Project Report

Florida Department of Environmental Protection (FDEP)

Project Title: Ultraviolet deactivation of coral disease lesions.

Principal Investigator(s):
Ian C. Enochs,
Graham Kolodziej

Project Dates:
4/23/18-6/30/18

Report:

Task 1: *Modification of existing infrastructure at the UM/CIMAS Experimental Reef Lab (ERL) to maintain 32 diseased coral fragments with constant temperature, light, and fresh seawater input.*

Experimental treatments were carried out in two temperature-controlled fiberglass raceways (Figure 1). Rectangular acrylic sheets (1/4" thickness, clear) were laser cut to allow the suspension of six rectangular aquaria (two liter) per sheet, as well as to include drainage holes between aquaria. Three sheets were utilized per raceway for a total of 36 aquaria. Support legs were designed, 3D printed, and attached to each acrylic sheet to suspend them at water level within the raceway. This fixed the top edges of the individual aquaria at 1 cm above the water line and eliminated the potential for cross contamination while still submerging the bulk of each aquaria in the water bath for temperature regulation (Figure 1). Acrylic support beams were cut and cemented to the sheets to minimize flex and maintain all aquaria in a horizontal position.

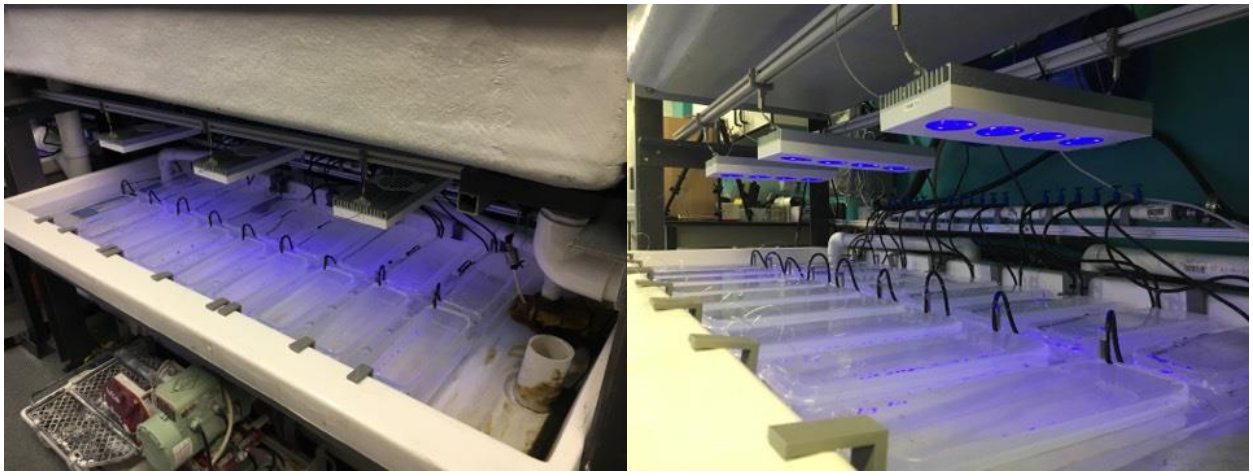

Figure 1. Experimental setup showing individual aquaria suspended in temperature-controlled water bath, including supporting infrastructure.

Valve-manifolds were constructed to allow for individual fresh seawater input to the aquaria, utilizing ERL's seawater delivery system as the water source. Each manifold consisted of 18 needle valves with black airline tubing cut to length and routed from the manifold to its corresponding aquaria (Figure 2). Tubing holders were designed and 3D printed to ensure the tubing was secured above the acrylic sheets. This ensured that no part of the tubing that contacted the aquaria's overflowing water would subsequently contact the contaminated bath water upon removal of the aquaria for photography/treatment/maintenance. Incoming water flows were directed down with the ends of the tubing just penetrating the surface of the water in the aquaria to maximize circulation and eliminate the possibility of splashing. Incoming seawater flow rates were set to 500 mL min^{-1} to each aquaria to maximize flow while still maintaining temperature regulation. Temperature baths were set to 25.3 ºC to match that at the collection site.

Figure 2. Valve manifolds for delivery of incoming seawater to each experimental aquaria, including 3D printed tubing holders.

