Fall 2017

Bacteria Mitigation in Sponge Mariculture, Jambiani Zanzibar

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SIT Study Abroad

BACTERIA MITIGATION IN SPONGE MARICULTURE, JAMBIANI ZANZIBAR

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Coastal Ecology and Natural Resource Management, Zanzibar: Fall 2017

Table of Contents

I. Acknowledgments:

Thank you to Marine Cultures for welcoming me into Jambiani and assisting me every single day. Thank you to Dr. Narriman Jiddawi and Said H. Omar for their guidance and advice. Thank you to Ali Said Omar and Vaui for their assistance and company. Most importantly, thank you to Dr. Richard Walz for his help and unending support. He has been an invaluable teacher and friend.

II. Abstract:

The declining seaweed industry coupled with efforts by the NGO Marine Cultures have led to the establishment of sponge mariculture in Jambiani, Zanzibar. However, growing cyanobacteria levels have substantially increased sponge mortality rates. In order to determine successful cyanobacteria mitigation treatments, six populations of farm ropes, support lines, and sponges were (a) untreated, (b) manually cleaned or submerged in solutions of (c) 2% hydrogen peroxide, (d) 4% hydrogen peroxide, (e) 50 g/L salt, or (f) 70 g/L salt. No conclusions were drawn from rope treatments, no techniques were effective for support line treatments, and both concentrations of hydrogen peroxide were successful for sponge treatments. Additionally, unstructured interviews were conducted with sponge farmers to ascertain longitudinal climate change and bacteria growth data in Jambiani.

III. Introduction:

Sponge farming was introduced to Zanzibar in 2009 by the NGO Marine Cultures. Sponges provide an alternative to the declining seaweed industry, considering their compatibility with warming waters combined with high global prices and demand (Vaterlaus and Bumbak, 2011). Both deep water (floating) and shallow water (off bottom) farms have been established in Jambiani. While off-bottom farms are more economically and logistically feasible for community members, particularly women, they face a number of obstacles associated with the shallow water environments (Hamad, 2017). According to conversations with staff at Marine Cultures and sponge farmers, cyanobacteria bio-fouling has destroyed a large percentage of off-bottom sponge crops in the last two years. One woman claimed that if she has 2,000 sponges, 1,300 of them will be killed by bacteria (Informant 1). Currently, bacteria growth is addressed by manually cleaning the ropes, support lines and sponges on the farm everyday. However, problems with this treatment method arise due to the physical limitations of farmers. Since a number of women cannot swim, the farmers are only able to access the farms at spring and neap tides, leaving the sponges without maintenance for days at a time (Informant 2). Alternate mitigation techniques are needed that address this treatment gap into account.

In order to mitigate mortality in off-bottom farms due to cyanobacteria, five new antifouling techniques were implemented in Jambiani. Methods included 15-second daily submersion in solutions of a) 2% hydrogen peroxide, (b) 4% hydrogen peroxide, (c) 50 g/L salt, or (d) 70 g/L salt. These new treatments were compared with the manual removal treatment and a control.

Considering the anticipated continued increase in bacteria populations with climate change, longitudinal interviews focusing on the relationship between climate change and cyanobacteria were conducted with Marine Cultures staff and sponge farmers (Paerl et al., 2016). Questions focused on how employment, industries and individuals had been affected by climate change and bacteria. The interviews were used to create a short documentary that shares oral histories of coastal erosion, seaweed farming and the impacts of bacteria on sponge farming and farmers in warming waters of the Western Indian Ocean.

IV. Background

i. Study Area:

Jambiani is a small village located on the east coast of Unguja, Zanzibar-Tanzania with a population of 6,060 (Tanzania NBS, 2012). A recent Institute of Marine Sciences (IMS) dissertation found "locals are primarily engaged in tourism activities, coconut rope making, fishing, seaweed farming, octopus hunting, shell collecting, small-scale agriculture, livestock keeping and small businesses" (Ahmada Hamad, 2017). The average annual temperature in Jambiani is 27.7 °C and the average yearly rainfall is 1518 mm (Climate-Data.org., 2015).

Studies were conducted at the Marine Cultures sponge farms in Jambiani. There are two farms, the first operated by economically independent farmers and the second used as training sites led by Marine Cultures staff. The farms are located approximately 500 meters from the

Jambiani coastline (Figure 1). Depth differed substantially with tide, ranging from 0.5-5 meters, and water temperature was recorded at 29 °C.

Figure 1: Map of Jambiani village and sponge farms. Site 1 is located in the bottom grid and site 2 is located in the top grid.

ii. Key Concepts:

Seaweed farming is one of the primary employment opportunities for women in Jambiani. However, the industry has seen a decrease in global demand over the last decade. According to Msuya (2011) "when seaweed farming started in Tanzania in 1989, the two species that are farmed commercially…were thriving" (p. 34). However, a global decline in seaweed prices combined with higher water temperatures has resulted in a drastic decrease in production. In Jambiani's neighboring village, Paje, there were 1,400 farmers (both men and women) during the seaweed production peak (1990-1998). However, in 2010, this was reduced to less than 200 farmers, all of whom were women (Msuya, 2011).

Marine Cultures began experimentation with sponge farming in 2009 in order to establish an alternative industry for women, modeled after farms established in Polynesia and Australia.

The founder of Marine Cultures, Christian Vaterlaus, cites the higher earnings, increasing demand, lower initial costs and simple methods as comparative advantages of sponge farming.

Sponges, from the phylum porifera "exhibit a wide variety of forms, their size and shape frequently being determined by the nature of the material which they are growing on and by the water currents flowing over them" (Karleskint et al., 2012). They are known for their unique anatomy consisting of "a system of water canals… through which large amounts of water circulate" (Karleskint et al., 2012). Sponges are extremely absorbent, holding "as much as 35 times their weight in liquid" and in high demand considering consumers are willing to pay a higher price for "a sponge that is superior to anything synthetic" (Karleskint et al., 2012). According to Connie Vauterlaus, co-founder of Marine Cultures, Callyspongiidae sp. was selected because it grows and reaches maturity at a faster rate than other sponges.

Since 2009, two farms have been established; "The first farm is located at the tail of a channel at an operational depth of 4 m to 9 m (due to the tidal range) and the second farm is located in a shallow area (1m to 5m)" (Vaterlaus and Bumbak, 2011). Experimental phases of the project determined the most successful species for production at Jambiani (Ageleas Mauritiana var. oxeata and Callyspongiidae sp)., as well as the most successful method (threaded line). Additionally, multiple studies, including an ISP from 2011 and an IMS dissertation, have compared success in off-bottom farming and floating farms. Ahmada Hamad (2017) concluded that the "floating method is the best method…for commercial production" and "innovative techniques are needed to make the off-bottom method more efficient" (pg. 45). It is vital to make progress in off-bottom technology, considering the floating method requires swimming, a boat and scuba equipment, which is unrealistic for local women.

A discussion with Marine Cultures staff revealed cyanobacteria growth is one of the largest barriers to off bottom farm success. Cyanobacteria are photosynthetic organisms that proliferate in warm and nutrient rich waters (Speer, 1995). Cyanobacteria growth restricts food availability and water flow in organisms, referred to as "bio-fouling." Cyanobacteria mitigation has been studied in both freshwater and saltwater contexts, especially Cyanobacteria Harmful Algal Blooms (CyanoHAB's). Watershed/Airshed controls have been implemented in regions with extensive capital and political resources. These controls rely heavily on the regulation of non-point source pollution or expensive treatments, such as sediment dredging and flushing. Chemical mitigation strategies have been implemented in less commercialized regions lacking extensive funds, such as Zanzibar (Paerl et al., 2016).

Figure 2: Example of red algae growing on sponge surface.

Figure 3: Cyanobacteria growing on sponges and support line.

Most chemical mitigation techniques have been applied in the context of bivalve mariculture. Three studies show potential for application in Jambiani sponge farms. According to a study conducted in 2011 of triple salt based disinfectant as an antifouling mechanism, "immersion in 3% disinfectant for 30 s reduced the biomass of fouling material by up to 89% and would be feasible in field applications using existing treatment equipment" (Paetzold and

Davidson, 2011). A second study, conducted in 2007, concluded that mussels exposed to 4% acetic acid mixed with seawater for one minute could eliminate soft bodied organisms in biofouling (Forrest et al., 2007). A third study from 2011 did not address biofouling in farms, but analyzed the application of hydrogen peroxide in waste ponds for the removal of cyanobacteria. Removal in field conditions was 78% successful (Barrington et al., 2011). Considering treatment development methodology from this study was not transferable, a secondary study (based on zebra mussel biofouling) was utilized to determine hydrogen peroxide concentrations (Petraille and Miller, 2000).

