MICROBIAL COMMUNITY STRUCTURE IN THE NORTHWEST ATLANTIC OCEAN AS DETERMINED BY FLUORESCENCE IN SITU HYBRIDIZATION

KIMBERLEY F. KEATS

MICROBIAL COMMUNITY STRUCTURE IN THE NORTHWEST ATLANTIC OCEAN AS DETERMINED BY FLUORESCENCE *IN SITU* HYBRIDIZATION

by

© Kimberley F. Keats

A thesis submitted to the

School of Graduate Studies

in partial fulfilment of the

requirements for the degree of

Master of Science

Environmental Science Programme

Memorial University of Newfoundland

December 2006

St. John's Newfoundland and Labrador

Your file Votre référence
ISBN: 978-0-494-31263-6
Our file Notre référence
ISBN: 978-0-494-31263-6

Abstract

Fluorescence *in situ* hybridization (FISH) is widely used to characterize the phylogenetic diversity of microbial communities. Here I present the results of a laboratory-based study of the effects of sample storage on the detection of bacterial cells using FISH, as well as a field- and laboratory-based study of changes in the phylogenetic diversity of the microbial community in biogeochemical provinces of the Northwest Atlantic Ocean over three consecutive seasons, and how these changes relate to biogeochemical and ecosystem processes on a large geographical and temporal scale. Results from the 12-month time-course study of storage effects indicate that samples can be stored for up to six months with no significant change in target cell detection, and stored for up to 12 months with a minimal (< 7%) effect of storage. Results from the Northwest Atlantic study show variations in microbial community structure between biogeochemical provinces, as well as seasonally within each province. From summer to fall, there was a noticeable decrease in the proportion of *Cytophaga-Flavobacteria*, and the α-, β-, and γ-*Proteobacteria* dominated the fall community at all stations. Seasonally, the most noticeable shift in the relative abundances of specific phylogenetic groups occurred from summer to fall. Spatially, the greatest differences in bacterial community composition were observed between the North Atlantic Subtropical Gyre and the Atlantic Arctic province.

Acknowledgements

First and foremost, I would like to thank my supervisor, Richard Rivkin. Thank you for your invaluable advice, support, and encouragement, as well as your intellectual input and editing assistance. Thank you for always believing in my capabilities as a student and as a researcher.

Many thanks to my supervisory committee members, David Schneider and John Gow. Thanks you for your useful comments and editing assistance. Dave, thanks especially for your invaluable statistical advice!

Thanks to the Rivkin lab: Michelle Hale, Heather Evans, Diane Horlick, Ashley Callahan, and Paul Matthews. Thank you all for your assistance with sample collection and analyses.

Thank you to Chris Parrish, Chair of the Environmental Science Programme, and Gail Kenny, secretary for the interdisciplinary science programmes.

Thanks to everyone involved in the Canadian Surface Ocean - Lower Atmosphere Study (C-SOLAS). Thanks especially to the captain and crew of the CCGS *Hudson* and NGCC *Martha L. Black*; Maurice Levasseur, C-SOLAS project leader; Sylvie Roy, cruise coordinator; as well as Bill Miller, Michel Gosselin, and Michael Scarratt, chief scientists.

I would like to thank Bill Li for providing the flow cytometry data; Michael Scarratt and Sonia Michaud for the DMS(P) data; Michel Gosselin, Zhi-Ping Mei, and Julien Pommier for the chlorophyll *a* data, and Zhi-Ping also for the surface mixed layer calculations. I would also like to thank Emmanuel Devred, Shuba Sathyendranath, Trevor Platt, and César Fuentes-Yaco for providing the updated biogeochemical province designations.

Thanks to our funding sources. This research was supported in part by a fellowship from the School of Graduate Studies, Memorial University of Newfoundland, and grants to Dr. Rivkin from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the C-SOLAS Research Network. Funding was provided to C-SOLAS by NSERC and the Canadian Foundation for Climate and Atmospheric Sciences.

Many, many thanks to my family. Mom, Dad, Bert, Nan, Denise, Aunt Jessie, and the rest of the crew. I am truly blessed to have such a wonderful and supportive family. I love you all dearly. Thank you for your advice and encouragement.

Many thanks to my favourite gals - Denise, Steph, Michelle, Sarah, Ruth, Tara, Vanessa, Lene, and Bronwyn. Thank you for always being there for a good conversation, a lot of laughs, and a break from thesis writing!

Thanks to my fellow students in the 2006 Agouron Institute – University of Hawaii Summer Course in Microbial Oceanography. Thank you for reminding me of the reasons that I chose to study oceanography… for all of the engaging discussions, and your intellectual input and endless enthusiasm. Thanks to Matt Church, Dave Karl, and the entire HOT (Hawaii Ocean Time-Series) team, as well as Mel Simon and the Agouron Institute.

My list of thanks would not be complete if I did not acknowledge Moire Wadleigh, who passed away in the fall of 2004. Moire was my co-supervisor and Chair of the Environmental Science Programme when I began my degree. Moire was a beautiful person, a caring professor, and a dedicated scientist. The Moire A. Wadleigh Graduate Award for Excellence in Environmental Science has been established at Memorial University in her memory.

To everyone who has helped me complete my Master's thesis – whether by assisting with sample collection and analyses; providing additional data, intellectual input, and editing assistance; or giving advice, encouragement, and moral support…

THANK YOU.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1: Introduction

It is widely recognized that global climate is changing. However, a major uncertainty in climate models is the prediction of future levels of atmospheric greenhouse gases, such as CO_2. As a result, it is difficult to predict future climate change (Hansen et al. 1998). Determining the role of the oceans in the uptake or release of CO_2 is fundamental to understanding the relationship between anthropogenic emissions of CO_2 and atmospheric levels (Schimel 1998). The World Ocean is an important sink for climate-active gases, such as CO_2, but ocean-carbon models generally assume that ocean circulation and biogeochemical processes remain unchanged over time (Sarmiento et al. 1998). Other modeling studies have suggested that the capacity of the oceans to sequester CO_2 is largely determined by biogeochemical processes and that changes in physical and biological processes would thus influence ocean carbon uptake (Sarmiento et al. 1998). One of the primary goals of the suite of projects sponsored by the International Geosphere-Biosphere Programme (IGBP), including AIMES (Analysis, Integration and Modeling of the Earth System), GLOBEC (Global Ocean Ecosystem Dynamics), IMBER (Integrated Marine Biogeochemistry and Ecosystem Research), and SOLAS (Surface Ocean Lower Atmosphere Study), is to improve model parameterizations and reduce uncertainties about the role of the oceans in mediating changes in atmospheric concentrations of greenhouse gases.

The World Ocean can be partitioned into ecological regions; the primary compartments being the Polar, Westerlies, Trades, and Coastal Boundary Zone biomes (Longhurst 1998). Within each of these biomes, the oceans can be further partitioned into

biogeochemical provinces, which are defined on the basis of physical processes and the biological response to spatial and temporal variations in physical forcing (Platt and Sathyendranath 1999). The partitioning of the oceans into biogeochemical provinces by Longhurst (1998) relied on global surface chlorophyll field data determined by the Coastal Zone Colour Scanner (CZCS). The critical conditions for defining biogeochemical provinces include the subsurface chlorophyll profile, values for photosynthetic parameters, and depths of the mixed layer and nutricline (Longhurst 1998). The boundaries of biogeochemical provinces are not discrete and thus, vary at interannual and seasonal time scales (Platt and Sathyendranath 1999). However, identifying water masses by a defined set of characteristics is useful in determining regional variations in the structure of the pelagic food web, specifically the autotrophic phytoplankton and heterotrophic bacteria (Li and Harrison 2001). The structure of the microbial food web is controlled largely by biogeochemical processes in the upper ocean, resulting in phylotypes and trophic levels with different physiological characteristics and metabolic capacities, and thus, different roles in ecosystem function. Variations in the structure and dynamics of the microbial community in contrasting biogeochemical provinces of the oceans will lead to distinct patterns in the cycling of climate-active gases (e.g. CO_2, volatile sulphur, and halogenated compounds) which, in turn, will have important feedbacks on climate.

The importance of microbial food webs in carbon cycling and their possible role in the export, and sequestration, of biogenic carbon have been studied more in recent years, particularly since it has been recognized that microorganisms have a greater role in

pelagic food webs than was previously thought (Legendre and Le Fèvre 1995). Heterotrophic microbial metabolism is an important pathway for the transformation of organic matter in the oceans and is responsible for a majority of the remineralization of biogenic carbon in the upper ocean (Williams 2000). Thus, spatial and temporal variability in microbial community structure may also influence the export, and sequestration, of biogenic carbon in the oceans.

1.1 Role of *Bacteria* and *Archaea* in Ocean Biogeochemistry

The past three decades have seen major advances in the field of marine microbial ecology, beginning with the landmark paper by Pomeroy (1974) identifying bacteria as highly metabolically active components of marine food webs. Although earlier studies implicated bacteria in the utilization of particulate and dissolved organic matter in the oceans, the role of bacteria in marine ecosystems remained poorly understood (Azam and Fuhrman 1984). Azam et al. (1983) suggested that bacteria constitute a significant portion of the total biomass of the oceans and that bacterial biomass had been largely underestimated, due to the small size and low culturability of marine bacteria. It is now well recognized that bacteria are highly abundant in the oceans, often dominating the biomass of planktonic food webs (Azam 1998) and providing a major metabolic pathway for the transformation of dissolved organic matter (Williams 2000).

Dissolved organic matter (DOM) represents one of the largest sinks of organic carbon in the biosphere and is of critical importance in determining global carbon budgets (Azam 1998). Since heterotrophic microbial metabolism is the major pathway for the

transformation of DOM in the oceans, understanding the central role of heterotrophic bacteria in carbon cycling has become increasingly important as it is now accepted that global climate is changing with increasing levels of carbon dioxide (Azam 1998). In order to determine how bacterial metabolism influences biogeochemical processes in the oceans, it is important to study bacterial communities on an appropriate habitat scale and incorporate factors that control their growth in the water column (Azam and Worden 2004). The growth of bacteria is controlled by a myriad of factors, including temperature (Rivkin et al. 1996; Kirchman and Rich 1997), salinity (Hobbie 1988; Gonzalez and Moran 1997), pH (Hiorns et al. 1997), radiation (Herndl et al. 1993; Aas et al. 1996), and trace metal supply (Tortell et al. 1996). The most important, and variable, factors are water temperature and the availability of inorganic and organic nutrients in the water column. The distribution of marine bacteria is also determined by depth, particle association, predation (grazing) and virus-mediated mortality (Nold and Zwart 1998). The environmental variables that govern the growth of bacteria also determine the extent to which bacteria are able to metabolize DOM, and thus have important implications for the cycling and remineralization of biogenic carbon.

1.2 Microbial Community Structure and Function

Until recently, studies of heterotrophic bacteria in mediating water-column biogeochemical processes have focused on these bacteria as a single group, neglecting the phylogenetic diversity of bacterial communities (Kirchman 2002). Although there has been some progress in characterizing the diversity of marine *Bacteria* and *Archaea*, very little is known about the function of specific bacterial phylotypes (Giovannoni and Rappé

2000). Molecular phylogenetic analyses of prokaryotic assemblages have indicated that representatives from nearly all of the major divisions of *Bacteria* and *Archaea* can be found in aquatic ecosystems (Kirchman 2002). As with marine microbial ecology, the past three decades have seen major advances in molecular phylogenetics, the study of genetic relationships and evolutionary history using the primary structure of informational macromolecules, i.e. DNA, RNA and proteins (DeLong 1998b). With the development of a molecular sequence-based phylogenetic tree (Woese and Fox 1977), which related all organisms through comparison of ribosomal RNA (rRNA) sequences, the three primary domains of life were defined: *Eucarya* (eukaryotes), *Bacteria* (eubacteria), and *Archaea* (archaebacteria). The universal phylogenetic tree provides a reference framework for identifying organisms through the use of gene sequences (Pace 1997) and has become a useful tool in the study of microbial diversity (Head et al. 1998) since rRNA sequences are used to design oligonucleotide probes for the detection of individual species, genera and families of microorganisms (DeLong et al. 1989).

Prior to the use of rRNA sequences in establishing the phylogenetic diversity of bacteria, studies relied heavily on morphological and metabolic distinctions of specific bacterial groups (Pace 1997). The low culturability of marine bacteria (<< 1%) has not allowed for the laboratory isolation and characterization of specific bacterial phylotypes on the basis of optimal growth conditions, therefore recent studies have relied on cultivation-independent methods of determining microbial community structure and function (Macalady and Banfield 2003). The extraction and amplification of gene sequences directly from the natural environment has resulted in the detection of whole groups of

previously unrecognized *Bacteria* and *Archaea* (DeLong 1998b; Hugenholtz et al. 1998; Rappé and Giovannoni 2003; Venter et al. 2004; DeLong and Karl 2005), and has motivated new studies to determine the metabolic capacities of specific bacterial groups (Macalady and Banfield 2003).

1.2.1 Dimethylsulphide and the Microbial Food Web

Dimethylsulphide (DMS) is the principal volatile sulphur-containing compound in the oceans and the major natural source of sulphur to the atmosphere (Denman et al. 1996). Recent estimates of the marine emission flux of DMS range from 15×10^{12} to 33×10^{12} g S y^{-1} (Simó 2001). DMS emissions from the world's oceans are equivalent to one-third of global anthropogenic sulphur emissions and compensate for a sulphur deficiency on the continents that is the result of runoff losses to the oceans (Simó 2001). In the atmosphere, DMS is oxidized to produce sulphur aerosols that, either directly or indirectly though the development of clouds, scatter solar radiation and affect the radiative balance of the earth, thus influencing climate (Denman et al. 1996).

Oceanic production of atmospheric sulphur is a function of phytoplankton biomass, diversity, and activity. DMS is produced by the enzymatic breakdown of dimethylsulphoniopropionate (DMSP), which is abundant in phytoplankton and a major sulphur carrier for the transfer of DMS through the marine food web (Simó 2001). DMSP, the biological precursor of DMS, contains a methylated, reduced sulphur component and is thus prone to microbial degradation (Yoch 2002). Particulate DMSP,

when released, either by direct excretion, grazing or viral lysis, as dissolved DMSP, is readily metabolized by heterotrophic bacteria to sulphate, DMS and methanethiol (Zubkov et al. 2001). Dissolved DMSP can satisfy from 1 to 15% of the total bacterial carbon demand and nearly 100% of the total bacterial sulphur demand (Simó et al. 2002; Malmstrom et al. 2004b).

Recent studies indicate that bacteria are a major sink for DMS (González et al. 2000; Kiene et al. 2000; Simó et al. 2002; Malmstrom et al. 2004b). When bacterial sulphur demand is high, more DMSP will be used as a sulphur source for biomass production, and less will be released as DMS (Simó 2001). Thus, bacteria have a controlling role in the amount of DMS that is released into the atmosphere by switching DMSP degradation towards more or less DMS production (Yoch 2002). Consequently, factors that control bacterial activity, such as UV-B radiation, temperature, nutrients, and dissolved organic matter, are also important in controlling DMS concentrations (Simó 2001).
Although it is known that DMSP is either assimilated into biomass or metabolized to DMS by bacteria, little is known about the specific bacterial groups involved in DMSP consumption and net DMS production (Zubkov et al. 2001). Recent studies of the functional diversity of bacteria, through the use of molecular techniques, indicate that DMSP is not a universal growth substrate for heterotrophic bacteria (González et al. 1999; González et al. 2000; Moran et al. 2003; Malmstrom et al. 2004b). Conversely, these studies indicate that DMSP degradation is characteristic of only a few bacterial metabolisms, but that these bacterial groups may constitute a large fraction of total bacterial activity (Simó 2001).

The production and consumption of volatile sulphur-containing compounds in the oceans is largely influenced by microbial metabolism, yet little is known about the structure and function of specific bacterial groups or the factors that control their distribution and activity (Simó 2001). It appears that not all heterotrophic bacteria can assimilate DMSP into biomass and form DMS, but that few bacterial groups, such as the *Roseobacter* clade of α-*Proteobacteria,* can both utilize DMSP as a growth substrate and metabolize it to DMS (Moran et al. 2003). Thus, the phylogenetic structure of bacterial communities is an important factor to consider in determining the role of bacteria in DMSP consumption and net DMS production.

1.2.2 Linking Phylogeny to Function in Microbial Communities

Although there has been some recent progress in characterizing the diversity of marine bacterial assemblages, linking community structure with specific biogeochemical processes has proven to be difficult (Kirchman 2002). Only a small fraction of naturally occurring bacteria, as identified by rRNA clones, appear to be closely related to cultivated bacteria from the same environments (DeLong 1998b). This indicates that the use of culture-based methods in modelling specific biogeochemical processes does not provide an accurate representation of natural ecosystem dynamics (Zehr and Voytek 1998). Determining the abundance, biomass, phylogenetic diversity and metabolic activity of bacteria is fundamental to linking the dynamics of microbial communities with larger scale ecosystem processes (Kirchman 2002). Bacterial parameters, such as biomass, growth rate and growth efficiency are determined by the availability of DOM, and are likely to differ among bacterial phylotypes (Kirchman 2003). Given the tremendous

phylogenetic diversity of marine microbial communities, it is a vast assumption that all bacteria are metabolically active or functioning equally in the biogeochemical cycling of DOM. It is most likely that different bacterial phylotypes also differ in their growth capacities and contribution to the cycling and transformation of DOM (Kirchman 2003). A study by Cottrell and Kirchman (2000b) of the relative contributions of specific bacterial phylotypes to the utilization of DOM in the Delaware Bay estuary, using microautoradiography combined with fluorescence *in situ* hybridization (Micro-FISH), determined that all phylotypes do not utilize DOM equally. The *Cytophaga-Flavobacteria* cluster comprised the largest fraction of cells assimilating chitin and protein, but the smallest fraction consuming amino acids. In contrast, the assimilation of amino acids was dominated by the α-*Proteobacteria*, for which protein consumption was lowest. Thus, understanding the role of bacteria in biogeochemical models, such as those for ocean-carbon cycling, requires knowledge of not only the ecology but also the phylogenetic diversity and metabolic activity of microbial communities.

1.3 Molecular Approaches to Characterizing Microbial Community Structure

Analyses of small-subunit (16S or 18S) rRNA and other gene sequences have allowed for the development of molecular methods for characterizing the diversity of microbial assemblages (Hugenholtz et al. 1998). Gene sequence information has facilitated the design of rRNA-targeted oligonucleotide probes to identify microorganisms at target taxonomic levels through fluorescence *in situ* hybridization (FISH) (Glöckner et al. 1996; Hugenholtz et al. 2001; Pernthaler et al. 2001), as well as the development of specific primers for Polymerase Chain Reaction (PCR)-based approaches (Pace et al. 1986;

Fuhrman et al. 1993; Zehr and Voytek 1999). PCR-based approaches involve the isolation of total community DNA, which is used as a template for the amplification of 16S rRNA genes with specific primers (Liu et al. 1997). The PCR amplification of 16S rRNA genes is followed by the standard cloning of community gene products. Comparative sequence analyses of individual clones provide the basis for development of clone libraries, which are then used for probe design (Egert and Freidrich 2003). Other PCR-based techniques, such as Denaturing Gradient Gel Electrophoresis (DGGE), Single-stranded Site Conformational Polymorphism (SSCP), and Terminal Restriction Fragment Length Polymorphism (T-RFLP), involve the separation of amplified community gene products through methods of DNA melting, single-strand DNA conformation, and labelling of terminal restriction fragments, respectively (Liu et al. 1997; Egert and Freidrich 2003; Dorigo et al. 2005; Spiegelman et al. 2005).

1.3.1 Fluorescence *In Situ* Hybridization

In contrast to the PCR-based fingerprinting techniques, the whole cell *in situ* hybridization techniques, such as FISH, involve the hybridization of fluorescently-labelled rRNA-targeted oligonucleotide probes to group specific gene sequences, and thus identify cells (i.e. unicellular microorganisms) at target taxonomic levels (Glöckner et al. 1996). The FISH method has been employed in a number of studies of microorganisms, primarily from the domains *Bacteria* and *Archaea*, but also from the domain *Eucarya*, for the detection of nano- and picophytoplankton (Simon et al. 1995), nano-planktonic protists (Lim et al. 1993; Lim et al. 1995) and photosynthetic pico-heterokonts (stramenopiles) (Massana et al. 2002). FISH has been used to characterize the

phylogenetic composition of microbial assemblages in lakes and estuaries (Pernthaler et al. 1997, Glöckner et al. 1999; Bouvier and del Giorgio 2002; Heidelberg et al. 2002; Cottrell and Kirchman 2003; Zwisler et al. 2003), limnetic organic aggregates (lake snow) (Weiss et al. 1996; Schweitzer et al. 2001), soil (Hahn et al. 1992; Christensen et al. 1999), activated sludge flocs and biofilms (Wagner et al. 1994; Amann et al. 1996; Daims et al. 2001), ice and snow (Alfreider et al. 1996; Brinkmeyer et al. 2003), as well as coastal and oceanic marine habitats (Karner and Fuhrman 1997; DeLong et al. 1999; Glöckner et al. 1999; Cottrell and Kirchman 2000a, 2000b; Wells and Deming 2003).

Although FISH is now widely used for determining the phylogenetic composition of microbial communities, this technique does have both advantages and disadvantages. The major advantage of the FISH method is that it allows for a more quantitative description of the microbial community structure than PCR-based approaches since total cell counts, as determined by 4', 6'-diamidino-2-phenylindol (DAPI), and probe-positive counts for target phylogenetic groups are used to determine the relative abundances of those groups (Glöckner et al. 1999). However, one of the major limitations of the FISH technique is that the effectiveness of target cell detection varies widely (Bouvier and del Giorgio 2003). The percentage of cells detected with the Eubacteria probe (EUB338) varies from 1% in soil to 100% in enriched culture, with an average of 56% for all ecosystem types (Bouvier and del Giorgio 2003). In oceanic environments, detection with the EUB338 probe ranged from 39% at 1 m in the North Sea to 96% at 0 m in the Antarctic Ocean (Glöckner et al. 1999). Although results from studies of microbial community composition have been compiled for nearshore (< 5 miles offshore) and

continental shelf (5-50 offshore) environments (Glöckner et al. 1999; Bouvier and del Giorgio 2003), there have been very few studies of open ocean environments.

A number of methodological factors contributing to the variation in target cell detection have been identified, and these include: cell fixation, cell storage, hybridization conditions, mounting solutions, fluorochrome type, and counting methods (Bouvier and del Giorgio 2003; Williams et al. 2004). Other factors that may contribute to a low detection signal using FISH are poor accessibility to probe target sites (rRNA) and low ribosome content (Wagner et al. 2003). The physiological state of target cells appears to have a significant effect on target cell detection, since faster-growing and highly-active cells have more ribosomes and bind more to probe molecules, resulting in a stronger detection signal (Bouvier and del Giorgio 2003). As well, it is now recognized that certain phylogenetic groups may differ in their metabolic capacities and functional roles in biogeochemical cycling (Cottrell and Kirchman 2000b; Kirchman 2002, 2003) and this may influence the detection of these cells using FISH (Bouvier and del Giorgio 2003).

1.4 Major Groups of Marine *Bacteria*

The most widely studied groups of marine *Bacteria* include the *Cytophaga-Flavobacteria* cluster and the alpha, beta, and gamma subclasses of *Proteobacteria.* Thus, rRNA-targeted oligonucleotide probes have been designed for the phylogenetic analysis of microorganisms at the highest taxonomic level: domain *Bacteria* (EUB338); as well as the intermediate taxonomic levels: alpha (ALF968), beta (BET42a) and gamma

(GAM42a) subclasses of *Proteobacteria*, and the *Cytophaga-Flavobacteria* (CF319a) cluster (Glöckner et al. 1999).

1.4.1 *Cytophaga-Flavobacteria* Cluster

Heterotrophic bacteria belonging to the *Cytophaga-Flavobacteria* cluster of the *Cytophaga-Flavobacterium-Bacteroides* phylum (*CFB*-phylum) are numerically abundant in a range of habitats, including ocean sediments (Llobet-Brossa et al. 1998), polluted river waters (Kenzaka et al. 1998), hydrothermal vents (Sievert et al. 2000), and Antarctic rocks and sea ice (Smith et al. 2000). This group includes the unicellular, gliding, nonspore-forming, gram-negative, rod-shaped bacteria of the *Cytophaga* and *Flavobacterium* genera (Weller et al. 2000; Kirchman 2002). Most *Cytophaga-Flavobacteria* are aerobic and chemoorganotrophic, functioning in the degradation of biopolymers such as cellulose, chitin, and pectin (Giovannoni and Rappé 2000; Kirchman 2002). Uncultured *Cytophaga-Flavobacteria* are widespread in the marine environment and have been found associated with marine organic aggregates as well as free-living in seawater (Giovannoni and Rappé 2000). The *Cytophaga-Flavobacteria* cluster is generally the most numerically abundant phylogenetic group in oceanic habitats (Glöckner et al. 1999), often constituting as much as half of the total bacteria identified by FISH (Kirchman 2002).

1.4.2 *Proteobacteria*

The *Proteobacteria* group of *Bacteria* includes the alpha, beta, gamma, delta, and epilson subclasses, and is considered to be the most diverse and successful taxon in the oceans

(Giovannoni and Rappé 2000). The *Proteobacteria* are gram-negative and are all thought to have derived from an ancestral purple photosynthetic bacteria (Dyer 2003).

1.4.2.1 Alpha-*Proteobacteria*

The alpha subclass of *Proteobacteria* is widespread and includes both culturable and nonculturable members. The culturable α-*Proteobacteria* include representatives from the *Roseobacter* and *Sphingomonas* clades, however a majority of the marine α-*Proteobacteria* appear to be highly specialized and unculturable species (Giovannoni and Rappé 2000). Members of the *Roseobacter* clade, of the α-3 subclass of *Proteobacteria*, are prevalent in marine and hypersaline environments, but absent from freshwater and terrestrial soil environments (Buchan et al. 2005). In contrast, the *Roseobacter* clade is ubiquitous in marine habitats, and is most often associated with marine algae, particularly during phytoplankton blooms (González et al. 2000; Zubkov et al. 2001, 2002). *Roseobacter* populations have also been associated with sea ice (Brown & Bowman 2001; Brinkmeyer et al. 2003), diseased corals (Cooney et al. 2002; Pantos et al. 2003), sponges (Taylor et al. 2004; Webster et al. 2004), microbial mats (Jonkers and Abed 2003), sea grasses (Weidner et al. 2000), coastal biofilms (Dang and Lovell 2000, 2002), and hypersaline lakes (Suzuki et al. 1999; Lebrenz et al. 2000).

The *Roseobacter* clade has been identified as a key participant in the metabolism of DMSP, a precursor of DMS. A study by González et al. (2000), characterizing the bacterial community structure associated with a DMSP-producing phytoplankton bloom in the North Atlantic, has provided evidence that the marine *Roseobacter* clade of the α-

subclass of *Proteobacteria* is numerically abundant in the heterotrophic bacterial community and that *Roseobacter* phylotype abundance is positively correlated with DMSP concentrations. Other studies of bacteria isolated from DMSP enrichments of coastal seawater (González et al. 1999) and DMSP-producing algal blooms (González et al. 2000; Zubkov et al. 2002) have also implicated the α-*Proteobacteria*, which are abundant in both coastal and open-ocean waters (Giovannoni & Rappé 2000), in the transformation of DMSP to DMS and other volatile dissolved organic sulphur compounds (González et al. 1999; Moran et al. 2003; Malmstrom et al. 2004b). A recent study by Malmstrom et al. (2004b) also identified the *Roseobacter* clade as the key participant in the biogeochemical transformation of dissolved DMSP during non-bloom conditions. In both the Sargasso Sea and the Gulf of Marine, the α-*Proteobacteria* accounted for approximately 40% of the DMSP-assimilation, followed by the *Cytophaga-Flavobacteria* cluster in the Sargasso Sea and the γ-*Proteobacteria* in the Gulf of Maine.

