ACTIVITY AND ABUNDANCE OF BACTERIAL GROUPS IN THE SARGASSO SEA AND MID-ATLANTIC BIGHT

by

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ABSTRACT

Microbial communities are essential to the processing of dissolved organic matter and nutrients in the ocean. Molecular methods are used to assess phylogenetic structure and activity within marine bacterial populations. However, types of activity and varying abundances can affect bacterial contributions to biogeochemical and organic matter fluxes. The goals of this study were to evaluate activity levels of rare and abundant bacterial groups in the Mid-Atlantic Bight and Sargasso Sea, and to compare shifts in the active and total bacterial populations on a short temporal scale with changing activity levels.

Bacteria that are replicating their DNA are active members of the community. Thus, a metric for bacterial activity is to monitor DNA synthesis. I assessed bacterial activity via DNA replication using incorporation of a thymidine analog, 5-bromo-2'-deoxyuridine, into bacterial DNA. Denaturing gradient gel electrophoresis and sequencing were used to generate profiles of the total and active populations in the Sargasso Sea and Mid-Atlantic Bight in spring and summer. Total and active communities were 65-95% similar within seasons, indicating that most bacteria are actively dividing. Bacterial groups varied in activity within each sample. SAR11 (*Alphaproteobacteria*) was active and abundant throughout communities. Another alphaproteobacterial member, a *Rhodobacter* sp., was enriched

only in active communities. Additionally, representatives of *Bacteroidetes* and *Gammaproteobacteria* were more active than their abundances suggested.

In marine systems, abundances of bacterial members may not be indicative of their activity levels. I used tag pyrosequencing of the 16S rDNA V1-V2 region to compare the active and total communities from spring and summer 2009 and 2010 in the Mid-Atlantic Bight and Sargasso Sea. Activity levels of a majority of bacterial taxa were proportional to their abundances in the total community. However, I observed differential activity in bacteria of varying abundances and activity. *Flavobacteria* (*Bacteroidetes*) were between 5 and 20% of the total community in both environments, but were consistently more active than suggested by their abundances. Rhodobacterial (*Alphaproteobacteria*) ribotypes were active, rare members of the Mid-Atlantic Bight bacterial community. In the Sargasso Sea, SAR86 (*Gammaproteobacteria*) was more active when rare. SAR11 was consistently abundant in both locations and seasons, but had discrepancy in activity levels relative to abundance. Changes in the active bacterial community also influenced the composition of the total community in spring 2009 of the Mid-Atlantic Bight and spring 2010 of the Sargasso Sea. Rare and active bacteria, such as unclassified bacteria in the Sargasso Sea and rhodobacters in the Mid-Atlantic Bight, become more abundant members of the microbial population within 12-96 hours of sampling.

The research in this thesis investigated differential activity of bacterial groups in the Sargasso Sea and Mid-Atlantic Bight. I analyzed bacterial activity at fine phylogenetic and temporal scales. I identified rare and abundant bacterial taxa with higher or lower activity levels than expected. Furthermore, I observed shifts in

community structure with changes in bacterial activity and environmental conditions. By profiling changing activity levels of bacterial taxa and rapid responses in community structure, this study examined the interplay of diversity and activity in microbial assemblages, and contributed to knowledge of the ecology of marine microbes.

Chapter 1

INTRODUCTION

Bacterial activity significantly influences biogeochemical cycles and the flux of organic matter in the ocean (Azam, 1998). Heterotrophic bacteria are responsible for releasing inorganic nutrients and dissolved organic matter, which are in part from autotrophic bacteria. Thus assessments of bacterial activity are critical insights into the microbial loop in marine ecosystems (Cole and Pace, 1995; Sherr and Sherr, 2000). Historical assessments treat microbial communities as a "black box", in which the internal structure and composition are not accounted for (Kirchman and Williams, 2000). More recent analyses of microbial activity reveal the inner workings of the "black box", by describing how and which bacteria contribute to organic matter cycling. Even still, the routes by which heterotrophic bacteria transfer organic material vary and are dependent on independent activity. Microbial activity is quantified via genetic-based means in measurements of rRNA or functional gene expression (Karner and Fuhrman, 1997). These assessments can be quite specific and identify phylogenetic groups, yet methods may have inherent biases that discriminate against specific bacterial groups (Drenovsky *et al.*, 2008). Despite these inherent and encountered difficulties, examining community structure and activity is crucial to understanding the impact of marine heterotrophic bacterial assemblages on their environment. The research presented in this thesis addresses part of the

knowledge gap by evaluating shifts in marine bacterial activity in the Sargasso Sea and Mid-Atlantic Bight.

1.1 Bacterial Diversity in Marine Systems

Advances in microbial ecology have revealed diverse assemblages of bacteria across marine and aquatic environments. The bacterial community is composed of several major phylogenetic divisions (Ciccarelli *et al.*, 2006), yet a small pool of bacterial groups seeds a majority of all bacterial ribotypes in the oceans (Giovannoni and Rappé, 2000). Dominant ribotypes include representatives in the *Alphaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*, *Cyanobacteria*, and *Bacteroidetes*, among other groups (Biers *et al.*, 2009). In total, bacteria can comprise over half to a substantial majority of the surface marine prokaryotic community (Bouvier and del Giorgio, 2003).

Members of the *Alphaproteobacteria* class are phenotypically diverse and global players in the ocean. They constitute nearly half of the marine microbial genetic pool (Biers *et al.*, 2009). Within the *Alphaproteobacteria*, constituents of the SAR11 clade are numerically dominant and ecologically significant. SAR11 was first identified through cloning and sequencing of rRNA in the Sargasso Sea (Giovannoni *et al.*, 1990), but members have since been cultured using dilution-to-extinction methods (Connon and Giovannoni, 2002; Rappé *et al.*, 2002). The SAR11 clade is highly diverse and abundant in marine systems, consisting of several subclades found at different depths and habitat types, and reported to comprise between 25-30 percent of marine communities in the euphotic and mesopelagic zones (Glockner *et al.*, 1999;

Brown and Fuhrman, 2005; Giovannoni and Stingl, 2005; Morris *et al.*, 2002, Carlson *et al.*, 2009).

Another example of an important marine phylum is *Bacteroidetes*, which is also globally distributed and ecologically diverse. In the North Atlantic Ocean, members of *Bacteroidetes* comprise up to 20% of the surface bacterial community (Gómez-Pereira *et al.*, 2010). Some representatives of *Bacteroidetes* are less abundant but more active than members of the SAR11 clade in both the Mid-Atlantic Bight and Sargasso Sea (Campbell *et al.*, 2009; Vila-Costa *et al.*, 2010; Campbell *et al.*, 2011). Activity data suggest that *Cytophaga*-like bacteria of the *Bacteroidetes* group account for more consumption of high-molecular weight organic matter than *Alphaproteobacteria* members (Kirchman, 2002; Cottrell and Kirchman, 2003). Pure-culture studies concluded that *Flavobacteria* are able to degrade a variety of complex macromolecules (Bernardet *et al.*, 1996; Glockner *et al.*, 1999). In addition, abundance and activity of *Bacteroidetes* are linked to phytoplankton blooms in that they can degrade released polysaccharide and protein molecules (O'Sullivan *et al.*, 2004, Gómez-Pereira *et al.*, 2010). Consequently, in the marine environment where there is a greater supply of low-molecular weight DOM such as in the Sargasso Sea, the contribution of *Bacteroidetes* to bacterial production may not be consistent over time (Cottrell and Kirchman, 2003).

1.2 Bacteria in the Sargasso Sea and Mid-Atlantic Bight

The Sargasso Sea, the large western North Atlantic subtropical gyre, is a well-characterized oceanographic region. Bounded to the west and northwest by the

Gulf Stream and to the south by the North Atlantic equatorial current, the oligotrophic, low-nutrient Sargasso Sea is isolated from nutrient-rich coastal waters (Steinberg *et al.*, 2001, Venter *et al.*, 2004). Scientists have extensively studied the seasonal physical, biological and chemical features of the Sargasso Sea. Hydrostation 'S' has been sampled biweekly since 1954 (Schroede and Stommel, 1969) and the Bermuda Atlantic Time-series Study (BATS) site has been sampled monthly since 1988 (Steinberg *et al.*, 2001). In the Sargasso Sea, subtropical mode water forms each winter when cold fronts disperse the seasonal thermocline and lead to convective mixing (Halliwell *et al.*, 1994). Nutrient-rich deep water is introduced to the surface and results in a phytoplankton bloom and net autotrophy. The onset of summer and high-pressure systems that prevent frontal passage leads to the formation of a shallow, brightly-lit mixed layer (Steinberg *et al.*, 2001). Autumn brings tropical storm activity and increased wind to the region, altering the thermal and physical profiles and changing the ecosystem to net heterotrophy (Nelson, 1998).

Diverse microbial communities are found in the Sargasso Sea, and include prokaryotic phytoplankton, eukaryotic phytoplankton, photoautotrophic bacterioplankton, and heterotrophic bacterioplankton. Studies using flow and image cytometry show that heterotrophic bacterioplankton dominate the prokaryotic community and make significant contributions to organic carbon cycling (Sieracki *et al.*, 1995; Buck *et al.*, 1996). Through molecular and culturing techniques, scientists have identified members from SAR11, *Bacteroidetes*, *Cyanobacteria* (including notable taxa *Prochlorococcus* and *Synechococcus*), and all five groups of *Proteobacteria*, among others (Venter *et al.*, 2004). In net heterotrophic periods,

bacterial growth rates are higher compared to net autotrophic periods, yet bacterial biomass is correspondingly lower (Caron *et al.*, 1999). Consequently, average bacterial production is constant between the two seasons in this oligotrophic environment (Caron *et al.*, 1999). Because of significant changes in the environment, this consistency in average bacterial production implies that important players in the microbial community also significantly change, whether in abundance or activity.

The Mid-Atlantic Bight (MAB) is the oceanic region between Cape Hatteras and the southwest edge of Georges Bank. This area, which contains the Delaware Bay estuary, is significantly influenced by the mixing and exchange of MAB shelf, slope, Gulf Stream and Sargasso Sea waters (Churchill *et al.*, 1993). Water mass exchange can not only transport plankton in this area but also alter productivity; released Gulf Stream water tends to increase near-surface nutrient concentrations over the continental slope (Churchill and Cornillon, 1991). Like the Sargasso Sea, the MAB region also experiences net autotrophy in the marine plankton ecosystem during the spring, and net heterotrophy in the summer/autumn. The Delaware Coastal Current is the major persistent water transport system on the Delaware shelf. It originates from the efflux of bay water low in salinity, into higher salinity coastal waters (Skrabal, 2006).

The unique features of the MAB adjacent to the Delaware Bay help shape the microbial community in the area. The most abundant bacterial groups in the Delaware Bay are the *Alphaproteobacteria*, *Betaproteobacteria*, *Cytophaga*-like bacteria of the *Bacteroidetes* phylum, and *Actinobacteria* (Kirchman *et al.*, 2005). At the mouth of the estuary, *Alphaproteobacteria* are more abundant than *Bacteroidetes*

(Campbell *et al.*, 2011). In addition, bacterial activities of these groups shift over time; higher percentages of both groups are more active in the summer than in the spring (Cottrell and Kirchman, 2003). SAR11 members comprise over 25% of the total community at all times, but certain flavobacterial members cycle between being rare and abundant, or are always rare (Campbell *et al.*, 2011). As in the Sargasso Sea, the bacterial community of the MAB adjacent to the Delaware Bay experiences temporal changes in composition that could influence organic cycling and biogeochemical fluxes.

Long-term, comprehensive studies such as the Bermuda Atlantic Time-series Study (BATS) and the Mid-Atlantic Bight Microbial Observatory for Photoheterotroph Exploration at site FB (FB) (Figure 1.1) are intended to assess shifts in bacterial community composition and structure and assess individual and group activity. In these studies, combinations of activity and molecular methods are used to quantify relative contributions of bacterial groups to biogeochemical processes (Cottrell and Kirchman, 2003, 2004, Campbell *et al.*, 2009, Agogué *et al.* 2011, Teira *et al.* 2010). The research presented in this thesis examines the changing bacterial community structure and activity in these two environments.

1.3 Assessments of Bacterial Activity and Community Structure

A critical topic in microbial ecology is the influence of community composition in bacterial assemblages on production and other aspects of microbial activity. Given that bacterial groups such as SAR11 and *Flavobacteria* are both cosmopolitan and metabolically diverse, the potential of bacterial activity to impact

oceanographic and biogeochemical processes is apparent, yet not fully understood. Similarly, individual members of microbial ecosystems, and not just different groups, have differing levels of activity. Thus evaluations of community structure need to also assess individual contributions to total activity.

One method to analyze community structure and activity is to target bacterial 16S rRNA. Because 16S rRNA content is regulated in growing, hence active, cells regardless of metabolisms, quantification of cell-specific rRNA content is a useful index of cell activity (Karner and Fuhrman, 1997, Nocker *et al.*, 2007). Polymerase chain reaction (PCR) amplified bacterial 16S rRNA genes are the input for DNA-fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE) and amplicon pyrosequencing (Smith and Osborn, 2009). DGGE of PCR products provides a genetic profile, and is appropriate for assessing changes in community structure over time in response to environmental perturbation (Nocker *et al.*, 2007). Sequencing 16S rRNA genes can also provide a more developed picture of bacterial community structure. The sequence tag approach of Sogin *et al.* (2006) uses high-throughput pyrosequencing of hypervariable regions in 16S rRNA to resolve individual ribotypes (Huber *et al.*, 2007). Furthermore, tag sequencing with 454 technology avoids cloning bias and artifacts, and is a cost effective method of analyzing microbial diversity (Sogin *et al.*, 2006).

The assimilation of 5-bromo-2'-deoxyuridine (BrdU), an analog of thymidine, into 16S rRNA genes (rDNA) is parallel to the incorporation of thymidine in bacterial production assays. BrdU incorporation can be combined with aforementioned 16S rRNA genetic approaches to analyze bacterial activity

(Hamasaki, 2006). BrdU-labeled DNA can be detected by a wide range of cytochemical and immunochemical techniques (Moran *et al.*, 1985; Steward and Azam, 1999). In addition, BrdU-labeled DNA can be separated from and compared to unlabeled DNA due to the compound's antigenicity (Haider *et al.*, 1997). BrdU incorporation has been used to examine bacterial growth in soils (Borneman, 1999; Yin *et al.*, 2000), rhizospheres (Artursson and Jansson, 2003; Artursson *et al.*, 2005), sediments (Edlund and Jansson, 2008), and lakes and oceans (Urbach *et al.*, 1999; Pernthaler *et al.*, 2002, Mou *et al.*, 2007; Hamasaki *et al.*, 2007; Taniguchi and Hamasaki, 2008).

The relationship between bacterial community structure and activity can be assessed with BrdU-labeling in conjunction with sequencing and molecular methods. DGGE paired with BrdU incorporation has been used to profile the metabolically active bacterial community of coastal and open oceans (Hamasaki *et al.*, 2007; Taniguchi and Hamasaki, 2008). In addition, BrdU-incorporating community profiles can include bacterial ribotypes not seen in the total community (Hamasaki *et al.*, 2007, Taniguchi and Hamasaki, 2008). Chapter 2 of this thesis focuses on the use of BrdU-labeling with DGGE profiling in identifying active fractions of bacteria in the Mid-Atlantic Bight and Sargasso Sea.

In combination with activity measures, pyrosequencing can evaluate activity levels and community structures of bacterial assemblages (Jones and Lennon, 2010, Campbell *et al.*, 2011). Evaluation of abundances of 16S rDNA describes total bacterial communities (Muyzer *et al.*, 1993), while 16S rRNA analysis can characterize the active fraction of bacterial communities (Moeseneder *et al.*, 2001). In

the Mid-Atlantic Bight adjacent to the Delaware Bay, comparison of 16S rRNA and rDNA libraries revealed higher representation of rare bacteria than abundant bacteria in the active community (Campbell *et al.*, 2011). Combining this index with substrate addition yields an examination of specific activity and genetic expression. The addition of dimethylsulfoniopropoionate (DMSP) in the Sargasso Sea generated a shift in the bacterial community to heterotrophic activity, with relative increases of *Gammaproteobacteria* and *Bacteroidetes* transcripts (Vila-Costa *et al.*, 2010). In a coastal environment, *Gammaproteobacteria* was also enriched in the active DMSP-degrading community; yet both SAR11 and *Bacteroidetes*, though active, were not substantial contributors (Mou *et al.*, 2008). The active bacterial community labeled with BrdU can be sequenced and compared to the total bacterial community to provide a more developed picture of bacterial community structure. Chapter 3 discusses the combination of BrdU-labeling of active bacteria with 454 tag pyrosequencing to examine activity and abundance within bacterial communities.

The main goal of this research was to examine the link between marine bacterial activity and community composition across a spatiotemporal scale. I assessed marine bacterial activity and community shifts in coastal and open ocean environments through the uptake of BrdU and subsequent amplicon sequencing of labeled bacterial DNA. The examination of bacterial activity using BrdU-labeling aids in the evaluation of the ecological roles of active bacteria in the marine environment, and provides further knowledge of the interplay between community diversity and activity.

1.4 References

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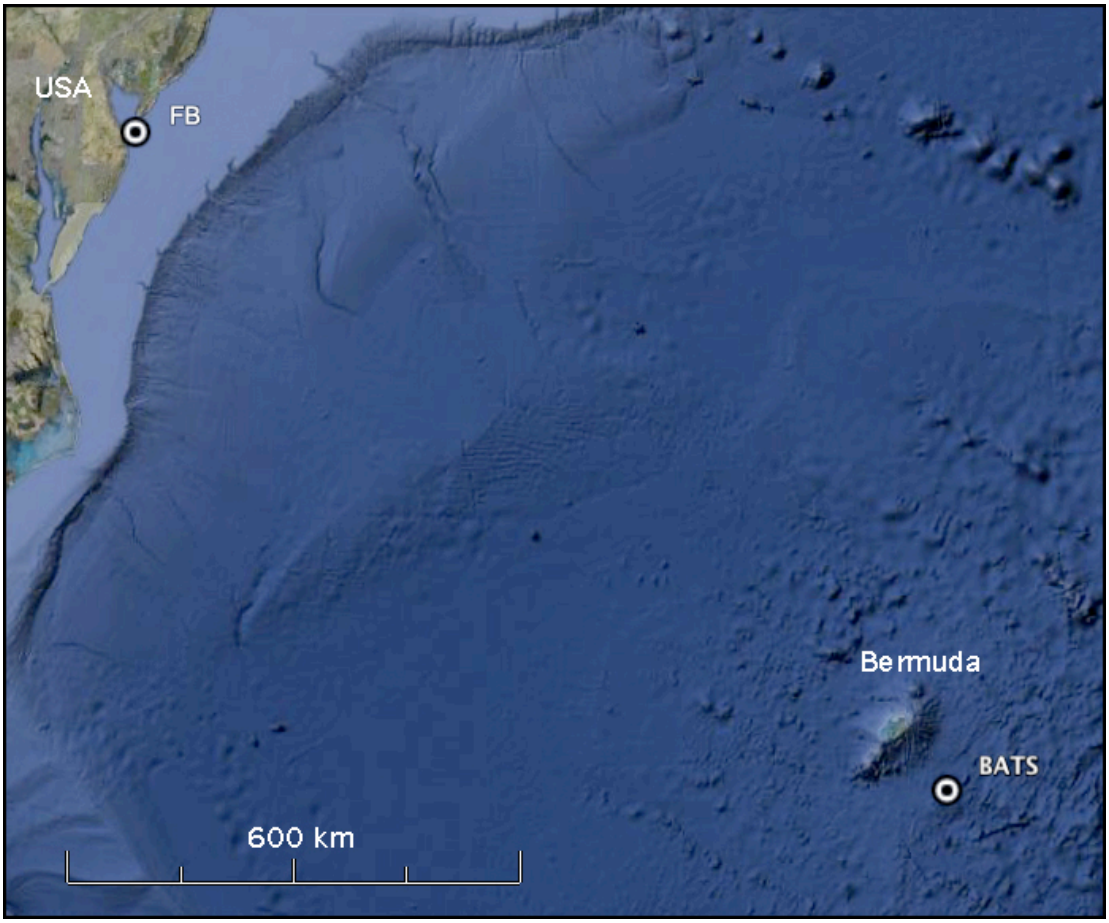

Figure 1.1: The Mid-Atlantic Bight Microbial Observatory at site FB (FB) and Bermuda Atlantic Time-series Study (BATS) are both long-term studies of the biogeochemical processes occurring at each site.

Chapter 2

ACTIVELY REPLICATING BACTERIA IN THE SARGASSO SEA AND MID-ATLANTIC BIGHT

2.1 Abstract

A diverse assemblage of bacterial ribotypes comprises the active bacterial community in marine environments. In order to study the association of activity and community structure, labeling of bacterial DNA with 5-bromo-2'-deoxyuridine (BrdU) was used to isolate the active fractions, and denaturing gradient gel electrophoresis (DGGE) and sequencing of ribotype bands were performed to obtain community profiles of particle-associated and free-living active and total bacterial assemblages. These assessments were used in the Mid-Atlantic Bight and Sargasso Sea during spring and summer over two years to analyze shifts in community structure. I hypothesized that the BrdU-labeled bacterial community would differ from the total community in both locations and seasons. I found that samples followed a temporal trend, in that both active and total bacterial communities clustered by season and by year. Within samples, dissimilarities between communities incorporating BrdU and total communities ranged from 5% to 35%. The active and seasonal response was seen on a phylogenetic level as well. Some alphaproteobacterial members such as SAR11 were ubiquitous in active and total communities, whereas a Rhodobacter-like ribotype was specific to active

communities. Additionally, representatives of *Bacteroidetes* and *Gammaproteobacteria* were enriched in the active communities. BrdU-labeling used with phylogenetic assessments identified active bacterial members in these marine ecosystems, emphasized overall similarities between the active and total communities, and demonstrated variation in the activity of rare members on a spatiotemporal scale. The examination of bacterial activity using BrdU-labeling will help evaluate the ecological roles of active bacteria in the marine environment, and will provide further knowledge of the interplay between community composition and activity.

2.2 Introduction

Bacteria are essential participants in marine dissolved organic matter (DOM) and nutrient cycles (Ducklow, 2000). Concurrent with fluxes in physical parameters and DOM, bacterial activity also varies seasonally and spatially. In situ phytoplankton-derived dissolved organic carbon (DOC) can sustain bacterial activity in spring in a coastal eutrophic environment, but bacterial activity shifts to incorporation of both residual DOM and in situ phytoplankton-derived DOC in late summer and autumn (Sintes *et al.*, 2010). In the oligotrophic areas of the central Atlantic Ocean, bacterial activity was an order of magnitude higher in the autumn than in the spring (Gasol *et al.*, 2009), and in the autumn both in situ phytoplankton-derived DOC and additional allochthonous or autochthonous carbon sources can support bacterial activity (Teira *et al.*, 2003). Characterizing bacterial activity relative

to location and season is important to understanding bacterial contribution to biogeochemical cycling.

In addition to examining the 'where' and 'when' of bacterial activity, it is also useful to describe 'who' in the bacterial community and 'how much' is active by studying composition in conjunction with activity. Previous studies have examined the relative activities of bacterial groups. *Sphingobacter-Flavobacteria* was found to preferentially consume high-molecular weight DOM in a coastal Mid-Atlantic Bight estuary whereas *Alphaproteobacteria* are responsible for more low-molecular weight DOM mineralization (Cottrell and Kirchman, 2000). In the coastal North Atlantic Ocean, various carbon and carbon/nitrogen sources favored the proliferation of *Gammaproteobacteria* and *Alphaproteobacteria* (Alonso-Sáez *et al.* 2009). However, in many environments the active bacterial community is composed of a diverse assemblage of bacterial groups. Furthermore, it is hypothesized that active bacteria are abundant, whereas rare bacteria are slow-growing or inactive (Pedrós-Alió, 2006). It is thus necessary to study the association of bacterial community structure and activity to obtain a clearer picture of the ecological roles of bacteria.

Several tactics have been used to examine bacterial activity relative to community composition. Common methods to assess bacterial activity on a phylogenetic level include microautoradiography combined with fluorescence in situ hybridization (micro-FISH) (Cottrell and Kirchman, 2004, Malmstrom *et al.*, 2007), and comparison of 16S rRNA and 16S rRNA gene libraries (Lami *et al.*, 2009, Campbell *et al.*, 2009). Another method uses the incorporation of 5-bromo-2'-deoxyuridine (BrdU) into bacterial DNA during DNA replication (Steward and

Azam, 1999, Nelson and Carlson, 2005). BrdU-labeled DNA can be separated from unlabeled, or inactive, bacterial DNA with the use of specific antibodies (Haider *et al.*, 1997), and used in molecular diversity assessments. Coupled with sequencing and community fingerprinting techniques, BrdU-labeling successfully detected active bacteria in soils, sediments and aquatic ecosystems (Pernthaler *et al.*, 2002, Hamasaki *et al.*, 2007, Edlund and Jansson, 2008). In addition, BrdU-labeling revealed rare, active bacterial groups in the oligotrophic west Pacific Ocean (Taniguchi and Hamasaki, 2008). Actively growing bacteria in the oligotrophic Sargasso Sea of the North Atlantic Ocean and the coastal Mid-Atlantic Bight have not been described. Community profiles of actively replicating bacteria in these regions would explore the complex association of environment, community structure, abundance and varying types of activity.

The goal of this study was to assess marine bacterial activity and community shifts in coastal and open ocean environments on a spatiotemporal scale. The active fractions of particle-associated and free-living bacteria from the Mid-Atlantic Bight and Sargasso Sea were labeled with BrdU. They were compared to the total community using the community profiling technique of denaturing gradient gel electrophoresis (DGGE). BrdU-labeling and DGGE enriched for active bacterial ribotypes that were at times not apparent in the total community. Community profiles between total and active bacterial communities were similar within a season, but varied between years in both locations.

2.3 Materials and Methods

Samples were obtained in spring and summer of 2009 and 2010 from the BATS site (31º40'N, 64º10'W) and the FB site (38º46'N, 74º55'W) (Table 2.1). Seawater was collected 50 m below the surface at BATS and 1 m below the surface at FB. At FB, both night and day samples 12 hours apart from a single sampling trip were collected (two sampling events, T_0 and T_{12}, per cruise). At BATS, samples were collected daily over four days (four sampling events, T_0 through T_{96}, per cruise). Various standard oceanographic parameters were measured, including water temperature, salinity, bacterial production via the microcentrifugation protocol, and nutrients, as described previously (Tables 2.2 and 2.3) (Steinberg *et al.*, 2001; Cottrell *et al.*, 2006; Campbell *et al.*, 2009).

2.3.1 BrdU-labeling

Duplicate 10 L samples at BATS and 2 L samples at FB were incubated for 6 hours with a final concentration of 10 μM BrdU at in situ light and temperatures. Duplicate controls were incubated without BrdU. Particle-associated bacteria were collected on 0.8 μm polycarbonate filters (Isopore; Millipore), and the filtrate was passed through 0.22 μm filters (Durapore; Millipore) to collect free-living bacteria. Filters were stored at -80ºC until processed.

2.3.2 DNA Extraction

DNA was extracted using either the potassium ethyl xanthogenate-sodium dodecyl sulfate protocol (PEX-SDS) described by Tillett and Neilan (2000) with modifications or acetyltrimethylammonium bromide-polyvinylpyrrolidone-β-

mercaptoethanol (CTAB/PVP/β-ME) method (Dempster *et al.*, 1999). Samples from March 2009 at BATS were extracted with PEX-SDS; all other samples used in this study were extracted with the CTAB protocol. For PEX-SDS, thawed filters were incubated in 1 mL of 1% PEX-SDS buffer (1% potassium ethyl xanthogenate, 100 mM Tris-HCl [pH 7.5], 20 mM EDTA [pH 8.0], 1% sodium dodecyl sulfate, 800 mM ammonium acetate) at 70ºC for 120 min. The filters were vortexed for 10 seconds, and the lysate was eluted into 1.5 mL microcentrifuge tubes. The tubes were placed on ice for 30 min then cellular debris was pelleted by centrifugation for 45 min at 14,000 rpm at 4ºC. The supernatant was halved and transferred to two clean 1.5 mL microcentrifuge tubes and mixed with equal volumes of isopropanol. After a room temperature incubation of 10 min, DNA was pelleted by centrifugation at 14,000 rpm for 45 min at room temperature. The pellet was washed once with 70% ethanol and air dried. For CTAB, thawed filters were incubated with 1 mL of CTAB and 8 µl of β-mercaptoethanol at 65ºC for 15 min with occasional inversion of the tube. The lysate was transferred to a 2 mL microcentrifuge tube, then mixed with an equal volume of chloroform/isoamyl alcohol (24:1) and incubated for 20 min at room temperature with constant agitation. The tubes were centrifuged for 15 min at 12,500 rpm, and the top aqueous layer was transferred to a clean 2 mL tube for a second chloroform/isoamyl alcohol extraction. The second aqueous layer was split and transferred to two new 1.5 mL microcentrifuge tubes then ½ volume of 5 M NaCl and 1 volume of isopropanol was added to each tube. After 1 h incubation at -80ºC, the sample tube was centrifuged at 12,500 rpm for 45 min. The DNA pellet was washed with 70% ethanol and air dried. Following both extractions, DNA was resuspended in

50 µl of sterile H_2O. The DNA concentration was quantified via a Picogreen assay (Invitrogen) as previously described (Campbell *et al.*, 2009).

2.3.3 BrdU Immunocapture

Immunocapture was performed as detailed by Urbach *et al.* (1999), with modifications by Hamasaki *et al.* (2007). All incubations were done at room temperature in the dark with constant agitation. Herring sperm DNA (10 mg mL^{-1} in PBS; Trevigen Inc., Gaithersburg, MD) was denatured at 95ºC for 7 min, frozen in dry-ice ethanol and thawed. The solution was mixed 9:1 with anti-BrdU monoclonal mouse antibody (1:10 in PBS; Sigma-Aldrich, St. Louis, MO) and incubated for 30 min. Extracted DNA samples (0.1 µg) were denatured for 7 min, frozen in dry-ice ethanol and thawed. The denatured DNA sample was mixed with 10 µL of herring sperm DNA-antibody mixture and incubated for 30 min. Paramagnetic beads with goat anti-mouse immunoglobulin G (Dynabeads; Dynal Biotech, Oslo, Norway) were washed with PBS with acetylated bovine serum albumin (BSA) (1 mg mL^{-1}; Sigma Aldrich, St. Louis, MO) using a magnetic concentrator (MagnaRack, Invitrogen) and resuspended in PBS-BSA at the initial concentration. Each sample mixture was incubated with 25 µL of bead suspension for 4 h. The beads were washed seven times with 100 µL of PBS-BSA. Initial and final washes were saved in equal volume isopropanol at -80ºC to verify complete removal of non-BrdU-labeled DNA. The beads were resuspended in 20 µL of sterile H_2O and stored at -20ºC until PCR amplification and DGGE. Unlabeled environmental samples as well as a blank H_2O-only negative control were processed in the same manner. Low recovery of unlabeled

DNA after immunocapturing was confirmed with a Picogreen assay (Campbell *et al.*, 2009).

