The Analysis of the Microbiome of *S. Natans* and *S. Fluitans*

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Abstract

The Sargasso Sea is an oligotrophic gyre poor with the phosphorous nutrient. There are two species of *Sargassum* within this body of water; *Sargassum Natans* and *Sargassum Fluitans*. Both of these types of *Sargassum* are pelagic and act as a host for macrobiota and microbial communities. Certain bacterial lineages are known to be able to cleave the C-P bond of methylphosphonate, a compound abundant in the water, in order to produce a phosphorous nutrient. This study focused on identifying the microbiome of *S. Natans* and *S. Fluitans* and determine what bacterial lineages were enhanced by the addition of methylphosphonate. To study the *Sargassum* microbiome, a reproducible and accurate method of removing the microbiome from *Sargassum* samples was developed. This method involved using a water pick to power wash the *Sargassum* and a sonicating bath to disperse the cells. The microbiome cell abundance and community structure were analysed using microscopy protocols. The microbiome data gathered aligned with previous data confirming the accuracy of the method and several trails were performed showing reproducibility. The microbiome of *S. Fluitans* was higher in abundance than *S. Natans* but the community contributions were similar with *Roseobacter* being the dominant species. The response of the microbiome to methylphosphonate additions was investigated using incubation experiments. After seven days, the bacterial abundance increased with increasing concentration of methylphosphonate and the lineages *Vibrio* and *Alteromonas* increased in abundance.

Introduction

The waters within the North Atlantic Gyre are characterized as being nutrient poor where very little life should exist (Steinberg et al., 2001). However, this gyre is home to a large variety of both flora and fauna found within the Sargasso Sea (Laffoley et al., 2011). The Sargasso Sea is the home to many creatures that can only live within the large rafts of the macroalgae and it also acts as a nursery to many pelagic fish, sea turtles and other wildlife (Casazza and Ross, 2008; Trott et al., 2010). As a result of its important role regarding this biota, it is vital to understand how *Sargassum* can survive in nutrient deficient waters. The main species of *Sargassum* in the Sargasso Sea are *S. Natans* and *S. Fluitans* (Moreira and Alfonso, 2013). S. Natans and S. Fluitans from the Gulf, Caribbean, and Sargasso Sea shared five common forms (Martin, 2016).

It is thought that bacteria on the *Sargassum* can cleave the C-P bond of methylphosphonate, a compound that is abundant in the water column, producing methane and phosphonate. This provides a source of phosphorous to the microbial community and an outgassing of methane to the atmosphere (Karl et al., 2008). It is known that in the nutrient deficient waters of the Sargasso Sea it is nitrogen and phosphorous that limits growth so that

added phosphonate to the surface waters allows for the *Sargassum* to grow larger (Cotner et al., 1997; Lomas et al., 2010).

To best understand the relationship between *Sargassum* and its microbiome, all the different organisms involved must be identified. Research done in 1972 into the *Sargassum* microbiome has proved that the bacteria *Dichthrix*, a nitrogen-fixer, is a member of this microbiome (Capone et al., 2008). This proves that nitrogen fixing bacteria are present within the microbiome. As nitrogen is one of the growth limiting nutrients in the Sargasso Sea, the presence of nitrogen-fixing bacteria is significant as it shows that both the bacteria and *Sargassum* colonies have found a way to coexist in such a way that both organisms are able to thrive (Capone et al., 2008).

Other research has found *Roseobacter* bacteria in surface seawater from the North Atlantic Gyre including the Sargasso Sea (Parsons et al., 2012; Sosa et al., 2017). These bacteria may cleave the C-P bond in methylphosphonate and release P nutrients into the water column. These bacteria are one of many different lineages which have the ability to break this bond. A 2017 study conducted on waters near Hawaii found that *Sulfitobaccter*, a bacteria closely related to *Roseobacter*, is also able to break down the C-P bond in Methylphosphonate (Sosa et al., 2017). A study of *S. Muticum* in Portugal revealed substantial temporal shifts in the microbiome with large abundance of *Rhodobacteraceae* (including *Roseobacter*) and *Loktanella* in September-March but prevalence of *Pirellulales* during the summer months (Serebryakova et al., 2018).

This study is focused on identifying the bacteria within the microbiome and which of the lineages are responsible for adding additional phosphorous into the water. This study will improve upon previous study (Klein, 2018) and develop a reproducible method for removing the microbiome from *Sargassum*. (Klein, 2018) found that blasting the *Sargassum* twice with a pressured blast from a syringe removed the greatest number of bacteria. This study tested sonication, power washing with a water pick and power washing with the water pick followed by sonication. The water pick with sonication method was hypothesised to be the best method as it would have the cells evenly distributed throughout the sample due to the sonication (Klein, 2018).

Once this method development was completed and the best sampling method was found, the experiments' focus shifted to the overall goal of classifying the different bacterial lineages and identifying which are responsible for the breakdown of methylphosphonate. This study was able to determine the microbiome of *S. Natans* and *S. Fluitans* and investigate how the microbiome changes with additions of methylphosphonate.

Methods

Method Development for removing *Sargassum* Microbiome

The first test run had the four methods, sonication, Syringe, water pick and water pic with sonication, and compared the amount of bacteria and archaea removed by each method. For all

methods a 5g *Sargassum* sample was used with 40mL of sterile seawater. For the sonication, the *Sargassum* was placed in a plastic bag with the water and placed in a sonicating bath for 30 minutes. The syringe method used a 40mL syringe to shoot water at the *Sargassum*. This was repeated twice. The water pick method used the sterile seawater in a similar fashion to the syringe method but with a more consistent, higher water pressure. The method water pick and sonication repeated the same steps as the water pick but with the sample then placed in the sonicating bath for 30 minutes to disperse any bacteria cells throughout the sample. Table 1 summarizes all the different methods tested.

			
Method: Sonicate (Son)	Method: Syringe (Syr)	Method: Water pick (WP)	Method: Water pick and Sonication (WP + Son)
Sample: 5g *Sargassum* 40mL Seawater	Sample: 5g *Sargassum* 40mL Seawater	Sample: 5g *Sargassum* 40mL Seawater	Sample: 5g *Sargassum* 40mL Seawater
Steps: Sonicate *Sargassum* in water for 30mins	Steps: Blast *Sargassum* with water using syringe	Steps: Blast *Sargassum* w/ WP	Steps: Blast *Sargassum* w/ WP Sonicate for 30mins
Table 1: Four methods tested for removing *Sargassum* microbiome			

Method Development Results

The bacteria and archaea were then counted for all the different. As shown in Figure 2, it was found that the water pick with sonication removed the most bacteria and archaea from the *Sargassum*. Not only did it have a higher count of bacteria cells, with 4.0E +06 bacterial cells per mL compared to the next highest with 3.2E+06 bacterial cells per mL, but there was a more even distribution of cells across the entire sample (See Figure 2 Images).

