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Microbial Diversity in Seafoam at the SIO Pier

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Marine Biology

by

Siyun Luo

Committee in charge:

Professor Brian Palenik, Chair
Professor Bianca Brahamsha
Professor Paul Jensen

2022

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The thesis of Siyun Luo is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2022

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ABSTRACT OF THE THESIS

Microbial Diversity in Seafoam at the SIO Pier

by

Siyun Luo

Master of Science in Marine Biology

University of California San Diego, 2022

Professor Brian Palenik, Chair

Seafoam is a common occurrence where floating bubble patches are found on the sea surface or moved on to the beach. The injection of air into the sea surface causes bubbles, and surface-active compounds, also known as surfactants, are of vital importance in the formation and maintenance of the bubbles. The sources of surfactants can be bacteria, algae, and other marine organisms in the sea surface layer. However, the environmental mechanisms behind the foam formation in different occurrences are unclear, including what roles bacteria and eukaryotes might

play. After analyzing microbial communities of the seawater and foam samples using 16S and 18S rRNA sequencing, *Pseudoalteromonas* species were characterized as the most abundant bacterial species enriched in foam over seawater. *Cercozoa* were detected as the most abundant eukaryote in seafoam, while *Stramenopiles* were another dominant group in all samples. The microbial diversity in different foam samples varied greatly, which suggests the sources of surfactant may have been different in each foam event. Both bacterial strains and diatom strains were isolated from foam samples and cultured in the lab. *Pseudoalteromonas, Cobetia, Vibrio,* and *Winogradskyella* were isolated and are known biosurfactant producers. Qualitative and quantitative analyzes of the surfactant composition in foam samples and microbial isolate cultures should be conducted in the future to determine the capacities of bacteria and diatoms to affect foam production.

1 Introduction

1.1 Foam and physical process

Sea foam is a common phenomenon in the marine environment. Foam exists as the collection of bubble patches floating on the sea surface or set down on the beach with the movement of surf. The formation of sea foam can be attributed to the agitation of seawater. When waves break, air is injected into the sea surface, causing the formation of bubbles. However, pure water is unable to form foam without the presence of surface-active compounds that stabilize the bubbles(Schilling and Zessner 2011). Under laboratory observation, the decay time for foam with surfactant added is significantly longer than the decay time for a surfactant-free condition(Callaghan, Deane and Stokes 2013). In addition, the breaking wave slope and scale increase the foam lifetime. Field data shows wind speed will change the foam decay time, but the mechanism is still uncertain (Callaghan et al. 2012).

1.2 Sea Surface Microlayer

The sea surface microlayer (SML) is the air-water boundary with less than 1mm of thickness(Wurl et al. 2017). It is a biofilm-like layer formed from a complex structure of polysaccharides, proteins, and lipids(Wurl and Holmes 2008). It is known as the key boundary for transporting organic and inorganic materials between the atmosphere and water(Napolitano and Cicerone 1999). Also it is a site for high rates of heterotrophic bacterial activity (Obernosterer et al. 2005) and the turnover of organic matter (Reinthaler, Sintes and Herndl 2008). A viscous sublayer, thermal sublayer and salinity diffusion sublayer are suggested to make up the physical structure of

the SML (Soloviev et al. 2014). Natural substances like oils and proteins which are incompatible with the aquatic and atmospheric ecosystem will preferentially accumulate in the sea surface microlayer (Schilling and Zessner 2011). Compounds resulting from human activities, for example, pesticides and petrol may also accumulate in the layer (Napolitano and Richmond 1995). The organisms associated with the air-water interface are known as neuston, ranging from bacteria to large siphonophores. Studies have shown the composition of microorganisms in the SML were significantly different from that of underlying seawater (Cunliffe, Upstill-Goddard and Murrell 2011). Foams were suggested to be the essentially concentrated SML such that 1L of foam water would represent 2 m^2 of SML (Napolitano and Cicerone 1999). A recent study (Rahlff et al. 2021) compared the bacterial community composition in seafoam, SML water and underlying water. The results showed the foam environment selects for bacterial taxa common to the SML; typical neuston such as *Pseudoalteromonas* and *Vibrio* were highly abundant in foam which confirmed that foam is a highly compressed version of the SML.

1.3 Ecological impact of sea foam

Materials and compounds are transported to the sea surface where foam forms by physical processes. As a consequence, the concentration of pollutants and toxins in foam is likely to be higher than those in underlying seawater, which could make foam harmful to organisms in the ocean. The antibiotic acrylic acid was measured in the sea foam event produced by a *Phaeocystis pouchetii* bloom in North Sea (Eberlein et al. 1985). According to (Craig, Ireland and Bärlocher 1989), the phenolic content in foam collected in New Brunswick was toxic to a macro-invertebrate

amphipod *Corophuim volutator*. The bioaccumulation capacity of foam can cause it to be potentially toxic to human beings. It is possible for humans to have direct contact with sea foam on the beach and nearshore sea area. The toxic materials in foam will also be transported to humans via the food web. The pollutants enriched in sea foam can be released into air and propagated as aerosols by breaking foam bubbles, which creates another pathway for human exposure to toxins and pathogens via foam (Maynard 1968).

1.4 Surface active compounds

Surfactants are the amphiphilic organic compounds often with hydrophobic hydrocarbon chains and hydrophilic head groups which decrease the surface tension of the water. Surface-active agents also include protein, lipids, and carbohydrates that can be released from seaweeds, broken phytoplankton etc.(Velimirov 1980). Studies also confirm that some surfactants are "biosurfactants" specifically produced by various microorganisms. Compared with chemical surfactants, biosurfactants possess the properties of lower toxicity, higher degradability and specific activity under extreme conditions of pH, salinity and temperature(Makkar and Cameotra 2002). Biosurfactants reduce the surface tension at the air-water interface, exhibiting emulsifying capacity(Rizzo et al. 2013). Biosurfactants were suggested to be divided into low-molecular-mass ones that will efficiently reduce air-water interfacial tension mainly including glycolipids, lipopeptides, phospholipids; and high-molecular-mass biosurfactants that are more efficient emulsifiers including protein, lipoprotein, amphipathic polysaccharides, liposaccharides and the complex of these polymers (Rosenberg and Ron 1999).

1.5 Eukaryotes as a source of surfactants

The sources of surfactants fall into several groups, being accidentally or specifically produced by bacteria, algae, and other marine organisms in the sea surface layer. Those produced below the sea surface can be transported by several physical process such as convection, upwelling, and bubble scavenging(Kurata et al. 2016). In the oceanic environment, large scale formation of sea foam is more common after algal blooms such as the foam events in East Frisian coastal waters. Two *Phaeocystis pouchetii* blooms happened in the spring of 1982 and 1983. During the peak and after the breakdown of the bloom, the water turned reddish-brown. The blooms resulted in high concentration of both particulate and dissolved organic matter on the sea surface. With wave action, the sea surface was turbulent, and foam washed ashore. In these unusual foam events, the beach was covered by foam up to one meter high (Bätje and Michaelis 1986). Several dinoflagellate blooms of *Cochlodinium catenatum* on the coast of eastern pacific islands were reported to cause viscous foam(Guzmán et al. 1990), and were associated with the mortality of reef organisms. Seaweeds could exude water-soluble mucilage spontaneously or by being agitated by breaking waves, and this mucilage is a vital surface-active compound for foam formation(Velimirov 1980).

1.6 Bacteria as a source of surfactants

Bacteria could also be a source of biosurfactants that lead to sea foam. Bacterial strains belong to *Pseudomonas* (Rizzo et al. 2013) and *Vibrio* (Hu, Wang and Wang 2015) are widely confirmed as biosurfactant producing microorganisms. A short chain rhamnolipid was found to be produced by a strain MCTG214(3b1) of *Pseudomonas* from coastal seawater in Florida(Twigg et

al. 2018). In the study of(Kurata et al. 2016), some biosurfactant producers were first reported belonging to *Cellulophaga, Cobetia, Cohaesibacter, Idiomarina, Pseudovibrio* and *Thalassospira*. *Actinomycetes* are also producers and the strain *Nocardiopsis* alba MSA10 of *Actinomycetes* isolated from marine sponges in coastal India was reported to produce lipopeptide biosurfactant(Gandhimathi et al. 2009). Thus, biosurfactant production is not universal but relatively common in marine bacteria.

1.7 Thesis goal

The goal of this thesis is to answer the following questions: what is the 16S rRNA and 18S rRNA diversity of the microbes in foam? Are there distinct bacteria or eukaryotes in the foam that are different from or enriched from seawater? Can bacteria and diatoms isolated from foam and cultured in the lab represent the major types found in foam samples? Do bacteria or diatoms potentially control the formation of foam? To answer these questions, comparative amplicon sequence analysis between foam samples collected from the beach next to the SIO pier and seawater samples collected from the SIO pier were conducted. Bacteria and diatoms were isolated from foam samples and compared to amplicon data.

2 Methods

2.1 Sample collection

Samples were collected at Scripps Institution of Oceanography (SIO) on eight different days (Table 2) when thick foam existed. Foam resting on the beach was carefully scooped into 50mL sterile falcon tubes. After foam had settled into liquid, used a certain amount of sample for

bacterial and diatom culturing, and the rest of the samples were centrifuged at 7000 rpm for 10 min before removing the supernatant. The pellets were frozen at -80°C for future DNA extraction. Seawater samples were collected from the end of SIO pier, approximately 1000 ft from the beach. 500mL seawater was filtered onto 0.2 μm Supor filters and frozen at -80°C.

2.2 Foam enrichment

Foam samples were diluted 1:1, 1:10, 1:100 in Hyclone water, and 100μL of each were immediately spread on 2216 agar plates (MA 2216; Difco, Detroit, Mich.). Bacteria strains grew separately from each other and were isolated by their morphology. Bacteria isolates were cultured routinely on 2216 plates. 50μL of foam sample were added into 50 mL f/2 media (Ryther and Guillard 1962) for enrichment for diatom isolation. Enrichment cultures were grown on agarose plates by being serial diluted into 1:1, 1:10, 1:100 for diatom isolation. Isolated diatoms were transferred to f/4 media for long term culture.

2.3 DNA extraction and sequencing of isolated strains

For bacterial samples, well isolated colonies were selected from plates. Each colony was picked up and resuspended in 10μL of Hyclone water. Then samples were incubated at 95 °C for 10 minutes. Extracted DNA was frozen at -20°C before PCR thermocycling. For diatom samples, f/4 diatom cultures were centrifuged at 7000rpm for 10 minutes. Pellets were resuspended in 180μL of enzymatic lysis buffer (90μL mixture of 40ul of 1M stock Tris-HCl, 8μL of 0.5M EDTA, 24μL of Triton X-100, and 928μL of Hyclone water; 90μL 100mg/mL lysozyme) and incubated at 37 °C for 30 min. Subsequently, samples were eluted using **Qiagen DNeasy** Blood and Tissue kit based

on the manufacturer's instruction. PCR thermocycling conditions were set for specific primers. For single diatoms and bacterial samples, 18S and 16S rRNA PCR products were used to identify the strains using Sanger sequencing methods by Eton Biosciences. The primers 27F-1492R and 515F-806R were used for 16S RNA sequencing(Amin et al. 2015); Primers Moon A-Moon B were used for 18S rRNA sequencing(Moon-van der Staay, De Wachter and Vaulot 2001). CLC Genomics Workbench was used to align sequences of each strand for consensus sequences. Alignments were compared with 16S/18S rRNA sequences in NCBI GenBank nucleotide database by BLAST research(Altschul et al. 1990) to identify the most closely related isolates.