Of the uncultured α-*Proteobacteria*, the SAR11 cluster is the most abundant and ubiquitous (Giovannoni & Rappé 2000). Within heterotrophic marine bacterioplankton, the SAR11 clade accounts for 26% of the derived rRNA gene clones, whereas the *Roseobacter* clade accounts for 16% (Selje et al. 2004). In subtropical regions, such as the Sargasso Sea, the members of SAR11 clade often dominate the surface bacterial community and may constitute as much as half of the identified cells (Morris et al. 2002; Selje et al. 2004). Despite their widespread abundance in marine environments, little is known about the physiology and biogeochemical role of SAR11 clade (Morris et al. 2002;

Malmstrom et al. 2004a). However, a recent study by Malmstrom et al. (2004a), in the coastal North Atlantic Ocean and the Sargasso Sea, found that bacteria belonging to the SAR11 clade were highly metabolically active in surface waters, accounting for approximately 50% of the amino acid assimilation and 30% of the DMSP consumption. These results indicate that the SAR11 clade may outcompete other bacterial groups for dissolved free amino acids and DMSP, and thus may play a significant role in the cycling of carbon, nitrogen, and sulphur in the oceans (Malmstrom et al. 2004a).

1.4.2.2 Beta-*Proteobacteria*

The beta subclass of *Proteobacteria* accounts for only about 2.5% of all environmental 16S rDNA clones; the majority of these having been recovered from freshwater and terrestrial habitats (Suzuki and DeLong 2002). The β-*Proteobacteria* are prevalent in freshwater lakes (Pernthaler et al. 1997, 1998), limnetic organic aggregates (Schweitzer et al. 2001), activated sludge flocs (Snaidr et al. 1997) and Arctic melt pools (Brinkmeyer et al. 2004), but are relatively uncommon in marine environments, where they have only been identified in samples from coastal seawater and not the oligotrophic ocean (Cottrell and Kirchman 2000b; Rappé et al. 2000). In a comparison of the phylogenetic composition of freshwater and marine bacterioplankton communities, the β-subclass of *Proteobacteria* accounted for 16% of the cells identified by FISH in freshwater samples, but only 4% of the cells in sample from the coastal Pacific; β-*Proteobacteria* were completely absent in samples from the Antarctic Ocean and North Sea (Glöckner et al. 1999). It has been suggested that although the β- and γ-*Proteobacteria* coexist in coastal environments (Rappé et al. 1997), these subclasses may differ in their capacities to

metabolize DOM and thus, differences in the quality and supply of labile DOM in freshwater and marine habitats would determine the distribution of these groups, particularly since the growth of entire bacterial assemblages is often determined by the availability of DOM (Williams 2000). However, a study by Cottrell and Kirchman (2000b) indicated that the β- and γ-*Proteobacteria* have similar capacities to utilize DOM, accounting for the smallest fraction of bacteria consuming chitin and N-acetylglucosamine, and 19 to 29% of the cells assimilating protein and amino acids. Although the γ-*Proteobacteria* are generally displaced by the β-*Proteobacteria* in freshwater habitats (Glöckner et al. 1999), the β-*Proteobacteria* must be restricted from the open ocean by a selective variable other than the availability of DOM (Cottrell and Kirchman 2000b).

1.4.2.3 Gamma-*Proteobacteria*

The gamma subclass of *Proteobacteria* contains the majority of culturable marine bacteria, which are divided into two related clades: the first includes the *Alteromonas, Pseudoaltermonas, Marinomonas, Shewanella*, and *Glacieola* genera; and the second, the *Oceanospirillum* and *Marinobacter* genera (Giovannoni & Rappé 2000). Generally, the γ-*Proteobacteria* are more frequently detected in culture collections than in cultivation-independent surveys of microbial diversity (Suzuki and DeLong 2002). Only one clade of γ-*Proteobacteria* has been identified from small-subunit rRNA analyses of marine bacterioplankton communities, the SAR86 cluster (Giovannoni & Rappé 2000). Members of this group appear to be widespread and have been identified in seawater from the Atlantic and Pacific oceans, in both coastal and open ocean habitats (Suzuki and

DeLong 2002). Studies of bacterioplankton have revealed low relative abundances (< 4%) of γ-*Proteobacteria* in freshwater systems (Glöckner et al. 1999) but higher abundances in marine environments, where they have been found to constitute up to 30% of the bacterial community in some coastal areas (Cottrell and Kirchman 2000a, 2000b). These results are consistent with the γ-*Proteobacteria* being copiotrophs and well-adapted to high nutrient concentrations (Glöckner et al. 1999).

Along with the *Roseobacter* clade of α-*Proteobacteria*, members of the γ-*Proteobacteria,* including the SAR86 group, have also been identified as important participants in the assimilation of DMSP (Malmstrom et al. 2004b; Vila et al. 2004). A significant discovery involving the γ-*Proteobacteria* occurred when Béjà et al. (2000) uncovered a bacterial rhodopsin, subsequently called proteorhodopsin, encoded in the genome of an uncultivated member of the SAR86 cluster. The presence of proteorhodopsin, a light-driven proton pump, in certain members of SAR86 cluster provides evidence of a previous unrecognized phototrophic pathway in the oceans (Béjà et al. 2001). Since its discovery, proteorhodopsin has been identified in members of the SAR86 cluster from the Eastern Pacific, Central Northern Pacific, Southern Ocean (de la Torre et al. 2003), and the Mediterranean and Red Seas (Sabehi et al. 2003).

1.5 Marine *Archaea*

Prior to the development of the universal phylogenetic tree (Woese and Fox 1977; Woese et al. 1990), the *Archaea* were thought to be highly atypical bacterial species, inhabiting

only extreme environments, such as hydrothermal vents and hot springs (DeLong 2003). Although *Archaea* are known to thrive in habitats of extremely high temperatures, low pH, high salinity or strict anoxia (DeLong 1998a), the use of molecular techniques has revealed the widespread presence of *Archaea* in temperate (DeLong 1992; Fuhrman and Davis 1997; Massana et al. 1997; Ouverney and Fuhrman 2000; Karner et al. 2001) and polar oceans (DeLong et al. 1994; Murray et al. 1998; Church et al. 2003). Moreover, studies indicate that *Archaea* can constitute a considerable fraction of the marine prokaryotic community, particularly in the deep (> 200 m) ocean (Fuhrman and Davis 1997; Karner et al. 2001), and are actively involved in the heterotrophic uptake of DOM in the form of dissolved amino acids (Ouverney and Fuhrman 2000; Teira et al. 2004). Within the domain *Archaea*, there are two major lineages: the *Euryarchaeota*, containing the methanogens, extreme halophiles, and sulphur reducers; and the *Crenarchaeota*, containing the extreme thermophiles (Woese et al. 1990; McInerney et al. 2002). Studies have indicated that *Archaea* can comprise as much as 20% of the total rRNA in marine planktonic communities, particularly at depths greater than 100 m (Giovannoni & Rappé 2000; DeLong and Pace 2001).

1.6 Objectives and Justification for Study

This thesis has two main objectives and is organized into two manuscript chapters, Chapter 2: Effects of long-term sample storage on the detection of bacterial cells using fluorescence *in situ* hybridization and Chapter 3: Seasonal and spatial patterns of microbial community structure in the Northwest Atlantic Ocean.

Chapter 2 examines the results of a laboratory-based study of the effects of both short- and long-term storage of preserved FISH filters on the detection of bacterial cells using four rRNA-targeted oligonucleotide probes. Replicate seawater samples were prepared (fixed and filtered) and stored frozen (-20°C) for up to 12 months. During this time course, samples were hybridized, counterstained, and counted at time intervals of 1.5, 3, 6, and 12 months to determine the effects of storage on the detection of bacterial cells using FISH.

Chapter 3 examines the results of a field- and laboratory-based study of changes in the phylogenetic diversity of the microbial community in biogeochemical provinces of the Northwest Atlantic Ocean over three consecutive seasons and how these changes influence biogeochemical and ecosystem processes on a large geographical and temporal scale. The phylogenetic diversity of the microbial community was characterized using the FISH method, and these characterizations were related to a number of concurrently measured ecological and climate-related parameters, namely measures of bacterial dynamics (bacterial abundance, bacterial biomass, bacterial production and bacterial growth rate); distinguishing features of the biogeochemical provinces, specifically temperature and chlorophyll *a*; and the climate-active trace gas, dimethylsulphide (DMS) and its biological precursor, dimethylsulphoniopropionate (DMSP).

As samples were collected during three cruises in the Northwest Atlantic Ocean over a sampling period of six months, during the spring (April 25 – May 14), summer (July 8 - 22) and fall (October 13 – 27) of 2003, and stored for varying lengths of time before

examination, the storage effects study (Chapter 2) was designed and implemented to determine if there were any effects of long-term sample storage and to provide a baseline for results from the Northwest Atlantic study (Chapter 3).

1.7 Literature Cited

Aas, P., Lyons, M. M., Pledger, R., Mitchell, D. L., and Jeffrey, W. H. 1996. Inhibition of bacterial activities by solar radiation in nearshore waters and the Gulf of Mexico. Aquat. Microb. Ecol., 11: 229-238.

Afreider, A., Pernthaler, J., Amann, R., Sattler, B., Glöckner, F. O., Wille, A., and Psenner, R. 1996. Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. Appl. Environ. Microbiol., 62: 2138-2144.

Amann, R., Snaidr, J., Wagner, M., Ludwig, W., and Schleifer, K.-H. 1996. In situ visualization of high genetic diversity in a natural microbial community. J. Bacteriol., 178: 3496-3500.

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

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, F. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser., 10: 257-263.

Azam, F., and Fuhrman, J. A. 1984. Measurement of bacterioplankton growth in the sea and its regulation by environmental conditions. In: Hobbie, J. E., and Williams, P. J. leB. Heterotrophic Activity in the Sea. Plenum Press, New York: 179-192.

Azam, F., and Worden, A. Z. 2004. Microbes, molecules and marine ecosystems. Nature, 303: 1622-1624.

Béjà, O., Aravind, L., Koonin, E. V., Suzuki, M. T., Hadd, A., Nguyen, L. P., Jovanovich, S. B., Gates, C. M., Feldman, R. A., Spudich, J. L., Spudich, E. N., and DeLong, E. F. 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science, 289: 1902-1906.

Béjà, O., Spudich, E. N., Spudich, J. L., Leclerc, M., and DeLong, E. F. 2001. Proteorhopdopsin phototrophy in the ocean. Nature, 411: 786-789.

Bouvier, T. C., and del Giorgio, P. A. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol. Oceanogr., 47: 453-470.

Brinkmeyer, R., Glöckner, F. O., Helmke, E., and Amann, R. 2004. Predominance of β-proteobacteria in summer melt pools on Arctic pack ice. Limnol. Oceanogr., 49: 1013-1021.

Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R., and Helmke, E. 2003. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl. Environ. Microbiol., 69: 6610-6619.

Brown, M. V., and Bowman, J. P. 2001. A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). FEMS Microbiol. Ecol., 35: 267-275.

Buchan, A., González, J. M., and Moran, M. A. 2005. Overview of the marine *Roseobacter* lineage. Appl. Environ. Microbiol., 71: 5665-5677.

Christensen, H., Hansen, M., and Sørensen, J. 1999. Counting and size classification of active soil bacteria by fluorescence in situ hybridization with an rRNA oligonucleotide probe. Appl. Environ. Microbiol., 65: 1753-1761.

Church, M. J., DeLong, E. F., Ducklow, H. W., Karner, M. B., Preston, C. M., and Karl, D. M. 2003. Abundance and distribution of planktonic *Archaea* and *Bacteria* in the Western Antarctic Peninsula. Limnol. Oceanogr., 48: 1893-1902.

Cooney, R. P., Pantos, O., Le Tissier, M. D. A., Barer, M. R., O'Donnell, A .G., Bythell, J. C. 2002. Characterization of the bacterial consortium associated with black band disease in coral using molecular microbiological techniques. Environ. Microbiol., 4: 401-413.

Cottrell, M. T., and Kirchman, D. L. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnol. Oceanogr., 48: 168-178.

Cottrell, M. T., and Kirchman, D. L. 2000a. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl. Environ. Microbiol., 66: 5116-5122.

Cottrell, M. T., and Kirchman, D. L. 2000b. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high- molecular-weight dissolved organic matter. Appl. Environ. Microbiol., 66: 1692-1697.

Daims, H., Ramsing, N. B., Schleifer, K.-H., and Wagner, M. 2001. Cultivation independent, semiautomatic determination of absolute bacterial cell numbers in environmental samples by fluorescence in situ hybridization. Appl. Environ. Microbiol., 67: 5810-5818.

Dang, H. Y., and Lovell, C. R. 2000. Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. Appl. Environ. Microbiol., 66: 467-475.

Dang, H. Y., and Lovell, C. R. 2002. Seasonal dynamics of particle-associated and free-living marine Proteobacteria in a salt marsh tidal creek as determined using fluorescence in situ hybridization. Environ. Microbiol., 4: 287-295.

de la Torre, J. R., Christianson, L. M., Béjà, O., Suzuki, M. T., Karl, D. M., Heidelberg, J., and DeLong, E. F. 2003. Proteorhopdopsin genes are distributed among divergent marine bacterial taxa. Proc. Natl. Acad. Sci., 100: 12830-12835.

DeLong, E. F. 1992. Archaea in coastal marine environments. Proc. Natl. Acad. Sci., 89: 5685-5689.

DeLong, E. F. 1998a. Everything in moderation: Archaea as 'non-extremophiles'. Curr. Opin. Genet. Dev., 8: 649-654.

DeLong, E. F. 1998b. Molecular phylogenetics: new perspective on the ecology, evolution and biodiversity of marine organisms. In: Cooksey, K. E., ed. Molecular Approaches to the Study of the Ocean. Chapman & Hall, London: 1-28.

DeLong, E. F. 2003. Oceans of *Archaea*. ASM News, 69: 503-511.

DeLong, E. F., and Karl, D. M. 2005. Genomic perspectives in microbial oceanography. Nature, 437: 336-342.

DeLong, E. F., and Pace, N. R. 2001. Environmental diversity of Bacteria and Archaea. Syst. Biol., 50: 470-478.

DeLong, E. F., Taylor, L. T., Marsh, T. L., and Preston, C. M. 1999. Visualization and enumeration of marine planktonic Archaea and Bacteria by using polyribonucleotide probes and fluorescent in situ hybridization. Appl. Environ. Microbiol., 65: 5554-5563.

DeLong, E. F., Wickham, G. S., and Pace, N. R. 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. Science, 243: 1360-1363.

DeLong, E. F., Wu, K. Y., Prézelin, B. B., and Jovine, R. V. M. 1994. High abundance of Archaea in Antarctic marine picoplankton. Nature, 371: 695-697.

Denman, K., Hofmann, E., and Marchant, H. 1996. Marine biotic responses to environmental change and feedbacks to climate. In: Houghton, J.T. Climate Change 1995: The Science of Climate Change. Cambridge University Press, Cambridge: 485-516.

Dorigo, U., Volatier, L., and Humbert, J.-F. 2005. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. Water Res., 39: 2207-2218.

Dyer, B. D. 2003. A Field Guide to Bacteria. Cornell University Press, Ithaca, 355 pp.

Egert, M., and Freidrich, M. W. 2003. Formation of pseudo-terminal restriction fragments, a PCR-related bias affecting terminal restriction fragment length polymorphism analysis of microbial community structure. Appl. Environ. Microbiol., 69: 2555-2562.

Fuhrman, J. A., and Davis, A. A. 1997. Widespread Archaea and novel Bacteria from the deep sea as shown by 16S rRNA gene sequences. Mar. Ecol. Prog. Ser., 150: 275-285.

Fuhrman, J. A., McCallum, K., and Davis, A. A. 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific oceans. Appl. Environ. Microbiol., 59: 1294-1302.

Giovannoni, S., and Rappé, M. 2000. Evolution, diversity and molecular ecology of marine prokaryotes. In: Kirchman, D. L., ed. Microbial Ecology of the Oceans. John Wiley & Sons, New York: 47-84.

Glöckner, F. O., Amann, R., Alfreider, A., Pernthaler J., Psenner, R., Trebesius, K., and Schleifer, K.-H. 1996. An in situ hybridization protocol for detection and identification of planktonic bacteria. Syst. Appl. Microbiol., 19: 403-406.

Glöckner, F. O., Fuchs, B. M., and Amann, R. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl. Environ. Microbiol., 65: 3721-3726.

González, J. M., Kiene, R. P., and Moran, M. A. 1999. Tranformation of sulfur compounds by an abundant lineage of marine bacteria in the α-subclass of the class *Proteobacteria.* Appl. Environ. Microbiol., 65: 3810-3819.

González, J. M., and Moran, M. A. 1997. Numerical dominance of a group of marine bacteria in the α-subclass of the class *Proteobacteria* in coastal seawater. Appl. Environ. Microbiol., 63: 4237-4242,

González, J. M., Simó, R., Massana, R., Covert, J. S., Casamayor, E. O., Pedrós-Alió, C., and Moran, M. A. 2000. Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. Appl. Environ. Microbiol., 66: 4237-4246.

Hahn, D., Amann, R., Ludwig, W., Akkermans, A. D. L., and Schleifer, K.-H. 1992. Detection of micro-organisms in soil after in situ hybridization with rRNA targeted, fluorescently labelled oligonucleotides. J. Gen. Microbiol., 138: 879-887.

Hansen, J., Sato, M., Lacis, A., Reudy, R., Tegen, I., and Matthews, E. 1998. Climate forcings in the industrial era. Proc. Natl. Acad. Sci., 95: 12753-12758.

Head, I. M., Saunders, J. R., and Pickup, R. W. 1998. Microbial evolution, diversity, and ecology: a decade of ribosomal RNA analysis of uncultivated organisms. Microb. Ecol., 35: 1-21.

Heidelberg, J. F., Heidelberg, K. B., and Colwell, R. R. 2002. Seasonality of Chesapeake Bay bacterioplankton species. Appl. Environ. Microbiol., 68: 5488-5497.

Herndl, G., Müller-Niklas, G., and Frick, J. 1993. Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean. Nature, 361: 717-719.

Hiorns, W. D., Methé, B. A., Nierzwickibauer, S. A., and Zehr, J. P. 1997. Bacterial diversity in Adirondack mountain lakes as revealed by 16S rRNA gene sequences. Appl. Environ. Microbiol., 63: 2957-2960.

Hobbie, J. E. 1988. A comparison of the ecology of planktonic bacteria in fresh and saltwater. Limnol. Oceanogr., 33: 750-764.

Hugenholtz, P., Goebel, B. M., and Pace, N. R. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol., 180: 4765-4774.

Hugenholtz, P., Tyson, G. W., and Blackall, L. L. 2001. Design and evaluation of 16S rRNA-targeted oligonucleotide probes for fluorescence in situ hybridization. In: Lieberman, B. A., ed. Molecular Biology, vol. 179: Steroid Receptor Methods: Protocols and Assays. Humana Press, Totowa NJ: 29-42.

Jonkers, H. M., and Abed, R. M. M. 2003. Identification of aerobic heterotrophic bacteria from the photic zone of a hypersaline microbial mat. Aquat. Microb. Ecol., 30: 127-133.

Karner, M. B., DeLong, E. F., and Karl, D. M. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature, 409: 507-510.

Karner, M., and Fuhrman, J. A. 1997. Determination of active marine bacterioplankton: a comparison of universal 16S rRNA probes, autoradiography, and nucleoid staining. Appl. Environ. Microbiol., 63: 1208-1213.

Kenzaka, T., Yamaguchi, N., Tani, K., and Nasu, M. 1998. rRNA-targeted fluorescent in situ hybridization analysis of bacterial community structure in river water. Microbiol. U. K., 144: 2085-2093.

Kiene, R. P., Linn, L. J., and Bruton, J. A. 2000. New and important roles for DMSP in marine microbial communities. J. Sea Res., 43: 209-224.

Kirchman, D. L. 2003. The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic material in aquatic ecosystems. In: Findlay, S. E. G., and Sinsabaugh, R. L., eds. Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Academic Press, San Diego: 217-241.

Kirchman, D. L. 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiol. Ecol., 39: 91-100.

Kirchman, D. L., and Rich, J. H. 1997. Regulation of bacterial growth rates by dissolved organic carbon and temperature in the Equatorial Pacific Ocean. Microb. Ecol., 33: 11-20.

Lebrenz, M., Tindall, B. J., Lawson, P. A., Collins, M. D., Schumann, P., and Hirsch, P. 2000. *Staleya guttiformis* gen. nov. and *Sulfitobacter brevis* sp. nov., α-3-Proteobacteria from hypersaline, heliothermal and meromictic Antarctic Ekho Lake. Int. J. Syst. Evol. Microbiol., 50: 303-313.

Legendre, L., and Le Fèvre, J. 1995. Microbial food webs and the export of biogenic carbon. Aquat. Microb. Ecol., 9: 69-77.

Li, W. K. W., and Harrison, W. G. 2001. Chlorophyll, bacteria and picophytoplankton in ecological provinces of the North Atlantic. Deep-Sea Res. II, 48: 2271-2293.

Lim, E. L., Amaral, L. A., Caron, D. A., and DeLong, E. F. 1993. Application of rRNA-based probes for observing nanoplanktonic protists. Appl. Environ. Microbiol., 59: 1647-1655.

Lim, E. L., Caron, D. A., DeLong, E. F. 1995. Development and field application of a quantitative method for examining natural assemblages of protists with oligonucleotide probes. Appl. Environ. Microbiol., 62: 1416-1423.

Liu, W., Marsh, T. L., Cheng, H., and Forney, L. J. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl. Environ. Microbiol., 63: 4516-4522.

Llobet-Brossa, E., Rossello-Mora, R., and Amann, R. 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. Appl. Environ. Microbiol., 64: 2691-2696.

Longhurst, A. 1998. Ecological Geography of the Sea. Academic Press, San Diego, 398 pp.

Macalady, J., and Banfield, J. F. 2003. Molecular geomicrobiology: genes and geochemical cycling. Earth. Planet. Sci. Lett., 209: 1-17.

Malmstrom, R. R., Kiene, R. P., Cottrell, M. T., and Kirchman, D. L. 2004a. Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the North Atlantic Ocean. Appl. Environ. Microbiol., 70: 4129-4135.

Malmstrom, R. R., Kiene, R. P., Kirchman, D. L. 2004b. Identification and enumeration of bacteria assimilating dimethylsulfoniopropionate (DMSP) in the North Atlantic and Gulf of Mexico. Limnol. Oceanogr., 49: 597-606.

McInerney, J. O., Mullarkey, M., Wernecke, M. E., and Powell, R. 2002. Bacteria and Archaea: molecular techniques reveal astonishing diversity. Biodiversity, 3: 3-10.

Massana, R., Guillou, L., Díez, B., and Pedrós-Alió, C. 2002. Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean. Appl. Environ. Microbiol., 68: 4554-4558.

Massana, R., Murray, A. E., Preston, C. M., and DeLong, E. F. 1997. Vertical distribution and phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara Channel. Appl. Environ. Microbiol., 63: 50-56.

Moran, M. A., González, J. M., and Kiene, R. P. 2003. Linking a bacterial taxon to sulfur cycling in the sea: studies of the marine *Roseobacter* group. Geomicrobiol. J., 20: 375-388.

Morris, R. M., Rappé, M. S., Connon, S. A., Vergin, K. L., Siebold, W. A., Carlson, C. A., and Giovannoni, S. J. 2002. SAR11 clade dominates ocean surface bacterioplankton communities. Nature, 420: 806-810.

Murray, A. E., Preston, C. M., Massana, R., Taylor, L. T., Blakis, A., Wu, K., and DeLong, E. F. 1998. Seasonal and spatial variability of Bacterial and Archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl. Environ. Microbiol., 64: 2585-2595.

Nold, S. C., and Zwart, G. 1998. Patterns and governing forces in aquatic microbial communities. Aquat. Ecol., 32: 17-35.

Ouverney, C. C., and Fuhrman, J. A. 2000. Marine planktonic Archaea take up amino acids. Appl. Environ. Microbiol., 66: 4829-4833.

Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. Science, 276: 734-740.

Pace, N. R., Stahl, D. J., Lane, D. J., and Olsen, G. J. 1986. The analysis of natural microbial populations by ribosomal RNA sequences. Adv. Microbiol. Ecol., 9: 1-55.

Pantos, O., Cooney, R. P., Le Tissier, M. D. A., Barer, M. R., O'Donnell, A. G., Bythell, J. C. 2003. The bacterial ecology of a plague-like disease affecting the Caribbean coral *Montastrea annularis.* Environ. Microbiol., 5: 370-382.

Pernthaler, J., Alfreider, A., Posch, T., Andreatta, S., and Psenner, R. 1997. In situ classification and image cytometry of pelagic bacteria from a high mountain lake (Gossenköllesee, Austria). Appl. Environ. Microbiol., 63: 4778-4783.

Pernthaler, J., Glöckner, F. O., Unterholzner, S., Alfreider, A., Psenner, R., and Amann, R. 1998. Seasonal community and population dynamics of pelagic Bacteria and Archaea in a high mountain lake. Appl. Environ. Microbiol., 64: 4299-4306.

Pernthaler, J., Glöckner, F. O., Schönhuber, W., and Amann, R. 2001. Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. In: Paul, J., ed. Methods in Microbiology: Marine Microbiology, vol. 30. Academic Press, London: 207-226.

Platt, T., and Sathyendranath, S. 1999. Spatial structure of pelagic ecosystem processes in the global ocean. Ecosystems, 2: 384-394.

Pomeroy, L. R. 1974. The ocean's food web: a changing paradigm. BioScience, 24: 409-504.

Rappé, M. S., and Giovannoni, S. J. 2003. The uncultured microbial majority. Annu. Rev. Microbiol., 57: 369-394.

Rappé, M. S., Kemp, P. F., and Giovannoni, S. J. 1997. Phylogenetic diversity of marine coastal picoplankton 16S rRNA cloned from the continental shelf off Cape Hatteras, North Carolina. Limnol. Oceanogr., 42: 811-826.

Rappé, M. S., Vergin, K., and Giovannoni, S. J. 2000. Phylogenetic comparisons of a coastal bacterioplankton community with its counterparts in open ocean and freshwater systems. FEMS Microbiol. Ecol., 33: 219-232.

Rivkin, R. B., Anderson, M. R., and Lajzerowicz, C. 1996. Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. Aquat. Microb. Ecol., 10: 243-254.

Sabehi, G., Massana, R., Bielawski, J. P., Rosenberg, M., DeLong, E. F., and Béjà, O. 2003. Novel proteorhopdopsin variants from the Mediterranean and Red Seas. Environ. Microbiol., 5: 842-849.

Sarmiento, J. L., Hughes, T. M. C., Stouffer, R. J., and Manabe, S. 1998. Simulated response of the ocean carbon cycle to anthropogenic climate warming. Nature, 393: 245-249.

Schimel, D. S. 1998. The carbon equation. Nature, 393: 208-209.

Schweitzer, B., Huber, I., Amann, R., Ludwig, W., and Simon, M. 2001. α- and β-proteobacteria control the consumption and release of amino acids on lake snow aggregates. Appl. Environ. Microbiol., 67: 632-645.

Selje, N., Simon, M., and Brinkhoff, T. 2004. A newly discovered *Roseobacter* cluster in temperate and polar oceans. Nature, 427: 445-448.

