Bacterioplankton community structure and enzymatic activity in Barkley Sound, Canada, and its response to a simulated diatom bloom

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Non-Technical Summary

Bacteria serve as the nutrient recyclers of the ocean. They are responsible for recovering important nutrients back into the water column by breaking down organic matter in the oceans that would otherwise sink and be lost in the surface waters. These recycled nutrients may then be used again by the marine primary producers, phytoplankton. Diatoms, a marine phytoplankton, and bacteria have been found to be directly linked in the world's oceans and bacterioplankton community structure can change in response to large diatom blooms. This project examines the bacterioplankton community in Barkley Sound, located on Vancouver Island Canada, and the extent to which the community structure would change with a simulated diatom bloom. Bottle incubations were conducted from two locations inside of Barkley Sound with two experimental treatments, the first consisted of an added food source called a diatom lysate. The second was a control with no added food source. The relative bacterial abundance was monitored with relative cell counts obtained using a DAPI stain that binds to DNA. The bacterial growth and activity was also monitored by examining their enzymatic activity. The bacterial abundance increased in both the experimental treatments but more so in the lysate bottle incubations. The enzymatic activity was also elevated in the lysate bottles versus the controls, with lipase as the preferred substrate showing the highest rate of decomposition. It was found that bacterioplankton abundance was on the same order of magnitude as similar estuarine systems in the Pacific North West, and that like in the bottle incubations lipase had the highest enzymatic activity overall. Cellulase activity was also found to be confined to the surface samples except in stations sampled in Effingham Inlet.

Abstract

This study examines the bacterioplankton community dynamics in Barkley Sound, located on Vancouver Island Canada, and the extent to which the community structure would change with the occurrence of a simulated diatom bloom. Bottle incubations were conducted using a diatom lysate to replicate bloom conditions and bacterial relative abundance was monitored with DAPI cell counts along with enzymatic activity using fluorogenic methylumbellifery (MUF)-compounds. The community structure was also examined using T-RFLP techniques to show the community dynamics. Within Barkley Sound, the bacterioplankton relative cell abundance was an average of $1.1*10^6$ cells/ml at 5m, and $1.2*10^6$ cells/ml at 10m from the bottom. Bacterioplankton enzymatic activity was the greatest for lipase at all stations sampled, along with in the bottle incubations. The lysate bottles after the incubation displayed the highest rates of hydrolysis, along with the greatest increase in bacterial relative abundance. When the enzymatic activity was normalized by the abundances however, the lipase use per cell was less in the lysate then in the control bottles. The bacterioplankton community at the stations chosen for the bottle inoculations was dominated by two atypical fragment lengths 481 and 283 and clades typical of surface water samples were absent from the data including SAR11 and SAR 202.After the incubations, the same two fragments dominated the community, but their relative percent compositions changed. The lysate bottle community was much more diverse than the inoculate station sample, with 5% of the community represented by five distinct fragment lengths in the Effingham lysate and four distinct fragment lengths in the Imperial Eagle lysate bottles.

Acknowledgments

I would like to start off by thanking the University of Washington Oceanography program for giving me an opportunity such as this. To be able to conduct research like this is an extraordinary experience. I would also like to thank both of my advisors, Robert Morris and Rick Keil. Whether it was late night hours in the Bamfield Marine Science lab working on Enzyme incubations, or my many trips to their respective offices, they stuck by me and made this project manageable. I would also like to thank my friends who put up with my constant stressed banter over the past few months. I'm sure they have all heard enough about bacterioplankton, enzymatic activity, and cell counting.

Introduction

Bacteria and phytoplankton have been understood to be directly linked in the world's oceans (Bird et al. 1984), and it has been shown that bacterioplankton community structure can change in response to diatom blooms in marine settings (Morris et al. 2006). It has also been shown in lab that marine bacteria display elevated DNA synthesis, a proxy for growth rate, when grown in culture with marine diatoms who's exudates serve as the bacteria's source of carbon (Murray et al. 1986). When bacteria break down the post bloom diatoms and other phytoplankton in the water column they reintroduce nutrients back into the system to be used again. Much of this organic material originates from the phytoplankton primary production and the zooplankton communities sloppy feeding (Baltar et al 2009). This microbial loop is particularly important in recycling nutrients in the surface ocean where bacteria break down large particles and phytoplankton cells that would otherwise sink out of the mixed layer (Azam et al. 1983, Pomeroy et al. 2007). When bacteria break down these larger particles their autolytic enzymes play a role, and this activity can be monitored by the use of fluorogenic methylumbellifery (MUF)-compounds (Hoppe 1983). Although (MUF)-compounds are not directly the same as those in the environment, they have been shown to be analogous in marine settings, and a good indicator of the enzymatic activity and substrate turnover in nature (Hoppe 1983, Kim et al. 1986). Pronounced changes in bacterioplankton abundance, productivity and enzymatic activity have been shown to occur in seawater mesocosms used to simulate naturally occurring diatom blooms (Riemann et al, 1999).

Barkley Sound, a temperate estuary consisting of a glacially carved fjord system, lies on the southwest side of Vancouver Island, Canada. Three main channels make up the outer part

of the embayment, Loudon to the north, Imperial Eagle in the center, and Trevor Channel to the south. Each channel presents a slightly different environment. Imperial Eagle Channel is approximately 100m deep and is exposed to the open ocean so it often has characteristics similar to water offshore (Taylor 1996). Effingham Inlet, a steep walled glacially carved fjord lies at its head. It is divided up into three distinct basins separated by 40m and 60m sills, and has been known to become anoxic in the to most northern for much of the year (Nuwer 2005). Trevor Channel to the south is narrower and is influenced by the extensive Alberni Inlet at its head, and can receive a large amount of freshwater input. This estuarine system acts as a good representative study location of costal estuaries typical of North America costal systems because of the large diversity of costal estuarine system landscapes and fjords it encompasses. This project examines the bacterioplankton community in Barkley Sound, Canada, and the extent to which the community structure, enzymatic activity, and bacterial abundance would change within a simulated diatom bloom. An understanding of the bacterioplankton community and how a diatom bloom affects its community structure would enhance our understanding of bloom dynamics and the recycling of post bloom nutrients.

Methods

The cruise began in Bamfield Canada on March 25th, 2010 aboard the Bamfield Marine Science Center vessel the Barkley Star. One liter bottles were filled from stations throughout the Barkley Sound, particularly Effingham (Ef), Imperial Eagle (IE) and Admiralty Inlets (AI), Trevor Channel (TC) Sarita Bay (SB), and Sanmateo Bay (SMB) (Fig 1).

