Establishing a link between bacterial distribution and nutrient variation

Jeff Eaton

University of Washington Oceanography 444
Eatonj2@u.washington.edu
May 7, 2010

Non-technical summary

Bacteria are the most abundant living organisms on the planet. Ocean bacteria account for as much carbon as all of the land plants and ten times the amount of nutrients. Despit their obvious importance very little is know about them. This is due in the most part from a lack of technology and the ability to study them. With the creation of DNA fingerprinting and gene sequencing it became possible to study this once unknown group. It was discovered that bacteria are incredibly diverse and are believed to be performing critical roles in the oceans. This brings up the current question of identifying which bacteria are where and what are they doing? By studying the variation in communities along with variation in the natural environment it becomes possible to establish connections between different bacteria species and what role they perform. DNA fingerprinting methods were used to survey the variation in marine bacteria communities from varying locations within Barkley Sound. DNA fingerprinting works by observing a piece of DNA from each bacterium in the sample and cutting it at different lengths depending upon the DNA code. Two different lengths mean that the bacteria present are different species. Results showed three distinct groups independent of geographical location. There was a surface community, a deep oxygenated water community and a deep anoxic community. Environmental analysis showed that surface communities had higher levels of oxygen while communities at depth had higher levels of phosphate, silicate and ammonia. It is possible that the bacteria that dominate in each area are in part responsible for the environmental variation.

Acknowledgements

I would like to thank the people at Bamfield Marine Science Center for hosting us on such short notice and allowing us to utilize their facilities. A thank you to the UW nutrient analysis group for processing all samples on such short notice. To my class mates and professors for helping out with the field work and keeping the last couple quarters interesting and lively.

Thank you Robert Morris and Chris Frazar for all of your help and guidance throughout the T-RFLP and clone library process. A special thanks to a handful of people in CEG lab (you know who you are). To the long days in lab and all the failures… THANK YOU. If I had a chance to do it all over again I would say there is a 70% I would.

Abstract

Bacteria are the most abundant and diverse living organisms on earth. All of the carbon contained within ocean bacteria is equal to that of all the land plants and estimates place the amount of nutrients stored in ocean bacteria as ten times the amount found within land plants. With the rapid turnover rates on the order of days to weeks bacteria play a critical role in the ocean carbon and nutrient cycles. Bacteria are not only a vast nutrient pool but are also responsible for recycling nutrients. Other studies have found that bacteria are also capable of being the primary producers in environments such as anoxic communities or communities lacking in light. Despite their importance it is still unknown which specific bacteria are where and what they are doing. By studying the variation in communities along with variation in the natural environment it becomes possible to establish connections between different bacteria species and what role they perform. DNA and nutrient samples were taken throughout Barkley Sound and compared using T-RFLP. T-RFLP analysis resulted in three distinct groups focusing around the surface, oxygenated depth and anoxic communities. T-RFLP and nutrient assay results indicate that the surface dominate bacteria (481bp) may be phototrophic or coupled with surface plankton. Other T-RFLP data indicates that higher NO_3 levels in Sarita Bay may be contributed to a bacterium with a fragment length of 418 or 430bp. SAR11 subgroup Ib was discovered in anoxic waters.

Introduction

Global estimates of total prokaryotic cells are around 4-6 x 10^{30} and carbon estimates are around 350-550 Pg of carbon (1Pg = 10^{15}grams), 85-130Pg of nitrogen and 9-14 Pg of phosphorous (Whitman et al. 1998). These estimates place prokaryotic carbon loads equal to that of plants and nutrient loads 10 times higher then plants (Whitman et al. 1998). A majority of prokaryotes are found in the surface and subsurface oceans. Surface ocean prokaryotes are dominated by bacteria and have a turnover rate of 6-25 days (Whitman et al. 1998). The large estimates of carbon and nutrients bound with in bacteria along with the quick turnover rates make bacteria a key player in the carbon and nutrient cycles.

The carbon flux out of the surface ocean is a balance between carbon uptake from photosynthesis and the community respiration. Autotrophic bacteria, such as Cyanobacteria, are able to perform photosynthesis and sequester carbon (Puddu et al. 1997). Estimates for the top 200m of the ocean and coastal waters place autotrophic prokaryote counts around 2.9 x 10^{27} (Whitman et al. 1998). Not all bacteria are autotrophic. Heterotrophic marine bacteria respiration rates can account for 50 to 90% of the total community respiration (Rivkin & Legendre 2001). Heterotrophic bacteria are able to utilize both dissolved and particulate organic carbon (DOC and POC). They can aid in the breakdown of POC that would otherwise be exported into the benthos (Jahnke & Craven1995). This maintains higher levels of carbon in the upper water column.

Despite their small size prokaryotes are the world's largest nutrient pool in living organisms (Zehr and Ward 2002). Bacteria capture particulate organic matter from river inputs that contain vital nutrients that would normally sink through the water column and deposit on the ocean floor. When other organisms die or excrete waste that results in POC, bacteria are able to quickly absorb and break it down. The POC is not only carbon but also contains vital nutrients.

When the bacteria are eaten or die via viral lyses they release the stored nutrients such as phosphorus back into the water column (Zehr and Ward 2002). They act as a

In anoxic waters bacteria and archaea are the main source of primary production. By performing a process called denitrification, nitrogen is reduced from a very complex organic form such as NH_4 (ammonia) into a simpler more usable form such as N_2O (Codispoti 1985). Denitrification is a process that only occurs in the absence of oxygen creating high levels of reduced nutrients that feed the surface during mixing events. Other processes such as sulfate reduction is also only done in anoxic conditions. It results in reduced sulfates and a thriving microbial community. This is often seen in hydrothermal systems and through symbiosis allows for the very complex biomes to arise. Methanogenesis also drive the communities of anoxic zones. By utilizing high levels of CO_2 and hydrogen in the absence of oxygen microbes can gain energy by creating methane. This has been observed in anoxic zones and is an area of concern for climate change.

While bacteria are responsible for many vital roles in the ocean it is not clear which bacteria fill those roles. The bacteria community structure is known to vary depending upon salinity and temperature as well as nutrient concentrations (Puddu et al. 1997). This community variation is what determines what is being recycled or sequestered and how efficiently. Barkley Sound is an area of high variability in a small area. A few studies have looked at the bacteria in the sediments of the anoxic basins in Effingham Inlet but no known studies have been done on the bacterial communities in the water column throughout the sound.

