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Tentacle Extension in *Pachyclavularia* Coral Under Deoxygenated Conditions

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ABSTRACT

*Coral reefs are ecosystems that provide shelter to a variety of marine life, but they are deteriorating at an unprecedented rate. While factors like pollution and climate change have long been recognized as stressors to coral, the effects of ocean deoxygenation on coral have not been as deeply studied. Many commercially important fish, such as grouper and snapper, are dependent on the coral reef. Reef-related fisheries in the United States are valued at more than $100 million. The purpose of this study was to examine the effects of deoxygenation on the coral species Pachyclavularia violacea. As an indicator of coral health, tentacle extension was monitored using mobile timelapse-photography recordings taken over a period of 5 hours. Marine water from the tank was deoxygenated using the Gast*$_{TM}$ *DOA-P704-AA High-Capacity Vacuum Pump. Two hundred (200) milliliters of water was added to the tank in which the coral was stationed; the control group receiving oxygenated water and the experimental group receiving deoxygenated water. It was anticipated that there would be some tentacle retraction due to the disturbance of the water column, unrelated to the oxygen saturation. The control group had a 32.5% reduction in tentacle extension compared to the experimental group at 59% reduction immediately following addition of the water. The average recovery was 20.9 minutes for the control group and 53.1 minutes for the experimental group. Based on these results, the conclusion that can be drawn is that coral fragments (P. violacea) are impacted by deoxygenation of immediate surrounding water. Future research will focus on completing additional trials to minimize confidence intervals and determine the hypoxia threshold for the recovery of P. violacea fragments. Long-range growth studies of corals grown in deoxygenated water should be a follow-up study.*

INTRODUCTION

Scientists predict that there will be a 70 to 90 percent decrease in live coral on reefs by 2050 (UNEP, n.d.). The impact of coral reefs is not limited to the environment; the degradation of coral reefs also affects people. Coral reef fisheries depend on reef-associated fish, but catches of reef-associated fish have been steadily declining as the condition of coral reefs continues to deteriorate (Eddy et al., 2021). According to the National Oceanic and Atmospheric Association (NOAA), the economic value of tourism, commercial fisheries, and coastal development sectors currently totals to about $6.2 billion per annum in the Mesoamerica Reef in the Caribbean and $13.9 billion per annum in the Coral Triangle in Southeast Asia (UNEP, n.d.). NOAA also found that achieving the predicted benefits of healthy coral reefs will bring $34.6 billion and $36.7 billion to each location, respectively (UNEP, n.d.).

While pollution and warming ocean temperatures have long been recognized as key factors in the destruction of coral reefs, a few recent studies have identified deoxygenation as a notable contributor (Nelson & Altieri, 2019). The International Union for Conservation of Nature (IUCN) reports that the volume of ocean waters completely exhausted of dissolved oxygen has quadrupled since the 1960s (IUCN, 2020). Additionally, the levels of oxygen in the ocean are expected to decline on average by 3 to 4 percent by 2100 (IUCN, 2020). The declining availability of dissolved oxygen negatively impacts reef fisheries by encouraging reef fish to avoid the growing hypoxic zones and harming their shelters, the coral reefs (Hughes et al., 2020).

In reef ecosystems, soft corals provide shelter to a multitude of marine organisms including fish, shrimps, and snails (American Friends of Tel Aviv University, 2011). *P. violacea*, commonly known as green star polyps, are a species of soft coral found in the shallow to moderate depths along the upper reef edges of the Indo-Pacific Ocean (Page, 2020). Recent studies of the Indo-Pacific Ocean have found that a large pool of warm water has doubled in size since 1900 and increased in temperature (Stevens, 2020). This finding along with the green star polyps' sensitivity to water conditions made *P. violacea* an attractive option for this study.

Previous studies that have examined the impact of deoxygenation on coral have found that limited availability of dissolved oxygen negatively impacts coral reef ecosystems. However, these studies focus on evaluating the entire ecosystem rather than a specific coral species, and they do not measure the short-term impact of deoxygenation. The primary goal of this study is to observe the short-term impact of deoxygenated water on *P. violacea* coral fragments by evaluating immediate reactions as well as quantifying the average time needed for complete recovery.

MATERIALS AND METHODS

This study uses a two-group experimental design to compare the effects of deoxygenated water on coral. The control group was tested with regular tank water while the experimental group was tested with tank water containing a lower concentration of dissolved oxygen (DO).

P. violacea or green star polyps were used as the test subjects. The coral was maintained in two 40-liter tanks, as seen in Figure 3. The tanks were constructed before the experiment.

