Effects of environmental and anthropogenic influences on bacterial growth and community structure in Newfoundland coastal waters

by © Lindsey C. Hemphill A Thesis submitted

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Abstract

The release of petroleum hydrocarbons into coastal Newfoundland waters is an environmental and economic concern. Placentia and St. Mary's Bays, Newfoundland have contrasting anthropogenic uses and environmental conditions, with greater shipping and traffic in the former site. Bacteria have a key role in marine food web transformations; and petroleum hydrocarbons have been reported to both stimulate and inhibit marine plankton. Here, we examine the influence of temperature and the addition of various combinations of petroleum hydrocarbons, inorganic nutrients and organic carbon, on bacterial growth and composition in Placentia and St. Mary's Bays, Newfoundland. Organic carbon plus inorganic nutrients and elevated temperature generally increased bacterial growth while petroleum hydrocarbons did not influence growth in Placentia Bay and inhibited growth in St. Mary's Bay. High-throughput metagenomics analysis examined the response of the bacterial community and showed that the relative abundance of known hydrocarbon degraders increased in the presence of petroleum hydrocarbons, with greater numbers in Placentia than St. Mary's Bay following incubation. The bacterial community of Placentia Bay, where concentrations of petroleum hydrocarbons are higher, may be preconditioned to favour hydrocarbon degraders compared to St Mary's Bay.

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+[N]: The *in situ* treatment from the growth and community structure experiments.

+[C]: The control treatment from the growth and community structure experiments.

+[I]: The inorganic nutrient treatment from the growth and community structure experiments.

+[O]: The organic carbon treatment from the growth and community structure experiments.

+[I+O]: The inorganic nutrient plus organic carbon treatment from the growth and community structure experiments.

+[H]: The hydrocarbon treatment from the growth and community structure experiments.

+[H+I]: The hydrocarbon plus inorganic nutrient treatment from the growth and community structure experiments.

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Chapter 1: Introduction

Bacteria are ubiquitous in the open ocean, and oceans are estimated to have 10^{29} bacteria (Whitman *et al*., 1998). They are important players in biogeochemical processes of the oceans (Falkowski *et al*., 2008), such as carbon, sulfur and nitrogen cycles (Kellogg *et al*., 1972; Ward, 1996; Azam, 1998; González *et al*., 2000; Pomeroy *et al*., 2007). Bacteria take up nutrients for their own growth and metabolism, and transform carbon, nitrogen and phosphorus compounds, sometimes making them more bioavailable and influencing nutrient cycles (Strom, 2008). Bacteria are responsible for the majority of respiration and remineralization of carbon in the ocean as major components in the biological pump (Ducklow *et al*., 2001). Bacteria determine the fate of organic carbon in the ocean by respiring carbon dioxide in either the upper or deep ocean, or transferring it to higher trophic levels through grazing (Kirchman, 2002; Pomeroy *et al*., 2007). The transformation of carbon by bacteria is an important biogeochemical process, as they can utilize carbon that would otherwise be unavailable to higher trophic levels or sequestered in the ocean, or by respiration which makes carbon dioxide in the upper ocean readily transferable to the atmosphere (Sherr and Sherr, 1988; Pomeroy *et al*., 2007). Through their transformations in the ocean, bacteria regulate carbon dioxide concentrations ultimately influencing atmospheric composition (Falkowski, 1998).

Variation in the structure, function or growth of the bacterial community therefore influences the fate of carbon in the ocean, and potentially climate change. The world is undergoing global warming, and the International Panel on Climate Change (2007) predicts that temperatures may increase by 2 to 4^{o}C. All of the feedback loops between

bacteria and climate, and the impact on biogeochemical processes however, are still not fully understood (Doney *et al*., 2004; Jiao *et al*., 2010). Thus, to be able to determine the effect of bacterial function on biogeochemical processes, we must include ecological factors that in turn influence bacterial growth.

There are multiple factors that influence bacterial growth, including temperature (Rivkin *et al*., 1996; Kirchman and Rich, 1997), the availability of inorganic nutrients such as nitrogen and phosphorous (Chrzanowski *et al*., 1995; Rivkin and Anderson, 1997), and organic carbon (Rivkin and Anderson, 1997; Carlson *et al.,* 2002; Cuevas *et al*., 2011). The growth and abundance of marine bacteria are suggested to be positively related to temperature (Hoch and Kirchman, 1993; Shiah and Ducklow, 1994; Ochs *et al.*, 1995), with low temperatures increasing the response time of growth compared to higher temperatures (Kirchman and Rich, 1997; Granéli *et al*., 2004). Temperature has been suggested to be a more influential factor on the production and growth of bacteria than the supply of substrates (Hoch and Kirchman, 1993; Shiah and Ducklow, 1994). Overall bacterial production and growth was found to be lower in the Ross Sea, and western Arctic Ocean than in lower latitude oceans, however, individual samples showed they were capable of growth rates just as high as those in the warmer waters, implying a controlling variable in addition to temperature (Kirchman *et al*., 2005, 2009). Kirchman *et al*. (2009) suggested that the supply of dissolved organic matter (DOM) was this other influential factor. Other studies have found the effect of nutrients to be greater than that of temperature, with warming significantly increasing the effect of nutrients, indicating the effects of temperature may be more influential in nutrient rich environments

(Christoffersen *et al*., 2006; Kirchman *et al*., 2009; Degerman *et al.* 2013; Özen *et al.,* 2013). The direct effect of temperature may be an overestimate because of the ability of bacterial communities to adapt rapidly, and imposed limitations by other factors such as nutrients (Kirchman *et al*., 2009).

Many studies have examined the availability of inorganic nutrients and organic carbon as a controlling factor of bacterial growth (Kirchman, 1990; Cotner *et al*., 1997; Meon and Amon, 2004). Bacteria utilize available organic nutrients in the ocean for their own growth. Growth is stimulated when glucose or other carbon substrates are added (Kirchman, 1990; Carlson and Ducklow, 1996; Kirchman and Rich, 1997; Church *et al*., 2000; Carlson *et al*., 2002; Cuevas *et al*., 2011). Nitrogen (N) and phosphorous (P) are often the limiting inorganic nutrients for bacterial growth in seawater (Vrede *et al*., 1999; Cuevas *et al*., 2011). Heterotrophic bacterial growth rate appears to be the greatest when both energy (carbon) and limiting nutrients (N and P) are available (Rivkin and Anderson, 1997; Shiah *et al*., 1998; Church *et al*., 2000; Carlson *et al*., 2002; Cuevas *et al*., 2011; Sebastián and Gasol, 2013). The response of bacterial communities to nutrient amendments however, depends on the original limitation in their environment (Thingstad *et al*., 2008). Bacteria are able to respond quickly to their environments, remaining at low metabolic activity in limited environments, and responding when conditions become favourable (Pomeroy *et al*., 2007). The environmental factors that influence the growth of bacteria therefore, indirectly influence the fate and the cycling of carbon in the ocean.

Two biota on earth that have large scale influence on cycling and climate are bacteria, and more recently, humans. The concentration of carbon dioxide has increased

by approximately 40% since 1750 due to human activity and the burning of fossil fuels, influencing the climate (Ducklow, 2008). Human activity may not only directly affect climate by the release of carbon dioxide into the atmosphere, but can potentially also indirectly affect it through their impacts on the bacterial community. A major impact human activity has on the environment is through the release of petroleum hydrocarbons. Petroleum hydrocarbons have been suggested to be one of the major contaminants in marine environments (Windom, 1992), with anthropogenic activity responsible for approximately 50% of the petroleum hydrocarbon entering these systems (Kvenvolden and Cooper, 2003). Petroleum hydrocarbon release into seawater continues to increase as marine traffic increases. It is estimated that each year about 0.1% of total petroleum production ends up the marine environment (Vila *et al*., 2010).

The release of petroleum hydrocarbons into coastal Newfoundland waters is a concern, as production and movement of petroleum hydrocarbons is significant in this area. Newfoundland began producing crude petroleum hydrocarbon in 1996, becoming a major supplier in eastern Canada (Hughes, 2010; Canada-Newfoundland and Labrador Offshore Petroleum Board, 2014). Newfoundland produces most petroleum hydrocarbon from three fields: Hibernia, Terra Nova and White Rose, all offshore on the Grand Banks (Hughes, 2010). In 2007, about 134.5 million barrels of crude petroleum were produced in Newfoundland (Hughes, 2010). The production and movement of petroleum hydrocarbons puts this environment at risk for catastrophic events (i.e. shipping accidents or pipe bursts), as well as chronic input (i.e. ships, refineries). It has been well documented that large scale petroleum hydrocarbon spills have significant impacts, such

as increasing abundance and changing community structure, on the ambient bacterial communities (Bragg *et al*., 1994; Alonso-Gutiérrez *et al*., 2009; Hazen *et al*., 2010; Valentine *et al*., 2012; Acosta-González *et al*., 2013).

The addition of petroleum hydrocarbons appears to increase the abundance and growth of bacteria (Head *et al*., 2006; Cappello *et al.,* 2007; Yakimov *et al.,* 2007), as well as significantly altering the community structure (McKew *et al*., 2007; Valentine *et al*., 2012; Rodríguez-Blanco *et al*., 2013). Studies of the DNA of bacterial communities contaminated with petroleum hydrocarbons suggest that the community structure was changed, either by selection of hydrocarbon degrading bacteria (Harayama *et al*., 1999; Kasai *et al.,* 2001; Yakimov *et al*., 2005) or from toxic effect of petroleum hydrocarbons on certain species (Grötzschel *et al*., 2002).

Some bacteria (hydrocarbon degrading bacteria) utilize petroleum hydrocarbons as their source of carbon and energy (Yakimov *et al*., 2007). The introduction of petroleum hydrocarbons into pristine environments appears to stimulate the growth of the small population of indigenous hydrocarbon degrading bacteria (Røberg *et al*., 2011), tending to shift the bacterial community towards these species (MacNaughton *et al*., 1999; Röling *et al.,* 2002; Head *et al*., 2006; Yakimov *et al*., 2007). The removal of petroleum hydrocarbons through biodegradation by bacteria has been observed in multiple environments, from temperate (Cappello *et al*., 2007; Paissé *et al*., 2010; Dubinsky *et al*., 2013), to cold seawater (Siron *et al*., 1995; Garrett *et al.,* 2003; Coulon *et al*., 2007). At least 79 species of bacteria have been determined to be hydrocarbon degraders (Prince, 2005). Most of these species are from the Phylum *Proteobacteria*, but

some are also from the Phyla *Firmicutes*, *Actinobacteria* and *Bacteroidetes* (Prince, 2005). In petroleum hydrocarbon-laden environments, the composition of the bacterial community is influenced by environmental factors, the composition and concentration of the petroleum hydrocarbons, and the indigenous species present (Røberg *et al*., 2011).

There have been a number of studies on the effects of petroleum hydrocarbons on the bacterial community, as related to large scale petroleum hydrocarbon spills. Most have employed relatively high concentrations of petroleum hydrocarbons added to the seawater enrichment experiments, with ranges from 100 mgL^{-1} to 17 000 mgL^{-1} of crude petroleum hydrocarbons (Kasai *et al*., 2002ab; Cappello *et al*., 2007; Coulon *et al*., 2007; Paissé *et al*., 2010; Jiménez *et al*., 2011; Baelum *et al.,* 2012). Such concentrations are relevant to catastrophic petroleum spills but do not reflect concentrations relevant to low level chronic input. The study reported here focused on the response of the bacterial community structure, activity and growth to chronic low concentrations of petroleum hydrocarbons as there appears to be little published information of these effects at low concentrations (Paissé *et al*., 2008). This study tested the hypothesis that chronic low concentration of petroleum hydrocarbon additions will act as a carbon source, inducing growth of hydrocarbon degrading bacteria, and creating a shift in the bacterial community composition. To fully understand the response of the bacterial community to these petroleum hydrocarbon inputs, and provide framework for their ultimate consequences on the large scale biogeochemical processes, this study determined both the total and functional responses of the bacterial community, and utilized genomic techniques to understand the community structure associated with these responses.

Molecular and genomic techniques have increased information available on marine bacterial genomics and diversity (Doney *et al*., 2004; Strom, 2008). High-throughput sequencing of bacterial DNA enables us to begin to understand the community processes that underlie ecosystem function and biogeochemical processes (Doney *et al*., 2004; Strom, 2008). Many strains of bacteria occupy different ecological niches (Cullen *et al*., 2007) and genomic data has revealed that most bacteria can only execute one step in the degradation of a substrate, so overall transformations require consortia (Strom, 2008). Many studies on bacteria use cultured species, but to fully understand the roles of bacterial consortia, determining both the function and response to ecological factors, and the composition of the community *in situ* are needed (Ducklow, 2008). Bacterial productivity and community structure reflect ecological factors, such as the supply of nutrients that vary within and among oceanic regions (Fung *et al.*, 2000). The integration of community composition, community function, and the biogeochemical processes will allow us to understand observed responses to disturbances such as petroleum hydrocarbon additions and to make more realistic predictions of the consequences of these disturbances.

The objectives of this study were to determine if low concentrations of petroleum hydrocarbons can be a carbon source and to determine their effects on the bacterial community by analysing the communities function and response to both petroleum hydrocarbons and ecological factors, such as nutrients and temperature, alongside the analysis of the bacterial community composition. The goal is to understand further the large scale implications on the biogeochemical and carbon cycles. This study was carried

out in two cold bays of coastal Newfoundland Canada, with differing intensity of marine traffic and associated petroleum hydrocarbon input. The ecological properties were characterized and the effects of temperature (ambient and ambient increased by 2^{o}C), inorganic nutrients (ammonium and phosphorous) and organic carbon (glucose), and petroleum hydrocarbons on bacterial community growth were studied in incubations. High-throughput 16SV6 rDNA sequence analysis was used to determine the community structure, and to test for effects of the addition of nutrients and petroleum hydrocarbons on the structure as well as function of the bacterial communities in either bay.

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Chapter 2: Ecological context and enrichment experiment

2.1 Introduction

The availability of inorganic nutrients and organic carbon, and temperature are some of the main factors that control bacterial growth (Kirchman, 1990; Rivkin *et al*., 1996; Cotner *et al*., 1997; Meon and Amon, 2004) and the response of the bacterial communities to amendments depends on the original limitation in their environment (Thingstad *et al*., 2008). Nitrogen (N) and phosphorous (P) are the main inorganic nutrients that are often a limiting factor to bacterial growth in seawater (Vrede *et al*., 1999; Cuevas *et al*., 2011), shown to stimulate growth and production (Caron *et al*., 2000; Thingstad *et al*., 2008; Cuevas *et al*., 2011). Maximum increases in the bacterial growth rates however, occur when both organic (carbon) and inorganic nutrients (N and P) are present (Rivkin and Anderson, 1997; Shiah *et al*., 1998; Church *et al*., 2000; Carlson *et al*., 2002; Cuevas *et al*., 2011; Sebastián and Gasol, 2013). Carbon limitation has been demonstrated in cold environments, such as the Arctic and Antarctic (Wedborg *et al.,* 1998; Church *et al*., 2000; Granéli *et al*., 2004; Meon and Amon, 2004; Kirchman *et al*., 2005). Increased bacterial abundance and activity in Arctic and subarctic waters has been found to be related to phytoplankton blooms, suggested to be in response to the enrichment of the environment with organic carbon (Yager *et al.*, 2001; Sherr and Sherr, 2003). When bacterial activity and growth are carbon limited, activity can be stimulated when glucose or other carbon substrates are added (Kirchman, 1990; Carlson and Ducklow, 1996; Kirchman and Rich, 1997; Church *et al*., 2000; Carlson *et al*., 2002; Cuevas *et al*., 2011).

The addition of petroleum hydrocarbons into marine systems can potentially act as an alternative carbon source for bacteria, with observed increases in abundance and growth (Head *et al*., 2006; Cappello *et al.,* 2007; Yakimov *et al.,* 2007), as well as significantly altering the community structure (McKew *et al*., 2007; Valentine *et al*., 2012; Rodríguez-Blanco *et al*., 2013). Bacteria that can utilize petroleum hydrocarbons as their source of carbon and energy are referred to as hydrocarbon degrading bacteria (Yakimov *et al*., 2007), suggested to be ubiquitous even in areas with no petroleum hydrocarbon contamination (Lo Giudice *et al.,* 2010). Previous studies of bacterial communities contaminated with petroleum hydrocarbons suggest the community structure changed, either by selection of hydrocarbon degrading bacteria (Harayama *et al*., 1999; Kasai *et al.,* 2001; Yakimov *et al*., 2005) or from toxic effect of petroleum hydrocarbons on certain species (Grötzschel *et al*., 2002), influencing growth and total abundance. Some of the toxic components of petroleum hydrocarbon, such as polycyclic aromatic hydrocarbons (PAHs), can inhibit some bacterial taxa, and as a result there may be a shift in the bacterial community structure towards those which either have the ability to tolerate or degrade these compounds (MacNaughton *et al*., 1999; Röling *et al.,* 2002; Head *et al*., 2006; McKew *et al*., 2007; Valentine *et al*., 2012; Rodríguez-Blanco *et al*., 2013). The removal of petroleum hydrocarbons through biodegradation by bacteria has been observed in multiple environments, from temperate (Cappello *et al*., 2007; Paissé *et al*., 2010; Dubinsky *et al*., 2013), to cold seawater (Siron *et al*., 1995; Garrett *et al.,* 2003; Coulon *et al*., 2007; Lo Giudice *et al.,* 2010). The population sizes of marine petroleum hydrocarbon degraders are typically low in unpolluted environments but appear to increase significantly in response to large scale petroleum hydrocarbon spills, such as the

Deepwater Horizon or Prestige spill (Cappello *et al.,* 2007; Hazen *et al.,* 2010; Jiménez *et al*., 2011; Kostka *et al.,* 2011). It has been well documented that large scale petroleum hydrocarbon spills have significant impacts on the ambient bacterial communities (Bragg *et al*., 1994; Alonso-Gutiérrez *et al*., 2009; Hazen *et al*., 2010; Valentine *et al*., 2012; Acosta-González *et al*., 2013). Previous studies have examined the impact of petroleum hydrocarbon pollution on bacterial abundance or the biodegradation of these compounds, with a relatively high concentration range from 100 mgL^{-1} to 17 000 mgL^{-1} of crude petroleum hydrocarbons (Kasai *et al*., 2002ab; Cappello *et al*., 2007; Coulon *et al*., 2007; Paissé *et al*., 2010; Jiménez *et al*., 2011; Baelum *et al.,* 2012). Particulate aliphatic hydrocarbon concentration in the Bedford Basin, Nova Scotia was 8.1 to 9.5 μgL^{-1} in an industrial area with significant marine traffic (Parrish, 1987; Parrish *et al*., 1988) and suggested by Parrish (1987) to be contaminated. There is little information on these chronic low level petroleum hydrocarbon contamination effects on bacterial communities and growth, particularly in cold subarctic environments (Paissé *et al*., 2008), and whether the concentration will have toxic or stimulatory effects on bacterial growth.

A major contaminant in marine environments are petroleum hydrocarbons (Windom, 1992), with approximately 50% of the petroleum hydrocarbons entering these systems from anthropogenic activity (Kvenvolden and Cooper, 2003). Petroleum hydrocarbon release into seawater continues to increase as marine traffic increases. The release of petroleum hydrocarbons into coastal Newfoundland waters is a concern, as production and movement of petroleum hydrocarbons is significant in this area. Well development and production of petroleum hydrocarbons in the Newfoundland offshore

began in 1997 (Canada-Newfoundland and Labrador Offshore Petroleum Board, 2014), and continues to be a major industry. The majority of petroleum hydrocarbon production occurs from three fields offshore on the Grand Banks: Hibernia, Terra Nova and White Rose (Hughes, 2010). The cumulative production of crude petroleum hydrocarbons in Newfoundland, as of 2014, was approximately 913 million barrels (Canada-Newfoundland and Labrador Offshore Petroleum Board, 2014). There is also a petroleum hydrocarbon refinery at Come By Chance in Placentia Bay, with constant commercial movement of tankers to the head of the bay, as well as a ferry and dockyard at Argentia (Khan, 2003). The production and movement of petroleum hydrocarbons puts this environment at risk for catastrophic events (i.e. shipping accidents or pipe bursts), as well as chronic input (i.e. ships and refineries). We propose that the constant movement of ships, and petroleum hydrocarbon usage in Placentia Bay, has influenced the bacterial community due to chronic low level inputs of petroleum hydrocarbons which have also been expressed in negative health effects (i.e. lesions and low lymphocyte levels) for the flatfish population (Khan, 2003). We also propose that the adjacent St. Mary's Bay with little to no commercial activity and no refinery has limited petroleum hydrocarbon input in comparison to Placentia Bay.

We carried out survey and manipulation experiments in Placentia and St. Mary's Bays, Newfoundland, to understand the response of bacterial growth to the inputs of organic carbon and inorganic nutrients, and determine if low concentrations of petroleum hydrocarbons acted as a toxic substance or an alternative carbon source. This study assessed the response of the bacterial populations from two bays in Newfoundland with

differing marine traffic use to determine if previous exposure to petroleum hydrocarbons influences bacterial growth, and we utilized an original experimental set up allowing direct comparisons between all treatments. In addition the experiments were incubated at two temperatures to evaluate potential climate forcing on these interactions.

2.2 Methods

2.2.1 Field Site

Water samples were collected in Placentia and St. Mary's Bays, which are situated between the Burin and Avalon Peninsulas, on the southeast coast of Newfoundland. Sampling was carried out during August 2012, and April, June and August or September 2013. Generally, during each sampling period, water samples were taken at multiple locations in each bay (Figure 2.1): i.e. samples that were taken at the mouth, middle, and head of each bay, referred to as "ecological samples". Water samples were either collected by a boat or off a dock (Tables 2.1 and 2.2). During each sampling period, surface water was collected for experimental manipulations from the middle of each bay.

2.2.2 Ecological Samples

A Seabird 19S CTD was deployed to measure vertical profiles of temperature and conductivity for two sampling depths (Appendix A). The CTD data provided the temperature for all ecological samples, as well as the reference temperature for the incubation experiments (Tables 2.1, 2.2, 2.3 and 2.4). At each site, water samples were collected at two depths using 5 L Niskin bottles and approximately 2 L of seawater was

transferred into Nalgene bottles. Water samples were always collected at the surface, and when taken off the dock at the deepest possible depth. When the samples were collected off the boat the second depth was taken at the bottom of the mixed layer. All Nalgene bottles for samples and subsamples were rinsed with 5% hydrochloric acid (HCl), deionized water, and sample water. The Nalgene bottles were placed in a cooler with ice from time of collection, until returned to the laboratory for sample processing. In the laboratory subsamples were taken for dissolved organic carbon and nitrogen (DOC and DON), particulate organic carbon and nitrogen (POC and PON), chlorophyll a, and bacterial abundance. Particulate hydrocarbons were also determined during November 2013. In this study, particulate hydrocarbons include all hydrocarbon components suspended in the sampled water greater than 7.0 μm. Dissolved hydrocarbons were not measured during this study due to analysis constraints.

2.2.2.1 Sample Collection and Analysis in Laboratory

Dissolved Organic Carbon and Nitrogen (DOC and DON)

Subsamples were collected from the Nalgene bottles taken from only sites PB2 and SMB2 at surface depth (Tables 2.3 and 2.4). Fifty milliliters of water was filtered through a sterile Acrodisc (syringe filter, 25 mm diameter, 0.2 μm pore) into acid-washed 100 mL Boston rounds and stored at -20^{o}C until analysis. Seawater samples were thawed in a refrigerator and then brought to room temperature before analysis. Before subsampling, the samples were mixed well, and 25 mL was transferred to clean glass 40 mL vials. All glassware was washed with dish liquid and rinsed with distilled water, then combusted for >5 hours (h), at 450°C. The pH of the samples was adjusted to pH 2, with

the addition of 2M HCl. MilliQ water sample blanks were run before, during and after the seawater samples with the same method. The duplicate samples were analyzed on a Shiadzu TOC-V_{CPH} machine, with an ASI-V auto sampler and a connect TNM-1 analyser. Standards of potassium hydrogen phthalate (NPOC) were dried for one hour at 105-120^{o}C and cooled in a desiccator, and potassium nitrate (TN) were dried for 3 hours at 105-110 and cooled in a desiccator before being run and were within 5% of reference standards (bought from University of Miami).

Chlorophyll a

Duplicate 300 mL of seawater from the Nalgene bottles were filtered (5 psi) onto 25 mm GF/F filters (Whatman). The filtering units were rinsed with 5% HCl, deionized water and sample water before filtering sample water. The filters were stored at -20^{o}C until analysis. The GF/F filters were placed into 90% acetone washed, glass vials with 5 mL of 90% acetone, shaken, and incubated overnight in the dark at -20^{o}C. Florescence was determined before and after acidification with 5% HCl using a Turner designs 10-AU Fluorometer (Strickland and Parsons, 1972) for analysis. The fluorometer has a detection limit of 0.025 μgL^{-1} (range 0-250 μgL^{-1}) for extracted chlorophyll a and sensitivity was set at 30-50% of 900.00 at high (Turner designs). Calibration was done using a 200 μgL^{-1} chlorophyll a standard in 90% acetone, and a blank of 90% acetone.

Particulate Organic Carbon and Nitrogen (POC and PON)

Water samples from only sites PB2 and SMB2 at surface depth (Tables 2.3 and 2.4) were used for analysis. Three hundred milliliters of seawater from the Nalgene

bottles were filtered (5 psi) onto precombusted (at 500ºC for 12 hours) GF/F filters (25mm, Whatman). One filter was obtained per Niskin bottle. The filters were placed in precombusted aluminum foil squares (500ºC for 12 hours), and stored at -20ºC until analysis. The filtering units were rinsed with 5% HCl, deionized water and sample water before filtering sample water. The samples were thawed, and moved to glass scintillation vials (previously combusted at 450ºC for 8 hours), dried at 80ºC for 24 hours, and placed in a desiccator saturated with HCl fumes for 24 hours to remove inorganic carbon. Samples were then returned to the 80ºC oven for at least 24 hours or until the filters were packaged into tin discs and analysed on a Perkin Elmer 2400 Series II CHNS/O elemental analyzer. The detection limits were 0.001 to 3.6 mgs for POC and 0.001 to 6.0 mgs for PON. The precision of the instrument was $\leq$ 0.2% and calibration was done using acetanilide (71.09% carbon, 6.71% hydrogen, 10.36% nitrogen) standards.

Bacterial abundance

Subsamples of fifty milliliters were taken from the Nalgene bottles from each depth for bacterial enumeration. The subsamples were placed into falcon tubes with 1.5% final concentration of glutaraldehyde and stored at 4ºC. Before sample water filtration blanks were created using deionized water utilizing the following method. Duplicate seawater samples were filtered within five days of collection, onto black 0.2 µm, 25 mm diameter, polycarbonate filters (GE Water & Process Technologies) and stained with 0.0468 gL^{-1} 3, 6-tetramethyl diaminoacridine (Acridine Orange). Prior to use, the filtering tower was rinsed with 5% HCl, deionized water and sample water before filtering sample water. The filters were mounted on glass slides with Cargille Type A immersion oil and

stored at -20ºC until counting. Slides were viewed using an Olympus epifluorescent microscope (100 x 1.30 oil objective) equipped with blue excitation (BP440, DM455, AFC + Y475) (Hobbie *et al*., 1977). A minimum of 600 cells per filter was enumerated.