2.4 DNA extraction and amplicon sequencing of foam and seawater samples

For seawater samples, frozen filtered samples were cut into pieces and divided into centrifuge tubes. For foam samples, pellets were added into the tubes. after adding 80μL of 100mg/mL lysozyme and 560μL TE(50mM Tris,20mM EDTA) , each tube was vortexed and incubated at 37°C for 30 minutes. Next, 80μL 10% Sodium Dodecyl Sulfate (SDS) and 80μL 10mg/mL Proteinase K were added to each tube and tubes were vortexed and incubated at 55°C for 2.5 hour. Then 16μL RNAseA was added into each tube and tubes were then vortexed and incubated at 37°C for 30 min. 800μL of Phenol:Chloroform:Isoamyl Alcohol(25:24:1) was added to samples. After being centrifuged at 13500rpm for 1 minute, aqueous phase was pipetted into new tubes for repeat using Phenol:Chloroform:Isoamyl Alcohol(25:24:1) and another repeat using Chloroform:Isoamyl Alcohol (24:1). Finally, samples were eluted using **Qiagen DNeasy** Blood and Tissue kit based on the manufacturer's instruction. To identify the 16S and 18S

rRNA diversity in the samples, extracted DNA was sent for Illumina amplicon sequencing by RTL Genomics (Lubbock TX). Primer set EUK1319-EUKbr was used for 18S rRNA sequencing; 515yF-806bR and 28F-388R were used for 16S rRNA sequencing.

2.5 Data analysis

The amplicon sequencing data were processed using QIIME2 2021.4(Bolyen et al. 2019). The paired-end reads were denoised with DADA2 through QIIME2. Filtered sequences from DADA2 were assigned taxonomic identity by the classifier based on SILVA 132 database(Quast et al. 2013). Alpha diversity was calculated within QIIME2 using Faith's Phylogenetic Diversity, Shannon's diversity index, and Observed Features. Beta diversity was calculated within QIIME2 using Bray-Curtis distance and weighted UniFrac distance. The results of beta diversity were visualized through Principal coordinate analysis (PCoA) and the significance was calculated using PERMANOVA in QIIME2. Differentially abundant microbial taxa were identified by Analysis of Composition of Microbiomes (ANCOM) in QIIME 2.

3 Results

3.1 Isolates

A total of 17 phenotypically different bacterial strains were isolated directly from three foam samples or from foam enrichments (Table 1). Bacterial strains displayed different colors, shapes, and textures on 2216 marine agar plates. The strains were analyzed by 16S rRNA sequencing and then identified by top BLAST hits against the NCBI 16S rRNA gene database (https://BLAST.ncbi.nlm.nih.gov/BLAST.cgi). SLBAC203 and SLBAC301, which are two strains

from different sampling days, show the same yellow fluorescence and gliding characteristics and were both identified as *Cellulophaga*. Strains SLBAC101 and SLBAC311 which had white, round, smooth and sticky morphology were found to be *Pseudoalteromonas*. All bacterial isolates and their top hits from BLAST are listed in Table 1and represent diverse bacterial lineages.

Table 1: Identification of bacteria(a) and eukaryote(b) strains using top BLAST hits against the NCBI 16S and 18S rRNA gene database.

(a)

Date of sample	Isolate code	Top BLAST hit	Accession	Per. ident
05/04/20	SLBAC101	*Pseudoalteromonas sp*. S32CA	KF188511	99.26%
05/22/20	SLBAC201	*Vibrio parahaemolyticus isolate* VP328	JF779837	98.49%
05/22/20	SLBAC202	*Phaeobacter sp.* strain 6D	MK719858	97.98%
05/22/20	SLBAC203	*Cellulophaga lytica* strain FPBB1	MK346082	100.00%
05/22/20	SLBAC204	*Vibrio parahaemolyticus* strain TV18	MT549167	96.46%
05/22/20	SLBAC205	*Dietzia maris* strain Ba2SD-27	MT373593	99.24%
11/09/20	SLBAC301	*Cellulophaga lytica* strain FPBB1	MK346082	100.00%
11/09/20	SLBAC302	*Winogradskyella rapida* strain SCB36	NR_118846	99.87%
11/09/20	SLBAC303	*Aquimarina latercula* strain DL3-7-2	KF146503	100%
11/09/20	SLBAC304	*Phaeobacter gallaeciensis* strain P75	KY357427	100.00%
11/09/20	SLBAC305	*Zobellia russellii* strain KMM 3677	NR_024828	99.83%
11/09/20	SLBAC306	*Tenacibaculum* sp. MAR_2010_175	JX854354	99.85%
11/09/20	SLBAC307	*Shewanella* sp. strain 137A-1	MK143355	99.85%
11/09/20	SLBAC308	*Bacillus* sp. strain F3	MK568387	100.00%
11/09/20	SLBAC309	*Halomonas* sp. strain MC16	MF431785	99.50%
11/09/20	SLBAC310	*Cobetia* marina strain W1B	MN326584	100.00%
11/09/20	SLBAC311	*Pseudoalteromonas* sp. strain L24	MN889166	99.69%

Table 1: Identification of bacteria(a) and eukaryote(b) strains using top BLAST hits against the NCBI 16S and 18S rRNA gene database, continued.

(b)

Date of sample	Isolate code	Top BLAST hit	Accession	Per. ident
091919	SLDIA001	*Nitzschia* sp. SZCZCH845	KT943641	99.53%
091919	SLDIA002	*Nitzschia dubia* strain TA37	KY320381	99.38%
121719	SLDIA101	*Nitzschia capitellata* strain UPMC-A0077	MK834583	99.44%
121719	SLDIA102	*Bacillariophyta* sp. A2	FR744763	99.89%
121719	SLDIA103	*Nitzschia* sp. isolate KSA0120	KX981849	99.76%
052220	SLDIA201	*Nitzschia dubia* strain TA37	KY320381	99.42%
052220	SLDIA202	*Nitzschia* sp. SZCZCH658	KT943651	99.04%

Seven diatom strains were isolated from f/2 foam enrichment cultures by spreading on f/2 agar plates. Strain identity was determined using microscopy and 18S rRNA Sanger sequencing. Under microscopy, all diatom strains were pennates with a *Nitzschia*-like morphology, and after sequencing six out of seven strains have *Nitzschia* as the top BLAST hit and one is *Bacillariophyta* (Table 1). They were not however identical.

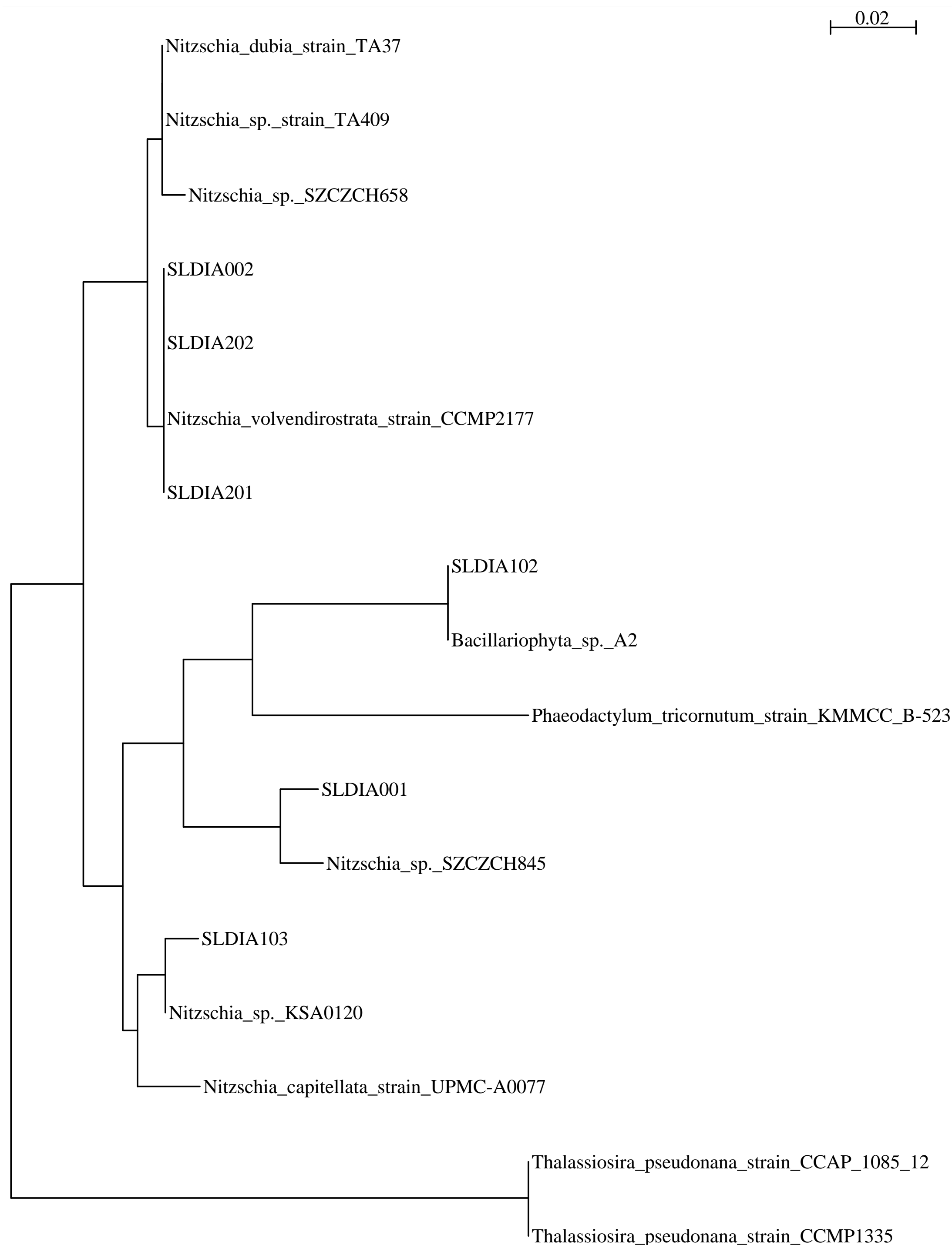
3.2 Phylogenetic analysis

Phylogenetic analysis was conducted to show the relationships among the 17 bacteria isolates and related cultured strains using SeaView (Gouy, Guindon and Gascuel 2010) (Figure 1). As expected from the BLAST study, strains SLBAC203 and SLBAC301 were identified to be from the same genus and most similar to five identified *Cellulophaga* strains. While both SLBAC101

and SLBAC311 were define as *Pseudoalteromonas* strains, SLBAC101 was closest to strain *Pseudoalteromonas*_sp._93(2013) which is a biosurfactant producing isolate(Malavenda et al. 2015) and SLBAC311 was closest to *Pseudoalteromonas* sp. 1400 which showed alginolytic activity in a study on the disruption of biofilms(Daboor et al. 2019).