2.3.4 DGGE

Bacterial 16S rRNA genes were amplified from 1.0 μL of immunocaptured, BrdU-labeled or unlabeled DNA-bead mixture, as well as 1.0 ng of nonimmunocaptured unlabeled DNA, from either the particle-associated (≥ 0.8 μm) size fraction or the free-living (0.8 - 0.22 μm) size fraction. The variable V3 region of the bacterial 16S rRNA gene was amplified in a touchdown PCR protocol using GC358F, a forward primer complementary to positions 341 to 358 with a 40-bp GC clamp (5'-CCTACGGGAGGCAGCAG-3') and Univ517R, a universal reverse primer complementary to positions 517 to 534 (5'-ATTACCGCGGCTGCTGG-3') (Muyzer *et al.*, 1993, Kirchman *et al.*, 2001). All PCR reactions contained 1.0 μL of sample, 0.5 μL of each primer (10 μM) and 12.5 μL of Bio-X-Act 2X Short Mix (Bioline), in UV-treated PCR water to a final volume of 25 μL. The touchdown protocol consisted of a decreasing annealing temperature from 65ºC to 54ºC by 0.5ºC per cycle for 22 cycles, followed by 10 cycles at 55ºC, each for 1 minute. The denaturing step was 5 minutes at 94ºC, extension was for 1 minute at 72ºC, and a final extension step was 5 minutes at 72ºC. For each community, 20 μL of PCR product was loaded onto 8% polyacrylamide gels in 1x TAE (20 mM Tris, 10 mM acetate, 0.5 mM Na_2EDTA) with a denaturing gradient from 22-55%. The gels were subjected to electrophoresis for 5 hours at 150 V and 60ºC (DcodeTM, BioRad). Gels were stained for 10 min in fresh ethidium bromide (1 μg mL^{-1} final concentration) and destained for 20 min in

fresh H2O. The gels were visualized on a UV table (Gel Logic 100 Imaging System; Kodak MI; Fisher Scientific). Banding patterns were characterized using Phoretix 1D Advanced (TotalLab, Ltd), and dendrograms and principal component analyses of community structure were assembled using Primer6 (Primer-E, Ltd). For sequencing, DGGE bands were excised, eluted in 20 μL sterile H_2O and PCR reamplified for single bands. DGGE bands were sequenced with capillary electrophoresis (ABI Prism 310 Genetic Analyzer, Applied Biosystems) using a BigDye terminator sequencing kit (Applied Biosystems) as previously described (Campbell and Cary, 2001). Phylogenetic classification was performed using BLAST (www.ncbi.nlm.nih.gov/BLAST) and RDP (Cole *et al.*, 2007).

2.4 Results

2.4.1 Extraction Optimization

To test the variability in community structure due to different extraction protocols, I extracted two samples from August 2009 at the BATS site with the PEX-SDS protocol and analyzed the community profiles on a DGGE alongside analogous CTAB-extracted samples (Figure 2.1). No differences in community structure due to extraction method are apparent via banding patterns. Additionally, the dendrogram of community similarities had consensus between analogous samples regardless of extraction protocol (Figure 2.2).

2.4.2 Unique Bands in Total and Active Communities

I used denaturing gradient gel electrophoresis to evaluate total and BrdU-labeled bacterial DNA from: a) the particle-associated bacterial community in the Sargasso Sea; b) the free-living bacterial community in the Sargasso Sea; c) the particle-associated bacterial community in the Mid-Atlantic Bight; and d) the free-living bacterial community in the Mid-Atlantic Bight (Figures 2.3-2.6). The community collected on the 0.8 μm filter includes particles colonized by bacteria, filamentous bacteria, and other larger cells. Bacteria that pass through the 0.8 μm filter and are collected on the 0.22 μm size filter are considered free-living and not associated with particles. Community structures from two sampling events from each cruise were analyzed. From the Sargasso Sea, samples collected at times T_0 and T_{96} representing the beginning and end of each sampling cruise (first and second triplet of lanes of each set, respectively) were evaluated via DGGE. In the Mid-Atlantic Bight, samples collected at time T_0 at night (first triplet of lanes of each set) and T_{12} in the day (second triplet of lanes of each set) were subjected to DGGE (Table 2.4). In each triplet of lanes, the first lane is the untreated, nonimmunocaptured community (UW); the second lane is the untreated, immunocaptured community (UI); and the third lane is the labeled, immunocaptured community (LI). The UI community profile is considered as background noise for the immunocapture treatment.

I evaluated the four gels for presence/absence of unique bands to characterize community structures (Tables 2.6-2.9). Within a cruise, bacterial ribotype bands that occurred in LI communities but not in UW were considered unique to the labeled community. Bands that occurred in UW communities but not in

LI were considered unique to the total community. Across all samples, no bands were found that occurred in UI communities but not in either UW or LI.

2.4.3 Similarities in Community Structure

I evaluated similarities in community structure by constructing dendrograms from Bray-Curtis similarities calculated from banding patterns in DGGE gels. Community structures of Sargasso Sea particle-associated bacterial assemblages were 50% or more similar to each other (Figure 2.7). Overall, samples clustered with other representatives from the same sampling event. The two spring 2009 labeled communities clustered together at over 80% similarity; a similar trend was observed in summer 2009 communities. On the contrary, each spring 2010 labeled community clustered with their respective total communities at 80-85% similarity. These observations were confirmed in the principal component analysis graph (Figure 2.11). All summer samples grouped together at over 70% similarity, and communities from both spring 2009 samples were also over 70% similar. In addition, the active communities of both spring 2010 were similar to each other and to their associated total communities.

In the free-living bacterial fraction of the Sargasso, community structures were clustered overall by season (Figure 2.8). In the summer, total communities were 100% similar to each other, and over 85% similar to labeled communities. The labeled communities in spring and summer 2009 were not as similar to the total communities of those samples. However, labeled communities in the two spring 2010 samples were identical to each other. Both total communities of spring 2010 also had

100% consensus. Summer samples clustered away from spring samples based on similarity of community structure (Figure 2.12). Two clusters of spring samples were 80% similar. BrdU-labeled bacterial assemblages in the two samples from spring 2009 were different from each other and from the total communities of spring 2009. The overall and labeled communities in the two samples from spring 2010 were highly similar to each other, and clustered with one set of spring 2009 samples. As seen in the dendrogram, the total communities of both summer 2009 samples were identical, and their labeled fractions clustered with the total communities.

Community structure similarities in the particle-associated bacterial populations of the Mid-Atlantic Bight were separated by season as well (Figures 2.9 and 2.13). Both of the total communities in the spring resembled each other, and were grouped with the labeled fractions. Overall, they clustered at 60% similarity separately from the two spring unlabeled, immunocaptured communities. Summer samples were more variable, in that while the structures of the two total communities resembled each other, only the day summer labeled community was related to the total communities at more than 60% similarity. The summer night labeled community and its associated unlabeled, immunocaptured community were less than 10% similar to other samples in the particle-associated fraction.

I observed a seasonal trend in the clustering of free-living bacterial community structures of the Mid-Atlantic Bight (Figure 2.10). Within the spring cluster, the total communities formed one branch, and were highly similar to the structure of the labeled fraction from the night sample. The structure of the day labeled fraction was more dissimilar to the other spring samples, and clustered

separately from them (Figure 2.14). Community structures of the summer samples were more also more related by labeling than by sample. Within the summer samples, one cluster contained the labeled fractions of both samples, and the total community of the day sample. Furthermore, the structure of the total bacterial assemblage from the night sample was a distinct cluster and did not resemble the labeled community.

2.4.4 Variation in Phylogenetic Community Structure

To examine community structure, I excised and sequenced bacterial ribotype bands based on presence in gel lanes, relative intensity and uniqueness. Some bands were unable to be sequenced due to impurity in the sample; of the 33 bands excised, only 16 were successfully sequenced and identified by RDP and BLAST analyses (Table 2.5). Sequencing data was paired with banding matrices to evaluate presence of ribotypes across samples (Tables 2.6-2.9).

In particle-associated communities of the Sargasso Sea, ribotypes related to *Synechococcus* (ribotypes 1 and 4) and *Vibrionaceae* (5) were observed across all samples (Table 2.6). A majority of bands were found throughout spring and summer samples as well. *Pseudoalteromonas* (ribotype 2) was found only in the labeled communities of 2009, and not identified in either the total or labeled fractions of spring 2010. Bands 3, 6, 8, and 19 were also found in 2009 samples and not in 2010 samples. A sequenced *Flavobacteriaceae* (ribotype 3) was present in the total and labeled communities in summer samples, but not present in the labeled communities of three of the four spring samples. Six bands (12, 27-31) were also unique to summer samples and compared to the summer total community, more unique bands were

observed in the labeled fraction. Some bands were common to spring samples exclusively (bands 4 and 7); however, communities in spring 2010 had additional unique bands (bands 1, 2, 11, 18, 20, and 26).

The free-living bacterial communities of the Sargasso Sea also had phylogenetic differences in BrdU-labeling (Table 2.7). Unclassified alphaproteobacterial ribotypes (6 and 7) and two SAR11 types (8 and 9) were common to labeled and total communities in all samples. Other bands were shared between spring and summer samples (bands 12, 13, 16, 17, 19, 21). A gammaproteobacterium (ribotype 12) was observed in the summer total and labeled communities, but found only in the labeled communities of spring samples. One *Bacteroidetes* member (ribotypes 11 and 14), and bands 5, 10 and 15 were identified in spring total and labeled communities, and not evident in summer samples. Another *Bacteroidetes* representative (ribotypes 10 and 13) was unique to the labeled and total bacterial communities of spring 2010 and not found elsewhere in the community profiles. Some bands were present in both samples of 2009 and not in 2010 (bands 4 and 14), and band 1 was exclusive to the summer total and labeled communities. More unique bands were resolved in the BrdU-labeled fractions of the spring samples than in the total communities. On the other hand, in the summer samples compared to the total community no bands were unique to the labeled fraction.

In the profiles of the particle-associated bacterial communities of the Mid-Atlantic Bight (Table 2.8), a majority of bands were common to both seasons. Bands 3, 6 and 27 were unique to the spring samples, while several bands were found only in summer samples (bands 2, 4, 8, 10, 13, 16, 29). A majority of ribotypes that

were present in the labeled communities was also present in the total communities. Certain ribotypes were present in the spring total community, which were not represented in the labeled community (bands 15, 18, 19, 20). On the other hand, no ribotypes were unique to the summer active bacterial fraction, which were not apparent in the total community. Labeled ribotypes appeared to increase in abundance between spring and summer (bands 1, 5, 22).

In the free-living bacterial profiles, more unique bands were observed in the total communities than in the labeled fractions across both seasons. Two ribotypes from the free-living bacterial profiles were identified on a phylogenetic level (Table 2.9). A flavobacterium (ribotype 15) was ubiquitous to Mid-Atlantic Bight free-living communities, in both the total and labeled fractions of spring and summer samples. Other bands were also found in both sets of samples (bands 2, 4, 8, 10, 12, 14, 17, and 18). Two bands were present in the spring labeled and total communities, but not apparent in the summer labeled community (bands 3 and 11). Unique bands were also found in summer samples (bands 1, 5 and 7). In spring samples, a member of the *Rhodobacter* order (ribotype 16) and band 15 were observed in the active communities and not represented in the total community, but was common to total and active fractions in summer samples. On the other hand, band 9 was found in active and total bacterial fractions of summer samples and the total communities of the spring samples. Additionally, while unique ribotypes were observed in the spring active communities, no unique bands were identified in the active fractions of summer samples.

2.5 Discussion

The activity and composition of bacterial assemblages are influenced by a variety of factors, including season, location, and types of activity (Vázquez-Domínguez *et al.*, 2008, Alonso-Sáez *et al.*, 2009, Vila-Costa *et al.*, 2010, Campbell *et al.*, 2011). Subsequently, compositions of the active and total bacterial communities may differ. In this study, I described community structure in the active and total bacterial assemblages of the Mid-Atlantic Bight and Sargasso Sea on a temporal scale by using BrdU-labeling of replicating DNA. Bacteria that incorporate BrdU into their DNA are thus active, and can be separated from the total community. I observed clustering of sample community structures according to the sampling event in which they were taken. In addition, samples from the free-living fraction were grouped into spring and summer clusters, whereas seasonality was less apparent in the particle-associated assemblages. The active bacterial fraction was similar in structure to the total community in a majority of samples from both locations. Exceptions to this conclusion occurred in three sampling events from the Sargasso Sea, where I observed more agreement between active communities sampled three days apart than between the active and total communities of each sample. Furthermore, specific ribotypes were unique to either the active or total communities in each sampling event. In the Mid-Atlantic Bight, half of the observed bacterial ribotypes cycled in abundance and activity (Campbell *et al.*, 2011). Similarly, in the Sargasso Sea populations of certain bacterial ribotypes were observed to increase subsequent to the spring phytoplankton bloom (Treusch *et al.*, 2009). Uniqueness of

bacterial ribotypes to season and/or the active fraction implies a small but important shift in community structure in both locations.

Several abundant bacterial ribotypes were also active in this study. The active bacterial fraction resembled the total community within each sampling event. Alphaproteobacterial and SAR11 ribotypes were common to the active and total free-living communities of the Sargasso Sea. In addition, ribotype bands were observed in total and active communities of the Mid-Atlantic Bight that resembled SAR11 subclades identified previously in DGGE analysis of the Mid-Atlantic Bight bacterial community (Campbell *et al.*, 2009). Representatives of *Alphaproteobacteria* are both active and abundant in the Sargasso Sea and Mid-Atlantic Bight (Malmstrom *et al.*, 2005, Agogué *et al.*, 2011, Campbell *et al.*, 2011). In the present study I observed a consistent representation of flavobacterial ribotype bands in both active and total fractions. In both oceanographic regions, flavobacterial representatives constitute between 5 and 20% of the bacterial community (Cottrell and Kirchman, 2004, Gómez-Pereira *et al.*, 2010). In the Sargasso Sea and Mid-Atlantic Bight, abundances of *Bacteroidetes* and *Alphaproteobacteria* ribotypes, among other well-represented groups, are also indicative of their activity levels.

Activity of individual bacterial members can shape community structure and overall bacterial activity. In this study, I observed ribotype-specific differences in the composition of the active and total bacterial communities. While many bacterial ribotypes were common to the total and active fractions in the Sargasso Sea and Mid-Atlantic Bight, up to 26% of observed bacterial ribotypes in each season were either active but not abundant or abundant but not active. In some cases, active bacteria are

not necessarily the most abundant members in the microbial community. In the Mid-Atlantic Bight, a significant portion of the community has been shown to be more active when rare (Campbell *et al.*, 2011). This observation of less abundant ribotypes that are still active explains why DGGE did not resolve abundance in the total community structure yet saw presence in the active fraction for a *Rhodobacter* sp. in the Mid-Atlantic Bight spring, and a *Pseudoalteromonas* (*Gammaproteobacteria*) member in the Sargasso Sea.

Environmental factors are also important influences that shape bacterial community structure. The Mid-Atlantic Bight adjacent to the Delaware Bay and the Sargasso Sea are two well-characterized and contrasting oceanographic regions (Churchill *et al.*, 1993, Steinberg *et al.*, 2001). In both the coastal Mid-Atlantic Bight and open-ocean Sargasso Sea, spring is associated with nutrient-rich waters and phytoplankton primary production. In the summer and late fall, nutrient depletion and stratification of the water column alters the ecosystem to net heterotrophy. In this study I observed ribotypes specific to either spring or summer in each oceanographic region. Two ribotypes belonging to *Bacteroidetes* were found only in the spring Sargasso free-living bacterial community, though other *Bacteroidetes* (*Flavobacteria*) members were ubiquitous. The sequenced flavobacterial ribotype from the Mid-Atlantic Bight was consistently represented in spring and summer communities. However it is likely that some of the season-specific ribotypes that were not sequenced may be *Flavobacteria*. Some flavobacterial clades have predictable abundances and distributions. In the Delaware Estuary adjacent to the Mid-Atlantic Bight, they were most abundant at the mouth of the estuary where salinity was

highest (Kirchman *et al.*, 2005). Gómez-Pereira *et al.* (2010) identified a correlation between temperature and abundance of *Polaribacter* spp. in the North Atlantic. Salinity and temperature, among other physical parameters, have been used to characterize the seasonal environments in both the Mid-Atlantic Bight and Sargasso Sea. With a supported relationship between seasonality of bacterial taxa and environmental parameters, the activity and abundance of season-specific ribotypes in the bacterial community may be influenced by environmental shifts.

The sampling effort of this study was not fine enough to resolve seasonal patterns in community structure in the Sargasso Sea and Mid-Atlantic Bight. I saw clustering of active and total communities within a season in both locations. Though I was unable to discern a seasonal pattern in the Mid-Atlantic Bight due to fewer samples taken, communities of the spring clustered together separately from summer samples. Community composition in the Mid-Atlantic Bight shifted slightly between years, hence consistent temporal patterns may not be apparent with additional sampling (Campbell *et al.*, 2009). However, others were able to discern seasonal trends in community structure. Fuhrman *et al.* (2006) saw high predictability in bacterial distribution and abundance in a coastal northeast Pacific site. In other short-term (1-2 years) seasonal studies, similarities were seen in bacterial communities from the same sampling season (Schauer *et al.*, 2003, Alonso-Sáez *et al.*, 2008, Campbell *et al.*, 2009).

Bacterial community composition in the Sargasso Sea was similar based on season and/or activity level. I observed higher similarity between active communities collected within 4 days of each other in the Sargasso Sea, than between

active and total communities from the same sample. The active communities from the two particle-associated samples resembled each other in the spring and summer of 2009, as did the two active free-living fractions in spring 2010. Additionally, while particle-associated samples of the Sargasso were more similar on a yearly and not seasonal scale, the free-living samples grouped into distinct spring and summer clusters. Consistency in community structure in both the active and total fractions of the Sargasso indicates a stable microbial community in this environment. The Sargasso Sea experiences predictable shifts in environmental parameters and the microbial community. Abundances of specific bacterial groups in the Sargasso were related to seasonality (Treusch *et al.*, 2009). Furthermore, summer samples may cluster together due to similar community structures (Morris *et al.*, 2005).

Denaturing gradient gel electrophoresis has been used in previous studies to analyze changes in microbial community structure (Murray *et al.*, 1996, Schauer *et al.*, 2003, Alonso-Sáez *et al.*, 2007, Campbell *et al.*, 2009). Taking into consideration the associated methodological problems and biases (von Witzingerode *et al.*, 1997), DGGE is useful for comparative, not quantitative, assessments of community structure (Nocker *et al.*, 2006). I used BrdU-labeling in conjunction with DGGE to provide the fingerprint of the active bacterial community (Hamasaki *et al.*, 2007, Taniguchi and Hamasaki, 2008). Additionally, DGGE enables excision and sequencing of ribotype bands of interest (Nocker *et al.*, 2006). Thus the combination of BrdU and DGGE was able to identify potentially influential members in the active and total communities.

This study emphasizes the importance of examining the link between activity and community structure. I described this relationship in the spring and summer of the Mid-Atlantic Bight and Sargasso Sea. The active bacterial community bore strong resemblance to the total community in composition and structure. While I was able to resolve broad community profiles of both the active and total fractions, additional research is needed to examine seasonal patterns in activity and community composition. Furthermore, finer resolution is necessary to observe changes in the active bacterial community due to the contributions of individual taxa. To address these questions, in chapter 3 I analyzed the free-living bacterial communities of the Sargasso Sea and Mid-Atlantic Bight for individual differences in ribotype and activity using BrdU and 454 pyrosequencing. The active and total communities of spring and summer over two years were analyzed for activity and abundance of bacterial taxa. Such examinations will help to provide improved understanding of the impact of bacterial assemblages on their environment.

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Table 2.1: Sampling cruises at the BATS and FB sites in 2009 and 2010.

Date	Location	Season	Site	Sampling events
17-20 March 2009	Sargasso Sea	Spring	BATS	4
17-18 April 2009	Mid-Atlantic Bight	Spring	FB	4
6-8 July 2009	Mid-Atlantic Bight	Summer	FB	2
14-17 August 2009	Sargasso Sea	Summer	BATS	4
24-27 March 2010	Sargasso Sea	Spring	BATS	4

Table 2.2: Environmental parameters measured at FB. All measurements were obtained at the time of sampling 12 hours after the initial sample.

Sample	Temper-ature (ºC)	Salinity (psu)	Bacterial production (pmol $L^{-1}h^{-1}$)	Chl a (µg L^{-1})	NO_3 (µM)	NH4 (µM)	PO_4 (µM)	Si (µM)
Spring 2009	8.31	31.6	67.8 ± 6.32	3.92 ± 0.165	0.158 ± 0.007	1.26 ± 0.011	0.045 ± 0.0002	1.31 ± 0.042
Summer 2009	19.5	30.5	133 ± 3.41	0.713 ± 0.008	Below detection	0.924 ± 0.004	0.302 ± 0.004	6.88 ± 0.455

Table 2.3: Environmental parameters measured at BATS for samples taken initially at time T_0 and 96 hours later at T_{96}. Temperature and salinity were obtained at the time of sampling. Nutrient data is the average ± standard deviations of historically recorded measurements taken between 40 and 60 meters below surface in the same seasons from 2005-2008.

Season	Sample time	Temper-ature (ºC)	Salinity (psu)	Bacterial production (pmol L^{-1} h^{-1})	Chl a (μg kg^{-1})	NO_3 (μmol kg^{-1})	NO_2 (μmol kg^{-1})	PO_4 (μmol kg^{-1})	Si (μmol kg^{-1})
Spring 2009	T_0	19.7	36.6	9.72 ± 0.897	0.232 ± 0.126	0.060 ± 0.099	0.075 ± 0.051	0.000 ± 0.000	0.839 ± 0.090
	T_{96}	19.7	36.7	13.8 ± 1.06					
Spring 2010	T_0	18.0	36.6	7.03 ± 0.437					
	T_{96}	18.8	36.6	6.76 ± 0.678					
Summer 2009	T_0	23.6	36.7	10.1 ± 1.13	0.100. ± 0.064	0.006 ± 0.032	0.008 ± 0.012	0.000 ± 0.000	0.581 ± 0.220
	T_{96}	24.3	36.6	14.5 ± 1.32					

Table 2.4: Samples analyzed in in subsequent figures and tables. SS = Sargasso Sea, MAB = Mid-Atlantic Bight. PA = particle-associated (≥ 0.8 µm) size fraction, FL = free-living (0.8 – 0.22 µm) size fraction. U = unlabeled with BrdU, L = labeled. W = not immunocaptured, U = immunocaptured. T_x = sample taken x hours into sampling cruise.

Code	Location	Season	Year	Size fraction	Labeled	Immunocaptured	Sampling Event
A1	SS	Spring	2009	PA	U	W	T_0
A2	SS	Spring	2009	PA	U	I	T_0
A3	SS	Spring	2009	PA	L	I	T_0
A4	SS	Spring	2009	PA	U	W	T_{96}
A5	SS	Spring	2009	PA	U	I	T_{96}
A6	SS	Spring	2009	PA	L	I	T_{96}
A7	SS	Summer	2009	PA	U	W	T_0
A8	SS	Summer	2009	PA	U	I	T_0
A9	SS	Summer	2009	PA	L	I	T_0
A10	SS	Summer	2009	PA	U	W	T_{96}
A11	SS	Summer	2009	PA	U	I	T_{96}
A12	SS	Summer	2009	PA	L	I	T_{96}
A13	SS	Spring	2010	PA	U	W	T_0
A14	SS	Spring	2010	PA	U	I	T_0
A15	SS	Spring	2010	PA	L	I	T_0
A16	SS	Spring	2010	PA	U	W	T_{96}
A17	SS	Spring	2010	PA	U	I	T_{96}
A18	SS	Spring	2010	PA	L	I	T_{96}
B1	SS	Spring	2009	FL	U	W	T_0
B2	SS	Spring	2009	FL	U	I	T_0
B3	SS	Spring	2009	FL	L	I	T_0
B4	SS	Spring	2009	FL	U	W	T_{96}
B5	SS	Spring	2009	FL	U	I	T_{96}
B6	SS	Spring	2009	FL	L	I	T_{96}

Table 2.4 continued.

Code	Location	Season	Year	Size fraction	Labeled	Immunocaptured	Sampling Event
B7	SS	Summer	2009	FL	U	W	T_0
B8	SS	Summer	2009	FL	U	I	T_0
B9	SS	Summer	2009	FL	L	I	T_0
B10	SS	Summer	2009	FL	U	W	T_{96}
B11	SS	Summer	2009	FL	U	I	T_{96}
B12	SS	Summer	2009	FL	L	I	T_{96}
B13	SS	Spring	2010	FL	U	W	T_0
B14	SS	Spring	2010	FL	U	I	T_0
B15	SS	Spring	2010	FL	L	I	T_0
B16	SS	Spring	2010	FL	U	W	T_{96}
B17	SS	Spring	2010	FL	U	I	T_{96}
B18	SS	Spring	2010	FL	L	I	T_{96}
C1	MAB	Spring	2009	PA	U	W	T_0
C2	MAB	Spring	2009	PA	U	I	T_0
C3	MAB	Spring	2009	PA	L	I	T_0
C4	MAB	Spring	2009	PA	U	W	T_{12}
C5	MAB	Spring	2009	PA	U	I	T_{12}
C6	MAB	Spring	2009	PA	L	I	T_{12}
C7	MAB	Summer	2009	PA	U	W	T_0
C8	MAB	Summer	2009	PA	U	I	T_0
C9	MAB	Summer	2009	PA	L	I	T_0
C10	MAB	Summer	2009	PA	U	W	T_{12}
C11	MAB	Summer	2009	PA	U	I	T_{12}
C12	MAB	Summer	2009	PA	L	I	T_{12}

Table 2.4 continued.

Code	Location	Season	Year	Size fraction	Labeled	Immunocaptured	Sampling Event
D1	MAB	Spring	2009	FL	U	W	T_0
D2	MAB	Spring	2009	FL	U	I	T_0
D3	MAB	Spring	2009	FL	L	I	T_0
D4	MAB	Spring	2009	FL	U	W	T_{12}
D5	MAB	Spring	2009	FL	U	I	T_{12}
D6	MAB	Spring	2009	FL	L	I	T_{12}
D7	MAB	Summer	2009	FL	U	W	T_0
D8	MAB	Summer	2009	FL	U	I	T_0
D9	MAB	Summer	2009	FL	L	I	T_0
D10	MAB	Summer	2009	FL	U	W	T_{12}
D11	MAB	Summer	2009	FL	U	I	T_{12}
D12	MAB	Summer	2009	FL	L	I	T_{12}

Table 2.5: Sequencing results of BrdU-labeled DGGE bands.

Ribotype	RDP Classification	Nucleotide sequence
1	*Synechococcus* Group IIa	TGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGC AACGCCGCGTGAGGGATGAAGGCCTCTGGGCTGTAAAC CTCTTTTATCAAGGAAGAAGATCTGACGGTACTTGATGA ATAAGCCNCGG
2	*Pseudoalteromonas*	TGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGC CATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAG CACTTTCAGTAAGGAGGAAAGGTTAAGTGTTAATAGCA CTTAGCTGTGACGTTACTTACAGAAGAAGCACCGGCTAA
3	*Flavobacteriaceae*	TGAGGAATATTGGGCAATGGAGGCAACTCTGACCCAGC CATGCCGCGTGCAGGAAGACGGCCCTATGGGTTGTAAA CTGCTTTTATACAGGAAGAACACCACCCACGTGTGGGTG ACTGACGGTACTGTAAGAANAAGGACCGGCT
4	*Synechococcus* Group IIa	TGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGC AACGCCGCGTGAGGGATGAAGGCCTCTGGGCTGTAAAC CTCTTTTATCAAGGAAGAAGATCTGACGGTACTTGATGA ATAAGCCACGG
5	*Vibrionaceae*	TGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGC CATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAG TACTTTCAGTTGTGAGGAAGGGGGTGTCGTTAATAGCG GNNNNCTNNNNNTTAGCAACAGAA
6	*Alphaproteobacteria*	TANGGAATATTGGACAATGGGGGAAACCCTGATCCAGC AATGCNGNGTGAGTGAAGAAGGCTTNGGGTTGTAAAAC TCTTTCATCAATNATNATAATNACATNANTTNAAGAAGAAG
7	*Alphaproteobacteria*	TAGGGAATATTGGACAATGGGGGAAACCCTGATCCAGC AATGCCGCGTGAGTGAAGAAGGCCTTCGGGTTGTAAAAC TCTTTCATCAATNANNATAATGACA

Table 2.5 continued.

Ribotype	RDP Classification	Nucleotide sequence
8	SAR11 *Pelagibacter*	TAGGGAATATTGCACAATGGAGGAAACTCTGATGCAGC GATGCCGCGTGAGTGAAGAAGGCCTTTGGGTTGTAAAG CTCTTTCGTCGGGGAAGAAAATGACTGTACCCGAATAAGAA
9	SAR11 *Pelagibacter*	TGGGGAATCTTGCACAATGGGGGAAACCCTGATGCAGC GATGCCGCGTGAGTGAAGAAGGCCCTTGGGTTGTAAAA CTCTTTCGTCGGGGAAGAAAATGACTGTACCCGAATAA GAAG
10, 13	*Bacteroidetes*	TAAGGAATATTGGTCAATGGACGAAAGTCTGAACCAGC CATGCCGCGTGCAGGATGACTGCCCTATGGGTTGTAAA CTGCTTTTATATAGGAAGAAAAACTTNNTCGTGAGAAA GCTTGACGGT
11, 14	*Bacteroidetes*	TAAGGAATATTGGTCAATGGACGAAAGTCTGAACCAGC CATGCCGCGTGCAGGATGACTGCCCTATGGGTTGTAAA CTGCTTTTATATAGGAAGAAAAACTTTCTCGTGAGAAA GCTTGACGGTACTATAAGA
12	*Gammaproteobacteria*	TGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGC CATACCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAG CACTTTAAGCAGGGAGAAAAAGTTATAAGTTAATACCT TATAACCGTGATGTTACCTGCAGAATAAGCACCGGCTA
15	*Flavobacteriaceae*	TGAGGAATATTGGACAATGGAGGCAACTCTGATCCAGC CATGCCGCGTGCAGGATGACGGCCNTATGGGTTGTAAA CTGCTTTTATGTAGGAAGAAACACNCTCACGTGTGAGA GCTTGACGGTNCTACAAGAATAAG
16	*Rhodobacteraceae*	TAGGGAATCTTAGACAATGGGGGCAACCCTGATCTAGC CATGCCGCGTGTGTGATGAAGGCCCTAGGGTCGTAAAG CACTTTCGCCAGAGATNATAATGACAGTATC

Table 2.6: Matrix of banding patterns from the particle-associated community profile of the Sargasso Sea. Sample key is in Table 2.4.

Sequenced ribotype	Band	Samples																	
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18
	1													+		+			
	2													+		+			
	3	+						+			+								
	4						+									+	+		+
	5						+			+	+		+			+	+		+
	6	+								+	+		+						
	7					+	+							+		+			
	8						+	+		+	+	+	+						
	9	+					+	+		+	+	+	+			+			+
	10	+		+			+	+		+				+		+			+
	11													+	+	+	+		+
	12							+			+								
2	13			+			+			+			+						
	14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15	+		+	+	+	+	+		+	+	+	+	+		+	+		
1, 4	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	18													+			+		+
	19	+	+	+	+	+	+	+	+	+	+	+	+						

Table 2.6 continued.

Sequenced ribotype	Band	Samples																	
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18
	20													+		+	+		+
	21	+	+	+	+	+	+		+	+		+	+	+		+	+		
	22	+		+			+						+	+	+	+	+	+	+
3	23	+			+	+		+	+	+	+	+	+				+	+	+
5	24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
	26																+		+
	27							+	+	+	+	+	+				+		
	28							+			+	+	+						
	29							+	+	+	+	+	+						
	30							+	+	+	+	+	+						
	31							+	+	+	+	+	+						

Table 2.7: Matrix of banding patterns from the free-living community profile of the Sargasso Sea. Sample key is in Table 2.4.