Particulate Hydrocarbon

One and a half liters of seawater from the Nalgene bottles were filtered with a vacuum (5 psi) onto precombusted (500ºC for 12 hours) GF/F filters (25 mm diameter, Fisher). The filters were placed in precombusted aluminum foil squares (500ºC for 12 hours), and stored in the dark at -20ºC. Prior to use, the filtering tower was rinsed with 5% HCl, deionized water and sample water before filtering sample water. The samples then underwent sonicating extraction, using 100% dichloromethane and chloroform extracted water, to separate out the hydrocarbons. The recovered hydrocarbons were suspended in 500 µL of hexane, and transferred into gas chromatography vials. Two µL was injected into the GC-MS (6890N/5973inert, Agilent) by an auto-sampler. Compounds were separated using a DB-5ms column (0.25mm x 30 mm x 0.25 µm, Agilent 122-5532) with helium as the carrier gas (flow rate 1.3 $mLmin^{-1}$). The oven temperature (injection temperature) initially was 60ºC (held for 1 min) and programmed to increase at $8ºCmin^{-1}$ to 290ºC and held for 15 minutes. Typical operating conditions of MS with the scan acquisition mode were a 3 min solvent delay, ionization energy 70 eV, and scan per second over range electron m/z= 50 to 550 amu. A calibration curve was created with a range of standards from 0.4 $\mu g\mu L^{-1}$ to 6.4 $\mu g\mu L^{-1}$ of petroleum hydrocarbon (Hibernia crude oil, API 34.6) suspended in hexane. Each sample was then run at least three times, and an average was used for discussion. The particulate

hydrocarbon concentration obtained from a deionized water blank was removed from the sample concentrations before analysis.

2.2.3 Growth Studies

Approximately 18 L of seawater was collected using a 5 L Niskin bottle and transferred into insulated containers that had been serially rinsed with 5% hydrochloric acid, deionized water and sample water, and returned to the laboratory for processing. At the laboratory, particle free water was prepared by gravity filtering 14.4 L of sample seawater through a 0.2 µm polycarbonate membrane filter (Pall Life Sciences). A grazer-free <1.0 µm fraction of bacteria was prepared by filtering 3.6 L of seawater through 1.0 µm polycarbonate membrane filters (GE Water & Process Technologies) at low pressure (5 psi). All glassware and filtration apparatuses were 5% HCl washed and rinsed with deionized water. The filtered water was then combined in a ratio of 4:1 (0.2 µm membrane filtered: 1.0 µm membrane filtered) and placed into acid-washed 2 L Nalgene containers with each container receiving 1.5 L of the seawater dilution culture. Each Nalgene container had one of six substrate treatments for each temperature manipulation. The treatments were an unamended control (**+[C]**), the addition of inorganic nutrients (1 µM Na_2HPO_4 and 16 µM $NaNO_3$; "**+[I]**"), organic carbon (25 µM glucose; "**+[O]**"), inorganic nutrients plus organic carbon (1 µM Na_2HPO_4, 16 µM $NaNO_3$ and 25 µM glucose; "**+[I+O]**"), petroleum hydrocarbon (95.9 μgL^{-1} Hibernia crude oil, API 34.6; "**+[H]**") and the addition of 95.9 μgL^{-1} petroleum hydrocarbon (Hibernia crude oil, API 34.6) plus inorganic nutrients (1 µM Na_2HPO_4, and 16 µM $NaNO_3$; "**+[H+I]**"). After the addition of the nutrients, subsamples of fifty milliliters were taken from the 1.5 L

containers for initial bacterial abundance, and the remaining seawater was subdivided into 500 mL aliquots, and placed in three replicate 500 mL Nalgene incubation bottles. All incubation bottles were then placed in a constant temperature water bath and incubated in the dark, with one set of the six treatments at the ambient temperature at the time of collection and a second set at ambient temperature plus 2^{o}C. Subsamples of fifty milliliters were taken from each of the triplicate incubation bottles at time final, and all bacterial abundance samples were analysed as previously reported. Experiments during April and June were incubated for 72 hours, and incubations during August lasted 48 hours.

2.2.4 Statistical Analyses

Particulate hydrocarbon concentrations were determined from the GC-MS analysis using the following equation:

(Peak area of hydrocarbon$_x$/slope correction of hydrocarbon$_x$)*final volume = particulate concentration of hydrocarbon$_x$

Slope correction for each hydrocarbon compound was determined from the calibration curve. Total particulate hydrocarbon concentration was the sum of all hydrocarbon compound concentrations. The chromatographic peaks were discriminated and identified by comparing the mass spectra with those in the NIST MS library. The carbon preference index (CPI) the ratio of odd to even carbons present, was calculated from (Bakhtiari *et al*., 2009), as modified from Bray and Evans (1961):

$$\text{Overall CPI} = (\sum \text{odds } C_{15\text{-}33} + \sum \text{odds } C_{17\text{-}35})/2(\sum \text{evens } C_{16\text{-}34})$$

The CPI allows us to infer the potential source of the hydrocarbon, whether from natural biota (greater number of odd carbons; CPI >1) or anthropogenic input (greater number of even carbons; CPI <1).

All statistical analysis was done in MiniTab 16 statistical software (2010). General linear models and multi-factor ANOVAs were run first for both the ecological sample and growth study analyses, and every variable was found to have significant interactions and violated the assumptions of the model. Therefore, one-way ANOVAs were used to determine the effect of bay (Placentia and St. Mary's Bay), and sampling period, on the DOC and DON concentrations and chlorophyll a concentration, and to determine the effect of bay on the concentration of particulate hydrocarbon. For the growth study, multiple individual one-way ANOVAs were conducted to determine the effect of the five treatments (**+[C]**, **+[I]**, **+[O]**, **+[I+O]**, **+[H]**, **+[H+I]**), sample period/season (August 2012, April 2013, June 2013, and August 2013), temperature (ambient and ambient increased by 2^{o}C), and bays (Placentia and St. Mary's Bays), on bacterial growth rates. Tukey pairwise comparison test was used for post-hoc comparisons of response of the bacteria among treatments, within a sampling period, temperature and single bay.

For ecological samples without replicate measurements (i.e. POC, PON and bacterial abundance) from the same Niskin bottle, it is assumed that these samples were representative of the environment from which they were collected. The analytical precision of analysis of POC and PON was 0.4%, and the absence of replicate measurements meant comparison of the standard deviation from the enumeration of

bacterial abundance was used to determine the effect of bay, as well as sampling period, on the bacterial abundance.

2.3 Results

2.3.1 Ecological Samples

There was a higher concentration of both POC and DOC in St. Mary's than Placentia Bay for all sampling periods (Table 2.5), except during September when the concentration was greater in Placentia Bay (Tables 2.3 and 2.4). Generally, there was a significantly greater concentration of DON and PON in Placentia Bay than St. Mary's Bay, except during August and September (Tables 2.3, 2.4 and 2.5).

Generally, the DOC, and DON concentrations in both bays, were significantly greater ($p < 0.001$) and POC and PON concentrations were higher during September than during August or June (Tables 2.3 and 2.4).

The surface chlorophyll a concentrations were not significantly different between the two bays ($p = 0.279$), or among sampling periods ($p = 0.474$). The only significant difference (Table 2.6) for the chlorophyll a concentrations were at depth, between the bays during June 2013, where Placentia Bay had a higher concentration (Tables 2.3 and 2.4). For the deep collected chlorophyll a samples, there was no significant difference among sampling periods in St. Mary's Bay ($p = 0.295$). However, in Placentia Bay, the chlorophyll a concentration was significantly greater during September, and lower during April (Tables 2.3 and 2.6), than all other sampling periods.

There was generally a greater bacterial abundance in St. Mary's than Placentia Bay for all sampling periods, and depths, except during April and June when the abundance from the middle of the bay was greater in Placentia than St. Mary's Bay (Tables 2.1 and 2.2). Generally, in Placentia Bay, the bacterial abundance from the middle of the bay, was greater than the other sites within the bay (Table 2.1) whereas, in St. Mary's Bay, the bacterial abundance from the head of the bay, was greater than at the other sites within the bay (Table 2.2). The maximum bacterial abundances occurred during August and September in St. Mary's Bay, and during April and September in Placentia Bay (Tables 2.1 and 2.2).

Particulate hydrocarbon concentrations were 20.1 ± 2.0 μgL^{-1} (mean ± SD) in Placentia Bay (Station PB2*), significantly greater ($p = 0.005$) than the 6.0 ± 0.56 μgL^{-1} in St. Mary's Bay (Station SMB2*) (Figure 2.1, Table 2.7). The hydrocarbons found in both bays were aliphatic and between $C_{24}H_{50}$- $C_{32}H_{66}$. The mean CPI for Placentia Bay was <1 and for St. Mary's Bay was >1 (Table 2.7).

2.3.2 Growth Studies

2.3.2.1 Comparison of response to treatments

The response of bacteria to nutrients and petroleum hydrocarbons varied with bay, season, and treatment (Tables 2.8 and 2.9). In both bays, the bacterial growth rate in the **+[I]** treatment was never significantly different from the control (Figure 2.2), except in St. Mary's Bay, during April (Figure 2.2 D) where at ambient and elevated temperature, the growth rate in the control was significantly greater ($p= 0.005$ and $p= 0.006$, respectively) than in the **+[I]** treatment. In Placentia Bay, the growth rate in the **+[O]**

treatment was not significantly different from that in the control (Figure 2.2 A, C, E, G), except at elevated temperature, during August 2012, and June where it was significantly greater ($p < 0.001$ for both months). In St. Mary's Bay, the growth rate of the **+[O]** treatment was always significantly greater than in the control (Figure 2.2 B, D, F, H), except at ambient temperature during August 2012, where it was significantly lower ($p= 0.01$), and at elevated temperature, during June and August 2013, where rates were not significantly different from the control. In both Placentia and St. Mary's Bays, the bacterial growth rate in the **+[I+O]** treatment was significantly greater than the control (Table 2.8, Figure 2.2), except in Placentia Bay during April, at ambient temperature (Figure 2.2 C). In both bays, the growth rate in the **+[H]** and **+[H+I]** treatments were either not significantly different, or significantly lower than in the control, except during April (Figure 2.2 C, D). During April, at elevated temperature, the **+[H]** treatment, and **+[H+I]** treatment were significantly greater than the control ($p= 0.018$ and $p < 0.001$, respectively) in Placentia and St. Mary's Bays, respectively.

2.3.2.2 Comparison of the effects of temperature, within a treatment

The bacterial growth rate changed in response to incubations at different temperatures (Table 2.10, Figure 2.2). Table 2.10 shows the difference between temperatures from the one-way ANOVA, and the temperature at which the greater growth was observed. In Placentia Bay, there was a significant difference between the temperatures in at least one of the organic carbon treatments (**+[O]** or **+[I+O]**) during each of the sampling periods (Table 2.10). During all sampling periods, the growth rate in the organic carbon treatments was significantly greater at the elevated (i.e. ambient +

2°C) temperature than the ambient temperature, except during August 2013, where the growth rate was greater at ambient temperature (Table 2.10). For all other treatments and sampling periods, if there was a significant difference between temperatures, the growth rates were greater at ambient than elevated temperatures (Table 2.10), except during April, where the growth rate in the **+[H]** treatment was greater at elevated temperature.

In St. Mary's Bay, there was a significant difference between the two temperatures for all treatments, except during June and August 2013 (Table 2.10). During June, the bacterial growth rate in the **+[O]** treatment was not significantly different at the two temperatures. During August 2013, bacterial growth rate in only the **+[H]** treatment was significantly different ($p = 0.009$) at the two temperatures. During all sampling periods, the growth rate was greater at elevated than ambient temperature, except during August 2012, in the **[C]** and **+[I]** treatments, which were greater at ambient temperature (Table 2.10).

2.3.2.3 Comparison of response of treatments to sample period

The response of bacteria to the nutrient and hydrocarbon treatments was complex and changed with season and location (Tables 2.8 and 2.9, Figure 2.2). The addition of inorganic nutrients (**+[I]**) did not increase the rates of bacterial growth, relative to the control in any of the 16 experiments (i.e. four experiments, two bays, and two temperatures). During April, the growth rate during one experiment in St. Mary's Bay, at both temperatures, was significantly lower than the control when inorganic nutrients were added (Figure 2.2 D). The pattern of response was similar in both bays (Figure 2.3).

The addition of organic carbon only (+**[O]**) increased the growth rate relative to the control, in six of the 16 experiments. The growth rate during one experiment in August, in St. Mary's Bay was lower than the control. In the remaining experiments, the addition of organic carbon did not influence bacterial growth, relative to the control (Figure 2.3). The pattern of response was similar in both bays during August 2012, and 2013 at elevated temperature (Figure 2.3 A, B). The pattern changed between the two bays during April, June and August 2013 at ambient temperature (Figure 2.3 C, D, E, F, G, H).

A consistent pattern during all sample periods, was enhanced growth rates in the **+[I+O]** treatments relative to the control, in both Placentia and St. Mary's Bays and at both temperatures (Figure 2.3 A, B, C, D, E, F, G, H), except in Placentia Bay, during April, at ambient temperature, where the bacterial growth rates were not significantly different in any treatment (Figure 2.2 C). The pattern of response was generally similar in both bays (Figure 2.3).

There were 24 experiments in total for bacterial growth rates that included the addition of petroleum hydrocarbons, either alone (+**[H]**) or in combination with inorganic nutrients (+**[H+I]**) i.e. three experiments, two bays, two hydrocarbon treatments, and two temperatures. During four experiments (two each in both Placentia and St. Mary's Bays), the growth rates were lower in the presence than absence of petroleum hydrocarbons (Figure 2.3 D, E, H). In Placentia Bay, both of these experiments were during June, at both ambient and elevated temperature. In St. Mary's Bay, the two experiments were during April, and August 2013, both at ambient temperature. In the remaining

experiments, there was no significant effect, relative to the control, of the petroleum hydrocarbons on bacterial growth rates (Figure 2.3 C, D, F, G, H). Generally, the pattern of response differed between both bays, except during August 2013 (Figure 2.3 G, H).

The bacterial growth rate in each nutrient and hydrocarbon treatment was significantly different between sampling periods (Table 2.11, Figure 2.3). Table 2.11 shows the difference between sample periods, from the one-way ANOVA. In Placentia Bay, for all nutrient and hydrocarbon treatments, at ambient and elevated temperature, the growth rates were generally the greatest during August (1.4 to 1.6 d^{-1}) and the lowest growth rates were during April for all treatments (no growth to 0.3 d^{-1}). In St. Mary's Bay, at ambient temperature, the growth rate was the greatest during June and August at both ambient (~0.7 to 1.7 d^{-1}) temperatures and elevated (~0.9 to 1.7 d^{-1}) temperatures. The lowest growth rates were during April (Table 2.11, Figure 2.2).

2.3.2.4 Comparison of response to treatments, between Placentia and St. Mary's Bays

The response of bacterial growth rate to nutrient and hydrocarbon additions differed in Placentia and St. Mary's Bays (Table 2.12, Figures 2.2 and 2.3). Table 2.12 shows the difference between the two bays, from the one-way ANOVA, and the bay in which the greater growth was observed. During August 2012, the growth rate was 1.4- to 3-fold greater for all treatments in Placentia Bay than St. Mary's Bay (Figure 2.2 A, B). The bacterial growth rate was generally greater in St. Mary's Bay than Placentia Bay, for all treatments (Figure 2.2 C, D, E, F) during April (2- to 35-fold) and June (2.5- to 5-fold). During August 2013, the pattern was more complex and treatment dependent.

Bacterial growth rates in the **+[O]** and **+[I+O]** treatments were greater in St. Mary's than Placentia Bay (1.1- to 1.2-fold), whereas for the **+[H]** and **+[H+I]** treatments, the growth rates were generally greater in Placentia, than St. Mary's Bay (1.2- to 1.8-fold, Table 2.12).

2.4 Discussion

2.4.1 Response of bacterial growth to inorganic nutrient, organic carbon and petroleum hydrocarbon additions

This study appears to be unique in its approach on the growth experiments, by combining the identification of multiple nutrient, petroleum hydrocarbon, and temperature effects on the same bacterial populations, enabling direct comparisons. Our study found that the addition of both inorganic nutrients and organic carbon had the greatest effect on the bacterial community, stimulating growth, while the effect of organic carbon only appears to be influenced by temperature. We have shown that small inputs of petroleum hydrocarbons into subarctic marine systems appear to either have no influence or inhibit bacterial growth over the short term.

The addition of only organic carbon generally did not appear to increase bacterial growth rates in Placentia Bay (Figure 2.2), except during August 2012, and June 2013 at elevated temperatures (Figure 2.2 A, E), suggesting that it was the interaction of organic carbon and temperature that increased the growth rate, as it was only at elevated temperature that enhanced growth occurred. Glucose is a bioavailable energy and carbon source for bacteria, and various factors can control bacteria utilization of bioavailable

carbon. These factors include temperature (Pomeroy *et al*., 1991; Shiah and Ducklow, 1994), and inorganic nutrient availability (Rivkin and Anderson, 1997; Thingstad *et al*., 1998). Our results suggest that both temperature and carbon were limiting factors to bacterial growth in this bay. The POC and DOC concentrations were the lowest during these two months (Table 2.3) suggesting that the bay was potentially limited in the availability of organic carbon. In St. Mary's Bay, the addition of only organic carbon generally increased the bacterial growth rates, except during August 2012. These results suggest that this bay may have been initially limited in organic carbon (Kirchman, 1990; Carlson and Ducklow, 1996; Church *et al*., 2000; Carlson *et al*., 2002; Kirchman *et al*., 2005).

Our study suggests that nutrient loading in marine systems can have major effects on the growth of the bacterial community. The addition of both inorganic nutrients and organic carbon (+**[I+O]**) generally led to the greatest response, and increase in the bacterial growth rates in both bays supporting the hypothesis that bacteria are co-limited by organic carbon and inorganic nutrients. Similar increases in the bacterial growth rates with a maximum when both energy (organic carbon) and limiting nutrients (such as inorganic nutrients) are present, have been noted elsewhere (Rivkin and Anderson, 1997; Shiah *et al*., 1998; Church *et al.,* 2000; Carlson *et al*., 2002; Cuevas *et al*., 2011; Sebastián and Gasol, 2013).

The results from our study suggest that the addition of low level petroleum hydrocarbons did not stimulate bacterial growth during short term incubations, and suggests that the toxic nature of petroleum hydrocarbon may be inhibitory to some of the

subarctic bacterial communities. The addition of low levels of petroleum hydrocarbons, either alone (**+[H]**) or with inorganic nutrients (**+[H+I]**), appeared to either have had no effect on the bacterial growth or to inhibit growth rates (Figure 2.2). The bacterial communities were either unable to utilize the hydrocarbon compounds as a carbon source, their growth was inhibited by the toxicity, or the concentration was too little and the community remained carbon limited. Petroleum hydrocarbons can be toxic to marine organisms, such as phytoplankton, with aromatic hydrocarbons more toxic than aliphatic compounds (Nicodem *et al*., 1997; Harayama *et al*., 1999; Megharaj *et al*., 2000; Pumphrey and Madsen, 2007; Bacosa *et al*., 2012; Ozhan *et al*., 2014). In this study we used petroleum hydrocarbon concentrations of 95.9 μgL^{-1}, a much smaller concentration in comparison to other studies that used a range from 100 mgL^{-1} to 17 000 mgL^{-1} of crude petroleum hydrocarbons (Kasai *et al*., 2002ab; Cappello *et al*., 2007; Coulon *et al*., 2007; Paissé *et al*., 2010; Jiménez *et al*., 2011; Baelum *et al.,* 2012). It has been suggested composition of the petroleum hydrocarbons rather than the absolute concentrations may determine the effect and potential toxicity to phytoplankton communities (Shailaja, 1988). In contrast to our results, bacterial abundance and growth have been reported to be stimulated by the input of petroleum hydrocarbon, including large scale petroleum spills (Delille and Vaillant, 1990; Siron *et al*., 1995; Delille *et al*., 1997; Kasai *et al*., 2001; Delille and Pelletier, 2002; Harayama *et al.,* 2004; Røberg *et al*., 2007; Brakstad *et al*., 2008; Lo Giudice *et al*., 2010; Kostka *et al*., 2011; Valentine *et al*., 2012). The growth has been suggested to be from an increase in the abundance of hydrocarbon degrading bacteria (MacNaughton *et al*., 1999; Yakimov *et al*., 2004; Head *et al*., 2006; Cappello *et al*., 2007; Atlas and Hazen, 2011; Jiménez *et al*., 2011; Baelum *et al*., 2012; Jurelevicius

et al., 2013). When Arctic sea ice was contaminated in the field with crude petroleum hydrocarbons, bacterial growth was stimulated (Brakstad *et al*., 2008). The enrichment of Antarctic seawater with diesel hydrocarbons also showed the growth of bacteria at low temperatures (4^{o}C; Lo Giudice *et al*., 2010). The Deepwater Horizon spill stimulated bacterial abundance in contaminated sands (Kostka *et al*., 2011), as well as feeding a bloom of hydrocarbon degrading bacteria in the seawater (Valentine *et al*., 2012).

We have shown that small inputs of petroleum hydrocarbons into subarctic marine systems can have inhibitory effects on bacterial growth. Recently, there has been a proposal of trans-Arctic shipping routes, because of the decreased sea ice coverage (Smith and Stephenson, 2013; Stephenson *et al*., 2013). The movement of tankers and ships in this sensitive ecosystem will have significant impacts on the bacterial communities, specifically on the abundance and growth characteristics and inhibition may occur. The potential inhibition could have a negative effect on the carbon cycle, as bacteria are main players in this marine cycle (Kirchman, 2000). Bacteria influence the movement of carbon through the microbial loop, as they incorporate organic carbon in their own biomass, as well as remineralize carbon releasing inorganic carbon back into the ocean (Pomeroy *et al*., 2007). The exact consequences can be difficult to predict, however, a reduction in bacterial growth suggests that there will be less organic carbon available to be returned to higher trophic levels, such as grazers that feed on bacteria, as well as a loss of remineralized carbon in the ocean.

2.4.2 Response of bacterial growth to temperature

Our results suggest that climate change has potentially large implications in Newfoundland waters and elsewhere, with observable stimulation of bacterial growth with the small increase of just 2°C in the ocean. Our results show the bacterial growth response to temperature is influenced by limiting nutrients, and potentially suggest that Placentia Bay may be responding to extra organic carbon loading. An increased temperature of 2°C generally increased the bacterial growth rates for all treatments in St. Mary's Bay, and for organic carbon treatments in Placentia Bay (Figures 2.3 A, B and 2.4 A, B). In Placentia Bay, the 2°C increase in temperature appeared to only have an effect on growth when organic carbon was present, with or without inorganic nutrients, suggesting that availability of organic carbon was a crucial factor in determining bacterial growth rates. At 8°C and above inorganic nutrients plus organic carbon (+**[I+O]**) were required for stimulated growth, with the influence diminishing at 19°C in both bays (Figure 2.4 A, B). At 19°C organic carbon, and petroleum hydrocarbons plus inorganic nutrients are required to stimulate growth in Placentia Bay. In St. Mary's Bay, bacterial growth begins to diminish at a lower temperature, around 11°C, and stimulation of growth required organic carbon (with or without inorganic nutrients) until 19°C (Figure 2.4 B). Bacteria are important in biogeochemical cycles in the ocean, and their response to a small change in temperatures in ecologically sensitive regions such as the Arctic, can influence the overall response of the oceans to climate change (Kirchman *et al*., 2009). The temperature in the Arctic Ocean has been increasing over the past 100 years, with a peak increase of approximately 5°C observed in 2007 (Steele *et al*., 2008). Models predict the Arctic can be ice free by 2040 (Holland *et al*., 2006). Greater bacterial growth in cold

marine environments in response to increasing temperature particularly when supplied with nutrients, could increase bacterial respiration and reduce the efficiency of the biological pump, potentially influencing climate change through the movement of carbon dioxide. Increased respiration can release a greater amount of carbon dioxide that is in contact with and transferred to the atmosphere, reducing the amount of carbon available to be exported and stored in the deep ocean by the biological pump (Ducklow *et al*., 2001).

Our results suggest a more complicated and integrated relationship between temperature and nutrients with bacterial growth than some previous studies. It has been suggested that the affinity for the uptake of substrates decreases with decreasing temperature, when the temperature is below the bacteria's optimum (Nedwell, 1999), and bacterial growth has been positively related to increasing temperature (Hoch and Kirchman, 1993; Shiah and Ducklow, 1994; Ochs *et al.*, 1995; Felip *et al*., 1996). The findings of our study support recent studies that indicated that even at low temperatures, and in cold water environments, temperature and nutrients have a similar influence on bacterial species in both polar and temperate regions (Garrett *et al*., 2003; Rodríguez-Blanco *et al*., 2013). Polar systems (Arctic Ocean and Ross Sea) were found to have lower average bacterial production rates than more temperate ocean systems, although the ranges were similar (Kirchman *et al*., 2009). Differences and patterns in the rates were not explained by temperature effect alone, rather by an interaction of temperature, dissolved organic matter, and other environmental factors (Kirchman *et al*., 2009). The effects of nutrients have even been suggested to be greater than temperature, with

maximum influence when both high nutrient and temperature were present, suggesting the effects of temperature can be more influential in nutrient rich environments (Christoffersen *et al*., 2006; Kirchman *et al*., 2009; Degerman *et al*., 2013; Özen *et al*., 2013). Biodegradation of hydrocarbons has also been found to occur at low temperatures ($\leq 5^{o}C$), and in cold climates such as the Arctic and Antarctic by adapted bacterial communities (Whyte *et al*., 1998; Deppe *et al*., 2005; Gerdes *et al*., 2005; Brakstad and Bonaunet, 2006).

2.4.3 Response of bacterial growth to sampling period

This study suggests that the introduction of shipping, or increase in anthropogenic activities that include petroleum hydrocarbon input into the seawater, can have significant effects on the seasonal variation of the bacterial growth. The response of bacterial growth to petroleum hydrocarbons determined in this study appears to be the opposite of previously observed seasonal activity. We observed the stimulation of bacterial growth in the presence of petroleum hydrocarbons during April only (Figure 2.2 C, D). Therefore, sampling period appears to significantly affect the response of bacterial growth to petroleum hydrocarbon addition, and suggests that petroleum hydrocarbons may have a greater influence at lower temperatures. In contrast, bacterial productivity has been shown to be high in the summer, related to phytoplankton blooms and DOM (Ducklow *et al*., 2001; Yager *et al*., 2001; Sherr and Sherr, 2003), depleting the water mass of nutrients and DOM by late summer (Ducklow *et al*., 2001; Holmes *et al*., 2008). Our results also contrast some previous studies that have suggested bacteria from cold environments have decreased metabolism and petroleum hydrocarbon degradation rates in the low water

temperatures (Nedwell, 1999; Margesin *et al*., 2003; Michaud *et al*., 2004). It has also been suggested that petroleum hydrocarbon degradation in seawater in the winter is slower than in the summer (Siron *et al*., 1995; Piehler and Paerl, 1996). However, bacteria from cold water environments can still have significant potential for biodegradation at low temperatures, and recent studies on Arctic and Antarctic seawater determined that indigenous bacteria can degrade petroleum at temperatures as low as 0-7^{o}C (Delille and Vaillant, 1990; Delille *et al*., 1997; Yakimov *et al*., 2004; Deppe *et al*., 2005; Gerdes *et al*., 2005; Brakstad and Bonaunet, 2006; Coulon *et al*., 2007; Brakstad *et al*., 2008; Lo Giudice *et al*., 2010), suggesting that the influence of petroleum hydrocarbons determined in our study may be because the bacteria are already cold adapted.