However, this method also removed a large amount of background material that made it difficult to analyse the slide. To counteract this, the water sample was put through a 200micron filter to get rid of large particles using a funnel. The *Sargassum* was placed directly on top of the filter and was then blasted with a water pick. The difference between the filtered water samples and non-filtered water samples is shown in Figure 3.

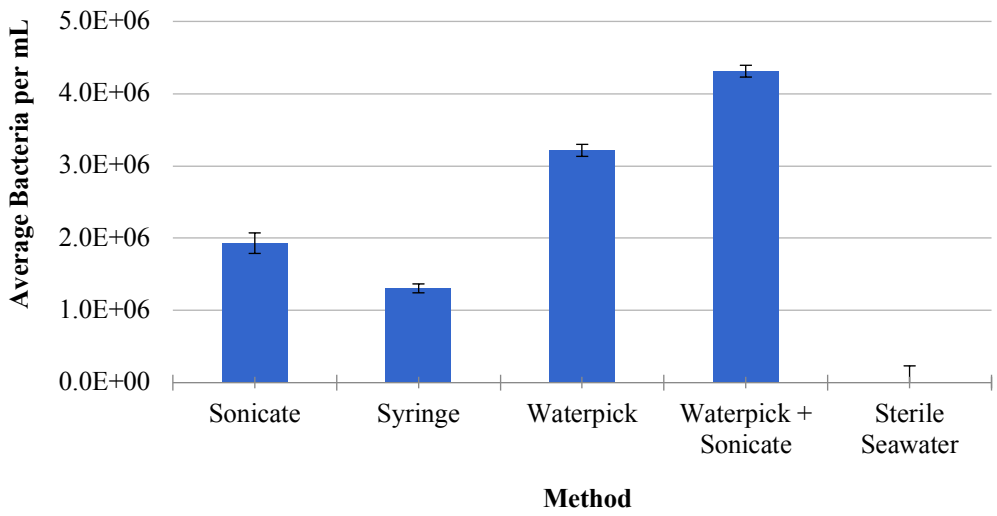


Figure 1: Bar Graph to show the average bacterial counts per mL on the y axis for each of the methods on the x axis error is shown as standard error (n=3).

The *Sargassum* sizes were cut down to 2g of *Sargassum* and 20mL of sterile seawater in order to reduce the sample size. It was determined that sonication should be done immediately prior to slide making.

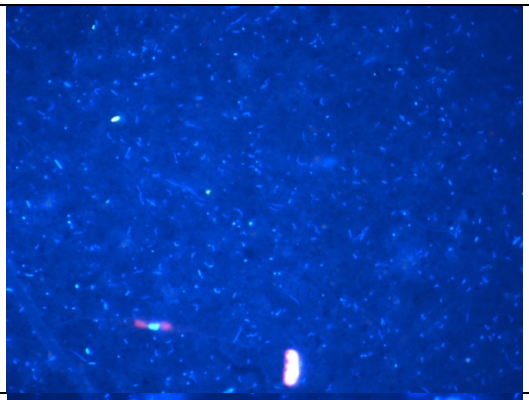
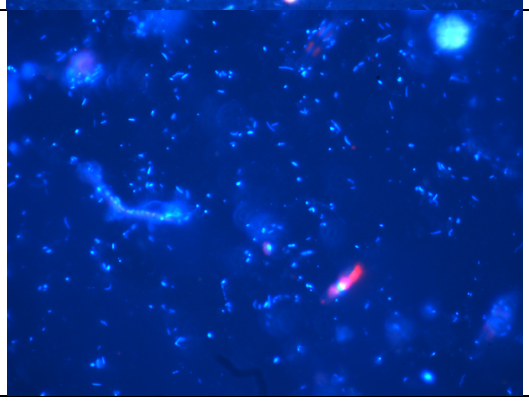
Non-filtered water sample (Trial 1)	
Filtered water sample (Trial 2)	

Figure 2: Images from both filtered and non-filtered water samples

To prevent any contamination with bacterial cells, all materials were sterilised using the autoclave or ethanol. The *Sargassum* used was only handled with gloves. The water pick was placed under a UV light for 15 minutes, it was then rinsed with ethanol, sterile water and sterile seawater to ensure there was no carry over of cells. A water pick control was added with no *Sargassum* to detect any cell carry over. The control was 21 times lower than when *Sargassum* was added (Figure 3, p=0.0175 T.Test).

