Project title: Investigation of the transmission, infectivity and differential host specificity of a disease outbreak on the Florida reefs

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Disease virulence

To determine the virulence of the disease *in-situ*, 20 *M. cavernosa* colonies with subacute tissue loss disease (Fig. 1) were mapped, tagged and photographed at site FtL5 (July 10, 2017). To help visualize tissue loss, bands of marine epoxy were placed on the dead part of the colonies, parallel and approximately 5cm, from lesion edges. Colonies were relocated and photographed on approximately monthly intervals until Nov. 2017. Visual estimates on colony health (% of colony dead, diseased, and healthy) were recorded *in-situ* and from photo review.

Fig. 1. Example of *M. cavernosa* with sub-acute tissue loss disease on a reef off Ft. Lauderdale

Results

Tagged diseased colonies had a starting amount of healthy tissue of 20-99% (avg. = 51.3%) of the colony. Colonies continued to lose tissue throughout the four-month period with an average loss of 6.4% of the initial live tissue per month. There was variability among colonies in amount of healthy tissue lost during the 4-month period ranging from <1% to 100% (Fig. 2). Virulence of tissue lesions varied through time, appearing to wax and wane, but there was no apparent seasonality in tissue loss between July and November, 2017 (Fig. 3).

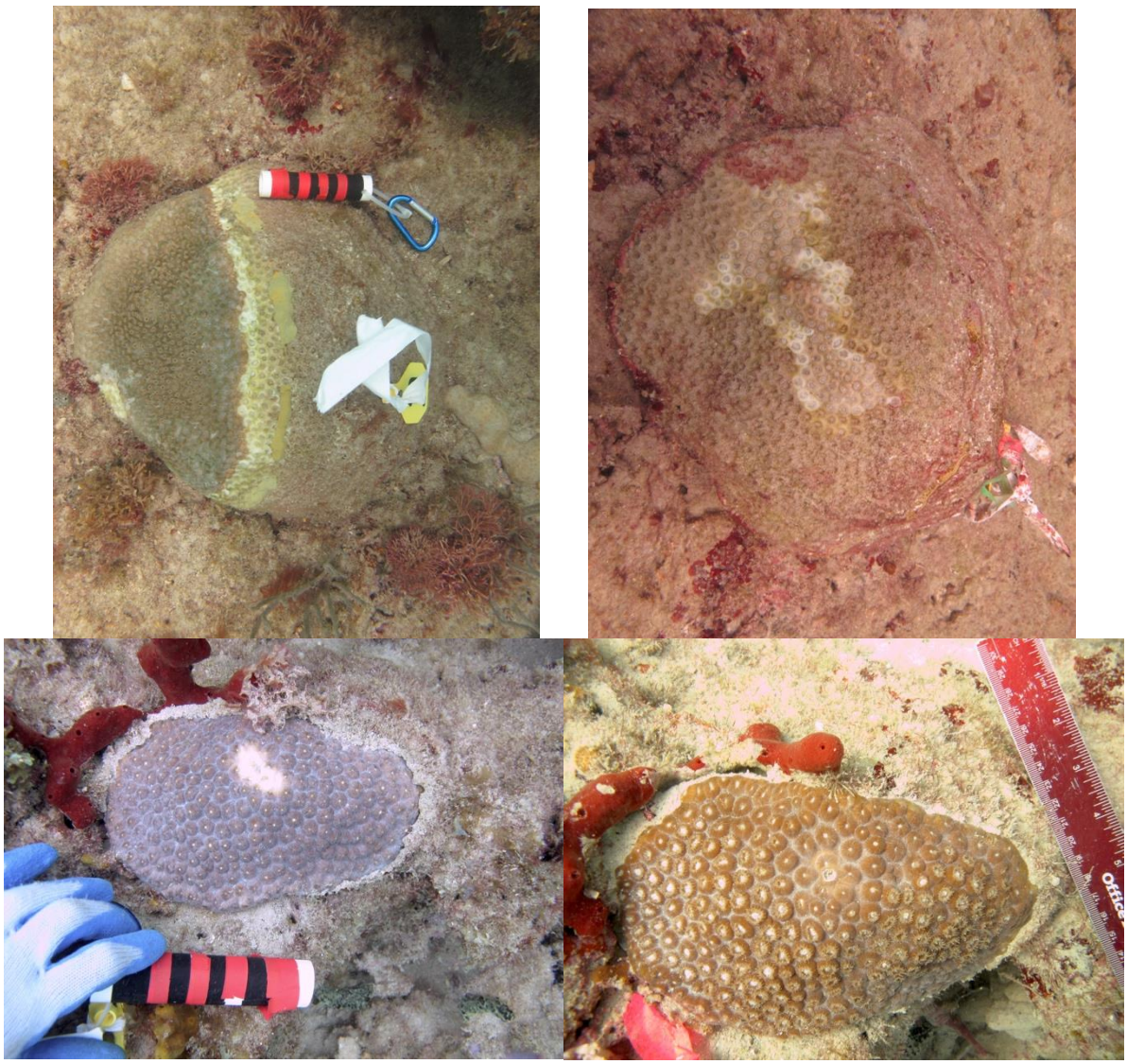

Fig. 2. Examples of differences in rates of tissue loss in tagged *M. cavernosa* with sub-acute tissue loss disease. Top photos show a colony with 100% loss of healthy tissue between July (left) and Nov. (right). Bottom photos show a colony with <1% loss between July (left) and Nov. (right).