Seasonal variation has not been well documented in most ocean regions, and is much better known in coastal regions where there are significant seasonal changes in the abundance and growth of the bacterial community (Nikrad *et al.*, 2012; Chen *et al*., 2014). The highest bacterial growth rates in Placentia Bay were during August, and in St. Mary's Bay they were during August and June (Figure 2.2 F, G, H). The lowest bacterial growth rates were during April in both bays, when temperature was the lowest (Figure 2.2 C, D). This is consistent for Newfoundland waters, where bacterial abundances were found to increase from July to October, and growth was at a seasonal minimum from January to April, during periods of low sea water temperatures (< 0^{o}C) (Putland, 2000). In Newfoundland waters, the spring phytoplankton bloom occurs during April and May (Navarro and Thompson, 1995; Choe and Deibel, 2008; Thompson *et al*., 2008), and

although bacterial production was found to remain low during this bloom (Pomeroy and Deibel, 1986), this may not be a consistent feature in Newfoundland coastal waters (Rivkin *et al*., 1996) as local factors such as currents and freshwater input can vary. The chlorophyll a concentration was the greatest during August and September (Tables 2.3 and 2.4), indicating a large fall bloom of phytoplankton. The chlorophyll a concentration does not appear to suggest a spring bloom during April and May, though the low sampling frequency may have missed the bloom. The POC and DOC concentrations also suggest a fall bloom with the highest concentration in both bays during August and September, as well as a spring bloom during April, with the second highest concentrations for both bays (Tables 2.3 and 2.4). The chlorophyll a concentrations and bacterial growth rates appeared to be the highest during the same sampling period (August; Tables 2.3 and 2.4, Figure 2.2), suggesting bacterial growth may be related to phytoplankton blooms, consistent with previous studies (Sherr and Sherr, 2003; Buchan *et al*., 2014), or both may be controlled by the same factors (Rivkin and Anderson, 1997; Kirchman, 2000).

2.4.4 Response of bacterial growth to location (bays)

Our study indicates that there is petroleum hydrocarbon contamination in Placentia Bay, and the source is suggested to be from the movement of tankers and shipping, or the activity associated with the petroleum hydrocarbon refinery in Placentia Bay. The bacterial growth rates with the addition of petroleum hydrocarbons were significantly greater in Placentia Bay, than St. Mary's Bay, during August (Table 2.12), and there was a significantly greater concentration of particulate hydrocarbons (20.1 ± 2.0

μgL^{-1}) in Placentia Bay than in St. Mary's Bay (6.0 ± 0.56 μgL^{-1}). The concentration of total n-alkanes was 8.2 μgL^{-1} in uncontaminated Antarctic waters, which were similar or lower than values for the open ocean (Cripps and Priddle, 1991; Cripps, 1992). The particulate aliphatic hydrocarbon concentration from the Scotian Shelf to the Bedford Basin, Nova Scotia was 0.35 to 2.8 μgL^{-1} on the shelf increasing to 8.1 to 9.5 μgL^{-1} in the Bedford Basin, close to an industrial area with suggested contamination (Parrish, 1987; Parrish *et al*., 1988). In the geographically close region of Conception Bay, Newfoundland, low concentrations of particulate hydrocarbons (<10 kDa) were found from 0.28 to 3.0 μgL^{-1} and colloidal hydrocarbons from 0.01 to 0.04 μgL^{-1} (Liu *et al.*, 1998; Liu and Parrish 1996). Low concentrations of particulate hydrocarbons were also found in the relatively uncontaminated Cape Hatt off of Baffin Island, from 0.2 to 0.5 μgL^{-1} (Cretney *et al*., 1987). These reports provide a baseline for uncontaminated waters. The particulate hydrocarbon concentrations from this study suggest that both Placentia and St. Mary's Bays have relatively high particulate hydrocarbon concentrations, and potentially indicating a greater input of hydrocarbons in Placentia Bay. The CPI for Admirals Beach (SMB2), from St. Mary's Bay was close to or higher than 1 (Table 2.7), indicating a greater number of odd over even carbon homologs. This ratio suggests the hydrocarbons are predominantly from algae and cyanobacteria or terrestrial plants (Punyu *et al*., 2013). The CPI for Argentia (PB2), from Placentia Bay was less than 1 (Table 2.7), indicating a greater number of even over odd carbon homologs. This ratio suggests the hydrocarbons are predominantly from microorganisms including diatoms and petroleum hydrocarbons (Punyu *et al*., 2013). The CPI value along with the particulate hydrocarbon concentration suggests that Placentia Bay has anthropogenic petroleum hydrocarbon

contamination. In contrast, the hydrocarbon concentration may be slightly elevated in St. Mary's Bay, but does not appear to be from petroleum contamination.

The bacterial response to nutrient and petroleum hydrocarbon additions differed between the two bays (Figure 2.3) with significantly greater growth rates in Placentia Bay with the addition of petroleum hydrocarbons, likely due to different nutrient limitations and function of the community enabling a different response. During August 2012, the growth rates in all treatments and the control were greater in Placentia Bay than St. Mary's Bay (Figure 2.2 A, B), while for April and June, they were greater in St. Mary's Bay than Placentia Bay (Figure 2.2 C, D, E, F). During all three of these sampling periods the carbon (POC and DOC) and nitrogen (PON) concentrations were greater in St. Mary's Bay during August 2012 (Tables 2.3 and 2.4), potentially explaining the greater growth rates for this bay, including the control (Tables 2.3 and 2.4). During August 2013, the bacterial response in the two bays was different. The bacterial growth rates with the addition of organic carbon was significantly greater in St. Mary's Bay (Table 2.12), suggesting that this bay was limited in organic carbon, allowing a greater increase in bacterial growth when it was supplied. These results are supported by a significantly lower concentration of both POC and DOC in St. Mary's Bay for this month (Table 2.4). Salinity at all sites during all sampling periods were close to previously reported values from Newfoundland waters (Choe and Deibel, 2008), except at site SMB1 at the top of the bay in St. Mary's Bay during June and September (Appendix A), indicating a small freshwater influence at this site. The bacterial growth results suggested that overall the response to petroleum hydrocarbons was similar in both bays (Figure 2.2). However,

during a single sample period the growth rates were always greater in one bay over the other for all treatments, except during August, where the bacterial growth rates were greater in Placentia Bay for the petroleum hydrocarbon treatments only (Table 2.12). These results combined with the suggestion that there was a low concentration of petroleum hydrocarbons in Placentia Bay suggests that the anthropogenic input may have primed the community for hydrocarbon degradation during August only, allowing greater bacterial growth rates. It has been well documented that large petroleum hydrocarbon spills, from ships or wells, such as the Exxon Valdez, Deepwater Horizon, and Prestige spills, have major effects on the marine ecosystem (Bragg *et al*., 1994; Alonso-Gutiérrez *et al*., 2009; Hazen *et al*., 2010; Valentine *et al*., 2012; Acosta-González *et al*., 2013). We suggest that even without the risk of large scale petroleum hydrocarbon spills, the constant movement and low input of petroleum hydrocarbons from tankers and ships will affect the bacterial community's growth and abundance. This study provides a framework for the potential short term effects of low concentrations of petroleum hydrocarbons on cold water bacterial communities.

It has been shown that bacterial community structure changes with latitude, and oceanic regions show distinct bacterial communities (Wietz *et al*., 2010; Ghiglione and Murray, 2012). Our results suggest that even close geographical regions can have differing bacterial community functions and responses. Therefore, environmental factors, such as global warming, or nutrient loading, may invoke a different response from the bacterial communities within a region. We suggest that the bacterial community may not respond similarly within oceanic regions. Rather, the bacterial consortium and potentially

even the bacterial food web have the ability to respond differently, or more individually than originally thought. Future studies should not rely on a single community to be representative of the examined ocean, or region.

2.4.5 Conclusion and future directions

There have been few studies on the effect of chronic low levels of petroleum hydrocarbons on marine bacterial communities (Paissé *et al*., 2008), and this study is the first to directly compare these effects to those observed from temperature, and nutrient additions. The incubation experiments conducted in this study indicated that organic carbon and inorganic nutrient enrichment can increase bacterial growth. However, low level petroleum hydrocarbon addition generally does not increase bacterial growth, sometimes even inhibiting growth. Global warming has been suggested to increase the nutrient supply in the Arctic by thawing of frozen tundra releasing more nutrients into the Arctic Ocean from run off or rivers (IPCC, 2007; Kirchman *et al*., 2009), or the loss of ice coverage may increase upwelling from the wind, bringing nutrient rich deep water to the surface (Carmack and Wassmann, 2006), or from the potential increase in anthropogenic activity and fertilizer use in the area. We suggest that this will have a large impact on the marine bacterial community in the sensitive Arctic and subarctic regions, likely increasing the bacterial growth and abundance. The general absence of a response of the bacterial community to petroleum hydrocarbon input in either bay indicates that the suggested contamination in Placentia Bay may not be great enough to stimulate growth of hydrocarbon degraders to an observable level, or the potential toxic effects on other species outweighed this growth. These results can have broad implications, especially for

the recently proposed trans-Arctic shipping routes, as we suggest there will be significant effects on the bacterial community with simple everyday movement of tankers.

Global warming has already occurred in the Arctic, with a peak increase of 5ºC observed during 2007 (Steele *et al*., 2008), and our results suggest that changes in temperature will also have significant impacts on the bacterial growth. Bacteria incorporate organic carbon in their own biomass, and remineralize carbon releasing inorganic carbon back into the ocean (Pomeroy *et al*., 2007). Bacteria are a major part of the carbon cycle, and could change the overall patterns of carbon flux in the ocean (Azam and Malfatti, 2007) with changes in temperature.

Sampling period, and location also have significant effects on the response of bacteria. We suggest that future studies will need to include a range of locations to sample the bacterial community within oceans or regions, as the community response may vary within the same region. The response of one community cannot represent the entire region, and makes responses to environmental factors even more difficult to predict.

Some studies report that the effect of petroleum hydrocarbon inputs may not be observable immediately, but can occur after 14 days (Paissé *et al*., 2010). The current experiment was incubated for 48 to 72 hours to reduce potential bottle effects, therefore, we may have not observed the full response of the community to hydrocarbon input, and could be a future consideration, to observe the response over a longer period of time. This study was only able to report particulate hydrocarbon concentration from one sampling period. Future studies could focus on the hydrocarbon concentrations over multiple sampling periods, as there is greater activity in Placentia Bay during the summer months

due to ferry activity. It has also been reported that hydrocarbon concentration appears to increase during winter months, as fuel use increases (Parrish, 1987; Liu *et al*., 1998). This study only used one petroleum hydrocarbon source. Future studies could utilize a range of petroleum hydrocarbon types as it appears in this study that the composition could have a greater effect on the response of the bacterial community than the amount. The current experiment also removed grazers from the incubations, suggesting the bacterial growth and abundance observed may be an overestimate of *in situ* communities. Therefore, the full extent of anthropogenic change may not be represented here, and this study may be showing a larger influence from these inputs than if grazing interactions were included.

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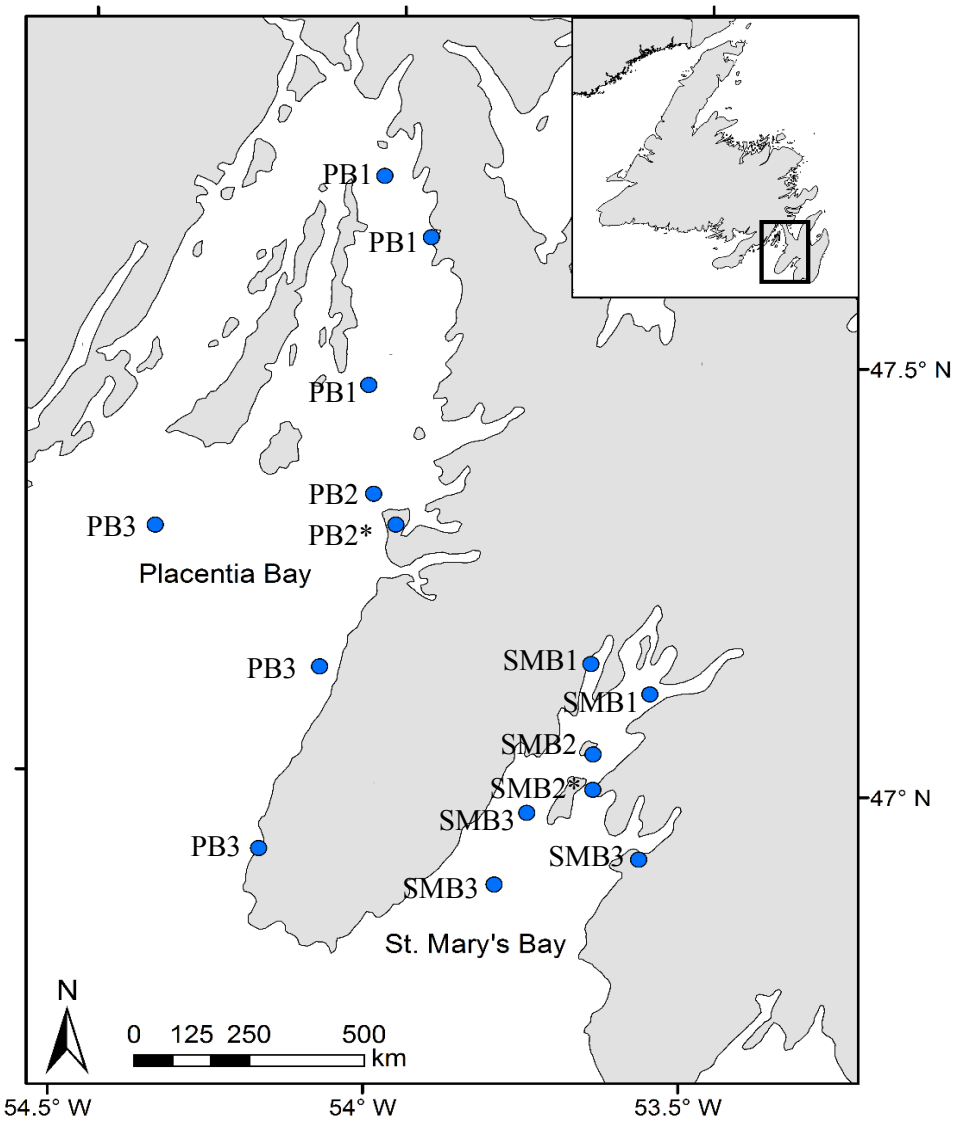


Figure 2.1. Sample site locations in Placentia and St. Mary's Bays, Newfoundland. Growth study samples were collected from sites PB2* and SMB2*, further described in Tables 2.3 and 2.4.

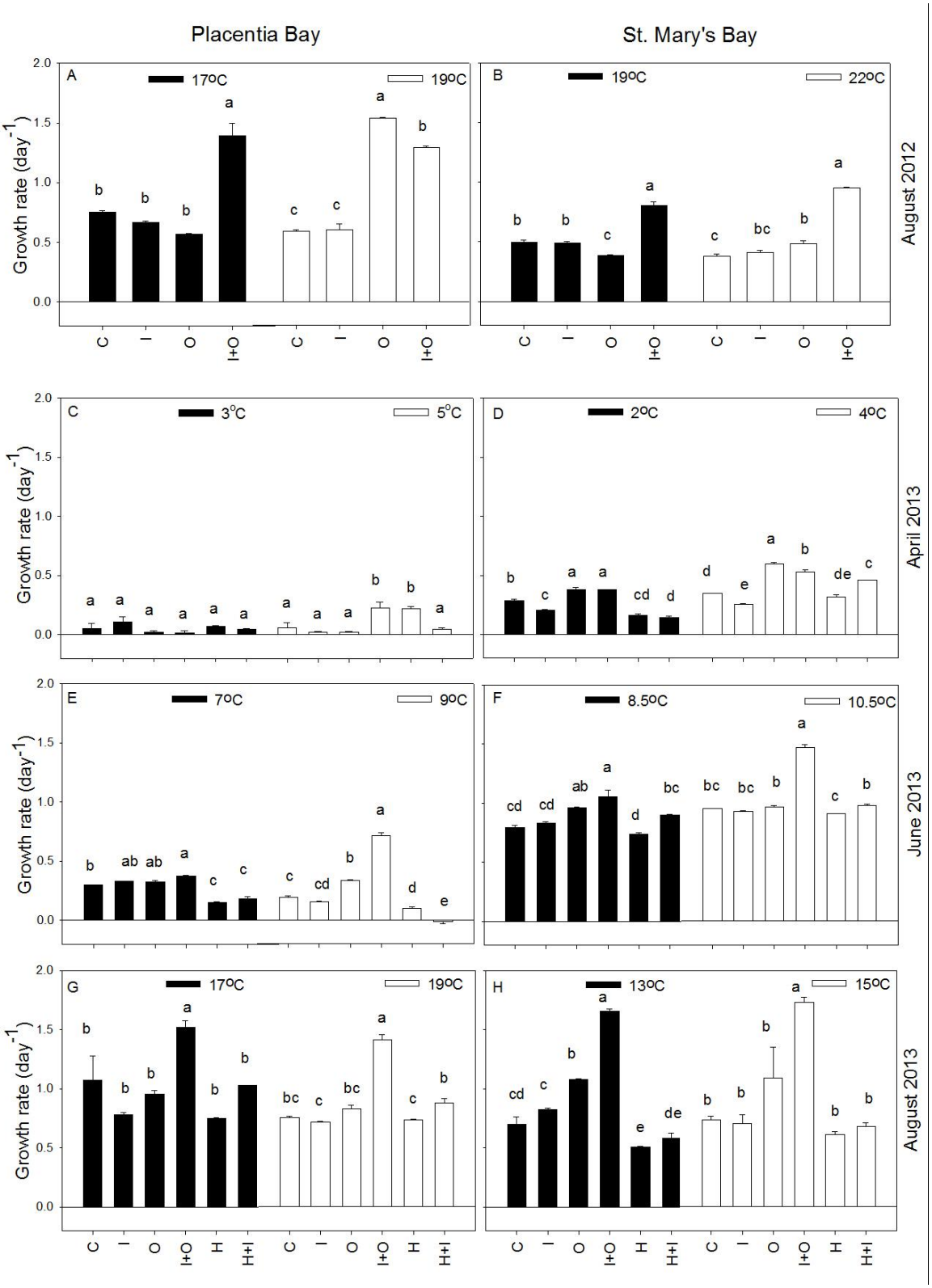

Placentia Bay
St. Mary's Bay
August 2012
April 2013
June 2013
August 2013
Growth rate (day-1)
A
17ºC
19ºC
B
19ºC
22ºC
C
3ºC
5ºC
D
2ºC
4ºC
E
7ºC
9ºC
F
8.5ºC
10.5ºC
G
17ºC
19ºC
H
13ºC
15ºC
C
I
O
I+O
H
H+I

Figure 2.2. Growth rate (d^{-1}) of bacteria during 48 or 72h incubations with inorganic nutrient, organic carbon and petroleum hydrocarbon additions at two temperatures, from Placentia Bay and St. Mary's Bay, during August 2012 (Panels A and B), April (Panels C and D), June (Panels E and F) and August 2013 (Panels G and H).

Vertical bars are the mean growth rates (n= 3). Different letters above the vertical bars represent mean growth rates that are significantly different (determined by Tukey's pairwise comparison test). Vertical bars that have two letters (ex. 'ab') are not significantly different from either letter (both 'a' and 'b') and are only compared within a single temperature. Error bars are standard deviation. The treatments were an unamended control (C), and additions of inorganic nutrients (I), organic carbon (O), inorganic nutrients plus organic carbon (I+O), hydrocarbons (H) and hydrocarbons plus inorganic nutrients (H+I).

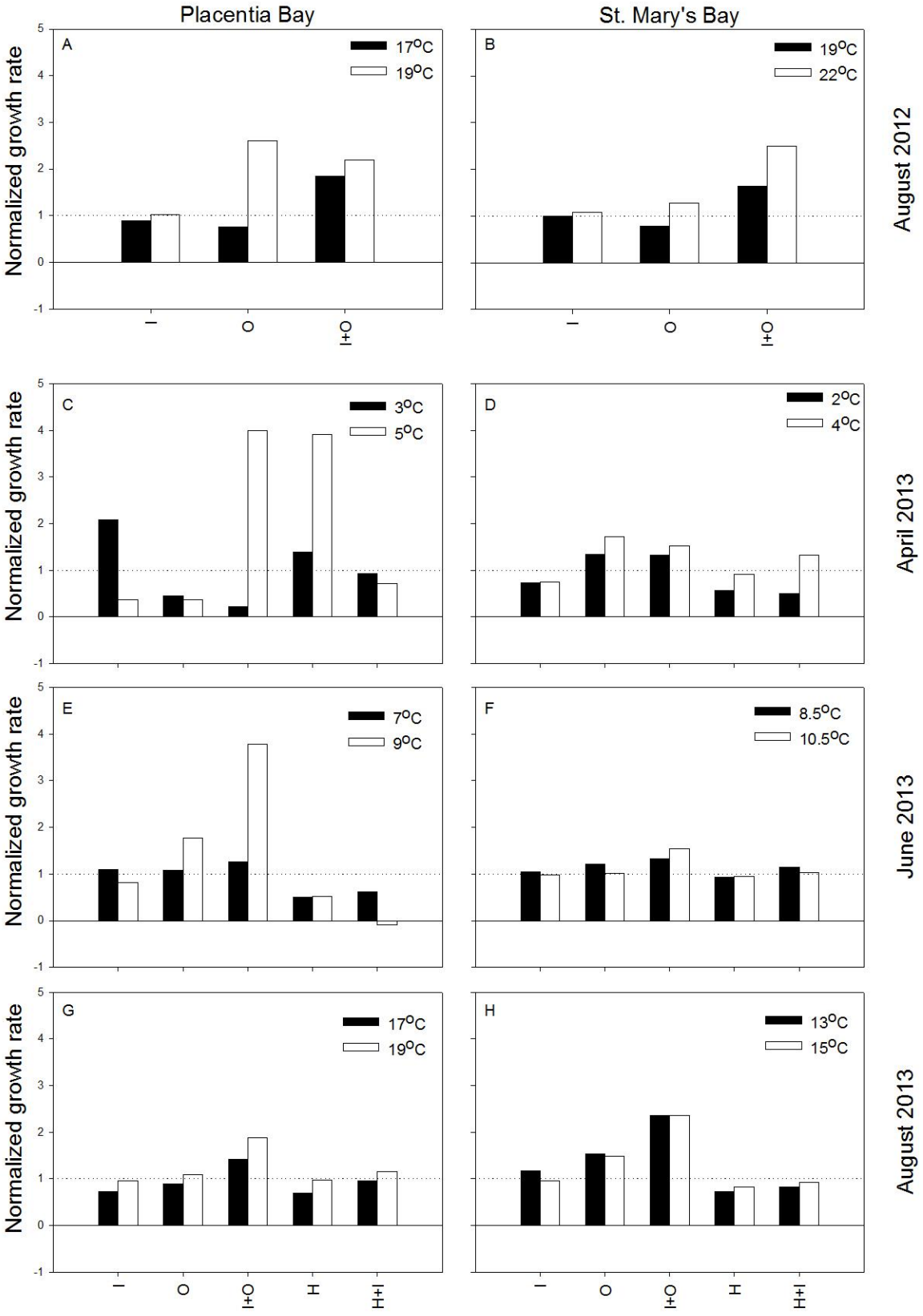
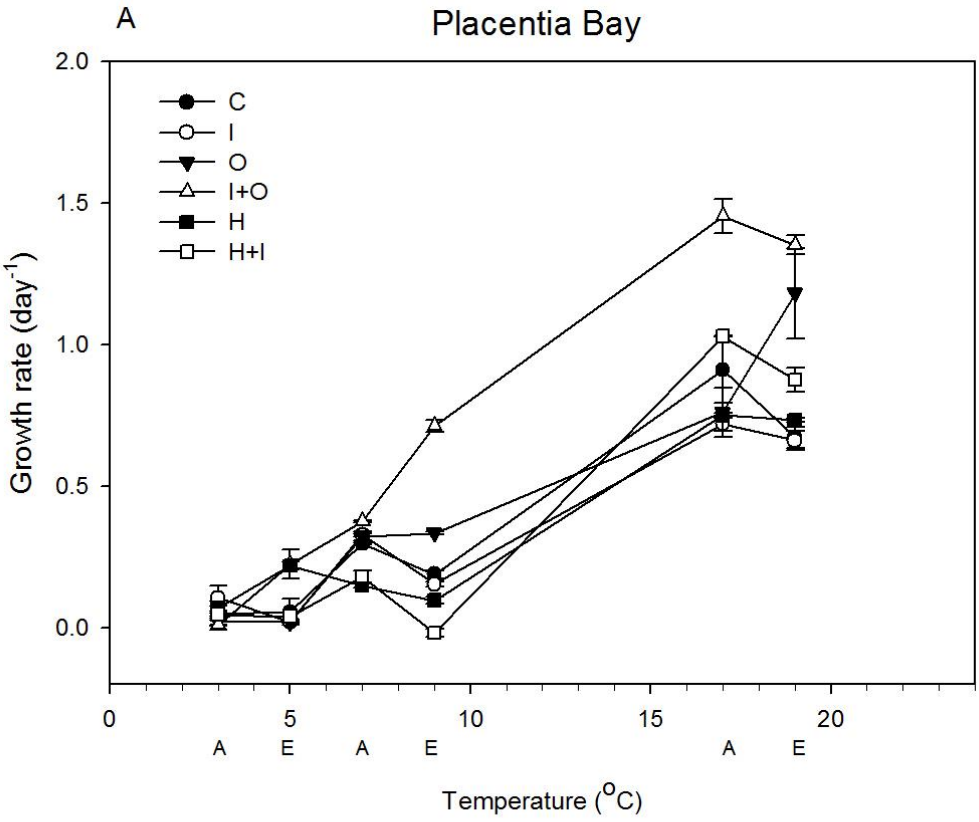

Placentia Bay
St. Mary's Bay
Normalized growth rate
A
17°C
19°C
B
19°C
22°C
August 2012
C
3°C
5°C
D
2°C
4°C
April 2013
E
7°C
9°C
F
8.5°C
10.5°C
June 2013
G
17°C
19°C
H
13°C
15°C
August 2013
I
O
I+O
H
H+I

Figure 2.3. Effect of inorganic nutrient, organic carbon and petroleum hydrocarbon additions on bacterial growth rate (d^{-1}) from Placentia and St. Mary's Bay during August 2012 (Panels A and B), April (Panels C and D), June (Panels E and F) and August 2013 (Panels G and H).

The nutrient, carbon and hydrocarbon addition growth rates are normalized to the control (growth rate in treatment/growth rate in control). The horizontal line denotes a normalized growth rate of 1.0 (control = treatment).