Sequenced ribotype	Band	Samples																	
		B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18
	1							+		+	+		+						
10, 13	2													+		+	+		+
11, 14	3			+	+		+							+		+	+		+
	4			+	+			+	+	+	+	+	+						
	5			+	+		+									+			+
12	6			+			+	+		+	+	+	+			+			+
6	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	9							+			+	+	+	+	+	+	+		+
	10	+	+			+	+							+	+	+	+	+	+
	11							+		+	+			+		+	+	+	+
	12	+	+		+	+	+	+		+	+			+	+	+	+	+	+
	13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	14	+			+			+			+								
	15	+	+	+	+	+	+							+			+		
	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2.8: Matrix of banding patterns from the particle-associated community profile of the Mid-Atlantic Bight. Sample key is in Table 2.4. No ribotypes bands from this profile were sequenced.

Band	Samples											
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
1			+	+			+			+	+	+
2							+			+	+	+
3	+		+	+								
4							+			+	+	+
5						+	+			+	+	+
6	+		+	+		+						
7	+		+	+		+	+			+	+	+
8										+	+	+
9	+	+	+	+	+	+	+			+	+	+
10										+	+	+
11	+		+	+		+	+			+		+
12	+		+	+			+				+	+
13										+	+	+
14	+	+	+	+	+	+	+					+
15				+			+			+	+	+
16							+			+	+	+
17	+		+	+	+	+	+			+		
18	+			+			+			+		
19	+			+			+			+		+
20	+			+	+		+			+		+
21	+		+	+	+	+	+			+	+	+
22			+	+			+				+	+
23	+	+	+	+	+	+	+			+	+	+
24			+	+		+	+		+	+	+	+
25	+	+	+	+	+	+	+			+	+	+
26				+			+			+	+	+
27	+	+	+	+	+	+						
28	+	+		+		+	+			+		
29										+	+	+

Table 2.9: Matrix of banding patterns from the free-living community profile of the Mid-Atlantic Bight. Sample key is in Table 2.4.

Sequenced ribotype	Band	Samples											
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
	1							+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+			+		
	4	+	+	+	+	+		+	+	+			
	5							+	+	+	+	+	+
15	6	+	+	+	+	+	+	+		+	+	+	+
	7							+	+				
	8	+	+	+	+	+	+	+	+		+	+	+
	9	+	+		+	+		+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+	+	+	+
	11	+	+	+	+			+					
	12	+	+	+	+	+	+	+	+	+	+	+	+
16	13			+			+	+	+	+	+	+	+
	14	+	+	+	+	+		+	+	+	+	+	+
	15		+	+		+	+	+	+	+	+	+	+
	16	+						+	+	+	+	+	+
	17	+	+	+	+	+	+	+	+	+	+	+	+
	18	+	+	+	+	+	+	+	+	+	+	+	+

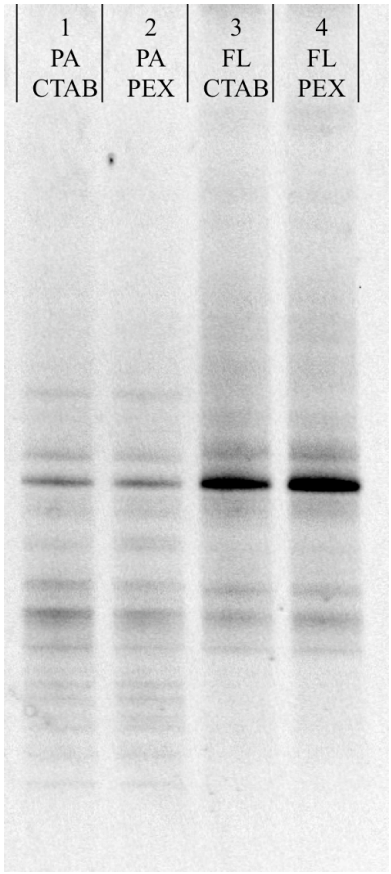

Figure 2.1: Structures of bacterial communities extracted with different protocols. DNA of particle-associated bacteria (≥ 0.8 µm in size) and free-living bacteria (0.8 – 0.22 µm) was extracted with CTAB and compared to analogous samples extracted with PEX-SDS using DGGE analysis. Lanes 1 and 2 are the particle-associated (PA) communities; lanes 3 and 4 are the free-living (FL) communities. Lanes 1 and 3 were extracted with CTAB. Lanes 2 and 3 were extracted with PEX-SDS.

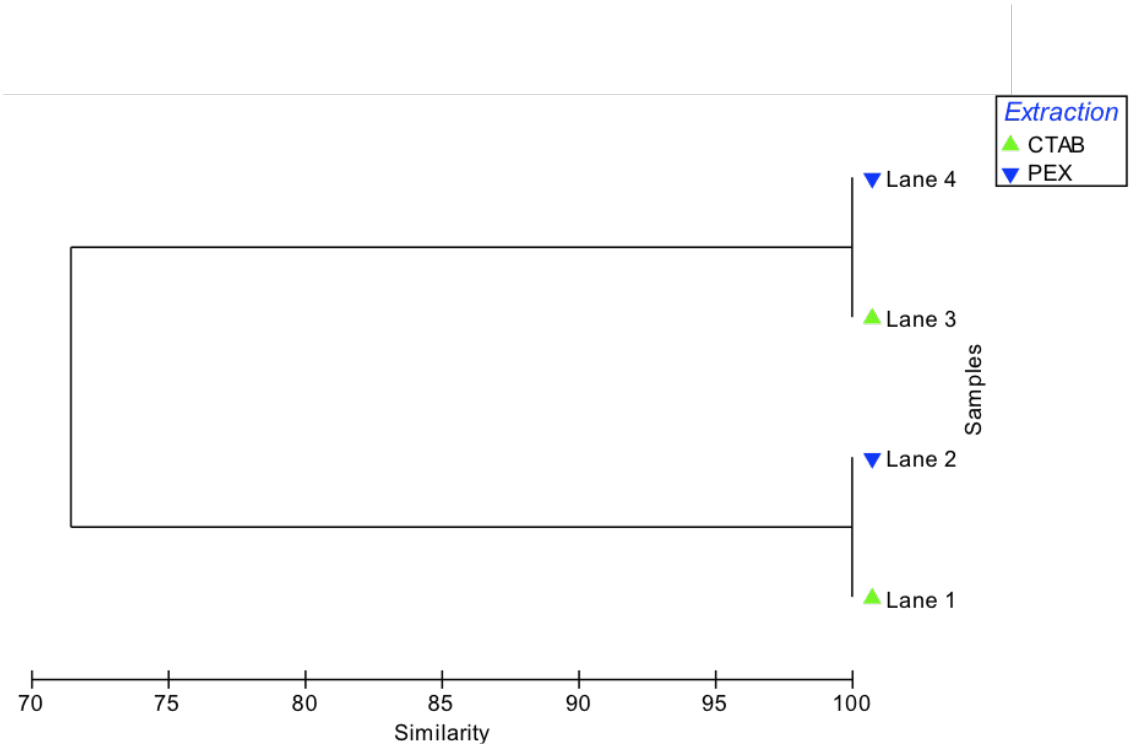

Figure 2.2: Bray-Curtis similarity dendrogram showing the relationships of microbial community structures obtained from two different extraction procedures. Lanes 1 and 2 are the particle-associated communities, and lanes 3 and 4 are the free-living communities. CTAB-extracted DNA was used for lanes 1 and 3, and PEX-SDS-extracted DNA was loaded into lanes 2 and 4.

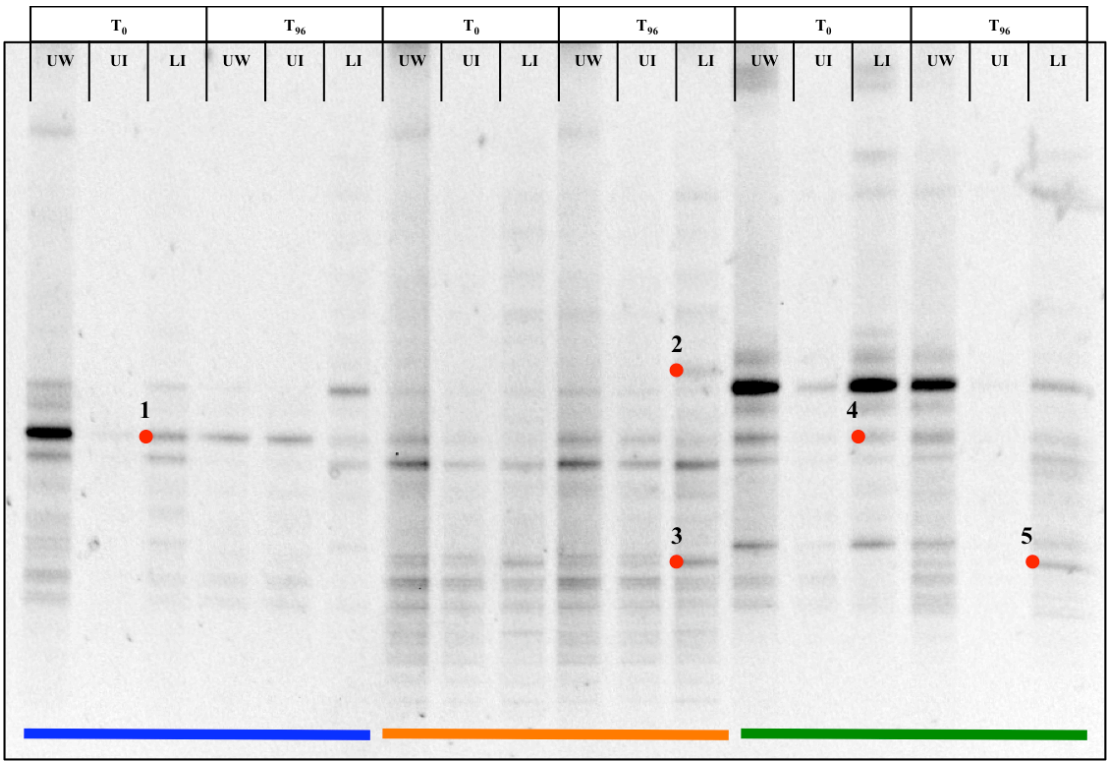

Figure 2.3: Structures of active and total particle-associated (≥ 0.8 µm) bacterial communities from 2009 and 2010 in the Sargasso Sea. Unlabeled bacterial DNA was compared with BrdU-labeled and immunocaptured bacterial DNA using DGGE analysis. Spring 2009 samples are indicated with a blue bar, summer 2009 samples are orange, and spring 2010 samples are green colored. Sample T_0 was taken initially in each cruise; sample T_{96} was taken 96 hours later. UW indicates unlabeled, not immunocaptured; UI is unlabeled, immunocaptured; and LI is labeled, immunocaptured DNA. Excised and sequenced bands are numbered.

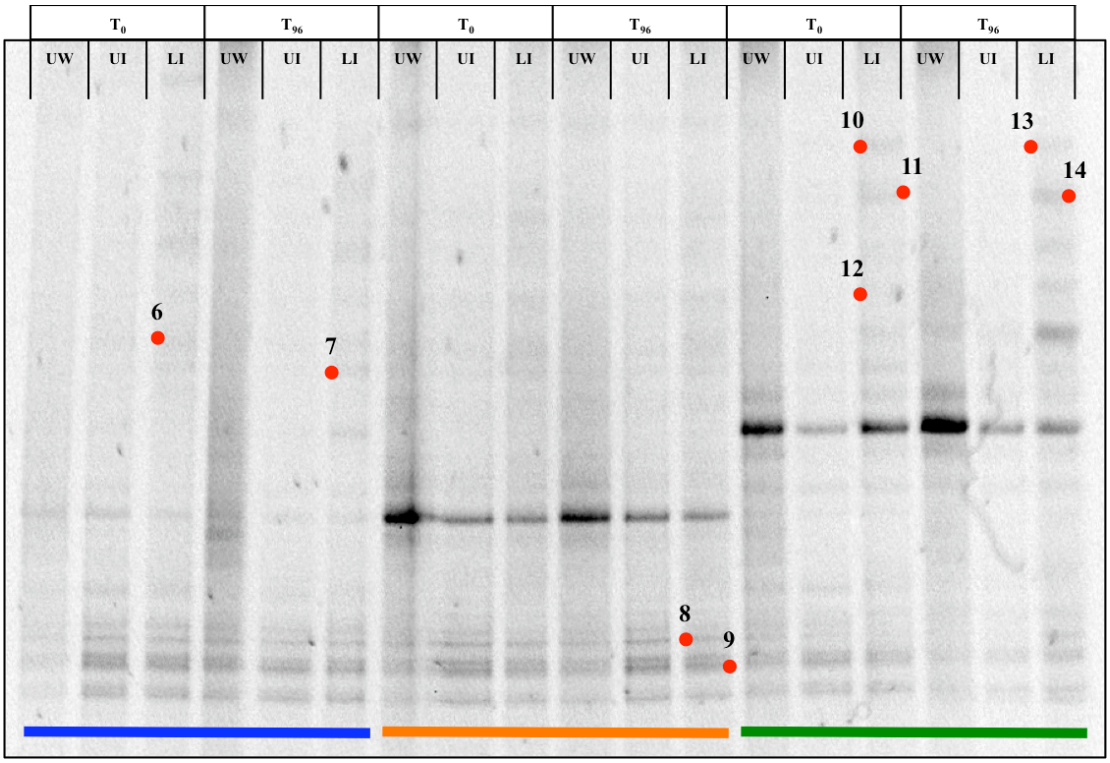

Figure 2.4: Structures of active and total free-living (0.8 – 0.22 µm) bacterial communities from 2009 and 2010 in the Sargasso Sea. Refer to the caption from Figure 2.3 for explanation of colors and abbreviations.

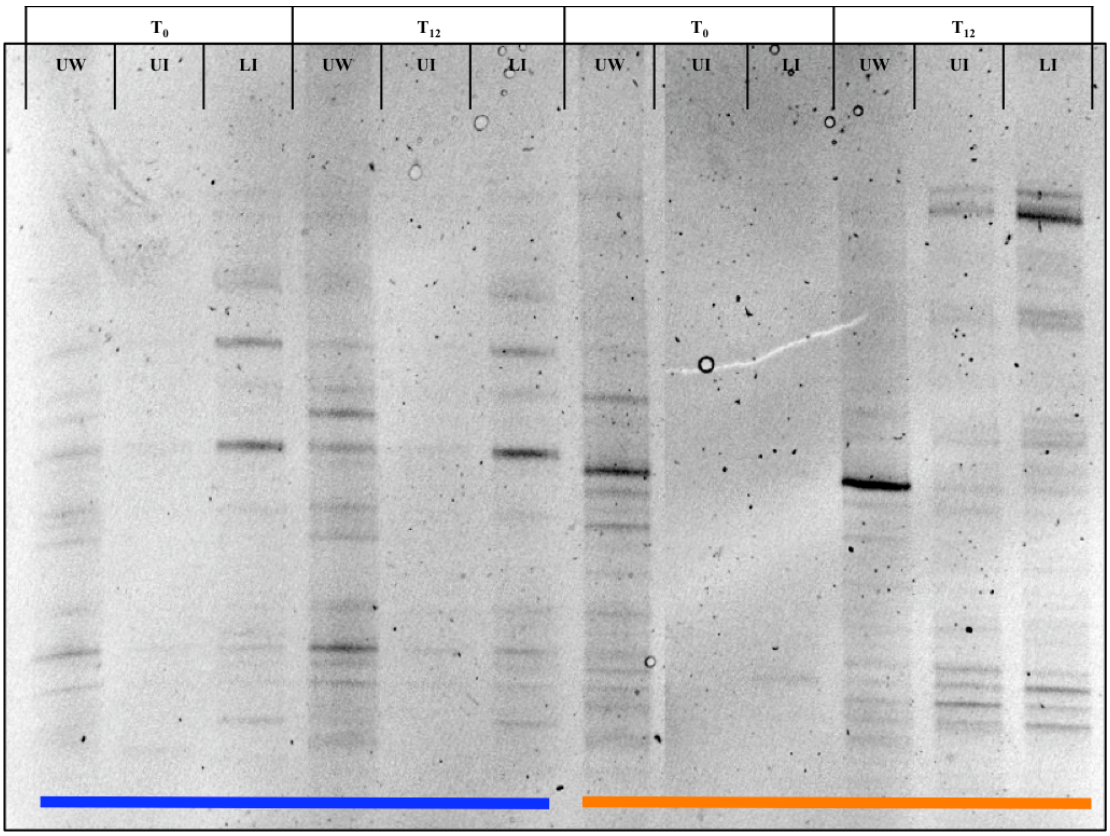

Figure 2.5: Structures of active and total bacterial particle-associated (≥ 0.8 µm) communities from 2009 in the Mid-Atlantic Bight. Unlabeled bacterial DNA was compared with BrdU-labeled and immunocaptured bacterial DNA using DGGE analysis. Spring samples are indicated with a blue bar, and summer samples are orange. Sample T_0 was taken initially in each cruise; sample T_{12} was taken 12 hours later. UW indicates unlabeled, not immunocaptured; UI is unlabeled, immunocaptured; and LI is labeled, immunocaptured DNA. Excised and sequenced bands are numbered.

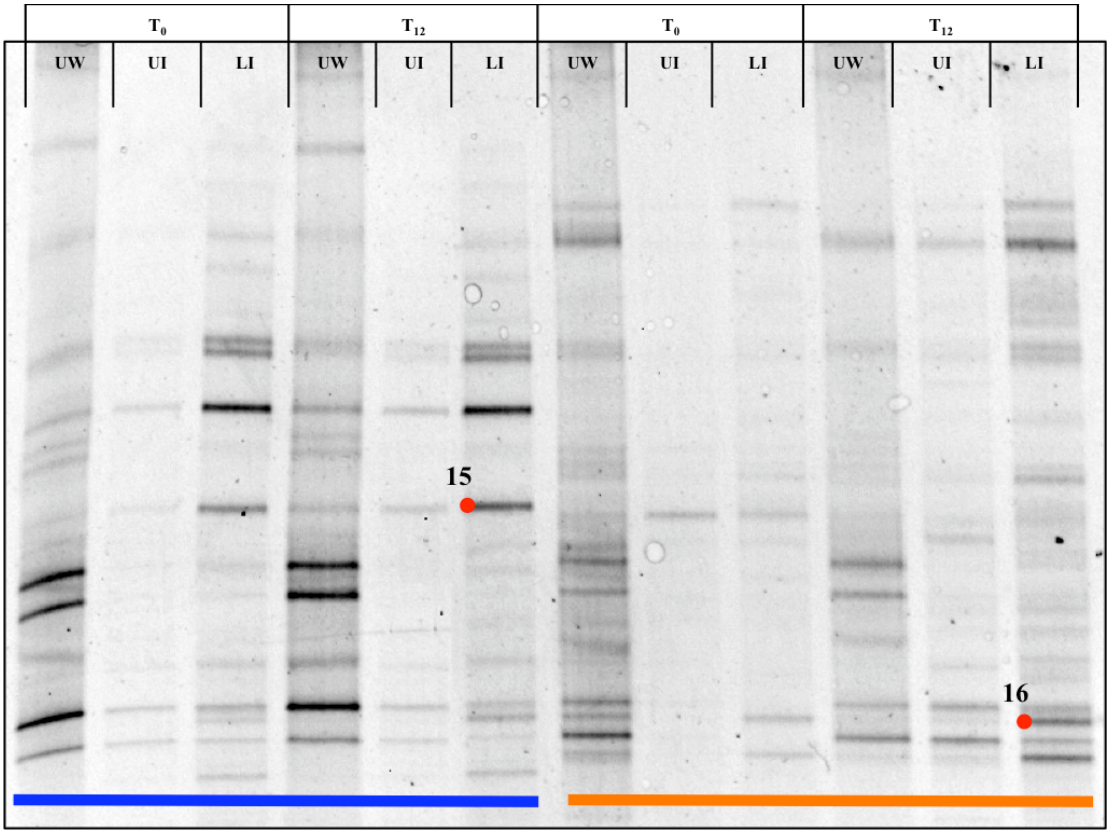

Figure 2.6: Structures of active and total free-living (0.8 – 0.22 µm) bacterial communities from 2009 in the Mid-Atlantic Bight. Refer to the caption from Figure 2.5 for explanation of colors and abbreviations.

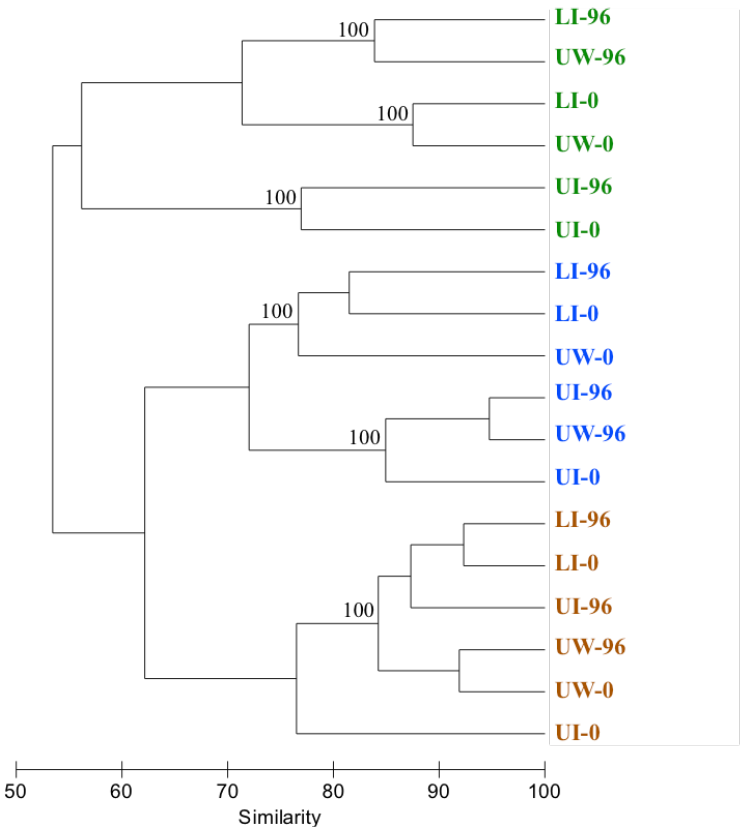

Figure 2.7: Bray-Curtis similarity dendrograms of particle-associated community structures of the Sargasso Sea. Presence/absence of ribotype bands characterized bacterial communities from each treatment. Spring 2009 samples are indicated with blue font, summer 2009 samples are orange, and spring 2010 samples are green colored. UW indicates unlabeled, not immunocaptured; UI is unlabeled, immunocaptured; and LI is labeled, immunocaptured DNA. Samples taken initially and 96 hours later in each cruise are marked with '0' or '96' respectively. Significant clusters ($p<0.05$) are presented with percent agreement.

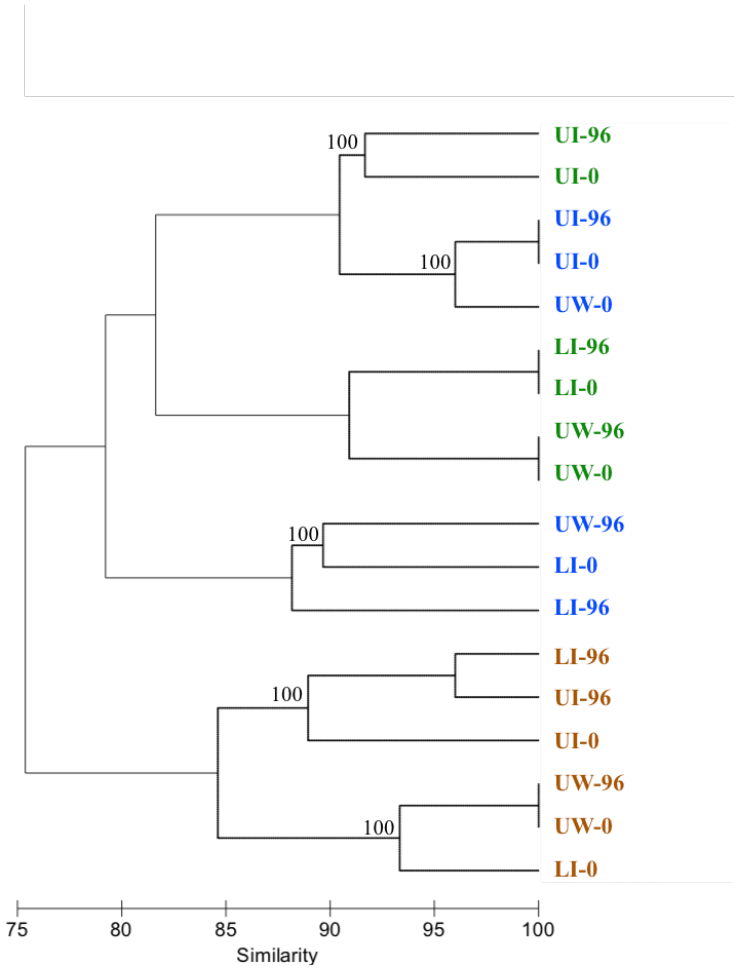

Figure 2.8: Bray-Curtis similarity dendrograms of free-living community structures of the Sargasso Sea. Refer to the caption from Figure 2.7 for explanation of colors and abbreviations.

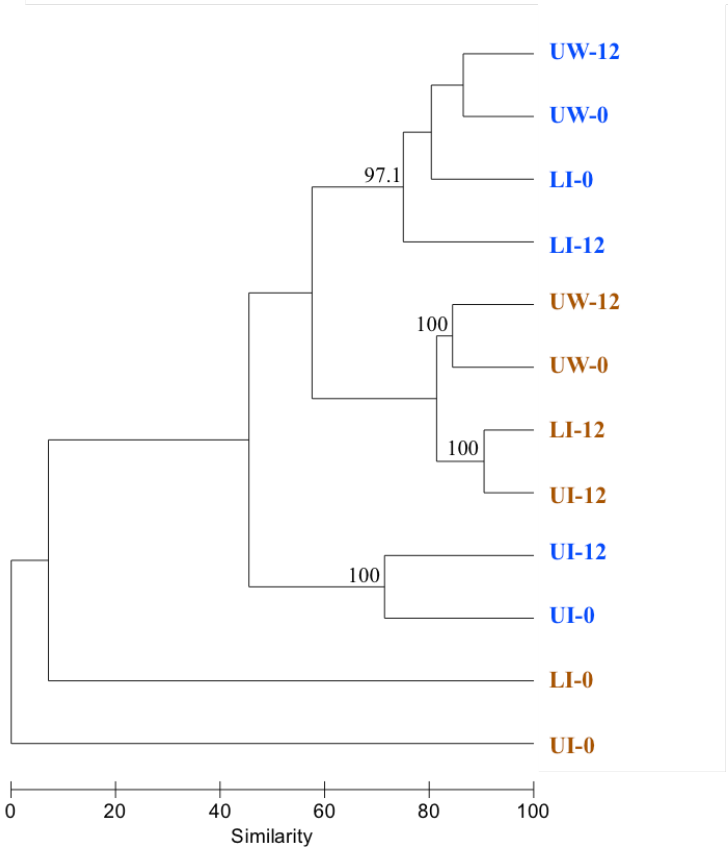

Figure 2.9: Bray-Curtis similarity dendrograms of particle-associated community structures of the Mid-Atlantic Bight. Presence/absence of ribotype bands characterized bacterial communities from each treatment. Spring 2009 samples are indicated with blue font, and summer 2009 samples are orange. UW indicates unlabeled, not immunocaptured; UI is unlabeled, immunocaptured; and LI is labeled, immunocaptured DNA. Samples taken initially and 12 hours later in each cruise are marked with '0' or '12' respectively. Significant clusters ($p<0.05$) are presented with percent agreement.

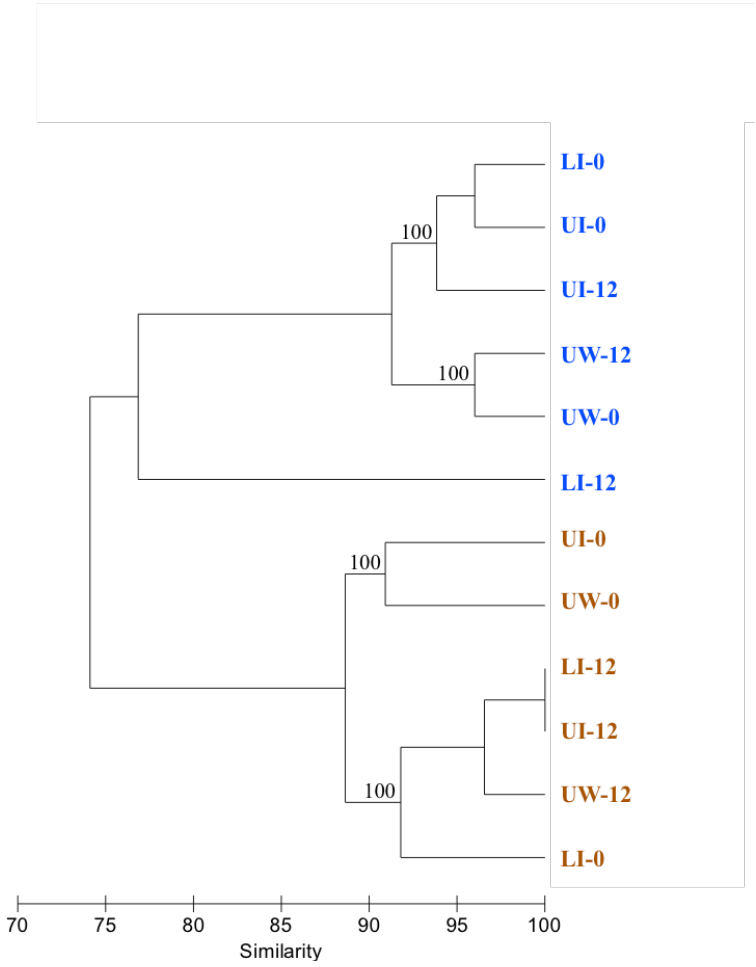

Figure 2.10: Bray-Curtis similarity dendrograms of free-living community structures of the Mid-Atlantic Bight. Refer to the caption from Figure 2.9 for explanation of colors and abbreviations.