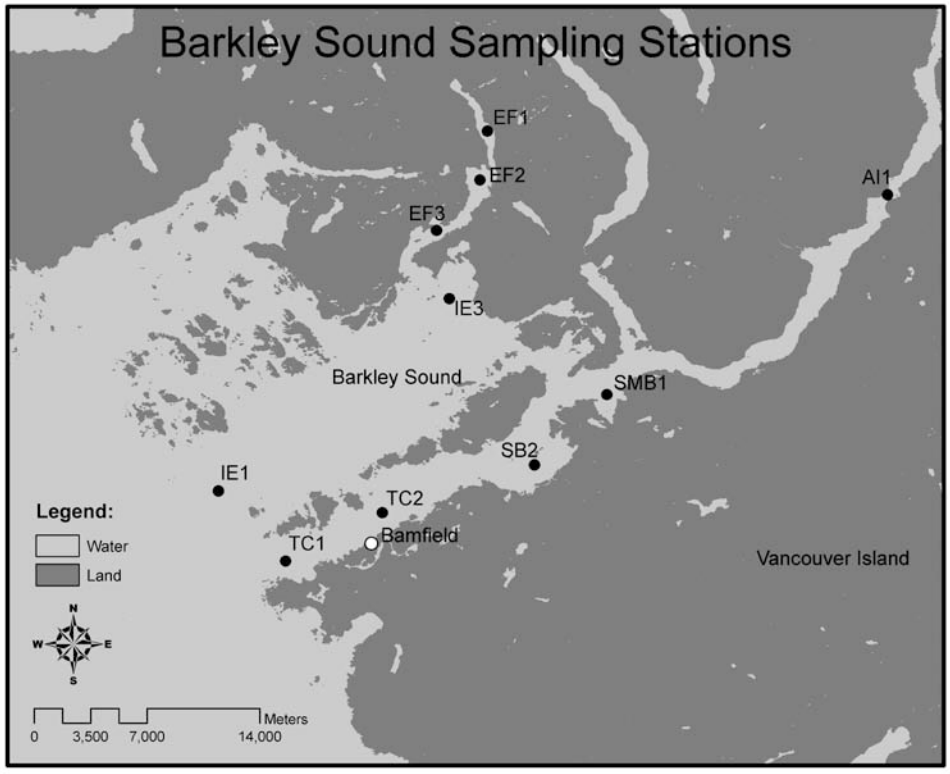

Figure 1: Displays the sampling stations inside Barkley Sound, Vancouver Island Canada.

Water samples were used for both the enzymatic activity analysis and to determine the relative bacterial abundances.

Methods: Incubation Experiment

To set up the incubations, a ten-liter carboy was filled from a hand Niskin deployed at a depth of 5m at stations Effingham Central (EF2) and Imperial Eagle South (IE1) (Fig 1). The incubations were carried out inside two-liter polycarbonate bottles, and prepared similarly to a previous described method (Carlson et al.1996). To set up the dilution, 1.5L of water was added each two liter bottle from the ten liter carboy through a 0.2-micron filter to sterilize the water sample. The remaining 0.5L from each bottle was filled from the carboy with unfiltered whole

water. This whole water contained the bacterioplankton, and was diluted with the filtered seawater to remove grazing pressure from the incubations. This study focused on two experimental treatments each done with a replicate. A resuspended pellet of diatom exudates was added to the first duplicate bottles to replicate conditions of a post diatom bloom. The steps taken to culture and make the lysate are described in the next section. The second set of bottles was a control treatment, to which no diatom exudates were added. The bottles were placed inside an unlit refrigerator maintained at a constant 10°C for the entire 70hr incubation. In order to get a baseline bacterioplankton population cell concentration, 25ml of unfiltered media was taken from each 10L carboy and 5ml of 5% formalin solution was added to fix the samples. They were then stored at 4°C until analyzed.

Methods: Diatom Lysate

To obtain the diatom lysate for use in the incubation, a 5L axenic culture of *Thalassiosira pseudonana* was grown in the Morris Lab in Ben Hall at the University of Washington in a 10L carboy. A growth medium containing a trace metal and vitamin solution was used to culture the diatoms. The culture was grown in lab for a week in a walk in 16-20 degree Celsius refrigerator under fluorescent lights. The culture was continuously aerated to keep the diatoms suspended. After a week, the sample was split into equal one-liter volumes and centrifuged at 1000rpm for twenty minutes. The supernatant was then decanted and the pellet resuspended and centrifuged again for another twenty minutes. The pellet was rinsed, and frozen to freeze fracture the cells at -20 degrees Celsius. This technique was adapted from a similar study done by Carlson et al. (1996).

Methods: Cell Counts

The following method for filtering and preparation of samples is adapted from that previously described in Morris et al. (2004). From each station's one liter bottle, a 25ml sample was collected and 5ml of 5% formalin solution was added to fix the samples. A 25ml sample was also taken initially, and after the 70hr incubation for initial and final relative bacterioplankton abundances for each of the incubation bottles. The fixed samples were then stored at 4°C until analyzed. The fixed samples were filtered on a 0.2 μM polycarbonate filter backed by a 0.8 μM support filter on a filter tower. 5ml of sample was added to the tower, and filtered using an electric vacuum. The top 0.2 μM filter was removed using adhesive tape and attached to the corresponding slide. The slides were placed in a slide box and refrigerated until they were transported back to the Morris lab in Ben Hall, at the University of Washington. Each filter was quartered using a razor blade and attached to another labeled slide. To prepare the slides, a drop of Citifluor mounting oil containing 5 mg mL^{-1} DAPI (4', 6-diamidino-2-phenylindole) solution was placed on each slide before the filter was attached to cause the bacterial cells DNA to fluoresce. The finished slides were stored at 4°C until analyzed. Each slide was imaged and bacterioplankton cells counted using a Nikon 80i microscope equipped with a CoolSNAP HQ2 Camera (Photometrics, Tucson, AZ) at a magnification of 1000X. Fifteen fields were imaged from each slide and counted using the NIS Elements Basic Research software (Nikon Instruments, Melville, NY).