A
Placentia Bay
C
I
O
I+O
H
H+I
Growth rate (day-1)
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
A E A E A E
Temperature (OC)

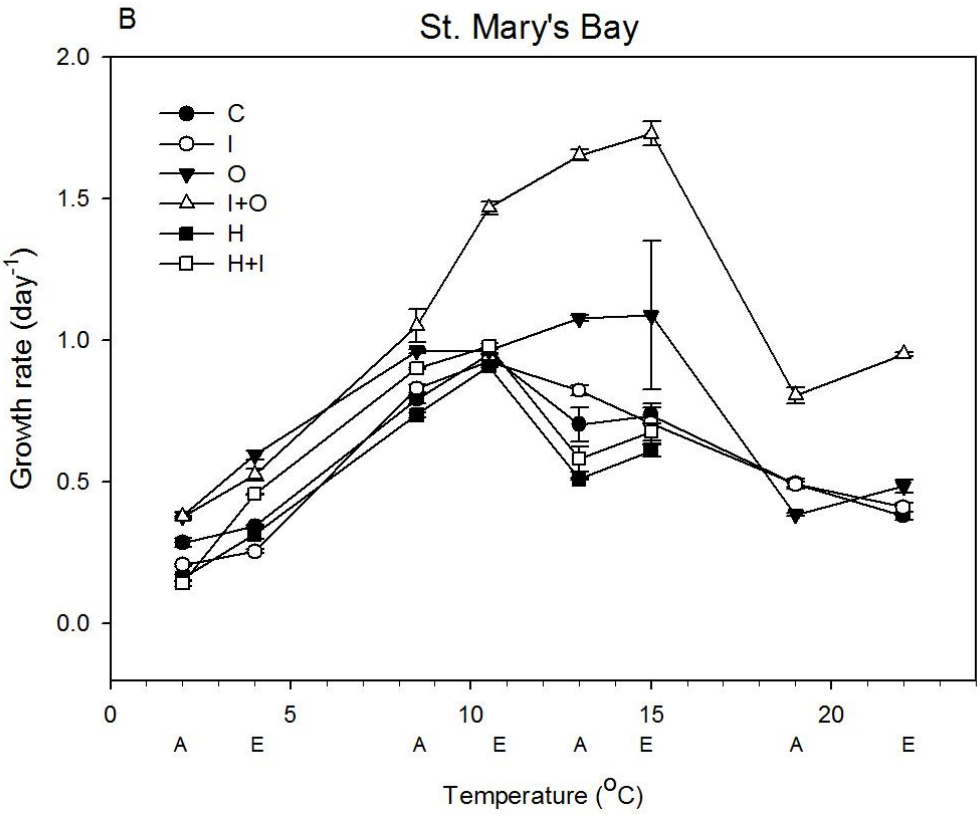

B
St. Mary's Bay
C
I
O
I+O
H
H+I
Growth rate (day-1)
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
A E A E A E A E
Temperature (OC)

Figure 2.4. Temperature dependence of bacterial growth rate (d^{-1}) by treatment in Placentia (Panel A) and St. Mary's Bays (Panel B).

A and E represent the ambient and elevated temperatures, respectively, for the manipulations. Error bars are standard deviation (n=3).

Table 2.1. Ecological information collected from all sample sites in Placentia Bay.

Date	Latitude (°N)	Longitude (°W)	Station No.	Collection Depth (m)	Collection Mode	[a](°C)	[b]Chl a Mean	± SD	[c]BA Mean	± SD	Salinity (psu)
August 9, 2012	46.933	54.200	PB3	1	Boat	18	0.08	0.01	764	107	31.8
				15		9	0.20	0.07	760	57	32.2
August 9, 2012	47.133	54.088	PB3	1	Boat	18	0.12	0.02	927	130	31.8
				15		15	0.12	0.01	790	59	32.1
August 9, 2012	47.275	54.029	PB2	1	Boat	18	0.12	0.01	669	94	31.9
				15		17	0.14	<0.01	796	60	31.9
August 9, 2012	47.423	54.013	PB1	1	Boat	18	0.09	<0.01	473	66	31.9
				15		7	0.11	<0.01	528	40	32.6
April 30, 2013	47.642	53.936	PB1	1	Dock	4	0.09	0.01	418	52	33.1
				3		4	0.09	0.01	386	52	32.7
April 30, 2013	47.292	53.990	PB2	1	Dock	5.5	0.05	<0.01	1047	131	32.1
				3		5.5	0.05	0.01	1113	149	32.1
April 18, 2013	47.166	55.148	PB3	1	Boat	1	1.14	0.06	753	94	*
				9		1	0.24	0.01	718	96	*
June 25, 2013	46.919	54.186	PB1	1	Boat	10	0.19	0.11	609	94	32.1
				8		10	0.09	0.01	482	75	32.3
June 25, 2013	47.340	53.984	PB2	1	Boat	11	0.06	0.01	799	123	31.9
				10		10	0.19	0.03	873	135	32.3
June 25, 2013	47.642	53.936	PB1	1	Dock	9.5	0.27	0.14	1011	156	31.7
September 5, 2013	47.292	53.990	PB2	1	Dock	12.5	0.34	0.11	833	124	31.6
				2		12.5	0.34	0.12	820	113	31.6
September 5, 2013	47.642	53.936	PB1	1	Dock	17	0.07	0.05	1126	167	28.9
				2.5		15	0.17	0.01	1047	145	31.1

[a]Water temperature (°C) at time of collection.

[b]Chlorophyll a concentration from duplicate samples (Chl a; μgL^{-1}; mean ± standard deviation).

[c]Bacterial abundance from duplicate samples (BA; bacteria 10^6 $cellsL^{-1}$; mean ± standard deviation).

*Machine error with CTD, therefore salinity was not recorded.

Table 2.2. Ecological information collected from all sample sites in St. Mary's Bay.

Date	Latitude (°N)	Longitude (°W)	Station No.	Collection Depth (m)	Collection Mode	[a](°C)	[b]Chl a Mean	± SD	[c]BA Mean	± SD	Salinity (psu)
				3		18	0.18	<0.01	1347	110	31.7
August 10, 2012	46.886	53.800	SMB3	20	Boat	7	0.24	0.01	819	86	32.4
				3		19	0.11	0.11	2170	177	31.5
August 10, 2012	46.973	53.755	SMB3	20	Boat	5	0.43	0.15	1007	106	32.7
				3		19	0.22	0.06	2418	198	31.6
August 10, 2012	47.041	53.653	SMB2	20	Boat	5	0.41	0.13	1020	107	32.3
				3		21	0.33	0.08	2067	169	30.7
August 10, 2012	47.114	53.565	SMB1	20	Boat	5	0.29	<0.01	1045	110	32.0
				1		5.5	0.27	0.23	1223	196	32.4
May 2, 2013	46.920	53.580	SMB3	2	Dock	5.5	0.30	0.02	1253	238	32.0
				1		2.5	0.20	0.01	353	57	32.7
May 2, 2013	47.007	53.644	SMB2	3	Dock	2.5	0.19	0.01	277	53	32.2
				1		5	0.54	0.06	1058	170	31.9
May 2, 2013	47.158	53.660	SMB1	4	Dock	4	0.68	0.04	1126	213	31.7
				1		10.5	0.67	0.04	441	73	32.0
June 24, 2013	46.920	53.580	SMB3	3	Dock	10.5	0.97	0.10	393	73	31.5
				1		10	0.28	0.01	420	69	32.2
June 24, 2013	47.007	53.644	SMB2	5	Dock	10	3.24	4.36	394	73	32.0
				1		13	0.07	0.01	1681	278	29.1
June 24, 2013	47.158	53.660	SMB1	2.5	Dock	13	0.06	0.01	1507	280	29.4
				1		14.5	0.18	0.05	1379	170	31.4
September 4, 2013	46.920	53.580	SMB3	1.5	Dock	14.5	0.05	0.04	1614	195	31.4
				1		14.5	0.11	0.02	1548	191	31.4
September 4, 2013	47.007	53.644	SMB2	4.5	Dock	14.5	0.09	0.02	1477	179	31.4
				1		15	0.16	0.08	1896	234	24.9
September 4, 2013	47.158	53.660	SMB1	3.5	Dock	15	0.13	0.06	1541	186	27.3

[a]Water temperature (^{o}C) at time of collection.
[b]Chlorophyll a concentration from duplicate samples (Chl a; μgL^{-1}; mean ± standard deviation).
[c]Bacterial abundance from duplicate samples (BA; bacteria 10^{6} $cellsL^{-1}$; mean ± standard deviation).

Table 2.3. Summary of complete ecological sample analysis from Argentia, Placentia Bay, the site of sample collection for the growth studies.

Date	**Latitude (°N)**	**Longitude (°W)**	**Station No.**	**[a]Depth (m)**	**[b](°C)**	**[c]Chl a Mean ± SD**		**[d]BA Mean ± SD**		**[e]POC**	**[e]PON**	**[f]DOC Mean ± SD**		**[f]DON Mean ± SD**	
August 9, 2012	47.275	54.029	PB2	1	18	0.12	0.01	669	94	0.122	0.017	1.05	<0.01	0.06	0.01
				15	17	0.14	<0.01	796	60	-	-	-	-	-	-
April 30, 2013	47.292	53.990	PB2	1	5.5	0.05	<0.01	1047	131	0.189	0.026	1.58	0.01	0.12	<0.01
				3	5.5	0.05	0.01	1113	149	-	-	-	-	-	-
June 25, 2013	47.340	53.984	PB2	1	11	0.06	0.01	799	123	0.103	0.022	1.10	0.01	0.08	<0.01
				10	10	0.19	0.03	873	135	-	-	-	-	-	-
September 5, 2013	47.292	53.990	PB2	1	12.5	0.34	0.11	833	124	0.299	0.048	1.78	0.01	0.10	<0.01
				2	12.5	0.34	0.12	820	113	-	-	-	-	-	-

[a]Collection depth of water samples (m).
[b]Water temperature (°C) at time of collection.
[c]Chlorophyll a concentration from duplicate samples (Chl a; μgL^{-1}; mean ± standard deviation).
[d]Bacterial abundance from duplicate samples (BA; bacteria 10^6 $cellsL^{-1}$; mean ± standard deviation).
[e]Particulate organic carbon and nitrogen (POC and PON) concentrations (mgL^{-1}). No replicates were collected (see methods for details).
[f]Dissolved organic carbon and nitrogen (DOC and DON) concentrations from duplicate samples (mgL^{-1}; mean ± standard deviation).

Table 2.4. Summary of complete ecological sample analysis from Admirals Beach, St. Mary's Bay, the site of sample collection for the growth studies.

Date	**Latitude (°N)**	**Longitude (°W)**	**Station No.**	**[a]Depth (m)**	**[b](°C)**	**[c]Chl a Mean ± SD**		**[d]BA Mean ± SD**		**[e]POC**	**[e]PON**	**[f]DOC Mean ± SD**		**[f]DON Mean ± SD**	
August 10, 2012	47.041	53.653	SMB2	3	19	0.22	0.06	2418	198	0.156	0.030	1.14	0.01	0.07	<0.01
				20	5	0.41	0.13	1020	107	-	-	-	-	-	-
May 2, 2013	47.007	53.644	SMB2	1	2.5	0.28	0.01	353	57	0.190	0.020	1.65	0.01	0.10	0.01
				3	2.5	-	-	277	53	-	-	-	-	-	-
June 24, 2013	47.007	53.644	SMB2	1	10	0.11	0.02	420	69	0.119	0.018	1.14	0.01	0.07	<0.01
				5	10	0.09	0.02	394	73	-	-	-	-	-	-
September 4, 2013	47.007	53.644	SMB2	1	15	0.17	0.01	1548	191	0.220	0.033	1.28	0.01	0.15	0.01
				3.5	15	0.26	0.05	1477	179	-	-	-	-	-	-

[a]Collection depth of water samples (m).
[b]Water temperature (°C) at time of collection.
[c]Chlorophyll a concentration from duplicate samples (Chl a; μgL^{-1}; mean ± standard deviation).
[d]Bacterial abundance from duplicate samples (BA; bacteria 10^6 $cellsL^{-1}$; mean ± standard deviation).
[e]Particulate organic carbon and nitrogen (POC and PON) concentrations (mgL^{-1}). No replicates were collected (see methods for details).
[f]Dissolved organic carbon and nitrogen (DOC and DON) concentrations from duplicate samples (mgL^{-1}; mean ± standard deviation).

Table 2.5. Summary of one-way ANOVA results determining the effect of bay on DON and DOC concentrations (mgL^{-1}).

Response variable	[b]**Date**	**F**	[a]***p***
DON	August 2012	4.08	0.113
	April 2013	33.27	0.004
	June 2013	3.02	0.157
	September 2013	161.17	<0.001
DOC	August 2012	445.39	<0.001
	April 2013	92.92	0.001
	June 2013	31.66	0.005
	September 2013	9180.88	<0.001

[a]Significant differences had a value of $p < 0.05$.
[b]The total degrees of freedom = 5 for each sample period.

Table 2.6. Summary of one-way ANOVA results for effect of sampling period within each bay, and the effect of bays (Placentia and St. Mary's Bay), on concentration of chlorophyll a (μgL^{-1}) at deep depth (2m-20m).

Date	**Location**	[a]***df***	**F**	[b]***p***
-	St. Mary's Bay	9	1.65	0.295
-	Placentia Bay	9	7.33	0.025
August 2012	-	3	2.11	0.283
April 2013	-	3	7.40	0.113
June 2013	-	3	26.49	0.036
September 2013	-	3	2.88	0.232

[a]Degrees of freedom (*df*) were reported.
[b]Significant differences had a value of $p < 0.05$.

Table 2.7. Summary of particulate hydrocarbon analysis, from St. Mary's and Placentia Bays.

Location	**Latitude (°N)**	**Longitude (°W)**	**Station No.**	[a]**Particulate Hydrocarbon (µg L^{-1})**	[b]**CPI**
St. Mary's Bay	47.007	53.644	SMB2	7.30	1.02
				5.65	0.99
				5.02	1.28
Placentia Bay	47.292	53.990	PB2	15.33	0.71
				23.63	0.76
				21.34	0.74

[a]The deionized water (DI) blank was subtracted from the total concentration.
[b]Carbon preference index (CPI) = ($\sum$ odds C_{15-33} + $\sum$ odds C_{17-35})/2($\sum$ evens C_{16-34}).

Table 2.8. Summary of one-way ANOVA analysis to determine if there was a significant effect of treatment (inorganic nutrient, organic carbon and petroleum hydrocarbon addition) on bacterial growth rates, calculated at both ambient and elevated (ambient + 2°C) temperature.

Location	[a]**Date**	**Temperature**	**F-value**	***p***
Placentia Bay	August 2012	Ambient (17°C) Elevated (19°C)	47.69 353.30	<0.001 <0.001
St. Mary's Bay	August 2012	Ambient (19°C) Elevated (22°C)	105.78 255.29	<0.001 <0.001
Placentia Bay	April 2013	Ambient (3°C) Elevated (5°C)	1.56 11.22	0.243 <0.001
St. Mary's Bay	April 2013	Ambient (2°C) Elevated (4°C)	84.02 92.08	<0.001 <0.001
Placentia Bay	June 2013	Ambient (7°C) Elevated (9°C)	59.03 363.85	<0.001 <0.001
St. Mary's Bay	June 2013	Ambient (8.5°C) Elevated (10.5°C)	19.30 332.71	<0.001 <0.001
Placentia Bay	August 2013	Ambient (17°C) Elevated (19°C)	9.58 89.23	0.001 <0.001
St. Mary's Bay	August 2013	Ambient (13°C) Elevated (15°C)	168.54 14.07	<0.001 <0.001

[a]The total degrees of freedom for all sampling periods during 2012 was 11, and for all sampling periods during 2013 was 17.

Table 2.9. Summary of the pattern of response of bacterial growth to treatments in Placentia and St. Mary's Bays.

	Placentia Bay		**St. Mary's Bay**	
	Ambient	**Elevated**	**Ambient**	**Elevated**
August 2012	I+O>O=I=C	O>I+O>I=C	I+O>I=C>O	I+O>O=I; I=C
April 2013	C=I=O=I+O=H=H+I	I+O=H>C=I=O=H+I	I+O=O>C>I=H; H=H+I	O>I+O>H+I>C=H; C=H; H=I
June 2013	I+O=O=I; I=C>H=H+I	I+O>O>C=I; C>H>H+I	I+O=O; O=H+I; H+I=I=C; C=I=H	I+O>O=H+I=I=C; C=I=H
August 2013	I+O>C=I=O=H=H+I	I+O>H+I=O=C; O=C=I=H	I+O>O>I; I=C; C=H+I; H+I=H	I+O>O=C=I=H=H+I

Table 2.10. Summary of one-way ANOVA to determine if there was a significant effect of temperature on bacterial growth rates, for each treatment.

Location	[a]**Date**	**Treatment**	**F-value**	[b]***p***	[c]**Maximum Growth**
Placentia Bay	August 2012	C	88.09	0.001	Ambient
		O	13327.67	<0.001	Elevated
St. Mary's Bay	August 2012	C	23.67	0.008	Ambient
		I	18.36	0.013	Ambient
		O	17.36	0.014	Elevated
		I+O	23.96	0.008	Elevated
Placentia Bay	April 2013	I+O	15.14	0.018	Elevated
		H	76.21	0.001	Elevated
St. Mary's Bay	April 2013	C	14.40	0.019	Elevated
		I	33.13	0.005	Elevated
		O	97.89	0.001	Elevated
		I+O	40.33	0.003	Elevated
		H	51.13	0.002	Elevated
		H+I	830.82	<0.001	Elevated
Placentia Bay	June 2013	C	54.93	0.002	Ambient
		I	314.04	<0.001	Ambient
		I+O	236.03	<0.001	Elevated
		H	15.16	0.018	Ambient
		H+I	59.09	0.002	Ambient
St. Mary's Bay	June 2013	C	95.81	0.001	Elevated
		I	40.88	0.003	Elevated
		I+O	42.33	0.003	Elevated
		H	275.75	<0.001	Elevated
		H+I	52.51	0.002	Elevated
Placentia Bay	August 2013	I	10.26	0.033	Ambient
		O	8.99	0.040	Ambient
		H+I	13.16	0.022	Ambient
St. Mary's Bay	August 2013	H	22.57	0.009	Elevated

[a]The degrees of freedom for both bays during all sampling periods was five.
[b]Only significant (p value < 0.05) results have been included. All ANOVA results are in Appendix B.
[c]Maximum growth is the temperature, at which, the maximum growth was observed for that treatment.

Table 2.11. Summary of one-way ANOVA results to determine if there was a significant effect of sampling period on bacterial growth rates, for each treatment.

Location	**Temperature**	**Treatment**	[a]***df***	**F-value**	***p***
Placentia Bay	Ambient	C	17	24.34	<0.001
		I	14	210.74	<0.001
		O	14	310.05	<0.001
		I+O	17	198.22	<0.001
		H	11	483.12	<0.001
		H+I	11	535.62	<0.001
	Elevated	C	14	151.43	<0.001
		I	14	275.96	<0.001
		O	14	801.52	<0.001
		I+O	14	375.38	<0.001
		H	8	814.56	<0.001
		H+I	8	345.96	<0.001
St. Mary's Bay	Ambient	C	17	144.39	<0.001
		I	14	1102.93	<0.001
		O	14	1450.55	<0.001
		I+O	17	508.30	<0.001
		H	11	1311.40	<0.001
		H+I	11	314.00	<0.001
	Elevated	C	14	397.88	<0.001
		I	14	115.72	<0.001
		O	14	8.47	0.003
		I+O	14	537.19	<0.001
		H	8	347.94	<0.001
		H+I	8	196.80	<0.001

[a]Total degrees of freedom (*df*).

Table 2.12. Summary of one-way ANOVA results to determine if there was a significant effect of bay (Placentia vs St. Mary's Bay) on bacterial growth rates, for each treatment.

[a]**Date**	[b]**Temperature**	**Treatment**	**F-value**	[c]***p***	[d]**Maximum Growth**
August 2012	Ambient	C	123.82	<0.001	Placentia Bay
		I	80.19	0.001	Placentia Bay
		O	614.96	<0.001	Placentia Bay
		I+O	28.59	0.006	Placentia Bay
	Elevated	C	147.02	<0.001	Placentia Bay
		I	15.21	0.018	Placentia Bay
		O	1850.68	<0.001	Placentia Bay
		I+O	324.97	<0.001	Placentia Bay
April 2013	Ambient	C	25.65	0.007	St. Mary's Bay
		O	350.47	<0.001	St. Mary's Bay
		I+O	346.09	<0.001	St. Mary's Bay
		H	38.56	0.003	St. Mary's Bay
		H+I	60.26	0.001	St. Mary's Bay
	Elevated	C	40.10	0.003	St. Mary's Bay
		I	1029.90	<0.001	St. Mary's Bay
		O	1362.26	<0.001	St. Mary's Bay
		I+O	28.64	0.006	St. Mary's Bay
		H	16.79	0.015	St. Mary's Bay
		H+I	1056.54	<0.001	St. Mary's Bay
June 2013	Ambient	C	861.73	<0.001	St. Mary's Bay
		I	1265.92	<0.001	St. Mary's Bay
		O	1415.17	<0.001	St. Mary's Bay
		I+O	127.17	<0.001	St. Mary's Bay
		H	2251.64	<0.001	St. Mary's Bay
		H+I	1038.29	<0.001	St. Mary's Bay
	Elevated	C	2895.55	<0.001	St. Mary's Bay
		I	4635.83	<0.001	St. Mary's Bay
		O	2223.10	<0.001	St. Mary's Bay
		I+O	577.68	<0.001	St. Mary's Bay
		H	4896.28	<0.001	St. Mary's Bay
		H+I	3486.08	<0.001	St. Mary's Bay
August 2013	Ambient	O	14.95	0.018	St. Mary's Bay
		H	499.22	<0.001	Placentia Bay
		H+I	101.01	0.001	Placentia Bay
	Elevated	I+O	27.95	0.006	St. Mary's Bay
		H	29.44	0.006	Placentia Bay
		H+I	14.30	0.019	Placentia Bay

[a]The total degrees of freedom for both bays during all sampling periods was five.
[b]See Table 2.8 for exact temperature values for each bay.
[c]Only significant (p value < 0.05) results have been included. All ANOVA results are in Appendix C.
[d]The bay in which the maximum growth was observed for that treatment.

Chapter 3: Environmental and anthropogenic influences on marine bacterial community composition

3.1 Introduction

Bacteria are ubiquitous in marine environments and are capable of adapting quickly to their environments (Wiebinga *et al*., 1997). The activity and growth of bacterial populations is often carbon limited in marine systems, and these processes are generally increased when glucose or other labile organic compounds are added (Kirchman *et al*., 1990; Carlson and Ducklow, 1996; Kirchman and Rich, 1997; Church *et al*., 2000; Carlson *et al*., 2002; Cuevas *et al.,* 2011). Carbon and energy limitations of bacteria appear to be the most profound in cold marine environments, such as the Arctic and Antarctic (Wedborg *et al.,* 1998; Church *et al*., 2000; Granéli *et al*., 2004; Meon and Amon, 2004; Kirchman *et al*., 2005). Inorganic nutrients, nitrogen (N) and phosphorous (P) are often a second or co-limiting factor to bacterial growth in seawater (Cotner *et al*., 1997; Vrede *et al*., 1999; Pinhassi *et al*., 2006; Cuevas *et al*., 2011; Nelson and Carlson, 2011). When both energy (carbon) and limiting nutrients (N and P) are supplied together, the growth and production of bacteria are maximally stimulated (Rivkin and Anderson, 1997; Shiah *et al.,* 1998; Caron *et al*., 2000; Church *et al*., 2000; Carlson *et al*., 2002; Thingstad *et al.,* 2008; Cuevas *et al*., 2011; Sebastián and Gasol, 2013).

Bacterial communities can rapidly respond to changes in their environments, such as fluxes in nutrient availabilities, with observable changes in growth characteristics and community composition (Cappello *et al*., 2007). The communities' adaptation to their environment is driven by the selection and propagation of the taxa best able to either

survive or grow rapidly in the environment, either by utilization of the available substrates or ability to tolerate harsh environments. Knowledge of the bacterial community composition therefore, can be extremely important and can allow us to infer not only environmental conditions but potentially the communities function and response. Generally, the Class *Gammaproteobacteria* have been reported to be opportunistic and grow quickly when nutrient levels are high, and are often capable of dominating the bacterial community (Pinhassi and Berman, 2003; Khandeparker *et al*., 2013; Eronen-Rasimus *et al*., 2014), while the *Alphaproteobacteria* Class appear to be abundant in environments with low nutrient concentrations (Pinhassi and Berman, 2003; Eronen-Rasimus *et al*., 2014). These relationships indicate shifts in the bacterial composition with the ability to thrive in contaminated or extreme systems (MacNaughton *et al*., 1999).

Petroleum hydrocarbons have been suggested to be one of the major contaminants in the marine environment (Windom, 1992), with estimates that each year about 0.1% of total petroleum production ends up in these systems (Vila *et al*., 2010). The toxic components of petroleum hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs), have been reported to inhibit some bacterial species, resulting in a shift in the bacterial community structure towards species which either have the ability to tolerate or degrade these compounds (MacNaughton *et al*., 1999; Röling *et al.,* 2002; Head *et al*., 2006; McKew *et al*., 2007; Valentine *et al*., 2012; Rodríguez-Blanco *et al*., 2013). Bacteria with the ability to obtain their carbon from petroleum hydrocarbons, or utilize these substrates as sources of energy, are classed as hydrocarbonoclastic bacteria, or hydrocarbon degraders (Head *et al*., 2006; Yakimov *et al*., 2007). The structure of

bacterial communities has been reported to change when contaminated with petroleum hydrocarbons, either by selection of hydrocarbon degrading bacteria (Harayama *et al*., 1999; Kasai *et al.,* 2001; Yakimov *et al*., 2005) or from toxic effect of petroleum hydrocarbons on certain species (Grötzschel *et al*., 2002). The addition of large concentrations of petroleum hydrocarbons into seawater microcosms, or *in situ* by spills, are often observed to cause rapid and significant increases in bacterial abundance as well as hydrocarbon degradation (Delille and Vaillant, 1990; Otremba and Toczek, 2002). However, there is little information on whether the input of low concentrations of petroleum hydrocarbons, such as that from marine traffic, would evoke the same responses in the bacterial communities (Paissé *et al*., 2008). Biodegradation of petroleum hydrocarbon by bacteria reduced the concentration in seawater (Atlas and Hazen, 2011), and the addition of nitrogen and phosphorous increased the total amount of degradation, suggesting biodegradation is limited by these nutrients (Röling *et al*., 2002; Garrett *et al*., 2003). Therefore, the community response of bacteria to petroleum hydrocarbon input can be variable. The abundance of marine hydrocarbon degraders is typically low in unpolluted waters and increases rapidly and significantly in response to the introduction of petroleum hydrocarbons (Head *et al*., 2006; Lo Giudice *et al.,* 2010), where they have been reported to grow and dominate the population (Cappello *et al.,* 2007; Yakimov *et al.,* 2007; Valentine *et al*., 2012). A wide range of at least 79 bacterial species have been reported to be hydrocarbon degraders (Prince, 2005). Most are from the Phylum *Proteobacteria*, with some from the Phyla *Firmicutes*, *Actinobacteria* and *Bacteroidetes* (Prince, 2005). Antarctic seawater isolates appear to act as a consortium with different substrate preferences, and shifts occurred in the Antarctic bacterial community when

petroleum hydrocarbons are present (Lo Giudice *et al.,* 2010). This shift in the community resulted in the predominance of *Proteobacteria*, mainly belonging to *Gamma*- and more rarely *Alpha*- subclasses (Lo Giudice *et al.,* 2010). The dominance of *Alphaproteobacteria* and *Gammaproteobacteria* was observed in biodegradation experiments and petroleum hydrocarbon polluted polar environments, and these two Classes are reported to be the major hydrocarbon degrading taxonomic groups, as they both contain multiple species capable of hydrocarbon degradation (Brakstad and Bonaunet, 2006; McKew *et al*., 2007; Jiménez *et al*., 2011). Thus, there are anthropogenic factors, such as petroleum hydrocarbons, as well as ecological factors that can influence the composition of the bacterial community.

