A comparison of Bacterial Community Diversity between Tahsis Inlet and the Open Ocean

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Abstract

This study aimed to ascertain bacterial community diversity in the coastal North Pacific Ocean during the beginning of winter. The hypotheses were that the coastal ocean would be more diverse than the open ocean, and that the contributors to the diversity would be different between the two. To this end, T – RFLP paired with a literature search of papers using molecular techniques was used to identify the organisms present in the samples. Between 145 and 175 Operational Taxonomic Units (OTUs) were identified in coastal waters in this study, and many of the abundant organisms were successfully identified.

Introduction

Prokaryotes are the most important group of organisms in the ocean. They are responsible for most nutrient cycling in the ocean, fueling all eukaryotic production in addition to their own carbon fixation. They are responsible for all nitrogen fixation that occurs in the ocean as well as the primary chemical losses (denitrification and anammox) of nitrogen from the ocean. This is significant due to nitrogen's role as the limiting nutrient for most of the ocean. However not all bacteria are capable of all metabolic actions, and the old microbiological adage of "everything is everywhere, but the environment selects" does not appear to be true (Perez- del-Olmo et al., 2009). As a result there is biogeographic heterogeneity, and in turn community heterogeneity in the microbial world that mirrors that found in the macroscopic world (Pommier et al., 2007).

Answering questions of biogeography with any culture dependent method is simply not viable for bacteria. This was evidenced by the so called “great plate count anomaly”, where the number of cultivable cells was far exceeded by the total number of bacterial cells as determined by epifluorescence microscopy, indicating we cannot rely on cultured organisms alone to answer biogeographic questions. However, microscopy, even when paired with culture dependent methods is not sufficient for answering questions of species distribution, as it can only differentiate between domains in most instances. When it can be more specific it is still not sufficient to resolve questions of species, even when microscopic techniques are combined (Zettler et al., 2013). Only the various molecular methods are able to resolve these questions when asked about bacteria, and sequencing based approaches are best able to resolve questions of bacterial identity (De Long, 2005). This is because a bacterial species is defined to have 97% or greater identity in the 16S rRNA gene.

All aquatic habitats have unique characteristic microbial populations (Crump et al., 1999; Simo et al., 2000; Zwart et al., 2002; Biers et al., 2009). For instance, the bacterioplankton population is measurably different between freshwater, the coastal ocean, and the open ocean (Rappe et al., 2000). This is a result of each of these habitats having its own unique set of physical and chemical parameters that result in unique niches. These niches in turn result in the expression of different metabolic enzymes. This sort of enzymatic variance is responsible for the six different carbon fixation pathways, the myriad of redox pairs used by bacteria in the ocean, and all extremophilic adaptations, which in turn make their own niches. Where there are more niches, there is a corresponding increase in biodiversity, as more organisms are able to live in the same place at the same time without competing for resources with one another. This can be through exploiting different resources, or exploiting the same resources at different times, or any number of other competition limiting measures. Thus, questions of diversity inform about biogeographical trends, and vice versa.

Tahsis Inlet is an estuarine fjord with a settlement (the village of Tahsis) at the mouth of the Tahsis river with a population of 316 people (Figure 1). Near the town, there is a fish farm potentially affecting the bacterial population as well; there may be fish pathogens, or organisms exploiting their waste and egesta. The two sources of surface water to the inlet are the Tahsis River, and the Tahsis Narrows, which connects to the neighboring Esperanza Inlet. The fresher water found at the surface is much less dense, so it will stay at the surface. The area is prone to landslides so, combined with the river, there is a large flux of terrigenous sediments to the area. There is strong salinity induced stratification, as seen by a strong halocline beginning at ~4m. Temperature is essentially uniform at 10°C in this depth range.

Figure 1. An image of Tahsis Inlet. North is at the top of the figure. The marker indicates the location of sample collection for nutrients and water.

This study aims to resolve the bacterial diversity in the surface waters of Tahsis Inlet (an estuarine fjord fed by the Tahsis river) and a portion of Nootka Sound in the coastal North East Pacific ocean through terminal restriction fragment length polymorphisms (T – RFLP). I selected the surface waters as I hypothesize they would be the most diverse of all possible depths due to the presence of more niches. During the sample period the irradiance was rather low, due to it being winter when the samples were collected, so any photosynthetic organisms will be limited to the surface ocean. This study will also compare coastal ocean bacterial diversity to that found in the open ocean and postulate reasons for any differences.

The first hypothesis is that the surface waters of Tahsis Inlet will have more bacterial diversity than the open ocean, due to a greater number of available niches. I expect there to be a greater number of niches in this inlet than the open ocean because of the influence of land and freshwater. The second hypothesis is that the drivers of this diversity will differ between these two habitats, given the physical and chemical differences between Tahsis Inlet and the open ocean. This would be demonstrated by components of microbial diversity varying between the coastal and open ocean, as evidenced by different relative abundances of bacterial clades.

T- RFLP is a sequencing technique targeting the bacterial 16S gene that cleaves it after certain base pair sequences, so each species will have a particular length of their 16S once split. This method is specifically intended to obtain a community fingerprint, and is therefore uniquely suited to answer this question. In fact, it has been used to answer similar questions in the past (Moss et al., 2006; Stoica, 2009; Zhang et al., 2011; Yeo et al., 2013).

Methods

Sample collection and filtration

A total of 5 samples were collected from Niskin bottles on a Sea-bird SBE-9 CTD rosette deployed over the side from 1.3m depth. All samples were collected on December 15th, 2015 at night from 49°51.394 N and 126°39.5070 W in Tahsis Inlet, Nootka Sound, British Columbia, Canada, aboard the *RV Thomas G Thompson* (Figure 1). Samples were then filtered through 0.2μm polyethersulfone filter (Supor-200, Pall Corp, Ann Arbor, MI) filters in amber rigs. Each sample was connected to an air pump to speed the filtration process. Once each filter was dry, it was inserted into and stored in a cryotube, and placed in a shipboard -80°C freezer. Nutrient samples were collected from a Niskin bottle mounted on the same CTD rosette used above, from the same depth. Samples were filtered through a .45μm filter, frozen on board and transported back to the University of Washington and analyzed by the Oceanography Marine Chemistry Lab.

