ABSTRACT

Title of Document: LINKING PHYLOGENY AND LIPID COMPOSITION OF NATURAL BACTERIAL COMMUNITIES IN ARCTIC WATERS

Rachael Y. Dyda, M.S., 2005

Directed By: Professor H. Rodger Harvey, Marine Estuarine Environmental Sciences

The effects of organic matter on Arctic bacterioplankton community composition and lipid composition were studied in regrowth experiments amended with peat, ice algae and ice-rafted debris. Bacterial community composition was examined using length heterogeneity PCR, individual fatty acid, and intact phospholipid (IPL) analyses. The Inoculum contained rRNA genes identified as *Alphaproteobacteria* and *Gammaproteobacteria*, while control and substrate amended incubations were dominated by *Gammaproteobacteria* or *Bacteroidetes*. *Alphaproteobacteria* dominated extended peat-amended incubations, with DNA sequences 99% similar to the Arctic Ocean *Sulfitobacter* (ARK10278). Fatty acids synthesized in incubations overlapped, with 16:0n and 16:1Δ9 and 18:0n dominating, excluding the extended peat incubation where 16:1Δ9, 18:1Δ11 and 16:0n dominated. Phosphatidylglycerol and phosphatidylethanolamine were the only phospholipids observed using liquid chromatography mass spectrometry, with only subtle differences among distinct

bacterioplankton communities in regrowth experiments. These results indicate that *Sulfitobacter* may be important in the degradation of terrestrial organic matter in the Arctic Ocean.

LINKING PHYLOGENY AND LIPID COMPOSITION OF NATURAL BACTERIAL COMMUNITIES IN ARCTIC WATERS

By

Rachael Y. Dyda

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2005

Advisory Committee:
Professor H. Rodger Harvey, Chair
Professor Roberta Marinelli
Assistant Professor Marcelino Suzuki

Dedication

This thesis is dedicated in loving memory of my grandparents, Delberta Bondie and Anthony J. and Mildred A. Dyda.

Acknowledgements

First of all, I would like to extend my deepest gratitude to my advisor, Dr. H. Rodger Harvey, for all of his guidance, patience and support over the past 5+ years. I have truly enjoyed working with him and learning from him. He has been most gracious in providing me with both educational and professional opportunities and I am thankful for his financial assistance through my graduate career. I wish him the best of luck in all of his future endeavors and will look back on my "CBL years" with fondness. I would also like to thank the other members of my committee, Drs. Marcelino Suzuki and Roberta Marinelli, for their expertise, knowledge and assistance during the research for my Master's thesis. I am particularly indebted to Marcelino, for many fruitful discussions and for an introduction to microbial ecology with great patience, especially considering the barrage of questions which ensued. Marcelino also deserves credit for tirelessly reviewing many earlier versions of this thesis, providing substantial feedback and direction.

I would also like to thank the past and present members of the "Harvey Lab": Antonio Mannino, who was instrumental in my early training and education when I first arrived at CBL; Se-Jong Ju, for his zest for life and research and for asking just the right questions and for assistance with TLC-FID analyses; Laura Belicka, for being a wonderful friend and labmate over the years and for aiding in my training and in the collection of samples for this work; Lori Roth, who has been a good friend and has provided me with many great memories and Angela Squier, for her outstanding LC/MS expertise and knowledge, good laughs and a quirky Brit humor. I would like to thank all of my labmates for providing years of constructive feedback and wonderful memories. I

wish them all future success, and most of all, happiness. I would also like to thank Karen Eisenreich for her assistance with the ANOVA analysis.

I am especially grateful to my parents, Richard Dyda and Aimee Fluharty and my brother, Ronald Dyda, for their love, support and encouragement and for helping me to be the woman I am today. Furthermore, I would like to thank Jen Kilpatrick (Dickson), for being my "best good friend" through the years and across the miles. We are joined by a bond that is indescribable; Christie-Sue Decker (Cheely), for her support, honesty and wit; Shani Russell and Janna Owens for their advice and wisdom; Susan Klosterhaus and Heather Stapleton, as our friendships have endured ups and downs and have perservered. You have all enriched my life with friendship, laughter and humor, and the occasional glass of wine. I am honored and blessed to know all of you. I would also like to thank Bianca Witkowski, for her patience, support, love, friendship, humor, understanding and encouragement.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction and Overview

Background

Microbes are ubiquitous in the environment and despite their small sizes, they represent the largest pool of living biomass in aquatic systems (Sherr and Sherr 2000; Whitman et al. 1998). Microbes are metabolically diverse and play crucial roles in many biogeochemical cycles: Sulfur oxidation and reduction, nitrogen fixation, nitrification, denitrification, and fermentation are a few of the processes that are bacterially mediated (Friedrich et al. 2001; González et al. 1999; Sherr and Sherr 2000; Zehr and Ward 2002). The recognition of the microbial loop in aquatic food webs further emphasizes the importance of microbes as primary producers and the critical role they perform in the transfer and regeneration of nutrients such as nitrogen and phosphorus (Sherr and Sherr 2000).

Bacteria are also key players in the cycling of carbon, as dissolved organic matter (DOM) is the largest reservoir of reduced carbon in the oceans and bacteria play an important role in its formation and transformation (Ogawa et al. 2001). For example, during bacterial utilization and transformation of DOM, labile components are removed, resulting in DOM that is more homogeneous and refractory (Amon et al. 2001). In addition, approximately one-half of the oceanic primary production is channeled into the microbial loop (Azam 1998). Previous studies have shown that cultured bacteria differ from one another in their uptake of DOM (Martinez et al. 1996). This seems particularly true for members of the *Cytophaga-Flavobacter* group which utilize high molecular

weight (HMW) DOM (Kirchman 2002) and *Alphaproteobacteria*, which have been shown to degrade amino acids (Cottrell and Kirchman 2000b) as well as salt marsh grass detritus (Buchan et al. 2003). SAR11, a clade of the *Alphaproteobacteria* group, are ubiquitous in marine environments and have recently been shown to assimilate low molecular weight (LMW) compounds such as glucose and amino acids (Malmstrom et al. 2005). Furthermore, a diverse assemblage of bacteria in marine systems are responsible for uptake of dimethyl sulfoniopropionate (DMSP), including the *Roseobacter* clade of the *Alphaproteobacteria* as well as *Gammaproteobacteria* and *Bacteroidetes* (González et al. 1999; Mou et al. 2005; Vila et al. 2004). These broad phylogenetic groups also comprise multiple species (Giovannoni and Rappé 2000) which likely differ in their metabolic capabilities and whose members vary spatially and temporally. Nonetheless, it is well known that both the amount and type of DOM can be an important factor structuring aquatic bacterial community compositions (Covert and Moran 2001; Kirchman et al. 2004) and it has been recently proposed that two functionally divergent groups of aquatic bacterioplankton comprise *in-situ* communities. Generalists are capable of rapid response to organic matter and are characterized as having a more opportunistic life history strategy, while specialists are dependent on more particular environmental and/or nutrient conditions (Crump et al. 2003; Langenheder et al. 2005).

In addition to DOM composition and concentration, other factors may play a role in both the bacterial species diversity and abundance in aquatic systems. It has been shown that salinity can be an important factor structuring bacterial communities, with *Betaproteobacteria* and *Actinobacteria* dominating in freshwater systems; *Alphaproteobacteria* dominating in the oceans and *Bacteroidetes* present in both marine

and freshwater systems (Glöckner et al. 1999; Glöckner et al. 2000). Other important environmental factors influencing bacterial community composition include temperature, nutrient availability and dissolved oxygen (Carlson et al. 2002; Schultz et al. 2003). Furthermore, predation, competition, and viral infection are also known to affect bacterial community composition in aquatic systems (Long and Azam 2001; Pernthaler et al. 1996; Šimek et al. 2001; Wommack and Colwell 2000).

Identification, enumeration, and knowledge of bacterial community composition and their metabolic capabilities are crucial to fully understand the role of bacteria in the marine environment. In classic studies, growth in culture was often used to identify and enumerate members of the bacterial community. However, it is well known that only 0.01% to 0.1% of oceanic bacteria produced colonies using standard plating techniques (Kogure et al. 1979) and the term "the great plate anomaly" was used to describe the discrepancy between counts performed on bacteria grown on agar media versus bacterial counts via microscopic examination (Staley and Konopka 1985). Furthermore, it was determined that cultured species made up approximately 1% of the natural community (Staley and Konopka 1985) and therefore cultured species were not major contributors to bacterial diversity in the natural environment (Amann et al. 1995; Giovannoni et al. 1990). Since standard culturing methods were unsuccessful for the majority of bacteria in natural environments, other methodologies were developed and employed to identify and quantify bacterial communities without cultivation. Sequencing and comparison of 16S rRNA genes provided an improved way to determine evolutionary relationships among microorganisms (Woese and Fox 1977), independent of physiological and morphological characters, and cloning of these genes from environmental samples

enabled the identification of *in-situ* microbial communities (Schmidt et al, 1991; Giovannoni et al. 1990). These approaches revealed that microbes are the most phylogenetically diverse group of organisms on Earth (Hugenholtz et al. 1998) and that bacteria characterized in culture studies were not representative of the breadth of bacteria present in aquatic environments (Giovannoni and Rappé 2000).

As universally present cellular components, lipids have been utilized as indicators of the presence of bacteria and algae in natural environments (Kaneda 1991; Meyers 1997). Lipids play critical roles in the structure of cell membranes as well as energy storage and metabolism (Ratledge and Wilkinson 1988). Lipids form a bilayer in most cell membranes, with the polar (hydrophilic) head groups oriented towards the extracellular and cytosolic aqueous phases, and the nonpolar (hydrophobic) region (Cullis et al. 1996) facing each other between the head groups. Phospholipids are the dominant lipids in prokaryotic membranes and consist of a glycerol backbone which forms the connection to the esterified fatty acid side chains at the *sn*-1 and *sn*-2 positions and the phosphate-containing head group at the *sn*-3 position (Ratledge and Wilkinson 1988). Phospholipids which commonly occur in prokaryotic cells include phosphatidylcholine (PC), phosphatidylphosphatidic acid (PA), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidyl-glycerol (DPG) (Rütters et al. 2002b; Sturt et al. 2004), with PE and PG commonly observed in most bacterial cell membranes (Fang et al. 2000b; Lechevalier and Lechevalier 1988).

The type and amount of lipid in bacterial cell membranes is dependent on several factors, including growth rate and stage, temperature, pressure, pH, and nutrient and

oxygen availability (Rose 1988). Membrane fluidity and the maintenance of a lamellar liquid crystalline phase are affected by changes in the phospholipid headgroup as well as alterations in the fatty acid chains (Cronan 2002; Rilfors and Lindblom 2002; Russell 1988). Most cell membranes are preferentially maintained in the lamellar liquid-crystalline phase, with the lamellar gel phase occurring at lower temperatures (Gennis 1989). Several parameters are known to influence main phase transition (i.e. gel to liquid-crystalline phase) temperatures, including the location of the double bond(s) as well as branching points, acyl chain length, chain length difference between the *sn*-1 and *sn*-2 acyl chains and headgroup structures (Huang and Li 1999). For example, relative to saturated acyl chains, a decreased main phase transition temperature occurs with increased degree of unsaturation in fatty acids (Rose 1988; Yano et al. 1998), usually occurring as either a single or multiple *cis*-double bonds in the *sn*-2 acyl chain (Huang and Li 1999). The effect on membrane fluidity is especially pronounced when the double bond(s) occur near the center of the chain (Huang and Li 1999). Furthermore, mixtures of lipids result in a broadening of the phase transition temperature range over which the phase transition occurs (Gennis 1989; Russell 1988).

Fatty acids have received considerable attention as bacterial biomarkers (Harvey and Macko 1997; Ratledge and Wilkinson 1988; Wakeham et al. 2003) with odd and branched fatty acids traditionally assigned as indicators of the presence of bacteria (Kaneda 1991). In addition, fatty acid composition appears to reflect recent history of nutrient uptake, as dissolved organic matter substrates have been shown to dramatically influence fatty acid composition (Harvey et al. 2005). Harvey and coworkers examined the effect of DOM substrates on bacterioplankton community composition and cell

membrane fatty acids and found that when grown on protein, the fatty acid distribution was dominated by 15:0 iso and 17:0 iso whereas 16:1Δ9, 16:0n, 18:1Δ11 and 18:0n were the dominant fatty acids synthesized when grown on either natural DOM and ultrafiltered DOM (Harvey et al. 2005). The main conclusions from this study were that phylogenetically differing groups of bacteria from several temperate estuarine environments overlapped in their cell membrane fatty acid composition and that fatty acid distribution was dependent on the organic matter substrate (Harvey et al. 2005).

Recently, the development of approaches combining high performance liquid chromatography (HPLC) and mass spectrometry to have allowed the analysis of intact phospholipids (IPLs) in bacterial membranes in cultures as well as *in-situ* microbial communities (Rütters et al. 2002b; Zink et al. 2003). Since IPLs rapidly degrade upon cell death, they can be used as indicators of lipids in viable bacteria and archaea cells in natural environments (Harvey et al. 1986; White et al. 1979), and have the potential to reflect the recent history of substrate utilization as well as infer the presence and significance of specific bacterioplankton groups. Moreover, IPL analysis provides detailed structural information available for both headgroup and associated fatty acid side chains, thereby providing a greater possibility of correlations between specific microbial lipids with *in-situ* microbial communities (Fang and Barcelona 1998; Rütters et al. 2002b).

Study Site

The Arctic Ocean is unique as it receives approximately 10% of the global freshwater and riverine DOM input while only representing 1% of the global ocean volume (Opsahl et al. 1999). It is estimated that in Arctic surface waters, 14-24% of the

dissolved organic carbon (DOC) is terrestrially derived (Benner et al. 2005) with terrestrial POC and DOC accounting for 12.6×10^6 t y^{-1} and 28.1×10^6 t y^{-1} of the organic carbon input (Stein and Macdonald 2004). Despite being seasonally covered in ice and light limited, the Arctic Ocean is a productive ecosystem. Labile carbon sources include ice algae as well as high planktonic primary production over continental shelves during spring blooms, and thus the organic carbon source is dominated by marine primary production, accounting for 329×10^6 t y^{-1} (Stein and Macdonald 2004). The microbial loop efficiently utilizes this labile carbon with bacterial production consuming between 35% to 150% of primary production (Wheeler et al. 1996). Furthermore, it has been documented that cell-specific rates of extracellular enzyme activity in the Arctic Ocean are similar to those measured in warmer waters, such as coastal southern California and the Caribbean Sea (Huston and Deming 2002), suggesting that bacterially mediated organic matter degradation is not limited by the low polar temperatures. Polar environments represent a significant proportion of global primary production and also likely to be the most dramatically affected by global climate change (Holland and Curry 1999; Vavrus and Harrison 2003). Therefore, determining the sources, transport processes and fate of carbon in the Arctic Ocean in order to better understand its biogeochemical cycling, as well as to examine any potential changes in the cycling of carbon due to global warming is paramount.

Microbes who thrive under extreme conditions of temperature and/or pressure are deemed extremophiles. *Bacteria* and *Archaea* have developed mechanisms to thrive in very cold environments such as the deep ocean and polar seas as well as in sea ice brine pockets. In order to live in such environments, bacteria have specially adapted enzymes

that function at low temperatures (Van Den Burg 2003). As previously mentioned, another adaptation of *Bacteria* to live in a cold environment is higher percentages of unsaturated fatty acids in cell membranes to maintain cell wall fluidity and to transport substances and nutrients (Deming 2002). *Archaea* have also adapted to living in extreme environments by utilizing alkyl ether lipids to stabilize their cell membranes (Hoefs et al. 1997). Alterations in both headgroup and fatty acid side chains have been attributed to the maintenance of membrane fluidity (Cronan 2002; Rilfors and Lindblom 2002; Russell 1988), as the compound geometry and headgroup size and charge affect packing characteristics in the cell membrane (Gennis 1989). In addition, microbes have been shown to alter their metabolism in response to seasonal changes in the availability of substrates, becoming less active during times of substrate limitation (Sherr et al. 2003). Furthermore, in order to respond to the strong seasonality of substrate availability, Huston and Deming (2002) noted that bacteria exhibit a differential expression of bacterial extracellular enzymes in response to changes in the quality of particulate organic matter (Huston and Deming 2002).

To date, few studies have examined the *in-situ* microbial community composition of the Arctic Ocean. Bano and Hollibaugh evaluated the phylogenetic diversity of bacteria in the Arctic Ocean water column during three seasons by sequencing both cloned fragments of 16S rRNA genes and bands obtained from denaturing gradient gel electrophoresis (DGGE) (Bano and Hollibaugh 2002). Based on the clone library results from all samples, the most commonly retrieved clones were *Alphaproteobacteria*, in particular members of the SAR11 clade, and *Gammaproteobacteria* belonging to a number of cultured, as well as uncultured clades. Members of the SAR11 clade have

been shown to utilize LMW compounds such as amino acids and glucose as well as DMSP (Malmstrom et al. 2005; Malmstrom et al. 2004a). Seasonal changes in the bacterioplankton distribution were not dramatic, with *Gammaproteobacteria* and *Deltaproteobacteria* more prevalent in winter and *Bacteroidetes* only retrieved in summer and fall (Bano and Hollibaugh 2002). Depth-dependent distributions were also found, with *Alphaproteobacteria* dominating the mixed layer and *Gammaproteobacteria* dominating at halocline depths. Bano and Hollibaugh attributed the seasonal differences observed in baceterioplankton community composition to organic matter availability, with *Bacteroidetes* prevalent in post-phytoplankton bloom conditions, characterized by increased availability of labile organic matter substrates. The importance of *Bacteroidetes* in fall samples was corroborated by Wells and Deming who used fluorescent *in situ* hybridization to show that members of the *Bacteroidetes* cluster consisted a large proportion of hybridizable cells in surface waters in the Northwest Passage (Wells and Deming 2003). Finally, a phylogenetic examination of summer pack sea ice Arctic communities, both by cultivation and 16S rRNA gene cloning showed that *Gammaproteobacteria* including several cultured genera (*Colwellia* spp., *Glaciecola* spp. and *Marinobacter* spp.), the *Roseobacter* clade (particularly *Octadecabacter* spp) and the *Bacteroidetes* were dominant phylotypes, which was attributed to the observance of these groups in association with surfaces such as microalgae or particles (Brinkmeyer et al. 2003). Noteworthy, the same study used FISH probes to corroborate the dominance of the same phylotypes, and also showed that a novel group of organisms closely related to the *Roseobacter* strain *Shippagan* (*Sulfitobacter* ARK10278 and related strains) were relatively rare (<1% of FISH counts).

Rationale

It is expected that global warming will cause shifts in precipitation, wind and weather patterns, and that the polar regions will experience increased melting or thawing during the summer months and less extensive freezing during the winter months, thereby impacting the Earth's albedo and resulting in changes in the freshwater and organic matter input to oceanic systems (Grebmeier et al. 1998). As a result, it is predicted that rising global temperatures and the changes associated with global warming will considerably alter the world ocean through modifications in food web structure and dynamics, nutrient concentrations, freshwater input, circulation patterns, organic matter input and upwelling and in turn will have drastic effects on the carbon cycle. In fact, there is data which indicates that global warming has already affected the Arctic, resulting in increased sea surface temperature, changes in precipitation, sea-ice extent and duration, changes in permafrost temperature and shifts in the distribution of vegetation (Moritz et al. 2002).

Although the direct effects of global warming on microbial communities have yet to be resolved, increasing temperatures will influence stratification and circulation with changes to nutrient and carbon cycles (Deming 2002). Given that DOM composition and concentration plays a role in structuring bacterial communities, that bacteria affect DOM composition by preferentially utilizing labile DOM, and that different members of the community are responsible for the degradation of specific types of organic matter (Malmstrom et al. 2005; Martinez et al. 1996), it is expected that changes in organic matter inputs to the Arctic Ocean will affect bacterial community composition. Shifts in microbial community composition may have important consequences for the cycling of

carbon within the microbial loop, the transfer of carbon to higher trophic levels, and the balance of carbon export and microbial respiration.

Studies examining bacterial lipids, microbial phylogeny and metabolism have historically relied on pure cultures or isolates of bacteria (Giovannoni and Rappé 2000). Several issues arise from examining microbial community composition and metabolism using culture-dependent studies. First, the bacteria studied may not represent major contributors to *in-situ* microbial communities. Secondly, changes in growth conditions (i.e. media components, temperature, pH and dissolved oxygen) are known to affect cell membrane lipid composition, thereby limiting the extrapolation of the results to natural communities. With the advances in current phylogenetic methodology, identification of natural microbial community diversity can be employed in conjunction with cell membrane lipid analysis to examine naturally occurring bacterial communities and to determine microbial contribution to the oceanic DOM pool.

The goals of this research are to determine whether Arctic bacteria differ in their capabilities to utilize various types of organic matter and to determine whether shifts in bacterial membrane lipids are representative of either changes in bacterial community composition or are a community response to the differing carbon sources. The possibility of assigning specific lipid biomarkers to either groups or individual bacteria will also be evaluated.

To address these questions, experiments were conducted to monitor the response of *in-situ* bacterial communities to a diverse array of Arctic organic matter substrates, including peat, ice algae and ice-entrained debris. Bacterial regrowth experiments involve the dilution and subsequent regrowth of *in-situ* bacteria communities and provide

an advantage over traditional culturing or isolation techniques in that *in-situ* bacterial communities are used and growth conditions closely mimic those of natural environments, increasing the applicability of the results. To date, no studies have examined the response of *in-situ* Arctic bacteria communities to carbon additions. Chapter 2 of this thesis describes the results of the phylogenetic response of the Arctic bacterial communities to the carbon additions using length-heterogeneity polymerase chain reaction (LH-PCR). Community composition shifted in all of the incubations, including no-addition Controls. The greatest phylogenetic shift occurred in extended incubations involving the addition of the peat substrate and a clone library was constructed from this sample. Chapter 3 describes the results of the fatty acid and intact phospholipid analyses of the bacterial communities in the bacterial regrowth experiments. Fatty acid distributions overlapped among the incubations, with 16:0n and 16:1Δ9 making up the dominant fatty acids in most of the incubations. Intact phospholipid analysis revealed that PG and PE headgroups were present and corroborated the fatty acid results, with 16:0n and 16:1Δ9 commonly observed. No substantial shift in either fatty acid or IPL distribution was detected as a function of organic matter substrate. Results of studies with a *Roseobacter* clade isolate revealed that organic matter greatly affected both fatty acid and intact phospholipid distributions. Finally, chapter 4 integrates the results of the bacterial phylogeny with the lipid compositions of the regrowth experiments. Fatty acids and IPLs overlapped among phylogenetically distinct groups of bacteria. Potential exceptions were the dominance of 16:1Δ9 in the second regrowth of the control incubation, dominated by LH-PCR fragments of 351 base pairs (bp) and the presence of 18:1Δ11 as the second most abundant fatty acid in the extended Peat incubation, in which

Alphaproteobacteria, specifically ARK10278, an Arctic *Roseobacter* clade member closely related to strain *Sulfitobacter* dominated the community composition.

Chapter 2: Phylogenetic responses of Arctic bacterioplankton communities to organic matter additions

Synopsis

Organic matter (OM) composition can play a significant role in structuring bacterial community composition in aquatic systems (Jardillier et al. 2004; Pinhassi et al. 2004) and bacteria play an important role in the formation and transformation of dissolved organic matter (DOM) (Amon et al. 2001; Zou et al. 2004). The goal of this research was to investigate the phylogenetic responses of bacterioplankton to organic matter additions in bacterial regrowth experiments. Arctic bacterioplankton were diluted 10-fold with bacteria-free water and growth was followed in 20 L duplicate carboys, after the addition of different organic matter substrates (Experiment 1). Organic matter additions included peat, ice algae and ice rafted debris, and a no-addition control was also set-up. Bacterial incorporation of [^{3}H]-leucine into bacterial protein (i.e. bacterial production) was used as a proxy of bacterial growth. Upon early stationary phase growth at 11 days as determined by bacterial production, samples from all replicates were taken for phylogenetic (16S rRNA gene) and lipid analyses. Water (2 L) leftover from the first regrowth of the no addition-control and the peat-amended incubations were subject to a second 1:10 dilution, and growth was followed for an additional 12 days (Experiment 2). Phylogenetic changes in community composition were monitored by length heterogeneity-PCR (LH-PCR), in which the natural length variation of the 5' end of the

small subunit (SSU) ribosomal RNA (rRNA) gene is used estimate community composition. The Inoculum water was dominated by rRNA genes putatively identified as *Alphaproteobacteria* and *Gammaproteobacteria*. Community composition shifted over time in all of the incubations, including no addition Controls. The Controls from both experiments were dominated by larger (349-354 bp, 352 in particular) LH-PCR fragments than the Peat, Ice algae and Debris additions, which were dominated by somewhat shorter LH-PCR fragments (344-352 bp, particularly 347 bp). In all Experiment 1 incubations, *Alphaproteobacteria* (312-318 bp) fragments decreased in relative abundance, while fragments increasing in relative abundance in all incubations corresponded mostly to *Gammaproteobacteria* and *Bacteroidetes*. A considerably different shift in bacterial community composition was observed in Peat enrichments at the end of Experiment 2 with dominant LH-PCR fragments of 316-317 bp, corresponding to *Alphaproteobacteria*. A ribosomal RNA operon clone library was constructed for this sample and analysis of 62 clones by ITS-LH-PCR (internal transcribed spacer-length heterogeneity-polymerase chain reaction) and DNA sequencing revealed that over half of the clones had 99% sequence similarity to the *Sulfitobacter* strain ARK10278, a member of the *Roseobacter* clade. This strain was previously isolated from the Arctic Ocean and likely contributed to the observed increase in the *Alphaproteobacteria*. Interestingly, the ITS-LH-PCR analysis showed that these ARK10278 – related clones belonged to 14 distinct phylotypes. The similarity of the bacterioplankton community composition observed in Experiment 1 among all incubations was probably due to the rapid response of select members of the bacterioplankton community, in this case, *Gamma-proteobacteria* and/or *Bacteroidetes* to the organic matter additions. Based on the LH-

PCR results, the shift in bacterioplankton community composition in Experiment 1 appears to be independent of organic matter substrate and is likely a reflection of the ability of these groups of bacteria to rapidly respond to substrate additions and to outgrow other members of the bacterioplankton during the time frame of these incubations. However, the dominance of *Alphaproteobacteria*, specifically *Sulfitobacter*, in the second regrowth of the Peat incubation, not observed in the Control, indicates that these bacteria responded to the peat substrate additions. These findings suggest that the *Roseobacter* clade in the Arctic Ocean may be involved in the degradation of terrestrially derived organic matter. The results of this study corroborate recently proposed models in which aquatic bacterioplankton communities are comprised of generalists, characterized by successful growth under a wide range of conditions that enable them to quickly respond to available substrate, and specialists, with more specific environmental and/or nutritional growth requirements (Crump et al. 2003; Langenheder et al. 2005).

Introduction

Marine bacterioplankton are abundant, ubiquitous and are primary mediators of biogeochemical processes in aquatic systems (Sherr and Sherr 2000). Yet most naturally occurring marine bacterioplankton are not well-represented in culture collections (Giovannoni and Rappé 2000) and as a result, little is known regarding the role that the majority of environmentally significant bacteria play in marine biogeochemical cycles. The application of culture-independent phylogenetic techniques utilizing the extraction of nucleic acids directly from the environment facilitates the determination of *in-situ* microbial community composition, their metabolic capabilities and their response to substrates (Cottrell et al. 2000c; Giovannoni and Rappé 2000). These techniques have

shown that microbial diversity and function are far greater than previously estimated using standard culture methodologies (Doney et al. 2004; Suzuki et al. 1997) and provide opportunities to investigate *in-situ* microbial community composition and to link bacterioplankton diversity and ecological function.

It is known that bacterial diversity in natural environments may be controlled by a number of factors, including grazing, viral lysis, salinity and DOM quality and quantity (Findlay et al. 2003; Jardillier et al. 2004; Kirchman et al. 2004; Schultz et al. 2003). Studies using natural bacterial communities have shown that bacteria differ in their uptake capabilities of organic matter (Cottrell and Kirchman 2000b; Malmstrom et al. 2005). Some groups of bacteria have been implicated with the utilization of specific DOM types. For example, *Bacteroidetes* have been linked to the uptake and degradation of lignin, chitin, proteins and other high molecular weight (HMW) DOM (Cottrell and Kirchman 2000b; Kirchman 2002; Pinhassi et al. 1997), and are frequently associated with the decay of phytoplankton blooms (Fandino et al. 2001). On the other hand, *Alphaproteobacteria* have been shown to utilize amino acids and other low molecular weight DOM (Cottrell and Kirchman 2000b; Ouverney and Fuhrman 1999), although bacteria in the *Roseobacter* lineage have also been shown to use lignin (Buchan et al. 2001; Buchan et al. 2004), sulfur organic compounds (Buchan et al. 2004; Covert and Moran 2001; Mou et al. 2005) and even hydrocarbons (Brakstad and Lodeng 2005). Enrichment studies have shown that marine *Gammaproteobacteria* utilize glucose (Øvreås et al. 2003) as well as HMW dissolved organic carbon (DOC) (Covert and Moran 2001). Furthermore, SAR86, a ubiquitous, yet uncultured *Gammaproteobacteria* (Eilers et al. 2000a; Eilers et al. 2000b) has been shown to play an important role in the

cycling of dimethylsulfoniopropionate (DMSP) (González et al. 2000). Other culturable members of the *Gammaproteobacteria* such as *Vibrio* spp. and *Alteromonas* and *Colwellia* are known copiotrophs, i.e. they are able to rapidly respond to changes in substrate and successfully grow under high nutrient conditions (Eilers et al. 2000a; Pinhassi and Berman 2003; Thompson et al. 2004). This type of metabolic response has been observed in marine, estuarine and freshwater systems and it has recently been proposed that aquatic bacterioplankton communities are made up of two groups that differ in their physiology: generalists which are metabolically flexible and quickly respond to new substrates (opportunistic) and specialists which have more specific environmental and/or nutritional growth requirements (Crump et al. 2003; Langenheder et al. 2005).

In the aforementioned studies, the phylogenetic resolution was broadly defined (phylum to class), complicating the interpretation of the results and leading to generalization of uptake patterns. Thus in the current study we examined the response of bacterioplankton communities to organic matter additions at both broad and finer phylogenetic resolutions. Organic matter utilization was examined using two sets of Arctic bacterioplankton regrowth incubations. These experiments consisted of 1:10 dilutions of bacterivore-free (<1.0μm filtered) water with bacterioplankton-free (0.2 μm filtered) water amended with different organic matter including: 1) frozen (-80°C) and thawed *Melosira arctica*, a sea ice associated diatom prevalent in the Arctic (Booth and Horner 1997; Falk-Petersen et al. 1998) 2) ice-rafted debris, (a mixture of ice algae and ice entrained sediment, and 3) peat from the water's edge of the Ikpikpuk River. Since it has previously been shown that some bacteria respond favorably to enclosure (Ferguson

et al. 1984; Suzuki 1999), no-addition controls were also employed to evaluate changes in the microbial community due to experimental manipulation.

The phylogenetic diversity of bacterioplankton in the incubations was assessed by length-heterogeneity-PCR (LH-PCR), a culture-independent technique that utilizes fluorescently labeled PCR primers to detect PCR amplicons that are identified based the natural length variation found in 5' end of the small subunit rRNA genes (Suzuki et al. 1998). Compared to methods such as fluorescent restriction fragment length polymorphism and terminal restriction fragment length polymorphism, that use restriction endonuclease site variability to distinguish PCR amplicons (Bruce 1997; Liu et al. 1997), the sizes of LH-PCR amplicons are somewhat congruent with phylogeny. This enables fast (albeit putative) identification of qualitative diversity of a mixed bacterial population while peak intensities are assumed to reflective of the relative contributions of different community members.

In addition, intact phospholipid and fatty acid analyses were also performed on the bacterial communities (see Chapter 3) and the relationship between bacterial phylogeny and cell membrane lipid distribution explored (Chapter 4).

Methods

Overview of Arctic experiment set-up

Arctic bacterioplankton (<1.0μm filtered water) were diluted 10-fold with bacteria-free water (<0.2μm filtered water) supplemented with organic matter additions. Organic matter substrates consisted of *Melosira arctica*, a sea ice associated diatom

prevalent in the Arctic (Booth and Horner 1997; Falk-Petersen et al. 1998). This fresh algal sample was collected 15 June 1998, on a SHEBA/JOIS cruise, rinsed with filtered Seawater, promptly frozen and stored at -80ºC until thawed for utilization in the regrowth experiments. Additional substrates included ice-rafted debris, (a mixture of ice algae and ice entrained sediment, collected June 2002 on the HLY02-01 cruise near station 22 at 72°24N 159°77W) and peat from the water's edge of the Ikpikpuk River during the HLY02-01 cruise. Table 2.1 gives the amount of substrates added to the incubations. The carbon content of the substrates was determined by drying the substrates overnight at 50°C. Samples were homogenized with a mortor and pestle and stored in combusted amber vials sealed with Teflon caps until analyis. The carbon of the final algae and debris substrates was determined by filtering subsamples of the sample water onto precombusted (450°C, 4-6 hours) GF/F filters. Filters were dried overnight at 50°C and $CaCO_3$ was removed by acidification. Total organic carbon and nitrogen of both substrates and filtered samples was determined with an Exeter Analytical 440-XA elemental analyzer. A no-addition control was also used to discern shifts in community composition due to experimental manipulation. Hereafter, these samples will be referred to as Ice algae, Debris, Peat and Control. All incubations were conducted in duplicate in the dark at –1°C. Bacterial regrowth was followed until early stationary phase, as determined by bacterial production using leucine incorporation (D.L. Kirchman personal comm.). At this point samples from all replicates were collected for phylogenetic (16S rRNA gene) and lipid analyses. For the first experiment (Experiment 1), samples were taken after 11 days, and a volume of 2 liters of water from the no-addition Control and the peat amended incubations was left over in the carboys and subject to a second 1:10

dilution, with growth for an additional 12 days (Experiment 2). Phylogenetic changes in community composition in all incubations were monitored by LH-PCR (Suzuki et al. 1997). Samples for DNA from the Peat incubation were screened through 10µm mesh to remove the peat substrate.