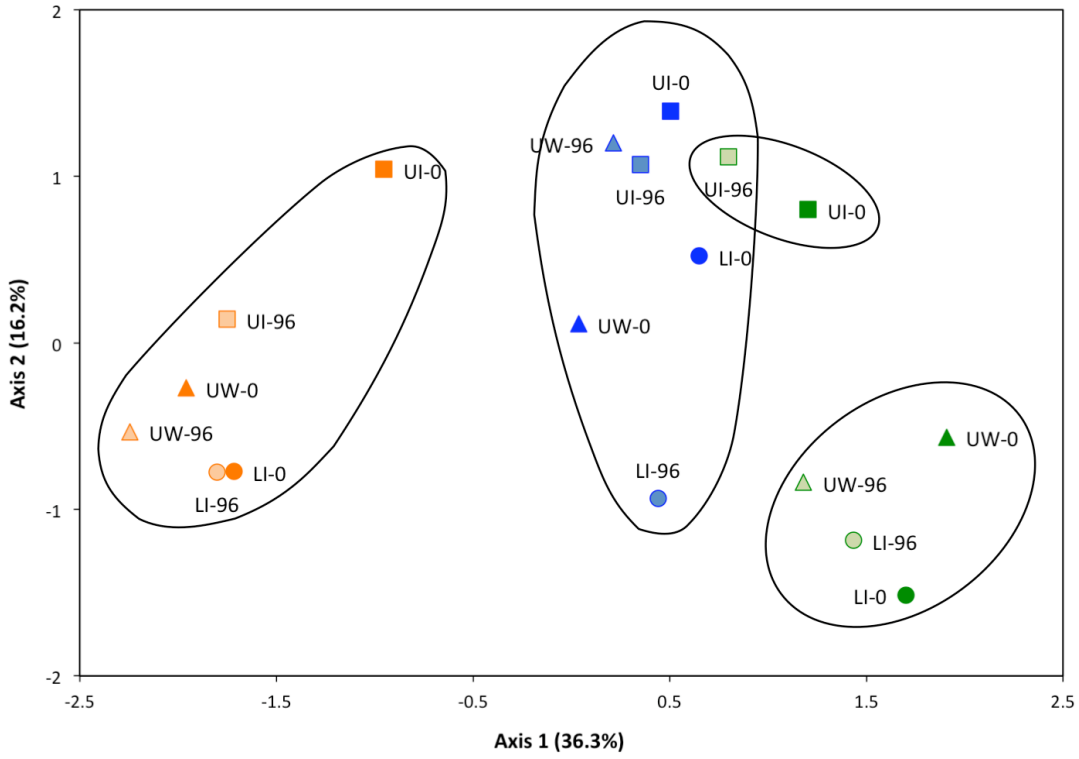

Figure 2.11: Principal component analysis (PCA) of particle-associated bacterial community similarities in the Sargasso Sea. Bray-Curtis similarity matrices were constructed from presence/absence of ribotype bands. Spring 2009 samples are indicated with blue font, summer 2009 samples are orange, and spring 2010 samples are green colored. UW and the triangle symbol indicates unlabeled, not immunocaptured; UI and square symbol is unlabeled, immunocaptured; and LI and circular symbol is labeled, immunocaptured DNA. The sample taken initially in each event is marked with '0' and a filled symbol; the sample taken 96 hours later is marked with '96' and an empty symbol. Samples are clustered at 70% similarity.

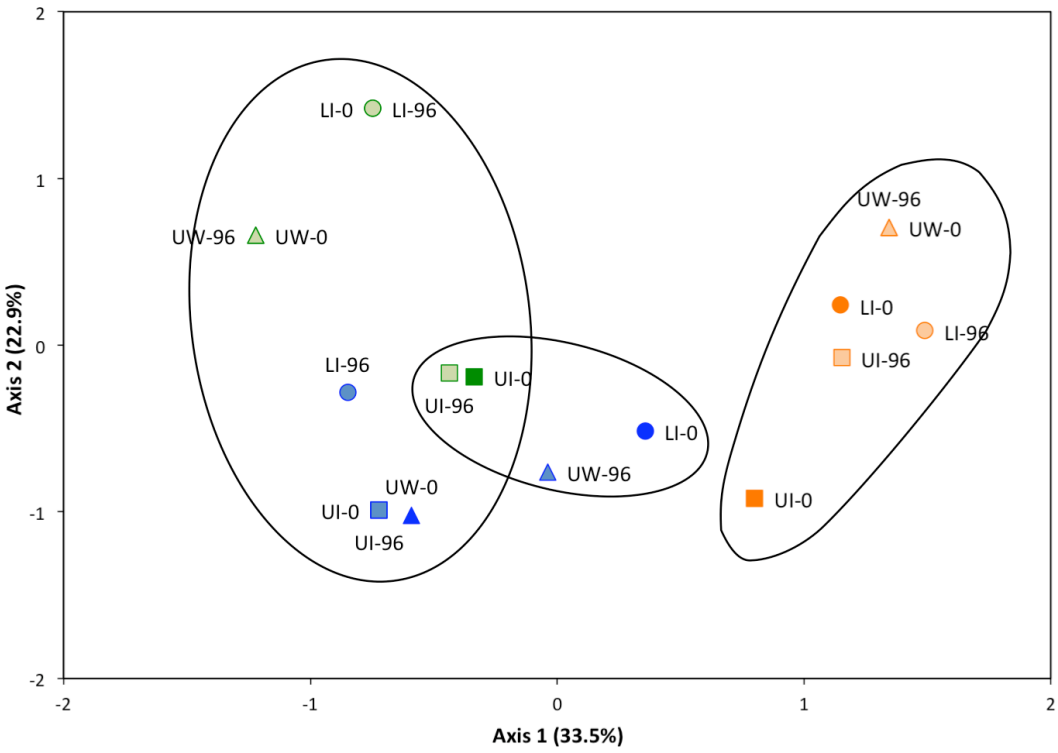

Figure 2.12: Principal component analysis (PCA) of free-living bacterial community similarities in the Sargasso Sea. Refer to the caption from Figure 2.11 for explanation of colors and abbreviations. Samples are clustered at 80% similarity.

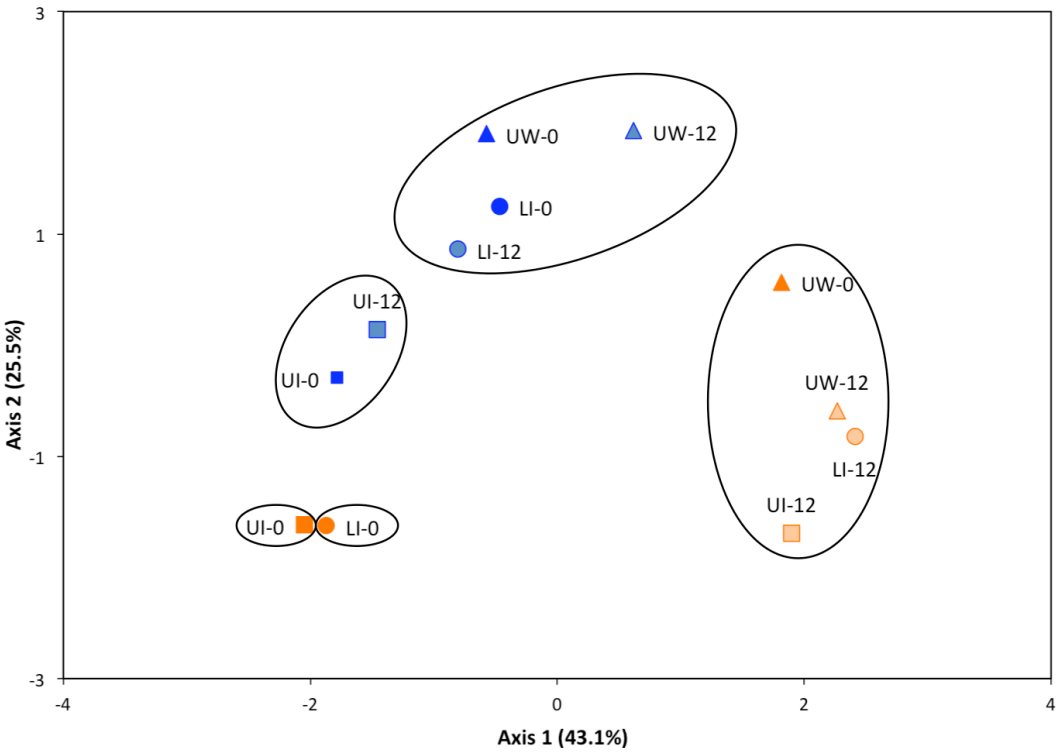

Figure 2.13: Principal component analysis (PCA) of particle-associated bacterial community similarities in the Mid-Atlantic Bight. Bray-Curtis similarity matrices were constructed from presence/absence of ribotype bands. Spring 2009 samples are indicated with blue font, and summer 2009 samples are orange. UW and the triangle symbol indicates unlabeled, not immunocaptured; UI and square symbol is unlabeled, immunocaptured; and LI and circular symbol is labeled, immunocaptured DNA. The sample taken initially in each event is marked with '0' and a filled symbol; the sample taken 12 hours later is marked with '12' and an empty symbol. Samples are clustered at 60% similarity.

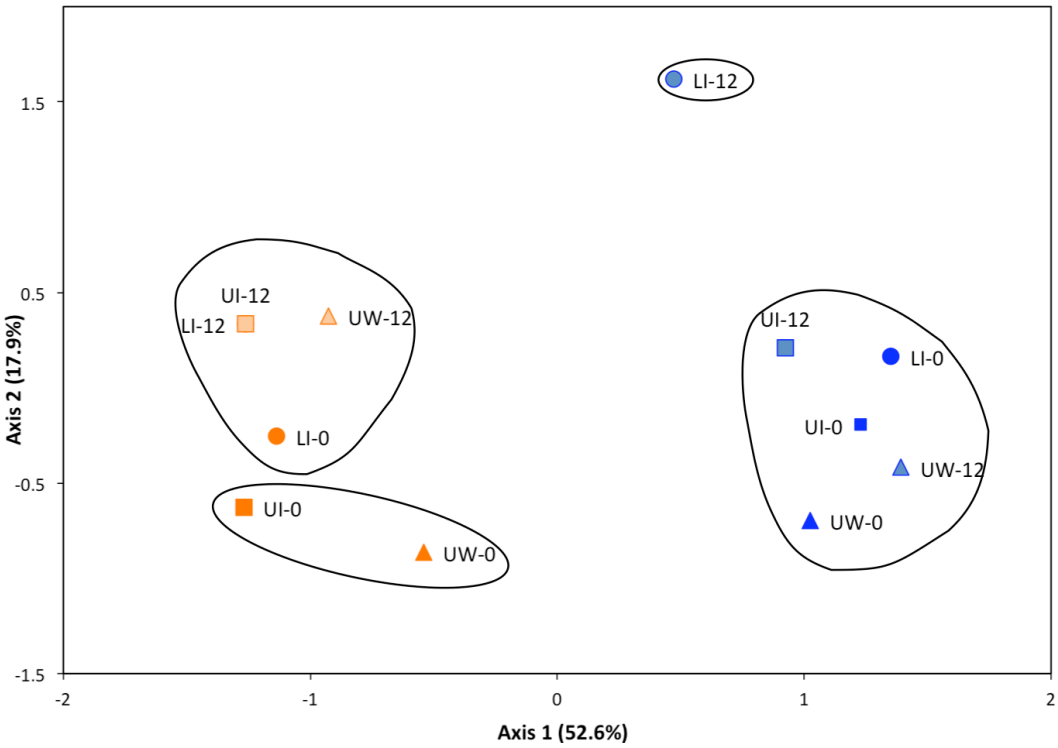

Figure 2.14: Principal component analysis (PCA) of free-living bacterial community similarities in the Mid-Atlantic Bight. Refer to the caption from Figure 2.13 for explanation of colors and abbreviations. Samples are clustered at 90% similarity.

Chapter 3

ACTIVITY AND STRUCTURE OF BACTERIAL ASSEMBLAGES IN THE SARGASSO SEA AND MID-ATLANTIC BIGHT

3.1 Abstract

In the aquatic environment, bacterial communities are diverse in their structures and activities. With bacteria playing such an important role in biogeochemical processes, assessing both structure and activity of marine bacterial communities is important to understanding the impact of these assemblages on their environment. This study evaluated the linkage between bacterial activity and community composition. Active bacteria were identified via labeling of bacterial rRNA genes with 5-bromo-2'-deoxyuridine (BrdU), and the active and total communities collected over two years from the Mid-Atlantic Bight off the Delaware Coast and the Sargasso Sea were compared via tag pyrosequencing analysis of 16S rDNA V1-V2 region. I found that activity levels of most bacterial taxa were representative of their abundances in the total community. However, many bacteria that had significant differences in activity were also rare. In the Sargasso Sea, SAR86 (*Gammaproteobacteria*) was highly active in the spring when members were less abundant. Rhodobacterial types (*Alphaproteobacteria*) were less than 5% of the total community in the Mid-Atlantic Bight, but were regularly more active than their abundances indicated. In both environments, ribotypes identified as *Flavobacteria*

(*Bacteroidetes*) were consistently more active than suggested by their abundances. Environmental conditions that timed spring mixing and the subsequent phytoplankton bloom, as well as activity levels of rare bacteria, influenced the composition of the active and total bacterial community in two sampling events. Some abundant bacteria, including SAR11 and cyanobacterial types, were not observed to have proportionally high activity levels, which may be due to varying types of activity in the community that are not targeted seen in this study. In both environments and sampling seasons I observed abundant and rare bacterial ribotypes with varying levels of activity. This study compared activity levels and abundances of bacterial groups on fine phylogenetic and temporal scales. These results provide insight into the complex relationship between bacterial taxa, individual activity and community structure in the marine environment.

3.2 Introduction

The marine microbial population consists of innumerable taxa with a suite of metabolisms available to them (Kirchman, 2008). Activity of the bacterial community can be described with cellular characteristics such as replication, substrate uptake, and nucleic acid content, among others (del Giorgio and Gasol, 2008). Recent efforts have been taken to explore the link between activity and structure of marine bacterial communities (Alonso-Sáez *et al.*, 2009, Campbell *et al.*, 2009, Teira *et al.*, 2010, Campbell *et al.*, 2011). Abundant bacteria are hypothesized to contribute more to bacterial activity than rare bacteria; for example, SAR11 is one of the most abundant clades in aquatic systems with growth rates on par with other bacteria (Teira

et al., 2010, Malmstrom *et al.*, 2005, Morris *et al.*, 2002). On the other hand, some rare bacteria may be slow growing or inactive (Pedrós-Alió, 2006). With these discrepancies apparent yet not fully understood, the relationship between bacterial community composition and activity needs further exploration.

The marine bacterial community consists of a comparatively small number of very abundant taxa, and several rare taxa (Sogin *et al.*, 2006, Pedrós-Alió, 2007, Gilbert *et al.*, 2009). Conventional molecular techniques build upon the current knowledge of bacterial ribotypes—that is, the most abundant types—to characterize the community, and thus fail to pick up rare bacterial taxa (Pedrós-Alió, 2006). Massively parallel tag pyrosequencing is appropriate for characterizing the low-abundance, high-diversity bacterial ribotypes. High-throughput pyrosequencing of the hypervariable 16S rRNA regions provides the basis for examining the community structure, species richness and taxonomy of marine microbial communities (Sogin *et al.*, 2006, Huber *et al.*, 2007, Mou *et al.*, 2008). This approach can be used to parameterize not only the total community, via evaluation of 16S rRNA genes (Muyzer *et al.*, 1993), but also the active bacterial community. Sequencing the 16S rRNA gene (rDNA) and 16S rRNA yields a comparison of the growth rates of bacterial ribotypes (Jones and Lennon, 2010). Using this metric in the Delaware Bay, Campbell *et al.* found that while abundance correlated with activity overall, several rare bacteria, including members of SAR11, *Gammaproteobacteria*, and *Bacteroidetes*, were very active (Campbell *et al.*, 2011). Another approach to evaluating activity is to sequence gene expression profiles in combination with addition of a substrate. This method measures bacterial activity from substrate

incorporation or degradation. In the Sargasso Sea *Gammaproteobacteria* and *Bacteroidetes* were more actively degrading dimethysulfoniopropionate (DMSP) than other known DMSP-degraders such as *Roseobacter* and SAR11 (Vila-Costa *et al.*, 2010). Sequencing the total and active bacterial community is a comprehensive approach to address the relationship between bacterial community structure and activity.

Uptake of 5-bromo-2'-deoxyuridine (BrdU) into marine bacterial rDNA can be combined with community profiling techniques to describe the active bacterial fraction of the total community (Steward and Azam, 1999). Bacteria that incorporate BrdU are actively replicating their DNA. The active community as assessed with BrdU-labeling is often different from the total community, and from the profiles obtained with other activity proxies (Malmstrom *et al.*, 2005, Hamasaki *et al.*, 2007, Taniguchi and Hamasaki, 2008). However, these approaches have analyzed activities of bacterial groups that are relatively abundant at 5% or more of the community. The activity of less abundant and rare bacterial groups has not been assessed using BrdU-labeling. As inputs into pyrosequencing, BrdU-labeled rDNA sequences would provide insight into an active population of bacteria that are in low abundance in the total rDNA sequenced community.

The goal of this study was to examine the relationship between bacterial community structure and activity by sequencing 16S rDNA and BrdU-labeled rDNA of bacterial taxa sampled over two years in different seasons and locations. I hypothesized that the active community structure would differ from the total community structure, and that some bacterial groups would be more active than their

abundances in the total community would indicate. The compositions of total and active spring and summer communities from a microbial observatory in the Mid-Atlantic Bight adjacent to the Delaware estuary and from the Bermuda Atlantic Time-series Study (BATS) station in the Sargasso Sea were evaluated via BrdU-labeling and 454 tag pyrosequencing. Within each location, community composition in both the active and total communities varied between seasons and between years. Additionally, while some bacterial groups were more active than others, the overall structures between the active and total communities were similar.

3.3 Materials and Methods

3.3.1 Sample Collection

Samples were obtained in spring and summer of 2009 and 2010 from the Bermuda Atlantic Time-series Study (BATS) site (31º40'N, 64º10'W) and from a microbial observatory site FB at the Mid-Atlantic Bight adjacent to the Delaware Bay (38º46'N, 74º55'W) (Table 3.1). BATS is located in the oligotrophic Sargasso Sea, whilst FB is in a coastal ocean system. Seawater was collected 50 m below the surface at BATS and 1 m below the surface at FB. Various standard oceanographic parameters were measured, including water temperature, salinity, bacterial production via the microcentrifugation method, and nutrients (Tables 3.2 and 3.3), as described previously (Steinberg *et al.*, 2001, Cottrell *et al.*, 2006, Campbell *et al.*, 2009).

3.3.2 BrdU-labeling

Duplicate 10 L samples at BATS and 2 L samples at FB were incubated for 6 h with a final concentration of 10 μM BrdU at in situ light and temperatures. Duplicate controls were incubated without BrdU. At FB, samples collected from night and day from a single sampling trip were labeled with BrdU. At BATS, samples were collected daily at 0600 local time over four days. Water was prefiltered through 0.8 μm polycarbonate filters (Isopore; Millipore), and the filtrate was passed through 0.22 μm filters (Durapore; Millipore) to collect free-living bacteria. Filters were stored at -80ºC until processed.

3.3.3 Extraction and Immunocapture of BrdU-labeled DNA

DNA from the 0.22 μm size fraction was extracted as described in Chapter 2, using either the potassium ethyl xanthogenate-sodium dodecyl sulfate protocol (PEX-SDS) described by Tillett and Neilan (2000) with modifications or acetyltrimethylammonium bromide-polyvinylpyrrolidone-β-mercaptoethanol (CTAB/PVP/β-ME) method (Dempster *et al.*, 1999). Samples from March 2009 at BATS were extracted with PEX-SDS; all other samples used in this study were extracted with the CTAB protocol. After extraction, 0.1 μg of DNA was subjected to BrdU immunocapture, as detailed in Chapter 2. Night and day samples from FB (T_0 and T_{12}), and samples from the first and last day from BATS (T_0 and T_{96}) were immunocaptured. Unlabeled environmental samples from the same sampling events as well as a blank H_2O-only negative control were processed in the same manner.

3.3.4 PCR Amplification

Bacterial 16S rRNA genes were amplified from 1.0 μL of immunocaptured, BrdU-labeled or unlabeled DNA-bead mixture, as well as 1.0 ng of nonimmunocaptured unlabeled DNA. Pre-designed primer sets were used to amplify a fragment of the hypervariable V1 through V3 regions of the 16S rRNA genes (Hamady *et al.*, 2008; Chun *et al.*, 2010). The composite forward primer was 5'-CGTATCGCCTCCCTCGCGCCATCAG-X-**AGAGTTTGATCMTGGCTCAG**-3'; primer A is the underlined sequence, the bold sequence is universal bacterial primer 27F, and in between is a 7- or 8-base sequence (X). Each sample was designated a unique barcode sequence X which was used to identify amplified sequences by sample (Table 3.4) (Hamady *et al.*, 2008). The reverse primer was 5'-CTATGCGCCTTGCCAGCCCGCTCAG-**ATTACCGCGGCKGCTGG**-3', consisting of the underlined sequence primer B and the bold sequence the broad range bacterial primer 519R. All PCR reactions contained 1.0 μL of sample, 0.25 μL of the reverse primer (stock concentration of 10 μM), 2.5 μL of the forward primer (1.0 μM), 0.5 μL of dNTPs (10 mM), 1.5 μL of $MgCl_2$ (25 mM), 0.5 μL of bovine serum albumin (10 mg mL^{-1}), 2.5 μL of AmpliTaq 10X PCR Buffer II, and 0.625 units of AmpliTaq DNA polymerase, in UV-treated PCR water to a final volume of 25 μL. The buffer, Taq polymerase, and $MgCl_2$ were from Applied Biosystems. Cycle number was optimized to minimize amplification of immunocaptured, unlabeled DNA, plateau effects and PCR artifacts, and was generally between 24-32 cycles. The denaturing step was 5 minutes at 95ºC, annealing was at 56ºC for 30 seconds, extension was for 1.5 minutes at 72ºC, and a final extension step was 7 minutes at

72ºC. Samples were amplified in triplicate in 25 μL reactions and pooled. The amplicons of total rRNA genes and BrdU-labeled rRNA genes were gel-purified of primers and salts using the QIAQuick Gel Extraction Kit (Qiagen), pooled with other tag-amplified sequences, and sent to Research and Testing Laboratory (Lubbock, Texas) to be pyrosequenced on the 454 GS/FLX Titanium Sequencing System (Roche).

3.3.5 Sequence Analyses

The total number of sequences from the pooled amplicons was 713,833. Quality filtering and denoising was performed in QIIME (Caporaso *et al.*, 2010). Sequences were quality filtered to the following parameters: minimum length = 200 bp, maximum length = 700 bp, minimum average quality score = 5, sliding window test of quality score = 25, maximum homopolymers = 6, maximum number of Ns = 0, and no barcode correction was allowed. After quality filtering and denoising, 250,034 sequences remained. In mothur (Schloss *et al.*, 2009), sequences were further trimmed, dereplicated, aligned against the Silva bacterial template, and screened for chimeras. The resulting pool of sequences was 165,987, from which datasets for each location were extracted. The number of sequences assigned to samples taken from the Mid-Atlantic Bight (MAB) was 49,931, and 75,187 sequences were assigned to samples from the Sargasso Sea (SS). To remove non-overlapping sequences and facilitate constructing distance matrices, MEGA 5.0 (Tamura *et al.*, 2011) was used to remove nucleotides after position 1207 in the aligned MAB sequence set, and after position 1233 in the SS sequences set, generating an average number of bases of 277

and 290, respectively, covering the V1-V2 region. The sequence sets were dereplicated and preclustered to remove noise, yielding 21,355 for the MAB set and 33,102 sequences for the SS set. Distance matrices of 0.25 dissimilarity were constructed from the resulting sets, and operational taxonomic units (OTUs) were clustered at 0.03 distance using the average neighbor algorithm. Sequences from OTUs were classified using the Silva taxonomy and Bayesian classifier, and those identified as chloroplasts were eliminated from further analyses. To compare samples at equal sample size, the number of sequences per sample was rarefied in QIIME, and the resulting OTU table was resampled 100 times and averaged to generate a mean OTU table. The Sargasso sample set was rarefied to 1100 sequences; three samples contained fewer than 1100 sequences and were excluded from the final OTU table. Samples taken in spring 2010 from the Mid-Atlantic Bight were rarefied to 613 sequences, and the remaining MAB samples were rarefied to 1146 sequences; two samples contained fewer than 613 sequences and were excluded from the dataset. I calculated percent composition on varying levels of taxonomic classification in QIIME. I used QIIME to calculate rarefaction curves for alpha diversity, and mothur to calculate nonparametric θ_{YC} (Yue and Clayton, 2005) for similarities in community structure, and to generate principal component analyses.

3.3.6 Statistical Analyses

Reduced major axis regressions (RMA) were calculated on the percent compositions of OTUs in each rarefied and averaged community, with the total community as the x-variable and either the unlabeled, immunocaptured or the active

community as the y-variable. The frequencies of OTUs in the active and total communities were tested if they were significantly different ($p < 0.01$) from each other as described in Campbell *et al.* (2009) with modification. Briefly, the statistical test presented in Audic and Claverie (1997) was used for comparisons where the OTU was rare in both populations (≤ 1% community composition). The standard two-population proportions test (Christensen, 1992) was used in all other instances. $P < 0.01$ was chosen for stringency in these tests.

3.4 Results

3.4.1 Sampling Effort

Two sampling events per season for four seasons from each location were evaluated for community composition, activity and efficiency from BrdU immunocapturing, for a total of 48 samples (Table 3.4). Bacterial incorporation of BrdU approximates DNA replication, thus for these analyses the labeled community represents the active bacterial fraction. Samples from the Sargasso Sea had on average 3544 sequences representing 326 observed OTUs at 3% dissimilarity (Table 3.5). The nonparametric Chao1 species richness estimator predicted an average number of 833 OTUs per sample. Samples were relatively even with an average reciprocal Simpson index of 9.5. From the Mid-Atlantic Bight, samples had on average 2606 sequences representing 264 observed OTUs at 3% dissimilarity (Table 3.6). The Chao1 richness estimator predicted an average number of 674 OTUs per sample. The average reciprocal Simpson index was 5.4, indicating higher species unevenness compared to the Sargasso Sea.

The rarefaction curves (Figures 3.1-3.2) suggest that sampling did not exhaust bacterial richness in these samples. Additionally, in the Sargasso Sea relatively more OTUs were observed in spring than in the summer. The opposite trend was observed in the Mid-Atlantic Bight, with summer samples having more OTUs than spring samples. OTU evenness (Figures 3.3-3.4) was well-estimated with sampling effort. In general, active communities in the Sargasso Sea were less even than their total community counterparts. Some seasonal trends were observed, in that summer 2010 samples were more even than spring 2010 samples. However, both sample sets in 2009 had similar species evenness. In the Mid-Atlantic Bight, I also observed that active communities were generally more uneven than their total community counterparts. A pattern of seasonality was less obvious in the coastal samples.

3.4.2 Variation in Community Structure

I described variation in community structure by evaluating principal coordinate analyses (PCoA) from dissimilarity matrices based on θ_{YC} (Yue and Clayton, 2005). Samples from the Sargasso Sea were different in community structures between treatments and sampling events (Figure 3.5). Bacterial communities in spring 2009 clustered together and away from other sampling events. Summer samples from 2009 and 2010 grouped together as well. On the other hand, community structures of samples from spring 2010 were different from each other and samples from other events. Furthermore, communities from sample T_0 of that spring were distinctly separated from those of sample T_{96}. In general the structures of

active bacterial communities were relatively dissimilar to their respective total communities, but were not similar to each other in each season.

Community structures in the Mid-Atlantic Bight also varied between season and sample (Figure 3.6). Structures tended to cluster together based on sampling event. In addition, there was more dissimilarity between samples of summer 2009 and the following summer, than between spring and summer samples from 2010. Samples from spring 2009 were highly dissimilar in community structure between sampling events and treatments. Structures of the labeled fractions were distinctly separate from the total and unlabeled communities. However, the total communities of spring 2009 were comparable in structure to those of spring 2010. Overall the active communities were different in structure from their complementary total and unlabeled communities, with higher distinction between the labeled communities of spring 2009 and other samples.

3.4.3 Regressing the Active and Total Communities

Sample sets from each location were rarefied to a prescribed sequence number to incorporate a majority of samples. Rarefied data was resampled 100 times, and the resulting data was averaged for each sample. I calculated percent composition of each OTU in the rarefied, averaged communities. To model similarity in community structure, I analyzed paired community compositions using reduced major axis regression on the percent contribution of OTUs in each community.

In the Sargasso Sea community profile (Table 3.7), I calculated RMA regression on the unlabeled, immunocaptured (U) samples against the total,

nonimmunocaptured (W) communities, for five pairwise comparisons. Contributions of OTUs to the W community were on par with their representation in the U community (average slope = 0.99 ± 0.14, y-intercept = $1.0x10^{-3}$ ± $6.0x10^{-3}$, R^2 = 0.92 ± 0.07). As a result, the U community will not be considered further. I also regressed the labeled, immunocaptured (L) community against the total community (average slope = 0.91 ± 0.12, y-intercept = $4.0x10^{-3}$ ± $6.0x10^{-3}$, R^2 = 0.91 ± 0.07). The regressions for spring 2010 sample T_{96} are shown with their respective pairwise community comparisons in Figures 3.7-3.8. These equations suggest that in general activity of individual taxa can be predicted based on their abundance, with more abundant taxa having more representation in the active community and rare taxa having low contribution to the active community. However, not all OTUs had equal abundance in the active and total communities.

Using RMA regression I evaluated U vs. W and L vs. W communities in the Mid-Atlantic Bight sample set as well (Table 3.8). Percentage composition of OTUs to the W community was similar to that of the U community (average slope = 1.0 ± 0.21, y-intercept = $-1.0x10^{-3}$ ± $9.0x10^{-3}$, R^2 = 0.99 ± 0.01). Therefore the U community will not be considered further. Regressions were also calculated on the pairwise comparisons of the active against the total community (average slope = 0.91 ± 0.26, y-intercept = $-6.6x10^{-3}$ ± $15x10^{-3}$, R^2 = 0.69 ± 0.31). The regressions for spring 2009 sample T_0 are shown with their respective pairwise community comparisons in Figures 3.9-3.10. Based on these equations, RMA regression was unable to correlate activity due to abundance. Consequently the communities of the

Mid-Atlantic Bight were dissimilar in the representation of OTUs between the active and total communities.

3.4.4 Rapid Change in Activity over Time

To analyze short-term temporal change in the active and total bacterial communities between sampling times, I conducted RMA regressions on the total communities of the second sample against the first sample in each sampling event. I also evaluated changes in activity related to taxonomic composition of the community by regressing the active communities of the second sample against sample T_0. The equations are presented in Table 3.9 for the Sargasso Sea and Table 3.10 for the Mid-Atlantic Bight. I used percent compositions of OTUs in rarefied, averaged sample sets, and rarefaction removed samples with low sequence numbers. As a result, some comparisons could not be conducted.

In the Sargasso, except for spring 2010 RMA regressions explained almost all of the observed variation (average slope = $0.96 \pm 3.4\text{x}10^{-3}$, y-intercept = $1.1\text{x}10^{-3} \pm 2.4\text{x}10^{-3}$, $R^2 = 0.95 \pm 0.08$). Consequently, the total communities of sample T_{96} were very similar to those of sample T_0 in spring and summer 2009, and summer 2010. For the remaining spring of 2010, RMA regression did not explain more than 33% of variation in the total communities (slope = 0.76 ± 0.01, y-intercept = $6.7\text{x}10^{-3} \pm 5.4\text{x}10^{-3}$, $R^2 = 0.33$). Therefore the total community shifted between sampling events. Regression analysis of the active community of sample T_{96} against that of T_0 was conducted as well. For spring 2009, summer 2009 and summer 2010 the regression explained 97% of observed variation, and was a good fit (average slope =

0.90 ± 2.4x10^{-3}, y-intercept = 2.7x10^{-3} ± 1.6x10^{-3}, R^2 = 0.97 ± 0.04). With a slope of less than one, the active communities changed between sampling points in these seasons. In addition, active OTUs in sample T_0 were predicted to have increased activity in sample T_{96}. For spring 2010 the regression explained 70% of variation between the active communities of T_0 and T_{96} (slope = 0.79 ± 7.3x10^{-3}, y-intercept = 6.1x10^{-3} ± 3.0x10^{-3}, R^2 = 0.70). However, due to the discrepancy between the total communities in spring 2010, changes in activity independent of shifts in total community structure were not easily resolved.