DNA extraction and PCR

DNA extraction and amplification occurred as per Marshall and Morris, 2012. Briefly, bacterial genomic DNA was extracted with a DNeasy Blood and Tissue Kit (QIAGEN, Germantown, MD, USA) according to the manufacturer’s instructions (Marshall and Morris 2012).

Polymerase Chain Reaction (PCR) is a method in molecular biology to synthetically make many copies of certain genes in a sample. In this study, it amplified the 16S gene of all bacteria in environmental samples. 16S rRNA genes were amplified using a semi-nested PCR reaction with *Taq* polymerase (Fermentas, Hannover, MD, USA) and bacterial primers. PCR amplifications were performed according to Marshall and Morris, 2012, with the following modifications; the master mix was 178.4μL filtered water, 32μL 10X buffer, 32μL 2mM dNTP, 16μL $MgCl_2$, 32μL 50% acetamide, 6.4μL 27F primer, 6.4μL 1492R primer, and 0.8μL Taq Polymerase. There were 5 samples, and two controls, one positive and one negative. Amplifications were performed in a

C1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) using the following conditions: denaturation at 94°C for 30 seconds, hybridization at 55°C for 60 seconds, and elongation at 72°C for 120 seconds. PCR was run for 3.5 hours with only 27f and 1492r primers.

Gel electrophoresis and restriction digest

Two restriction digests were conducted in this study. The purpose of this gel electrophoresis was to determine if PCR successfully amplified any genetic material present in the sample. If electrophoresis was successful, there would be bands of fluorescently labeled DNA that matches the ladder in size and shape. The first gel was run directly after PCR to test for success. The gel was made by combining 40mL TRE buffer and .4g molecular agarose. This mixture was heated until the agarose was evenly dissolved. At this point, the solution was cooled until all boiling stopped, and was then poured slowly into the center of the gel tray. The gel was evenly distributed and there were no bubbles. Before the gel cooled, a well maker was used to make 10 wells at one end of the gel, which was then allowed to cool until solid. In the well farthest to the left, a dye ladder was added. This process was repeated for the seven tubes from the thermocycler, in the order of NC, PC, and samples 1-5 in order. A second dye ladder was added to the last (10th) well. The tray was covered with opaque plastic and the gel was run at 100 volts for 60 minutes.

The 16S PCR products were digested with 5' labeled 6-FAM, with the same thermocycler settings as above, followed by an inactivation phase at 80°C for 20 minutes, and amplicons were restricted with the *Hae*III enzyme. This enzyme splits the

PCR products at GGCC after the second nucleotide, and cuts both strands of DNA, making a pair of identical, inverted fragments each time it cuts. The fragments were sequenced by the Fred Hutch Cancer Center, and the sequence data were returned electronically. For each sample, the fragment length in nucleotides, and the fluorescent intensity in relative units were graphed (Figures 2 and 3). Length values lower than 80 nucleotides were discounted as fragments that small are almost certainly errors in this method. The length in nucleotides was used to identify individual operational taxonomic units (OTUs), as each organism has a characteristic length based on the primers and restriction enzyme used. The fluorescence units in relative units portray the number of pieces of DNA of that length in the sample after PCR, and therefore the abundance of the organism with that fragment length. However, this method is not perfect. Multiple OTUs can have the same fragment length, potentially artificially inflating apparent abundance and decreasing diversity. Additionally, PCR does not amplify all DNA equally, so the fluorescence data can only be compared quantitatively between samples in the same run, not samples from other papers because individual runs always have slightly different parameters.

The purpose of the second gel was to test for digests occurring, and followed the same procedure, except 4μl ethidium bromide was added to the buffer/agarose solution, and the loading dye in the second gel contained no SYBR. The ladder was 100 base pair. The gel was run at 70 volts for 40 minutes.

Results

Results of the Restriction Digest and Nutrient Analysis

Two of the five samples failed at either the DNA extraction or amplification steps, and a third failed the quality control step in sequencing, leaving samples 1 and 3 to be analyzed. The files were opened and processed with PeakScanner v1.0 (Applied Biosystems). Sample 1 (Figure 2) had a total of 175 OTUs. Sample 3 (Figure 3) had a total of 145 OTUs. Nutrient measurements deviated from the Redfield ratio, with 1.25μmol kg^{-1} PO_4 to 12.37μmol kg^{-1} total Nitrogen summed across NO_3, NO_2, and NH_4. This indicates either a degree of oligotrophy, or nutrient depletion from the autumn bloom or both.