Barkley Sound is located along the south western coast of Vancouver Island. Within Barkley Sound four distinct regions can be identified. Effingham Inlet is located in the central northern section of Barkley Sound. It has several sills and two deep basins that have been shown

to be anoxic and a location of sulfur reduction (Hurtgen 1999). Imperial Eagle Channel is connected straight with the ocean and leads up into Effingham Inlet. It is strongly influenced by the ocean and is the only outlet of Effingham Inlet. Trevor Channel is separated by a chain of islands from Imperial Eagle Channel and makes up the eastern side of Barkley Sound. It connects the Pacific Ocean and Alberni Inlet. Alberni Inlet is located in the north eastern section of the sound and has larger freshwater inputs then Effingham Inlet and no known anoxic basins.

The purpose of this study was twofold. The first goal was to address the lack of information regarding the bacterial community compositions in Barkley Sound and how they varied. This was done by using terminal restriction fragment length polymorphism (T-RFLP) to compare communities in a similar way done by Morris et al. (2005) and Moesender et al. (2001). The second purpose of this study is to tentatively link the bacteria with environmental variables such as high phosphate or oxygen levels.

Methods

1 liter water samples were collected at 5m and 5m from the bottom throughout the varying areas in Barkley Sound (Fig 1). 3 stations were set in Effingham Inlet, 2 in Imperial Eagle Channel, 2 in Trevor Channel, 1 in Sarita bay, 1 in San Mateo Bay and 1 in Alberni Inlet. Station locations where picked to search for similarities due to mixing and survey the anoxic waters of Effingham Inlet and other areas of variability. Nutrient samples were also collected at each sample location. Nutrients were pre filtered using a .45µm syringe filter.

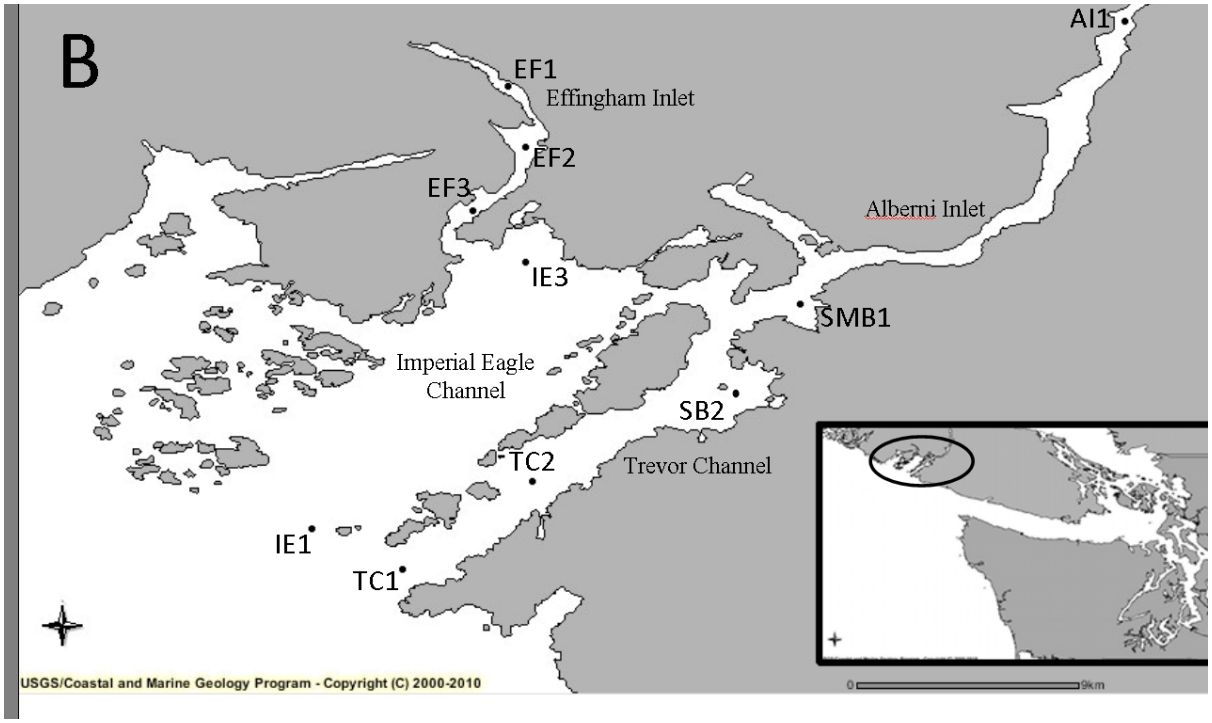

Figure 1: Map of Barkley Sound courtesy of Diana Haring. Stations in Effingham Inlet have been named EF, Imperial Eagle Channel IE, Trevor Channel TC, Sarita Bay SB, San Mateo Bay SMB and Alberni Inlet as AI. Samples taken at 5m depth have been labeled with an S at the end of the name and samples taken at 5m from the bottom have been labeled with a D.

Due to lack of ship space cells were concentrated at the Bamfield Marine Science Lab by filtering the 1 liter water samples onto 45mm 0.2µm filters, 1ml of sucrose lysis buffer was added to each sample and stored at -80ºC. Anoxic water samples were taken 1st from the niskins bottles, filled to overflowing for 5 seconds and caped with minimal air bubbles. They were also filtered 1st to minimize any oxygen contamination.

Cells were lysed by adding 200µl of 10% sodium disulfide solution and 10µl of protease K then placed in a shaking incubator at 37ºC for 30min followed by a 30min incubation at 55ºC while vortexing every 5-10minutes. DNA extractions were performed using a Qiagen DNA extraction kit (Valencia, CA). The final DNA elution was 50µl.

For T-RFLP analysis, DNA was diluted in tris to a range of 0.5-1.5 ng/µl. Polymerase chain reaction (PCR) (protocol: 94°C 30sec, 59°C 30sec, 72°C 60sec, 32cycles) was done using primers 27F-fam ([G-Fam] AGRGTTYGATYMTGGCTCAG) and 519R (GWATTACCGCGGCKGCTG). These primers only target the first ~500bp of the 16S rDNA. Enzyme cutting was performed with Hae III for a minimum of 16 hours at 37°C.