V. Methodology:

i. Site Preparation:

Experiments were conducted at two sites located on the Marine Cultures sponge farm. Site one consisted of four 230 cm rope segments while site two consisted of two 230 cm segments. Site one was located on the northeastern side of the farm and site two was located on the mid-northwestern side of the farm (Figure 1). The rope segments were located at a fixed height and position in the water column, held by concrete bases on each corner of the farm. Each rope contained four sponges, spaced 46 centimeters apart. Sponges were approximately 6cm x 6cm x 6cm, however each varied in specific shape and maximum diameter. Sponges were attached to the farm ropes using fishing line, approximately 28 cm in length. Each segment of fishing line was inserted through the rope and tied, ensuring sponges would remain in constant positions throughout the treatment period. Locations and lengths were selected following conversations with Marine Cultures staff and farmers, based on availability of resources and space.

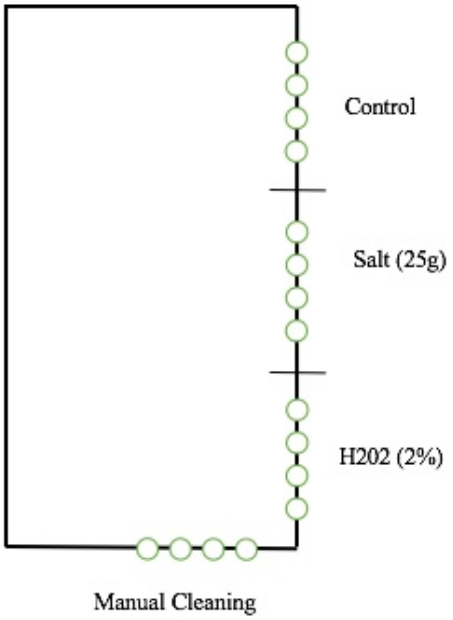

Figure 3: Site 1 held four treatment methods including manual cleaning and control.

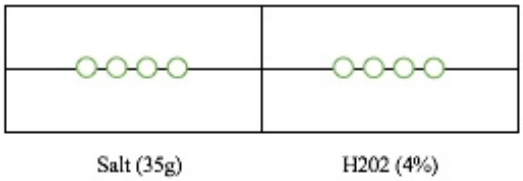

Figure 5: Site 2 held two treatment methods, including increased Hydrogen Peroxide and Salt.

ii. Materials

Stationary materials, including rope, line, and sponges, remained at the site throughout the treatment period. Observation supplies included one wetsuit, dry boots, one mask/snorkel, one GoPro and a 10-meter tape measure. In order to produce solutions, 565 grams of salt, 300mL

Figure 6: Sponge farmers cleaning ropes near site 1.

Figure 7: An overview of site 1 at high tide.

of 6% strength H_2O_2, and 14.4 mL of 50% strength H_2O_2 were used throughout the treatment period. A 10cm segment of synthetic rope and a small knife were used during manual cleaning. Supplies were transported to the farm in separate 800 mL airtight containers. All supplies were carried in a 7 L bucket, and a rectangular personal flotation device (PFD) was used to stabilize materials on site.

iv. Trial Treatments

Initial treatments included control, manual cleaning, vinegar, salt and hydrogen peroxide. Six sponges of similar size (6 x 6 x 6 cm) were collected from the nursery farm and transported to the site 1 in the farm. Two sponges were submerged in 8% hydrogen peroxide solution for one minute, and attached in site one. The same procedure was repeated with two sponges in 250g/L saline solution and two sponges in 100% vinegar solution. The sponges were attached to site one and observed after 24 hours.

The second round of trial treatments was conducted at the same site, using new sponges from the nursery farm. Two sponges were submerged in 6% hydrogen peroxide solution for one minute, and attached to site 1. The same procedure was repeated with two sponges in 3% saline solution and two sponges in 50% vinegar solution. The sponges were observed after 24 hours.

v. Final Treatments

Treatments included manual cleaning, 2% H_2O_2, 5% salt, control, 7% salt, and 4% H_2O_2. Treatments were applied November 5-10 and 15-18 at low tide. While exact time of application and tidal range varied every day, application occurred at low tide every day.

Salt treatments were weighed on a scale, 25g and 35g respectively, and were sealed in 800 mL airtight containers. Hydrogen peroxide treatments, 10 mL and 20 mL of 6% strength H_2O_2, were measured in a graduated cylinder and sealed in identical 800mL containers. Solutions were transported to the site in a 7 L bucket using the PFD for stability and flotation. Supplies were secured to the farm perimeter, and observations were taken for the first immediately before application 500 mL of seawater was added and mixed until homogenous in each 800 mL container. Water was added to each solution on site.

However, following a labeling error on November 8, 6% strength hydrogen peroxide was replaced by 50% strength. During treatment on November 8, 10 mL and 20 mL 50% strength hydrogen peroxide was applied but did not kill the sponges. In order to create a concentration equivalent to the original solution, 2.5 liters of seawater were combined with 6 mL of H_2O_2 for the 2% solution, and 2.5 liters seawater were combined with 12 mL H_2O_2 for the 4% solution. This solution was applied for the rest of the treatment period.

Observations and measurements were taken immediately upon arrival at the site. Three categories were created to record growth for each section. First, vertical growth in centimeters was measured along each section of the rope. The rope was held above the water line and measured from the bottom of the rope to its tip of apparent growth. Second, growth in centimeters was recorded along the fishing line attaching each sponge to the rope. The fishing line was held above the water, and length was recorded using the tape-measure. Third, approximate percentage coverage and mortality were recorded for each sponge. The sponge was assessed both above and below water to increase visual clarity.

During solution application, the container was unsealed and each sponge was fully submerged in the solution for 15 seconds. Remaining solution was evenly applied to the rope and

fishing line until all 500 mL had been used. During manual cleaning, a 5 cm piece of rope was wrapped around the main line, and moved up and down vigorously until all bacteria was removed from the line. No treatment was applied to the control line.

VI. Results

i. Trial Treatment Results

Trial one resulted in 100% sponge mortality. All vinegar treated sponges, all salt treated sponges and all H_2O_2 treated sponges were found lifeless following the 24-hour trial period. The second trial resulted in 33% sponge mortality. All vinegar sponges showed 100% bleaching and were dead after 24 hours. Both H_2O_2 sponges survived, but showed 30% and 50% bleaching respectively. Salt sponges appeared to be healthy.

ii. Final Treatment Results

Results were divided into three groups: rope growth, support line growth and sponge percent coverage. Identical analysis was conducted for each group using Excel, ANOVA and Tukey Honest Significant Difference (HSD) Post-Hoc software.

a. Rope Growth

Daily growth in the five segments of each treatment area was averaged and graphed using Excel (Table 1 & Figure 8). Control had the highest daily average (3.68 cm), while H_2O_2 (4%), manual cleaning and salt (35g) shared the lowest daily average (0 cm).

Additionally, treatment averages were split into two categories based on site location and graphed. Site 1 had a maximum daily average of 3.68 cm in the control treatment group, and a

minimum of 0 cm in the manual cleaning treatment group. Site 2 had a maximum of 0.95 cm in the H_2O_2 (4%) treatment group and a minimum of 0 cm in both groups (Figures 9 and 10).

Table 1: Average daily vertical bacteria growth on rope in centimeters.

Date	Manual Cleaning	H_2O_2 (2%)	Salt (25g)	Control	Salt (35g)	H_2O_2 (4%)
5-Nov	0.366	0.34	0.12	0.71		
6-Nov	0	0.37	0.31	0.8	0	0
7-Nov	0.22	0.47	0.4	0.95	0.13	0.1
8-Nov	0.3	0.48	0.48	0.86	0.32	0.17
9-Nov	0.24	0.75	0.81	1.5	0.21	0.1
10-Nov	0.20	0.68	1.12	1.66	0.22	0.14
15-Nov	3.2	2.1	2.5	3	0.47	0.95
16-Nov	0.34	1.6	2.46	3.1	0.54	0.65
17-Nov	0.23	1.92	2.56	3.68	0.82	0.45
18-Nov	0.15	2.35	2.67	3.47	0.82	0.48