Table 2.1. Amount of substrates added to regrowth experiments.

		Substrate organic carbon content (%)	Mass of substrate added (g)	Incubation organic carbon content (mg/L)
<1.0 um				0.016
Ice algae*	Carboy 1			
	Initial†	13.10	0.79	0.53
	Final‡			0.40
	Carboy 2			
	Initial†	13.10	0.93	0.62
	Final‡			0.35
Debris**	Carboy 1			
	Initial†	1.96	17.43	4.84
	Final‡			2.51
	Carboy 2			
	Initial†	1.96	19.15	5.32
	Final‡			3.29
Peat ***	Carboy 1			
	Initial†	32.10	3.00	48.14
	Final‡	37.96		56.92
	Carboy 2			
	Initial†	32.10	3.00	48.19
	Final††	37.96		56.98

*collected during SHEBA/JOIS cruise, 15 June 1998
**collected during HLY02-01 cruise, June 2002 near stn 22.
***collected during HLY02-01 cruise, 04 June 2002, Ikpikpuk River, water's edge, screened to >35 um.
† calculated from mass of substrate added and initial organic carbon content
‡ measured from partculate carbon/partculate nitrogen (PC/PN) analysis
†† calculated from mass of substrate added and final organic carbon content

The water sample used for Experiment 1 was collected near the Arctic Ocean station HLY 04-02-007 (HV2, 70°13.117N 167°38.653W) on May 20, 2004 from the science seawater system (inlet at c.a. 8.3 meter depth) aboard the USCGC Healy. Water used to dilute Peat and Control incubations in Experiment 2 was also collected from the seawater system on 31 May 2004 near station HLY04-02-017 (EHS5, 72°43.810N 158°25.475W). Site specific information is given in Table 2.2. The seawater system was flushed for approximately 5 minutes prior to collection to discard fouling organisms and obtain fresh subsurface water samples. The water samples were filtered through a series of inline 3.0 μm, 1.0 μm and 0.2 μm cartridge filters (Pall Life Sciences) into 20 liter carboys using a peristaltic pump. Each carboy was rinsed three times with 2 liters of < 0.2 μm water and was then filled with 18 liters of < 0.2 μm water. The 0.2 μm cartridge filter was removed and 2 liters of < 1.0 μm filtered water added to each carboy for a 10% bacterial inoculum.

Table 2.2. Sampling locations and characteristics for water used in both bacteria regrowth experiments.

Station Number	Sample Location	Location	Site Water Depth (m)	Sampling Water Depth (m)	Salinity	Temperature (°C)	Oxygen (ml/l)
7	Herald Valley	70° 64'N, 167° 28'W	56	8.3	32.84	-1.77	8.60
17	East Hannah Shoal	72° 72'N, 158° 40'W	247	8.4	29.51	-1.58	9.46

Bacterial counts and cell volumes

Samples for bacterial counts for Experiments 1 and 2 were taken daily and preliminary counts were conducted onboard to monitor growth in all incubations. Slides were frozen and later recounted with bacterial volumes calculated using semi-automated

microscopy as described by Cottrell and Kirchman (Cottrell and Kirchman 2004a). For bacterial counts, a modified protocol based on Velji and Albright (Velji and Albright 1993) was followed. Briefly, sample water was fixed with 5% (v/v) 37% formaldehyde and stored in the dark at 4ºC. Bacteria in fixed samples were filtered onto 25 mm 0.2-µm Isopore GTBP02500 black polycarbonate membranes (Millipore Billerica, MA) with a 25 mm 0.45-µm pore size MF HAWP02500 nitrocellulose backing filter (Millipore) using gentle vacuum filtration. With approximately 1 mL of sample water remaining in the filter tower, vacuum pressure was released and the fluorescent DNA-binding stain, 4',6-diamindino-2-phenylindole (DAPI, Sigma, St Louis, MO), was added, for a concentration of approximately 2.5 µg/mL. The sample was incubated for 10 minutes, filtered, and the filter mounted on a microscope slide (FisherBrand, 3" × 1" × 1 mm, part number 12-549); a drop of immersion oil was placed on top of the filter and covered with a 25-mm coverslip (FisherBrand, 22-mm square, part number 12-542B). Slides were allowed to settle horizontally at 4ºC for several hours prior to upright storage in the slide box and were stored frozen until analysis. Onboard counts were conducted using an Olympus BH2 with a 100× objective. A 100W-mercury lamp for was used for UV excitation at 365 nm and emission wavelength was > 400 nm. Counts were performed using a 10×10 ocular grid and at least 10 randomly chosen fields of view were counted per slide. Bacterial cell volumes were obtained for conversion of cell abundances into bacterial biomass estimates.

DNA collection, extraction and quantification

Subsamples of water from the Inoculum and all incubation carboys was collected into 2L LDPE bottles with spigots (Nalgene, Rochester, NY) and filtered using a

peristaltic pump onto 13 mm Supor 200 (Gelman, Ann Arbor, MI) 0.2 µm membrane filters encased in a Swinnex filter holder (Millipore). After sample collection, the filters were transferred using forceps and stored at –20 °C in 130 µL Qiagen lysis buffer (Qiagen, Valencia, CA) in 0.6 mL microcentrifuge tubes until sample analysis. Gloves and isopropanol-cleaned forceps were employed to minimize contamination. The sampling apparatus (tubing, carboys, Swinnex filter holders) was rinsed with nanopure water between samples.

DNA was extracted and purified using the Qiagen DNeasy Tissue Kit (Qiagen; http://www1.qiagen.com/literature/protocols/DNeasyTissue.aspx) with a modified protocol (Suzuki et al. 2001b). To minimize contamination, benchtop and pipetors were cleaned with a 2% bleach solution. Briefly, the 13 mm Supor filters were thawed, 5 µL of RNAse I (Sigma, 1:5 dilution) and 50 µL of lysozyme solution (73 mg/mL) were added to the 0.6-mL microcentrifuge tubes. The tubes were vortexed and incubated for 1 hour at 37ºC. 5 µL of Proteinase K solution (Qiagen kit) was added and the samples were vortexed and incubated at 70 °C for 30 minutes. The entire volume from each tube was then transferred to 1.5-mL microcentrifuge tubes and 410 µL AL/E buffer (205 µL AL Buffer + 205 µL 95% ethanol) was added and the tubes were vortexed for approximately 15 seconds. The mixture was pipetted onto a DNeasy spin column, in 2 mL collection tubes and centrifuged at 16000 rpm for 1 minute to bind the DNA to the DNeasy matrix. The spin columns were washed with 500 µL of buffers AW1 and AW2 and centrifuged at 16000 rpm for one minute between washings. The DNeasy membranes were dried by centrifugation at 16000 rpm for 5 minutes and DNA was incubated with a volume of 200 µL of DEPC treated TE Buffer (Ambion, Austin, TX),

followed by centrifugation at 16000 rpm for 1 minute to elute the DNA. Samples were stored at -20ºC until DNA concentrations were determined using the PicoGreen dsDNA Quantitation Assay and Kit (Molecular Probes, Eugene, OR catalog number P-7589, http://probes.invitrogen.com/servlets/publications?id=137). A standard curve in a concentration range of 1000 to 5.252 pg/µL was performed using a series of dilutions in TE of the supplied 100 µg/mL lambda dsDNA standard. Sample DNA (6 µL) was diluted 1:10 with TE buffer (54 µL). 50 µl of diluted standards and samples were transferred to a 96 well low fluorescence microplate (Nalge Nunc Inc Rochester, NY) and 50 µL of 1:200 PicoGreen reagent in TE buffer was added to each sample and standard well. Fluorescence of the standards and DNA samples was measured on a Spectra MAX Gemini microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA), with excitation and emission wavelengths at 485 nm and 538 nm, respectively, in duplicate fluorescence readings. The Control Carboy 1, Experiment 2 sample was lost during DNA extraction, therefore data is not available for comparisons between replicate Control carboys of Experiment 2.

LH-PCR

Purified DNA samples were diluted to 0.07 ng/µl (the lowest concentration among the samples) and used as a template in PCR reactions using primer 27F (Giovannoni 1991) labeled with the fluorochrome NED and the unlabeled reverse primer 338R (Amann et al. 1995), both primers are specific for the domain *Bacteria*. In a final reaction volume of 10 µl, reaction mixtures contained 1× PCR buffer, 0.2 mM each deoxynucleoside triphosphate (Promega, Madison WI), 1.5 mM $MgCl_2$, primers (0.5 µM each) and 0.03 units of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA). All

reactions were conducted in an AB9700 thermal cycler (Applied Biosystems, Foster City, CA), programmed with an initial denaturation at 94ºC for 2 minutes and 25 to 32 cycles (depending on the sample), 30 seconds denaturation at 96ºC for, 30 seconds annealing at 55ºC , and 30 seconds extension at 72ºC. Upon cycle completion, reactions were maintained at 4-6ºC. Agarose gel electrophoresis was conducted on an aliquote of the PCR product to estimate product concentration and to check for contamination using a 1% (w/v) agarose gel in 1× TBE buffer stained with ethidium bromide (Sigma, 0.5 µg/ml). Gels were run at 100 V for 1 hour. LH-PCR products (1 µl) were combined with 8 µl of 3% (v/v) of GS2500 size standard (Applied Biosystems) in highly deionized Formamide (Applied Biosystems) and denatured for 2 minutes at 94ºC. Fragments were separated by capillary electrophoresis in an AB3100 Genetic analyzer (Applied Biosystems) using 36 cm capillaries and POP4. Fragment sizes and relative peak heights were analyzed using the Genescan package (Applied Biosystems). The software outputs fluorescence data into electropherograms with the peak positions representing different sizes (Suzuki et al. 1998). Peak heights were used as a proxy of the abundance of each fragment. Fragment lengths were compared with known size fragments (Suzuki et al. 1998) to determine community composition in all experimental incubations. Data for Carboy 2 from the Ice algae incubation are not available due to difficulties during sample processing.

Peat2exp2f Clone Library Construction and analysis

A long-fragment cloning protocol was employed to amplify fragments containing the 16S rRNA, the 23S rRNA as well as the naturally variable internal transcribed spacer (ITS) region in order to determine diversity within a mixed microbial community (Béjà et

al. 2000; Suzuki et al. 2004). This reaction utilized Platinum *Taq* Hi Fidelity, which is a mixture of thermostable DNA polymerases, combining *Taq* and a proofreading DNA polymerase for a balance of fidelity and processivity (Invitrogen, http://www.invitrogen.com/content/sfs/brochures/711_022289_B_PCRRTPCR_bro.pdf).

Purified DNA (100 µl) from Peat, Carboy 2, Experiment 2, screened through 10 µm mesh, (abbreviated Peat2exp2f) was used as a template for the PCR reaction. To decrease PCR contamination effects, the purified Peat2exp2f DNA was concentrated using a Microcon 100 (Millipore) centrifugal filter from 100 µl to 8.0 µl prior to the reaction. PCR reactions employed primers 27F (Giovannoni 1991) and 1933R (Amann et al. 1995). In a final reaction volume of 25 µl, reaction mixtures contained 1× Platinum HiFi PCR buffer, 0.2 mM each deoxynucleoside triphosphate (Promega, Madison WI), 3.0 mM $MgSO_4$, primers (0.5 µM each), and 0.025 units of Platinum *Taq* Hi Fidelity enzyme (Invitrogen, Carlsbad, CA). All reactions were conducted in a Robocycler Gradient 96 (Stratagene, La Jolla, CA), programmed with an initial 2 minutes enzyme activation step at 94ºC, and 30 cycles, of 56 seconds denaturation at 94ºC, 69 seconds annealing at 55ºC, and 3 minutes 30 seconds extension at 68ºC. Upon cycle completion, reactions were initially lowered to 25ºC and then maintained at 4-6ºC. A reconditioning step was conducted in order to reduce heteroduplex formation during the PCR reaction (Thompson et al. 2002). 2.5 µl from the first 30-cycle PCR was mixed with 22.5 µl of the same PCR cocktail used for the original PCR and reactions were run in the Robocycler with 2 minutes enzyme activation at 94ºC followed by 5 cycles as above and one hold at 68ºC for 7 minutes. PCR products were run in a 1% agarose gel to check for size of PCR fragments and contamination in negative controls.

Since the long-fragment PCR reaction utilized the Platinum *Taq* Hi Fidelity enzyme, which produces some blunt-ended PCR products, PCR products were A-tailed with the Qiagen A addition kit (http://www1.qiagen.com/literature/handbooks/PDF/Cloning/A-Addition/1020787_HB_CLON_A-Ad_072002.pdf). 8 µl of long fragment PCR product and 2 µl of 5× Qiagen A addition were mixed and incubated for 30 minutes at 37ºC on a heating block. A-tailed products were immediately cloned with the TOPO TA® Cloning Kit for Sequencing (Invitrogen): http://www.invitrogen.com/content/sfs/manuals/topotaseq_man.pdf. 3 µl of PCR product from the A-Tailing step, 1 µl salt solution (Invitrogen) and 1 µl TOPO vector (Invitrogen) were combined and incubated at room temperature for 5 minutes. Since the presence of salts would result in arcing during the electroporation step, drop dialysis of the ligation products was employed to decrease salts in the ligation solution. DNA free water (5 µl) was added to the ligation products and the combined 10 µl were pipetted onto a 0.025 µM Millipore VSWP membrane filter (Millipore) floating on 0.5× TE buffer and dialyzed for 1 hour.

Transformation via electroporation:

Electroporation is a transformation method which introduces foreign DNA into competent cells (*E. coli* in this case) using a high-voltage electric pulse. This pulse induces pore formation in the cell wall and increases permeability, enabling the introduction of DNA in the cell (Lurquin 1997). Electroporation was carried out as described in Béjà and coworkers (Béjà et al. 2000). Briefly, ligation products were used to transform electrocompetent *E.coli* DH10B™ cells via electroporation using a Gene

Pulser (Bio-Rad, Hercules, CA) with the following settings: 2500 V, 25 µF and 100 ohms. Cells were recovered for one hour at 37ºC at 225 rpm using an shaker incubator (Lab-Line® Orbit-Environ Shaker, Lab-Line, Melrose Park, IL). 100 µl of recovered cells were plated onto LB (BD Difco LB agar, Miller, 2007-03-01) agar plates with 50 µg/ml of Kanamycin. Plates were incubated overnight at 37ºC and 96 colonies were picked into microtiter dishes with 180 µl of LB media containing 7% glycerol and 50 µg/ml of Kanamycin and allowed to grow overnight at 37ºC before storage at -80ºC.

Screening for full sized inserts:

In order to separate circular plasmid DNA from cellular debris, plasmids from clones were purified by alkaline lysis using a modified protocol by Ausubel and coworkers (Ausubel et al. 1988). Briefly, clones were grown overnight in 1 ml of 2× LB with 50 µg/ml Kanamycin at 37ºC at 325 rpm in 96 well block plates covered with micropore tape (3M). Cells were resuspended by pipetting up and down and 300 µl were transferred to a U-bottom microplate and pelleted in a Sorvall Legend T plate centrifuge (Kendro, Asheville, NC) at 1500 G for 5 minutes and supernatant was decanted by flickering onto bleach. 50 µl of GTE buffer (Sambrook et al. 1989) and 2 µg of RNAse I (Sigma, 1:5 dilution) were added to each well using a repeater pipettor. Plates were covered and pellets were resuspended by vortexing for 1 minute at setting 4 and/or by pipetting up and down. 100 µl of NaOH/SDS (solution P2, Sambrook et al. 1989) was added to each well; plate was covered and inverted three times and allowed to sit for 2 minutes at room temperature. 50 µl potassium acetate (solution P3, Sambrook et al. 1989) was added to each well and the plate was covered and vortexed for 1 minute at setting 4 and then spun for 10 minutes at 3000 rpm in a Sorvall Legend T plate centrifuge

(Kendro). 180 µl of supernatant was aspirated from the pelleted debris using a Hydra Microfluidic Dispenser machine (Robbins) and transferred to a new U-bottom shaped microtiter dish. 126 µl of isopropanol was added to each well and the plate was sealed and inverted 5 times to mix. The solution was chilled at -20ºC for 30 minutes and then spun at 3000 rpm for 30 minutes; supernatant was discarded with a flicker. Pellets were washed with 300 µl of 70% and 100% ethanol, discarding the supernatant between each wash with a flicker, dried at 37ºC and DNA was resuspended in 50 µl of TE. The plate was sealed and stored at -20ºC.

Next, a PCR was conducted to screen for long inserts. The conditions for this reaction were the same used for cloning, with the exception that this reaction employed vector primers M13F (GTAAAACGACGGCCAG) and M13R (CAGGAAACAGCTATGAC), Platinum *Taq*® polymerase (Invitrogen) and 3.0 mM $MgCl_2$. All reactions were conducted in a Robocycler Gradient 96 (Stratagene, La Jolla, CA), with the same PCR parameters as outlined in the clone library construction and analysis section, with the exception that the reaction was run for 25 cycles. A 1% agarose gel stained with 0.5 µg/ml of ethidium bromide (100V, 1 hour) in TBE was used to visually identify clones with full inserts.

Based on the gel results, 82 clones from the Peat2exp2f sample with full inserts were selected for screening using a novel method based on LH-PCR and ITS-LH-PCR, (Suzuki et al. 2004). This method that utilizes the natural heterogeneity of the 5' end of the 16S rRNA and the internal transcribed spacer (ITS) region, as well as the presence and location of the tRNA-alanine gene is used to identify closely related phylotypes. PCR reactions were performed using 3 sets of primers: (1) 27F (NED) (Giovannoni

1991) and 338R (Amann et al. 1995), this primer set was used to compare with the previous results of the LH-PCR; (2) 1406F-FAM (Suzuki et al. 2004) and 66R (Suzuki et al. 2004); and (3) 1406F-HEX (Suzuki et al. 2004) and tRNAalaR (Suzuki et al. 2004). PCR reactions were conducted under the same conditions as described for the LH-PCR, with the exception that the reactions were run for 15 cycles and 72ºC extension for 1 minute. Fragments were separated by capillary electrophoresis on an Applied Biosystems 3100 Genetic Analyzer, with POP4 (Applied Biosystems) and 36-cm capillaries. Fragment sizes were estimated by the Genescan software (Applied Biosystems) and the GS2500 size standard as above (Applied Biosystems). Fragment sizes for the three primer sets (27F-NED and 338R; 1406F-FAM and 66R; 1406F-HEX and tRNAalaR) were used in conjunction, to putatively identify the clones and to provide an estimate of the bacterial diversity of the sample, and clones were grouped or distinguished based on the sizes of the three fragments. Each unique combination of fragment sizes was identified as a phylotype. Phylotypes differing by a one base pair for any of the three fragments were combined, as the difference likely represents slight variations estimated by the size-calling algorithm. Based on the phylotype groupings, a select number of clones from the Peat2exp2f sample were chosen for sequence analysis.

Sequencing:

Sequencing was used to determine the phylogenetic diversity of the clones with a greater resolution. Given that many clones formed groups based on the fragment lengths obtained by the ITS-LH-PCR, at least one clone from each group was partially sequenced. Since the *Alphaproteobacteria* appeared to dominate the community, the phylogenetic affiliation of these clones was of particular interest, and therefore, full

sequencing was employed for these clones. Plasmid DNA was purified using the Montage 96 Plasmid kit, (Millipore, http://www.millipore.com/userguides.nsf/dda0cb48c91c0fb6852567430063b5d6/a66dd8ce90690d8685256b4a0059d7b5/$FILE/P36315Rev.-Plasmidweb.pdf). Full sequencing employed primers 27F (Giovannoni 1991), 1100R (Suzuki et al. 2004), 1074F (Suzuki et al. 2004) and 1541R (Suzuki et al. 2000), while primers 27F and 519R (Amann et al. 1995) were used for partial sequencing. Ribosomal RNA genes were sequenced using the Big Dye v3.1 protocol, employing dideoxynucleotide termination reactions with 1:32 dilutions (Applied Biosystems, http://docs.appliedbiosystems.com/pebiodocs/04337035.pdf). The PCR reactions for the 27F primer were as follows: 40 cycles on an AB9700 thermal cycler consisting of 10 seconds denaturation at 96ºC, 5 seconds annealing at 50ºC, and 2 minutes 30 seconds extension at 60ºC. Reactions with 1074F, 1100R and 1541R primers were conducted as above, with the extension temperature lowered from 60ºC to 55ºC. Reactions using 519R primer were run at 60ºC annealing and 65ºC extension. Some plasmids with poor sequence were also purified using the FastPlasmid Mini protocol (Eppendorf, http://www.eppendorf.jp/product/molecular/fastplasmid_manual(English).pdf). These plasmids were then used in 27F and 519R reactions for partial sequencing.

Sequences were initially aligned edited and assembled the using the Staden Package (Sanger Institute). Sequences were then imported and automatically aligned by the ARB package (Ludwig et al. 2004). Alignments were manually checked and sequences were added to a ca. 35,000 sequences tree using ARB_PARSIMONY and a filter excluding highly variable positions. Near relatives were used to identify possible

base miscalls and sequences were removed from, and re-added to the tree using phyla specific filters. Phylogenetic distances to nearest relatives were determined using the program *dnadist* of PHYLIP package (Felsentein 1989) in the ARB software using filters that excluded highly variable positions and ambiguities and the Kimura two-parameter model method (Kimura 1980). Clones were classified according to the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu) scheme.

Results

Cell counts and cell volume

All statistical comparisons were conducted using a repeated measures ANOVA (SAS Institute, Cary, NC) with a Tukey-Kramer adjusted p value and comparisons were made at a 95% confidence level.

Experiment 1

At the onset, the Control, Peat and Ice algae incubations had comparable cell numbers. However, by day 7, cell abundances were significantly higher in the Ice algae (8.4 to 9.6 $\times 10^5$ cells ml^{-1}) relative to the Control (0.64 to 0.68 $\times 10^5$ cells ml^{-1}) (ANOVA, $p < 0.0001$). The Peat (3.4 $\times 10^5$ cells ml^{-1}) incubations were significantly different than the Control by day 9 (ANOVA, $p = 0.0056$) (Figure 2.1). There was a similar, albeit less obvious trend in cell volumes in the incubations, with cell volumes very similar in all incubations until day 7, when the bacterial cell volumes in the Peat and Ice algae incubations were slightly higher relative to the Controls, but were not significantly different (ANOVA, $p = 0.9999$ and $p = 1$, respectively) (Figure 2.1). By the end of Experiment 1 (day 11), the Ice algae incubations had the highest cell abundance

(6.8 and 7.6 × 10^5 cells ml^{-1}) and cell abundances in the Ice Algae incubation were statistically different than the Peat (ANOVA, $p = 0.0041$) and the Control (ANOVA, $p < 0.0001$). The Ice Algae also had the highest cell volume by the end of Experiment 1 (0.166 and 0.294 μm^3) and it was significantly different than the Peat incubation (ANOVA, $p = 0.0124$) but not the Control (ANOVA, $p = 0.0609$) (Figure 2.1).

Experiment 2

Like Experiment 1, cell abundances for Experiment 2 remained similar until day 8, with the Peat bacterial abundances slightly higher than the Control by the end of Experiment 2 (day 12) but were not significantly different from one another (ANOVA, $p = 0.4194$) (Figure 2.1). Bacterial abundances in the Control differed between experiments and were approximately 2-3 times higher at the end of Experiment 2 in comparison with Experiment 1. This trend was not observed in the Peat incubation, where bacterial abundances were nearly equal at the conclusion of both experiments. Although no clear trends were observed in the cell volume data for Experiment 2, by day 12, the Control incubation had slightly higher cell volumes than the Peat incubation from Experiment 2, as well as the Experiment 1 Control at day 11 used as its inoculum (Figure 2.1). However, no significant differences were observed among the Peat and Control cell volumes for the duration of Experiment 2. Final cell volumes in Peat incubations were approximately equal in Experiments 1 and 2 (Figure 2.1).

Bacterial carbon was calculated for all incubations assuming 65 $fgC/\mu m^3$ and using cell abundance and cell volume at endpoints (day 11, Experiment 1; day 12, Experiment 2) when samples were collected for DNA and lipid analyses. A wide range of values was observed, from 0.512 µg C/L in the Control Carboy 1 from

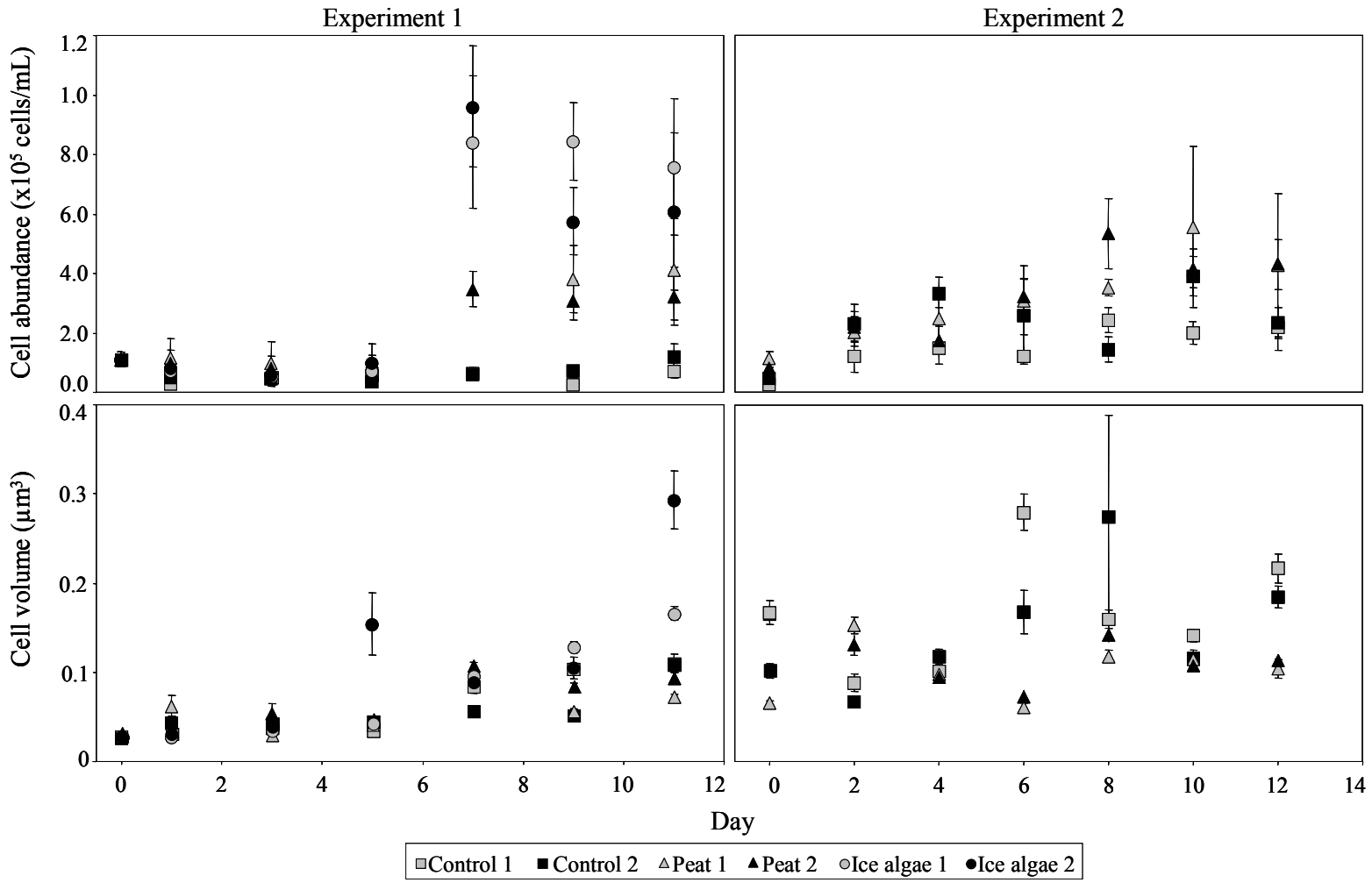

Figure 2.1. Bacterial counts and cell volumes for Experiments 1 and 2. Error bars represent the standard deviation and standard error of the cell abundance and cell volumes, respectively.

Experiment 1 to 11.623 µg C/L in Carboy 2 of the Ice algae incubation although most of the values ranged from 2 – 3.2 µg C/L (Table 2.3).

Table 2.3. Bacterial carbon contents for Experiments 1 and 2, calculated using cell abundance (cells/mL) and cell volume (μm^3/cell), assuming 65 fg C/μm^3.

Experiment 1

	<1.0 µm	Control Carboy 1	Control Carboy 2	Peat Carboy 1	Peat Carboy 2	Ice algae Carboy 1	Ice algae Carboy 2
Bacterial Carbon (µg C L^{-1})	0.199	0.512	0.863	1.963	1.993	8.175	11.623

Experiment 2

	Control Carboy 1	Control Carboy 2	Peat Carboy 1	Peat Carboy 2
Bacterial Carbon (µg C L^{-1})	3.064	2.780	2.886	3.160

LH-PCR

Experiment 1

The bacterial community composition based on LH-PCR electropherograms is shown as histograms, with the bars representing the relative peak height of the sized fragments (Figure 2.2). In the Inoculum water (Figure 2.2), fragments sized 315-317 base peaks (bp) collectively represented approximately 30% of the amplified fragments. In marine plankton these shorter fragments generally correspond to the SSU rRNA genes of *Alphaproteobacteria* and likely represent several different clades (Suzuki et al. 1998). The Inoculum water also contained fragments sized 344 (2.8%), 346 (6%), 348 (9.7%), 349 (10.8%), 350 (16.7%) and 351 (17%) bp. Most of these fragments can represent different phylogenetic groups in marine plankton, including *Gamma*-, *Beta*-and *Delta*-subdivisions of the *Proteobacteria* and the *Bacteroidetes* phylum. In marine plankton the fragment sized 350 bp corresponds mostly to the *Gammaproteobacteria* and, like the

315-317 fragments, probably represents several clades of this proteobacterial class (Suzuki et al. 1998).

Relative to the Inoculum water, the bacterial community composition shifted in all incubations. The Peat, Debris and Ice algae treatments had similar bacterial distributions after 11 days, with a prevalent fragment sized 347 bp, ranging from 14 to 53% (Figure 2.2). In marine plankton these fragment sized 344-354 represent *Gammaproteobacteria* and *Bacteroidetes*. In the Control incubations the prevalent fragment was sized 352 bp (38-63%, Figure 2.2), likely representing *Gammaproteobacteria* and possibly *Betaproteobacteria*.

Overall, replicate carboys for each of the treatments and Controls had similar bacterial community composition, although some minor discrepancies were seen between carboys. In the Control incubations, a fragment sized 350 bp (20%) was present in Carboy 1 and not in Carboy 2. Also, Control Carboy 2 had a higher relative proportion of fragments sized 352 bp (Figure 2.2). Furthermore, in Carboy 1, the fragment sized 349 bp comprised a higher percentage of the relative distribution than in Carboy 2 (Figure 2.2). The bacterial community composition in the Peat incubations differed slightly between replicate carboys, as seen in Carboy 1, which contained a fragment sized 346 bp which was not present in Carboy 2 (Figure 2.2) and 347 made up a higher percentage of the relative distribution in Carboy 1 of the Peat incubation.

