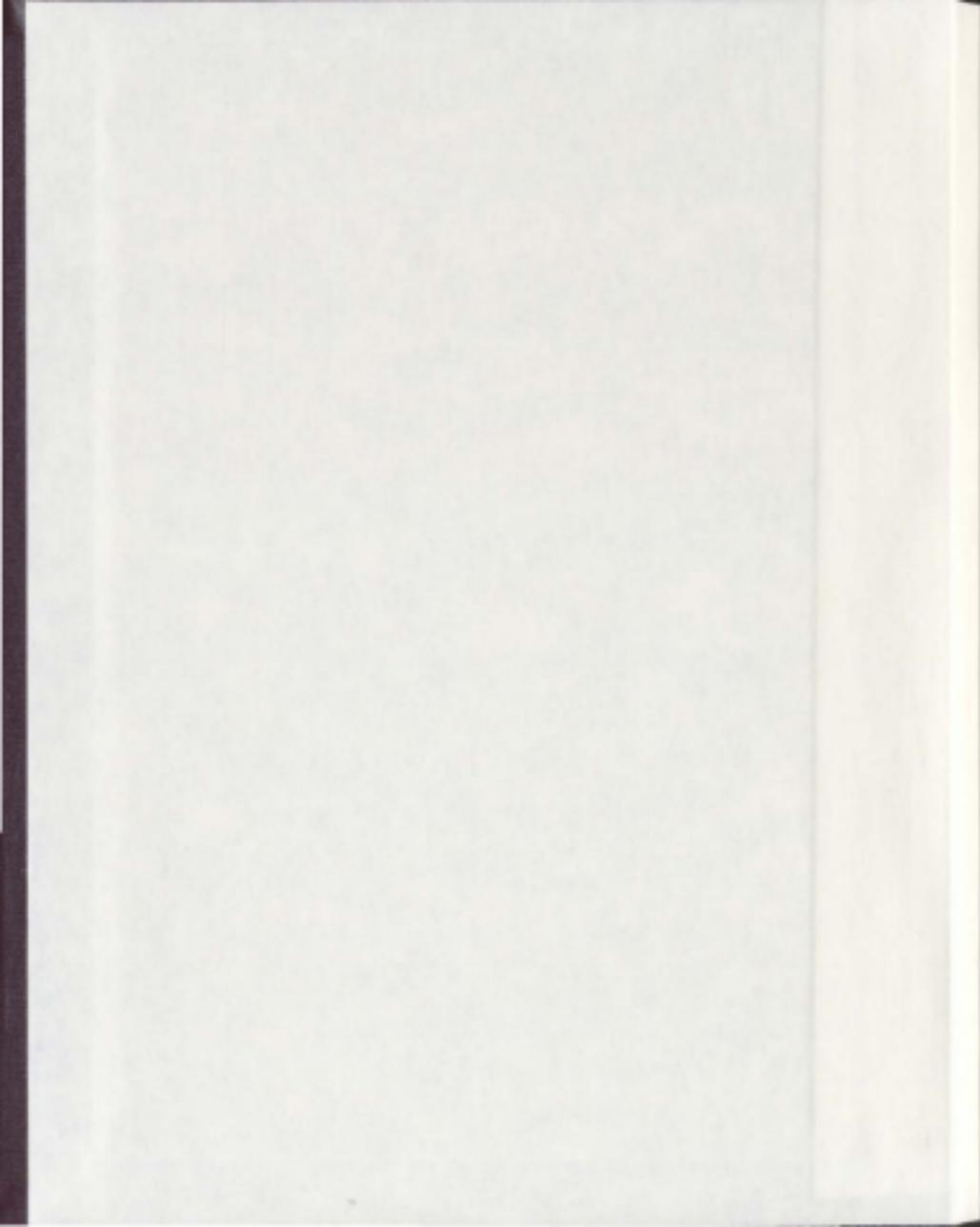


SPATIAL AND TEMPORAL VARIATION IN THE  
POPULATION STRUCTURES, CARBON USE AND  
CLIMATE RESPONSES OF HETEROTROPHIC  
MICROBIAL COMMUNITIES IN COASTAL AND  
OFFSHORE NORTHWEST ATLANTIC SITES

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**Spatial and Temporal Variation in the Population Structures,  
Carbon Use and Climate Responses of Heterotrophic Microbial  
Communities in Coastal and Offshore Northwest Atlantic Sites**

by

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**Abstract:**

Heterotrophic marine microbes were studied across spatial and temporal scales in the northwest Atlantic to investigate microbial communities' structural and functional responses to climate-relevant environmental forcings. Cellular abundance, morphometric, and 16S RNA-targeted Fluorescent In Situ Hybridization (FISH) analyses were used to examine variation in microbe-mediated carbon flow as it pertained to grazing pressure, temperature-shifts, and dissolved organic matter (DOM) availability. Significant spatial differences in growth and biomass production *versus* experimental manipulations indicate climate-driven physical changes in the upper ocean may influence future basin-scale patterns of the biogeochemical cycling of carbon. Seasonal variation of cell size and growth during grazer-exclusion experiments points to the increasing importance of inorganic nutrient limitation on plankton dynamics in a warming ocean. Analysis of grazing control on microbial communities relative to current and predicted ocean temperatures also suggests impacts of a warming ocean on spring phytoplankton bloom initiation and on carbon cycling in the upper ocean.

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## List of Abbreviations and Symbols

### Incubation Treatments:

- MSW – modified seawater
- RAW – whole seawater (200 $\mu$ m-filtered)
- +C – glutamate nutrient enrichment
- +T – positive temperature-shift
- +C+T – both a nutrient enrichment and temperature-shift

### Acronyms and Abbreviations:

- AO – Acridine orange
- AODC – Acridine orange direct counts
- BP – Bacterial Production
- CCGS – Canadian Coast Guard Ship
- chl. a – Chlorophyll a
- CTD – Conductivity, Temperature, Depth instrument
- DAPI – 4',6-diamidino-2-phenylindole
- DMSF – dimethyl sulphonium propionate
- DOC – dissolved organic carbon
- DOM – dissolved organic matter
- DON – dissolved organic nitrogen
- ERD – Ecosystem Research Division
- FISH – fluorescent in situ hybridization
- GLU – glutamate
- HR – hybridization ratio
- IPCC – Intergovernmental Panel on Climate Change
- Leu – leucine
- LSE – Labrador Sea Expedition
- NL – Newfoundland and Labrador
- POM – particulate organic matter
- TdR – tritiated thymidine
- TOC – total organic carbon

### Oceanography:

- BGCP – biogeochemical province
- ARCT – Atlantic Arctic province
- BPLR – Boreal Polar province
- NWCS – Northwest Atlantic Shelves province
- NCW – Newfoundland coastal waters

### Statistics:

- ANOVA – analysis of variance
- MANOVA – multivariate analysis of variance

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Appendix 1: Protocols and Methods

Appendix 2: Raw Data and Calculations

### **1.1 Central Problem**

The dynamic nature of marine microbial communities results from the interaction of biotic and abiotic factors that change in space and time. Spatial and temporal changes occur and interact with changes in global climate and a wide range of associated forcing functions such as temperature, nutrient availability, and grazing pressure. Annually recurring community structures and variation in community function have been observed and studied in natural and artificial environments (see Penthaler & Amann 2005 for a review), however studies following changes in both community structure and function have yet to determine whether or not these shifts represent physiological plasticity or succession of important phylotypes (Alonso & Penthaler 2006). A central tenet of community ecology is that strong spatial and temporal patterns link environmental processes, ecosystem function, and biological diversity (Sterner & Elser 2002; Hooper *et al.* 2005), and it is these linkages that are the focus of my research. My ultimate goal is to advance understanding of marine microbial community structures, their associated function(s), and how these relationships change in response to environmental forcings across temporal and spatial scales. This study and others like it are important since climate change-induced variation in the structure and activities of microbial communities will not only affect nutrient and energy transfer in food webs, but will alter biogeochemical cycles as well.

Despite the central role of the microbial loop in oceanic ecosystems, methodological limitations of autecological studies (Schäfer *et al.* 2000; Kan *et al.* 2006; Mary *et al.* 2006) have favored synecological studies of microbial community function (Pace & Cole 1994; Gasol & Moran 1999; Riemann *et al.* 2000). These traditional 'black box' approaches have produced important data quantifying fluxes of nutrients and energy through the microbial system, yet have yielded limited insight into how distinct populations function and interact with one another (Massana *et al.* 2001; Carlson *et al.* 2004; Cho & Giovannoni 2004). Description of the functional activities of complex microbial communities can lead to sound ecological hypotheses; testing of these hypotheses will require information on how the components of these communities respond to environmental perturbations through the application of both spatial-temporal field analyses and experimental manipulations (Begon *et al.* 1996; Gray & Head 2001).

Recently, advanced molecular techniques adapted to work with marine microbes allow in-depth perception of the dynamic nature and regulation mechanisms of microbial food webs (Penthaler *et al.* 2002b; Teira *et al.* 2004). It is in this larger context of both bottom-up and top-down regulation that 'Gleasonian' studies (Gleason 1926; Penthaler & Amann 2005), which focus on discrete and quantifiable microbial populations, can reveal how these populations interact with their competitors, predators, and their physicochemical environment (Penthaler & Amann 2005). The research described here measured the relative abundances of planktonic members of the Archaea and two Eubacterial groups ( $\alpha$ -proteobacteria and  $\gamma$ -proteobacteria) in the Northwest

Atlantic. Neither of these groups can be delimited by unique metabolic capabilities; however they represent a large proportion of most marine surface water prokaryotic communities (Cottrell & Kirchman 2000b), are likely major components in secondary production, and embody a wide-range of metabolic diversity in the marine surface layers.

My work aims to characterize the relationship between bacterioplankton community structure and its ecological and biogeochemical *function* by studying physiological activities of heterotrophic picoplankton communities and enumerating dominant groups in the marine bacterioplankton from *in situ* and experimental samples<sup>1</sup>. Chapter Two provides an overview of marine bacterial ecology, and sets the context for this study. Chapter Three presents the results of a Temperate-Arctic transect in the Northwest Atlantic during May-June 2006 where the spatial variation in microbial community structure and growth rates were measured during nutrient enrichment and temperature-shift experiments. Chapter Four presents the results of experiments designed to evaluate temporal changes in the microbial community structure and growth rates of Logy Bay, NL. Central to this work is the idea that incubation experiments which combine food web manipulations (i.e. Gasol *et al.* 2002) with water transplanted from spatially or temporally disparate points will reveal new information concerning the complex top-down and bottom-up control of bacterioplankton populations.

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<sup>1</sup> For simplicity, the term *community structure* will denote the relative (measured) or absolute (theoretical) abundances of the phylotypes (phylogenetically-related groups) being studied. *Functions of capacity* will refer to both quantifiable biophysical processes and metabolic potential that influence the community's ability to carry out the function of DOM uptake and assimilation.

## 1.2 Research Questions

This research in microbial oceanography sought first to characterize the carbon use and 16S ribotype population distributions of spatially and temporally separated marine bacterioplankton communities in the Labrador Sea and Logy Bay, Newfoundland and Labrador (NL), Canada to answer:

- 1a. Do different locations in the Northwest Atlantic support phylogenetically- and/or functionally-distinct bacterioplankton communities?
- 1b. Do *in situ* microbial communities change their phylogenetic composition and/or function over time?

Second, analysis of the paired community structure and function datasets generated by this survey may detect important ecological identities or relationships relevant to the questions:

- 2a. Can communities with disparate structures have similar functions and/or *vice versa*?
- 2b. Are changes observed in function linked to phenotypic plasticity or to a shift in community structure?

Finally, the spatial and temporal variation in the reaction of marine bacterioplankton communities to perturbations to environmental forcings remains an important area of study and invites inquiry concerning:

- 3a. What effect do environmental forcings (nutrients, temperature, and grazing) have on bacterial communities (cell size, growth rates, and group abundances)?

**3b.** Do these controlling factors change in significance at different spatial or temporal scales?

To answer these questions, three specific objectives were identified to:

1. Evaluate short- and long-term variation in planktonic community structure and growth rates as they relate to temperature, nutrient availability, and location. (Chapters 3 & 4)
2. Determine if communities with similar function (growth rates, abundance, cell size) have disparate structures (group abundances) or vice versa. (Chapter 4)
3. Determine what effects available dissolved organic carbon (DOC) in the form of glutamate (GLU), water temperature, and grazing have on bacterioplankton community structure and function across spatial and temporal scales. (Chapters 3 & 4)

### **1.3 Overview**

#### 1.3.1 Research Context

Since 70% of the World's Oceans are at or below 5°C (Baross & Morita 1978), studies of the distribution and physiology of marine microbes in low-temperature environments like the North Atlantic are important for understanding global biogeochemical cycles. With this in mind, my field work in this cold ocean was carried out in two phases: 1) a spatial transect in the Labrador Sea, conducted on the CCGS Hudson in May-June 2006, and 2) a temporal transect in Logy Bay, Newfoundland from February to October 2007. In these

areas, the surface layer is generally dominated by mesophilic and psychrotrophic, rather than psychrophilic bacteria (Morita 1975; Ferroni & Kaminski 1980; Gow & Mills 1984). As well, evidence from flow cytometry indicates that the bacterioplankton of the Labrador Sea are biochemically heterogeneous (Li *et al.* 1995), however whether this is a consequence of phylogenetic diversity or phenotypic plasticity remains to be seen.

### **Labrador Sea**

The Labrador Sea (geographic centre: 60°00'N, 55°00'W) is situated between Labrador and Greenland, and is the coldest basin in the subpolar North Atlantic (Yashayaev *et al.* 2003). It is significant locally as the source region of the Labrador Current that strongly affects the hydrography of shelf ecosystems downstream (Talley & McCartney 1982), and globally as a principal component in the global ocean conveyor belt system (Dickson *et al.* 2002). The Labrador Sea is also the site of many international oceanographic surveys focusing on Northern Ocean processes (Yashayaev 2006).

The 2006 Labrador Sea Expedition (LSE) 2006019 conducted by the Bedford Institute of Oceanography ran from May 25 to June 4, a period when the Labrador Sea is capped by a shallow, low salinity and gradually warming surface mixed layer. A rapid decrease in surface CO<sub>2</sub> concentrations there coincides with a decrease in nutrient concentrations, indicating rapid growth of phytoplankton in the spring bloom (Takahashi *et al.* 1993). The biological program of LSE 2006019 measured variability in plankton biomass, productivity and biogenic carbon inventories as a continuation of studies begun in 1994 to provide descriptions of large-scale spatial and temporal variation in biogeochemical

processes to the Ecosystem Research Division's (ERD) climate-studies initiative. Spatial and temporal variation in microbial biomass in the Labrador Sea is relatively small, with bacterial stocks increasing only 2-4 fold from winter to summer (Li & Dickie 2003). Nanozooplanktonic grazers may keep bacterial stocks below carrying capacity (Fenchel 1986); however the low average temperatures (4-8°C) (Parson & Lalli 1988) do reduce bacterial demand for DOC and may limit microbial growth as well.

### **Logy Bay**

Logy Bay (location: 47°38'N, 52°39'W) is a small coastal bay north of St. John's, NL on the Avalon Peninsula. Logy Bay opens to the east to the North Atlantic Ocean. Its waters are dominated by coastal diversions of the Labrador Current. At depth, the Labrador Current keeps the waters of Logy Bay at -2 to -1°C, while the mixed layer (5 to 65m) warms seasonally from -1.8 to 14°C (Kendaris 1980; Putland 2000). This site was an ideal location for temporal variation studies in heterotrophic bacterial communities because of its location convenient to the Ocean Sciences Centre of Memorial University. During my studies the mean annual water temperature for Logy Bay was 6.1°C in 2005 and 5.6°C in 2006 (unpublished data), conditions similar to that of Labrador Sea and such that one would expect bacterial demand for DOC to be reduced. Past studies of Newfoundland coastal waters (NCW) have focused on the urochordate *Oikopleura vanhoeffeni* (Knoechel & Steel-Flynn 1989; Deibel & Lee 1992), however microzooplankton are important grazers in NCW and may play important roles in carbon flow through pelagic food webs at cold temperatures (Putland 2000).

### 1.3.2 Incubation Experiments

Incubation or mesocosm experiments (Pace & Cole 1994) are commonly employed research tools that have been used for decades to complement descriptive field studies (Perthaler & Amann 2005). Incubations involve whole (RAW) or size-fractionated (MSW) water samples from the environment of interest that may be experimentally manipulated with respect to nutrient availability or temperature (Eilers *et al.* 2000; Jürgens *et al.* 1999b; Lebaron *et al.* 1999; Suzuki 1999; Massana *et al.* 2001; Beardsley *et al.* 2003; Ovreås *et al.* 2003; Winter *et al.* 2004). The incubation experiments performed in my study were short-term (96 hours) and of small volumes (0.5-1L), and share the same reasoning of tracer uptake experiments, that a measurable change from *in situ* conditions in response to the experimental manipulation provides information on the original state of the community assemblage, or components thereof. The experiments provided information on microbial communities in their original state, during and at the end of incubation periods to evaluate how these assemblages respond to climate-driven environmental factors. Incubation experiments are not without their problems however, as the handling and manipulation of the mesocosms no doubt irreversibly destroys important characteristics of the *in situ* condition (Azam 1998; Perthaler & Amann 2005). Furthermore, large comprehensive datasets are required in marine microbial ecology to sufficiently gauge system complexity. Spatial and/or temporal sampling restrictions and limited experimental evidence can only do so much to inform on ecosystem-scale processes and their links to biogeochemical cycles, however incubation treatments present a research tool that can be standardized to compare intrinsic microbial responses between systems.

### 1.3.3 Direct Counts and Size Estimation

Acridine Orange (AO) is a nucleic acid selective metachromatic stain that interacts with DNA by intercalation after which it fluoresces green at 525nm. The use of AO epifluorescence microscopy for direct enumeration of microbes was introduced by Hobbie and colleagues in 1977 and has been reported to yield better estimates of microbial size and abundance than the newer DAPI method (Suzuki *et al.* 1993). For this reason, AO direct counts (AODC) and image capture were used to estimate changes in microbial abundance and cell size (both population and biomass growth rates). Image analysis was conducted with Image Pro Plus v.6.2 (Media Cybernetics, MD) to obtain Norland estimates of cell carbon.

### 1.3.4 Statistical Methods

The structure and function data collected from the LSE 2006019 were correlated with available ancillary data including: TOC profile, temperature, salinity, chlorophyll a, primary production, as well as phytoplankton abundance and biomass measures. Temperature and salinity data were also gathered with the Logy Bay samples.

The sampling and experimental treatments were structured to suit exploratory MANOVA analyses between clade abundances and other measured variables. The incubation experiments also provided a useful structure of Control/Treatment and Before/After data points for confirmatory MANOVA analyses. Relationships determined from these first two analyses were then employed to interpret the interactions between biology, space and time. Statistical analyses were carried out with version 14 of the Minitab software

package. Analyses of variance and covariance were executed via the General Linear Model—with appropriate checks for normality, independence, and homogeneity—to test specific hypotheses (Seber 1977). Specific regression and ANOVA analyses were also performed. A significance criterion of 5% was chosen for all analytical decisions.

The material set forth in this overview chapter outlines the broad context for this study within the field of marine bacterial ecology. Readers familiar with the subject are invited to move on to Chapter 3 where I continue with a description of the present study. Because both Chapters 3 and 4 are written to stand on their own, some of the material presented here will be of necessity repeated.

### 2.1 Marine Bacterial Ecology

Heterotrophic Archaeal and Eubacterial cells range in abundance from  $10^8$  —  $10^{10}$  cells  $L^{-1}$  in marine systems, comprise up to 70% of the biomass in marine surface waters (Fuhrman *et al.* 1989), process around 50% of primary production (Fuhrman & Azam 1982; Azam *et al.* 1983), and are therefore important components of the global carbon cycle (Kirschman *et al.* 1991; Legendre & Rassoulzadegan 1995; ). Investigations into the structure and function of microbial communities seek answers to the ecological roles played by specific microbial taxa (Pace 1988; Pernthaler & Amann 2005), including the ecological contribution bacterioplankton make to larger food webs in the marine system. However, it remains to be understood how variation in microbial communities on both spatial and temporal scales affects important pathways in marine ecosystem functioning (Sarmiento & LeQuéré 1996; Thingstad & Lignell 1997). Recent advances in molecular techniques based on 16S ribosomal DNA (rDNA) sequences (Acinas *et al.* 1999; Cottrell & Kirschman 2000b; Moesender *et al.* 2001) have enabled studies to address important ecological questions by examining the biogeographical patterns in bacterial community

structure, as well as investigating which environmental factors are important in community function (Papke & Ward 2004; Horner-Devine *et al.* 2004).