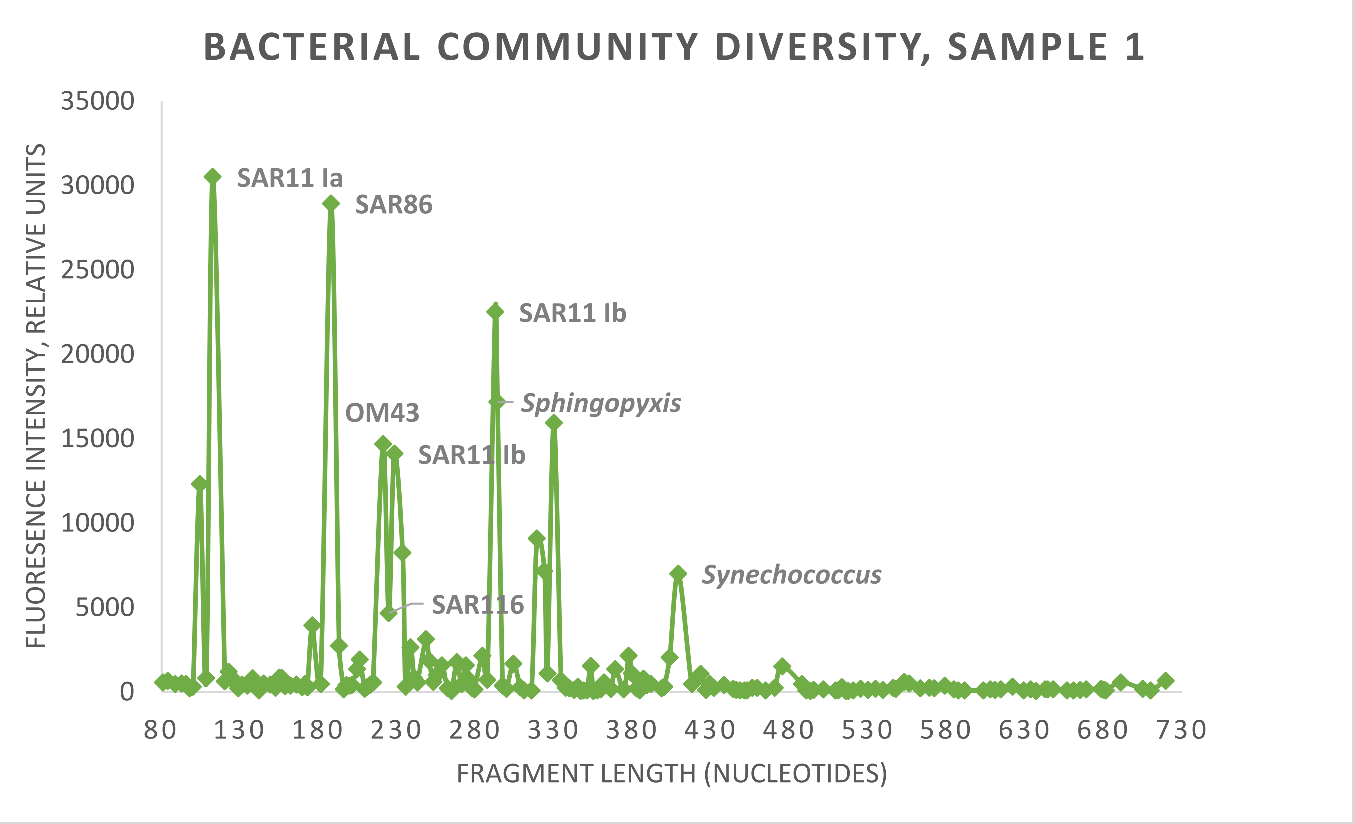

Figure 2. Bacterial community structure via T-RFLP for sample 1. Each data point is a discrete OTU. OTUs identified successfully in both samples, and with more than 5000 units of fluorescent intensity are identified on the graph by the most specific taxonomic level possible. Larger peaks indicate more abundant organisms.

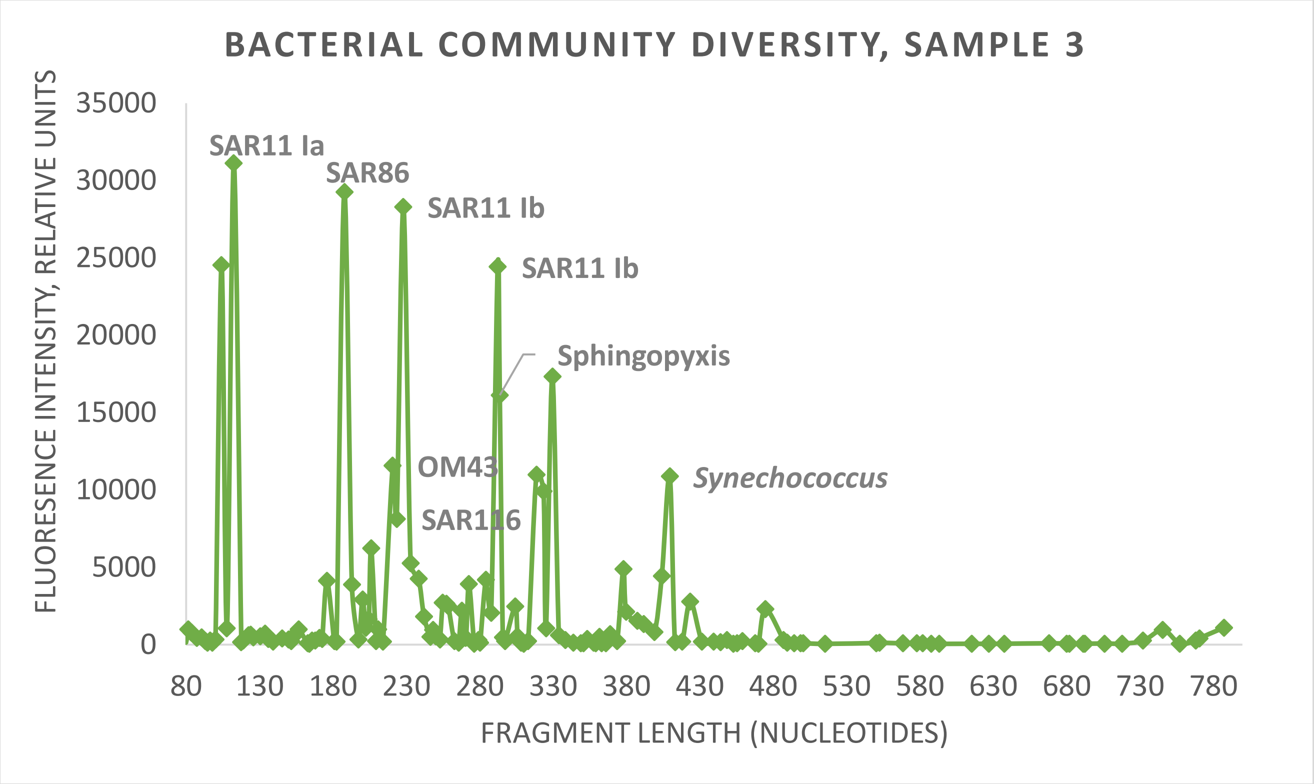

Figure 3. Bacterial community structure via T-RFLP for sample 3. Each data point is a discrete OTU. OTUs identified successfully in both samples, and with more than 5000 units of fluorescent intensity are identified on the graph by the most specific taxonomic level possible. Larger peaks indicate more abundant organisms

