# DISCERNING THE MECHANISMS OF COEXISTENCE BETWEEN MARINE CREN/THAUMARCHAEA AND MARINE BACTERIA VIA STABLE ISOTOPE PROBING

by

LAUREN MARIE SEYLER

A dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Oceanography

Written under the direction of

Lee J. Kerkhof

And approved by

_____

_____

_____

_____

_____

New Brunswick, New Jersey

October, 2015

ABSTRACT OF THE DISSERTATION

DISCERNING THE MECHANISMS OF COEXISTENCE BETWEEN MARINE CREN/THAUMARCHAEA AND MARINE BACTERIA VIA STABLE ISOTOPE PROBING

by LAUREN MARIE SEYLER

Dissertation Director:

Lee J. Kerkhof

Marine mesophilic archaea were discovered two decades ago. However, the role of these culture-resistant organisms in marine nutrient cycling and the nature of their relationship with bacteria have yet to be elucidated. Current thinking is that thaumarchaea may dominate ammonia oxidation in sediments and oxygen minimum zones, based on the ubiquity of archaeal ammmonium monooxygenase genes. Yet, ammonia-oxidation (or other forms of autotrophy/mixotrophy) may only represent a subset of the full metabolic capability of the phylum. The purpose of this Ph.D. research was to assess whether estuary and marine archaea are autotrophic or heterotrophic and if they compete with bacteria for the same electron donors and acceptors. Salt marsh sediments from a New Jersey state park were screened for heterotrophy using stable isotope probing, by amending with a single $^{13}$C-labeled compound (acetate, glycine, or urea), a complex $^{13}$C-biopolymer (lipids, proteins, or growth medium), or autotrophy using $^{13}$C-bicarbonate.

$^{13}$C-labeled DNA was analyzed by TRFLP analysis of 16S rRNA genes. SIP analyses indicated salt marsh thaumarchaea and crenarchaea are heterotrophic, double within 2–3 days and often compete with heterotrophic bacteria for the same organic substrates. A clone library of $^{13}$C-amplicons was screened to find matches to the $^{13}$C-TRFLP peaks. Some of these archaea displayed a preference for particular carbon sources, whereas others incorporated nearly every $^{13}$C-substrate provided. Resource partitioning of proteins, and urea at low concentrations, was also observed. SIP was also performed in the open waters of the Sargasso Sea at multiple depths and latitudes, using $^{13}$C-labeled acetate, urea, and bicarbonate. In this environment, mixotrophy appeared to be the dominant metabolic strategy, though some heterotrophic OTUs (operational taxonomic units) were observed. No exclusively autotrophic OTUs were detected. Urea was also a competitive substrate in deep waters, with archaea outcompeting bacteria for its uptake in the majority of microcosms. Archaea-specific and bacteria-specific predators were detected via incorporation of $^{13}$C into eukaryotic OTUs. This research demonstrates the significance of organic carbon uptake and selective predation on the structuring of marine archaeal communities.

# ACKNOWLEDGEMENTS

First and foremost, I wish to thank my advisor Lee Kerkhof, for his wisdom, advice, and patient good humor, and for reminding me that "you can't win if you don't play." I'm thankful to Jen Biddle and Jack Gilbert for providing career guidance, furthering my career in countless ways, and challenging my ideas. I would also like to thank Costa Vetriani, Peter Morin, and Bob Chant, for serving on my committee and providing valuable insight and constructive comments throughout my graduate career.

I would be remiss not to also acknowledge the many contributions of Steve Tuorto and Lora McGuinness to this dissertation, and all of my graduate work. Whenever I was confused by my data or unsure what to do next, they were always available with advice, ideas, or sympathy. To the many other Rutgers professors and staff who have inspired me, mentored me, or aided in my career development, I am eternally grateful.

My career in science truly began with the guidance and inspiration of my father Brian Seyler, who taught me a love of learning, a great respect for academia, and a stubborn resolve to do anything I put my mind to. Thank you for the field trips, experiments, documentaries, and discussions that sparked my fascination with the natural world.