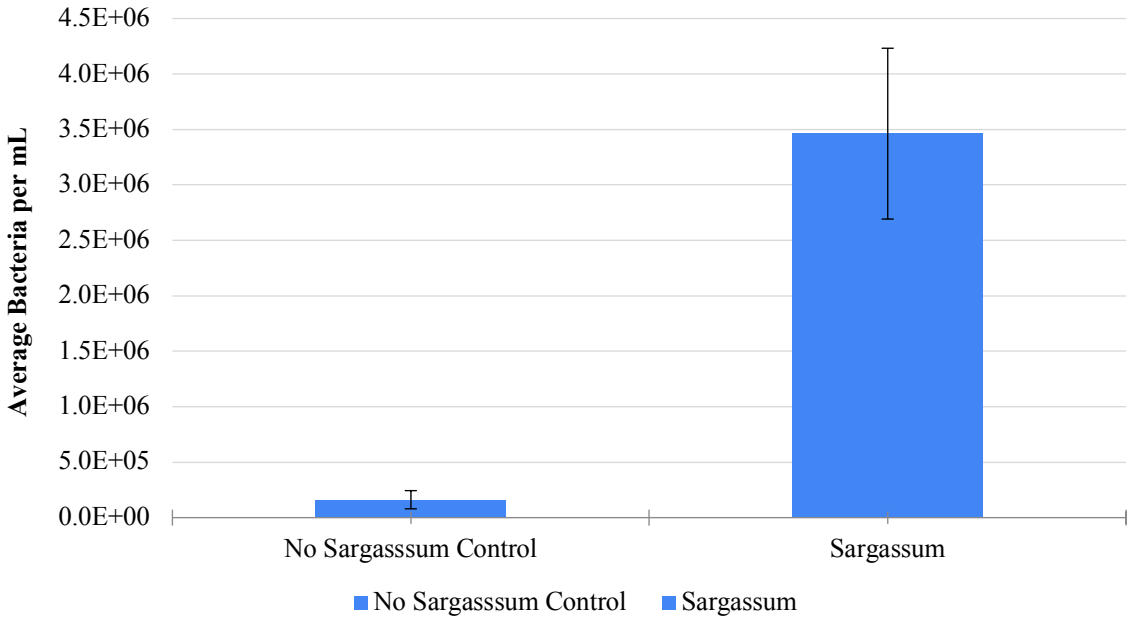


Figure 3: Bar Graph to show how bacteria counts increased with the addition of *Sargassum.* The average bacteria per mL of seawater is on the y axis and the treatments with and without *Sargassum* are on the x axis. Error is shown as standard deviation (n=6).

Methylphosphonate Additions

To test how the microbiome was changed due to the addition of Methylphosphonate, a series of trials were conducted to test how differing amounts of methylphosphonate would affect the bacterium abundance on *Sargassum*. To do this samples of *Sargassum* weighing approximately 2g were added to serum bottles filled with 100mL of water. Different amounts of methylphosphonate were added to each sample set and the bottles were over pressured by adding 20mL of air. The bottles were then left to incubate for 3 days.

Bacterioplankton abundance (DAPI)

Seawater (10-20 mL) was filtered through a polycarbonate 0.2µm 25mm filter previously stained with Irgalan black (SIGMA-Aldrich, St. Louis, MO USA) under light vacuum (5-7 mm Hg). The filters were then stained in darkness with 4', 6-di-amidino-2-phenylindole dihydrochloride (5 ug ml^{-1} DAPI, SIGMA-Aldrich, St. Louis, MO USA) and placed on a microscope slide using Resolve immersion oil and stored at -20C until further use (Porter and Feig, 1980). Total bacterioplankton abundance was determined using an AX70 epifluorescent

microscope (Olympus, Tokyo, Japan) under ultraviolet excitation at 100x magnification. At least 400 cells (10 fields) were counted for bacterioplankton abundance and normalized per mL of seawater (Steinberg et al., 2001).

Fluorescence *in situ* hybridization (FISH)

The abundance of *Alteromonas, Roseobacter* and SAR202 was quantified using the FISH method in (Parsons et al., 2012) adapted from (Morris et al., 2002; Morris et al., 2004). Following filtration through 0.2um polycarbonate filters, samples were submerged in 95% ethanol to dehydrate prior to probing for bacteria including *Alteromonas, Roseobacter* and SAR202 using probe sequences specific to each bacteria listed in table 1. Filters were washed twice at 10 minutes per wash using SET 1 buffer solution (20 $mmol^{-1}$ Tris-HCL, 70 $mmol^{-1}$ NaOH, 5 $mmol^{-1}$ EDTA, pH 7.4) heated to 52°C. Filters were then placed on slides to which 20 ul of 6-diamidino-2-phenylindole dihydrochloride (DAPI, SIGMA-Aldrich, St. Louis, MO USA) in citiflour solution was added. Filters were analysed under Cy3 (550 nm) and UV wavelength sets adapted from the methods of Carlson (2009).

Bacteria	**Probe sequence**	**Wash buffer**	**Wash Temperature [°C]**
Alteromonas	5'- TGTTATCCCCCTCGCAAA-3'	SET 1	52
Roseobacter	5'- CAACGCTAACCCCCTCCG-3'	SET 1	52
Vibrio	5'-CCCCACATCAAGGCAATTTC-3'	SET 1	45
SAR202	5'- GTTACTCAGCCGTCTGCC-3'	SET 1	52
	5'-TGTCTCAGTCCCCCTCTG-3'		

Table 2: FISH bacteria probe sequences, and wash buffer temperatures for *Alteromonas, Roseobacter, Vibrio* and *SAR202*

Catalyzed Reporter Deposition Fluorescence *in situ* hybridization (CARD-FISH)

Methodology for CARD-FISH conducted to probe for SAR11 (Thaumarcheota, Euryarcheota and SAR324) was adapted from (Parsons et al., 2015). Polycarbonate filters containing samples were embedded in agarose prior to permealization with 0.5M lysozyme. Washes were conducted in SET 2 buffer solution (20 $mmol^{-1}$ Tris-HCL 70 $mmol^{-1}$ NaOH, 5 $mmol^{-1}$ EDTA, pH 7.4) at 52C for 10 minutes followed by a wash in PBS for 10 minutes. DAPI citiflour was added as described in the FISH protocol and imaged under Cy3 (550 nm) and UV light, using methods adapted from Carlson (2009).

Image Analysis

Image analysis using an AX70 epifluorescent microscope (Olympus, Tokyo, Japan) was used to process FISH and CARD-FISH slides excited with Cy3 (550 nm) and UV wavelengths

as previously described. The image capturing was performed using a Toshiba (Irvine, CA, USA) CCD video camera with a Pro-series capture kit version 4.5 (Media Cybernetics, Bethesda, MD, USA) and processed with Image Pro software (version 4.5; Media Cybernetics) as previously described (Carlson et al., 2009; Parsons et al., 2015).

Results

The *Sargassum* Microbiome

Using the data collected from the initial analysis of the microbiome, it was determined that the results were consistent with previous data collected (Klein, 2018). These results confirmed that the water pick removal method was a reliable method, and the data collected for the *Sargassum* microbiome was reproducible (Figure 4). The new data set also shows the reproducibility of the water pick method with the microbiome staying relatively constant across different samples with *Alteromonas* being the dominant lineage.

