SCTLD - Not Treated	50	39	30	50	25	5/11/2022	5/20/2022	6/10/2022
415	MCAV	SCTLD - Not Treated	95	50	85	40	24	5/9/2022	5/18/2022	6/9/2022
344	MCAV	SCTLD - Not Treated	64	62	40	45	20	5/8/2022	5/17/2022	6/8/2022
569	MCAV	SCTLD - Treated	39	37	27	20	25	5/11/2022	5/19/2022	6/9/2022
1542	MCAV	SCTLD - Treated	28	28	32	65	26	5/10/2022	5/19/2022	6/9/2022
7120	MCAV	SCTLD - Treated	82	110	175	60	18	5/10/2022	5/19/2022	6/9/2022
496	MCAV	SCTLD - Treated	48	48	33	75	25	5/9/2022	5/18/2022	6/9/2022
7116	MCAV	SCTLD - Treated	31	25	42	85	18	5/9/2022	5/18/2022	6/8/2022
293	MCAV	SCTLD - Treated	168	83	99	30	24	5/6/2022	5/18/2022	6/8/2022
7129	MCAV	SCTLD - Treated	138	100	94	10	25	5/8/2022	5/18/2022	6/8/2022
34	OFAV	Healthy	155	108	118	95	24	5/5/2022	5/16/2022	6/1/2022
30	OFAV	Healthy	60	58	61	98	24	5/5/2022	5/16/2022	6/1/2022
32	OFAV	Healthy	207	121	112	98	21	5/6/2022	5/17/2022	6/8/2022
28	OFAV	Healthy	162	108	130	98	24	5/8/2022	5/17/2022	6/1/2022
31	OFAV	Healthy	158	72	107	94	20	5/6/2022	5/17/2022	6/1/2022
7115	OFAV	SCTLD - Not Treated	150	140	205	70	19	5/10/2022	5/19/2022	6/9/2022
441	OFAV	SCTLD - Not Treated	208	195	180	30	15	5/10/2022	5/19/2022	6/9/2022
371	OFAV	SCTLD - Not Treated	158	156	140	70	25	5/9/2022	5/18/2022	6/9/2022
7201	OFAV	SCTLD - Not Treated	110	80	108	25	20	5/9/2022	5/18/2022	6/8/2022
7130	OFAV	SCTLD - Not Treated	158	154	130	38	10	5/8/2022	5/18/2022	6/8/2022
684	OFAV	SCTLD - Treated	225	165	262	80	18	5/12/2022	5/20/2022	6/10/2022
665	OFAV	SCTLD - Treated	180	150	185	15	26	5/12/2022	5/20/2022	6/10/2022
613	OFAV	SCTLD - Treated	178	158	180	8	23	5/11/2022	5/19/2022	6/10/2022
1161	OFAV	SCTLD - Treated	79	38	100	50	21	5/8/2022	5/18/2022	6/8/2022

Appendix II: ANOVA tables

O. faveolata **respiration**

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Treatment	5	979299.720	195859.944	1.419	0.232
Time	2	6243943.240	3121971.620	22.613	<0.001
Treatment x Time	10	1182214.325	118221.432	0.856	0.578
Residual	56	7731325.116	138059.377		
Total	73	16316190.652	223509.461		

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
T0 vs. month	750.806	6.691	<0.001	Yes
T0 vs. week	381.678	3.549	0.002	Yes
week vs. month	369.128	3.171	0.002	Yes

O. faveolata **photosynthesis**

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Treatment	5	2457378.949	491475.790	1.601	0.175
Time	2	11854613.506	5927306.753	19.303	<0.001
Treatment x Time	10	3054277.748	305427.775	0.995	0.459
Residual	56	17195298.904	307058.909		
Total	73	33274627.575	455816.816		

Comparisons for factor: **Time**

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
T0 vs. month	1016.124	6.072	<0.001	Yes
T0 vs. week	618.067	3.853	<0.001	Yes
week vs. month	398.058	2.293	0.026	Yes

O. faveolata **P / R**

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Treatment	5	79.369	15.874	0.836	0.530
Time	2	95.988	47.994	2.528	0.089
Treatment x Time	10	66.474	6.647	0.350	0.962
Residual	56	1063.259	18.987		
Total	73	1340.409	18.362		

M. cavernosa **respiration**

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Treatment	5	306290.395	61258.079	1.716	0.143
Time	2	602782.999	301391.499	8.444	<0.001
Treatment x Time	10	308673.455	30867.345	0.865	0.570
Residual	66	2355800.265	35693.943		
Total	83	3611014.641	43506.200		

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
T0 vs. month	217.109	4.108	<0.001	Yes
week vs. month	131.466	2.424	0.036	Yes
T0 vs. week	85.643	1.726	0.089	No

M. cavernosa **photosynthesis**

Source of Variation	DF	SS	MS	F	P
Treatment	5	2292271.929	458454.386	3.077	0.014
Time	2	2260476.617	1130238.309	7.586	0.001
Treatment x Time	10	331205.597	33120.560	0.222	0.993
Residual	70	10428719.548	148981.708		
Total	87	15192595.038	174627.529		

Comparisons for factor: **Time**

Comparison	Diff of Means	t	P	P<0.050
T0 vs. month	396.986	3.890	<0.001	Yes
T0 vs. week	201.940	2.012	0.094	No
week vs. month	195.046	1.874	0.065	No

M. cavernosa **P / R**

Source of Variation	DF	SS	MS	F	P
Treatment	5	79.369	15.874	0.836	0.530
Time	2	95.988	47.994	2.528	0.089
Treatment x Time	10	66.474	6.647	0.350	0.962
Residual	56	1063.259	18.987		
Total	73	1340.409	18.362		