I am grateful to all the friends and family who have offered their help and encouragement over the years. Most of all, I wish to thank my husband Keith Stratton, for his unending patience, love, and support. Your belief in me gives me strength. I promise to always try to live up to it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# Chapter 1. Introduction

## Abstract

The existence of mesophilic and low-temperature archaea in the world's oceans was discovered two decades ago (Delong, 1992; Fuhrman et al., 1992). While much work has been done to characterize these culture-resistant organisms, their role in marine nutrient cycling and the nature of their relationship with bacteria occupying the same environments have yet to be elucidated. Because these microorganisms are both abundant and ubiquitous, one may surmise that they play a variety of roles in the marine ecosystem. For example, current thinking is that members of the phylum Thaumarchaeota (Brochier-Armanet et al., 2008) may dominate ammonia oxidation, the first step of nitrification, in sediments and oxygen minimum zones, based on the ubiquity of archaeal ammmonium monooxygenase genes (Francis et al., 2005). The concept that thaumarchaea are chemoautotrophic is supported by isotopic signatures associated with $CO_2$ uptake (Ingalls et. al., 2006). Conversely, earlier research indicated that open ocean thaumarchaea (Ouverney and Fuhrman, 2000; Hallam et al., 2006; Martin-Cuadrado et al., 2008) and deep sea sediment bathyarchaea and crenarchaea (Biddle et al., 2006) were heterotrophic. A recent study found that two cultured strains of ammonia-oxidizing thaumarchaea were obligate mixotrophs, requiring the addition of a low concentration of α-ketoglutaric acid to the inorganic basal medium for growth (Qin et al., 2014). At present, we know very little about what marine archaea use as carbon and energy sources, and whether they compete with heterotrophic or nitrifying bacteria that are using the

same electron donors and acceptors. This Ph.D. research assesses whether coastal and pelagic thaumarchaeal, crenarchaeal, and bathyarchaeal populations are autotrophic or heterotrophic, and what substrates and growth conditions marine archaea can utilize in a natural setting. The research also provides some information on the competitive relationship between archaea and bacteria co-existing in the same environment.

**Overall Objective**

Previous studies have attempted to elucidate the life strategies of marine archaea (chiefly belonging to the phylum Thaumarchaeota) by molecular ecology approaches. Initially, CARD-FISH experiments utilizing $^{3}H$-labeled amino acids were performed on open ocean archaea (Herndl et. al., 2005; Varela et. al., 2008). Subsequently, metagenomic studies have been employed to reveal the genetic capabilities of the thaumarchaeal community. The $\delta\ ^{13}C$ signatures of archaeal membrane lipids have also been used to explore archaeal metabolic capability (e.g. Varela et. al., 2008). For example, heterotrophy is considered the dominant life strategy for bathyarchaea and crenarchaea in deep sea sediment (Biddle et al., 2006). Marine Group II euryarchaea appear to be motile photoheterotrophs, based on a genome assembled from surface seawater (Iverson et al., 2012). Unfortunately, all approaches have limitations that do not allow for the direct testing of competition between archaea and bacteria. Specifically, $\delta\ ^{13}C$ methods provide only general information about the source of the carbon (i.e., whether the carbon was from an organic or inorganic source), MARFISH experiments may confound cellular uptake with cell "stickiness," and the possession of a particular

gene by a metagenomic study does not necessarily predicate its expression (Dubnau and Losick, 2006; Smits et al., 2006).

In this project, I used stable isotope probing experiments (Radajewski et al., 1999; Gallagher et al., 2005) to assess if marine archaeal populations were heterotrophic or autotrophic, how long it took for DNA synthesis and cell division to occur, if there were preferences for specific electron donors, and if there was direct competition between specific archaea and bacteria for specific substrates. In contrast to the approaches listed above, these stable isotope probing (SIP) experiments demonstrate substrate uptake and assimilation, by tracking carbon incorporation into newly synthesized DNA. In the literature, the first SIP experiment performed on archaea focused mostly on fermenters and methanogens, and was conducted in an acidic fen (Hamberger et. al., 2008). In this Ph.D. research study, SIP methodology was used to examine carbon uptake by marine archaea, in order to provide answers to the following questions:

1) "who" is eating "what" in a given environment,

2) "which" domain is best at consuming specific organic substrates at a given concentration, and

3) whether co-existence of archaea and bacteria is a function of "who" is eaten by "which" microbial predator.