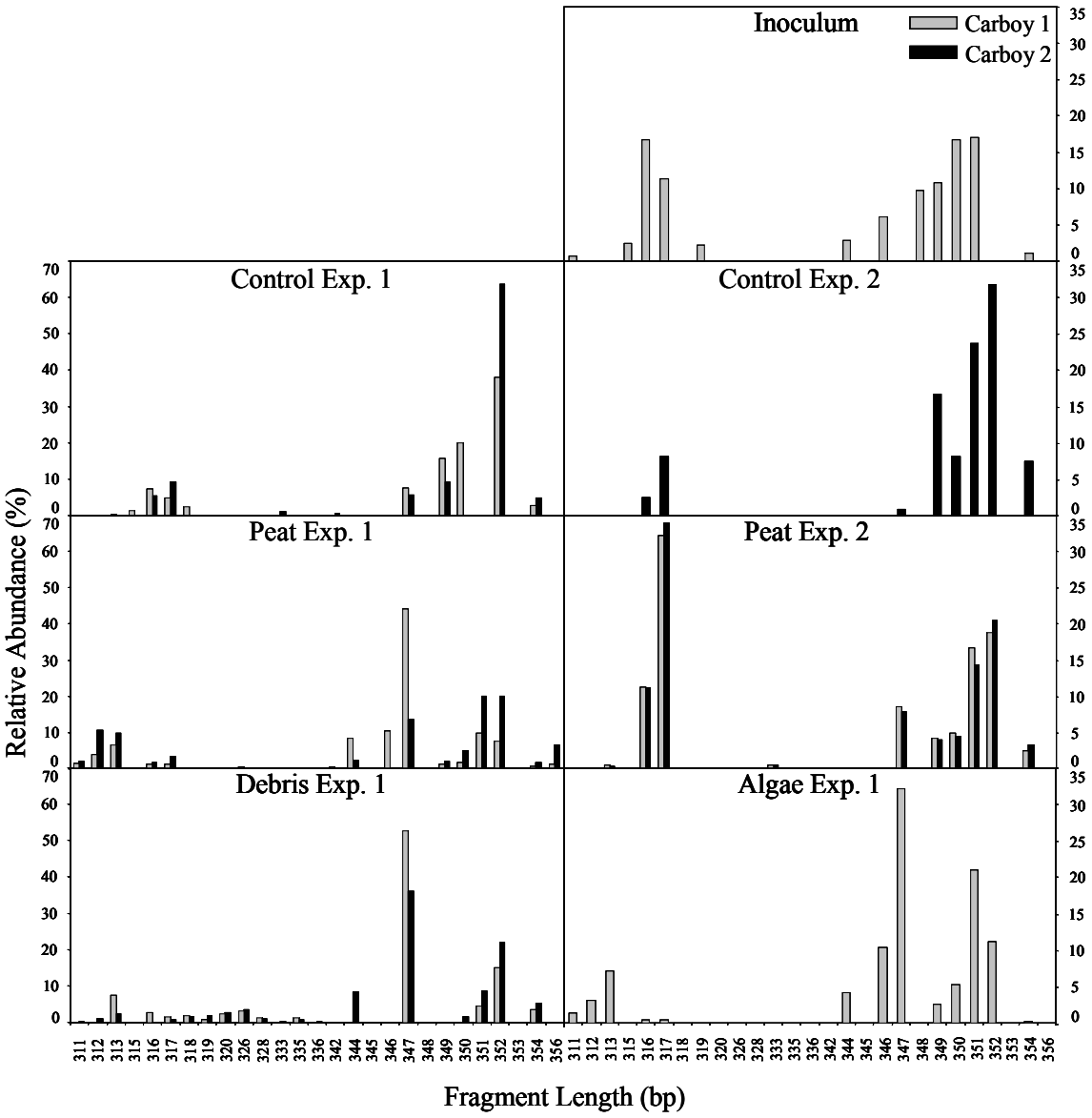

Figure 2.2. Relative abundances of DNA fragments representing >1% of the total peak heights from LH-PCR from the Inoculum and all regrowth experiments with histogram bars representing duplicate carboys.

Experiment 2

At the end of the extended incubation (23 days total, including Experiments 1 and 2; 12 day incubation time for Experiment 2), Controls in Experiment 2 were dominated by fragments sized 351 and 352 bp (Figure 2.2), mostly corresponding to *Gamma*- and

Beta- subdivisions of the *Proteobacteria* as well as the *Bacteroidetes* (Suzuki et al. 1998). The bacterial community composition in the Control from Experiment 2 shifted relative to its inoculum (Day 11, Experiment 1, Carboy 2), notably by the large proportion of fragments sized 351 bp (Figure 2.2).

The Peat incubation showed a unique shift in bacterial community composition over time, with a fragment sized 317 bp dominating the relative peak height distribution (32-34%, Figure 2.2). In marine plankton this size corresponds mostly to the SSU rRNA genes of *Alphaproteobacteria* (Suzuki et al. 1998). Dominant fragments (347-352 bp) found in the inoculum for this incubation (Experiment 1, Peat, day 11) were also present in at the end of Experiment 2, although in much lower relative proportions (Figure 2.2). Carboys 1 and 2 of the Peat incubation in Experiment 2 had nearly identical bacterial communities.

Clone library

Based on the LH-PCR results, the Peat incubation from Experiment 2 was chosen for clone library analysis since the community composition in this sample was clearly different. Of 96 picked clones, 82 were identified as having full inserts during the M13F screening. Of the 82 clones chosen for sequencing, 7 did not yield fragments when analyzed by Genescan and 11 had a 351 bp 27F-338R fragment with no detectable tRNA or ITS fragments and those clones were also excluded from further analysis. Of the remaining 64 clones, 2 were overlooked during sequencing analysis. Therefore, 62 clones were used for phylogenetic reconstruction.

Screening:

Based on the fragment sizes of the three primer sets, clones from the Peat incubation from Experiment 2 were grouped and identified (Table 2.4). These clones

Table 2.4. Phylotypes from Peat, Carboy 2 Experiment 2, 10µm screened sample (Peat2exp2f), their measured rRNA (27F-338R), ITS and tRNA LH-PCR fragments and the number of clones per phylotype. bp: base pairs; No.: number; nd: not determined.

Phylotype	Representative sequence	rRNA fragment (bp)	ITS fragment (bp)	tRNA fragment (bp)	No. of clones
Alphaproteobacteria					
1	peC06	318	882	359	2
2	peF05	317	942	359	9
3	peD04	318	945	359	3
4	peE05	317	953	359	1
5	peE08	318	959	359	5
6	peG04	318	966	359	2
7	peD03	318	1037	360	7
8	peG01	318	1074	359*	5
9	peD02	318	1095	359*	3
10	peG06	317	986	359	1
11	peE10	318	1131	360	1
12	peH07	317	892	340	1
13	peF11	320	914	365	1
14	peF09	320	987	367	2
Betaproteobacteria					
15	peA02	nd	nd	nd	1
Gammaproteobacteria					
16	peA06	349	827	359	3
17	peF04	351	720		1
18	peF01	320	915	427	1
19	peE03	352	952	428	1
20	peH01	nd	nd	nd	1
21	peA03	349.59	806.93	476	1
Firmicutes					
22	peC03	357	437	no	4
23	peH11	358	445	no	1
24	peC10	355	461	no	1
25	peB10	344	440	no	1
Bacteroidetes					
26	peC02	348	no	418	1
27	peD01	348	754	421	1
28	peG12	347	943	439	1

*Not observed in this specific clone, inferred from other clones with same ITS fragment size

were added into a phylogenetic tree containing previously published sequences. The *Alphaproteobacteria* made up the largest and most diverse group of identified clones, accounting for 43 of the 62 clones represented by 14 different phylotypes, with *Sulfitobacter* representing 12 of the 14 phylotypes (Table 2.5). The majority of these clones (34 out of 43) were very closely related (>99%) to a previously published sequence, ARK10278, an Arctic member of the *Roseobacter* clade (see Chapter 1) (Brinkmeyer et al. 2003) (Table 2.5). *Gammaproteobacteria* were the second most abundant bacteria and were also very diverse, contributing 8 clones, with 6 phylotypes, including *Psychrobacter*, *Stenotrophomonas*, *Colwellia*, *Pseudomonadaceae, Methylophaga and Alteromonadaceae* (Tables 2.4 and 2.5). *Firmicutes* consisted of 7 clones and 4 phylotypes, all of which grouped with *Bacillus* (>96%) (Tables 2.4 and 2.5). Three clones grouped with *Bacteroidetes*, each representing a different *Polaribacter* phylotype and one clone grouped with *Betaproteobacteria*, specifically *Janthinobacterium* (Tables 2.4 and 2.5).

Table 2.5. Clones from Peat, Carboy 2 Experiment 2, 10µm screened sample (Peat2exp2f), their phylogenetic affiliation, the number of phylotypes, grouping based on the Ribosomal Database Project (RDP) classification, sequence similarities to, and accession numbers of the closest relative and the closest cultured relative measured by dnadist. bp: base pairs; nd: not determined; str.: strain; T: type.

Phylotype	*RDP group*	Closest relative	Accession number	% Similarity	bp	Closest cultured relative	Accession number
Alphaproteobacteria							
1	*Sulfitobacter*	str. ARK10278	AF468378	100	1317	str. ARK10278	AF468378
2	*Sulfitobacter*	str. ARK10278	AF468378	99.85	1326	str. ARK10278	AF468378
3	*Sulfitobacter*	sea ice clone ANT1/4_14-133	AY165563	99.64	276	str. ARK10278	AF468378
4	*Sulfitobacter*	sea ice clone ARKICE-75	AF468302	98.11	532	str. ARK10278	AF468378
5	*Sulfitobacter*	str. ARK10278	AF468378	99.6	1317	str. ARK10278	AF468378
6	*Sulfitobacter*	str. ARK10278	AF468378	99.6	1326	str. ARK10278	AF468378
7	*Sulfitobacter*	str. ARK10278	AF468378	99.9	1317	str. ARK10278	AF468378
8	*Sulfitobacter*	str. ARK10278	AF468378	99.77	1327	str. ARK10278	AF468378
9	*Sulfitobacter*	str. ARK10278	AF468378	100	1339	str. ARK10278	AF468378
10	*Sulfitobacter*	str. ARK10278	AF468378	100	816	str. ARK10278	AF468378
11	nd	nd	nd	nd	nd	nd	nd
12	*Rhodobacteraceae*	*Loktanella salsilacus* LMG 22002	AJ582229	99.77	863	*Loktanella salsilacus* LMG 22002	AJ582229
13	*Sulfitobacter*	str. ARK10207	AF468373	99.7	312	str. ARK10207	AF468373
14	*Sulfitobacter*	str. ARK9990	AY167339	99.45	913	str. ARK9990	AY167339
Betaproteobacteria							
15	*Janthinobacterium*	str. R-7687	AJ440985	100	228	str. R-7687	AJ440985
Gammaproteobacteria							
16	*Psychrobacter*	str. R-8160	AJ440989	100	523	str. R-8160	AJ440989
17	*Stenotrophomonas*	*Stenotrophomonas maltophilia* str. e-p13	AJ293473	98	397	*Stenotrophomonas maltophilia* str. e-p13	AJ293473
18	*Colwellia*	sea ice clone ARKICE-87	AF468313	100	447	str. ANT9207	AY167299
19	*Alteromonadaceae*	sea ice clone ARKXV/1-96	AY165593	99.4	419	*Colwellia* sp. IE1-3	AY829232
20	*Pseudomonadaceae*	str. IsoE3	AF150718	99.8	459	str. IsoE3	AF150718

Table 2.5. continued

21	*Methylophaga*	sea ice clone ARKIA-123	AF468294	99.8	915	*Methylophaga thalassica*	X87339
Firmicutes							
22	*Bacillus*	*Bacillus jeotgali* (T) YKJ-10	AF221061	99.1	1451	*Bacillus jeotgali* (T) YKJ-10	AF221061
23	*Bacillus*	*Bacillus jeotgali* YKJ-11	AF221062	99.1	903	*Bacillus jeotgali* YKJ-11	AF221062
24	*Bacillus*	*Bacillus* sp. 19498	AJ315066	97.6	454	*Bacillus* sp. 19498	AJ315066
25	*Bacillus*	soil clone BSV71	AJ229215	96	246	*Bacillus jeotgali* (T) YKJ-10	AF221061
Bacteroidetes							
26	*Polaribacter*	*Polaribacter irgensii* ANT9044	AY167314	98	426	*Polaribacter irgensii* ANT9044	AY167314
27	*Polaribacter*	clone ZD0255	AJ400343	97	453	*Polaribacter* sp. SW019	AF493675
28	*Polaribacter*	*Polaribacter irgensii* 23-P	M61002	98.9	684	*Polaribacter irgensii* 23-P	M61002

Discussion

Phylogenetic techniques utilizing 16S rRNA genes enable the identification of *in-situ* bacterial communities and aids in understanding the metabolic capabilities of naturally occurring bacteria. While the factors structuring *in-situ* microbial communities are complex, studies have shown that DOM composition can play a role in bacterial community composition, with certain bacteria linked to preferential uptake of various types of DOM (Cottrell and Kirchman 2000b; Covert and Moran 2001; Kirchman et al. 2004; Pinhassi and Berman 2003). The substrates used in this experiment were selected based on differing lability and organic matter composition which, based on previous studies, could potentially have profound effects on bacterial community composition. However, the LH-PCR results from Experiment 1 indicate that no particular phylogenetic group of bacteria responded preferentially to the substrates, although it is very possible that different organisms represented the dominant fragments observed (344-354 bp) in each of the different additions. Since differences in the bacterial community compositions of the Control and treatment carboys based on LH-PCR were observed, changes in the bacterial community composition of the substrate-amended incubations were likely not solely due to manipulation and confinement as previously observed in similar experiments (Suzuki 1999). Furthermore, additional environmental factors known to affect bacterial community composition such as temperature, salinity, pressure, and grazing were not likely to be the cause of the observed shift in bacterial community composition in Experiments 1 and 2. Unfortunately, due to the fact that some LH-PCR fragments overlap in size among the different groups of bacteria, it is difficult to determine which specific members of the bacterial community were responsible for the

shifts in community composition observed in the organic matter additions of Experiment 1, and this remains a subject for further investigation.

Viral induced mortality could be one cause for the shift observed in community composition between Experiments 1 and 2, as it is known that viruses play key roles in bacterioplankton community composition, with viruses selectively infecting bacterioplankton which have either reached a certain growth rate or density (Wommack and Colwell 2000). This could partially explain the decrease in the dominant peaks (352 for the Control, and 347 for the Peat) observed in Experiment 1 relative to Experiment 2. However, the disparate community composition between the Control and Peat incubations in Experiment 2 and the prevalence of *Alphaproteobacteria* and in particular, *Roseobacter* in the Peat incubations from Experiment 2, indicates that the change in community composition cannot be attributed to bacteriophage infection alone.

One potential explanation for the similarity of the community shifts observed using LH-PCR among the incubations from Experiment 1 may be that certain bacteria have greater metabolic flexibility and are able to rapidly respond to organic matter and therefore outgrow other members of the bacterioplankton (Langenheder et al. 2005). This type of response has been observed in the *Gammaproteobacteria*, which have been shown to utilize a wide spectrum of DOM sources (Covert and Moran 2001; Ivanova et al. 2002) and some members of this group are said to be opportunistic, exhibiting rapid growth responses during enrichment experiments, thereby outgrowing and outcompeting other bacteria (Eilers et al. 2000a; Fuchs et al. 2000; Pinhassi and Berman 2003). Recently, it has been proposed that bacterioplankton communities are comprised of physiological divergent bacteria: generalists, which are characterized by successful

growth under a variety of conditions and specialists, which have more specific environmental and/or nutritional growth requirements (Crump et al. 2003; Langenheder et al. 2005; Miki and Yamamura 2005). This perspective on bacterioplankton community dynamics may explain the overlap in bacterial community composition observed in Experiment 1 when organic matter substrates of differing labilities were used. Under this premise, the results of this study suggest that some group(s) of bacteria with an opportunistic life history strategy initially responded to labile fractions of all organic matter substrate additions. Subsequently, some limiting condition, proposed here as the exhaustion of the labile fraction of the Peat DOM, resulted in the decline of such species, thereby enabling the *Alphaproteobacteria* to become the dominant group of bacteria in the extended Peat incubation. It is proposed that the labile component of the Peat was fully utilized by the end of Experiment 1, and that the dominance of the *Alpha-proteobacteria*, in particular *Roseobacter*, in the extended Peat incubation can be attributed the ability of *Roseobacter* to utilize more refractory components organic matter such as lignin and related aromatic compounds via the beta-ketoadipate pathway (Buchan et al. 2000; Buchan et al. 2004; Covert and Moran 2001). The peat substrate itself likely consists of a complex mixture of terrestrially-derived compounds such as lignins, humics, cellulose, phenolics and polysaccharides (Killops and Killops 1993), supporting this hypothesis.

The LH-PCR and clone library results from the Peat2exp2f sample from Experiment 2 are in good agreement and show that the *Alphaproteobacteria* dominated the community, with 14 different phylotypes contributing to the observed change in community composition. The majority of these phylotypes were closely related (>99%

similarity) to ARK10278 found in Arctic sea-ice (Brinkmeyer et al. 2003), indicating that the bacteria present in this incubation represented naturally occurring members of Arctic microbial assemblages and was not an introduced organism. Since this organism is in pure culture, studies on the ability of this organism to grow on peat could be attempted in the future.

While no clear phylogenetic response to organic matter substrates was detected in Experiment 1, the lability of the added substrate affected the metabolic response of the bacterial community. The bacterial production rates (D.L. Kirchman personal comm.) mirrored the lability of the substrates, with the highest bacterial production supported by the labile carbon from the Ice algae incubation followed by the Debris and Peat substrates, with the Control incubation having the lowest bacterial production. The Ice algae incubation also supported the highest cell abundances and cell volumes, resulting in the highest bacterial carbon values. Combined these results agree with previous observations that organic matter lability affects microbial growth and metabolism (Fuchs et al. 2000; Lennon and Pfaff 2005).

In conclusion, organic matter quality played an important role in bacterial growth and cell volumes, with more labile substrates supporting higher bacterial abundances and cell volumes. Manipulation and confinement are probably the cause of the changes observed in the bacterial community in the Control incubations relative to the Inoculum water, while the shifts observed in the Peat, Ice algae and Debris incubations are likely responses of the bacterial community to the labile fraction of the organic matter substrates. Since many LH-PCR size fragments overlap, it was difficult to infer which specific bacteria were responsible for the community shifts observed in Experiment 1,

although the similarity of the community composition in these incubations indicates that certain bacteria are adapted to be able to quickly respond to diverse organic matter substrates and can therefore successfully outcompete other bacteria. During the timeframe of these experiments, the results shown here support previous observations that bacteria, namely members of the *Gammaproteobacteria* as well as *Bacteroidetes* (Bano and Hollibaugh 2002; Brinkmeyer et al. 2003; Yager et al. 2001), preferentially respond to labile components of DOM and that community response as detected by LH-PCR was independent of organic matter source in this study. Furthermore, it is likely that the bacteria in these incubations transform the DOM via the uptake and removal of the labile components. Clone library analysis of the Peat incubation from Experiment 2 supported LH-PCR results and showed that the majority of the clones (69%) belonged to the *Alphaproteobacteria*, particularly *Sulfitobacter*, with nearly all of these clones (79%) closely related (>99% similarity) to ARK10278, a member of the *Roseobacter* clade. The dominance of these bacteria in the Peat incubations suggests that they could be important in the uptake and cycling of terrestrially-derived organic matter in the Arctic Ocean, although this contrasts to previous results, which found that a novel group of organisms closely related to ARK10278 were relatively rare (<1% of FISH counts) (Brinkmeyer et al. 2003). Therefore, increases input of terrestrially derived organic matter into the Arctic Ocean may result in changes in bacterioplankton community structure, with bacteria previously observed to be uncommon comprising a larger proportion of the bacterioplankton community.

Chapter 3: Using lipid biomarkers to monitor the response of in-situ Arctic microbial communities to organic matter substrate additions

Synopsis

Bacterial regrowth experiments were employed to examine the response of *in-situ* Arctic bacteria communities to a variety of organic matter substrates, including peat, ice algae and ice-entrained debris. Cell membrane lipid composition in enrichments and controls was examined using both fatty acid and intact phospholipid (IPL) analyses to determine the effect of the organic matter substrates on lipid distribution. Fatty acid distributions among the treatments showed significant overlap, with 16:1Δ9, 16:0n and 18:0n comprising the three major acids in most cases, with the exception of the extended Peat incubation, where the distribution was dominated by 16:1Δ9, 18:1Δ11 and 16:0n, corresponding to a shift in the bacterial community. Fatty acids typically attributed to bacteria such as branched- and odd-chain fatty acids, were not major contributors to the fatty acid distribution observed in the regrowth experiments. Intact phospholipids (IPLs) can be used to distinguish living prokaryotes and are valuable markers for viable bacteria in complex matrices such as sediments. IPLs were successfully employed here in identifying phospholipid-associated fatty acids in the ice algae and debris-amended incubations where the presence of substrate complicated the interpretation of the fatty acids methyl ester (FAME) analysis. Prior to analysis of IPLs in the bacterial regrowth

experiments, cultures of R2A84, a marine member of the *Roseobacter* clade isolated off the coast of Oregon, were used to refine LC/MS methodology. Cultures were grown on 2 modified versions of media, one with, and one without peptone in order to determine the effect of organic matter substrate on cell membrane lipid composition. When grown on the media containing peptone, C-15 and C-17 branched- and odd-chain fatty acids dominated the fatty acid distribution of R2A84. However, when grown in the absence of peptone, 18:1Δ11 comprised over 85% of the total fatty acids. Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) represent common bacterial cell membrane constituents and were the only IPLs detected in the cultures and regrowth experiments. Analysis of IPLs in positive ion mode resulted in protonated headgroup species and fragmentation occurred at the glycerol-phosphate bond, revealing headgroup structures. Complementary structural information was provided by running the samples in negative ion mode resulting in a deprotonated compound with fragmentation at the glycerol-fatty acid bond, enabling identification of the fatty acid tail groups. PG and PE were present in nearly equal amounts in the culture grown on broth containing peptone, while only PG was detected in the culture grown on the media without peptone. In the regrowth experiments, PE generally dominated the IPL distribution. Fatty acids detected during IPL analysis were analogous to those found in individual fatty acid analysis in both of the R2A84 cultures as well as in the regrowth experiments. The fatty acids 15:0 and 17:0 were commonly observed in the culture grown on broth containing peptone while 18:1 was prevalent in the media without peptone. In the case of the Arctic regrowth experiments, fatty acid and IPL synthesis overlapped when bacteria were grown on natural organic matter substrates, with 16:1Δ9, 16:0n, 18:1Δ11 and 18:0n prevalent. The

results of this study, in particular the R2A84 cultures, indicate that both fatty acid and IPL distribution are influenced by organic matter substrate. Furthermore, when grown on natural organic matter substrates, bacterial fatty acids and intact phospholipids of phylogenetically distinct Arctic bacterioplankton overlapped. Branched- and odd-chain carbon fatty acids, used as bacterial markers in natural systems, were not major contributors to the Arctic bacterioplankton fatty acids. Therefore, the exclusive use of odd- and branched-chain fatty acids may underestimate bacterial presence and/or biomass in natural systems. The results of this research indicate that knowledge of bacterial utilization of organic matter is critical for interpretation of cell membrane lipid composition.

Introduction

Bacteria comprise the largest pool of living biomass in aquatic systems (Whitman et al. 1998), yet knowledge of *in-situ* microbial community composition and metabolic capabilities is relatively limited. Only 1% of bacteria are generally considered culturable, and recent phylogenetic efforts to examine natural environments has shown that cultured species are generally not major contributors to microbial diversity in natural systems (Giovannoni and Rappé 2000). Bacteria play primary roles in many biogeochemical cycles, ranging from the repackaging and transfer of elements into higher trophic levels and nutrient regeneration via the microbial loop as well as nitrification, denitrification, sulfur oxidation and methanogenesis, among other processes (Sherr and Sherr 2000). In the carbon cycle, microbes are responsible for the uptake, transformation and release of organic matter in aquatic systems (Amon et al. 2001). Dissolved organic matter (DOM) in oceanic systems represents the largest pool of reduced carbon in the marine

environment and is generally considered autochthonous and largely resistant to degradation (Benner 2002; Ogawa et al. 2001). Bacteria contribute to the DOM pool via release of cellular components (Wakeham et al. 2003; Zou et al. 2004) and play important roles in its composition by preferentially utilizing labile components, resulting in the production of refractory DOM (Ogawa et al. 2001; Zou et al. 2004). Studies have shown that bacteria differ in their uptake ability of organic matter (Kirchman 2002; Malmstrom et al. 2005; Martinez et al. 1996), hence, organic matter composition can play an important role in structuring aquatic bacterial communities. Knowledge of bacterial community composition and their metabolic capabilities is therefore crucial to understanding their role in organic matter cycling.

Cellular components such as lipids have long been employed as indicators of bacteria in the aquatic environment (Ratledge and Wilkinson 1988). In particular, phospholipid ester-linked fatty acids have received considerable attention as bacterial biomarkers (Guckert et al. 1985; Ratledge and Wilkinson 1988; White et al. 1979), especially odd- and branched-chain fatty acids which are exclusively synthesized by *Bacteria* (Kaneda 1991). Additional lipids such as the hydroxy fatty acids have been attributed to bacteria (Wakeham et al. 2003) as well as methylhopanoids, which have been found in gram-negative bacteria, methanotrophs, cyanobacteria, acetic acid bacteria, nitrogen fixers and "purple non-sulfur bacteria" (Farrimond et al. 2004; Talbot et al. 2003). Furthermore, unique ladderane lipids have been found only in *Bacteria* capable of the annamox reaction (Damsté et al. 2005). Archaea typically do not contain significant amounts of fatty acids, but can be differentiated from bacteria by the presence of ether-linked isoprenoidal side chains in their cell membrane lipids (Boucher et al. 2004). To

date, there is little information regarding the lipid composition of natural bacterial communities in the marine environment.

Recent analytical advances have enabled researchers to examine the presence of *in-situ* bacteria using high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI-MS) of intact phospholipids (IPLs) (Sturt et al. 2004; Zink and Mangelsdorf 2004). Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) are two of the most commonly encountered IPLs in bacterial cell membranes (Fang et al. 2000b; Lechevalier and Lechevalier 1988). The measurement of IPLs is advantageous because these compounds are generally dominant components of cell membranes (Zink and Mangelsdorf 2004) and degrade upon cell death, therefore representing living biomass (Harvey et al. 1986; White et al. 1979). Furthermore, identification of headgroup and associated fatty acid tail group combinations provides an opportunity for improved taxonomic association of lipids (Fang et al. 2000a) and *in-situ* microbial communities (Rütters et al. 2002b; Zink and Mangelsdorf 2004).

This study utilized bacterial regrowth experiments to examine the response of *in-situ* Arctic bacterial communities to an array of Arctic organic matter substrates including peat, ice algae and ice rafted debris using 16S rRNA phylogenic methods (refer to Chapter 2 of this thesis) and cell membrane lipid composition. Fatty acid and IPL analyses were conducted to determine the effect of organic matter addition on the cell membrane lipid synthesis as well as to assess the relationship between phylogeny and cell membrane lipids (refer to Chapter 4 of this thesis). Since previous work has shown that environmentally significant bacteria may be able to quickly and preferentially utilize a variety of primers present in protein substrates for synthesis of odd- and branched-chain

fatty acids (Harvey et al. 2005), bacterial cultures were employed to further elucidate the effect of organic matter substrate on cell membrane lipid composition.

Methods

Arctic experiment overview

A more detailed overview of the Arctic regrowth experiments is given in Chapter 2. Briefly, the water sample used for Experiment 1 was collected near the Arctic Ocean station HLY 04-02-007 (HV2, 70°13.117N 167°38.653W) on May 20, 2004 from the science seawater system (inlet at c.a. 8.3 meter depth) aboard the USCGC Healy. Site specific information is given in Table 2.2. Water used to dilute Peat and Control incubations in Experiment 2 was also collected from the seawater system on 31 May 2004 near station HLY04-02-017 (EHS5, 72°43.810N 158°25.475W). Water was collected after 5 minutes of flushing to discard fouling organisms in the system and to obtain fresh subsurface water (inlet at c.a. 8.3 meter depth). Sample water was filtered through a series of inline 3.0 μm, 1.0 μm and 0.2 μm cartridge filters (Pall Life Sciences) into 20 liter carboys using a peristaltic pump, with 3 rinses of 2 liters of < 0.2 μm filtered water prior to collection of water for initiation of experiments. Arctic bacterioplankton (2 liters of <1.0μm filtered water) were diluted 10-fold with bacteria-free water (18 liters of <0.2 μm filtered water) and supplemented with organic matter additions. Organic matter substrates consisted of *Melosira arctica*, a sea ice associated diatom prevalent in the Arctic (Booth and Horner 1997; Falk-Petersen et al. 1998), ice rafted debris, (a mixture of ice algae and ice entrained sediment) and peat from the water's edge of the Ikpikpuk River. A no-addition control was also used to discern shifts in community composition

due to experimental enclosure. Hereafter, these samples will be referred to as Ice algae, Debris, Peat and Control. All incubations were conducted in duplicate in the dark at –1°C and bacterial regrowth to early stationary phase occurred (monitored using bacterial production, see Chapter 2) at which time samples from all replicates were taken for bacterial abundance, biomass and production as well as phylogenetic (16S rRNA gene, see Chapter 2) and lipid analyses. For this first experiment (Experiment 1), samples were taken after 11 days, and a volume of 2 liters of water from the no-addition control and the Peat amended incubations remained in the carboys and were subject to a second 1:10 dilution with a < 0.2 μm water sample, and growth occurred for an additional 12 days (Experiment 2). At the conclusion of both experiments, bacterioplankton were sampled for bacterial abundance, biomass, production, phylogeny and lipids. Phylogenetic changes in community composition were monitored as described in chapter 2.

R2A84 bacteria cultures

Given that members of the *Roseobacter* clade became a dominant component of community composition in the extended Peat incubation (see chapter 2), R2A84, a *Roseobacter* originally isolated from coastal Oregon seawater (Suzuki et al. 1997) was chosen as a model organism to refine the LC/MS methodology. Furthermore, this culture was easily accessible and grew readily in culture. R2A84 was inoculated into 2 mL of sterilized marine R2A media (Suzuki et al. 1997). Media sterilization was conducted by autoclaving, using an AMSCO Stage 3 autoclave, 30 minutes of steam at 121°C. R2A84 cultures were grown at ambient room temperature (approximately 20°C) and were gently shaken daily to ensure aeration. A control (media only) was used to monitor the possibility of culture contamination. When significant turbidity was observed in the 2

mL culture, cultures were streaked onto modified marine R2A (marine R2A media with 0.25 g/l yeast extract, termed mR2A-1) agar plates to assure culture purity. Plates were grown overnight at 37°C, single colonies were picked into 2 mL of mR2A-1 media and the liquid growth repeated. Once turbidity was observed, the 2 mL culture was used to inoculate 2 x 500 mL flasks, each containing 250 mL of autoclaved mR2A-1 broth. Flasks were covered with Bio-Shield sterilization wrap (Allegiance) and foil to prevent culture contamination during growth. When the culture in each flask became turbid, the two flasks were pooled; 950 µL was removed for bacterial counts and preserved with 37% formaldehyde (5% of total volume) and stored at 4°C in the dark. Counts were conducted using semi-automated microscopy as described by Cottrell and Kirchman (Cottrell and Kirchman 2004b). The remainder of the culture (approximately 499 mL) was used in lipid analysis as described below. To determine the effect of organic matter substrate on cell membrane lipid composition, the aforementioned process was repeated using a modified marine R2A broth (0.25 g/l yeast extract) that excluded peptone from the recipe. This broth recipe will be referred to as mR2A-2.

Lipid extraction

A. R2A84 bacterial cultures

After culture turbidity was observed, cells were separated from the media by centrifugation at 12000 rpm until cells were pelleted (20-100 min) in Sorvall polypropylene Dry-Spin 250-mL capacity centrifuge bottles, at 10-15°C using a Sorvall RC 24 centrifuge and rotor SLA 1000 (Kendro, Asheville, NC). The supernatant was decanted from the pellet and was properly disposed. The extraction protocol from Sturt et al. (Sturt et al. 2004) was used with slight modifications and all glassware was solvent

rinsed prior to being used in the extraction procedure. Briefly, the pelleted cells were resuspended using 50mM phosphate buffer, pH 7.4 and transferred to a glass test tube with a Teflon-lined cap. Dichloromethane (DCM) and methanol (MeOH) were added to the phosphate buffer in the following ratio: 1:2:0.8 (DCM/MeOH/phosphate buffer). Samples were sonicated at output 3, duty cycle 70% with a Branson Sonifier 250 probe for 5 minutes and DCM and buffer were added to achieve a final ratio of 1:1:0.8 (DCM/MeOH/phosphate buffer). Samples were vigorously shaken and the lower organic phase containing the total lipid extract (TLE) was transferred to a rotary evaporator flask. DCM was added to the sample to achieve separation of the organic and water phases and again vigorously shaken, allowed to separate, and the lower organic layer containing the TLE was removed. A total of 3 extractions, removing the TLE and organic phase each time, were performed and extracts were combined and evaporated to dryness with reduced pressure, with the water bath temperature maintained at 30°C. TLEs were redissolved in 4 aliquots of 2 mL of 2:1 (DCM/MeOH), pooled, evenly distributed to four 2-mL amber vials, flushed with nitrogen, capped, and stored at -70°C until analysis. Sub-samples of the TLEs were utilized for fatty acid (GC and GC/MS) and intact phospholipid analyses (LC/MS).

B. Arctic incubations

Samples for lipid analysis of the Arctic bacterial regrowth experiments were collected by vacuum filtration of sample water onto precombusted (450°C) 47-mm GF/F filters (0.7μm nominal pore size). Incubations with peat as the organic substrate were screened through 10μm Nitex mesh prior to filtration to minimize collection of peat particles and all lipid samples were frozen at -70°C until analysis. Given low expected

bacterial concentrations, a single filter was obtained per carboy for the Control and Peat incubations, with fatty acid and intact phospholipid (IPL) analyses conducted on separate filters (i.e. carboys). Therefore, statistical analyses such as ANOVA or standard deviations are not possible for either intact phospholipid or individual fatty acid analyses. Lipids were extracted as outlined in the R2A84 lipid extraction methodology, with the exception that initial centrifugation for cell pelleting was not employed, and the entire filter was placed in the glass test tube.

Fatty acids in total extracts by GC and GC/MS

For the determination of individual fatty acids in the total extract, a 2 mL aliquot of the R2A84 TLE in 2:1 (DCM/MeOH) was dried down under N_2. The remainder of the method was followed as outlined in Mannino and Harvey (Mannino and Harvey 1999). Briefly, the internal standard nonadecanoic acid was added prior to alkaline hydrolysis. Alkaline hydrolysis was conducted at 70°C for 30-60 minutes. Fatty acids were methylated using boron trifluoride in methanol at 70°C for 30 minutes. Polar lipids were quantified using capillary gas chromatography (HP5890 II, Hewlett Packard). Lipids were separated using a J&W Scientific ZB-5 fused silica column (60 m length x 0.32 mm i.d. x 0.25 μm film thickness) with quantification by flame ionization detection. Samples were injected in the splitless mode (oven temp 50°C; injector at 225°C), with hydrogen as the carrier gas. An Agilent 6890 GC coupled to an Agilent 5973N mass selective detector was used for structural identification. Sample runs were conducted using identical conditions as above but with He as the carrier gas. Double-bond positions of monounsaturated fatty acids were identified by GC/MS analysis as the dimethyl disulfide (DMDS) adducts (Nichols et al. 1986a).