In the Mid-Atlantic Bight, the RMA regression explained on average 98% of variation between the total communities of samples in the same sampling event (average slope = 1.0 ± 2.3x10^{-3}, y-intercept = -0.67x10^{-3} ± 1.9x10^{-3}, R^2 = 0.98 ± 0.01). The total community structure observed at time T_{12} resembled that of T_0. I consequently regressed the active community of sample T_{12} against that of sample T_0 to evaluate change in activity between sampling events. The resulting RMA regression explained on average 87% of change in the active bacterial community (average slope = 0.84 ± 5.5x10^{-3}, y-intercept = 5.1x10^{-3} ± 3.7x10^{-3}, R^2 = 0.87 ± 0.10). Furthermore, with a slope of less than one, in general the active OTUs at time T_0 were more active in sample T_{12}. Overall the regression shows that the total bacterial community remained consistent, and the active community changed between sampling times

3.4.5 Taxonomic Composition of Bacterial Communities

I analyzed the phylogenetic structure at 97% similarity of the rarefied, resampled and averaged active and total bacterial community profiles in the Sargasso Sea and Mid-Atlantic Bight. The OTUs are specified with respect to their environment of origin; hence OTU 5 in the Sargasso Sea sample set is not necessarily the same as taxon 5 in the Mid-Atlantic Bight. *Proteobacteria* was the most abundant phylum in the Sargasso Sea, accounting for over 50% of bacterial sequences in a majority of samples (Figure 3.11). Sample T_{96} in spring 2010 was the exception, with *Proteobacteria* constituting 48% and 36% in the active and total communities respectively. Bacterial samples that could not be classified into phyla were regularly less than 5% in all samples except in spring 2010. Unclassified bacteria represented between 32% and 39% of the active communities in spring 2010, and increased from less than 1% to 52% of the total community between samples T_0 and T_{96}. Other abundant phyla in these samples include *Cyanobacteria* (up to 37%), *Bacteroidetes* (up to 10%), *Actinobacteria* (up to 7.8%), and *Deferribacteres* (up to 6.6%). Less abundant phyla were *Chloroflexi*, *Firmicutes*, and *Planctomycetes*, making up less than 0.30% of the community in each sample.

A majority of *Bacteroidetes* members from the Sargasso Sea samples belonged to the flavobacterial class (Table 3.11). Some sequences were identified as *Sphingobacteria* or were not classified, and appeared only in the total communities of spring 2010 and one summer 2009 total community. Overall, *Bacteroidetes* was less abundant in the summer compared to the spring. Prevalent cyanobacterial sequences were *Prochlorococcus* and *Synechococcus* OTUs (Table 3.12). One OTU,

Prochloro_2, was the dominant member in the *Prochlorococcus* group. Similarly, a majority of *Synechococcus* sequences belonged to a single OTU, Synecho_5. Cyanobacterial communities in the summer were dominated by Prochloro_2, while Synecho_5 and other cyanobacterial OTUs dropped below 6%. *Cyanobacteria* composed less than 0.01% to 9.7% of spring samples, but increased to over 30% in some summer samples.

Of the *Proteobacteria* in the Sargasso Sea communities, the major members were *Alphaproteobacteria*, followed by *Gammaproteobacteria* and *Deltaproteobacteria* (Table 3.13). Abundances of *Alphaproteobacteria* were almost a magnitude more than other proteobacterial members. SAR11 clades contributed most to alphaproteobacterial dominance (Table 3.14). Between 2.1-20% of *Alphaproteobacteria* was made up by members of *Rickettsiales*, *Rhodobacterales*, and OCS116, among others. OCS116 members decreased in abundance between spring and summer in both years, from 2.5% on average to below 0.50% of the community. There were many more *Rhodobacterales* and *Rickettsiales* sequences in the active communities compared to the total communities of summer 2010 samples. Similarly, *Rhodospirillales* members had higher representation in the active fraction than in total communities of spring samples.

Within the gammaproteobacterial class, the most abundant orders were *Oceanospirillales*, *Salinisphaerales* and *Alteromonadales* (Table 3.15). Some *Gammaproteobacteria* were unable to be classified further than class level, and constituted up to 20% of the class. These unclassified gammaproteobacterial sequences comprised more of the community in the summer samples. In addition,

members of clade KI89A were unique to summer samples. On the other hand, *Salinisphaerales* was observed only in spring samples. Members of *Vibrionales* were typically not abundant, however they were almost 10% of the active community in spring 2010 sample T_{96}.

As in the Sargasso Sea, *Proteobacteria* were abundant members of Mid-Atlantic Bight bacterial communities (Figure 3.12). Except for the active communities of spring 2009, they were between 70 – 90% of bacterial sequences. They dropped to 57% and 38% of bacterial sequences in the active communities of spring 2009 samples T_0 and T_{12}, respectively. *Bacteroidetes* became the next most abundant bacterial sequences in those samples, with 42% and 61% contribution to the active communities respectively. In other sampling events, *Bacteroidetes* was less than 23% of bacterial communities, including the total profiles of spring 2009. The next most abundant bacterial phyla I observed were *Deferribacteres* (up to 5.4%), *Cyanobacteria* (5.1%), *Verrucomicrobia* (3.0%), and *Actinobacteria* (2.9%). Other bacterial phyla included members of *Planctomycetes*, *Lentisphaerae*, and *Acidobacteria*, comprising no more than 1% of the sequence sets. Some bacteria could not be classified; these unclassified bacteria were up to 7.8% of summer samples, but 2.3% or less of spring samples. Due to low sequence number, the active community of spring 2010 sample T_{12} was not described.

Flavobacteria were the most abundant *Bacteroidetes* representatives in Mid-Atlantic Bight samples (Table 3.16). *Sphingobacteria* were less than 1% of spring 2009 samples, but comprised between 4.2% and almost 20% of *Bacteroidetes* in the other samples. Sequences belonging to neither class were below 5.8% of this

phylum. As seen in Figure 3.12, generally there were more *Bacteroidetes* sequences in the active fraction than in total communities of each sampling event. In spring samples, *Sphingobacteria* were more represented in the active community compared to their abundance in the total community. This trend did not carry over to summer samples, though overall *Sphingobacteria* was more represented in the *Bacteroidetes* phylum in summer samples.

Alphaproteobacteria were abundant in Mid-Atlantic Bight samples, with over 75% of proteobacterial sequences assigned to this class (Table 3.17). *Gammaproteobacteria* constituted less than 6% of *Proteobacteria* in spring 2009, but was more abundant in other samples. I also observed *Betaproteobacteria* (2.2% or less), *Deltaproteobacteria* (also 2.2% or less), and sequences that could not be assigned to the class level (under 3% of proteobacterial sequences). *Deltaproteobacteria* were more abundant in sequence sets from the summer compared to spring.

I analyzed the contributions of groups to *Alphaproteobacteria* sequences (Table 3.18). SAR11 clades were responsible for over 70% of sequences to this class. *Rhodobacterales* and *Rickettsiales* were also substantial contributors, with sometimes up to 31% of sequences assigned to either of these orders. Other *Alphaproteobacteria* (less than 6%) included *Sphingomonodales*, *Rhizobiales*, and those that could not be classified further. Members of OCS116 were less than 1% of sequences in this class, and observed only in summer samples. Additionally, in most samples *Rhodobacterales* contributed more to the active community compared to the total

community. *Rickettsiales* members also had more representation in the active fractions than in the total community profiles of spring 2009 samples.

The most prominent members of *Gammaproteobacteria* were *Oceanospirillales* and *Alteromonodales* (Table 3.19). Sequences assigned to *Oceanospirillales* were between 40-95% of all gammaproteobacterial representatives. Similarly, alteromonads had a wide range of composition, with up to 61% of *Gammaproteobacteria* in some samples and below 1% in other samples. Fewer alteromonads were identified in the active communities of summer samples, while at the same time more sequences in the summer active communities were attributed to *Oceanospirillales*. Unclassified *Gammaproteobacteria* were generally not abundant, though in summer 2010 they were about 13% and 4% of the total community. *Thiotrichales* sequences were observed only in the whole communities of sample T_{12} in both spring 2009 and 2010, in low representation. Almost 14% of sequences in *Gammaproteobacteria* were identified as belonging to the KI89A clade in one summer 2010 total community, though normally they were about 4% or less. Pseudomonads were not abundant in these samples and were observed only in the active community of spring 2009 sample T_0, with less than 5% of gammaproteobacterial sequences assigned to them.

3.4.6 Significant Differences in Activity Level of OTUs

RMA regression analysis of active versus total community profiles suggested that activity of OTUs follows their abundances. However, some OTUs were not on the regression line. Furthermore, RMA equations showed a shift in

compositions of active communities between samples in the Mid-Atlantic Bight, and between two total community samples in the Sargasso Sea. Some trends were observed in class and order-level abundances of bacterial groups in the active and total communities. I used statistical tests to conduct pairwise comparisons of each OTU's percentage contributions to the active and total communities, to evaluate a significant difference between activity level and abundance. An OTU is significantly less active than its abundance suggests if it was statistically overrepresented in the total community compared to the active community. An OTU has a significantly higher activity level than its abundance would indicate if it was overrepresented in the active fraction compared to the total community profile.

In the Sargasso Sea, 44 OTUs had significant difference in activity levels compared to their abundances in the total communities. In spring 2009, a *Synechococcus*-assigned OTU Synecho_5 was less active than abundant in both samples (Tables 3.20 and 3.21). Another OTU in both samples, SAR86_16 (*Gammaproteobacteria*), had higher than expected activity. An active flavobacterial OTU was observed in sample T_{96}, and sample T_0 had active OTUs that were assigned to *Flavobacteria* (OTU 14), *Alphaproteobacteria* (82), *Actinobacteria* (123), and *Gammaproteobacteria* (129). Though Synecho_5 was not as active in spring 2009, it had higher activity than abundance in spring 2010 (Tables 3.22 and 3.23). The phylogenetic profiles of OTUs with significantly higher or lower activity in spring 2010 were different between samples as well (Figures 3.13-3.14). Several unclassified OTUs were highly active in sample T_0 but one such OTU, unc_8, was less active in sample T_{96}, and the remaining unclassified OTUs from sample T_0 were

not significantly different in activity levels compared to their abundances in sample T_{96}. NS2b_14, a flavobacterial OTU that was active in spring 2009 sample T_{96}, was less active in spring 2010 sample T_0 but returned to higher activity levels in sample T_{96}. OTUs that were less active in the first sample belonged to SAR11 clades (OTUs 1, 3 and 30), *Flavobacteria* (79), *Deferribacteres* (13), *Actinobacteria* (56 and 92), *Deltaproteobacteria* (119), and *Gammaproteobacteria* (129) (Table 3.22). Active OTUs in spring 2010 also included alphaproteobacterium 82, which was common to both samples, NS2b_46 (*Flavobacteria*), and five gammaproteobacterial OTUs (35, 49, 138, 705, and 710) (Table 3.23).

The summer 2009 communities of the Sargasso had fewer OTUs with differing activity levels compared to their abundances (Tables 3.24-3.25). Prochloro_2 assigned to *Prochlorococcus* was significantly less active in all four sequence sets. SAR11_1 and 6, OTUs belonging to the SAR11 clade, were highly active members of the summer 2009 sample T_0 (Table 3.24). Alpha_82, previously observed in spring samples as being more active than its abundance suggested, was also more active in sample T_{96} of summer 2009 (Table 3.25). SAR11_6 as well as 17 had higher activity levels than indicated by abundances in summer 2010 (Tables 3.26-3.27). Also in both summer 2010 samples, two actinobacterial taxa (31 and 36) were less active than expected from abundances, and SAR116_42, a *Rickettsiales* member, was more represented in the active fractions compared to the total communities. The second sample had additional OTUs that had differences in activity levels (Table 3.27). One SAR11 OTU (4) was less active than its abundance would indicate, though two additional SAR11 taxa (19 and 25) were more active. Other taxa with higher-

than-expected activities included representatives of SAR406 (*Deferribacteres*, 13 and 26) and an unclassified alphaproteobacterium (145).

In the Mid-Atlantic Bight sequence sets, 31 taxa had significantly different activity levels compared to their abundances in the total communities. Spring 2009 contained low-activity taxa such as SAR11_1 and SAR116_8 (Tables 3.28 and 3.29). In addition, SAR86_2 in sample T_0, and SAR116_3 and SAR11_4 in sample T_{12}, did not have as high activity levels as expected. Active taxa common to both samples were members of *Bacteroidetes* (9, 16, 19, 25 and 26) and a *Roseobacter* member (*Alphaproteobacteria*, 13) (Figures 3.15-3.16). The first sample had an additional three taxa that were more active and belonged to *Flavobacteria* (50, 73 and 98) (Table 3.28). Similarly, other high-activity OTUs in sample T_{12} were assigned to *Flavobacteria* as well (23 and 30) (Table 3.29). Differentially active OTUs in spring 2010 were dissimilar to those presented in spring 2009 (Table 3.30). SAR86_2, having low activity levels in the previous year, was more active with respect to its abundance in the first sample of spring 2010. I also observed increased activity in SAR11_1 and Colwellia_20 (*Gammaproteobacteria*), and decreased activity in Roseobacter_7. Both SAR11_1 and SAR86_2 were active in spring 2010, yet less active in sample T_0 of 2009; similarly, Roseobacter_7 had significant activity in the first spring and was not very active in the second spring.

Active OTUs in summer samples of the Mid-Atlantic Bight included representatives of *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, *Cyanobacteria* and *Deferribacteres*. In summer samples of 2009, I observed increased activity in Roseobacter_7 and decreased activity in Litoricola_17

(*Gammaproteobacteria*) (Tables 3.31 and 3.32). Other taxa that were more active than expected in the first sample were SAR86_2 and Owenweeksia_41 (*Flavobacteria*) (Table 3.31). SAR116_8, which was previously not active with respect to its abundance in spring 2009, had low activity levels in this sample as well. In sample T_{12}, a *Marinomonas* taxon (*Gammaproteobacteria*, 35) had low activity (Table 3.32). Three alphaproteobacterial taxa had higher activity levels than abundances suggested, including a rhodobacterial OTU (11) and two unclassified members (27 and 106). In summer 2010 communities, SAR86_2 was again more active than expected (Tables 3.33 and 3.34). The night sample had a significantly less active unclassified cyanobacterial OTU (37) and a significantly more active SAR406 member (*Deferribacteres*, 51) (Table 3.33). Sample T_{12} also had a low-activity cyanobacterial OTU, though the OTU was different from the first sample (SubsectionI_18) (Table 3.34). Other less active OTUs in the second sample were SAR11_1, and *Rickettsiales* members SAR116_3 and SAR116_8. Taxa with higher activity levels than suggested by abundances in sample T_{12} were Rhodobacter_11, Owenweeksia_41, SAR86_58, Oceanospirillales_130, and unc_5, an unclassified bacterium. Comparing summer samples to each other, Owenweeksia_41 and Rhodobacter_11 were more active than expected in 'T_{12}' samples but did not have significant differences in activity in 'T_0' samples.

3.5 Discussion

Evaluations of activity and abundance of individual bacteria provide insight into how community structure can impact bacterial contributions to

biogeochemical fluxes. In this study I assessed variation in activity in the marine bacterial assemblage by comparing the abundances of individual ribotypes in the active community to their representation in the total community. In general activity of bacterial taxa was proportional to their abundance in the total community. I observed that in both environments, some taxa were significantly different in activity levels compared to their abundance. In each environment and season, over 80% of bacteria with significant discrepancies in activity each comprised less than 5% of sequences in the total community. Similarly, some abundant bacteria were not always the most active members. In addition to discrepancies relative to abundance, I also observed changes in bacterial activity over time. In several samples, community structure of the total bacterial assemblage was consistent in short time frames of hours to days. However, the active community rapidly changed between samples, thereby influencing the total community composition.

Abundances of most bacterial taxa were indicative of their activity levels in these environments. Using reduced major axis (RMA) regression to analyze the relationship between abundance and activity, I observed high correlation between contributions of bacterial taxa to the active and total communities in three of four sample sets in both the Mid-Atlantic Bight and Sargasso Sea. For major phylogenetic groups in these sampling events, such as *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, and *Cyanobacteria*, the difference in contribution was less than 10% between active and total communities. Furthermore, of the nearly 3,000 taxa analyzed in each environment, over 98% of taxa did not differ significantly between percentage contributions to the total and active fractions.

It is hypothesized that many abundant bacteria are actively growing and participate in biogeochemical processes, whereas rare bacteria are less active or not growing at all (Pedrós-Alió, 2006). In the coastal Bay of Biscay, *Bacteroidetes*, *Alphaproteobacteria*, and *Gammaproteobacteria* were abundant and active metabolizers in the bacterial community (Alonso-Sáez *et al.*, 2009). Similarly, in the northeastern Atlantic Ocean abundant bacteria such as members of *Bacteroidetes* were also enzymatically active (Davey *et al.*, 2001).

Though I saw a linear relationship between abundance and activity for a majority of bacterial taxa, I also observed bacterial members of disproportionate activity and abundance. In the Sargasso Sea, an unclassified alphaproteobacterium was rare but active throughout samples. Additionally, compared to abundance I observed higher activity of a SAR116 (*Alphaproteobacteria*) taxon in summer 2010, and no difference in activity in summer 2009 in the Sargasso Sea. An OTU belonging to SAR86 (*Gammaproteobacteria*), though not abundant in spring Sargasso samples, was nevertheless active. Similarly, in the Mid-Atlantic Bight taxa identified as SAR86 were active but in low abundance in summer samples. Rhodobacterial types in the Mid-Atlantic Bight were also more active than their abundances suggested in sampling events from spring and summer 2009, and summer 2010. While not much is known about the abundance of SAR86 and SAR116 in the Mid-Atlantic Bight, SAR86 and SAR116 reach their highest abundances of 4% and 25% respectively in summer months in the Sargasso (Morris *et al.*, 2005, Treusch *et al.*, 2009). In the Mid-Atlantic Bight, ribotypes belonging to *Rhodobacteraceae* had significantly higher growth rates when rare (Campbell *et al.*, 2011).

Seasonality was observed in the bacterial communities of the Sargasso Sea. Cyanobacterial taxa such as *Prochlorococcus* were abundant in summer samples, whereas *Synechococcus* and *Bacteroidetes* members were numerous in spring samples. I also observed clustering of bacterial communities in the summer samples. Compared to the spring, assemblages in the summer contained relatively fewer bacterial taxa and were more even in distribution. Other abundant bacteria that were seen in all summer Sargasso samples were from SAR11 and *Oceanospirillales* (e.g. SAR86), among others. Other studies also saw clustering of bacterial communities in the summer of the Sargasso Sea due to similar structures (Morris *et al.*, 2005, Treusch et al., 2009).

Bacterial community structure in the Mid-Atlantic Bight did not follow a seasonal trend. I found that SAR11 taxa were abundant but not active in spring 2009 and summer 2010 in the Mid-Atlantic Bight, though they were active in the other samples. In addition, some rhodobacterial types had changes in activity relative to abundance. Many taxa belonging to SAR11 and *Rhodobacteraceae* were observed to vary in abundance and activity in the Mid-Atlantic Bight (Campbell *et al.*, 2011). In the total assemblages and the bacterial communities of spring 2010 and all four of the summer samples, *Bacteroidetes* members had predictable abundances between 5-20% of sequences. *Bacteroidetes* was more than twice as abundant in the active assemblages compared to the total communities in spring 2009 of the Mid-Atlantic Bight, with flavobacterial members replacing SAR11 clades as the dominant active taxa. Their substantial increase in activity in spring 2009 may be related to timing of the spring bloom in the Mid-Atlantic Bight. Compared to the following year, the

sampling event in spring 2009 was in warmer waters and had higher measured chlorophyll a concentrations and lower nutrient concentrations. Furthermore, bacterial production using leucine incorporation was lower. In this sampling event, actively growing *Flavobacteria* may have been consuming autochthonous macromolecules in lieu of the added compounds. Flavobacterial ribotypes have increased activity in response to phytoplankton blooms (Riemann *et al.*, 2000, Gómez-Pereira *et al.*, 2010). Furthermore, *Flavobacteria* are able to consume complex polysaccharides such as those released by phytoplankton (Kirchman, 2002).

Bacterial community structures observed in three of four sampling events in each location resembled each other, similar to what others have observed in marine environments (Schauer *et al.*, 2003, Alonso-Sáez *et al.*, 2008, Campbell *et al.*, 2009). The abundant and active members were consistent between these samples as well. However, I observed changes on a short temporal scale in the active community in spring 2009 of the Mid-Atlantic Bight and the total community in spring 2010 of the Sargasso Sea. RMA analyses indicated that the active communities of spring 2009 in the Mid-Atlantic Bight and spring 2010 in the Sargasso Sea were dissimilar to their respective total communities. In these sampling events, community structure of the active fraction was not representative of the total communities. The total community of the Mid-Atlantic Bight remained the same in spring 2009 between samples, but within 12 hours of sampling the constituents changed in the active communities. *Bacteroidetes* was more enriched in the active community in the second sample compared to that of the first. Rhodobacterial types were also more abundant in the active fraction of the second sample. SAR11 ribotypes experienced a decline in

activity levels between samples in spring 2009 of the Mid-Atlantic Bight. Though they originated from similar total bacterial communities, the active fractions of bacterial communities from the same sampling event did not resemble each other. Over a span of four days the total communities in the Sargasso Sea experienced a significant shift as well. Unclassified bacteria, including some potential chloroplasts, were over 30% of the active community of the first sample, but less than 5% in the total community. In the second sample, they constituted nearly 40% and 50% of the active and total fractions, respectively. In four days these actively replicating bacteria had a quantitative response by becoming the dominant members in both the active and overall communities. Change in environmental conditions can promote rapid responses in marine bacteria (Lauro *et al.*, 2009, Yooseph *et al.*, 2010). Coastal waters in the Mid-Atlantic Bight experience seasonally dynamic conditions and high variability in environmental parameters (Churchill *et al.*, 1993). In addition, community structure in this environment may shift between years, obscuring reliable temporal patterns (Campbell *et al.*, 2009). On top of immediate changes in environmental conditions, timing of deep mixing and the spring phytoplankton bloom may subsequently shift the spring bacterial community in the Sargasso Sea. Fluxes in light, temperature and nutrients can influence the active community in both environments, which can subsequently shape the composition of the total bacterial assemblage.

I observed a high correlation between bacterial activity and community structure in this study. However, some abundant bacterial members did not have activity levels as high as their abundances would indicate. SAR11 and *Synechococcus*

composed a large fraction of the total bacterial community in spring of the Sargasso Sea, but the BrdU-labeled community did not have comparable representation of these taxa. In the summer of the Sargasso, *Synechococcus* and SAR11 were not as abundant as *Prochlorococcus*, but they were enriched in the active community. In summer, *Prochlorococcus* became abundant but were not proportionally active. Similarly, in the Mid-Atlantic Bight SAR11 members were less active in spring 2009 and summer 2010, but increased in activity level in spring 2010. In the Sargasso Sea, *Cyanobacteria* and *Proteobacteria* are typically the dominant members of the bacterial community (Treusch *et al.*, 2009). The major cyanobacterial members reach their maximum abundances in the Sargasso at different times of the year, with *Synechococcus* abundant in spring and *Prochlorococcus* dominant in summer and fall (DuRand *et al.*, 2001). In the Sargasso Sea, *Synechococcus* and *Prochlorococcus* members had high rates of cellular phosphate uptake (Zubkov *et al.*, 2007), but *Cyanobacteria* had low incorporation rates of tritiated thymidine in the oligotrophic subtropical North Pacific Ocean (Church *et al.*, 2006). SAR11 actively incorporated amino acids and glucose in the Northwest Atlantic Ocean, yet showed selectivity in assimilating other DOM sources (Malmstrom *et al.*, 2005). The observed discrepancies between abundance and activity for certain bacterial groups may be due in part to the methodology of this study. Because BrdU is a thymidine analog, sufficient labeling with the compound may be seen only in bacteria capable of incorporating exogenous nucleosides or coding for thymidine kinases (Jeffrey and Paul, 1990, Urbach *et al.*, 1999). Genomes of *Prochlorococcus* and other *Cyanobacteria* lack nucleoside transporters or thymidine kinases (Jeffrey and Paul,

1990). Furthermore, a cultured *Prochlorococcus* strain replicated DNA in dark to avoid damage from UV radiation (Kolowrat *et al.*, 2010), and my experiments in the Sargasso Sea were conducted in the light. Furthermore, BrdU-labeling relies on DNA replication as a proxy for activity. Other activity assays include measuring enzymatic activity (Davey *et al.*, 2001, Kirchman *et al.*, 2004, Dimitriu *et al.*, 2010), 16S rRNA/rRNA gene ratios (Buckley and Schmidt, 2003, Lami *et al.*, 2009, Campbell *et al.*, 2011), or metatranscriptomic libraries (Poretsky *et al.*, 2005, Mou *et al.*, 2008, Gilbert *et al.*, 2009, Vila-Costa *et al.*, 2010). Use of BrdU-labeling captured active bacterial members, however it did not exhaust the scope of activities in the bacterial assemblage. The suitability of this method needs to be further examined in comparison with other assays.

This study examined bacterial contributions to the active and total communities at phylogenetic levels to understand the link between activity and community structure. I confirmed a typical trend between abundance and activity in the Mid-Atlantic Bight and Sargasso Sea. Investigating community composition of active and total communities revealed significant differences in activity levels of both abundant and rare individual taxa. I observed several rare bacterial ribotypes that were more active than expected; at the same time, some abundant taxa were not proportionally active. While I was able to observe the dynamics of active and total bacterial communities on different time scales, further research is necessary to link immediate and long-term environmental shifts to responses in active bacterial assemblages. Seasonality in bacterial activity and structure continues to be examined, however more frequent sampling is needed to capture rapid changes in bacterial

activity due to environmental perturbation. Furthermore, evaluating growth and community composition using a suite of metrics, such as 16S rRNA/rRNA gene ratios, metatranscriptomics, DNA replication, and enzymatic activity, will improve understanding of the metabolic capabilities of bacterial assemblages. Spatiotemporal monitoring of abundant and rare bacteria, and varying types of activity of bacterial communities will contribute to our knowledge of the relationship between bacterial diversity and activity, and biogeochemical cycles.

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Table 3.1: Sampling cruises at the BATS and FB sites in 2009 and 2010.

Date	Location	Sampling events
17-20 March 2009	BATS	4
17-18 April 2009	FB	4
6-8 July 2009	FB	2
14-17 August 2009	BATS	4
8 March 2010	FB	2
24-27 March 2010	BATS	4
20-23 August 2010	BATS	4
17 September 2010	FB	2

Table 3.2: Environmental parameters measured at FB. Temperature, salinity, bacterial production and Chl a were obtained at the time of sample T_{12}. Bacterial production, chl a and nutrients for samples collected at FB in 2009 and spring 2010, as well as production and chl a for summer 2010, are provided as measured ± standard deviation. Remaining nutrient data for summer 2010 is the average ± standard deviations of historically recorded measurements taken in the same months from 2006-2009.

Sample	Temperature (^oC)	Salinity (psu)	Bacterial production (pmol L^{-1} h^{-1})	Chl a (µg L^{-1})	NO_3 (µM)	NH_4 (µM)	PO_4 (µM)	Si (µM)
Spring 2009	8.31	31.6	67.8 ± 6.3	3.92 ± 0.17	0.158 ± 0.007	1.26 ± 0.01	0.045 ± 0.0002	1.31 ± 0.04
Summer 2009	19.5	30.5	133 ± 3	0.713 ± 0.008	Below detection	0.924 ± 0.004	0.302 ± 0.004	6.88 ± 0.46
Spring 2010	3.70	31.4	112 ± 6	2.48 ± 0.55	0.863 ± 0.005	1.42 ± 0.01	0.095 ± 0.001	3.25 ± 0.22
Summer 2010	21.5	30.7	208 ± 12	5.01 ± 0.53	1.60 ± 1.56	2.25 ± 1.99	0.544 ± 0.166	10.2 ± 3.3

Table 3.3: Environmental parameters measured at BATS for samples T_0 and T_{96}. Temperature and salinity were obtained at the time of sampling. Nutrient data is the average ± standard deviations of historically recorded measurements taken between 40 and 60 meters below surface in the same seasons from 2005-2008.

Season	Sample	Temperature (°C)	Salinity (psu)	Bacterial production (pmol L^{-1} h^{-1})	Chl a (ng kg^{-1})	NO_3 (µmol kg^{-1})	NO_2 (µmol kg^{-1})	PO_4 (µmol kg^{-1})	Si (µmol kg^{-1})
Spring 2009	T_0	19.7	36.6	9.72 ± 0.90	232 ± 126	0.060 ± 0.099	0.075 ± 0.051	0.000 ± 0.000	0.839 ± 0.090
	T_{96}	19.7	36.7	13.8 ± 1.1					
Spring 2010	T_0	18.0	36.6	7.03 ± 0.44					
	T_{96}	18.8	36.6	6.76 ± 0.68					
Summer 2009	T_0	23.6	36.7	10.1 ± 1.1	100. ± 64	0.006 ± 0.032	0.008 ± 0.012	0.000 ± 0.000	0.581 ± 0.220
	T_{96}	24.3	36.6	14.5 ± 1.3					
Summer 2010	T_0	23.9	36.7	4.48 ± 0.36					
	T_{96}	23.2	36.7	5.54 ± 0.79					

Table 3.4: Barcodes specific to samples used to identify the tag-amplified V1-V3 region of bacterial 16S rRNA genes. Samples are indicated as 'Location-SeasonYear-Labeling-Immunocapturing-Collection'. Location is either BATS (B) or FB (M), season is spring (SP) or summer (SU), and year is 2009 or 2010. Labeled samples are indicated with L, and unlabeled with U. Immunocaptured samples are indicated with I, while nonimmunocaptured samples are W. Collection A or B refers to when the sample was collected; for FB, A indicates a night collection (T_0) and B a day-time collection (T_{12}); and for BATS, sample A is from the first day (T_0) and B is from the last day (T_{96}) of the sampling event.

Sample	Barcode
B.SP09.U.W.A	AAGCCGC
B.SP09.U.I.A	ACCTAATA
B.SP09.L.I.A	ACCTCTAA
B.SP09.U.W.B	CAAGAAC
B.SP09.U.I.B	ACCTTCCA
B.SP09.L.I.B	ACGAATGA
B.SU09.U.W.A	AGTTGGC
B.SU09.U.I.A	ACGAGCTA
B.SU09.L.I.A	ACGCAAAA
B.SU09.U.W.B	TATCAAC
B.SU09.U.I.B	ACGCCGCA
B.SU09.L.I.B	ACGCTAGA
B.SP10.U.W.A	AGGCGGC
B.SP10.U.I.A	ACTAAGAA
B.SP10.L.I.A	ACTAGACA
B.SP10.U.W.B	CGGTATC
B.SP10.U.I.B	ACTATGGA
B.SP10.L.I.B	ACTCCATA
B.SU10.U.W.A	TGACGAC
B.SU10.U.I.A	ACTCGTAA
B.SU10.L.I.A	ACTGACCA
B.SU10.U.W.B	ACAAGGC
B.SU10.U.I.B	ACTGCTGA
B.SU10.L.I.B	ACTGTCTA

Sample	Barcode
M.SP09.U.W.A	ACTTCAAA
M.SP09.U.I.A	AGAGCGAA
M.SP09.L.I.A	AGAGTACA
M.SP09.U.W.B	ACTTGGCA
M.SP09.U.I.B	AGATAGGA
M.SP09.L.I.B	AGATGATA
M.SU09.U.W.A	AGAAAAGA
M.SU09.U.I.A	AGATTTAA
M.SU09.L.I.A	AGCACCCA
M.SU09.U.W.B	AGAACGTA
M.SU09.U.I.B	AGCAGTGA
M.SU09.L.I.B	AGCCACTA
M.SP10.U.W.A	AGAATCAA
M.SP10.U.I.A	AGCCGAAA
M.SP10.L.I.A	AGCCTGCA
M.SP10.U.W.B	AGACATCA
M.SP10.U.I.B	AGCGCAGA
M.SP10.L.I.B	AGCGGGTA
M.SU10.U.W.A	AGACGCGA
M.SU10.U.I.A	AGCTACAA
M.SU10.L.I.A	AGCTCTCA
M.SU10.U.W.B	AGACTTTA
M.SU10.U.I.B	AGCTTCGA
M.SU10.L.I.B	AGGAATTA

Table 3.5: Analysis of sequences in each Sargasso Sea sample at 97% similarity with alpha diversity measures. T_x indicates time of sampling. L indicates a labeled sample, and U indicates an unlabeled sample. I indicates immunocapturing was performed on sample, and W marks samples not immunocaptured. Chao1 estimates species richness with predicting the total number of OTUs in the community. Inverse Simpson is a species evenness index, where a higher number indicates more evenness in the sample.