### **2.1.1 Functional Roles:**

Bacteria and other micro-organisms are recognized as important components of marine ecosystem processes (Sorokin 1981; Azam *et al.* 1983; Ducklow & Carlson 1992). Seawater contains a significant pool of both particulate and dissolved organic matter (POM and DOM), though most organic material in the ocean surface layer is found as DOM (Hagström *et al.* 1988; Dafner & Wangersky 2002). Bacterioplankton consume both forms of organic matter to produce biomass and ATP energy, remineralizing the organic matter with variable efficacy to CO<sub>2</sub>, and they may thus impact the efficiency of carbon export from the surface ocean via both physical and biological pathways (Pomeroy 1974; Azam *et al.* 1983; Oliver *et al.* 2004). These pathways have been shown to be important yet variable and poorly understood aspects of marine carbon cycling and food web dynamics (Fuhrman & Azam 1982; Sherr *et al.* 1988; Legendre & Rassoulzadegan 1995). However, as the main consumers of DOM in seawater, heterotrophic marine bacteria do mediate biogeochemical carbon fluxes via CO<sub>2</sub> exchange between the lower atmosphere and upper ocean layers, and therefore elicit important questions concerning their globally-significant, carbon-fueled metabolism (Azam 1998; Malmstrom *et al.* 2005; Manganello, *et al.* 2009).

Bacterial uptake of DOM and incorporation into biomass is largely dependant on the chemical composition (quality) of the material (Moriarty & Bell 1993). While DOM

pools in the water column may be relatively high, the labile (bioavailable) component of this pool can be quickly exhausted, limiting bacterial production, and resulting in a buildup of stable DOM in surface waters (Carlson & Ducklow 1995; Thingstad *et al.* 1997; Penthaler *et al.* 2001b). Limitation of microbial metabolism by inorganic nutrients such as orthophosphate and nitrogen can also restrict DOM use by and other metabolic processes of heterotrophic bacteria (Hoerrihan *et al.* 1988; Zweifel *et al.* 1993; Kirchman 1994). These limitations—which are heterogeneous across spatial and temporal scales—permit an essentially diverse array of DOM-consuming bacterioplankton that fill these dynamic niche spaces (Hutchinson 1961; Cottrell & Kirchman 2000a; Johnson *et al.* 2006). Taxon-specific activities then can be critical to ecosystem processes, and are critical to a complete understanding of structure-function relationships in microbial ecology.

### **2.1.2 Community Structure:**

Despite the long-standing assertion that “everything is everywhere” ( Beijerinck 1913), and the limitations of current community structure metrics, studies have found that distinct marine bacterioplankton populations vary through space and time (Ghiglione *et al.* 2005; Morris *et al.* 2005; Penthaler & Amann 2005; Fuhman *et al.* 2006; Martiny *et al.* 2006). Accordingly, studies of the relationship between bacterioplankton community structure and function must recognize that neither aspect is static on either spatial or temporal scales. This is not a minor consideration, as strikingly dissimilar community structures have been found just 5 km apart, while community compositions have remained relatively constant over distances of 10 km in the same area of study (Suzuki *et*

*et al.* 2001). Temporal changes in community structure and function have been related to environmental variations that occur on seasonal scales (Ghiglione *et al.* 2005; Morris *et al.* 2005; Fuhrman *et al.* 2006), however the environmental conditions that give rise to disparate population distributions, and the functional capabilities of these diverse community structures are poorly understood (Schink 2000; Fuhrman 2002). It is therefore a major objective of marine microbial ecologists to account for these heterogeneous distribution patterns of microbial population structures and to determine what effects these forcings have on ecosystem function (Pernthaler & Amann 2005).

The *functional capacity* of microbial communities to process DOM is dependant on both the composition of the communities and the environmental forcings acting upon them (Felip *et al.* 1996; Bell *et al.* 2005). In marine communities, bacterioplankton populations exhibit variation within and between taxonomic groups in terms of cell size (Lee & Fuhrman 1987; Beardsley *et al.* 2003), metabolic activity (Cottrell & Kirchman 2000a; Schink 2000; Fuhrman 2002), growth strategies (Pernthaler *et al.* 2001b), and niche-partitioning (Johnson *et al.* 2006). On a strictly morphological level where bacterial cells range in size from equivalent spherical radii of 0.1 to 2  $\mu\text{m}$  (Lee & Fuhrman 1987), it is evident that the abundance and activity of specific populations can contribute disproportionately to changes in community biomass and therefore total carbon flux (Pernthaler *et al.* 1996; Jürgens *et al.* 1999a). However, studies have just begun to articulate important links between community structure and function of marine bacterioplankton (Jürgens *et al.* 1999b; González *et al.* 2000; Gray & Head 2001).

Therefore, a major challenge in aquatic microbial ecology is to understand the functional roles of phylogenetically-defined bacterial populations in marine ecosystems with respect to elemental fluxes (Zubkov *et al.* 2001a,b; Pernthaler & Amann 2005). And while the specific rates of consumption of DOM by phylogenetically-distinct bacterioplankton groups are currently under investigation (Zubkov *et al.* 2003; Malmstrom *et al.* 2005; Yokokawa & Nagata 2010), the relative importance of these groups as mediators of specific element fluxes across relevant environmental conditions remains to be determined on a global scale.

## **2.2 Environmental Control of Bacterioplankton Communities**

### **What are the factors controlling bacterial populations and processes?**

Phylogenetic-specific cellular activities are but one factor shaping the ecological function of bacterioplankton communities. Environmental factors can also impact bacterial communities' biogeochemically-important cycling of energy and materials in the ocean by affecting cell physiology as well as influencing the outcomes of interspecific competition. (Hale *et al.* 2004; Chiu *et al.* 2005). Thus, an identification and a thorough understanding of the factors that control the distribution, abundance, and activity of marine microbial populations is critical to predicting ecosystem function and response to environmental change (Chapin *et al.* 2000; Seymour *et al.* 2010). Strong spatial and temporal patterns have been observed in the structure and/or function of marine bacterioplankton communities, patterns which illustrate that these organisms are governed by environmental (i.e. abiotic and biotic) processes (Morin 1999; Sterner & Elser 2002), and that these processes govern the link between ecosystem function and biological

diversity (Loreau *et al.* 2001; Hooper *et al.* 2005). In marine environments, bacterioplankton community structure is thought to be influenced by nutrient availability (Torsvik *et al.* 2002), temperature (Thompson *et al.* 2004; Chiu *et al.* 2005), salinity (Suzuki *et al.* 2001), and selective losses to grazers and viral infections (Fuhrman 1992; Thingstad & Lignell 1997), while community function is known to be influenced by seasonal and latitudinal changes in temperature and irradiance (Madigan *et al.* 2003; Hewson *et al.* 2006), as well as variable sources and quality of substrates (Cho & Azam 1988; Ducklow & Carlson 1992; Hewson *et al.* 2006). A central goal of microbial ecology then is a partitioning of these bottom-up (resource limitation), and top-down (grazing) influences on both microbial community function and structure, as the dominance of any one factor can result in differential responses to ecosystem change (Sala & Graham 2002). As both nutrients and temperature have been shown to limit microbial growth rates, it is believed that experiments proposed here will produce similar results. Top-down pressure from grazers is expected to limit microbial abundances, but not growth rates.

#### **Bottom-up regulation:**

Bottom-up forces such as temperature and nutrient availability play important roles in shaping microbial communities and their activities. As a general rule, bacterial population growth rates are temperature-dependant and temperature has a greater impact on microbial activity in resource-limited environments (Pomeroy *et al.* 1995; Felip *et al.* 1996). At low temperatures, prokaryotic cells allocate proportionally more cellular energy and resources to biomass production, and therefore have greater gross growth

efficiencies (GGEs) than cells in warmer waters (Christian & Weibe 1974; Rivkin *et al.* 1996). It should not be surprising then that bacterial cells are generally larger in cold rather than warm water environments (Chrzanowski *et al.* 1988; Hagstrom *et al.* 1988; Wiebe *et al.* 1992). These compensatory interactions that occur at low temperatures characteristic of the Labrador Sea (-1 to 5°C) can result in significant carbon flux from microbial to metazoan trophic levels (Rivkin *et al.* 1996). Currently, our understanding of temperature effects on prokaryotic community function is still incomplete, as fundamental questions remain concerning the relationship between temperature dependency of bacterial growth and prokaryotic community structure under different environmental conditions. For this reason, the research presented in the next two chapters examines the temperature dependency of prokaryotic specific growth rates, whether this temperature dependency changes seasonally, and whether the relationship between temperature and metabolism varies with organic carbon enrichment.

Concentration gradients of dissolved organic carbon and inorganic nutrients are another feature of pelagic environments important to microbial communities (Alonso & Perthaler 2006). Seasonal variation in nutrient availability is thought to transiently limit or encourage activity and growth of populations of the heterotrophic picoplankton (Cottrell & Kirchman 2000a; Kuosa & Kaartokallio 2006), while inter-taxonomic variation in substrate uptake and incorporation rates may explain the coexistence of diverse microbial communities in oligotrophic habitats (Alonso & Perthaler 2006). It has also been proposed that heterotrophic picoplankton enter a dormant/starvation phase

when nutrient limitation results in unfavorable conditions for cellular activities (Stevenson 1978; Roszak & Colwell 1987).

The research conducted as part of this thesis examined the specific growth rates of heterotrophic marine prokaryotes under *in situ* and DOC/N-enriched conditions across both spatial and temporal scales. As physiochemical conditions change in the Northwest Atlantic both seasonally and spatially, it is expected that prokaryotic communities of different functionalities will be observed. Relative population abundances were also measured during nutrient-limited and nutrient-replete incubation experiments to study the response of oligotrophic and eutrophic populations to spatially- and temporally-variant environmental forcings. Changes in community structure are thought to represent a succession of community members, while relatively static community structures may indicate metabolic plasticity of the microbial flora under study.

#### **Loss Processes:**

Bottom-up factors such as temperature and substrate availability regulate microbial communities, but it is important to recognize that these communities do not exist or function in isolation from other biological components of the environment. Viral abundance is known to play a key role in structuring microbial communities and is an important loss process to consider in community-level analyses (Longnecker *et al.* 2010). While top-down regulatory factors (Pace & Cole 1994) can be considered to start at any trophic level, this study's scope is limited to what impact metazoan and protozoan grazers have on bacterioplankton community structure and function.

Top-down regulation by grazers can maintain bacterioplankton abundances below carrying capacity (Li *et al.* 2001), resulting in a substantial fraction of bacterial biomass being transferred to higher trophic levels (Sherr *et al.* 1987; Pernthaler & Amann 2005). However, grazing mortality does not simply regulate bacterial biomass, but can act as a selective removal process that directly structures bacterial communities (González *et al.* 1990), while actually augmenting nutrient pools for the ungrazed members of the microbial assembly (Sherr *et al.* 1988; Weisse & Scheffel-Möser 1991; Glibert 1998). Predation then, is believed to be another principal biological process that balances microbial production in marine environments (Azam *et al.* 1983; Sherr *et al.* 1987; Fuhrman & Noble 1995), though the extent to which grazing pressure and/or nutrient availability regulate bacterioplankton communities may depend on such things as ambient temperature (Glibert 1998) and the community structure that these forcings are acting upon.

To investigate community-wide and group-specific effects of top-down and bottom-up regulatory factors, acute though environmentally-relevant temperature-shift incubations were performed at 7 stations located throughout the Labrador Sea. Acute, climate change-relevant (IPCC 2001) temperature-shift and nutrient-manipulation experiments were completed at the Ocean Sciences Centre in Logy Bay. These experiments sought to reveal the importance of temperature, C/N limitation, and grazing pressure as environmental forcings on microbial community structure and function.

### 2.3 Bacterioplankton Populations

The major bacterioplankton clades (see Figure 2.1) show heterogeneous distribution over large spatial scales (Giovannoni & Stingl 2005), while populations within clades are known to vary in distribution and abundance over small spatial scales (Suzuki *et al.* 2001). However, while biogeographical patterns of both high- and low-level groupings have been observed and studied, the underlying mechanisms driving these relationships remain undefined (Kirchman *et al.* 2005). This study chose a Gleasonian perspective to investigate environmental forcings on discrete microbial populations and their ecological functions. For this purpose, members of the Archaea and Eubacteria were selected for study as they represent a large proportion of the marine surface water prokaryotic community (Cottrell & Kirchman 2000b). As yet, there is little information regarding the metabolic function of specific bacterial populations in natural assemblages (Boschker *et al.* 1998).



with other marine bacteria, classification of  $\alpha$ -proteobacteria is based solely on 16S ribosomal DNA sequence, as no known biochemical or molecular feature can distinguish them from other groups (Gupta 2005).

### **$\gamma$ -proteobacteria**

Members of the  $\gamma$ -proteobacteria subdivision have been detected in the Atlantic and Pacific oceans from both coastal and open ocean habitats (Suzuki & DeLong 2002), where they have been shown to constitute up to 30% of picoplanktonic cells in marine samples (Cottrell & Kirchman 2000a). Despite the majority of cultivable marine bacteria belonging to this subdivision, little is known concerning the ecological roles played by  $\gamma$ -proteobacteria in marine systems. However,  $\gamma$ -proteobacteria have been identified as copiotrophs that are adapted to high nutrient concentrations (Glöckner *et al.* 1999); although DOM availability has not yet explained  $\gamma$ -proteobacterial distributions in aquatic environments (Cottrell & Kirchman 2000a). Bacteria of the as yet uncultivated SAR86 cluster are a widely distributed and globally abundant population belonging to the  $\gamma$ -proteobacteria subdivision. As with the *Roseobacter* of the  $\alpha$ -proteobacteria, several  $\gamma$ -proteobacteria populations, including SAR86, have been identified as important assimilators of DMSP (Malmstrom *et al.* 2004; Vila *et al.* 2004).

**Table 2.1: A summary of genome size data for alpha- and gamma-proteobacteria, as well as Crenarchaeota and Euryarchaeota.**

	Alpha-	Gamma-	Euryarchaeota	Crenarchaeota
<b>Count</b>	n = 82	n = 163	n = 40	n = 8
<b>Range</b>	8822	5552	4641	1380
<b>Size</b>	3434 ± 2421	3349 ± 1434	2393 ± 1057	2452 ± 465

These data were obtained from The Institute of Genomic Research's Comprehensive Microbial Resource<sup>1</sup> and lists compiled by Fogel *et al.* 1999 and Islas *et al.* 2004. Note that genome sizes are reported as mean ±  $\sigma$  in kilobase pairs.

### Archaea

Archaea, one of the three domains of life, comprise an abundant and well-studied heterotrophic picoplankton group that may represent 30% of prokaryotic cells in marine systems (Pernthaler *et al.* 2002a). The Archaea comprise two major pelagic lineages, the Crenarchaeota and Euryarchaeota that show differential vertical and seasonal distributions in the water column (Dolan 2005; Pernthaler & Amann 2005). Current evidence suggests that the Archaea are involved in N-cycling (Cabello *et al.* 2004), however their contributions to biogeochemical cycles remain unknown.

### 2.3.2 Identification and Delineation of Bacterial Populations

Various methods exist that allow for the identification of different microbial taxa from environmental samples (Pernthaler & Amann 2005). The methods of this study are based

<sup>1</sup> [http://cmr.tigr.org/tigr-scripts/CMR/shared/Genomes.cgi?complete\\_only=1](http://cmr.tigr.org/tigr-scripts/CMR/shared/Genomes.cgi?complete_only=1)

on Fluorescent In Situ Hybridization (FISH), a semi-quantitative cytogenetic technique. FISH employs oligo- or polynucleotide-based probes intended to be specific to targeted cells (Giovannoni *et al.* 1988; DeLong *et al.* 1989; Amann *et al.* 1990; Amann *et al.* 1995; Pernthaler *et al.* 2004), and can be used to complement, or in place of, marine bacterial 16S rDNA clone libraries or other community structure metrics (Pernthaler & Amann 2005). Using FISH with epifluorescence microscopy (Hobbie *et al.* 1977; Porter & Feig 1980), relative abundances of targeted cells can be calculated by comparing the number of cells detected by the group-specific probes to the total number of cells detected using a general stain such as DAPI. The FISH technique is generally used by marine ecologists to describe community structure (Cottrell & Kirchman 2000b) and detect seasonal variation in community structure (Burkert *et al.* 2003; Mary *et al.* 2006), while nested probe sets allow hierarchical studies that can detect niche-partitioning within groups (Eilers *et al.* 2000; Beardsley *et al.* 2003; Massana & Jürgens 2003). Studies employing FISH have also shown spatial variation in subgroups of the proteobacteria (Glöckner *et al.* 1999), regulation of bacterial community structure by protistan and metazoan grazing pressure (Simek *et al.* 1997; Jürgens *et al.* 1999a; Langenheder & Jürgens 2001; Simek *et al.* 2001), community transitions in aquatic environments (Bouvier & del Giorgio 2002; del Giorgio & Bouvier 2002; Kirchman *et al.* 2004), and that 16S rDNA clone libraries can fail to detect important members of microbial assemblages by misrepresenting population abundances (Cottrell & Kirchman 2000b; Eilers *et al.* 2001; Pernthaler & Amann 2005).

FISH is not without its limitations. Inadequate fluorescence intensity is one critical shortcoming of mono-labeled oligonucleotide-based FISH. When cells are small and cellular ribosome content is low—as is the case with slow-growing bacteria from natural water samples (Morita 1997; Fegatella *et al.* 1998; Pernthaler *et al.* 2002a)—cell detection and population enumeration may vary with the physiological status of each individual cell (Pernthaler & Amann 2005). An important consequence of this failing is that inherently slow-growing and/or small cells (such as marine members of the SAR86 clade) will not be detected with the same efficiency, thus biasing community structure estimates (Pernthaler *et al.* 2002b). Another limitation of the FISH technique is the prevalence of false positives that result from non-specific binding of probe molecules to non-target cells and microscopic debris. In environmental samples it is often impossible to enumerate populations that comprise less than 10% of the total sample because of false-positives and counting errors.

### 3.1 Abstract:

Global climate change will affect temperature and nutrient supply patterns in the World's Oceans. Heterotrophic bacteria are important in the cycling and transformation of organic carbon and their growth and production are controlled by both temperature and nutrient availability. To determine environmental factors that control bacterial dynamics and community structures temperature-shift, Dissolved Organic Matter- (DOM-) enrichment, and grazer-exclusion experiments were conducted with microbial assemblages collected from six locations, representing three biogeochemical provinces in the Labrador Sea (northwest Atlantic). Cellular abundance and morphometrics were determined from time series samples by flow cytometry, microscopy, and image analysis to examine variation of microbe-mediated carbon-flow. Relative abundances of Archaea,  $\alpha$ -, and  $\gamma$ -proteobacteria populations were determined for *in situ* and incubated microbial communities by standard 16S rRNA-targetted FISH. The results show significant differences within and between provinces in microbial communities' responses to incubation treatments with respect to growth and biomass production. Thus, climate-driven physical changes in the upper ocean can have spatially variable effects on biological systems with concomitant implications for the biogeochemical cycling of carbon.

### 3.2 Introduction:

Thesis: As marine bacteria are important biological components of the biogeochemical cycling of carbon through the World Ocean, and there exists regional variation in the bottom-up and top-down controls of bacterial communities, significant geographic patterns in the functional and structural response of microbial communities may arise in response to increased temperature and nutrient availability.

The World Ocean plays a critical role in the carbon cycle and is a global sink of the climate-active gases (i.e. CO<sub>2</sub>) (Sarmiento 1993; Ducklow & Field 2000). The ocean's ability to sequester CO<sub>2</sub> is determined by both physical and biological processes and when taken together, these biogeochemical processes provide important two-way coupling between the marine carbon cycle and global climate. While marine carbon cycle processes have been documented to respond to short-term climatic anomalies such as El Nino and the Arctic Oscillation, it is unknown how biological feedback mechanisms will function long-term in a warming World Ocean (Solomon 2007). Currently, the marine carbon cycle is functioning to mitigate CO<sub>2</sub>-induced climate change, but will this always be so?