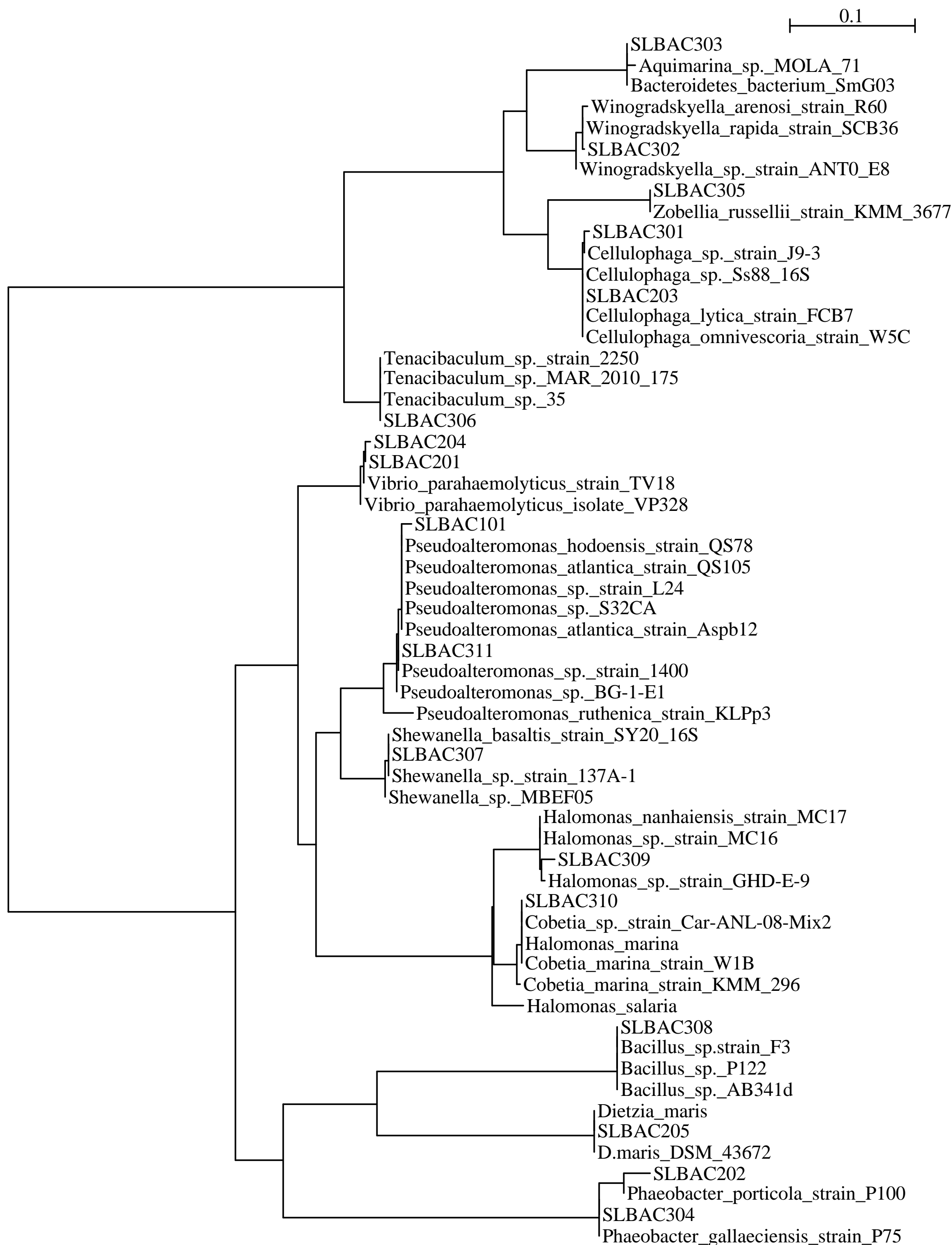
Each single sequence of the diatom isolates and their top BLAST hits were used for phylogenetic analyses. SLDIA001 has the identical sequence as *Nitzschia* sp. SZCZCH845 isolated from littoral zone of Bohai and Yellow Seas in Yantai Region of Northeast China(Witkowski et al. 2016) and SLDIA002, SLDIA201, and SLDIA202 has the identical sequence as *Nitzschia volvendirostrata* strain CCMP2177 from benthic samples from Lockeport in Canada (Lobban et al. 2021). Strain SLDIA102 shows the same sequence as Bacillariophyta sp. A2 which was isolated from seawater of North Sea in a study about marine microbes producing omega-3 fatty acids(Zhang 2011). SLDIA101 was dropped out from the phylogenetic tree because of the short read length of the sequence.

Figure 1: Consensus maximum-likelihood tree based on 18S rRNA(a) or 16S rRNA(b) and sequences of microbial isolates. The top BLAST hits of isolates and related strains from previous studies were incorporated along with the isolates.



(a)

Figure 1: Consensus maximum-likelihood tree based on 18S rRNA(a) or 16S rRNA(b) and sequences of microbial isolates. The top BLAST hits of isolates and related strains from previous studies were incorporated along with the isolates.



(b)

Figure 1: Consensus maximum-likelihood tree based on 18S rRNA(a) or 16S rRNA(b) and sequences of microbial isolates. The top BLAST hits of isolates and related strains from previous studies were incorporated along with the isolates, continued.

3.3 Amplicon sequencing of foam and pier samples

We used 16S rRNA amplicon sequencing to compare the relative abundance of bacterial taxa in foam and nearby SIO pier water. For one set of foam/pier samples, we used primers 515yF-806bR. We obtained a total of 260416 sequences and found 638 ASVs. For another set of foam/pier samples we used primers 28F388R from which 180953 sequences and 747 ASVs were found (Table 2). The relative abundance of bacterial taxa at the phylum and genus levels are shown in the Figure 2 below. The relative abundance of taxa varied greatly between different sampling dates.

Table 2: Overview of foam samples and 16S rRNA(a) 18S rRNA(b) amplicon sequence results
(a)

Sample ID	Total sequences	Total sequences after quality control	Percentage after quality control	Primer set	Temperature (°C)	Chla (μg/L)
Foam121719	54041	35439	65.58	515yF-806bR	15.94	26.66
Foam020420	50398	40665	80.69	515yF-806bR	14.86	1.13
Foam050420	9581	7680	80.16	515yF-806bR	20.08	35.16
Foam052220	10206	7283	71.36	515yF-806bR	15.24	4.82
Foam110920	97340	86504	88.87	515yF-806bR	17.78	13.24
Pier050420	10479	8658	82.62	515yF-806bR	20.08	35.16
Pier052520	15535	9839	63.33	515yF-806bR	17.04	3.63
Pier110920	12836	10390	80.94	515yF-806bR	17.78	13.24
Foam040918	24037	7953	33.09	28F-388R	16.98	2.7
Foam052118	39997	17139	42.85	28F-388R	17.25	5.07
Foam021819	32761	22601	68.99	28F-388R	14.79	49.65
Pier040918	46515	19824	42.62	28F-388R	16.98	2.7
Pier052118	23228	10286	44.28	28F-388R	17.25	5.07
Pier021819	14416	10032	69.59	28F-388R	14.79	49.65

Table 2: Overview of foam samples and 16S rRNA(a) 18S rRNA(b) amplicon sequence results, continued (b)

Sample ID	Total sequences	Total sequences after quality control	Percentage after quality control	Primer set	Temperature (°C)	Chla (μg/L)
Foam121719	29606	18447	62.31	EUK1391-EUKbr	15.94	26.66
Foam020420	31387	11985	38.93	EUK1391-EUKbr	14.86	1.13
Foam050420	51655	43039	40.05	EUK1391-EUKbr	20.08	35.16
Foam052220	76719	67935	91.16	EUK1391-EUKbr	15.24	4.82
Foam110920	51818	34998	67.82	EUK1391-EUKbr	17.78	13.24
Pier050420	51655	43039	85.43	EUK1391-EUKbr	20.08	35.16
Pier052520	36980	29858	80.87	EUK1391-EUKbr	17.04	3.63
Pier110920	45573	26867	59.75	EUK1391-EUKbr	17.78	13.24
Foam040918	36929	24035	69.05	EUK1391-EUKbr	16.98	2.7
Foam021819	17351	13427	78.16	EUK1391-EUKbr	14.79	49.65
Pier040918	32262	24035	77.64	EUK1391-EUKbr	16.98	2.7
Pier021819	18973	9418	49.97	EUK1391-EUKbr	14.79	49.65

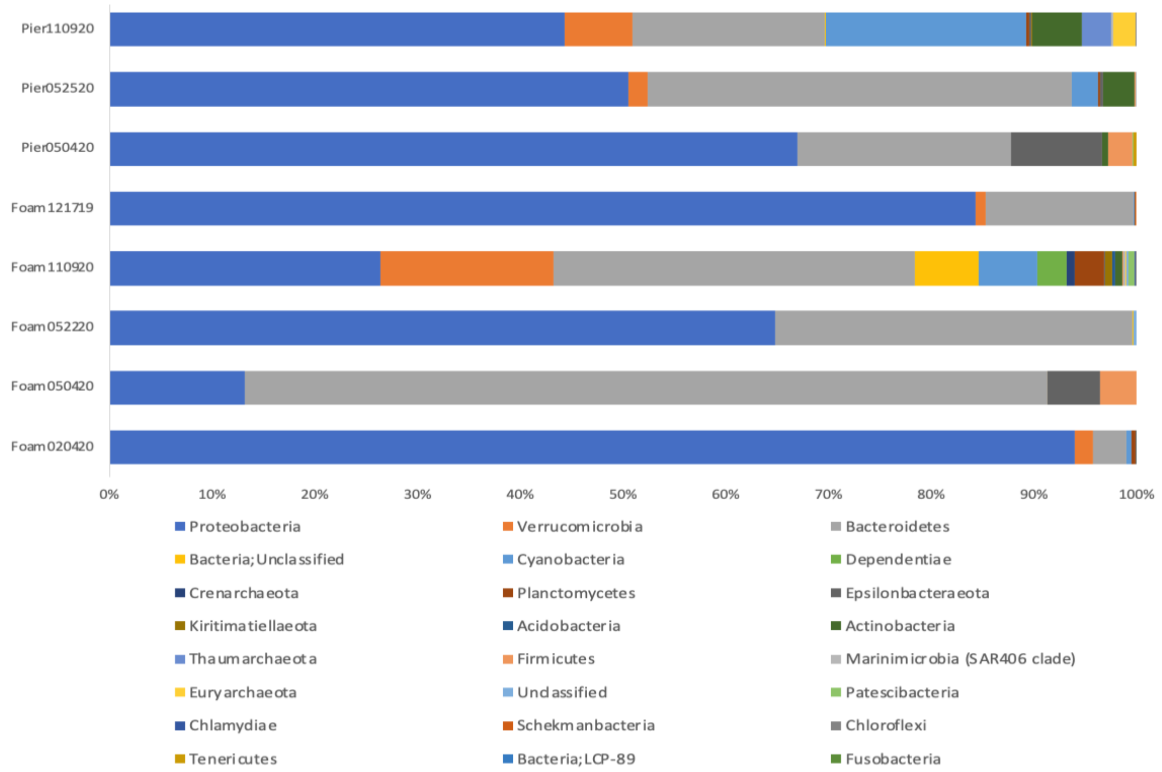
Proteobacteria and *Bacteroidetes* are the major phyla among all samples, adding up to more than 70% of the bacterial sequence abundance. *Bacteroidetes* showed significant abundance in foam samples. *Cyanobacteria* were the next most abundant microbe among most samples except samples from 050420 and 052220. *Verrucomicrobia* was present in both samples from 110920 with a little higher relative abundance in foam (16.828%) than in seawater (6.583%), and it was also found in other three foam samples from 052520(1.870%), 020420(1.773%), and 1221719(0.934%).