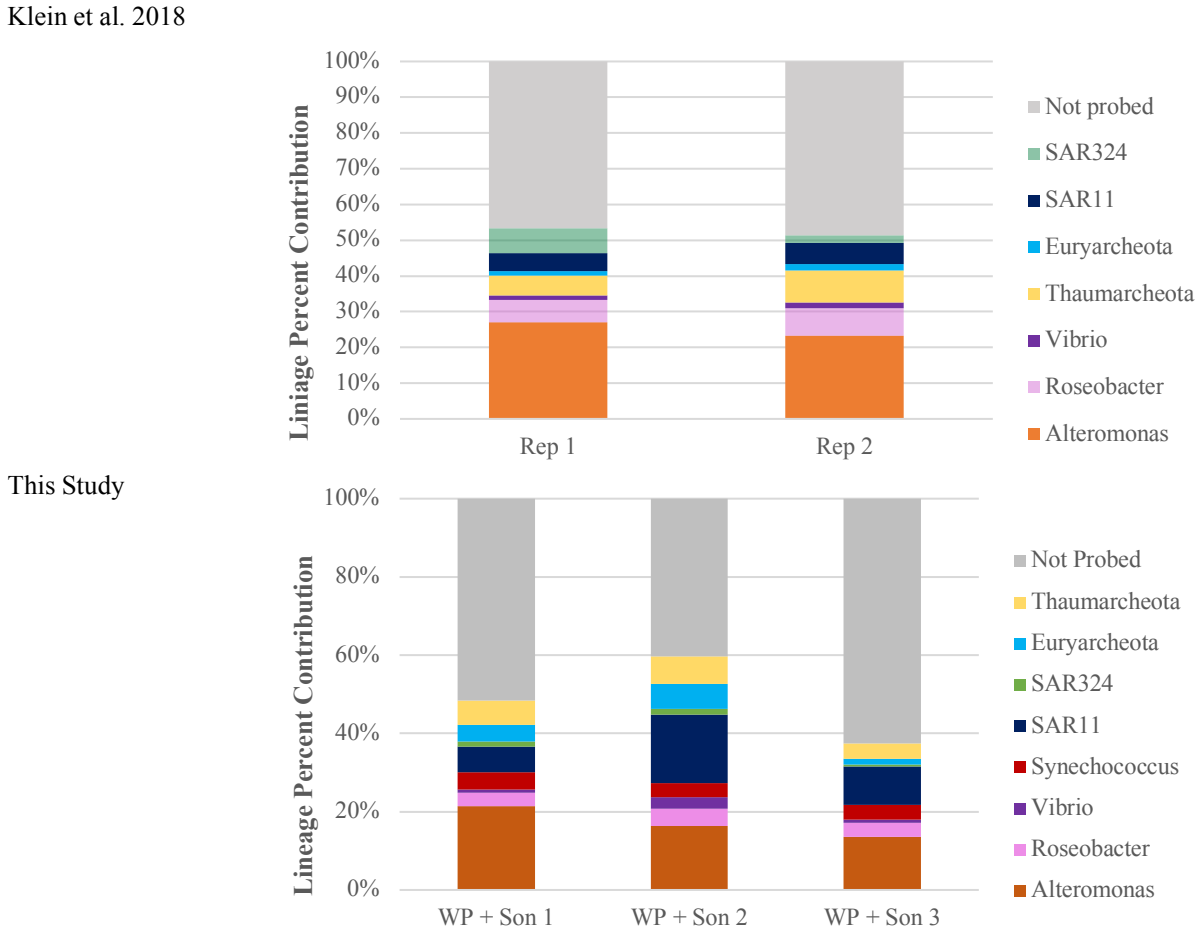


Figure 4: Comparison between the results gathered by Klein et al. 2018 and this study. The stacked bar graph show the percent contribution of each lineage to the total bacteria on the y axis and the replicates on the x axis.

Once a reproducible method was determined, the microbiome of the two *Sargassum* species, *Sargassum Natans* and *Sargassum Fluitans,* was studied. The bacterial abundance found within the microbiome of *S. Fluitans* were higher than with *S. Natans* (Figure 5A, p=0.0350 T.Test, n=2). The bacterial lineages within the microbiomes of both species were similar with a significant strong positive correlation (r=0.697, p=0.034, n=14). Both species showed a dominance of *Roseobacter* (Figure 5B) with smaller contributions from the six other lineages analysed. There were more SAR11 (4% difference) and *Euryarcheota* (2% difference) in the microbiome of *S. Fluitans* than in the microbiome of *S. Natans.*