Sievert, S. M., Kuever, J., and Muyzer, G. 2000. Identification of 16S ribosomal DNA-defined bacterial populations at a shallow submarine hydrothermal vent near Milos Island (Greece). Appl. Environ. Microbiol., 66: 3102-3109.

Simó, R., Archer, S. D., Pedrós-Alió, C., Gilpin, L., Stelfox-Widdicombe, C. E. 2002. Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic. Limnol. Oceanogr., 47: 53-61.

Simó, R. 2001. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. Trends Ecol. Evol., 16: 287-294.

Simon, N., LeBot, N., Marie, D., Partensky, F., and Vaulot, D. 1995. Fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes to identify small phytoplankton by flow cytometry. Appl. Environ. Microbiol., 61: 2506-2513.

Snaidr, J., Amann, R., Huber, I., Ludwig, W., and Schleifer, K.-H. 1997. Phylogenetic analysis and in situ identification of bacteria in activated sludge. Appl. Environ. Microbiol., 63: 2884-2896.

Smith, M. C., Bowman, J. P., Scott, F. J., and Line, M. A. 2000. Sub-lithic bacteria associated with Antarctic quartz stones. Antarct. Sci., 12: 177-184.

Spiegelman, D., Whissell, G., and Greer, C. W. 2005. A survey of the methods for the characterization of microbial consortia and communities. Can. J. Microbiol., 51: 355-386.

Suzuki, M. T., and DeLong, E. F. 2002. Marine prokaryotic diversity. In: Staley, J. T., and Reysenbach A.-L., eds. Biodiversity of Microbial Life. John Wiley & Sons, New York: 209-234,

Suzuki, T., Muroga, Y., Takahama, M., and Nishimura, Y. 1999. *Roseivivax halodurans* gen. nov., sp. nov. and *Roseivivax halotolerans* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from a saline lake. Int. J. Syst. Bacteriol., 49: 629-634.

Taylor, M. W., Schupp, P. J., Dahllöf, I., Kjelleberg, S., and Steinberg, P. D. 2004. Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. Environ. Microbiol., 6: 121-130.

Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G. J. 2004. Combined catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. Appl. Environ. Microbiol., 70: 4411-4414.

Tortell, P. D., Maldonado, M. T., and Price, N. M. 1996. The role of heterotrophic bacteria in iron-limited ocean ecosystems. Nature, 383: 330-332.

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., Wu, D., Paulsen, I., Nelson, K. E., Nelson, W., Fouts, D. E., Levy, S., Knap, A. H., Lomas, M .W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkock, C., Rogers, Y., and Smith, H. O. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science, 304: 66-74.

Vila, M., Simó, R., Kiene, R. P., Pinhassi, J., González, J. M., Moran, M. A., Pedrós-Alió, C. 2004. Use of microautoradiography combined with fluorescence in situ hybridization to determine dimethylsulfoniopropionate incorporation by marine bacterioplankton taxa. Appl. Environ. Microbiol., 70: 4648-4657.

Wagner, M.-H., Erhart, R., Manz, W., Amann, R., Lemmer, H., Wdei, D., and Schleifer, K.-H. 1994. Development of an rRNA-targeted oligonucleotide probe specific for the genus Actinobacter and its application for in situ monitoring in activated sludge. Appl. Environ. Microbiol., 60: 792-800.

Wagner, M., Horn, Matthias., and Daims, Holger. 2003. Fluorescence *in situ* hybridisation for the identification and characterisation of prokaryotes. Curr. Opin. Microbiol., 6: 302-309.

Webster, N. S., Negri, A. P., Munro, M. M. H. G., and Battershill, C. N. 2004. Diverse microbial communities inhabit Antarctic sponges. Environ. Microbiol., 6: 288-300.

Weidner, S., Arnold, A., Stackebrandt, E., and Pühler, A. 2000. Phylogenetic analysis of bacterial communities associated with leaves of the seagrass *Halophila stipulacea* by culture-independent small-subunit rRNA gene approach. Microb. Ecol., 39: 22-31.

Weiss, P., Schweitzer, B., Amann, R., and Simon, M. 1996. Identification in situ and dynamics of bacteria on limnetic organic aggregates (Lake Snow). Appl. Environ. Microbiol., 62: 1998-2005.

Weller, R., Glöckner, F. O., and Amann, R. 2000. 16S rRNA-targeted oligonucleotide probes for the *in situ* detection of members of the phylum *Cytophaga-Flavobacterium-Bacteroides*. Syst. Appl. Microbiol., 23: 107-114.

Wells, L. E., and Deming, J. W. 2003. Abundance of Bacteria, the Cytophaga-Flavobacterium cluster and Archaea in cold oligotrophic waters and nepheloid layers of the Northwest Passage, Canadian Archipelago. Aquat. Microb. Ecol., 31: 19-31.

Williams, P. J. leB. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material. In: Kirchman, D. L., ed. Microbial Ecology of the Oceans. John Wiley & Sons, New York: 153-200.

Williams, S. C., Heaton, D. P., Hristov, R., Frischer, M. E., and Verity, P. G. 2004. Methodological factors that contribute to variability using fluorescence in situ hybridization (FISH) in marine planktonic environments. [Online.] http://www.skio.peachnet.edu/research/veritylab/biocomp/presentations/methods_factors/methods_factors.pdf. Accessed 17 November 2005.

Woese, C. R., and Fox, G. E. 1977. Phylogenetic structure of the prokaryotic domain: The primary kingdoms. Proc. Natl. Acad. Sci., 74: 5088-5090.

Woese, C. R., Kandler, O., Wheelis, M. L. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci., 87: 4576-4579.

Yoch, D. C. 2002. Dimethylsulfoniopropionate: its sources, role in the marine food web, and biological degradation to dimethylsulfide. Appl. Environ. Microbiol., 68: 5804-5815.

Zehr, J. P., and Voytek, M. A. 1999. Molecular ecology of aquatic communities: reflections and future directions. Hydrobiologia, 401: 1-8.

Zubkov, M.V., Fuchs, B. M., Archer, S. D., Kiene, R. P., Amann, R., and Burkill, P. H. 2001. Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulphoniopropionate in an algal bloom in the North Sea. Environ. Microbiol., 3: 304-311.

Zubkov, M.V., Fuchs, B. M., Archer, S. D., Kiene, R. P., Amann, R., and Burkill, P. H. 2002. Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. Deep-Sea Res. II, 49: 3017-3038.

Zwisler, W., Selje, N., and Simon, M. 2003. Seasonal patterns of the bacterioplankton community in a large mesotrophic lake. Aquat. Microb. Ecol., 31: 211-225.

Chapter 2: Effects of Long-Term Sample Storage on the Detection of Bacterial Cells using Fluorescence *In Situ* Hybridization

2.1 Abstract

Fluorescence *in situ* hybridization (FISH) is a popular molecular tool for characterizing the phylogenetic diversity of microbial communities. Although widely used, a major limitation of the FISH technique is that the effectiveness of target cell detection varies widely, over ecosystem types and with differences in methodology. While several of these factors have been investigated, the effect of long-term sample storage on the efficiency of target cell detection with rRNA-targeted oligonucleotide probes is unknown. Samples for FISH may be filtered and stored for many weeks or months before analysis. Therefore, quantifying the effects of storage on the detection of bacterial cells is crucial in comparing studies where bacterial community structure has been characterized from seawater samples that have been collected and analyzed over a long period of time. We present results from a 12 month time-course study during which replicate seawater samples were prepared, stored frozen, and hybridized after 1.5, 3, 6, and 12 months to determine the effects of long-term sample storage. The time-dependent slope of the probe for *Eubacteria*, but not the *Cytophaga-Flavobacteria* cluster or the alpha and gamma subclasses of *Proteobacteria*, differed significantly from zero, with a percent change in target cell detection of 6.3% per year. This change in target cell detection was small and within the error of bacterial counting. Thus, we conclude that during this 12-month time-course study there was a minimal effect of long-term storage on the detection of bacterial cells using FISH.

2.2 Introduction

Bacteria are ubiquitous and abundant in diverse habitats, ranging from nutrient-rich soils, freshwater ecosystems and oceans, to extreme environments, such as hydrothermal vents and sea-ice (Whitman et al. 1998; Stackebrandt and Embley 2000; Horner Devine et al. 2003). Bacteria also have a central role in the mediation of biogeochemical processes, including the cycling of organic carbon, nitrogen, sulphur, phosphorus and inorganic elements (Fenchel et al. 1998; Newman and Banfield 2002). Despite the high abundances and productivity of bacteria, characterization of bacterial diversity has been limited by the difficulty of isolating the vast majority of bacterial cells in laboratory culture (Zengler et al. 2002). Recent advances in field of molecular microbial ecology, particularly the development of cultivation-independent methods of determining microbial community structure and function, have provided new insight into the importance and extent of microbial diversity. Prior to the use of molecular tools in establishing the taxonomy of microorganisms, studies relied heavily on morphological and metabolic distinctions of specific groups (Pace 1997; Venter et al. 2004; Giovannoni and Stingl 2005). The low culturability of marine bacteria (<< 1%) has allowed for laboratory isolation and characterization of few bacterial groups, and generally only under optimal growth conditions (Macalady and Banfield 2002). As a result, relatively little is known about both the composition of *in situ* bacterial communities and their specific molecular, physiological, and ecological characteristics.

Recent studies have used cultivation-independent methods (i.e. analyses of small-subunit rRNA (16S or 18S) and other gene sequences) to determine microbial community

structure (Head et al. 1998; Hugenholtz et al. 1998; Pernthaler and Amann 2005). The extraction and amplification of gene sequences directly from the natural environment has resulted in the detection of entire groups of previously unrecognized *Bacteria* and *Archaea* (DeLong 1998; Hugenholtz et al. 1998; Rappé and Giovannoni 2003; Venter et al. 2004; DeLong and Karl 2005). Gene sequence information has facilitated the development of specific primers for polymerase chain reaction (PCR) based approaches (Pace et al. 1986; Fuhrman et al. 1993; Zehr and Voytek 1999) as well as the design of rRNA-targeted oligonucleotide probes for fluorescence *in situ* hybridization (FISH) (Glöckner et al. 1996; Hugenholtz et al. 2001; Pernthaler et al. 2001), all of which are useful in characterizing the diversity of bacterial assemblages. The conceptual basis of the FISH technique is relatively straightforward; fluorescently-labelled, rRNA-targeted oligonucleotide probes are hybridized to group specific gene sequences and thus, identify cells, i.e. unicellular microorganisms, at target taxonomic levels (Glöckner et al. 1996). The FISH method has been employed in a number of studies of microorganisms, most commonly from the domains *Bacteria* and *Archaea* as well as *Eucarya*, where it has been used for the detection of nano- and pico-phytoplankton (Simon et al. 1995), nano-planktonic protists (Lim et al. 1993; Lim et al. 1995), and photosynthetic pico-heterokonts (stramenopiles) (Massana et al. 2002). FISH has been used to characterize the phylogenetic composition of microbial assemblages in lakes and estuaries (Pernthaler et al. 1997, Glöckner et al. 1999; Bouvier and del Giorgio 2002; Heidelberg et al. 2002; Cottrell and Kirchman 2003; Zwisler et al. 2003), limnetic organic aggregates (lake snow) (Weiss et al. 1996; Schweitzed et al. 2001), soil (Hahn et al. 1992; Christensen et al. 1999), activated sludge flocs and biofilms (Wagner et al. 1994; Amann et al. 1996; Daims

et al. 2001), ice and snow (Alfreider et al. 1996; Brinkmeyer et al. 2003), as well as coastal and oceanic marine habitats (Karner and Fuhrman 1997; DeLong et al. 1999; Glöckner et al. 1999; Cottrell and Kirchman 2000a, 2000b; Wells and Deming 2003).

Although FISH is now a generally accepted tool for characterizing bacterial community structure, one of the major limitations of this technique is that the effectiveness of target cell detection varies widely (Bouvier and del Giorgio 2003). The percentage of cells detected with the Eubacteria probe (EUB338) varies from 1% in soil to 100% in enriched culture, with an average of 56% for all ecosystem types (Bouvier and del Giorgio 2003). A number of methodological factors contributing to this variation have been identified, and they include: cell fixation, cell storage, hybridization conditions, mounting solutions, fluorochrome type, and counting methods (Bouvier and del Giorgio 2003; Williams et al. 2004). Williams et al. (2004) recently assessed selected factors to develop a standard protocol for FISH and recommended that oligonucleotide probes be labelled with the fluorochrome Cy3, and that samples be fixed with formaldehyde (final concentration 3.7%), stored at 4°C after fixation (30 minutes to 24 hours), filtered onto white polycarbonate filters and stored at -20°C until analysis, incubated for 2 hours at 46°C for optimal hybridization, and mounted with glycerol-medium, Citifluor #4 (Citifluor Ltd., Canterbury, UK).

Another important, yet often neglected, factor is the effect of sample storage on the efficiency of target cell detection with rRNA-targeted oligonucleotide probes. As with samples prepared for direct counting of bacterial abundance in seawater (Turley and

Hughes 1992; Turley 1993; Gundersen et al. 1996), samples for FISH may be filtered and stored for many weeks or months before analysis. Thus, it is essential that the effects of storage, particularly changes in cell reactivity to oligonucleotide probes and subsequent visualization, be quantified in order to accurately compare studies where bacterial community structure has been characterized using samples that have been stored for varying lengths of time.

Previous studies of the effects of sample storage on the detection of bacteria (Turley and Hughes 1992, 1994; Turley 1993; Gundersen et al. 1996) and photosynthetic picoplankton (Booth 1987; Hall 1991; Putland and Rivkin 1999) have shown that long-term storage of seawater samples results in a significant reduction in the abundance of cells detected using epifluorescence microscopy (i.e. apparent cell loss). Studies of the effects of storage on direct counting of bacterial abundances have shown that prepared samples, which have been filtered, stained, and mounted, can be stored frozen (-20°C) for up to 70 days with no significant loss of cells (Turley and Hughes 1992; Turley 1993). When stained with DAPI, cell numbers were consistent for up to 24 weeks when stored at 4°C, but decreased significantly after being stored for 36 weeks (Porter and Feig 1980). An average 39% decrease in bacterial cell numbers was reported after 40 days of storage for samples preserved with gluteraldehyde and stained with Acridine Orange (AO) (Turley and Hughes 1992). A 56% decrease in bacterial cell numbers was observed in gluteraldehyde preserved seawater samples after they had been stored for 99 days at 4°C, compared with gluteraldehyde-fixed samples that were immediately stained with AO and counted (Turley and Hughes 1994). Significant cell loss has also been observed in

seawater samples preserved with Lugol's solution (Nishino 1986) and formaldehyde (Spinrad et al. 1989). Seawater samples for analyses of bacterial community structure using FISH differ from those prepared for either DAPI or AO direct counts (Kepner and Pratt 1994) in that FISH samples are initially fixed and then filtered onto 0.2 μm white polycarbonate filters, after which they are stored at -20°C until they are hybridized with oligonucleotide probes, then counterstained with DAPI and counted immediately or stored for 3 to 4 days at -20°C. Therefore, the effects of a delay between sample collection, hybridization, and DAPI staining, may differ from those observed with seawater samples that have been stained and mounted before storage, and thus cannot be predicted based upon previous published studies on the effects of storage duration on bacterial counting.

This study examines the effects of both short- and long-term storage of preserved FISH filters on the detection of bacterial cells using four rRNA-targeted oligonucleotide probes. Replicate seawater samples were prepared (fixed and filtered) and stored frozen (-20°C) for up to one year. During this time course, samples were hybridized, counterstained and counted at time intervals of 1.5, 3, 6, and 12 months to determine the effects of storage on the detection of bacterial cells using FISH.

2.3 Materials and Methods

2.3.1 Sampling, Cell Fixation, and Storage

Seawater was collected on 28 May 2004 at Tapper's Cove, Newfoundland, Canada (49°39'54"N, 52°43'38"W). The experimental design replicated field conditions where

samples were collected and processed through to the storage stage. To ensure a sufficient number of replicates for statistical analyses, twenty replicate filters (i.e. four filters for each of five time points) were prepared from a single seawater sample. For each 90-mL replicate seawater sample, two 45-mL aliquots were placed into sterile 50-mL falcon tubes and fixed with 10 mL of 37% formalin (5 mL in each falcon tube). After one to six hours, the fixed samples were filtered on white 0.2-μm polycarbonate membrane filters (Millipore GTTP04700) that were placed over a cellulose prefilter (Millipore AP1504700). The polycarbonate filters were then washed with 50 mL of phosphate buffered saline (sterile and prefiltered), and air-dried over absorbent paper in individual sterile petri dishes. One set of four filters was hybridized and counted immediately, and the other four filter sets were stored at -20°C until analysis at 1.5, 3, 6, and 12 months.

2.3.2 Oligonucleotide Probes

In this study, fluorescently-labelled rRNA-targeted oligonucleotide probes (MOBIX Lab, Hamilton, Ontario, Canada) were used for the phylogenetic analysis of microorganisms at one of the highest taxonomic levels: *Bacteria* (EUB338); as well as the intermediate taxonomic levels: alpha (ALF968) and gamma (GAM42a) subclasses of *Proteobacteria*, and the *Cytophaga-Flavobacteria* (CF319a) cluster. The nonsense probe (NON338), which is complementary to the EUB338 probe, was used as a negative control for the detection of non-specific binding and background fluorescence (Glöckner et al. 1996). The target bacterial groups and gene sequences for the oligonucleotide probes employed in this study are shown in Table 2.1. All oligonucleotide probes were labelled at the 5' end position with the sulfoindocyanine dye, indocarbocyanine (Cy3).

2.3.3 Fluorescence *In Situ* Hybridization and DAPI Staining

The method of FISH that was employed in this study is a modification of the protocol of Glöckner et al. (1996). Each filter was cut into five triangular pieces, and each piece was placed on a microscope slide. Before beginning the procedure, the hybridization buffer was prepared. The hybridization buffer required 360-µL NaCl 5M, 40-µL Tris-HCl 1M pH 8, and 700-µL formamide for all probes except ALF968, which required 400-µL formamide. This was completed to 2 mL with 0.2-µm filtered Milli Q water, after which 2-µL sodium dodecyl sulphate (SDS) 10% was added.

After the hybridization buffer was prepared, the hybridization solution was mixed by adding 6 µL (50 ng/µL) of probe to 48 µL of hybridization buffer. The filters were hybridized by adding 20 µL of the hybridization solution to each filter section. The slide was then placed in a hybridization chamber containing a piece of absorbing paper and 1 mL of hybridization buffer (to create a humid atmosphere). The chamber was closed, sealed and incubated in the dark for two hours at 46°C. This procedure was repeated for each of the five filter pieces, one for each of the oligonucleotide probes.

During the incubation, the washing buffer was prepared in individual sterile 50-mL falcon tubes. The washing buffer consisted of 1-mL Tris-HCl 1M pH 8, 500-µL EDTA, and 700 -µL NaCl 5 M for all probes except ALF968, which required 2150-µL NaCl; this was brought up to 50 mL with filtered Milli Q water, after which 50 µL SDS 10% was added. The washing buffer was also placed in the incubator at 46°C. After two hours, the hybridization chamber and the washing buffer were removed from the incubator, and

filter pieces were placed in individual falcon tubes of washing buffer and incubated in the dark for 15 minutes at 46°C. Each filter piece was then dried over absorbent paper at room temperature, placed on a glass slide and counter-stained with 50 μL of DAPI (1 μg/mL) for 1 minute (on ice). After staining, each filter piece was washed with 1 mL of filtered Milli Q water and dried over absorbent paper. Each filter piece was mounted on a glass slide in glycerol medium (Citifluor #1; Citifluor Ltd., Canterbury, UK). The slides were then immediately observed under the microscope or stored at –20°C for a maximum of two days before microscopic analysis.

2.3.4 Epifluorescence Microscopy

The prepared slides were observed using an Olympus BH-2 epifluorescent microscope, equipped with a 100x 1.30 oil objective (1250x total magnification), Cy3 filter (41007-HQ) and DAPI filter (UG-1). All probes were fluorescently-labelled with the sulfoindocyanine dye Cy3, which absorbs at 552 nm (green light) and emits at 565 nm (orange light). As Cy3 fluorescence fades much more rapidly than DAPI fluorescence (Pernthaler et al. 2001), direct counts of hybridized (group specific) cells were completed first, followed by DAPI counts (under UV light), for total bacteria, in the same field of view. At least 500 hybridized cells were enumerated for each of the filters or, at low abundances of hybridized cells, at least 1,000 DAPI stained cells were counted (Pernthaler et al. 1998). At each time point (t = 0, 1.5, 3, 6 and 12 months), counts were completed for four phylogenetic groups, as well as for the negative control probe, on each of four filters.

2.3.5 Determination of Relative Abundances of Hybridized Cells

From the direct counts of hybridized and DAPI stained bacteria, relative abundances of hybridized cells were determined by dividing the number of hybridized cells for each target group by the total number of DAPI stained cells in each field of view. All values were corrected for non-specific binding and background fluorescence by subtracting counts obtained with the NON338 probe.

2.3.6 Statistical Treatment and Models

The assumptions (Seber 1977) of normality, independence, and homogeneity of the residuals were checked and found to hold. Linearity was also confirmed, and a two-way Analysis of Variance (ANOVA) was employed to determine an overall time effect, followed by an Analysis of Covariance (ANCOVA) to determine if the time-dependent slopes (i.e. percent change per month) differed significantly for each probe group, and further regression analyses for the individual probe groups. The response variable was the proportion of bacterial cells detected with target oligonucleotide probes, reported as a percentage of the total bacterial cells determined by DAPI counts. All statistical analyses were performed using Minitab Release 14 and SPSS 13.0.

2.4 Results and Discussion

The overall objective of this study was to determine the effects of sample storage on the characterization of bacterial community structure using FISH as the analytical tool. To achieve this goal, we carried out a time course study using four rRNA-targeted oligonucleotide probes to quantify the effects of storage on the detection of bacterial cells

at the highest taxonomic level, the domain *Bacteria*; and the intermediate levels, alpha (ALF968) and gamma (GAM42a) subclasses of *Proteobacteria*, and the *Cytophaga-Flavobacteria* (CF319a) cluster. Replicate seawater samples were fixed, filtered and either counted immediately (i.e. time zero) or stored frozen at -20°C for up to one year. During this time course, four replicate samples were thawed, hybridized with target oligonucleotide probes, counterstained with DAPI and counted at 1.5, 3, 6, and 12 months. The structure of the bacterial community, as characterized by FISH, was determined at each time point in order to determine how storage influenced the hybridization and detection of target cells.

The proportions of bacterial cells detected with each of the target oligonucleotide probes are shown in Figure 2.1. A sequential statistical analysis was employed to determine if target cell detection (%) changed over time. Firstly, a two-way ANOVA was used to examine if all probe groups exhibited the same response; 'time' was the independent variable and 'oligonucleotide probe' was the fixed factor. A significant interaction between 'time' and 'oligonucleotide probe' ($F_{12,71} = 2.83$, $p = 0.004$, $n = 78$) indicated that the probe groups responded differently. Secondly, an ANCOVA was used to determine whether the slopes of the regression of target cell detection (%) versus time differed for the probe groups. The results of the ANCOVA were also statistically significant ($F_{3,71} = 4.92$, $p = 0.004$, $n = 78$), indicating that the probe groups responded differently over time. Thirdly, regression analyses were conducted to assess the effects of storage time within each probe group (Table 2.2). No significant differences in target cell detection were observed for the CF319a, ALF968, or GAM42a probes. Only the

EUB338 probe had a time-dependent slope that was statistically significant (slope = 0.529, $p = 0.037$, $n = 20$); the percent change in target cell detection was 6.3% per year, which is small relative to the typical counting error of ±10% for FISH (reported by Glöckner et al. 2000, Pernthaler et al. 2001), and ±15% for Acridine Orange or DAPI direct counts (reported by Ducklow and Dickson 1994). We conclude that during this 12-month time course study, there was a minimal effect of long-term sample storage on target cell detection using FISH and the resultant analyses of bacterial community structure. A statistically significant effect was only observed for the EUB338 probe, and there was no statistically significant difference in target cell detection with the EUB338 probe over the first 6 months ($p = 0.870$, $n = 16$; Table 2.3).

The results of this study are an important validation of field studies where bacterial community structure has been characterized from seawater samples that have been fixed, filtered and stored for up to 12 months. Although there have been several studies of the effects of sample storage on direct counts of bacteria (Turley and Hughes 1992, 1994; Turley 1993; Gundersen et al. 1996) and photosynthetic picoplankton (Booth 1987; Hall 1991; Putland and Rivkin 1999), we are not aware of any previous studies quantifying the effects of long-term sample storage on the efficiency of target cell detection using FISH. The results of prior studies of storage effects cannot be applied to samples stored for FISH since these studies assessed the effects of storage on the detection of DAPI- or AO-stained cells, but not the detection of cells using rRNA-targeted oligonucleotide probes. Our results show that the effects of storage on samples prepared for FISH differ from those observed with seawater samples for DAPI or AO direct counts. As there was no

statistically significant difference in target cell detection over the first 6 months of the time course, samples for FISH can be prepared (fixed and filtered) and stored at -20°C for up to 6 months with no significant change in target cell detection, and stored for up to 12 months with a very small (< 7%) effect of storage. Thus, we recommend that, when possible, preserved FISH samples should be analyzed within 6 months of sample collection.

Accurate estimates of both total bacterial numbers and the relative abundances of specific bacterial phylotypes are crucial to quantify both bacterial trophodynamics and the relationship between bacterial community structure and ecosystem function. Estimates of bacterial abundance, with appropriate conversion factors, are used to calculate bacterial biomass and estimate the flux of carbon through the microbial food web and quantify the role of bacteria in mediating biogeochemical processes (Pernthaler and Amann 2005). Although there has been a great deal of progress in characterizing the phylogenetic diversity of marine bacterial assemblages, through the use of FISH (Glöckner et al. 1999; Amann et al. 2001; Wagner et al. 2003) and other molecular methods (Pace et al. 1986; Fuhrman et al. 1993; Zehr and Voytek 1999; Spiegelman et al. 2005), linking bacterial community structure with specific biogeochemical processes has proven to be difficult (Kirchman 2002). Only a small fraction of naturally occurring bacteria, as identified by rRNA clones, appear closely related to cultivated bacteria from the same environments (DeLong 1998). Recently developed molecular techniques combining catalyzed reporter deposition with FISH (CARD-FISH) (Pernthaler et al. 2002) and microautoradiography (MICRO-CARD-FISH) (Teira et al. 2004; Herndl et al. 2005) allow for the

characterization of the phylogenetic diversity of bacteria as well as the measurement of substrate utilization by specific bacterial phylotypes. A current constraint of the FISH technique is that the percentage of cells detected with the Eubacteria probe varies widely with ecosystem type; however, there are a number of methodological factors that may also contribute variation in results, including cell fixation, hybridization conditions, mounting solutions, fluorochrome type and counting methods (Bouvier and del Giorgio 2003; Williams et al. 2004). Moreover, since these methodological factors may contribute to variation in the detection of bacterial cells using FISH (and perhaps other techniques using similar methodology, such as MICRO-CARD-FISH), these factors must be addressed to accurately compare studies of the phylogenetic diversity of bacteria from different ecosystem types as well as samples from the same ecosystem that have been analyzed at different times. Although future research should also examine the effects of storage on loss of fluorescence when using the sulfoindocyanine dye, indocarbocyanine (Cy3), as well as the length of time that prepared slides may be stored without a significant reduction in cell detection using epifluorescence microscopy, the results reported here provide a baseline for the length of time that seawater samples for FISH may be stored before significant changes in target cell detection are observed.