Identification of organisms

Identifying individual OTUs was done by reviewing literature in which T-RFLP was conducted with the same PCR primers and restriction enzyme as this study in parallel with a genomic technique to identify individual OTUs, such as clone libraries, pyrosequencing, or both (Ivanova et al., 2000; Morris et al., 2004; Kibe et al., 2005; Morris et al., 2005; Stepanasukas and Sieracki, 2007; Scheubulin et al., 2010; Marietou and Bartlett, 2014; Salter et al., 2014). Papers were included if they passed all of the above criteria and had a published table or figure representing fragment length and identity. Results of this literature search are included in Tables 1 and 2, though only positive matches are included. All identifications are as taxonomically specific as possible based on the level of specificity in the source papers. After all possible organisms were identified their phylum or subphylum was also recorded for later analysis. Where possible, broad metabolic requirements, such as autotroph vs heterotroph, and aerobe vs anaerobe, were assigned to each identified OTU through either *The Encyclopedia of Life* page on the given organism or the digital edition of *Bergey's Manual of Systematics of Archaea and Bacteria*.

Table 1. A more holistic view of diversity in samples 1. ND indicates insufficient specificity of fragment identity for any knowledge of metabolism and/or taxonomy. A "?" following an identification indicates rounding was necessary to arrive at that identification.

Fragment Length	Fluoresecent Intensity	Identity	Phylum	Metabolism
211.0364	7022	Synechcoccus	Cyanobacteria	Autotroph
212.5568	418	Comamonadcae?	Betaproteobacteria	Autotroph
220.97	2158	Bacillariophyta Chloroplast	Bacillariophyta	Autotroph
224.6544	411	Prasynophyte Cholroplast	Chlorophyta	Autotroph
193.0669	399	Pelomonas	Betaproteobacteria	Symbiont, associated with animal kidneys
233.488	205	Flavobacterium	Bacteroidetes	Symbiont, fish pathogen
253.0242	160	Flavobacterium	Bacteroidetes	Symbiont, fish pathogen
299.5547	187	Coriobacteriaceae	Actinobacterium	Symbiont, gut bacterium
228.1616	428	*Methylobacterium*	Alphaproteobacteria	Symbiont, methyltroph
262.0698	14691	OM43?	Betaproteobacteria	Symbiont, methyltroph
279.1575	197	*Marinosulfonomonas methylotropha*	Alphaproteobacteria	Symbiont, methyltroph
293.9097	17191	*Sphingopyxis*	Alphaproteobacteria	Oligotroph
165.7429	28948	SAR86	Gammaproteobacteria	Oligotroph, aerobic heterotroph
366	372	SAR116?	Alphaproteobacteria	Oligotroph, photoheterotroph
374.4615	4671	SAR116?	Alphaproteobacteria	Oligotroph, photoheterotroph
377.5385	30525	SAR 11 Ia	Alphaproteobacteria	Oligotroph, photoheterotroph
388.7692	14110	SAR11 Ib	Alphaproteobacteria	Oligotroph, photoheterotroph

409.0769	22518	SAR11 Ib	Alphaproteobacteria	Oligotroph, photoheterotroph
112.6104	8254	Bacillus	Firmicutes	Soil, aerobe or facultative anaerobe
187.8919	603	Acinetobacter	Gammaproteobacteria	Soil, aerobic heterotroph
197.5841	154	Clostridium	Firmicutes	Soil, anaerobic Heterotroph
134.503	99	Alcanivorax	Gammaproteobacteria	Aerobic alkane and pyrene degraders
196.1333	2137	Ulvibacter	Bacteroidetes	Aerobic, organochemotrophs
284.3811	1110	Oceanospirillales	Gammaproteobacteria	Heterotroph
292.4227	141	*Tenacibaculum mesophilum*	Bacteroidetes	Heterotroph, iron chlating siderophore
310.8905	2731	Gammaproteobacterium	ND	ND
323.8499	295	Gammaproteobacterium	ND	ND
325.6091	7183	Bacteroidetes?	ND	ND
342.7852	133	Bacteroidetes?	ND	ND

Table 2. A more holistic view of diversity in samples 3. ND indicates insufficient specificity of fragment identity for any knowledge of metabolism and/or taxonomy. A “?” following an identification indicates rounding was necessary to arrive at that identification.

Fragment Length	Fluorescent Intensity	Identity	Genus	Metabolism
377.9661	4872	Bacillariophyta Chloroplast	Bacillariophyta	Autotroph
409.661	10871	*Synechcoccus*	Cyanobacteria	Autotroph
197.4301	332	Comamonadcae?	Betaproteobacteria	Autotroph, chemoautotroph
279.1382	304	Flavobacterium	Bacteroidetes	Symbiont, fish pathogens
165.6766	260	*Methylobacterium*	Alphaproteobacteria	Symbiont, methyltroph
220.8743	11567	OM43	Betaproteobacteria	Symbiont, methyltroph
366.1017	86	Coriobacteriaceae	Actinobacterium	Symbionts, gut bacterium
187.8299	29269	SAR86	Gammaproteobacteria	Oligotroph, aerobic chemoheterotroph
293.7955	16125	*Sphingopyxis*	Alphaproteobacteria	Oligotroph, heterotroph
112.5172	31132	SAR11 Ia	Alphaproteobacteria	Oligotroph, photoheterotroph
133.9036	727	SAR116	Alphaproteobacteria	Oligotroph, photoheterotroph
192.974	3886	SAR116	Alphaproteobacteria	Oligotroph, photoheterotroph
223.5289	8132	SAR116	Alphaproteobacteria	Oligotroph, photoheterotroph