Season	Time	Labeled	Immuno-captured	Number of Sequences	Observed OTUs	Chao1	Inverse Simpson
Spring 2009	T_0	L	I	3374	365	861	7.35
		U	I	1821	258	475	7.39
		U	W	7913	638	1586	7.30
	T_{96}	L	I	3603	392	965	7.83
		U	I	866	149	356	6.07
		U	W	5660	547	1656	8.23
Spring 2010	T_0	L	I	1100	230	629	16.85
		U	I	288	76	123	10.58
		U	W	3232	372	883	11.07
	T_{96}	L	I	1165	221	443	27.47
		U	I	1121	203	458	19.84
		U	W	3817	455	1086	18.89
Summer 2009	T_0	L	I	4854	411	1049	7.13
		U	I	3877	343	956	6.61
		U	W	4458	393	1152	6.81
	T_{96}	L	I	7742	600	1368	9.44
		U	I	6474	447	1220	6.74
		U	W	3391	369	1195	8.65
Summer 2010	T_0	L	I	4520	331	995	5.32
		U	I	20	8	13	5.14
		U	W	4119	237	569	4.97
	T_{96}	L	I	4724	372	1018	7.00
		U	I	3795	264	613	6.12
		U	W	3113	132	343	4.45

Table 3.6: Analysis of sequences in each Mid-Atlantic Bight sample at 97% similarity with alpha diversity measures. See caption from table 3.5 for explanation of time, labeled and immunocaptured columns.

Season	Time	Labeled	Immuno-captured	Number of Sequences	Observed OTUs	Chao1	Inverse Simpson
Spring 2009	T_0	L	I	5209	387	1007	6.35
		U	I	8033	412	1413	2.62
		U	W	5510	359	830	2.73
	T_{12}	L	I	1561	184	470	8.87
		U	I	1959	202	429	4.76
		U	W	7234	490	1426	3.31
Spring 2010	T_0	L	I	613	71	134	3.32
		U	I	82	25	55	5.62
		U	W	1215	172	358	4.50
	T_{12}	L	I	216	44	117	3.67
		U	I	747	99	191	3.02
		U	W	1143	120	231	3.19
Summer 2009	T_0	L	I	2887	322	873	9.18
		U	I	2967	308	864	6.59
		U	W	2621	287	563	8.32
	T_{12}	L	I	1225	183	461	8.57
		U	I	1942	229	520	4.80
		U	W	4242	385	991	6.76
Summer 2010	T_0	L	I	2692	382	932	4.65
		U	I	2171	319	786	3.30
		U	W	1146	242	869	4.62
	T_{12}	L	I	3311	528	1351	11.07
		U	I	2372	322	670	3.97
		U	W	1435	265	638	6.13

Table 3.7: Equations generated in RMA regression analysis of Sargasso Sea bacterial communities. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Within each sample, percent contributions of each OTU to the active community (L) were regressed against its contribution to the total community (W) using reduced major axis regression. The unlabeled, immunocaptured (U) community was also compared against the total community (W). Due to removal of samples with sequences below the rarefaction threshold, some pairwise comparisons could not be conducted.

Season	Sample	Comparisons	Intercept ($\times 10^{-3}$)	St. error of intercept ($\times 10^{-3}$)	Slope	St. error of slope ($\times 10^{-3}$)	R^2
Spring 2009	T_0	L vs. W	0.093	1.6	1.0	2.6	0.98
		U vs. W	5.6	1.3	1.0	2.0	0.99
	T_{96}	L vs. W	-0.86	1.6	1.0	2.8	0.97
		U vs. W	n/a				
Spring 2010	T_0	L vs. W	5.4	2.8	0.81	5.4	0.85
		U vs. W	n/a				
	T_{96}	L vs. W	4.7	1.7	0.83	4.4	0.90
		U vs. W	0.63	2.2	0.98	5.6	0.89
Summer 2009	T_0	L vs. W	0.67	2.5	0.98	4.0	0.94
		U vs. W	-0.49	2.8	1.0	4.4	0.94
	T_{96}	L vs. W	1.3	1.5	0.96	2.7	0.97
		U vs. W	-3.8	1.8	1.1	3.2	0.97
Summer 2010	T_0	L vs. W	0.80	3.7	0.97	4.9	0.91
		U vs. W	n/a				
	T_{96}	L vs. W	5.7	4.3	0.80	5.4	0.84
		U vs. W	4.2	4.5	0.85	5.7	0.85

Table 3.8: Equations generated in RMA regression analysis of Mid-Atlantic Bight bacterial communities. Samples were rarefied 100 times for 613 and 1146 sequences for spring 2010 and the remaining samples respectively, then frequencies for each OTU were averaged for a mean representation. Within each sample, percent contributions of each OTU to the active community (L) were regressed against its contribution to the total community (W) using reduced major axis regression. The unlabeled, immunocaptured (U) community was also compared against the total community (W). Due to removal of samples with sequences below the rarefaction threshold, some pairwise comparisons could not be conducted.

Season	Sample	Comparisons	Intercept ($\times 10^{-3}$)	St. error of intercept ($\times 10^{-3}$)	Slope	St. error of slope ($\times 10^{-3}$)	R^2
Spring 2009	T_0	L vs. W	16	6.2	0.65	5.7	0.77
		U vs. W	-0.76	1.1	1.0	1.0	1.0
	T_{12}	L vs. W	13	8.8	0.61	8.9	0.38
		U vs. W	5.5	2.5	0.83	2.5	0.97
Spring 2010	T_0	L vs. W	-5.5	3.1	1.2	3.6	0.97
		U vs. W	n/a				
	T_{12}	L vs. W	n/a				
		U vs. W	-0.81	1.4	1.0	1.4	0.99
Summer 2009	T_0	L vs. W	1.5	2.9	0.95	4.6	0.93
		U vs. W	-4.2	1.6	1.1	2.5	0.99
	T_{12}	L vs. W	3.7	1.6	0.89	2.3	0.98
		U vs. W	-6.2	1.7	1.2	2.4	0.99
Summer 2010	T_0	L vs. W	0.17	1.5	0.99	1.7	0.99
		U vs. W	-5.9	1.5	1.2	1.8	0.99
	T_{12}	L vs. W	8.6	2.3	0.74	3.2	0.95
		U vs. W	-8.1	1.9	1.2	2.6	0.99

Table 3.9: Equations generated in RMA regression analysis of bacterial communities from Sargasso Sea samples T_0 and T_{96}. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Percent contributions of each OTU to the total community in sample T_{96} were regressed against its contribution to the total community in sample T_0 using reduced major axis regression.

Sample	Comparisons	Intercept ($\times 10^{-3}$)	St. error of intercept ($\times 10^{-3}$)	Slope	St. error of slope ($\times 10^{-3}$)	R^2
Spring 2009	Whole (T_{96}) vs. whole (T_0)	1.7	1.1	0.94	1.8	0.99
	Active (T_{96}) vs. active (T_0)	0.83	1.3	0.97	2.2	0.98
Summer 2009	Whole (T_{96}) vs. whole (T_0)	3.2	1.1	0.89	1.7	0.99
	Active (T_{96}) vs. active (T_0)	3.8	0.64	0.87	1.0	1.0
Spring 2010	Whole (T_{96}) vs. whole (T_0)	6.7	5.4	0.76	1.1	0.33
	Active (T_{96}) vs. active (T_0)	6.1	3.0	0.79	7.3	0.70
Summer 2010	Whole (T_{96}) vs. whole (T_0)	-1.7	5.0	1.1	6.7	0.86
	Active (T_{96}) vs. active (T_0)	3.6	2.9	0.87	3.9	0.93

Table 3.10: Equations generated in RMA regression analysis of bacterial communities from Mid-Atlantic Bight samples T_0 and T_{12}. Samples were rarefied 100 times for 613 and 1146 sequences for spring 2010 and the remaining sampling events respectively, then frequencies for each OTU were averaged for a mean representation. Percent contributions of each OTU to the total community in sample T_{12} were regressed against its contribution to the total community in sample T_0 using reduced major axis regression.

Sample	Comparisons	Intercept ($\times 10^{-3}$)	St. error of intercept ($\times 10^{-3}$)	Slope	St. error of slope ($\times 10^{-3}$)	R^2
Spring 2009	Whole (T_{12}) vs. whole (T_0)	3.1	1.5	0.91	1.4	0.99
	Active (T_{12}) vs. active (T_0)	4.8	5.3	0.85	7.5	0.77
Summer 2009	Whole (T_{12}) vs. whole (T_0)	-3.8	2.1	1.1	3.4	0.97
	Active (T_{12}) vs. active (T_0)	-1.2	3.9	1.0	6.6	0.88
Spring 2010	Whole (T_{12}) vs. whole (T_0)	-6.4	2.1	1.2	2.4	0.99
	Active (T_{12}) vs. active (T_0)					
Summer 2010	Whole (T_{12}) vs. whole (A)	4.4	1.8	0.87	2.1	0.98
	Active (T_{12}) vs. active (T_0)	12	1.9	0.64	2.3	0.96

Table 3.11: Percent composition of phylogenetic classes to *Bacteroidetes* in Sargasso Sea bacterial communities. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the class level were summed, and the sum was used to calculate percent composition. The number of sequences in each sample assigned to *Bacteroidetes* is presented in the last column.

Season	Time	Sample	Class of *Bacteroidetes*			Number of sequences
			Flavobacteria	***Sphingobacteria***	**Unclassified**	
Spring 2009	T_0	Active	100	0	0	47
		Total	100	0	0	15
	T_{96}	Active	100	0	0	57
		Total	100	0	0	20
Spring 2010	T_0	Active	100	0	0	58
		Total	97.1	1.94	0.97	103
	T_{96}	Active	100	0	0	101
		Total	96.4	1.19	2.38	84
Summer 2009	T_0	Active	100	0	0	16
		Total	100	0	0	3
	T_{96}	Active	100	0	0	33
		Total	85.7	14.3	0	28
Summer 2010	T_0	Active	100	0	0	5
		Total	0	0	0	0
	T_{96}	Active	100	0	0	1
		Total	0	0	0	0

Table 3.12: Percent composition of phylogenetic groups to *Cyanobacteria* in Sargasso Sea bacterial communities. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the order level were summed, and the sum was used to calculate percent composition. Percent composition for the dominant *Prochlorococcus* and *Synechococcus* OTUs are presented separately from other assigned *Cyanobacteria*. The number of sequences in each sample assigned to *Cyanobacteria* is presented in the last column.

Season	Time	Sample	*Cyanobacteria*						Number of sequences
			Other *Prochloro-coccus*	Prochloro-coccus_2	Other *Synecho-coccus*	Synecho-coccus_5	Unclassified	Other	
Spring 2009	T_0	Active	0	85.7	0	11.4	0	2.86	35
		Total	0	50.6	6.49	42.9	0	0	77
	T_{96}	Active	0	53.7	0	41.5	0	4.88	41
		Total	0	33.6	9.35	57.0	0	0	107
Spring 2010	T_0	Active	3.33	16.7	3.33	56.7	10.0	10.0	30
		Total	0	71.4	0	28.6	0	0	7
	T_{96}	Active	0	15.8	10.5	42.1	0	31.6	19
		Total	0	55.6	0	0	0	44.4	9
Summer 2009	T_0	Active	0	95.7	2.16	2.16	0	0	185
		Total	0	95.2	2.60	2.23	0	0	269
	T_{96}	Active	0	95.2	3.61	1.20	0	0	166
		Total	0	97.6	1.46	0.98	0	0	205
Summer 2010	T_0	Active	0	92.9	5.36	1.79	0	0	112
		Total	0	100	0	0	0	0	217
	T_{96}	Active	0	98.0	1.53	0.51	0	0	196
		Total	0.77	96.9	1.80	0.52	0	0	388

Table 3.13: Percent composition of phylogenetic classes to *Proteobacteria* in Sargasso Sea bacterial communities. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the class level were summed, and the sum was used to calculate percent composition. The number of sequences in each sample assigned to *Proteobacteria* is presented in the last column.

Season	Time	Sample	Class of *Proteobacteria*				Number of sequences
			α	δ	γ	Other	
Spring 2009	T_0	Active	89.7	0.46	9.62	0.23	863
		Total	95.5	0.75	3.76	0	797
	T_{96}	Active	91.6	0.24	7.41	0.73	823
		Total	93.3	1.05	5.64	0	763
Spring 2010	T_0	Active	86.9	0.48	11.3	1.27	628
		Total	87.0	1.34	11.2	0.40	747
	T_{96}	Active	58.7	0.42	39.6	1.26	475
		Total	82.6	1.16	16.2	0	345
Summer 2009	T_0	Active	92.8	1.19	5.27	0.79	759
		Total	91.1	1.31	6.69	0.87	688
	T_{96}	Active	86.6	1.64	10.4	1.37	730
		Total	87.7	2.34	8.18	1.75	685
Summer 2010	T_0	Active	96.7	0.22	1.80	1.24	890
		Total	93.0	0	5.92	1.05	760
	T_{96}	Active	93.8	0.77	4.37	1.03	778
		Total	98.8	0	1.20	0	582

Table 3.14: Percent composition of phylogenetic groups to class *Alphaproteobacteria* in Sargasso Sea bacterial communities. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the order level were summed, and the sum was used to calculate percent composition. The number of sequences in each sample assigned to *Alphaproteobacteria* is presented in the last column.

Season	Time	Sample	Order of *Alphaproteobacteria*								Number of sequences
			Caulo-bacterales	OCS116	*Rhodo-bacterales*	*Rhodo-spirillales*	*Rickett-siales*	SAR11	Unclass-ified	Other	
Spring 2009	T_0	Active	0.26	1.29	0.39	0.13	1.55	94.4	1.81	0.13	774
		Total	0	1.84	0.39	0	2.37	95.3	0.13	0	761
	T_{96}	Active	0.27	1.33	1.19	0.13	2.12	93.5	1.46	0	754
		Total	0.14	2.25	0.84	0	3.51	92.8	0.42	0	712
Spring 2010	T_0	Active	0	2.93	1.10	0.37	1.10	90.3	4.03	0.18	546
		Total	0	2.92	1.08	0	1.54	94.2	0.31	0	650
	T_{96}	Active	0	3.58	6.09	0.72	4.30	79.9	5.38	0	279
		Total	0	3.51	6.67	0	2.81	87.0	0	0	285
Summer 2009	T_0	Active	0.28	0	0.99	0	4.97	91.5	2.27	0	704
		Total	0	0.48	1.12	0.16	6.06	91.5	0.64	0	627
	T_{96}	Active	0	0	1.74	0.16	5.70	89.7	2.37	0.32	632
		Total	0.17	0.50	0.83	0.33	6.99	89.9	1.00	0.33	601
Summer 2010	T_0	Active	0.46	0	0.46	0	5.46	92.5	0.70	0.46	861
		Total	0.28	0	0	0	0.57	97.5	1.27	0.42	707
	T_{96}	Active	0.41	0	0.27	0	5.34	91.1	1.92	0.96	730
		Total	0	0	0	0	0.87	97.9	0.87	0.35	575

Table 3.15: Percent composition of phylogenetic groups to class *Gammaproteobacteria* in Sargasso Sea bacterial communities. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the order level were summed, and the sum was used to calculate percent composition. The number of sequences in each sample assigned to *Gammaproteobacteria* is presented in the last column.

Season	Time	Sample	Order of *Gammaproteobacteria*						Number of Sequences
			Altero-monadales	KI89A_ clade	*Oceano-spirillales*	*Salini-sphaerales*	*Vibrionales*	Other	
Spring 2009	T_0	Active	0	0	75.9	21.7	0	2.41	83
		Total	6.67	0	80.0	13.3	0	0	30
	T_{96}	Active	3.28	0	90.2	6.56	0	0	61
		Total	4.65	0	86.0	9.30	0	0	43
Spring 2010	T_0	Active	0	0	87.3	9.86	0	2.82	71
		Total	0	0	78.6	19.0	0	2.38	84
	T_{96}	Active	3.72	0	81.9	4.79	9.57	0	188
		Total	1.79	0	92.9	5.36	0	0	56
Summer 2009	T_0	Active	0	0	82.5	0	0	17.5	40
		Total	34.8	0	56.5	0	0	8.70	46
	T_{96}	Active	0	0	80.3	0	0	19.7	76
		Total	0	3.57	78.6	0	0	17.9	56
Summer 2010	T_0	Active	0	0	81.3	0	0	18.8	16
		Total	0	0	91.1	0	0	8.89	45
	T_{96}	Active	0	5.88	79.4	0	0	14.7	34
		Total	0	0	100	0	0	0	7

Table 3.16: Percent composition of phylogenetic classes to *Bacteroidetes* in Mid-Atlantic Bight bacterial communities. Samples were rarefied 100 times for 613 and 1146 sequences for spring 2010 and the other seasons respectively, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the class level were summed, and the sum was used to calculate percent composition. The number of sequences in each sample assigned to *Bacteroidetes* is presented in the last column.

Season	Time	Sample	Class of *Bacteroidetes*				Number of sequences
			Other *Bacteroidetes*	*Flavo-bacteria*	*Sphingo-bacteria*	Unclassified	
Spring 2009	T_0	Active	0.23	97.9	0.46	1.38	434
		Total	0.97	97.1	0	1.94	103
	T_{12}	Active	0.31	97.6	0.78	1.26	637
		Total	0	99.4	0.57	0	175
Spring 2010	T_0	Active	3.64	89.1	5.45	1.82	55
		Total	0	94.2	0	5.77	52
	T_{12}	Active	0	0	0	0	0
		Total	0	100	0	0	33
Summer 2009	T_0	Active	0.42	93.6	4.24	1.69	236
		Total	1.43	90.7	6.43	1.43	140
	T_{12}	Active	0	94.5	3.66	1.83	164
		Total	0	91.8	8.20	0	61
Summer 2010	T_0	Active	0	94.3	5.71	0	35
		Total	0	88.1	8.47	3.39	59
	T_{12}	Active	0	93.8	6.19	0	113
		Total	0	76.2	19.0	4.76	42

Table 3.17: Percent composition of phylogenetic classes to *Proteobacteria* in Mid-Atlantic Bight bacterial communities. Samples were rarefied 100 times for 613 and 1146 sequences for spring 2010 and the other seasons respectively, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the class level were summed, and the sum was used to calculate percent composition. The number of sequences in each sample assigned to *Proteobacteria* is presented in the last column.

Season	Time	Sample	Class of *Proteobacteria*						Number of sequences
			α	β	δ	γ	Unclassified	Other	
Spring 2009	T_0	Active	94.2	1.87	0	3.75	0.17	0	587
		Total	92.4	1.94	0	5.59	0.11	0	930
	T_{12}	Active	93.2	2.02	0	4.79	0	0	397
		Total	94.1	1.77	0	3.78	0.35	0	847
Spring 2010	T_0	Active	78.6	0.37	0	20.4	0.56	0	538
		Total	88.0	0.87	0	10.3	0.44	0.44	458
	T_{12}	Active	0	0	0	0	0	0	0
		Total	85.9	1.35	0	12.5	0	0.19	518
Summer 2009	T_0	Active	72.4	0.95	0	26.6	0	0	733
		Total	76.0	1.36	0.37	22.3	0	0	808
	T_{12}	Active	88.2	2.01	0	9.18	0.63	0	795
		Total	80.5	1.98	0	16.7	0.81	0	860
Summer 2010	T_0	Active	84.8	1.10	1.10	12.0	0.98	0	817
		Total	82.7	2.16	2.16	10.3	2.73	0	878
	T_{12}	Active	79.9	1.02	1.31	16.8	1.02	0	685
		Total	87.9	1.73	2.59	6.29	1.48	0	811

Table 3.18: Percent composition of phylogenetic groups to class *Alphaproteobacteria* in Mid-Atlantic Bight bacterial communities. Samples were rarefied 100 times for 613 and 1146 sequences for spring 2010 and the other seasons respectively, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the order level were summed, and the sum was used to calculate percent composition. The number of sequences in each sample assigned to *Alphaproteobacteria* is presented in the last column.

Season	Time	Sample	Order of *Alphaproteobacteria*						Number of sequences
			OCS116	*Rhodo-bacterales*	*Rhodo-spirillales*	*Rickettsiales*	SAR11	Other	
Spring 2009	T_0	Active	0	9.95	0	12.3	77.6	0.18	553
		Total	0	1.86	0	9.55	88.1	0.47	859
	T_{12}	Active	0	30.8	0	10.8	58.4	0	370
		Total	0	4.52	0	8.91	86.3	0.25	797
Spring 2010	T_0	Active	0	14.9	0	2.13	83.0	0	423
		Total	0	20.8	0.25	3.47	75.4	0	403
	T_{12}	Active	0	0	0	0	0	0	0
		Total	0	13.3	0	4.27	82.2	0.22	445
Summer 2009	T_0	Active	0	10.2	0	14.9	72.9	2.07	531
		Total	0.16	4.89	0.33	22.6	70.7	1.30	614
	T_{12}	Active	0	11.3	0	20.8	63.2	4.71	701
		Total	0	4.77	0	21.0	73.4	0.87	692
Summer 2010	T_0	Active	0.29	3.90	0	8.37	84.0	3.46	693
		Total	0	2.48	0.28	10.3	81.4	5.51	726
	T_{12}	Active	0	10.4	0.37	16.1	68.2	4.94	547
		Total	0	2.81	0.28	19.4	72.8	4.77	713

Table 3.19: Percent composition of phylogenetic groups to class *Gammaproteobacteria* in Mid-Atlantic Bight bacterial communities. Samples were rarefied 100 times for 613 and 1146 sequences for spring 2010 and the other seasons respectively, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the order level were summed, and the sum was used to calculate percent composition. The number of sequences in each sample assigned to *Gammaproteobacteria* is presented in the last column.

Season	Time	Sample	Order of *Gammaproteobacteria*						Number of sequences
			Altero-monadales	KI89A_ clade	*Oceano-spirillales*	*Pseudo-monadales*	*Thiotrichales*	Other	
Spring 2009	T_0	Active	36.4	0	59.1	4.55	0	0	22
		Total	11.5	3.85	84.6	0	0	0	52
	T_{12}	Active	15.8	0	84.2	0	0	0	19
		Total	28.1	6.25	62.5	0	3.13	0	32
Spring 2010	T_0	Active	60.9	0	38.2	0	0	0.91	110
		Total	48.9	0	48.9	0	0	2.13	47
	T_{12}	Active	0	0	0	0	0	0	0
		Total	56.9	0	41.5	0	1.54	0	65
Summer 2009	T_0	Active	7.69	0.51	91.8	0	0	0	195
		Total	18.9	1.11	80.0	0	0	0	180
	T_{12}	Active	4.11	0	95.9	0	0	0	73
		Total	20.1	2.08	77.8	0	0	0	144
Summer 2010	T_0	Active	0	2.04	93.9	0	0	4.08	98
		Total	6.67	0	80.0	0	0	13.3	90
	T_{12}	Active	0.87	2.61	95.7	0	0	0.87	115
		Total	3.92	13.7	78.4	0	0	3.92	51

Table 3.20: Significantly differently represented OTUs ($p < 0.01$) in the active vs. total community of spring 2009 sample T_0 from the Sargasso Sea. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. OTUs with sequences that comprised ≤1 % of the total community were considered rare. If the OTU was rare in both populations the differential representation was calculated using the method presented in Audic and Claverie (1997). Otherwise, the differential representation was calculated using the two-population proportions test. Figures represented are the percentage contribution of the OTU to the population in consideration.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	0.39	2.92	Synecho_5	*Synechococcus* (*Cyanobacteria*)
Active	1.74	0.65	SAR86_16	SAR86 (γ)
Active	1.92	0.04	Flavo_44	*Flavobacteria* (*Bacteroidetes*)
Active	0.93	0.08	Alpha_82	Unclassified *Alphaproteobacteria*
Active	0.46	0.07	Acidimicrobineae_123	*Acidimicrobineae* (*Actinobacteria*)
Active	1.61	0.34	Salinisphaeraceae _129	*Salinisphaeraceae* (γ)

Table 3.21: Significantly differently represented OTUs in the active vs. total community of spring 2009 sample T_{96} in the Sargasso Sea. See caption from table 3.20 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	1.52	5.39	Synecho_5	*Synechococcus* (*Cyanobacteria*)
Active	1.81	0.52	NS2b_14	NS2b_marine_group (*Flavobacteria*)
Active	1.56	0.78	SAR86_16	SAR86 (γ)

Table 3.22: Significantly differently represented OTUs in the active vs. total community of spring 2010 sample T_0 in the Sargasso Sea. See caption from table 3.20 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	16.9	21.5	SAR11_1	SAR11 (α)
Total	13.5	18.6	SAR11_3	SAR11
Active	1.52	0.17	Synecho_5	*Synechococcus* (*Cyanobacteria*)
Active	3.92	0	unc_7	Unclassifed, potentially chloroplast
Active	5.53	0.09	unc_8	Unclassifed, potentially chloroplast
Active	2.67	0	unc_9	Unclassifed, potentially chloroplast
Active	1.34	0.09	unc_11	Unclassifed
Active	2.05	0.08	unc_12	Unclassifed
Total	0.36	2.59	SAR406_13	SAR406 (*Deferribacteres*)
Total	2.41	4.29	NS2b_14	NS2b_marine_group (*Flavobacteria*)
Active	3.30	0.06	unc_23	Unclassifed
Active	0.80	0.03	unc_27	Unclassifed, potentially chloroplast
Total	1.07	2.78	SAR11_30	SAR11

Table 3.22 continued.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Active	1.25	0	unc_32	Unclassifed
Active	0.80	0	unc_33	Unclassifed, potentially *Micromonas* sp. RCC299 mitochondrion 16S
Active	0.71	0.03	unc_39	Unclassifed
Active	1.16	0	unc_40	Unclassifed
Total	0.09	0.99	Acidimicrobineae_56	Acidimicrobineae (*Actinobacteria*)
Active	0.53	0	unc_69	Unclassifed, potentially chloroplast
Total	0.09	0.73	NS4_79	NS4 (*Flavobacteria*)
Active	0.89	0	Alpha_82	Unclassified alphaproteobacterium
Total	0.09	0.69	Acidimicrobineae_92	*Acidimicrobineae*
Total	0.09	0.76	SAR324_119	SAR324 (δ)
Total	0.45	1.42	Salinisphaeraceae_129	*Salinisphaeraceae* (γ)

Table 3.23: Significantly differently represented OTUs in the active vs. total community of spring 2010 sample T_{96} in the Sargasso Sea. See caption from table 3.20 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Active	0.68	0.03	Synecho_5	*Synechococcus* (*Cyanobacteria*)
Total	10.3	15.1	unc_8	Unclassified, potentially chloroplast
Active	5.11	2.73	NS2b_14	NS2b_marine_group (*Flavobacteria*)
Active	2.18	0.81	ZD0405_35	ZD0405 (γ)
Active	0.93	0.13	NS2b_46	NS2b_marine_group
Active	1.35	0.39	SAR86_49	SAR86 (γ)
Active	0.58	0	Alpha_82	Unclassified alphaproteobacterium
Active	2.71	0.74	SAR86_138	SAR86
Active	0.84	0.02	Vibrio_705	*Vibrio* sp. (γ)
Active	0.59	0	Vibrio_710	*Vibrio* sp.