At the genus level, *SAR 11* clades show much more abundance in seawater as expected

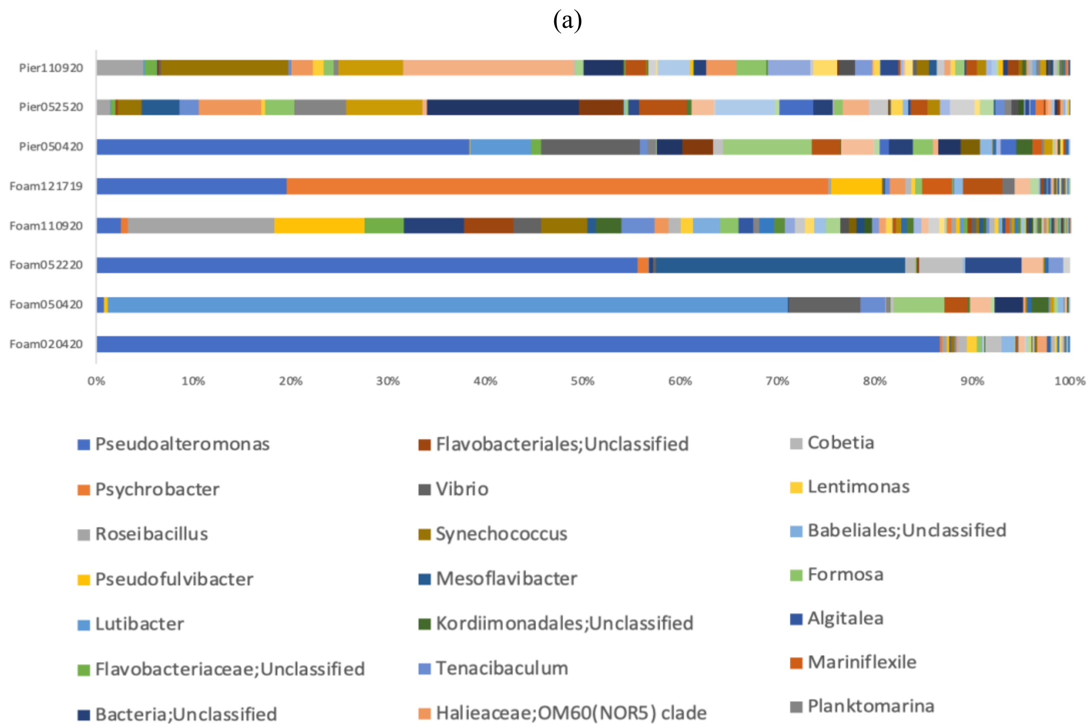
since SAR 11 is the most abundant bacterioplankton in the oceans (Giovannoni 2017). SAR11 clades were barely found in foam. For each seawater sample, the combined relative abundance of SAR11 clades are 53.218%, 44.129%, 43.079%, 19.625%, 0.539% and 0.468%. Among the 8 foam samples, SAR11 clades were only detected in 2 samples with relative abundances of 1.484% and 0.887%. Interestingly, the two seawater samples with low SAR11 abundance were collected at the peak and end of the *Lingulodinium* bloom event in 2020.

Pseudoalteromonas is ubiquitous in both seawater and foam samples, but it tended to be more abundant in foam samples when comparing between the two samples collected on the same date. The relative frequency of *Pseudoalteromonas* in the 020420 foam sample was 86.347%, which was extremely high, while the second most abundant genus, *Psychromonas,* was 1.712%. *Psychromonas* was found in seven foam samples among eight in total and only one out of seven seawater samples. However, the samples from 050420 were opposite in which it was 0.807% in foam and 37.849% in seawater. *Psychrobacter* has 55.874% relative abundance in 121719 foam sample and was detected in a small amount in the other four foam samples. *Flavobacteriaceae (Pseudofulvibacter, Lutibacter, and Formosa*) were commonly found genera in foam samples with relatively higher abundance than in seawater samples. *Lutibacter* existed only in samples from 050420 and was high in foam (69.466%) and lower 6.179% in seawater. *Cobetia* was presented in all foam samples and was not detected in any seawater samples. Its presence in 020918 foam is 26.154% and ranged from 0.438% to 1.253% in other foam samples.

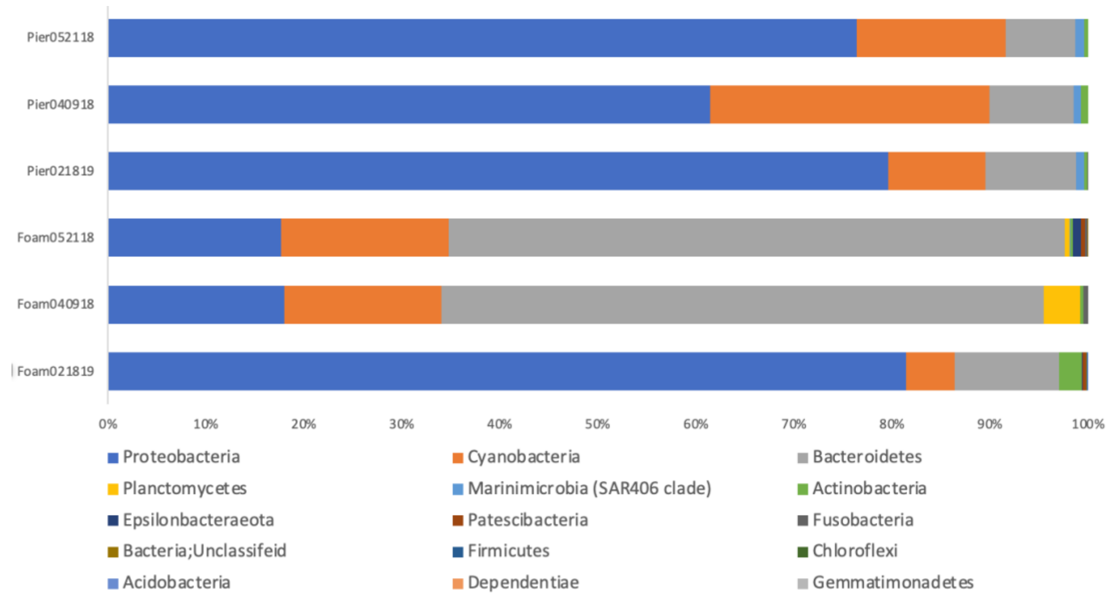
Figure 2: Relative sequence abundance of bacterial taxa in seawater and foam samples using primer set 515yF-806bR at phylum(a) and genus(b) levels and samples using 28F-388R at phylum(c) and genus(d) levels



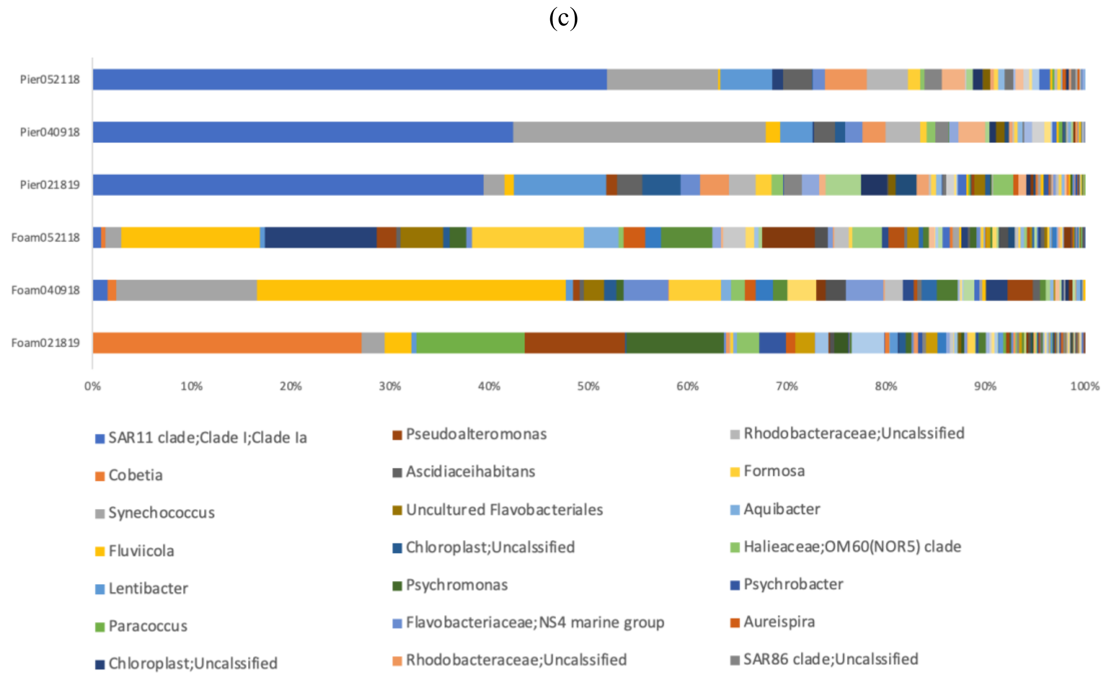
(a)



(b)



(c)



(d)

Figure 2: Relative sequence abundance of bacterial taxa in seawater and foam samples using 16S primer set 515yF-806bR at phylum(a) and genus(b) levels and samples using 28F-388R at phylum(c) and genus(d) levels, continued.