Existing LED aquarium lights (AquaIllumination Hydra 52 HD) were placed above each of the six acrylic sheets to allow one light per six aquaria. Light levels were adjusted using a submersible PAR meter (Apogee MQ-200) and set to an average PAR value of 275 with a 12-hour photoperiod from 0700-1900, increasing from off-to-full-intensity from 0700-1000 and decreasing from full-intensity-to-off from 1600-1900.

Rectangular acrylic platforms were cut to fit in the bottom of each aquaria to allow for the coral sample to be raised to the surface for uniform treatment with UV light. Fishing line loops were attached to these platforms for this purpose, and loops were also attached to the aquaria for ease of removing them from the raceways without cross-contamination.

Task 2: *Construction of a lab-based UVC treatment source.*

An acrylic chamber was designed and constructed to accommodate a dual-lamp 6W 254 nm UVC laboratory lamp (Cole Parmer EW-97620-20), including a guillotine-style door to restrict UV exposure only to diseased areas and proximate live coral tissue (Figure 3). A UVC meter (General Tools UV512C) was used to measure dosages, which were in turn used to calculate treatment times. Utilizing the acrylic sample platforms, the meter was raised to the surface of the aquaria and placed underneath the light to mimic the same manner and location in which the samples would be treated. This was done first in air, then with the meter sealed in a plastic bag, then again submerging the sealed meter under a surficial layer of water to ascertain the amount of UV light attenuated by that depth of water. This step was repeated to determine a mean treatment intensity (7100 microwatts cm^{-2}). 50,000 microwatt-seconds cm^{-2} of UVC light (8 seconds of exposure) was selected as it exceeds twice the treatment necessary for complete destruction of more UV tolerant bacteria species (Ultra-Violet Products bacterial destruction chart).

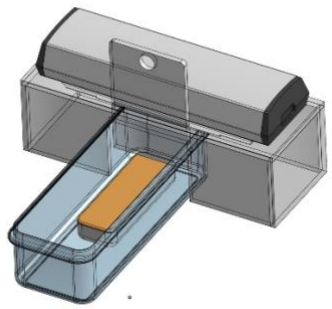

Figure 3. CAD design of acrylic UV-exposure chamber with light.

Task 3: *Sample collection.*

Samples were collected on 5/24/18 at Cheeca Rocks Reef in the Upper Florida Keys utilizing permit FKNMS-2018-007. 10 colonies in total (four *Pseudodiploria strigosa*, six *Colpophyllia natans*) were collected from three sites at Cheeca Rocks (Table 1).

Site	Latitude	Longitude	# *P. strigosa*	# *C. natans*
3	24.89742	- 80.61573	1	5
6	24.89685	- 80.61997	1	1
2	24.89666	- 80.61693	2	0

Table 1. Number of diseased colonies collected of each species at specific GPS coordinates.

Samples were collected using hammer and chisel, transported to the surface, double-bagged in three mil contractor trash bags, placed inside YETI coolers filled with seawater, then filled with their own seawater and sealed to avoid cross-contamination (Figure 4). Samples were transported back to ERL and placed in raceways. There, they were separated by genus in well-flushed holding tanks and allowed to acclimate overnight (Figure 5).

Figure 4. Collected *C. natans* colony in collection bag for transport to ERL.

Figure 5. Collected *C. natans* colonies in overnight holding tank.

Figure 6. *C. natans* colony being cut into samples using a tile saw.

Colonies were cut the following day (5/25/18) using a tile saw (MK Diamond MK-101), one genus and colony at a time, to roughly 15 cm long by 5 cm wide rectangles (Figure 6). Samples were cut to have no more than 25% total area of dead skeleton before the disease margin, with the remaining live tissue visibly healthy and unaffected by disease. Each sample was then rinsed diseased-end-down to clear off any flocculent from the cutting process, assigned a unique ID tag number and placed randomly into its own aquaria (Figure 7). The tile saw and ruler were washed between colonies to minimize possibility of cross-contamination.