Marine heterotrophic bacteria constitute an abundant and active planktonic group whose study is of paramount importance in determining the future biological uptake and release of CO<sub>2</sub> by the World Ocean. These heterotrophic bacteria consume and degrade dissolved organic matter (DOM)—an important pool of marine carbon—influencing on a

global scale the export and sequestration of biogenic carbon in the marine environment (Azam 1998; Williams 2000; Pearce *et al.* 2007).

The metabolic activity and phylogenetic composition of marine bacterioplankton communities is subject to bottom-up (i.e. temperature and nutrient supply), as well as top-down (i.e. grazing) control (Shiah & Ducklow 1994; Suzuki 1999; Cottrell & Kirchman 2000a). These biological and physical regulatory factors vary across spatial scales, and thus their influence will have varying importance in geographically distinct regions of the World Ocean. Biogeochemical provinces (BGCPs) are spatial partitions of different oceanic ecological regions based upon the depths of the nutricline and the subsurface chlorophyll profile (Longhurst 1998). Variation between BGCPs in the structure and function of microbial communities leads to regionally distinct patterns in carbon cycling which have important feedbacks on climate.

The Labrador Sea (geographic centre: 60°00' N, 55°00' W) is situated between Labrador and Greenland, and contains three BGCPs: the Boreal Polar (BPLR), Atlantic Arctic (ARCT), and Northwest Atlantic Shelves (NWCS) provinces. Current climate change models predict an increase of between 1.1 and 6.4°C in sea surface temperatures by 2100, as well as more rainfall and associated terrestrial run-off in polar regions (IPCC 2007). These climate changes will create warmer, less saline, and more nutrient replete surface waters across the Labrador Sea; conditions whose impact on the two-way coupling between climate and marine heterotrophic bacterial communities is not fully understood.

This study investigated the structural and functional responses of microbial communities from contrasting regions within Labrador Sea to proxy conditions for climate change using incubation experiments. Changes in microbial abundance and cell morphometrics were used to track changes in bacterial carbon use, while standard 16S rRNA fluorescent *in situ* hybridization (FISH) was employed to detect changes in the phylogenetic composition in the microbial communities. Incubation experiments were performed with grazer-reduced seawater cultures (MSW) designed to reveal the intrinsic microbial response to treatments independent of grazer control.

### **3.3 Materials and Methods:**

#### **3.3.0 Protocols and chemical recipes**

Additional protocol details and complete chemical recipes are included in Appendix 1.

#### **3.3.1 Seawater sampling**

Seawater samples were collected from May 25 to June 3, 2006 at six stations representing three biogeochemical provinces across the Labrador Sea (see Table 3.2 for details). These samples were obtained from depths of 5m or less by a Seabird CTD with a 24 bottle rosette (General Oceanics) aboard the CCGS Hudson and were stored for less than 2 h in opaque 25 L sterile carboys below deck before processing. Seawater chlorophyll *a* and salinity were measured by a CTD-mounted Deep fluorometer and Autosal salinometer respectively. Nitrate, phosphate and silicate concentration data were provided by other members of the Labrador Sea Expedition.

### 3.3.2 Incubation treatments

Four dilution treatments of 1 part 1.0  $\mu\text{m}$ -filtered to 4 parts 0.2  $\mu\text{m}$ -filtered seawater (MSW) were prepared for all 6 stations sampled. The treatments consisted of a non-manipulated control (MSW), a temperature-shift incubated at *in situ* +5.0°C (MSW+T), DOM-enriched incubations with +10  $\mu\text{M}$  glutamate over *in situ* concentrations (MSW+C), and a combined temperature-shift and DOM-enriched treatment (MSW+C+T). An additional 1.0  $\mu\text{m}$ -filtered seawater (RAW) treatment was incubated for 3 of the 6 stations. Each treatment was performed in triplicate in 1 L polycarbonate bottles and incubated in the dark for 96 hours. Incubations took place in either water baths below deck or a flow-through seawater bath on deck that was covered with a neutral-density filter to reduce incident illumination.

Marine viruses can influence microbial abundance and community structure (Wilhelm & Suttle 1999). Each incubation treatment was performed in triplicate to control for viral impacts between replicates. Mortality processes are known to vary between ecosystems of different trophic state, however this work did not seek to measure if viral losses varied between treatments or sampling sites.

### 3.3.3 Bacterial growth and biovolume

Samples of 10mL were collected from individual bottles after vigorous shaking immediately prior to (t=0) and every 24 hours until the end of the incubation period (t=96). These samples were fixed with glutaraldehyde (final concentration, 2% [vol/vol]), for determination of cell titer by Acridine Orange direct counts (Hobbie, 1977). These

samples were also used to measure biovolume by epifluorescent microscopy and image analysis with Image Pro Plus v.6.2 software (Media Cybernetics, Bethesda, MD). Estimates of bacterial biomass were derived from biovolume measurements (image analysis) multiplied by a carbon conversion equation (i.e.  $C = 120 \cdot V^{0.72}$ ; Norland 1993).

$^3\text{H}$  leucine incorporation rates from whole seawater as well as flow cytometry (FCM) data were provided by Dr. W. Li of the Bedford Institute of Oceanography. *In situ* bacterial growth rates were derived from estimates of bacterial production, by conversion of the radioisotope uptake rates to cell carbon production assuming 3 kg carbon per mol leucine incorporated (Kirchman *et al.* 1982). FCM data was used in conjunction with Acridine Orange direct counts to give greater certainty to microbial cell abundances.

Cyanobacteria are impossible to distinguish from heterotrophic bacteria when using Acridine Orange direct counts. It is expected that their presence led to a relatively small and consistent overestimation of heterotrophic cell abundances in *in situ* samples, however experimental incubations performed in the dark sought to minimize their impact on community dynamics.

### 3.3.4 Bacterial community structure

Bacterial community structure for *in situ* and incubation samples was measured using a modification of the classical Fluorescent In Situ Hybridization (FISH) protocol from Glöckner *et al.* (1996). A hierarchical suite of eight 16S rRNA-targeted probes (7 targeted and 1 nonsense control) was employed as per Table 3.1 (Mobix). These

oligonucleotide probes were all labelled with indocarbocyanine (Cy3) for fluorescent detection of targeted cells.

**Table 3.1: 16S rRNA-targeted oligonucleotide probes employed in this study**

Target	Name	5'-3' Sequence
Archaea	ARCH915	GTG CTC CCC CGC CAA TTC CT
Eubacteria	EUB338	GCT GCC TCC CGT AGG AGT
$\alpha$ -proteobacteria	ALF968	GGT AAG GTT CGT CGC GTT
SAR11 cluster	SAR11-441	TAC AGT CAT TTT CTT CCC CGA C
Roseobacteria	ROS537	CAA CGC TAA CCC CCT CC
$\gamma$ -proteobacteria	GAM42a	GCC TTC CCA CAT CGT TT
SAR86 cluster	SAR86-1249	GGC TTA GCG TCC GTC TG
N/A	NON338	ACT CCT ACG GGA GGC AGC

Samples of 45 mL for FISH analysis were collected from individual bottles after vigorous shaking into 50 mL falcon tubes at the start ( $t=0$ ) and finish ( $t=96$ ) of the incubation period. 5 mL of 57% formalin was added to each falcon tube and the samples were left to fix for 2 hours, after which they were filtered onto 0.2  $\mu$ m polycarbonate membrane filters (Millipore GTTP04700) supported on a cellulose prefilter (Millipore AP1504700). Filters were then washed with 50 mL of sterile phosphate buffered saline (PBS) and allowed to air-dry in individual sterile petri dishes. Dried filters were stored at  $-20^{\circ}\text{C}$  until analysis.

Filters were thawed at room temperature while the hybridization buffer was prepared. Hybridization solution was prepared by adding 6  $\mu\text{L}$  (50 ng/ $\mu\text{L}$ ) of probe to 48  $\mu\text{L}$  of hybridization buffer. Filters were cut into triangular eighths and sections were placed on microscope slides to which 20  $\mu\text{L}$  of hybridization solution was added. Slides were placed in hybridization chambers with 1 mL of hybridization buffer to ensure a humid atmosphere and incubated in the dark for two hours at 46°C. Washing buffer was prepared during this incubation and warmed to 46°C. After two hours, filter sections were incubated in washing buffer for 15 minutes at 46°C and then dried at room temperature. Filter sections were then counter-stained with 50  $\mu\text{L}$  of DAPI (1  $\mu\text{g}/\text{mL}$ ) for 1 minute on ice, washed with 1 mL of MilliQ water and re-dried over absorbent paper. Dried filter pieces were mounted on glass slides in Citifluor #1, a glycerol medium (Citifluor Ltd., Canterbury, UK). Slides were stored at -20°C overnight before microscopic analysis.

### 3.3.5 Epifluorescent microscopy

FISH filters were observed with a 100x 1.30 oil objective lens (total magnification 1250X) on an Olympus BH-2 epifluorescent microscope, using Cy3 (41007-HQ) and 4',6-diamidino-2-phenylindole (DAPI) UG-1 filters. Since DAPI fluorescence persists longer than Cy3 fluorescence (Pentthaler *et al.* 2001), counts of hybridized cells were completed before total cell enumeration for each field of view. Either a minimum of 500 hybridized cells, or at least 1000 DAPI-positive cells were counted for each sample as per Pentthaler *et al.* (1998). Counts were completed for Archaea, Eubacteria,  $\alpha$ - and  $\gamma$ -

proteobacteria, as well as for the negative control probe for all analyzed samples. Counts of SAR11, *Roseobacter*, and SAR86 were completed for a subset of analyzed samples. Relative abundances of hybridized cells were determined by dividing the Cy3 counts by the DAPI counts. These relative abundances were corrected for background fluorescence and non-specific binding by subtracting count values from the NON338 negative control probe.

### 3.4 Results

#### 3.4.1 Biogeochemical province designations

The six stations sampled across the Labrador Sea are mapped with their LSE ID numbers in Figure 3.1. Since BGCP boundaries are complex and seasonally variable, *post hoc* analysis was performed to determine if the stations' BGCP designations were correct. Table 3.2 presents a subset of oceanographic data used to characterize each station. Table 3.3 shows that the statistical inspection of *de facto* biogeochemical province designations for the 6 experimental stations was significant with respect to ocean chemistry and biology.

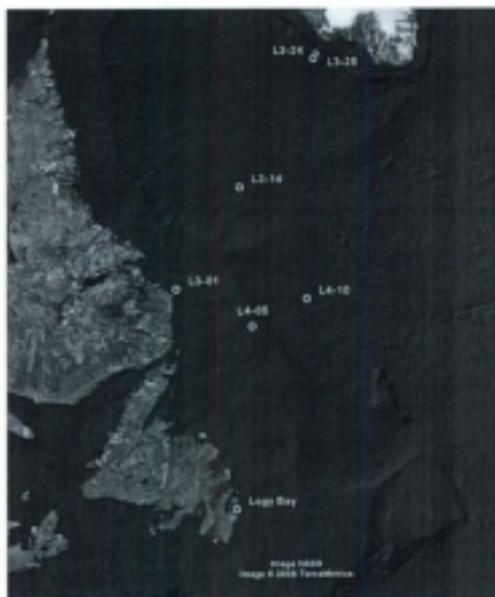


Figure 3.1: Station map for Labrador Sea Expedition samples.

Table 3.2: Selected physiochemical properties of sampled LSE stations.

	BOCP designation					
	ARCT			BPLR	NWCS	
	L3-24	L3-25	L4-10	L3-14	L3-01	L4-05
Chl. a µg/L	1.17	1.47	2.24	5.90	3.17	1.62
Temperature °C	4.4	2.5	6.6	4.7	1.7	3.3
Salinity ppm	34.39	34.45	34.57	34.69	31.73	32.62
NO <sub>3</sub> mg/L	2.68	3.38	5.41	4.70	0.17	0.16

**Table 3.3: Significance of biogeochemical province groupings of sampled LSE stations for selected biological and chemical properties**

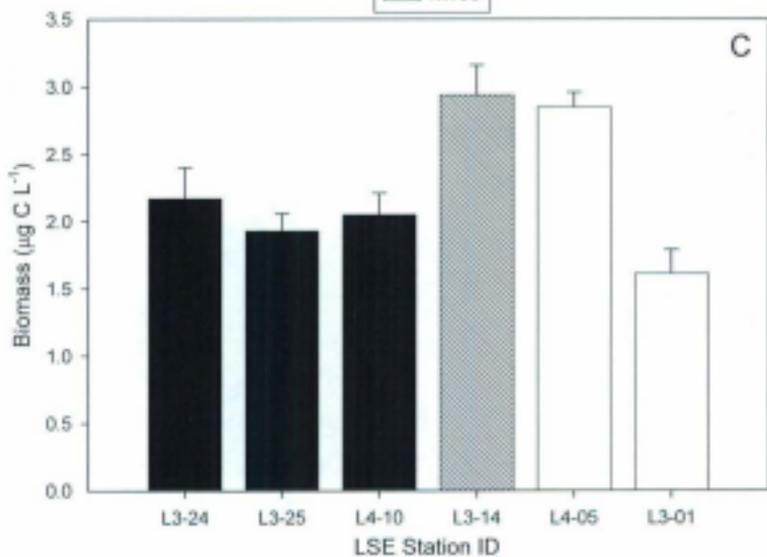
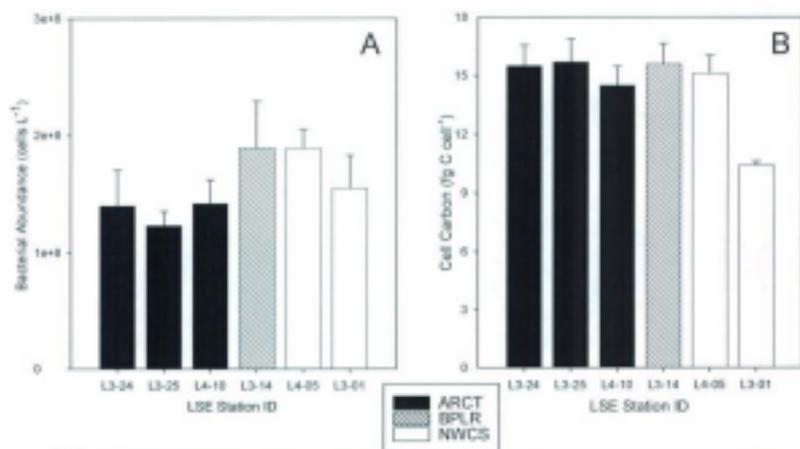
Property	Significance
Bacterial abundance	$p = 0.005, F_{2,17} = 7.56$
Cell carbon	$p = 0.024, F_{2,17} = 4.87$
Microbial biomass	$p = 0.066, F_{2,17} = 3.27$
Specific Growth Rate	$p = 0.738, F_{2,17} = 0.31$
Chlorophyll a	$p = 0.040, F_{2,5} = 11.41$
Phaeophytin	$p = 0.024, F_{2,5} = 16.54$
Salinity	$p = 0.012, F_{2,5} = 26.85$
Nitrate	$p = 0.066, F_{2,5} = 7.68$

#### 3.4.2 *In situ* community structure and function

Significant differences in microbial abundance, cell carbon and biomass between sampling sites are illustrated in Figure 3.2. Spatial variation in microbial communities' abilities to incorporate available DOM is illustrated in Figure 3.3. These heterogeneous distribution patterns of cellular morphometrics and incorporation rates point to bacterioplankton communities with distinct biogeochemical functions within the Labrador Sea. However, when DOM incorporation rates are considered with the carbon requirements required for cell growth in Figure 3.4, it is seen that these communities exhibit more similar growth rates. This homogeneity of *in situ* growth rates across biogeochemical provinces in the Labrador Sea demonstrates that there are important distinctions to be made between the ecological and biogeochemical functions of microbial communities.

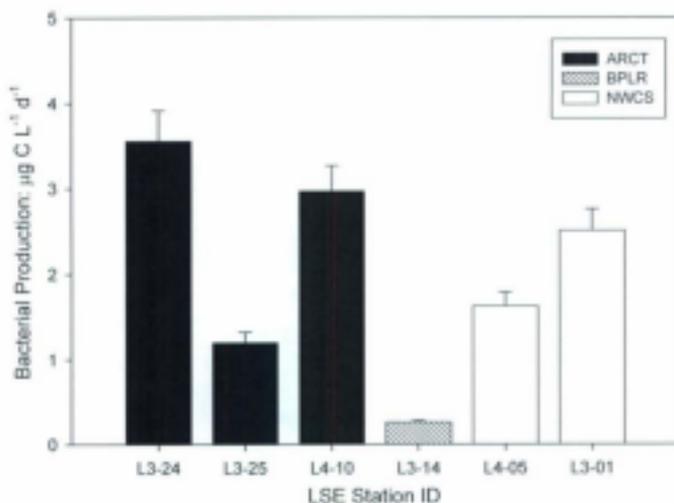
Comparison of *in situ* community structures shown in Figure 3.5 produced no significant link between or within BGCPs. However, *in situ* temperature may play an important role in determining population abundances of Eubacterial and Archaeal cells. Temperature relationships between the Crenarchaeota and Euryarchaeota may explain this result for ARCH915-positive cells. However, when the EUB338 result is considered, temperature appears to have less significance with respect to population structures. Cell detection and population enumeration can vary with the physiological status of each cell (Perthaler & Amann 2005), and with a range of temperatures this variation in probe-positive cells may be more representative of active populations in the microbial communities as opposed to straight quantification of populations. Further analysis of *in situ* community structures

showed a positive connection between chl. *a* concentrations and  $\alpha$ -proteobacteria. This result matches others that point to a significant ecological relationship between members of the  $\alpha$ -proteobacteria and phytoplankton (Gonzalez *et al.* 2000).

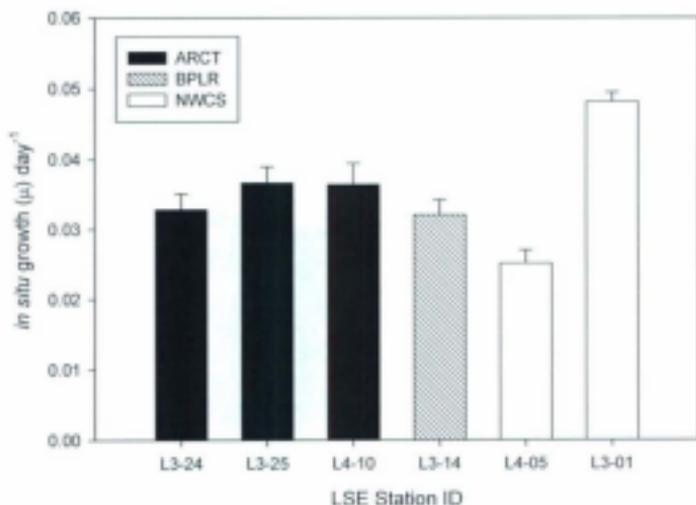


**Figure 3.2: A comparison of *in situ* heterotrophic bacterial abundance (A), cell carbon (B), and biomass (C) from Labrador Sea stations.** Bacterial abundance was determined from Acridine Orange direct counts. Cell carbon was estimated from cell volumes determined by Image Analysis. Biomass was calculated as the product of bacterial abundance and average cell carbon. Error bars delimit the upper symmetrical 95% confidence limit of: (A) counts among replicate fixed samples ( $n=3$ ) and (B) measurements of individual cells ( $n > 1000$ ); and (C) the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.

Within the Labrador Sea, there was significant variation between biogeochemical provinces in: bacterial abundance ( $p = 0.005$ ,  $F_{2,17} = 7.56$ ), cell carbon ( $p = 0.024$ ,  $F_{2,17} = 4.87$ ), and biomass ( $p = 0.003$ ,  $F_{2,17} = 10.13$ ). Stations within the NWCS biogeochemical province were significantly different with respect to cell carbon ( $p < 0.001$ ,  $F_{1,5} = 257.34$ ) and biomass ( $p = 0.004$ ,  $F_{1,5} = 36.75$ ), but not abundance. Within the ARCT biogeochemical province, stations were not significantly different with respect to any of these three measures.