The salinity in the tanks was maintained using marine salt to a concentration of approximately 35 parts-per-trillion (ppt) and a refractometer. The refractometer was used to measure the concentration of salinity, and its glass plate was routinely cleaned with distilled water and a screen cloth.

200 milliliters of aquarium water were retrieved from the constructed tanks and placed inside a vacuum desiccator connected to the Gast$_{TM}$ DOA-P704-AA High-Capacity Vacuum Pump. The water was degassed for approximately 15 minutes or until the pressure inside the desiccator reached approximately -24 Hg.

To allow the fragment enough time to recover from each trial, the entire experiment took place over the course of approximately six months. In each trial, one healthy fragment of *P. violacea* was removed from the maintenance tanks and placed inside a separate 2.5-gallon tank filled with aquarium water from the maintenance tanks. An optical dissolved oxygen sensor was used to measure the level of dissolved oxygen in the tanks throughout each trial of the experiment. Using a mobile timelapse-photography application and the 3D-printed camera tool, the coral fragment was photographed every thirty seconds. The camera tool held the camera and the coral at an equal distance from each other throughout the trials, ensuring that the angle and perspective of the photographs was consistent throughout the time lapse, as seen in Figure 2. After about 5 minutes of photography, the deoxygenated water was applied on top of the coral fragment. The mobile application continued to take pictures of the coral fragment for 5 hours.

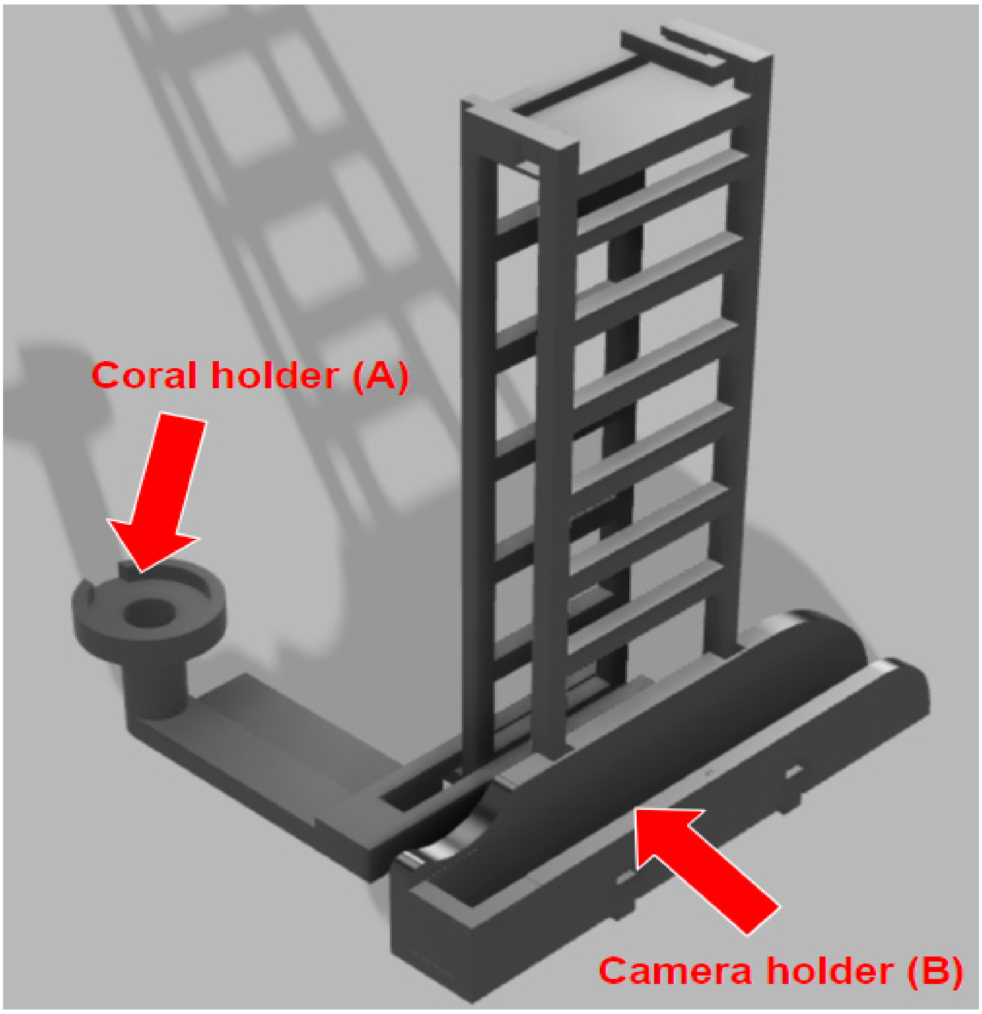

Figure 1. Fusion 360 digital rendering of 3D printed tool; camera tool printed using Cura (printing software).