Table 3.24: Significantly differently represented OTUs in the active vs. total community of summer 2009 sample T_0 in the Sargasso Sea. See caption from table 3.20 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Active	23.2	18.4	SAR11_1	SAR11 (α)
Total	15.7	22.8	Prochloro_2	*Prochlorococcus* (*Cyanobacteria*)
Active	6.45	4.15	SAR11_6	SAR11

Table 3.25: Significantly differently represented OTUs in the active vs. total community of summer 2009 sample T_{96} in the Sargasso Sea. See caption from table 3.20 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	14.1	17.8	Prochloro_2	*Prochlorococcus* (*Cyanobacteria*)
Active	0.73	0	Alpha_82	Unclassifed alphaproteobacterium

Table 3.26: Significantly differently represented OTUs in the active vs. total community of summer 2010 sample T_0 in the Sargasso Sea. See caption from table 3.20 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	9.25	19.3	Prochloro_2	*Prochlorococcus* (*Cyanobacteria*)
Active	8.06	1.99	SAR11_6	SAR11 (α)
Active	1.41	0.33	SAR11_17	SAR11
Total	0.02	1.49	Acidimicrobineae_31	*Acidimicrobineae* (*Actinobacteria*)
Total	0.28	2.33	Acidimicrobineae_36	*Acidimicrobineae*
Active	2.64	0.15	SAR116_42	SAR116 (α)

Table 3.27: Significantly differently represented OTUs in the active vs. total community of summer 2010 sample T_{96} in the Sargasso Sea. See caption from table 3.20 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	17.1	33.5	Prochloro_2	*Prochlorococcus* (*Cyanobacteria*)
Total	3.24	7.07	SAR11_4	SAR11 (α)
Active	7.38	0.45	SAR11_6	SAR11
Active	0.80	0.03	SAR406_13	SAR406 (*Deferribacteres*)
Active	1.27	0.04	SAR11_17	SAR11
Active	1.10	0.04	SAR11_19	SAR11
Active	0.85	0	SAR11_25	SAR11
Active	0.54	0	SAR406_26	SAR406
Total	0.20	2.95	Acidimicrobineae_31	*Acidimicrobineae* (*Actinobacteria*)
Total	0.15	4.11	Acidimicrobineae_36	*Acidimicrobineae*
Active	2.13	0.44	SAR116_42	SAR116 (α)
Active	0.71	0.09	Alpha_82	Unclassified alphaproteobacterium
Active	0.48	0.03	Alpha_145	Unclassified alphaproteobacterium

Table 3.28: Significantly differently represented OTUs ($p < 0.01$) in the active vs. total community of spring 2009 sample T_0 in the Mid-Atlantic Bight. Samples were rarefied 100 times for 613 sequences for spring 2010 samples and 1146 for all other samples, then frequencies for each OTU were averaged for a mean representation. OTUs with sequences that comprised ≤1 % of the total community were considered rare. If the OTU was rare in both populations in a pair of samples the differential representation was calculated using the method presented in Audic and Claverie (1997). Otherwise, the differential representation was calculated using the two-population proportions test. Figures represented are the percentage contribution of the OTU to the population in consideration.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	32.2	58.9	SAR11_1	SAR11 (α)
Total	1.02	2.90	SAR86_2	SAR86 (γ)
Total	0.51	1.56	SAR116_8	SAR116 (α)
Active	19.8	4.25	Ulvibacter_9	*Ulvibacter* (*Bacteroidetes*)
Active	2.76	0.72	Roseobacter_13	*Roseobacter* (α)
Active	3.20	0.41	Ulvibacter_16	*Ulvibacter*
Active	3.47	0.70	Flavobacter_19	*Flavobacter* (*Bacteroidetes*)
Active	2.36	0.55	NS2b_25	NS2b_marine_group (*Flavobacteria*)
Active	0.81	0.18	Ulvibacter_26	*Ulvibacter*
Active	1.16	0.25	NS5_50	NS5 (*Flavobacteria*)
Active	0.81	0.20	NS5_73	NS5
Active	0.53	0.06	Flavobacter_98	*Flavobacter*

Table 3.29: Significantly differently represented OTUs in the active vs. total community of spring 2009 sample T_{12} in the Mid-Atlantic Bight. See caption from table 3.28 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	16.0	53.0	SAR11_1	SAR11 (α)
Total	1.65	3.87	SAR116_3	SAR116 (α)
Total	2.59	5.46	SAR11_4	SAR11
Active	4.09	0.93	Roseobacter_7	*Roseobacter* (α)
Total	0.06	1.29	SAR116_8	SAR116
Active	26.6	7.39	Ulvibacter_9	*Ulvibacter* (*Flavobacteria*)
Active	4.69	1.80	Roseobacter_13	*Roseobacter*
Active	4.22	0.95	Ulvibacter_16	*Ulvibacter*
Active	6.86	1.95	Flavobacter_19	*Flavobacter* (*Bacteroidetes*)
Active	2.56	0.62	Flavobacter_23	*Flavobacter*
Active	2.40	0.70	NS2b_25	NS2b_marine_group (*Flavobacteria*)
Active	1.51	0.40	Ulvibacter_26	*Ulvibacter*
Active	1.85	0.45	Owenweeksia_30	*Owenweeksia* (*Flavobacteria*)
Active	0.62	0.03	Bacteroidetes_83	Unclassified *Bacteroidetes*

Table 3.30: Significantly differently represented OTUs in the active vs. total community of spring 2010 sample T_0 in the Mid-Atlantic Bight. See caption from table 3.28 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Active	52.6	45.5	SAR11_1	SAR11 (α)
Active	4.05	1.70	SAR86_2	SAR86 (γ)
Total	0.49	2.10	Roseobacter_7	*Roseobacter* (α)
Active	10.4	2.91	Colwellia_20	*Colwellia* (γ)

Table 3.31: Significantly differently represented OTUs in the active vs. total community of summer 2009 sample T_0 in the Mid-Atlantic Bight. See caption from table 3.28 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Active	13.1	7.93	SAR86_2	SAR86 (γ)
Active	2.68	1.02	Roseobacter_7	*Roseobacter* (α)
Total	1.76	5.87	SAR116_8	SAR116 (α)
Total	0.65	2.73	Litoricola_17	*Litoricola* (γ)
Active	6.69	3.53	Owenweeksia_41	*Owenweeksia* (*Flavobacteria*)

Table 3.32: Significantly differently represented OTUs in the active vs. total community of summer 2009 sample T_{12} in the Mid-Atlantic Bight. See caption from table 3.28 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Active	2.50	0.75	Roseobacter_7	*Roseobacter* (α)
Active	3.48	1.64	Rhodobacter_11	*Rhodobacter* (α)
Total	0.08	0.97	Litoricola_17	*Litoricola* (γ)
Active	1.46	0.45	Alpha_27	Unclassified alphaproteobacterium
Total	0.49	1.61	Marinomonas_35	*Marinomonas* (γ)
Active	0.72	0.05	Alpha_106	Unclassified alphaproteobacterium

Table 3.33: Significantly differently represented OTUs in the active vs. total community of summer 2010 sample T_0 in the Mid-Atlantic Bight. See caption from table 3.28 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Active	4.33	2.32	SAR86_2	SAR86 (γ)
Total	0.93	2.66	Cyano_37	Unclassified cyanobacterium
Active	3.17	1.03	SAR406_51	SAR406 (*Deferribacteres*)

Table 3.34: Significantly differently represented OTUs in the active vs. total community of summer 2010 sample T_{12} in the Mid-Atlantic Bight. See caption from table 3.29 for explanation of table.

Community in which OTU is overrepresented	Active	Total	OTU ID	Assigned taxonomy
Total	27.7	38.5	SAR11_1	SAR11 (α)
Active	5.02	1.17	SAR86_2	SAR86 (γ)
Total	1.50	3.78	SAR116_3	SAR116 (α)
Active	2.13	0.71	unc_5	Unclassified bacterium
Total	2.32	5.04	SAR116_8	SAR116
Active	4.09	1.56	Rhodobacter_11	*Rhodobacter* (α)
Total	0.53	2.13	SubsectionI_18	Unclassified cyanobacterium
Active	2.27	1.02	Owenweeksia_41	*Owenweeksia* (*Flavobacteria*)
Active	0.98	0.20	SAR86_58	SAR86
Active	0.80	0.20	Oceanospirillalles_130	*Oceanospirillales* (γ)

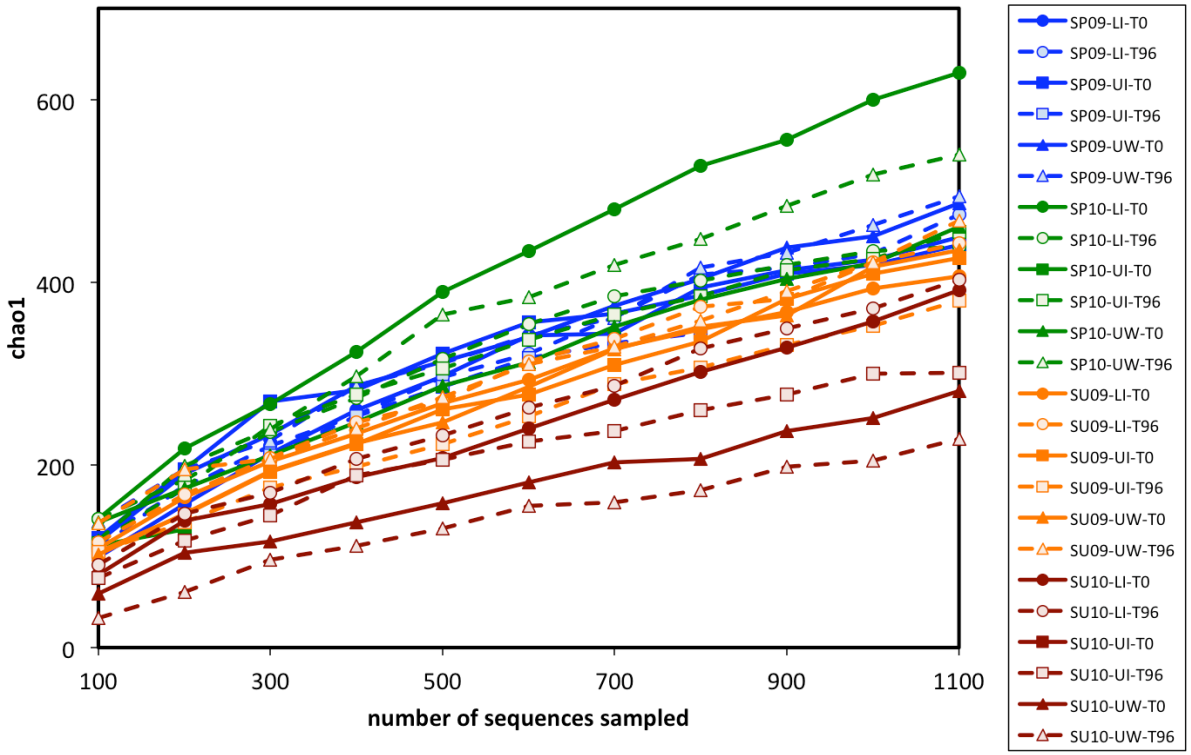

Figure 3.1: **Species richness curves of rarefied bacterial communities from the Sargasso Sea. Spring 2009 samples are indicated with blue, summer 2009 samples are orange, spring 2010 samples are green, and summer 2010 samples are red. Triangle symbols represent unlabeled, immunocaptured communities; squares are unlabeled, immunocaptured; and circles are labeled, immunocaptured communities. Sample T_0 is marked with a filled symbol, and sample T_{96} is an empty symbol.**

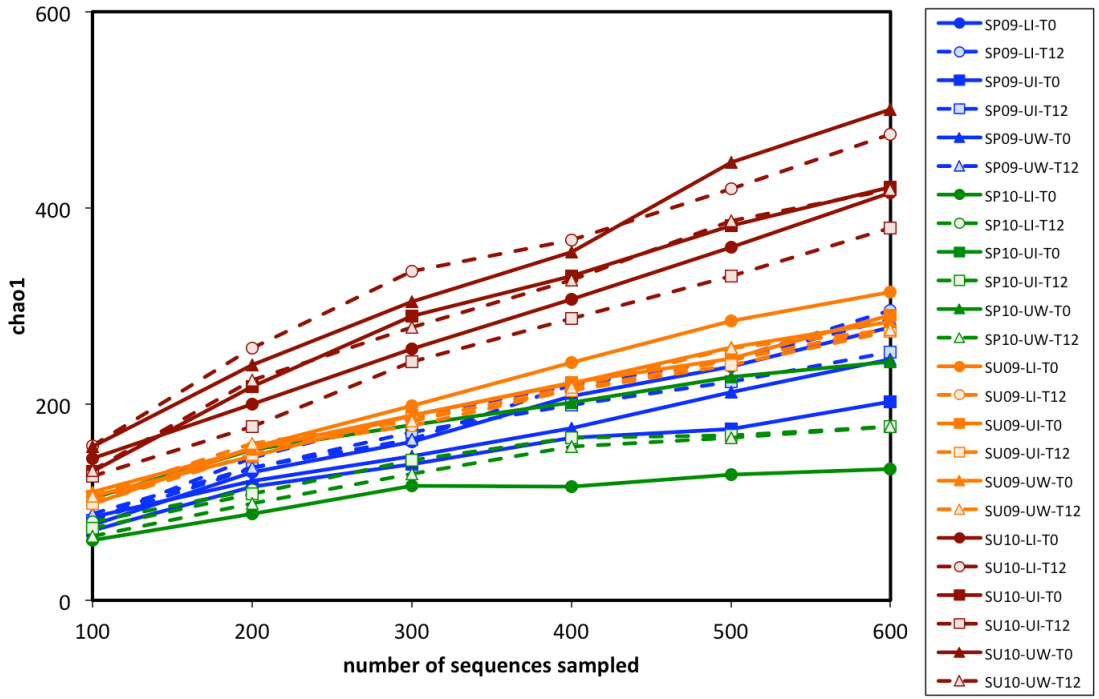

Figure 3.2: Species richness curves of rarefied bacterial communities from the Mid-Atlantic Bight. Sample T_0 is marked with a filled symbol, and sample T_{12} is an empty symbol. See caption from figure 3.1 for explanation of legend.

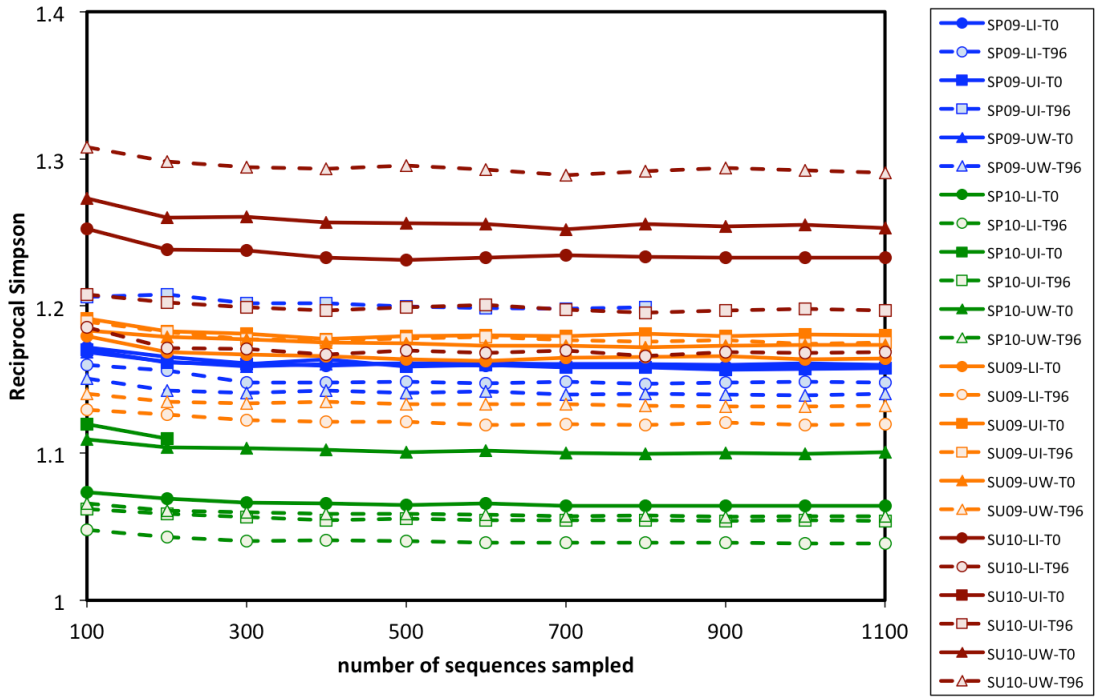

Figure 3.3: **Species evenness curves of bacterial communities from the Sargasso Sea. Spring 2009 samples are indicated with blue, summer 2009 samples are orange, spring 2010 samples are green, and summer 2010 samples are red. Triangle symbols represent unlabeled, immunocaptured communities; squares are unlabeled, immunocaptured; and circles are labeled, immunocaptured communities. Sample T_0 is marked with a filled symbol, and sample T_{96} is an empty symbol.**

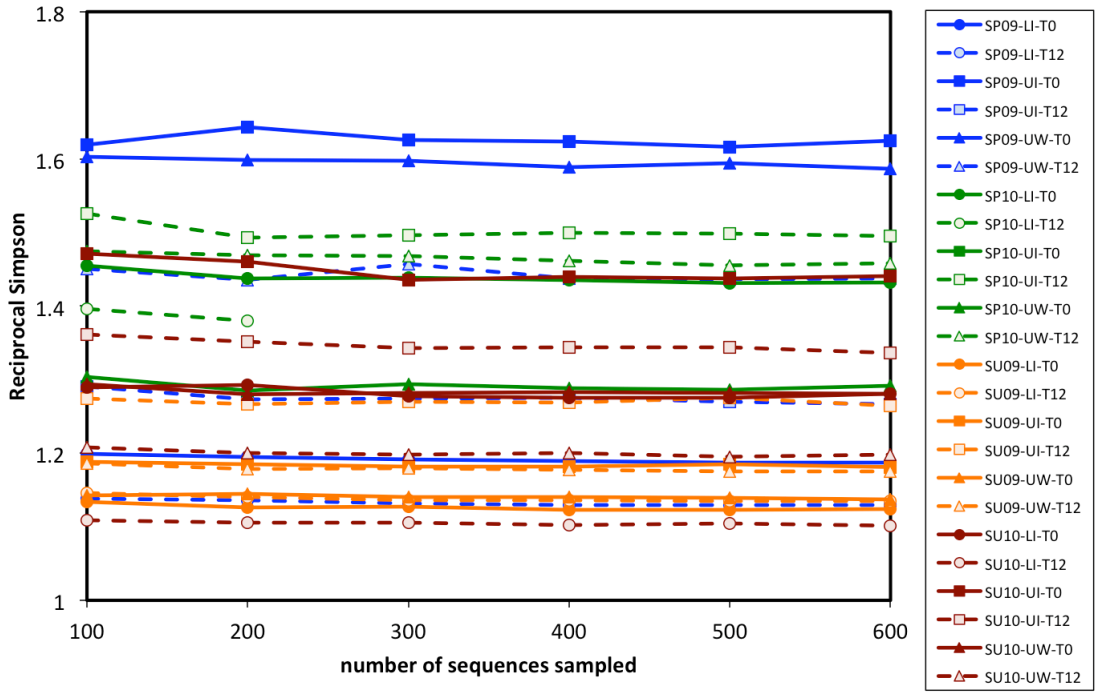

Figure 3.4: **Species evenness curves of bacterial communities from the Mid-Atlantic Bight. Sample T_0 is marked with a filled symbol, and sample T_{12} is an empty symbol. See caption from figure 3.3 for explanation of legend.**

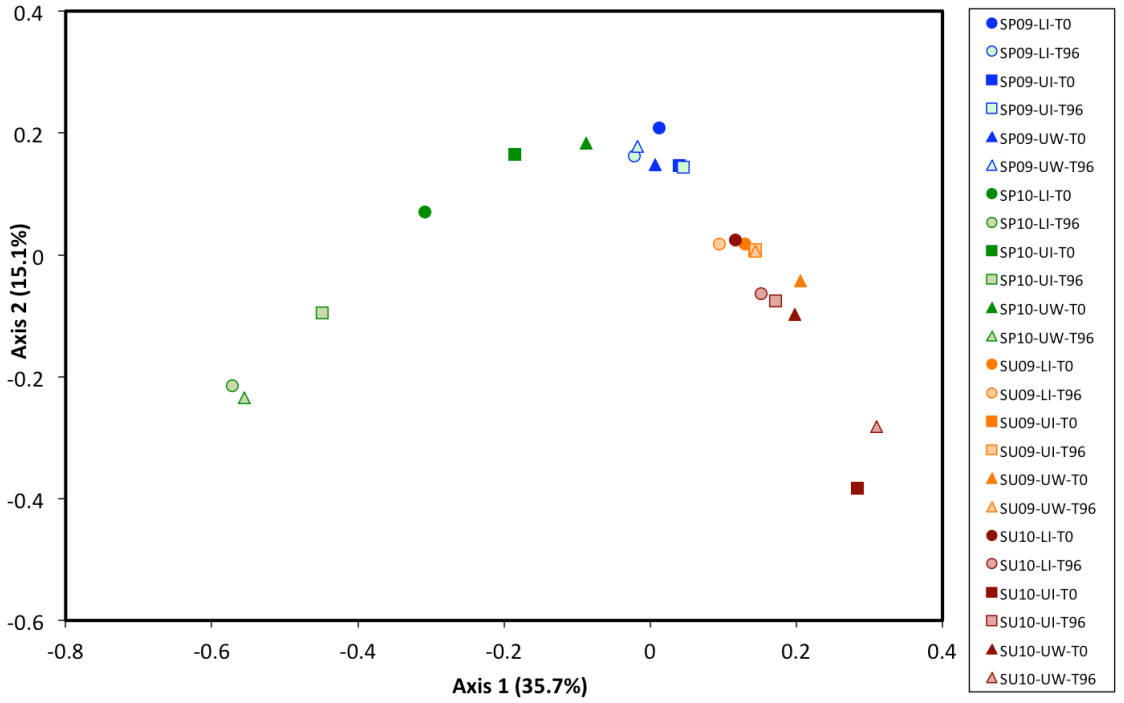

Figure 3.5: **Principal component analysis (PCA) of bacterial community structure from the Sargasso Sea Similarity matrices were calculated based on Yue and Clayton's nonparametric θ. Spring 2009 samples are indicated with blue, summer 2009 samples are orange, spring 2010 samples are green, and summer 2010 samples are red. Triangle symbols represent unlabeled, immunocaptured communities; squares are unlabeled, immunocaptured; and circles are labeled, immunocaptured communities. Sample T_0 is marked with a filled symbol, and sample T_{96} is an empty symbol.**

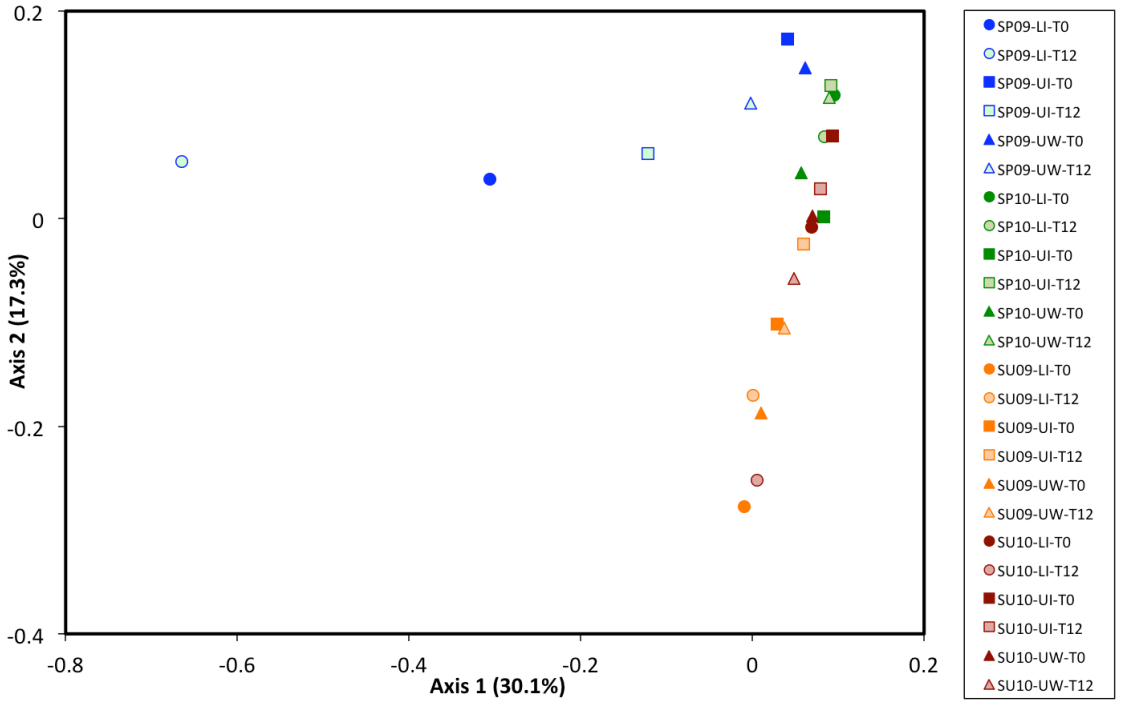

Figure 3.6: Principal component analysis (PCA) of bacterial community structure from the Mid-Atlantic Bight. Sample T_0 is marked with a filled symbol, and sample T_{12} is an empty symbol. See caption from figure 3.5 for explanation of legend.

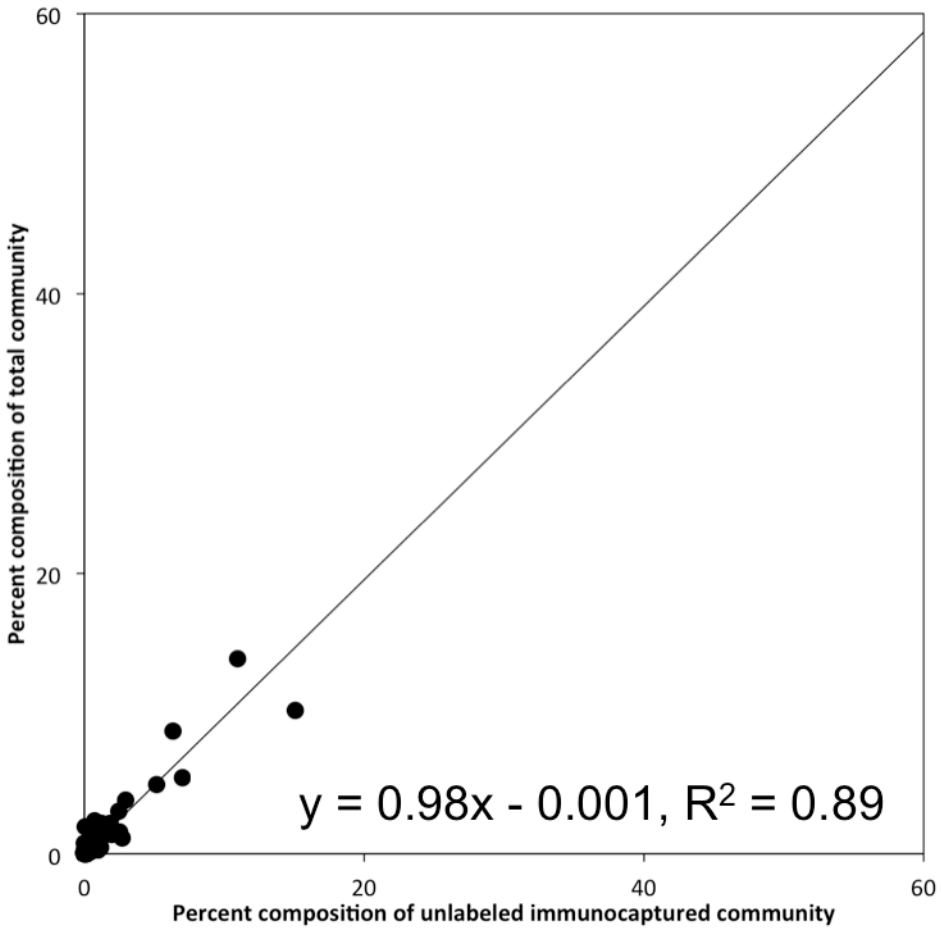

Figure 3.7: Contribution of each OTU to the unlabeled, immunocaptured vs. total communities of the Sargasso Sea spring 2010 sample T_{96}. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Reduced major axis (RMA) regression was conducted on the percent contribution of each OTU to the unlabeled, immunocaptured community against its contribution to the total community.

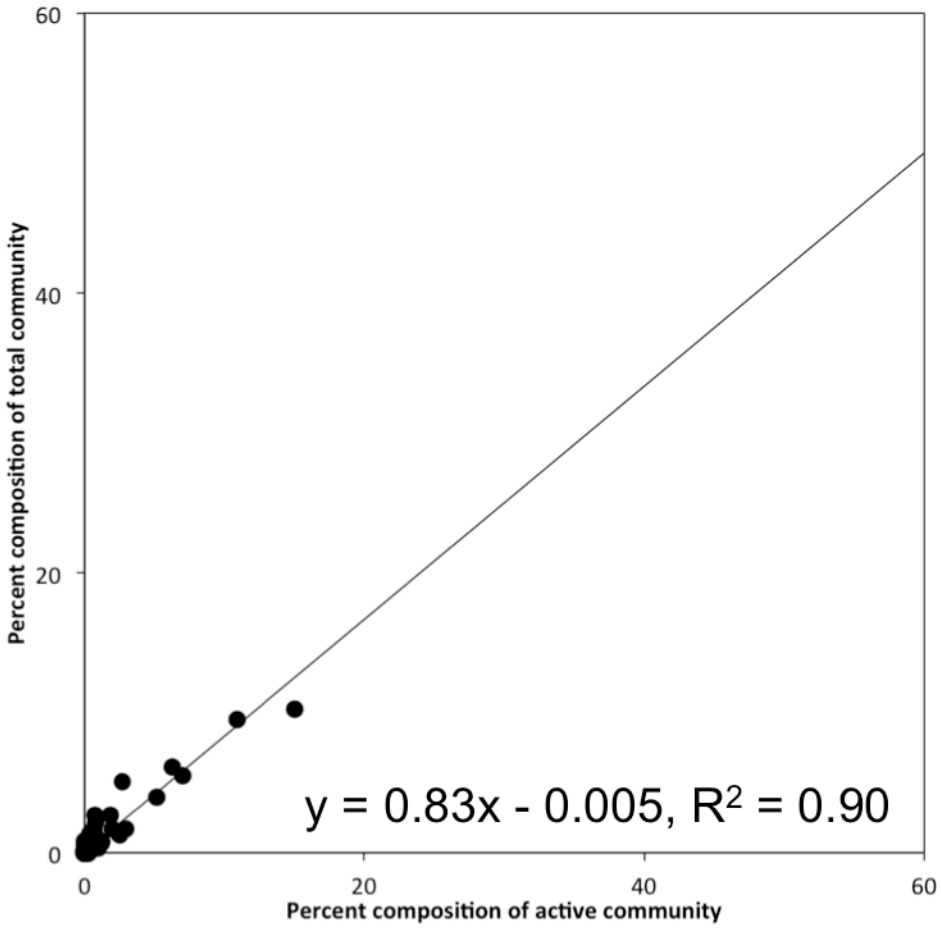

Figure 3.8: Contribution of each OTU in the active vs. total communities of the Sargasso Sea spring 2010 sample T_{96}. See caption from figure 3.7 for explanation.

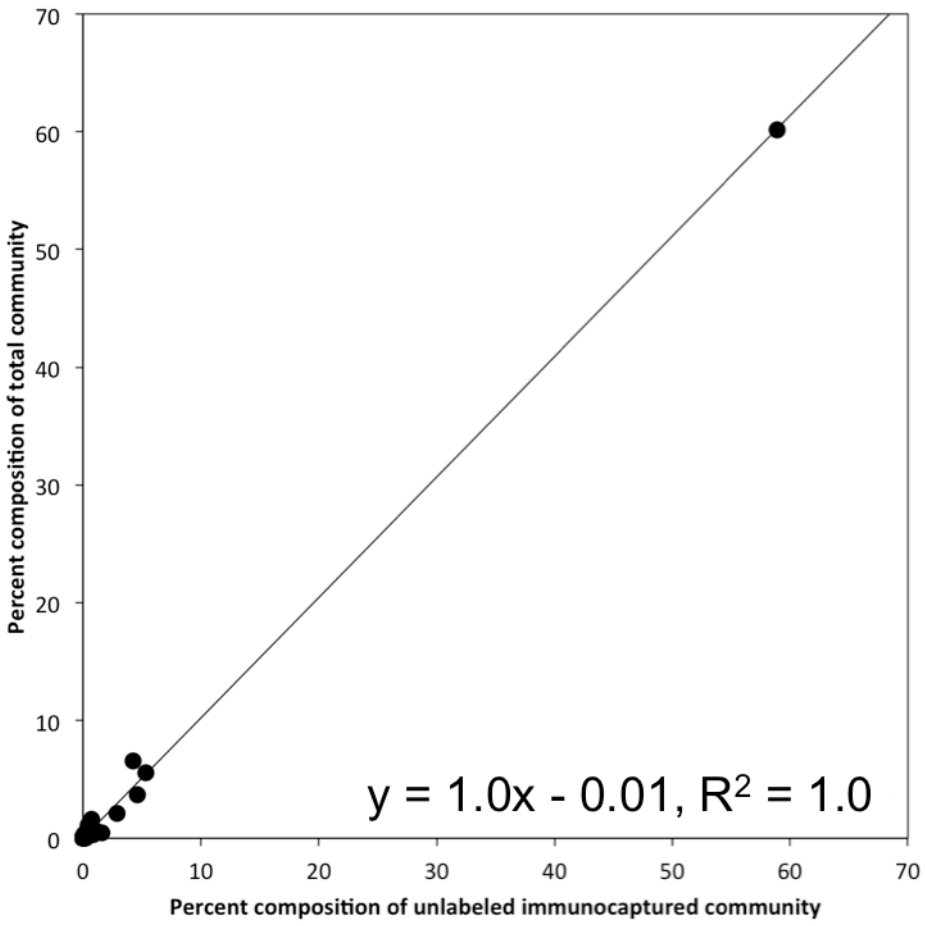

Figure 3.9: Contribution of each OTU to the unlabeled, immunocaptured vs. total communities of the Sargasso Sea spring 2009 sample T_{12}. Samples were rarefied 100 times for 1146 sequences, then frequencies for each OTU were averaged for a mean representation. Reduced major axis (RMA) regression was conducted on the percent contribution of each OTU to the unlabeled, immunocaptured community against its contribution to the total community.