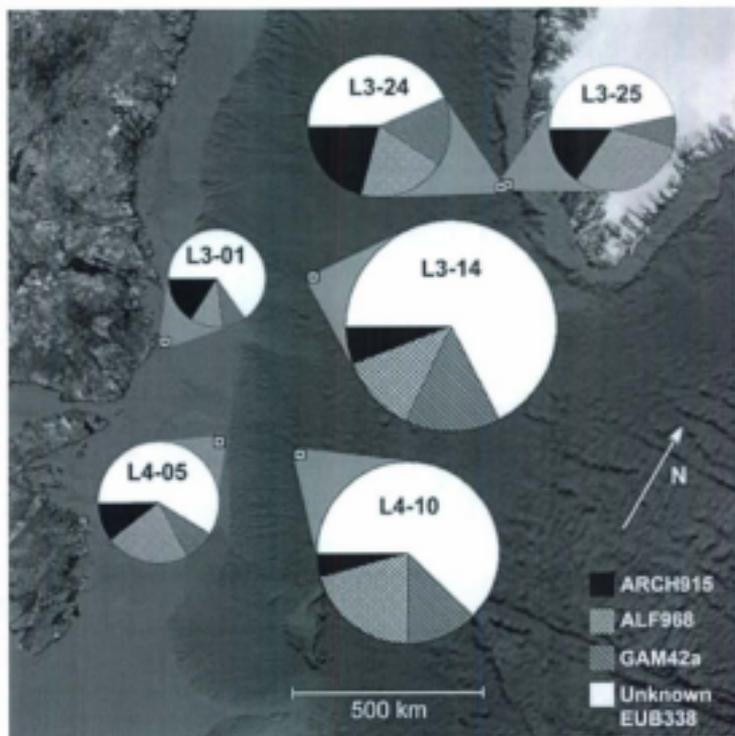


**Figure 3.3:** *In situ* Bacterial Production ( $\mu\text{g C L}^{-1} \text{d}^{-1}$ ) for all experimental stations in the Labrador Sea. Production estimates for  $^{14}\text{C}$  Leucine incorporation assays assume 3 kg/mol Leu incorporated. The stations are coded by biogeochemical province: black, shaded, and white for the Arctic Shelves (ARCT), Boreal Polar (BPLR), and Northwest Atlantic Shelves (NWCS) provinces respectively. Error bars represent the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.



**Figure 3.4:** *In situ* growth ( $\mu$  d<sup>-1</sup>) for all experimental stations in the Labrador Sea. Growth rate estimates utilize bacterial production estimates from <sup>14</sup>C Leucine incorporation assays that assume 3 kg/mol Leu incorporated and cell carbon estimates from cell volumes determined by Image Analysis. The stations are coded by biogeochemical province: black, shaded, and white for the Arctic Shelves (ARCT), Boreal Polar (BPLR), and Northwest Atlantic Shelves (NWCS) provinces respectively. Error bars delimit the upper symmetrical 95% confidence limit of counts among replicate treatment bottles (n=3).

*In situ* growth rates were significantly different between stations ( $p < 0.001$ ,  $F_{5,17} = 34.62$ ), but not within the ARCT province ( $p = 0.201$ ,  $F_{2,8} = 2.12$ ).



**Figure 3.5: *In situ* community structures from experimental stations in the Labrador Sea.** Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARCH915, ALF968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALF968- nor GAM42a-positive. Pie size is scaled relative to the microbial biomass (range 1.5–3.3  $\mu\text{g C/L}$ ).

Bacterioplankton communities sampled showed significant differences in phylogenetic composition on spatial scales. Between BGCPs, there were significant differences in community proportions for both ARCH915- and ALF968-positive cells ( $p < 0.001$ ,  $F_{3,47} = 9.79$ ; and  $p < 0.001$ ,  $F_{3,47} = 7.22$ ). Within the ARCT province, there are also significant differences for both ARCH915- and ALF968-positive cells ( $p = 0.003$ ,  $F_{2,8} = 18.50$ ; and  $p = 0.032$ ,  $F_{2,8} = 6.46$ ). Temperature is significant in explaining variation in HR for EUB338 and ARCH915 between stations ( $p = 0.037$ ,  $F_{1,47} = 4.61$ ;  $p < 0.001$ ,  $F_{1,47} = 13.72$  respectively). Concentration of chl. *a* was significant in explaining variation in HR for ALF968 ( $p = 0.001$ ,  $F_{1,47} = 13.41$ ).

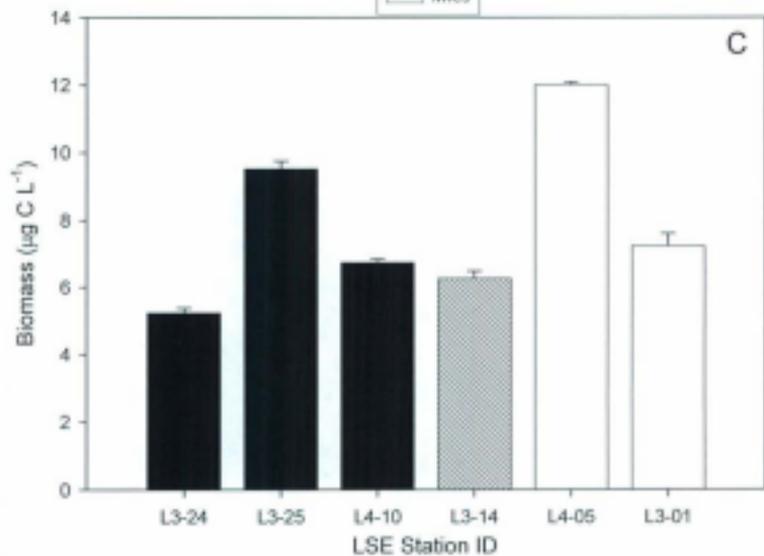
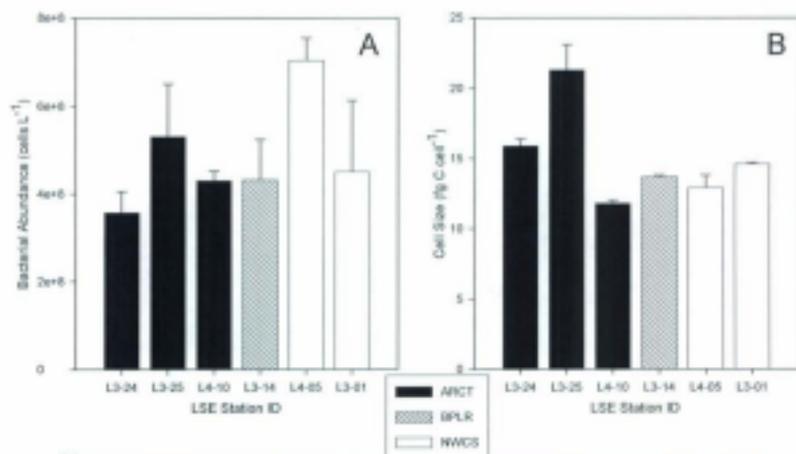
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### 3.4.3 Impact of environmental forcings on bacterioplankton communities

Temperature-shift experiments showed significant spatial variation on the impact of bacterioplankton community function (Figure 3.6), however BGCP designation was not significant in explaining variation of most parameters. DOM-enrichment experiments (Figure 3.7) demonstrated spatial variability of the limitation of bacterial abundance, cell

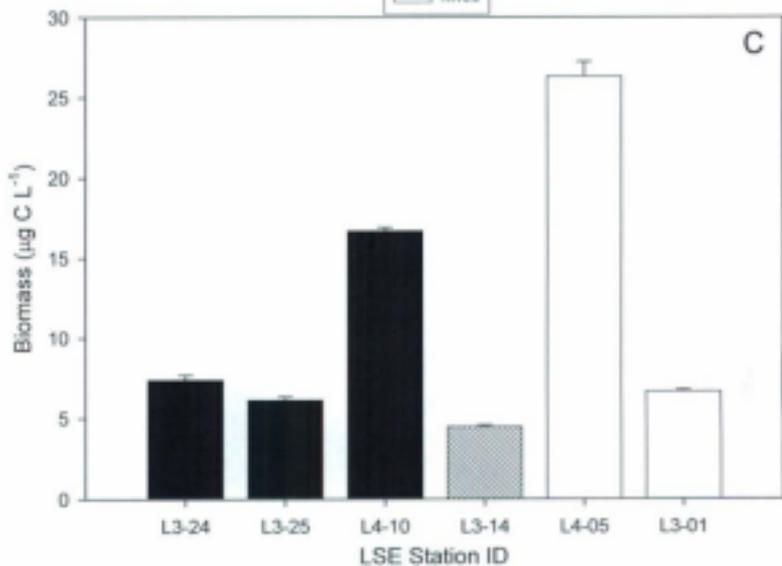
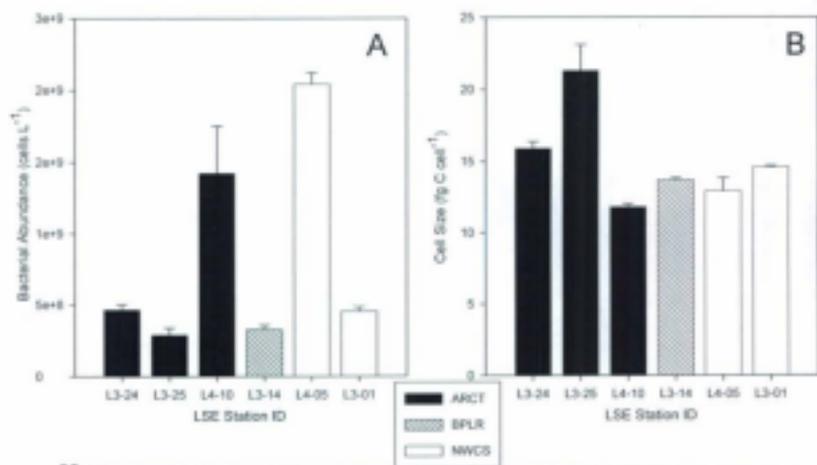
size, and total biomass by available DOM concentrations among BGCPs in the Labrador Sea. Figure 3.8 shows that communities exhibit a broad range of biomass production relieved of both temperature- and DOM-limitation, however this variation is not explained by measured physiochemical parameters, BGCP categories, microbial cellular morphometrics, or *in situ* community structures. Collectively, these figures present significant differences in the way existent microbial communities respond to local environmental forcings. Temperature-limitation of microbial function is not homogeneous across the Labrador Sea, thus a system-wide warming as predicted by IPCC climate change models may not be accurately calculated using current biogeochemical province theory. DOM-limitation of microbial function also shows spatial variation, however climate change will have a spatially variable effect on DOM input rates in the Labrador Sea through increased terrigenous inputs and seasonal melt cycles. Thus, no geographic-based predictions can be satisfied with this experiment.

Incubation experiments of whole seawater (RAW) and grazer-excluded modified seawater (MSW) show achieved ( $\mu$ ) and intrinsic ( $\mu_i$ ) growth rate estimates from which grazing rates are inferred (Table 3.4). Microbial communities sampled in the Labrador Sea showed significant spatial variation in the importance of grazing as a loss process, while relatively homogeneous achieved bacterial growth rates measured at all stations indicate the importance of other biological and physiochemical controls.



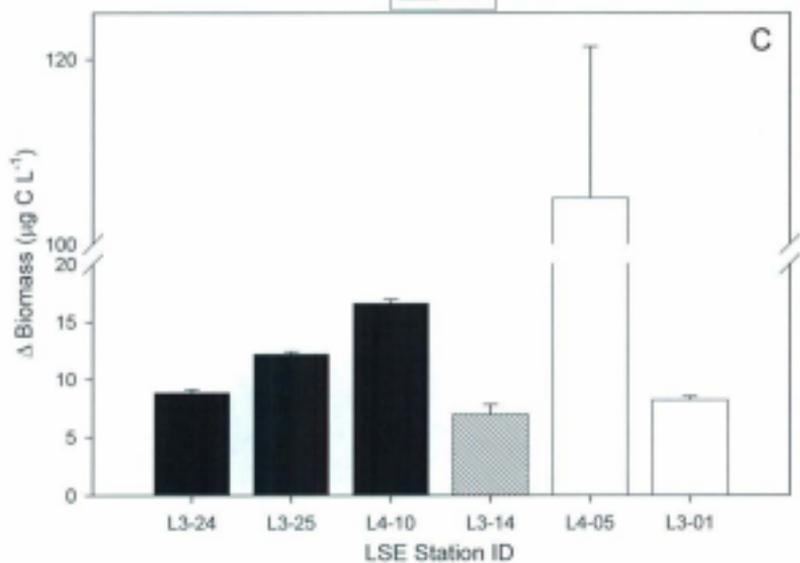
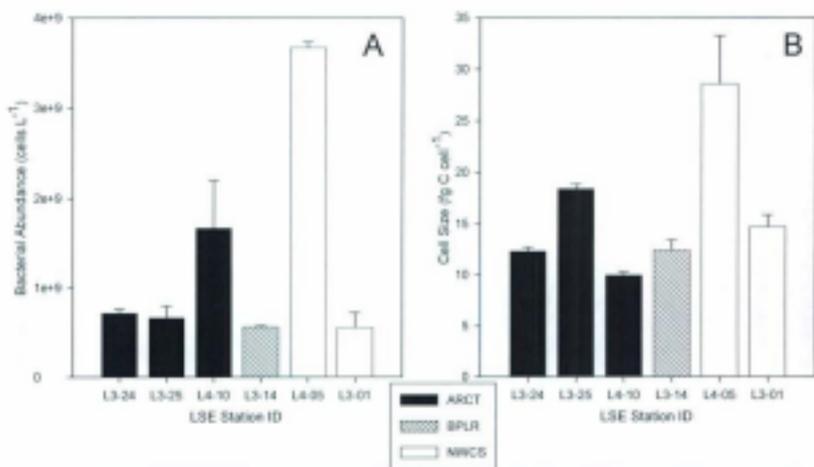
**Figure 3.6: A comparison of heterotrophic bacterial abundance (A), cell carbon (B), and biomass (C) at the end of temperature-shifted incubations from Labrador Sea stations.** Bacterial abundance was determined from Acridine Orange direct counts. Cell carbon was estimated from cell volumes determined by Image Analysis. Biomass was calculated as the product of bacterial abundance and average cell carbon. Error bars delimit the upper symmetrical 95% confidence limit of: (A) counts among replicate fixed samples ( $n=3$ ) and (B) measurements of individual cells ( $n > 1000$ ); and (C) the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.

There was no significant difference between biogeochemical provinces with respect to bacterial abundance or biomass achieved at the end of temperature-shifted incubations, however differences in cell carbon were significant ( $p = 0.027$ ,  $F_{2,17} = 4.63$ ). Within the ARCT province, there was significant variation between stations in: bacterial abundance ( $p = 0.001$ ,  $F_{2,8} = 25.5$ ), cell carbon ( $p = 0.043$ ,  $F_{2,8} = 5.56$ ), and biomass ( $p < 0.001$ ,  $F_{2,8} = 1828.63$ ). As well, within the NWCS province, there was significant variation in biomass between stations ( $p = 0.001$ ,  $F_{1,5} = 97.39$ ). Significant spatial variation in growth rates ( $\mu$ ) and increase in biomass between MSW controls and MSW+T temperature-shifted experiments was present among BGCs ( $p < 0.001$ ,  $F_{5,17} = 17.48$ ;  $p < 0.001$ ,  $F_{5,17} = 13.28$ ) as well as within the ARCT province ( $p = 0.001$ ,  $F_{2,8} = 32.89$ ;  $p < 0.01$ ,  $F_{2,8} = 46.86$ ).



**Figure 3.7: A comparison of heterotrophic bacterial abundance (A), cell carbon (B), and biomass (C) at the end of DOM-enriched incubations from Labrador Sea stations.** Bacterial abundance was determined from Acridine Orange direct counts. Cell carbon was estimated from cell volumes determined by Image Analysis. Biomass was calculated as the product of bacterial abundance and average cell carbon. Error bars delimit the upper symmetrical 95% confidence limit of: (A) counts among replicate fixed samples ( $n=3$ ) and (B) measurements of individual cells ( $n >1000$ ); and (C) the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.

Within the Labrador Sea, there was significant variation between biogeochemical provinces in: bacterial abundance ( $p = 0.017$ ,  $F_{2,17} = 5.40$ ), cell carbon ( $p < 0.001$ ,  $F_{2,17} = 64.86$ ), and biomass ( $p = 0.044$ ,  $F_{2,17} = 3.89$ ) achieved during DOM-enriched incubations. Within the ARCT province, there was significant variation between stations in: bacterial abundance ( $p < 0.001$ ,  $F_{2,8} = 74.95$ ) and biomass ( $p < 0.001$ ,  $F_{2,8} = 1528.33$ ), but not cell carbon. Within the NWCS province, there was significant variation in biomass between stations ( $p = 0.011$ ,  $F_{1,5} = 20.03$ ). Variation in growth rates ( $\mu$ ) and increase in biomass between MSW controls and MSW+C DOM-enriched experiments was significantly different across BGCPs ( $p < 0.001$ ,  $F_{3,17} = 170.46$ ;  $p = 0.006$ ,  $F_{3,17} = 5.92$ ). Variation in growth rates (but not biomass) was significant within the ARCT province ( $p < 0.001$ ,  $F_{2,8} = 121.72$ ;  $p = 0.816$ ;  $F_{2,8} = 0.21$ ).



**Figure 3.8: A comparison of heterotrophic bacterial abundance (A), cell carbon (B), and biomass (C) at the end of temperature-shifted, DOM-enriched incubations from Labrador Sea stations.** Bacterial abundance was determined from Acridine Orange direct counts. Cell carbon was estimated from cell volumes determined by Image Analysis. Biomass was calculated as the product of bacterial abundance and average cell carbon. Error bars delimit the upper symmetrical 95% confidence limit of: (A) counts among replicate fixed samples ( $n=3$ ) and (B) measurements of individual cells ( $n > 1000$ ); and (C) the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.

There was a significant difference between provinces with respect to bacterial abundance ( $p = 0.045$ ,  $F_{2,17} = 3.83$ ), but not cell carbon or biomass achieved during temperature-shifted, DOM-enriched incubations. Bacterial abundance ( $p < 0.001$ ,  $F_{2,8} = 100.35$ ), cell carbon ( $p < 0.001$ ,  $F_{2,8} = 52.37$ ), and biomass ( $p < 0.001$ ,  $F_{2,8} = 125.99$ ) were significantly different between stations of the ARCT province. As well, cell carbon ( $p = 0.019$ ,  $F_{1,5} = 14.54$ ) and biomass ( $p = 0.047$ ,  $F_{1,5} = 8.01$ ) were significantly different between the two NWCS stations sampled. Variation in bacterial abundance and biomass at the end of incubation experiments showed significant differences between sampling location ( $p < 0.001$ ,  $F_{3,71} = 121.88$ ;  $p < 0.001$ ,  $F_{3,71} = 475.98$ ), incubation treatment ( $p < 0.001$ ,  $F_{3,71} = 99.91$ ;  $p < 0.001$ ,  $F_{3,71} = 349.28$ ), and an interaction term between the two is observed ( $p < 0.001$ ,  $F_{15,71} = 32.82$ ;  $p < 0.001$ ,  $F_{15,71} = 225.50$  respectively). This result means that the heterotrophic bacterial response to treatment conditions was dependent on location

sampled. No other physiochemical or biological variables measured showed these broad significant trends, so the ecological basis for this interaction term remains unknown. Variation in community structures detectable only at a great resolution of analysis may dictate important structure-function relationships not apparent in this study.

**Table 3.4: Spatial variation in bacterial growth rate ( $\mu$ ) during grazer-manipulation experiments conducted with Labrador Sea surface waters as determined by Acridine Orange direct counts.**

	Temperature	RAW growth ( $\mu$ )	MSW growth ( $\mu^3$ )	Grazing rate
L3-24	4.2 $\pm$ 1.2°C	0.056 $\pm$ 0.064	0.064 $\pm$ 0.012	0.008 $\pm$ 0.065
L3-25	4.0 $\pm$ 1.0°C	0.018 $\pm$ 0.088	0.016 $\pm$ 0.075	-0.002 $\pm$ 0.116
L4-05	5.0 $\pm$ 1.0°C	0.092 $\pm$ 0.045	0.235 $\pm$ 0.037	0.143 $\pm$ 0.058

Achieved bacterial growth rates ( $\mu$ ) for RAW treatments were not significantly different between the ARCT and NWCS provinces ( $p = 0.164$ ,  $T_4 = 1.58$ ), however grazing rates did vary significantly between the two provinces ( $p = 0.021$ ,  $T_4 = 3.31$ ).