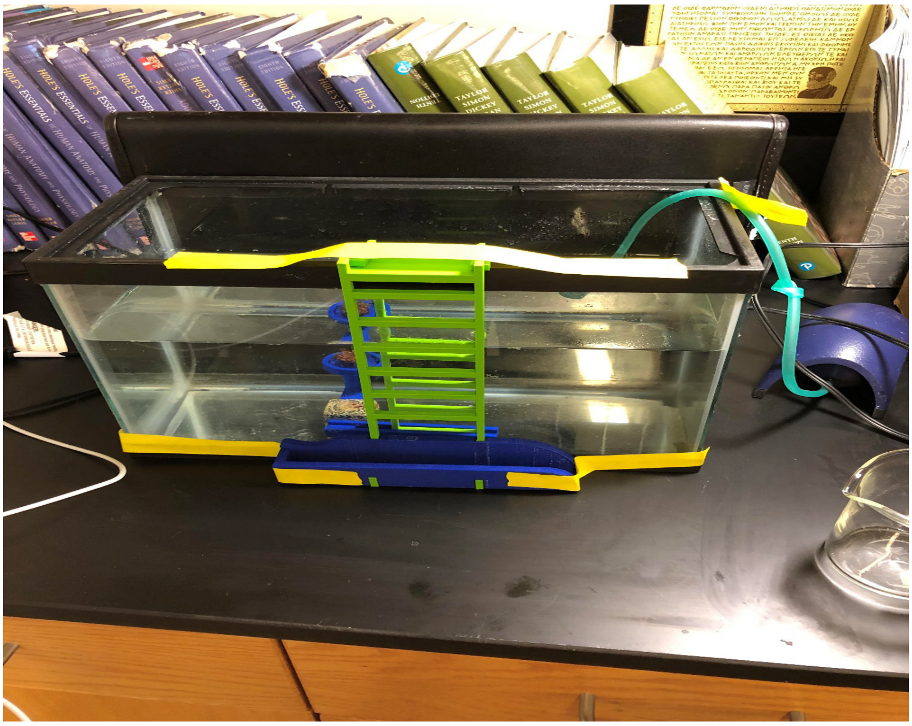

Figure 2: Test setup of experiment.

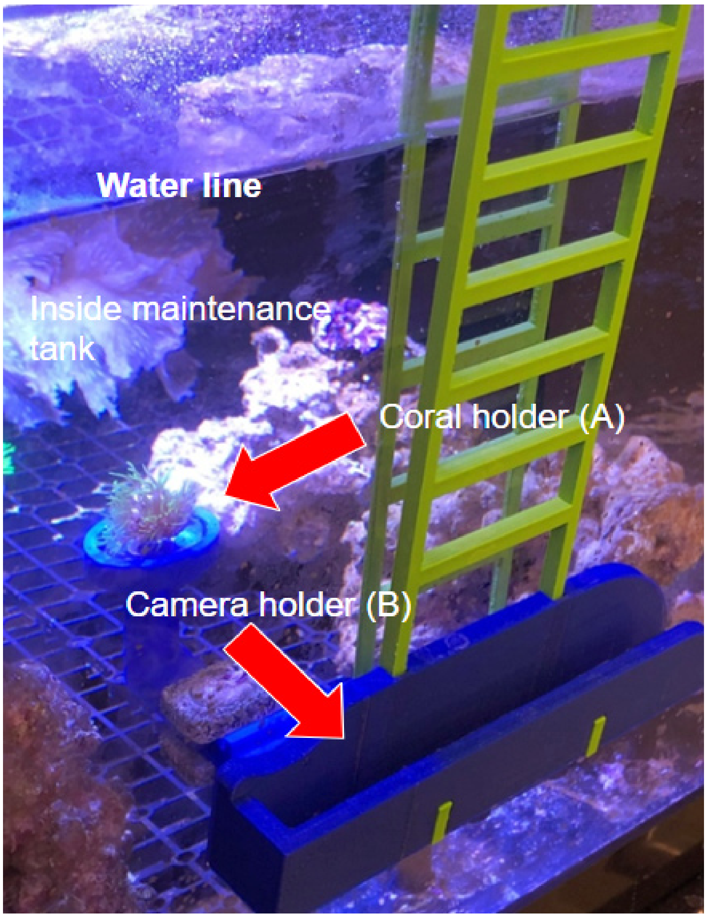

Figure 3. Coral inside maintenance tank on camera tool.