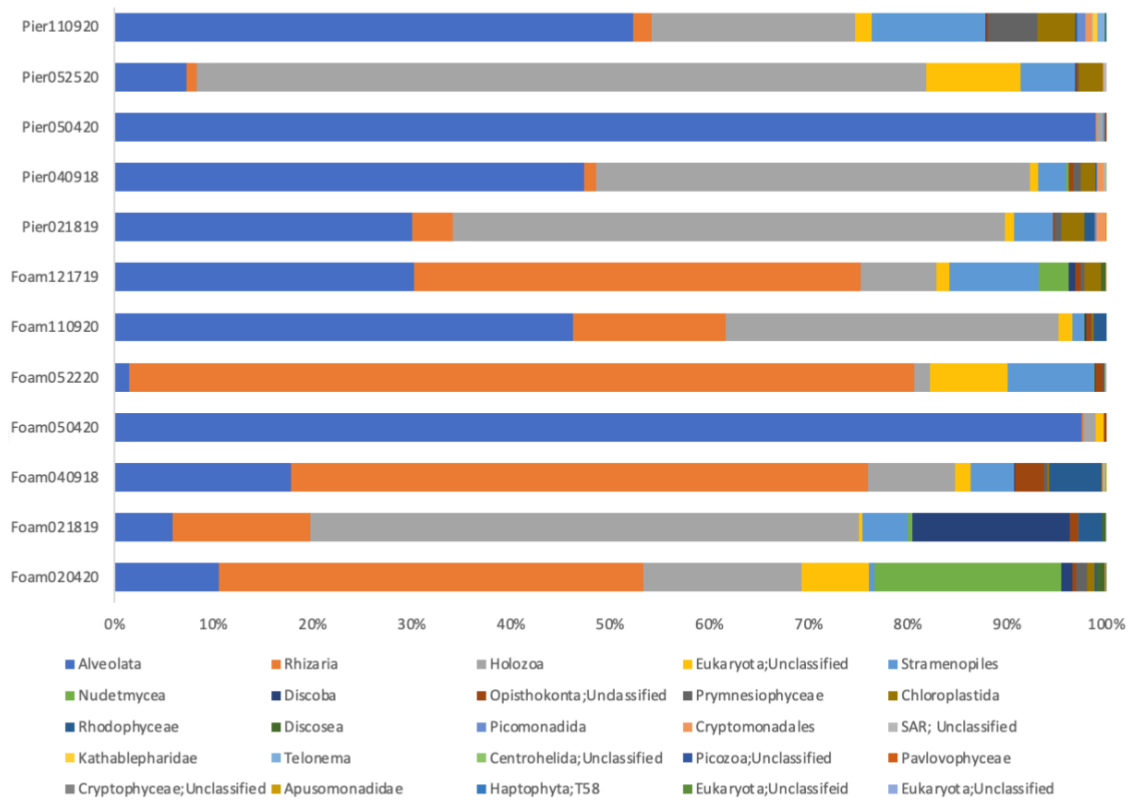
The 18S rRNA amplicon sequence analysis of 12 samples yielded 491758 sequence and 1332 ASVs (Table 2). At the class level, the dominant composition of foam and seawater are similar. *Alveolata*, *Rhizaria*, and *Holozoa* are the most abundant groups in all samples. *Rhizaria* contents in foam are 79.146%, 58.193%, 45.043%, 42.829%, 15.395%, and 13.918% respectively which are much higher than those in seawater (4.099%, 1.816%,1.248% and 1.018%). *Stramenopiles* is another dominant group that existed in all samples except both the seawater and foam samples from 050420 (Figure 3).

At the genus level, *Lingulodinium* has the highest frequency of 63.373% in 050420 foam sample and 88.104% in 050420 seawater sample which was at the peak of a *Lingulodinium* bloom event in 2020. *Protaspidae* showed 61.596% abundance in the 052220foam sample and is occasionally presented in other samples. A group of *Dinophyceae* was abundant in the 110920foam sample (17.812%) and 110920 seawater sample (13.198%) while having less than 5% abundance in every other sample. After extracting the sequence of this group of *Dinophyceae* and using BLAST against the NCBI Genbank database, the result showed the most similar classified strain is a genus of parasitic dinoflagellates *Oodinium* with 90% identity.

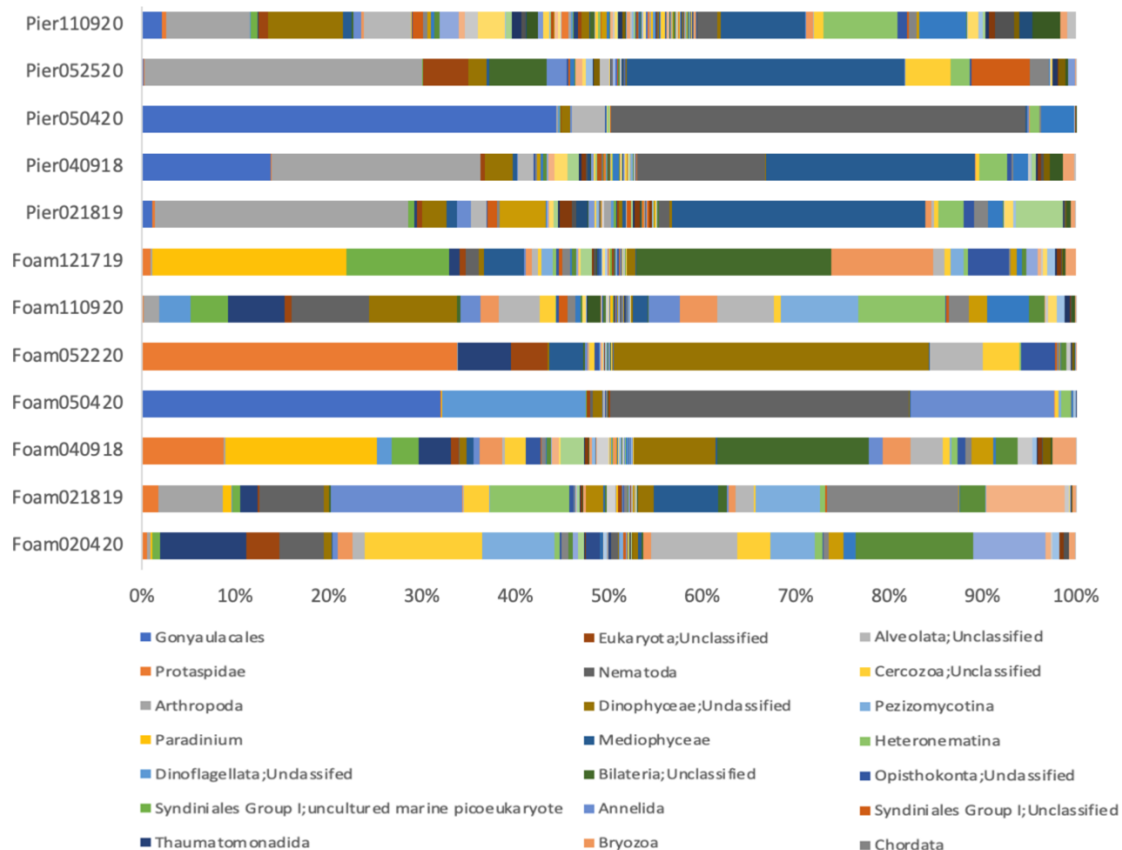
Interestingly, the *Rhizaria* in different foam samples were from different genera. On 052220, *Protaspidae* account for 66.761% of abundance on 052220 and 16.540% on 040918. The amoeboid flagellate *Thaumatomonadida* is the second abundant group of *Rhizaria* in foam samples with the relative abundance of 17.380% (020420), 11.504% (110920), 11.200% (052220), 6.419%(040918). *Paradinium* was the dominant genus in 040918 (30.612%) and 121719 (39.996%)

foam samples. Paradinium is known as a parasitic protist that infects marine, planktonic copepods (Skovgaard and Daugbjerg 2008). A group of unclassified Rhizaria is abundant in 020420 foam samples with 23.746% relative frequency. Sequence of the unclassified Rhizaria was extracted and analyzed using BLAST. Results show that the most closely related strain is biflagellate gliding bacterivorous protozoan *Cercomonas* celer strain C-51 but with only 87% identity.

Figure 3: Relative sequence abundance of eukaryotic taxa in seawater and foam samples using primer set EUK1319-EUKbr at phylum(a) and genus(b) levels and samples using 28F-388R at phylum(c) and genus(d) levels



(a)



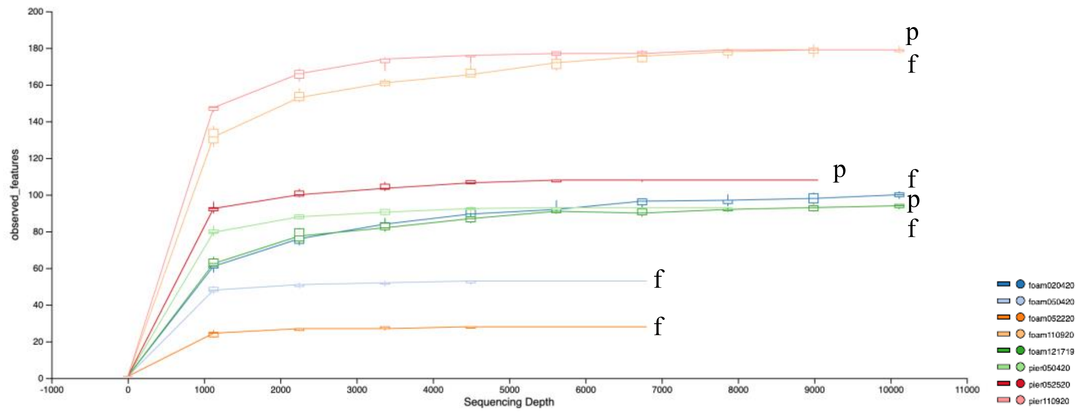
(b)

Figure 3: Relative sequence abundance of eukaryotes taxa in seawater and foam samples using 18S primer set EUK1319-EUKbr at phylum(a) and genus(b) levels and samples using 28F-388R at phylum(c) and genus(d) levels, continued.