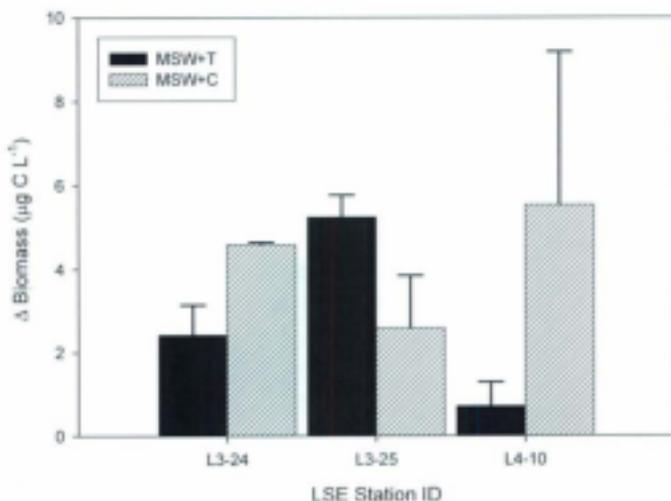
#### 3.4.4 Community dynamics within a biogeochemical province

The strong physiochemical similarities between stations sampled in the ARCT province (see Table 3.2) belied the variation in microbial communities' functional responses to increased temperature and DOM. Likewise, phytoplankton communities within biogeochemical provinces showed remarkable homogeneity in their response to seasonal environmental changes, such as the bloom initiation that follows salinity-driven water

column stratification in the ARCT province in April and early May (Longhurst 1998). Figure 3.9 illustrates intra-biogeographical province variation in both temperature- and DOM-limitation of microbial biomass.

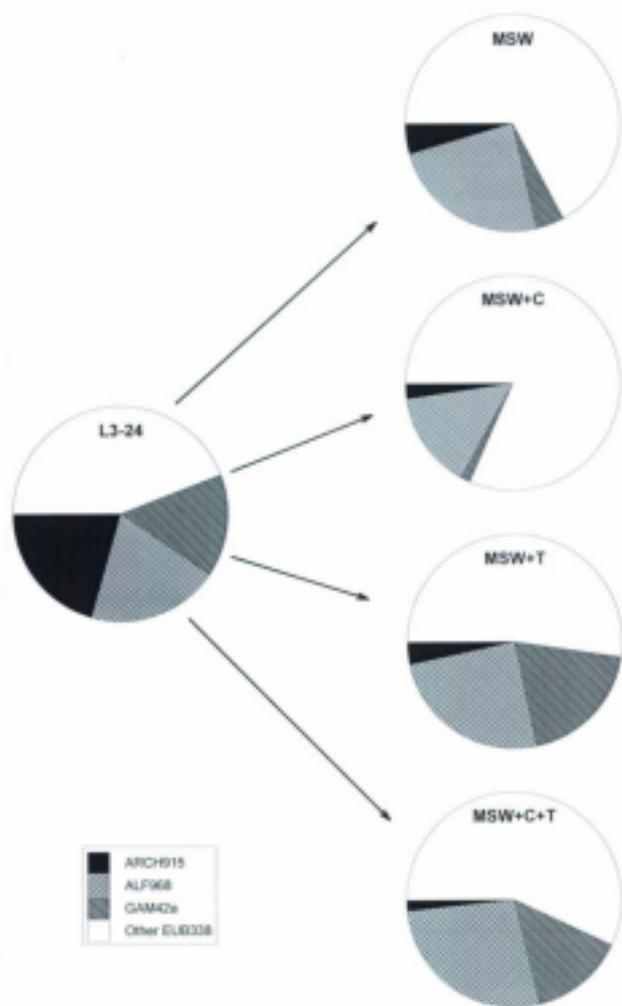
Figures 3.10 to 3.12 below depict incubation treatment-induced community structure changes for each ARCT station. Within the ARCT province, the three sampled stations showed variable responses to incubation conditions in terms of relative population abundances. One of three stations showed an increase in GAM42a-positive cells as the only significant detectable community structure response to the removal of grazing pressure. Both the increased temperature and the enriched DOM experiments showed significant changes in the ALP968-positive cells at two stations, and GAM42a-positive cells at the third.

Figures 3.13 and 3.14 show a significant connection between whole community and population-specific growth rates. In both cases, the increase of ALP968-positive cells was significant relative to total increase of biomass. This is evidence for a structure-function link from these experiments.



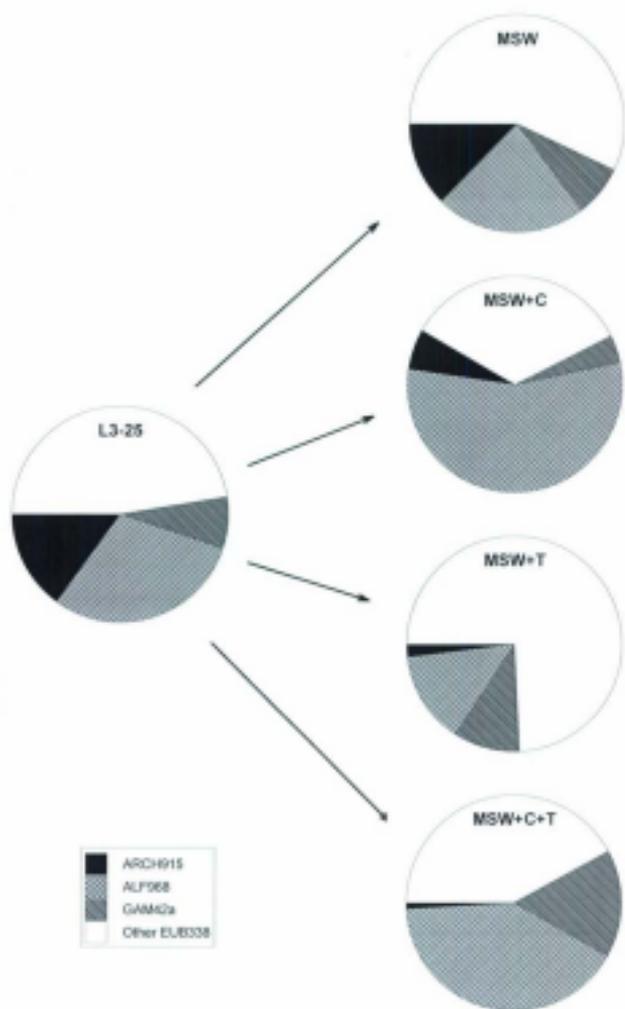
**Figure 3.9: Biomass change during incubations from the Arctic Shelves (ARCT) province.** The dark and shaded bars represent the difference in biomass ( $\mu\text{g C/L}$ ) from MSW controls for Temperature-shifted (MSW+T) and DOM-enriched (MSW+C) incubations respectively. Biomass was calculated as the product of bacterial abundance and average cell carbon. Cell carbon was estimated from cell volumes determined by Image Analysis. Error bars delimit the upper symmetrical 95% confidence limit of counts among replicate treatment bottles ( $n=3$ ).

Increase in biomass production by bacterioplankton communities in the ARCT province to temperature and DOM-enrichment manipulations was significantly different among stations ( $p < 0.001$ ,  $F_{2,8} = 171.27$ ;  $p < 0.001$ ,  $F_{2,8} = 227.44$  respectively). Biomass limitation by temperature was similar at stations L3-24 and L3-25, however there was a significant difference in DOM limitation of bacterioplankton biomass between these two communities.



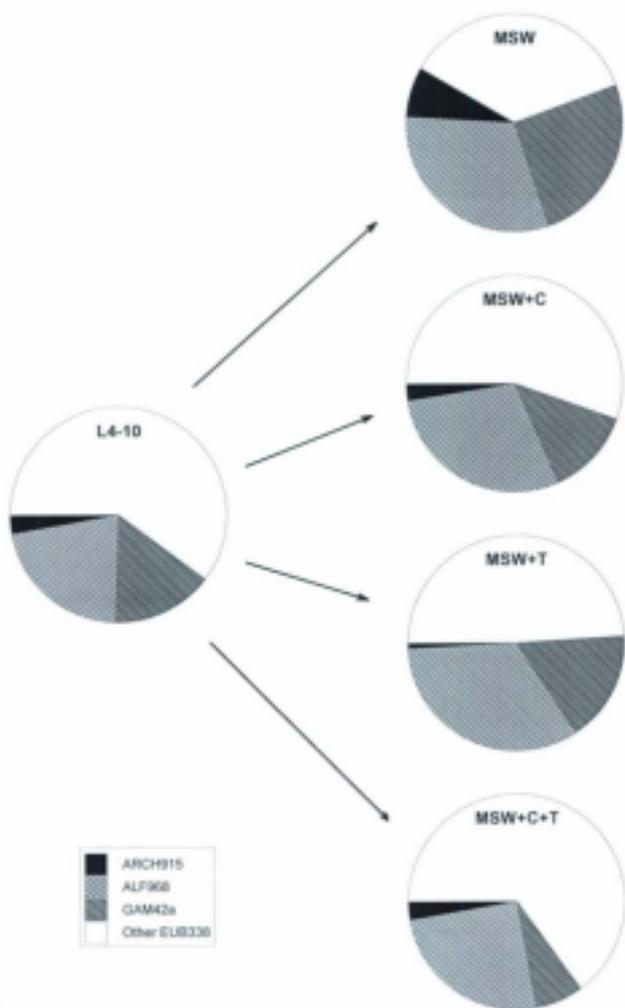
**Figure 3.10: Community structure changes in response to incubation treatments from L3-24.** Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARCH915, ALF968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALF968- nor GAM42a-positive. The circle on the left shows the initial MSW batch community, and the circles to the right show the communities after 96 hours at the specified treatment conditions.

For the MSW treatment, there was a significant decrease in community proportion between  $t=0$  and  $t=96$  of GAM42a-positive cells ( $p = 0.045$ ,  $T_4 = 4.54$ ). For the DOM-enriched treatment, there was a significant decrease in community proportion with respect to the MSW  $t=96$  control for ALF968-, as well as a concomitant increase in proportion of unidentified EUB338-positive cells ( $p = 0.004$ ,  $T_4 = 15.27$ ; and  $p = 0.013$ ,  $T_4 = 8.59$ ). For temperature-shifted treatment, there was a significant increase in GAM42a-positive cells relative to MSW  $t=96$  controls ( $p = 0.012$ ,  $T_4 = 9.13$ ). For the combined DOM-enriched, temperature-shifted treatment, there was also a significant increase in community proportion between  $t=0$  and  $t=96$  for GAM42a-positive cells relative to MSW  $t=96$  controls ( $p = 0.011$ ,  $T_4 = 9.34$ ).



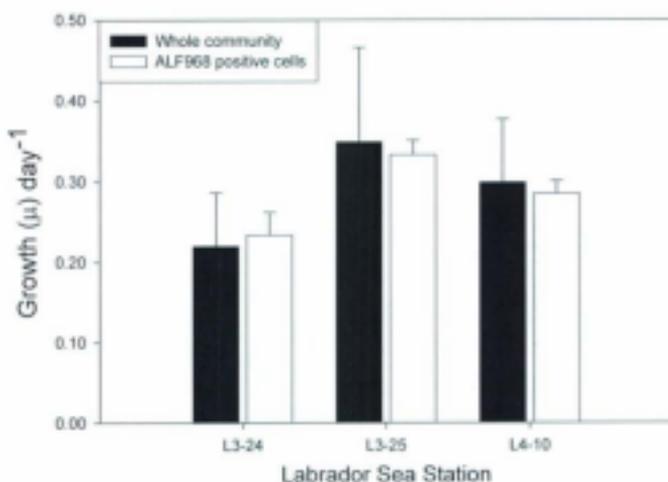
**Figure 3.11: Community structure changes in response to incubation treatments from L3-25.** Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARCH915, ALF968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALF968- nor GAM42a-positive. The circle on the left shows the initial MSW batch community, and the circles to the right show the communities after 96 hours at the specified treatment conditions.

For the MSW treatment, there were no significant differences in community proportion with respect to MSW t=0 controls for any probe-positive cells. For the DOM-enriched treatment, there was a significant increase in the community proportion with respect to the MSW t=96 control for ALF968-, as well as a concomitant decrease in the proportion of unidentified EUB338-positive cells ( $p = 0.007$ ,  $T_4 = 11.96$ ; and  $p = 0.016$ ,  $T_4 = 7.86$ ). For temperature-shifted treatment, there was a significant decrease in community proportion with respect to the MSW t=96 control for ALF968-, as well as an increase in the proportion of EUB338-positive cells ( $p = 0.008$ ,  $T_4 = 10.97$ ; and  $p = 0.005$ ,  $T_4 = 14.79$ ). For the combined DOM-enriched, temperature-shifted treatment, there was a significant increase in community proportion between t=0 and t=96 for both ALF968- and GAM42a-positive cells ( $p = 0.009$ ,  $T_4 = 10.61$ ;  $p = 0.005$ ,  $T_4 = 13.90$ ).



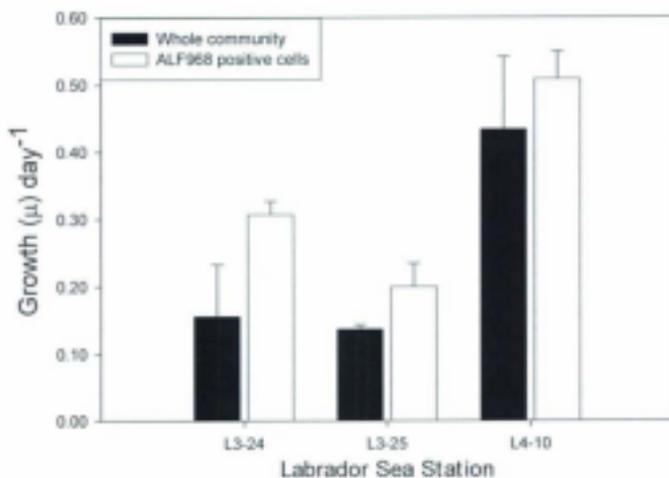
**Figure 3.12: Community structure changes in response to incubation treatments from L4-10.** Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARC18915, ALP968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALP968- nor GAM42a-positive. The circle on the left shows the initial MSW batch community, and the circles to the right show the communities after 96 hours at the specified treatment conditions.

For the MSW treatment, there were no significant differences in community proportion with respect to MSW t=0 controls for any probe-positive cells. For the DOM-enriched treatment, there was a significant decrease in community proportion with respect to the MSW t=96 control for GAM42a-, as well as a concomitant increase in the proportion of EUB338-positive cells ( $p = 0.005$ ,  $T_4 = 13.51$ ; and  $p = 0.007$ ,  $T_4 = 12.21$ ). For temperature-shifted treatment, there was a significant increase in community proportion with respect to the MSW t=96 control for ALP968-positive cells ( $p = 0.019$ ,  $T_4 = 7.08$ ). For the combined DOM-enriched, temperature-shifted treatment, there was a significant decrease in community proportion with respect to the MSW t=96 control for GAM42a- as well as an increase in the proportion of EUB338-positive cells ( $p = 0.016$ ,  $T_4 = 7.69$ ; and  $p = 0.013$ ,  $T_4 = 8.77$ ).



**Figure 3.13:** A comparison of growth rates for the whole community and  $\alpha$ -proteobacteria in ARCT province temperature-shift experiments. Whole community growth rates were calculated from the change in biomass from *in situ* over the 96-hour incubations.  $\alpha$ -proteobacteria growth rates were estimated from the hybridization ratios of the ALF968 probe at  $t=0$  and  $t=96$  with the change in bacterial abundance determined by Acridine Orange direct counts.

Variation in  $\alpha$ -proteobacteria growth rates was significant in explaining variation in whole community growth rates between stations in the ARCT province ( $p < 0.001$ ,  $F_{1,8} = 544.29$ ).



**Figure 3.14: A comparison of growth rates for the whole community and  $\alpha$ -proteobacteria in ARCT province DOM-enrichment experiments.** Whole community growth rates were calculated from the change in biomass from *in situ* over the 96-hour incubations.  $\alpha$ -proteobacteria growth rates were estimated from the hybridization ratios of the ALP968 probe at  $t=0$  and  $t=96$  with the change in bacterial abundance determined by Acridine Orange direct counts.

Variation in  $\alpha$ -proteobacteria growth rates was significant in explaining variation in whole community growth rates between stations in the ARCT province ( $p = 0.026$ ,  $F_{1,3} = 9.87$ ).

### 3.5 Discussion

Measurements of *in situ* bacterial production, as well as microbial cell size and abundance depict significant spatial variation in microbial community function. Biogeochemical province designation explained variation between stations in biological systems, however inspection of community structures showed significant spatial variation of community structures both between and within biogeochemical provinces. These results suggest a multitude of possible community structures are able to perform similar ecological functions under current marine physiochemical conditions.

The measured responses of microbial communities via changes in cell size and abundance at the end of incubation experiments showed significant spatial variation in the impact on bacterial community function by grazing, temperature, and available DOM. The impact of these environmental forcings on the microbial community structures was also measured. This analysis showed no significant trends between BGCPs with respect to functional or structural responses to predicted environmental changes. If these results are representative of larger areas have important implications for future biogeochemical cycling in the Labrador Sea and indicate current compartmentalization of oceanic regions may be inadequate for understanding ecosystem change in a warming ocean.

Looking further into the responses of microbial communities within a specific oceanic region, there was no significant observed pattern of community structure changes in response to tested environmental forcings within the ARCT biogeochemical province.

Given the strong physiochemical similarities of the stations sampled in the ARCT province, these results indicate a deterministic element where the community structures on which environmental forcings act affect the resulting post-perturbation phylogenetic composition of microbial communities. Analysis of population-specific growth rates did show significant variation of ALF968-positive cells relative to total community biomass production for some temperature-shifted and DOM-enriched experiments. Two scenarios can explain this result: 1) if correlative, the  $\alpha$ -proteobacteria detected by ALF968 are metabolically representative of the total microbial community in these systems and their growth was observed to keep pace with community averages; or 2) if causative,  $\alpha$ -proteobacteria detected by ALF968 show the most active initial response to these experimental perturbations in environmental conditions. Recent studies (Mou *et al.* 2008; Newton *et al.* 2010) have identified  $\alpha$ -proteobacteria of the *Roseobacter* lineage as successful ecological generalists. The greater niche breadth of these microbes may explain a more active response of ALF968-positive cells to these incubation conditions.

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### **3.6 Conclusion**

Six locations from three biogeochemical provinces in the Labrador Sea were represented in this study. Climate-relevant physiochemical conditions of temperature and available DOM were manipulated for incubation experiments, during which significant geographic variation in microbial community structural and functional responses was observed. These stochastic changes in environmental forcings produced no clear spatial patterns in community responses, however ribosome-based population analyses point to community

composition as an important regulator of biomass dynamics. Chapter 4 investigates how microbial communities respond to temporal (seasonal) variation in the physicochemical environment.

Chapter 4: Temporal variation of microbial communities of Logy Bay, NL in their structural and functional responses to environmental change

**4.1 Abstract:**

Temperature-shift, dissolved organic matter (DOM)-enrichment, and grazing experiments were conducted with microbial assemblages collected at four seasonally distinct points from Logy Bay, Newfoundland (northwest Atlantic) to investigate temporal variation in the communities' functional and structural responses to environmental forcings. Cellular abundance and morphometrics were determined from time series samples by epifluorescence microscopy and image analysis to examine spatial variation of microbe-mediated carbon-flow with respect to climate change-relevant environmental factors. Relative abundances of Archaea as well as the  $\alpha$ - and  $\gamma$ -proteobacteria were determined for *in situ* and experimental microbial communities by standard 16S rRNA-targeted FISH. This study reports a seasonal cycle of community structures dominated by unidentified Eubacteria with relatively static  $\alpha$ - and  $\gamma$ - proteobacteria populations that increase as an overall proportion of the community only during the seasonal temperature maximum. Cell size and growth showed seasonally variable responses to consistent environmental forcings during grazer-excluded incubations, responses that point to the importance of inorganic nutrient limitation in a warming ocean. Comparison of the importance of grazing control on microbial communities at current and warmer predicted temperatures suggests ecosystem-scale impacts of a warming ocean on spring phytoplankton bloom initiation and DOM concentrations in the upper ocean.

#### 4.2 Introduction

Thesis: Marine bacteria are important biological components of the biogeochemical cycling of carbon through the World Ocean that are subject to temporally-variable bottom-up and top-down controls. Seasonal variation in the nutritional status, temperature-limitation, and phylogenetic composition of microbial communities will force these communities to exhibit temporally-variable structural and functional changes in response to consistent environmental forcings such as increased water temperature and availability of organic matter as predicted for the North Atlantic in a warming ocean.