Methods: Enzymatic Activity

Along with the DAPI counts, samples were taken at each station and used with florogenic model substrates for extracellular activity (EEA) measurements of bacteria as

described in Hoppe (1993). Three fluorogenic methylumbellifery (MUF)-compounds are used for this method: lipase, chitinase, and cellulase. In order to obtain an idea of the enzymatic activity during the lysate incubation, three samples were taken o0ver the course of the experiment. One sample initially, before the addition of the lysate to get the baseline activity, one halfway through the incubation, and one at its completion. At each sampling time, four 3ml samples were taken from each bottle and a pre-determined concentration of one of the different florogenic model substrates will be added to each. The concentration of each substrate to be added was determined using the substrate saturation technique also outlined in Hoppe (1993). The initial fluorescence was measured using a TD-700 laboratory fluorometer by Turner Designs at 455nm under 365nm excitation, and then at one hour intervals for three hours. The samples were stored in a dark fridge, at 10 degrees Celsius between sampling times. To obtain the rate of hydrolyzation, the change in florescence was divided by the time the sample incubated in hours, and then by a conversion factor determined using the concentration technique outlined in Hoppe (1993). This conversion calculates the rate of the hydrolyzation of the respective substrate in units of uM substrate/L/hr. Along with the samples taken from the bottle incubations; this procedure was preformed with water samples collected at each station in Barkley Sound to resolve the spatial variation of the bacterial use of these compounds (Fig 1).

Methods: T-RFLP

To understand how the bacterioplankton community responds to the addition of diatom exudates it is important to also understand what makes up the bacterioplankton population at the sampling locations, and how this community changes over the course of the incubations. In order to understand this, terminal restriction fragment length polymorphism (T-RFLP) was used

as outlined in Marsh (1999). Samples were collected from the 10L carboys for the initial community composition, along with when the incubations were completed for comparison using this technique. All T-RFLP work was completed in the Morris Lab at the University of Washington's Ben Hall, following the methods described in Morris et al. 2005. A chromatogram of the resulting data for each station incubation was then compared to determine the community structure, and if the addition of a diatom lysate altered it in any way.

Results: Station Data

Bacterioplankton cell counts were done for each sampling station to get relative abundances throughout Barkley Sound. Abundances were within normal estuarine levels and there was little variation between locations sampled with an average of $1.1*10^6$ cells/ml in the surface samples, and $1.2*10^6$ cells/ml in the bottom samples. The Effingham Inlet Central (EF2) station deep sample displayed elevated bacterioplankton abundance with $3.6*10^6$ cells/ml. This area is anoxic during much of the year, and was at the time of sample collection.

The enzymatic hydrolyzation rate for model substrates was determined at each sampling station to determine the spatial variability of the bacterial use of these compounds (Fig 2). The rate of hydrolysis for the lipase substrate was the highest at all stations sampled, with a station wide average of 3.06 uM released/L/hr, maximum of 7.2 uM released/L/hr at Imperial Eagle North (IE3) surface and a minimum of 0.61 uM released/L/hr at station Effingham South (EF3) deep. Chitinase and cellulase both displayed much lower activities with averages of 0.049 and 0.040 uM released/L/hr respectively. Chitinase enzymatic activity was highest at station Imperial Eagle North (IE3) in both the surface and deep locations, at 0.329 uM and 0.176 uM released/L/hr respectively. The next highest station activity was at Effingham

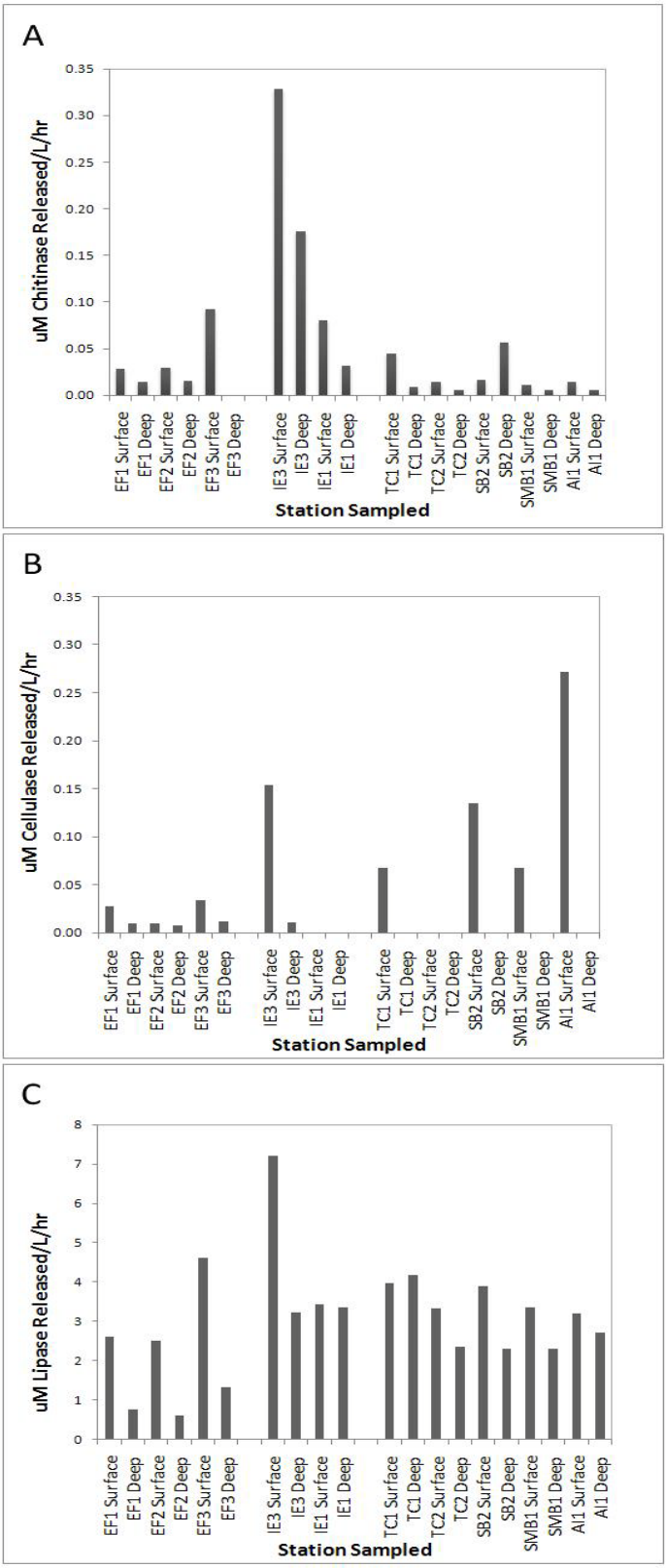

Figure 2: Bacterial enzymatic activity as shown by uM substrate released/L/hr. A) Chitinase activity, B) Cellulase activity, and C) Lipase activity by station.