Polar lipid classes by TLC-FID

Thin-layer chromatography with flame ionization detection (TLC-FID) employed an Iatroscan MK-V TLC/FID Analyzer (Iatron Laboratories Inc., Tokyo) to separate and quantify IPLs (Volkman et al. 1986). A multi-step solvent separation procedure was used to separate IPLs from other lipid classes using a method adapted from Parrish and Ackman (Parrish and Ackman 1983). Aliquots of samples (1-2µL) were manually spotted with a 5 µL syringe (SGE International) onto S-III Chromarods (Iatron Laboratories Inc., Tokyo). The origin was focused using a 1:1 mixture of DCM/MeOH followed by development of neutral lipids using hexane/diethyl ether/formic (85:15:0.2). Using this procedure, phospholipids remained at the origin and partial scanning of the rods was conducted to remove neutral components. Chromarods were developed a second time in acetone with partial scanning to remove non-phospholipid components. Development in DCM/MeOH/H_2O (50:20:1) enabled separation of individual phospholipids. Individual phospholipids in samples were identified and quantified by comparison with PG and PE phospholipid standards similar to the method described in Ju and coworkers (Ju et al. 1997).

IPL structures and fatty acid linkages by LC/MS

Intact phospholipids (IPLs) present in lipid extracts were separated by high performance liquid chromatography followed by electrospray ionization mass spectrometry (HPLC-ESI-MS) using a method adapted from Sturt et al. (Sturt et al. 2004). IPLs were separated using normal-phase HPLC on an Agilent 1100 Series HPLC system equipped with a Luna 5µ Silica column (250 mm × 4.60 mm, 5µm; Phenomenex, part number 00G-4274-E0) with a 4.0 mm × 3.0 mm Silica guard column (Phenomenex,

kit part number KJ0-4282, cartridge part number AJO-4348). The solvent systems used at a flow rate of 0.5 ml/min included 100% A (79:20:0.12:0.034 of hexane/2-propanol/formic acid/17.6 M NH_{3aq}) for 1 hour to equilibrate the column prior to initial TLE sample injection. During sample runs, the solvent gradient proceeded as follows: 100% A to 20% A:80% B (88:10:0.12:0.034 of 2-propanol/water/formic acid/17.6% M NH_{3aq}) over 55 minutes, a hold for 10 minutes, then back to 100% A for 1 hour to re-equilibrate the column. Solvent blanks (2:1 dichloromethane/methanol) were run prior to sample analysis. A total lipid extract volume of 10 µl was injected per sample.

TLE samples were analyzed separately in both positive and negative ion modes on an Agilent 1100 Series ion trap mass spectrometer with an electrospray ionization (ESI) interface. In positive ion mode, the fragmentation pattern reveals the phospholipid headgroup and in negative ion mode, the fragmentation pattern reveals which fatty acid side chains are associated with each headgroup as well as which fatty acids are associated with each other, enabling full identification of the compound (Figure 3.1) (Sturt et al. 2004). The nitrogen drying gas was set to 6 L/min and its temperature was 325°C. Parameters for sample runs were optimized during direct infusion of phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) standards into the ESI source, (15 µg/ml concentration at an infusion rate of 12 µl/min). For sample runs, the capillary and end plate voltages were set at 3500 and 500 V, respectively.

Results

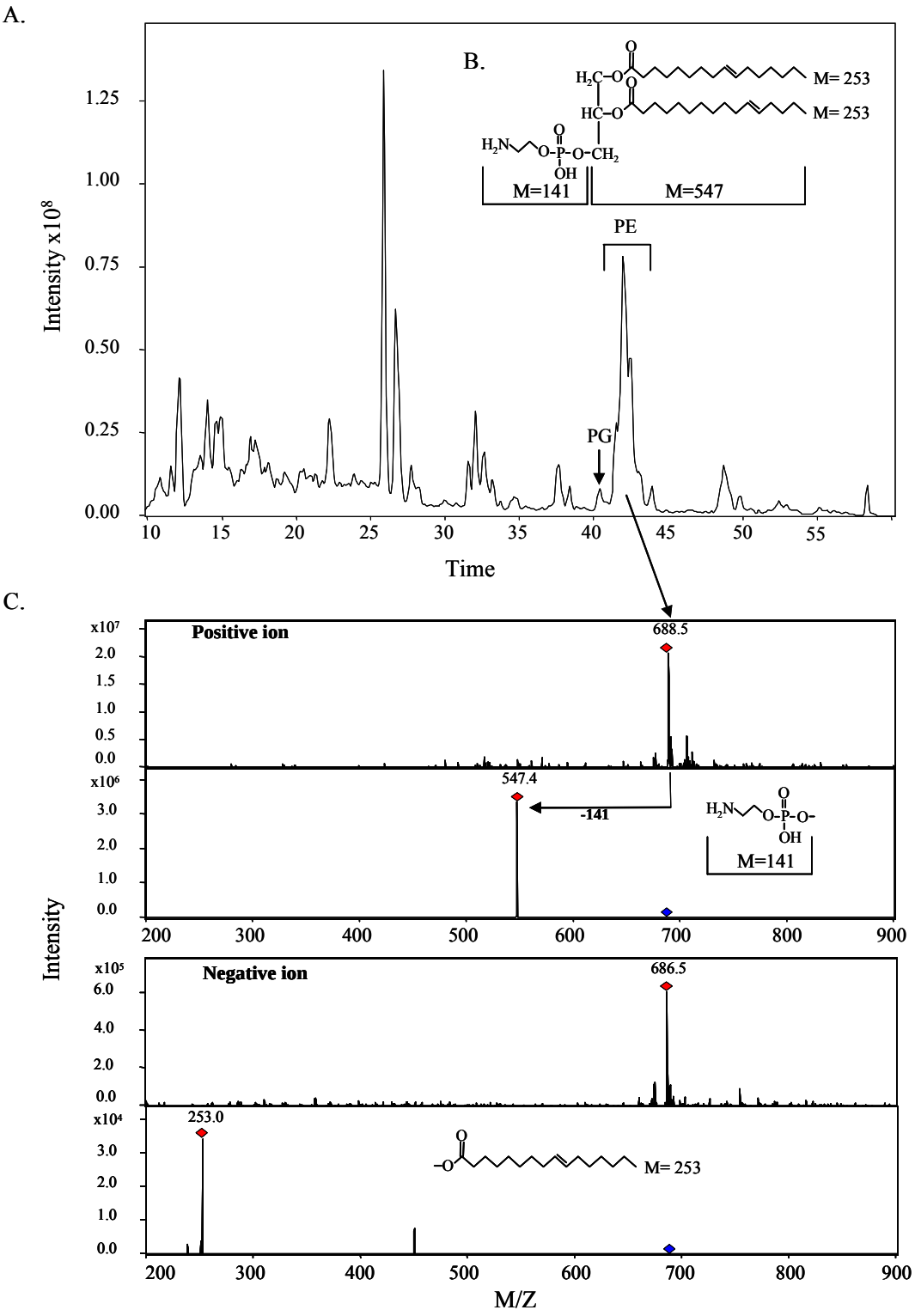

Figure 3.1. Example of an LC/MS chromatogram (A) and spectra (C) of PE-16:1/16:1 from Control, Experiment 2. Fragmentation (C) in positive ion mode shows the loss of the PE headgroup while negative ion mode shows the individual fatty acids. The PE structure (B) is given to show fragmentation of the compound.

R2A84 fatty acids

Total fatty acids in strain R2A84 grown on the two media are shown in Table 3.1. Despite the fact that cell abundances were higher in the mR2A-1 culture ($2.28x10^8$ versus $8.57x10^7$) and cell volumes in the two cultures were similar (Table 3.1), the measured fatty acid concentration for the R2A84 culture grown on mR2A-2 (exclusion of peptone) was approximately 1.5 times higher than that of the R2A84 culture grown on mR2A-1 where peptone was included in the media (Table 3.1). The lower cell abundance in the culture grown on mR2A-2 broth also resulted in lower estimates of bacterial carbon (calculated from cell abundances, cell volumes and assuming 65 fg C/μm^3). Bacterial fatty acid cellular content was calculated using a regression obtained from work conducted in the Delaware Bay using several estuarine habitats (Harvey et al. 2005), where the relationship between bacterial carbon and bacterial fatty acids was determined to be: Fatty acid methyl esters (FAME; µg/L) = 0.2662*Bacterial carbon (µg C/L) + 0.3383, R^2=0.608. In determining the aforementioned relationship, polyunsaturated fatty acids (PUFAs) containing greater than 2 double bonds and dicarboxylic acids (DCAs) were excluded, and the same was done for this study. In addition to differences in the fatty acid concentrations between the two cultures, the fatty acid distributions were vastly different (Figure 3.2). When grown on peptone-containing mR2A-1 media, odd and branched fatty acids dominated, specifically the 15:0 anteiso, (15:0a, 39.8%); 17:0 anteiso (17:0a, 26.5%); 15:0 iso, (15:0i, 11.6%) and 17 iso (17:0i, 9.12%). On the other hand, when grown on peptone-free mR2A-2, 18:1Δ11 made up over 85% of the fatty acids.

Table 3.1. Cell abundances, cellular carbon values, likely and estimated fatty acid concentration and cellular fatty acid content for R2A84 cultures grown on mR2A-1 (+ peptone) and mR2A-2 (- peptone) and Arctic incubations.

Treatment	R2A84 + peptone	R2A84 - peptone	Inoculum	Control		Peat		Ice algae
				Exp 1	Exp 2	Exp 1	Exp 2	
Cell Abundance ($_{x}10^{5}$ cells mL^{-1})	2278.78	856.76	1.11	0.72	2.18	4.13	4.30	7.57
Cell volume (μm^3)	0.04	0.03	0.03	0.11	0.22	0.07	0.11	0.17
Bacterial C (µg C/L)*	635.81	178.11	0.20	0.51	3.06	1.96	3.16	8.18
Likely Bacterial FA (µg/L)**	291.74	465.46	0.84	0.21	1.33	0.50	0.75	15.83
Estimated Bacterial FA (µg/L)***	169.59	47.75	0.39	0.47	1.15	0.86	1.18	2.51
Cellular FA content (fg FA/cell)	1.28	5.43	7.54	2.97	6.10	1.21	1.75	20.89
Cellular FA content (fg FA/μm^3)	0.05	0.17	0.21	0.33	1.32	0.09	0.20	3.47

*Assuming 65fgC/μm^3

**excluding PUFA>2 dbl bonds and DCA's

***calculated using Delaware Bay regression: FAME (µg/L) = 0.2662*Bacterial Carbon (µg C/L) + 0.3383 (Harvey et al., 2005)

R2A84 intact phospholipids

In the R2A84 culture grown on media containing peptone (mR2A-1 culture), PG and PE contributed approximately equally to the IPLs, while in the absence of peptone, the IPLs in the mR2A-2 culture consisted solely of PG (Figure 3.2). In addition to differences in the IPL distribution, R2A84 grown on the mR2A-2 broth had nearly four times more IPL per cell volume than in the mR2A-1 culture (1.14 versus 0.31 fg IPL/μm^3, Table 3.2). These results indicate that organic matter substrate greatly influences both IPL composition and content.

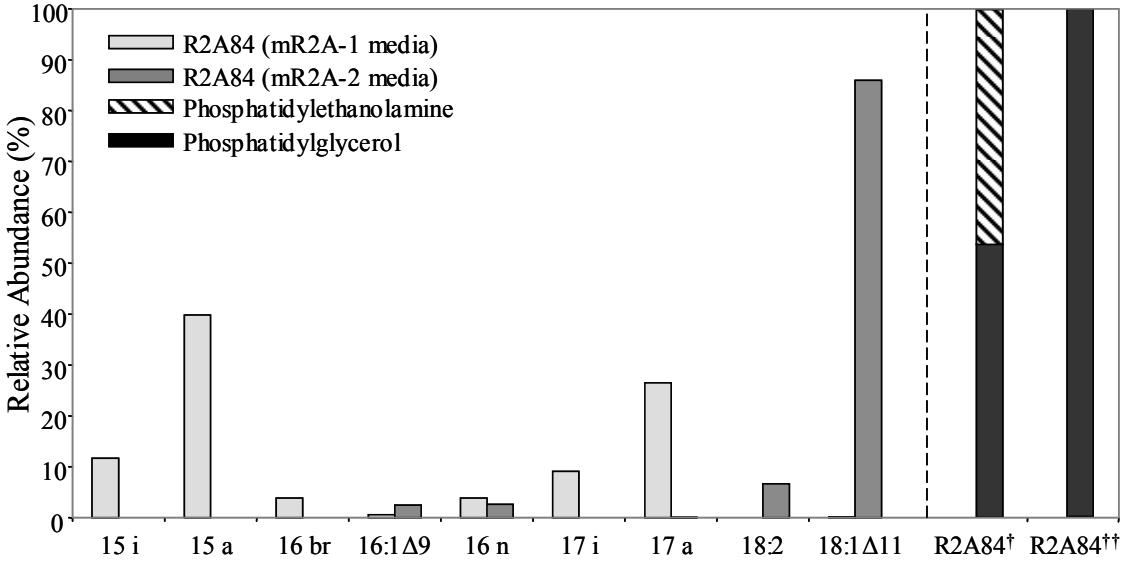

Figure 3.2. Relative abundance of total fatty acids (>1%) and intact phospholipids (IPLs) in R2A84 cultures. The position of the double bond numbered from the carboxylic end is given by Δ. The histograms represent the two cultures with the fatty acid distribution of R2A84 grown on mR2A-1 (+ peptone) and mR2A-2 (- peptone) medias. The two histogram bars on the right show the relative IPL abundance in R2A84 cultures, as calculated using TLC-FID results. † is the R2A84 culture grown on the mR2A-1 media (+ peptone) and †† denotes the R2A84 culture grown on mR2A-2 media (- peptone).

Table 3.2. Cellular content of PG, PE and total IPL in the R2A84 cultures and Arctic incubations.

Treatment	R2A84 + peptone	R2A84 - peptone	Inoculum	Control		Peat		Ice algae
				Exp 1	Exp 2	Exp 1	Exp 2	
Cell Abundance ($_{x}10^{5}$ cells mL^{-1})	2278.78	856.76	1.11	1.22	2.32	3.25	4.27	7.57
Cell volume (μm^{3})	0.04	0.03	0.03	0.11	0.18	0.09	0.10	0.17
Cellular PG content (fg PG/cell)	3.88	35.53	29.18	117.26	13.72	31.94	8.05	16.50
Cellular PG content (fg PG/μm^{3})	0.17	1.14	0.81	12.72	2.52	3.02	0.84	2.74
Cellular PE content (fg PE/cell)	3.35	nf	315.05	134.55	202.38	13.13	69.58	300.83
Cellular PE content (fg PE/μm^{3})	0.14	nf	8.69	14.60	37.23	1.24	7.23	49.96
Cellular IPL content (fg IPL/cell)	7.23	35.53	344.24	251.81	216.09	45.07	77.63	740.44
Cellular IPL content (fg IPL/μm^{3})	0.31	1.14	9.50	27.33	39.75	4.26	8.07	122.95

nf: not found

Since phospholipids are the dominant lipids in the majority of prokaryotic cell membranes (Gennis 1989), it was expected that the dominant fatty acids observed during IPL analysis would be associated with phospholipids. LC/MS intact phospholipid analysis of R2A84 grown in the presence and absence of peptone supports this, with the major fatty acids observed in fatty acid analysis for the two cultures also observed to be associated with IPLs (Table 3.3). In the culture grown on mR2A-1 broth, 17:0 and 15:0

Table 3.3. Intact phospholipid headgroups and associated fatty acid side chain combinations in R2A84 cultures grown on mR2A-1 (+ peptone) and mR2A-2 (- peptone).

	R2A84 + peptone	**R2A84 - peptone**
PG	17:0/18:1; 17:0/17:0; 17:0/16:0; 17:0/15:0; 16:0/15:0; 15:0/15:0	18:1/19:0; 18:1/18:1; 18:1/16:1; 18:1/16:0; 16:1/16:1
PE	17:0/17:0; 17:0/16:0; 17:0/15:0; 16:0/15:0; 15:0/15:0; 15:0/14:0; 14:0/14:0	nf

nf: not found

were the most commonly observed fatty acids detected by LC/MS, with the following fatty acid combinations observed in association with both PG and PE: 17:0/17:0, 17:0/16:0, 17:0/15:0, 16:0/15:0, and 15:0/15:0. There were also some differences in the fatty acids associated with each headgroup in this culture. For example, 17:0/18:1 was only observed associated with PG and only 15:0/14:0 and 14:0/14:0 were associated with PE, indicating that some specific combinations of headgroup and associated fatty acid side chain might exist, with shorter (≤ C-14) fatty acid side chains associated with PE and

longer fatty acid side chains associated with PG. Notably, in the mR2A-2 culture, 18:1 was observed in all but one of the fatty acid side chain combinations (Table 3.3).

Fatty acid analysis of Arctic microbial regrowth incubations

Based on the results of the R2A84 cultures, it was evident that organic matter played a substantial role cell membrane fatty acid and intact phospholipid compositions of this bacterium. Therefore, given that the natural organic matter substrates utilized in the Arctic bacteria regrowth experiments were of differing organic matter compositions and labilities, it was expected that an effect of organic matter substrate on both cell membrane lipid composition and bacterial community composition would also be observed. In general, measured bacterial fatty acid values were in good agreement with bacterial fatty acids estimated from conversion factors obtained from bacterial regrowth experiments in a variety of estuarine environments (Harvey et al. 2005), with differences ranging from a factor of 1.16X-2.24X between the measured and estimated values (Table 3.1). The Ice algae incubation was the exception, differing by a factor of 6.3X between observed and estimated values (Table 3.1). Cellular fatty acid content of bacteria in the Inoculum waters and the Control and Peat incubations (0.09 to 0.33 fg FA/μm^3) was in general comparable to the range observed for the R2A84 cultures (0.05 to 0.17 fg FA/μm^3), with the exception of the Control from Experiment 2 (1.32 fg FA/μm^3). The cellular fatty acid content in the Ice algae incubation was much higher than observed in the cultures or the Arctic experiments, and this is likely due to the confounding presence of ice algae substrate on the filter during sample collection. These calculations are not available for the Debris incubation, as bacterial cell counts could not be performed due to the presence of substrate on the filter.

In order to compare the fatty acid composition of bacterioplankton in different treatments, as well as the added organic substrates, relative distributions were used. Bacterial community fatty acid distributions changed from those in the Inoculum in both Controls, and the distribution in the Controls was different at the conclusion of Experiments 1 (day 11) and 2 (day 12). The bacteria in Inoculum waters were dominated by 16:0n (30.3%) and 18:0n (16.9% of the total fatty acids) (Table 3.4). At the conclusion of Experiment 1 (day 11), the Control was dominated by 16:0n (24.4%) as well as 16:1Δ9, (19%) and by the end of Experiment 2 (day 12), 16:1Δ9 comprised over 64% of the total fatty acid distribution (Table 3.4).

Table 3.5 gives the results of the fatty acid distribution of Peat Experiments 1 and 2 as well as the Peat substrate. The Peat substrate consisted mainly of 16:0n (20.9%), 22:0n (14.4%), and 24:0n (13.8%). Similar fatty acid distributions were observed in the bacterial community grown with peat and the Control in Experiment 1, with 16:0n and 16:1Δ9 the dominant fatty acids in the Peat incubation at the end of Experiment 1 (day 11), but the dominant fatty acids in the Peat incubation were reversed relative to the Control, with 16:1Δ9 (24.3%) dominating, followed by 16:0n (14.4%). Peat incubation fatty acid distribution also changed between Experiments 1 and 2, with 16:1Δ9 accounted for the majority of the relative fatty acid distribution (34.7%) followed by 18:1Δ11 (17.8%) and 16:0n (10.7%) by the end of Experiment 2 (day 12).

Table 3.4. Fatty acid concentration and relative distribution in the Inoculum water and the Control incubations.

Treatment	Inoculum		Control Exp 1		Control Exp 2	
Fatty Acid	µg/L	(%)	µg/L	(%)	µg/L	(%)
12 n	0.00	0.5	0.00	0.2	0.00	0.1
C 9 DCA	nf	nf	nf	nf	nf	nf
13 i	0.00	0.1	nf	nf	nf	nf
13 a	0.00	0.1	nf	nf	nf	nf
13 n	0.00	0.0	0.00	0.2	0.00	0.1
14 i	nf	nf	nf	nf	nf	nf
14 a	0.00	0.3	0.00	0.5	0.00	0.0
14:2	nf	nf	nf	nf	nf	nf
14:3	nf	nf	nf	nf	nf	nf
14:1	0.00	0.0	0.00	0.6	0.00	0.1
14:1	0.00	0.4	0.00	1.1	0.04	3.0
14:1	nf	nf	nf	nf	nf	nf
14 n	0.04	5.2	0.01	3.9	0.07	5.5
15:1	0.00	0.4	nf	nf	nf	nf
15 br	0.01	0.9	nf	nf	nf	nf
15:1	0.00	0.0	nf	nf	nf	nf
15 i	0.01	0.9	0.00	0.6	0.00	0.1
15 a	0.01	0.8	0.00	0.6	0.00	0.1
15:1	0.00	0.4	0.01	4.4	0.03	1.9
15:1	nf	nf	nf	nf	nf	nf
15:1	0.00	0.0	0.00	0.4	0.00	0.2
15 n	0.02	2.8	0.01	3.5	0.01	1.0
16:1	0.00	0.2	nf	nf	nf	nf
16:1	0.00	0.1	nf	nf	nf	nf
16 br	0.00	0.0	nf	nf	nf	nf
16 br	0.00	0.1	0.00	0.7	0.00	0.2
16:2	nf	nf	nf	nf	nf	nf
16:4	nf	nf	nf	nf	nf	nf
16:2*	nf	nf	nf	nf	nf	nf
16:0 br	0.00	0.4	0.00	0.4	0.00	0.0
16:2	nf	nf	0.00	0.4	nf	nf
16:1Δ7	0.04	4.4	0.01	4.2	0.01	0.4
16:1Δ9	0.06	7.3	0.04	19.1	0.86	64.6
16:2**	0.00	0.1	0.00	0.3	0.01	0.7
16:1Δ11	0.00	0.2	0.00	0.4	0.00	0.3
16 br***	nf	nf	0.00	0.2	0.00	0.0
16 n	0.26	30.3	0.05	24.4	0.08	6.1
17 br	0.00	0.1	nf	nf	nf	nf
17 br	0.00	0.1	nf	nf	nf	nf
17 br	0.00	0.3	nf	nf	nf	nf
17 i	0.00	0.2	0.00	0.2	0.00	0.0
17 a	0.00	0.4	0.00	0.5	0.00	0.2

Table 3.4. continued

17:1Δ9	0.01	0.6	0.01	4.7	0.02	1.8
17:1Δ11	nf	nf	0.00	0.6	0.00	0.2
17 n	0.01	1.6	0.00	1.8	0.01	0.5
18:3	nf	nf	nf	nf	nf	nf
18:4	nf	nf	nf	nf	nf	nf
18 br	nf	nf	nf	nf	0.00	0.1
18:2	0.00	0.4	nf	nf	0.02	1.8
18 br	0.00	0.1	0.00	0.1	0.00	0.0
18:2	0.01	1.1	0.00	1.7	0.00	0.4
18:1Δ9	0.05	5.9	0.01	3.9	0.02	1.5
18:1Δ11	0.02	2.8	0.01	4.2	0.09	6.8
18:1	nf	nf	0.00	0.1	0.00	0.0
18:2	0.00	0.1	0.00	0.1	0.00	0.0
18:1Δ13	0.00	0.0	0.00	0.1	0.00	0.0
18 n	0.14	16.9	0.02	9.1	0.02	1.6
19:1	0.00	0.1	0.00	0.3	0.00	0.2
19 br	0.00	0.1	nf	nf	nf	nf
C 16 DCA	nf	nf	nf	nf	nf	nf
20:4	nf	nf	nf	nf	nf	nf
20:5	0.00	0.3	nf	nf	nf	nf
20:3	nf	nf	nf	nf	nf	nf
20:2****	coel	coel	nf	nf	nf	nf
20 br	0.00	0.1	0.00	1.7	nf	nf
20:1Δ11	coel	coel	nf	nf	nf	nf
20:1Δ13	0.00	0.1	nf	nf	nf	nf
20 n	0.01	1.3	0.00	0.6	0.00	0.2
C 17 DCA	nf	nf	nf	nf	nf	nf
21 br	0.00	0.1	0.00	0.4	nf	nf
21:1	nf	nf	nf	nf	nf	nf
21 n	0.00	0.2	0.00	0.1	0.00	0.0
22:6	0.00	0.1	nf	nf	nf	nf
C 18 DCA	nf	nf	nf	nf	nf	nf
22:3	0.00	0.1	nf	nf	nf	nf
22:2	0.00	0.0	nf	nf	nf	nf
22 br	0.00	0.1	0.00	1.1	nf	nf
22:1	0.00	0.1	nf	nf	nf	nf
22:1	nf	nf	nf	nf	nf	nf
22 n	0.01	1.3	coel	coel	coel	coel
23 i	0.00	0.1	nf	nf	nf	nf
23 a	0.00	0.1	nf	nf	nf	nf
C 19 DCA	nf	nf	nf	nf	nf	nf
23:1	nf	nf	nf	nf	nf	nf
23 n	0.00	0.5	0.00	0.2	0.00	0.0
C 20 DCA	nf	nf	nf	nf	nf	nf
24 i	0.00	0.4	0.00	0.2	0.00	0.0
24 a	0.00	0.0	nf	nf	nf	nf
24:1	nf	nf	nf	nf	nf	nf
24:1	nf	nf	nf	nf	nf	nf

Table 3.4. continued

24:1	nf	nf	nf	nf	nf	nf
24 n	0.03	3.1	0.00	0.9	0.00	0.1
C 21 DCA	0.00	0.0	nf	nf	nf	nf
25 i	0.00	0.3	0.00	0.1	nf	nf
25 a	0.00	0.6	0.00	0.3	0.00	0.1
25 n	0.01	0.8	0.00	0.3	0.00	0.0
C 22 DCA	0.00	0.0	nf	nf	nf	nf
26 i	0.00	0.3	0.00	0.2	0.00	0.0
26:1	0.00	0.0	nf	nf	nf	nf
26 n	0.01	1.6	0.00	0.4	0.00	0.1
C 23 DCA	0.00	0.0	nf	nf	nf	nf
27 i	0.00	0.0	nf	nf	nf	nf
27 a	0.00	0.2	nf	nf	nf	nf
27 n	0.00	0.2	nf	nf	nf	nf
C-24 DCA	0.00	0.1	nf	nf	nf	nf
28 br	0.00	0.0	nf	nf	nf	nf
28 n	0.00	0.6	nf	nf	nf	nf
C-25 DCA	0.00	0.0	nf	nf	nf	nf
29 br	0.00	0.0	nf	nf	nf	nf
29 n	0.00	0.1	nf	nf	nf	nf
C 26 DCA	nf	nf	nf	nf	nf	nf
30 n	0.00	0.2	nf	nf	nf	nf
C 27 DCA	nf	nf	nf	nf	nf	nf
31 n	0.00	0.0	nf	nf	nf	nf
C 28 DCA	nf	nf	nf	nf	nf	nf
32 n	0.00	0.0	nf	nf	nf	nf
C 29 DCA	nf	nf	nf	nf	nf	nf

DCA=Dicarboxylic acid; D, no. double bond from carboxylic acid end; br, branched; n, normal; I, iso; a, anteiso; nf, not found; coel, coeluted.

* is 16:2+16:3 in Ice Algae Substrate

**is 16:1 in Peat incubations and Peat Substrate

***is 16:1 in Ice Algae Substrate

****is 20:4 in Ice Algae incubation and Ice Algae Substrate

Table 3.5. Fatty acid concentration and relative distribution in the Inoculum water the Peat incubations and the Peat substrate.

Treatment	Inoculum		Peat Exp 1		Peat Exp 2		Peat Substrate	
Fatty Acid	µg/L	(%)	µg/L	(%)	µg/L	(%)	µg/L	(%)
12 n	0.00	0.5	0.00	0.2	0.00	0.2	0.01	0.6
C 9 DCA	nf	nf	nf	nf	nf	nf	0.01	0.5
13 i	0.00	0.1	0.00	0.5	0.00	0.1	0.00	0.1
13 a	0.00	0.1	0.00	0.2	0.00	0.0	nf	nf
13 n	0.00	0.0	0.00	0.2	0.00	0.1	0.00	0.1
14 i	nf	nf	0.00	0.1	0.00	0.0	nf	nf
14 a	0.00	0.3	0.00	0.5	0.00	0.2	0.00	0.3
14:2	nf	nf	nf	nf	nf	nf	nf	nf
14:3	nf	nf	nf	nf	nf	nf	nf	nf
14:1	0.00	0.0	0.00	0.4	0.00	0.1	nf	nf
14:1	0.00	0.4	0.01	2.4	0.02	2.0	nf	nf
14:1	nf	nf	0.00	0.5	0.00	0.1	nf	nf
14 n	0.04	5.2	0.02	3.4	0.03	4.1	0.03	2.1
15:1	0.00	0.4	0.01	1.1	0.00	0.4	nf	nf
15 br	0.01	0.9	nf	nf	nf	nf	nf	nf
15:1	0.00	0.0	nf	nf	nf	nf	nf	nf
15 i	0.01	0.9	0.01	1.3	0.00	0.5	0.02	1.2
15 a	0.01	0.8	0.01	1.4	0.00	0.4	0.03	1.9
15:1	0.00	0.4	0.02	4.4	0.02	2.4	nf	nf
15:1	nf	nf	nf	nf	nf	nf	nf	nf
15:1	0.00	0.0	0.00	0.9	0.00	0.4	nf	nf
15 n	0.02	2.8	0.01	2.3	0.01	1.3	0.02	1.2
16:1	0.00	0.2	nf	nf	nf	nf	nf	nf
16:1	0.00	0.1	nf	nf	nf	nf	nf	nf
16 br	0.00	0.0	nf	nf	nf	nf	nf	nf
16 br	0.00	0.1	0.00	0.2	0.00	0.2	nf	nf
16:2	nf	nf	nf	nf	nf	nf	nf	nf
16:4	nf	nf	nf	nf	nf	nf	nf	nf
16:2*	nf	nf	nf	nf	nf	nf	nf	nf
16:0 br	0.00	0.4	0.00	0.4	0.00	0.1	0.01	0.4
16:2	nf	nf	0.00	0.2	0.00	0.1	nf	nf
16:1Δ7	0.04	4.4	0.02	4.3	0.02	2.5	0.00	0.2
16:1Δ9	0.06	7.3	0.12	24.4	0.26	34.7	0.02	1.4
16:2**	0.00	0.1	0.00	0.5	0.00	0.6	0.00	0.1
16:1Δ11	0.00	0.2	0.00	0.9	0.00	0.4	0.01	0.4
16 br***	nf	nf	0.00	0.1	0.00	0.1	nf	nf
16 n	0.26	30.3	0.07	14.4	0.08	10.7	0.30	21.0
17 br	0.00	0.1	0.00	0.5	0.00	0.1	nf	nf
17 br	0.00	0.1	0.00	0.3	0.00	0.1	nf	nf
17 br	0.00	0.3	0.00	0.2	0.00	0.1	0.00	0.3
17 i	0.00	0.2	0.00	0.1	0.00	0.1	0.00	0.3
17 a	0.00	0.4	0.00	0.4	0.00	0.3	0.01	0.4

Table 3.5. continued

17:1Δ9	0.01	0.6	0.02	3.2	0.02	2.2	0.00	0.2
17:1Δ11	nf	nf	0.00	0.7	0.00	0.2	0.00	0.3
17 n	0.01	1.6	coel	coel	0.00	0.2	0.01	0.8
18:3	nf	nf	nf	nf	nf	nf	nf	nf
18:4	nf	nf	nf	nf	nf	nf	nf	nf
18 br	nf	nf	nf	nf	0.00	0.1	nf	nf
18:2	0.00	0.4	0.00	0.5	0.03	4.5	nf	nf
18 br	0.00	0.1	0.00	0.3	0.00	0.0	nf	nf
18:2	0.01	1.1	0.00	0.8	0.00	0.5	0.01	0.9
18:1Δ9	0.05	5.9	0.01	2.5	0.02	2.6	0.04	2.9
18:1Δ11	0.02	2.8	0.03	5.8	0.13	17.8	0.03	1.8
18:1	nf	nf	0.00	0.1	0.00	0.1	nf	nf
18:2	0.00	0.1	nf	nf	nf	nf	nf	nf
18:1Δ13	0.00	0.0	nf	nf	nf	nf	nf	nf
18 n	0.14	16.9	0.03	6.5	0.03	4.0	0.06	4.2
19:1	0.00	0.1	0.00	0.2	0.00	0.3	0.00	0.3
19 br	0.00	0.1	nf	nf	nf	nf	nf	nf
C 16 DCA	nf	nf	nf	nf	nf	nf	0.02	1.5
20:4	nf	nf	nf	nf	nf	nf	nf	nf
20:5	0.00	0.3	0.00	0.3	0.00	0.3	0.00	0.2
20:3	nf	nf	nf	nf	nf	nf	nf	nf
20:2****	coel	coel	0.00	0.1	0.00	0.1	nf	nf
20 br	0.00	0.1	0.01	1.9	0.00	0.0	0.00	0.1
20:1Δ11	coel	coel	nf	nf	nf	nf	0.01	0.8
20:1Δ13	0.00	0.1	nf	nf	nf	nf	0.00	0.3
20 n	0.01	1.3	0.00	0.9	0.01	0.7	0.07	4.8
C 17 DCA	nf	nf	nf	nf	nf	nf	0.00	0.2
21 br	0.00	0.1	0.00	0.2	0.00	0.1	nf	nf
21:1	nf	nf	nf	nf	nf	nf	nf	nf
21 n	0.00	0.2	0.00	0.2	0.00	0.1	0.02	1.4
22:6	0.00	0.1	nf	nf	nf	nf	nf	nf
C 18 DCA	nf	nf	nf	nf	nf	nf	0.02	1.6
22:3	0.00	0.1	nf	nf	nf	nf	nf	nf
22:2	0.00	0.0	nf	nf	nf	nf	nf	nf
22 br	0.00	0.1	0.00	0.9	nf	nf	nf	nf
22:1	0.00	0.1	nf	nf	0.00	0.0	0.01	0.7
22:1	nf	nf	nf	nf	nf	nf	0.00	0.2
22 n	0.01	1.3	0.01	2.3	coel	coel	0.21	14.4
23 i	0.00	0.1	0.00	0.1	0.00	0.0	nf	nf
23 a	0.00	0.1	coel	coel	0.00	0.1	nf	nf
C 19 DCA	nf	nf	nf	nf	nf	nf	0.00	0.0
23:1	nf	nf	nf	nf	nf	nf	0.00	0.3
23 n	0.00	0.5	0.00	0.6	0.00	0.3	0.04	2.7
C 20 DCA	nf	nf	nf	nf	0.00	0.0	0.03	1.7
24 i	0.00	0.4	0.00	0.1	0.00	0.1	0.00	0.1
24 a	0.00	0.0	nf	nf	nf	nf	nf	nf
24:1	nf	nf	nf	nf	nf	nf	0.01	1.0
24:1	nf	nf	nf	nf	nf	nf	nf	nf

Table 3.5. continued

24:1	nf	nf	nf	nf	nf	nf	0.00	0.2
24 n	0.03	3.1	0.01	2.9	0.01	1.5	0.20	13.8
C 21 DCA	0.00	0.0	nf	nf	nf	nf	0.00	0.1
25 i	0.00	0.3	0.00	0.1	0.00	0.1	0.00	0.2
25 a	0.00	0.6	0.00	0.1	0.00	0.2	0.00	0.2
25 n	0.01	0.8	0.00	0.4	0.00	0.3	0.02	1.2
C 22 DCA	0.00	0.0	nf	nf	0.00	0.0	0.04	2.7
26 i	0.00	0.3	0.00	0.1	0.00	0.1	nf	nf
26:1	0.00	0.0	nf	nf	nf	nf	0.00	0.2
26 n	0.01	1.6	0.01	1.3	0.01	0.8	0.05	3.4
C 23 DCA	0.00	0.0	nf	nf	nf	nf	0.00	0.1
27 i	0.00	0.0	nf	nf	nf	nf	nf	nf
27 a	0.00	0.2	nf	nf	0.00	0.0	nf	nf
27 n	0.00	0.2	0.00	0.1	0.00	0.0	0.00	0.2
C-24 DCA	0.00	0.1	nf	nf	0.00	0.0	0.01	0.6
28 br	0.00	0.0	nf	nf	nf	nf	nf	nf
28 n	0.00	0.6	0.00	0.4	0.00	0.2	0.01	0.9
C-25 DCA	0.00	0.0	nf	nf	nf	nf	0.00	0.1
29 br	0.00	0.0	nf	nf	nf	nf	nf	nf
29 n	0.00	0.1	nf	nf	0.00	0.0	0.00	0.1
C 26 DCA	nf	nf	nf	nf	0.00	0.0	0.00	0.1
30 n	0.00	0.2	nf	nf	0.00	0.1	nf	nf
C 27 DCA	nf	nf	nf	nf	0.00	0.0	nf	nf
31 n	0.00	0.0	nf	nf	nf	nf	nf	nf
C 28 DCA	nf	nf	nf	nf	nf	nf	nf	nf
32 n	0.00	0.0	nf	nf	nf	nf	nf	nf
C 29 DCA	nf	nf	nf	nf	nf	nf	nf	nf

DCA=Dicarboxylic acid; D, no. double bond from carboxylic acid end; br, branched; n, normal; I, iso; a, anteiso; nf, not found; coel, coeluted.
* is 16:2+16:3 in Ice Algae Substrate
**is 16:1 in Peat incubations and Peat Substrate
***is 16:1 in Ice Algae Substrate
****is 20:4 in Ice Algae incubation and Ice Algae Substrate

Ice algae and Debris substrates themselves were also collected on the filter during collection of bacterioplankton from these incubations and thus it was not possible to easily distinguish fatty acids attributable to the bacteria versus the substrate. This was evident by the similarity of the fatty acid distribution of the Ice algae incubation and ice algae substrate as well as the Debris incubation and debris substrate (Tables 3.6 and 3.7, respectively), with the incubations and substrates dominated by the same fatty acids.