Both physical and biological processes determine the function of the World Ocean in the carbon cycle and as a global sink of CO<sub>2</sub>. Taken together, these biogeochemical processes enable important two-way coupling between marine carbon cycling and global climate. While marine carbon cycle processes such as the sequestration of CO<sub>2</sub> have been shown to respond to short-term climatic anomalies, the long-term effects of a warming World Ocean on biological feedback mechanisms are unknown (Solomon 2007). Will a warmer World Ocean still function to mitigate CO<sub>2</sub>-induced climate change?

Marine heterotrophic bacteria constitute an abundant and active planktonic group whose study is of paramount importance in determining the future biological uptake and release of CO<sub>2</sub> by the World Ocean. These heterotrophic bacteria consume and degrade dissolved organic matter (DOM)—an important pool of marine carbon—influencing on a global scale the export and sequestration of biogenic carbon in the marine environment (Azam 1998; Williams 2000; Pearce *et al.* 2007).

The activity and composition of marine microbial communities is subject to bottom-up (i.e. temperature and nutrient supply), as well as top-down (i.e. grazing) control (Shiah & Ducklow 1994; Suzuki 1999; Cottrell & Kirchman 2000a). These biological and physical regulatory factors vary across temporal scales, and thus their influence may have varying importance at different periods throughout the annual cycle. In regions such as the Northwest Atlantic Shelves biogeochemical province that exhibit strong seasonal variation in physical parameters, significant succession in microbial communities is observed (Pernthaler *et al.* 1998; Pinhassi & Hagstrom 2000; Keats *et al.* 2005). Neither the functional consequences of this structural shift nor the repercussions of new carbon and temperature regimes in a warming ocean are fully understood.

The study site in Logy Bay was chosen as a coastal proxy for the Northwest Atlantic Shelves biogeochemical province and was therefore expected to show a seasonal succession of microbial communities. However, the ecological functions of these communities and whether or not they would exhibit seasonally variable, climate-relevant responses to consistent environmental forcings of increased temperature and available DOM was unknown.

Chapter 3 examined spatial variation in the structural and functional responses of microbial communities. This study investigated seasonal variation in the same responses of microbial communities from Logy Bay to IPCC-predicted climate change-based incubation manipulations. Experiments were performed during the seasonal temperature

maximum and minimum (August and February respectively), as well as mid-warming (May) and mid-cooling (November) periods during the annual cycle. Variation of bacterial abundance and cell morphometrics were used to track changes in microbial carbon use, while standard 16S rRNA fluorescent in situ hybridization (FISH) was employed to detect changes in the phylogenetic composition in the microbial communities. Incubation experiments were performed with grazer-reduced seawater cultures (MSW) designed to reveal the intrinsic microbial response to treatments independent of grazer control. Bottom-up control on microbial communities was quantified with paired whole seawater (RAW) and grazer-reduced (MSW) treatments. Neither viral loads nor rates of viral lysis were quantified, however incubation experiments did not appear to show a significant viral impact on the results presented here.

### **4.3 Materials and Methods**

#### **4.3.0 Protocols and chemical recipes**

Additional protocol details and complete chemical recipes are included in Appendix 1.

#### **4.3.1 Seawater sampling**

Seawater was collected at four seasonally distinct time points from Logy Bay, NL at 5 m depth. This water was stored for less than 2 h in opaque 25 L sterile carboys at the Ocean Sciences Centre before processing.

#### **4.3.2 Incubation treatments**

6 L seawater was collected into an acid-rinsed carboy. 4 L seawater was filtered through 200  $\mu\text{m}$  screen and Gelman capsule (0.2  $\mu\text{m}$ ) added to 1 L of 1  $\mu\text{m}$ -filtered SW to

produce 5 L modified seawater (MSW). 500 mL of batch MSW was added to 3 x 500 mL incubation bottles kept at ambient temperatures for 96 hours as control treatments. 3 x 500 mL incubation bottles of MSW were kept at *in situ* +2.5°C for 96 hours for temperature-shift treatments. 10 mL of 500 µM Glu solution was added to 3 x 500 mL incubation bottles of MSW and kept at ambient temperatures for 96 hours for DOM-enrichment treatments.

3 L 1.0µm-filtered seawater was collected into acid-rinsed carboy to produce 3 L of RAW seawater. 3 x 500 mL incubation bottles of RAW were kept at ambient temperatures for 96 hours with an additional 3 x 500 mL incubation bottles of RAW kept at +2.5°C for 96 hours for temperature controls and treatment for grazing experiments.

Marine viruses are known to influence microbial abundance and community structure. Incubation treatments were performed in triplicate to control for viral impacts between replicates. This work did not measure if viral loss processes varied between treatments or sampling points.

#### **4.3.3 Bacterial growth and biovolume**

10 mL drawn directly from MSW (RAW for grazing experiments) reservoir (t=0), and from individual incubation bottles (t=24, 48, 72, 96) was preserved in glutaraldehyde 2% final concentration [vol/vol]. Cells filtered onto Irgan-black-stained 0.2 µm filters, post-stained with Acridine Orange and stored on slides at -20°C (Hobbie *et al.* 1977).

AODC samples were counted and cell size was measured by epifluorescent microscopy. Bacterial cells in ~20 randomized fields of view were counted and photographed (image capture from video). Image Analysis was performed on 500-1500 cells per sample. Estimates of bacterial biomass were derived from these biovolume measurements multiplied by a carbon conversion factor (i.e.  $C = 120 \cdot V^{0.72}$ ; Norland 1993).

Cyanobacteria, impossible to distinguish from heterotrophic bacteria when using Acridine Orange direct counts, are thought to have presented a relatively small and consistent overestimation of heterotrophic cell abundances in *in situ* samples. Experimental incubations performed in the dark sought to minimize their impact on community dynamics.

#### **4.3.4 Fluorescent In Situ Hybridization:**

50 mL samples were fixed with 37% formalin for 1-2 hours at 4°C, filtered through 47mm 0.2 µm isopore polycarbonate membrane filters (Millipore GTTP04700) mounted on cellulose prefilters (Millipore AP1504700), and washed with sterile phosphate buffered saline solution (PBS) for t=0 (batch) and t=96 (each incubation bottle). Filters were left to air dry for 1 hour and stored in sterile petri dishes sealed with parafilm at -20 °C. Filters were cut into pieces and stained with Cy3-labelled oligonucleotide probes (Mobix) as per Glöckner *et al.* 1999. Following hybridization, filters were washed and counterstained with 4',6-diamidino-2-phenylindole (DAPI). Filters were then tried and

mounted on glass slides with Citifluor oil and inspected at 1000X magnification with specific light filter sets for DAPI and Cy3 fluorophores. Microscopic fields were first viewed with the Cy3 filters before switching to the DAPI filters, to minimize photo-bleaching of the Cy3 fluorophore.

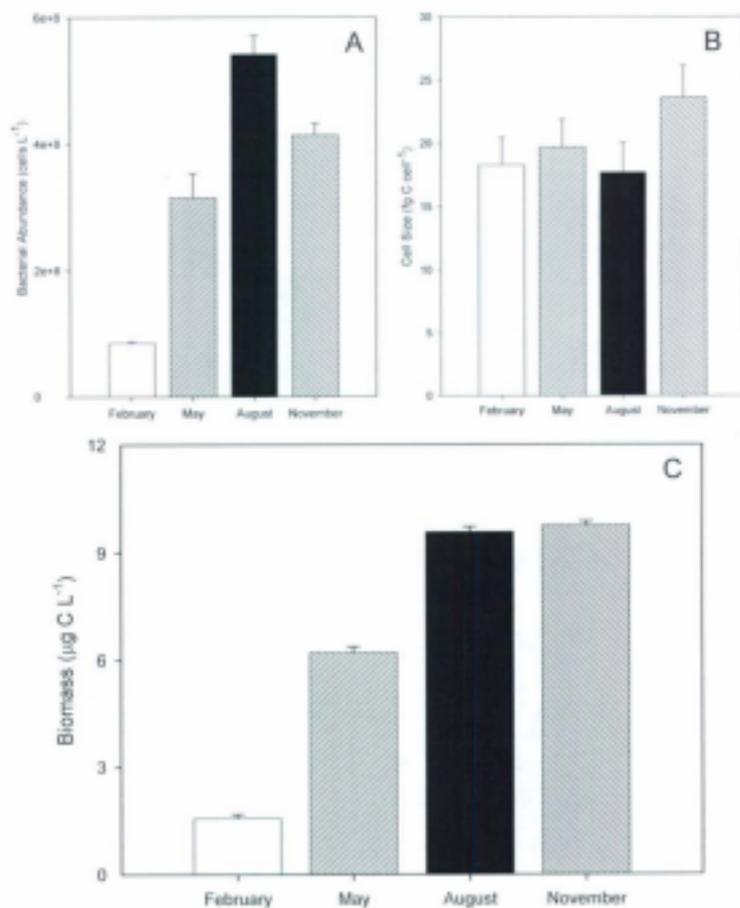
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## 4.4 Results

### 4.4.1 *In situ* community structure and function

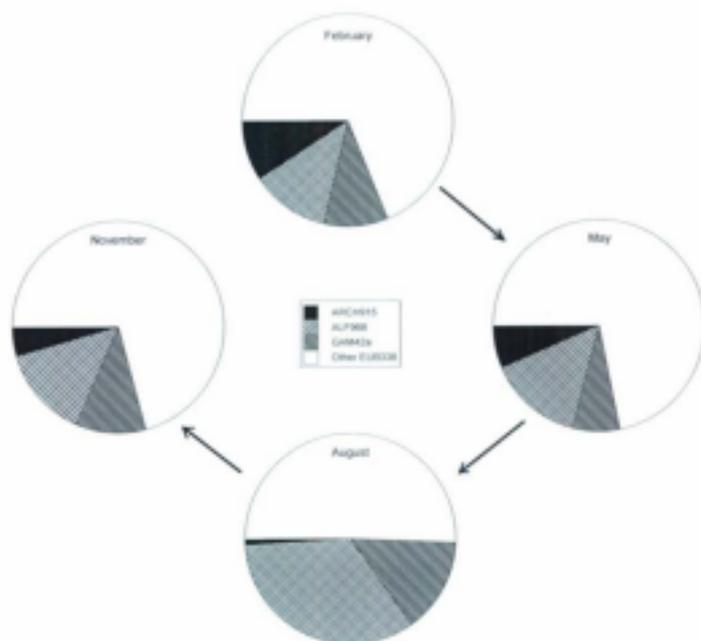
The Logy Bay time series consisted of four sampling periods: the seasonal temperature minimum and maximum (February and August), as well as warming and cooling transition times (May and November). Sea surface temperatures ranged from -1.0 to 9.7°C. Bacterial abundance ranged under one order of magnitude from  $8.5 \times 10^7$  to  $5.4 \times 10^8$  cells/L. Microbial biomass was least in February at 1.6 µg C/L and greatest in November at 9.8 µg C/L. Significant seasonal variation in bacterial abundance, cell carbon and biomass for Logy Bay is illustrated in Figure 4.1. This temporal variation in microbial standing stock is consistent with annual convective turnover patterns observed elsewhere (Morris *et al.* 2005).

*In situ* community structures shown in Figure 4.2 were relatively homogeneous during the sampling period, changing significantly in August with an increase in ALF968-positive cells at the expense of ARCH915 abundance before returning to the annual standard again in November.



**Figure 4.1:** A comparison of heterotrophic bacterial abundance (A), cell carbon (B), and biomass (C) from Modified Seawater (MSW) samples prepared after *in situ* collection for the Logy Bay time series. Bacterial abundance was determined from Acridine Orange direct counts and ranged from  $8.5 \times 10^7$  to  $5.4 \times 10^8$ . Cell carbon was estimated from cell volumes determined by Image Analysis. Biomass was calculated as the product of bacterial abundance and average cell carbon. Error bars delimit the upper symmetrical 95% confidence limit of: (A) counts among replicate fixed samples ( $n=3$ ), (B) measurements of individual cells ( $n > 1000$ ), and (C) the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.

Image analysis of microbial cells from *in situ* samples demonstrated significant differences between sampling months with respect to: bacterial abundance ( $p < 0.001$ ,  $F_{3,31} = 215.07$ ), cell carbon ( $p < 0.001$ ,  $F_{3,31} = 26.71$ ), and biomass ( $p < 0.001$ ,  $F_{3,31} = 73.49$ ).



**Figure 4.2:** Community structures from each Logy Bay sampling period. Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARCH915, ALF968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALF968- nor GAM42a-positive.

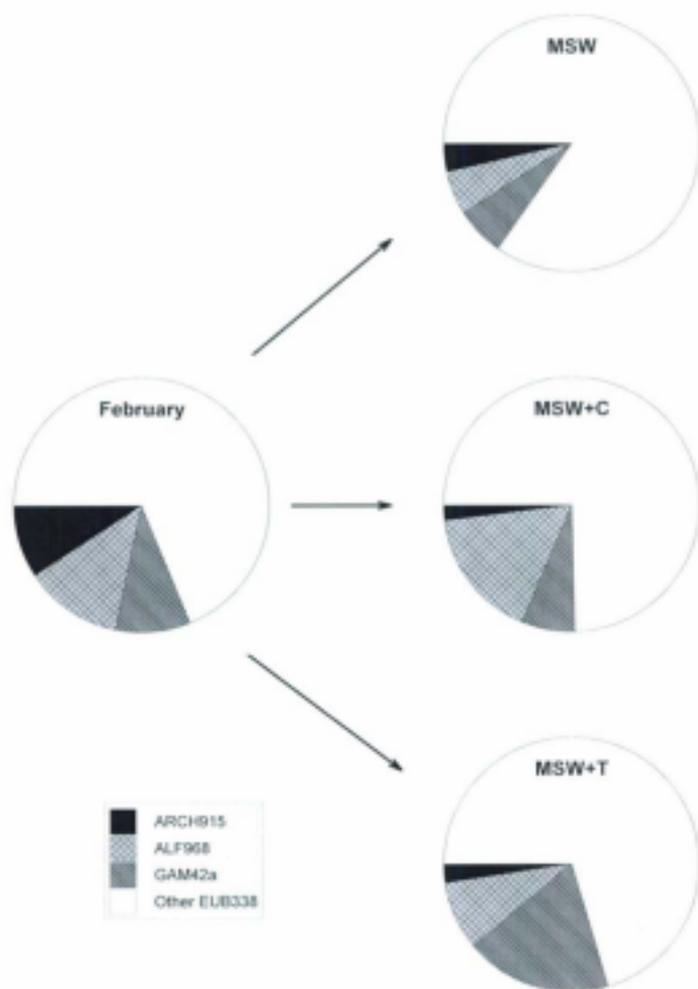
The community composition of samples taken from Logy Bay was not significantly different for the February, May, and November sampling periods. The August community contained a significantly smaller proportion of ARCH915-positive cells ( $p = 0.020$ ,  $F_{3,11} = 5.92$ ) and a significantly greater proportion of ALF9168-positive cells ( $p = 0.001$ ,  $F_{3,11} = 14.81$ ) compared to the other sampling periods. Although it appears that the August community consisted of fewer unidentified EUB338 cells, this result was not statistically significant.

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#### 4.4.2 Grazer-excluded community dynamics

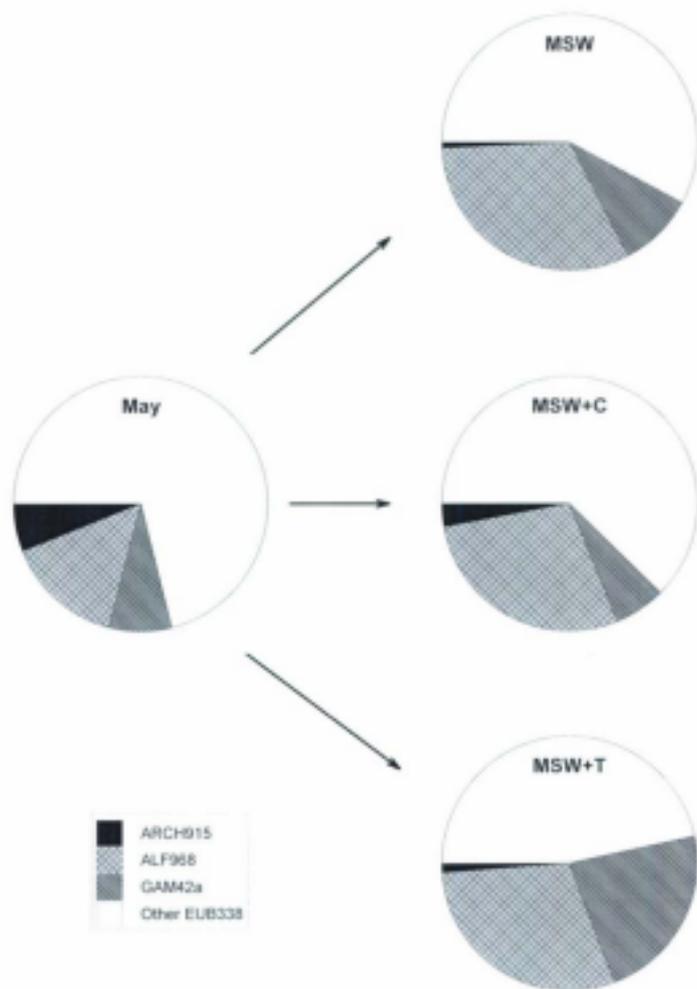
FISH analysis of community structure changes during modified seawater manipulation experiments for the Logy Bay time series are presented in Figures 4.3 to 4.6. These simple grazer-excluded MSW experiments showed increases in ALP968-positive cells for three of the four sampling periods, with a decrease during February experiments relative to *in situ* controls. DOM-enriched (MSW+C) experiments showed increases of ALP968-positive cells at the end of the incubation period relative to time final controls for three of the four sampling periods, with no response during the May experiments. Temperature-shift (MSW+T) experiments showed increases of GAM42a-positive cells relative to time final controls for three of the four sampling periods, with no response during the February experiments.

Variation in achieved bacterial abundance, cell carbon, and biomass at the end of incubation periods are depicted for DOM-enrichment and temperature-shift experiments in Figures 4.7 and 4.8 respectively. Both bacterial abundance and cell carbon functional responses showed significant seasonal variation to consistent environmental forcings. Figure 4.9 illustrates changes in microbial biomass in response to increased temperature or DOM during the Logy Bay time series. This figure shows that only during the August sampling period did microbes experience simple temperature- or DOM-limitation of biomass production free of grazing losses.



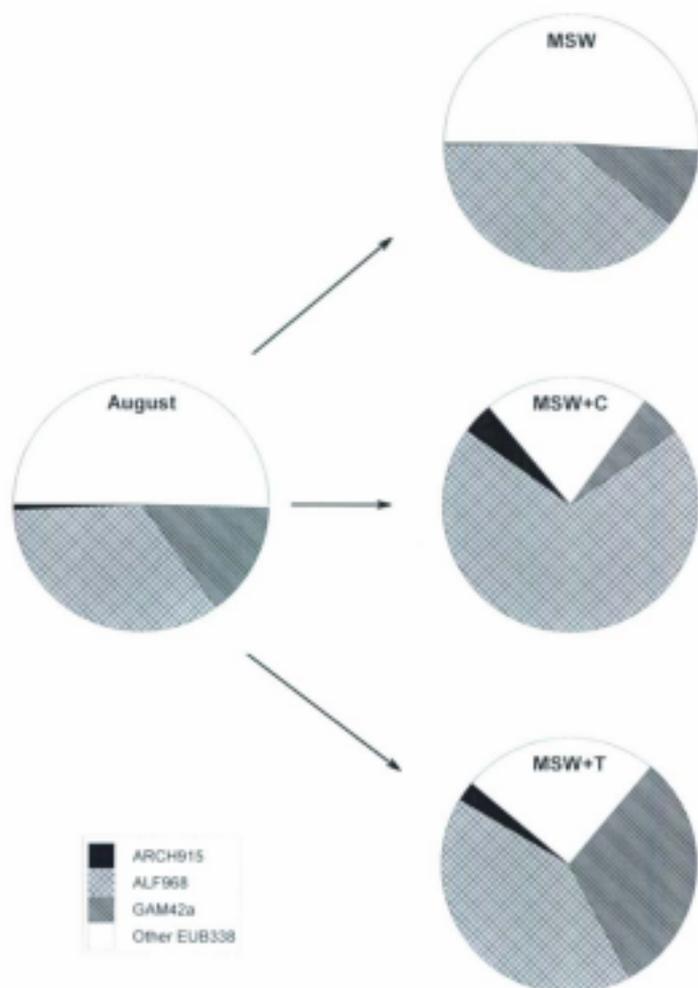
**Figure 4.3:** Community structure changes in response to February incubation treatments. Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARCH915, ALF968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALF968- nor GAM42a-positive. The circle on the left shows the initial MSW batch community, and the circles to the right show the communities after 96 hours at the specified treatment conditions.