South (EF3) surface at 0.092 uM released/L/hr. Cellulase activity was highest at stations Alberny Inlet (AI1) surface at 0.271 uM released/L/hr and Imperial Eagle North (IE3) surface at 0.153 uM released/L/hr. Cellulase was the least active substrate, and displayed the greatest number of stations without any detectable activity. Of the stations that displayed activity, only those in Effingham Inlet and station Imperial Eagle North (IE3) did so in both the surface and at deep samples. All other stations only displayed cellulase activity in the surface water sample.

Results: Bottle Incubations

Effingham Central (EF2) and Imperial Eagle South (IE1) were chosen as the locations to conduct the bottle incubations (Fig 1). Both locations had similar initial

bacterioplankton abundances of$1.0*10^6$ and $9.8*10^5$ cells/ml respectively. The relative abundances for the duplicate bottles were averaged for each sample location by treatment, and increased in all incubation bottles over the course of the experiment. When sampled at the end of the incubation, the Imperial Eagle control bottles had an average of $1.8*10^6$ cells/ml while the lysate bottles had an average of $1.1*10^7$ cells/ml (Fig 3).

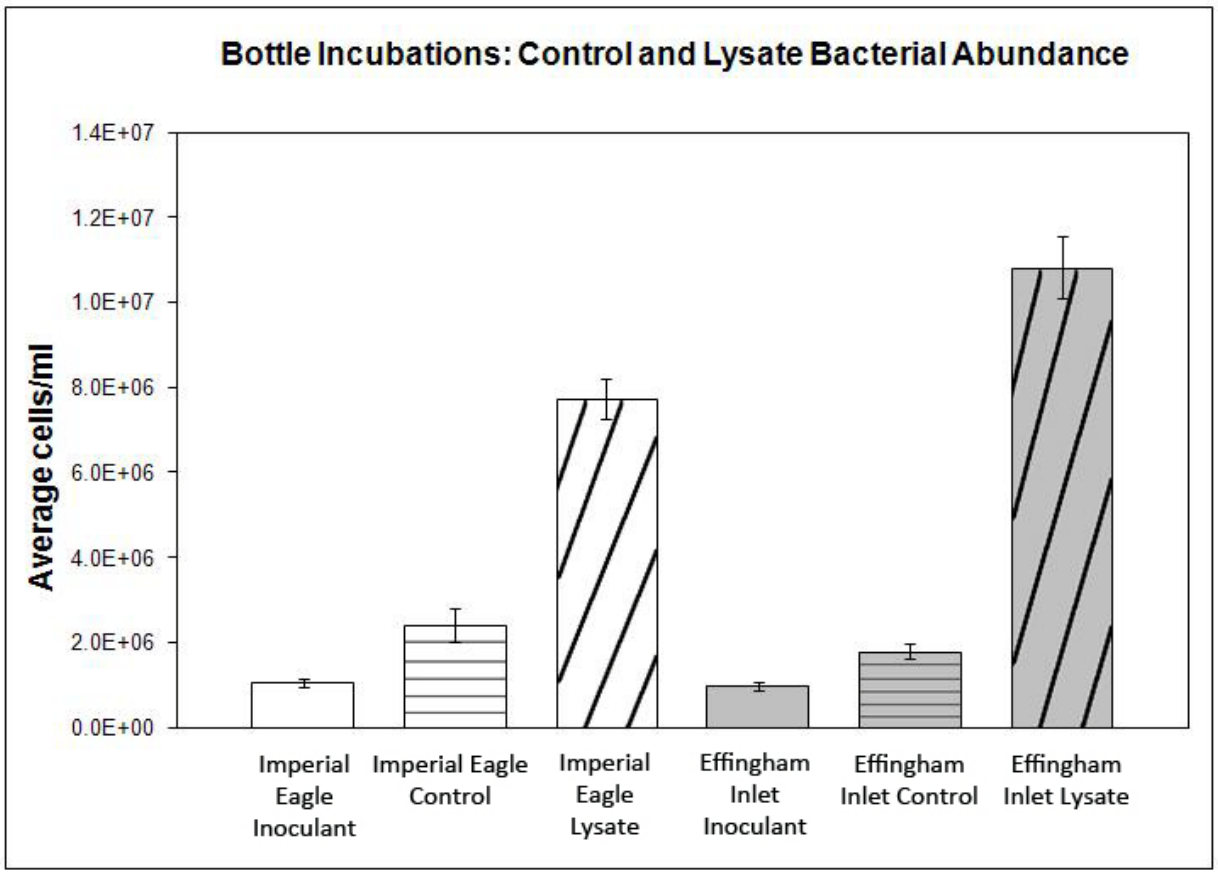

Figure 3: The relative bacterioplankton abundances as determined by DAPI cell counts over the course of the bottle incubations. The inoculants used in the bottles displayed similar relative abundances to begin the incubations. After the 70hr incubation, both the control and lysate bottle treatments displayed increased relative bacterioplankton abundance.

The Effingham Inlet control bottles had an average of $2.4*10^6$ cells/ml, while the Lysate bottles had an average of $7.7*10^6$ cells/ml (Fig 3). In both locations, the lysate bottles had higher

bacterioplankton relative abundances than the control bottles. The control bottles also had higher relative abundances than the source stations that were used as inoculants.

The bacterial enzymatic activity was monitored over the course of the incubations by use of lipase, cellulase and chitinase fluorogenic methylumbellifery (MUF)-compounds. The initial and final enzymatic activity after the 70hr incubation was averaged between the