Table 3.6. Fatty acid concentration and relative distribution in the Inoculum water, the Ice algae incubation and the Ice algae substrate.

Treatment	Inoculum		Ice Algae		Ice Algae Substrate	
Fatty Acid	µg/L	(%)	µg/L	(%)	µg/L	(%)
12 n	0.00	0.5	0.01	0.1	3.74	1.2
C 9 DCA	nf	nf	nf	nf	nf	nf
13 i	0.00	0.1	0.02	0.1	nf	nf
13 a	0.00	0.1	nf	nf	nf	nf
13 n	0.00	0.0	0.01	0.0	0.30	0.1
14 i	nf	nf	nf	nf	nf	nf
14 a	0.00	0.3	0.02	0.1	nf	nf
14:2	nf	nf	0.00	0.0	nf	nf
14:3	nf	nf	0.00	0.0	nf	nf
14:1	0.00	0.0	0.01	0.1	nf	nf
14:1	0.00	0.4	0.39	2.4	nf	nf
14:1	nf	nf	0.01	0.0	nf	nf
14 n	0.04	5.2	2.02	12.2	44.01	13.6
15:1	0.00	0.4	0.00	0.0	nf	nf
15 br	0.01	0.9	0.03	0.2	nf	nf
15:1	0.00	0.0	0.00	0.0	nf	nf
15 i	0.01	0.9	0.06	0.4	nf	nf
15 a	0.01	0.8	0.04	0.2	nf	nf
15:1	0.00	0.4	0.32	1.9	nf	nf
15:1	nf	nf	0.01	0.1	nf	nf
15:1	0.00	0.0	0.02	0.1	nf	nf
15 n	0.02	2.8	0.27	1.6	0.85	0.3
16:1	0.00	0.2	nf	nf	nf	nf
16:1	0.00	0.1	nf	nf	nf	nf
16 br	0.00	0.0	nf	nf	nf	nf
16 br	0.00	0.1	nf	nf	nf	nf
16:2	nf	nf	0.01	0.0	nf	nf
16:4	nf	nf	0.04	0.3	14.13	4.4
16:2*	nf	nf	0.01	0.0	4.98	1.5
16:0 br	0.00	0.4	nf	nf	nf	nf
16:2	nf	nf	nf	nf	nf	nf
16:1Δ7	0.04	4.4	0.19	1.2	nf	nf
16:1Δ9	0.06	7.3	5.20	31.5	84.17	26.1
16:2**	0.00	0.1	0.17	1.0	9.35	2.9
16:1Δ11	0.00	0.2	0.05	0.3	3.85	1.2
16 br***	nf	nf	0.20	1.2	6.38	2.0
16 n	0.26	30.3	4.60	27.8	52.87	16.4
17 br	0.00	0.1	nf	nf	nf	nf
17 br	0.00	0.1	nf	nf	nf	nf
17 br	0.00	0.3	nf	nf	nf	nf
17 i	0.00	0.2	0.00	0.0	nf	nf
17 a	0.00	0.4	0.00	0.0	nf	nf

Table 3.6. continued

17:1Δ9	0.01	0.6	0.18	1.1	nf	nf
17:1Δ11	nf	nf	0.02	0.1	nf	nf
17 n	0.01	1.6	0.10	0.6	0.47	0.1
18:3	nf	nf	0.01	0.1	0.89	0.3
18:4	nf	nf	0.03	0.2	3.17	1.0
18 br	nf	nf	nf	nf	nf	nf
18:2	0.00	0.4	nf	nf	nf	nf
18 br	0.00	0.1	nf	nf	nf	nf
18:2	0.01	1.1	0.07	0.4	2.98	0.9
18:1Δ9	0.05	5.9	0.28	1.7	4.78	1.5
18:1Δ11	0.02	2.8	0.30	1.8	0.58	0.2
18:1	nf	nf	nf	nf	nf	nf
18:2	0.00	0.1	0.02	0.1	0.25	0.1
18:1Δ13	0.00	0.0	0.02	0.1	0.27	0.1
18 n	0.14	16.9	0.58	3.5	23.28	7.2
19:1	0.00	0.1	0.01	0.0	nf	nf
19 br	0.00	0.1	nf	nf	nf	nf
C 16 DCA	nf	nf	nf	nf	nf	nf
20:4	nf	nf	0.01	0.1	0.73	0.2
20:5	0.00	0.3	0.54	3.3	52.82	16.4
20:3	nf	nf	0.01	0.0	1.01	0.3
20:2****	coel	coel	0.02	0.2	4.23	1.3
20 br	0.00	0.1	nf	nf	nf	nf
20:1Δ11	coel	coel	0.02	0.1	nf	nf
20:1Δ13	0.00	0.1	nf	nf	nf	nf
20 n	0.01	1.3	0.04	0.3	0.59	0.2
C 17 DCA	nf	nf	nf	nf	nf	nf
21 br	0.00	0.1	nf	nf	nf	nf
21:1	nf	nf	coel	coel	nf	nf
21 n	0.00	0.2	0.00	0.0	nf	nf
22:6	0.00	0.1	0.04	0.3	2.14	0.7
C 18 DCA	nf	nf	nf	nf	nf	nf
22:3	0.00	0.1	nf	nf	nf	nf
22:2	0.00	0.0	nf	nf	nf	nf
22 br	0.00	0.1	nf	nf	nf	nf
22:1	0.00	0.1	0.20	1.2	nf	nf
22:1	nf	nf	0.01	0.0	nf	nf
22 n	0.01	1.3	0.04	0.2	nf	nf
23 i	0.00	0.1	nf	nf	nf	nf
23 a	0.00	0.1	nf	nf	nf	nf
C 19 DCA	nf	nf	nf	nf	nf	nf
23:1	nf	nf	0.01	0.0	nf	nf
23 n	0.00	0.5	0.00	0.0	nf	nf
C 20 DCA	nf	nf	nf	nf	nf	nf
24 i	0.00	0.4	0.00	0.0	nf	nf
24 a	0.00	0.0	nf	nf	nf	nf
24:1	nf	nf	0.07	0.4	nf	nf
24:1	nf	nf	nf	nf	nf	nf

Table 3.6. continued

24:1	nf	nf	0.15	0.9	nf	nf
24 n	0.03	3.1	0.03	0.2	nf	nf
C 21 DCA	0.00	0.0	nf	nf	nf	nf
25 i	0.00	0.3	nf	nf	nf	nf
25 a	0.00	0.6	0.00	0.0	nf	nf
25 n	0.01	0.8	0.00	0.0	nf	nf
C 22 DCA	0.00	0.0	nf	nf	nf	nf
26 i	0.00	0.3	0.00	0.0	nf	nf
26:1	0.00	0.0	nf	nf	nf	nf
26 n	0.01	1.6	0.01	0.0	nf	nf
C 23 DCA	0.00	0.0	nf	nf	nf	nf
27 i	0.00	0.0	nf	nf	nf	nf
27 a	0.00	0.2	nf	nf	nf	nf
27 n	0.00	0.2	nf	nf	nf	nf
C-24 DCA	0.00	0.1	nf	nf	nf	nf
28 br	0.00	0.0	nf	nf	nf	nf
28 n	0.00	0.6	0.00	0.0	nf	nf
C-25 DCA	0.00	0.0	nf	nf	nf	nf
29 br	0.00	0.0	nf	nf	nf	nf
29 n	0.00	0.1	nf	nf	nf	nf
C 26 DCA	nf	nf	nf	nf	nf	nf
30 n	0.00	0.2	nf	nf	nf	nf
C 27 DCA	nf	nf	nf	nf	nf	nf
31 n	0.00	0.0	nf	nf	nf	nf
C 28 DCA	nf	nf	nf	nf	nf	nf
32 n	0.00	0.0	nf	nf	nf	nf
C 29 DCA	nf	nf	nf	nf	nf	nf

DCA=Dicarboxylic acid; D, no. double bond from carboxylic acid end; br, branched; n, normal; I, iso; a, anteiso; nf, not found; coel, coeluted.

* is 16:2+16:3 in Ice Algae Substrate

**is 16:1 in Peat incubations and Peat Substrate

***is 16:1 in Ice Algae Substrate

****is 20:4 in Ice Algae incubation and Ice Algae Substrate

Table 3.7. Fatty acid concentration and relative distribution in the Inoculum water, the Debris incubation and the Debris substrate.

Treatment	Inoculum		Debris		Debris Substrate	
Fatty Acid	µg/L	(%)	µg/L	(%)	µg/mg OC	(%)
12 n	0.00	0.5	0.14	0.1	nf	nf
C 9 DCA	nf	nf	nf	nf	nf	nf
13 i	0.00	0.1	0.06	0.1	nf	nf
13 a	0.00	0.1	0.02	0.0	nf	nf
13 n	0.00	0.0	0.11	0.1	nf	nf
14 i	nf	nf	nf	nf	nf	nf
14 a	0.00	0.3	0.09	0.1	nf	nf
14:2	nf	nf	nf	nf	nf	nf
14:3	nf	nf	nf	nf	nf	nf
14:1	0.00	0.0	0.02	0.0	nf	nf
14:1	0.00	0.4	0.47	0.4	nf	nf
14:1	nf	nf	0.03	0.0	nf	nf
14 n	0.04	5.2	9.92	8.8	3.52	13.0
15:1	0.00	0.4	0.13	0.1	nf	nf
15 br	0.01	0.9	nf	nf	nf	nf
15:1	0.00	0.0	nf	nf	nf	nf
15 i	0.01	0.9	0.38	0.3	nf	nf
15 a	0.01	0.8	0.41	0.4	nf	nf
15:1	0.00	0.4	0.31	0.3	nf	nf
15:1	nf	nf	0.02	0.0	nf	nf
15:1	0.00	0.0	0.08	0.1	nf	nf
15 n	0.02	2.8	1.69	1.5	0.39	1.5
16:1	0.00	0.2	nf	nf	nf	nf
16:1	0.00	0.1	nf	nf	nf	nf
16 br	0.00	0.0	nf	nf	nf	nf
16 br	0.00	0.1	nf	nf	nf	nf
16:2	nf	nf	nf	nf	nf	nf
16:4	nf	nf	nf	nf	nf	nf
16:2*	nf	nf	nf	nf	nf	nf
16:0 br	0.00	0.4	nf	nf	nf	nf
16:2	nf	nf	nf	nf	nf	nf
16:1Δ7	0.04	4.4	0.28	0.2	nf	nf
16:1Δ9	0.06	7.3	16.16	14.4	7.40	27.4
16:2**	0.00	0.1	0.65	0.6	nf	nf
16:1Δ11	0.00	0.2	0.14	0.1	0.07	0.3
16 br***	nf	nf	nf	nf	nf	nf
16 n	0.26	30.3	60.60	54.0	13.70	50.7
17 br	0.00	0.1	nf	nf	nf	nf
17 br	0.00	0.1	nf	nf	nf	nf
17 br	0.00	0.3	nf	nf	nf	nf
17 i	0.00	0.2	0.21	0.2	0.14	0.5
17 a	0.00	0.4	0.08	0.1	0.01	0.0

Table 3.7. continued

17:1Δ9	0.01	0.6	0.25	0.2	0.02	0.1
17:1Δ11	nf	nf	0.07	0.1	0.01	0.0
17 n	0.01	1.6	0.33	0.3	0.05	0.2
18:3	nf	nf	nf	nf	nf	nf
18:4	nf	nf	nf	nf	nf	nf
18 br	nf	nf	nf	nf	nf	nf
18:2	0.00	0.4	nf	nf	nf	nf
18 br	0.00	0.1	nf	nf	nf	nf
18:2	0.01	1.1	0.03	0.0	nf	nf
18:1Δ9	0.05	5.9	2.40	2.1	0.33	1.2
18:1Δ11	0.02	2.8	0.69	0.6	0.19	0.7
18:1	nf	nf	nf	nf	nf	nf
18:2	0.00	0.1	0.07	0.1	0.08	0.3
18:1Δ13	0.00	0.0	0.04	0.0	nf	nf
18 n	0.14	16.9	2.08	1.9	0.32	1.2
19:1	0.00	0.1	0.04	0.0	nf	nf
19 br	0.00	0.1	nf	nf	nf	nf
C 16 DCA	nf	nf	0.02	0.0	nf	nf
20:4	nf	nf	nf	nf	nf	nf
20:5	0.00	0.3	0.29	0.3	0.13	0.5
20:3	nf	nf	nf	nf	nf	nf
20:2****	coel	coel	nf	nf	nf	nf
20 br	0.00	0.1	nf	nf	nf	nf
20:1Δ11	coel	coel	0.12	0.1	0.03	0.1
20:1Δ13	0.00	0.1	0.03	0.0	nf	nf
20 n	0.01	1.3	0.92	0.8	0.01	0.0
C 17 DCA	nf	nf	nf	nf	nf	nf
21 br	0.00	0.1	nf	nf	nf	nf
21:1	nf	nf	nf	nf	nf	nf
21 n	0.00	0.2	0.41	0.4	0.04	0.1
22:6	0.00	0.1	0.17	0.2	0.00	0.0
C 18 DCA	nf	nf	0.03	0.0	nf	nf
22:3	0.00	0.1	nf	nf	nf	nf
22:2	0.00	0.0	nf	nf	nf	nf
22 br	0.00	0.1	nf	nf	nf	nf
22:1	0.00	0.1	0.08	0.1	nf	nf
22:1	nf	nf	nf	nf	nf	nf
22 n	0.01	1.3	1.68	1.5	0.11	0.4
23 i	0.00	0.1	nf	nf	nf	nf
23 a	0.00	0.1	nf	nf	nf	nf
C 19 DCA	nf	nf	nf	nf	nf	nf
23:1	nf	nf	nf	nf	nf	nf
23 n	0.00	0.5	0.84	0.8	0.06	0.2
C 20 DCA	nf	nf	0.04	0.0	0.00	0.0
24 i	0.00	0.4	0.01	0.0	nf	nf
24 a	0.00	0.0	nf	nf	nf	nf
24:1	nf	nf	0.04	0.0	nf	nf
24:1	nf	nf	0.06	0.1	nf	nf

Table 3.7. continued

24:1	nf	nf	0.06	0.1	nf	nf
24 n	0.03	3.1	2.98	2.7	0.15	0.5
C 21 DCA	0.00	0.0	0.02	0.0	0.00	0.0
25 i	0.00	0.3	coel	coel	nf	nf
25 a	0.00	0.6	coel	coel	nf	nf
25 n	0.01	0.8	0.78	0.7	0.05	0.2
C 22 DCA	0.00	0.0	0.09	0.1	0.01	0.0
26 i	0.00	0.3	nf	nf	nf	nf
26:1	0.00	0.0	0.05	0.0	nf	nf
26 n	0.01	1.6	2.21	2.0	0.08	0.3
C 23 DCA	0.00	0.0	0.04	0.0	0.00	0.0
27 i	0.00	0.0	nf	nf	nf	nf
27 a	0.00	0.2	nf	nf	nf	nf
27 n	0.00	0.2	0.54	0.5	0.02	0.1
C-24 DCA	0.00	0.1	0.09	0.1	0.01	0.0
28 br	0.00	0.0	nf	nf	nf	nf
28 n	0.00	0.6	1.45	1.3	0.04	0.1
C-25 DCA	0.00	0.0	0.05	0.0	0.00	0.0
29 br	0.00	0.0	nf	nf	nf	nf
29 n	0.00	0.1	0.23	0.2	0.01	0.0
C 26 DCA	nf	nf	0.12	0.1	0.00	0.0
30 n	0.00	0.2	0.36	0.3	0.01	0.0
C 27 DCA	nf	nf	0.09	0.1	0.01	0.0
31 n	0.00	0.0	0.05	0.0	nf	nf
C 28 DCA	nf	nf	0.09	0.1	nf	nf
32 n	0.00	0.0	0.06	0.1	nf	nf
C 29 DCA	nf	nf	0.02	0.0	nf	nf

DCA=Dicarboxylic acid; D, no. double bond from carboxylic acid end; br, branched; n, normal; I, iso; a, anteiso; nf, not found; coel, coeluted.
* is 16:2+16:3 in Ice Algae Substrate
**is 16:1 in Peat incubations and Peat Substrate
***is 16:1 in Ice Algae Substrate
****is 20:4 in Ice Algae incubation and Ice Algae Substrate

To isolate the bacterioplankton fatty acid distribution, visual comparisons between the fatty acid distributions of the Control and Peat incubations, which were not affected by the presence of substrate and were therefore assigned a bacterioplankton source; and the fatty acid distributions of the Ice algae and Debris incubations and substrates were made. The results of these comparisons are shown in Table 3.8. The dominant fatty acids (14:0n, 16:0n, 16:1Δ9) observed in the Ice algae and Debris incubations were found in

both the Control and Peat incubations, as well as in the organic matter substrates and were therefore deemed mixed (M) in origin. However, we were able to assign several fatty acids to bacterioplankton, including 14:1, 15:0i, 15:0a, 15:1, 16:1Δ7 and 17:1Δ9, since these fatty acids were present in the incubations but were not observed in the substrates.

Table 3.8. Fatty acid origin (those >1% of the total) determined via visual comparison of Control and Peat incubations, identified as bacterial fatty acids and Algae, Peat and Debris substrates.

Origin				
B	**M**	**A**	**T**	**M-2**
14:1	14 n	16:4	C16 DCA	20 n
15:1	15 n	20:5	21 n	22:1
15 i	16:1Δ9		C18 DCA	24 n
15 a	16:2*		22 n	
15:1	16 br		23 n	
16:1Δ7	16 n		C20 DCA	
17:1Δ9	17 n		24:1	
18:2	18:2		25 n	
20 br	18:1Δ9		C22 DCA	
22 br	18:1Δ11		26 n	
	18 n		28 n	

A=algal, B=bacterial, M=mixed (bacterial+algal),
M-2=mixed (bacterial+terrestrial), T=terrestrial
* is 16:1 in Peat incubation

Intact phospholipids in Arctic microbial regrowth incubations

With the exception of the Peat and Control incubations of Experiment 1, PE was the dominant IPL observed, making up 89 to 94.8% of the total IPLs (Figure 3.3). The Peat incubation from Experiment 1 was dominated by PG (70.9%) while the Control

incubation from Experiment 1 was comprised of nearly equal amounts of PG and PE (46.6% and 53.4%, respectively) (Figure 3.3). Total cellular IPL contents are shown in

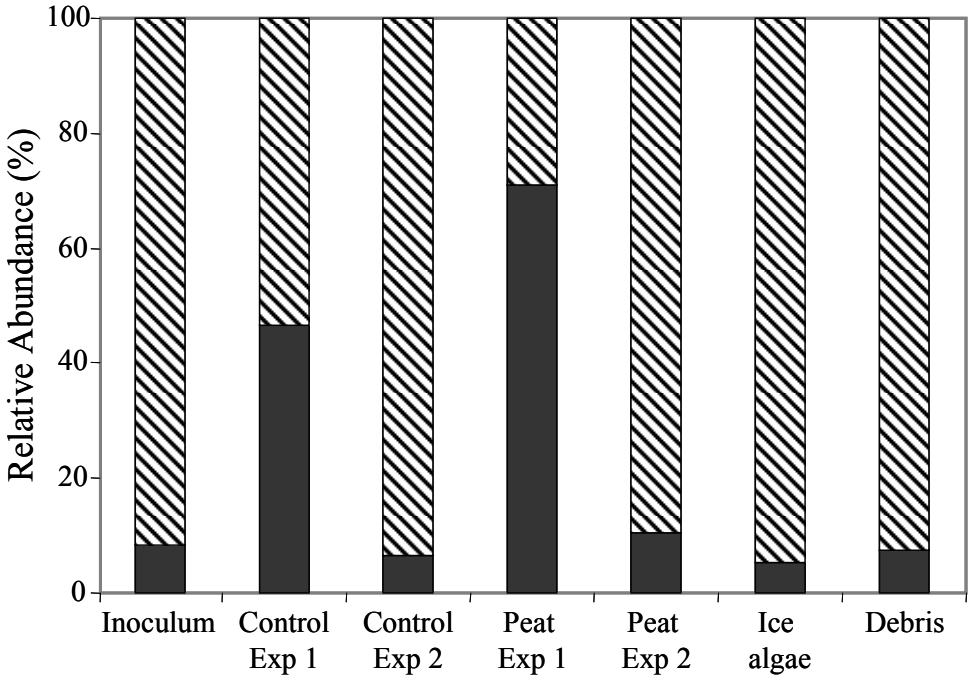

Figure 3.3. Relative abundance of PG (black) and PE (hatched bars) in the Arctic incubations; calculated using TLC-FID results.

Table 3.2; there was a nearly 30-fold variation in these values, from 4.26 fg IPL/μm^3 in the Peat Experiment 1 to 122.95 fg IPL/μm^3 in the Ice algae incubation. All of the cellular IPL values in the Arctic experiments were higher than those observed in the R2A84 cultures. In general, fatty acids observed in total fatty acid analysis were also seen during IPL analysis, with 18:1, 16:0 and 16:1 fatty acids commonly present (Table 3.9). One exception to this trend was the Inoculum water where 18:0n was not associated with either PE or PG, yet it was one of the dominant fatty acids. No major shifts in either headgroup or fatty acid side chains were detected upon the addition of the various organic matter substrates. Finally, based on these results and the results of the R2A84 cultures

Table 3.9. Intact phospholipid headgroups and associated fatty acid side chain combinations in Arctic incubations.

	Inoculum	Control		Peat		Ice algae	Debris
		Exp 1	Exp 2	Exp 1	Exp 2		
PG	18:1/18:1; 18:1/16:1; 16:0/16:1; 16:1/16:1	16:0/16:1; 16:1/16:1	18:1/18:1; 18:1/18:2; 18:1/16:1; 16:0/16:1; 16:1/16:1	18:1/16:1; 17:1/16:1; 16:0/16:1; 16:1/16:1	18:1/18:1; 18:1/18:2; 18:1/16:1; 16:0/16:1; 16:1/16:1	18:1/16:1; 16:0/16:1; 16:1/16:1	18:1/18:1; 18:1/16:1; 16:0/16:1; 16:1/16:1
PE	18:1/16:1; 16:0/16:1; 16:1/16:1; 16:1/14:0; 16:1/14:1	18:1/16:1; 17:1/16:1; 16:0/16:1; 16:1/16:1; 16:1/15:1; 16:1/14:0; 15:1/14:0	16:0/16:1; 16:1/16:1; 16:1/14:0; 16:1/14:1	18:1/16:1; 17:1/16:1; 16:0/16:1; 16:1/16:1; 16:1/15:0; 16:1/15:1; 16:1/14:0; 16:1/14:1	16:1/16:1; 16:1/14:0; 16:1/14:1	16:0/16:1; 16:1/16:1; 16:0/14:1; 16:1/14:1; 15:0/14:1; 14:0/14:1	16:0/16:0; 16:0/16:1; 16:1/16:1; 16:1/15:1; 16:0/14:1; 16:1/14:1; 15:0/14:1

grown on mR2A-1 broth, it appears that fatty acid side chains of 14 or less carbons in length are preferentially associated with PE.

Discussion

Dissolved organic matter quality and composition is one of the many factors known to influence bacterial community composition in aquatic systems (Eiler et al. 2003; Kirchman et al. 2004). Conversely, bacteria contribute to the carbon pool in oceanic systems via the release of cellular components (Wakeham et al. 2003; Zou et al. 2004) and transform the DOM pool via preferential uptake of labile DOM, resulting in DOM that is more homogeneous and refractory (Amon et al. 2001; Ogawa et al. 2001). The results from the R2A84 cultures suggest that the cellular fatty acid and intact phospholipid composition is highly dependent upon organic matter source when bacteria are grown on pure substrates. A dramatic difference was observed with 15 and 17-chain fatty acids dominating when peptone was included in the media and almost exclusively 18:1Δ11 when peptone was removed. The fatty acid distribution of R2A84 grown on the media containing peptone was similar to the trends observed in Harvey et al. for temperate estuaries, where the cellular lipid composition of bacterial communities grown on protein (bovine serum albumin aka BSA) consisted mainly of 15- and 17-carbon chain fatty acids regardless of the microbial community composition (Harvey et al. 2005). In bacteria, the synthesis of odd- and branched-chain fatty acids is rather unique with branched-chain compounds such as methylbutyrate acting as primers. These compounds are often formed via the deamination and oxidative decarboxylation of the amino acids leucine, valine and isoleucine (Fulco 1983). Yet utilization of these primers alone for synthesis of branched and odd fatty acid does not explain the observed differences in the

R2A84 cultures since both media recipes included casamino acids which contain higher amounts of leucine, isoleucine and valine amino acids than peptone (see Becton, Dickinson and Company analysis: http://www.bd.com/ds/technicalCenter/typicalAnalysis/typ-casamino_acids.pdf and http://www.bd.com/ds/technicalCenter/typicalAnalysis/typ-bacto_peptone.pdf). Furthermore, we ruled out direct uptake of the media substrates, as fatty acid analysis conducted on individual media components revealed that peptone and casamino acids were dominated by 16 and 18-carbon chain fatty acids (data not shown). The results shown here and those from Harvey and coworkers suggest that environmentally significant bacteria may be able to quickly and preferentially utilize a variety of primers present in protein substrates for synthesis of odd- and branched-chain fatty acids (Harvey et al. 2005).

Compared to the bacterioplankton community analysis (Chapter 2), the results of this study suggest that phylogenetically diverse bacterial communities overlap in their fatty acid distributions and this has previously been observed (Harvey et al. 2005). For example, although the Control and Peat incubations from Experiment 1 showed the greatest similarity in fatty acid relative distribution, the bacterial communities differed in these incubations. Furthermore, in the Peat Experiment 2 incubation, where the largest shift in community composition occurred, while there was an increase in the relative abundance of 18:1Δ11, 16:1Δ9 remained as the dominant fatty acid despite the dramatic change in community composition. On the other hand, there was an observed increase in 16:1Δ9 in the Control Experiment 2 incubation relative to the Control in Experiment 1. The bacterial community did shift between these two incubations with a decrease in the

relative peak height of 350 and 352 base pairs (bp) and the appearance of a fragment sized 351 bp. However, attributing fatty acids to particular bacteria based on LH-PCR results is difficult as the aforementioned fragments could represent different phylogenetic groups in marine plankton, including the *Gamma*- and *Beta*- subdivisions of the *Proteobacteria* and the *Bacteroidetes* phylum (Suzuki et al. 1998). The comparison between bacterial phylogeny and cell lipid distribution will be further explored in Chapter 4 of this thesis.

Generally, bacterial presence has been inferred through the presence of 18:1Δ11 as well as iso and anteiso forms of C15 and C17, as branched- and odd-chain fatty acids are synthesized only by *Bacteria* (Gillan and Hogg 1984; Guckert et al. 1985; Perry et al. 1979; Smith et al. 1986; Volkman et al. 1980). However, branched- and odd-chain fatty acids were not major contributors to the fatty acid distribution in the Control and Peat incubations and this is in agreement with the results of Harvey and Macko who found a lack of correlation between bacterial biomass and branched and odd fatty acid concentration (Harvey and Macko 1997). Overall, the trends in fatty acid distributions observed are similar to the results reported by Harvey and coworkers, where 16:1Δ9, 16:0n and 18:1Δ11 were the dominant fatty acids in bacterial regrowth experiments in the no addition controls and incubations supplemented with >1kDa (ultrafiltered DOM >1kDa) in a variety of temperate estuarine environments (Harvey et al. 2005). In aquatic environments, 16:1Δ9, 16:0, 14:0 as well as 20:5 have been shown to be associated with diatoms (Gillan et al. 1981; Nichols et al. 1986b; Viso and Marty 1993) and the observance of these fatty acids in the bacterial regrowth experiments indicates that multiple sources for these fatty acids occur in natural environments. Therefore, the

contribution of bacteria to the organic matter pool in aquatic systems may be underestimated by using only odd and branched fatty acids as indicators of bacteria.

Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) are two of the most commonly observed phospholipids in bacterial cell membranes (Fang et al. 2000b; Lechevalier and Lechevalier 1988) and these were the only phospholipids observed in the R2A84 cultures as well as in the Arctic incubations. Compared to fatty acid analysis alone, detailed structural information available for headgroups and fatty acid side chains offers a greater possibility to the discovery of correlations between specific microbial lipids with *in-situ* microbial communities (Fang and Barcelona 1998; Rütters et al. 2002b), yet no distinct trends in either IPL headgroup and/or fatty acid side chains were detected among the Arctic incubations in association with phylogenetic changes in community composition. However, the R2A84 culture work showed that IPL composition was greatly affected by organic matter substrate. Several mechanisms might explain changes in microbial phospholipids as alterations in both phospholipid headgroup and fatty acid side chains have been observed in cultured bacteria when exposed to differing culture conditions such as temperature and media components (Russell 1988) as well as the addition of various substrates and solvents (Johnston and Goldfine 1992; Macdonald and Goldfine 1991; Ramos et al. 2002; Rose 1988). Changes in fatty acid chains and phospholipid headgroups are often attributed to maintenance of membrane fluidity (Cronan 2002; Rilfors and Lindblom 2002; Russell 1988), as different headgroup and associated side chains exhibit differential packing characteristics in the cell membrane due to their geometry and headgroup size and charge (Gennis 1989). In the R2A84 culture grown without peptone, where 18:1Δ11 comprised over 85% of the

relative distribution of fatty acids, only PG was detected and the shift in phospholipid headgroup distribution and fatty acid side chains observed in this culture were replicated in a different R2A84 culture. Therefore, growth phase and environmental culture conditions such as temperature were not likely the cause of the observed fatty acid distribution. The association of unsaturated fatty acids with PG has been observed before, in a previous study where in some cultured bacteria that produce poly-unsaturated fatty acids (PUFAs), 20:5Δ17 and monounsaturated fatty acids (MUFAs) were mainly found associated with PG, while branched fatty acids were mainly associated with PE (Nichols et al. 1997). This association of unsaturated fatty acids with PG was attributed to a modification in order to maintain a lamellar lipid phase (Nichols et al. 1997), which is the preferred cell membrane state (Gennis 1989). It remains unclear why only PG was synthesized in the mR2A-2 culture, although it is apparent that organic matter substrate played a significant role in both IPL and fatty acid compositions.

Several parameters are known to influence main phase transition (i.e. gel to liquid-crystalline phase transition) temperatures, including the location of the double bond(s) as well as branching points, acyl chain length, chain length difference between the *sn*-1 and *sn*-2 acyl chains and headgroup structures (Huang and Li 1999). For example, relative to saturated acyl chains, a decreased main phase transition temperature occurs with both the inclusion of either a single or multiple *cis*-double bonds in the *sn*-2 acyl chain especially when the double bond(s) occur near the center of the chain (Huang and Li 1999). Furthermore, mixtures of lipids result in a broadening of the temperature range over which the phase transition occurs (Gennis 1989; Russell 1988). At low temperatures, cell membrane fluidity is maintained largely through modifications of the

fatty acid side chains, via branching, increased level of unsaturation, or decreased acyl chain length in response to low temperatures, with these modifications resulting in a lowered liquid-crystalline to gel phase transition temperature (Russell 1988). *E. coli* has been shown to modify cell membrane lipid composition mainly via changes in the degree of unsaturation in the fatty acid side chains when exposed to decreased temperature and this response was attributed to maintenance of a lamellar liquid crystalline cell membrane phase (Rilfors and Lindblom 2002). Based on published phase transition temperatures for PE, a gel to liquid crystalline transition temperature of -2.4°C was observed for C(9):C(10)PE where C(X):C(Y) represent the fatty acids in the sn-1 and sn-2 positions, respectively (Huang and Li 1999). With increasing length of acyl chains, the transition temperature increases, with a calculated value of 74.4°C for C(18):C(18)PE (Huang and Li 1999). Therefore, it would be expected that in order to maintain a liquid crystalline phase, short chain and/or highly unsaturated fatty acids would be observed in the cell membrane lipids of Arctic bacterioplankton growing at -1°C. However, such fatty acids were not observed in these samples, either during FAME or IPL analyses, indicating that cell membrane fluidity for these bacteria is probably affected either by factors such as the lipid/protein ratio as well as the presence of glycolipids, sterols and/or hopanoids in the cell membrane (Russell 1988).

Interpretation of the fatty acid and head group associations in the Arctic incubations is difficult since the lipids observed are derived from a community of bacteria. In general, fatty acids 16, 17 and 18 carbons in length were found to be associated with PG, while greater diversity in fatty acid distribution (C-14 to C-18) was observed in the fatty acids associated with PE (Table 3.9), indicating the potential for

some specificity of phospholipid headgroup and fatty acid side chains. However, compared to the R2A84 culture grown without peptone where a monounsaturated fatty acid dominated (18:1Δ11, 86%) and only PG was observed, the Control incubation from Experiment 2 had the highest monounsaturated concentration (16:1Δ9, 65%, with total monounsaturates making up 80%) among all of the Arctic regrowth incubations, yet PE was the dominant phospholipid (93.6%), thereby complicating interpretation of headgroup and fatty acid side chain associations. Such discrepancies between the lipid results of the cultures and the Arctic incubations highlights the difficulty in extrapolating the response of cultured organisms to natural bacterial communities.

In the Ice algae and Debris incubations, interpretation of the fatty acid analysis was problematic since it was not possible to separate fatty acids in substrate from those in the bacterioplankton. Since IPLs hydrolyze rapidly after cell death (Harvey et al. 1986; White et al. 1979), these compounds were employed to identify fatty acids associated with living bacterial biomass in the Algae and Debris incubations. Some fatty acids observed during FAME analysis were not detected in IPL analysis. For example, 18:0n accounted for 17% of the relative fatty acid distribution in the Inoculum water, yet not found to be associated with either PG or PE. This may be due to several reasons, one of which is the fact that fatty acids observed in our FAME analysis are not exclusively derived from phospholipids and could be associated with other compounds such as glycerides, glycolipids and free-fatty acids (Ratledge and Wilkinson 1988; Rütters et al. 2002b). Another reason for the disparities between fatty acids detected during FAME and IPL analysis may be that specific IPLs and associated fatty acids were present below the LC/MS instrument detection limits.

Conclusions

These results show that when grown on natural organic matter substrates, bacterial fatty acids and intact phospholipids of Arctic bacterioplankton tend to overlap. Culture work with a member of the *Roseobacter* clade shows that both the fatty acid and IPL composition change dramatically when specific primers are available for lipid synthesis. These results suggest:

1. Organic matter can play a significant role in bacterial cell membrane lipid composition, as seen with the R2A84 cultures where 15 and 17-chain carbon fatty acids dominated when peptone was included in the media. Upon the exclusion of peptone, 18:1Δ11 comprised over 85% of the total fatty acid distribution. In addition, consistent trends in fatty acid distribution among the Control and Peat incubations suggest that bacterial lipid synthesis overlaps among phylogenetically distinct groups.
2. The dominant fatty acids of bacteria growing on natural organic matter include 16:1Δ9, 16:0n, 18:1Δ11 and 18:0n. The exclusive use of odd and branched fatty acids may underestimate bacterial presence and/or biomass in natural systems.
3. IPL analysis allows the discrimination of fatty acids attributable to living biomass, as in the case of the Ice algae and Debris incubations, where substrate obscured fatty acid analysis.

Chapter 4: The phylogenetic response and cell membrane lipid composition of *in-situ* Arctic microbial communities to organic matter substrates

Synopsis

Bacteria play a role in controlling the character of organic matter and in turn organic matter quantity and composition structures aquatic microbial communities. This study employed bacterial regrowth experiments to examine the response of *in-situ* Arctic bacterial communities to native organic matter substrates, including peat, ice algae and ice-rafted debris. The phylogenetic response of bacteria was monitored using length heterogeneity polymerase chain reaction (LH-PCR) with a clone library constructed in order to further resolve community composition. Cell membrane fatty acids (FA) and intact phospholipid (IPL) analyses were conducted to explore the effect of organic matter substrate on lipid composition as well as to examine the relationship between phylogeny and cell membrane lipids. The bacterial community of starting (Inoculum) waters consisted of rRNA genes putatively identified as *Alphaproteobacteria* and *Gammaproteobacteria*. The community of Control and substrate-amended incubations were dominated by fragments corresponding to *Gammaproteobacteria* or *Bacteroidetes*, with the Control dominated by longer LH-PCR fragments (352 base pairs or bp) than the substrate-amended incubations (347 bp). In a first regrowth of all incubations (11 days, Experiment 1), the *Alphaproteobacteria* (312-318 bp) decreased in relative abundance.

Peat amended incubations after a second regrowth (Experiment 2) were dominated by LH-PCR fragments corresponding to *Alphaproteobacteria* (316-317 bp). Clone library construction and analysis of 62 clones by ITS-LH-PCR (internal transcribed spacer-length heterogeneity polymerase chain reaction) for the Peat incubation from Experiment 2 showed that over half of the clones (34 or 55%) had 99% sequence similarity to *Sulfitobacter* previously isolated from the Arctic Ocean. A more detailed phylogenetic analysis showed that 14 different phylotypes contributed to the observed increase in the *Alphaproteobacteria*.

Similar to bacterial community composition, fatty acid distribution showed commonality among the treatments, with 16:0n, 16:1Δ9 and 18:0n the dominant fatty acids in most incubations. The exception to this trend was the extended Peat incubation, where 18:1Δ11 became the second most abundant fatty acid. Phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) were the only headgroups observed, and no shift in headgroup was observed upon either the addition of organic matter or with shifts in community composition as detected by LH-PCR.

Introduction

Microbes are ubiquitous in the environment and are metabolically diverse, playing crucial roles in biogeochemical cycles (Sherr and Sherr 2000). In the organic carbon cycle of aquatic systems, microbes are largely responsible for the uptake, regeneration and transformation of dissolved organic matter (DOM). DOM represents the largest pool of reduced carbon in the marine environment (Benner 2002; Ogawa et al. 2001). Bacteria alter this reduced carbon pool by preferentially utilizing labile components of DOM, resulting in DOM that is more homogeneous and refractory (Amon

et al. 2001; Ogawa et al. 2001). In addition to affecting the DOM composition, studies have shown that bacteria differ in their uptake capabilities of dissolved organic matter substrates (Buchan et al. 2004; Cottrell and Kirchman 2000b; Kirchman 2002; Pinhassi et al. 1997). The differential response of bacteria to organic matter substrates has led to the hypothesis that there are two functionally divergent groups of bacteria. The first are the generalists, characterized by metabolic flexibility and the ability to quickly respond to new substrates. The second are the specialists; requiring more specific nutritional and/or environmental conditions for growth (Crump et al. 2003; Langenheder et al. 2005). In the Arctic Ocean, primary production is the dominant organic matter source, followed by terrestrial input (Benner et al. 2005; Stein and Macdonald 2004) with organic matter inputs varying seasonally and spatially. The bacterioplankton community in the Arctic Ocean has been shown to be consisted mainly of *Gammaproteobacteria*, *Alphaproteobacteria* and *Bacteroidetes* (Bano and Hollibaugh 2002; Brinkmeyer et al. 2003; Wells and Deming 2003). Therefore, application of the current theory regarding bacterial communities and organic matter uptake would indicate that in the Arctic Ocean, generalists would quickly respond and dominate the bacterioplankton community when labile organic matter is available and utilize the labile fraction of the organic matter, yielding DOM that is more homogeneous and refractory. On the other hand, specialists would become dominant after the utilization of the labile fraction of organic matter, via the uptake of the refractory components of the organic matter pool.

To date, few studies have examined the *in-situ* microbial community composition of the Arctic Ocean. Bano and Hollibaugh evaluated the phylogenetic diversity of bacteria in the Arctic Ocean water column during three seasons by sequencing both

cloned fragments of 16S rRNA genes and bands obtained from denaturing gradient gel electrophoresis (DGGE) (Bano and Hollibaugh 2002). Based on the clone library results from all samples, the most commonly retrieved clones were *Alphaproteobacteria*, in particular members of the SAR11 clade, and *Gammaproteobacteria* belonging to a number cultured, as well as uncultured clades. Seasonal changes in the bacterioplankton distribution were not dramatic, with *Gammaproteobacteria* and *Deltaproteobacteria* more prevalent in winter and *Bacteroidetes* only retrieved in summer and fall. While no correlations were made regarding the seasonality of bacterioplankton community, such changes could be due to seasonal variations in organic matter content and quality, especially with regard to phytoplankton bloom events. Depth-dependent distributions were also found, with *Alphaproteobacteria* dominating the mixed layer and *Gammaproteobacteria* dominating at halocline depths (Bano and Hollibaugh 2002). The importance of *Bacteroidetes* in fall samples was corroborated by Wells and Deming who used fluorescence *in situ* hybridization to show that members of the *Bacteroidetes* cluster consisted a large proportion of hybridizable cells in surface waters in the Northwest Passage (Wells and Deming 2003). Finally, a phylogenetic examination of summer pack sea ice Arctic communities, both by cultivation and 16S rRNA gene cloning showed that *Gammaproteobacteria* including several cultured genera (*Colwellia* spp., *Glaciecola* spp. and *Marinobacter* spp.), the *Roseobacter* clade (particularly *Octadecabacter* spp) and the *Bacteroidetes* were dominant phylotypes (Brinkmeyer et al. 2003). The same study used FISH probes to corroborate the dominance of the same phylotypes, and also showed that a novel group of organisms similar to strain ARK10278 and closely related to the

Roseobacter strain *Shippagan*, were relatively rare (<1% of FISH counts) (Brinkmeyer et al. 2003).

Despite the fact that prokaryotes make up the largest pool of living biomass in aquatic systems (Whitman et al. 1998), full understanding of *in-situ* microbial community structure, diversity and function is limited by the fact that only about 1% of bacteria are culturable (Giovannoni and Rappé 2000). The recent application of 16S rRNA phylogenetic techniques have revealed that the phylogenetic and metabolic diversity of microbes is much greater than previously estimated using culture-dependent methods (Doney et al. 2004; Suzuki et al. 1997). The ability to extract nucleic acids directly from the environment has enabled the investigation of *in-situ* microbial community composition and metabolic capabilities as well as their response to substrates without cultivation (Béjà et al. 2002; Gich et al. 2005). Identification of *in-situ* bacterial assemblages and knowledge of their metabolic capabilities are essential to understanding the role of bacteria in organic matter cycling and how organic matter composition might affect microbial community composition.

As critical components of cellular membranes, lipids have often been used as bacterial indicators in aquatic systems. For example, the presence of odd- and branched-chain fatty acids, hydroxy fatty acids and methylhopanoids have been used as markers of the *Bacteria* (Härtner et al. 2005; Kaneda 1991; Wakeham et al. 2003), and crenarchaeol is a glycerol dibiphytanyl glycerol tetraether lipid that is specific to the *Crenarchaeota* (Pearson et al. 2004). However, conflicting results have been obtained in comparisons of bacterial fatty acids and bacterial phylogeny. Mergaert and coworkers compared phylogenetic and fatty acid analysis of 137 bacterial isolates, and found differing fatty

acid compositions among closely related bacteria (Mergaert et al. 2001) and were therefore able to distinguish closely related organisms based on their fatty acid distributions. This is in contrast to Harvey and Macko who noted a lack of concordance between branched and odd fatty acid concentrations and bacterial carbon during experimental microbial degradation of phytoplankton (Harvey and Macko 1997). Furthermore, in field incubations, Harvey and coworkers found that fatty acid distributions overlapped among phylogenetically distinct groups of bacteria and that the fatty acid composition of bacterial communities was strongly influenced by organic matter substrate (Harvey et al. 2005). These discrepancies suggest that the relationship between cell membrane lipids and microbial community composition is complex and has yet to be resolved.

While fatty acids have traditionally received much of the focus as bacterial markers in natural environments (Ratledge and Wilkinson 1988; Ringelberg et al. 1997), recently intact phospholipids (IPLs) have been used as indicators of living microbial biomass in complex matrices such as sediments (Rütters et al. 2002b; White et al. 1996; Zink and Mangelsdorf 2004). IPLs consist of two fatty acids found in the *sn*-1 and *sn*-2 positions and a phosphate-containing headgroup in the *sn*-3 position, all linked via ester bonds to a glycerol backbone (refer to Figure 4.1) (Fang and Barcelona 1998). IPLs are major components of lipids in bacterial cell membranes (Schweizer 1988) and given that they rapidly degrade upon cell death (Harvey et al. 1986; White et al. 1979), they are useful indicators of living biomass. Recent analytical advances using high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI-MS) (Sturt et al. 2004; Zink and Mangelsdorf 2004), now allow the entire structure of an IPL to be

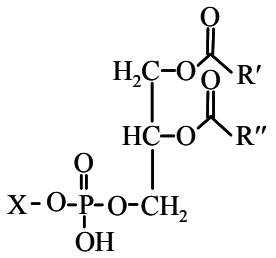

Figure 4.1. Phospholipid structure, where R′ and R″ indicate two esterified fatty acids in the *sn*-1 and *sn*-2 positions and X is the phosphate containing headgroup esterified at the *sn*-3 position.

determined, providing an opportunity for greater taxonomic linkage of lipids and in-situ microbial communities (Fang et al. 2000b; Sturt et al. 2004). However, currently little information is available regarding the lipid composition of naturally occurring marine bacterioplankton communities.

The Arctic Ocean is unique in that it receives approximately 10% of the global freshwater and riverine dissolved organic matter (DOM) input, while representing only 1% of the global ocean volume (Opsahl et al. 1999). Terrestrially derived dissolved carbon is estimated to comprise 14-24% of the dissolved organic carbon (DOC)in Arctic surface waters (Benner et al. 2005) with terrestrial POC and DOC accounting for 12.6×10^6 t y^{-1} and 28.1×10^6 t y^{-1} of the organic carbon input (Stein and Macdonald 2004). Labile carbon sources in the Arctic Ocean include ice algae as well as high primary production over continental shelves during spring blooms, with organic carbon dominated by marine primary production, accounting for 329×10^6 t y^{-1} (Stein and Macdonald 2004). The microbial loop efficiently utilizes this labile carbon with bacterial production consuming between 35% to 150% of primary production (Wheeler et al. 1996).

The polar regions are expected to be the first regions impacted by the effects of global warming, with changes in quantity and quality of nutrients and alterations in stratification and circulation, affecting aquatic food web structure and dynamics (Deming 2002; Grebmeier et al. 1998). Shifts have already been observed in terrestrial vegetation distribution, precipitation, circulation patterns, and sea-ice coverage and duration (Moritz et al. 2002). Given that experiments have shown that bacteria differ in their ability to utilize DOM (Cottrell and Kirchman 2000b; Malmstrom et al. 2005; Martinez et al. 1996), changes in DOM quality or quantity in aquatic systems are expected to affect *in-situ* bacterial community composition, which will in turn affect carbon cycling via the microbial loop and the transfer of carbon and nutrients into higher trophic levels.

This study employed bacterial regrowth incubations to examine the response of *in-situ* Arctic microbial communities to diverse Arctic organic matter substrates. These experiments consisted of a 1:10 dilution of < 1.0μm filtered water with < 0.2μm filtered water supplemented with various organic matter substrates with bacterial regrowth occurring to early steady state conditions. Organic matter additions consisted of 1) *Melosira arctica*, a sea ice associated diatom prevalent in the Arctic (Booth and Horner 1997; Falk-Petersen et al. 1998), 2) ice-rafted debris consisting of a mixture of ice algae and ice entrained sediment, 3) peat collected from the water's edge of the Ikpikpuk River and 4) no-addition controls. To determine bacterial response to organic matter additions, the diversity of 16S rRNA genes was examined using length-heterogeneity polymerase chain reaction (LH-PCR), a culture-independent technique which employs a fluorescently labeled primer to detect PCR amplified fragments from differing organisms using the natural length variation found in the 5' end of the small subunit rRNA genes (Suzuki et

al. 1998). Amplicon lengths are phylogenetically informative and peak intensities produced are assumed to reflect gene abundance. Intact phospholipid and fatty acid analyses were determined from parallel samples to study the effect of the addition of organic matter on the cell membrane lipid composition as well as to assess the relationship between phylogeny and cell membrane lipid composition.

Methods

Arctic experiment overview

Two sets of incubations were performed; in the first set (Experiment 1), Arctic bacterioplankton (<1.0 μm filtered water) were diluted (1:10) with bacteria-free (0.2 μm filtered) water followed by the addition of various organic matter substrates. The organic matter substrates consisted of *Melosira arctica*, a sea-ice associated diatom common in the Arctic (Booth and Horner 1997; Falk-Petersen et al. 1998). This fresh algal sample was collected 15 June 1998, on a SHEBA/JOIS cruise, rinsed with filtered seawater and promptly frozen and was stored at -80ºC until thawed for utilization in the regrowth experiments. Additional substrates included ice-rafted debris, (a mixture of ice algae and ice entrained sediment), collected June 2002 on the HLY02-01 cruise near station 22 at 72°24N 159°77W and peat, collected from the water's edge of the Ikpikpuk River during the HLY02-01 cruise (refer to Table 2.1 for the amount of substrates added to the incubations and details regarding carbon content determination). A no-addition control was employed to detect shifts in the bacterioplankton community composition due to manipulation and enclosure (Ferguson et al. 1984; Suzuki 1999). Hereafter these treatments will be referred to as Control, Peat, Debris and Ice algae. Incubations were

conducted in duplicate 20 liter carboys in the dark at -1°C and bacterial regrowth allowed to proceed to early stationary phase occurred as measured by bacterial production using leucine incorporation (D.L. Kirchman personal comm.), at which time samples from all replicates were taken for phylogenetic (16S rRNA gene) and lipid analyses. Sample water for Experiment 1 was collected near Arctic Ocean station HLY 04-02-007 (HV2) (70°13.117N 167°38.653W) on May 20, 2004 from the science seawater system (inlet at c.a. 8.3 meter depth) aboard the USCGC Healy. Refer to Table 2.2 for site characteristics. Sample water was then filtered through a series of inline 3.0 μm, 1.0 μm and 0.2 μm cartridge filters into 20 liter carboys using a peristaltic pump. Each carboy was rinsed three times with 2 liters of < 0.2 μm water and filled with 18 liters of < 0.2 μm water containing the natural dissolved organic matter (DOM) from the site. The 0.2 μm cartridge filter was removed and 2 liters of < 1.0 μm filtered water was added to each carboy for a 10% bacterial inoculum. For this first experiment (Experiment 1), samples were taken after 11 days, and a volume of 2 liters of water from the no-addition control and the Peat amended incubations which remained in the carboys and was subjected to a second 1:10 dilution, with growth occurring for an additional 12 days (Experiment 2). Sample water for this extended incubation was collected on 31 May 2004 near station HLY04-02-017 (EHS5) at 72°43.810N 158°25.475W. For both Experiments 1 and 2, the seawater system was flushed for approximately 5 minutes prior to collection to obtain fresh subsurface sample water and to remove fouling organisms. Bacterioplankton abundance and production were followed daily until early stationary growth occurred at days 11 and 12 for Experiments 1 and 2, respectively, at which point samples were taken for phylogenetic and lipid analyses. Prior to bacterial collection by filtration, Peat

incubations were screened through 10 μm mesh to prevent the collection of peat substrate during sampling.

Bacterial counts and cell volumes

Samples for bacterial counts for Experiments 1 and 2 were taken daily and initially counted onboard to monitor growth in all incubations. For bacterial counts, a modified protocol of Velji and Albright (Velji and Albright 1993) was followed. Briefly, sample water was fixed with 5% (v/v) 37% formaldehyde and stored in the dark at 4ºC. Bacteria in fixed samples were filtered and the fluorescent DNA-binding stain, 4',6-diamindino-2-phenylindole (DAPI), was added at a concentration of 2.5 μg/mL and incubated for 10 minutes. Onboard counts were conducted using an Olympus BH2 with a 100× objective. A 100W-mercury lamp for UV excitation at 365 nm and emission wave length at > 400 nm. Counts were performed using a 10×10 ocular grid and at least 10 randomly chosen fields of view were counted per slide. Slides were frozen and later recounted and bacterial volumes were calculated using semi-automated microscopy as described by Cottrell and Kirchman (Cottrell and Kirchman 2004a). This procedure is described with more details in Chapter 2.

DNA collection, extraction and quantification

Subsamples of water from all incubation carboys and the Inoculum water was filtered using a peristaltic pump onto 13 mm Supor 200 (Gelman) 0.2 μm membrane filters encased in Swinnex filter holders (Millipore). After sample collection, the filters were stored at –20°C in 130 μL Qiagen lysis buffer until sample analysis. DNA was extracted and purified using the Qiagen DNeasy Tissue Kit with a modified protocol

(Suzuki et al. 2001b). Briefly, the 13 mm Supor filters were thawed, 5 µL of RNAse I (Sigma, 1:5 dilution) and 50 µL of lysozyme solution (73 mg/mL) was added to 0.6-mL microcentrifuge tubes, the tubes were vortexed and incubated for 1 hour at 37ºC. 5 µL of Proteinase K solution (Qiagen kit) was added and the samples were again vortexed and incubated at 70°C for 30 minutes. The entire volume from each tube was then transferred to 1.5-mL microcentrifuge tubes and 410 µL AL/E buffer (205 µL AL Buffer + 205 µL 95% ethanol) was added and vortexed for approximately 15 seconds. The DNeasy Tissue Kit protocol was followed, with the following modifications: the DNeasy colums were dried by centrifugation at 16000 rpm for 5 minutes and DNA was incubated with a volume of 200 µL of DEPC treated TE Buffer (Ambion) instead of the supplied buffer, followed by centrifugation at 16000 rpm for 1 minute to elute the DNA. DNA samples were stored at -20ºC. The Control Carboy 1, Experiment 2 sample was lost during extraction of DNA, and data is not available for comparisons between replicate carboys.

DNA concentration was determined using the PicoGreen dsDNA Quantitation Assay and Kit (Molecular Probes). A standard curve in a concentration range of 1000 to 5.252 pg/µL was performed using a series of dilutions in TE of the supplied 100 µg/mL lambda dsDNA standard. 50 µl of diluted standards and samples were transferred to a 96 well low fluorescence microplate (Nalge Nunc Inc Rochester, NY) and 50 µL of 1:200 PicoGreen reagent in TE buffer was added to all sample and standard wells. Fluorescence of the standards and DNA samples was measured on a Spectra MAX Gemini microplate spectrofluorometer (Molecular Devices), with excitation and emission wavelengths at 485 nm and 538 nm, respectively in duplicate fluorescence readings.

LH-PCR

Purified DNA was diluted to 0.07 ng/µl (the lowest concentration among the samples) and was used as a template in the PCR reaction using 27F (Giovannoni 1991) labeled with the fluorochrome NED and unlabeled reverse primer 338R (Amann et al. 1995), both primers are specific for the domain *Bacteria*. In a final reaction volume of 10 µl, reaction mixtures contained 1× PCR buffer, 0.2 mM each deoxynucleoside triphosphate (Promega), 1.5 mM $MgCL_2$, primers (0.5 µM each) and 0.03 units of Platinum *Taq* DNA polymerase enzyme (Invitrogen). All reactions were conducted in a AB9700 thermal cycler (Applied Biosystems), programmed with an initial denaturation at 94ºC for 2 minutes and 25 to 32 cycles (depending on the sample), 30 seconds denaturation at 96ºC, 30 seconds annealing at 55ºC , and 30 seconds extension at 72ºC. Upon cycle completion, reactions were maintained at 4-6ºC. Agarose gel electrophoresis (1% w/v in 1× TBE buffer) stained with ethidium bromide (0.5 µg/ml) was conducted on aliquotes of the PCR products to estimate product concentrations and to check for contamination. Gels were run at 100 V for 1 hour. For LH-PCR, products (1 µl) were combined with 8 µl of 3% (v/v) GS2500 marker (Applied Biosystems) in highly deionized Formamide (Applied Biosystems) and denatured for 2 minutes at 94ºC. Fragments were separated by capillary electrophoresis in an AB3100 Genetic analyzer (Applied Biosystems) using 36 cm capillaries and POP4. Fragment sizes and relative concentrations were analyzed using the Genescan package (Applied Biosystems). The software outputs fluorescence data into electropherograms with the peak positions representing different sizes (Suzuki et al. 1998). Peak heights were used as a proxy of the abundance of each fragment. Fragment lengths were compared with known size

fragments (Suzuki et al. 1998) to determine community composition in all experimental incubations. Data for Carboy 2 from the Ice algae incubation are not available due to difficulties during sample processing.

Peat2exp2f Clone Library Construction and analysis

A long-fragment cloning protocol was employed to amplify fragments containing the 16S rRNA, the 23S rRNA as well as the naturally variable internal transcribed spacer (ITS) region in order to determine diversity within a mixed microbial community (Béjà et al. 2000; Suzuki et al. 2004). Purified DNA (100 µl) from Peat, Carboy 2, Experiment 2, screened through 10 µm mesh, (abbreviated Peat2exp2f) was used as a template for the PCR reaction. Contamination during the PCR reaction was problematic and this was in part due to the low concentration of the purified DNA sample. Therefore, to decrease PCR contamination effects, the purified Peat2exp2f DNA was concentrated using a Microcon 100 (Millipore) centrifugal filter from 100 µl to 8.0 µl for this reaction. PCR reactions employed primers 27F (Giovannoni 1991) and 1933R (Amann et al. 1995). In a final reaction volume of 25 µl, reaction mixtures contained 1× HiFi PCR buffer, 0.2 mM each deoxynucleoside triphosphate (Promega), 3.0 mM $MgSO_4$, primers (0.5 µM each), and 0.025 units of Platinum *Taq* Hi Fidelity enzyme (Invitrogen). All reactions were conducted in a Robocycler Gradient 96 (Stratagene), programmed with an initial 2 minutes enzyme activation step at 94ºC, and 30 cycles, of 56 seconds denaturation at 94ºC, 69 seconds annealing at 55ºC and 3 minutes 30 seconds extension at 68ºC. Upon cycle completion, reactions were initially lowered to 25ºC and then maintained at 4-6ºC. A reconditioning step was conducted in order to reduce heteroduplex formation during the PCR reaction (Thompson et al. 2002). 2.5 µl of PCR product from the first 30-cycle

PCR was mixed with 22.5 µl of the same PCR cocktail used for the original PCR and reactions were run in the Robocycler with 2 minutes enzyme activation at 94ºC followed by 5 cycles as above and one hold at 68ºC for 7 minutes. PCR products were run in a 1% agarose gel to check for size and contamination.

Long-fragment PCR products were A-tailed with the Qiagen A addition kit. A volume of 3 µL of A-tailed products was immediately cloned with the TOPO TA® Cloning Kit for Sequencing. Drop dialysis of the ligation products was employed to decrease salts in the ligation solution prior to electroporation. DNA free water (5 µl) was added to the ligation products and the combined 10 µl was pipetted onto a 0.025 µM Millipore VSWP membrane filter (Millipore) floating on 0.5× TE buffer for 1 hour.

Transformation via electroporation:

Electroporation was carried out as described in Béjà and coworkers (Béjà et al. 2000). Briefly, ligation products were used to transform into electrocompetent *E.coli* DH10B™ cells via electroporation using a Gene Pulser (Bio-Rad) with the following settings: 2500 V, 25 µF and 100 ohms. Cells were incubated for one hour at 37ºC at 225 rpm using an shaker incubator (Lab-Line® Orbit-Environ Shaker). 100 µl of recovered cells was plated onto LB (BD Difco LB agar) agar plates with 50 µg/ml of Kanamycin, spread with 40 µl of X-gal (40 mg/ml). Plates were incubated overnight at 37ºC and colonies were picked into microtiter dishes with 180 µl of LB media containing 7% glycerol and 50 µg/ml of Kanamycin and growth occurred overnight at 37ºC followed by storage at -80ºC.

Screening for full sized inserts:

In order to separate circular plasmid DNA from cellular debris, plasmids from clones were purified by alkaline lysis using a modified protocol by Ausubel and coworkers (Ausubel et al. 1988), with plasmid storage at -20ºC. The procedure is described in depth in Chapter 2. Next, a PCR was conducted to screen for long inserts. The conditions for this reaction were the same was for the LH-PCR, with the exception that this reaction employed universal primers M13F (GTAAAACGACGGCCAG) and M13R (CAGGAAACAGCTATGAC) and 3.0 mM $MgCl_2$. All reactions were conducted in a Robocycler Gradient 96 (Stratagene), with the same PCR parameters as outlined in the clone library construction and analysis section, with the exception that the reaction was run for 25 cycles. Upon cycle completion, reactions were initially maintained at 68ºC for 7 minutes and then lowered to 25ºC. A 1% agarose gel stained with 0.5 µg/ml of ethidium bromide (100V, 1 hour) in TBE was used to visually identify clones with full inserts.

Based on the gel results, 82 clones from the Peat2exp2f sample with full inserts were selected for screening using a novel method based on ITS-LH-PCR (Suzuki et al. 2004), in which the natural heterogeneity of the 5' end of the 16S rRNA and the internal transcribed spacer (ITS) region and the presence and location of the tRNA-alanine gene is used to identify closely related phylotypes. PCR reactions were performed using 3 sets of primers: (1) 27F (NED) (Giovannoni 1991) and 338R (Amann et al. 1995), this primer set was used to compare with the previous results of the LH-PCR; (2) 1406F-FAM (Suzuki et al. 2004) and 66R (Suzuki et al. 2004); and (3) 1406F-HEX (Suzuki et al. 2004) and tRNAalaR (Suzuki et al. 2004). PCR reactions were conducted under the same conditions as described for the LH-PCR, with the exception that the reactions were run

for 15 cycles and 72ºC extension for 1 minute. Fragments were separated by capillary electrophoresis on an Applied Biosystems 3100 Genetic Analyzer with POP4 and 36-cm capillaries. Fragment sizes were estimated by the Genescan software (Applied Biosystems) and the GS2500 size standard (Applied Biosystems). Fragment sizes for the three primer sets (27F-NED and 338R; 1406F-FAM and 66R; 1406F-HEX and tRNAalaR) were used in conjunction, to putatively identify the clones and to provide an estimate of the bacterial diversity of the sample, and clones were grouped or distinguished based on the sizes of the three fragments. Each unique combination of fragment sizes was identified as a phylotype. Phylotypes differing by a one base pair for any of the three fragments were combined, as the difference likely represents slight variations estimated by the size-calling algorithm. Based on the groupings, a select number of clones from the Peat2exp2f sample were chosen for sequence analysis.