228.1192	28309	SAR11 Ib	Alphaproteobacteria	Oligotroph, photoheterotroph
292.3631	24416	SAR11 Ib	Alphaproteobacteria	Oligotroph, photoheterotroph
233.047	5264	Bacillus	Firmicutes	Soil, aerobe or facultative anaerobe
252.8929	343	Acinetobacter?	Gammaproteobacteria	Soil, aerobic heterotroph
170.8337	431	Actinobacterium	Actinobacterium	Soil, heterotrophic, some tiny marine representatives
200.1797	2913	Massillia	Betaproteobacteria	Aerobic heterotroph
313.1261	244	Polaromonas	Betaproteobacteria	Aerobic heterotrophs, psychrophiles
284.3998	4203	Ulvibacter	Bacteroidetes	Aerobic, organochemotrophs
498.9831	90	Roseobacter?	Alphaproteobacteria	Anaerobic heterotrophs
257.3875	2659	SAR202	Chloroflexi	Anoxygenic phototrophs, anaerobic heterotrophs
325.309	1052	Oceanospirillales	Gammaproteobacteria	Heterotroph
413.2203	162	Oceanospirillales	Gammaproteobacteria	Heterotroph
373.7288	218	*Tenacibaculum mesophilum*	Bacteroidetes	Heterotroph, iron chlating siderophore
423.7288	2791	Rhodobacteracaea?	Alphaproteobacteria	Heterotroph, photoheterotroph, anoxygenic photoautotroph
202.514	1076	SAR316	ND	ND
210.9211	998	Uncultured Gammaproteobacteria	ND	ND
262.8369	222	Bacteroidetes?	ND	ND
265.7184	121	Bacteroidetes?	ND	ND
439.4915	184	Bacteroidetes?	ND	ND
206.0976	6244	Ralstonia	Betaproteobacteria	Probably a contaminant

Relative Diversity

To visually represent sample diversity, Figures 4 and 5 were assembled from phylum or subphylum level diversity in samples 1 and 3, as that was the lowest level that could be identified for all OTUs. A probable contaminant and all chloroplasts were removed from the set that was used to construct these figures, due to not representing bacterial diversity. The phylum Proteobacteria was split into its component subphyla to better represent the diversity, as phylum Proteobacteria made up a majority of the organisms in both samples, and in fact much of the ocean.

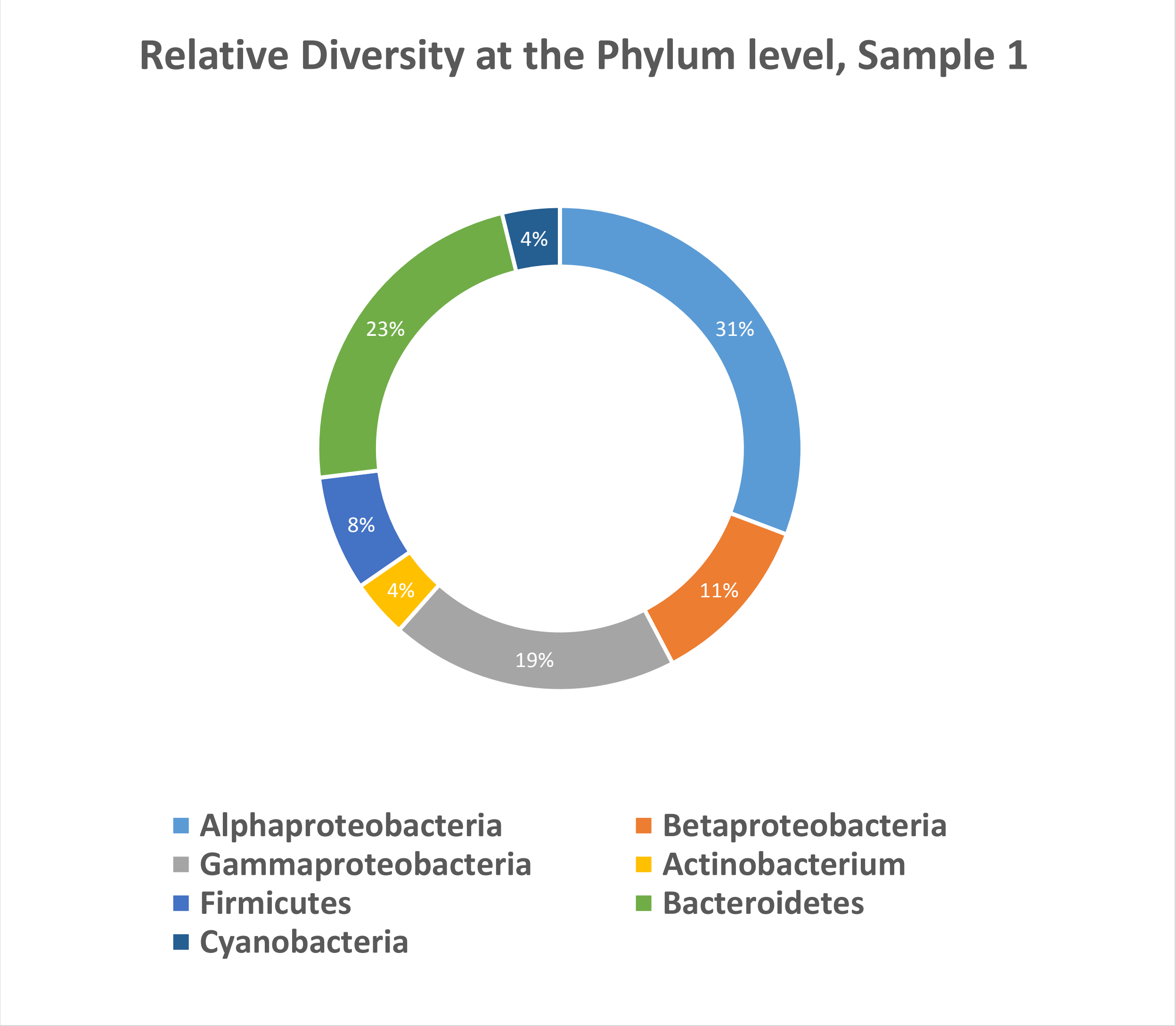

Figure 4. Relative numbers of identifications in each phylum or subphylum in sample 1. The chloroplasts were removed, as they are not bacteria. The Proteobacteria are responsible for 62% of the diversity at the phylum level.