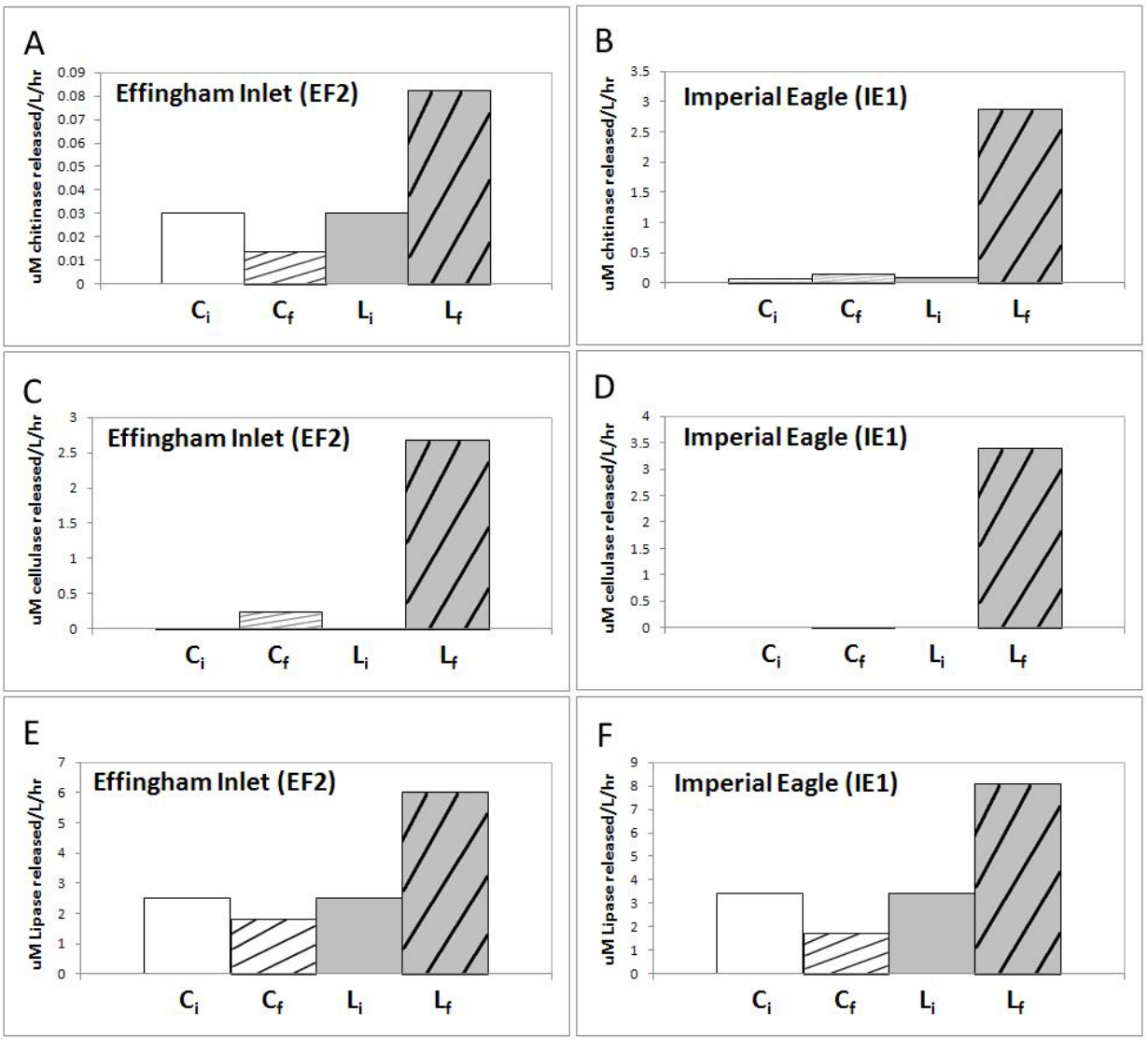

Figure 4: The enzymatic activity for A), B) chitinase, C),D) cellulase, and E), F) lipase for the initial control (C_i) and lysate (L_i) bottles and after a 70hr incubation, final control (C_f) and lysate (L_f) bottles.

duplicate bottle treatments (Fig 4). Overall the lysate bottles (L_f) after the incubation displayed higher enzymatic activity then the control bottles (C_f) from the same location. The Imperial Eagle South (IE1) bottles also exhibited higher enzymatic activity for all three compounds than that of the Effingham Central (EF2) bottles (Fig 4). The cellulase enzymatic activity in both stations control bottles increased over the course of the incubation as seen by the elevated C_f rates (Fig 4C, D). The opposite trends were seen in both the chitinase and lipase activity in the control bottles. The enzymatic activity decreased in both stations lipase bottles over the course of the incubations, and in the chitinase bottle for Effingham Central (EF2) (Fig 4E, F, A). The enzymatic activity in all the lysate bottles increased over the incubation period for each of the substrates used. The Lipase activity doubled for both stations in the lysate bottles after the incubation (Fig 4E, F). The chitinase activity doubled in the Effingham Central (EF2) lysate bottles, and increased from 0.08 to 2.873uM released/L/hr in Imperial Eagle South (IE1) (Fig 4A, B). The cellulase activity in the lysate bottles for both stations showed the greatest increase in activity during the incubation, where there was no initial activity in any of the initial bottles. Effingham Central (EF2) increased to 2.686 uM released/L/hr, and Imperial Eagle South (IE1) increased to 3.4uM released/L/hr (Fig 4C, D).

The bacterioplankton enzymatic activity was normalized to the bacterial abundances for each for each of the substrates for the bottle incubations by dividing the final enzymatic activity (uM substrate released/L/hr) by the bacterial relative abundances changes during the incubation (cells/L/hr) to give the bacterial use per cell (uM released/cell) (Fig 5). Cellulase was the most actively degraded substrate in the lysate bottles with 0.028 uM released/cell in the Effingham Central (EF2) station and 0.024 uM released/cell in Imperial Eagle South (IE1) (Fig 5B).

The bacterioplankton substrate use per cell for chitinase was much lower in both the Effingham Central (EF2) bottles than the respective Imperial Eagle South (IE1) bottles (Fig 5A). The bacterial substrate use per cell was greater in the lysate bottles at both stations when compared to their respective controls for chitinase and cellulase (Fig 5A, B). The lipase use per cell, however, was less in the lysate bottles for both stations than in the control bottles (Fig 5C).