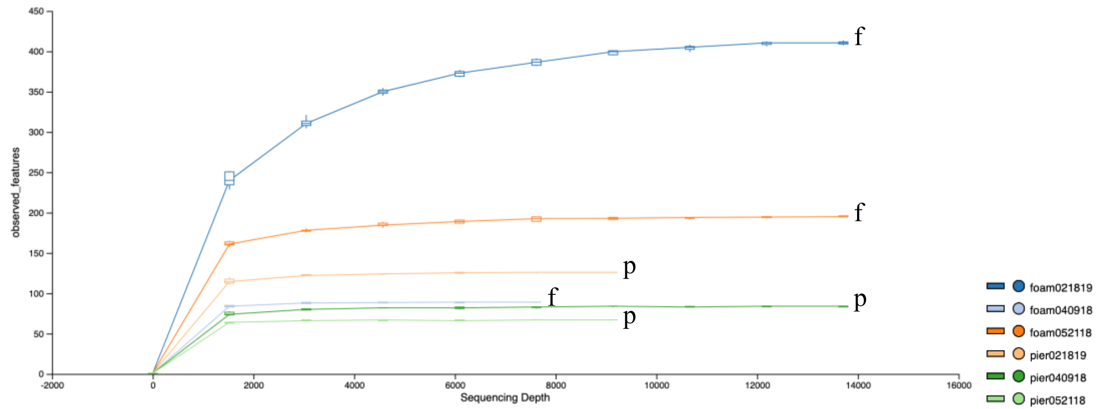
3.4 Alpha diversity

Rarefaction analysis was carried out at the ASVs level and is shown in Figure 4. Most rarefaction curves appeared to reach a plateau with sampling depth along the x-axis sample, implying the recovered sequences commendably represented the eukaryotes and bacterial communities. For a few samples, the rarefaction curves still intend to increase at the level of subsampling, suggesting that greater sequencing depth could be chosen to represent the entire microbial community for them. Alpha diversity estimates were calculated within QIIME 2 using Shannon indexes and observed features number metrics at specific rarefaction depths. No

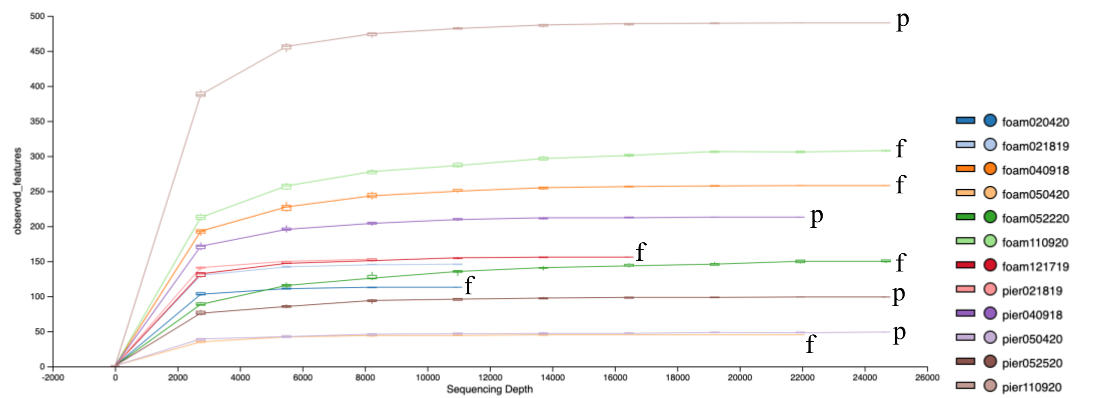
significant difference was observed between foam samples and seawater samples.



(a)



(b)



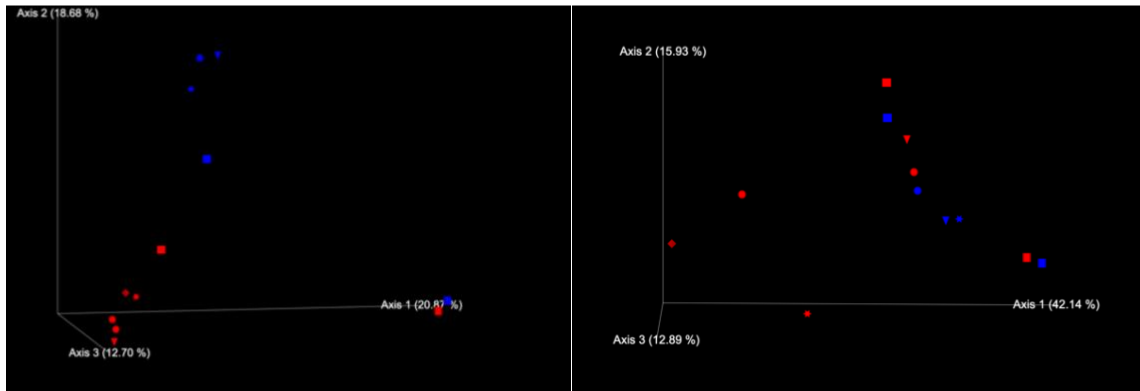
(c)

Figure 4: Alpha rarefaction of foam and seawater samples. (a)16S rRNA sequence using primers 515yF-806bR; (b) 16S rRNA sequence using primers 28F-388R; (c) 18S rRNA sequence using primers EUK1319-EUKbr.

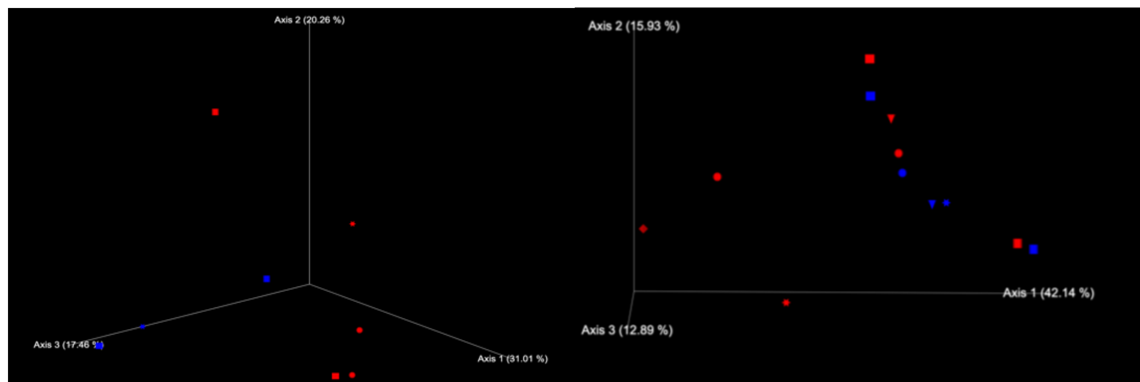
3.5 Beta diversity

Weighted Unifrac distance and Bray Curtis distances were used to measure the beta diversity between the samples and perform principal coordinate analyses (PCoA). Separation between 18S rRNA sequences of foam samples and seawater samples was found on PCoA plot. For the Bray Curtis measure, the first axis, which had 20.87% variation, divided the water and foam samples except for the 050420 samples (fig.5(a)). The second axis, with 18.86% variation, separated the foam samples and water samples again except for the samples from 050420. PERMANOVA test was applied based on the distance matrix and indicated a significant difference in the composition of eukaryotes between foam samples and seawater samples($q=0.013<0.05$).

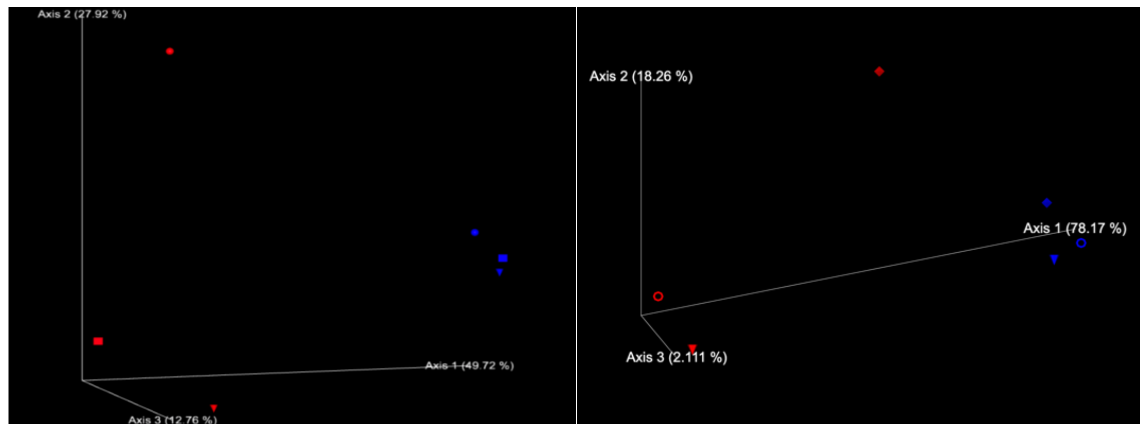
The same beta diversity analyses were conducted for the 16S rRNA sequencing results (figure 5(C)-(F)). For the samples from 021819, 040918 and 051819, both Bray Curtis and Weighted-Unifrac measures of beta diversity showed obvious separation between foam samples and seawater samples on axis1 with 49.72% and 78.17% variation respectively. These were data from primers 28F-388R. Bacterial communities in seawater and foam are highly different according to this separation. There were no significant differences in beta diversity of samples from other dates using primer set 515yF-608bR. Foam and seawater samples were not well separated on the plot while the PERMANOVA test showed the p values were all higher than 0.05.



(a) (b)



(c) (d)



(e) (f)

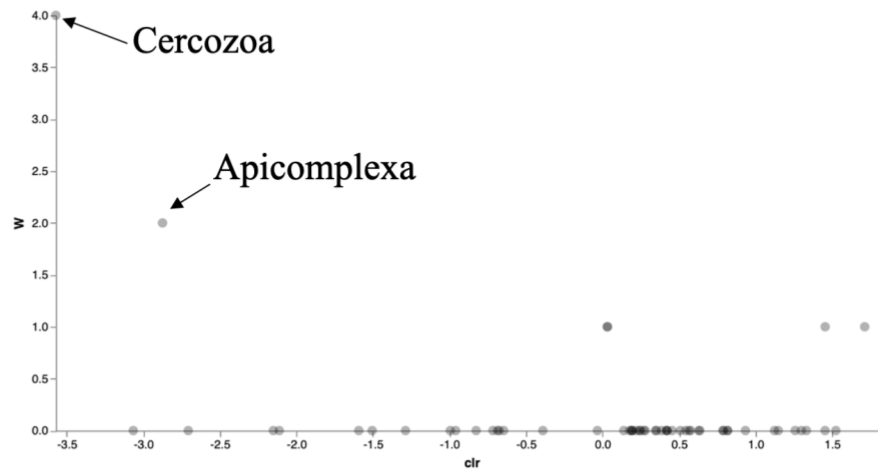
Figure 5: Beta diversity of foam (red) and seawater (blue) samples.(a)Bray-Curtis principal coordinates analysis plot of 18S rRNA sequencing; (b)Weighted-Unifrac principal coordinates analysis plot of 18S rRNA sequencing;(c) Bray-Curtis principal coordinates analysis plot of 16S rRNA sequencing of samples using primer set 515yF-806bR;(d) Weighted-Unifrac principal coordinates analysis plot of 16S rRNA sequencing using primer set 515yF-806bR;(e)Bray-Curtis principal coordinates analysis plot of 16S rRNA sequencing of samples using primer set28F-388R;(f) Weighted-Unifrac principal coordinates analysis plot of 16S rRNA sequencing using primer set set 28F-388R

3.6 Differential abundance

The differential abundance of microbes was characterized using ANCOM in Qiime2. For eukaryotes, according to the ANCOM volcano plot (Figure 6), *Cercozoa* were the only differentially abundant group with high relative abundance at the phylum level in foam. At the genus level, *Peregriniidae* and *Copelata* were found to be differentially abundant between foam and seawater. No taxa of phytoplankton or macroalgae were found to be differentially abundant with high abundance in foam samples.