The composition of bacterial communities differs among oceanic regions (Wietz *et al*., 2010), possibly because of ecological or physical factors such as water temperature (Baldwin *et al*., 2005; Fuhrman *et al*., 2008; De Maayer *et al*., 2014), nutrient availability (Abell and Bowman, 2005), and water masses (Teira *et al*., 2006; Galand *et al*., 2010). In both the Arctic and Antarctic, *Alphaproteobacteria* and *Gammaproteobacteria* dominate the bacterial community (Brown and Bowman, 2001; Kasai *et al*., 2001; Brinkmeyer *et al*., 2003; Prabagaran *et al*., 2007; Agogué *et al*., 2011; Williams *et al.,* 2012; Sebastián and Gasol, 2013; Xu *et al*., 2014; Kim *et al*., 2014). Bacterial communities from mid latitude and equatorial regions are more similar in composition to each other than to those in cold waters (Baldwin *et al*., 2005). The composition of bacterial communities however, can also depend on the geographical location within these regions (Fuhrman and Steele, 2008), as local environmental conditions can differ between regions, influencing the

composition of the community (Ghiglione *et al*., 2012). In the study reported here two geographically similar locations in coastal Newfoundland waters with differing nutrient and petroleum hydrocarbon inputs were included to expand the robustness of this study as this scale appears to be unique.

Environmental conditions in Newfoundland coastal waters are highly seasonal, with temperatures ranging from -1.5^{o}C during the winter, to 18^{o}C during summer and fall (Putland, 2000; Choe and Deibel, 2008; Fu *et al*., 2010), and are considered to have the oceanographic characteristics of subarctic oceans (de Young and Sanderson, 1995). Placentia Bay is an ice-free, deep-water inlet (Khan, 2003), with significant anthropogenic activity. It has ferry traffic, a terminal and dockyard, the Marystown shipping company, and the Come by Chance petroleum refinery (Khan, 2003). Much of the petroleum extracted from offshore fields around Newfoundland is trans-shipped and stored in Placentia Bay until loaded onto tankers and shipped to other locations (Higgins, 2009). It has been estimated that there were 8, 286 vessel movements per year in Placentia Bay, including tankers, the ferry, and fishing vessels (Transport Canada, 2007). It also supports a significant fishing industry. Based on the presence of shipping we hypothesize that the petroleum hydrocarbon usage in Placentia Bay will lead to chronic, low concentrations of petroleum hydrocarbons (Chapter 2), and this may be manifested in negative health effects (i.e. lesions and low lymphocyte levels) for the flatfish population (Khan, 2003). The adjacent St. Mary's Bay with little to no commercial activity and no refinery will limit petroleum hydrocarbon input in comparison to Placentia Bay (Chapter 2).

There is little information on the effects of chronic low concentrations of petroleum hydrocarbon contamination on the composition of bacterial communities, and whether they act as a toxic substance or carbon source, including in cold subarctic environments (Paissé *et al*., 2008). We carried out manipulation experiments to understand the effects of low concentrations of petroleum hydrocarbons on the bacterial communities' composition, in a unique set-up enabling the direct comparison to the effects of organic carbon and inorganic nutrient input. This study evaluated the composition of the bacterial community of two different populations of bacteria by 16SV6 rDNA analysis and high-throughput sequencing to compare the response of the communities in the two different bays that are environmentally distinct, and during cold and warm sampling periods.

3.2 Methods

3.2.1 Field Site

Water samples were collected in Placentia (47.103^{o}N, 54.186^{o}W) and St. Mary's (47.022^{o}N, 53.754^{o}W) Bays, which are situated between the Burin and Avalon Peninsulas, on the southeast coast of Newfoundland. Sampling was carried out during April and August 2013. During each sampling period, surface water was collected for experimental manipulations from the centre point of each bay.

3.2.2 Community Composition Studies

These experiments were run in conjunction with the 'growth studies' described in Chapter 2.

Approximately 18 L of seawater was collected using a 5 L Niskin bottle and transferred into insulated containers, and returned to the laboratory for processing. All glassware, filtration apparatuses, and sample containers were serially rinsed with 5% hydrochloric acid (HCl), deionized water and sample water before sample collection. At the laboratory, particle free water was prepared by gravity filtering 14.4 L of sample seawater through a 0.2 µm polycarbonate membrane filter (Pall Life Sciences). A grazer-free <1.0 µm fraction of bacteria was prepared by filtering 3.6 L of seawater through 1.0 µm polycarbonate membrane filters (GE Water & Process Technologies) at low pressure (5 psi). The filtered water was then combined in a ratio of 4:1 (0.2 µm membrane filtered: 1.0 µm membrane filtered) and placed into acid-washed 2 L Nalgene containers with each container receiving 1.5 L of the seawater dilution culture. Each Nalgene container had one of six substrate treatments for each temperature manipulation. The treatments were an unamended control (+**[C]**), the addition of inorganic nutrients (1 µM Na_2HPO_4 and 16 µM $NaNO_3$; "+**[I]**"), organic carbon (25 µM glucose; "+**[O]**"), inorganic nutrients plus organic carbon (1 µM Na_2HPO_4, 16 µM $NaNO_3$ and 25 µM glucose; "+**[I+O]**"), petroleum hydrocarbon (95.9 μgL^{-1} Hibernia crude oil, API 34.6; "+**[H]**") and the addition of 95.9 μgL^{-1} petroleum hydrocarbon (Hibernia crude oil, API 34.6) plus inorganic nutrients (1 µM Na_2HPO_4, and 16 µM $NaNO_3$; "+**[H+I]**"). After the addition of the nutrients, the 1.5 L containers were subdivided into 500 mL aliquots, and placed in three replicate 500 mL Nalgene incubation bottles. All incubation bottles were then placed in a constant temperature water bath and incubated in the dark, at the ambient temperature at the time of collection. Experiments during April were incubated for 72 hours, and incubations during August were incubated for 48 hours.

3.2.2.1 Sample Collection and Analysis in Laboratory

Microbial communities from undiluted and unfiltered 500 mL samples of surface seawater from each bay were collected onto 0.22 µm pore size polycarbonate filters (47 mm diameter, Millipore) from time zero and are referred to throughout the text as *in situ* community (**+[N]**). Samples (500 mL) of each treatment were taken at the end of the incubation (72 hours in April, 48 hours in August), and these are referred to as final samples. Each filter was then placed in a petri dish (Fisher, 50 mm diameter, with pad) and sealed with parafilm. Samples were stored at -80^{o}C until analysis.

DNA extraction:

Filters were thawed on ice, and in a microfuge tube, each filter was combined with 1 mL pre-lysis buffer (10 mM Tris-HCl, 1 mM EDTA, 0.8 M Sucrose, pH 8.0) and 5 µL lysozyme (200 mgmL^{-1}) and incubated at 37^{o}C for 30 minutes. Five µL of proteinase K (20 mgmL^{-1}) and 110 µL of 10% SDS were then added and the tubes were incubated at 37^{o}C overnight. After incubation, 50 µL of 10% cetyltrimethylammonium bromide (CTAB) in 1.4 M NaCl was added to each microfuge tube, and incubated again at 65^{o}C for 30 minutes. The samples were serially extracted using phenol-chloroform-isoamyl alcohol (25:24:1) and then chloroform-isoamyl alcohol (24:1). Forty µL of sodium acetate (3 M, pH 5.2) was added to the sample, followed by a volume of isopropanol equal to the total volume (440 µL) and then the samples were placed at -80^{o}C for 20 minutes to precipitate the DNA. The DNA was pelleted by centrifugation and the pellets were washed with 70% ethanol and air dried, and then dissolved in 30 µL of Tris-EDTA (TE) buffer.

PCR amplification:

The 16SV6 primers were based on those designed by researchers at the Marine Biological Laboratory at the Josephine Bay Paul Center, USA (Huber *et al*., 2007) and flanked the V6 hypervariable region of bacterial 16S rDNA. The PCR was performed using Phusion High-Fidelity DNA polymerase, High-Fidelity (HF) buffer and deoxynucleotide triphosphates (dNTPs) from New England BioLabs. The primers used were synthesized at Life Technologies (Thermo Fisher Scientific). The format of the barcoded, adaptor-fused primers was IonTorrent A-Adaptor-barcode-(16SV6 reverse primer), designed by Yunyun Fu (Fu, Rivkin and Lang, unpublished). Reverse primers were barcoded, and each reaction used only one reverse primer. All barcode sequences used in this study are listed in Table 3.1. Forward primers were a combination of four sequences combined in equal portions before making the PCR master mix; format was IonTorrent P1-Adaptor(16SV6 forward primer). The sequences were:

P1-Forward1:
CCTCTCTATGGGCAGTCGGTGAT(CTAACCGANGAACCTYACC)
P1-Forward2:
CCTCTCTATGGGCAGTCGGTGAT(ATACGCGARGAACCTTACC)
P1-Forward3:
CCTCTCTATGGGCAGTCGGTGAT(CNACGCGAAGAACCTTANC)
P1-Forward4:
CCTCTCTATGGGCAGTCGGTGAT(CAACGCGMARAACCTTACC)

The PCR master mix (for one sample) contained 33.6 µL PCR H_2O, 10 µL 5xHF buffer, 0.75 µL forward primer, 1 µL $MgCl_2$, 1 µL dNTPs, and 0.4 µL phusion. Each sample then had 0.75 µL of the barcode primer, and 2.5 µL of sample template added to the master mix. Each sample had a PCR amplicon library generated. Cycling conditions were

98ºC for 30 seconds, 30 cycles at 98ºC for 10 seconds and 72ºC for 30 seconds, and a final 2 minute extension at 72ºC. The products were cleaned with Agencourt AMPure XP reagent (1.4 x the volume of PCR product) and washed with 500 µL of 70% ethanol twice, and suspended in 10 µL of PCR H_2O (Sogin *et al*., 2006). The quality of the product was determined by using an Agilent 2100 BioAnalyser.

High-throughput sequencing:

The amplicon product was deposited in wells of an Ion 316 V2 chip for high-throughput sequencing on an IonTorrent PGM sequencer. The OneTouch kit used was Ion PGM Template OT2 200 Kit, standard procedure with no deviation. The sequencing kit was Ion PGM Sequencing 200 Kit V2, standard procedure for 100bp sequencing with no deviation. There were 2350856 total sequences recovered.

3.2.3 Sequence Analyses

Classification of sequences was determined by Mothur program v.1.33.0, and compared to the silva.bacteria database (Schloss *et al*., 2009). The total number of sequences detected for each treatment varied, therefore, the total percentage of each Phylum out of the total number of sequences for that treatment was determined and compared. Any Phylum that was <1% of the total number of sequences for each treatment was designated as 'other'. The total percentage of each Class and Order was determined out of the total number of sequences for that Phylum, or Class, respectively, in that treatment. Any Class or Order that was <1% of the total number of sequences of the Phylum or Class, respectively, was designated 'other'. The dominant Phyla, Class, and Orders were determined in this way, for each treatment. The most abundant Genera (>4%

of the total number of sequences in each treatment, in at least one treatment per bay) from only the three most relatively abundant Classes were analyzed in greater detail. We have adopted the terminology of Engel and Gupta (2014) and Eronen-Rasimus *et al*. (2014), using relative abundance to be synonymous with the proportion of total number of sequences. At the Genus level, the percent change of the relative abundance from the control was calculated as follows:

Equation 1. ((Relative abundance of Treatment – relative abundance of control)/relative abundance of control)*100

Equation 2. -((Relative abundance of control – relative abundance of treatment)/relative abundance of treatment)*100

Equation 1 was used when there was a higher relative abundance in the treatment than the control, and equation 2 was used when there was lower relative abundance in the treatment, then in the control. The percent change for the control was also calculated, but in comparison to the *in situ* community using the same equations.

3.3 Results

The potential response of bacterial taxa to the petroleum hydrocarbon and nutrient treatments was characterized using 16SV6 rDNA sequence analysis. Results are reported from April and August 2013, as they represent the lowest and highest ambient temperatures, respectively, and would likely represent the community responses at the 'end members' of the environmental range for the region. Any stated change in the

relative abundance (i.e. increase or decrease) in the control was in comparison to relative abundance *in situ*. Changes in all nutrient and petroleum hydrocarbon treatments were in comparison to the control.

Classification of sequences and comparison of bacterial communities

The relative abundance of the dominant Phyla, Classes, Orders and Genera all varied with incubation condition, nutrient and petroleum hydrocarbon treatments, and with sampling period (Figures 3.1 to 3.5, Tables 3.2 and 3.3). The dominance of *Proteobacteria* (*Alpha-* and *Gamma-*) was consistent for all nutrient and petroleum hydrocarbon treatments (Figures 3.1 and 3.2) although varying in the magnitude of change of relative abundance, and differed in the dominant Genus dependent on the type of treatment (Figures 3.6, 3.7 and 3.8, Table 3.2).

3.3.1 Phylum

At the Phylum level, *Proteobacteria* dominated (67.2 to 99.7% of the total number of sequences) the community *in situ*, in the control, and in all nutrient and hydrocarbon treatments in both bays, and during both sampling periods. Generally the relative abundance of *Proteobacteria* was greater during August than April (Figure 3.1). The second most abundant Phylum, *Bacteroidetes* (<1 to 27.1%) generally was lower during August than April (Figure 3.1).

To further explore the community composition of the incubations, multiple (i.e. Class, Order and Genera) taxonomic levels were examined.

3.3.2 Class

The dominant Phylum, *Proteobacteria* was divided into *Alphaproteobacteria* and *Gammaproteobacteria,* the two Classes that dominated all incubations (Figure 3.2). *Alphaproteobacteria* dominated the total number of sequences of *Proteobacteria* (52.2 to 86.7%) *in situ*, in the control, and in all nutrient and hydrocarbon treatments in both bays, during April (Figure 3.2 A, C). During August, *Alphaproteobacteria* dominated the relative abundances *in situ,* but decreased in the control and the nutrient and hydrocarbon treatments (Figure 3.2 B, D). *Gammaproteobacteria,* dominated (11.5 to 98.2%) in the control, and nutrient and hydrocarbon treatments in both bays, during August, and generally was lower during April than August (Figure 3.2).

The Phylum *Bacteroidetes* was dominated by the Classes *Flavobacteria* and *Sphingobacteria* (Figure 3.3). *Flavobacteria* dominated the relative abundances of the total number of sequences of *Bacteroidetes* (50.3 to 98.0%) *in situ*, in the control, and in all nutrient and hydrocarbon treatments in both bays, during both sampling periods (Figure 3.3). This was followed by *Sphingobacteria* (1.2 to 49.0%) which was similar in the control and nutrient and hydrocarbon treatments during both sampling periods, and greater *in situ* during August*,* in both bays (Figure 3.3 B, D).

3.3.3 Order

Within the Class *Alphaproteobacteria,* the dominant Orders were *Rhizobiales*, SAR 11 clade, and *Rhodobacterales*. Within the Class *Gammaproteobacteria*, the dominant Orders were *Altermonadales*, *Oceanospirillales*, *Vibrionales, Pasteurellales*, and *Enterobacteriales. Rhodobacterales* dominated the relative abundances of the total

number of sequences of *Alphaproteobacteria* (22.5 to 83.8%) *in situ*, in the control, and in all nutrient and hydrocarbon treatments in both bays, during both sampling periods, and it was greater during August than April (Figure 3.4). This was followed by SAR 11 clade (6.5 to 45.9%), which was lower during August than April (Figure 3.4 B, D). *Rhizobiales* generally dominated the relative abundances of the total number of sequences of *Alphaproteobacteria* (<1 to 65.1%) in the nutrient and hydrocarbon treatments in St. Mary's Bay, during April only (Figure 3.4 A, C). *Rhodospirillales* was not detectable >1% in either bay during April, but the proportions of this Order increased during August in Placentia Bay only (<1 to 13%), *in situ,* and in the nutrient and hydrocarbon treatments (Figure 3.4 B, D).

Altermonadales generally dominated the relative abundances of the total number of sequences of *Gammaproteobacteria* (4.2 to 84.4%) *in situ*, in the control, and in all nutrient and hydrocarbon treatments in both bays, during both sampling periods and was highly variable (Figure 3.5). *Vibrionales* generally dominated the relative abundances (<1 to 89.9%) in the nutrient and hydrocarbon treatments in both bays during August (Figure 3.5 B, D). This was followed by *Oceanospirillales* (<1 to 51.3%) which was generally greater during April than August, for both bays (Figure 3.5 A, C). The relative abundance of *Enterobacteriales* (<1 to 28.7%) was greater during August for both bays, *in situ*, in the control, and in all nutrient and hydrocarbon treatments (Figure 3.5 B, D). *Pasteurellales* was generally <1% of the total number of sequences of *Gammaproteobacteria*, in both bays during April (Figure 3.5 A, C). *Pasteurellales* was

greater (<1 to 29%) in the control and nutrient and petroleum hydrocarbon treatments during August and was highly variable (Figure 3.5 B, D).

3.3.4 Genus

We examined the response of the Genera (>4% of the total number of sequences) to the nutrient and petroleum hydrocarbon treatments from the three most abundant Classes, *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* (Table 3.2). The relative abundances of these Genera, in all nutrient and hydrocarbon treatments, were compared to the control, and any change greater than 25% from the control is discussed. The control was compared to the *in situ* community (Figure 3.6 A, B, Table 3.3).

The relative abundance of *Ancalomicrobium* (Class *Alphaproteobacteria*), *Glaciecola, Teredinibacter, Colwellia, Pseudoaltermonas, Psychromonas, Oleispira, Saccharospirillum, Phocoenobacter, Pseudomonas, Cycloclasticus, Vibrio,* and *Providenicia* (Class *Gammaproteobacteria*), and *Polaribacter* (Class *Bacteroidetes*), in both bays, and during both sampling periods were greater in the control than in the *in situ* community (Figure 3.6 A, B, Table 3.3).

The relative abundance of the *Roseobacter* clade (Class *Alphaproteobacteria*), *Teredinibacter, Saccharospirillum,* and *Pseudomonas* (Class *Gammaproteobacteria*), and *Polaribacter* (Class *Bacteroidetes*) in the **+[I]** treatment was greater than in the control (Figure 3.6 C, D, Table 3.3). Generally, the increase in relative abundance was greater during August than April (Table 3.3), and *Saccharospirillum, Pseudomonas* and *Polaribacter* increased only in Placentia Bay, while the *Roseobacter* clade increased only in St. Mary's Bay (Table 3.3).

The relative abundance of the *Roseobacter* clade (Class *Alphaproteobacteria*), *Glaciecola, Pseudoaltermonas, Psychromonas, Teredinibacter, Saccharospirillum, Phocoenobacter, Pseudomonas, Cycloclasticus,* and *Vibrio* (Class *Gammaproteobacteria*), and *Polaribacter* (Class *Bacteroidetes*) in the **+[O]** treatment was greater than in the control (Figure 3.7 A, B, Table 3.3). Generally, the increase in relative abundance was greater during April than August (Table 3.3), and *Saccharospirillum, Pseudomonas* and *Polaribacter* increased only in Placentia Bay, while the *Roseobacter* clade and *Glaciecola* increased only in St. Mary's Bay (Table 3.3).

The relative abundance of *Glaciecola, Teredinibacter, Psychromonas, Oleispira, Saccharospirillum, Pseudomonas* and *Vibrio* (Class *Gammaproteobacteria*), and *Polaribacter* (Class *Bacteroidetes*) in the **+[I+O]** treatment was greater than in the control (Figure 3.7 C, D, Table 3.3). Generally, the increase in relative abundance was greater during April than August, and in Placentia Bay than St. Mary's Bay (Table 3.3).

The relative abundance of *Ancalomicrobium* (Class *Alphaproteobacteria*), and in *Glaciecola, Teredinibacter, Oleispira,* and *Phocoenobacter* (Class *Gammaproteobacteria*), and *Polaribacter* (Class *Bacteroidetes*) in the **+[H]** treatment was greater than in the control (Figure 3.8 A, B, Table 3.3). Generally, the increase in relative abundance was greater during August than April, and *Ancalomicrobium,* and *Glaciecola* increased only in Placentia Bay, and *Phocoenobacter* only increased in St. Mary's Bay (Table 3.3).

The relative abundance *Glaciecola, Teredinibacter, Colwellia, Oleispira, Saccharospirillum, Phocoenobacter,* and *Vibrio* (Class *Gammaproteobacteria*), as well as

Polaribacter (Class *Bacteroidetes*) in the **+[H+I]** treatment was greater than in the control (Figure 3.8 C, D, Table 3.3). Generally, the increase in relative abundance was greater during August than April, and an equal number of Genera increased in either only Placentia Bay or only St. Mary's Bay (Table 3.3).

3.4 Discussion

3.4.1 Effect of treatment on the bacterial community composition

Several potential ecological and anthropogenic factors may influence the community structure of subarctic marine bacterial communities. Short term incubations showed that the bacterial community structure in subarctic waters at both the Class and Genus level, was generally influenced by all enrichment treatments. The bacterial community composition shifted to the dominance by a few Genera by all nutrient and petroleum hydrocarbon additions, regardless of growth rate (see Chapter 2). The type of addition determined which Genus dominated the community.

The addition of nutrients, and low concentrations of petroleum hydrocarbons resulted in a shift in the community structure. Bacterial taxa responded differently to additions of inorganic nutrients and/or organic carbon. Although *Gammaproteobacteria, Alphaproteobacteria,* and *Bacteroidetes* did not increase in relative abundance when inorganic nutrients were added, we observed an increase in the relative abundance of several Genera from these Classes (Table 3.3, Figure 3.6 C, D). This suggests differences in the community structure at the Genera but not at the Class level, and implies that metagenomics data should be analysed at the Genera level because analysis at higher taxa

levels may miss responses to the treatment. Shifts at the Genera level were observed without a change in cell growth (Chapter 2, Figure 2.2 C, D, G, H) suggesting either the enhancement of growth (i.e. increase of cell abundance) of these Genera was too small to be observed or growth was balanced by a decrease in growth (or cell abundance) of other Genera.

The availability or supply of organic carbon was the most growth limiting factor to *Gammaproteobacteria* followed by the availability of inorganic nutrients, therefore when these nutrients were present *Gammaproteobacteria* could outcompete or inhibit *Alphaproteobacteria.* For all incubations that were enriched with organic carbon, the relative abundance of *Gammaproteobacteria* increased, and *Alphaproteobacteria* decreased (Figure 3.2), and the magnitude of the response was greater in both Classes when inorganic nutrients and organic carbon were supplied together (Figure 3.2). *Gammaproteobacteria* dominated the **+[O]** treatment, while a similar response was not observed in the **+[I]** treatment and the maximum relative abundance was observed when both nutrients were present (i.e. **+[I+O]** treatment), suggesting both carbon and inorganic nutrient limits. *Gammaproteobacteria* appears to outcompete or inhibit *Alphaproteobacteria,* in both the **+[O]** and **+[I+O]** treatments. These results are consistent with previous reports that *Gammaproteobacteria* are capable of rapid growth in response to high organic nutrient concentrations and are capable of dominating the bacterial community (Fuchs *et al*., 2000; Pinhassi and Berman, 2003; Alonso-Sáez *et al*., 2007; Eronen-Rasimus *et al*., 2014), while *Alphaproteobacteria* have been reported to

outcompete other bacteria and dominate the community at low nutrient levels (Pinhassi and Berman, 2003; Eronen-Rasimus *et al*., 2014).

The Genera *Psychromonas* and *Vibrio* were limited by both organic carbon and inorganic nutrients, with *Vibrio* capable of dominating the bacterial community when these nutrients were available (Table 3.2), suggesting that the growth of the bacterial population reported in Chapter 2 was likely due to these two Genera. The addition of organic carbon (i.e. **+[O]** and **+[I+O]** treatments) stimulated multiple Genera (Figure 3.7), with the greatest increase in relative abundance generally by Genera *Psychromonas* and *Vibrio* (Figure 3.7, Table 3.2 and 3.3). This stimulation was simultaneous with increases in the population growth rates for these treatments (Chapter 2, Figure 2.2 C, D, G, H). *Vibrio* also dominated the **+[O]** treatment in Placentia Bay, however, bacterial population growth was not observed in this treatment (Chapter 2, Figure 2.2 C, G), suggesting that there was likely viral mortality that was important in controlling the relative abundance of other Genera in Placentia Bay (Figure 3.7 B).

Vibrio and *Psychromonas* were able to use glucose as a carbon source to support growth and dominate the bacterial community. The specific relationship between *Psychromonas* and organic carbon and inorganic nutrients has not been previously reported. We suggest that *Psychromonas* is able to utilize glucose, stimulating their growth at low temperatures. *Psychromonas* has been reported in coastal waters from the Antarctic (Lo Giudice *et al*., 2012) and the Arctic (Canion *et al*., 2013), as well as in sediments near Kongsfjorden in the Arctic Sea (Tian *et al*., 2009), consistent with our findings in coastal subarctic waters. Bowman *et al*. (2003) found the relative abundance

of *Psychromonas* in Antarctic sediments to be 2-5% from clone libraries, a greater abundance than our findings *in situ,* suggesting the capability of the Genus to survive in cold but subarctic environments such as Newfoundland, while preferring or thriving in polar environments. The abundances of *Vibrio* increased with the addition of nutrients, and were high *in situ* when ambient nutrient concentrations are high (Pinhassi and Berman, 2003; Rehnstam-Holm *et al*., 2010; Oberbeckmann *et al*., 2012; Khandeparker *et al*., 2013). Eilers *et al*. (2000) stated it was the addition of carbon compounds that stimulated *Vibrio* growth, and the Genus' abundance has been suggested to be related to phytoplankton and diatom abundance due to the release of nutrients and organic matter from these sources (Lobitz *et al*., 2000; Rehnstam-Holm *et al*., 2010; Khandeparker *et al*., 2013), supporting our results. *Vibrio* spp. have been found to be ubiquitous in marine environments, and are commonly found in coastal waters, ranging in locations from the Adriatic Sea (Baffone *et al*., 2006), the North Sea (Vezzulli *et al*., 2012), the English Channel (Tall *et al*., 2013), and the Arabian Sea (Rehnstam-Holm *et al*., 2014). The relative abundance of *Vibrio* appears to vary, as it was found to be 2.2% of microbial counts in the coastal waters of the North Sea (Oberbeckmann *et al*., 2012), and the relative abundance was 0.02% in the Baltic Sea (Eiler *et al*., 2006), and 3.2% on the coast of Africa (Wietz *et al*., 2010). Our results *in situ* are consistent with the proportions found in the colder latitude water of the Baltic Sea.