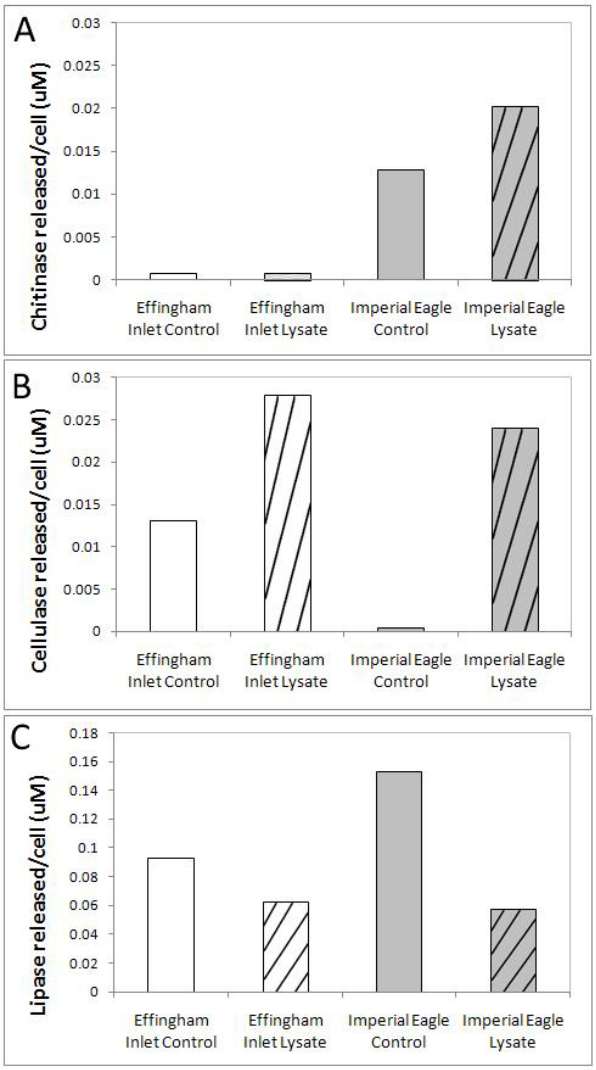

Figure 5: Bacterioplankton enzymatic activity normalized on a per cell basis for A) chitinase, B) celulase, and C) lipase activity (uM substrate released/cell).

The terminal restriction fragment length polymorphism (T-RFLP) data was analyzed by relative fragment lengths, and averaged between the two duplicate bottle incubations done for each station and treatment (Fig 6). The bacterioplankton community present at stations Effingham (EF2) and Imperial Eagle (IE1) are very similar. There are peaks at fragment length 481 representing 38.74% and 35.29% and 283 representing 6.74% and 4.80% respectively that make up a majority of the populations indicating that the initial inoculants used for the bottle incubations were very similar. Imperial Eagle (IE1) had a unique peak at the F48 that represented 10.86% of the sample community that was not present in the

Effingham Central (EF2) sample (Fig 6A, B). Over the course of the incubation, the community in the control bottles stayed similar in their structure, but the relative percent of each bacterioplankton present shifted. The peak at fragment length 283 makes up the majority of the sample at both locations at 38.71% of the community in the Effingham (EF2) control bottles and 28.57% in the Imperial Eagle (IE1) control bottles. The bacterioplankton represented at fragment length 481 now was 15.77% of the community in the Effingham (EF2) control and 24.29% in the Imperial Eagle (IE1) control bottles (Fig. 5C, D). Again there was one unique peak present in the Imperial Eagle (IE1) control sample that was at least 5% of the community, at fragment length 295. The lysate bottles also displayed a different community final then the inoculant samples, but they were much more diverse then the control bottles (Fig 6E, F). The two largest peaks were at fragment length 324 with 18.09% in Effingham (EF2) lysate and 20.93% in Imperial Eagle (IE1) lysate, and at length 326 with 9.19% at Effingham (EF2) lysate and 15.18% in the Imperial Eagle (IE1) lysate bottle. The two dominant peaks in the inoculant samples were still present but composed a different percent of the total community. The bacterioplankton represented by fragment length 283 did not increase in abundance to dominate the community as greatly as it did in the control bottles, making up 8.64% of the community in the Effingham (EF2) lysate bottle and 4.32% in the Imperial Eagle (IE1) lysate bottle (Fig 6E, F). The bacterioplankton signified by fragment length 481 decreased in its abundance in the lysate incubation bottles as it did in the controls, comprising only 1.28%

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in Effingham (EF2) lysate and 2.10% in the Imperial Eagle (IE1) lysate bottles. Fragment length 407 was also present in each lysate bottle, encompassing 7.56% of the community in the Effingham (EF2) lysate incubation bottles, and 6.71% in the Imperial Eagle (IE1) lysate bottles. This fragment was present in the other samples, but not at a level above 5%.

Discussion

Effingham inlet displayed enzymatic activity at all stations at both surface and depth for all three substrates except chitinase its southern station (EF3) in the deep sample (Fig 2A). This can most likely be attributed to the fact that the inlet is poorly flushed and suspended particles would have a higher residence time allowing for the bacterioplankton to break them down more effectively. The Cellulase data displays this situation clearly (Fig 2B). The stations in Effingham Inlet along with Imperial Eagle North (IE3) at its mouth are the only locations that there was any observed enzymatic activity in the deep samples. All other stations solely display cellulose activity in the surface samples. This is most likely due to the fact that the rest of Barkley sound is relatively well flushed and mixed, so the woody debris that cellulase is derived from is primarily retained in the surface waters were it is degraded by the bacteroplankton community. In Effingham Inlet, woody debris is able to become waterlogged and sink to be broken down at depth because of the slow flushing rate. The highest cellulase activity was seen in Alberni Inlet at station AI1 (Fig 2B). This station was directly outside of the Franklin River that drains a large portion of the mountains nearby (Cameron Carter, personal correspondence). The river transports large amounts of organic debris including woody fragments accounting for the elevated cellulase activity. This debris would be transported out of the inlet to nearby stations including San Mateo Bay (SMB1), and Sarita Bay (SB2) accounting for the surface

cellulase activity seen there. Imperial Eagle North (IE3) displayed the highest enzymatic activity for both chitinase and Lipase, along with the second largest activity for cellulase in the surface waters. This could be due to a number of environmental factors including an elevated phytoplankton population, convergence of water masses or organic mater runoff from land but the data presented by this study does not provide any definite answer. The dinoflagellate community at this station consisted of four distinct species, making it for one of the more diverse locations sampled in Barkley Sound and this may have also been a contributing factor (Ashley Leung, personal communication). The lipase enzymatic activity was greater than all other substrates at all stations sampled. Lipids are a much simpler compounds when compared to cellulose and chitin, so bacterioplankton preferentially break it down when available accounting for the higher rate of enzymatic activity. This has been noted in similar studies using with florogenic model substrates including Hoppe 1983.