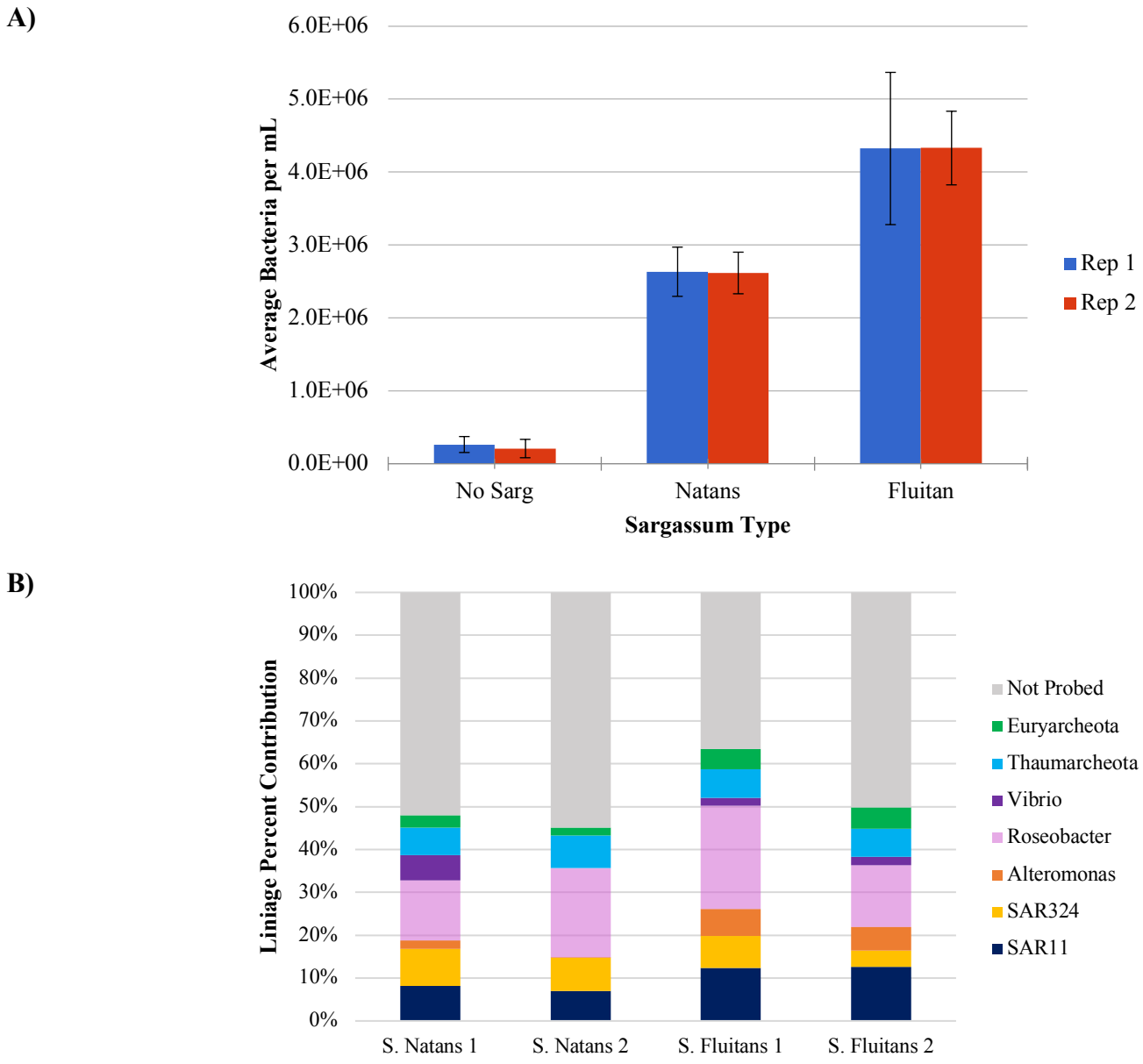


Figure 5: Comparison between *Sargassum* Species; *S. Natans* and *S. Fluitans.* A) Bar Chart of bacterial abundance on the y axis and the replicates on the x axis and B) Stacked Bar Chart of the lineage percent contribution to total bacteria on the y axis and the replicates on the x axis.

Methylphosphonate Additions

At the start of the experiment the bacterial abundance was an order of magnitude lower than the bacterial abundance after seven days in the control treatment that included *S. Natans* but no methylphosphnate addition. The addition of methylphosphonate to the samples caused an increase in bacteria cells (Figure 6). Bacterial abundance in the *Sargassum* microbiome increased with the addition of methylphosphonate (Figure 6 blue line). As the phosphorous nutrient changes with increased methylphosphonate additions, the *Sargassum* microbiome responds to the addition of phosphate from the cleavage of the C-P bond of methylphosphonate until the phosphate produced is no longer a limiting factor and bacterial growth starts to plateau (MPn > 570nM). The water counts were not expected to follow the trend of the *Sargassum*. The water used was completely sterile so any bacteria cells would have come from the *Sargassum* in the sample and the number of bacteria cells remained constant with increasing methylphosphonate additions (Figure 6 red line).

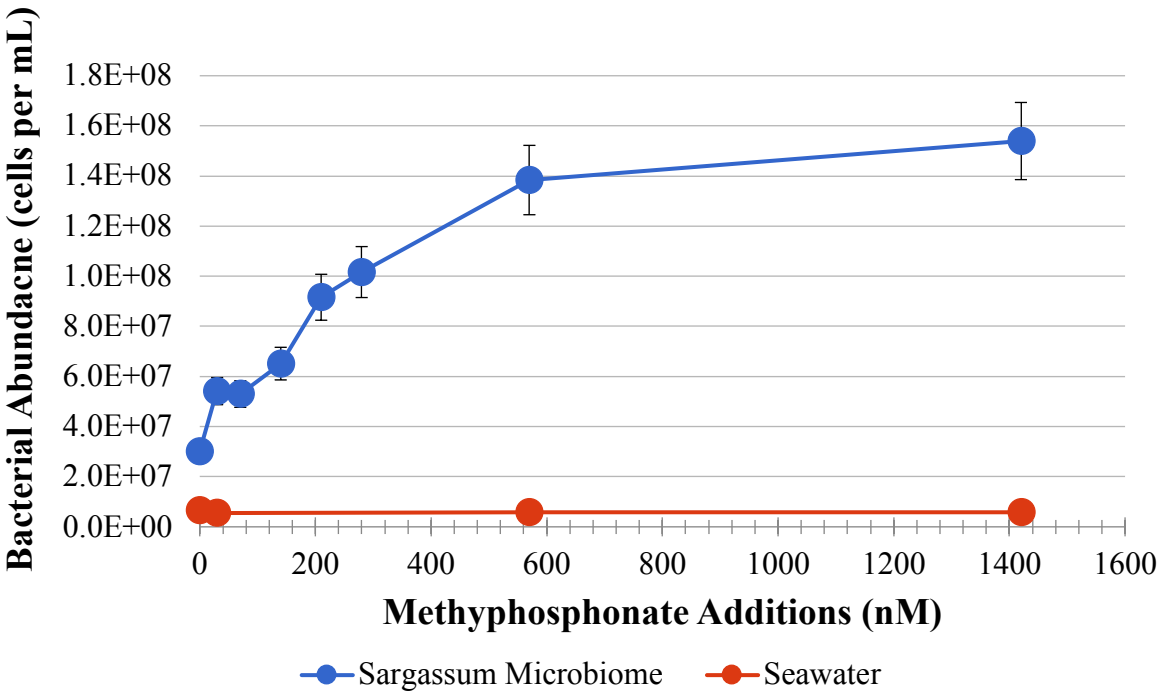


Figure 6: Line graph showing the increasing concentrations of methylphosphonate (nM) on the x axis and bacterial abundance (cells per mL) on the y axis.

There was a significant weak negative correlation between the bacterial abundance in the *Sargassum* microbiome when compared to the water sampled at the end of the experiment suggesting that these two communities were significantly different ($r = -0.183$, $p < 0.001$, $n = 6$). The bacterial abundance in the *Sargassum* microbiome was an order of magnitude higher than in

the seawater. The highest methylphosphonate addition of 1420nM had 124 million more bacterial cells in the *Sargassum* microbiome than the control with no methylphosphonate added.