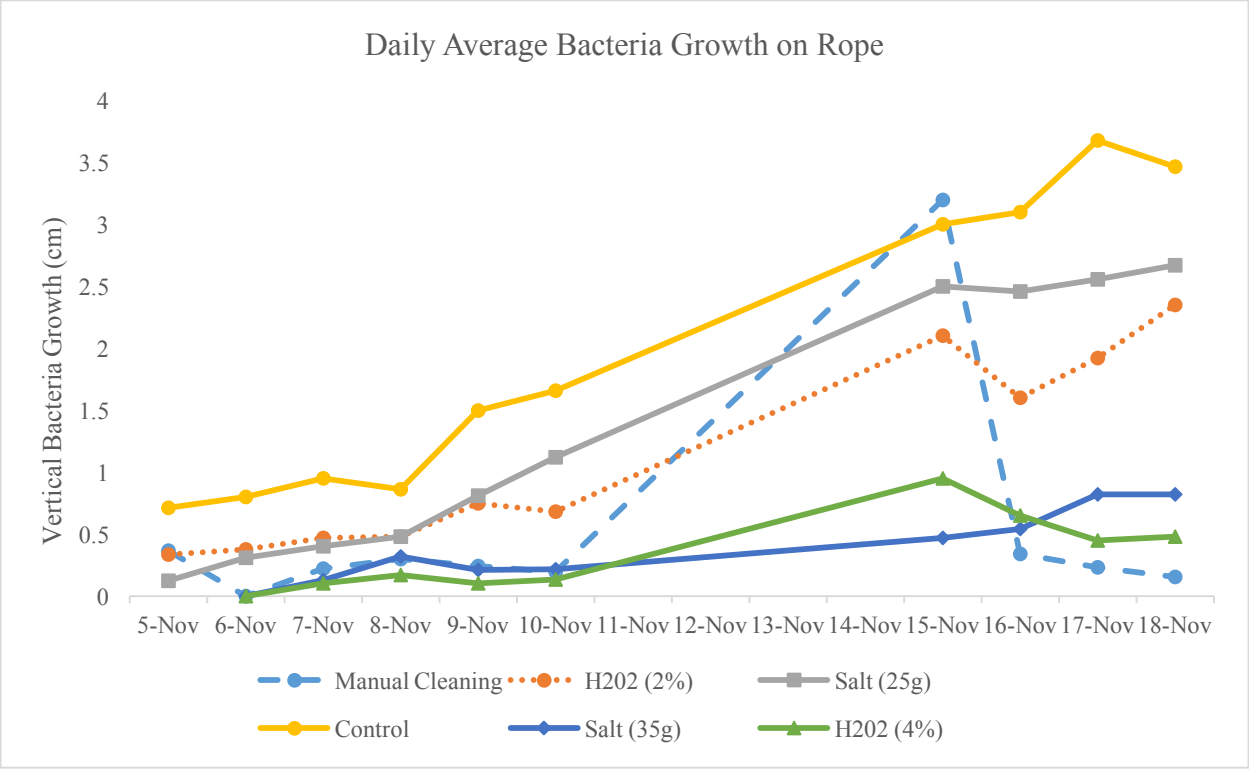

Figure 5: Daily average vertical bacteria growth on rope compared among treatment methods.

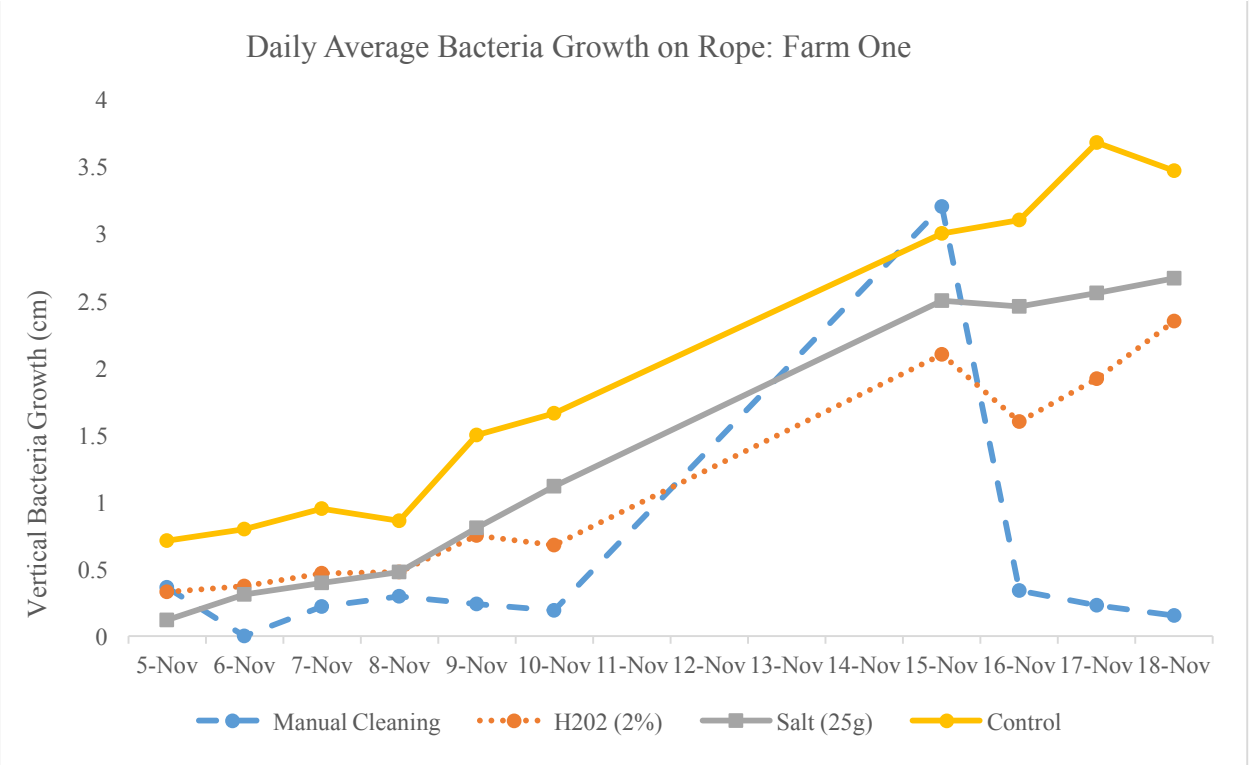

Figure 9: Daily average vertical bacteria growth on rope compared among treatment methods located at site one.

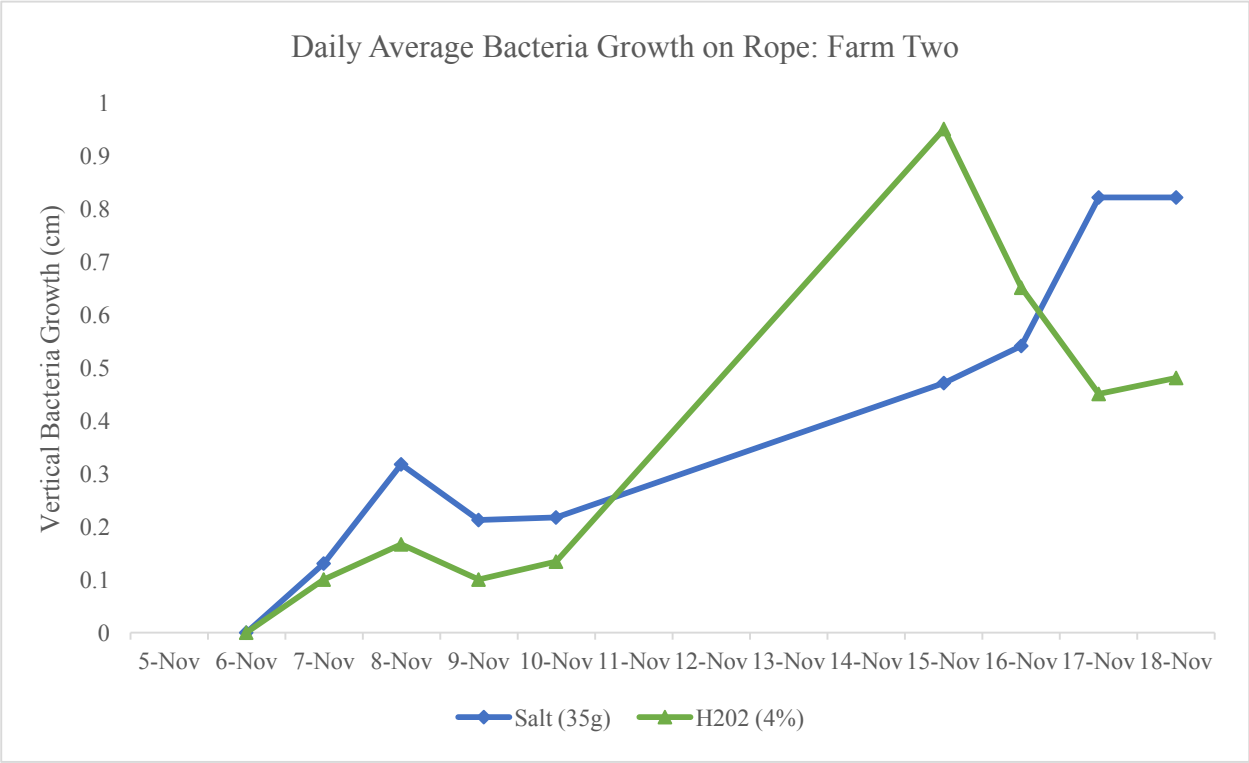

Figure 6: Daily average vertical bacteria growth on rope compared among treatment methods located at site two.