With the addition of a diatom lysate to replicate the conditions of a post diatom bloom, the bacterioplankton community structure shifted along with the enzymatic activity and bacterial abundance over the course of the bottle incubations. The results supported the hypothesis that the bacterial abundance would increase within the lysate incubation bottles. The bacterioplankton abundance also increased in the control bottles, but this was most likely due to the removal of grazing pressure and top down control limitations (Billen et al.1990, Smith et al. 1995). With the addition of a food source for the bacterioplankton, in this case the diatom lysate, they were no longer limited by bottom-up controls and could increase in abundance (Fig 3). The enzymatic activity for lipase was also highest in the bottle incubations when compared to the other substrates, analogous to the station data collected throughout

Barkley Sound. The Lysate bottles after the 70hr incubation displayed the greatest enzymatic activity for all substrates at both stations sampled (Fig 4). The relative lipase released/cell however decreased over the course of the incubation in both the Imperial Eagle and Effingham Inlet Lysate bottles (Fig 5C). Both other substrates displayed increasing activity in the lysate bottles when compared by substrate released/cell however. With the addition of a food source, the bacterioplankton community that responded had a higher efficiency while using the cellulase and chitinase substrates when compared to lipase in the lysate bottles. This is most likely due to the shift in community structure that occurred during the incubation as seen by the T-RFLP data (Fig 6). Following a diatom bloom, enzymatic activities generally increase, and can vary or peak at different times, along with a corresponding changing bacterioplankton community (Riemann et al. 2000). As a bacterial population changes, so do their metabolic capabilities and what organic matter they target.

Although the same bacterioplankton were present in the final incubation samples as the initial inoculation, their percent of the total community had shifted greatly (Fig 6). In the control incubations, the dominant bacterioplankton represented by fragment lengths 481 and 283 switched from the initial community relative abundances. Fragment 481 decreased in abundance while fragment length 283 increased to dominate both locations communities in the control bottles. The bacterioplankton represented by fragment length 283 is most likely an opportunistic, fast growing, r-selected group. Such species trade off the ability to compete for limited resources for the ability to reproduce quickly and respond to environmental changes (Pianka 1970). In the case of the control bottles, the bacterioplankton was no longer limited by grazing pressure, and introduced to a new environment that it was more adept to take

advantage of. With this said, the bacterioplankton represented by fragment length 481 that declined in relative abundance in the control bottles is most likely a k-selected group. Such species trade off fast replication, for the ability to compete at lower nutrient or other environmental restraints. It was not as quick to respond to the lifted grazing pressure, and did not increase in abundance as much as the other bacterium, resulting in a lower total percentage of the overall final control bottle community. The lysate community structure also shifted greatly from the initial community composition over the course of the incubation (Fig 6E, F). The lysate incubation incurred both the removal of grazing pressures top down controls, and the addition of a food source, the removal of bottom down controls. This enabled a differing bacterial community response and a dissimilar bacterioplankton to take advantage to the lifted restrictions. Instead of a simple switch in the community structure as in the control bottles, a whole new set of bacterioplankton were able to increase their relative abundances (Fig 6E, F). This can be seen by the increase in the relative percentage of the bacterioplankton represented by fragment lengths 324 and 326 that were almost completely absent from both the control bottles and the initial inoculations. Along with this, the lysate bottle community was much more diverse than the control bottles. Because of the removal of both top-down and bottom-up restrictions, a more expansive quick responding r-selected bacterioplankton community responded. With the removal of both pressures a wider range of bacterioplankton where able to respond, and take advantage of the new conditions. Because the diatom lysate consisted of fractured cells in suspension, much of the bacteria population would have most likely consisted of attached or colonizing bacterioplankton versus the control bottles that would be comprised more of free-living species. It has been shown that there are phylogenetic differences between

free living and attached bacteria by DeLong et al 1993, and such differences could lead to a differing growth response. This also helps explain why the community would change over the course of the incubation. If the initial inoculant samples were comprised mainly of free-living adapted strains of bacteria, then with the addition of the lysate, the community would shift to a more aggregate based community. Based off this, it is most likely that the 324 and 326 fragment lengths that dominated both the lysate incubations represent a bacterioplankton that generally aggregates or colonizes on particulate matter. This can also possibly help explain why the control bottles had the same two main peaks at 283 and 481 base pairs as the initial inoculant samples. These are most likely free-living bacterioplankton species that shifted in their composition due to the removal of grazing pressure, but were still well adapted to the free floating niche.

It should be noted that the bacterial community as seen by the T-RFLP analysis at the locations chosen for the incubation inoculants differ greatly from similar studies done (Gonzalez et al. 2000, Morris et al. 2005). The marine bacterioplankton SAR 11 Clade, SAR 86 and SAR 202 clusters have been shown to be the most prevalent groups in seawater (Giovannoni et al. 2000). SAR 11 has been shown to account for upward of 35% of surface water bacterioplankton communities, and the SAR 202 groups represents 10% of all DAPI staining cells in the Pacific Ocean (Morris et al. 2002, 2004). Both the SAR 11 and SAR 202 clades were examined in Morris et al. 2005 using the same T-RFLP methods as this study and have been found to be represented by fragment lengths not seen in the inoculants samples used. Due to their usual relative abundances in marine ecosystems this was quite unexpected, and would point to the fact that the inoculants samples used for the bottle incubations were most likely dissimilar of

commonly occurring bacterioplankton communities. The common SAR 86 group was present in the community sampled, but in levels far below what is generally seen. Represented by fragment length 188 from Morris et al. 2005, the SAR 86 group accounted for only 4.61% of the community in the Effingham Inlet Central (EF2), and 2.23% in the Imperial Eagle South (IE1) locations used as inoculants to start the incubations. This bacterioplankton however was not one that responded and remained or decreased in relative abundance during incubations. The bacterioplankton represented by fragment length 324 that responded in the lysate incubation bottles to dominate the community can be identified by similar T-RFLP analysis as Arctic96BD-19 (Katie Marshall, unpublished data). This bacterioplankton is related to SUP05, an uncultured oxygen minimum zone microbe closely related to chemoautotrophic symbiotic gill microbes found in the gills of vent clams and mussels (Walsh et al. 2009). Saanich Inlet, a nearby anoxic fjord in British Columbia was examined by Zaikova et al. 2010, to understand the seasonal microbial dynamics in a low oxygen system. Both Arctic96BD-19 and SUP05 were found, with SUP05 comprising 73% and 99% of SSU rRNA clones recovered in the deep water, anoxic zone libraries. Arctic96BD-19 was found primarily in surface 10m samples, and in oxygen transition zones. This is supportive of the fact that Arctic96BD-19 was found in the surface samples in Effingham Inlet, a commonly anoxic fjord and the nearby Imperial Eagle Inlet. SUP05 using similar T-RFLP analysis has been shown to be represented by fragment length 193 (Katie Marshall, unpublished data). This fragment length was absent from both surface inoculant samples and the resulting incubations as this clade is found in anoxic, deep samples.