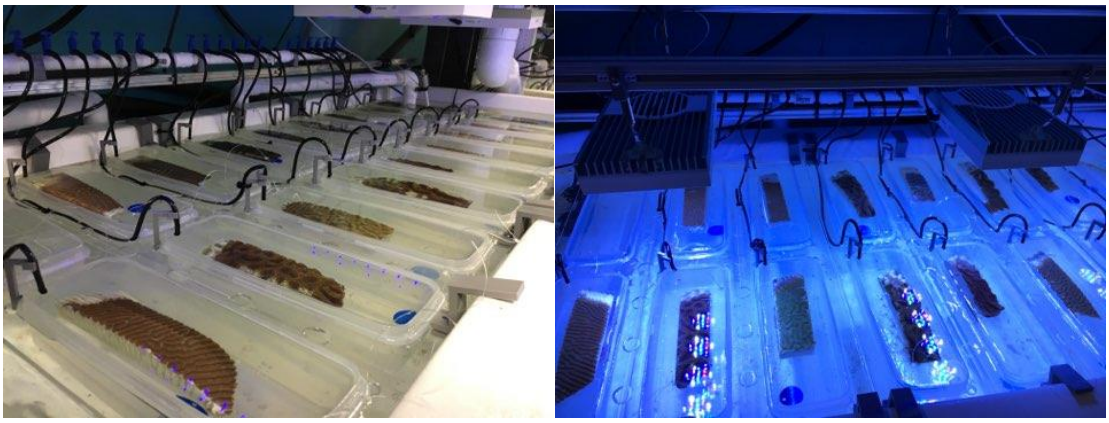

Figure 7. Samples housed in their experimental aquaria.

Task 4: *Evaluation of the effectiveness of UVC for arresting the spread of coral disease across previously healthy tissue.*

Initial treatments were conducted on the same day the samples were cut (5/25/18). Each sample and aquaria was removed from its water bath. The sample was positioned immediately below the water line via the underlying acrylic platform, and placed into the UV treatment chamber to expose an area extending one centimeter beyond the disease margin. A photo was subsequently taken from a fixed top-down orientation to measure the exact amount of live tissue exposed (Figure 8). The sample was then treated with UVC light for eight seconds to equate to 50,000 microwatt-seconds cm^{-2}. Following, the treated sample was removed from the chamber and lowered back down to the bottom of the aquaria (Figure 9). Samples were then placed under a photography rig to take a high-definition image of its current disease state with a scale bar. Treatments were done to 25 of the 33 total samples (12 of 16 *P. strigosa* samples treated, 13 of 17 *C. natans* samples treated). One sample from each colony was not treated and served as control.

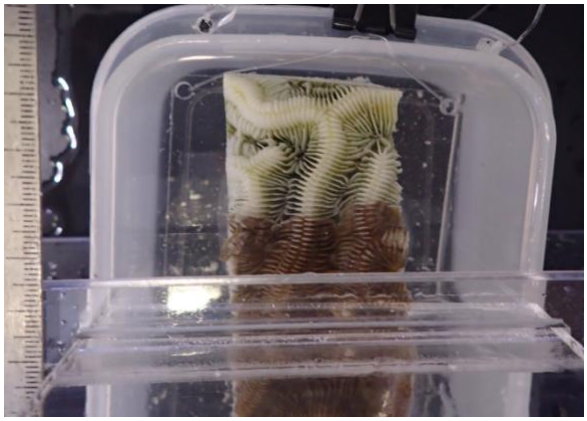

Figure 8. Sample prepared for UV treatment.