Stations EF1S (5m) and EF1D (108m) where selected for clone libraries due to their high levels of variation but similar geographical location. DNA extraction samples from EF1S and EF1D and the first ~500 base pairs of the 16s amplicon were cloned using PCR (protocol: 94°C 30sec, 59°C 30sec, 72°C 60sec, 35cycles) primers 27FB (AGRGTTYGATYMTGGCTCAG) and 519R (GWATTACCGCGGCKGCTG). DNA was purified via standard Quiagen gel extraction and gel purification(Valencia, CA). Ligation was performed using pGem vectors (Wisconsin). Vector incorporation was done using undiluted ligation and 100µl of one shot Topo one cells. They were incubated on ice for 30 min, heat shocked at 42°C for 45sec, 250µl of SoC medium was added then put in a 37°C shaker for 1hour. 1/10 and 1/100 dilutions where plated on ampicillin plates to check vector incorporation. Upon confirmation DNA was sent to a genome lab to be sequenced. Unfortunately attempts to clone failed and clone libraries will not be addressed.

Results

Oxygen and nutrient measurements showed general trends in decreasing oxygen with depth while phosphate, silicate and salinity increased with depth. Phosphate levels varied between 7.86µM measured at EF1D to 0.63µM at station IE3S (Fig. 2). Silicate varied from 112.56µM at station EF1D to 11.15µM at station IE3S (Fig. 2). Nitrate levels varied from 19.45µM at station EF3D to 0.03µM at station EF1D (Fig. 2). Nitrite was highest at station

EF2D at 3.55µM while all other stations were below 0.4 µM (Fig. 3). Ammonium was highest at station EF1D (36.87 µM) and was the lowest at SB2D (0.11 µM) (Fig. 3).

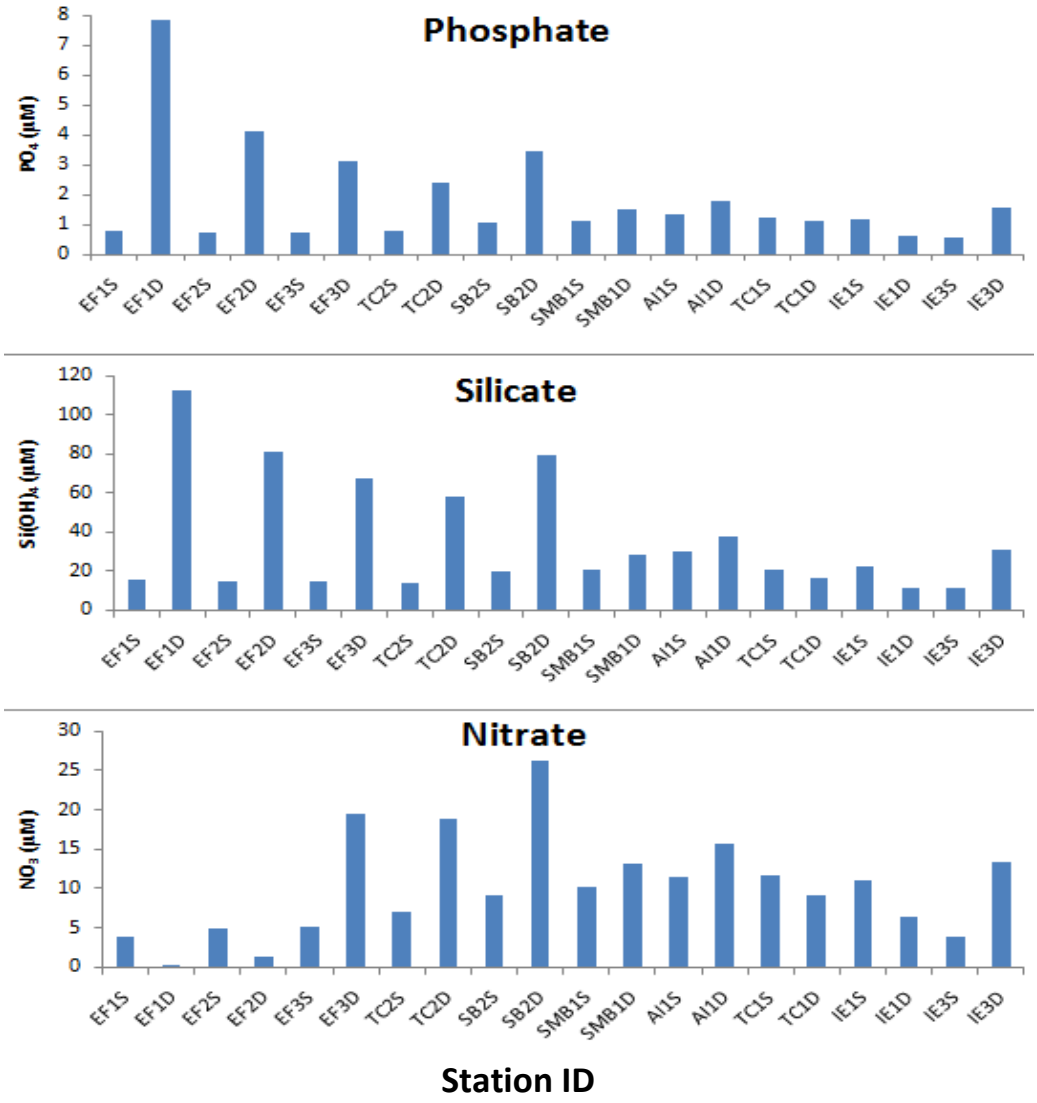

Figure 2: Nutrient measurements taken throughout Barkley Sound.