ANOVA single-factor analysis was conducted and the null hypothesis was rejected. Data displayed a significant p-value of 0.0004. Tukey Honest Significant Difference (HSD) Post-Hoc analysis resulted in three significant comparisons. The manual cleaning, salt (35g), and H_2O_2 (4%) treatments had p-values <0.01 when compared to the control (Appendix). Following Post-Hoc analysis, average growth of statistically significant groups was graphed. Control maintained the highest daily average (3.68 cm), while H_2O_2 (4%), manual cleaning and salt (35g) maintained the lowest daily average (0 cm). Additionally, average rope growth of statistically significant treatments was compared before and after the treatment gap. Manual cleaning had the highest growth during this period (3.06 cm), followed by control (1.34 cm) and H_2O_2 (4%) (0.82 cm). Salt (35g) had the least growth (0.2525 cm).

Table 2: ANOVA single-factor average daily vertical bacteria growth on rope results.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	20.24	5	4.05	5.45	0.0004180 4	2.39
Within Groups	38.63	52	0.73			
Total	58.87	57				

Table 3: Tukey Post-Hoc analysis for average bacteria growth on rope among (a) manual cleaning, (b) H2O2 (2%), (c) salt (25g), (d) control, (e) salt (35g), and (f) H2O2 (4%).

Pair	Q statistic	p-value	Inference
A vs B	2.1322	0.6411986	insignificant
A vs C	3.0028	0.2918162	insignificant
A vs D	5.3152	0.0055096	** p<0.01
A vs E	0.4741	0.8999947	insignificant
A vs F	0.6711	0.8999947	insignificant
B vs C	0.8705	0.8999947	insignificant
B vs D	3.183	0.2330699	insignificant
B vs E	2.5494	0.4740864	insignificant
B vs F	2.7465	0.3905308	insignificant
C vs D	2.3124	0.5695777	insignificant
C vs E	3.3967	0.1747429	insignificant
C vs F	3.5938	0.1308126	insignificant
D vs E	5.6475	0.0026904	** p<0.01
D vs F	5.8446	0.0017359	** p<0.01
E vs F	0.1921	0.8999947	insignificant

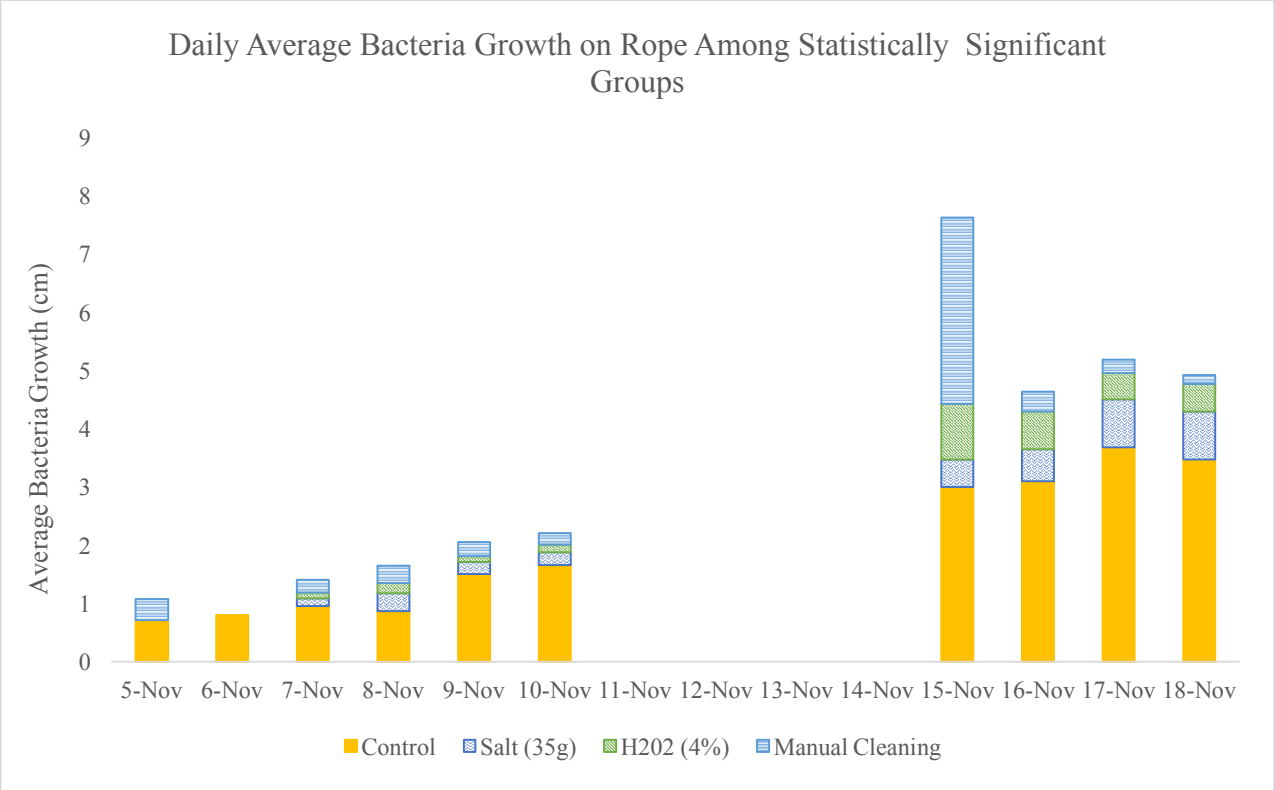

Figure 7: Daily average vertical bacteria growth on rope compared among statistically significant treatment methods.

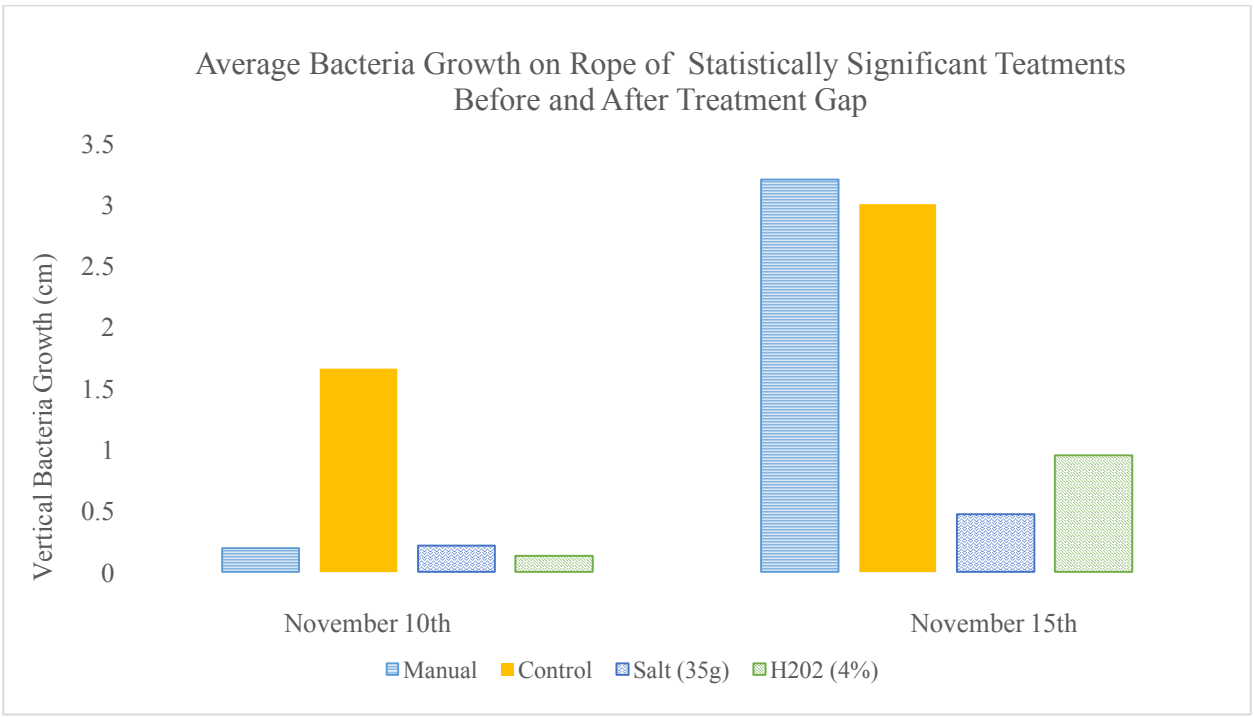

Figure 12: Vertical bacteria growth on rope among statistically significant treatment methods compared before and after the treatment gap.

b. Support Line Growth

Daily growth on the five support lines of each treatment area was averaged and graphed using excel (Table 4 & Figure 13). Manual cleaning had the highest daily average (16.3 cm), while H_2O_2 (4%) had the lowest daily average (0 cm).