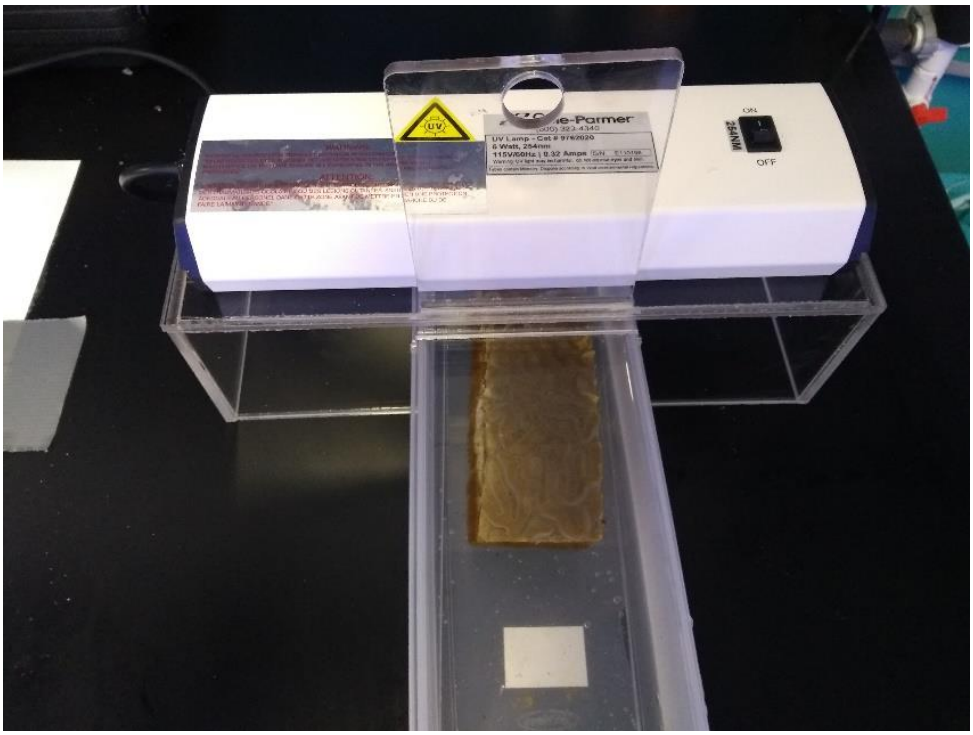

Figure 9. Sample being treated with UV light.

Photographs were taken periodically, every 24 hours for one week to track disease progression. This was done by removing each aquaria individually from its raceway, placing it under the photography rig to take the picture, placing it back in the raceway, then sterilizing gloves using 95% ethanol between each sample. On the second day post-treatment (5/27/18), a decision was made to flush the disease margins of flocculent/necrotic tissue that had accumulated overnight before taking photographs.

Figure 10. Example of how measurements were taken in ImageJ.

At the end of week one, photographs were analyzed for linear progression of the disease margin using ImageJ. This was done by taking measurements along the top, middle, and bottom of the sample from the end of dead skeletal material to the disease margin, averaging them together to get a linear distance, and repeating this process using the same skeletal end points for each sample's pictures (Figure 10). The measurements from the first day (5/25/18) were used as a zero value, and the previous day's values subtracted from the current to assess the incremental linear mortality each day.

It was concluded that the disease had not been deactivated after the first week utilizing the current treatment methodology, with *C. natans* experiencing rapid advancement of the disease throughout the week. *P. strigosa*, while still infected, seemed to be more resistant to the progression of the disease and was therefore chosen to continue with new treatments. The *C. natans* samples were left as-is and consolidated into the same raceway, while repeating daily photographs to track the disease progression.

Figure 11. Acrylic "firebreak" sheet in place on coral about to be treated.

Of the 14 remaining *P. strigosa* samples, six were treated again using the same methodology as the initial treatment, however exposure time was increased to 15 seconds (100,000 microwatt-seconds cm^{-2}) and a two cm region beyond the disease margin was treated rather than one in the previous application of UV.

The remaining eight samples were treated instead by attempting to create a "firebreak" in the live tissue by exposing it to UV light long enough to render it dead to emulate the physical "trenching" method of creating a gap between the disease margin and the remaining live tissue. To do this, rectangular pieces of acrylic were cut to cover the sample entirely with the exception of a one cm-wide strip parallel to the disease margin. This piece was placed so that the parallel strip was located three cm away from the disease margin on healthy tissue, and exposed area was then treated with 30 seconds of UV light (200,000 microwatt-seconds cm^{-2})

(Figure 11). The acrylic holding sheets were removed from the raceway and all 14 *P. strigosa* samples were placed on the bottom of the same raceway to allow for greater flow over the samples. These treatments were all done on 5/31/18. The following day (6/1/18), distressed tissue was visible where the treatment had occurred in the form of pale lesions, but no mortality was visible on any of the eight "firebreak" exposure areas. A decision was made to treat the same areas again with an additional 60 seconds of UV light (400,000 microwatt-seconds cm^{-2}), which was done on 6/1/18, which resulted in noticeable lesion progression within 24 hours and initial mortality of the treatment areas on some samples within 48-72 hours (Figure 12). Photographs were taken of all samples every 24 hours for one week to assess disease progression. Photos were then analyzed using the same methodology as before in ImageJ to assess the progression of the disease.