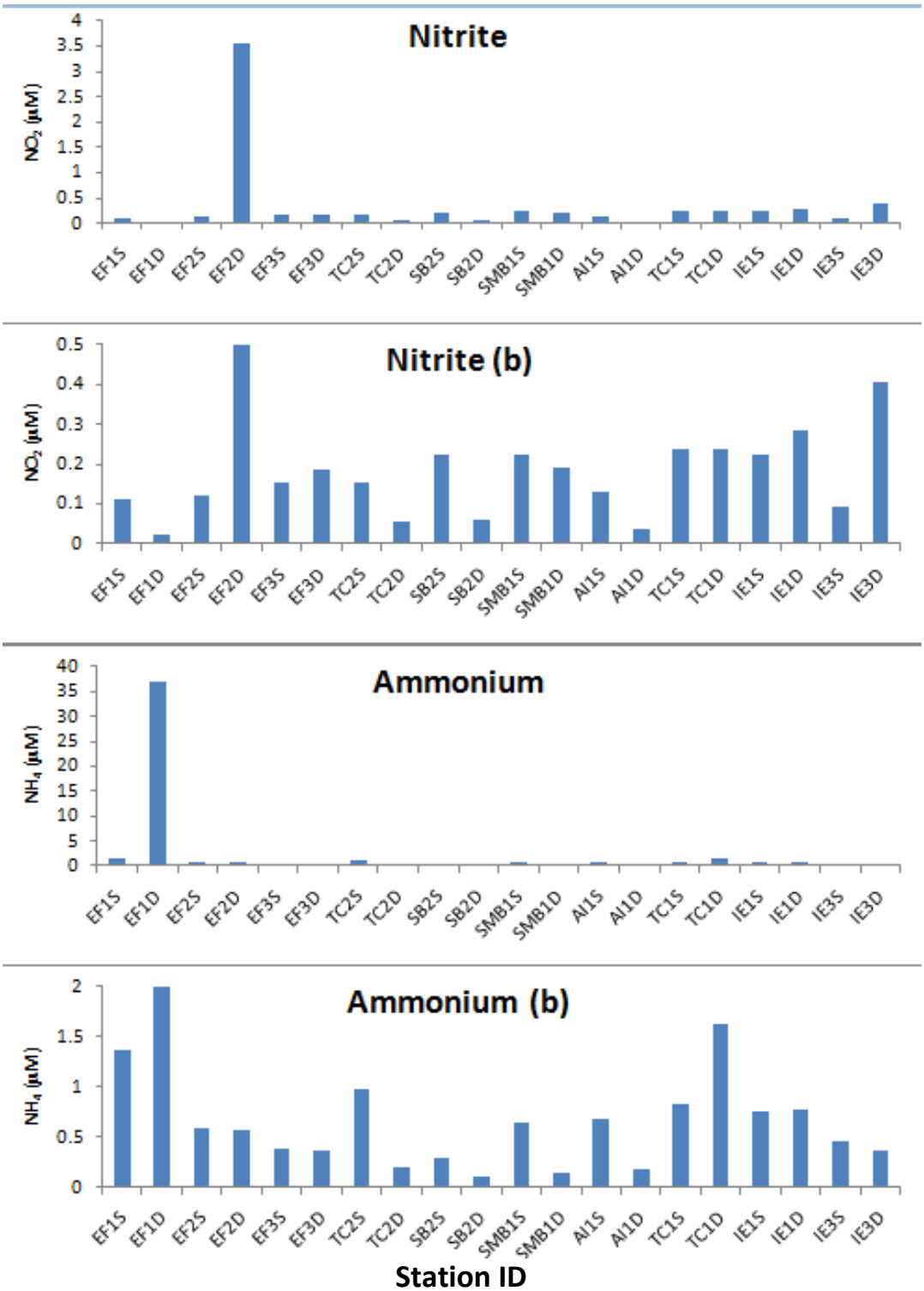

Figure 3: Nitrite and ammonium measurements taken at Barkley Sound. Nitrate (b) and ammonium (b) have been rescaled to observe small scale variation between the samples.