In terms of 16S rRNA, *Flavobacterium* and *Planktomarina* were the differentially more abundant genera in seawater. Others were also differentially abundant in foam but with lower W value. Notably, *Cobetia, Psychrobacter*, *Winogradskyella*, and *Pseudoalteromonas* were found on the end of the x axis, which suggests they had significant impact on the composition of bacterial communities, but their W values are low (Figure 6).

Figure 6: Differentially abundant microbial taxa identified by Analysis of Composition of Microbiomes (ANCOM). (a)18S rRNA at phylum level;(b)18S rRNA at genus level; (c)16S rRNA using 28F-388R at genus (d)16S rRNA using 515yF-806bR at genus level, continued.

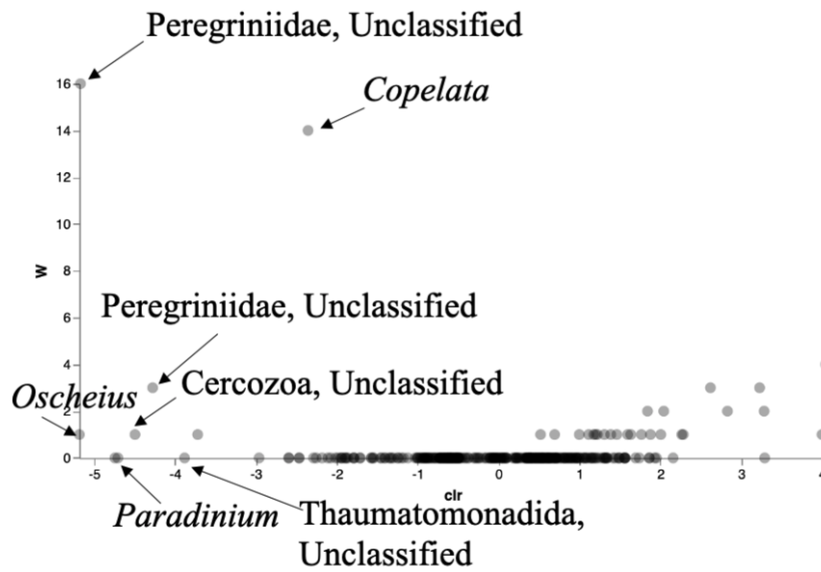


18S rRNA at phylum level

abundant in foam

abundant in seawater

(a)

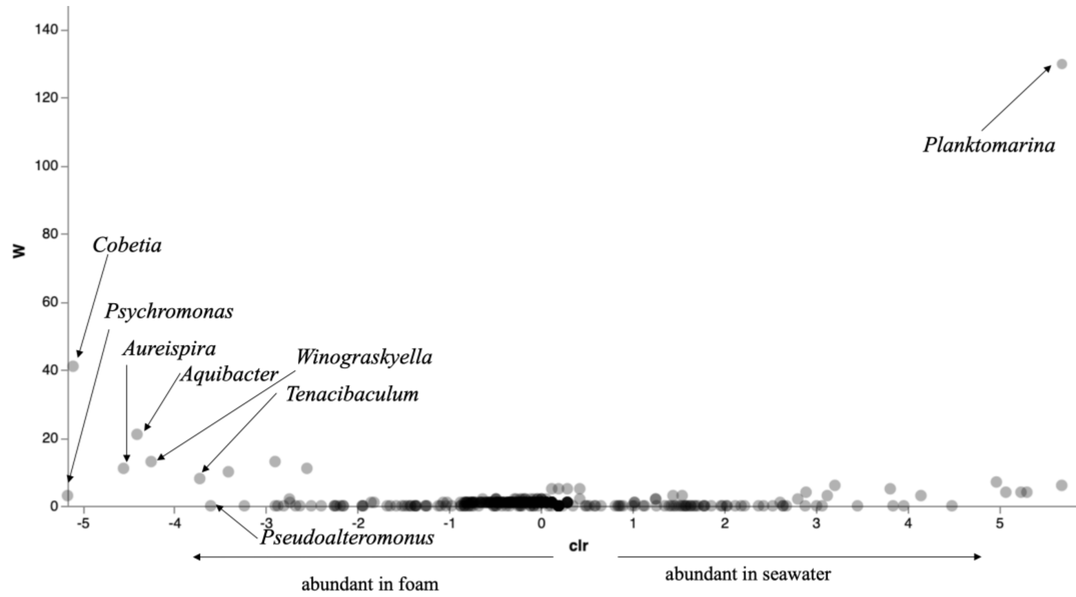


18S rRNA at genus level

abundant in foam

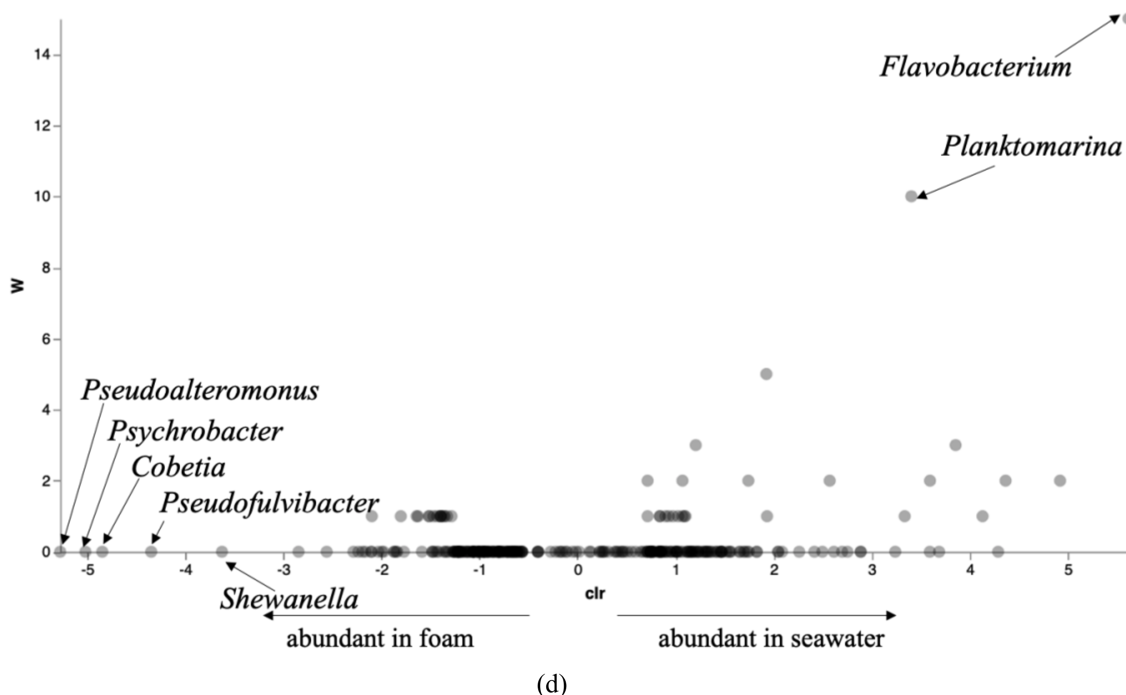
abundant in seawater

(b)



(c)

Figure 6: Differentially abundant microbial taxa identified by Analysis of Composition of Microbiomes (ANCOM). (a)18S rRNA at phylum level;(b)18S rRNA at genus level; (c)16S rRNA using 28F-388R at genus (d)16S rRNA using 515yF-806bR at genus level, continued.



(d)

Figure 6: Differentially abundant microbial taxa identified by Analysis of Composition of Microbiomes (ANCOM). (a)18S rRNA at phylum level;(b)18S rRNA at genus level; (c)16S rRNA using 28F-388R at genus (d)16S rRNA using 515yF-806bR at genus level, continued.

3.7 Isolates in amplicon sequencing

To examine the potential abundance of our isolates in foam samples more generally, 16S rRNA and 18S rRNA amplicon sequence results of the environmental samples used as a BLAST database. Isolates were analyzed using BLAST against the amplicon sequence data to determine if the lab isolates are representative of the major microbial types in foam samples.

For the eukaryotic strains, 5 of 7 diatom isolate sequences were found in the amplicon sequencing results. All of the most relevant ASVs of the isolates were identified as *Bacillariophytina* while they were identified as *Nitzschia* using BLAST against the NCBI nucleotide database. See Table 3. Interestingly these diatoms did not appear to be more abundant in foam than at the pier.

As for the bacterial isolates, a total of 17 isolates were analyzed. Most of the isolates have the same annotated phylogenetic identities based on NCBI nucleotide database and amplicon sequencing results. However, SLBAC203 and SLBAC301 which were identified as mostly likely *Cellulophaga* using the NCBI nucleotide database have the same top BLAST hit as *Sediminicola* ASV which also belongs to family *Flavobacteriacea* but with only 93.28% identities. SLBAC205 *Dietzia maris* was not found in the amplicon sequences and is either rare in foam or a contaminant. SLBAC101 and SLBAC311 have 100% identities as two different *Pseudoalteromonas* ASVs. On the sample date they were collected, top BLAST hit of SLBAC101 has 0.807% relative abundance in 050420 foam sample and 36.70% relative abundance in seawater sample; top BLAST hit of SLBAC311 has 2.52% relative abundance in 110920 foam sample and not presented in seawater.

SLBAC201 and SLBAC204 both have the same 100% identities of a Vibrio ASV. SLBAC202 and SLBAC304 have 100% identities to two different Rhodobacteraceae ASVs. The top BLAST hit of SLBAC304 was one of the most abundant ASVs with 1.4% abundance in 110920 foam samples while Rhodobacteraceae one of the top five abundant groups in both 110920 seawater and foam samples. SLDIA002, SLDIA201 and SLDIA202 have 100% identities as three different diatom strains of genus *Bacillariophytina.* The other four strains were not identified in the amplicon sequencing results using BLAST.