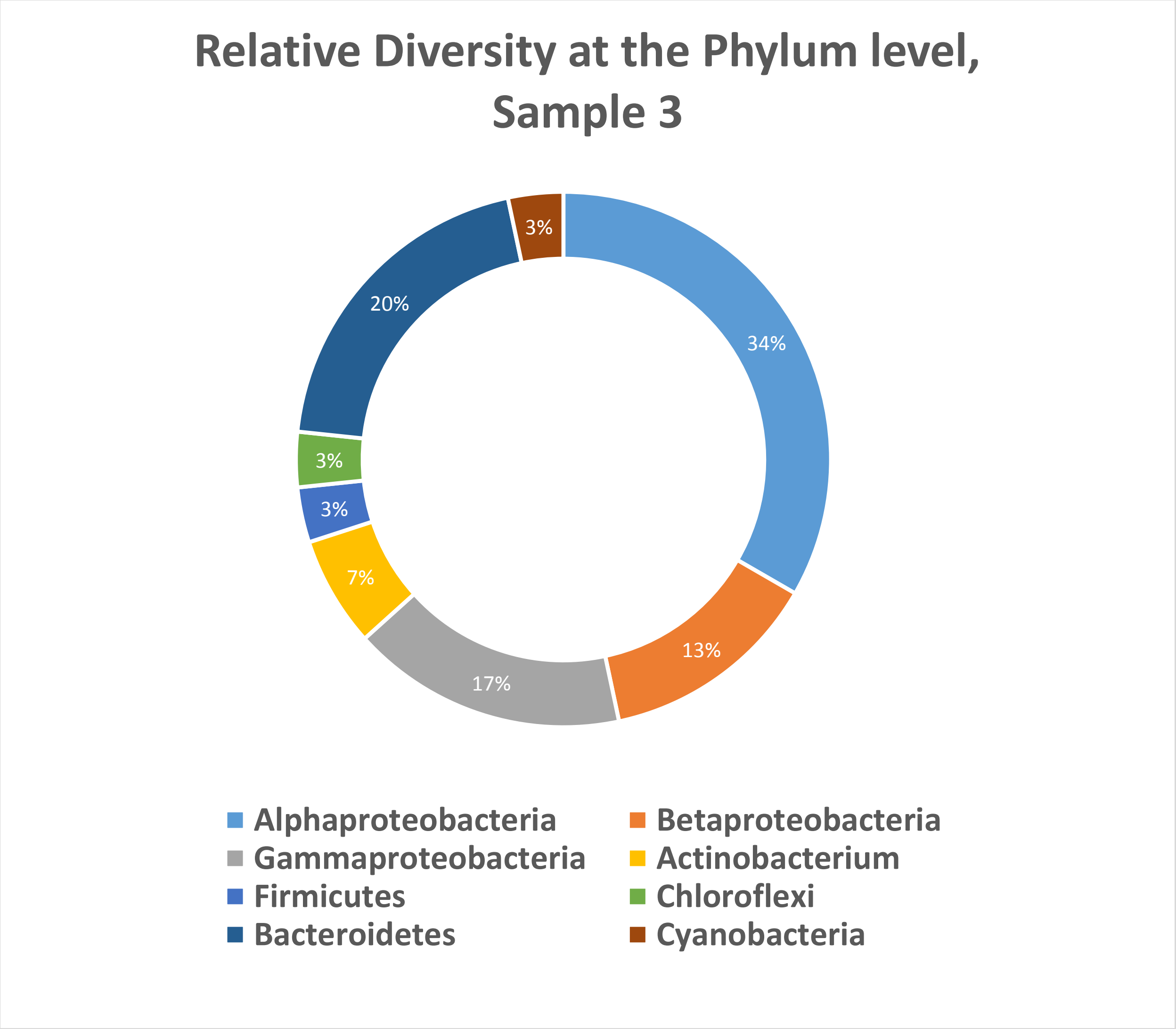

Figure 5. Number of identifications in each phylum or subphylum in sample 3. The chloroplast and *Ralstonia* were removed. The latter as it is probably a contaminant, and the former as it is not a bacterium. The Proteobacteria account for 65% of the sample diversity at the phylum level.

To provide quantitative analysis of sample diversity, average peak height, standard deviations, Shannon Diversity Indices and Evenness were calculated for both samples. For sample 1, the average peak height was 1576.322 fluorescent intensity units, the standard deviation was 4393.888, H = 3.671688, and E = 0.293221. For sample 3, the average peak height was 2280.389 fluorescent intensity units, the standard deviation was 5583.413, H = 3.562333, and E = 0.280456. Total number of organisms was assumed to be the sum of all fluorescence units in each sample. Shannon Index values greater than 3 are indicative of relatively high biodiversity in a sample. Evenness values have a maximum value of 1, indicating the sample diversity is perfectly even, in which all species would have the same number of representatives in the sample. So, the lower the E value, the less even the sample is.

Discussion

Hypotheses

The hypotheses for this study were that the surface waters of the coastal ocean would be more diverse than the surface waters of the open ocean, and that the contributors to said diversity would be different. The first will be supported by evidence from filled niches, Shannon Index, and Evenness. The second will be supported by filled niches and relative abundance of different clades.

Community Metabolic Niches

With the metabolic data from Tables 1 and 2, it is possible to postulate why each identified organism with any known metabolic capabilities was present in the sample at the time of sampling, and therefore to know which niches were filled.