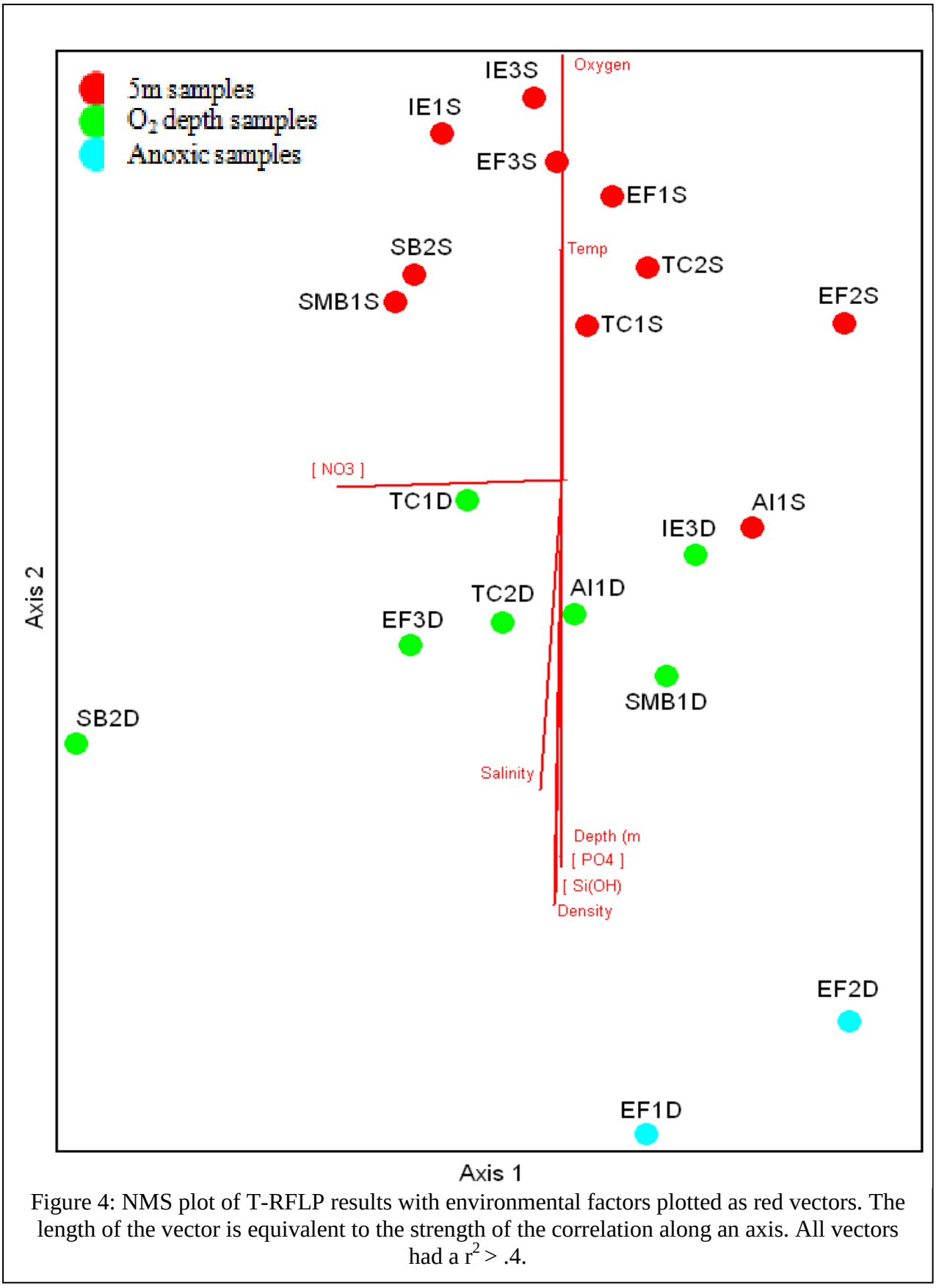

Figure 4: NMS plot of T-RFLP results with environmental factors plotted as red vectors. The length of the vector is equivalent to the strength of the correlation along an axis. All vectors had a $r^2 > .4$.

T-RFLP analysis resulted in three distinct groupings of bacteria (Fig. 4). All 5m station samples with the exception of AI1S grouped together. All deep oxygenated samples grouped together with station SB2D as a slight outlier. The two anoxic stations EF1D and EF2D form the last group. The surface group showed strong peaks at 481base pairs (bp) and 283bp (Fig. 5). Surface samples also showed the highest concentrations of several low abundance fragments such as fragment 520 (Fig. 6).

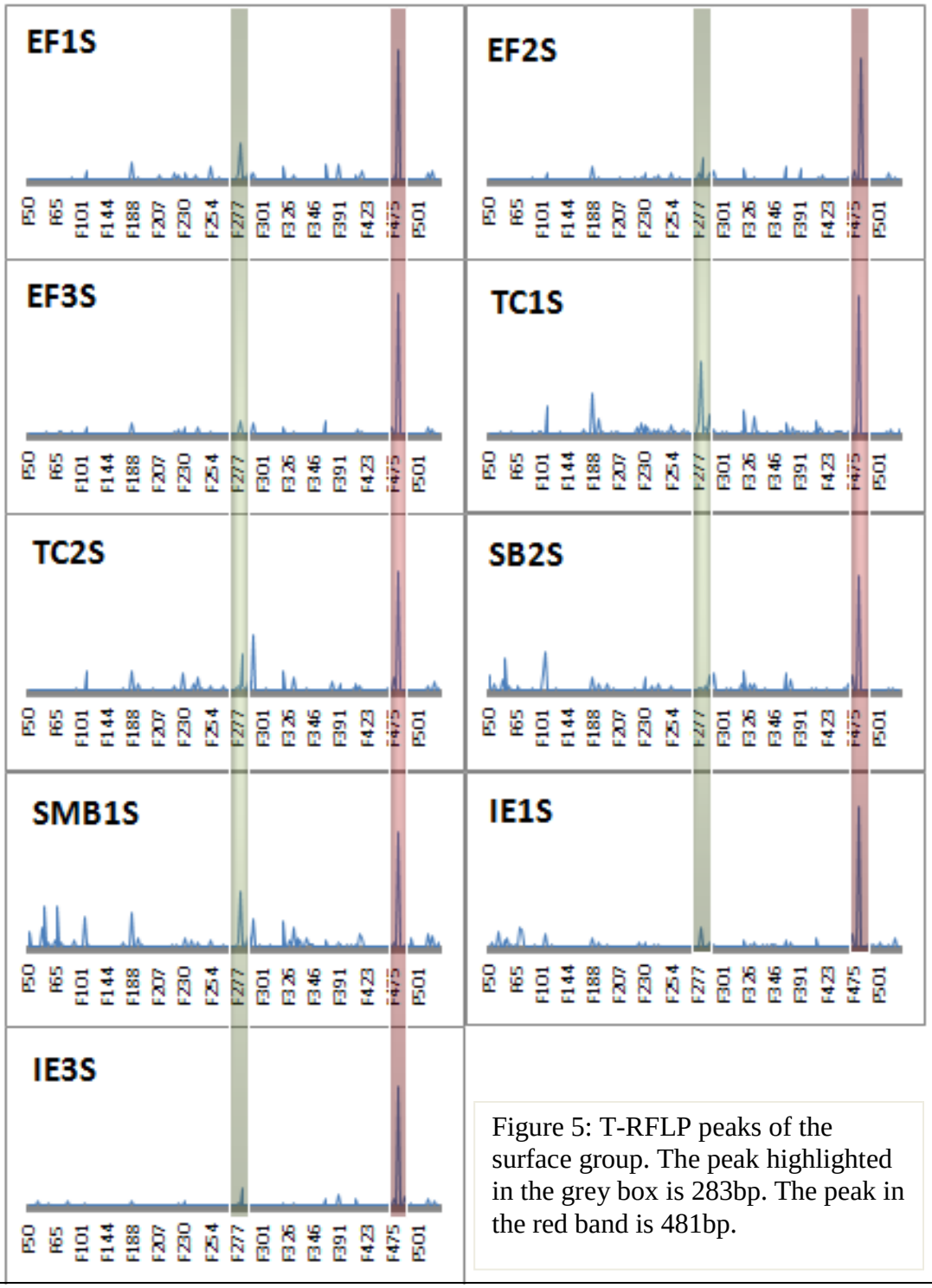

Figure 5: T-RFLP peaks of the surface group. The peak highlighted in the grey box is 283bp. The peak in the red band is 481bp.