**Significance**

This Ph.D. research provides a basis of understanding the competitive/cooperative relationship between bacteria and archaea, not only in a marine context but potentially in

other environments as well. Understanding archaeal/bacterial interactions represents an excellent model system for studying microbial competition in general by establishing paradigms for microbial competition/coexistence in other environments. Finally, understanding the life strategy of archaea *in situ* may also make them easier to cultivate in the laboratory. To date, only four thaumarchaea have successfully been isolated in culture, all of them ammonia oxidizers. This Ph.D. research promises to reveal how marine archaea respond to various electron donors, and which conditions are most optimal for their growth. If these organisms can be isolated in pure culture, classical microbiology techniques can then be used to properly classify them and further our understanding of their growth, metabolism, and ecology.

**Specific Hypotheses**

$H_1$**)** Marine archaea and bacteria coexist through resource partitioning. That is, the archaea use electron donors and/or acceptors that are not readily incorporated by the bacteria, so there is no direct competition between the two groups.

$H_2$**)** Archaea use the same or similar electron donors and acceptors as bacteria, but at different concentrations (e.g., Martens-Habbena et al., 2009).

$H_3$**)** Archaea and bacteria use the same electron donors and/or acceptors, but are preyed upon by different groups of predators ("top-down" versus "bottom-up" population control).

$H_0$**)** The null hypothesis is the observed coexistence of the archaea and the bacteria results from some form of physical forcing (such as advective transport from one

location to another) and is responsible for the apparent co-location and activity of archaea and bacteria during random sampling.

**Background**

Classical methods in microbiology rely on isolation and laboratory cultivation for the characterization of microorganisms. This approach is inherently limited, however, since many of the species directly observed in the environment cannot currently be cultivated in the laboratory. Direct counts of prokaryotes from environmental samples exceed the number yielded by cultural methods by up to nearly four orders of magnitude (Jannasch and Jones, 1959). To date, roughly 7,500 species of prokaryote have been characterized, but the total number of species in existence is difficult to estimate- though it could well be in the millions (Ward, 2002; Torvisk et. al., 2002).

The technique of isolating and characterizing pure cultures of organisms also inherently ignores the interactions that occur between microbial groups *in situ*. While single strain approaches have provided researchers with invaluable information regarding the physiology and metabolic capability of many different types of organisms, these same approaches do not adequately represent complex microbial communities, where metabolic intermediates can be shared, resources can be competed for, and interspecies communication may take place, etc. Our knowledge of microbes at the individual level, therefore, is far greater than our understanding of their ecology.

Advances in molecular techniques, however, have provided researchers with culture-independent methods of classification of microorganisms. These techniques allow

for the study of organisms that are resistant to laboratory cultivation, and preclude the biases inherent in studying organisms in pure culture (Amann et al., 1995; Head et al., 1998; Hugenholtz et al., 1998; McInerney et al., 2001). Such methods also provide insights into the evolutionary (Woese, 1987) and metabolic (Hug et al., 2013) relatedness of species.

One such molecular technique is the use of ribosomal RNA to identify different species of microorganisms (Olsen and Woese, 1993). It was using this approach that led Carl Woese to conclude that the traditional Linnean system of taxonomy used to describe relationships among species was inadequate. All prokaryotic organisms were grouped into one kingdom, the *Monera*, which included both bacteria and archaea. The other four kingdoms contained all the eukaryotic organisms: *Animalia*, *Plantae*, *Fungae*, and *Protista*. Woese's work with 16S rRNA revealed that all of these eukaryotic organisms were more closely related to each other than the bacteria were to the archaea. He proposed a new system using three "domains," the Eucarya, the Bacteria, and the Archaea (Woese et. al., 1990).

Archaea share some characteristics with bacteria; for example, they are both prokaryotic, may contain plasmids, and have 70S ribosomes (as opposed to the 80S ribosomes of eukaryotes). They have circular genomes and use operons to control gene transcription, and they lack the mRNA introns seen in eukaryotes. However, archaea also have several physiological traits in common with eukaryotes: their DNA is packaged with histones, they have several RNA polymerases (bacteria only have one), and they lack peptidoglycan in their cell walls. Like eukaryotes, the archaeal initiator tRNA is

methionine, not formyl-methionine as in bacteria, and they use transcription factors to regulate the transcription of DNA into mRNA. Archaea also have a structural attribute that neither of the other two domains shares: branched, ether-linked lipids in their cell membranes. This distinguishing feature is often exploited by researchers who wish to study only the archaea of a particular system. For example, there have been a number of studies using the composition of archaeal lipids to determine their metabolic capabilities (e.g., Biddle et. al., 2006).