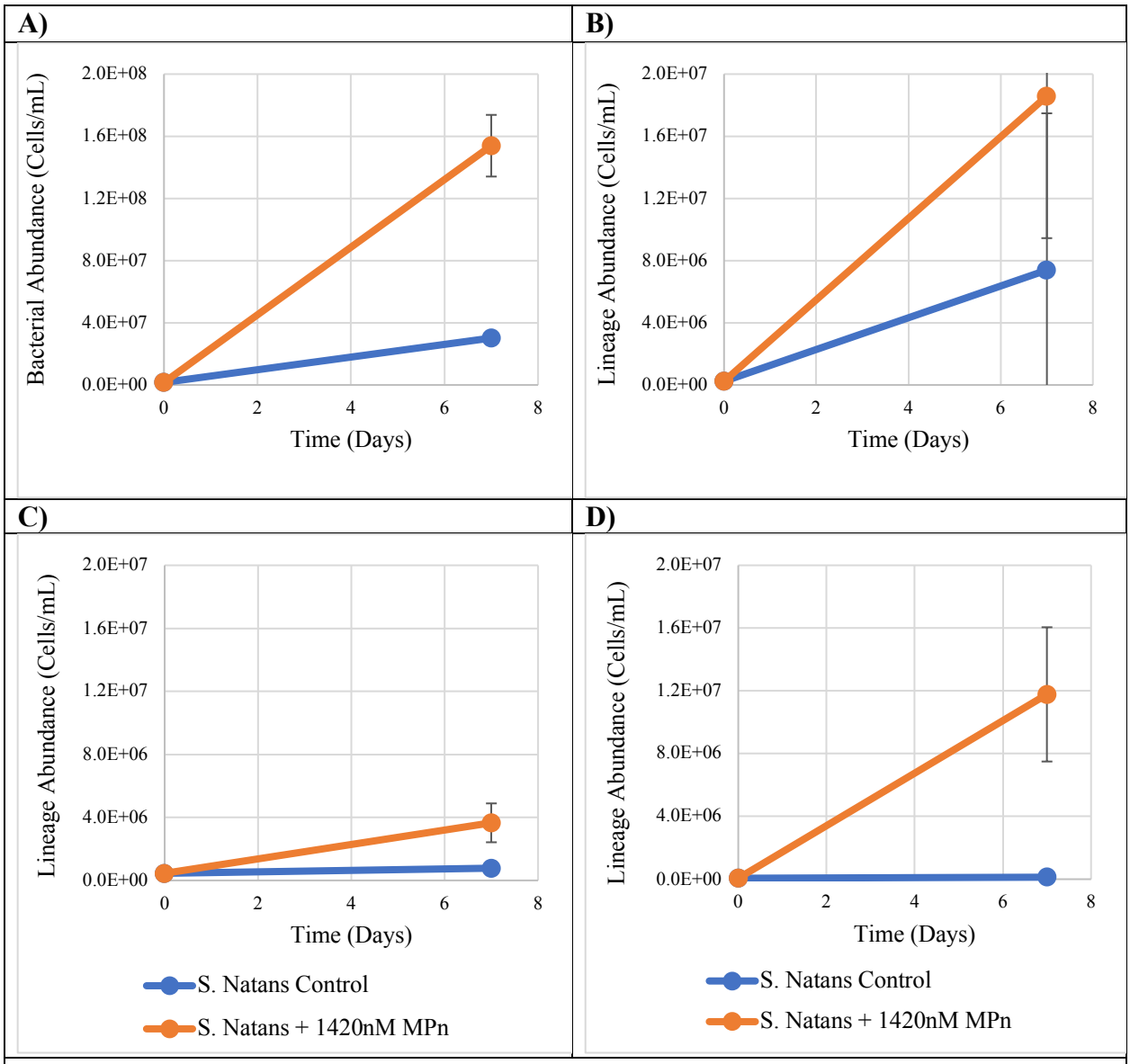


Figure 7: Bacteria cell counts for different bacterial lineages A) shows a line graph with the total bacteria abundance (cells/mL) on the y axis and the time (days) on the x axis B) shows a line graph with the *Alteromonas* lineage abundance (cells/mL) on the y axis and time (days) on the x axis C) shows a line graph with the *Roseobacter* lineage abundance (cells/mL) on the y axis and time (days) on the x axis D) shows a line graph with the *Vibrio* lineage abundance (cells/mL) on the y axis and time (days) on the x axis.

The difference in bacterial abundance of the *Sargassum* microbiome between the control with no methylphosphonate and the highest methylphosphonate addition was 124 million cells (Figure 7A). Based on the FISH analysis, the highest lineage increase between the control with no methylphosphonate and the highest methylphosphonate addition was 12 million *Vibrio* cells

(Figure 7D) followed by 11 million *Alteromonas* cells (Figure 7B) with only 3 million *Roseobacter* cells (Figure 7C).

At the start of the experiment, the *Sargassum* microbiome was dominated with *Roseobacter* being 27% of total bacteria (Figure 8B). After 7 days, The percent contribution of *Roseobacter* in the *Sargassum* microbiome was only around 2% of total bacteria (Figure 8B). *Alteromonas* contributed to 14% of the total bacteria on day 0, increased to 25% in the control treatment after 7 days and was 12% in the highest methylphosphonate addition but it's abundance increased in both the control and methylphosphonate addition (Figure 8). In contrast, *Vibrio* was barely detected on day 0, decreased in the control after 7 days and increased to 8% of the total bacteria and by 12 million cells in the highest methylphosphonate addition (Figure 8).

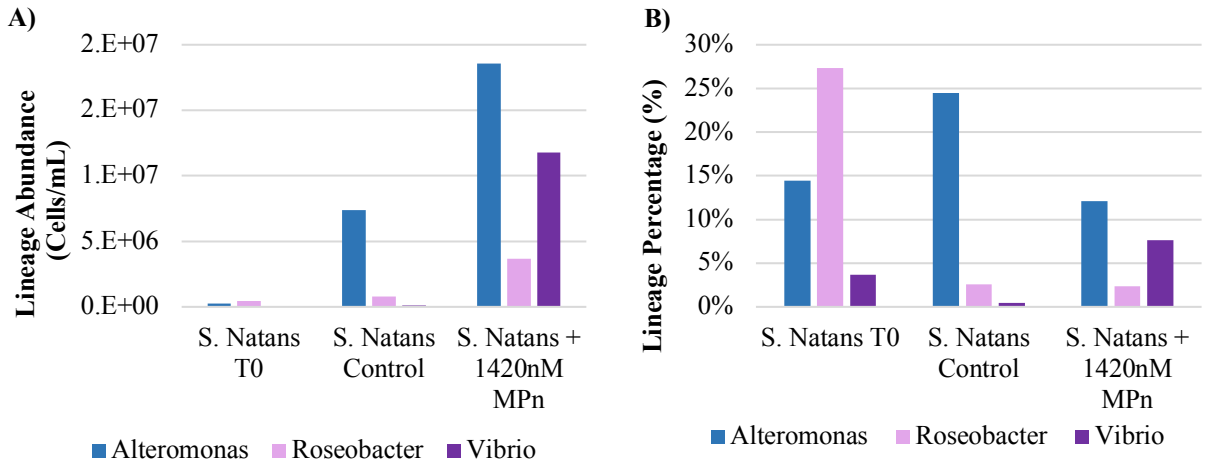


Figure 8: Bar charts to showing A) the lineage abundance (cells/mL) and B) the percent contribution of the lineages to total bacteria on the y axis and the sample time and treatment on the x axis. Lineages are Alteromonas (blue), Roseobacter (pink) and Vibrio (purple).