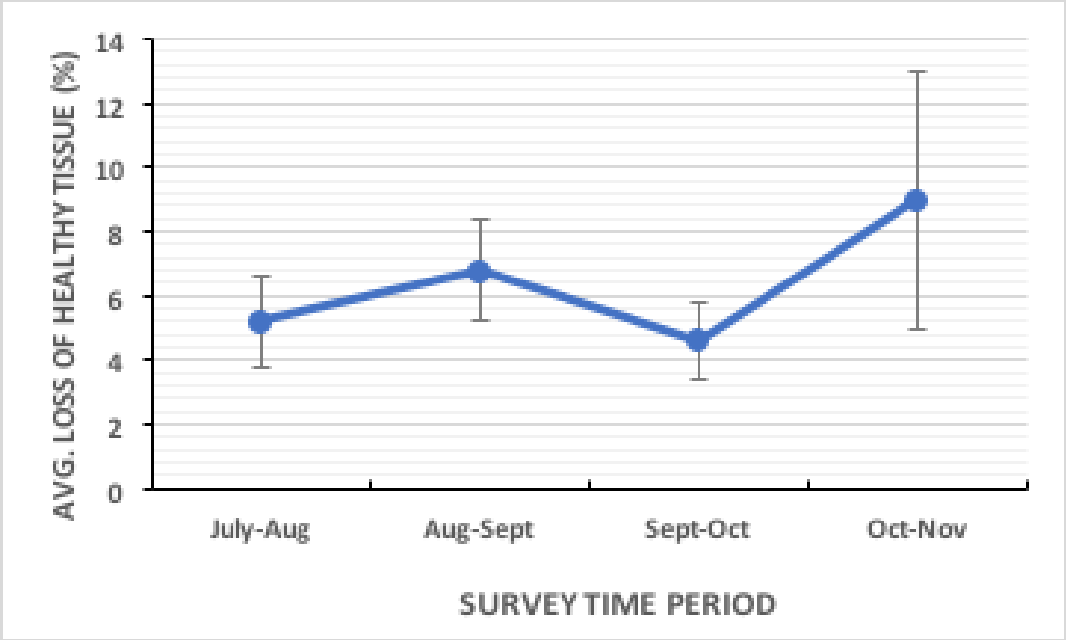

Fig. 3. Average loss of healthy coral tissue on 20 tagged *M. cavernosa* with sub-acute tissue loss disease. Data reflect mean and standard error.

Therapeutic diagnosis with antibiotic treatment

Fragments of diseased corals from the field were treated with cocktails of antibiotics in a therapeutic diagnosis approach to determine if the lesions are caused by bacterial agent. If the disease lesions are significantly slowed or stopped by antibiotics compared to untreated diseased corals from the same colony, then that suggest the infectious agent is a bacterium and not a virus or eukaryotic parasite. For each experimental block, a larger fragment with a disease lesion was cut in half so that each smaller fragment had a portion of the lesion. Each fragment was housed in its own aquarium in filter-sterilized seawater with a bubbler to create water motion. All aquaria were placed in a heated water table to maintain water temperatures at summer ambient levels (29-30C). As a control, one fragment from each set was not treated with antibiotics, while the experimental fragments were treated with a mixture of amoxicillin (50 µg/ml of tank water) and kanamycin (50 µg/ml of tank water) added directly to the aquaria water. Partial water changes were conducted daily to maintain water quality. During water changes, experimental tanks received water already dosed with antibiotics. A total of 21 sets of diseased fragments from *M. cavernosa* were tested. 13 of the 21 sets were run in July, 2017 for 10-11 days and 8 sets were run in November, 2017 for 14 days. Only pairs of fragments whose controls showed progressive tissue loss (active disease) were used in the analysis.