2.5 Acknowledgements

This research was supported in part by a fellowship to KFK from the School of Graduate Studies, Memorial University of Newfoundland, and grants to RBR from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Surface Ocean Lower Atmosphere Study (C-SOLAS) Research Network. Funding was provided to C-SOLAS by NSERC and the Canadian Foundation for Climate and Atmospheric Sciences. We thank H. Evans for assistance with sample collection.

2.6 Literature Cited

Afreider, A., Pernthaler, J., Amann, R., Sattler, B., Glöckner, F. O., Wille, A., and Psenner, R. 1996. Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. Appl. Environ. Microbiol., 62: 2138-2144.

Amann, R., Fuchs, B. M., and Behrens, S. 2001. The identification of microorganisms by fluorescence in situ hybridisation. Curr. Opin. Microbiol., 12: 231-236.

Amann, R., Snaidr, J., Wagner, M., Ludwig, W., and Schleifer, K.-H. 1996. In situ visualization of high genetic diversity in a natural microbial community. J. Bacteriol., 178: 3496-3500.

Booth, B. C. 1987. The use of autofluorescence for analyzing oceanic phytoplankton communities. Bot. Mar., 30: 101-108.

Bouvier, T. C., and del Giorgio, P. A. 2003. Factors influencing the detection of bacterial cells using fluorescence in situ hybridization (FISH): a quantitative review of published reports. FEMS Microbiol. Ecol., 44: 3-15.

Bouvier, T. C., and del Giorgio, P. A. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol. Oceanogr., 47: 453-470.

Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R., and Helmke, E. 2003. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl. Environ. Microbiol., 69: 6610-6619.

Christensen, H., Hansen, M., and Sørensen, J. 1999. Counting and size classification of active soil bacteria by fluorescence in situ hybridization with an rRNA oligonucleotide probe. Appl. Environ. Microbiol., 65: 1753-1761.

Cottrell, M. T., and Kirchman, D. L. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnol. Oceanogr., 48: 168-178.

Cottrell, M. T., and Kirchman, D. L. 2000a. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl. Environ. Microbiol., 66: 5116-5122.

Cottrell, M. T., and Kirchman, D. L. 2000b. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and

high- molecular-weight dissolved organic matter. Appl. Environ. Microbiol., 66: 1692-1697.

Daims, H., Ramsing, N. B., Schleifer, K.-H., and Wagner, M. 2001. Cultivation independent, semiautomatic determination of absolute bacterial cell numbers in environmental samples by fluorescence in situ hybridization. Appl. Environ. Microbiol., 67: 5810-5818.

DeLong, E. F. 1998. Molecular phylogenetics: new perspective on the ecology, evolution and biodiversity of marine organisms. In: Cooksey, K. E., ed. Molecular Approaches to the Study of the Ocean. Chapman & Hall, London: 1-28.

DeLong, E. F., and Karl, D. M. 2005. Genomic perspectives in microbial oceanography. Nature, 437: 336-342.

DeLong, E. F., Taylor, L. T., Marsh, T. L., and Preston, C. M. 1999. Visualization and enumeration of marine planktonic Archaea and Bacteria by using polyribonucleotide probes and fluorescent in situ hybridization. Appl. Environ. Microbiol., 65: 5554-5563.

Ducklow, H., and Dickson, A., eds. June 1994. Protocols for the Joint Global Ocean Flux Studies (JGOFS) core measurements. [Online.] http://www.jodc.go.jp/JGOFS_DMO/JGOFS_Protocols.pdf

Fenchel, T., King, G. M., and Blackburn, T. H. 1998. Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling, 2nd ed. Academic Press, San Diego, London; 307 pp.

Fuhrman, J. A., McCallum, K., and Davis, A. A. 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific oceans. Appl. Environ. Microbiol., 59: 1294-1302.

Glöckner, F. O., Amann, R., Alfreider, A., Pernthaler J., Psenner, R., Trebesius, K., and Schleifer, K.-H. 1996. An in situ hybridization protocol for detection and identification of planktonic bacteria. Syst. Appl. Microbiol., 19: 403-406.

Glöckner, F. O., Fuchs, B. M., and Amann, R. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl. Environ. Microbiol., 65: 3721-3726.

Giovannoni, S. J., and Stingl, U. 2005. Molecular diversity and ecology of microbial plankton. Nature, 437: 343-348.

Gundersen, K., Bratbak, G., and Heldal, M. 1996. Factors influencing the loss of bacteria in preserved seawater samples. Mar. Ecol. Prog. Ser., 137: 305-310.

Hahn, D., Amann, R., Ludwig, W., Akkermans, A. D. L., and Schleifer, K.-H. 1992. Detection of micro-organisms in soil after in situ hybridization with rRNA targeted, fluorescently labelled oligonucleotides. J. Gen. Microbiol., 138: 879-887.

Hall, J. A. 1991. Long-term preservation of picoplankton for counting by epifluorescence microscopy. Br. Phycol. J., 26: 169-174.

Head, I. M., Saunders, J. R., and Pickup, R. W. 1998. Microbial evolution, diversity, and ecology: a decade of ribosomal rRNA analysis of uncultivated microorganisms. Microb. Ecol., 35: 1-21.

Heidelberg, J. F., Heidelberg, K. B., and Colwell, R. R. 2002. Seasonality of Chesapeake Bay bacterioplankton species. Appl. Environ. Microbiol., 68: 5488-5497.

Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., and Pernthaler, J. 2005. Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol., 71: 2303-2309.

Horner-Devine, M. C., Carney, K. M., and Bohannan, B. J. M. 2003. An ecological perspective on bacterial biodiversity. Proc. R. Soc. Lond. B, 271: 113-122.

Hugenholtz, P., Goebel, B. M., and Pace, N. R. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bacteriol., 180: 4765-4774.

Hugenholtz, P., Tyson, G. W., and Blackall, L. L. 2001. Design and evaluation of 16S rRNA-targeted oligonucleotide probes for fluorescence in situ hybridization. In: Lieberman, B. A., ed. Molecular Biology, vol. 179: Steroid Receptor Methods: Protocols and Assays. Humana Press, Totowa NJ: 29-42.

Karner, M., and Fuhrman, J. A. 1997. Determination of active marine bacterioplankton: a comparison of universal 16S rRNA probes, autoradiography, and nucleoid staining. Appl. Environ. Microbiol., 63: 1208-1213.

Kepner, R. L., Jr., and Pratt, J. R. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol. Rev., 58: 603-615.

Kirchman, D. L. 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiol. Ecol., 39: 91-100.

Lim, E. L., Amaral, L. A., Caron, D. A., and DeLong, E. F. 1993. Application of rRNA-based probes for observing nanoplanktonic protists. Appl. Environ. Microbiol., 59: 1647-1655.

Lim, E. L., Caron, D. A., and DeLong, E. F. 1995. Development and field application of a quantitative method for examining natural assemblages of protists with oligonucleotide probes. Appl. Environ. Microbiol., 62: 1416-1423.

Macalady, J., and Banfield, J. F. 2003. Molecular geomicrobiology: genes and geochemical cycling. Earth Plant. Sci. Lett., 209: 1-17.

Massana, R., Guillou, L., Díez, B., and Pedrós-Alió, C. 2002. Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean. Appl. Environ. Microbiol., 68: 4554-4558.

Newman, D. K., and Banfield, J. F. 2002. Geomicrobiology: how molecular-scale interactions underpin biogeochemical systems. Science, 296: 1071-1077.

Nishino, S. F. 1986. Direct acridine orange counting of bacteria preserved with acidified Lugol iodine. Appl. Environ. Microbiol., 52: 602-604.

Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. Science, 276: 734-740.

Pace, N. R., Stahl, D. J., Lane, D. J., and Olsen, G. J. 1986. The analysis of natural microbial populations by ribosomal RNA sequences. Adv. Microbiol. Ecol., 9: 1-55.

Pernthaler, A., Pernthaler, J., and Amann, R. 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol., 68: 3094-3101.

Pernthaler, J., Alfreider, A., Posch, T., Andreatta, S., and Psenner, R. 1997. In situ classification and image cytometry of pelagic bacteria from a high mountain lake (Gossenköllesee, Austria). Appl. Environ. Microbiol., 63: 4778-4783.

Pernthaler, J., and Amann, R. 2005. Fate of heterotrophic microbes in pelagic environments: focus on populations. Microbiol. Mol. Biol. Rev., 69: 440-461.

Pernthaler, J., Glöckner, F. O., Schönhuber, W., and Amann, R. 2001. Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. In: Paul, J., ed. Methods in Microbiology: Marine Microbiology, vol. 30. Academic Press, London: 207-226.

Pernthaler, J., Glöckner, F. O., Unterholzner, S., Alfreider, A., Psenner, R., and Amann, R. 1998. Seasonal community and population dynamics of pelagic Bacteria and Archaea in a high mountain lake. Appl. Environ. Microbiol., 64: 4299-4306.

Porter, K. G., and Feig, Y. S. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr., 25: 943-948.

Putland, J. N., and Rivkin, R. B. 1999. Influence of storage mode and duration on the microscopic enumeration of Synechococcus from a cold coastal ocean environment. Aquat. Microb. Ecol., 17: 191-199.

Rappé, M. S., and Giovannoni, S. J. 2003. The uncultured microbial majority. Annu. Rev. Microbiol., 57: 369-394.

Schweitzer, B., Huber, I., Amann, R., Ludwig, W., and Simon, M. 2001. α- and β-proteobacteria control the consumption and release of amino acids on lake snow aggregates. Appl. Environ. Microbiol., 67: 632-645.

Seber, G. A. F. 1977. Analysis of residuals. In: Seber, G. A. F. Linear Regression Analysis. John Wiley & Sons, New York: 162-172.

Simon, N., LeBot, N., Marie, D., Partensky, F., and Vaulot, D. 1995. Fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes to identify small phytoplankton by flow cytometry. Appl. Environ. Microbiol., 61: 2506-2513.

Spiegelman, D., Whissell, G., and Greer, C. W. 2005. A survey of the methods for the characterization of microbial consortia and communities. Can. J. Microbiol., 51: 355-386.

Spinrad, R. W., Glover, H., Ward, B. B., Codispoti, L. A., and Kullenberg, G. 1989. Suspended particle and bacterial maxima in Peruvian coastal waters during a cold water anamoly. Deep-Sea Res., 36: 715-733.

Stackebrandt, E., and Embley, T. M. 2000. Diversity of uncultured organisms in the environment. In: Colwell, R. R., and Grimes, D. J., eds. Nonculturable Micro-Organisms in the Environment. ASM Press, Washington: 57-75.

Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G. J. 2004. Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. Appl. Environ. Microbiol., 70: 4411-4414.

Turley, C. M. 1993. Direct estimates of bacterial numbers in seawater samples without incurring cell loss due to sample storage. In : Kemp, P. F., Sherr, B. F., Sherr, E. B., and Cole, J .J., eds. Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton: 143-147.

Turley, C. M., and Hughes, D. J. 1992. Effects of storage on direct estimates of bacterial numbers of preserved seawater samples. Deep-Sea Res., 39: 375-394.

Turley, C. M., and Hughes, D. J. 1994. The effect of storage temperature on the enumeration of epifluorescence-detectable bacterial cells in preserved sea-water samples. J. Mar. Biol. Ass. U. K., 74: 259-262.

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., Wu, D., Paulsen, I., Nelson, K. E., Nelson, W., Fouts, D. E., Levy, S., Knap, A. H., Lomas, M.W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkock, C., Rogers, Y., and Smith, H. O. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science, 304: 66-74.

Wagner, M.-H., Erhart, R., Manz, W., Amann, R., Lemmer, H., Wdei, D., and Schleifer, K.-H. 1994. Development of an rRNA-targeted oligonucleotide probe specific for the genus Actinobacter and its application for in situ monitoring in activated sludge. Appl. Environ. Microbiol., 60: 792-800.

Wagner, M., Horn, M., and Daims, H. 2003. Fluorescence in situ hybridisation for the identification and characterisation of prokaryotes. Curr. Opin. Microbiol., 6: 302-309.

Weiss, P., Schweitzer, B., Amann, R., and Simon, M. 1996. Identification in situ and dynamics of bacteria on limnetic organic aggregates (lake snow). Appl. Environ. Microbiol., 62: 1998-2005.

Wells, L. E., and Deming, J. W. 2003. Abundance of Bacteria, the Cytophaga-Flavobacterium cluster and Archaea in cold oligotrophic waters and nepheloid layers of the Northwest Passage, Canadian Archipelago. Aquat. Microb. Ecol., 31: 19-31.

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

Williams, S. C., Heaton, D. P., Hristov, R., Frischer, M. E., and Verity, P. G. 2004. Methodological factors that contribute to variability using fluorescence in situ hybridization (FISH) in marine planktonic environments. [Online.] http://www.skio.peachnet.edu/research/veritylab/biocomp/presentations/methods_factors/methods_factors.pdf. Accessed 17 November 2005.

Zehr, J. P., and Voytek, M. A. 1999. Molecular ecology of aquatic communities: reflections and future directions. Hydrobiologia, 401: 1-8.

Zengler, K., Toledo, G., Rappé, M., Elkins, J., Mathur, E. J., Short, J. M., and Keller, M. 2002. Cultivating the uncultured. Proc. Natl. Acad. Sci., 99: 15681-15686.

Zwisler, W., Selje, N., Simon, M. 2003. Seasonal patterns of the bacterioplankton community in a large mesotrophic lake. Aquat. Microb. Ecol., 31: 211-225.

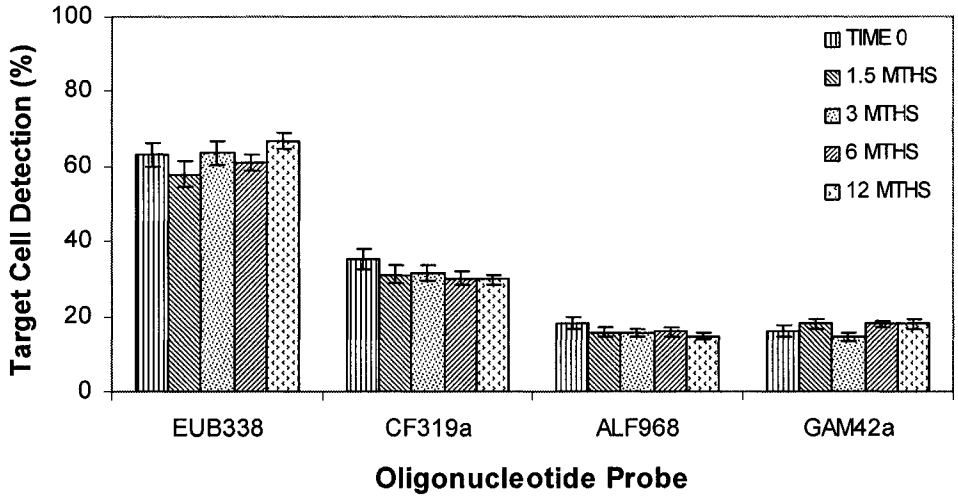


Figure 2.1. Mean percentage (%) of bacterial cells detected with target oligonucleotide probes during a one-year time course. Target groups are the *Eubacteria* (EUB338), alpha- (ALF968) and gamma- (GAM42a) *Proteobacteria*, and the *Cytophaga-Flavobacteria* (CF319a) cluster. All probe counts were corrected by subtracting counts obtained with the negative control probe NON338. Percentages were computed as cells hybridizing with the target oligonucleotide probe divided by the total cells as determined from DAPI counts, and averages were calculated from four replicate filters at each time point. Error bars indicate 95% confidence intervals.

Table 2.1. Oligonucleotide probe sequences and target bacterial groups.

Probe	**Sequence**	**Specificity**
EUB338	GCTGCCTCCCGTAGGAGT	Most *Bacteria*; not Planctomyces
CF319a	TGGTCCGTGTCTCAGTAC	*Cytophaga-Flavobacteria*
ALF968	GGTAAGGTTCTGCGCGTT	α-*Proteobacteria*
GAM42a	GCCTTCCCACATCGTTT	γ-*Proteobacteria*
NON338	ACTCCYACGGGAGGCAGC	Negative control probe; complementary to EUB338

Table 2.2. Results of Model I regression analysis for each target probe group (percent target cell detection) as a function of time (month) for a one-year time course.

Probe	**Slope**	**p**	**n**
EUB338	0.529	0.037	20
CF319a	-0.314	0.076	19
ALF968	-0.207	0.144	20
GAM42a	0.188	0.192	20

Table 2.3. Results of Model I stepwise regression analysis of percent target cell detection for the EUB338 probe as a function of time (months) during a one-year time course. Values reported are the slopes of the regression (percent change per month) with p and n in parentheses. Slopes that are significantly different (p = 0.05) from zero are underlined.

	1.5 mths	**3 mths**	**6 mths**	**12 mths**
Initial	-3.480	0.260	-0.086	<u>0.529</u>
	(0.145; 8)	(0.883; 12)	(0.870; 16)	(0.037; 20)
1.5 mths	-	3.990	0.404	<u>0.769</u>
		(0.063; 8)	(0.557; 12)	(0.007; 16)
3 mths	-	-	1.030	0.563
			(0.302; 8)	(0.104; 12)
6 mths	-	-	-	<u>1.200</u>
				(0.021; 8)

Chapter 3:

Seasonal and Spatial Patterns of Microbial Community Structure in the Northwest Atlantic Ocean

3.1 Abstract

The phylogenetic diversity of the microbial community was characterized during three seasons (spring, summer, and fall 2003) in contrasting biogeochemical provinces of the Northwest Atlantic Ocean, as part of the Study of Air-sea Biogeochemical Interactions in the Northwestern Atlantic (SABINA), a Canadian SOLAS (Surface Ocean Lower Atmosphere Study) network project. Microbial community structure was characterized using fluorescence *in situ* hybridization (FISH) with rRNA-targeted oligonucleotide probes, and related to a number of concurrently-measured environmental parameters. The proportion of cells detected with the *Eubacteria* probe ranged from 23 to 76% at the surface, with a mean of 45%, and from 1 to 17% at 200 m, with a mean of 11%. Fewer cells were detected with the *Archaea* probe; relative abundances ranged from 0 to 10% at the surface, with a mean of 1%, and from 0 to 9% at 200 m, with a mean of 3%. The *Cytophaga-Flavobacteria* cluster dominated the spring and summer bacterial communities at most stations, except station T2, in the Sargasso Sea, where the α-*Proteobacteria* was the dominant phylogenetic group. From summer to fall, there was a noticeable decrease in the proportion of *Cytophaga-Flavobacteria*, and the α-, β-, and γ-*Proteobacteria* dominated the fall community at all stations. Seasonally, the most noticeable shift in the relative abundances of specific phylogenetic groups occurred from summer to fall. Spatially, the greatest differences in bacterial community composition

were observed between the North Atlantic Subtropical Gyre and the Atlantic Arctic province.

3.2 Introduction

The World Ocean can be partitioned into distinct ecological regions; the primary ones being the Polar, Westerlies, Trades, and Coastal Boundary Zone biomes (Longhurst 1998). Within each of these biomes, the oceans can be further partitioned into biogeochemical provinces, which are defined on the basis of physical processes and the biological response to spatial and temporal variations in physical forcing (Platt and Sathyendranath 1999). The critical criteria for defining biogeochemical provinces include the subsurface chlorophyll profile, values of the photosynthetic parameters, and depths of the mixed layer and nutricline (Longhurst 1998). The boundaries of these biogeochemical provinces are not fixed or discrete and vary at interannual and seasonal time scales (Platt and Sathyendranath 1999). Identifying water masses by a defined set of physicochemical characteristics is useful in assessing regional variations in the structure of the pelagic food web, particularly the autotrophic phytoplankton and heterotrophic bacteria (Li and Harrison 2001), as the ratio of bacterial biomass to phytoplankton biomass generally increases along a gradient of primary productivity (Fuhrman et al. 1989; Cho and Azam 1990).

Heterotrophic bacteria are ubiquitous and abundant in the World Ocean and often dominate the biomass of planktonic food webs, particularly in oligotrophic ocean regions (Williams 2000). Bacteria also have a central role in mediating much of the water

column biogeochemical processes, including the cycling of organic carbon, nitrogen, sulphur, phosphorus and inorganic elements (Fenchel et al. 1998; Newman and Banfield 2002). Until recently, marine bacteria have been considered as a single functional group with unknown diversity and variation (Kirchman 2002). However, it is now recognized that the abundance and activity of different phylogenetic groups within the bacterial assemblage and their role in the cycling, transformation, and remineralization of dissolved organic matter (DOM) may vary both spatially and temporally (Cottrell and Kirchman 2000; Kirchman 2003; Malmstrom et al. 2005). Bacterial parameters, such as biomass, specific growth rate, and growth efficiency are controlled, in part, by the availability of organic and inorganic nutrients. Thus, it is likely that specific bacterial phylotypes also differ in their growth capacities and contribution to the cycling and transformation of DOM (Kirchman 2003). A study by Cottrell and Kirchman (2000b) of the relative contributions of specific bacterial phylotypes to the utilization of DOM in the Delaware Bay estuary indicated that all bacterial groups do not use all components of DOM equally. The *Cytophaga-Flavobacteria* cluster comprised the largest fraction of cells assimilating chitin and protein, but the smallest fraction consuming amino acids. In contrast, the assimilation of amino acids was dominated by the α-*Proteobacteria*, for which protein consumption was lowest.

The planktonic *Archaea*, previously thought to thrive only in extreme environments, e.g. high temperature, low pH, high salinity, or strict anoxia (Delong 1998), are now estimated to account for as much as one-third of all prokaryotic cells in the World Ocean (Karner et al. 2001). Moreover, recent studies have shown that *Archaea* are actively

involved in the heterotrophic uptake of DOM, in the form of dissolved amino acids, particularly in the deep (> 200 m) ocean (Ouverney and Fuhrman 2000; Teira et al. 2004). Thus, understanding the role of *Bacteria* and *Archaea* in biogeochemical models, such as those for ocean-carbon cycling, requires knowledge not only of the ecology but also the phylogenetic diversity and metabolic activity of microbial communities.

The structure of the microbial food web is controlled largely by biogeochemical processes in the upper ocean, resulting in phylotypes and trophic levels with different physiological characteristics and metabolic capacities, and thus, different roles in ecosystem function. Moreover, variations in the structure and dynamics of the microbial community in contrasting biogeochemical provinces of the oceans will lead to distinct patterns in the cycling of climate-active gases (CO_2, volatile sulphur, and halogenated compounds) which, in turn, will have important feedbacks on climate. Recent studies indicate that bacteria are a major sink for dimethylsulphide (DMS) (González et al. 2000; Kiene et al. 2000; Simó et al. 2000; Malmstrom et al. 2004b), the principal volatile sulphur-containing compound in the oceans and the major source of sulphur to the atmosphere (Denman et al. 1996). When bacterial sulphur demand is high, more dimethylsulphoniopropionate (DMSP), the biological precursor of DMS, is used as a sulphur source for biomass production and less is released as DMS (Simó 2001). Thus, bacteria have a controlling role in the amount of DMS that is released into the atmosphere by switching DMSP degradation towards more or less DMS production (Yoch 2002).

Within the α-*Proteobacteria*, the SAR11 clade has been identified as a highly metabolically-active group in the surface waters of the coastal North Atlantic Ocean and Sargasso Sea, accounting for as much as 50% of the amino acid assimilation and 30% of DMSP consumption (Malmstrom et al. 2004a). Thus, the α-*Proteobacteria* may outcompete other bacterial phylotypes for dissolved free amino acids and DMSP, and play a significant role in the cycling of carbon, nitrogen, and sulphur in the oceans (Malmstrom et al. 2005). Members of the *Cytophaga-Flavobacteria* cluster are also widespread in the marine environment, functioning primarily in the degradation of biopolymers such as cellulose, chitin, and pectin (Giovannoni and Rappé 2000; Kirchman 2002). The *Cytophaga-Flavobacteria* cluster is often the most numerically abundant phylogenetic group in oceanic habitats (Glöckner et al. 1999), and may constitute as much as half of the bacterial assemblage (Kirchman 2002).

In the North Atlantic Ocean, there is a seasonal progression from light to nutrient limitation of phytoplankton growth (Townsend et al. 1994). During the spring bloom, there is a succession from large diatoms to small eukaryotic and prokaryotic autotrophs and heterotrophs (Ducklow and Harris 1993; Longhurst 1998). Depth-integrated bacterial abundances vary little across biogeochemical provinces of the Northwest Atlantic, with the exception of the Atlantic Arctic province, where bacterial abundances have been documented as two-fold higher than in the North Atlantic Subtropical Gyre (Li and Harrison 2001). Seasonal cycles of carbon production and nutrient utilization have been studied in certain regions of the Northwest Atlantic (Wu et al. 2000; Bates 2001; Cavender-Bares et al. 2001; Gruber et al. 2002). However, little is known about the

seasonal dynamics of climate-active trace gases. High emissions of climate-active trace gases, such as DMS, have been observed during all seasons, not only during the spring phytoplankton bloom (Malmstrom et al. 2004b). In addition, very little is known about the phylogenetic diversity of the bacterial community in the Northwest Atlantic or the biological and physicochemical forcings that influence the community structure. Prior studies of bacterial diversity in the Northwest Atlantic have been limited to estuaries and bays (Cottrell and Kirchman 2000; Bouvier and del Giorgio 2002), and the Sargasso Sea, primarily at the Bermuda Atlantic Time-series Study (BATS) site (Fuhrman et al. 1993; Mullins et al. 1995; Malmstrom et al. 2004b; Morris et al. 2005). Moreover, since seasonal cycles are difficult to study in the open ocean, mainly due to limited ship time and financial resources, there have been no prior seasonal studies of bacterial community structure in the Atlantic Ocean. Similar studies have been undertaken in freshwater (Lindström 1998; Pernthaler et al. 1998) and coastal (Murray et al. 1998; Pinhassi & Hagström 2000; Schauer et al. 2003; Zwisler et al. 2003; Mary et al. 2006) environments, but there have been no studies of the seasonality of bacterial community structure in an open ocean environment.

Here we examine seasonal changes in the phylogenetic diversity of the bacterial community in contrasting biogeochemical provinces of the Northwest Atlantic Ocean, and how these changes relate to biogeochemical and ecosystem processes on a large geographical and temporal scale. These results are used to test the general hypothesis that bacterial community structure is controlled by environmental conditions, particularly variations in factors such as temperature, chlorophyll *a*, and nutrient availability

(Fuhrman et al. 2006). The phylogenetic diversity of the bacterial community was characterized during the Study of Air-sea Biogeochemical Interactions in the Northwestern Atlantic (SABINA), using fluorescence *in situ* hybridization (FISH), and related to a number of concurrently measured ecological and climate-related parameters, namely measures of bacterial dynamics (bacterial abundance, bacterial biomass, bacterial production and bacterial growth rate); distinguishing features of the biogeochemical provinces, specifically temperature and chlorophyll *a*; and the climate-active trace gas, dimethylsulphide (DMS) and its biological precursor, dimethylsulphoniopropionate (DMSP).

3.3 Materials and Methods

3.3.1 Sampling Area

This research was undertaken during three SABINA cruises in the Northwest Atlantic Ocean, during the spring (April 25 – May 14, CCGS *Hudson*), summer (July 8 – 22, NGCC *Martha L. Black*), and fall (October 13 – 27, NGCC *Martha L. Black*) of 2003. During each cruise, samples were collected from eight stations within five ecological provinces, as defined by Longhurst (1998); Coastal biome: Northwest Atlantic Shelves Province (NWCS); Westerlies biome: Gulf Stream province (GFST), North Atlantic Drift Province (NADR), North Atlantic Subtropical Gyral West Province (NASW); and Polar biome: Atlantic Arctic Province (ARCT). (Figure 3.1 gives geographic locations and coordinates of the stations sampled).