The results from our study suggest that the addition of low concentrations of petroleum hydrocarbons did not alter the bacterial community composition at the Class level (Figures 3.2 and 3.3), but did shift the composition towards either hydrocarbon

tolerant or degrading Genera either capable of surviving the toxicity or utilizing the petroleum hydrocarbons as a carbon source. Our results appear to contrast previous studies that reported a shift in the bacterial communities to the dominance of Class *Gammaproteobacteria*, and to a lesser extent Class *Alphaproteobacteria*, when petroleum hydrocarbons were present (Röling *et al*., 2002; Yakimov *et al.,* 2004; Deppe *et al*., 2005; Gerdes *et al*., 2005; Prabagaran *et al*., 2007; Brakstad *et al*., 2008; Alonso-Gutiérrez *et al*., 2009; Lo Giudice *et al*., 2010; Paissé *et al*., 2010; Vila *et al*., 2010; Kostka *et al*., 2011; Jurelevicius *et al*., 2013). The Genera *Colwellia, Oleispira, Vibrio,* and *Polaribacter* increased in relative abundance (Figure 3.8, Table 3.3) and have been reported to either be enriched in petroleum hydrocarbon contaminated environments, or capable of degrading petroleum hydrocarbons, and are all considered to be hydrocarbon degrading bacteria (Hedlund and Staley, 2001; Melcher *et al*., 2002; Yakimov *et al*., 2004, 2007; Brakstad *et al*., 2008; Hazen *et al*., 2010; Valentine *et al*., 2012; Redmond and Valentine, 2012; Gutierrez *et al.,* 2013; Liu and Liu, 2013; Lin *et al*., 2014; Wang *et al*., 2014). *Glaceicola,* however, has been found in both seawater and petroleum hydrocarbon contaminated seawater, suggesting the Genus is hydrocarbon tolerant and not a hydrocarbon degrader (Prabagaran *et al*., 2007; Brakstad *et al*., 2008; Sauret *et al*., 2014). In addition the Genera *Ancalomicrobium, Teredinibacter, Saccharospirillum,* and *Phocoenobacter* increased in relative abundance (Figure 3.8, Table 3.3) but have not previously been reported in petroleum hydrocarbon contaminated environments, or to degrade these compounds. Our results suggest they may be hydrocarbon tolerant, allowing them to thrive over other Genera more sensitive to these compounds. We conclude that there was a small shift in the bacterial community composition at the Genus

level, in both bays, selecting for a few hydrocarbon degrading, or tolerant Genera, and as discussed below, there were differences between the two bays.

Bacterial population growth was not observed in Placentia Bay, and declined in St. Mary's Bay with the addition of petroleum hydrocarbons (Chapter 2, Figure 2.2 C, D, G, H), and our results suggest the petroleum hydrocarbons inhibited the growth of some Genera and shifted the community composition in both bays. We suggest the composition of the petroleum hydrocarbon compounds has an important influence on the response of the bacterial community by determining the direction the community will shift, as hydrocarbon degraders such as *Cycloclasticus* may only utilize specific petroleum compounds. In Placentia Bay, there was an increase of growth of some hydrocarbon degrading and tolerant Genera, however, there was no net population growth (Chapter 2, Figure 2.2 C, G) since the growth of other bacterial Genera were inhibited by the petroleum hydrocarbons. In St. Mary's Bay, *Providencia*, *Cycloclasticus,* and *Saccharospirillum* showed the greatest decline in relative abundance (Tables 3.3 and 3.4, Figure 3.8 A, C), suggesting these Genera were responsible for the net decline in population growth observed in Chapter 2 (Figure 2.2 D, H) due to either being outcompeted by other hydrocarbon tolerant Genera or by inhibition from the petroleum hydrocarbon addition. *Providencia,* and *Saccharospirillum* have not previously been found related to either the utilization or inhibition by petroleum hydrocarbons, and we suggest that aliphatic petroleum hydrocarbons have a negative effect on their growth. *Cycloclasticus* has been reported to utilize primarily single or multiple ring aromatics as the sole source of carbon and energy (Chung and King, 2001; Kasai *et al*., 2002; McKew

et al., 2007; Teira *et al*., 2006; Valentine *et al*., 2012), and considered to be a marine obligate hydrocarbonoclastic bacteria (Chung and King, 2001; Kasai *et al.*, 2002; Yakimov *et al.*, 2005; Teira *et al*., 2006; McKew *et al*., 2007; Yakimov *et al.,* 2007; Cui *et al*., 2008; Valentine *et al*., 2012). The petroleum hydrocarbon used in this study, and the hydrocarbons found in both bays were mainly aliphatic, and suggests the addition limited this Genus as it was unable to utilize aliphatic hydrocarbons as a carbon source.

The bacterial composition of subarctic Newfoundland coastal waters *in situ* was consistent with cold water bacterial communities. Previous incubation studies showed the dominance of both *Alphaproteobacteria* and *Gammaproteobacteria* in temperate and cold waters, including the Arctic and Antarctic (Brown and Bowman, 2001; Kasai *et al*., 2001; Brinkmeyer *et al*., 2003; Prabagaran *et al*., 2007; Agogué *et al*., 2011; Williams *et al.,* 2012; Sebastián and Gasol, 2013; Xu *et al*., 2014; Kim *et al*., 2014). *Flavobacteria* and *Sphingobacteria* have also been reported in the bacterial communities of cold waters, such as the German Bight in the North Sea or Antarctic (Abell and Bowman, 2005; Brakstad and Lodeng, 2005; Ghiglione and Murray, 2012; Grzymski *et al*., 2012; Buchan *et al*., 2014). The contribution of *Ancalomicrobium* and *Teredinibacter* to *in situ* bacterial communities is not well documented. The *Roseobacter* clade can comprise up to 25% of total bacteria (Buchan *et al.,* 2005), and *Polaribacter* have been found to dominate the surface water in Antarctic waters (Kim *et al*., 2014), consistent with our results showing these Genera dominated the incubations (Table 3.2).

Our results are consistent with an inorganic nutrient enrichment experiment that found *Alphaprotoebacteria* and *Gammaproteobacteria* dominated all microcosms (Xu *et al*., 2014). It is well known that populations of bacteria increase in growth and abundance when both organic carbon and inorganic nutrients are supplied (Rivkin and Anderson, 1997; Shiah *et al.,* 1998; Caron *et al*., 2000; Church *et al*., 2000; Carlson *et al*., 2002; Thingstad *et al.,* 2008; Cuevas *et al*., 2011; Sebastián and Gasol, 2013). We suggest that the changes in the observed population growth of the bacterial community (Chapter 2, Figure 2.2), may reflect the responses of a few key bacterial Genera. We determined that the population growth from the bacterial community in Newfoundland coastal waters, during the organic carbon additions was from *Vibrio,* and to a lesser extent *Psychromonas*. We observed an increase in the relative abundance of hydrocarbon degraders when petroleum hydrocarbons were added, however, there was an absence of net bacterial population growth (Chapter 2, Figure 2.2). This suggests either the concentration of petroleum hydrocarbons was not great enough to support high growth rates of these Genera, the toxic effects of the petroleum compounds on sensitive Genera was equal to, or greater than the growth of the hydrocarbon degraders, or alternatively the incubation time was too short to show the communities full response. It is well documented that large inputs of petroleum hydrocarbons shifted bacterial communities towards hydrocarbon degraders (Röling *et al.,* 2002; Head *et al*., 2006; McKew *et al*., 2007; Hazen *et al.,* 2010; Sheppard *et al*., 2012). The increase in relative abundance of these hydrocarbon degraders was not observed in either of the nutrient treatments, suggesting that even low concentrations of petroleum hydrocarbon input influenced the community structure. Our results suggest that the composition and concentration of the

petroleum hydrocarbons (i.e. mainly aliphatic) are important. Knowledge of the composition of the bacterial community at multiple phylogenetic levels can therefore help us understand and better predict the function and response of bacterial communities as we have observed distinctive patterns at different levels, with more subtle patterns observed at the Genus level. This study details the response of the bacterial community and specific Genera, to the addition of low concentrations of petroleum hydrocarbons, in a set up enabling direct comparison to the effects of organic carbon, and inorganic nutrients. Increasing anthropogenic activities, such as creating runoff, use of fertilizers, and chronic release of petroleum hydrocarbons through shipping will significantly affect bacterial communities.

3.4.2 Effect of location (bay) on the bacterial community composition

We observed differences in the bacterial community composition in Placentia and St. Mary's Bays, both *in situ* and in all of the enrichment treatments (Figure 3.2, Table 3.2). Bacterial population growth observed during the incubations (Chapter 2, Figure 2.2) appears to be due to the growth of a few Genera which differed between the two bays.

The bacterial taxa and population growth rates (Chapter 2, Figure 2.2) suggest these differences were due to greater ambient organic nutrient and carbon levels in St. Mary's Bay (Chapter 2, Tables 2.3 and 2.4). For the *in situ* conditions and control incubations, the relative abundance of *Gammaproteobacteria* was lower in Placentia than St. Mary's Bay, while in the enriched treatments the relative abundance was similar in both bays (Figure 3.2). This suggests this Class was able to utilize the nutrient and petroleum hydrocarbon inputs and increase to the same relative abundance in both bays

after addition. *Gammaproteobacteria* increase in the presence of nutrients (as discussed above) and the lower relative abundance in Placentia Bay suggests this bay was more depleted in nutrients, supported by the data in Chapter 2 (Tables 2.3 and 2.4), and explains the lower population growth observed in Placentia Bay (Chapter 2, Figure 2.2 C, D, G, H). For all incubations enriched with organic carbon (with and without inorganic nutrients), *Vibrio* and *Psychromonas* were the major contributors to population growth in both bays (as discussed above). The population growth rate of the bacterial community however, was found to be generally greater in St. Mary's Bay for both sampling periods (Chapter 2, Table 2.12), therefore suggesting the difference in growth rates between the two bays was due to greater growth of *Vibrio* and *Psychromonas* and suggesting a higher level of nutrients in St. Mary's Bay supported by *in situ* data (Chapter 2, Tables 2.3 and 2.4).

Our results suggest that there was an increase in relative abundances of previously identified hydrocarbon degrading bacteria in Placentia Bay in comparison to St. Mary's Bay. For all incubations enriched with petroleum hydrocarbons (with and without inorganic nutrients), the bacterial population growth was found to be greater in St. Mary's Bay during April, and greater in Placentia Bay during August (Chapter 2, Table 2.12). In petroleum hydrocarbon incubations during April the increases in the relative abundances of *Glaciecola*, *Ancalomicrobium* and *Teredinibacter* were greater in St. Mary's Bay than Placentia Bay (Table 3.2). During August, the increases in the relative abundances of *Oleispira*, *Glaceicola*, *Saccharospirillium*, *Vibrio,* and *Polaribacter* were greater in Placentia Bay than St. Mary's Bay (Table 3.2). Thus, these Genera were responsible for

the observed difference in population growth between the two bays. As previously discussed, of these Genera only *Oleisipira*, *Vibrio* and *Polaribacter* are hydrocarbon degraders (Hedlund and Staley, 2001; Melcher *et al*., 2002; Yakimov *et al*., 2004, 2007; Liu and Liu, 2013; Lin *et al*., 2014; Wang *et al*., 2014), and *Glaceicola* is hydrocarbon tolerant (Prabagaran *et al*., 2007; Brakstad *et al*., 2008; Sauret *et al*., 2014) suggesting an increase in relative abundances of hydrocarbon degrading bacteria in Placentia Bay that is not observed in St. Mary's Bay.

The response of the bacterial community composition to the enrichment treatments differed between the two bays, suggesting that even close geographical locations can have significant differences in the bacterial community. Even though these locations share some of the same ecological factors, such as climate and weather patterns, our results suggest there are differences in other ecological factors capable of influencing the bacterial composition and response to inputs. These results are supported by previous studies on bacterial communities at multiple latitudes. Although the Arctic and Antarctic regions were found to be more similar to each other in bacterial production than to lower latitude regions, and have similar climate drivers, there is a clear difference in the bacterial communities (Ghiglione *et al*., 2012). The results from Chapter 2 (Figure 2.2) suggested the bacterial population growth responded to petroleum hydrocarbons addition only during August, in Placentia Bay suggesting the community may have begun to become preconditioned towards hydrocarbon degraders which was not observed in St. Mary's Bay. The results from the current Chapter lend support to this hypothesis, however they also suggest that there was a greater increase in relative abundance of

hydrocarbon degrading Genera in Placentia Bay than St. Mary's Bay during both sampling periods, and it appears that a change in community structure precedes a change in functional response. The lack of population growth in the overall bacterial community appears not to be due to the lack of an increase in the relative abundance of hydrocarbon degrading bacteria, but from the inhibition of bacteria sensitive to the potentially toxic compounds of petroleum hydrocarbon (discussed above), or the concentration of petroleum hydrocarbons was not great enough to remove carbon limitation on growth of those species able to utilize it. These data suggest that a preconditioned hydrocarbon degrading community may be present in Placentia Bay, developed from previous exposure to petroleum hydrocarbons from anthropogenic activity (Kappell *et al*., 2014), as the same shift to hydrocarbon degraders was not observed in St. Mary's Bay. We suggest that to fully understand the response of bacterial communities, and to create accurate predictions and models, the structure of the bacterial community should be determined at multiple taxonomic levels and must be considered alongside the community's function.

3.4.3 Effect of sampling period on the bacterial community composition

Sampling period appeared to have the greatest effect on community composition of all studied factors; inorganic nutrient, organic carbon and petroleum hydrocarbon additions, and location (Figures 3.2, 3.7 and 3.8). Temperature was determined to be an important factor during each sampling period that influenced bacterial community composition. Our study suggests that *Gammaproteobacteria* dominated the community during the warmer month of August, while *Alphaproteobacteria* dominated during the

colder month of April, with this growth contributed by only a few key Genera. *Gammaproteobacteria* have been demonstrated to be variable in relative abundance during both summer and winter in subantarctic and Antarctic coastal regions (Ghiglione and Murray, 2012), suggesting the response to season observed in our study was related to the Genera present.

Generally, we found a greater increase in relative abundance in Genera reported to grow better in warmer waters, during August, and in those reported to be psychrophilic or psychrotolerant during April (Figure 3.6, 3.7 and 3.8), suggesting temperature is a strong influence of season on bacterial community composition. The increase in relative abundances of *Pseudomonas* and *Psychromonas* in the **+[C],** and **+[O]** treatments, respectively, was greater during April, than August (Figures 3.6 A, B, and 3.7 A, B, Tables 3.2 and 3.3). Multiple strains or species from *Pseudomonas* are psychrotolerant, capable of growing at low temperatures (Choo, 1998; Carrión *et al*., 2011; Pesciaroli *et al*., 2012). *Pseudomonas* has been found in multiple cold environments, including Arctic soils and sediments (Choo *et al*., 1998; Canion *et al*., 2013), sea ice (Gerdes *et al*., 2005), and seawater from the White Sea (Pesciaroli *et al*., 2012) and Antarctic (Lo Giudice *et al*., 2010; Carrión *et al*., 2011). *Psychromonas* are psychrophilic, and grow best at low temperatures, and some of the species in this Genus have cold shock proteins (Jung *et al*., 2010). *Psychromonas* have also been found in cold environments such as sea ice, seawater, and sediments (Groudieva *et al*., 2003; Auman *et al*., 2006; Nogi *et al*., 2007) from both Arctic fjords (Corsaro *et al*., 2008; Canion *et al*., 2013) and the Antarctic (Lo Giudice *et al*., 2012). The relative abundances of the Genera *Polaribacter*, *Vibrio*,

Oleispira, and *Phocoenobacter* in the **+[I], +[I+O], +[H],** and **+[H+I]** treatments, respectively, either decreased or remained similar during April, and increased during August (Tables 3.2 and 3.3) when both temperature and chlorophyll a concentrations were the highest (Chapter 2, Tables 2.3 and 2.4). *Phocoenobacter* have been isolated from porpoises and therefore may not be bacteria associated with open seawater communities (Foster *et al*., 2000). The Genus has been found capable of growth at 22ºC (Foster *et al*., 2000), and although the Genus' optimum temperature has yet to be reported we suggest that this Genus may prefer warmer temperatures. *Polaribacter* has been found to be dominant in the cold environments of Arctic and Antarctic coastal waters and sea ice, suggested to have a bipolar distribution (Gosink *et al*., 1998; Staley and Gosink, 1999; Brinkmeyer *et al*., 2003; Grzymski *et al*., 2012; Williams *et al*., 2012; Li *et al*., 2013). Gosink *et al*. (1998) described the Genus as psychrophilic, although recently Kim *et al*. (2014) have discovered some species to be mesophilic and present in temperate marine waters. *Polaribacter* have also been reported to be found only during the summer (11% of the bacterial community; 2ºC) in Antarctic seawater, and absent during the winter (-1.7ºC; Grzymski *et al*., 2012), showing a similar pattern to our results. *Vibrio* generally grows well in warm (>18ºC) seawater (Vezzulli *et al*., 2013), and their abundance peaked in lower latitude oceans (Wietz *et al.* 2010), suggested to be related to this preference for warm waters (Rehnstam-Holm *et al.,* 2014; Oberbeckmann *et al*., 2012). However, *Vibrio* are ubiquitous in marine seawater, also found in temperate marine waters including the North Sea (Oberbeckmann *et al*., 2012), Pacific Northwest (Turner *et al.,* 2013), and Southwest United Kingdom (Powell *et al*., 2013). *Oleispira* is another psychrophilic Genus, with optimal growth between 2 and 4ºC (Yakimov *et al*.,

2004, 2007), found in cold environments such as Antarctic and Arctic waters (Murray and Grzymski, 2007; Alonso-Sáez *et al*., 2010). Coulon *et al*. (2007) and Brakstad and Bonaunet (2006) reported *Oleispira* in the Thames Estuary, and a Norwegian fjord (Trondheimsfjord), and both studies therefore, suggested a broader distribution for the Genus than only in polar waters. Our results are consistent with these reports, except *Oleispira*, which was consistent with being found in colder water communities but contrasted reports that suggested low optimal temperatures for growth. Thus, we suggest that the higher temperature did not limit the growth of this Genus. A similar trend was observed with the other psychrophilic Genus, *Polaribacter*. These results suggest that increasing ocean temperature may support the spread and dominance of those bacteria that prefer warmer temperatures in the more northern latitudes.

Shifts in the bacterial community composition between summer and winter have previously been documented (Murray and Grzymski, 2007; Piquet *et al*., 2011; Ghiglione and Murray, 2012; Grzymski *et al.,* 2012), and we suggest temperature is a main seasonal factor on bacterial community structure. Global warming is already occurring, with the Arctic Ocean observing a peak increase in average temperature of 5^{o}C during 2007 (Steele *et al*., 2008), and already suggested to have significant effects on the present bacterial community composition (IPCC, 2007). Our study suggests that the warming of seawater will support the spread and dominance of those bacteria that prefer warmer temperatures such as *Vibrio*. Understanding the bacterial community composition and its response to changes in temperature is important not only to predict the effects of climate change, but also to determine possible impacts on human health. Some bacterial groups,

such as the Genus *Vibrio*, contain multiple species known to be human pathogens (Oliver, 2013; Vezzulli *et al*., 2013) and suggested to propagate in warmer waters, potentially exposing a greater population of humans to disease.

3.4.4 Conclusions and future directions

This study showed that the bacterioplankton communities *in situ* from both Placentia and St. Mary's Bays, during April and August 2013, contained members of several Classes and Genera, including *Alphaproteobacteria* (*Roseobacter* clade, *Ancalomicrobium*), *Gammaproteobacteria* (*Glaciecola*, *Tereinibacter*, *Colwellia*, *Pseudoaltermonas*, *Psychromonas*, *Oleispira*, *Saccharospirillum*, *Phocoenobacter*, *Pseudomonas*, *Cycloclasticus*, *Vibrio*, and *Providencia*) and *Bacteroidetes* (*Polaribacter*). The composition of the bacterial communities found in both bays was consistent with those found in cold waters, including the Arctic and Antarctic, often found to be dominated by *Gammaproteobacteria* and *Alpahproteobacteria* (Bowman *et al.,* 1997; Brown and Bowman, 2001; Junge *et. al.,* 2002; Brinkmeyer *et. al*., 2003; Prabagaran *et al*., 2007; Brakstad *et al.,* 2008; Kim *et al.,* 2014). We suggest that increasing ocean temperature may support the spread and dominance of those bacteria that prefer warmer temperatures, such as *Vibrio* to more northern latitudes. This study determined temperature influenced bacterial community composition during each sampling period. Our study provides insight on the response of cold water bacterial communities to sampling period and temperature, when other ecological or anthropogenic factors are also present.

In turn, the enrichment treatments were the most variable factor, and showcased how variable bacterial communities can be, and their quick adaptations to their environments and incubation. This study suggests that increasing anthropogenic activities, such as runoff, use of fertilizers, and chronic release of petroleum hydrocarbons through shipping can lead to shifts in the bacterial communities' composition. Nutrient addition showed the greatest increase in relative abundance, and a shift towards *Gammaproteobacteria* (*Vibrio*) members in the bacterial communities when organic carbon was supplied. This study provides a framework for the potential short term effects of low concentrations of petroleum hydrocarbons on cold water bacterial communities. The addition shifted the community towards some known hydrocarbon degraders (*Vibrio*, *Colwellia*, *Oleisipra*, *Polaribacter*) with a greater increase in Placentia Bay than St. Mary's Bay, suggesting that the community was preconditioned by the low concentrations of anthropogenic input of petroleum hydrocarbons from shipping movement. These results suggest that the recently proposed trans-Arctic shipping route will have significant implications on the bacterial community composition as a result of oil tanker traffic.

This study only considered the patterns of change in two Phyla and their associated Genera. There are possibly many interactions in the bacterial communities occurring not discussed here, and many taxa below the cut-off of 1-4% of the total number of sequences, that may have significant responses to the enrichment treatments or sampling period and locations. It has been suggested that light influences the bacterial community composition (Vila *et al*., 1998; Baptista *et al*., 2014) suggesting that the dark

incubations used in this study may have influenced the composition. However, neither counts or sequencing was done for photoautotrophic groups specifically, therefore this influence was not included in this study but may be considered for future studies. Generally, all Genera increased in relative abundance when incubated in the unamended control (Figure 3.6 A, B, Table 3.3), suggesting the removal of grazing, and competition for nutrients with algae was a significant influence on the relative abundances of most Genera. The results and relative abundances observed in this study may therefore be an overestimate from what would occur *in situ*. We only had access to one type of petroleum hydrocarbon, and utilized one concentration, where a gradient may have provided further insight into the responses of the bacterial communities as a whole. Our results suggested that overall the petroleum hydrocarbons used inhibited many Genera, and the response to different hydrocarbon compositions, at low concentrations, should also be quantified. Diversity indices and phylogenetic similarity trees were not made in this study, but could provide us with more details of the changes within the communities.

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Yakimov, M. M., Gentile, G., Bruni, V., Cappello, S., D'Auria, G., Golyshin, P. N., & Giuliano, L. (2004). Crude oil-induced structural shift of coastal bacterial communities of Rod Bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. *FEMS Microbiology Ecology*, *49*, 419–432.

Yakimov, M. M., Timmis, K. N., & Golyshin, P. N. (2007). Obligate oil-degrading marine bacteria. *Current Opinion in Biotechnology*, *18*, 257–266.

Table 3.1. All barcode sequences used with reverse primers for this study.

IonXpress_1	CTAAGGTAAC
IonXpress_2	TAAGGAGAAC
IonXpress_3	AAGAGGATTC
IonXpress_4	TACCAAGATC
IonXpress_5	CAGAAGGAAC
IonXpress_6	CTGCAAGTTC
IonXpress_7	TTCGTGATTC
IonXpress_8	TTCCGATAAC
IonXpress_9	TGAGCGGAAC
IonXpress_10	CTGACCGAAC
IonXpress_11	TCCTCGAATC
IonXpress_12	TAGGTGGTTC
IonXpress_13	TCTAACGGAC
IonXpress_14	TTGGAGTGTC
IonXpress_15	TCTAGAGGTC
IonXpress_16	TCTGGATGAC
IonXpress_17	TCTATTCGTC
IonXpress_18	AGGCAATTGC
IonXpress_19	TTAGTCGGAC
IonXpress_20	CAGATCCATC

Table 3.2. Summary of relative abundance (%) of Genera present in each treatment (out of total sequences for each microcosm), during April and August 2013.

[a]Genera	[b]Class	[c]Bay	[d]Relative Abundance (%)													
			N		C		I		O		I+O		H		H+I	
			Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013
Roseobacter_clade	Alpha	SM	23.14	17.51	11.13	16.00	11.68	22.26	18.66	7.46	7.86	1.31	9.69	14.90	11.49	14.77
		PB	32.82	38.93	20.70	41.16	21.60	40.11	15.01	8.37	19.18	2.78	16.45	37.10	18.26	16.45
Ancalomicrobium	Alpha	SM	12.96	0.96	27.75	1.34	33.32	1.43	1.84	0.47	27.04	0.07	20.70	1.22	32.05	1.20
		PB	14.03	0.32	15.42	0.48	1.30	0.45	6.45	0.10	7.67	0.03	25.58	0.47	12.60	0.27
Glaciecola	Gamma	SM	0.24	0.00	0.50	1.65	0.38	1.85	3.75	0.38	0.32	0.12	0.53	1.54	0.33	2.85
		PB	0.05	0.03	0.19	16.67	0.02	19.01	0.11	11.21	0.15	1.99	0.18	22.04	0.15	12.50
Teredinibacter	Gamma	SM	1.63	1.47	1.95	0.06	1.92	0.09	0.05	0.03	2.77	0.00	2.30	0.16	2.13	0.22
		PB	1.96	0.55	4.63	0.13	0.79	0.21	2.46	0.03	1.97	0.01	5.81	0.19	3.68	0.11
Colwellia	Gamma	SM	0.57	0.06	15.20	2.24	15.79	1.90	0.91	0.75	9.14	0.19	11.94	1.83	10.51	3.37
		PB	0.09	0.02	4.46	0.86	0.17	0.66	3.75	0.47	3.64	0.16	2.44	0.46	3.34	0.08
Pseudoalteromonas	Gamma	SM	0.30	0.82	0.16	5.67	0.10	5.72	1.76	8.20	0.03	1.66	0.12	4.96	0.11	7.10
		PB	0.04	0.42	0.10	2.82	0.00	2.46	0.48	2.67	0.11	0.64	0.03	2.69	0.07	2.00
Psychromonas	Gamma	SM	0.53	0.79	0.73	0.00	0.42	0.00	1.37	0.77	14.30	0.85	0.35	0.01	0.48	0.00
		PB	0.07	0.06	0.36	0.00	0.00	0.00	11.41	1.05	12.68	0.87	0.24	0.00	0.18	0.00
Oleispira	Gamma	SM	0.00	0.00	0.07	1.04	0.08	0.93	0.04	0.32	0.03	0.06	0.07	1.91	0.06	1.22
		PB	0.00	0.02	0.05	0.25	0.00	0.19	0.03	0.16	0.07	0.03	0.04	3.81	0.04	0.39
Saccharospirillum	Gamma	SM	0.01	0.00	0.26	5.34	0.24	3.94	0.00	1.69	0.09	0.98	0.21	0.06	0.08	0.14
		PB	0.00	0.01	0.15	0.07	0.00	0.10	0.17	0.09	0.25	0.05	0.16	0.05	0.19	0.24
Phocoenobacter	Gamma	SM	0.00	0.11	0.20	2.66	0.06	2.27	2.35	0.45	0.03	2.26	0.09	21.10	0.06	17.70
		PB	0.00	0.00	0.01	0.83	0.00	0.36	0.04	0.30	0.00	0.14	0.00	0.64	0.01	2.64
Pseudomonas	Gamma	SM	0.24	1.61	0.37	0.07	0.13	0.05	0.11	0.02	0.19	0.00	0.35	0.05	0.26	0.07
		PB	0.04	0.08	0.64	0.04	4.88	0.03	1.47	0.01	0.51	0.06	0.21	0.03	0.43	0.00
Cycloclasticus	Gamma	SM	0.00	0.14	0.00	7.41	0.01	7.47	2.80	11.53	0.00	2.52	0.01	5.34	0.01	7.65

[a]Genera	[b]Class	[c]Bay	[d]Relative Abundance (%)													
			N		C		I		O		I+O		H		H+I	
			Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013
		PB	0.00	0.07	0.00	5.99	0.01	6.90	0.00	7.82	0.00	1.78	0.00	0.10	0.00	4.07
Vibrio	Gamma	SM	0.00	2.80	0.00	17.06	0.15	14.01	6.60	55.19	0.00	85.36	0.02	21.00	0.01	13.68
		PB	0.00	0.14	0.00	3.97	0.16	3.45	0.10	55.35	0.00	84.69	0.01	3.87	0.00	5.65
Providencia	Gamma	SM	0.02	0.00	0.00	19.32	0.00	17.84	0.00	3.81	0.00	0.15	0.00	0.15	0.00	0.18
		PB	0.00	0.00	0.00	4.17	0.00	3.89	0.79	2.47	0.00	0.86	0.00	4.63	0.00	0.07
Polaribacter	Bacter	SM	2.75	0.31	11.26	1.39	6.78	1.30	0.40	0.30	16.88	0.08	21.95	1.25	17.59	1.07
		PB	1.60	0.40	2.41	1.69	1.10	2.26	3.10	0.80	2.90	0.04	9.91	1.61	10.17	3.04

[a]Only Genera that were >4% of the total number of sequences in at least one treatment for both bays and months, were included in the table.