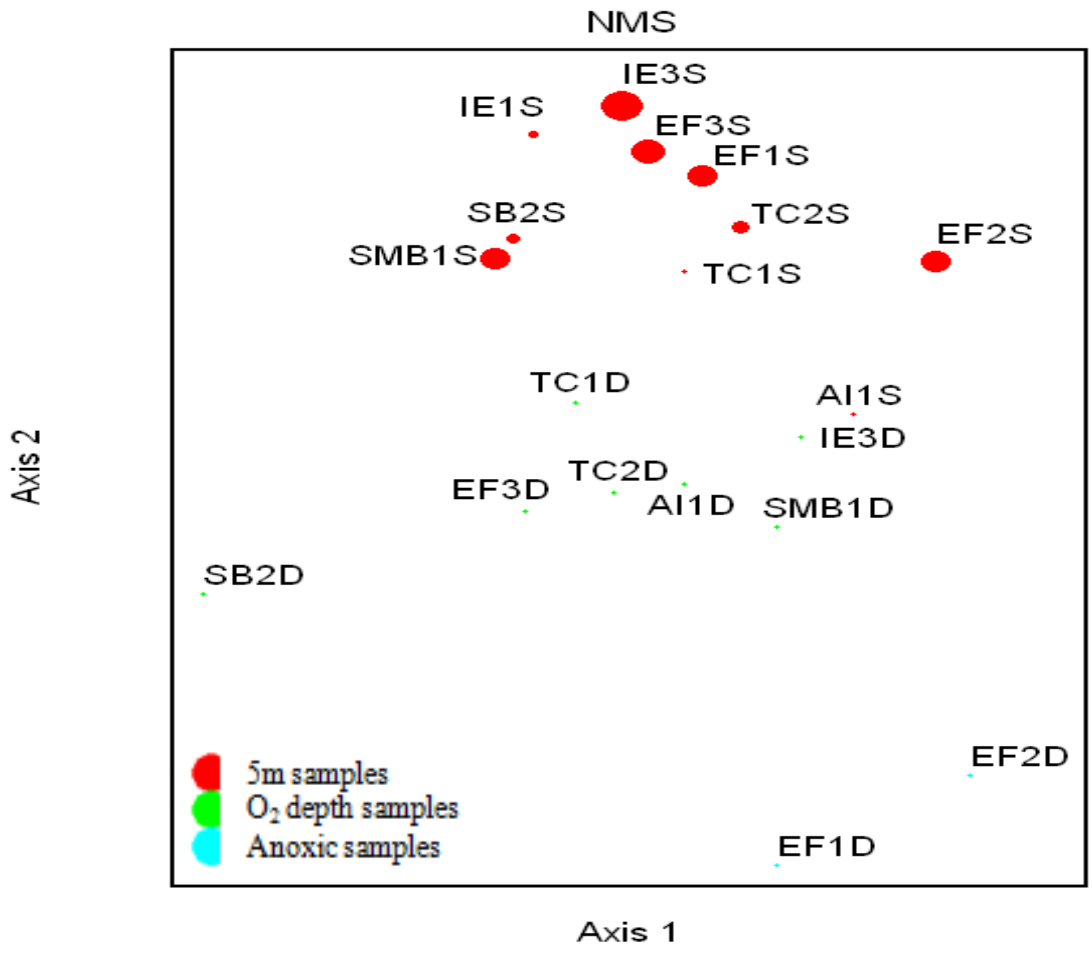

Figure 6: Concentration of fragment 520. Symbols size represents relative abundance of fragment 520. The larger the symbol the greater the concentration found.

Oxygenated depth samples were not dominated by any one fragment length like the surface group. The peak at 114bp was fairly prevalent in all samples (Fig. 7). The anoxic zones showed two matching peaks at 193bp and 228bp (Fig. 8). The inner basin EF1D showed 16 fragment lengths found only in it while EF1D had 2 site specific fragments (not shown). There were no fragments found in both of the anoxic zones that were not also found in oxygenated water.