Table 4: Average daily bacteria growth on support line in centimeters.

Date	Manual Cleaning	H_2O_2 (2%)	Salt (25g)	Control	Salt (35g)	H_2O_2 (4%)
5-Nov	2.67	0.25	2.125	2.75		
6-Nov	2	0.58	2.5	2.75	0.38	0
7-Nov	0.625	0.38	3	3	3.38	2.9
8-Nov	5.25	3.25	3.75	3	7	3.75
9-Nov	3	1.25	3.75	4	5.25	3.5
10-Nov	3	1.38	3.25	4.125	5.88	4.2
15-Nov	16.34	8.25	10.75	15.67	6	6
16-Nov	1.58	8.25	11	16	11	3
17-Nov	0.5	5.75	10.75	16	10.25	0.5
18-Nov	0.34	4.5	11.5	16	10.5	0.75

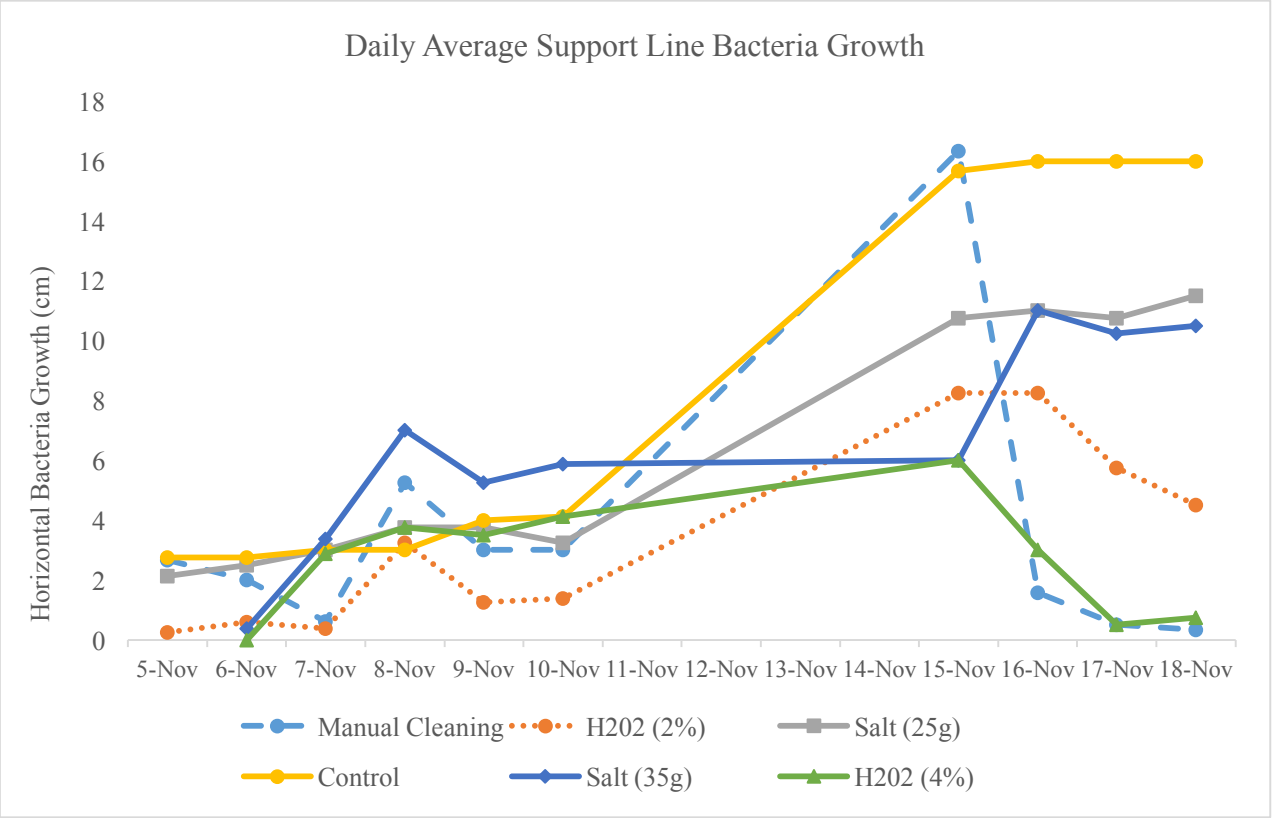

Figure 8: Daily average horizontal support line bacteria growth on rope measured in centimeters.

ANOVA single-factor analysis was conducted and the null hypothesis was rejected. Data displayed a significant p-value of 0.03. Tukey HSD Post-Hoc analysis resulted in no statistically significant comparisons.

Table 5: ANOVA single-factor results for average support line growth.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	243.01	5	48.60	2.63	0.03	2.39
Within Groups	961.90	52	18.49			
Total	1204.91	57				

Table 6: Tukey Post-Hoc analysis for average support line growth among (a) manual cleaning, (b) H2O2 (2%), (c) salt (25g), (d) control, (e) salt (35g), and (f) H2O2 (4%).

Pair	Q statistic	p-value	Inference
A vs B	0.1072	0.90	insignificant
A vs C	1.9913	0.70	insignificant
A vs D	3.5292	0.14	insignificant
A vs E	2.2155	0.61	insignificant
A vs F	0.5775	0.90	insignificant
B vs C	2.0985	0.65	insignificant
B vs D	3.6364	0.12	insignificant
B vs E	2.3199	0.57	insignificant
B vs F	0.4731	0.90	insignificant
C vs D	1.5379	0.88	insignificant
C vs E	0.2773	0.90	insignificant
C vs F	2.5157	0.49	insignificant
D vs E	1.2196	0.90	insignificant
D vs F	4.0126	0.07	insignificant
E vs F	2.72	0.40	insignificant

c. Sponge Percentage Cover

Average daily sponge percent coverage for each treatment area was calculated and graphed using excel (Table 7 and Figure 14). Control had the highest daily average (65%), while H_2O_2 (4%) and manual had the lowest daily average (0 cm).

Treatment averages were then separated into two categories based on site location and graphed. Site one had a maximum daily average of 65% growth in the control treatment group, and a minimum of 0% in the manual cleaning treatment group. Site 2 had a maximum of 5% in the Salt (35g) treatment group and a minimum of 0% in the H_2O_2 (4%) group (Figures 14 and 15).

Table 7: Daily average percent sponge coverage among all treatment methods.

Date	Manual Cleaning	H_2O_2(2%)	Salt (25g)	Control	Salt (35g)	H_2O_2 (35g)
5-Nov	4	1	2.5	10		
6-Nov	3	2.5	7	15.5	0.5	0.5
7-Nov	10	2.25	17.5	19.25	0.5	0
8-Nov	6.25	1.25	23.75	21.25	1.25	0
9-Nov	0	1.25	25	26.25	1.25	0
10-Nov	3.75	1.25	21.25	21.25	1.25	1.75
15-Nov	41.67	5	12.5	65	5	1.5
16-Nov	0	3.75	15	60	4.25	1.5
17-Nov	0	3.75	13	51.6	4.25	0.5
18-Nov	0	1.25	12.5	50	3.75	1.25