[b]Classes *Alphaproteobacteria* (Alpha), *Gammaproteobacteria* (Gamma), and *Bacteroidetes* (Bacter).

[c]St. Mary's Bay (SM), and Placentia Bay (PB).

[d]Relative abundance (%) for all incubation treatments; *in situ* community (N), control (C), inorganic nutrients (I), organic carbon (O), inorganic nutrients and organic carbon (I+O), petroleum hydrocarbon (H), petroleum hydrocarbon and inorganic nutrients (H+I) were reported. See methods for calculations.

Table 3.3. Summary of the percent change (%) in relative abundance for all Genera present from Table 3.2, in Placentia and St. Mary's Bays during April and August 2013.

Genera	[a]**Class**	[b]**Bay**	[c]**Percent Change (%)**											
			C		**I**		**O**		**I+O**		**H**		**H+I**	
			Apr 2013	**Aug 2013**	**Apr 2013**	**Aug 2013**	**Apr 2013**	**Aug 2013**	**Apr 2013**	**Aug 2013**	**Apr 2013**	**Aug 2013**	**Apr 2013**	**Aug 2013**
Roseobacter_clade	Alpha	SM	-108	-9	5	39	68	-114	-42	-1121	-15	-7	3	-8
		PB	-59	6	4	-3	-38	-392	-8	-1381	-26	-11	-13	-150
Ancalomicrobium	Alpha	SM	114	40	20	7	-1408	-185	-3	-1814	-34	-10	15	-12
		PB	10	50	-1086	-7	-139	-380	-101	-1500	66	-2	-22	-78
Glaciecola	Gamma	SM	108	[e]*	-32	12	650	-334	-56	-1275	6	-7	-52	73
		PB	280	55467	-850	14	-73	-49	-27	-738	-6	32	-27	-33
Teredinibacter	Gamma	SM	20	-2350	-2	50	-3800	-100	42	*	18	167	9	267
		PB	136	-323	-486	62	-88	-333	-135	-1200	25	46	-26	-18
Colwellia	Gamma	SM	2567	3633	4	-18	-1570	-199	-66	-1079	-27	-22	-45	50
		PB	4856	4200	-2524	-30	-19	-83	-23	-438	-83	-87	-34	-975
Pseudoalteromonas	Gamma	SM	-88	591	-60	1	1000	45	-433	-242	-33	-14	-45	25
		PB	150	571	*	-15	380	-6	10	-341	-233	-5	-43	-41
Psychromonas	Gamma	SM	38	*	-74	0	88	*	1859	*	-109	*	-52	0
		PB	414	*	*	0	3069	*	3422	*	-50	0	-100	0
Oleispira	Gamma	SM	*	*	14	-12	-75	-225	-133	-1633	0	84	-17	17
		PB	*	1150	*	-32	-67	-56	40	-733	-25	1424	-25	56
Saccharospirillum	Gamma	SM	2500	*	-8	-36	*	-216	-189	-445	-24	-8800	-225	-3714
		PB	*	600	*	43	13	29	67	-40	7	-40	27	243
Phocoenobacter	Gamma	SM	*	2318	-233	-17	1075	-491	-567	-18	-122	693	-233	565
		PB	*	*	*	-131	300	-177	*	-493	*	-30	0	218
Pseudomonas	Gamma	SM	54	-2200	-185	-40	-236	-250	-95	*	-6	-40	-42	0
		PB	1500	-100	663	-33	130	-300	-25	50	-205	-33	-49	*
Cycloclasticus	Gamma	SM	[d]0	5193	*	1	*	56	0	-194	*	-39	*	3
		PB	0	8457	*	15	0	31	0	-237	0	-5890	0	-47

Genera	[a]Class	[b]Bay	[c]Percent Change (%)											
			C		I		O		I+O		H		H+I	
			Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013
Vibrio	Gamma	SM	0	509	*	-22	*	224	0	400	*	23	*	-25
		PB	0	2736	*	-15	*	1294	0	2033	*	-3	0	42
Providencia	Gamma	SM	*	*	0	-8	0	-407	0	-12780	0	-12780	0	-10633
		PB	0	*	0	-7	*	-69	0	-385	0	11	0	-5857
Polaribacter	Bacter	SM	309	348	-66	-7	-2715	-363	50	-1638	95	-11	56	-30
		PB	51	323	-119	34	29	-111	20	-4125	311	-5	322	80

[a]Classes *Alphaproteobacteria* (Alpha), *Gammaproteobacteria* (Gamma), and *Bacteroidetes* (Bacter).

[b]St. Mary's Bay (SM), and Placentia Bay (PB).

[c]Percent change = [Equation 1. ((Relative abundance of Treatment – relative abundance of control)/relative abundance of control)*100, Equation 2. -((Relative abundance of control – relative abundance of treatment)/relative abundance of treatment)*100] for all incubation treatments; *in situ* community (N), control (C), inorganic nutrients (I), organic carbon (O), inorganic nutrients and organic carbon (I+O), petroleum hydrocarbon (H), petroleum hydrocarbon and inorganic nutrients (H+I). For all treatments, the percent change (%) is in comparison to the control, except the control which is in comparison to the *in situ* community (N).

[d, e]For some Genera, no change (0) was observed, or the percent change was not calculated (*), because of division by zero. Refer to Table 3.2 for absolute values.

Table 3.4. General patterns of the percent change (%) in relative abundance for all Genera present from Table 3.3, in Placentia and St. Mary's Bays during April and August 2013.

Genera	[a]**Class**	[b]**Bay**	[c]**Percent Change (%)**											
			[d]**C**		**I**		**O**		**I+O**		**H**		**H+I**	
			Apr 2013	**Aug 2013**	**Apr 2013**	**Aug 2013**	**Apr 2013**	**Aug 2013**	**Apr 2013**	**Aug 2013**	**Apr 2013**	**Aug 2013**	**Apr 2013**	**Aug 2013**
Roseobacter_clade	Alpha	SM	-	-	+	+	+	-	-	---	-	-	+	-
		PB	-	+	+	-	-	-	-	---	-	-	-	-
Ancalomicrobium	Alpha	SM	+	+	+	+	--	-	-	---	-	-	+	-
		PB	+	+	--	-	-	-	-	---	+	-	-	-
Glaciecola	Gamma	SM	+	*	-	+	++	-	-	---	+	-	-	+
		PB	+	+++	--	+	-	-	-	--	-	+	-	-
Teredinibacter	Gamma	SM	+	---	-	+	---	-	+	*	+	+	+	+
		PB	+	-	-	+	-	-	-	---	+	+	-	-
Colwellia	Gamma	SM	+++	+++	+	-	---	-	-	---	-	-	-	+
		PB	+++	+++	---	-	-	-	-	-	-	-	-	--
Pseudoalteromonas	Gamma	SM	-	++	-	+	+++	+	-	-	-	-	-	+
		PB	+	++	[e]*	-	+	-	+	-	-	-	-	-
Psychromonas	Gamma	SM	+	*	-	[e]0	+	*	+++	*	-	*	-	0
		PB	+	*	*	0	+++	*	+++	*	-	0	-	0
Oleispira	Gamma	SM	*	*	+	-	-	-	-	---	0	+	-	+
		PB	*	+++	*	-	-	-	+	--	-	+++	-	+
Saccharospirillum	Gamma	SM	+++	*	-	-	*	-	-	-	-	---	-	---
		PB	*	++	*	+	+	+	+	-	+	-	+	+
Phocoenobacter	Gamma	SM	*	+++	-	-	+++	-	--	-	-	++	-	++
		PB	*	*	*	-	+	-	*	-	*	-	0	+
Pseudomonas	Gamma	SM	+	---	-	-	-	-	-	*	-	-	-	0
		PB	+++	-	++	-	+	-	-	+	-	-	-	*
Cycloclasticus	Gamma	SM	0	+++	*	+	*	+	0	-	*	-	*	+
		PB	0	+++	*	+	0	+	0	-	0	---	0	-

Genera	[a]Class	[b]Bay	[c]Percent Change (%)											
			[d]C		I		O		I+O		H		H+I	
			Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013	Apr 2013	Aug 2013
Vibrio	Gamma	SM	0	++	*	-	*	+	0	+	*	+	*	-
		PB	0	+++	*	-	*	+++	0	+++	*	-	0	+
Providencia	Gamma	SM	*	*	0	-	0	-	0	---	0	---	0	---
		PB	0	*	0	-	*	-	0	-	0	+	0	---
Polaribacter	Bacter	SM	+	+	-	-	---	-	+	---	+	-	+	-
		PB	+	+	-	+	+	-	+	---	+	-	+	+

[a]Classes *Alphaproteobacteria* (Alpha), *Gammaproteobacteria* (Gamma), and *Bacteroidetes* (Bacter).
[b]St. Mary's Bay (SM), and Placentia Bay (PB).
[c]Percent change (%) summarizes the pattern of percent change from Table 3.3: < -1000% (---), -1000 to -500% (--), -500 to 0% (-), no change (0), 0-500% (+), 500-1000% (++), >1000% (+++) for all incubation treatments; *in situ* community (N), control (C), inorganic nutrients (I), organic carbon (O), inorganic nutrients and organic carbon (I+O), petroleum hydrocarbon (H), petroleum hydrocarbon and inorganic nutrients (H+I) were reported.
[d]For all treatments, the percent change (%) is in comparison to the control, except the control which is in comparison to the *in situ* community (N).
[e]For some Genera, no change (0) was observed, or the percent change was not calculated (*), because of division by zero. Refer to Table 3.2 for absolute values.

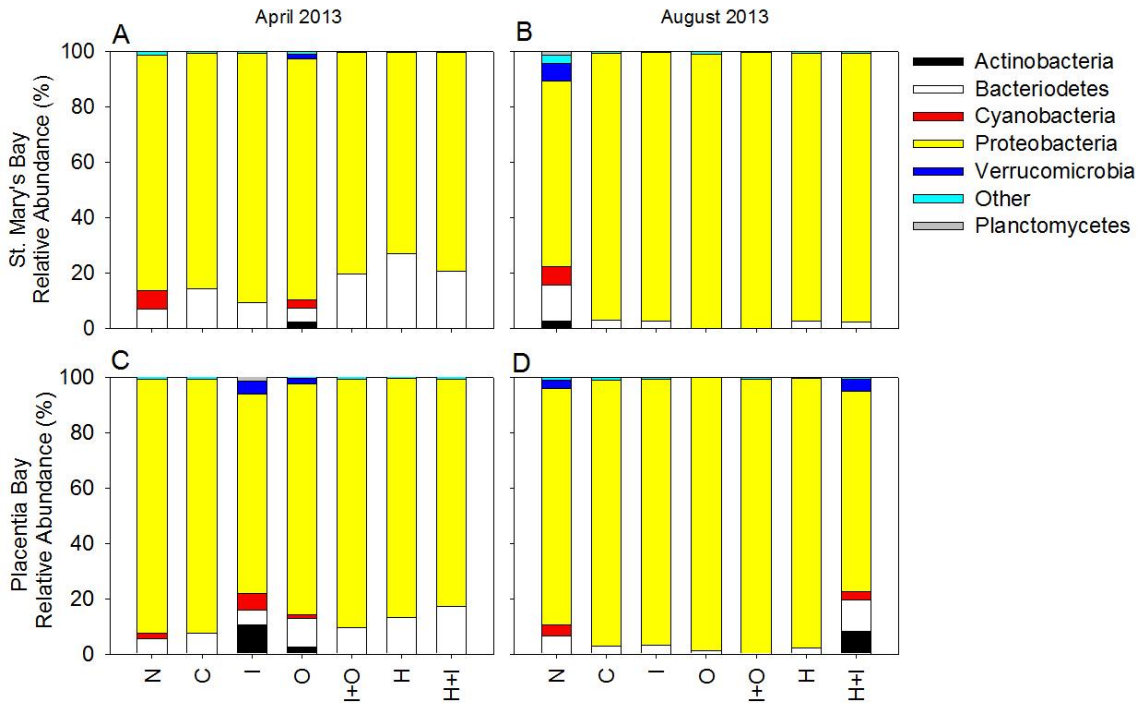

Figure 3.1. Relative abundance (%) of the total number of sequences of bacteria in the incubations collected from Placentia and St. Mary's Bay during April and August 2013. Phyla that accounted for less than 1% of the total number of sequences for each of the nutrient or hydrocarbon treatments were designated to 'Other'. The N community was collected from non-diluted sample water at the start of the incubation, all other conditions were collected from the bottles at the end of the incubation.

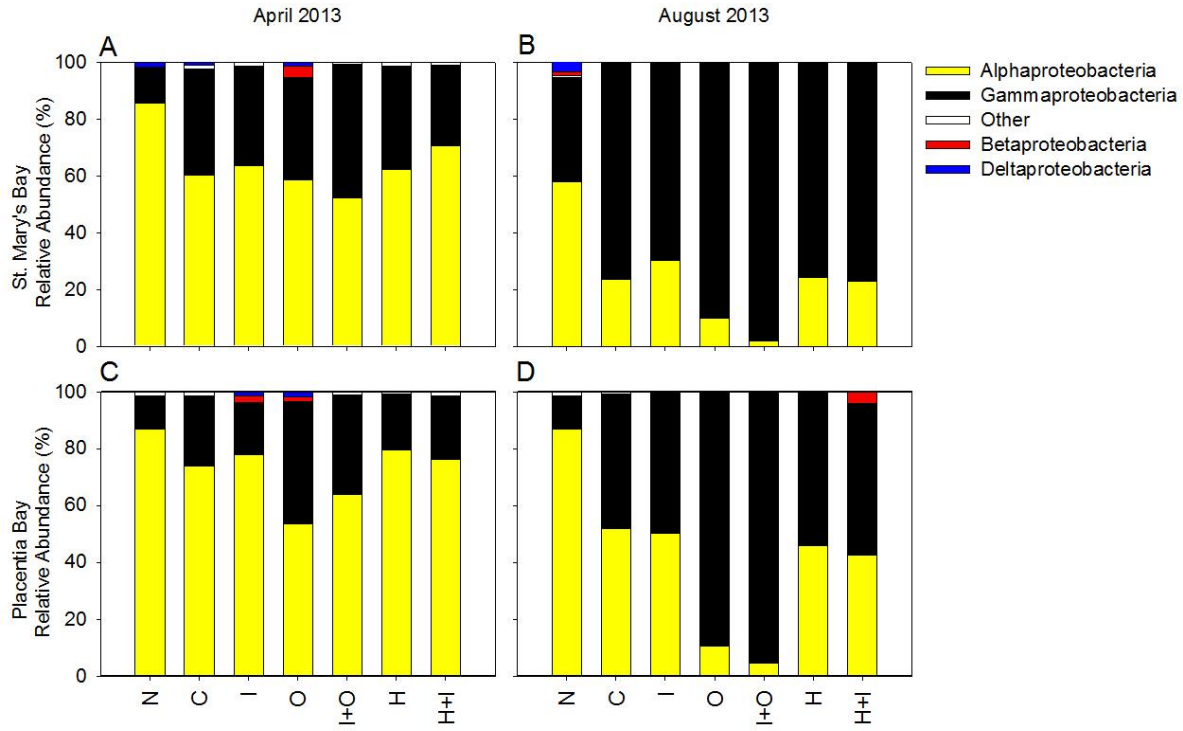

Figure 3.2. Relative abundance (%) of the number of sequences within the Phylum *Proteobacteria* in the incubations collected from Placentia and St. Mary's Bays during April and August 2013.

Classes that accounted for less than 1% of the number of sequences of *Proteobacteria* for each of the nutrient or hydrocarbon treatments were designated to 'Other'. The N community was collected from non-diluted sample water at the start of the incubation, all other conditions were collected from the bottles at the end of the incubation.

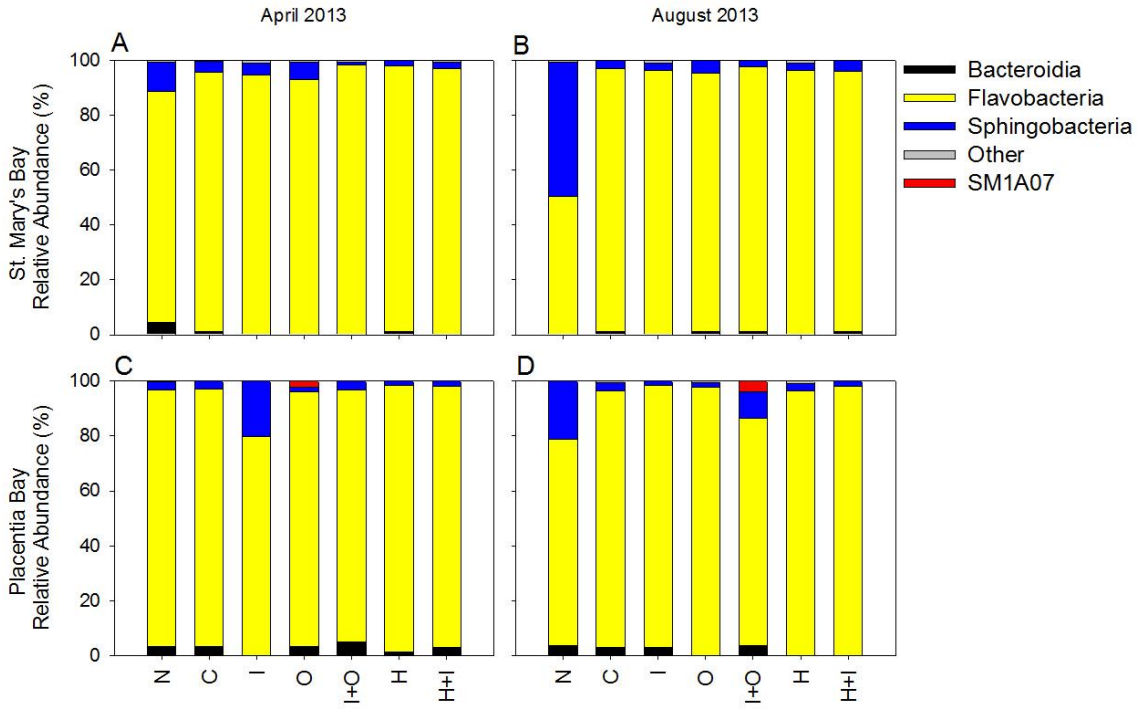

Figure 3.3. Relative abundance (%) of the number of sequences within the Phylum *Bacteroidetes* in the incubations collected from Placentia and St. Mary's Bays during April and August 2013.

Classes that accounted for less than 1% of the number of sequences of *Bacteroidetes* for each of the nutrient or hydrocarbon treatments were designated to 'Other'. The N community was collected from non-diluted sample water at the start of the incubation, all other conditions were collected from the bottles at the end of the incubation.

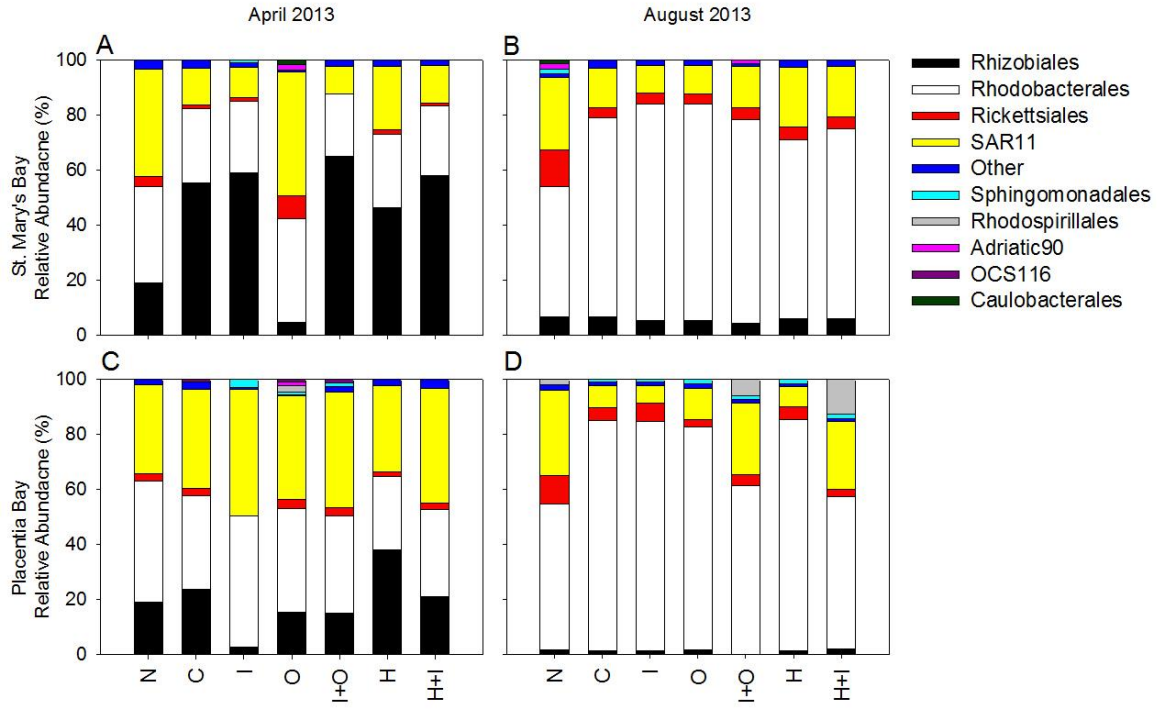

Figure 3.4. Relative abundance (%) of the number of sequences within the Class *Alphaproteobacteria* in the incubations collected from Placentia and St. Mary's Bays during April and August 2013.

Orders that accounted for less than 1% of the number of sequences of *Alphaproteobacteria* for each of the nutrient or hydrocarbon treatments were designated to 'Other'. The N community was collected from non-diluted sample water at the start of the incubation, all other conditions were collected from the bottles at the end of the incubation.

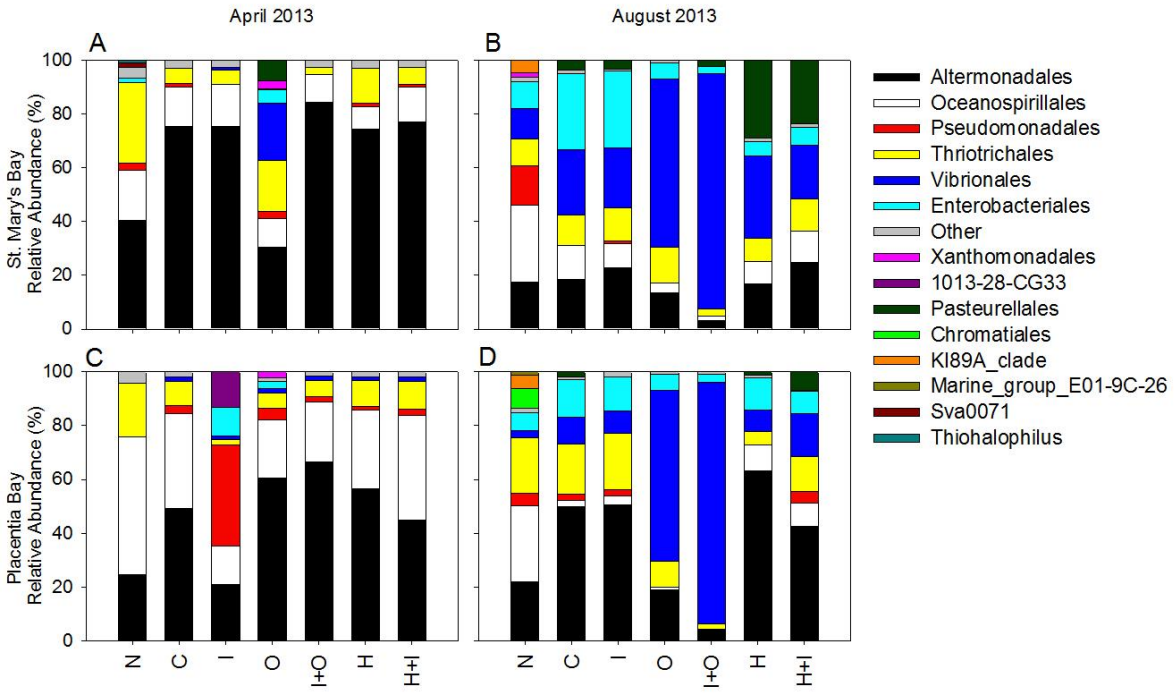

Figure 3.5. Relative abundance (%) of the number of sequences within the Class *Gammaproteobacteria* in the incubations collected from Placentia and St. Mary's Bays during April and August 2013.

Orders that accounted for less than 1% of the number of sequences of *Gammaproteobacteria* for each of the nutrient or hydrocarbon treatments were designated to 'Other'. The N community was collected from non-diluted sample water at the start of the incubation, all other conditions were collected from the bottles at the end of the incubation.

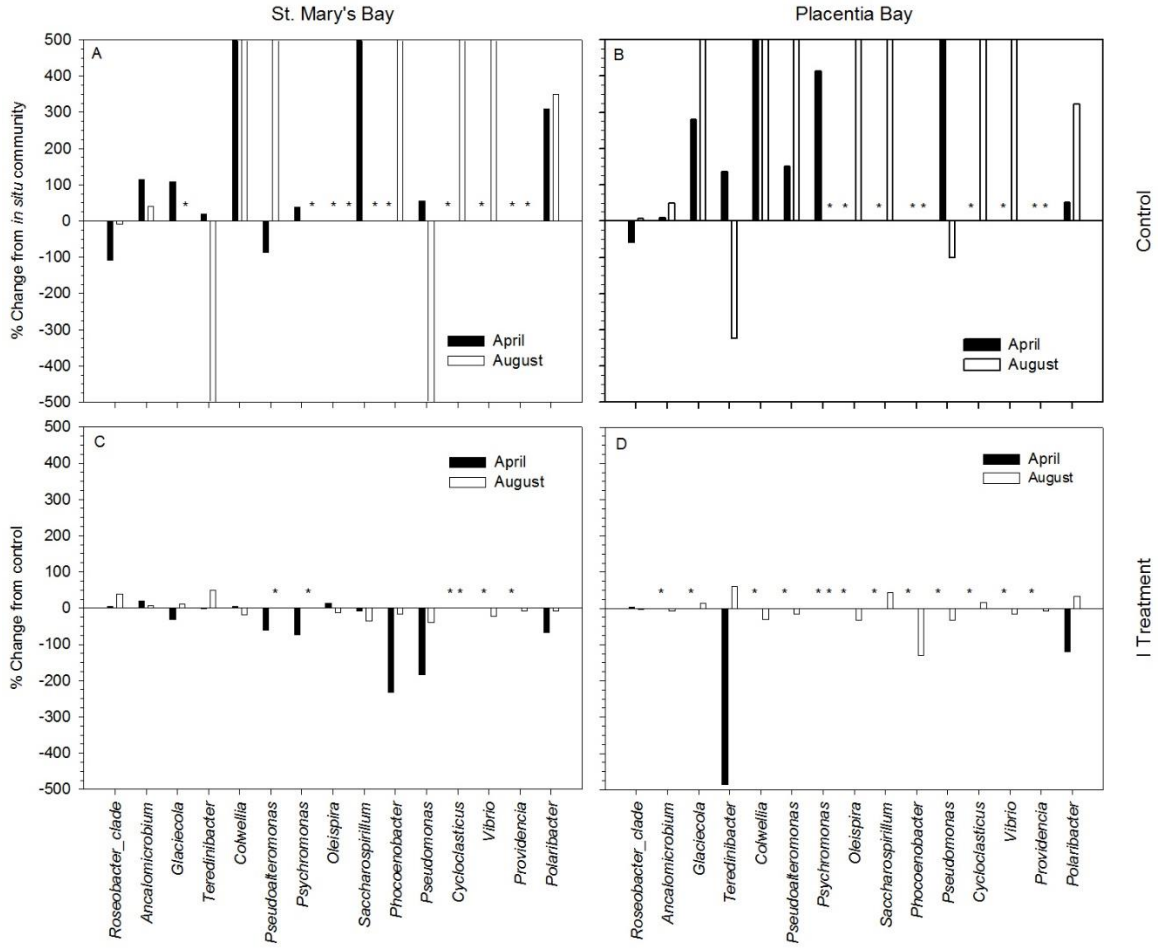

Figure 3.6. The percent change of each Genus in the control relative to the *in situ* community (Panels A and B), and in the inorganic (Panels C and D) nutrient addition treatment relative to the control, in both St. Mary's and Placentia Bay during April and August 2013.