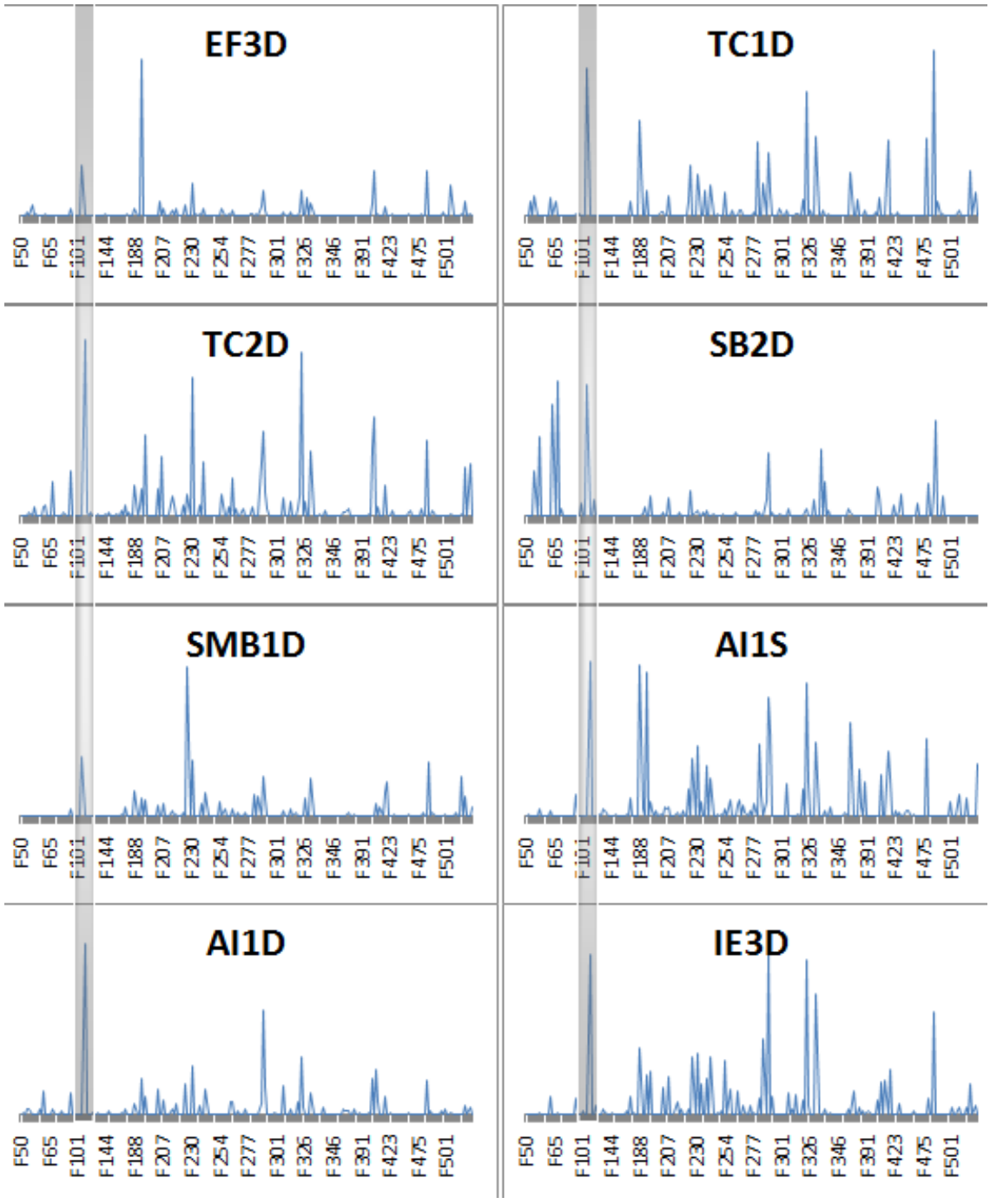

Figure 7: T-RFLP peaks of the surface group. The peak highlighted in the grey box is 113bp.

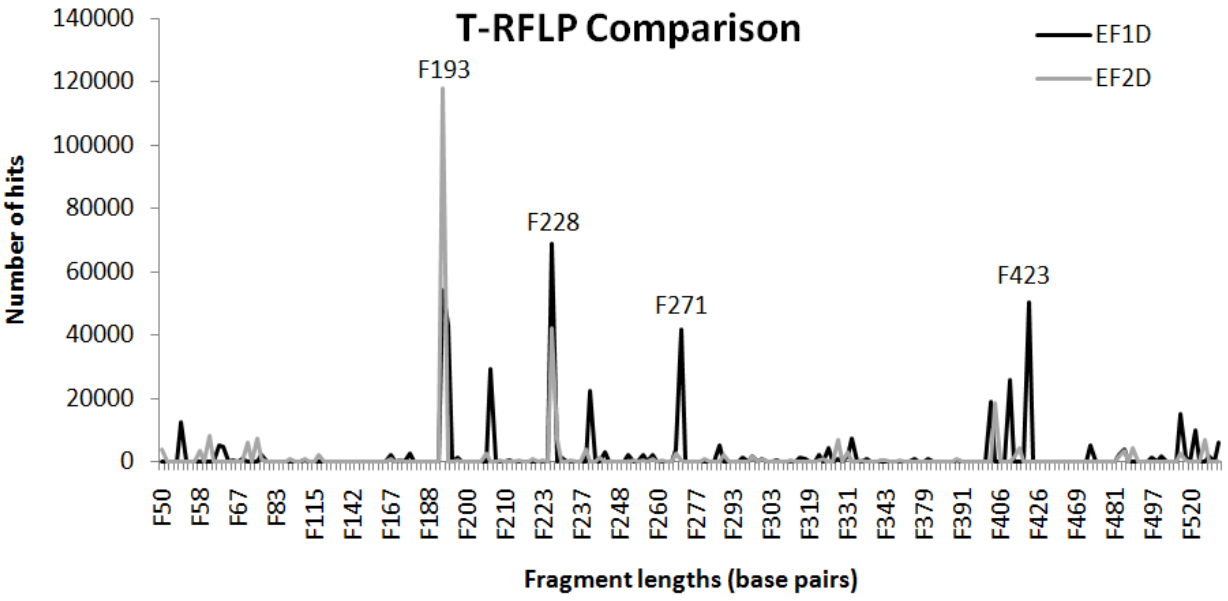

Figure 8: T-RFLP fragment length comparison of the anoxic bottom waters. Station EF1D in black and station EF2D in grey. The most dominate peaks are labeled with fragment lengths.

Discussion

T-RFLP analysis revealed very different community structures. The surface group was dominated by peak 481 but at depth samples showed very few traces of fragment 481. Instead depth samples varied on which peak dominated. This is likely due to the variety of nutrients and energy sources available to the bacteria. The surface waters have access to sunlight and the system is fed mainly via photosynthesis. The depth samples that have oxygen are dominated mainly by heterotrophic bacteria that are breaking down organics. Which bacterium is able to dominate in these zones is based upon a large variety of factors; what carbon source is available, which bacteria are transported into the area, what temperature and which nutrients are available. This makes the large diversity unsurprising. The anoxic zones showed a variety of unique peaks as well as very different dominating ones. The anoxic zone was dominated by fragment 193 which was found in almost every location but in orders of magnitude more as station EF2D. The

T-RFLP presented three very distinct groups based upon which groups dominate but several of the same fragments were found in a large portion of the samples. The variation in peaks and the overall groupings correspond with the belief that a majority of bacteria are everywhere but the environment determines which ones dominate.

The surface bacteria representing fragment 481 and other surface dominated peaks may be photosynthetic due to their high abundance in the surface and very low abundance at depth (Fig. 7&8). All surface dominated fragments could represent photoautotrophs or heterotrophic bacteria that are coupled with surface plankton. The surface waters throughout Barkley Sound at the time were heavily dominated by Skeletonema (Moreno C. unpubl.). Fragment 481 is not believed to be linked with the abundant diatom because the presence of Skeletonema in every surface site but the lack of peak 481 at every site. Fragment 283 and 520 appear to also be the most productive in the surface. Their numbers are highest in the surface waters and found in very low numbers at depth if at all. They may also represent photosynthetic bacteria or other surface species.