Data regarding the coral fragment's reaction was collected by counting the number of tentacles visible at the end of the trial and recording the time lapsed for the coral fragment to return to its original condition (when all the tentacles are fully extended as they were at the beginning of the trial). To account for differences in the number of tentacles on each fragment, the data took the percentage change in the number of tentacles visible between the number of tentacles visible before any treatment and the number of tentacles visible after treatment.

RESULTS

Table 1. Saturation Readings of Dissolved Oxygen

Trial	Control	Experimental
1	0.220	0.155
2	0.200	0.193
3	0.216	0.177
4	0.211	0.160
$\bar{x}$	0.21175	0.17125
Standard Error of Mean	0.00433	0.00864

Table 2. Percentage Change in Number of Tentacles Extended After Water Treatment

Trial	Control	Experimental
1	17	36
2	37	71
3	33	79
4	43	50
$\bar{x}$	32.5	59
Standard Error of Mean	5.56028	9.80646

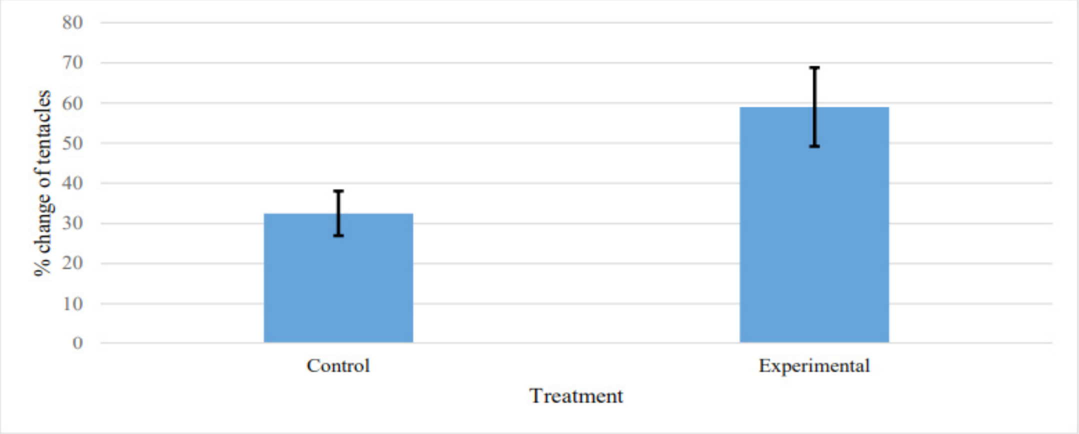

Figure 4. Percentage Change in Number of Tentacles Visible After Water Addition

The change in percentage of tentacles extended after water was added to the tank (*oxygenated water for the control group and deoxygenated water for the experimental group*). (n=4; +/- Standard error of the Mean). $p<0.05$. Figure was made by author.

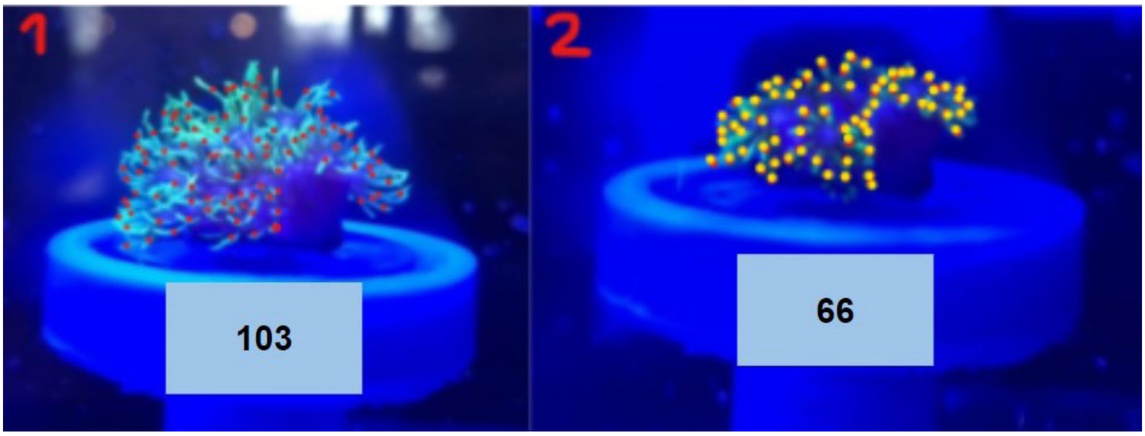

Figure 5. Number of Tentacles Visible in Trial 2 of the Experimental Group

This figure demonstrates the counting method used to quantify coral health. A desktop drawing software was used to mark the number of tentacles visible. Image #1 - extended tentacles marked red = 103; Image #2 extended tentacles marked yellow = 66.