Discussion

There has been an explosion in *Sargassum* densities in recent years that needs to be investigated by the scientific community in order to understand the causes of the increased abundance as there could be significant negative impacts on marine ecosystems and disrupting economic activities of local communities (Louime et al., 2017). The aim of this study was to develop a reliable, reproducible method of removing the microbiome from *Sargassum*, to identify the key components of the microbiome and find how those lineages react with added methylphosphonate in the reaction.

Sargassum Removal

This study improved upon sampling methods described in Klein, 2018. The best method to remove the *Sargassum* was by power washing using a water pick. The water pick removed the most bacterial cells. Several different methods were tested: syringe, sonication, water pick and

water pick with sonication as seen in Table 1. The water pick removed the most bacteria cells by a million cells out of all the methods (Figure 1). The addition of sonication helped to disperse the cells and ensured an even distribution for accurate counting using the DAPI stain and epifluorescent microscopy. This further increased the count by another million cells through the disruption of any bacteria aggregates. A preliminary filtering step was added to remove any large detritus and *Sargassum* cells from the sample. The initial hypothesis was that a method using the water pick would result in the highest bacteria counts. This was proved correct as the water pick method dislodged the most bacteria cell counts when compared with the previous methods (Figure 1).

The Sargassum Microbiome

There have not been many studies that have investigated the *Sargassum* microbiome. The bacteria *Dichthrix*, a nitrogen-fixer, can be found within the *Sargassum* microbiome (Capone et al., 2008). This study shows that *Alteromonas* was the dominant lineage and these results were consistent with previous data collected (Klein, 2018). Since *Alteromonas* are well-known copiotrophs that grow rapidly when labile organic substrates become available (Romera-Castillo et al., 2011; Wear et al., 2015), they may have responded to dissolved organic matter produced by the Sargassum as it reaches its later growth stages. As a result, Alteromonas may have out competed other lineages such as the slower growing archaea and oligotrophic bacteria (Figure 4). One such oligotrophic bacteria is SAR11, a free-living aerobic heterotrophic Alphaproteobacteria that account for 15-40% of all bacteria cells throughout the water column (Eiler et al., 2009; Giebel et al., 2011; Morris et al., 2012; Rappe et al., 2002; Schattenhofer et al., 2009). This lineage accounted for 11.5 ± 5.6% of the total bacteria within the *Sargassum* microbiome (Figure 4) and can be found contributing to ~40% of the total bacteria within the Sargasso Sea (Morris et al., 2002).

Sargassum Natans vs Sargassum Fluitans

Once the best method for removing the microbiome was found the focus of the experiment shifted from method development to data collection. The two *Sargassum* species, *S. Natans* and *S. Fluitans,* were compared. It was found that while the microbiome was fairly consistent between the two species (Figure 5, B), the bacteria cell counts were significantly higher in the samples with *S. Fluitans* (Figure 5, A). There is a significant different between the cell counts of the two samples (Figure 5, $p=0.001$, $n=2$); however, there was a strong, positive correlation between the microbiome (Figure 5, $r=0.697$, $p=0.034$, $n=14$). *S. Fluitans* was not collected in subsequent trials since it was not found within the Bermuda Platform. Thus, these results were not repeated. The difference in bacteria cell counts could have been due to factors such as *Sargassum* health, its stage in the life cycle or where the seaweed sample came from in the water.

The microbiome results differed from the previous trials. Since the comparison between *S. Natans* and *S. Fluitans* was carried out on freshly sampled Sargassum from off the Bermuda platform, this microbiome was more reflective of natural conditions. Roseobacter can break down the C-P bond in Methylphosphonate (Sosa et al., 2017) and there was a large abundance of

Rhodobacteraceae (including Roseobacter) found in another floating algaea; *S. Muticum* (Serebryakova et al., 2018). This study showed that *Roseobacter* contributed to 17.5 ± 4.8% of the total microbiome in S. Natans and contributed to 19.2 ± 6.9% of the total microbiome in S. Fluitans (Figure 5B). SAR11 contributed to 7.6 ± 0.8% of the total microbiome in S. Natans and contributed to 12.5 ± 0.2% of the total microbiome in S. Fluitans (Figure 5B). The other lineages were similar between the two species and included SAR324, *Alteromonas, Thaumarcheota* and *Euryarcheota*.

Methylphosphonate Additions

The next stage in research was to find how the addition of the compound methylphosphonate would affect both the bacteria cell counts and the microbiome. The data collected from this trial followed the trend that was expected to be seen with a steep curve eventually levelling off as the methylphosphonate addition became in excess (Figure 6). Samples were taken from both the sargassum and the seawater. This was done to see if the water bacteria counts would follow the same trend as that of the sargassum bacteria counts. The water counts stayed constant throughout all methylphosphonate concentrations (Figure 6).

There was a difference in the bacterial abundance of the *Sargassum* microbiome between the control with no methylphosphonate and the highest methylphosphonate addition (Figure 7A). Only three lineages were analysed as a result of time contraints. Of these lineages, Vibrio increased by the highest count between the control with no methylphosphonate and the highest methylphosphonate addition followed by *Alteromonas* with little change in *Roseobacter* (Figure 7). This was interesting since at the start of the experiment, the *Sargassum* microbiome was dominated with *Roseobacter* (Figure 8B). In contrast, *Vibrio* was barely detected at the start of the experiment, decreased in the control after 7 days and increased in the highest methylphosphonate addition (Figure 8). Since *Roseobacter* can break down the C-P bond in Methylphosphonate (Sosa et al., 2017), this result was counter intuitive. However, another study in the North Pacific Subtropical Gyre showed that additions of methylphosphonate, glucose and nitrate resulted in a bloom of *Vibrionales* followed by an increase in C-P lyase transcripts once phosphorus became limiting and a community shift to *Vibrionales* and *Rhodobacterales* (Martinez et al., 2013). This suggests that these two orders can utilize methylphosphonate aerobically under conditions of phosphorus limitation by using the C-P lyase pathway (Martinez et al., 2013).