Sequencing:

Sequencing was used to determine the phylogenetic diversity of the clones with greater resolution. Given that many clones formed groups based on the fragment lengths obtained by the ITS-LH-PCR, at least one clone from each group was partially sequenced. Since the *Alphaproteobacteria* dominated the community composition and was also very diverse, the phylogenetic affiliation of these clones was of particular interest, and therefore, full sequencing was employed for these clones. Plasmid DNA was purified using the Montage 96 Plasmid kit. Full sequencing employed primers 27F (Giovannoni 1991), 1100R (Suzuki et al. 2004), 1074F (Suzuki et al. 2004) and 1541R (Suzuki et al. 2000), while primers 27F and 519R (Amann et al. 1995) were used for partial sequencing. Ribosomal RNA genes were sequenced using Big Dye v3.1 protocol,

employing dideoxynucleotide termination reactions with 1:32 dilutions. The PCR reaction for the 27F primer was conducted as follows: 40 cycles on AB9700 thermal cycler consisting of 10 seconds denaturation at 96ºC, 5 seconds annealing at 50ºC and 2 minutes 30 seconds extension at 60ºC. Reactions using 1074F, 1100R and 1541R primers were run as above, with the extension temperature lowered from 60ºC to 55ºC. Reactions using the 519R primer were run at 60ºC annealing and 65ºC extension. Some plasmids with poor sequence were also purified using the FastPlasmid Mini protocol and partial sequencing of these clones was conducted.

Sequences were initially aligned edited and assembled the using the Staden Package (Sanger Institute). Sequences were then imported and automatically aligned by the ARB package (Ludwig et al. 2004). Alignments were manually checked and sequences were added to a ca. 35,000 sequences tree using ARB_PARSIMONY and a filter excluding highly variable positions. Near relatives were used to check for possible base miscalls and sequences were removed from and re-added to the tree using phyla specific filters. Phylogenetic distances to nearest relatives were determined using the program *dnadist* of PHYLIP package (Felsentein 1989) in the ARB software using filters that excluded highly variable positions and ambiguities and the Kimura two-parameter model method (Kimura 1980). Clones were classified according to the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu) scheme.

Total lipid extraction

Samples for lipid analysis of the Arctic bacterial regrowth experiments were collected by vacuum filtration of sample water onto precombusted (450°C) 47-mm GF/F filters. Incubations with peat as the organic substrate were screened through 10 μm Nitex

mesh prior to filtration to minimize collection of peat particles and all lipid samples were frozen at -70°C until analysis. Given low expected bacterial concentrations, only 1 filter per carboy was obtained for the Control and Peat incubations, with fatty acid and intact phospholipid (IPL) analyses conducted on separate filters (i.e. carboys). The extraction protocol from Sturt et al. was used with modifications (Sturt et al. 2004). All glassware was solvent rinsed prior to being used in the extraction procedure. Samples were sonicated with output 3, duty cycle 70% with a Branson Sonifier 250 probe for 5 minutes in DCM/MeOH/phosphate buffer (1:2:0.8; 50mM phosphate buffer, pH 7.4). DCM and buffer were added to achieve a final ratio of 1:1:0.8 (DCM/MeOH/phosphate buffer). Samples were vigorously shaken and the lower organic phase containing the total lipid extract (TLE) in DCM was removed and transferred to a rotary evaporator flask. DCM was added to the sample to achieve separation of the organic and water phases and again vigorously shaken and the organic layer containing the total lipids was removed. A total of 3 extractions, removing the TLE in the organic phase each time, were performed and TLEs were combined and evaporated to dryness with reduced pressure, with the water bath temperature maintained at 30°C. TLEs were redissolved in 4 aliquots of 2 mL of 2:1 (DCM/MeOH), pooled, evenly distributed to four 2-mL amber vials, flushed with nitrogen, capped, and stored at -70°C until analysis.

Fatty acids in total extracts by GC and GC/MS

Samples were processed as outlined in Mannino and Harvey (Mannino and Harvey 1999). Briefly, the internal standard nonadecanoic acid was added prior to alkaline hydrolysis. Alkaline hydrolysis was conducted at 70°C for 30-60 minutes. Fatty acids were methylated using boron trifluoride in methanol at 70°C for 30 minutes. Polar

lipids were quantified using capillary gas chromatography (HP5890 II, Hewlett Packard). Lipids were separated using a J&W Scientific ZB-5 fused silica column (60 m length x 0.32 mm i.d. x 0.25 μm film thickness) with quantification by flame ionization detection. Samples were injected in the splitless mode (oven temp 50°C; injector at 225°C), with hydrogen as the carrier gas. An Agilent 6890 GC coupled to an Agilent 5973N mass selective detector was used for structural identification. Sample runs were conducted using identical conditions as above but with He as the carrier gas. Double-bond positions of monounsaturated fatty acids were identified by GC/MS analysis as the dimethyl disulfide (DMDS) adducts (Nichols et al. 1986a).

Polar lipid classes by TLC-FID

Thin-layer chromatography with flame ionization detection (TLC-FID) using an Iatroscan MK-V TLC/FID Analyzer (Volkman et al. 1986) was employed to separate and quantify IPLs. A multi-step solvent separation procedure was employed to separate IPLs from other lipid classes using a method adapted from Parrish and Ackman (Parrish and Ackman 1983). Aliquots of samples (1-2μL) were manually spotted onto S-III Chromarods (Iatron Laboratories Inc., Tokyo) with a 5 μL syringe (SGE International). The origin was focused using a 1:1 mixture of DCM/MeOH followed by development of neutral lipids using hexane/diethyl ether/formic (85:15:0.2). Using this procedure, phospholipids remained at the origin and partial scanning of the rods was conducted to remove neutral components. Chromarods were developed a second time in acetone with partial scanning to remove non-phospholipid components. Development in DCM/MeOH/H_2O (50:20:1) enabled separation of individual phospholipids. Individual phospholipids in samples were identified and quantified by comparison with PG and PE

phospholipid standards similar to the method described in Ju and coworkers (Ju et al. 1997).

LC/MS

Intact phospholipids (IPLs) were separated by high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI-MS) using a method adapted from Sturt et al. (Sturt et al. 2004). IPLs were separated using normal-phase HPLC on an Agilent 1100 Series HPLC system equipped with a Luna 5µ Silica column 250 mm × 4.60 mm, 5µm; Phenomenex, part number 00G-4274-E0) with a 4.0 mm × 3.0 mm Silica guard column (Phenomenex, kit part number KJ0-4282, cartridge part number AJO-4348). The following solvent systems were used at a flow rate of 0.5 ml/min: 100% A for 1 hour to equilibrate the column prior to initial TLE sample injection, where A = 79:20:0.12:0.034 of hexane/2-propanol/formic acid/17.6 M NH_{3aq}. During sample runs, the solvent gradient proceeded as follows: 100% A to 20% A:80% B (88:10:0.12:0.034 of 2-propanol/water/formic acid/17.6% M NH_{3aq}) over 55 minutes, hold for 10 minutes, then back to 100% A for 1 hour to re-equilibrate the column. Solvent blanks (2:1 dichloromethane/methanol) were run prior to sample analysis. A TLE sample volume of 10 µl per sample was injected.

TLE samples were analyzed separately in positive and negative ion mode runs on an Agilent 1100 Series ion trap mass spectrometer with an electrospray ionization (ESI) interface. In positive ion mode, the fragmentation reveals the phospholipid headgroup and in negative ion mode, the fragmentation pattern reveals which fatty acid side chains are associated with each headgroup, enabling full identification of the compound (refer to Figure 3.1) (Sturt et al. 2004). The nitrogen drying gas was set to 6 L/min and its

temperature was 325°C. Parameters for sample runs were determined by optimization during direct infusion of phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) into the ESI source (15 μg/ml concentration at an infusion rate of 12 μl/min). For sample runs, the capillary and end plate voltages were set at 3500 and 500 V, respectively.

Results

Bacterial growth, cell volumes and cellular carbon

Bacterial cell counts and cell volumes in Experiment 1 were relatively similar across all incubations up to day 5 (0.37 to 1.5 × 10^5 cells ml^{-1} and 0.035 to 0.154 μm^3, respectively), with significantly differences observed by day 7 in the Ice Algae incubation relative to either the Control or Peat incubations (ANOVA, $p < 0.0001$ for both comparisons). The highest cell abundance and cell volumes (6.8 and 7.6 × 10^5 cells ml^{-1} and 0.166 to 0.294 μm^3) observed in the Ice algae amended incubation by the end of Experiment 1 (refer to Figure 2.1). In Experiment 2 the Peat additions showed higher cell abundances relative to the Control, but were not statistically different (ANOVA, $p = 0.4194$) and while cell volumes were lower in the Peat incubation relative to the Control, there was not a significant difference observed (ANOVA, $p = 0.5667$) (refer to Figure 2.1). Bacterial abundances in the Control were approximately 2-3 times higher at the end of Experiment 2 relative to Experiment 1. Cell volumes were also slightly higher in the Control from Experiment 2 compared with Experiment 1, while cell volumes in the Peat incubation were approximately the same between Experiments 1 and 2 (refer to Figure 2.1). Bacterial carbon was calculated for the Control, Peat and Ice algae incubations

from Experiment 1 and the Control and Peat incubations from Experiment 2 assuming 65 fgC/μm^3, using the cell abundance and cell volume at endpoints (day 11, Experiment 1; Day 12, Experiment 2) when samples were collected for DNA and lipid analyses. A wide range of carbon values were observed, from 0.512 $\mu C\ L^{-1}$ in the Control Carboy 1 from Experiment 1 to 11.623 $\mu C\ L^{-1}$ in Carboy 2 of the Ice algae incubation, with the majority of the values ranging from 2 – 3.2 $\mu C\ L^{-1}$ (see Table 2.3). All statistical comparisons were conducted using a repeated measures ANOVA (SAS Institute, Cary, NC) with a Tukey-Kramer adjusted p value and comparisons were made at a 95% confidence level.

Bacterial community composition

Experiment 1

The bacterial community composition based on the LH-PCR electropherograms data is shown as a histogram, with the bars representing the relative peak height of the sized fragments (refer to Figure 2.2). In the Inoculum water (refer to Figure 2.2), fragments sized 315-317 base peaks (bp) made up approximately 30% of the amplified fragments; in marine plankton these shorter fragments generally correspond to the SSU rRNA gene of *Alphaproteobacteria* but likely represent several different clades (Suzuki et al. 1998). The Inoculum water also contained fragments sized 344 (2.8%), 346 (6%), 348 (9.7%), 349 (10.8%), 350 (16.7%) and 351 (17%) bp. Most of these fragments can represent different phylogenetic groups in marine plankton, including *Gamma*-, *Beta*-and *Delta*-subdivisions of the *Proteobacteria* and the *Bacteroidetes* phylum. In marine plankton the fragment sized 350 bp corresponds mostly to *Gammaproteobacteria* and,

like the 315-317 fragments, probably encompasses several clades of this proteobacterial class (Suzuki et al. 1998).

Relative to the Inoculum water, the bacterial community composition shifted in all incubations. The Peat, Debris and Ice algae treatments had similar bacterial distributions after 11 days, with a prevalent fragment sized 347 bp, ranging from 14 to 53% (see Figure 2.2), which represents *Gammaproteobacteria* and *Bacteroidetes* in marine plankton. A similar distribution of peaks was observed in the Control incubations, with the prevalent fragment sized 352 bp (38-63%, see Figure 2.2). This fragment likely represents *Gammaproteobacteria*. Replicate carboys for each of the treatments and Controls had similar bacterial community composition, despite some minor discrepancies between carboys. In the Control incubations, a fragment sized 350 bp (20%) was observed in Carboy 1 and not in Carboy 2. Also, Control Carboy 2 had a higher relative proportion of fragments sized 352 bp (see Figure 2.2). Furthermore, in Carboy 1, the fragment sized 349 bp made up a higher percentage of the relative distribution than in Carboy 2 (refer to Figure 2.2). The bacterial community composition in the Peat incubations differed slightly between replicate carboys, as seen in Carboy 1 which contained a fragment sized 346 bp which was not present in Carboy 2 (see Figure 2.2) and 347 made up a higher percentage of the relative distribution in Carboy 1 of the Peat incubation.

Experiment 2

At the end of the extended incubation (23 days total including Experiments 1 and 2; 12 day incubation time for Experiment 2), Controls in Experiment 2 were dominated by fragments sized 351 and 352 bp (see Figure 2.2), corresponding mainly to the

Gamma- and *Beta-* subdivisions of the *Proteobacteria* as well as the *Bacteroidetes* (Suzuki et al. 1998). The bacterial community composition in the Control from Experiment 2 shifted relative to its inoculum (i.e. Day 11, Experiment 1, Carboy 2), notably by the large proportion of fragments sized 351 bp in Experiment 2 (see Figure 2.2).

Among all of the incubations from Experiments 1 and 2, the Peat incubation from Experiment 2 showed the most unique shift in bacterial community composition over time, with a fragment sized 317 bp dominating the relative peak height distribution (32-34%) (refer to Figure 2.2). In marine plankton this size corresponds mostly to the SSU rRNA genes of *Alphaproteobacteria* (Suzuki et al. 1998). Dominant fragments (347-352 bp) found in the inoculum water for this incubation (Peat day 11 Experiment 1) were also present at the end of Experiment 2, although in much lower relative proportions (see Figure 2.2). Carboys 1 and 2 of the Peat incubation in Experiment 2 had nearly identical bacterial community composition.

Clone library

Since the community composition of the Peat incubation from Experiment 2 differed from all of the other incubations, a clone library was constructed to refine the phylogeny of this community. Of the 96 picked clones, 82 were identified as having full inserts during the M13F screening. Of the 82 clones chosen for sequencing, 7 did not yield fragments when analyzed by Genescan and 11 had a 351 bp rRNA fragment but no detectable tRNA or ITS fragments, and those clones were excluded from further analysis. Of the remaining 64 clones, 2 were overlooked during sequencing analysis, leaving 62 clones for clone library construction.

Screening:

Based on the fragment sizes of the three primer sets, clones from the Peat incubation from Experiment 2 were grouped and identified (see Table 2.4). These clones were added into a phylogenetic tree consisting of previously published sequences. The *Alphaproteobacteria* made up the largest and most diverse group of identified clones, accounting for 43 of the 62 clones, with 14 different phylotypes, with *Sulfitobacter* spp representing the majority of the phylotypes, (12 out of 14) (see Table 2.5). The majority of the clones (34 out of 43) were very closely related (>99%) to a previously published sequence, ARK10278, an Arctic member of the *Roseobacter* clade (Brinkmeyer et al. 2003) (refer to Table 2.5). Based on fluorescence in situ hybridization (FISH) results, this group of bacteria in packed sea ice samples comprised less than 1% of FISH counts (Brinkmeyer et al. 2003). *Gammaproteobacteria* were the second most abundant bacteria, contributing 8 clones, and was also highly diverse, with 6 phylotypes, including *Psychrobacter*, *Stenotrophomonas*, *Colwellia*, *Pseudomonadaceae, Methylophaga and Alteromonadaceae* (see Tables 2.4 and 2.5). Seven clones were grouped with Firmicutes, with all 4 phylotypes grouped with Bacillus (>96%) (refer to Tables 2.4 and 2.5). Three clones grouped with *Bacteroidetes*, each representing a different *Polaribacter* phylotype and one clone grouped with *Betaproteobacteria*, specifically *Janthinobacterium* (see Tables 2.4 and 2.5).

Fatty acid analysis of Arctic incubations

Bacterial fatty acid cellular content was calculated using a regression obtained from work conducted in the Delaware Bay using several estuarine habitats (Harvey et al. 2005) and the relationship between bacterial carbon and bacterial fatty acids was

determined to be: Fatty acid methyl esters (FAME; $\mu g\ L^{-1}$) = 0.2662*Bacterial carbon ($\mu g\ C\ L^{-1}$) + 0.3383, R^2=0.608; bacterial carbon was calculated using the conversion of 65 fg C/μm^3. Polyunsaturated fatty acids (PUFAs) containing greater than 2 double bonds which are typically algal in origin (Falk-Petersen et al. 1998; Gillan et al. 1981) and dicarboxylic acids (DCAs) which can arise from a multitude of sources, including oxidation products in bacteria, as well as waxes and cutin (Wakeham 1999), were excluded from bacterial fatty acid calculations. In general, measured bacterial fatty acid values were in good agreement with calculated cellular bacterial fatty acids, with differences between the observed and estimated values ranging from a factor of 1.16X to 2.24X. The Ice algae incubation was the exception, differing by a factor of 6.3X between observed and estimated values (Table 3.1). Cellular fatty acid content in the Inoculum water and the Control and Peat incubations ranged from 0.09 to 0.33 fg FA/μm^3 with the exception of the Control from experiment 2 (1.32 fg FA/μm^3), and this is likely due to both the higher cell volumes and measured fatty acids in this sample. Much higher cellular fatty acid values were observed in the Algae incubation than in the cultures or the Arctic experiments, and this is likely due to the confounding presence of Algae substrate on the filter during sample collection. These calculations are not available for the Debris incubation, as bacterial cell counts could not be performed due to the presence of substrate on the filter.

In order to compare the fatty acid composition bacterioplankton in the different treatments as well as in the organic matter substrates, relative distributions were used, with fatty acids in Inoculum water shown for reference. Relative fatty acid distributions in the incubations changed both between Experiments 1 and 2 in the Control and Peat

incubations as well as between treatments. The Inoculum water was dominated by 16:0n (30.3%) and 18:0n (16.9%) (see Table 3.4). At the conclusion of Experiment 1 (day 11), the Control was dominated by 16:0n (24.4%) as well as 16:1Δ9 (19%) and by the end of Experiment 2, 16:1Δ9 made up over 64% of the total fatty acid distribution (see Table 3.4). Similar to the Control, the major fatty acids in the Peat incubation by the end of Experiment 1 were 16:1Δ9 (24.3%), followed by 16:0n (14.4%) (see Table 3.5). By comparison, the Peat substrate consisted mainly of 16:0n (20.9%), 22:0n (14.4%), and 24:0n (13.8%). Changes in fatty acid distribution also occurred between Experiments 1 and 2 in the Peat incubation. By the end of Experiment 2, the fatty acid distribution in the Peat incubation consisted of 16:1Δ9 (34.7%) followed by 18:1Δ11 (17.8%) and 16:0n (10.7%) (see Table 3.5).

During sample collection of the Ice algae and Debris incubations, it was not possible to completely separate the ice algae and debris substrates from growing bacteria and a fraction of the substrates were also collected on the filter. This made it difficult to assign which fatty acids were attributable to the bacteria versus the substrate itself. This is apparent by the similarity of the fatty acid distribution in the Ice algae incubation and ice algae substrate as well as the Debris incubation and debris substrate (refer to Tables 3.6 and 3.7, respectively). The fact that the dominant fatty acids in both the Ice algae and Debris incubations were also major contributors to the fatty acid distribution of the substrates also reflects this overlap. To determine the origin of the fatty acids in these incubations, visual comparisons were made between the fatty acid distributions of the Control and Peat incubations, deemed bacterially-derived fatty acids, and the Ice algae, Debris and Peat substrates. The results of these comparisons are shown in Table 3.8.

The dominant fatty acids (14:0n, 16:0n, 16:1Δ9) observed in the Ice algae and Debris incubations are likely derived from both the bacteria as well as the substrate and are assigned a mixed (M) origin. Several fatty acids were assigned bacterial in origin (B), including 14:1, 15:0i, 15:0a, 15:1, 16:1Δ7 and 17:1Δ9, as these fatty acids were present in the incubations and were not observed in the substrates.

Intact phospholipids in Arctic incubations

With the exception of the Peat and Control incubations from Experiment 1, phosphatidylethanolamine (PE) was the dominant IPL observed in the majority of the incubations, making up 89 to 94.8% of the total IPLs. The Peat incubation from Experiment 1 was dominated by phosphatidylglycerol (PG) (70.9%) while the Control incubation from Experiment 1 was made up of nearly equal amounts of PG and PE (46.6% and 53.4%, respectively) (Figure 3.3). The total cellular IPL content is given in Table 3.2. There was nearly a 30-fold variation in these values, from 4.26 fg IPL/μm^3 in the Peat Experiment 1 to 122.95 fg IPL/μm^3 in the Ice algae incubation. In general, the dominant fatty acids found during FAME analysis of the incubations and Inoculum water were also found during IPL analysis, with 18:1, 16:0 and 16:1 fatty acids commonly observed (refer to Table 3.9). The exception to this trend was that 18:0 was not associated with either PE or PG yet it was one of the dominant fatty acids in the analysis of the Inoculum water. No major shifts in either headgroup or fatty acid side chains were detected upon the addition of the various organic matter substrates.

Discussion

Many studies have shown that organic matter composition can affect microbial community composition and this includes the removal of labile components of DOM (Ogawa et al. 2001; Perez et al. 2003; Stoderegger and Herndl 1998) as well as preferential uptake of compounds by specific groups of bacteria (Abell and Bowman 2005; Buchan et al. 2003; Cottrell and Kirchman 2000b; Covert and Moran 2001; Kisand and Wikner 2003; Malmstrom et al. 2005). However, generalizations regarding substrate utilization is difficult, given that even phylogenetically related groups of bacteria consist of multiple species, which likely differ in their metabolic capabilities and their response to substrates (Giovannoni and Rappé 2000). The complex substrates used in this experiment were presumed to have differing labilities which could impact bacterial community composition. However, no clear trends in phylogenetic response to organic matter substrates was observed in Experiment 1 using LH-PCR, although it is very possible that the dominant fragments observed (344-354 bp) represent different organisms in the incubations. Comparison of the substrate-amended incubations and the no-addition Control indicate that experimental manipulation and confinement alone (Massana et al. 2001; Pernthaler et al. 2001a) are not the cause of the shift in community composition observed in the incubations. Moreover, through the use of a controlled environment and identical growing conditions, factors such as temperature, salinity or grazing which are known to play a role in structuring in-situ aquatic bacterial communities (Jardillier et al. 2004; Schultz et al. 2003) are not likely to be the cause of the observed changes in community composition. Viral induced mortality could be one cause for the shift observed in community composition between Experiments 1 and 2, as

it is known that viruses play key roles in bacterioplankton community composition, with viruses selectively infecting bacterioplankton which have either reached a certain growth rate or density (Wommack and Colwell 2000), and this could partially explain the decrease in the dominant peaks (352 for the Control, and 347 for the Peat) observed in Experiment 1 relative to Experiment 2. However, the disparate community composition between the Control and Peat incubations from Experiment 2 and the prevalence of *Alphaproteobacteria* and in particular, *Sulfitobacter* spp. in the Peat incubations from Experiment 2, indicates that the change in community composition cannot be attributed to bacteriophage infection alone.

Another potential explanation for the overlap in size fragments among the substrate-amended incubations from Experiment 1 is the recent suggestion that aquatic bacterioplankton communities are comprised of two functionally divergent groups of bacteria: generalists, who have greater metabolic flexibility and can respond quickly to changes in organic matter and specialists, which are successful under more specific environmental and/or nutritional growth requirements (Crump et al. 2003; Langenheder et al. 2005). For example, some members of the *Gammaproteobacteria* have a more opportunistic life history strategy as they have been shown to utilize a wide spectrum of DOM sources (Covert and Moran 2001; Ivanova et al. 2002) and members of this group respond favorably to experimental conditions and confinement and quickly outcompete other bacteria (Eilers et al. 2000a; Pinhassi and Berman 2003). Under this theory, the similarity observed in the substrate-amended incubations would be due to the ability of generalists/opportunists to rapidly respond to the labile fraction of the organic matter substrate additions, with some limiting factor, likely the full utilization of the labile

component of the organic matter, resulting in the decline of such species, thereby enabling the *Alphaproteobacteria*, especially *Sulfitobacter* spp., to dominate the extended Peat incubation. This is further supported by the fact that members of the *Roseobacter* clade have been shown to utilize lignin and related compounds via the beta-ketoadipate pathway (Buchan et al. 2000; Buchan et al. 2004). The peat substrate is probably comprised of a variety of refractory compounds such as lignins, humics, cellulose, phenolics and polysaccharides (Killops and Killops 1993), lending further support to this hypothesis. Due to the overlap in LH-PCR fragments among different groups of bacteria, it is difficult to determine which specific members of the bacterial community were responsible for the shifts in community composition observed in Experiment 1, and this remains a subject for further investigation.

In contrast to Experiment 1, the bacterial community in the Peat incubation from Experiment 2 was dominated by *Alphaproteobacteria*, and this was confirmed by clone library analysis. This shift in community composition was not observed in the Control incubation, indicating that the dominance of *Alphaproteobacteria* was in response to the peat substrate. This group of bacteria is phylogenetically diverse, with 14 different phylotypes, and the majority of the phylotypes were closely related (>99%) to an Arctic sea-ice associated *Sulfitobacter* spp. (Brinkmeyer et al. 2003), indicating that this is a naturally occurring member of the Arctic microbial community and is not an introduced organism. However, FISH results revealed that these organisms comprised less than 1% of FISH counts of bacteria in Arctic sea-ice (Brinkmeyer et al. 2003). Therefore, although the *Sulfitobacter* spp. dominated in the extended Peat incubation, this bacteria

may not be a major member of Arctic bacterioplankton. Furthermore, it appears that this bacteria could be important in the degradation of terrestrially derived organic matter.

In addition to occurring in Arctic sea-ice, members of the *Roseobacter* have been found in temperate coastal and open ocean, polar oceans and marine sediments (Buchan et al. 2005) and they are also metabolically diverse, as members have been shown to uptake HMW-DOM (González et al. 1996) as well as glucose under both oxic and anoxic conditions (Alonso and Pernthaler 2005) and some members have been found to be key players in the cycling of organic sulfur containing compounds such as dimethylsulfoniopropionate (DMSP) (González et al. 1999; Mou et al. 2005; Vila et al. 2004). ARK10278 is in pure culture and therefore, studies on the metabolic capabilities of this organism, especially with regard to peat utilization, could be attempted in the future.

Although no clear phylogenetic responses to the various organic matter substrates were detected using LH-PCR, it was clear that the lability of the organic matter resulted in a differential growth response: bacterial production values (D.L. Kirchman, personal comm.) mirrored the lability of the substrate, with highest bacterial production occurring in the Ice algae followed by the Debris, Peat, and Control incubations, respectively, by the end of Experiment 1 (day 11). The Ice algae incubation supported the highest cell abundances and cell volumes, resulting in the highest bacterial carbon values. Similar trends have been observed in previous studies, with substrate lability affecting microbial growth and metabolism (Fuchs et al. 2000; Lennon and Pfaff 2005).

Contrary to other studies which have distinguished bacterial groups based on fatty acid profiles as well as phylogenetic methods (Bühring et al. 2005; Mergaert et al. 2001;

Ritchie et al. 2000), the results from these experiments indicate that phylogenetically distinct groups of bacteria overlap in their cell membrane lipid composition when grown in the presence of natural organic matter substrates. This is in agreement with the results of Harvey and coworkers, where fatty acid synthesis overlapped among phylogenetically diverse groups of bacteria as defined by fluorescence in situ hybridization (FISH) in differing temperate estuarine environments (Harvey et al. 2005). Harvey and coworkers examined the effect of DOM substrates on bacterioplankton community composition and cell membrane fatty acids and they found that when grown on natural DOM and ultrafiltered DOM, the main fatty acids were 16:1Δ9, 16:0n, 18:1Δ11 and 18:0n regardless of the dominant bacterial group (Harvey et al. 2005). Similarly, 16:1Δ9, 16:0n and 18:0n generally dominated the fatty acid distribution in the Control and Peat incubations from this study. One exception to this trend was the observance of 18:1Δ11 as the second most abundant fatty acid in the Peat incubation from Experiment 2. This shift in fatty acid distribution coincided with the dominance of *Alphaproteobacteria*, and in particular, *Sulfitobacter* spp., in this incubation. Branched- and odd-chain fatty acids are typically used as indicators of bacteria in natural environments and although branched- and odd-chain fatty acids were present in the fatty acid distributions of the regrowth experiments, collectively these fatty acids made up a small fraction (generally less than 15%) of the total fatty acids. Based on the results of this experiment, studies which use only branched- and odd-chain fatty acids as bacterial markers will lead to underestimation of the contribution of bacteria to the organic matter pool in aquatic systems.

Utilization of intact phospholipid analysis and associated fatty acid side chains has been effectively employed to differentiate microorganisms (Fang et al. 2000b; Ivanova et al. 2000; Rütters et al. 2002b; Sturt et al. 2004) and represents a sophisticated approach to examine living biomass. Interpretation of the fatty acid and head group associations, however, require some caution and in this study are complicated by the fact that the lipids observed were derived from several groups of bacteria. Phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) were the only IPLs detected and they are known to be the primary phospholipids in bacterial cell membranes (Lechevalier and Lechevalier 1988; Suzumura 2005). There were no consistent trends in headgroups and the phylogenetic composition of the bacterial community in each of the amended incubations. The major fatty acids observed in fatty acid analysis were also observed in IPL analysis, and there appeared to be preferential fatty acid side chain combinations, notably 18:1/18:1, 18:1/16:1, 16:0/16:1, 16:1/16:1, 16:1/14:0 and 16:1/14:1. This is comparable to Zink and Mangelsdorf who found similar dominant fatty acid side chain combinations which was attributed to preferential biosynthesis of specific fatty acid combinations by bacteria (Zink and Mangelsdorf 2004). In general, fatty acids of 16, 17 and 18 carbons in length were found to be associated with PG and greater diversity in fatty acid distribution (C-14 to C-18) was observed in the fatty acids associated with PE, implying the potential for some specificity of phospholipid headgroup and fatty acid side chains. Differences in fatty acid side chains observed among the incubations may also be influenced by instrument detection limits, particularly for PG which comprised approximately 5-10% of the total phospholipid in the majority of the incubations.

This is the first study to explore the response of *in-situ* Arctic microbial communities to a variety of Arctic organic matter substrates. The results of this study clearly demonstrate that there was a preferential response of bacteria in terms of growth and production to organic matter substrates, with more labile substrates supporting both higher growth and production. Although disparate growth occurred among the substrate-amended incubations, community composition was remarkably similar as evaluated by LH-PCR. Based on current knowledge of bacterial life history strategies and metabolic capabilities, it is likely that the *Gammaproteobacteria* were responsible for the shift in community composition observed in Experiment 1, as this group has been shown to respond favorably to experimental conditions and can quickly outcompete other bacteria (Eilers et al. 2000a; Pinhassi and Berman 2003). Clone libraries for Experiment 1 incubations should be constructed in order to further refine the bacterial community composition. Most noteworthy was the growth of *Alphaproteobacteria*, particularly *Sulfitobacter*, in the extended Peat incubation. The *Sulfitobacter* which responded to the substrates in this experiment were closely related (>99%) to ARK10278, an Arctic sea-ice associated member of the *Roseobacter* clade, confirming that these bacteria may be ecologically significant in the cycling of terrestrially derived organic matter in the Arctic Ocean. The dominance of *Sulfitobacter* in this incubation corresponded with a shift in fatty acid composition, with 18:1Δ11 increasing in relative abundance, although no shift in phospholipid headgroup was detected. Furthermore, it appears that when grown on natural sources of DOM, Arctic bacteria preferentially synthesize 18:0n, 18:1Δ11, 16:0n and 16:1Δ9. The results shown here therefore indicate that organic matter plays an

important role in structuring both bacterioplankton community composition as well as cell membrane lipid distribution.

The effect of global warming on the Arctic region will likely result in decreased sea-ice extent and duration, concurrent with rising permafrost temperatures and increased freshwater input to the Arctic Ocean. Decreases in sea ice coverage and duration will result in reduced suitable habitat for sea-ice associated bacteria and algae but on the other hand will also likely increase the amount of light entering the water column. With more light, integrated water column primary productivity might increase. A reduction in sea ice may also result in more wind-driven mixing and this could counteract the effects of enhanced light penetration. However, due to the strong haline stratification in the Artic Ocean the balance appears to favor an increase of primary productivity and availability of labile organic matter.

Under this scenario, it could be envisioned that these increases in labile organic matter from phytoplankton blooms could support copiotrophs, such as *Gamma-proteobacteria* as well as *Bacteroidetes*, and that these bacteria could represent larger proportions of the community composition. Alterations in the supply of labile organic matter will also affect bacterial cell abundances and cell volumes, with more labile substrates supporting higher bacterial abundances, as observed in this experiment, although bacterivory and viral mortality will likely also increase with increasing bacterial abundances.

The terrestrial organic matter input to the Arctic Ocean will also be likely be affected by global climate change, via the warming of permafrost and melting of snow and glaciers. In response to increases in terrestrial organic matter inputs, bacteria with

the ability to degrade terrestrially derived organic matter would increase in significance in the bacterioplankton community. For example, the dominance of *Sulfitobacter* in the extended Peat incubation alludes to the possibility that this bacterial group may play an important role in the cycling of terrestrially derived organic matter, and therefore this specific group might thrive under a regime of enhanced terrestrial DOM. Thus, regardless of the outcome of global warming, the results of this study seem to indicate that shifts in Arctic bacterioplankton community composition could occur in response to alterations in organic matter inputs to the Arctic Ocean, in turn affecting the cycling and export of organic carbon.

Bibliography

ABELL, G. C. J., and J. P. BOWMAN. 2005. Colonization and community dynamics of class *Flavobacteria* on diatom detritus in experimental mesocosms based on Southern Ocean seawater. FEMS Microbiology Ecology **53:** 379-391.

ALONSO, C., and J. PERNTHALER. 2005. Incorporation of glucose under anoxic conditions by bacterioplankton from coastal North Sea surface waters. Applied and Environmental Microbiology **71:** 1709-1716.

AMANN, R. I., W. LUDWIG, and K. H. SCHLEIFER. 1995. Phylogenetic Identification and in-Situ Detection of Individual Microbial-Cells without Cultivation. Microbiological Reviews **59:** 143-169.