With the addition of a diatom lysate, the bacterioplankton community structure shifted along with the compounds they degrade. This is reflected in both the T-RFLP data showing the

community structure, and the extracellular enzymatic activity (EEA) on model fluorogenic substrates examined during the bottle incubations. This simulated post-bloom shift would affect the bacterioplankton recycling of important nutrients released during the degradation of organic matter. This alters the dynamics of the areas microbial loop, and the availability of these nutrients to the corresponding phytoplankton community. The importance of the bacterial breakdown of organic matter and the microbial loop to the marine ecosystem is significant, and our understanding of controlling factors to its dynamics is vital to our understandings of the ocean itself.

Conclusions

- The relative abundance of bacterioplankton in Barkley Sound and the surrounding fjords sampled was on the same order of magnitude of other similar estuarine systems in the northwest with an average of $1.1*10^6$ cells/ml in the surface samples, and $1.2*10^6$ cells/ml in the bottom samples.
- Bacterial extra cellular enzymatic activity was the greatest for lipase at all stations sampled with chitinase and cellulase substrates displaying significantly lower activities.
- The enzymatic activity was greatest in the lysate incubation bottles versus the control treatment for all substrates used with lipase having the highest rates of hydrolysis.
- Corresponding to the increased enzymatic activity was a higher relative bacterial abundance in the lysate bottles when compared to the controls.
- When normalized by the bacterial abundances, the lysate bottles displayed a reduced enzymatic activity for lipase when compared to the respective control bottles.
- The bacteria present in the initial locations chosen to serve as the inoculation for the bottle incubations were very similar as seen by the T-RFLP data, dominated by two bacterioplankton. These populations were very dissimilar from what is normally seen in similar studies done in the surface waters.
- Over the course of the incubations, the populations shifted in both the lysate and control bottles, with similar changes occurring in both station bottles.
- The same two representative bacterioplankton dominated the control bottle populations, but their relative populations reversed.
- The lysate bottles exhibited a much more diverse community then the initial inoculant community or the control bottles, with a total of five fragment lengths representing over 5% of the community in the Effingham lysate bottles and 4 in the Imperial Eagle lysate bottles.

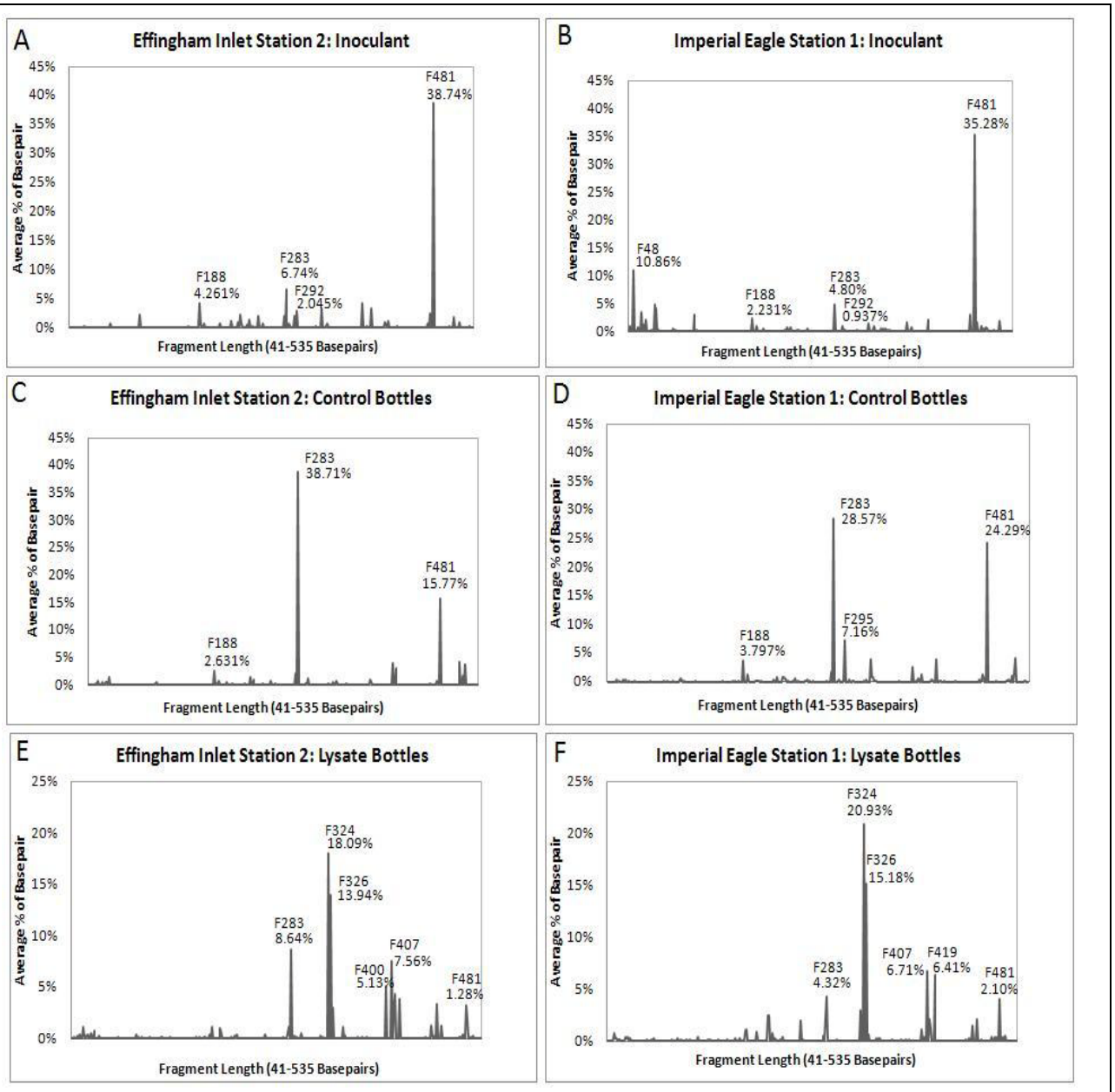

Figure 6: Chromatograms of the T-RFLP data from the bottle incubations from stations Effingham Inlet 2 and Imperial Eagle 1. Each peak represents a type of bacterioplankton present in the sample, denoted by the corresponding fragment length and the relative percent it makes up of the total community. Peaks that represent at least 5% of the community, or changed significantly over the course of the incubation are labeled for each sample.

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