Autotrophs

The season would suggest the primary producer niche is filled by few organisms due to the shortage of light and the oxidized state of the water column. The former will limit oxygenic photosynthesis and the latter will limit most chemosynthesis and anoxygenic photosynthesis. Nonetheless several were found in each sample, with sample 1 containing *Synechococcus* in abundance, and lower concentrations of Diatom and Prasynophyte chloroplasts. Either a denitrifying or chemosynthetic member of Comamonadcae from either the terrestrial soil or the town's waste water was also present. Sample 3 lacked the Prasynophyte chloroplast. While *Synechococcus* was one of the most abundant organisms in either sample it was not abundant enough to sustain all of the other organisms in the sample. This suggests there must be some allochthonous source of organic material for all the heterotrophs in the sample, which already sets the coastal ocean apart from the open ocean. We can rule out eukaryotes as this source due to the low abundance of chloroplasts. The other possible alternative is enhanced mixing during winter mixing up organic matter from the sediments, which also cannot happen in the open ocean, and would support the first hypothesis. However, this possibility may be unlikely if the salinity stratification persists through the winter.

Heterotrophs

Heterotrophic bacteria have two general solutions to avoiding this food limitation; symbiosis and oligotrophy. There is evidence for both of these in Tahsis Inlet. Each of these forms several additional niches organisms from the sample fell into. Symbiosis is the close association between two organisms in which at least one benefits. The common symbioses are mutualism, where both organisms benefit, commensalism, where one organism benefits and one is unaffected, and parasitism, where one organism benefits and the other is harmed. Oligotrophic organisms are organisms that thrive in low nutrient environments.

Symbioses

Symbioses appear common in the surface waters of Tahsis Inlet. Without physiological data it is in most cases impossible to determine what type of symbiosis two organisms exhibit. Fortunately, determining that a symbiosis is present is much simpler. All of the methyltrophs (*Mehtylobacterium*, OM43, and *Marinosulfonomonas methylotropha*) are likely symbionts, as they require C1 organic molecules, and are incapable of synthesizing them. It is a known fact algae, especially diatoms are able to synthesize compounds containing CH_3 functional groups, such as dimethylsulfoniopropionate (DMSP), and diatom chloroplasts were identified in this sample (Charlson et al., 1987). Another possibility is that these molecules are synthesized in the sediments and mixed upward during the annual stormy season, or that there is some combination of both sources. OM43 in particular likely acquires its methyl from sedimentary processes as it is common in coastal waters and virtually absent in the open ocean (Yeo et al., 2013). The other symbioses are all between animals and bacteria (Pelomonas, all of the Flavobacteria, Coriobacteriaceae, and potentially the Bacillus and Acinetobacter, though these two may also be soil bacteria). All of these organisms are associated with the gut or urinary tracks of mammals or fish. Flavobacteria are common fish parasites, and were likely in the sample as a result of the fish farm. The others are generally commensalists.

These organisms occupy niches that are absent or much reduced in the open ocean. Regardless of the source of C1 compounds, methyltrophs will always have more in coastal water due to the possibility of mixing and the greater abundance of large, eukaryotic algae in highly productive surface waters. The only source they would have access to in the open ocean would be diatoms during their annual bloom, and would necessarily exist in lower numbers in the rest of the year. None of the enteric microbes would be found reliably in the open ocean, as there are no towns or marine mammal hosts for them, and the paucity of large phytoplankton limit higher trophic levels from existing in consistent abundance in any one place.

Oligotrophs

The oligotrophic organisms (SAR11, SAR86, SAR116, and *Sphingopyxis*) can all be said to inhabit another niche, and these organisms would be the ones most expected from the open ocean. Another common trait among these organisms is photoheterotrophy, where light is used to generate ATP, but not fix carbon. In this way they can supplement their energetic requirements and use the minimal amounts of organic matter for cell maintenance and duplication without the energetic expense of maintaining a carbon fixation pathway. This also means their growth is less seasonal than photoautotrophs, since they can rely on dissolved organic matter as well. The other adaptation they have is their size. Oligotrophic bacteria are tiny ($<0.1\ \mu m^3$), which maximizes their surface area to volume ratio, which optimizes the rate of diffusion across their cell wall vs their metabolic rate.

Land Bacteria and others

A number of soil bacteria (Clostridium, Actinobacterium, and potentially Bacillus and Acinetobacter) were also identified. This area is subject to frequent landslides due to the very steep walls of the fjord. The Tahsis river also empties into the inlet, and brings sediments from upstream to the ocean. Lastly, runoff from rain can suspend sediments and bring them into the ocean as part of Hortonic flow. While all of these processes introduce bacteria attached to the sediments to the ocean, without some degree of halotolerance, these organisms tend to become dormant. Some members of Bacillus are known to be halotolerant, so they may not be inactive (Ivanova, 2000). Since there was no measurement of metabolic activity in this study, it is impossible to ascertain whether or not any of these organisms are contributing to the community metabolism. If they are, they will be another unique niche that is necessarily absent from the open ocean.

There were also a number of organisms with individual metabolisms that did not fit neatly into any other the other categories, and represent site specific diversity that would also be absent in the open ocean. Ulvibacter is a generalist heterotroph but not an oligotroph that requires a high concentration of Na^+ ions. The salinity of the samples was ~30psu, indicating water of a marine origin and explaining the presence of this organism. Alcanivorax obtains energy from aerobically oxidizing alkanes and pyrenes, hydrocarbons found in residues of boat fuels and other petrochemicals. Due to a lack of petrochemical exploration in Nootka Sound, boat fuels are the most likely source. *Tenacibaculum mesophilum* is an iron chelating organism, and has its maximum growth rate in mesophilic waters at ~10°C, which indicate this organism is probably more abundant in deeper waters where it can access the iron stored in the sediments, and where the water is at its optimum temperature. Its presence also suggests Tahsis Inlet is not iron limited, as this organism converts mineral iron to a form usable to most organisms. While *Polaromonas* is a psychrophile, the water temperature at the sample site is within its tolerable range, if only just. That it is an aerobic heterotroph is not enough to identify a niche for it.