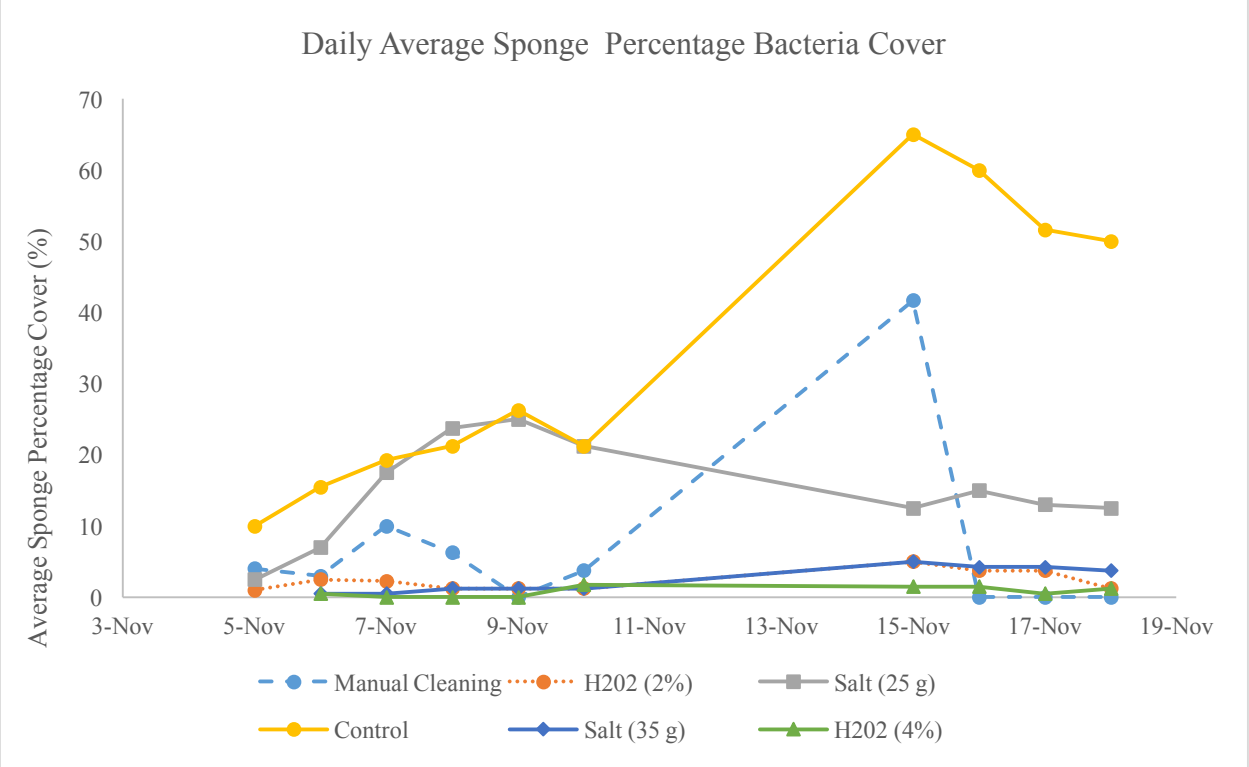

Figure 9: Daily average sponge percent bacteria coverage compared among treatment methods.

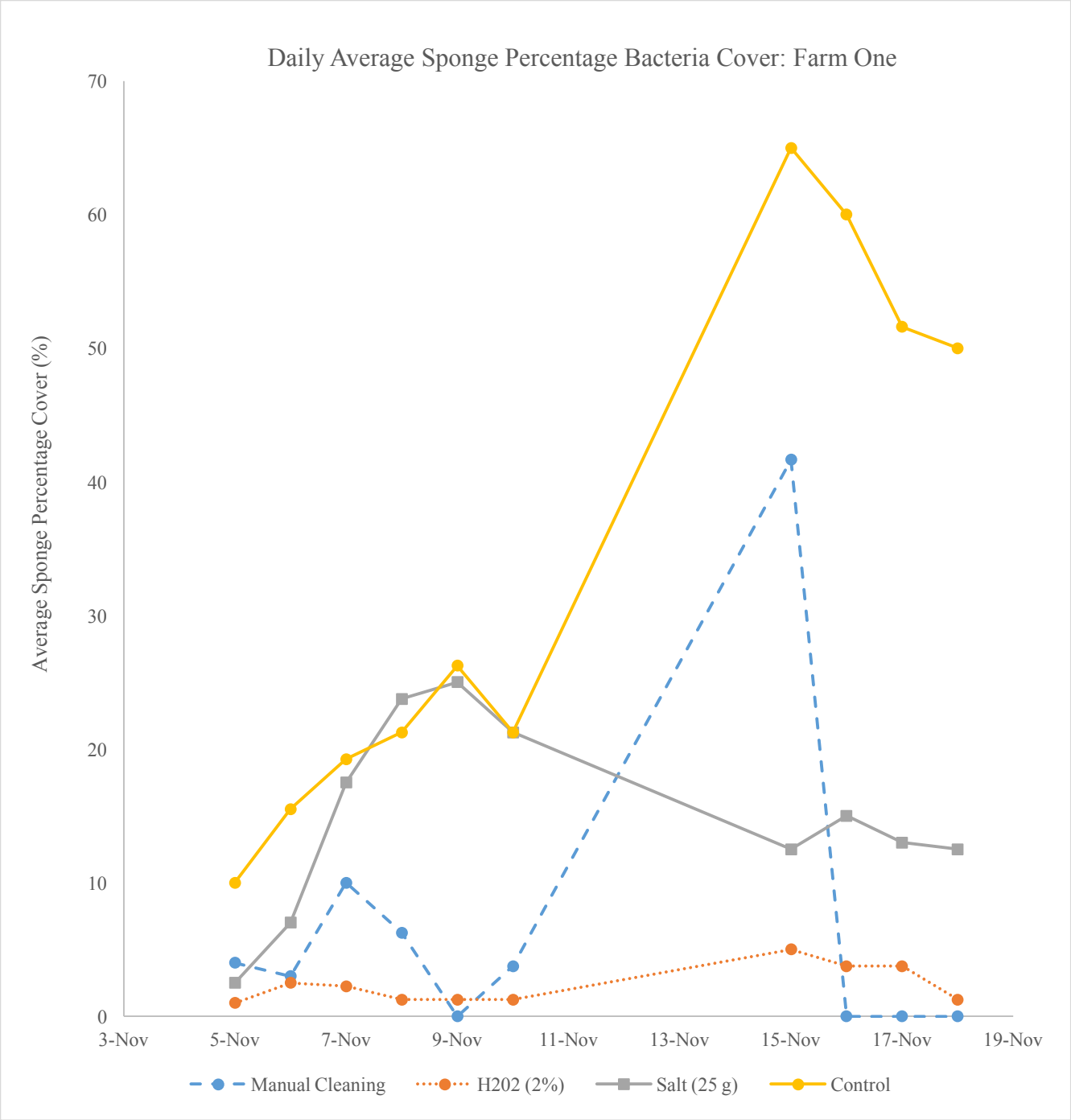

Figure 10: Daily average sponge percentage bacteria cover compared among treatment methods located at site one.

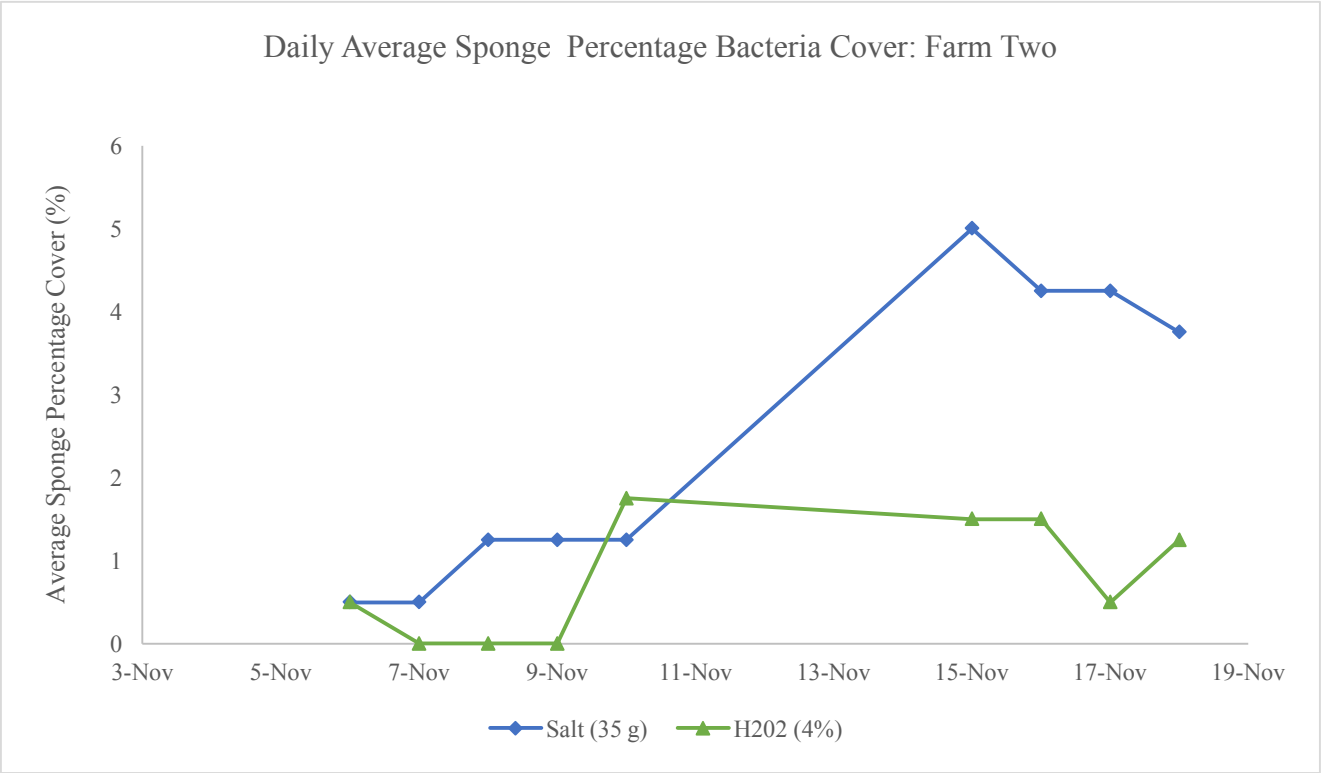

Figure 11: Daily average sponge percentage bacteria cover compared among treatment methods located at site two.