3.3.2 Sampling and Cell Fixation

At each of the eight stations, samples were collected using 10-L Niskin bottles mounted on a rosette sampler. Samples were collected at four depths, generally at the 50% and 30% light depths, the chlorophyll maximum, and one depth below the euphotic zone (generally 200 m). For each 90-mL seawater sample, two 45-mL aliquots placed into sterile 50-mL falcon tubes and fixed with 10 mL of 37% formalin (5 mL in each falcon tube). After one to six hours, the fixed samples were filtered on white 0.2-μm polycarbonate membrane filters (Millipore GTTP04700) that were placed over a cellulose prefilter (Millipore AP1504700). The polycarbonate filters were then washed with 50 mL of phosphate buffered saline (sterile and prefiltered), and air-dried over absorbent paper in individual sterile petri dishes. The samples were stored at -20°C until analysis.

3.3.3. Oligonucleotide Probes

In this study, fluorescently-labelled rRNA-targeted oligonucleotide probes (MOBIX Lab, Hamilton, Ontario, Canada) were used for the phylogenetic analysis of microorganisms at two of the highest taxonomic levels: *Archaea* (ARCH915) and *Bacteria* (EUB338); as well as the intermediate taxonomic levels: alpha- (ALF968), beta- (BET42a) and gamma- (GAM42a) *Proteobacteria*, and the *Cytophaga-Flavobacteria* (CF319a) cluster. The nonsense probe (NON338), which is complementary to the EUB338 probe, was used as a negative control for the detection of non-specific binding and background fluorescence (Glöckner et al. 1996). The target bacterial groups and gene sequences for the oligonucleotide probes employed in this study are shown in Table 2.1. All

oliognucleotide probes were labelled at the 5' end position with the sulfoindocyanine dye, indocarbocyanine (Cy3).

3.3.4 Fluorescence *In Situ* Hybridization and DAPI Staining

The method of FISH that was employed in this study is a modification of the protocol of Glöckner et al. (1996). Each filter was cut into seven triangular pieces, and each piece was placed on a microscope slide. Before beginning the procedure, the hybridization buffer was prepared. The hybridization buffer required 360-µL NaCl 5M, 40-µL Tris-HCl 1M pH 8, and 700-µL formamide for all probes except ALF968, which required 400-µL formamide. This was completed to 2 mL with 0.2-µm filtered Milli Q water, after which 2-µL sodium dodecyl sulphate (SDS) 10% was added.

After the hybridization buffer was prepared, the hybridization solution was mixed by adding 6 µL (50 ng/µL) of probe to 48 µL of hybridization buffer. The filters were hybridized by adding 20 µL of the hybridization solution to each filter section. The slide was then placed in a hybridization chamber containing a piece of absorbing paper and 1 mL of hybridization buffer (to create a humid atmosphere). The chamber was closed, sealed and incubated in the dark for two hours at 46°C. This procedure was repeated for each of the seven filter pieces, one for each of the oligonucleotide probes.

During the incubation, the washing buffer was prepared in individual sterile 50 mL falcon tubes. The washing buffer consisted of 1-mL Tris-HCl 1M pH 8, 500-µL EDTA, and 700-µL NaCl 5 M for all probes except ALF968, which required 2150-µL NaCl; this was

brought up to 50 mL with filtered Milli Q water, after which 50-μL SDS 10% was added. The washing buffer was also placed in the incubator at 46°C. After two hours, the hybridization chamber and the washing buffer were removed from the incubator, and filter pieces were placed in individual falcon tubes of washing buffer and incubated in the dark for 15 minutes at 46°C. Each filter piece was then dried over absorbent paper at room temperature, placed on a glass slide and counter-stained with 50 μL of DAPI (1 μg/mL) for 1 minute (on ice). After staining, each filter piece was washed with 1 mL of filtered Milli Q water and dried over absorbent paper. Each filter piece was mounted on a glass slide in glycerol medium (Citifluor #1; Citifluor, Canterbury, UK). The slides were then stored at -20°C (for a maximum of 48 hours) before microscopic analysis.

3.3.5 Epifluorescence Microscopy

The prepared slides were observed using an Olympus BH-2 epifluorescent microscope, equipped with a 100x 1.30 oil objective (1250x total magnification), Cy3 filter (41007-HQ) and DAPI filter (UG-1). All probes were fluorescently-labelled with the sulfoindocyanine dye Cy3, which absorbs at 552 nm (green light) and emits at 565 nm (orange light). As Cy3 fluorescence fades much more rapidly than DAPI fluorescence (Pernthaler et al. 2001), direct counts of hybridized (group specific) cells were completed first, followed by DAPI counts (under UV light), for total bacteria, in the same field of view. At least 500 hybridized cells were enumerated for each of the probes or, at low abundances of hybridized cells, at least 1,000 DAPI stained cells were counted (Pernthaler et al. 1998).

3.3.6 Determination of Relative Abundances of Hybridized Cells

From the direct counts of hybridized and DAPI stained bacteria, relative abundances of hybridized cells were determined by dividing the number of hybridized cells for each target group by the total number of DAPI stained cells in each field of view. The percentage of cells that were detected by the EUB338 probe but not by any of the probes for the intermediate taxonomic levels were cited as unidentified cells. All values were corrected for non-specific binding and background fluorescence by subtracting counts obtained with the NON338 probe.

3.3.7 Measurement of Other Water Column Properties

At each of the depths sampled for FISH, associated biological, chemical, and physical variables were also measured. Flow cytometry data was provided courtesy of Dr. W. K. W. Li of Bedford Institute of Oceanography, Department of Fisheries and Oceans Canada (DFO); Chl *a* data was provided courtesy of Drs. M. Gosselin and Z. P. Mei, and Mr. J. Pommier of Université du Quebec à Rimouski; DMS(P) data was provided courtesy of Dr. M. Scarratt and Ms. S. Michaud of Maurice Lamontagne Institute, DFO; and surface mixed layer depths were provided courtesy of Dr. Z. P. Mei.

Vertical profiles of temperature and *in vivo* chlorophyll fluorescence were obtained using a conductivity-temperature-depth (CTD) meter (equipped with an *in vivo* fluorometer) mounted on a rosette sampler. Seawater for the measurement of all other variables was collected from 10-L Niskin bottle samples. Chlorophyll *a* was determined fluorometrically after filtration using the method of Parsons et al. (1984). DMS and

DMSP analyses were conducted as described by Scarratt et al. (2002), except all samples were measured on a pulsed flame photometric detector (PFPD), and the total DMSPp was measured using a single filtration onto GF/F rather than from the sum of the different size fractions (M. Scarratt, personal communication). Bacterial abundances were determined by flow cytometry (Li 1995). Estimates of bacterial biomass were derived from biovolume measurements (image analysis) multiplied by a carbon conversion factor (i.e. $C = 120 \cdot V^{0.72}$; Norland 1993). Bacterial production was estimated from [^{3}H]-thymidine and [^{3}H]-leucine incorporation rates during 6-h dark incubations (Fuhrman and Azam 1982; Kirchman et al. 1985). Bacterial growth rates were derived from estimates of bacterial production, by conversion of the radioisotope uptake rates to cell carbon production (Kirchman et al. 1982).

Results are presented for stations L1, T2, T3, and T6 (originally designated Northwest Atlantic Shelves Province (NWCS), North Atlantic Drift Province (NADR), North Atlantic Subtropical Gyral West Province (NASW), and Atlantic Arctic Province (ARCT), respectively; Figure 2.1) as these stations had the most comprehensive coverage for all measured variables. Data for the relative abundances of hybridized cells and other variables for all stations can be found in the appendices.

3.3.8 Statistical Analyses

Relative abundances of hybridized cells were corrected for non-specific binding and background fluorescence by subtracting counts obtained with the NON338 probe, and the associated counting errors (standard deviations) were corrected using a propagation of

errors ($Z = A - B$, therefore $(\Delta Z)^2 = (\Delta A)^2 + (\Delta B)^2$; Taylor 1982). The mean standard deviation was calculated from counts of replicate fields for each of the probes (EUB338, ± 5.3%; CF319a, ± 2.8%; ALF968, ± 2.0%; BET42a, ± 1.3%; GAM42a, ± 1.5%; ARCH915, ± 1.4%). The general counting error associated with parallel counts of replicate filters, which are not practical for a large number of samples, has been cited as ± 10% (Glöckner et al. 2000; Pernthaler et al. 2001), and based on the results of Keats et al. (2006), the percent change in target cell detection is less than 7% for the duration of sample storage and analyses.

Correlation analyses were used to examine the relationships among the percentage of DAPI-stained cells detected with the *Eubacteria*, *Archaea*, and group-specific probes, and concurrently measured ecological and climate-related parameters. Since all variables were not measured on a continuous scale, Spearman's rank order correlation coefficients were computed. All statistical analyses were conducted using SPSS 13.0.

3.4 Results

3.4.1 Regional and Station Characteristics

The mean mixed layer temperatures, mixed layer depths, euphotic zone depths (depth of 1% incident irradiance), and maximum observed chlorophyll *a* concentrations, as well as the geographic locations, original and alternate biogeochemical province designations for Stations L, T2, T3, and T6, are shown in Table 3.2. The euphotic zone depth (z_{eu}) was generally deeper than the mixed layer depth (z_{mld}), except at stations L and T6 during the spring. The z_{mld} was greatest during the spring at stations L and T6, and during the fall at

stations T2 and T6. The z_{mld} remained shallow at all stations during the summer, ranging from 32 m at station T2 to 8 m at station L. Changes in the mean mixed layer temperature were much greater from spring to summer than from summer to fall for all stations; the lowest temperature was observed at station L during the spring (2.8°C), and the highest at station T2 during the fall (25.9°C). The Z_{eu} ranged from 30 m during the spring at station L to 110 m at station T2 during the summer. The highest observed chlorophyll concentrations ranged from a subsurface maximum of 16.13 μg L^{-1} at station L during the spring phytoplankton bloom, to a surface maximum of 0.33 μg L^{-1} at station T6 during the fall.

3.4.2 Heterotrophic Bacterial Abundance, Production, and Growth

Table 3.3 shows the bacterial abundances, production, and specific growth rates at the surface (50% or 30% light depth, depending where samples were collected) and 200 m depths. Bacterial abundances at the surface ranged from 0.18 x 10^9 cells L^{-1} at station T6 during the summer to 1.63 x 10^9 cells L^{-1} at station L during the spring phytoplankton bloom. At 200 m, bacterial abundances ranged from 0.10 x 10^9 cells L^{-1} at station L during the fall to 0.47 x 10^9 cells L^{-1} at station T2 during the spring. Bacterial abundances were generally three- to four-fold higher at the surface than at 200 m, except during the spring at stations T2 and T3, and during the summer at station T6, when bacterial abundances at 200 m were almost equivalent to those at the surface.

Bacterial production (calculated as the average of TdR- and Leu-incorporation rates) was also greatest at the surface during the spring phytoplankton bloom, with a value of 8.75

μg C L^{-1} d^{-1}, four-times higher than the other estimates of bacterial production. Otherwise, bacterial production at the surface ranged from 0.11 at station T2 during the spring to 2.12 μg C L^{-1} d^{-1} at station T2 during the summer. Bacterial production at 200 m ranged from 0.01 μg C L^{-1} d^{-1} at station L during the summer to 0.19 μg C L^{-1} d^{-1} at station T3 during the fall. Bacterial production was highest during spring, except at station T3, where bacterial production at 200 m was higher during fall.

Specific growth rate at the surface was highest at station L during spring, with a value of 0.17 d^{-1}. Specific growth rates at the surface were high during the spring at all stations, except at station T6. At 200 m, specific growth rates ranged from < 0.010 d^{-1} at station L during the summer to 0.068 d^{-1} at station T3 during the fall. Specific growth rates were greater at the surface than 200 m at all stations, except at station T3 during the summer, and stations T2 and T3 during the fall, when specific growth rates at 200 m exceeded those at the surface.

3.4.3 Microbial Community Structure (FISH)

3.4.3.1 Detection with the *Eubacteria* Probe

Overall, the proportion of cells detected with the *Eubacteria* probe ranged from 23 to 76 % at the surface (5-15 m depth), with a mean of 45% (95% CI, 38 to 52%), and 1 to 17% at 200 m, with a mean of 11% (95% CI, 8 to 14%). The proportion of cells detected with the *Eubacteria* probe (EUB338) at the surface (50% or 30% light depth, depending where samples were collected) and 200 m for stations L, T2, T3, and T6 are shown in Figure 3.2.

During spring at station L, the percentage of cells detected with the *Eubacteria* probe (% *Eubacteria*) ranged from 1% at 200 m to 76% at the surface. However, the % *Eubacteria* remained constant during the summer and fall, 44% at the surface and 7% at 200 m. At station T2, the % *Eubacteria* at 200 m ranged from 5% during the fall to 13% during the summer. At the surface, the highest proportion of cells detected with the *Eubacteria* probe at station T2 occurred during the spring at 53%, followed by a marked decrease to 23% during the summer, and an increase to 31% during the fall. A similar pattern was observed at station T3. The % *Eubacteria* at the surface was highest during the spring at 59%, decreased to 35% during the summer, and increased to 40% during the fall. A smaller range, 9 to 15%, was observed at 200 m. At station T6, the highest percentage of cells detected with the *Eubacteria* probe at the surface was also observed during the spring at 58%. Similarly to station L, the proportion of hybridized cells at station T6 remained relatively constant during the summer and fall at the surface, at 38 and 36%, respectively, and throughout all the seasons at 200 m, ranging from 15 to 17%. Overall, the *% Eubacteria* was highest during the spring at all stations, decreased from spring to summer, and remained relatively constant from summer to fall.

3.4.3.2 Detection with Group-Specific Probes

The percentage of bacterial cells detected with target oligonucleotide probes during the spring, summer, and fall is given for the surface (50% or 30% light depth, depending where samples were collected) only (Figure 3.3), as detection decreased significantly with depth and was less than 1% for most samples at 200 m.

At all stations, the highest relative abundances of *Cytophaga-Flavobacteria* occurred during the spring, ranging from 16% at station T2 to 27% at station T3. The *Cytophaga-Flavobacteria* accounted for the highest proportion of all identified cells at stations L, T3, and T6 during the spring and summer. Fewer cells belonging to the *Cytophaga-Flavobacteria* cluster were detected during the fall, ranging from 0% at station T2 to 15% at station L. The relative abundances of α-*Proteobacteria* ranged from 2% at station T6 to 26% at stations T2 and T3; all occurred during the spring. The proportion of α-*Proteobacteria* detected at station T6 was less than 4% during all seasons. At station T2, the α-*Proteobacteria* was the dominant group in both the spring and summer, whereas the *Cytophaga-Flavobacteria* cluster dominated at the other stations. The proportion of β-*Proteobacteria* was less than 2% at all stations and during all seasons, except at station T2 during the fall, when the β-*Proteobacteria* accounted for 11% of the bacterial assemblage. The relative abundances of γ-*Proteobacteria* were generally less than 5%, except at station L, where the proportion was highest during the spring at 21%, decreased to 4% during the summer, and increased to 15% during the fall. Cells from the *Archaea* domain were detected only during fall at stations T3 and T6, and accounted for 5 and 10%, respectively. The proportion of unidentified cells ranged from 0% at station T3 during the spring to 28% at station T6 during the fall. The highest proportions of unidentified cells were observed at station T6 during the spring and fall, followed by station T3 in the fall, and station L during the spring.

3.4.4 Relationships among Microbial Community Structure, Processes, and Environmental Factors

Spearman's rank order correlation coefficients were computed to examine the relationships among the proportion of cells detected with the *Eubacteria*, *Archaea*, and group-specific probes versus bacterial abundance, bacterial production, specific growth rate, bacterial cell volume, bacterial carbon, percentage of high-DNA cells, temperature, chlorophyll, and DMS(P) concentrations for both the mixed layer and 200 m.

Table 3.4 shows the results of correlation analyses for the percentage of cells detected with the *Eubacteria*, *Archaea*, and group-specific probes with BA, BP, and SGR within the mixed layer. Bacterial abundance, BP, and SGR were all strongly correlated with the % *Eubacteria.* Of the intermediate groups, only the % *Cytophaga-Flavobacteria* was significantly correlated with BA and BP. The % α-*Proteobacteria* was correlated with SGR, but not with BA or BP. No significant relationships were observed when % β-*Proteobacteria,* γ-*Proteobacteria*, and % *Archaea* were tested against BA, BP, and SGR. Within the mixed layer, no significant relationships were observed with bacterial cell volume, bacterial carbon, or % high-DNA cells. No significant correlations were observed with any of the variables at 200 m.

Table 3.5 shows the results of correlation analyses for BA, BP, SGR, and the proportions of cells detected with the *Eubacteria*, *Archaea*, and group-specific probes against temperature and chlorophyll. Results are given only for the mixed layer. Bacterial abundance, BP, SGR, *% Eubacteria* and % *Cytophaga-Flavobacteria* were all positively

correlated with chlorophyll, and inversely correlated with temperature. No significant relationships with chlorophyll or temperature were observed for the intermediate groups of *Proteobacteria* or the *Archaea*, or for any parameters at 200 m.

Table 3.6 shows the results of correlation analyses to examine the relationships among BA, BP, SGR, and % of DAPI-stained cells, including the specific phylogenetic groups, against DMSPd, and DMSPp concentrations for both the mixed layer and 200 m. Within the mixed layer, BA and BP were positively correlated with both DMSPd and DMSPp concentrations. However, at 200 m, BA was correlated only with DMSPp, and there were no significant correlations between BP and DMSPd or DMSPp. No significant relationships were observed with SGR or % *Eubacteria*. In the mixed layer, none of the phylogenetic groups were correlated with DMSPd, and only the % *Cytophaga-Flavobacteria* was correlated with DMSPp. At 200 m, the γ-*Proteobacteria* was positively correlated with both DMSPd and DMSPp, and there were no significant correlations with the other phylogenetic groups. No significant correlations with DMS were observed.

3.5 Discussion

In recent years, fluorescence *in situ* hybridization (FISH) has been widely used to characterize microbial community structure and had greatly advanced our knowledge of the abundance and distribution of *Bacteria* and *Archaea*. In the Northwest Atlantic Ocean, the proportion of cells detected with the *Eubacteria* probe ranged from 23 to 76 % in the surface mixed layer, with a mean of 45% (95% CI, 38 to 52%). These results are

comparable to previous studies of bacterial community structure in the North Sea (39 ± 3%, Glöckner et al. 1999; 55 ± 15%, Eilers et al. 2000), the Pacific Ocean (42 ± 3%, Glöckner et al. 1999), and the coastal waters of the Antarctic Peninsula (54 ± 9%; Murray et al. 1998), although only nearshore waters (< 5 miles from the coast) and the continental shelf (5-50 miles from the shoreline) were included in these studies. In the Northwest Atlantic, the proportion of cells detected with the *Archaea* probe ranged from 0 to 10% (mean = 1%; 95% CI, 0 to 2%) in the surface mixed layer, and 0 to 9% (mean = 3%; 95% CI, 2 to 4%) at 200 m. Across ecosystems, the *Archaea* have been shown to account for generally < 2% of cells detected using FISH (Bouvier and del Giorgio 2003). However, in the surface (< 100 m) waters of the Santa Barbara Channel, the relative abundances of *Archaea* ranged from 2 to 10%, with the highest proportions being observed during the winter (Massana et al. 1997).

Of the cells detected as *Bacteria*, about 71% (95% CI, 60 to 82%) could be identified as members of the *Cytophaga-Flavobacteria* cluster or the α-, β-, and γ-*Proteobacteria*. Generally, > 70% of the cells detected as *Bacteria* could be assigned to the intermediate phylogenetic groups; however, in some cases, a high proportion (0 to 28%) of cells could not be identified. This is most likely a result of the incomplete probe set used in this study; group-specific probes for the delta and epsilon subclasses of *Proteobacteria* were not used, and the CF319a probe has been shown to underestimate the abundance of *Cytophaga-Flavobacteria* because it does not recognize a number of groups in the *Bacterioidetes* genera and several members of the *Cytophaga-Flavobacteria* cluster, such as *Cytophaga hutchinsonii* (Weller et al. 2000; Kirchman 2002).

3.5.1 Seasonal Patterns in Microbial Community Structure

The highest proportion of cells detected with the *Eubacteria* probe was near the surface (5 m) at station L during the spring phytoplankton bloom, when bacterial abundance, production, and specific growth rates were also highest. In previous studies, high abundances of *Cytophaga-Flavobacteria* have been observed during phytoplankton blooms in spring and early summer, when labile organic matter, such as amino acids, proteins, and carbohydrates are produced (Zwisler et al. 2003; Mary et al. 2006). Of the 76% of cells detected as *Bacteria* at station L, 25% were identified as members of the *Cytophaga-Flavobacteria* cluster, 21% as γ-*Proteobacteria*, and 13% as α-*Proteobacteria*. The γ-*Proteobacteria* have also been hypothesized as having a competitive advantage in procuring limited inorganic nutrients as well as utilizing phytoplankton-derived DOM (Giovannoni and Rappé; Mary et al. 2006). Overall, the spring bacterial community was dominated by the *Cytophaga-Flavobacteria*, except at station T2, where it was dominated by the α-*Proteobacteria*. At station L, the γ-*Proteobacteria* was the second most abundant group, whereas at station T3, it was the α-*Proteobacteria*. At station T6, a high proportion of cells could not be identified. Previous studies of the seasonal succession in marine bacterioplankton have also shown the *Cytophaga-Flavobacteria* to be the most abundant phylogenetic group during the spring and early summer, accounting for up to 43% of the bacterial assemblage in the Baltic Sea (Pinhassi and Hagström 2000) and 46% in the English Channel (Mary et al. 2006). The success of the *Cytophaga-Flavobacteria* cluster is likely a result of their proficiency in utilizing the high molecular weight (HMW) fraction of DOM, which includes cellulose, chitin, and pectin, and their ability to degrade aerobically at low

temperatures (Reichenbach 1991; Kirchman 2002). The *Cytophaga-Flavobacteria* cluster is often the most abundant phylogenetic group throughout the year in both marine and freshwater systems, accounting for as much as half of the bacterial assemblage, but abundances have been shown to increase with the input of HMW DOM (Glöckner et al. 1999; Eilers et al. 2001; Kirchman 2002). Less is known about the characteristics of the *Proteobacteria*, although they have been shown to dominate the consumption of low molecular weight (LMW) DOM, and often account for a high proportion of cells utilizing dissolved free amino acids (Cottrell and Kirchman 2000).

The spring and summer bacterial communities were similar at station L, with high relative abundances of *Cytophaga-Flavobacteria*, and lower relative abundances of α- and γ-*Proteobacteria*. At stations T2 and T3, the *Cytophaga-Flavobacteria* and α-*Proteobacteria* decreased considerably from spring to summer. At station T6, the bacterial community remained much the same from spring to summer, although there were fewer unidentified cells. In general, the proportion of cells that hybridized with the *Eubacteria* probe was lower during the summer, when bacterial abundances were also low. Previous studies have also shown that the *Cytophaga-Flavobacteria* and the α-*Proteobacteria* often dominate the summer bacterial community in marine ecosystems (Pinhassi and Hagström 2000; Kelly and Chistoserdov 2001; Mary et al. 2006). The observed decrease in the percentage of hybridized cells during the summer may have been due to a temporal shift in substrate availability or temperature, and may have also been influenced by viral infection or selective grazing (Zwisler et al. 2003). Mesocosm nutrient enrichment and grazing studies have shown alterations in bacterial community

structure with changes in substrate supply and grazing intensity (Lebaron et al. 1999; Riemann et al. 2000).

Although the % *Eubacteria* remained relatively constant from summer to fall, the relative abundances of phylogenetic groups within the fall bacterial community differed from the spring and summer communities. At Station L during the fall, the relative abundances of *Cytophaga-Flavobacteria* and the α- and γ-*Proteobacteria* were essentially the same. At station T2, no cells were identified as *Cytophaga-Flavobacteria*, and the community was dominated by β-*Proteobacteria,* which had previously been absent. Stations T3 and T6 showed a similar pattern to station L, except for the presence of *Archaea* at these stations. In a recent study of bacterioplankton in the English Channel (Mary et al. 2006), the α-*Proteobacteria* constituted a large proportion of the bacterial community in late summer and fall, accounting for up to 36% of hybridized cells. In general, relative abundances of *Proteobacteria* have been reported to increase during the fall (Kelly and Christoserdov 2001), perhaps due to a shift in the source and/or lability of DOM, as has been shown in freshwater systems (Pernthaler et al. 1998; Crump et al. 2003), or selective grazing by protozoa (Jürgens et al. 1999; Šimek et al. 1999; Zwisler et al. 1999; Hahn and Höfle 2001). The α-*Proteobacteria*, in particular, have been identified as a highly-adaptive group that may out-compete other bacterial phylotypes for dissolved free amino acids and DMSP, and play a significant role in the cycling of carbon, nitrogen, and sulphur in the oceans (Malmstrom et al. 2005). The presence of *Archaea* in the surface waters of the northern stations during the fall is not surprising, as it is now known that the *Archaea* have a widespread presence in temperate (DeLong 1992; Fuhrman and Davis 1997;

Massana et al. 1997; Ouverney and Fuhrman 2000; Karner et al. 2001) and polar (DeLong et al. 1994; Murray et al. 1998; Church et al. 2003) oceans. Moreover, studies have indicated that *Archaea* can comprise as much as 20% of the total rRNA in marine microbial communities, although generally at depths greater than 100 m (Giovannoni and Rappé 2000; DeLong and Pace 2001).

3.5.2 Spatial Patterns in Environmental Parameters and Microbial Community Structure

The stations sampled during the SABINA cruises were chosen based upon the biogeochemical province designations of Longhurst (1998). Biogeochemical provinces are defined by the subsurface chlorophyll profile, values of photosynthetic parameters, and depths of the mixed layer and nutricline (Longhurst 1998), and are used to relate biological parameters, such as food web structure, to the physical environment, i.e. temperature, salinity, and incident irradiance (Devred et al. 2006). According to the Longhurst designations, station L is situated in the NWCS province, station T2 in the NASW province, station T3 in the NADR province, and station T6 in the ARCT province. However, following the SABINA cruises, Devred et al. (2006), used satellite observations of sea surface temperature and chlorophyll concentrations to redefine the boundaries of the Northwest Atlantic biogeochemical provinces. Since the boundaries of biogeochemical provinces are not discrete and vary at interannual and seasonal time scales (Platt and Sathyendranath 1999), the Longhurst (1998) designations are limited by the assumption of static boundaries. The updated biogeochemical province designations, shown in Table 3.2, consider dynamic boundaries for each of the provinces (Platt et al.

2005; Devred et al. 2006). An additional province, referred to here as the 'Slope' province, was identified to include the waters of the continental slope between the Gulf Stream and the continental shelf (Devred et al. 2006). As such, station L was within the NWCS during the spring, however during the summer and fall, it was classified as slope water (Devred et al. 2006). The NWCS is an extremely dynamic province, as a result of the conjunction of subpolar and subtropical conditions, and variable circulation due the influences of the Labrador Current and Gulf Stream (Longhurst 1998). Station T2, designated as NASW, was reclassified as being within the North Atlantic Subtropical Gyral East Province (NASE) during the summer, but was still classified as NASW during both the summer and fall. The characteristics of the NASE province are similar to the NASW province, with the primary difference being that the geographic location of the Sargasso Sea corresponds with the NASW province, which is typified by the formation of 18°C mode water, and floating masses of *Sargassum natans* are not observed to the east of the boundary (Longhurst 1998). Station T3, originally designated as NADR, was classified as slope water during the spring and summer, but as STGE during the fall. The NADR province is a frontal region, where a seasonal succession from deep winter mixing to vernal stratification is forced entirely by local wind stress and surface irradiation (Longhurst 1998). Station T6 was defined as ARCT during all seasons, in accordance with the original Longhurst (1998) designation. The ARCT province has some of the same characteristics as the NADR and Boreal Polar provinces, with a shallow euphotic zone depth, deep winter mixing, and rapid near-surface stabilization during the spring (Longhurst 1998).