One organism (*Ralstonia*) is probably a PCR contaminant in environmental samples (Salter et al., 2014). While this study identifies a number of potential contaminant organisms, there is reason to suspect they are not contaminants, as they all fit a possible niche in the ecosystem from the sample, with the exception of this organism. The presence of these contaminants is also predicated on low bacterial biomass, which is something this study did not ascertain, so it is impossible to be certain any of the organisms found in both this study and Salter et al., 2014 are or are not contaminants.

Lastly, a number of organisms were not identified to a sufficient level of specificity to know what niche they inhabit. Oceanospirillales are abundant marine heterotrophs, but was not identified with enough specificity to explain its presence. The *Massilia* genus is very metabolically diverse, so it is impossible to identify what niche this organism fills. SAR 202 is a green non sulfur bacterium, but there was not enough information to identify anything more specific about this bacterium. Rhodobacteracaea and Roseobacter are abundant in the ocean, but are very broad taxonomic categories, so it is impossible to ascribe niches to any of them as well. The uncultured Gammaproteobacteria also fall into this group.

Community Diversity

Both samples have high (>3.5), and nearly identical levels of biodiversity according to the Shannon Index. Both samples have an E value from .28 to .29, indicating very low evenness. Both of these are to be expected, as there were more than 100 OTUs per sample, and as some organisms have more than 31,000 reads, while others have fewer than 100. This heterogeneity is also demonstrated by the mean and standard deviation values. Since the standard deviation is more than 2.5 times the mean in either sample, it shows a great deal of variance in the data.

In sample 1, there were 12 very abundant (More than 5,000 fluorescence intensity units) OTUs identified, and together made up 68.48% of the total number of fluorescence intensity units in the sample. In sample 3, there were 15 very abundant OTUs, which together made up 71.29% of the fluorescence intensity units. Overall, sample 3 had larger peaks than sample 1, but the relative sizes and positions of the peaks indicate a great deal of similarity between the two samples. Given that they are from the same place and time, this confirms the utility of this method, as it returns the same values from the same inputs. This study also supports the trend in marine microbiology of a proportionally small number of OTUs dominating the diversity of the sample; in sample 1, ~7% of the diversity accounts for ~68% of the abundance, and in sample 3 ~10% of the diversity accounts for ~71% of the abundance.

At the phylum level, the largest difference between the 2 samples is the presence of the Chloroflexi SAR 202 in sample 3, and its absence in sample 1. Within the Proteobacteria phylum, sample 3 has a greater dominance of alpha- and beta-Proteobacteria, and sample 1 has a greater dominance of Gammaproteobacteria. This overall similarity also supports the validity of the method, because the two samples should be and are nearly identical. However, is also highlights the intense patchiness of the ocean, as this difference in diversity occurred on the order of meters. This is also why duplicates, or preferably triplicates are so crucial in oceanography, as a single sample would not have been able to pick up diversity on this spatial scale.

Comparison to the Open Ocean

Two papers were selected to compare the data in this study to; Simo et al., 2000, and Zhang et al., 2011. The former was selected specifically because it occurred during the north Atlantic phytoplankton bloom, so food and nutrients are abundant for all microbes, which should remove competitive pressure from the ecosystem, and result in maximum diversity. If the data in this study indicate greater diversity in surface coastal waters during their lowest point in diversity than the pinnacle of diversity in pelagic waters, the first hypothesis will be supported. The second study was selected because it occurred during an eddy induced period of upwelling in the South China Sea (SCS). This resulted in a smaller, and more localized phytoplankton bloom, but the presence of any bloom at all means light and nutrients are no longer limiting, so there should be maximum productivity, and therefore maximum diversity. So, if there is more diversity in the surface coastal ocean than the SCS, the first hypothesis will also be supported.

The first study found an average of ~84 OTUs in the surface ocean during the Atlantic phytoplankton bloom across their samples. As both samples in this study had more than 100 OTUs, the open ocean has less bacterial diversity than the coastal ocean. The second study found 18 – 38 OTUs in their samples of the euphotic zone in the SCS, indicating the surface coastal ocean is more diverse than the open ocean. Therefore, the first hypothesis appears to be supported.

The first study found 4.5% of their identified organisms were not members of phylum Proteobacteria. This is clear evidence for the second hypothesis, as both samples in this study had 35% or more of the sample accounted for by non-Proteobacteria. The second study found Cyanobacteria to make up nearly 70% of the diversity, followed by Alpha- and Gammaproteobacteria to make up approximately 10% of the sample each. This is more similar to the findings in this study, but there was more abundance in the non-dominant clades than found in the SCS. Therefore, the second hypothesis also appears to be supported.

Sources of Error

There are a number of potential sources of error in this study. PCR does not amplify all DNA equally, with some sequences inevitably being over or under represented in the data. Additionally, the Shannon Index requires the total number of organisms in the sample, and the sum of fluorescence units was used in this study, even though these are not the same thing. Additionally, the assumption that an ecosystem should be at its most diverse when competition is at its lowest is only one of the dominant theories on diversity, the other being that diversity is at its highest when competition is at its fiercest. However, there is not consensus on which (if either) of these theories is true, and I found no open ocean papers concerning nutrient limited, non-bloom pelagic waters. Lastly, there were only two samples in this study since one of the triplicate failed the quality control step in sequencing, indicating potential variation due to patchiness that this study did not capture.

Conclusion

This study aimed to determine the bacterial community diversity in the coastal North Pacific, and hypothesized that the diversity would be greatest in the surface coastal ocean than in the open ocean, and that the drivers of diversity would differ in the two habitats. The investigatory method was T – RFLP, paired with a literature search of other papers that had used either clone libraries or pyrosequencing. The T – RFLP analysis revealed between 145 and 175 OTUs in the two samples used in this study, and the identification method was successfully able to identify most of the most abundant organisms. The number and types of bacterial niches was larger and very different from what would have been expected in the open ocean, and was in fact quite different when compared to two papers based off open ocean data. Therefore, the hypotheses do appear to be supported.

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