For the MSW treatment, there were significant differences in community proportion between  $t=0$  and  $t=96$  for ALF968-, as well as the remaining proportion of EUB338-positive cells ( $p = 0.002$ ,  $T_4 = 24.79$ ; and  $p = 0.013$ ,  $T_4 = -8.55$ ). For the DOM-enriched treatment, there were significant differences in community proportion with respect to the MSW  $t=96$  control for ALF968-positive cells ( $p = 0.007$ ,  $T_4 = -11.66$ ). For temperature-shifted treatment, there were no significant differences in community proportion with respect to MSW  $t=96$  controls for any probe-positive cells.



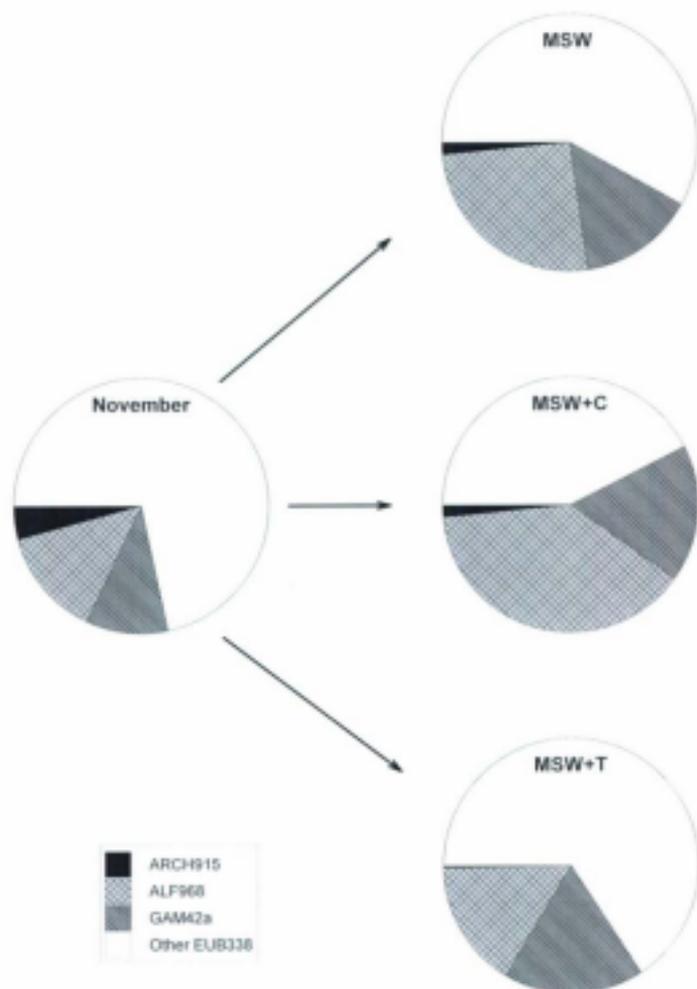
**Figure 4.4:** Community structure changes in response to May incubation treatments. Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARCH915, ALF968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALF968- nor GAM42a-positive. The circle on the left shows the initial MSW batch community, and the circles to the right show the communities after 96 hours at the specified treatment conditions.

For the MSW treatment, there were significant differences in community proportion between  $t=0$  and  $t=96$  for ALF968-, as well as the remaining proportion of EUB338-positive cells ( $p = 0.006$ ,  $T_4 = -13.42$ ; and  $p = 0.001$ ,  $T_4 = 27.39$ , respectively). For the DOM-enriched treatment, there were no significant differences in community proportion with respect to the MSW  $t=96$  control for any probe-positive cells. For temperature-shifted treatment, there were significant differences in community proportion with respect to the MSW  $t=96$  control for GAM42a, as well as the remaining proportion of EUB338-positive cells ( $p = 0.005$ ,  $T_4 = -13.68$ ; and  $p = 0.032$ ,  $T_4 = 5.43$  respectively).



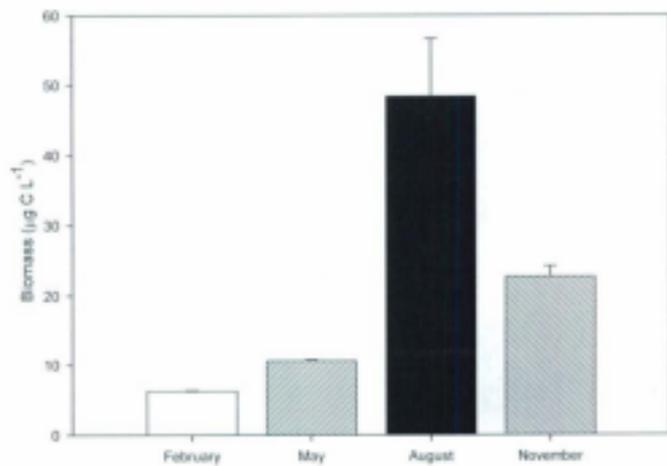
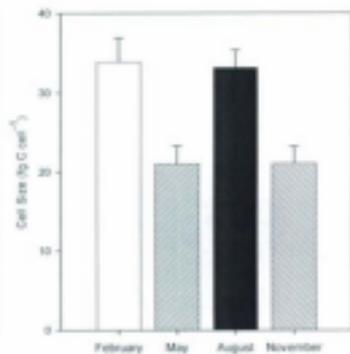
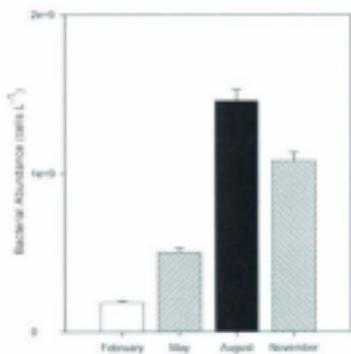
**Figure 4.5:** Community structure changes in response to August incubation treatments. Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARCH915, ALF968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALF968- nor GAM42a-positive. The circle on the left shows the initial MSW batch community, and the circles to the right show the communities after 96 hours at the specified treatment conditions.

For the MSW treatment, there were significant differences in community proportion between  $t=0$  and  $t=96$  for ALF968-, and GAM42a-positive cells ( $p = 0.042$ ,  $T_4 = -4.71$ ; and  $p = 0.045$ ,  $T_4 = 4.53$ , respectively). For the DOM-enriched treatment, there were significant differences in community proportion with respect to the MSW  $t=96$  control for ALF968-, as well as the remaining proportion of EUB338-positive cells ( $p = 0.011$ ,  $T_4 = -9.37$ ; and  $p = 0.009$ ,  $T_4 = 10.39$  respectively). For temperature-shifted treatment, there were significant differences in community proportion with respect to the MSW  $t=96$  control for ALF968-, and GAM42a, as well as the remaining proportion of EUB338-positive cells ( $p = 0.047$ ,  $T_4 = -4.46$ ;  $p < 0.001$ ,  $T_4 = -84.30$ ; and  $p < 0.001$ ,  $T_4 = 80.00$  respectively).



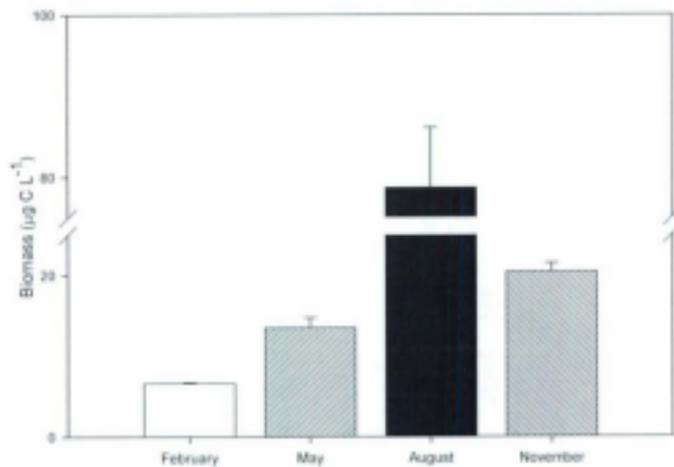
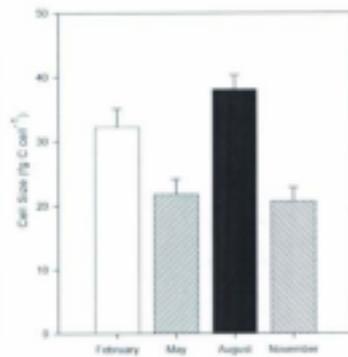
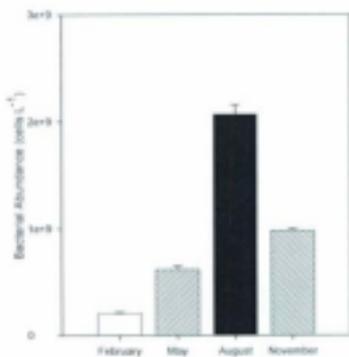
**Figure 4.6:** Community structure changes in response to November incubation treatments. Each circle represents the sum of the Archaeal and Eubacterial probe sets' Hybridization Efficiencies; the ARCH1915, ALF968, and GAM42a wedges are the proportions relative to this sum. The remainder is the proportion of EUB338-positive cells that are neither ALF968- nor GAM42a-positive. The circle on the left shows the initial MSW batch community, and the circles to the right show the communities after 96 hours at the specified treatment conditions.

For the MSW treatment, there were significant differences in community proportion between  $t=0$  and  $t=96$  for ALF968-, and GAM42a-, as well as the remaining proportion of EUB338-positive cells ( $p = 0.003$ ,  $T_4 = -18.47$ ;  $p = 0.003$ ,  $T_4 = -19.05$ ; and  $p < 0.001$ ,  $T_4 = 51.41$  respectively). For the DOM-enriched treatment, there were significant differences in community proportion with respect to the MSW  $t=96$  control for ALF968-, and GAM42a-, as well as the remaining proportion of EUB338-positive cells ( $p = 0.007$ ,  $T_4 = -11.59$ ;  $p = 0.027$ ,  $T_4 = -6.02$ ; and  $p = 0.004$ ,  $T_4 = 16.24$  respectively). For temperature-shifted treatment, there were significant differences in community proportion with respect to the MSW  $t=96$  control for ALF968-, and GAM42a, as well as the remaining proportion of EUB338-positive cells ( $p = 0.007$ ,  $T_4 = 11.83$ ;  $p < 0.001$ ,  $T_4 = -90.70$ ; and  $p = 0.012$ ,  $T_4 = -8.92$  respectively).



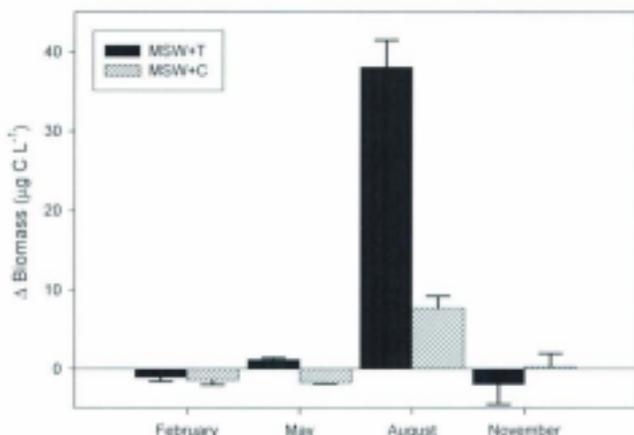
**Figure 4.7:** A comparison of heterotrophic bacterial abundance (A), cell carbon (B), and biomass (C) from DOM-enriched incubations during the Logy Bay time series. Bacterial abundance was determined from Acridine Orange direct counts. Cell carbon was estimated from cell volumes determined by Image Analysis. Biomass was calculated as the product of bacterial abundance and average cell carbon. Error bars delimit the upper symmetrical 95% confidence limit of: (A) counts among replicate fixed samples ( $n=3$ ), (B) measurements of individual cells ( $n > 1000$ ), and (C) the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.

Image analysis of microbial cells at the end of DOM-enrichment experiments demonstrated significant differences between sampling months with respect to: bacterial abundance ( $p < 0.001$ ,  $F_{3,11} = 575.01$ ), cell carbon ( $p < 0.001$ ,  $F_{3,11} = 93.75$ ), and biomass ( $p < 0.001$ ,  $F_{3,11} = 210.16$ ). Comparison to control incubations shows that the communities' response to increased DOM concentrations was significantly different between sampling months with respect to bacterial abundance, cell carbon, and biomass ( $p < 0.001$ ,  $F_{3,11} = 195.11$ ;  $p < 0.001$ ,  $F_{3,11} = 128.79$ ; and  $p < 0.001$ ,  $F_{3,11} = 49.23$ ). Excluding the August result, there was no significant difference in the communities' response to increased DOM availability via biomass production ( $p = 0.099$ ,  $F_{2,8} = 3.47$ ).



**Figure 4.8:** A comparison of heterotrophic bacterial abundance (A), cell carbon (B), and biomass (C) from temperature-shifted incubations during the Logy Bay time series. Bacterial abundance was determined from Acridine Orange direct counts. Cell carbon was estimated from cell volumes determined by Image Analysis. Biomass was calculated as the product of bacterial abundance and average cell carbon. Error bars delimit the upper symmetrical 95% confidence limit of: (A) counts among replicate fixed samples ( $n=3$ ), (B) measurements of individual cells ( $n > 1000$ ), and (C) the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.

Image analysis of microbial cells at the end temperature-shift experiments demonstrated significant differences between sampling months with respect to achieved: bacterial abundance ( $p < 0.001$ ,  $F_{3,11} = 997.54$ ), cell carbon ( $p < 0.001$ ,  $F_{3,11} = 136.45$ ), and biomass ( $p < 0.001$ ,  $F_{3,11} = 345.29$ ). Comparison to control incubations shows that the communities' response to increased temperature was significantly different between sampling months with respect to bacterial abundance, cell carbon, and biomass ( $p < 0.001$ ,  $F_{3,11} = 337.32$ ;  $p < 0.001$ ,  $F_{3,11} = 101.20$ ;  $p < 0.001$ ,  $F_{3,11} = 264.31$ ). Discounting the August result, there was no significant difference in the communities' biomass response to increased temperature free of grazing losses ( $p = 0.075$ ,  $F_{2,8} = 4.12$ ).



**Figure 4.9:** A comparison of biomass change during Logy Bay seasonal experiments. The dark and shaded bars represent the difference in biomass ( $\mu\text{g C/L}$ ) from MSW t-96 controls for Temperature-shifted (MSW+T) and DOM-enriched (MSW+C) incubations respectively. Biomass was calculated as the product of bacterial abundance and average cell carbon. Cell carbon was estimated from cell volumes determined by Image Analysis. Error bars delimit the upper or lower symmetrical 95% confidence limit of counts among replicate treatment bottles ( $n=3$ ).

Microbial community responses to either increased temperature or DOM-enrichment were not significantly different for the February, May, and November sampling periods ( $p = 0.075$ ,  $F_{2,8} = 4.12$ ;  $p = 0.099$ ,  $F_{2,8} = 3.47$  respectively). Both temperature and DOM

limitation of microbial biomass production were observed during the August sampling period, with a positive temperature shift of 2.5°C yielding approximately four times as much microbial biomass as from an addition of 10µM glutamate when compared to control conditions.

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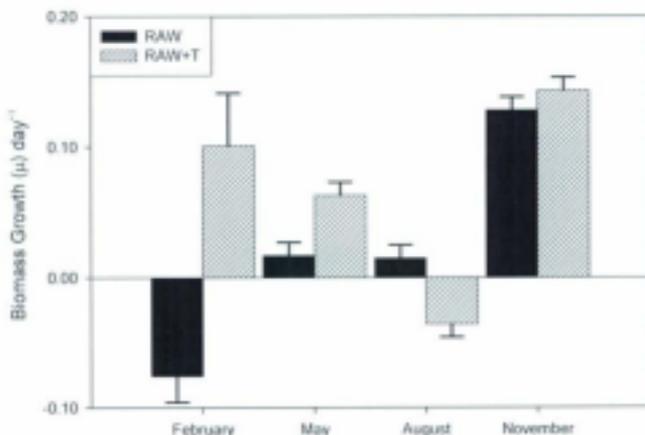
#### 4.4.3 Seasonal variation of grazing effects

Results of RAW incubation experiments conducted at *in situ* temperatures and at *in situ* temperatures +2.5°C for each sampling period presented in Figure 4.10 illustrate the seasonally variable effects of temperature on microbe-grazer interactions as they impact bacterial biomass production. Grazing rates shown in Figure 4.11 were calculated from whole water and grazer-excluded experiments conducted at *in situ* temperatures and *in situ* temperatures +2.5°C for each sampling period. Taken together, these results show a tight coupling of *in situ* microbial growth and grazing losses, however this relationship does not hold throughout the year for warmer predicted temperatures. An important result illustrated by these experiments is that with an increased ocean temperature, grazing rates are predicted to increase during the seasonal temperature maximum before decreasing during the cooling period. I hypothesize that this seasonally variable response will impact carbon flow and other sea surface processes mediated by heterotrophic bacterioplankton.

Image Analysis of microbial communities from the seasonal temperature minimum and maximum showed seasonally variable responses to grazing pressure. Changes in cell

carbon observed during grazing experiments were associated with differences in grazing rates and microbial biomass production at both *in situ* and increased temperatures. A positive temperature-shift of 2.5°C was observed to reverse the pattern of cell size responses between seasons, decoupling biomass production from grazing control during the seasonal temperature minimum while increasing grazing losses during the seasonal temperature maximum. If the results of these incubation experiments hold for the open ocean, climate warming in the Northwest Atlantic will have a significant impact on microbe-mediated nutrient cycles and may alter the initiation and maintenance of bloom events in the North Atlantic.

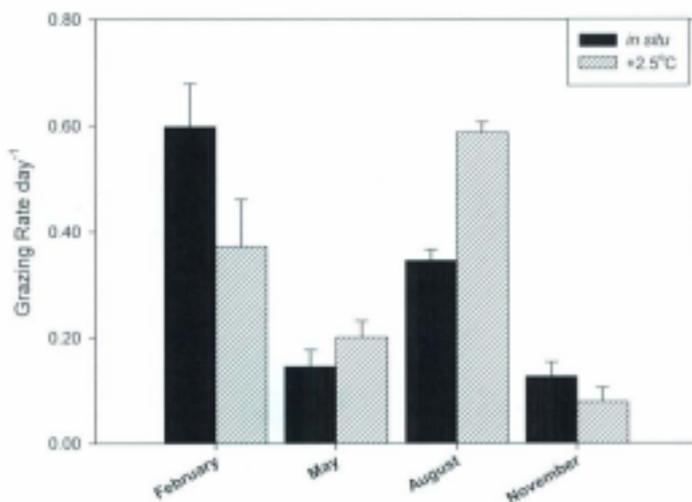
Figure 4.12 presents GAM42a population data for grazing experiments conducted at the temperature minimum and maximum. In February, whole community cellular abundance changed at a rate of  $0.03 \pm 0.08 \text{ day}^{-1}$  during RAW incubations while GAM42a-positive cells increased at a rate of  $0.61 \pm 0.38 \text{ day}^{-1}$ . In August, there was a whole community loss of cellular abundance at a rate of  $-0.02 \pm 0.02 \text{ day}^{-1}$  during RAW incubations while GAM42a-positive cells increased at a rate of  $0.57 \pm 0.22 \text{ day}^{-1}$ . These rates illustrate an increase in both the relative and absolute numbers of GAM42a-positive cells in heterotrophic bacterial communities experiencing grazing losses, a result that indicates both selective grazing (of GAM42a-negative cells) and consequent competitive advantage to the remaining GAM42a population over the 96 hour incubation period.



**Figure 4.10:** Biomass growth rates ( $\mu \text{ d}^{-1}$ ) for all grazing experiments conducted in Logy Bay. Biomass was calculated as the product of bacterial abundance from direct cell counts and average cell carbon as determined by Image Analysis. Experiments are coded by temperature regime: black bars for RAW experiments at *in situ* temperatures, shaded bars for RAW+T temperature-shifted experiments at 2.5°C above *in situ*. Error bars delimit the upper or lower symmetrical 95% confidence limit of the propagated uncorrelated uncertainty from the multiplication of two uncertain quantities.