Results: Nine of the 21 the diseased *M. cavernosa* fragments had controls with disease lesions that progressed through time. Of these 9 sets, 8 of the experimental fragments treated with amoxicillin/kanamycin had no further tissue loss (Fig 4). In the one experimental fragment that did show lesion progression, tissue loss did not occur until day 4 of the experiment whereas the untreated control lost tissue throughout (Fig 5). Cessation of lesion progression (n=8) suggests that the infectious agent(s) is bacterial. Of the 12 sets that that did not have progressive tissue loss, we observed that fragments from four sets started showing signs of re-pigmentation of the

bleached areas of the lesion. Antibiotic treated fragments appeared to re-pigment to a larger degree than control fragments (Fig 6) but this was not quantified.

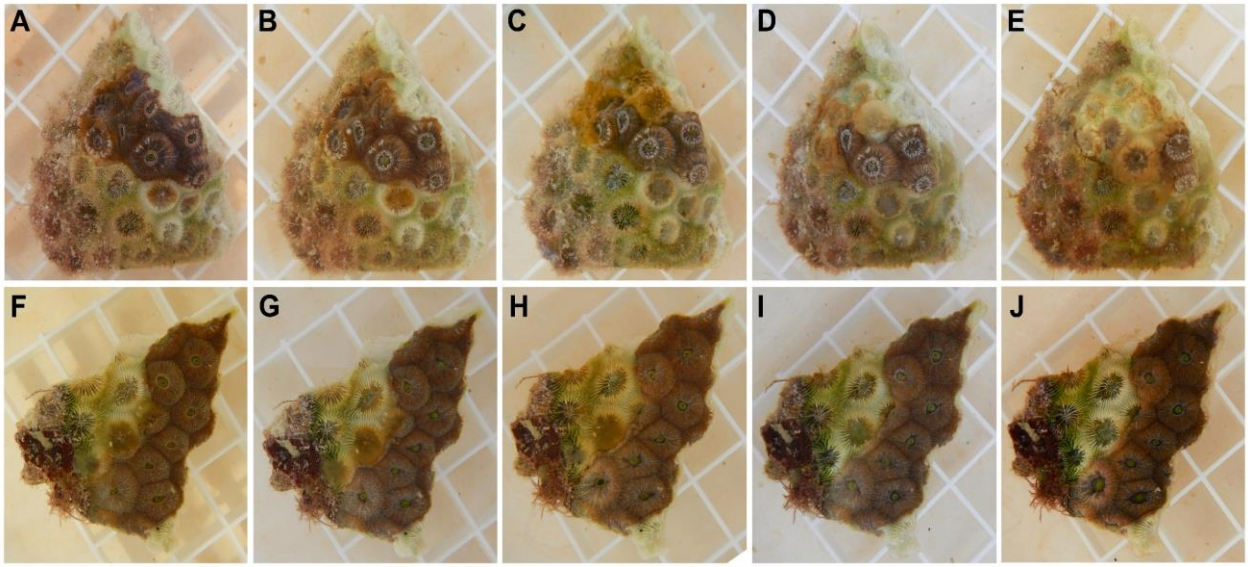

Figure 4. Antibiotic treatment stopped active disease lesions on M. cavernosa. A through E) Photos of a control fragment without antibiotic treatment A) before the start of the experiment, B) after 24 h, C) 48h, D) 72 h, and E) 96 h. F through J) Photos of an antibiotic-treated fragment F) before treatment, G) after 24 h, H) 48 h, I) 72 h, and J) 96 h. Each square on the grid is 1x1 cm.