The bacterial community structure at station T2 (NASW) was most noticeably different from the other stations, with the *α-Proteobacteria* being the dominant group as opposed to the *Cytophaga-Flavobacteria*. Station T6 (ARCT) had the highest proportions of unidentified cells and very few *Proteobacteria* as compared to the other stations. Stations L and T3 were the most similar in terms of bacterial community structure as well as environmental parameters, with both provinces reclassified as 'Slope' for two of the seasons.

3.5.3 Linking Phylogenetic Composition with Bacterial Processes

Although bacteria may be abundant, an uncertain fraction of these bacteria may be inactive and thus, not participating in the biogeochemical cycling of DOM (Gasol et al. 1999). The relative abundances of cells responding to target oligonucleotide probes should represent the proportion of metabolically active cells in the water column, as faster growing or highly active cells have a greater rRNA content, and thus may bind proportionally more to probe molecules, resulting in a stronger fluorescence signal (Bouvier and del Giorgio 2003). It has been hypothesized that the response of target groups to FISH is an indicator of the physiological state of cells, and that the observed phylogenetic shifts in community structure are accompanied by changes in both community and single-cell metabolic activity. It has also been suggested that bacterial abundance is not an indicator of the relative contribution of specific groups to the uptake of DOM (Cottell and Kirchman 2000; Malmstrom et al. 2005).

The *% Eubacteria* and % *Cytophaga-Flavobacteria* were positively correlated with chlorophyll, and inversely correlated with temperature. However, no significant relationships with chlorophyll or temperature were observed for the subclasses of *Proteobacteria* or the *Archaea*. Temperature is often used as a predictor for bacterial activity, and many studies have shown positive relationships between bacterial growth rates and temperature (White et al. 1991; Pomeroy et al. 1995). However, other studies have indicated that bacterial growth efficiency decreases with increasing temperatures, even though growth rates may increase (Chin-Leo and Benner 1992; Daneri et al. 1994;). In most cases, only a weak relationship between bacterial processes and temperature was observed (Rivkin et al. 1996; del Giorgio and Cole 2000). The inverse relationships with temperature observed in the Northwest Atlantic suggest that temperature control of bacterial parameters is influenced by other factors, such as substrate availability (Pomeroy and Wiebe 2001). It is premature to unequivocally relate environmental forcing to spatial variations in bacterial community structure; however, the results presented here indicate that temperature and chlorophyll are not the primary regulatory factors for the abundance and distribution of specific phylogenetic groups, particularly the *Cytophaga-Flavobacteria.*

Of the phylogenetic groups, only the % *Cytophaga-Flavobacteria* was significantly correlated with bacterial abundance and bacterial production. Members of the *Cytophaga-Flavobacteria* cluster are often found in high proportions in oceanic environments, with reported relative abundances of up to 70% (Glöckner et al. 1999; Simon et al. 1999). Cultured isolates as well as uncultured members of *Cytophaga-*

Flavobacteria are proficient in degrading the high molecular weight (HMW) fraction of DOM (Kirchman 2002). However, studies examining the growth rates of *Cytophaga-Flavobacteria* are somewhat contradictory. Jürgens et al. (1999) found that the net growth rate of *Cytophaga-Flavobacteria* cluster was two-fold higher than other phylogenetic groups, providing evidence that the high abundances are due to high growth rates. Other studies have indicated that the *Cytophaga-Flavobacteria* cluster does not grow faster than other bacterial groups, and may have a lower growth rate than both the α- and γ-*Proteobacteria* (Fuchs et al. 2000; Šimek et al. 2001). There was no observed relationship between % *Cytophaga-Flavobacteria* and specific growth rate in the North Atlantic, yet the % α-Proteobacteria was positively correlated with specific growth rate, indicating that the *Cytophaga-Flavobacteria* cluster did not have the highest growth rate.

Although the % α-*Proteobacteria* was positively correlated with specific growth rate, there were no correlations with bacterial abundance or bacterial production. The α-*Proteobacteria* can comprise a large fraction of heterotrophic marine bacteria, particularly the SAR11 and *Roseobacter* clades, which account for 26 and 16% of the derived rRNA gene clones, respectively (Selje et al. 2004). Both clades have been identified as key participants in the metabolism of DMSP, a precursor of DMS (González et al. 2000; Zubkov et al. 2001; Malmstrom et al. 2004ab; Vila et al. 2004). Thus, the α-*Proteobacteria* may mediate a large portion of the DOM flux in marine systems, particularly the transformation of organic sulphur compounds (Moran et al. 2003). In the Northwest Atlantic, the % α-*Proteobacteria* was correlated with SGR but not with DMSPp or DMSPd concentrations. The only observed correlations with DMSP

concentrations were the % *Cytophaga-Flavobacteria* and the % γ-*Proteobacteria*; both groups have the capacity to assimilate DMSP into biomass, but neither are able to cleave DMSP into DMS (Malmstrom et al. 2004b); thus, it is not surprising that there were no correlations with DMS concentrations. The ability to assimilate DMSP is common among phylogenetic groups because DMSP is a major sulphur source, and can satisfy > 90% of the total bacterial sulphur demand (Kiene and Linn 2000). The results presented here indicate that during sampling in the Northwest Atlantic, bacterial sulphur demand was high and more DMSP was used as a sulphur source for biomass production, therefore less was released as DMS (Simó 2001).

3.6 Conclusions and Future Directions

Here we presented results from a seasonal study of changes in the phylogenetic diversity of the microbial community in contrasting biogeochemical provinces of the Northwest Atlantic Ocean, and how these changes related to biogeochemical and ecosystem processes on a large geographical and temporal scale. Overall, the *% Eubacteria* was highest during the spring at all stations, decreased from spring to summer, and remained relatively constant from summer to fall. The relative abundances of the phylogenetic groups showed some seasonality, with the most noticeable shifts from the summer to fall. The *Cytophaga-Flavobacteria* cluster was the most abundant phylogenetic group during the spring and summer, particularly during the spring phytoplankton bloom, when there would have been a high input of labile organic matter, such as amino acids, proteins, and carbohydrates (Kirchman 2002). From summer to fall, there was a noticeable decrease in the proportion of *Cytophaga-Flavobacteria*, and the α-, β-, and γ-*Proteobacteria*

dominated the fall community at all stations. Spatially, the bacterial community composition differed most from the southernmost province, the North Atlantic Subtropical Gyre, to the northernmost province, the Atlantic Arctic province. The percentage of cells detected with the *Eubacteria* and group-specific probes decreased significantly with depth at all stations and was less than 1% for most samples at 200 m. Significant correlations for the percentage of cells detected with the *Eubacteria*, *Archaea*, and group-specific probes were observed with few biological and physico-chemical variables. A significant inverse relationship for % *Eubacteria* with temperature was observed, suggesting that temperature control of bacterial parameters is influenced by other factors, such as substrate availability.

Given that bacterial parameters, such as biomass, growth rate, and growth efficiency are determined in part by the availability of DOM, it is likely that specific bacterial phylotypes also differ in their growth capacities and contribution to the cycling and transformation of DOM (Kirchman 2003). A major assumption of biogeochemical models is that all heterotrophic bacteria participate equally in the uptake of DOM; however, there is now considerable evidence to suggest that the contributions of bacterial groups to DOM uptake differ with the availability and quality of DOM (Cottrell and Kirchman 2000; Schweitzer et al. 2001; Kirchman 2002). Thus, to improve model parameterizations, it would be useful to define bacterial groups on the basis of their respective roles in the cycling of DOM, either by classifying bacteria with the same distinct metabolic capabilities into 'functional groups' or by linking phylogenetic composition with substrate utilization (Kirchman 2003).

3.7 Acknowledgements

This research was supported in part by a fellowship to KFK from the School of Graduate Studies, Memorial University of Newfoundland, and grants to RBR from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Surface Ocean Lower Atmosphere Study (C-SOLAS) Research Network. Funding was provided to C-SOLAS by NSERC and the Canadian Foundation for Climate and Atmospheric Sciences. We wish to thank H. Evans, P. Matthews, and M. R. Anderson for their assistance with sample collection, as well as M. Levasseur, S. Roy, and the crew aboard the CCGS *Hudson* and NGCC *Martha L. Black*. We also thank W.K.W. Li of Bedford Institute of Oceanography, Department of Fisheries and Oceans Canada (DFO), for providing flow cytometry data, M. Gosselin, Z. P. Mei, and J. Pommier of Université du Quebec à Rimouski for providing Chl *a* data, M. Scarratt and S. Michaud of Maurice Lamontagne Institute, DFO, for providing DMS(P) data, Z. P. Mei for providing surface mixed layer depths, and D. C. Schneider and M. S. Hale, Memorial University of Newfoundland, for their invaluable statistical advice and editing assistance.

3.8 Literature Cited

Amann, R., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R., and Stahl, D. A. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol., 1919-1925.

Amann, R., Fuchs, B. M., and Behrens, S. 2001. The identification of microorganisms by fluorescence in situ hybridisation. Curr. Opin. Microbiol., 12: 231-236.

Bates, N. R. 2001. Interannual variability of the oceanic CO_2 and biogeochemical properties in the Western North Atlantic subtropical gyre. Deep-Sea Res., 48: 1507-1528.

Bouvier, T. C., and del Giorgio, P. A. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol. Oceanogr., 47: 453-470.

Bouvier, T. C., and del Giorgio, P. A. 2003. Factors influencing the detection of bacterial cells using the fluorescence in situ hybridization (FISH): A quantitative review of published reports. FEMS Microbiol. Ecol., 44: 3-15.

Cavender-Bares, K. K., Karl, D. M., and Chisholm, S. W. 2001. Nutrient gradients in the western North Atlantic Ocean: Relationship to microbial community structure and comparison to patterns in the Pacific Ocean. Deep-Sea Res., 48: 2373-2395.

Chin-Leo, G., and Benner, R. 1992. Enhanced bacterioplankton production and respiration at intermediate salinities in the Mississippi River plume. Mar. Ecol. Prog. Ser., 87: 87-103.

Cho, B. C., and Azam, F. 1990. Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. Mar. Ecol. Prog. Ser., 63: 253-259.

Church, M. J., DeLong, E. F., Ducklow, H. W., Karner, M. B., Preston, C. M., and Karl, D. M. 2003. Abundance and distribution of planktonic Archaea and Bacteria in the Western Antarctic Peninsula. Limnol. Oceanogr., 48: 1893-1902.

Cottrell, M. T., and Kirchman, D. L. 2000. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high- molecular-weight dissolved organic matter. Appl. Environ. Microbiol., 67: 1692-1697.

Crump, B. C., Kling, G. W., Bahr, M., and Hobbie, J. E. 2003. Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Appl. Environ. Microbiol., 69: 2253-2268.

Daneri, G., Riemann, B., and Williams, P. J. le B. 1994. In situ bacterial production and growth yield measured by thymidine, leucine and fractionated dark oxygen uptake. J. Plankton. Res., 16: 105-113.

del Giorgio, P. A., and Cole, J. J. 2000. Bacterial energetics and growth efficiency. In: Kirchman, D. L., ed. Microbial Ecology of the Oceans. John Wiley & Sons, New York: 289-325.

DeLong, E. F. 1992. Archaea in coastal marine environments. Proc. Natl. Acad. Sci., 89: 5685-5689.

DeLong, E. F. 1998. Everything in moderation: Archaea as 'non-extremophiles'. Curr. Opin. Genet. Dev., 8: 649-654.

DeLong, E. F., and Pace, N. R. 2001. Environmental diversity of Bacteria and Archaea. Syst. Biol., 50: 470-478.

DeLong, E. F., Wu, K. Y., Prézelin, B. B., and Jovine, R. V. M. 1994. High abundance of Archaea in Antarctic marine picoplankton. Nature, 371: 695-697.

Denman, K., Hofmann, E., and Marchant, H. 1996. Marine biotic responses to environmental change and feedbacks to climate. In: Houghton, J.T. Climate Change 1995: The Science of Climate Change. Cambridge University Press, Cambridge: 485-516.

Devred, E., Sathyendranath, S., and Platt, T. 2006. Dynamic assignment of biogeochemical provinces in the Northwest Atlantic. 13th Ocean Sciences Meeting, Honolulu, HI, abstr. OS32K-04.

Ducklow, H. W., and Harris, R. 1993. Introduction to the JGOFS North Atlantic Bloom Study. Deep-Sea Res., 32: 885-897.

Eilers, H., Pernthaler, J., and Amann, R. 2000. Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. Appl. Environ. Microbiol., 66: 4634-4640.

Eilers, H., Pernthaler, J., Peplies, J., Glöckner, F. O., Gerdts, G., and Amann, R. 2001. Isolation of novel pelagic bacteria from the German bight and their seasonal contributions to surface picoplankton. Appl. Environ. Microbiol., 67: 5134-5142.

Fenchel, T., King, G. M., and Blackburn, T. H. 1998. Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling, 2nd ed. Academic Press, San Diego, London; 307 pp.

Fuchs, B. M., Zubkov, M. V., Sahm, K., Burkill, P. H., and Amann, R. 2000. Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometry and molecular biological techniques. Environ. Microbiol., 2: 191-201.

Fuhrman, J. A., and Azam, F. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol., 66: 109-120.

Fuhrman, J. A., and Davis, A. A. 1997. Widespread Archaea and novel Bacteria from the deep sea as shown by 16S rRNA gene sequences. Mar. Ecol. Prog. Ser., 150: 275-285.

Fuhrman, J. A., Hewson, I., Schwalbach, M. S., Steele, J. A., Brown, M. V., Naeem, S. 2006. Annually reoccurring bacterial communities are predictable from ocean conditions. Proc. Natl. Acad. Sci., 103: 13104-13109.

Fuhrman, J. A., McCallum, K., and Davis, A. A. 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans. Appl. Environ. Microbiol., 59: 1294-1302.

Fuhrman, J. A., Sleeter, T. D., Carlson, C. A., and Proctor, L. M. 1989. Dominance of bacterial biomass in the Sargasso Sea and its ecological implications. Mar. Ecol. Prog. Ser., 57: 207-217.

Gasol, J. M., Zweifel, U. L., Peters, F., Fuhrman, J., and Hagström, Ă. 1999. Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl. Environ. Microbiol., 65: 4475-4483.

Glöckner, F. O., Amann, R., Alfreider, A., Pernthaler, J., Psenner, R., Trebesius, K., and Schleifer, K. 1996. An in situ hybridization protocol for detection and identification of planktonic bacteria. Syst. Appl. Microbiol., 19: 403-406.

Giovannoni, S. J., and Rappé, M. S. 2000. Evolution, diversity and molecular ecology of marine prokaryotes. In: Kirchman, D. L., ed. Microbial Ecology of the Oceans. John Wiley & Sons, New York: 47-84.

González, J. M., Kiene, R. P., and Moran, M. A. 1999. Tranformation of sulfur compounds by an abundant lineage of marine bacteria in the α-subclass of the class *Proteobacteria.* Appl. Environ. Microbiol., 65: 3810-3819.

González, J. M., Simó, R., Massana, R., Covert, J. S., Casamayor, E. O., Pedrós-Alió, C., and Moran, M. A. 2000. Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. Appl. Environ. Microbiol., 66: 4237-4246.

Gruber, N., Keeling, C. D., and Bates, N. R. 2002. Interannual variability in the North Atlantic Ocean carbon sink. Science, 298: 2374-2378.

Hahn, M. W., and Höfle, M. G. 2001. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol. Ecol., 35: 113-121.

Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., and Pernthaler, J. 2005. Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol., 71: 2303-2309.

Jürgens, K., Pernthaler, J., Schalla, S., and Amann, R. 1999. Morpological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. Appl. Environ. Microbiol., 65: 1241-1250.

Karner, M. B., DeLong, E. F., and Karl, D. M. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature, 409: 507-510.

Keats, K. F., Rivkin, R. B., Hale, M. S., and Schneider, D. C. 2006. Effects of long-term sample storage on the detection of bacterial cells using fluorescence *in situ* hybridization. Submitted December 2006, Limnology and Oceanography: Methods.

Kelly, K. M., and Chistoserdov, A. Y. 2001. Phylogenetic analysis of the succession of bacterial communities in the Great South Bay (Long Island). FEMS Microbiol. Ecol., 35: 85-95.

Kiene, R. P., and Linn, L. J. 2000. Distribution and turnover of dissolved DMSP and its relationship with bacterial production and dimethylsulfide in the Gulf of Mexico. Limnol. Oceanogr., 45: 849-861.

Kiene, R. P., Linn, L. J., and Bruton, J. A. 2000. New and important roles for DMSP in marine microbial communities. J. Sea Res., 43: 209-224.

Kirchman, D. L. 2003. The contribution of monomers and other low-molecular weight compounds to the flux of dissolved organic material in aquatic ecosystems. In: Findlay, S. E. G., and Sinsabaugh, R. L., eds. Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Academic Press, San Diego: 217-241.

Kirchman, D. L. 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiol. Ecol., 39: 91-100.

Kirchman, D., Ducklow, H., and Mitchell, R. 1982. Estimates of bacterial growth from changes in uptake rates and biomass. Appl. Environ. Microbiol., 44: 1296-1307.

Kirchman, D., K'nees, E., and Hodson, R. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural waters. Appl. Environ. Microbiol., 49: 599-607.

Lebaron, P., Servais, P., Trousselier, M., Courties, C., Vives-Rego, J., Muyzer, G., Bernard, L., Guindulain, T., Schäfer, H., and Stackebrandt, E. 1999. Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquat. Microb. Ecol., 19: 255-267.

Lindström, E. S. 1998. Bacterioplankton community composition in a boreal forest lake. FEMS Microbiol. Ecol., 27: 163-174.

Li, W. K. W. 1995. Composition of ultraphytoplankton in the central North Atlantic. Mar. Ecol. Prog. Ser., 122: 1-8.

Li, W. K. W., and Harrison, W. G. 2001. Chlorophyll, bacteria and picophytoplankton in ecological provinces of the North Atlantic. Deep-Sea Res. II, 48: 2271-2293.

Longhurst, A. 1998. Ecological Geography of the Sea. Academic Press, San Diego, 398 pp.

Malmstrom, R. R., Cottrell, M. T., Elifantz, H., and Kirchman, D. L. 2005. Biomass production and assimilation of dissolved organic matter by SAR11 bacteria in the Northwest Atlantic Ocean. Appl. Environ. Microbiol., 71: 2979-2986.

Malmstrom, R. R., Kiene, R. P., Cottrell, M. T., and Kirchman, D. L. 2004a. Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the North Atlantic Ocean. Appl. Environ. Microbiol., 70: 4129-4135.

Malmstrom, R. R., Kiene, R. P., Kirchman, D. L. 2004b. Identification and enumeration of bacteria assimilating dimethylsulfoniopropionate (DMSP) in the North Atlantic and Gulf of Mexico. Limnol. Oceanogr., 49: 597-606.

Manz, W., Amann, R., Ludwig, W., Vancanneyt, M., and Schleifer, K.-H. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum *Cytophaga-Flavobacter-Bacteroides* in the natural environment. Microbiology, 142: 1097-1106.

Manz, W., Amann, R., Ludwig, W., Wagner, M., and Schleifer, K.-H. 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of *Proteobacteria:* problems and solutions. Syst. Appl. Microbiol., 15: 593-600.

Mary, I., Cummings, D. G., Biegala, I. C., Burkill, P. H., Archer, S. D., and Zubkov, M. V. 2006. Seasonal dynamics of bacterioplankton community structure at a coastal station in the western English Channel. Aquat. Microb. Ecol., 42: 119-126.

Massana, R., Murray, A. E., Preston, C. M., and DeLong, E. F. 1997. Vertical distribution and phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara Channel. Appl. Environ. Microbiol., 63: 50-56.

Moran, M. A., González, J. M., and Kiene, R. P. 2003. Linking a bacterial taxon to sulfur cycling in the sea: studies of the marine *Roseobacter* group. Geomicrobiol. J., 20: 375-388.

Morris, R. M., Vergin, K. L., Cho, J.-C., Rappé, M. S., Carlson, C. A., and Giovannoni, S. J. 2005. Temporal and spatial response of bacterioplankton lineages to annual

convective overturn at the Bermuda Atlantic Time-series Study site. Limnol. Oceanogr., 50: 1687-1696.

Mullins, T. D., Britschgi, T. B., Krest, R. L., Giovannoni, S. J. 1995. Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnol. Oceanogr., 40: 148-158.

Murray, A. E., Preston, C. M., Massana, R., Taylor, L. T., Blakis, A., Wu, K., and DeLong, E. F. 1998. Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl. Environ. Microbiol., 64: 2585-2595.

Newman, D. K., and Banfield, J. F. 2002. Geomicrobiology: how molecular-scale interactions underpin biogeochemical systems. Science, 296: 1071-1077.

Norland, S. 1993. The relationship between biomass and volume of bacteria. In: Kemp, P. F., Sherr, B. F., Sherr, E. B., and Cole, J. J., eds. Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton: 303-307.

Ouverney, C. C., and Fuhrman, J. A. 2000. Marine planktonic Archaea take up amino acids. Appl. Environ. Microbiol., 66: 4829-4833.

Parsons, T. R., Maita, Y., and Lalli, C. M. 1984. A manual of chemical and biological methods of seawater analysis. Pergamon Press, Oxford, 173 pp.

Pernthaler, A., Pernthaler, J., and Amann, R. 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol., 68: 3094-3101.

Pernthaler, J., Glöckner, F. O., Schönhuber, W., and Amann, R. 2001. Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. In: Paul, J., ed. Methods in Microbiology: Marine Microbiology, vol. 30. Academic Press, London: 207-226.

Pernthaler, J., Glöckner, F. O., Unterholzner, S., Alfreider, A., Psenner, R., and Amann, R. 1998. Seasonal community and population dynamics of pelagic Bacteria and Archaea in a high mountain lake. Appl. Environ. Microbiol., 64: 4299-4306.

Platt, T., Bouman, H., Devred, E., Fuentes-Yaco, C., and Sathyendranath, S. 2005. Physical forcing and phytoplankton distributions. Sci. Mar., 69: 55-73.

Platt, T., and Sathyendranath, S. 1999. Spatial structure of pelagic ecosystem processes in the global ocean. Ecosystems, 2: 384-394.

Pinhassi, J., and Hagström, Å. 2000. Seasonal succession in marine bacterioplankton. Aquat. Microb. Ecol., 21: 245-256.

Pomeroy, L. R., Sheldon, J. E., Sheldon, W. M., and Peters, F. 1995. Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. Mar. Ecol. Prog. Ser., 117: 259-268.

Pomeroy, L. R., and Wiebe, W. J. 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquat. Microb. Ecol., 23: 187-204.

Rappé, M. S., Kemp, P. F., and Giovannoni, S. J. 1997. Phylogenetic diversity of marine coastal picoplankton 16S rRNA cloned from the continental shelf off Cape Hatteras, North Carolina. Limnol. Oceanogr., 42: 811-826.

Reichenbach, H. 1991. The order Cytophagales. In: Balows, A., Truper, H. G., Dworkin, M., Hardin, W., and Schleifer, K.-H., eds. The Prokaryotes, 2nd edn. Springer-Verlag, New York: 3631-3675.

Riemann, L., Steward, G., and Azam, F. 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl. Environ. Microbiol., 66: 578-587.

Rivkin, R. B., Anderson, M. R., and Lajzerowicz, C. 1996. Microbial processes in cold oceans. 1. Relationship between temperature and bacterial growth rate. Aquat. Microb. Ecol., 10: 243-254.

Rivkin, R. B., and Legendre, L. 2001. Biogenic carbon cycling in the upper ocean: Effects of microbial respiration. Science, 291: 2398-2400.

Scarratt, M. G., Levasseur, M., Michaud, S., Cantin, G., Gosselin, M., and de Mora, S. J. 2002. Influence of phytoplankton taxonomic profile on the distribution of dimethylsulfide and dimethylsulfoniopropionate in the northwest Atlantic. Mar. Ecol. Prog. Ser., 244: 49-61.

Schauer, M., Balagué, V., Pedrós-Alió, C., and Massana, R. 2003. Seasonal changes in the taxonomic composition of bacterioplankton in a coastal oligotrophic system. Aquat. Microb. Ecol., 31: 163-174.

Schweitzer, B., Huber, I., Amann, R., Ludwig, W., and Simon, M. 2001. α- and β-proteobacteria control the consumption and release of amino acids on lake snow aggregates. Appl. Environ. Microbiol., 67: 632-645.

Šimek, K., Kojecká, P., Nedoma, J., Hartman, P., Vrba, J., and Dolan, J. R. 1999. Shifts in bacterial community composition associated with different microzooplankton size fractions in a eutrophic reservoir. Limnol. Oceanogr., 44: 1634-1644.

Šimek, K., Pernthaler, J., Weinbauer, M .G., Hornak, K., Dolan, J. R., Nedoma, J., Masin, M., and Amann, R. 2001. Changes in bacterial community composition and dynamics and viral mortality rates associated with flagellate grazing in a mesoeutrophic reservoir. Appl. Environ. Microbiol., 67: 2723-2733.

Simó, R., Archer, S. D., Pedrós-Alió, C., Gilpin, L., and Stelfox-Widdicombe, C. E. 2002. Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic. Limnol. Oceanogr., 47: 53-61.

Simó, R. 2001. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. Trends Ecol. Evol., 16: 287-294.

Simon, M., Glöckner, F. O., and Amann, R. 1999. Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. Aquat. Microb. Ecol., 18: 275-284.

Stahl, D. A., and Amann, R. 1991. Development and application of nucleic acid probes. In: Stackebrandt, E., and Goodfellow, M., eds. Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Chichester, UK: 205-248.

Taylor, J. R. 1982. An introduction to error analysis: a study of uncertainties in physical measurements. University Science Books, Mill Valley, California, 270 pp.

Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G. J. 2004. Combined catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. Appl. Environ. Microbiol., 70: 4411-4414.

Townsend, D. W., Cammen, L. M., Holligan, P. M., Campbell, D. E., and Pettigrew, N. R. 1994. Causes and consequences of variability in the timing of spring phytoplankton blooms. Deep-Sea Res., 41: 747-765.

Wagner, M., Horn, M., and Daims, H. 2003. Fluorescence in situ hybridisation for the identification and characterisation of prokaryotes. Curr. Opin. Microbiol., 6: 302-309.

Wallner, G., Amann, R., Beisker, W. 1993. Optimizing fluorescent *in situ*-hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. Cytometry, 14: 136-143.

Weller, R., Glöckner, F. O., and Amann, R. 2000. 16S rRNA-targeted oliognucleotide probes for the *in situ* detection of members of the phylum *Cytophaga-Flavobacterium-Bacteroides*. Syst. Appl. Microbiol., 23: 107-114.

White, P. A., Kalff, J., Rasmussen, J. B., and Gasol, J. M. 1991. The effect of temperature and algal biomass on bacterial production and specific growth rates in freshwater and marine habitats. Microb. Ecol., 21: 99-118.

Williams, P. J. leB. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material. In: Kirchman, D. L., ed. Microbial Ecology of the Oceans. John Wiley & Sons, New York: 153-200.

Wu, J. F., Sunda, W., Boyle, E. A., and Karl, D. M. 2000. Phosphate depletion in the western North Atlantic Ocean. Science, 289: 759-762.

Yoch, D. C. 2002. Dimethylsulfoniopropionate: its sources, role in the marine food web, and biological degradation to dimethylsulfide. Appl. Environ. Microbiol., 68: 5804-5815.