Fragments found mainly in deep water oxygenated samples are most likely heterotrophic bacteria that are specialized for deep living or are out competed at the surface while fragments found in both locations equally such as fragment 324 most likely represent heterotrophic bacteria that are more generalized. Some fragments dominating at SB2D which showed high levels of NO_3 may be correlated with the high levels and possibly be denitrifies or converting NO_2 into NO_3. Fragments 418 and 430 were both found in higher levels at station SB2D then in any other locations indicating they may be responsible for the anomaly. Sample AI1S was grouped with the deep water oxygenated group because of the similarity of the bacteria found there (Fig. 7). The nutrients found at AI1S are more similar to those found at depth than those found in the

surface (Fig. 2). This may be due to mixing of the bottom and surface waters evenly distributing the nutrients and bacteria but further study regarding sills and wind pattern analysis is required.

By comparing with the fragment length identifications of past studies some fragments have been identified. Using the Morris et al. (2005) peak 228 has been identified as SAR11 subgroup b. Fragment 228 is one of the dominate bacterium found in the anoxic zones (Fig. 8). This is the first mention of subgroup Ib in anoxic waters. Other fragments have been identified using the Morris et al. (2005) fragment lengths. SAR11 subgroup a 113-117bp which is found in all samples had relatively low levels at the surface with high abundance at depth and insignificant values in the anoxic waters. This corresponds with a study done on the distribution of SAR11 conducted by Field et al (1997) which supports the ubiquity of SAR11 except it appears that in the surface waters SAR11 is being out competed by fragment 481. A study conducted in Saanich Inlet by Zaikova et al. (2009) identified SUP05 as the dominate bacteria in the anoxic bottom waters. Their sequence of SUP05 has a predicted Hae III fragment length 193bp. This corresponds with the peak fragment in EF2D and one of the dominate peaks in EF1D (Fig. 8). SUP05 is closely related to sulfur reducers found at hydrothermal vents and may also be responsible for sulfur reduction (Zaikova 2009).

Several peaks were anoxic specific and are not shown in figure 8. Since no one anoxic specific bacterium was found to be in both basins I believe that the interaction between the two basins is minimal or EF2 has undergone some disturbance that has killed its diversity. Anoxic fragments found in EF1D numbered 16 compared to only 2 found in EF2D. It is also possible that the diversity difference is not a lacking in EF2D but an excess in EF1D. EF1D was incredibly high in phosphate and ammonium which may be able to support a higher diversity of anoxic specific bacteria.

Clone lengths have shown to have varying fragment lengths. So even if the predicted length is 117bp long it is possible for the clone to only be 113bp but this can be corrected for by doing a clone library on the T-RFLP samples. Future studies would include this step to properly identify the T-RFLP fragments. Archaea diversity would also be a point of interest, especially in anoxic conditions. Further environmental measurements such as sulfur would also help in determining what the local bacteria are doing. Additional sampling to understand time variation would also help narrow down the possible roles of bacteria. Any further studies beyond these would be based upon isolating and culturing the bacteria or archaea and proving their speculative roles.

Conclusions

Bacteria are the most abundant and diverse living organisms on the planet but so little is known about them. By using DNA fingerprinting and measuring environmental variable such as nutrients it is possible to generate an idea on what each bacterium is doing. This was done in Barkley sound and resulted in the discovery of SAR11 subgroup b being found in anoxic waters for the first time. Fragments 481, 283, and 520 may possibly be photosynthetic or surface bound bacteria. Further support was founded for past research in SAR11 and its ubiquity. The dominate bacteria in the upper Effingham basin anoxic zone was concluded to be SUP05. Future study in this field is crucial in understanding what bacteria are doing and will help in defining future work.

Reference:

Codispoti, L.A. and J.P. Christensen. 1985. Nitrification, Denitrification and nitrous oxide cycling in the eastern tropical south Pacific Ocean. Marine chem. 16: 277-300.

Field, K.G., D. Gordon, T. Wright, M. Rappe, E. Urback, K. Vergin, and S.J. Giovannoni. 1997. Diversity and depth-specific distribution of SAR 11 cluster rRNA genes from marine planktonic bacteria. Appl. Environ. Microbiol. 63: 63-70.

Hurtgen, M.T., T.W. Lyons, E.D. Ingall and A.M. Cruise. 1999. Anomalous enrichments of iron monosulfide in euxinic marine sediments and the role of H_2S in iron sulfide transformation: examples from Effingham Inlet, Orca Basin, and the Black Sea. Amer. Journal of Sci. 299: 556-588.

Jahnke, R., B. B. Craven. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: a need for respiration rate measurements. Limnol. Oceanogr. 40. 2: 436-441.

Moesender, M.M, C. Winter and G.J. Herndl. 2001. Horizontal and vertical complexity of attached and free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints. Limnol. Oceanogr. 46: 95-107.

Morris, R.M, K.L. Vergin, J.C. Cho, M.S. Rappe, C.A. Carlson and S.J. Giovannoni. 2005. Temporal and spatial response of bacterioplankton lineages to annual convective overturn at the Bermuda Atlantic Time-series study sited. Limnol. Oceanogr. 50(5): 1687-1696.

Puddu, A., R. La Ferla, A. Allegra, C. Bacci, M. Lopez, F. Oliva, and C. Pierotti. 1997. Seasonal and spatial distribution of bacterial production and biomass along a salinity gradient (North Adriatic Sea). Hydrobiologia. 363.1-3: 271-282

Rivkin, R., L. Legendre, 2001. Biogenic carbon cycling in the upper ocean: effects of microbial respiration. Science. 291.5512: 2398-2400. http://www.jstor.org/stable/3082679

Whitman, W.B., D.C. Coleman, and W.J. Wiebe. 1998. Prokaryotes: The unseen majority. Proc. Natl. Acad. Sci. USA. 95: 6578-6583.

Zaikova, E., D.A. Walsh, C.P. Stilwell, W.W. Mohn, P.D. Tortell, and S.J. Hallam. 2009. Microbial community dynamics in a seasonally anoxic fjord: Saanich Inlet, British Columbia. Environmental Microbiology. 12(1): 172-191.

Zehr, J. P. and B. B. Ward. 2002. Nitrogen Cycling in the Ocean: New Perspectives on Processes and Paradigms. Applied and Environmental Microbiology. 68. 3: 1015-1024, DOI:10.1128/AEM.68.3.1015-1024.2002