Ordinal axis set to a maximum of 500%, and some percent change were unable to be calculated (*), because of division by zero. Refer to Table 3.2 for absolute values.

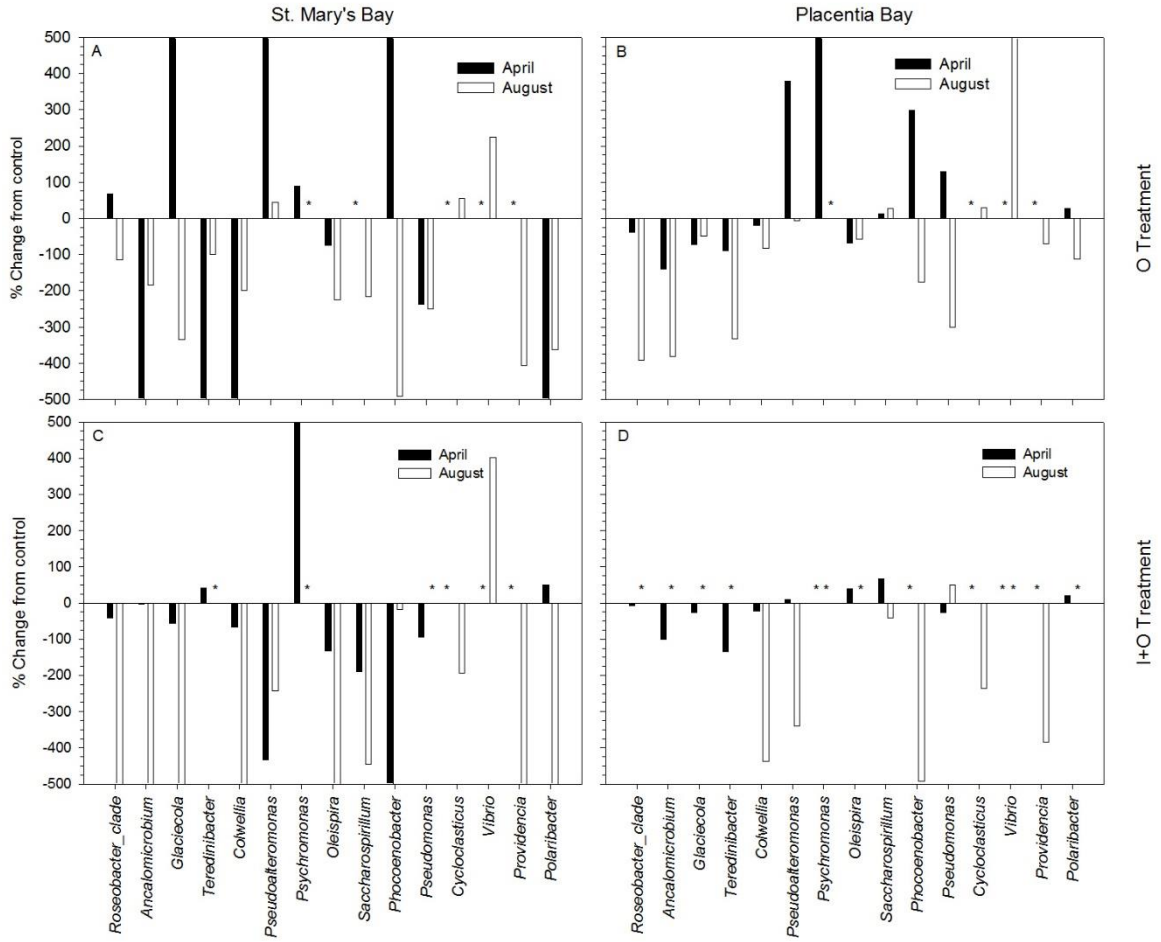

Figure 3.7. The percent change of each Genus in the organic carbon (Panels A and B), and inorganic nutrient plus organic carbon (Panels C and D) addition treatments relative to the control, in both St. Mary's and Placentia Bay during April and August 2013. Ordinal axis set to a maximum of 500%, and some percent change were unable to be calculated (*), because of division by zero. Refer to Table 3.2 for absolute values.

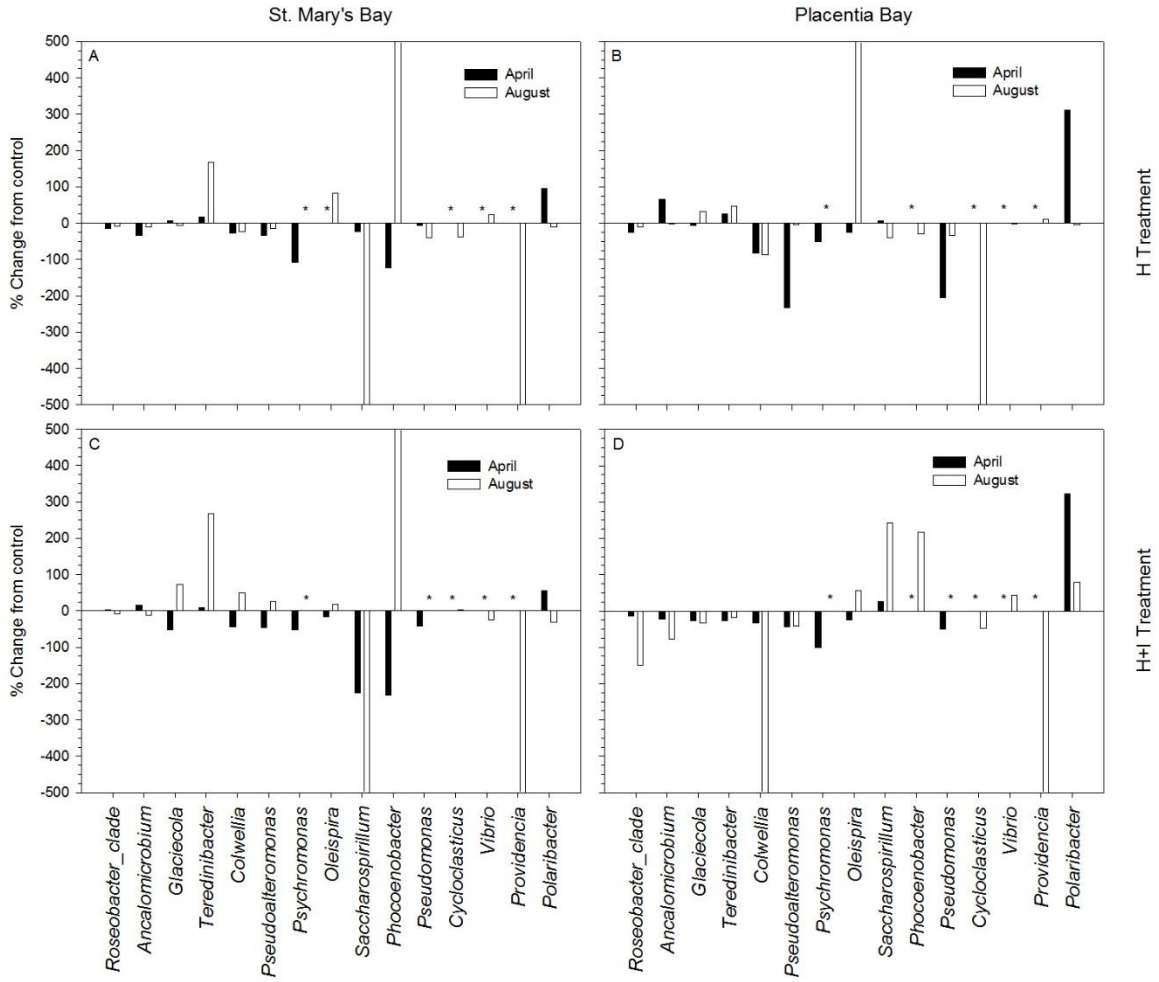

Figure 3.8. The percent change of each Genus in the hydrocarbon (Panels A and B), and hydrocarbon plus inorganic nutrient (Panels C and D) addition treatments relative to the control, in both St. Mary's and Placentia Bay during April and August 2013. Ordinal axis set to a maximum of 500%, and for some Genera the percent change was unable to be calculated (*), because of division by zero. Refer to Table 3.2 for absolute values.

Chapter 4: Conclusion

4.1 Overview of Chapters

Petroleum hydrocarbons are a significant contaminant in marine ecosystems (Windom, 1992) and can enter these systems through catastrophic events (shipping accidents, or pipe bursts), chronic input (ships, refineries, freshwater runoff) or from natural sources (Blumer *et al.,* 1971). The release of petroleum hydrocarbons into coastal Newfoundland waters is an environmental and economic concern. Petroleum hydrocarbons have been reported to both stimulate and inhibit marine bacteria and plankton (Grötzschel *et al*., 2002; Head *et al*., 2006; Cappello *et al.,* 2007; Yakimov *et al.,* 2007). Bacteria are important players in biogeochemical processes of the oceans (Falkowski *et al*., 2008), and play key roles in marine food web transformations (Kellogg *et al*., 1972; Ward, 1996; Azam, 1998; González *et al*., 2000; Pomeroy *et al*., 2007). The main process of natural removal of petroleum hydrocarbons from the ocean is biodegradation by bacteria, although there are other processes such as weathering (Cappello *et al*., 2007). Petroleum hydrocarbon degradation is influenced by which bacteria are already present in the marine environment (Röling *et al*., 2002; Yakimov *et al.,* 2004, 2005). The addition of petroleum hydrocarbons, such as during the Deepwater Horizon spill, has been shown to increase the abundance of hydrocarbon degrading bacteria that had been found to initially be present due to previous exposure to natural petroleum hydrocarbons seeps, and shift the community towards these species (Harayama *et al*., 1999; Kasai *et al.,* 2001; Head *et al*., 2006; Cappello *et al.,* 2007; Hazen *et al*., 2010; Kappell *et al*., 2014). It has been reported that the addition of inorganic nutrients

enhances the growth of hydrocarbon degrading bacteria (Atlas and Bartha, 1972). There appears to be little literature on the effects of chronic release of low concentrations of petroleum hydrocarbons on marine bacterial communities' growth or composition (Paissé *et al*., 2008). Quantifying the effects on both the bacterial growth, as well as community structure is needed to understand or predict responses to climate change or further anthropogenic activities.

Chapter 2 presented the results of an enrichment growth study to determine the effects of the addition of inorganic nutrients and organic carbon, low concentrations of petroleum hydrocarbons, and temperature on the growth of coastal bacteria during four different sampling periods. Replicate seawater samples were incubated at two temperatures, with five treatments (**+[I],** **+[O]**, **+[I+O]**, **+[H]**, **+[H+I]**) and an unamended control (**+[C]**). The results suggest that a continued increase in anthropogenic activities, such as the use of fertilizers and runoff, and release of low concentrations of petroleum hydrocarbons through shipping can significantly alter bacteria growth characteristics. The addition of both inorganic nutrients and organic carbon had the greatest stimulation of growth, while the effect of organic carbon alone was influenced by temperature. The results demonstrated that overall the addition of low concentrations of petroleum hydrocarbons did not stimulate bacterial growth except in Placentia Bay during August, and suggests that the toxic nature of petroleum hydrocarbon may be inhibitory to some of the subarctic bacterial communities. These results combined with the low concentration of petroleum hydrocarbons found to be from anthropogenic sources in Placentia Bay, suggests that shipping and refinery activity may have primed the community for

hydrocarbon degradation during August. Chapter 2 also suggests that climate change has potentially large implications on cold water bacteria with observable stimulation of bacterial growth with the small increase of just 2^{o}C in the ocean particularly when supplied with nutrients, and suggests that Placentia Bay may have greater organic carbon loading.

Chapter 3 presented the results of the nutrient and petroleum hydrocarbon additions described in Chapter 2 on bacterial community composition during spring and summer 2013. The bacterial community structure was characterized using 16SV6 rDNA analysis and high-throughput sequencing. Global warming has been suggested to increase the nutrient supply into the Arctic Ocean by thawing of frozen tundra and runoff (IPCC, 2007; Kirchman *et al*., 2009), as well as potential increase in petroleum hydrocarbon input because of the loss of ice coverage leading to the proposal of a trans-Arctic shipping routes (Smith and Stephenson, 2013; Stephenson *et al*., 2013). The results presented in this Chapter suggest that global warming can have significant impacts on cold water bacterial community compositions as the composition was shown to be influenced by nutrient and petroleum hydrocarbon additions, and combined with the results in Chapter 2 we showed that generally the growth observed in these incubations were from one or two key Genera as determined by the type of addition. The Genera *Psychromonas* and *Vibrio* (Class *Gammaproteobacteria*) were limited by both organic carbon and inorganic nutrients, with *Vibrio* capable of dominating the bacterial community. This study appears to be one of the first to determine the effects of low concentrations of petroleum hydrocarbons on bacterial community composition, and suggests that anthropogenic

activities related to shipping and refineries can shift the composition towards hydrocarbon degraders. The addition of low concentrations of petroleum hydrocarbons showed an increase in the relative abundance of known hydrocarbon degraders *Vibrio*, *Colwellia*, *Oleisipra*, and *Polaribacter*, with a greater number of hydrocarbon degraders, and greater increase in the relative abundance of *Vibrio, Oleisipra,* and *Polaribacter* in Placentia Bay. Some Genera such as *Polaribacter* or *Glaciecola* also increased in relative abundance in St. Mary's Bay in the presence of petroleum hydrocarbons, however they were either only hydrocarbon tolerant or not involved in hydrocarbon degradation (i.e. *Glaciecola*), or showed a smaller increase than in Placentia Bay (i.e. *Polaribacter*). These results combined with the growth rates determined in Chapter 2, suggest that community composition occurs before or in the absence of a functional response. Sampling period had the greatest influence on the community composition, and Chapter 3 suggested temperature acts as a significant seasonal factor indicating the warming of the oceans may support the spread of bacteria that prefer warmer temperatures in the more northern latitudes. Generally more psychrophilic and psychrotolerant Genera such as *Oleispira* increased in relative abundance during the spring (coldest period; Table 3.2 and 3.3), and Genera such as *Vibrio,* with warm water preferences either increased in relative abundance or to a greater magnitude during the summer (warmest period; Table 3.2 and 3.3).

4.2 Significance and future directions

The production and movement of petroleum hydrocarbons in Newfoundland coastal waters puts the area at risk for potential spills, but also for the chronic release of low levels of petroleum hydrocarbons due to shipping movements. The growth and community composition studies were necessary to determine if there were effects of these low inputs into the marine environment. Although there is considerable literature on the effects of petroleum hydrocarbons on the bacterial community (Head *et al*., 2006; Cappello *et al.,* 2007; Yakimov *et al.,* 2007), the concentrations of petroleum hydrocarbons are typically high (Kasai *et al*., 2002ab; Cappello *et al*., 2007; Coulon *et al*., 2007; Paissé *et al*., 2010) or are often related to spills (Jiménez *et al*., 2011; Baelum *et al.,* 2012). There have been few studies on the effects of chronic low concentrations of petroleum hydrocarbons on either bacterial community growth or composition (Paissé *et al*., 2008). It was unclear if petroleum hydrocarbons at low concentrations would be inhibitory or a carbon source to the bacterial communities, therefore organic carbon and inorganic nutrient additions were included to complete the experimental matrix. Although other studies have examined the effects of nutrients (Church *et al*., 2000; Carlson *et al*., 2002; Cuevas *et al*., 2011), petroleum hydrocarbons (Head *et al*., 2006; Cappello *et al.,* 2007; Yakimov *et al.,* 2007) and temperature (Rivkin *et al*., 1996; Kirchman and Rich, 1997) on bacterial communities' growth or structure, this study appears to be one of the first to combine the effects of these variables on the bacterial communities function, as well as their composition in a coastal cold water environment.

Climate warming significantly affects cold water oceans, as the temperature in the Arctic Ocean has been increasing over the past 100 years, with a peak increase of approximately 5^{o}C during 2007 (Steele *et al*., 2008). Bacteria are a large part of biogeochemical cycles in the ocean, and their response to a small change in water temperatures in ecologically sensitive regions, such as the Arctic, can have a huge influence in the overall response of the oceans to climate change (Kirchman *et al*., 2009). Our study provides insight on the response of cold water bacterial communities to elevated temperature and global warming, when other ecological or anthropogenic factors are also present. With climate warming, and decreased sea ice coverage in the Arctic, there has been a proposal of trans-Arctic shipping routes (Smith and Stephenson, 2013; Stephenson *et al*., 2013). Our study provides a framework for the potential short term effects of low concentrations of petroleum hydrocarbons on cold water bacterial communities, suggesting both potential inhibition of growth and a shift in community composition.

Relatively new genomic techniques such as high-throughput sequencing allows us to begin to understand the community processes that underlie ecosystem function and biogeochemical processes (Doney *et al*., 2004; Strom, 2008). Many studies on bacteria use cultured species, but to fully understand the roles of bacterial consortia, determining both the function and response to ecological factors, and the composition of the community *in situ* is needed (Ducklow, 2008). This study provides insight on how nutrient and petroleum hydrocarbon additions shift bacterial community structure to

bacteria capable of thriving in those environments, and how structure responds before or in absence of a functional response.

We show that petroleum hydrocarbons have significant effects on marine bacterial communities' structure and function even at low concentrations (Figure 2.2, Table 3.2). Some studies report that the effect of petroleum hydrocarbon inputs may not be observable immediately, but can occur after 14 days (Paissé *et al*., 2010). The current experiment was incubated for 48 to 72 hours to reduce potential bottle effects, therefore, we may have not observed the full response of the community to hydrocarbon input. Thus, a future consideration could be to observe the response over a longer period of time. Our results suggested that overall the petroleum hydrocarbons used inhibited many Genera, and that the composition could have a greater effect on the response of the bacterial community than the amount. Therefore, the response to a range of hydrocarbon compositions, at low concentrations, should also be quantified.

The response of the bacterial community can also be influenced by which bacteria are present in that region (Røberg *et al*., 2011). Therefore, the effects of low concentrations of petroleum hydrocarbons on consortia from multiple and differing oceanic regions should be examined. Understanding both the function and structure of the bacterial community is crucial for properly estimating or predicting the responses of these communities, and their impacts on biogeochemical processes in the ocean. Bacterial abundance can be used to determine growth rates, or estimate the flux of carbon through biomass, in the bacterial food web. However, it can be difficult connecting bacterial community structure to large scale biogeochemical processes in the ocean. Utilizing new

techniques, including the identification *in situ* through high-throughput sequencing, increases our ability to determine substrate usage by specific bacteria. Thus allowing us to investigate the relationship between community structure and ecosystem function, to understand how bacteria can influence the oceans' biogeochemical processes.

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Appendices

Appendix A: Salinity values (psu) taken from a Seabird 19S CTD at multiple depths, for all sites in both Placentia and St. Mary's Bay.

Date	Site	Latitude (°N)	Longitude (°W)	Depth (m)	Salinity (psu)
August 8, 2012	Placentia Bay	47.292	53.990	1	31.6
				2	31.9
				4	31.9
August 9, 2012	Placentia Bay	46.933	54.200	1	31.8
				2	31.8
				5	31.8
				20	32.2
August 9, 2012	Placentia Bay	47.133	54.088	1	31.8
				2	31.8
				5	31.8
				16	32.1
August 9, 2012	Placentia Bay	47.275	54.029	1	31.9
				2	31.9
				5	31.9
				16	31.9
August 9, 2012	Placentia Bay	47.423	54.013	1	31.9
				2	31.9
				5	31.9
				31	32.6
August 10, 2012	St. Mary's Bay	46.886	53.800	1	31.7
				2	31.7
				5	31.7
				31	32.4
August 10, 2012	St. Mary's Bay	46.973	53.755	1	31.5
				2	31.4
				5	31.5
				31	32.7
August 10, 2012	St. Mary's Bay	47.041	53.653	1	31.6
				2	31.5
				5	31.6
				31	32.3

August 10, 2012	St. Mary's Bay	47.114	53.565	1	30.7
				2	30.8
				5	31.2
				31	32.0
April 15, 2013	Placentia Bay	47.292	53.990	1	31.7
				2	31.7
				3	31.8
April 23, 2013	St. Mary's Bay	47.007	53.644	1	34.0
				2	32.4
				5	32.1
April 30, 2013	Placentia Bay	47.292	53.990	1	32.1
				2	32.1
April 30, 2013	Placentia Bay	47.642	53.936	1	33.1
				2	32.6
				3	32.7
May 2, 2013	St. Mary's Bay	46.920	53.580	1	32.4
				2	32.0
May 2, 2013	St. Mary's Bay	47.007	53.644	1	32.7
				2	32.2
				6	32.2
May 2, 2013	St. Mary's Bay	47.158	53.660	1	31.9
				2	31.7
				4	31.7
June 10, 2013	Placentia Bay	47.292	53.990	1	32.4
				2	32.5
June 17, 2013	St. Mary's Bay	47.007	53.644	1	33.7
				2	32.0
				5	32.0
June 24, 2013	St. Mary's Bay	46.920	53.580	1	32.0
				2	31.5
June 24, 2013	St. Mary's Bay	47.007	53.644	1	32.2
				2	31.9
				6	32.0
June 24, 2013	St. Mary's Bay	47.158	53.660	1	29.1
				2	29.2
				3	29.4
June 25, 2013	Placentia Bay	46.919	54.186	1	32.1
				2	32.2
				5	32.2
				10	32.3

June 25, 2013	Placentia Bay	47.340	53.984	1	31.9
				2	31.9
				5	32.1
				14	32.3
June 25, 2013	Placentia Bay	47.642	53.936	1	31.7
August 26, 2013	Placentia Bay	47.292	53.990	1	31.7
				2	31.2
				3	31.2
August 30, 2013	St. Mary's Bay	47.007	53.644	1	31.5
				2	31.5
				5	31.5
September 4, 2013	St. Mary's Bay	46.920	53.580	1	31.4
September 4, 2013	St. Mary's Bay	47.007	53.644	1	31.4
				2	31.4
				5	31.4
September 4, 2013	St. Mary's Bay	47.158	53.660	1	24.9
				2	27.3
September 5, 2013	Placentia Bay	47.292	53.990	1	31.6
September 5, 2013	Placentia Bay	47.642	53.936	1	28.9
				2	31.1

Appendix B: Results of one-way ANOVA to determine if there was a significant effect of temperature on bacterial growth rates, for each treatment.

Location	[a]**Date**	**Treatment**	**F-value**	***p***
Placentia Bay	August 2012	C	88.09	0.001
		I	1.37	0.307
		O	13327.67	<0.001
		I+O	0.86	0.406
St. Mary's Bay	August 2012	C	23.67	0.008
		I	18.36	0.013
		O	17.36	0.014
		I+O	23.96	0.008
Placentia Bay	April 2013	C	0.01	0.936
		I	3.66	0.128
		O	0.04	0.848
		I+O	15.14	0.018
		H	76.21	0.001
		H+I	0.25	0.640
St. Mary's Bay	April 2013	C	14.40	0.019
		I	33.13	0.005
		O	97.89	0.001
		I+O	40.33	0.003
		H	51.13	0.002
		H+I	830.82	<0.001
Placentia Bay	June 2013	C	54.93	0.002
		I	314.04	<0.001
		O	0.42	0.550
		I+O	236.03	<0.001
		H	15.16	0.018
		H+I	59.09	0.002
St. Mary's Bay	June 2013	C	95.81	0.001
		I	40.88	0.003
		O	0.02	0.899
		I+O	42.33	0.003
		H	275.75	<0.001
		H+I	52.51	0.002
Placentia Bay	August 2013	C	2.30	0.204
		I	10.26	0.033
		O	8.99	0.040
		I+O	2.12	0.219
		H	1.58	0.277
		H+I	13.16	0.022

St. Mary's Bay	August 2013	C	0.22	0.661
		I	2.53	0.187
		O	<0.01	0.967
		I+O	2.57	0.187
		H	22.57	0.009
		H+I	3.08	0.154

[a]The degrees of freedom for both bays during all sampling periods was five.

Appendix C: Results of one-way ANOVA to determine if there was a significant effect of bay (Placentia vs St. Mary's Bay) on bacterial growth rates, for each treatment.

[a]**Date**	[b]**Temperature**	**Treatment**	**F-value**	***p***
August 2012	Ambient	C	123.82	<0.001
		I	80.19	0.001
		O	614.96	<0.001
		I+O	28.59	0.006
	Elevated	C	147.02	<0.001
		I	15.21	0.018
		O	1850.68	<0.001
		I+O	324.97	<0.001
April 2013	Ambient	C	25.65	0.007
		I	5.09	0.087
		O	350.47	<0.001
		I+O	346.09	<0.001
		H	38.56	0.003
		H+I	60.26	0.001
	Elevated	C	40.10	0.003
		I	1029.90	<0.001
		O	1362.26	<0.001
		I+O	28.64	0.006
		H	16.79	0.015
		H+I	1056.54	<0.001
June 2013	Ambient	C	861.73	<0.001
		I	1265.92	<0.001
		O	1415.17	<0.001
		I+O	127.17	<0.001
		H	2251.64	<0.001
		H+I	1038.29	<0.001
	Elevated	C	2895.55	<0.001
		I	4635.83	<0.001
		O	2223.10	<0.001
		I+O	577.68	<0.001
		H	4896.28	<0.001
		H+I	3486.08	<0.001
August 2013	Ambient	C	2.87	0.166
		I	3.05	0.155
		O	14.95	0.018
		I+O	4.87	0.092
		H	499.22	<0.001
		H+I	101.01	0.001

	Elevated	C	0.38	0.570
		I	0.02	0.885
		O	0.99	0.376
		I+O	27.95	0.006
		H	29.44	0.006
		H+I	14.30	0.019

[a]The total degrees of freedom for both bays during all sampling periods was five.
[b]See Table 2.8 for exact temperature values for each bay.