Figure 12. Sample after second "firebreak" treatment showing visible lesion (top) and the same sample 72 hours later showing visible mortality at location of lesion (bottom).

Results:

After the first week, the disease had not been eradicated from the samples. *C. natans* samples were losing tissue at rates of 6.7 and 8.4 mm d^{-1} for treated and untreated samples, respectively. These numbers were 5.0 and 4.0 mm d^{-1} for *P. strigosa*. Though these numbers were decreasing throughout the week for both samples (from 8.3 to 4.8 and 11.2, to 3.9 mm d^{-1}, treated and untreated *C. natans*, respectively; 7.3 to 2.1 and 4.2 to 3.0 mm d^{-1}, treated and untreated *P. strigosa*, respectively), all samples showed continued mortality and progression of the disease margin.

This trend continued through the second week for *C. natans* samples, none of which had been further treated with UV radiation. The average untreated linear progression stayed remarkably similar to that of the first week at 8.3 mm d^{-1}, but concurrent with this was a marked jump back up in daily progression rates between experimental days seven and eight (post-treatment days six and seven) from 3.9 to 8.6 mm d^{-1} (Figure 13). The treated samples fared worse, with an increase in the weekly average to 11.6 mm d^{-1}. By the end of the two weeks, only five of the original 17 samples had living tissue remaining.

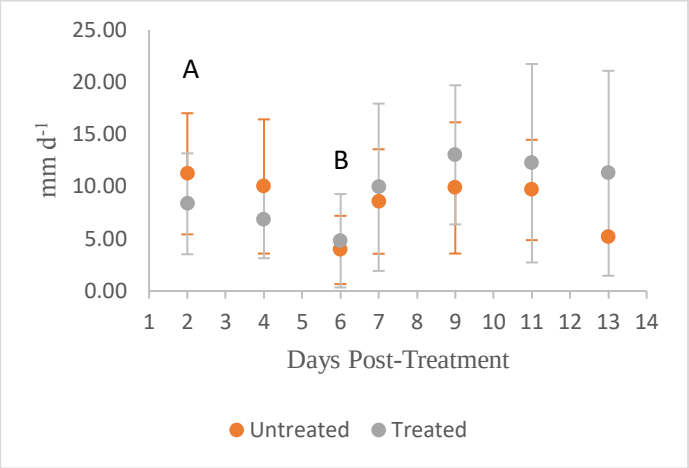

Figure 13. Graph of *C. natans* through two weeks post-treatment showing daily average linear disease progression for treated and untreated samples. Error bars shown are respective standard deviation of the sample set, with no bars possible for the single untreated sample left 13 days post-treatment. A. indicates the start of flushing the samples before photos to remove flocculent and necrotic tissues B. indicates the consolidation of all *C. natans* samples into the same raceway system.

A high degree of colony-specific and sample-specific responses were seen with regard to disease progression in *C. natans*, regardless of treatment. Two of the colonies sampled had linear progression averages of 10.1 and 9.2 mm d^{-1} over the first week, while the other two colonies' averages for the same period were 2.4 and 6.8 mm d^{-1}. Only one sample showed potential disease termination, with a weekly linear progression average of 0.4 mm d^{-1}.

Of the six *P. strigosa* samples that were treated with a second UV exposure, their average linear progression for the second week was comparable to that of the first at 4.4 mm d^{-1} (Figure 14). Again, these results were largely colony-specific with one colony averaging 2.3 mm d^{-1} over this week and the other averaging 6.5 mm d^{-1}. To note, both of these averages were lower than their previous week's values (3.5 and 8.7 mm d^{-1}, respectively). The values for both colonies also followed the same trend during this time. Their rates increased slightly from the previous day, decreased two days later, then continued increasing through the final day of measurement.