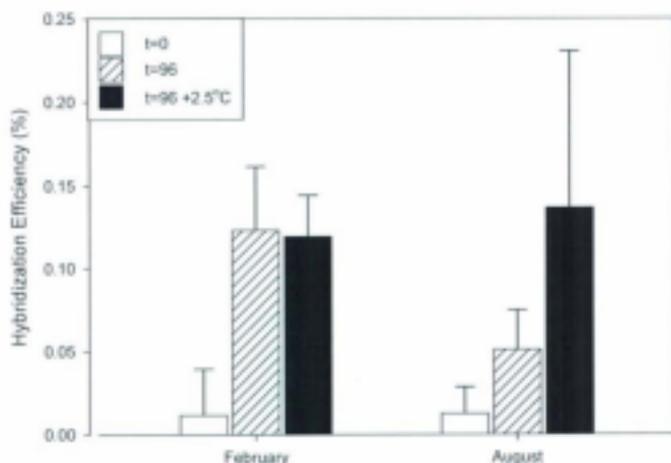
RAW biomass growth ( $\mu$ ) rates did vary significantly between sampled months ( $p < 0.001$ ,  $F_{3,11} = 61.74$ ), as did temperature-shifted RAW biomass growth rates ( $p < 0.001$ ,

$F_{3,31} = 27.19$ ). Biomass growth rates varied between sampling months for grazer-excluded experiments at both *in situ* and positively-shifted temperatures as well ( $p < 0.001$ ,  $F_{3,31} = 39.90$ ; and  $p < 0.001$ ,  $F_{3,31} = 41.77$  respectively. Data not shown). These data strongly suggest that temperature has a seasonally variable effect on both microbes and their protist grazers.



**Figure 4.11:** Mortality from grazing for all Logy Bay experiments. Grazing rates were estimated by calculating the difference between heterotrophic bacterial growth rates for whole (RAW) and grazer-excluded (MSW) treatments for both control and temperature-shifted seawater incubations. Growth rates were calculated for all treatments from the change in biomass over 96 hours. Biomass was calculated as the product of bacterial abundance and average cell carbon. Cell carbon was estimated from cell volumes determined by Image Analysis. Error bars represent the propagated uncorrelated uncertainty from the multiplication of these two uncertain quantities.

Grazing rates for both RAW and RAW+T treatments varied significantly between months ( $p < 0.001$ ,  $F_{3,11} = 67.70$ ; and  $p < 0.001$ ,  $F_{3,11} = 58.97$  respectively). Temperature-shifts did not make significant differences to grazing rates during February or May ( $p = 0.279$ ,  $T_4 = 1.47$ ; and  $p = 0.052$ ,  $T_4 = -2.74$  respectively) but did for both August and November experiments ( $p < 0.001$ ,  $T_4 = -21.07$ ; and  $p = 0.007$ ,  $T_4 = 5.14$  respectively). MSW experiments that removed grazers showed seasonally-variable increases in microbial cell carbon ( $p < 0.001$ ,  $F_{3,11} = 108.38$ ), with the smallest responses (percent increase) occurring during May and November when grazing rates were also observed to be at seasonal minimums.



**Figure 4.12:** GAM42a population response to RAW incubation treatments during February and August experiments from the Logy Bay time series. Each bar represents the proportion of GAM42a-positive cells relative to the sum of Archaeal and Eubacterial probe sets' Hybridization efficiencies for the given treatment. Error bars delimit the upper symmetrical 95% confidence limit of counts among replicate treatment bottles ( $n=3$ ).

Grazing resulted in significantly different community structures after incubation and between sampling periods. The proportion of GAM42a-positive cells at the end of RAW incubations was significantly different between the February and August experiments ( $p = 0.025$ ;  $T_4 = 6.29$ ). For both February and August grazing experiments, there was an increase in GAM42a-positive cells at the end of the incubation period. For February

grazing experiments, the proportion of GAM42a-positive cells was significantly greater at the end of both whole water incubations (RAW  $p = 0.005$ ,  $T_4 = -13.98$ ; RAW+T  $p < 0.001$ ,  $T_4 = -123.35$ ); there was no statistical difference between treatments. The proportion of GAM42a-positive cells did not change significantly during grazer-excluded incubations (see Figure 4.3), indicating that this community structure change is a response to grazing pressure. For August grazing experiments, the proportion of GAM42a-positive cells was significantly greater at the end of the RAW incubations ( $p = 0.042$ ,  $T_4 = 4.71$ ). The uncertainty surrounding the temperature-shifted result does not show significant differences from the  $t = 0$  control or the *in situ* temperature treatment. The proportion of GAM42a-positive cells decreased significantly during grazer-excluded incubations (see Figure 4.5), indicating that the increase of GAM42a-positive cells observed here is also a response to grazing pressure.

Image Analysis of the February and August experiments showed seasonally variable changes in cell size to both grazing pressure and temperature regime (*in situ*  $p = 0.003$ ,  $F_{1,5} = 38.40$ ;  $+2.5^\circ\text{C}$   $p < 0.001$ ,  $F_{1,5} = 228.63$ ). The February community lost  $6.1 \pm 0.2$  fg C/cell in average cell size during 96 hours of incubation at *in situ* temperature, while the August community gained on average  $3.5 \pm 0.2$  fg C/cell. This decrease in average cell size coincided with higher grazing losses and lower biomass production during February RAW experiments relative to those conducted in August. During the positive  $2.5^\circ\text{C}$  temperature-shift experiments the opposite pattern was observed with the February community gaining  $2.7 \pm 0.2$  fg C/cell in average cell size and the August community

losing  $-0.3 \pm 0.2$  fg C/cell. The changes in cell size observed for both temperature-shifted grazing experiments showed that the increase in temperature decoupled biomass production from grazing control during the seasonal temperature minimum while it increased grazing losses during the seasonal temperature maximum.

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#### 4.5 Discussion

The *in situ* measurements taken in Logy Bay show significant temporal variation in microbial community biomass and cellular morphometrics (Figure 4.1) and population structures (Figure 4.2). Comparison of relative probe-positive cellular abundances in Logy Bay during this time series shows not only that these communities change in phylogenetic composition over time, but also that there is some mechanism which returns population abundances to a relative norm after a period of change. These results further support the hypothesis raised in Chapter 3 that the microbes on which environmental forcings act does in part determine the resultant structures of post-perturbation communities. The relative importance of this structural control may be subject to environmental forcing thresholds, beyond which the balance between deterministic and stochastic processes is unknown.

The grazer-excluded incubation experiments confirm seasonally variable effects of temperature and DOM availability on cell size, cell growth, and population abundances (see Figures 4.7 and 4.8). FISH analyses showed that multiple microbial communities disparate in both structure and function can arise from comparable *in situ* communities

experiencing consistent environmental forcings. The results indicate that these microbial communities change via a succession of populations and that phenotypic plasticity has little if any effect on a community scale. Furthermore, communities with disparate structures were observed to possess similar functions in their abilities to produce biomass; however variation in other metabolic or ecological functions has not been ruled out. Taken together, the grazer-excluded treatment data show that communities of disparate structures—even under different DOM and temperature regimes—did not typically differ in their functional abilities to produce biomass (Figure 4.9). For all sampling periods, grazer-free DOM-enrichments and positive temperature-shifts were both observed to impact community structures, bacterial abundance, and cell carbon. As these changes were seldom accompanied by a net change in biomass, these structural and functional responses suggest a succession of populations during incubation, the ecological repercussions of which remain unknown. The seasonal variation in community structure responses to consistent environmental forcings is noteworthy given that, with the August exception, the *in situ* structure was not significantly different at this resolution of analysis. These variable responses indicate a community structure analysis with greater resolution would be needed to examine fine-scale effects of environmental forcings on seasonal population dynamics in Logy Bay.

Both community structure and function show seasonally variable responses to consistent environmental forcings observed with the grazing and temperature-shift experiments. Structurally, the increase in GAM42a populations during grazing mesocosm experiments for both temperature minimum and maximum periods shown in Figure 4.12 matches 7-

proteobacteria's reputation as an "opportunistic group" that can respond rapidly to newly available nutrients (Eilers *et al.* 2000; Yokokawa & Nagata 2005). However, Pernthaler and Amann (2005) suggest that size-selective grazing almost completely suppresses  $\gamma$ -proteobacteria abundance in coastal surface waters. Given this new result and that this group of bacterioplankton is also known to respond to temperature change, the response of  $\gamma$ -proteobacteria to environmental change deserves closer inspection to identify its important ecological traits.

With respect to community function, the results shown in Figures 4.10 and 4.11 raise important considerations concerning our understanding of microbe-mediated carbon fluxes in the upper ocean and how these processes and our climate predictions will change with a warming ocean. Bacterial activity in temperate and arctic regions is regulated in winter by a combination of low temperature and low DOM concentrations. With warming water and increased terrestrial input of organic material, I hypothesize that microbial prominence in the microbial loop will increase due to both a decreased significance of this bottom-up environmental control and diminished capacity of the top-down control by grazers. On a biogeochemical scale, this would mean an increased proportion of primary production being used to fuel secondary production by heterotrophic marine bacteria, with a concomitant shift towards increased ocean respiration and acidity. Viral control remain unaccounted for in this interpretation, and recent work (Longnecker *et al.* 2010) on both the presence and type of mortality processes indicates the trophic state of the ecosystem has a significant effect in

structuring microbial communities. Furthermore, these experiments were not designed to answer questions regarding transfer of microbial biomass to higher trophic levels. One important consideration for this community-scale physiological increase in microbial activity and ecosystem-scale shift away from the significance of grazing controls is the relative importance of inorganic nutrient limitation. With increased cell size and decreased grazing losses occurring in February, inorganic nutrient limitation may not only limit microbial activity but may also impact the initiation of the spring phytoplankton bloom. Additionally, increased grazing pressure in August will further decrease microbial biomass at this time of year and may result in increased DOM concentrations in the upper ocean.

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#### **4.6 Conclusion**

Microbes from a coastal northwest Atlantic site were sampled and incubated during a time series of manipulation experiments to study their structure and functional responses to grazers, increased available DOM, and increased temperature. Significant temporal variation in response to these consistent climate-relevant physiochemical conditions was observed. Variation of community properties and environmental regulatory factors did not fully explain differences in structural or functional responses observed during the time series, indicating the need for higher-resolution analyses to better understand the role and fate of bacterioplankton populations. The timing and magnitude of these variable functional responses may have ecosystem-scale implications with respect to DOM cycling in the upper ocean, and therefore biogeochemical cycling of carbon; this

consideration is especially important to the prediction of global impacts from a warming ocean.

## Appendix 1: Protocols and Methods

### A1.1: Seawater Collection

Seawater (SW) samples were obtained at 6 stations from two separate transects that included 3 Longhurstian biogeochemical provinces in the Labrador Sea from May 25 to June 7, 2006 (50.86–60.29° N 48.55–53.98° W, water temperature ranged 1.7 to 6.6°C). These samples were obtained from depths of <5 m by a Seabird CTD with a 24 bottle GO rosette aboard the CCGS Hudson and were stored for less than 2 h in opaque 25 l sterile carboys below deck before processing. Seawater chlorophyll a and salinity were measured by CTD-mounted Deep fluorometer and Autosal salinometer respectively. Other members of the LSE measured nitrate, phosphate and silicate concentrations. Seawater collection also occurred in February, May, August, and November by Niskin bottles from a zodiac in Logy Bay.

**Table A1-1:** A summary of ambient and incubated seawater temperatures from experiments conducted in the Labrador Sea (May 26-June 7, 2006)

Experiment Station	Location	Ambient Temperature	Incubation Temperature (range)	
			Control	Positive shift
2: L3-01	53.683 N, 55.557 W	1.7°C	2.7 ± 0.2°C	7.1 ± 1.2°C
3: L3-14	56.537 N, 52.679 W	4.7°C	4.2 ± 0.9°C	8.5 ± 0.0°C
4: L3-25	60.291 N, 48.549 W	2.5°C	4.0 ± 1.0°C	9.8 ± 1.3°C
5: L3-24	60.179 N, 48.677 W	4.4°C	4.2 ± 1.2°C	9.8 ± 1.3°C
6: L4-10	53.456 N, 49.503 W	6.6°C	5.5 ± 1.5°C	9.5 ± 1.5°C
7: L4-05	52.732 N, 51.999 W	3.3°C	5.0 ± 1.0°C	9.5 ± 1.5°C

**Table A1-2:** Physical distance (nautical miles<sup>a</sup>) between stations sampled in the Labrador Sea (May 26-June 7, 2006)

	L3-01	L3-14	L3-25	L3-24	L4-10	L4-05
L3-01	—	198.00	458.14	450.43	245.87	140.24
L3-14	198.00	—	200.29	252.57	215.07	229.94
L3-25	458.14	200.29	11.00 <sup>b</sup>	7.73	412.00	468.37
L3-24	450.43	252.57	7.73	—	404.99	460.87
L4-10	245.87	215.07	412.00	404.99	—	100.04
L4-05	140.24	229.94	468.37	460.87	100.04	—

- Assumes the earth is a perfect sphere with radius of 3443.9 nautical miles
- Distance between Station 4 (L3-25 BIO) where water for incubations experiments was collected and L3-27 where <sup>3</sup>H Leucine assays were performed

### **A1.2: Biomass and Abundance:**

#### Flow Cytometry:

3 x 1.8 mL samples collected from MSW reservoir (t=0) into 2 mL cryovials. Additional 1 x 1.8 mL samples collected from each incubation bottle at t=24 and t=96 for only DOM-enrichment and control treatments during the Labrador Sea Expedition. 0.2 mL of 10% paraformaldehyde added to each cryovial, vortexed and left to stand 10-45 min before flash freezing in liquid nitrogen. FCM samples were stored at -20°C until delivery to FCM counter at BIO.

### **A1.3: Reagents and Chemical Recipes:**

#### Rinsing Agents:

90% Acetone -

3600 mL Acetone; 400 mL dH<sub>2</sub>O

5% HCl -

1.4 L 37% HCl; 8.6 L dH<sub>2</sub>O

#### Stains:

Acridine Orange -

0.0117g AO; 25 mL dH<sub>2</sub>O; keep refrigerated

#### Fluorescent *in situ* Hybridization:

Phosphate Buffered Saline 10X -

75.972 g/L NaCl (0.13M); 7.098 g/L Na<sub>2</sub>HPO<sub>4</sub> (0.005M); 2.041 g/L KH<sub>2</sub>PO<sub>4</sub> (0.0015M);  
autoclave; to pH 7.2

Hybridization Buffer -

360 µL NaCl 5M; 40 µL Tris-HCl 1M pH 8; 700 µL formamide or 400 µL formamide  
(for ALF968 only); to 2 mL with 0.2 µm filtered Milli Q water; 2 µL 10% sodium  
dodecyl sulphate

Washing Buffer -

1 mL Tris-HCl 1M pH 8; 500 µL EDTA; 700 µL NaCl 5M or 2150 µL NaCl (for  
ALF968 only); to 50 mL with 0.2 µm filtered Milli Q water; 50 µL 10% sodium dodecyl  
sulphate

TE Buffer -

10 mM Tris-HCl pH 7.5; 1 mM EDTA pH 8.0

Probe Resuspension -

Probes were resuspended in TE Buffer as 5 g/L stocks; working probe solutions were  
diluted to 0.05 g/L.

**Table A1-3: FISH probe resuspension volumes**

Probe	Eub338	Non338	ALF968	GAM42a	ARCH915
MW	5998.2	6016.2	6068.2	5578.9	6471.5
nmol	16.4	10.1	17.9	14.8	22.8
umol	0.0164	0.0101	0.0179	0.0148	0.0228
mg	0.098370	0.060764	0.108621	0.082568	0.147550
M	8.3358E-06	8.3108E-06	8.2397E-06	8.9623E-06	7.7262E-06
uM	8.335834	8.31089	8.239676	8.962340	7.726184
10X uM	83.35834	83.1089	82.39676	89.62340	77.26184
TE					
Volume					
(mL)	0.196741	0.121527	0.217242	0.165135	0.29510

**A1.4: Statistical Treatment and Modeling**

All statistical analyses were performed using Minitab Release 14. Residuals were checked for assumptions of normality, independence, and homogeneity (Seber 1977) and found to hold for all reported tests.

Error Propagation:

**Table A1-4:** Error Propagation formulae

Type of Calculation	Form	Standard Deviation of $x$
Addition or Subtraction	$x = p + q - r$	$s_x = \sqrt{s_p^2 + s_q^2 + s_r^2}$
Multiplication or Division	$x = \frac{p \cdot q}{r}$	$s_x = x \sqrt{\left(\frac{s_p}{p}\right)^2 + \left(\frac{s_q}{q}\right)^2 + \left(\frac{s_r}{r}\right)^2}$

The calculations presented in this study often resulted in the propagation of uncertainty. Presented here is an example direct from Chapter 4 of the thesis: After Figure 4.11, cell carbon increase between  $t = 0$  and  $t = 96$  for grazer-excluded (MSW) experiments was calculated as a percentage change after 96 hours of incubation. Cell size had previously been determined using volumetric Image Analysis upon tens of thousands of individual cells. Cell carbon was calculated from cell size by a conversion equation (see thesis text). Cell carbon data (titled "Cell size") is presented in columns J & K in the Summary tab of the Data Master spreadsheet that accompanies this document. Data is presented there as the mean value  $\pm$  the 95% confidence limit (confidence limits for a mean: Snedecor, George W. and Cochran, William G. (1989), *Statistical Methods*, Eighth Edition, Iowa State University Press). Both initial and final cell carbon values are uncertain quantities, so the propagated uncertainty was calculated as per the formula found in Table A1-4, results shown in Table A1-5.

**Table A1-5:** Sample calculations of cell carbon increase (percent) and SD

		Mean	SD	$\bar{x}$	$S_x$
	t = 0	18.314	0.538708352		
February	t = 96	34.496	1.122623017	1.883537364	0.082625735
	t = 0	19.724	0.92271196		
May	t = 96	26.314	0.936390619	1.334099335	0.07841501
	t = 0	17.651	0.642743144		
August	t = 96	40.414	1.698401259	2.289537158	0.127312709
	t = 0	23.560	0.934033856		
November	t = 96	29.140	1.334573959	1.236859392	0.074922649

## Appendix 2: Raw Data and Calculations

The content of this appendix is found in the DATA\_Master.xls spreadsheet that accompanies this document. This material is presented to facilitate any additional analysis of the data collected during the Labrador Sea Expedition and Logy Bay studies.

The spreadsheet consists of 16 tabs as follows:

### Summary

All measures used in the writing of the thesis for both the Labrador Sea and Logy Bay experiments. Each column depicts a different measure, with the mean  $\pm$  95% CL (three measures) shown per sample.

### Figure Accounting

Data presented in graphical form in the body of the thesis is presented here tabulated for easy reference.

### LSE Profiles

All stations sampled during the Labrador Sea Expedition are presented here with their respective cruise track information and respective CTD sample numbers.

### Nutrient Results

This data provided by WOCE – HUDSON 2006-019 team shows Silicate, Phosphate, and Nitrate concentrations.

### Bottle Codes

Incubation bottles were numbered to facilitate sample-numbering schemes for bacterial abundance (AODC) and FISH analyses. This tab is a gazetteer of this labeling.

### AODC\_LS and AODC\_LB

Raw data of the Acridine Orange Direct Counts performed by microscopic analysis in the Rivkin Lab at the Ocean Sciences Centre, Memorial University.

### Image Analysis

Summaries of volume and carbon content estimates obtained through Image Analysis by Sabrina Penney and Ryan Murphy for both Labrador Sea and Logy Bay experiments. The original images and raw data (several gigabytes worth of information) can be found in the Rivkin Lab at the Ocean Sciences Centre, Memorial University.

### Growth

Biomass-based calculations of growth are presented for both Labrador Sea and Logy Bay experiments.

### FISH Counts

Raw data of the DAPI and Cy3 counts performed by microscopic analysis in the Rivkin Lab at the Ocean Sciences Centre, Memorial University.

TechValidation and SampValidation

Inter- and intra-experimental variation in FISH analysis is accounted in these two tabs.

BP Feb to BP Nov

These final four tabs present a full accounting of tritiated thymidine (TdR) and  $^{14}\text{C}$  Leucine incorporation assays conducted during the Logy Bay experiments. These data were of questionable merit to the thesis and excluded from further analysis.

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