Conclusions

This study showed that the water pick with sonication removal method removed the most bacteria with the best distribution of cells ensuring reproducible results. There was a difference in the abundance of bacteria in the microbiomes between *S. Fluitans* and *S. Natans*. This may be due to *S. Fluitans* having a larger surface area to host the bacteria. The microbiome was dominated by copiotrophic bacteria including *Alteromonas, Roseobacter and Vibrio*. This could be attributed by the labile dissolved organic matter produced by the *Sargassum* and consumed by these copiotrophs. The addition of methylphosphonate resulted in an increase of bacteria within

the *Sargassum* microbiome with a decrease in *Roseobacter* cells and increases in *Alteromonas* and *Vibrio* cells.

Further Research

In order to confirm several new theories, new data will need to be collected. With the methylphosphonate additions, it is crucial that the new trials be analysed with both DAPI counts, FISH and CARD-FISH. This will ensure that the unknown portion of the microbiome is reduced by adding data on SAR11, SAR324, *Thaumarcheota* and *Euryarcheota*. CARD-FISH needs to be carried out on the microbiome samples. In addition, *Sargassum* microbiome samples need to be extracted and the subsequent DNA analysed using Illumina sequencing of the V4 regions of the 16s ribosome. All trials will need to be repeated on *S. Fluitans* to see if the microbiome of this species changes with additions of methylphosphonate.

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Supplemental

The methods used to remove the microbiome from the *Sargassum* were done in triplicate with several controls used inclusing sterile seawater and a control with no *Sargassum*.

Slide ID	Method	Bact/mL	STD Bact/mL	Average Bact/mL	Average STD Bact/mL
Trial 1 Rep 1	Sonicate	2.6E+06	6.1E+05	1.9E+06	5.1E+05
Trial 1 Rep 2	Sonicate	1.4E+06	5.8E+05		
Trial 1 Rep 3	Sonicate	1.7E+06	3.4E+05		
Trial 1 Rep 1	Syringe	1.2E+06	3.3E+05	1.3E+06	3.8E+05
Trial 1 Rep 2	Syringe	1.0E+06	3.9E+05		
Trial 1 Rep 3	Syringe	1.7E+06	4.3E+05		
Trial 1 Rep 1	Waterpick	3.0E+06	8.5E+05	3.2E+06	6.2E+05
Trial 1 Rep 2	Waterpick	3.3E+06	6.3E+05		
Trial 1 Rep 3	Waterpick	3.4E+06	3.7E+05		
Trial 1 Rep 1	Waterpick + Sonicate	4.0E+06	4.3E+05	4.3E+06	4.3E+05
Trial 1 Rep 2	Waterpick + Sonicate	4.1E+06	1.6E+05		
Trial 1 Rep 3	Waterpick + Sonicate	4.8E+06	7.0E+05		
Trial 1 Rep 1	Sterile Seawater	1.2E+04	2.6E+04	7.8E+03	1.8E+04
Trial 1 Rep 2	Sterile Seawater	3.9E+03	1.2E+04		
Trial 1 Rep 3	Sterile Seawater	7.7E+03	1.6E+04		
Trial 2 Rep 1	Filtered Waterpick	7.7E+06	9.8E+05	8.4E+06	8.4E+05
Trial 2 Rep 2	Filtered Waterpick	7.2E+06	3.8E+05		
Trial 2 Rep 3	Filtered Waterpick	1.0E+07	1.2E+06		
Trial 2 Rep 1	Filtered Waterpick + Sonicate	5.0E+06	8.5E+05	4.7E+06	6.2E+05
Trial 2 Rep 2	Filtered Waterpick + Sonicate	5.2E+06	6.6E+05		
Trial 2 Rep 3	Filtered Waterpick + Sonicate	3.8E+06	3.4E+05		
Trial 3 Rep 1	No Sarg + WP	1.6E+05	1.1E+05	1.1E+05	7.6E+04
Trial 3 Rep 2	No Sarg + WP	1.3E+05	7.6E+04		
Trial 3 Rep 3	No Sarg + WP	4.5E+04	4.4E+04		
Trial 3 Rep 1	Waterpick	4.0E+06	6.8E+05	4.0E+06	5.3E+05
Trial 3 Rep 2	Waterpick	4.2E+06	4.6E+05		
Trial 3 Rep 3	Waterpick	3.9E+06	4.4E+05		
Trial 3 Rep 1	Waterpick + Sonicate	9.3E+06	1.7E+06	9.0E+06	1.2E+06
Trial 3 Rep 2	Waterpick + Sonicate	8.6E+06	9.3E+05		
Trial 3 Rep 3	Waterpick + Sonicate	9.2E+06	8.6E+05		

Table S1: Table showing the triplicate bacterial abundance data collected for all method trials analysed

Method for Removing the Sargassum Microbiome

The method for removing the microbiome from the *Sargassum* is described below.

Materials Needed:

- 50mL Falcon Tubes
- Falcon Tube Stand
- Cordless Water Pick
- Balance
- Weigh boats
- Funnel
- 200micron Filter
- Sonicating Bath
- Isopropanol Alcohol
- Distilled Water
- Sterilised Seawater
- Sargassum (*S. Natans* or/and *S. Fluitans*)
- Formalin

Method

- Ensure the funnel, Filter and Seawater have been autoclaved at 255°C for 30mins
- Disinfect the Waterpick by running isopropanol through
- Run the distilled water through and then the Sterile Seawater
- Put the funnel into the falcon tube and cover the opening with the filter (Tip: it works best when the filter has been wet)
- Place a 2g piece of Sargassum into the funnel and blast it with 20mL of Sterile Seawater (Tip: Hold the falcon tube about a foot away from the water pick to lessen the amount of water that splashes out)
- Rinse the filter piece with distilled water between each rep of the same algae species or rinse with Isopropanol and distilled water when different algae species are used
- Once all samples have been collected add 1mL of Formalin to every 10mL of sample (eg. 20mL of sample=2mL formalin, 40mL=4mL, etc)
- Sonicate in a sonicating bath for 30mins immediately before making slides