ANOVA single-factor analysis calculated a significant p-value of 6.20×10^{-9} and the null hypothesis was rejected. Tukey HSD Post-Hoc analysis resulted in four significant comparisons. The manual cleaning, salt (35g), and H_2O_2 (4%) and H_2O_2 (2%) treatments had p-values <0.01 when compared to the control (Table 9). Following Post-Hoc analysis, average sponge percent coverage of statistically significant groups was graphed. Control maintained the highest daily average (65%), while H_2O_2 (4%) and manual cleaning had the lowest daily average (0%). Additionally, average sponge percent coverage of statistically significant treatments was compared before and after the treatment gap. Manual cleaning had the highest growth during this period, increasing by 1009%. H_2O_2 (2%) increased by 30% and H_2O_2 (4) decreased by 0.14%.

Table 8: ANOVA single-factor results for average sponge percent bacteria growth.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7964.67	5	1592.93	14.60	6.20E-09	2.39
Within Groups	5673.51	52	109.11			
Total	13638.18	57				

Table 9: Tukey Post-Hoc analysis for average daily percent sponge bacteria growth among (a) manual cleaning, (b) H2O2 (2%), (c) Salt (25 g), (d) control, (e) Salt (35 g), and (f) H2O2 (4%).

Pair	Q statistic	p-value	Inference
A vs B	1.375	0.8999947	insignificant
A vs C	2.4623	0.510007	insignificant
A vs D	8.2175	0.0010053	** p<0.01
A vs E	1.3031	0.8999947	insignificant
A vs F	1.7942	0.7755354	insignificant
B vs C	3.8373	0.0895774	insignificant
B vs D	9.5924	0.0010053	** p<0.01
B vs E	0.0352	0.8999947	insignificant
B vs F	0.4559	0.8999947	insignificant
C vs D	5.7552	0.0021197	** p<0.01
C vs E	3.6997	0.1116862	insignificant
C vs F	4.1909	0.0494147	* p<0.05
D vs E	9.3014	0.0010053	** p<0.01
D vs F	9.7925	0.0010053	** p<0.01
E vs F	0.4787	0.8999947	insignificant

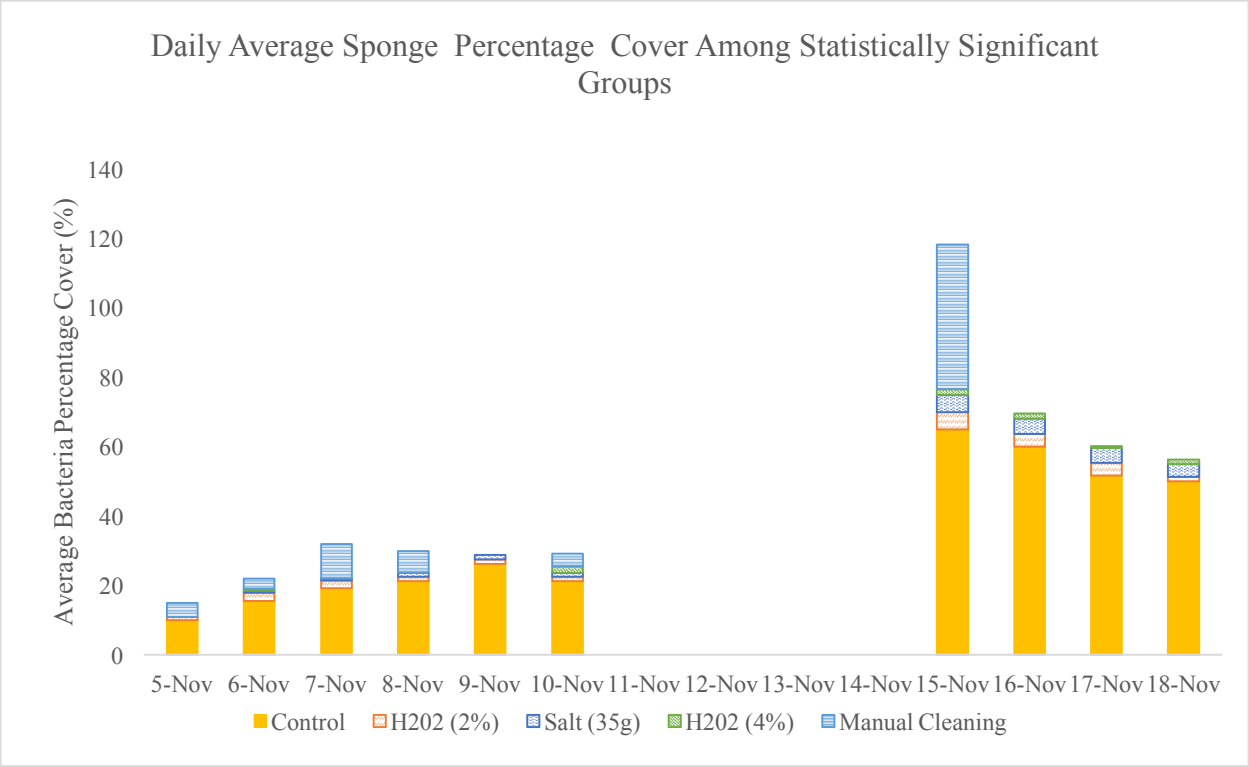

Figure 12: Daily average sponge percent bacteria coverage compared among statistically significant treatment methods.

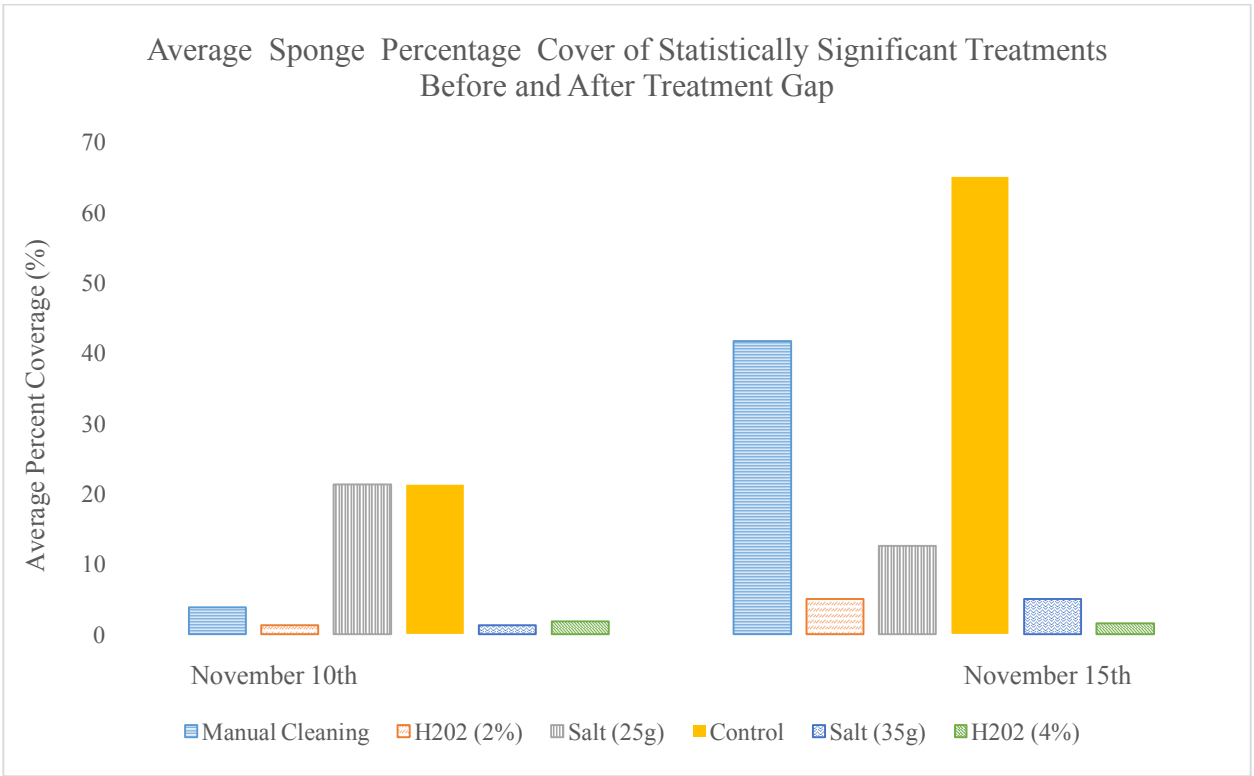

Figure 13: : Daily average sponge percent bacteria coverage among statistically significant treatment methods compared before and after the treatment gap.