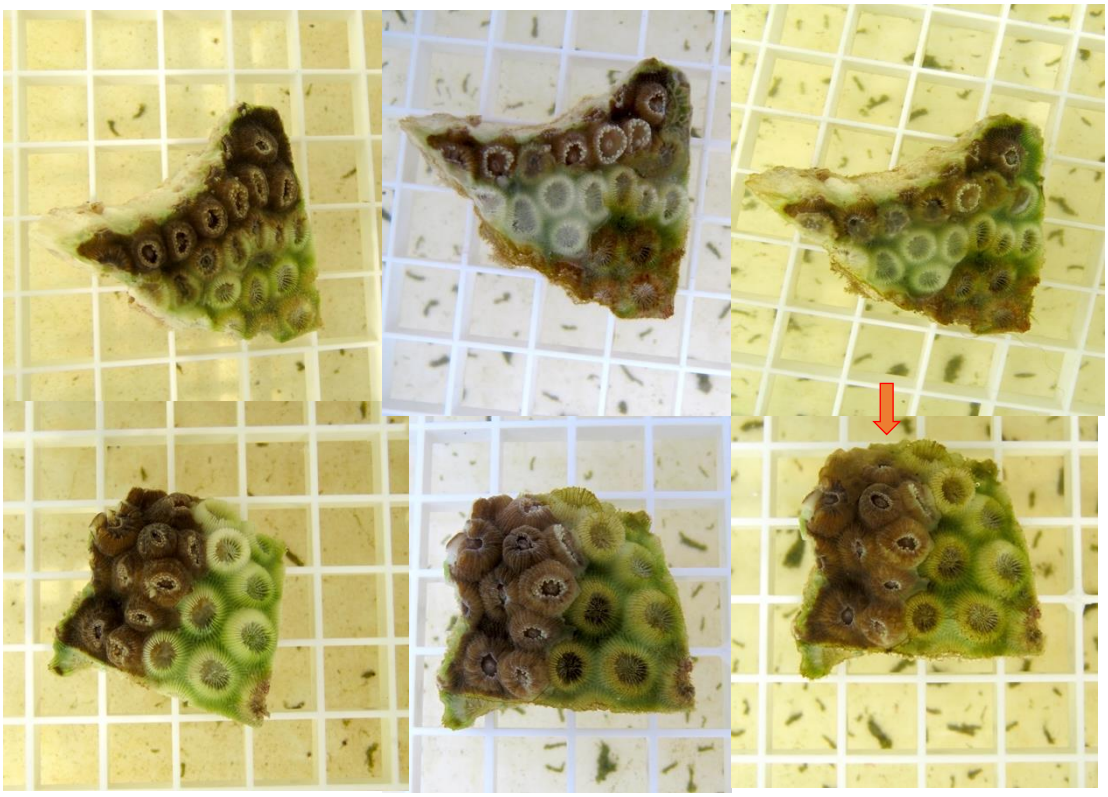

Fig 5. Antibiotic treatment slowed lesion progression on *M. cavernosa*. Top photos show control fragment with no antibiotics, from left to right, on day 0, day 3, day 4. Bottom photos show fragment treated with antibiotics, from left to right, on day 0, day 3, day 4. Red arrow indicates where tissue loss started on experimental fragment on day 4.

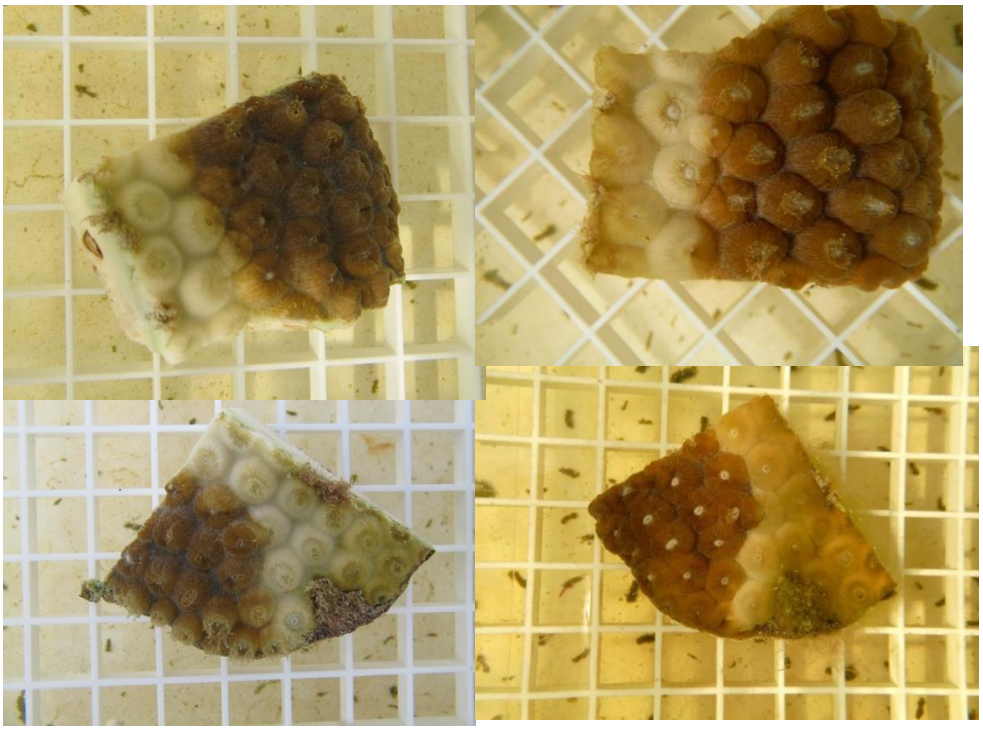

Fig. 6. Example of re-pigmentation of disease lesions through time. Top photos show the control fragment on day day 0 (left) and day 14 (right). Bottom photos show the experimental fragment with a larger degree of re-pigmentation on day 0 (left) and day 14 (right).