Table 3. Recorded Recovery Time in Minutes

Trial	Control	Experimental
1	29.5	39.5
2	22	65
3	15	71
4	17	37
x̄	20.875	53.125
Standard Error of Mean	3.22991	8.68997

The time elapsed in minutes from addition of water to the tank until the number of extended tentacles was equal to the extended tentacle number at time 0.

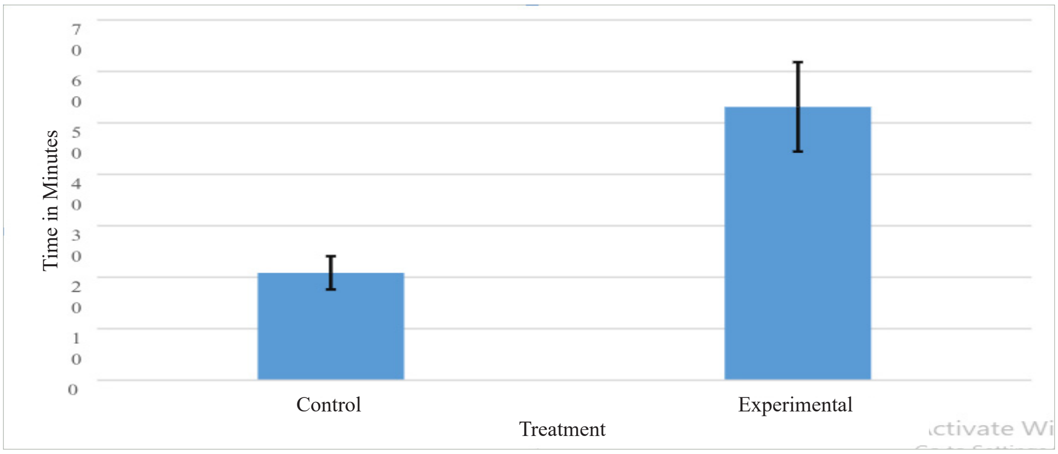

Figure 6. Average Recorded Recovery Time in Minutes

Average recovery time of coral tentacle extension. (n=4; +/- Standard error of the mean). p<0.02. Figure was made by author.

DISCUSSION AND CONCLUSIONS

The higher percentage change in the number of tentacles shown in the experimental group compared to the percentage change in the control group can be attributed to the change in the level of dissolved oxygen in the tank water. This likely contributed to the time required for full recovery, explaining the greater average recovery time in the experimental group than in the control group.

The percentage change in the number of tentacles in the experimental group was consistently higher than the percentage change in the number of tentacles in the control group (Table 2). In Table 2, the vertical height of the standard error bars reflects the variation or range of data. Non-overlapping Standard Error Bars provide a higher level of confidence that the difference in the number of tentacles was caused by the treatment, rather than by random variation. Like the percentage change in the number of visible extended tentacles, the average recovery time was also consistently longer in the experimental group than in the control group (Table 3). Again, there is more confidence that the difference is caused by the lowered levels of dissolved oxygen due to the non-overlapping Standard Error Bars. However, further research should focus on minimizing this confidence interval through a larger sample size and additional trials.

The results of this study align with previous findings that limited oxygen availability in the water affects the natural processes of coral (Nelson & Altieri, 2019; Hughes et al., 2020). Nevertheless, there is a need for more research regarding the extent to which deoxygenation affects coral respiration as the results from this study are nonspecific.

Research regarding the effects of deoxygenation on coral is important for the recovery of coral reef fisheries. High levels of hypoxia can cause reef-associated fish to avoid the affected region (Hughes et al., 2020). Depending on the severity, the limited accessibility of dissolved oxygen can also cause increases in fish and coral mortality rates (Hughes et al., 2020). These studies are valuable as coral reef fisheries depend on the existence and availability of reef-associated fish (The Nature Conservancy, n.d.).

Future research may involve observing the impact of different levels of deoxygenation on *P. violacea* over multiple weeks or years. This study used an optical dissolved oxygen sensor that measured the relative levels of dissolved oxygen in the water. Future studies may measure the exact levels of dissolved oxygen to determine the hypoxia threshold for the recovery of *P. violacea*.

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