VII. Discussion

i. Trial Treatments

While similar treatments have been applied to bivalves, no antifouling treatment had ever been attempted for marine sponges. As a result, there was little information to determine the affect of high concentrations of H_2O_2, vinegar, and salt on sponges. Trial treatment one was modeled after work with bivalves; however, the anatomical differences between bivalves and sponges were not taken into account. Considering sponges are soft bodied and extremely absorbent, they reacted to treatments differently than hard shelled bivalves. The 100% sponge mortality could be attributed to the high concentration and extended length of submersion, which is suited for bivalves.

Trial treatment two considered these anatomical differences into account, and reduced both concentration and length of sponge submersion for all treatments. However, since all vinegar and hydrogen peroxide sponges were negatively affected, concentration and submersion time were once again reduced to confirm final treatment methods. While salt treated sponges appeared healthy, submersion time was also lowered to 15 seconds in order to maintain uniformity across treatments.

ii. Final Treatments

a. Rope

Three rope treatments, manual cleaning, H_2O_2 (4%), and Salt (35 g), statistically treated bacteria more effectively than the control. All of these treatments were successful in controlling bacteria growth in relation to the control; however, the daily growth averages for H_2O_2 (4%) and salt (35 g) were significantly lower. Additionally, bacteria growth increased

drastically on the manual cleaning rope during the treatment gap, exceeding growth of the other methods by more than 80%. These differences could be attributed to the success of H_2O_2 (4%) and salt (35 g) in killing and/or preventing bacteria growth. However, there are multiple factors that call this conclusion into question.

Firstly, Site 1 and Site 2 were located approximately 50 meters apart on different farms. There were variances in seafloor topography, depth and current exposure at these locations, which could drastically alter bacteria growth rates. Secondly, the farm containing site two was constructed using new rope, which had barely been exposed to manual cleaning methods or natural weathering. As a result, the rope was unworn and almost perfectly smooth. Contrastingly, site 1 was constructed using older rope that had been vigorously cleaned and previously exposed to biofouling. The rope was frayed continuously along the line and hosted more organic material in unreachable areas of the rope, such as between threads. These factors created greater surface area and a more hospitable environment for bacteria growth.

A final consideration stems from the methodology of H_2O_2 (4%) and salt (35 g) application. While these methods did not involve the vigorous scraping that defines manual cleaning, they did involve contact with bacteria on the rope via the synthetic application sponge. While it appeared to be minimal, portions of bacteria were accidentally manually removed from H_2O_2 (4%) and Salt (35g) segments. This removal could have skewed results.

b. *Support Line*

While the ANOVA test resulted in a significant p-value, no direct Tukey HSD comparison was significant. Since none of the new treatments, or manual cleaning, differed from the control, there was no effective treatment within those applied. Considering multiple

treatments were efficient for both the ropes and the sponges, support lines were the only unsuccessful group. This could stem from both the location and composition of the line.

Since the support line is directly connected to both the sponge and the rope, it is extremely susceptible to any growth coming off of those sources. Frequently, intense growth was located close to the sponge and progressed down the line as it grew. Additionally, the support line was constructed of thick fishing line. This was extremely unabsorbent, and most likely failed to retain any of the solution treatments it was exposed to. Proximity to bacteria sources combined with ill suited materials could have restricted the effectiveness of all treatment methods.

c. *Sponges*

Sponges had the largest number of successful treatments. Manual Cleaning, H_2O_2 (2%), H_2O_2 (4%) and salt (25 g) were statistically significant, with a p-value of <0.01 when compared to the control. While all methods were successful, there was a striking difference in growth rate during the treatment gap. Manual cleaning increased by 1009%, while H_2O_2 (2%) only increased by 30% and H_2O_2 (4%) decreased by 0.14%. Manual cleaning had an extraordinarily high growth rate compared to other methods during the treatment break.

According to an interview with Okala, project manager of Marine Cultures, if bacteria attaches in "one or two days…the sponges [can] die." Therefore, it is extremely important to prioritize treatment methods that effectively address bacteria during treatment breaks. Considering both H_2O_2 treatment methods potentially kill or minimize growth over time, the hydrogen peroxide may be a preferable alternative to manual cleaning. Applying a method that restricts growth during treatment breaks could potentially decrease bacteria levels and mortality for sponges.

However, there are multiple variables to be considered. Like the rope group, it was apparent that average growth between Site 1 and Site 2 varied considerably. In general, growth was extremely low at Site 2 compared to Site 1. This could be attributed to any of the previously mentioned environmental variables, such as depth, temperature and current. Sponges may may have been especially impacted by current. Site 1 ropes were parallel to the current, while site 2 ropes were perpendicular, altering the surface area exposure as well as the frequency of contact with the ropes. Sponges parallel to the current would continuously bump into the rope when pushed back by strong currents or waves, potentially altering bacteria populations or harming the sponge.

While potential differences exist between the farms, both hydrogen peroxide treatments were successful in mitigating growth during the treatment break by an extraordinary margin.

d. Alternate Biofouling Mitigation

While salt treatments generally provided insignificant or inconclusive results in relation to bacteria mitigation, another use was discovered during the study. Upon contact with extremely saline water, a portion of microorganisms, plankton and juvenile organisms would detach from the sponge and float in the solution. They were likely shocked and affected by the high salinity. While not all biofouling organisms are destructive, some can be harmful to the sponge. The brittle star is known to restrict the oxygen intake of the sponge as it wraps around the interior. Crabs can feed on the sponge as they grow, or cut harmful holes as they burrow. It can be very difficult to find and manually remove these organisms, so salt treatments may provide an easier antifouling alternative.

e. Limitations

All groups faced a number of limitations, including sample size. Each treatment was limited to four sponges, due to resources available at the farm. In order to achieve more accurate and robust results, successful treatments should be reapplied with larger sample sizes.

IIX. Conclusion

As the first study on antifouling treatments on sponges in Zanzibar, the methodology and results of this study provide excellent background information. Ideally, these methods could serve as a model for future work in Jambiani, or alternatively, as a baseline for techniques in sponge antifouling

While it is possible that H_2O_2 (4%) and Salt (35g) are both effective treatments for bacteria growth on ropes, there are too many variables including location, rope material and cleaning methods to draw any distinct conclusions.

Post-Hoc analysis for the support line revealed that there was no effective treatment among those attempted.

Manual cleaning, H_2O_2 (2%), H_2O_2 (4%) and Salt (25g) were all effective when treating sponges. When the treatment break data was analyzed, there was a clear distinction in growth rate between both H_2O_2 methods and manual cleaning. While there are variables including location and farm construction that may effect this outcome, H_2O_2 and manual cleaning had a difference of over 900% in growth during the treatment window. It is very likely that H_2O_2 is a more effective treatment to stop growth during non-maintenance windows.

The effect of salt treatments on microorganisms and brittle stars was unexpected yet beneficial finding. While quantitative data was not tabulated in this study, qualitative information provides background information for further study.

IX. Recommendations

1. Continue experimenting with hydrogen peroxide treatments to (1) determine effects on sponge growth. Since the treatments were only applied for a total of 10 days, sponge growth was not observable. A study should be conducted to ascertain if sponges exposed to H_2O_2 have a different growth rate compared to the manual cleaning method. Additionally, (2) H_2O_2 concentration and frequency of application should be studied to determine ideal treatment strategies. Sponges were not killed by the accidental 15 second exposure to the concentrated 50% H_2O_2 on November 8. This indicates the potential for successful alternative methods.
2. Repurpose salt treatments to decrease brittle star and microorganism biofouling in sponges. This could be applied in the water, or it could be applied post harvest. Considering all brittle stars and other organisms must be manually removed from the sponge prior to sale, a salt solution before processing could significantly reduce processing time.
3. Use a larger sample size in future studies. Since sample size was limited to four sponges per treatment method, results may have been skewed.

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