Zubkov, M.V., Fuchs, B. M., Archer, S. D., Kiene, R. P., Amann, R., and Burkill, P. H. 2002. Rapid turnover of dissolved DMS and DMSP by defined bacterioplankton communities in the stratified euphotic zone of the North Sea. Deep-Sea Res. II, 49: 3017-3038.

Zwisler, W., Selje, N., and Simon, M. 2003. Seasonal patterns of the bacterioplankton community in a large mesotrophic lake. Aquat. Microb. Ecol., 31: 211-225.

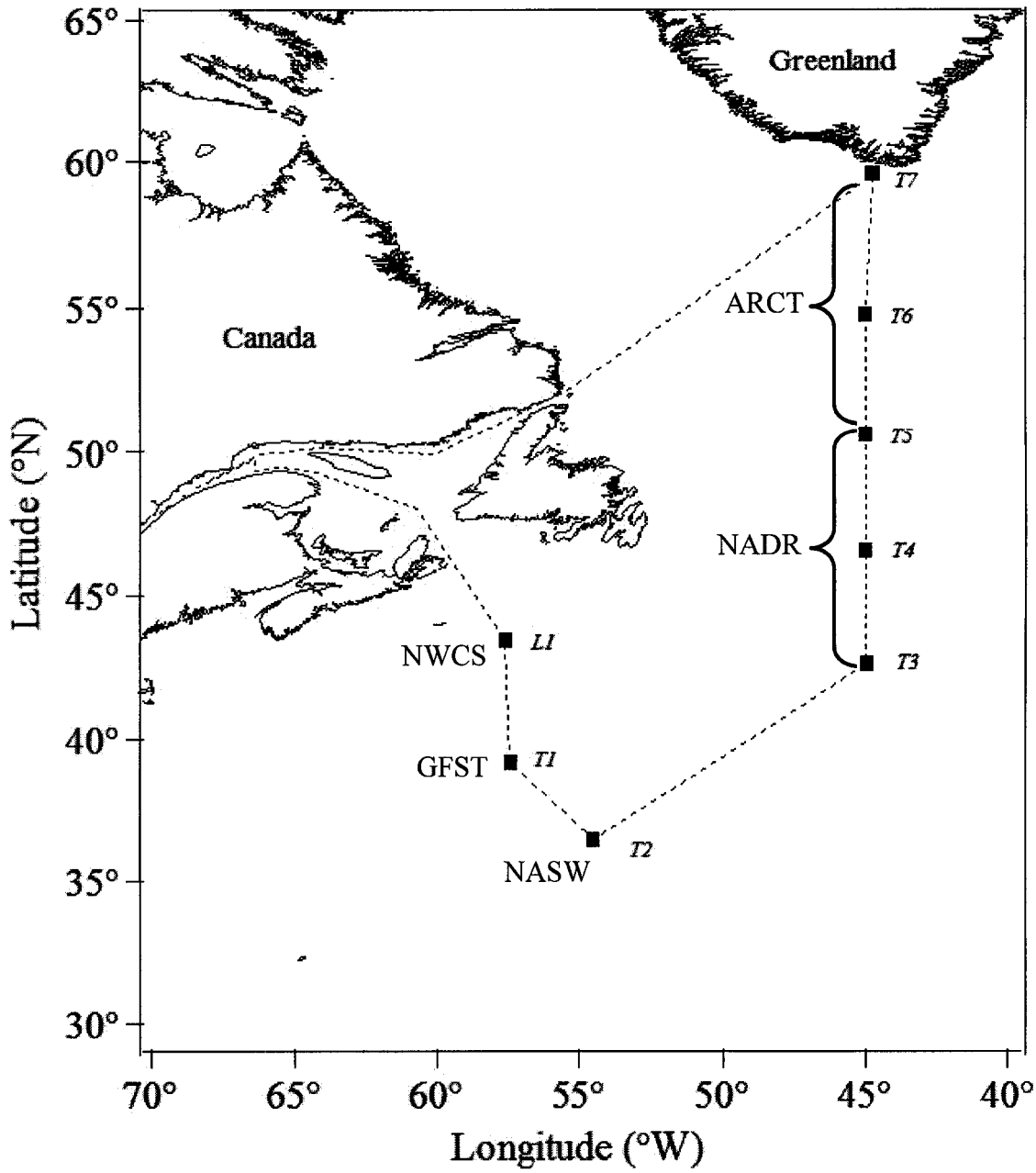
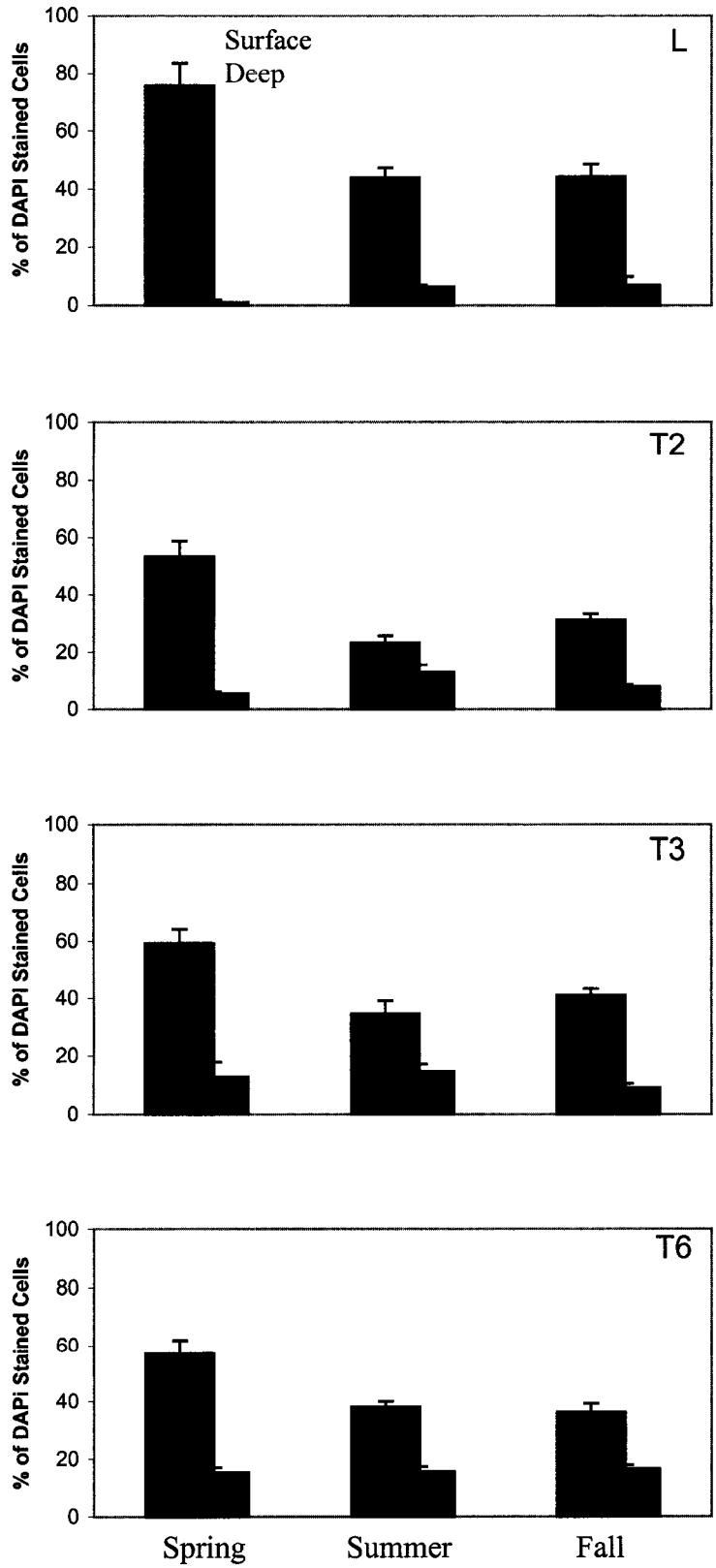


Figure 3.1. Station locations and biogeochemical provinces (NWCS – Northwest Atlantic Shelves province, GFST – Gulf Stream province, NASW – North Atlantic Subtropical Gyral West province, NADR – North Atlantic Drift province, and ARCT – Atlantic Arctic province) sampled during the C-SOLAS Northwest Atlantic expeditions.


% of DAPI Stained Cells
100
80
60
40
20
0
Surface
Deep
L
T2
T3
T6
Spring
Summer
Fall

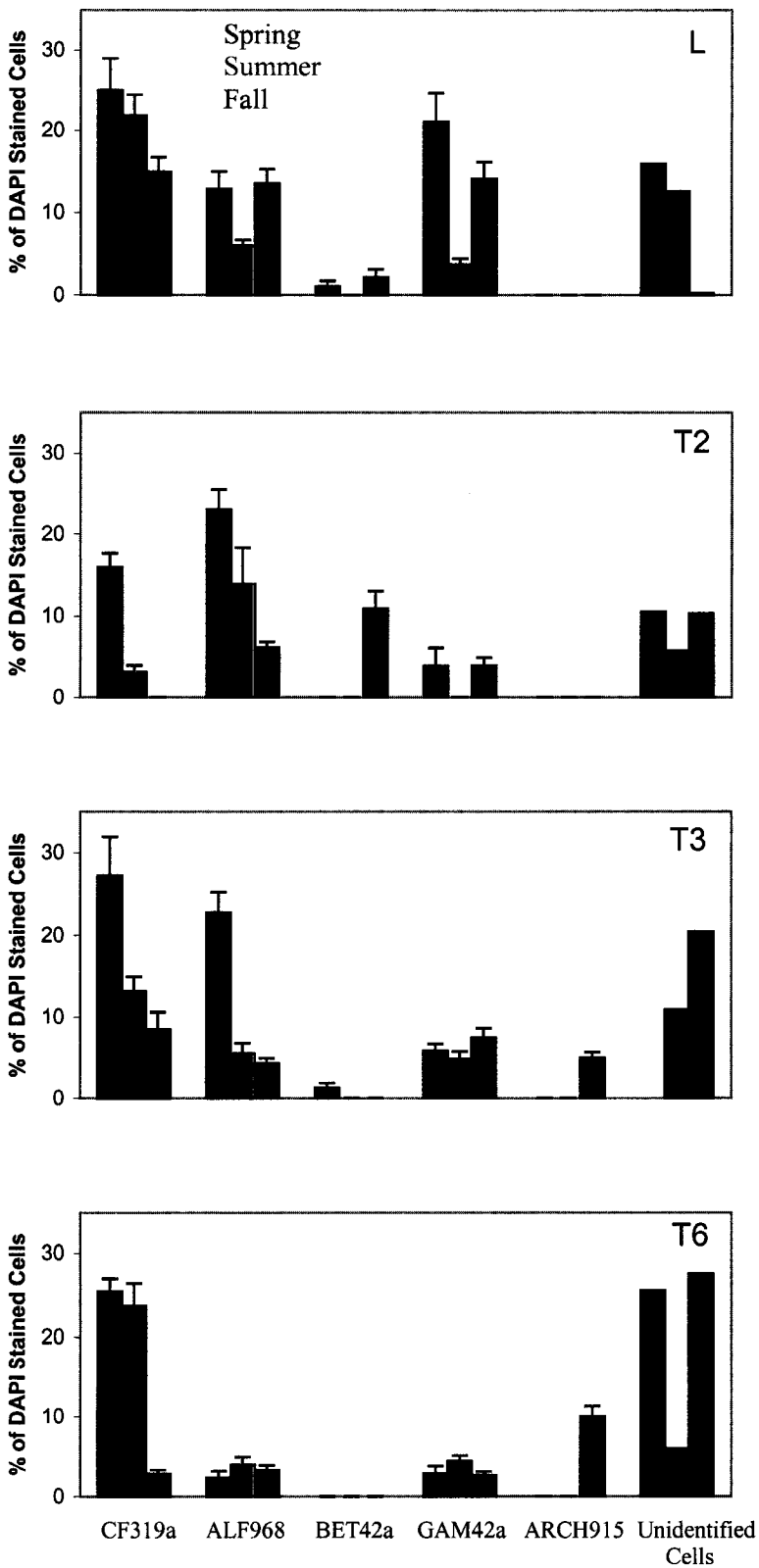

Spring
Summer
Fall
L
T2
T3
T6
% of DAPI Stained Cells
0
10
20
30
CF319a
ALF968
BET42a
GAM42a
ARCH915
Unidentified Cells

Table 3.1. Oligonucleotide probe sequences and target bacterial groups.

Probe	**Sequence (5'-3')**	**Specificity**	**Source**
EUB338	GCTGCCTCCCGTAGGAGT	Most *Bacteria*; not Planctomyces	Amann et al. 1990
CF319a	TGGTCCGTGTCTCAGTAC	*Cytophaga-Flavobacteria* cluster	Manz et al. 1996
ALF968	GGTAAGGTTCTGCGCGTT	α-*Proteobacteria*	Glöckner et al. 1999
BET42a	GCCTTCCCACTTCGTTT	β-*Proteobacteria*	Manz et al. 1992
GAM42a	GCCTTCCCACATCGTTT	γ-*Proteobacteria*	Manz et al. 1992
ARCH915	GTGCTCCCCCGCCAATTCCT	*Archaea*	Stahl and Amann 1991
NON338	ACTCCYACGGGAGGCAGC	Negative control probe; complementary to EUB338	Wallner et al. 1993

Table 3.2. Summary of station characteristics: geographic location, original biogeochemical province (BGP) designation (Longhurst 1998)[a], alternate biogeochemical province designation (Devred et al. 2006)[b], mean mixed layer temperature, mixed layer depth (calculated as the depth at which density is 0.125 kg/m^3 higher than at the surface ~5 m), euphotic zone depth (depth of 1% incident irradiance), and maximum observed chlorophyll *a* concentration for stations sampled in the Northwest Atlantic Ocean during the spring, summer, and fall of 2003.

Season	Station	Date 2003	Latitude (°N)	Longitude (°W)	BGP[a]	BGP[b]	Temp (°C)	Mixed Layer Depth (m)	Euphotic Zone Depth (m)	Chl *a* (μg L^{-1})
Spring	L	28-Apr	43.413	57.477	NWCS	NWCS	2.8	34	30	16.13
	T2	05-May	26.930	54.420	NASW	NASE	19.5	44	75	1.46
	T3	07-May	42.290	45.009	NADR	Slope	16.1	50	60	1.69
	T6	10-May	54.832	45.000	ARCT	ARCT	4.1	103	70	0.81
Summer	L	08-Jul	43.426	57.661	NWCS	Slope	19.3	8	50	1.38
	T2	11-Jul	36.809	54.404	NASW	GFST/NASW	25.7	32	110	0.65
	T3	14-Jul	42.338	45.006	NADR	Slope	23.3	13	85	0.38
	T6	20-Jul	54.846	45.003	ARCT	ARCT	8.6	15	78	0.38
Fall	L	13-Oct	43.422	57.683	NWCS	Slope	17.2	20	44	1.15
	T2	16-Oct	36.833	54.402	NASW	NASW	25.9	47	93	0.54
	T3	19-Oct	42.401	45.048	NADR	NASE	21.9	69	90	0.45
	T6	24-Oct	54.830	45.006	ARCT	ARCT	9.0	55	74	0.33

Table 3.3. Bacterial abundance, bacterial production, and specific growth rates at the surface (50% light depth, unless otherwise noted - *30% light depth) and 200 m for stations sampled in the Northwest Atlantic Ocean during the spring, summer, and fall of 2003.

Season	Station	Nominal Depth (m)	Bacterial Abundance (10^9 cells L^{-1})	Bacterial Production ($\mu g\ C\ L^{-1}\ d^{-1}$)	Specific Growth Rate (d^{-1})
Spring	L	5	1.62	8.75	0.17
		200	0.36	0.10	0.01
	T2	*20	0.63	2.12	0.15
		200	0.47	0.08	0.01
	T3	10	0.57	1.74	0.15
		200	0.43	0.09	0.01
	T6	10	0.96	0.54	0.03
		200	0.26	0.03	0.01
Summer	L	8	0.39	0.20	0.02
		200	0.15	0.01	0.00
	T2	15	0.40	0.11	0.01
		200	0.13	0.04	0.01
	T3	13	0.53	0.37	0.03
		200	0.13	0.18	0.05
	T6	12	0.18	0.23	0.06
		200	0.11	0.04	0.02
Fall	L	7	0.57	0.88	0.06
		200	0.10	0.06	0.03
	T2	14	0.42	0.14	0.02
		200	0.11	0.07	0.03
	T3	14	0.44	0.49	0.06
		200	0.12	0.19	0.07
	T6	11	0.70	0.30	0.02
		200	0.14	0.02	0.01

Table 3.4. Correlation coefficients (Spearman's rho) for bacterial abundance, bacterial production, and specific growth rate against the percentage of DAPI-stained cells detected with the *Eubacteria*, *Archaea*, and group-specific probes within the mixed layer (Table 3.2). All values are significant at $p < 0.05$, unless denoted as not significant (-), $n = 44$.

	Bacterial Abundance	Bacterial Production	Specific Growth Rate
% *Eubacteria*	0.348	0.483	0.484
% *Cytophaga-Flavobacteria*	0.320	0.378	-
% α-*Proteobacteria*	-	-	0.336
% β-*Proteobacteria*	-	-	-
% γ-*Proteobacteria*	-	-	-
% *Archaea*	-	-	-

Table 3.5. Correlation coefficients (Spearman's rho) for bacterial abundance, bacterial production, specific growth rate, and percentage of DAPI-stained cells against temperature and chlorophyll within the mixed layer (Table 3.2). All values are significant at $p < 0.05$, unless denoted as not significant (-).

	Temperature	n	Chlorophyll	n
Bacterial Abundance	-0.334	62	0.597	62
Bacterial Production	-0.310	61	0.720	61
Specific Growth Rate	-0.285	59	0.449	59
% *Eubacteria*	-0.479	48	0.430	48
% *Cytophaga-Flavobacteria*	-0.535	48	0.422	48
% α-*Proteobacteria*	-	48	-	48
% β-*Proteobacteria*	-	48	-	48
% γ-*Proteobacteria*	-	48	-	48
% *Archaea*	-	49	-	49

Table 3.6. Correlation coefficients (Spearman's rho) for bacterial abundance, bacterial production, specific growth rate, percentage of DAPI-stained cells (% *Eubacteria*), and relative abundances of hybridized cells for phylogenetic groups against concentrations of dissolved and particulate dimethylsulphoniopropionate (DMSPd and DMSPp, respectively) within the mixed layer (Table 3.2) and at 200 m. All values are significant at $p < 0.05$, unless denoted as not significant (-).

	DMSPd		DMSPp	
	Euphotic	200 m	Euphotic	200 m
Bacterial Abundance	0.419 (n = 44)	-	0.736 (n = 44)	0.700 (n = 15)
Bacterial Production	0.352 (n = 45)	-	0.750 (n = 45)	-
Specific Growth Rate	-	-	-	-
% *Eubacteria*	-	-	-	-
% *Cytophaga-Flavobacteria*	-	-	0.349 (n = 33)	-
% α-*Proteobacteria*	-	-	-	-
% β-*Proteobacteria*	-	-	-	-
% γ-*Proteobacteria*	-	0.618 (n = 14)	-	0.830 (n = 14)

Chapter 4: Summary

4.1 Synopsis of Manuscript Chapters

Fluorescence *in situ* hybridization (FISH) is widely used to characterize the phylogenetic diversity of unicellular organisms at target taxonomic levels, and involves the hybridization of fluorescently-labelled rRNA-targeted oligonucleotide probes to group-specific gene sequences (Glöckner et al. 1996). The FISH technique has been frequently used to study heterotrophic prokaryotes, from the domains *Bacteria* and *Archaea*, and less frequently for the *Eucarya*, i.e. nano- and pico-phytoplankton and protists (Lim et al. 1993, Lim et al. 1995; Simon et al. 1995), and photosynthetic pico-heterokonts (stramenopiles; Massana et al. 2002). The phylogenetic composition of microbial assemblages in lakes and estuaries (Pernthaler et al. 1997, Glöckner et al. 1999; Bouvier and del Giorgio 2002; Heidelberg et al. 2002; Cottrell and Kirchman 2003; Zwisler et al. 2003), limnetic organic aggregates (lake snow) (Weiss et al. 1996; Schweitzer et al. 2001), soil (Hahn et al. 1992; Christensen et al. 1999), activated sludge flocs and biofilms (Wagner et al. 1994; Amann et al. 1996; Daims et al. 2001), ice and snow (Alfreider et al. 1996; Brinkmeyer et al. 2003), as well as coastal and oceanic marine habitats (Karner and Fuhrman 1997; DeLong et al. 1999; Glöckner et al. 1999; Cottrell and Kirchman 2000a, 2000b; Wells and Deming 2003) have been characterized using FISH.

A limitation of the FISH technique is that the effectiveness of target cell detection varies across ecosystem types (Bouvier and del Giorgio 2003). The percentage of cells detected with the *Eubacteria* probe (EUB338) ranges from 1% in soil to 100% in enriched culture, with an average of 56% across ecosystem types (Bouvier and del Giorgio 2003). This

variation is potentially influenced by a number of methodological factors, such as cell fixation, hybridization conditions, mounting solutions, fluorochrome type, counting methods, and sample storage (Bouvier and del Giorgio 2003, Williams et al. 2004). The latter is particularly important since samples that are collected at sea are often stored for many weeks or months before analysis using FISH. Therefore, quantifying the effects of storage on the detection of bacterial cells is crucial for comparing studies of bacterial community structure from diverse regions and ecosystems.

Chapter 2 presented the results of a 12-month time-course study to determine the effects of long-term sample storage on hybridization efficiency and the characterization of community structure. In this study, replicate seawater samples were prepared, stored frozen, and hybridized after 1.5, 3, 6, and 12 months. The time-dependent slope of the probe for *Eubacteria*, but not the *Cytophaga-Flavobacteria* cluster or the alpha and gamma subclasses of *Proteobacteria*, was significantly different from zero, with a percent change in target cell detection of 6.3% per year. The results for the *Eubacteria* probe indicated that samples for FISH can be prepared (fixed and filtered) and stored at -20°C for up to 6 months with no significant change in target cell detection, and stored for at least 12 months with a minimal (< 7%) effect of storage. These results provide a baseline for the length of time that seawater samples for FISH may be stored before significant changes in target cell detection are observed. Thus, it is recommended that, when possible, preserved FISH samples should be analyzed within 6 months of sample collection.

Chapter 3 presented the results of a field- and laboratory- based study characterizing the phylogenetic diversity of the bacterial community during the spring, summer, and fall (2003) in contrasting biogeochemical provinces of the Northwest Atlantic Ocean. Microbial community structure was characterized using FISH, and related to a number of concurrently-measured environmental parameters. The proportion of cells detected with the *Eubacteria* probe was, on average, three-fold higher in the surface mixed layer than at 200 m (45% and 17%, respectively). In contrast, although the proportion of cells detected with the *Archaea* probe was low, it was higher at depth than near the surface. The relative abundances of specific bacterial groups showed seasonality, with the most noticeable shifts from summer to fall. The *Cytophaga-Flavobacteria* cluster was the most abundant phylogenetic group during the spring and summer, except station T2, in the North Atlantic Subtropical Gyre province, where the α-*Proteobacteria* was the dominant group. From summer to fall, there was a decrease in the proportion of *Cytophaga-Flavobacteria*, and the α-, β-, and γ-*Proteobacteria* dominated the bacterial community during fall at all stations. Seasonally, the most noticeable shift in the relative abundances of specific phylogenetic groups occurred from summer to fall. Spatially, the greatest differences in bacterial community composition were observed between the North Atlantic Subtropical Gyre and the Atlantic Arctic province.

4.2 Overall Significance

As samples for SABINA were collected during three cruises in the Northwest Atlantic Ocean, over a sampling period of six months, and stored for varying lengths of time before hybridization, the storage effects study was necessary to determine if there were

effects of long-term sample storage and to provide a baseline for results from the Northwest Atlantic study. Although Pernthaler et al. (2001) suggested that filters prepared for FISH "can be stored frozen for several months without apparent loss of hybridization signal"; there have been no previous quantitative studies of the time-dependent effects of sample storage on the efficiency of target cell detection using FISH. Despite the limitations of the 'classical' FISH method (DeLong et al. 1989, Amann et al. 1995, Glöckner et al. 1996, Pernthaler et al. 2001), it has been widely used to characterize bacterial community structure in the published literature (e.g. Glöckner et al. 1999, Amann et al. 2001, Bouvier and del Giorgio 2003, Wagner et al. 2003). Thus, it was essential that methodological factors, such as storage duration, which could confound the characterization of community structure be addressed to accurately compare studies of the phylogenetic diversity of bacteria from different ecosystem types as well as samples from the same ecosystem that have been analyzed at different times.

The results from SABINA are an important contribution to our knowledge of the phylogenetic diversity of the bacterial community in the Northwest Atlantic Ocean. Prior studies of prokaryotic community structure in the Northwest Atlantic have been limited to estuaries and bays (Cottrell and Kirchman 2000b; Bouvier and del Giorgio 2002), and the Sargasso Sea (Fuhrman et al. 1993; Mullins et al. 1995; Malmstrom et al. 2004; Morris et al. 2005). Moreover, there have been no prior seasonal studies of bacterial community structure in the Atlantic Ocean. Similar studies have been undertaken in freshwater (Lindström 1998; Pernthaler et al. 1998) and coastal (Murray et al. 1998; Pinhassi & Hagström 2000; Schauer et al. 2003; Zwisler et al. 2003; Mary et al. 2006) environments;

however, this was the first study of bacterial community structure on a large spatial scale in the Northwest Atlantic, as well as the first study of the seasonality of specific phylogenetic groups in an open ocean environment.

4.3 Future Directions

Accurate estimates of both total bacterial numbers and the relative abundances of specific bacterial phylotypes are crucial to quantify both bacterial trophodynamics and the relationship between bacterial community structure and ecosystem function. Estimates of bacterial abundance, with appropriate conversion factors, are used to calculate bacterial biomass and estimate the flux of carbon through the microbial food web and quantify the role of bacteria in mediating biogeochemical processes (Pernthaler and Amann 2005). Although there has been considerable progress in characterizing the phylogenetic diversity of marine bacterial assemblages, through the use of FISH (Glöckner et al. 1999; Amann et al. 2001; Wagner et al. 2003) and other molecular methods (Pace et al. 1986; Fuhrman et al. 1993; Zehr and Voytek 1999; Spiegelman et al. 2005), linking bacterial community structure with specific biogeochemical processes has proven to be difficult (Kirchman 2002). Only a small fraction of naturally occurring bacteria, as identified by rRNA clones, appear closely related to cultivated bacteria from the same environments (DeLong 1998). Recently, FISH has been combined with catalyzed reporter deposition (CARD-FISH; Pernthaler et al. 2002) and microautoradiography (MICRO-CARD-FISH; Teira et al. 2004, Herndl et al. 2005), to enhance sensitivity and improve detection of small or slow-growing cells with low rRNA content, and to measure substrate utilization by specific bacterial and archaeal phylotypes. These techniques provide opportunities to

further examine the phylogenetic diversity and functional roles of *Bacteria* and *Archaea*, and explore how microbial metabolism influences larger scale biogeochemical oceanic processes.

4.4 Literature Cited

Afreider, A., Pernthaler, J., Amann, R., Sattler, B., Glöckner, F. O., Wille, A., and Psenner, R. 1996. Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. Appl. Environ. Microbiol., 62: 2138-2144.

Amann, R., Fuchs, B. M., and Behrens, S. 2001. The identification of microorganisms by fluorescence in situ hybridisation. Curr. Opin. Microbiol., 12: 231-236.

Amann, R., Ludwig, W., and Schleifer, K.-H. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev., 59: 143-169.