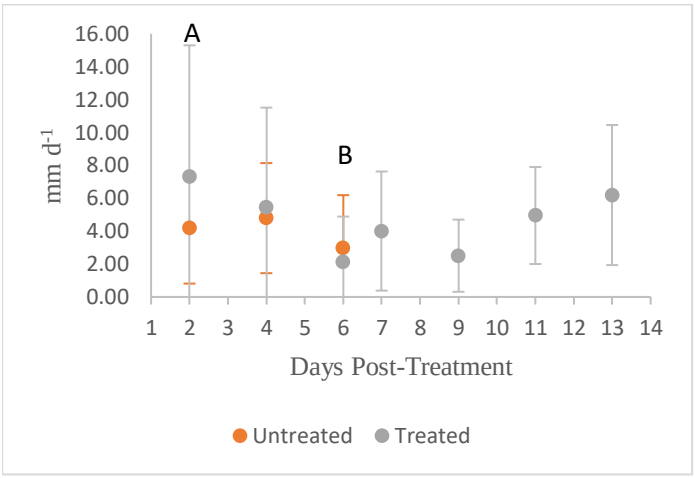

Figure 14. Graph of *P. strigosa* through two weeks post-treatment showing daily average linear disease progression for treated and untreated samples. Error bars shown are respective standard deviation of the sample set A. indicates the start of flushing the samples before photos to remove flocculent and necrotic tissues B. indicates the consolidation of all *P. strigosa* samples into the same raceway system as well as placement onto the bottom of the raceway system, and the second UV exposure treatment.

All *P. strigosa* samples still had living tissue at the conclusion of the second week, and one sample's linear progression appeared to have stopped entirely after the eighth day of the experiment. This same sample had initial linear progression rates of 9.0 and 6.0 mm d^{-1} on its second and fourth days post-treatment, respectively. That had dropped to a rate of only 0.2 mm d^{-1} on the sixth day post-treatment before it was treated with the second UV exposure.

The *P. strigosa* samples used with the "firebreak" treatment were qualitatively more successful in stopping the disease progression, though further monitoring/testing is needed. By the end of the second week of the experiment, all colonies were still alive and the disease had, at most, progressed to the treatment lesion. Because of this, the samples were removed from their holding tubs and placed directly in the bottom of the raceway, in order to maximize the effect of the circulatory flow in the system on the tissues and thereby minimize the artificial negative effects potentially exacerbating disease progression, and left for five additional days to assess progress from there. At this point, 19 days into the experiment, only one of the eight samples had perished entirely and only two additional samples' disease margins had proceeded past the treatment lesion. The remaining samples' disease margins had not progressed past or even up to the further end of the one cm treatment lesion. Additionally, there was no readily-apparent fresh mortality that could be identified through characteristic "white" skeletal areas that had not yet been colonized by algae. Finally, the sample that perished had a second disease margin start at its other end during the second experimental week, so it is believed this could have played a role in its mortality. These samples will continue to be monitored until their disease margins move past the lesion or the disease has been deemed halted.

Conclusions:

Though the direct UV treatment was unsuccessful at halting the progress of the disease, there is information to be gleaned. A colony or genotype-specific response was observed with both species of corals, and ranges of daily and weekly disease progression rates were ascertained for these species. On average the disease progressed much more rapidly through *C. natans* than *P. strigosa*. This could provide insight on the resistances of these species to this disease or the relationship of skeletal structure and porosity to disease progression. Additionally, it was seen that housing samples communally did not result in disease cropping up on areas of otherwise healthy tissue.

High flow was found to be important in clearing moribund tissues and flocculents produced at the disease margin. The removal of the acrylic racks holding the *P. strigosa* samples after the first week, to allow for an increase in circulatory flow experienced by the samples, helped in stemming buildup of flocculent material. Given the mixed results with halting or seemingly slowing the progress of the disease, it cannot be stated unequivocally that sterilization of the disease margin with UVC lighting is not a possible means of "curing" coral disease, however it may ultimately be a solution that is not feasible.

The "firebreak" samples have potentially provided the most hope of halting the progress of the disease akin to the trenching method currently being investigated and employed. Due to the promise seen, this will be a continued monitoring effort for these samples. Having a second, viable means of creating a gap between live, unaffected tissues and the disease margin to curb disease progression may prove to be beneficial to scientists and managers in combatting future disease outbreaks.