Table 3: Presence of isolates in ASV sequence data for different foam samples

(a)16S

Date of sample	Isolate code	ASV	Top BLAST hit	abundance in amplicon samples 515yF-806bR								Per. ident
				Foam 020420	Foam 050420	Foam 052220	Foam 110920	Foam 121719	Pier 050420	Pier 052520	Pier 110920	
050420	SLBAC 101	d7c175c5b206160e 3c14ffd3ae2019d7	*Pseudoalteromonas*	1826	62	2189	0	6267	3178	0	0	100
052220	SLBAC 201	0429a8a999c3238e 12bbfaa1714d385e	*Vibrio*	0	0	0	47	0	0	0	0	100
052220	SLBAC 202	3947f662165b8043 3b5e4724694196c7	*Rhodobacteraceae;* Unclassified	0	69	0	0	0	78	0	0	100
052220	SLBAC 203	73c21c4811ff72a51 d7e08c944be55c0	*Sediminicola*	0	0	0	0	15	0	0	0	93.68
052220	SLBAC 204	0429a8a999c3238e 12bbfaa1714d385e	*Vibrio*	0	0	0	47	0	0	0	0	100
052220	SLBAC 205	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
092011	SLBAC 301	73c21c4811ff72a51 d7e08c944be55c0	*Sediminicola*	0	0	0	0	15	0	0	0	93.28
092011	SLBAC 302	38bc5be59918b9e3 086bec0881545ea2	*Winogradskyella thalassocola*	161	0	0	0	286	0	0	0	100
092011	SLBAC 303	ed013b7287693531 a88dd54c3d9f4484	*Algitalea*	0	0	0	1062	0	0	0	0	93.68
092011	SLBAC 304	aba9e2d3c25ee990 d4df4b445d3a2113	*Rhodobacteraceae*	0	0	0	1239	0	0	0	0	100

Table 3: Presence of isolates in ASV sequence data for different foam samples, continued

Date of sample	Isolate code	ASV	Top BLAST hit	abundance in amplicon samples 515yF-806bR								Per. Ident
				Foam 020420	Foam 050420	Foam 052220	Foam 110920	Foam 121719	Pier 050420	Pier 052520	Pier 110920	
092011	SLBAC 305	cc246c2e29481ed5 cfcd612810ec2b33	*Maribacter*	13	0	0	0	31	0	0	0	97.23
092011	SLBAC 306	7cca75a624d36427 742f53dbcab8f084	*Flavobacteriaceae*	0	0	0	0	0	51	0	0	100
092011	SLBAC 307	fbd9b6f2c3b29c16b 2445d716b7919ae	*Shewanella*	35	0	0	0	0	0	0	0	100
092011	SLBAC 308	dd6549da7e42e03d a9992ef2db62b1c0	*Planococcaceae*	0	0	0	92	5	0	0	0	96.84
092011	SLBAC 309	5ee181e7af48fbffd8 5476b1eefd0472	*Halomonas*	0	0	0	34	0	0	0	0	97.72
092011	SLBAC 310	bf10e51940dbdbe7 2b5eec0d106e06c7	*Cobetia*	362	2	73	1084	248	0	0	0	97.66
092011	SLBAC 311	1b21d2b71f96b4a5 9b392aee9aa1d655	*Pseudoalteromonas*	16220	0	1840	2181	657	99	0	0	97.59
		Total frequency		40665	7680	7283	86504	35439	8658	9839	10390	

Table 3: Presence of isolates in ASV sequence data for different foam samples, continued

(b)18S

Date of sample	Isolate code	ASV	Top BLAST hit	abundance in amplicon samples EUK1319-EUKbr												Per. ident
				Foam 020420	Per. ident	Foam 040918	Foam 050420	Foam 052220	Foam 110920	Foam 121719	Pier 021819	Pier 040918	Pier 050420	Pier 052520	Pier 110920	
091919	SLDIA 001	136946233b35090c b6813adf456d5b80	*Bacillari -ophytina*	0	0	0	0	0	0	0	5	0	0	0	40	94.44
091919	SLDIA 002	7810612772c23d87 1e59a45d425b7d86	*Bacillari -ophytina*	0	0	0	0	0	0	0	0	0	0	0	29	100
121719	SLDIA 101	1d93b8c697b94f4a 1a114049bd9494ff	*Bacillari -ophytina*	0	0	0	0	0	0	15	0	0	0	0	12	89.19
121719	SLDIA 102	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
121719	SLDIA 103	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
052220	SLDIA 201	b6cbbb68fd9f7d1a0 fd4c291187a6069	*Bacillari -ophytina*	0	0	0	0	0	0	0	0	0	0	0	30	100
052220	SLDIA 202	5a3b7999e43f22d6f 12305af09030c22	*Bacillari -ophytina*	0	0	0	0	45	0	0	0	0	0	367	0	100
		Total frequency		11985	43039	67935	34998	18447	18447	11985	13427	24035	43039	29858	34998	

4 Discussion

We analyzed the 16S and 18S rRNA sequences of microbial communities from beach foam and seawater samples at the nearby Scripps pier to understand the differences between foam and seawater, and if distinct microbial groups are found in beach sea foam. Amplicon sequencing results showed that the microbial communities of foam and seawater are different. For 16S results, SAR11 clades are much more abundant in seawater than those in the foam; in contrast *Pseudoalteromonas*, *Psychromonas, Cobetia and Flavobacteriaceae (Pseudofulvibacter, Lutibacter, Formosa)* have relatively higher abundance in foam samples.

Although we found these differences between foam and seawater, the seafoam bacterial composition and relative abundances can be very different on different dates. Each foam event can have representative bacterial groups with disproportionally high abundance.

Similarly for eukaryotes, *Rhizaria* were relatively more abundant in foam than seawater, However, within the *Rhizaria* there were different dominant genera on different dates.

Foam events may be different from each other if they represent the decay of different algal blooms, and this decay may be associated with different but related bacterial taxa. For example, one of our sampling dates, 05042020 was at the peak of a *Lingulodinium* dinoflagellate bloom. According to the Scripps Pier Harmful Algae Bloom Report (https://sccoos.org/harmful-algal-bloom/), dark rust brown water was observed at Scripps Pier; there was new intense odorous sulfur smell from water; and foam was visible in the surf and along beaches. Along with the extremely

high eukaryotic abundance of *Lingulodinium,* the bacterial abundance of *Lutibacter* was extremely and unusually high. Other times of year at the SIO pier there are other dinoflagellate or diatom blooms and potentially associated bacteria in any foam present. In this model, the surfactants associated with foam are coming from phytoplankton lysis.

Foam samples were cultured in the lab for isolation of bacterial and diatom strains. A total of 13 genera of bacteria were isolated from foam samples while *Bacillariophytina* strains were the diatom group we obtained. All bacterial sequences have several identical or very closely related ASVs in the amplicon sequencing results except for *Cellulophaga*. Only one ASV out of all samples was classified as *Cellulophaga*, and the similarities with the strains we isolated in the lab was low.

In this study, some of the bacteria strains isolated from foam samples and the bacterial OTUs from amplicon sequencing results of foam samples were closely related to those previously identified as biosurfactant-producing bacteria (ie. *Cellulophaga, Cobetia*). Species *Cellulophaga lytica* has been widely isolated from the surface of marine animals(Kientz et al. 2016), sediments(Lee et al. 2018), and coastal shore (Gao et al. 2012). The colonies of *Cellulophaga lytica* grown in lab showed intense yellow color, iridescence, and gliding motility. Iridescence is a property of structural color due to the interaction between light and micron-sized periodic structures(Kientz et al. 2012). Gliding motility could be recognized with the spreading edge from the center of colonies. It has been found that the colonies' iridescence appearance is strongly correlated with gliding motility of bacterial strains (Kientz et al. 2012). The *C.lytica* strains isolated from filter-feeding *Sabellid Polychaetes* were confirmed to be biosurfactant producers for the first

time by testing the emulsification activity and surface tension reduction(Rizzo et al. 2013).

Pseudoalteromonas is one of the most abundant genera in our foam samples and two strains were isolated in the lab from foam samples. Several studies have reported it as a biosurfactant producer (Tripathi et al. 2018, Dang, Landfald and Willassen 2016). *Pseudoalteromonas* has been found in high concentration in diatom and dinoflagellate blooms and may be involved on algicidal functions when the blooms decline(Lee et al. 2000). *Vibrio* sp. was another abundant genus according to foam amplicon sequencing results and two strains of *Vibrio* were isolated in the lab. The high abundance of likely biosurfactant producers in our samples matches the results from previous foam stability studies that the formation and stability of foam is enhanced by surfactants(Heard et al. 2008). In this model, foam may be less due to the surfactants released by phytoplankton, but more due to the production of surfactants by bacteria in the surf zone as they degrade the phytoplankton.

While our study sampled beach sea foam, the results of amplicon sequencing and the bacteria isolated in lab correspond to the recent study(Rahlff et al. 2021) comparing the bacterial composition of sea surface foam with that of the SML and underlying water. The Rahlff study found that *Vibrio, Pseudoalteromonas, Winogradskyella,* and *Verrucomicrobiaceae* belonging to Gammaproteobacteria are typical sea foam colonizers. They confirmed that foams are enriched with surfactants by measuring the concentration in different marine layers. Our alpha diversity analysis showed no significant difference of richness and evenness of microbial diversity between foam and seawater which matches their result that no obvious difference in diversity between foam

and other layers was detected (Rahlff et al. 2021). Rahlff et al. classified the bacteria based on attachment status (particle-associated and free-living), and the number of particle-associated OTUs in foam was highly more abundant than in underlying water. They found a potential foam source in *Trichodesmium* sp. when obtaining a sample from the Timor Sea, but that cyanobacterium was not detected in our samples. While we see *Synechococcus* in foam, its role in foam formation is unknown.

Some of the eukaryotes of our samples were identified as parasites such as *Paradinium* (Skovgaard and Daugbjerg 2008). It was the dominant genus in two foam samples with about 35% relative abundance. Dinoflagellates are of vital importance in marine ecosystems, and some of them are known as parasites of marine animals and protists. The parasitic dinoflagellates have large impact on their hosts in several ways such as health condition, reproduction, and even biomass of the host population(Horiguchi 2015). Some harmful algal blooms have been reported to be affected by parasitic dinoflagellates groups. *Parvilucifera infectans* was found in a toxic dinoflagellate bloom of *Dinophysis* on the Swedish west coast, and then proved capable of killing the microalgae(Norén, Moestrup and Rehnstam-Holm 1999). The bloom of diatom *Guinardia delicatula* was controlled by nanoflagellate parasites *Cryothecomonas aestivalis*(Peacock, Olson and Sosik 2014). In our study, the abundant presence of parasites in foam samples may indicate that the possible source of the surfactants on certain days is from the lysis of dead phytoplankton and copepods caused by the parasites.

5 Conclusion

Using 16S rRNA microbial diversity analysis, it was found that *Pseudoalteromonas, Psychromonas, Cobetia* and Flavobacteriaceae (*Pseudofulvibacter, Lutibacter, Formosa)* have relatively high abundance in foam samples relative to seawater. Seafoam bacterial composition and abundance on different dates however varied greatly. Each foam event has representative bacterial groups with disproportionally high abundance. Most isolated bacteria from foam samples have matching ASVs in amplicon sequences among which *Pseudoalteromonas, Cobetia, Vibrio, Winogradskyella* were known biosurfactant producers. Easily isolated strains are abundant in foam except *Cellulophaga* strains.

For 18S rRNA microbial diversity, microbial composition was also different on different sampling dates. Cercozoa were the most common eukaryotic group in foam. The beta diversity analysis of eukaryotes showed significant differences between foam and seawater. *Bacillariophytina* and *Nitzschia* strains were the only diatom groups we obtained in lab and were not representative of foam eukaryotes as we had expected.

Flavobacteriaceae groups are abundant in foam amplicon sequencing, but we did not obtain any isolates. In the future, different protocols could be used to isolate Flavobacteriaceae groups characteristic of foam and to study their properties. The surfactant producing properties of these future isolates and the other bacteria reported here would be worth investigation. Characterizing the surfactants in foam of the environment samples and the surfactants produced by isolates could

help determine if bacteria are a common source of sea foam surfactants.

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