Amann, R., Snaidr, J., Wagner, M., Ludwig, W., and Schleifer, K.-H. 1996. In situ visualization of high genetic diversity in a natural microbial community. J. Bacteriol., 178: 3496-3500.

Bouvier, T. C., and del Giorgio, P. A. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol. Oceanogr., 47: 453-470.

Bouvier, T. C., and del Giorgio, P. A. 2003. Factors influencing the detection of bacterial cells using the fluorescence in situ hybridization (FISH): A quantitative review of published reports. FEMS Microbiol. Ecol., 44: 3-15.

Brinkmeyer, R., Knittel, K., Jürgens, J., Weyland, H., Amann, R., and Helmke, E. 2003. Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl. Environ. Microbiol., 69: 6610-6619.

Christensen, H., Hansen, M., and Sørensen, J. 1999. Counting and size classification of active soil bacteria by fluorescence in situ hybridization with an rRNA oligonucleotide probe. Appl. Environ. Microbiol., 65: 1753-1761.

Cottrell, M. T., and Kirchman, D. L. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnol. Oceanogr., 48: 168-178.

Cottrell, M. T., and Kirchman, D. L. 2000a. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl. Environ. Microbiol., 66: 5116-5122.

Cottrell, M. T., and Kirchman, D. L. 2000b. Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and

high- molecular-weight dissolved organic matter. Appl. Environ. Microbiol., 66: 1692-1697.

Daims, H., Ramsing, N. B., Schleifer, K.-H., and Wagner, M. 2001. Cultivation independent, semiautomatic determination of absolute bacterial cell numbers in environmental samples by fluorescence in situ hybridization. Appl. Environ. Microbiol., 67: 5810-5818.

DeLong, E. F., Taylor, L. T., Marsh, T. L., and Preston, C. M. 1999. Visualization and enumeration of marine planktonic Archaea and Bacteria by using polyribonucleotide probes and fluorescent in situ hybridization. Appl. Environ. Microbiol., 65: 5554-5563.

DeLong, E. F., Wickham, G. S., and Pace, N. R. 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. Science, 243: 1360-1363.

Fuhrman, J. A., McCallum, K., and Davis, A. A. 1993. Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific oceans. Appl. Environ. Microbiol., 59: 1294-1302.

Glöckner, F. O., Amann, R., Alfreider, A., Pernthaler J., Psenner, R., Trebesius, K., and Schleifer, K.-H. 1996. An in situ hybridization protocol for detection and identification of planktonic bacteria. Syst. Appl. Microbiol., 19: 403-406.

Glöckner, F. O., Fuchs, B. M., and Amann, R. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl. Environ. Microbiol., 65: 3721-3726.

Hahn, D., Amann, R., Ludwig, W., Akkermans, A. D. L., and Schleifer, K.-H. 1992. Detection of micro-organisms in soil after in situ hybridization with rRNA targeted, fluorescently labelled oligonucleotides. J. Gen. Microbiol., 138: 879-887.

Heidelberg, J. F., Heidelberg, K. B., and Colwell, R. R. 2002. Seasonality of Chesapeake Bay bacterioplankton species. Appl. Environ. Microbiol., 68: 5488-5497.

Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., and Pernthaler, J. 2005. Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol., 71: 2303-2309.

Karner, M., and Fuhrman, J. A. 1997. Determination of active marine bacterioplankton: a comparison of universal 16S rRNA probes, autoradiography, and nucleoid staining. Appl. Environ. Microbiol., 63: 1208-1213.

Kirchman, D. L. 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiol. Ecol., 39: 91-100.

Lim, E. L., Amaral, L. A., Caron, D. A., and DeLong, E. F. 1993. Application of rRNA-based probes for observing nanoplanktonic protists. Appl. Environ. Microbiol., 59: 1647-1655.

Lim, E. L., Caron, D. A., DeLong, E. F. 1995. Development and field application of a quantitative method for examining natural assemblages of protists with oligonucleotide probes. Appl. Environ. Microbiol., 62: 1416-1423.

Lindström, E. S. 1998. Bacterioplankton community composition in a boreal forest lake. FEMS Microbiol. Ecol., 27: 163-174.

Malmstrom, R. R., Kiene, R. P., Kirchman, D. L. 2004. Identification and enumeration of bacteria assimilating dimethylsulfoniopropionate (DMSP) in the North Atlantic and Gulf of Mexico. Limnol. Oceanogr., 49: 597-606.

Mary, I., Cummings, D. G., Biegala, I. C., Burkill, P. H., Archer, S. D., and Zubkov, M. V. 2006. Seasonal dynamics of bacterioplankton community structure at a coastal station in the western English Channel. Aquat. Microb. Ecol., 42: 119-126.

Massana, R., Guillou, L., Díez, B., and Pedrós-Alió, C. 2002. Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean. Appl. Environ. Microbiol., 68: 4554-4558.

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

Mullins, T. D., Britschgi, T. B., Krest, R. L., Giovannoni, S. J. 1995. Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnol. Oceanogr., 40: 148-158.

Murray, A. E., Preston, C. M., Massana, R., Taylor, L. T., Blakis, A., Wu, K., and DeLong, E. F. 1998. Seasonal and spatial variability of Bacterial and Archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl. Environ. Microbiol., 64: 2585-2595.

Pace, N. R., Stahl, D. J., Lane, D. J., and Olsen, G. J. 1986. The analysis of natural microbial populations by ribosomal RNA sequences. Adv. Microbiol. Ecol., 9: 1-55.

Pernthaler, A., Pernthaler, J., and Amann, R. 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol., 68: 3094-3101.

Pernthaler, J., Alfreider, A., Posch, T., Andreatta, S., and Psenner, R. 1997. In situ classification and image cytometry of pelagic bacteria from a high mountain lake (Gossenköllesee, Austria). Appl. Environ. Microbiol., 63: 4778-4783.

Pernthaler, J., and Amann, R. 2005. Fate of heterotrophic microbes in pelagic environments: focus on populations. Microbiol. Mol. Biol. Rev., 69: 440-461.

Pernthaler, J., Glöckner, F. O., Unterholzner, S., Alfreider, A., Psenner, R., and Amann, R. 1998. Seasonal community and population dynamics of pelagic Bacteria and Archaea in a high mountain lake. Appl. Environ. Microbiol., 64: 4299-4306.

Pernthaler, J., Glöckner, F. O., Schönhuber, W., and Amann, R. 2001. Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. In: Paul, J., ed. Methods in Microbiology: Marine Microbiology, vol. 30. Academic Press, London: 207-226.

Pinhassi, J., and Hagström, Å. 2000. Seasonal succession in marine bacterioplankton. Aquat. Microb. Ecol., 21: 245-256.

Schauer, M., Balagué, V., Pedrós-Alió, C., and Massana, R. 2003. Seasonal changes in the taxonomic composition of bacterioplankton in a coastal oligotrophic system. Aquat. Microb. Ecol., 31: 163-174.

Schweitzer, B., Huber, I., Amann, R., Ludwig, W., and Simon, M. 2001. α- and β-proteobacteria control the consumption and release of amino acids on lake snow aggregates. Appl. Environ. Microbiol., 67: 632-645.

Simon, N., LeBot, N., Marie, D., Partensky, F., and Vaulot, D. 1995. Fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes to identify small phytoplankton by flow cytometry. Appl. Environ. Microbiol., 61: 2506-2513.

Spiegelman, D., Whissell, G., and Greer, C. W. 2005. A survey of the methods for the characterization of microbial consortia and communities. Can. J. Microbiol., 51: 355-386.

Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G. J. 2004. Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. Appl. Environ. Microbiol., 70: 4411-4414.

Wagner, M.-H., Erhart, R., Manz, W., Amann, R., Lemmer, H., Wdei, D., and Schleifer, K.-H. 1994. Development of an rRNA-targeted oligonucleotide probe specific for the genus Actinobacter and its application for in situ monitoring in activated sludge. Appl. Environ. Microbiol., 60: 792-800.

Wagner, M., Horn, Matthias., and Daims, Holger. 2003. Fluorescence *in situ* hybridisation for the identification and characterisation of prokaryotes. Curr. Opin. Microbiol., 6: 302-309.

Weiss, P., Schweitzer, B., Amann, R., and Simon, M. 1996. Identification in situ and dynamics of bacteria on limnetic organic aggregates (Lake Snow). Appl. Environ. Microbiol., 62: 1998-2005.

Wells, L. E., and Deming, J. W. 2003. Abundance of Bacteria, the Cytophaga-Flavobacterium cluster and Archaea in cold oligotrophic waters and nepheloid layers of the Northwest Passage, Canadian Archipelago. Aquat. Microb. Ecol., 31: 19-31.

Williams, S. C., Heaton, D. P., Hristov, R., Frischer, M. E., and Verity, P. G. 2004. Methodological factors that contribute to variability using fluorescence in situ hybridization (FISH) in marine planktonic environments. [Online.] http://www.skio.peachnet.edu/research/veritylab/biocomp/presentations/methods_factors/methods_factors.pdf. Accessed 17 November 2005.

Zehr, J. P., and Voytek, M. A. 1999. Molecular ecology of aquatic communities: reflections and future directions. Hydrobiologia, 401: 1-8.

Zwisler, W., Selje, N., and Simon, M. 2003. Seasonal patterns of the bacterioplankton community in a large mesotrophic lake. Aquat. Microb. Ecol., 31: 211-225.

Appendix A: Summary of station characteristics: geographic location, original biogeochemical province (BGP) designation (Longhurst 1998)[a], alternate biogeochemical province designation (Devred et al. 2006)[b], mean mixed layer temperature, mixed layer depth (Z_{mld}; calculated as the depth at which density is 0.125 kg/m^3 higher than at the surface ~ 5 m), euphotic zone depth (Z_{eu}; depth of 1% incident irradiance), and maximum observed chlorophyll *a* concentration for stations sampled in the Northwest Atlantic Ocean during the spring, summer, and fall of 2003.

Season	Station	Date (2003)	Latitude (°N)	Longitude (°W)	BGP[a]	BGP[b]	Temp (°C)	Z_{mld} (m)	Z_{eu} (m)	Chl a ($\mu g\ L^{-1}$)
Spring	L	28-Apr	43.413	57.477	NWCS	NWCS	2.8	34	30	16.13
	T1	03-May	39.002	57.364	GFST	GFST	22.9	48	60	0.84
	T2	05-May	26.930	54.420	STGW	STGE	19.5	44	75	1.46
	T3	07-May	42.290	45.009	NADR	Slope	16.1	50	60	1.69
	T4	08-May	46.488	45.014	ARCT	NWCS	3.8	101	55	1.61
	T5	09-May	50.665	44.998	ARCT	NADR	8.5	124	80	0.73
	T6	10-May	54.832	45.000	ARCT	ARCT	4.1	103	70	0.81
	T7					not sampled				
Summer	L	08-Jul	43.426	57.661	NWCS	Slope	19.3	8	50	1.38
	T1	10-Jul	39.000	57.475	GFST	GFST/STGW	26.5	11	125	0.44
	T2	11-Jul	36.809	54.404	STGW	GFST/STGW	25.7	32	110	0.65
	T3	14-Jul	42.338	45.006	NADR	Slope	23.3	13	85	0.38
	T4	16-Jul	46.496	45.028	ARCT	NADR/ARCT	11.2	7	37	2.69
	T5	18-Jul	50.664	44.967	ARCT	NADR/ARCT	9.2	9	48	1.38
	T6	20-Jul	54.846	45.003	ARCT	ARCT	8.6	15	78	0.38
	T7	22-Jul	59.234	45.059	ARCT	ARCT	2.8	10	57	1.21
Fall	L	13-Oct	43.422	57.683	NWCS	Slope	17.2	20	44	1.15
	T1	15-Oct	39.027	57.518	GFST	GFST/STGW	25.8	70	110	0.35
	T2	16-Oct	36.833	54.402	STGW	STGW	25.9	47	93	0.54
	T3	19-Oct	42.401	45.048	NADR	STGE	21.9	69	90	0.45
	T4	21-Oct	46.500	45.001	ARCT	Slope	11.1	31	43	1.38
	T5	22-Oct	50.724	44.988	ARCT	NADR	12.4	65	50	0.77
	T6	24-Oct	54.830	45.006	ARCT	ARCT	9.0	55	74	0.33
	T7	27-Oct	59.571	44.794	ARCT	ARCT	3.6	9	73	0.46

Appendix B: Bacterial abundance, bacterial production, and specific growth rates for stations sampled in the Northwest Atlantic Ocean during the spring, summer, and fall of 2003 (*denotes the chlorophyll maximum).

Season	Station	Nominal Depth (m)	Light Depth (%)	Bacterial Abundance (10^9 cells L^{-1})	Bacterial Production ($\mu g\ C\ L^{-1}\ d^{-1}$)	Specific Growth Rate (d^{-1})
Spring	L	200		0.360	0.098	0.009
		50	*0.2	1.177	1.060	0.047
		20	5	1.416		
		5	50	1.618	8.747	0.168
	T1	200		1.069	0.650	0.038
		60	1	0.583		
		45	5	0.594	2.116	0.093
		10	50	0.564	0.663	0.090
	T2	200		0.470	0.084	0.013
		150		0.426	0.272	0.037
		75	1	0.471	0.164	0.020
		20	*30	0.634	2.117	0.150
	T3	200		0.425	0.091	0.011
		60	5	0.559	1.064	0.100
		25	30	0.615	2.583	0.222
		15	50	0.496		
	T4	200		0.438	0.047	0.007
		40	5	0.920	0.865	0.042
		15	30	0.661	0.560	0.043
		10	50	0.853	0.621	0.038
	T5	200		0.434	0.082	0.011
		40	*5	0.610	0.644	0.062
		15	30	0.509	0.618	0.057
		10	50	0.642		
	T6	200		0.257	0.031	0.007
		70	1	0.900	0.541	0.023
		30	15	0.995	0.869	0.039
		10	30	0.962	0.525	0.032
	T7			not sampled		

Season	Station	Nominal Depth (m)	Light Depth (%)	Bacterial Abundance (10^9 cells L^{-1})	Bacterial Production ($\mu g\ C\ L^{-1}\ d^{-1}$)	Specific Growth Rate (d^{-1})
Summer	L	200		0.151	0.008	0.003
		35	*	0.715	0.499	0.030
		15	30	0.617	0.890	0.046
		8	50	0.391	0.199	0.022
	T1	200		0.955	0.020	0.008
		95	*	0.248	0.006	0.001
		30	30	0.406	0.319	0.026
		15	50		0.081	
	T2	200		0.133	0.036	0.009
		85	*	0.414	0.149	0.017
		30	30	0.454	0.132	0.016
		15	50	0.396	0.111	0.013
	T3	200		0.127	0.176	0.055
		85	*1	0.553	0.140	0.013
		22	30	0.388	0.312	0.036
		13	50	0.521	0.371	0.030
	T4	250		0.227	0.217	0.028
		18	*	0.738	0.226	0.074
		10	30	0.769	0.313	0.063
		6	50	0.704	1.001	0.069
	T5	200		0.138	0.057	0.017
		30	*5	0.714	1.217	0.075
		13	30	0.951	1.752	0.078
		7	50	1.186	1.011	0.044
	T6	200		0.112	0.042	0.015
		32	15	0.164	0.155	0.038
		20	*30	0.249	0.354	0.060
		12	50	0.179	0.228	0.057
	T7	250		0.142	0.050	0.018
		37	5	0.263	0.194	0.034
		15	30	0.171	0.249	0.061
		9	*50	0.188	0.684	0.099

Season	Station	Nominal Depth (m)	Light Depth (%)	Bacterial Abundance (10^9 cells L^{-1})	Bacterial Production ($\mu g\ C\ L^{-1}\ d^{-1}$)	Specific Growth Rate (d^{-1})
Fall	L	200		0.097	0.058	0.030
		31	*5	0.836	0.567	0.032
		12	30	0.522	0.294	0.023
		7	50	0.566	0.875	0.063
	T1	200		0.151	0.031	0.010
		80	*	0.251	0.025	0.005
		18	30	0.325	0.440	0.055
		9	50	0.354	0.342	0.040
	T2	200		0.113	0.068	0.028
		64	*	0.291	0.220	0.037
		24	30	0.399	0.404	0.047
		14	50	0.419	0.143	0.015
	T3	200		0.123	0.191	0.068
		73	*	0.321	0.652	0.103
		23	30	0.467	0.635	0.083
		14	50	0.441	0.491	0.059
	T4	250		0.191	0.085	0.024
		28	5	1.232	0.903	0.032
		12	*30		0.784	
		6	50	1.458	0.592	0.025
	T5	200		0.143	0.038	0.016
		32	5	0.743	0.460	0.042
		13	30	0.752	0.512	0.036
		8	50	0.709	0.441	0.033
	T6	200		0.137	0.021	0.008
		31	15	0.724	0.411	0.025
		19	30	0.724	0.354	0.028
		11	*50	0.701	0.297	0.024
	T7	145		0.222	0.117	0.030
		48	5	0.268	0.440	0.100
		19	30	0.304	0.301	0.079
		11	50	0.352	0.135	0.063

Appendix C: Relative abundances (mean ± SD) of cells detected with target oligonucleotide probes for stations sampled in the Northwest Atlantic Ocean during the spring, summer, and fall of 2003. All probe counts were corrected by subtracting counts obtained with the negative control probe NON338. Percentages were computed as cells hybridized with the target oligonucleotide probe divided by the total cells determined from DAPI counts (~1000 DAPI-stained cells from 10 to 20 randomly chosen fields); nd, not detected.

Season	Station	Depth (m)	Proportion (%) of cells detected with probe					
			Eubacteria	*Cytophaga-Flavobacteria*	Alpha-*Proteobacteria*	Beta-*Proteobacteria*	Gamma-*Proteobacteria*	*Archaea*
Spring	L	200	1.0 ± 1.5	nd	nd	nd	nd	9.2 ± 7.1
		50	56.6 ± 9.2	31.8 ± 5.3	3.6 ± 1.5	8.3 ± 3.1	12.1 ± 4.1	2.2 ± 1.4
		20	71.9 ± 9.5	25.1 ± 4.4	4.4 ± 3.3	9.7 ± 4.7	12.3 ± 4.0	nd
		5	75.8 ± 13.5	25.0 ± 6.9	12.8 ± 3.7	1.0 ± 1.1	21.0 ± 6.4	nd
	T1	200	18.8 ± 3.1	4.6 ± 1.6	1.5 ± 0.5	nd	1.4 ± 1.2	2.0 ± 1.0
		60	58.3 ± 9.5	17.6 ± 1.9	10.4 ± 2.0	nd	nd	2.0 ± 1.5
		45						
		10						
	T2	200	5.3 ± 1.4	1.2 ± 0.7	nd	nd	nd	3.1 ± 1.1
		150	12.9 ± 2.7	nd	3.0 ± 0.8	nd	nd	nd
		75	11.1 ± 1.5	nd	nd	nd	1.8 ± 0.6	nd
		20	53.4 ± 9.5	16.0 ± 3.0	23.0 ± 4.3	nd	3.9 ± 1.8	nd
	T3	200	12.8 ± 8.9	1.2 ± 1.7	4.0 ± 2.4	nd	1.0 ± 2.0	4.6 ± 1.9
		60	63.1 ± 10.2	18.8 ± 4.0	15.3 ± 4.4	nd	1.3 ± 0.7	nd
		25	54.7 ± 4.4	12.6 ± 4.5	13.3 ± 3.3	1.1 ± 1.8	nd	1.2 ± 2.4
		15	59.2 ± 8.9	27.2 ± 4.3	22.8 ± 4.3	1.3 ± 1.4	5.9 ± 1.0	nd
	T4	200	nd	nd	nd	nd	nd	nd
		40						
		15	38.0 ± 10.8	22.9 ± 7.6	1.8 ± 2.7	nd	nd	nd
		10	49.7 ± 7.3	21.2 ± 3.8	nd	nd	nd	nd
	T5		not sampled for bacterial community structure					
	T6	200	15.4 ± 3.1	8.7 ± 2.2	nd	nd	0.9 ± 0.8	4.1 ± 1.2
		70						
		30	55.3 ± 12.4	17.3 ± 6.4	nd	nd	1.0 ± 1.3	nd
		10	57.1 ± 8.8	25.5 ± 2.6	2.3 ± 1.5	0.9 ± 1.5	2.8 ± 1.7	nd
	T7		not sampled					

Season	Station	Depth (m)	Proportion (%) of cells detected with probe					
			Eubacteria	*Cytophaga-Flavobacteria*	Alpha-*Proteobacteria*	Beta-*Proteobacteria*	Gamma-*Proteobacteria*	*Archaea*
Summer	L	200	3.7 ± 0.9	nd	nd	nd	nd	1.7 ± 0.4
		35						
		15						
		8	44.0 ± 5.8	21.9 ± 4.6	5.9 ± 1.3	nd	3.7 ± 1.3	nd
	T1	200						
		95						
		30						
		15						
	T2	200	13.1 ± 4.3	2.7 ± 1.7	2.3 ± 1.8	1.9 ± 1.8	nd	2.2 ± 1.7
		85	10.0 ± 4.8	2.8 ± 4.0	nd	nd	nd	nd
		30	16.9 ± 7.4	5.4 ± 3.4	1.3 ± 1.3	nd	nd	2.0 ± 6.8
		15	23.4 ± 4.1	3.2 ± 1.4	13.8 ± 7.8	nd	nd	nd
	T3	200	14.8 ± 4.2	7.2 ± 2.6	2.7 ± 1.3	1.9 ± 1.2	nd	0.7 ± 1.5
		85	9.5 ± 3.3	5.1 ± 1.7	5.5 ± 2.1	1.1 ± 1.0	2.1 ± 0.7	1.1 ± 0.7
		22	39.1 ± 5.3	11.5 ± 2.0	4.4 ± 2.0	0.7 ± 1.4	2.8 ± 1.8	nd
		13	34.6 ± 8.0	13.2 ± 3.2	5.5 ± 2.1	nd	4.9 ± 1.6	nd
	T4	250	3.7 ± 0.9	nd	nd	nd	nd	1.7 ± 0.4
		18						
		10	41.9 ± 5.1	8.8 ± 3.0	28.3 ± 3.5	3.8 ± 3.4	2.8 ± 2.2	nd
		6	58.2 ± 9.2	10.5 ± 3.5	22.8 ± 6.6	9.4 ± 2.5	5.7 ± 2.0	3.9 ± 1.8
	T5	200						
		30	27.5 ± 6.3	10.9 ± 2.2	4.3 ± 1.2	nd	1.2 ± 2.0	nd
		13	27.3 ± 5.7	15.9 ± 3.6	5.3 ± 1.8	3.3 ± 1.3	2.8 ± 0.9	nd
		7						
	T6	200	15.6 ± 3.3	4.8 ± 1.2	nd	nd	nd	nd
		32						
		20	50.9 ± 8.9	12.6 ± 3.0	11.2 ± 3.0	nd	2.2 ± 1.3	nd
		12	38.1 ± 3.3	23.7 ± 4.8	4.0 ± 1.7	nd	4.4 ± 1.2	nd
	T7	250	13.7 ± 3.1	nd	2.2 ± 1.2	nd	nd	1.9 ± 0.8
		37	12.6 ± 2.8	4.1 ± 0.6	5.1 ± 1.4	nd	0.8 ± 0.4	3.1 ± 0.4
		15	25.9 ± 7.2	12.9 ± 2.4	5.6 ± 1.5	nd	3.1 ± 1.4	nd
		9						

Season	Station	Depth (m)	Proportion (%) of cells detected with probe					
			Eubacteria	*Cytophaga-Flavobacteria*	Alpha-*Proteobacteria*	Beta-*Proteobacteria*	Gamma-*Proteobacteria*	*Archaea*
Fall	L	200	21.4 ± 4.7	5.2 ± 1.7	4.9 ± 0.9	nd	2.6 ± 1.3	4.7 ± 1.4
		31	38.0 ± 4.0	13.5 ± 2.4	5.7 ± 1.0	2.1 ± 0.8	1.9 ± 0.9	nd
		12	31.5 ± 3.5	13.0 ± 1.3	10.6 ± 2.7	nd	8.4 ± 3.0	nd
		7	44.4 ± 7.6	14.9 ± 3.2	13.5 ± 2.9	2.1 ± 1.8	14.1 ± 3.6	nd
	T1	200	4.9 ± 1.0	3.9 ± 1.0	1.1 ± 0.8	nd	nd	2.1 ± 1.1
		80						
		18	29.1 ± 3.8	16.3 ± 4.0	5.2 ± 1.3	nd	6.2 ± 1.6	nd
		9	35.3 ± 5.4	3.2 ± 2.2	2.0 ± 1.4	nd	8.4 ± 2.6	2.7 ± 1.8
	T2	200	7.9 ± 1.2	2.9 ± 1.1	0.8 ± 0.9	nd	1.4 ± 0.9	nd
		64	11.2 ± 4.5	2.5 ± 2.0	nd	nd	nd	1.0 ± 2.4
		24	25.2 ± 2.6	nd	6.5 ± 1.8	nd	1.1 ± 1.2	3.7 ± 1.2
		14	31.2 ± 3.8	nd	6.1 ± 1.9	10.9 ± 3.7	3.9 ± 1.7	nd
	T3	200	9.1 ± 2.4	nd	1.6 ± 0.9	nd	nd	1.9 ± 1.1
		73	10.4 ± 2.5	2.0 ± 1.8	nd	nd	nd	nd
		23	34.1 ± 6.0	3.4 ± 2.1	4.2 ± 1.4	1.5 ± 2.3	4.0 ± 2.7	6.7 ± 3.4
		14	40.9 ± 4.2	8.5 ± 3.7	4.3 ± 1.2	nd	7.5 ± 2.0	5.0 ± 1.2
	T4	250	13.6 ± 3.0	nd	1.9 ± 1.1	nd	nd	nd
		28						
		12	50.5 ± 6.2	25.7 ± 5.1	4.9 ± 1.3	nd	2.2 ± 0.9	nd
		6	42.9 ± 6.1	12.0 ± 3.1	10.6 ± 2.2	1.7 ± 0.9	2.5 ± 0.8	0.8 ± 0.6
	T5	200	20.1 ± 4.2	3.3 ± 1.0	1.6 ± 2.0	nd	nd	0.8 ± 1.2
		32	44.5 ± 5.3	8.0 ± 1.0	10.8 ± 2.1	nd	5.7 ± 2.0	nd
		13	42.8 ± 5.1	8.8 ± 1.4	6.9 ± 0.8	nd	3.4 ± 1.2	nd
		8	33.5 ± 3.3	8.5 ± 1.8	8.6 ± 1.3	0.7 ± 0.7	1.2 ± 0.3	nd
	T6	200	16.6 ± 2.5	3.2 ± 1.5	nd	nd	nd	1.5 ± 0.9
		31						
		19	39.6 ± 4.1	21.1 ± 3.1	7.9 ± 2.4	nd	4.0 ± 1.1	2.4 ± 0.8
		11	36.3 ± 5.2	2.8 ± 0.9	3.3 ± 1.1	nd	2.6 ± 0.8	10.0 ± 2.2
	T7	145	46.2 ± 5.3	17.3 ± 3.8	10.6 ± 1.7	0.9 ± 1.1	4.0 ± 1.1	2.1 ± 1.5
		48	50.9 ± 4.9	29.4 ± 13.1	12.8 ± 3.5	5.6 ± 1.4	1.9 ± 1.5	8.5 ± 1.9
		19	43.2 ± 5.8	8.3 ± 2.3	11.0 ± 1.8	1.1 ± 1.4	8.4 ± 3.0	4.6 ± 1.3
		11	60.5 ± 7.4	16.5 ± 6.6	17.1 ± 5.2	2.2 ± 1.2	2.3 ± 0.8	5.6 ± 2.0