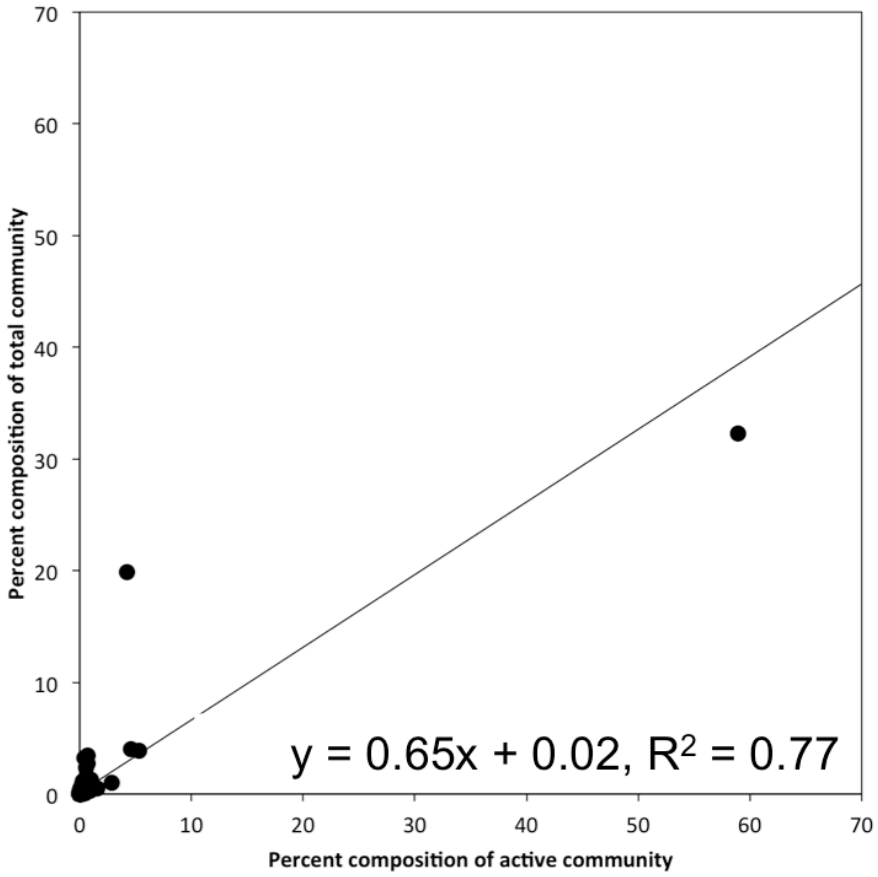

Figure 3.10: Contribution of each OTU in the active vs. total communities of the Mid-Atlantic Bight spring 2009 sample T_0. See caption from figure 3.9 for explanation.

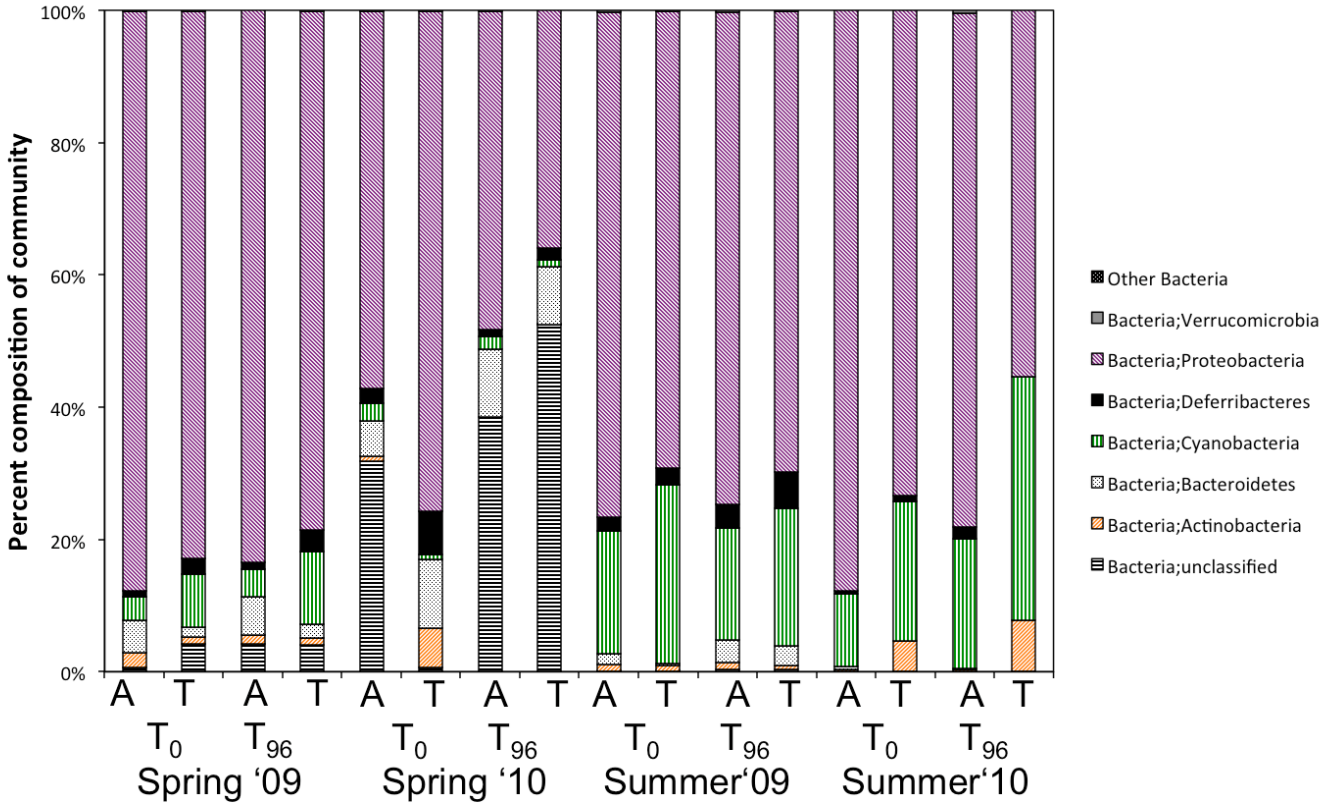

Figure 3.11: Percent composition of bacterial phyla to Sargasso Sea bacterial communities. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the phylum level were summed, and the sum was used to calculate percent composition.

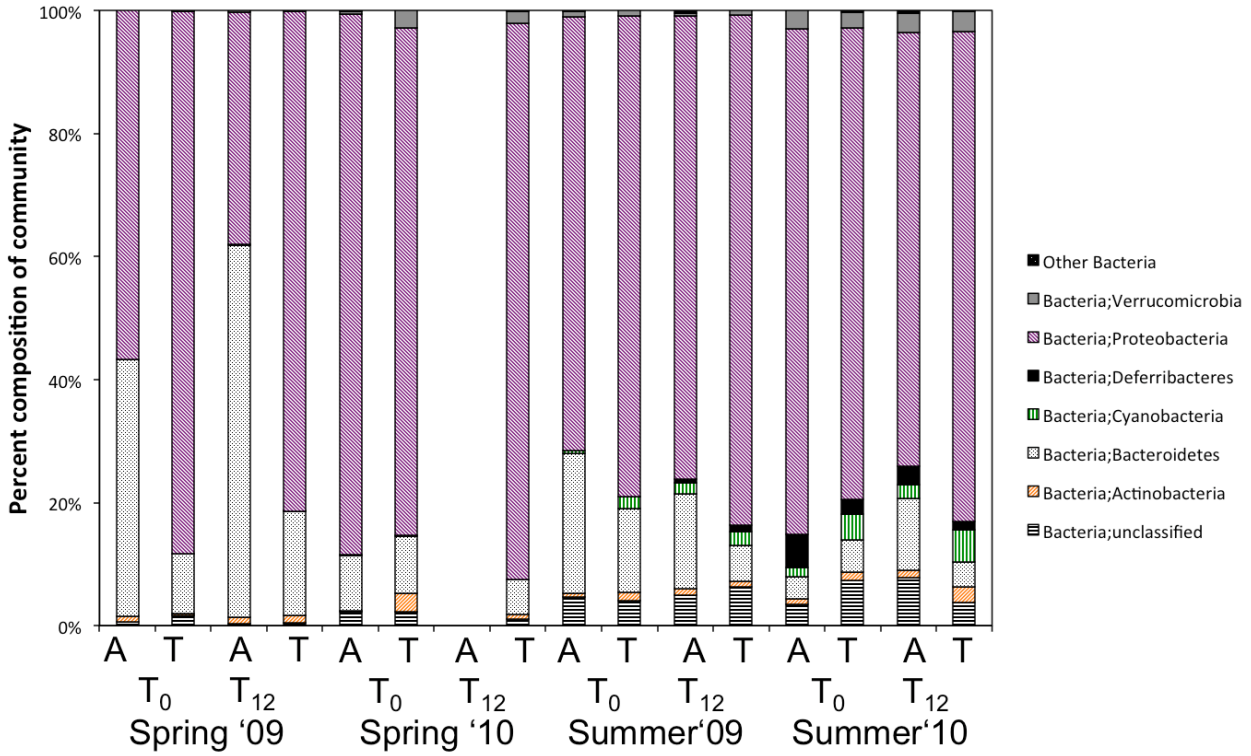

Figure 3.12: Percent composition of bacterial phyla to Mid-Atlantic Bight bacterial communities. Samples were rarefied 100 times for 613 and 1146 sequences for spring 2010 and the remaining samples respectively, then frequencies for each OTU were averaged for a mean representation. Frequencies of OTUs that agreed at the phylum level were summed, and the sum was used to calculate percent composition.

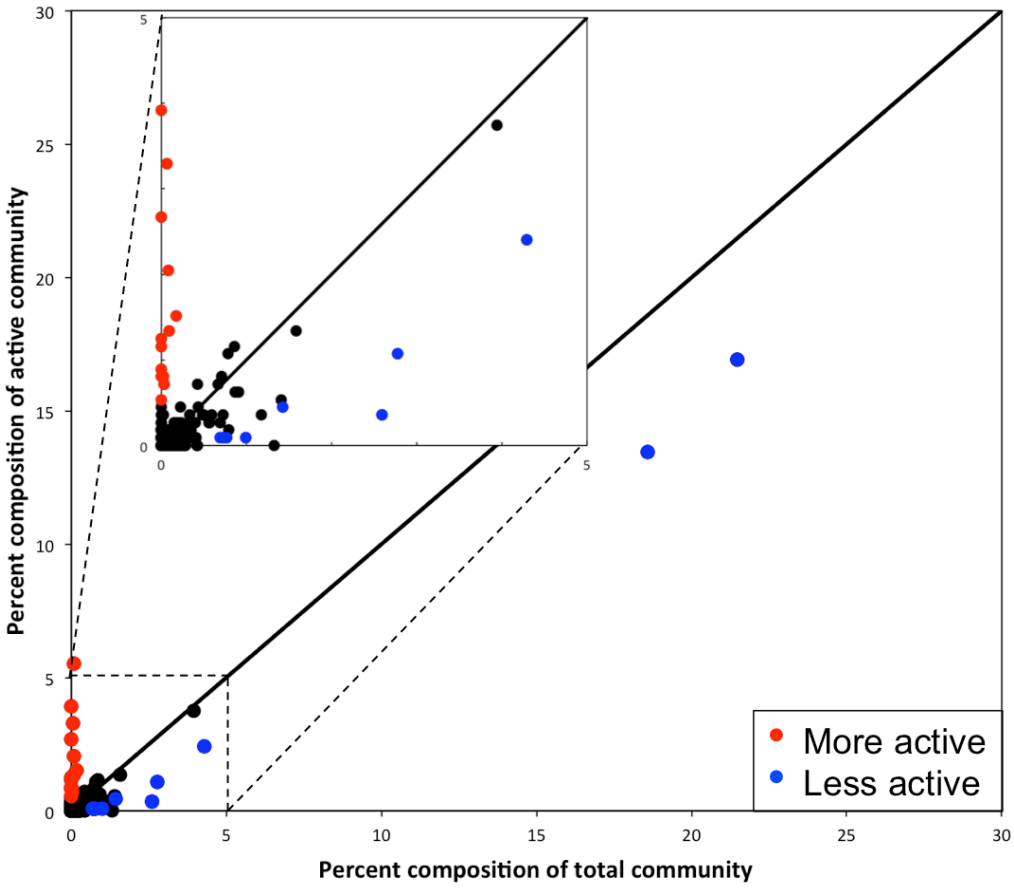

Figure 3.13: **Contributions of OTUs with significant difference in activity versus abundance to the active and total communities in the Sargasso Sea spring 2010 sample T_0. Samples were rarefied 100 times for 1100 sequences, then frequencies for each OTU were averaged for a mean representation. The percentage composition of each OTU in the active community was plotted against its percentage contribution to the total community. OTUs that had higher representation in the active community compared to the total community are plotted in red; those with lower activity than abundance are marked in blue. The 1:1 line is plotted in black. See table 3.18 for descriptions of the indicated OTUs.**

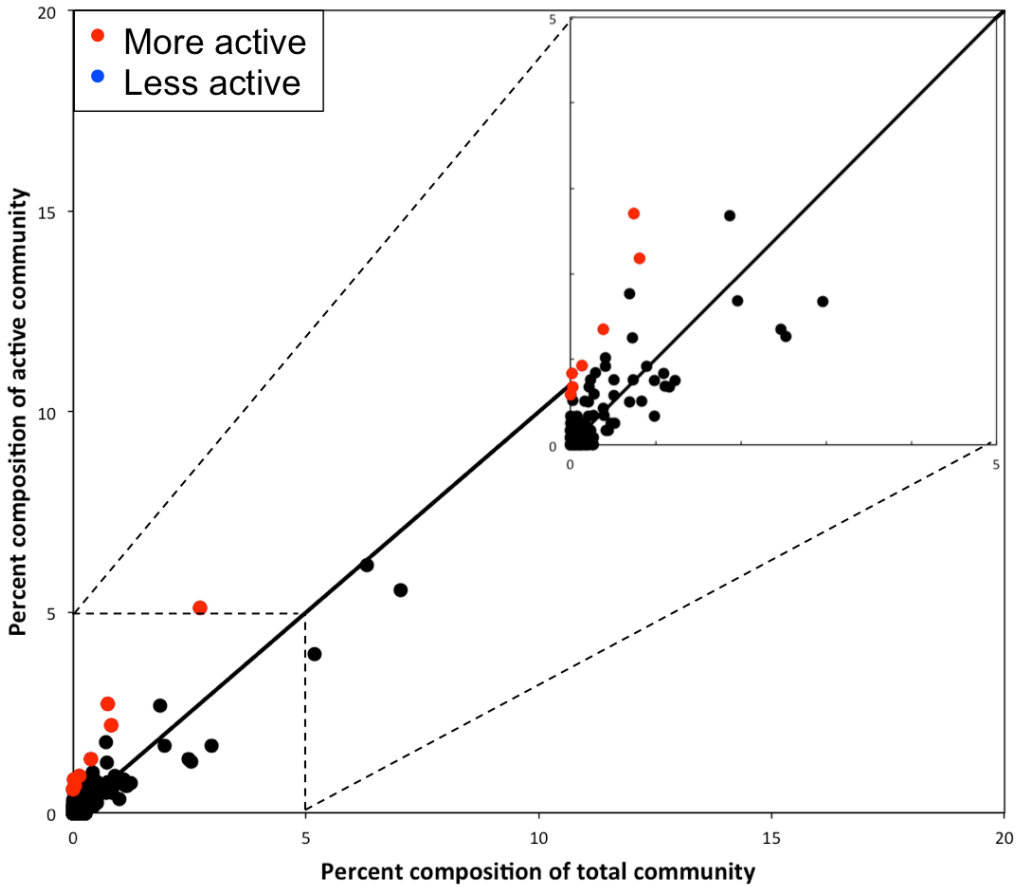

Figure 3.14: **Contributions of OTUs with significant difference in activity versus abundance to the active and total communities in the Sargasso Sea spring 2010 sample T_{96}. See caption from figure 3.13 for explanation of figure. See table 3.19 for descriptions of the indicated OTUs.**

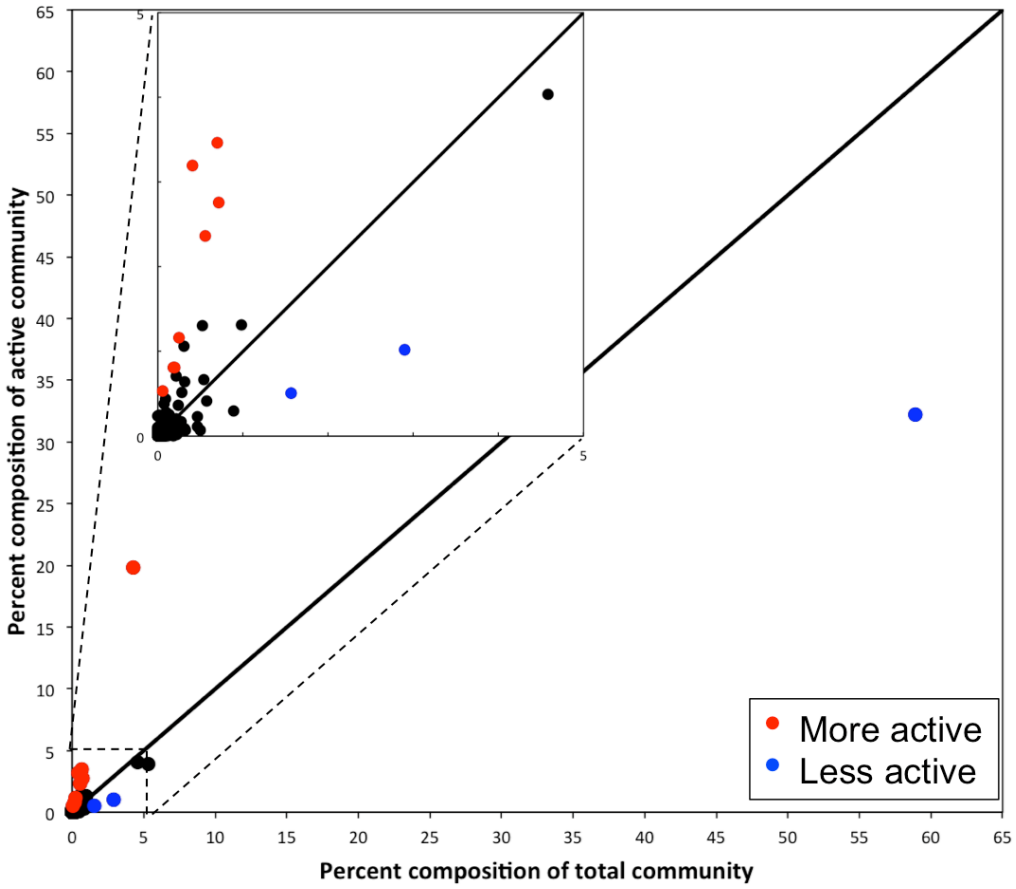
Percent composition of active community
Percent composition of total community
More active
Less active
0
5
10
15
20
25
30
35
40
45
50
55
60
65

Figure 3.15: **Contributions of OTUs with significant difference in activity versus abundance to the active and total communities in the Mid-Atlantic Bight spring 2009 sample T_0. Samples were rarefied 100 times for 1146 sequences, then frequencies for each OTU were averaged for a mean representation. The percentage composition of each OTU in the active community was plotted against its percentage contribution to the total community. OTUs that had higher representation in the active community compared to the total community are plotted in red; those with lower activity than abundance are marked in blue. The 1:1 line is plotted in black. See table 3.24 for descriptions of the indicated OTUs.**

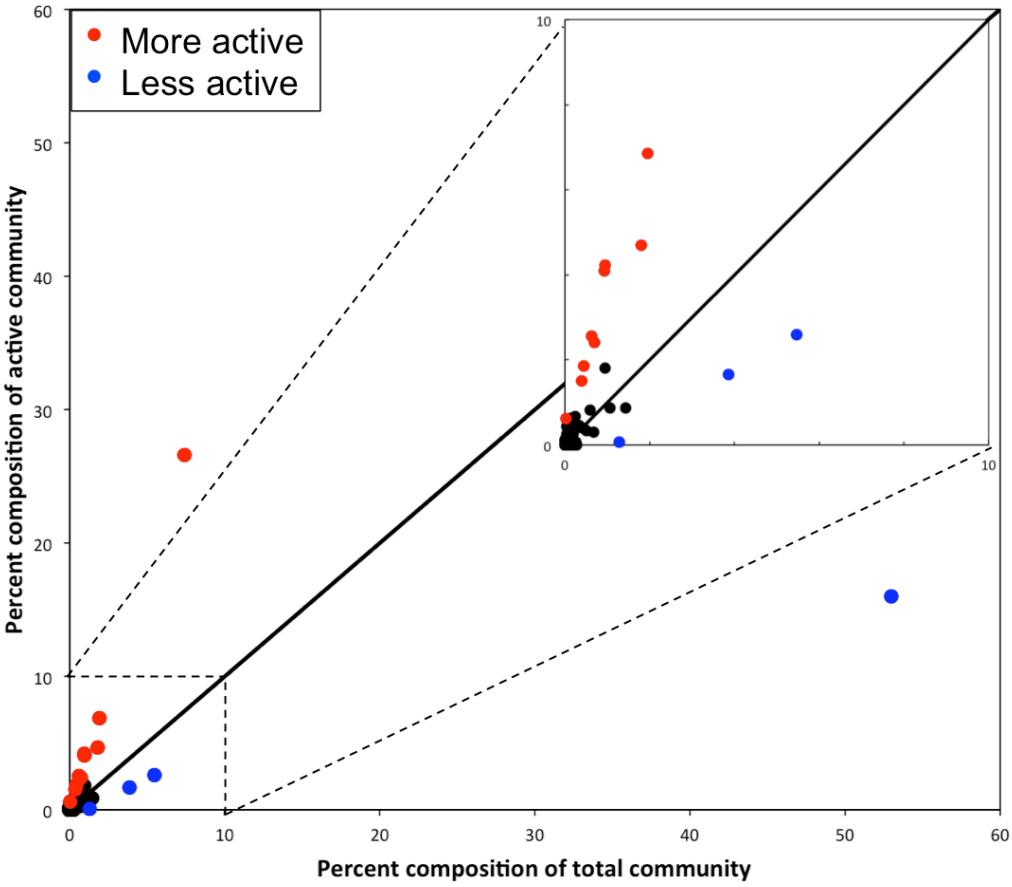

Figure 3.16: Contributions of OTUs with significant difference in activity versus abundance to the active and total communities in the Mid-Atlantic Bight spring 2009 sample T_{12}. See caption from figure 3.15 for explanation of figure. See table 3.25 for descriptions of the indicated OTUs.

Chapter 4

CONCLUSIONS

In microbial ecology, it is necessary to examine the relationship between microbial community composition and activity to better understand microbial impacts on biogeochemical processes. Traditional evaluations of activity simplified microbial populations by lumping them into the "black box." However, with an ever-expanding depth of phylogenetic and metabolic diversity, microbial communities must be examined with increased scrutiny. Furthermore, individual microbial members can vary in activity levels and shift overall community metabolism. Molecular methods can be used in conjunction with activity assays to catalogue microbial communities, and the metabolisms available to them, at fine phylogenetic scales.

Marine bacterial populations contain a select few high-abundance taxa and an immeasurable number of rare taxa (Sogin *et al.*, 2006, Pedrós-Alió, 2007, Gilbert *et al.*, 2009). Abundant bacteria are hypothesized to be actively growing, whereas rare constituents are thought to be slow growing or inactive (Pedrós-Alió, 2006). However, bacterial activity assays may generate conflicting results based on the type of activity measured and sensitivity to ecotypes. In many environments, bacterial taxa vary in abundance, distribution and activity. For example, SAR11 is one of the most abundant clades in aquatic systems, and studies have measured growth rates of SAR11 to be similar to those of other bacteria (Morris *et al.*, 2002, Malmstrom *et al.*, 2005). However, ecotypes of SAR11 cycle in abundance in the

euphotic zone of the Sargasso Sea (Carlson *et al.*, 2009). In addition, while SAR11 incorporated dissolved organic matter (DOM) in the Sargasso (Malmstrom *et al.*, 2005), they were not observed to be actively replicating their DNA in a coastal environment (Hamasaki *et al.*, 2007). The importance and impact of rare bacteria in the active microbial assemblage needs further examination as well. Members of SAR86 typically constitute between less than 1 to 10% of the bacterial community in the Sargasso and in the Mid-Atlantic Bight (Treusch *et al.*, 2009, Campbell *et al.*, 2009). However, they were actively degrading dimethysulfoniopropionate (DMSP) in the open ocean (Vila-Costa *et al.*, 2010) and engaged in DNA replication in a coastal bacterial population (Tada *et al.*, 2010). Community composition, types of activity and individual activity levels, among other factors, add complexity to the abundance/activity issue. To address this issue, we can evaluate bacterial activity levels in context of individual taxa and environmental changes, and determine how they shape total community structure.

4.1 Diversity in Activity Types

Synthesis of DNA is one type of activity in which bacterial populations engage. Labeling of bacterial DNA with 5-bromo-2'-deoxyuridine (BrdU) captures the fraction of the microbial population that is actively replicating DNA (Steward and Azam, 1999). BrdU-labeling detected active bacteria in aquatic ecosystems, soils and marine sediments (Pernthaler *et al.*, 2002, Hamasaki *et al.*, 2007, Edlund and Jansson, 2008, Dimitriu *et al.*, 2010). Bacterial ribotypes had varying levels of DNA replication and dissolved organic carbon (DOC) incorporation in the coastal Atlantic

(Mou *et al.*, 2007). Additionally, the DNA-synthesizing community was dissimilar from the total community and included several rare bacterial ribotypes (Hamasaki *et al.*, 2007). With such a broad range of activity levels and abundances in both the total and active bacterial communities, the link between abundance and activity is not always linear.

The research I presented in chapters 2 and 3 explored the relationship between activity and bacterial community composition in the Sargasso Sea and Mid-Atlantic Bight. Community structures of the active and total bacterial assemblages resembled each other in these different environments. However, some active bacterial ribotypes were in low abundance or not apparent in the total population. In addition, some ribotypes in the total community were not similarly represented in the active fraction. Members of SAR11 and *Cyanobacteria* had unexpected activity levels based on abundances; for example, some taxa of these groups had high abundances and relatively low activity levels. Based on this research, future descriptions of the active and total populations need to take into consideration the types of activity that are being targeted. In the coastal Pacific, representatives of SAR11 did not assimilate BrdU in proportion to their abundances (Hamasaki *et al.*, 2007). Additionally, sequenced genomes of cyanobacterial members do not contain thymidine kinases or exogenous nucleoside transporters (Jeffrey and Paul, 1990). Bacterial members can fall into a spectrum of activities, which may only be fully described using combination of methods (Smith and del Giorgio, 2003). Malmstrom *et al.* (2005) observed SAR11 members were responsible for more assimilation of glucose and amino acids than other bacterial types. However, SAR11 contributed only as much as,

or less than, expected to leucine turnover (Malmstrom *et al.*, 2005). Furthermore, abundances of SAR11 members were not indicative of their activity as measured via DNA synthesis, DMSP-degradation transcripts, or nutrient uptake (Hamasaki *et al.*, 2007, Vila-Costa *et al.*, 2010, Teira *et al.*, 2010). Active bacteria may be missed in the narrow scope of observing only DNA replication via BrdU incorporation. For a more comprehensive picture, different activity metrics should be used in future examinations of disproportionate activity with respect to abundance.

4.2 Individual Responses in Bacterial Activity

In Chapter 3 I combined BrdU-labeling with tag pyrosequencing to observe differential activity levels on an individual taxonomic scale, and compare shifts in the active bacterial community over short (hours and days) to long (seasonal) time scales. Pyrosequencing of the hypervariable 16S rRNA regions is a high-throughput method to analyze taxonomy, metabolic diversity and community structure in bacterial populations (Sogin *et al.*, 2006, Huber *et al.*, 2007). Bacterial taxa are often classified by clustering similar sequences into operational taxonomic units (Cohan, 2006). In marine microbial ecology, a standard cut-off of 3% sequence divergence is implemented for calling OTUs (Giovannoni and Stingl, 2005). At 97% similarity I observed high correspondence between the abundance of OTUs and their representation in the active community. Less than 2% of OTUs in the Mid-Atlantic Bight and Sargasso Sea had higher or lower activity levels than expected by their abundances. Only a handful of these taxa each constituted more than 5% of the total community, indicating that more active bacterial ribotypes were rare. However,

OTUs classified at 3% dissimilarity typically contain ecotypes with a wide range of metabolic capacities (Cohan, 2006). SAR11 as discussed above is comprised of multiple subclades with ecologically distinct characteristics (Morris *et al.*, 2005, Carlson *et al.*, 2009). *Synechococcus* and *Prochlorococcus* were not differentiated at 97% consensus in 16S rRNA sequences in the Sargasso Sea (Treusch *et al.*, 2009). Despite their apparent similarity, these two different cyanobacterial taxa reach their highest abundances at different times in the year (Treusch *et al.*, 2009). Further diversity exists even within these taxa. Ecotypes of *Prochlorococcus* can be described by light adaptation and chlorophyll b/chlorophyll a ratios, and *Synechococcus* clades have distinct phenotypic differences (Giovannoni and Stingl, 2005). OTU clusters I observed to have higher or lower activity levels could harbour bacterial ecotypes at greater than 97% similarity with even more variations in activity. Furthermore, rare ecotypes of differential activity would have been overlooked in OTUs with expected DNA synthesis levels. Given the discrepancy between ecotypes and phenotypes within even 97% similar clusters, we need to reconsider the resolution and depth at which we examine bacterial communities.

Microbial observatories provide useful time-series of bacterial community structure and environmental parameters. In the Mid-Atlantic Bight, many bacterial ribotypes cycled in abundance and activity throughout the year (Campbell *et al.*, 2009, Campbell *et al.*, 2011). Timing of maximum abundances of several taxa in the Sargasso Sea was relative to deep mixing events (Morris *et al.*, 2005, Treusch *et al.*, 2009). However, few examinations of microbial community structure are on temporal scales of hours to days. I observed differences in the active communities

twelve hours apart within one spring sampling event in the Mid-Atlantic Bight, and a shift over four days in the total communities due to changing activity levels in a spring sampling event from the Sargasso Sea. The DOC-metabolizing bacterial fraction in a coastal microbial observatory had different taxonomic distributions compared to the total community after 12 hours of incubation with an added DOC compound (Mou *et al.*, 2007). Vila-Costa *et al.* (2010) saw a shift in the DMSP-degrading community towards heterotrophic activity and enrichment in *Gammaproteobacteria* and *Bacteroidetes*, in as little as 30 minutes. Bacterial populations are capable of rapidly responding to changing environmental conditions, which can subsequently alter structure and production of the total community (Lauro *et al.*, 2009, Yooseph *et al.*, 2010). More frequent sampling of bacterial activity is suggested in order to establish a 'baseline' active community relative to environmental conditions, and capture changes in types of activity and shifts in active community structure.

With massive sequencing efforts generating high-resolution datasets, examinations of the ecological roles of marine bacteria need to consider the bacterial population at the level of individual taxon. I found that sequencing profiles in combination with activity assays are powerful insights into individual bacterial activity levels. In addition, microbial assemblages are capable of immediate shifts in structure and activity, but these are often hidden in the lapses between samplings in time-series studies. The research presented in this thesis demonstrated the usefulness of evaluating bacterial activity of individual taxa, and assessing shifts in the active and total bacterial community over time. Evaluating the abundances of active

bacterial taxa using different activity metrics and molecular genomic approaches on finer temporal scales will shed further light into the 'black box' of microbial communities, and provide better feedback into the role of individual bacteria in marine biogeochemical processes.

4.3 References

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