AMON, R. M. W., H. P. FITZNAR, and R. BENNER. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnology and Oceanography **46:** 287-297.

AUSUBEL, F. M., R. BRENT, R. E. KINGSTON, D. D. MOORE, J. G. SEIDMAN, and K. S. (EDS). 1988. Current Protocols in Molecular Biology. Wiley Interscience**:** 1.7.1-1.7.5.

AZAM, F. 1998. Microbial control of oceanic carbon flux: the plot thickens. Science **280:** 694-696.

BANO, N., and J. T. HOLLIBAUGH. 2002. Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean. Applied and Environmental Microbiology **68:** 505-518.

BÉJÀ, O. and others 2002. Unsuspected diversity among marine aerobic anoxygenic phototrophs. Nature **415:** 630-633.

---. 2000. Construction and analysis of bacterial artificial chromosome libraries from a marine microbial assemblage. Environmental Microbiology **2:** 516-529.

BENNER, R. 2002. Chemical Composition and Reactivity, p. 59-90. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of Marine Dissolved Organic Matter. Academic Press.

BENNER, R., P. LOUCHOUARN, and R. M. W. AMON. 2005. Terrigenous dissolved organic matter in the Arctic Ocean and its transport to surface and deep waters of the North Atlantic. Global Biogeochemical Cycles **19**.

BOOTH, B. C., and R. A. HORNER. 1997. Microalgae on the Arctic Ocean Section, 1994:

species abundance and biomass. Deep-Sea Research Part Ii-Topical Studies in Oceanography **44:** 1607-1622.

BOUCHER, Y., M. KAMEKURA, and W. F. DOOLITTLE. 2004. Origins and evolution of isoprenoid lipid biosynthesis in archaea. Molecular Microbiology **52:** 515-527.

BRAKSTAD, O. G., and A. G. G. LODENG. 2005. Microbial diversity during biodegradation of crude oil in seawater from the North Sea. Microbial Ecology **49:** 94-103.

BRINKMEYER, R., K. KNITTEL, J. JÜRGENS, H. WEYLAND, R. AMANN, and E. HELMKE. 2003. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Applied and Environmental Microbiology **69:** 6610-6619.

BRUCE, K. D. 1997. Analysis of mer gene subclasses within bacterial communities in soils and sediments resolved by Fluorescent-PCR-restriction fragment length polymorphism profiling. Applied and Environmental Microbiology **63:** 4914-4919.

BUCHAN, A., L. S. COLLIER, E. L. NEIDLE, and M. A. MORAN. 2000. Key aromatic-ring-cleaving enzyme, protocatechuate 3,4-dioxygenase, in the ecologically important marine *Roseobacter* lineage. Applied and Environmental Microbiology **66:** 4662-4672.

BUCHAN, A., J. GONZÁLEZ, and M. A. MORAN. 2005. An Overview of The Marine *Roseobacter* Lineage. Appl Environ Microbiol **71:** 5665-5677.

BUCHAN, A., E. L. NEIDLE, and M. A. MORAN. 2001. Diversity of the ring-cleaving dioxygenase gene *pcaH* in a salt marsh bacterial community. Applied and Environmental Microbiology **67:** 5801-5809.

---. 2004. Diverse organization of genes of the beta-ketoadipate pathway in members of the marine *Roseobacter* lineage. Applied and Environmental Microbiology **70:** 1658-1668.

BUCHAN, A., S. Y. NEWELL, M. BUTLER, E. J. BIERS, J. T. HOLLIBAUGH, and M. A. MORAN. 2003. Dynamics of bacterial and fungal communities on decaying salt marsh grass. Applied and Environmental Microbiology **69:** 6676-6687.

BÜHRING, S. I., M. ELVERT, and U. WITTE. 2005. The microbial community structure of different permeable sandy sediments characterized by the investigation of bacterial fatty acids and fluorescence in situ hybridization. Environmental Microbiology **7:** 281-293.

CARLSON, C. A. and others 2002. Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea. Aquatic Microbial Ecology **30:** 19-36.

COTTRELL, M. T., and D. L. KIRCHMAN. 2000b. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Applied and Environmental Microbiology **66:** 1692-1697.

---. 2004a. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. Aq. Microb. Ecol. **34:** 139-149.

---. 2004b. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. Aquatic Microbial Ecology **34:** 139-149.

COTTRELL, M. T., D. N. WOOD, L. Y. YU, and D. L. KIRCHMAN. 2000c. Selected chitinase genes in cultured and uncultured marine bacteria in the α- and γ-subclasses of the Proteobacteria. Applied and Environmental Microbiology **66:** 1195-1201.

COVERT, J. S., and M. A. MORAN. 2001. Molecular characterization of estuarine bacterial communities that use high- and low-molecular weight fractions of dissolved organic carbon. Aquatic Microbial Ecology **25:** 127-139.

CRONAN, J. E. 2002. Phospholipid modifications in bacteria. Current Opinion in Microbiology **5:** 202-205.

CRUMP, B. C., G. W. KLING, M. BAHR, and J. E. HOBBIE. 2003. Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Applied and Environmental Microbiology **69:** 2253-2268.

CULLIS, P. R., D. B. FENSKE, and M. J. HOPE. 1996. Physical properties and functional roles of lipids in membranes, p. 1-33. *In* D. E. Vance and J. E. Vance [eds.], Biochemistry of Lipids, Lipoproteins and Membranes. Elsevier Science.

DAMSTÉ, J. S. S., W. I. C. RIJPSTRA, J. A. J. GEENEVASEN, M. STROUS, and M. S. M. JETTEN. 2005. Structural identification of ladderane and other membrane lipids of planctomycetes capable of anaerobic ammonium oxidation (anammox). FEBS Journal **272:** 4270-4283.

DEMING, J. W. 2002. Psychrophiles and polar regions. Current Opinion in Microbiology **5:** 301-309.

DONEY, S. C., M. R. ABBOTT, J. J. CULLEN, D. M. KARL, and L. ROTHSTEIN. 2004. From genes to ecosystems: the ocean's new frontier. Frontiers in Ecology and the Environment **2:** 457-466.

EILER, A., S. LANGENHEDER, S. BERTILSSON, and L. J. TRANVIK. 2003. Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. Applied and Environmental Microbiology **69:** 3701-3709.

EILERS, H., J. PERNTHALER, and R. AMANN. 2000a. Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. Applied and Environmental Microbiology **66:** 4634-4640.

EILERS, H., J. PERNTHALER, F. O. GLÖCKNER, and R. AMANN. 2000b. Culturability and in situ abundance of pelagic bacteria from the North Sea. Applied and Environmental Microbiology **66:** 3044-3051.

FALK-PETERSEN, S., J. R. SARGENT, J. HENDERSON, E. N. HEGSETH, H. HOP, and Y. B. OKOLODKOV. 1998. Lipids and fatty acids in ice algae and phytoplankton from the Marginal Ice Zone in the Barents Sea. Polar Biology **20:** 41-47.

FANDINO, L. B., L. RIEMANN, G. F. STEWARD, R. A. LONG, and F. AZAM. 2001. Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. Aquatic Microbial Ecology **23:** 119-130.

FANG, J., M. J. BARCELONA, and J. D. SEMRAUB. 2000a. Characterization of methanotrophic bacteria on the basis of intact phospholipid profiles. FEMS Microbiology Letters **189:** 67-72.

FANG, J. S., and M. J. BARCELONA. 1998. Structural determination and quantitative analysis of bacterial phospholipids using liquid chromatography electrospray ionization mass spectrometry. Journal of Microbiological Methods **33:** 23-35.

FANG, J. S., M. J. BARCELONA, and P. J. J. ALVAREZ. 2000b. A direct comparison between fatty acid analysis and intact phospholipid profiling for microbial identification. Organic Geochemistry **31:** 881-887.

FARRIMOND, P., H. M. TALBOT, D. F. WATSON, L. K. SCHULZ, and A. WILHELMS. 2004. Methylhopanoids: Molecular indicators of ancient bacteria and a petroleum correlation tool. Geochimica Et Cosmochimica Acta **68:** 3873-3882.

FELSENTEIN, J. 1989. PHYLIP—Phylogeny Inference Package (version 3.2). Cladistics **5:** 164-166.

FERGUSON, R. L., E. N. BUCKLEY, and A. V. PALUMBO. 1984. Response of marine bacterioplankton to differential filtration and confinement. Appl Environ Microbiol **47:** 49-55.

FINDLAY, S. E. G., R. L. SINSABAUGH, W. V. SOBCZAK, and M. HOOSTAL. 2003. Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. Limnology and Oceanography **48:** 1608-1617.

FRIEDRICH, C. G., D. ROTHER, F. BARDISCHEWSKY, A. QUENTMEIER, and J. FISCHER. 2001. Oxidation of reduced inorganic sulfur compounds by bacteria: Emergence of a common mechanism? Applied and Environmental Microbiology **67:** 2873-2882.

FUCHS, B. M., M. V. ZUBKOV, K. SAHM, P. H. BURKILL, and R. AMANN. 2000. Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometric and molecular biological techniques. Environmental Microbiology **2:** 191-201.

FULCO, A. J. 1983. Fatty-Acid Metabolism in Bacteria. Progress in Lipid Research **22:** 133-160.

GENNIS, R. B. 1989. Biomembranes: molecular structure and function. Springer-Verlag.

GICH, F., K. SCHUBERT, A. BRUNS, H. HOFFELNER, and J. OVERMANN. 2005. Specific detection, isolation, and characterization of selected, previously uncultured members of the freshwater bacterioplankton community. Applied and Environmental Microbiology **71:** 5908-5919.

GILLAN, F. T., and R. W. HOGG. 1984. A Method for the Estimation of Bacterial Biomass and Community Structure in Mangrove-Associated Sediments. Journal of Microbiological Methods **2:** 275-293.

GILLAN, F. T., G. I. MCFADDEN, R. WETHERBEE, and R. B. JOHNS. 1981. Sterols and Fatty-Acids of an Antarctic Sea Ice Diatom, *Stauroneis amphioxys*. Phytochemistry **20:** 1935-1937.

GIOVANNONI, S. J. 1991. The Polymerase chain reaction, p. 177-203. *In* E. Stackebrandt and M. Goodfellow [eds.], Modern Microbiological Methods: Nucleic Acids Techniques in Bacterial Systematics. John Wiley and Sons.

GIOVANNONI, S. J., T. B. BRITSCHGI, C. L. MOYER, and K. G. FIELD. 1990. Genetic Diversity in Sargasso Sea Bacterioplankton. Nature **345:** 60-63.

GIOVANNONI, S. J., and M. S. RAPPÉ. 2000. Evolution, diversity and molecular ecology of marine prokaryotes, In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley Interscience**:** 47-84.

GLÖCKNER, F. O., B. M. FUCHS, and R. AMANN. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Applied and Environmental Microbiology **65:** 3721-3726.

GLÖCKNER, F. O. and others 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an

abundant group of actinobacteria. Applied and Environmental Microbiology **66:** 5053-+.

GONZÁLEZ, J. M., R. P. KIENE, and M. A. MORAN. 1999. Transformation of sulfur compounds by an abundant lineage of marine bacteria in the α-subclass of the class *Proteobacteria*. Applied and Environmental Microbiology **65:** 3810-3819.

GONZÁLEZ, J. M. and others 2000. Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. Applied and Environmental Microbiology **66:** 4237-4246.

GONZÁLEZ, J. M., W. B. WHITMAN, R. E. HODSON, and M. A. MORAN. 1996. Identifying numerically abundant culturable bacteria from complex communities: An example from a lignin enrichment culture. Applied and Environmental Microbiology **62:** 4433-4440.

GREBMEIER, J. M. and others 1998. Western Arctic Shelf-Basin Interactions (SBI) Science Plan in Arctic System Science-Ocean-Atmosphere-Ice Interactions, p. 1-12. National Science Foundation.

GUCKERT, J. B., C. P. ANTWORTH, P. D. NICHOLS, and D. C. WHITE. 1985. Phospholipid, Ester-Linked Fatty-Acid Profiles as Reproducible Assays for Changes in Prokaryotic Community Structure of Estuarine Sediments. FEMS Microbiology Ecology **31:** 147-158.

HÄRTNER, T., K. L. STRAUB, and E. KANNENBERG. 2005. Occurrence of hopanoid lipids in anaerobic *Geobacter* species. FEMS Microbiology Letters **243:** 59-64.

HARVEY, H. R., R. Y. DYDA, and D. L. KIRCHMAN. 2005. The Impact of DOM Composition on Bacterial Lipids and Community Structure in Estuaries. Aquatic Microbial Ecology **in press**.

HARVEY, H. R., R. D. FALLON, and J. S. PATTON. 1986. The effect of organic matter and oxygen on the degradation of bacterial membrane lipids in marine sediments. Geochimica et Cosmochimica Acta **50:** 795-804.

HARVEY, H. R., and S. A. MACKO. 1997. Catalysts or contributors? Tracking bacterial mediation of early diagenesis in the marine water column. Organic Geochemistry **26:** 531-544.

HOEFS, M. J. L., S. SCHOUTEN, J. W. DELEEUW, L. L. KING, S. G. WAKEHAM, and J. S. S. DAMSTÉ. 1997. Ether lipids of planktonic Archaea in the marine water column. Appl. Environ. Microbiol. **63:** 3090-3095.

HOLLAND, M. M., and J. A. CURRY. 1999. The role of physical processes in determining

the interdecadal variability of central Arctic sea ice. Journal of Climate **12:** 3319-3330.

HUANG, C.-H., and S. LI. 1999. Calorimetric and molecular mechanics studies of the thermotropic phase behavior of membrane phospholipids. Biochimica et Biophysica Acta **1422:** 273-307.

HUGENHOLTZ, P., B. M. GOEBEL, and N. R. PACE. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. Journal of Bacteriology **180:** 4765-4774.

HUSTON, A. L., and J. W. DEMING. 2002. Relationships between microbial extracellular enzymatic activity and suspended and sinking particulate organic matter. Deep-Sea Res. II **49:** 5211-5225.

IVANOVA, E. P. and others 2002. Two species of culturable bacteria associated with degradation of brown algae *Fucus evanescens*. Microbial Ecology **43:** 242-249.

---. 2000. Evaluation of phospholipid and fatty acid compositions as chemotaxonomic markers of *Alteromonas*-like proteobacteria. Current Microbiology **41:** 341-345.

JARDILLIER, L. and others 2004. Bottom-up and top-down control of bacterial community composition in the euphotic zone of a reservoir. Aquatic Microbial Ecology **35:** 259-273.

JOHNSTON, N. C., and H. GOLDFINE. 1992. Replacement of the Aliphatic Chains of *Clostridium acetobutylicum* by Exogenous Fatty-Acids - Regulation of Phospholipid and Glycolipid Composition. Journal of Bacteriology **174:** 1848-1853.

JU, S. J., J. R. KUCKLICK, T. KOZLOVA, and H. R. HARVEY. 1997. Lipid accumulation and fatty acid composition during maturation of three pelagic fish species in Lake Baikal. Journal of Great Lakes Research **23:** 241-253.

KANEDA, T. 1991. Iso-Fatty and Anteiso-Fatty Acids in Bacteria - Biosynthesis, Function, and Taxonomic Significance. Microbiological Reviews **55:** 288-302.

KILLOPS, S. D., and V. J. KILLOPS. 1993. An Introduction to Organic Geochemistry. Wiley.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol **16:** 111-120.

KIRCHMAN, D. L. 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiology Ecology **39:** 91-100.

KIRCHMAN, D. L., A. I. DITTEL, S. E. G. FINDLAY, and D. FISCHER. 2004. Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. Aquatic Microbial Ecology **35:** 243-257.

KISAND, V., and J. WIKNER. 2003. Combining culture-dependent and -independent methodologies for estimation of richness of estuarine bacterioplankton consuming riverine dissolved organic matter. Applied and Environmental Microbiology **69:** 3607-3616.

KOGURE, K., U. SIMIDU, and N. TAGA. 1979. Tentative Direct Microscopic Method for Counting Living Marine-Bacteria. Canadian Journal of Microbiology **25:** 415-420.

LANGENHEDER, S., E. S. LINDSTRÖM, and L. J. TRANVIK. 2005. Weak coupling between community composition and functioning of aquatic bacteria. Limnology and Oceanography **50:** 957-967.

LECHEVALIER, H., and M. P. LECHEVALIER. 1988. Chemotaxonomic use of lipids – an overview, p. 869-902. *In* C. Ratledge and S. G. Wilkinson [eds.], Microbial lipids. Academic Press.

LENNON, J. T., and L. E. PFAFF. 2005. Source and supply of terrestrial organic matter affects aquatic microbial metabolism. Aquatic Microbial Ecology **39:** 107-119.

LIU, W. T., T. L. MARSH, H. CHENG, and L. J. FORNEY. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Applied and Environmental Microbiology **63:** 4516-4522.

LONG, R. A., and F. AZAM. 2001. Antagonistic interactions among marine pelagic bacteria. Applied and Environmental Microbiology **67:** 4975-4983.

LUDWIG, W. and others 2004. ARB: a software environment for sequence data. Nucleic Acids Research **32:** 1363-1371.

LURQUIN, P. F. 1997. Gene transfer by electroporation. Molecular Biotechnology **7:** 5-35.

MACDONALD, D. L., and H. GOLDFINE. 1991. Effects of Solvents and Alcohols on the Polar Lipid-Composition of *Clostridium butyricum* under Conditions of Controlled Lipid Chain Composition. Applied and Environmental Microbiology **57:** 3517-3521.

MALMSTROM, R. R., M. T. COTTRELL, H. ELIFANTZ, and D. L. KIRCHMAN. 2005. Biomass production and assimilation of dissolved organic matter by SAR11 bacteria in the Northwest Atlantic Ocean. Applied and Environmental Microbiology **71:** 2979-2986.

MALMSTROM, R. R., R. P. KIENE, M. T. COTTRELL, and D. L. KIRCHMAN. 2004a. Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the North Atlantic ocean. Applied and Environmental Microbiology **70:** 4129-4135.

MANNINO, A., and H. R. HARVEY. 1999. Lipid composition in particulate and dissolved organic matter in the Delaware Estuary: Sources and diagenetic patterns. Geochimica Et Cosmochimica Acta **63:** 2219-2235.

MARTINEZ, J., D. C. SMITH, G. F. STEWARD, and F. AZAM. 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquatic Microbial Ecology **10:** 223-230.

MASSANA, R., C. PEDRÓS-ALIÓ, E. O. CASAMAYOR, and J. M. GASOL. 2001. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. Limnology and Oceanography **46:** 1181-1188.

MERGAERT, J., A. VERHELST, M. C. CNOCKAERT, T. L. TAN, and J. SWINGS. 2001. Characterization of facultative oligotrophic bacteria from polar seas by analysis of their fatty acids and 16S rDNA sequences. Systematic and Applied Microbiology **24:** 98-107.

MEYERS, P. A. 1997. Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. Organic Geochemistry **27:** 213-250.

MIKI, T., and N. YAMAMURA. 2005. Theoretical model of interactions between particle-associated and free-living bacteria to predict functional composition and succession in bacterial communities. Aquatic Microbial Ecology **39:** 35-46.

MORITZ, R. E., C. M. BITZ, and E. J. STEIG. 2002. Dynamics of recent climate change in the Arctic. Science **297:** 1497-1502.

MOU, X. Z., M. A. MORAN, R. STEPANAUSKAS, J. M. GONZÁLEZ, and R. E. HODSON. 2005. Flow-cytometric cell sorting and subsequent molecular analyses for culture-independent identification of bacterioplankton involved in dimethylsulfoniopropionate transformations. Applied and Environmental Microbiology **71:** 1405-1416.

NICHOLS, D. S., P. D. NICHOLS, N. J. RUSSELL, N. W. DAVIES, and T. A. MCMEEKIN. 1997. Polyunsaturated fatty acids in the psychrophilic bacterium *Shewanella gelidimarina* ACAM 456^{T}: molecular species analysis of major phospholipids and biosynthesis of eicosapentaenoic acid. Biochimica Et Biophysica Acta-Lipids and Lipid Metabolism **1347:** 164-176.

NICHOLS, P. D., J. B. GUCKERT, and D. C. WHITE. 1986a. Determination of Monounsaturated Fatty-Acid Double-Bond Position and Geometry for Microbial Monocultures and Complex Consortia by Capillary Gc-Ms of Their Dimethyl Disulfide Adducts. Journal of Microbiological Methods **5:** 49-55.

NICHOLS, P. D., A. C. PALMISANO, G. A. SMITH, and D. C. WHITE. 1986b. Microbial Ecology in Antarctic Sea Ice and Benthic Communities .1. Lipids of the Antarctic Sea Ice Diatom *Nitzschia cylindrus*. Phytochemistry **25:** 1649-1653.

OGAWA, H., Y. AMAGAI, I. KOIKE, K. KAISER, and R. BENNER. 2001. Production of refractory dissolved organic matter by bacteria. Science **292:** 917-920.

OPSAHL, S., R. BENNER, and R. M. W. AMON. 1999. Major flux of terrigeneous dissolved organic matter through the Arctic Ocean. Limnol. Oceanogr. **44:** 2017-2023.

OUVERNEY, C. C., and J. A. FUHRMAN. 1999. Combined Microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types in situ. Applied and Environmental Microbiology **65:** 1746-1752.

ØVREÅS, L. and others 2003. Response of bacterial and viral communities to nutrient manipulations in seawater mesocosms. Aquatic Microbial Ecology **31:** 109-121.

PARRISH, C. C., and R. G. ACKMAN. 1983. Chromarod separations for the analysis of marine lipid classes by iatroscan chromatography-flame ionization detection Journal of Chromatography A **262:** 103-112.

PEARSON, A. and others 2004. Nonmarine crenarchaeol in Nevada hot springs. Applied and Environmental Microbiology **70:** 5229-5237.

PEREZ, M. T., C. PAUSZ, and G. J. HERNDL. 2003. Major shift in bacterioplankton utilization of enantiomeric amino acids between surface waters and the ocean's interior. Limnology and Oceanography **48:** 755-763.

PERNTHALER, A., J. PERNTHALER, H. EILERS, and R. AMANN. 2001a. Growth patterns of two marine isolates: Adaptations to substrate patchiness? Applied and Environmental Microbiology **67:** 4077-4083.

PERNTHALER, J., B. SATTLER, K. ŠIMEK, A. SCHWARZENBACHER, and R. PSENNER. 1996. Top-down effects on the size-biomass distribution of a freshwater bacterioplankton community. Aquatic Microbial Ecology **10:** 255-263.

PERRY, G. J., J. K. VOLKMAN, R. B. JOHNS, and H. J. BAVOR. 1979. Fatty-Acids of Bacterial Origin in Contemporary Marine-Sediments. Geochimica Et Cosmochimica Acta **43:** 1715-1725.

PINHASSI, J., and T. BERMAN. 2003. Differential growth response of colony-forming α- and γ-proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. Applied and Environmental Microbiology **69:** 199-211.

PINHASSI, J. and others 2004. Changes in bacterioplankton composition under different phytoplankton regimens. Applied and Environmental Microbiology **70:** 6753-6766.

PINHASSI, J., U. L. ZWEIFEL, and Å. HAGSTRÖM. 1997. Dominant marine bacterioplankton species found among colony-forming bacteria. Applied and Environmental Microbiology **63:** 3359-3366.

RAMOS, J. L. and others 2002. Mechanisms of solvent tolerance in gram-negative bacteria. Annual Review of Microbiology **56:** 743-768.

RATLEDGE, C., and S. G. WILKINSON. 1988. Fatty acids, related and derived lipids, p. 23-53. *In* C. Ratledge and S. G. Wilkinson [eds.], Microbial lipids. Academic Press.

RILFORS, L., and G. LINDBLOM. 2002. Regulation of lipid composition in biological membranes - biophysical studies of lipids and lipid synthesizing enzymes. Colloids and Surfaces B-Biointerfaces **26:** 112-124.

RINGELBERG, D. B., S. SUTTON, and D. C. WHITE. 1997. Biomass, bioactivity and biodiversity: microbial ecology of the deep subsurface: analysis of ester-linked phospholipid fatty acids. FEMS Microbiology Reviews **20:** 371-377.

RITCHIE, N. J., M. E. SCHUTTER, R. P. DICK, and D. D. MYROLD. 2000. Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Applied and Environmental Microbiology **66:** 1668-1675.

ROSE, A. H. 1988. Influence of the environment on microbial lipid composition, p. 255-278. *In* C. Ratledge and S. G. Wilkinson [eds.], Microbial Lipids. Academic Press.

RUSSELL, N. J. 1988. Functions of lipids: Structural roles and membrane functions, p. 279-365. *In* C. Ratledge and S. G. Wilkinson [eds.], Microbial lipids. Academic Press.

RÜTTERS, H., H. SASS, H. CYPIONKA, and J. RULLKÖTTER. 2002b. Phospholipid analysis as a tool to study complex microbial communities in marine sediments. Journal of Microbiological Methods **48:** 149-160.

SAMBROOK, J., E. F. FRITSCH, and T. MANIATIS. 1989. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press.

SCHULTZ, G. E., E. D. WHITE, and H. W. DUCKLOW. 2003. Bacterioplankton dynamics in the York River estuary: primary influence of temperature and freshwater inputs. Aquatic Microbial Ecology **30:** 135-148.

SCHWEIZER, E. 1988. Biosynthesis of fatty acids and related compounds, p. 3-50. *In* C. Ratledge and S. G. Wilkinson [eds.], Microbial lipids. Academic Press.

SHERR, E., and B. SHERR. 2000. Marine microbes: an overview, In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley Interscience**:** 13-46.

SHERR, E. B., B. F. SHERR, P. A. WHEELER, and K. THOMPSON. 2003. Temporal and spatial variation in stocks of autotrophic and heterotrophic microbes in the upper water column of the central Arctic Ocean. Deep-Sea Research Part I-Oceanographic Research Papers **50:** 557-571.

ŠIMEK, K. and others 2001. Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. Applied and Environmental Microbiology **67:** 2723-2733.

SMITH, G. A., P. D. NICHOLS, and D. C. WHITE. 1986. Microbial Ecology in Antarctic Sea Ice and Benthic Communities .2. Fatty-Acid Composition and Microbial Activity of Benthic Marine Sediment from Mcmurdo Sound, Antarctica. FEMS Microbiology Ecology **38:** 219-231.

STALEY, J. T., and A. KONOPKA. 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu Rev Microbiol **39:** 321-346.

STEIN, R., and R. W. MACDONALD. 2004. Organic carbon budget: Arctic Ocean vs. global ocean, p. 315-322. *In* R. Stein and R. W. MacDonald [eds.], The organic carbon cycle in the Arctic Ocean. Springer-Verlag.

STODEREGGER, K., and G. J. HERNDL. 1998. Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. Limnol. Oceanogr. **43:** 877-884.

STURT, H. F., R. E. SUMMONS, K. SMITH, M. ELVERT, and K. U. HINRICHS. 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry - new biomarkers for biogeochemistry and microbial ecology. Rapid Communications in Mass Spectrometry **18:** 617-628.

SUZUKI, M., M. S. RAPPÉ, and S. J. GIOVANNONI. 1998. Kinetic bias in estimates of coastal picoplankton community structure obtained by measurements of small-

subunit rRNA gene PCR amplicon length heterogeneity. Applied and Environmental Microbiology **64:** 4522-4529.

SUZUKI, M. T. 1999. Effect of protistan bacterivory on coastal bacterioplankton diversity. Aquatic Microbial Ecology **20:** 261-272.

SUZUKI, M. T., C. M. PRESTON, O. BÉJÀ , J. R. DE LA TORRE, G. F. STEWARD, and E. F. DELONG. 2004. Phylogenetic Screening of Ribosomal RNA Gene-Containing Clones in Bacterial Artificial Chromosome (BAC) Libraries from Different Depths in Monterey Bay. Microbial Ecology **48:** 473-488.

SUZUKI, M. T., C. M. PRESTON, F. P. CHAVEZ, and E. F. DELONG. 2001b. Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey Bay. Aquatic Microbial Ecology **24:** 117-127.

SUZUKI, M. T. and others 1997. Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. Applied and Environmental Microbiology **63:** 983-989.

SUZUKI, M. T., L. T. TAYLOR, and E. F. DELONG. 2000. Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. Appl Environ Microbiol **66:** 4605-4614.

SUZUMURA, M. 2005. Phospholipids in marine environments: a review. Talanta **66:** 422-434.

TALBOT, H. M., A. H. SQUIER, B. J. KEELY, and P. FARRIMOND. 2003. Atmospheric pressure chemical ionisation reversed-phase liquid chromatography/ion trap mass spectrometry of intact bacteriohopanepolyols. Rapid Communications in Mass Spectrometry **17:** 728-737.

THOMPSON, F. L., T. IIDA, and J. SWINGS. 2004. Biodiversity of Vibrios. Microbiology and Molecular Biology Reviews **68:** 403-+.

THOMPSON, J. R., L. A. MARCELINO, and M. F. POLZ. 2002. Heteroduplexes in mixed-template amplifications: formation, consequence and elimination by 'reconditioning PCR'. Nucleic Acids Research **30:** 2083-2088.

VAN DEN BURG, B. 2003. Extremophiles as a source for novel enzymes. Curr. Opin. Microbiol. **6:** 213-218.

VAVRUS, S., and S. P. HARRISON. 2003. The impact of sea-ice dynamics on the Arctic climate system. Climate Dynamics **20:** 741-757.

VELJI, M. I., and L. J. ALBRIGHT. 1993. Improved sample preparation for enumeration of aggregated aquatic substrate bacteria, In: Kemp, P.F., B.F. Sherr, E.B. Sherr, and

J.J. Cole (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers**:** 139-147.

VILA, M. and others 2004. Use of microautoradiography combined with fluorescence in situ hybridization to determine dimethylsulfoniopropionate incorporation by marine bacterioplankton taxa. Applied and Environmental Microbiology **70:** 4648-4657.

VISO, A. C., and J. C. MARTY. 1993. Fatty-Acids from 28 Marine Microalgae. Phytochemistry **34:** 1521-1533.

VOLKMAN, J. K., D. A. EVERITT, and D. I. ALLEN. 1986. Some Analyses of Lipid Classes in Marine Organisms, Sediments and Seawater Using Thin-Layer Chromatography Flame Ionization Detection. Journal of Chromatography **356:** 147-162.

VOLKMAN, J. K., R. B. JOHNS, F. T. GILLAN, G. J. PERRY, and H. J. BAVOR. 1980. Microbial Lipids of an Intertidal Sediment -1. Fatty-Acids and Hydrocarbons. Geochimica Et Cosmochimica Acta **44:** 1133-1143.

WAKEHAM, S. G. 1999. Monocarboxylic, dicarboxylic and hydroxy acids released by sequential treatments of suspended particles and sediments of the Black Sea. Organic Geochemistry **30:** 1059-1074.

WAKEHAM, S. G., T. K. PEASE, and R. BENNER. 2003. Hydroxy fatty acids in marine dissolved organic matter as indicators of bacterial membrane material. Organic Geochemistry **34:** 857-868.

WELLS, L. E., and J. W. DEMING. 2003. Abundance of Bacteria, the Cytophaga-Flavobacterium cluster and Archaea in cold oligotrophic waters and nepheloid layers of the Northwest Passage, Canadian Archipelago. Aquatic Microbial Ecology **31:** 19-31.

WHEELER, P. A. and others 1996. Active cycling of organic carbon in the central Arctic Ocean. Nature **380:** 697-699.

WHITE, D. C., W. M. DAVIS, J. S. NICKELS, J. D. KING, and R. J. BOBBIE. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia **40:** 51-62.

WHITE, D. C., J. O. STAIR, and D. B. RINGELBERG. 1996. Quantitative comparisons of in situ microbial biodiversity by signature biomarker analysis. Journal of Industrial Microbiology **17:** 185-196.

WHITMAN, W. B., D. C. COLEMAN, and W. J. WIEBE. 1998. Prokaryotes: The unseen majority. Proceedings of the National Academy of Sciences of the United States of America **95:** 6578-6583.

WOESE, C. R., and G. E. FOX. 1977. Phylogenetic Structure of Prokaryotic Domain - Primary Kingdoms. Proceedings of the National Academy of Sciences of the United States of America **74:** 5088-5090.

WOMMACK, K. E., and R. R. COLWELL. 2000. Virioplankton: Viruses in aquatic ecosystems. Microbiology and Molecular Biology Reviews **64:** 69-+.

YAGER, P. L. and others 2001. Dynamic bacterial and viral response to an algal bloom at subzero temperatures. Limnology and Oceanography **46:** 790-801.

YANO, Y., A. NAKAYAMA, K. ISHIHARA, and H. SAITO. 1998. Adaptive changes in membrane lipids of barophilic bacteria in response to changes in growth pressure. Applied and Environmental Microbiology **64:** 479-485.

ZEHR, J. P., and B. B. WARD. 2002. Nitrogen cycling in the ocean: New perspectives on processes and paradigms. Applied and Environmental Microbiology **68:** 1015-1024.

ZINK, K. G., and K. MANGELSDORF. 2004. Efficient and rapid method for extraction of intact phospholipids from sediments combined with molecular structure elucidation using LC-ESI-MS-MS analysis. Analytical and Bioanalytical Chemistry **380:** 798-812.

ZINK, K. G., H. WILKES, U. DISKO, M. ELVERT, and B. HORSFIELD. 2003. Intact phospholipids - microbial "life markers" in marine deep subsurface sediments. Organic Geochemistry **34:** 755-769.

ZOU, L. and others 2004. Bacterial roles in the formation of high-molecular-weight dissolved organic matter in estuarine and coastal waters: Evidence from lipids and the compound-specific isotopic ratios. Limnology and Oceanography **49:** 297-302.