In terms of their physiology, archaea seem to represent a halfway point between the other two domains of life. They have been described as organisms that use eukaryotic proteins in a bacterial-like context (Myllykallio et al., 2000; Grabowski and Kelman, 2003). These observations have led to questions concerning the evolutionary relationship between the three domains (Gribaldo and Brochier-Armanet, 2006; Williams et al., 2012; Williams et al., 2013; Spang et al., 2015). Woese's work suggested a phylogenetic tree in which the bacteria descended directly from the last universal common ancestor (LUCA), while archaea and eukaryotes shared a common ancestor more recent than LUCA (Woese et. al., 1990). This would imply that the observed physiological similarities between the archaea and the eukaryotes are features shared with their last common ancestor, while the traits archaea share with bacteria are derived from LUCA (Gribaldo and Brochier-Armanet, 2006).

However, there are numerous artifacts that may be encountered when constructing phylogenetic trees that may make the results unreliable, and the root of the universal tree of life could well be found in any of the three domains (Forterre and Philippe, 1999;

Gribaldo and Philippe, 2002). It has also been suggested that the eukaryotes, rather than evolving from a common ancestor shared with the archaea, represent a fusion of the bacteria and the archaea through metabolic symbiosis (López-García and Moreira, 1999). If this were true, the tree of life would be better represented by a ring (Rivera and Lake, 2004). Alternatively, it has been suggested that the eukarya are not a separate domain at all, but rather a kingdom within the archaea, making all life as we know it either archaeal or bacterial in origin (Williams et al., 2012; Williams et al., 2013).

For many years it was assumed that all archaea were either methanogens or extremophiles: descendants of the prokaryotic inhabitants of a more extreme primordial world. However, not long after Woese suggested the three-domain system, the same ribosomal-RNA analysis techniques developed by Woese led to the discovery of archaea in mesophilic marine environments (Delong, 1992; Fuhrman et al., 1992). Since then archaea have been found in waters between 4-8 ºC (Massana et al., 1997), in polar seas (Murray et al., 1998), in soils (Birtrim et al., 1997), in caves (Gonzalez et al., 2006), in estuarine settings (Abreu et al., 2001; Nelson et al., 2009), and associated with multicellular organisms, such as sponges (Preston et al., 1996). Archaeal diversity has been studied in open ocean areas around the globe (Massana et al., 2000) and in deep-sea sediments (Vetriani et al., 1999; Teske et al., 2002; Sørenson and Teske, 2006; Biddle et al., 2008). The discovery of archaea in these locations is a milestone in marine microbiology. Here exists an entire clade of organisms that, up until a mere two decades ago, had gone completely unnoticed because of their resistance to laboratory culturing,

yet are apparently ubiquitous and frequently abundant in the ocean (DeLong et. al., 1994; Stein and Simon, 1996; Karner et. al., 2001).

The discovery of archaea in a multitude of environments has also greatly expanded our understanding of their diversity and phylogeny. Initially it was thought that the archaeal domain consisted of only two phyla, the Euryarchaeota and Crenarchaeota. However, 16S rRNA and metagenomic studies have led to the classification of many more archaeal phyla, including the Thaumarchaeota (Brochier-Armanet et al., 2008), Aigarchaeota (Nunoura et al., 2011), Korarchaeota (Barns et al., 1996), Bathyarchaeota (Meng, 2014), Parvarchaeota (Rinke et al., 2013), Nanoarchaeota (Huber et al., 2002) and several others (Rinke et al., 2013) (Figure 1.1). The Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota have been grouped into the TACK superphylum (Guy and Ettema, 2011).

Exactly what strategy the archaea are using to remain so ubiquitous and relatively abundant remains unknown. One possibility is that they play a role in ammonia oxidation, the first (and usually rate-limiting) step of nitrification. Nitrification is especially important in coastal marine environments, where it links the decomposition of nitrogenous organic matter to N loss via denitrification. The removal of a large percentage of anthropogenic N from estuaries and continental shelf regions effectively isolates the N cycle of the open ocean from the terrestrial N cycle (Seitzinger, 1988; Galloway et al., 2004). Each year as much as $4 \times 10^{11}$ kg of N cycles through the ocean (Galloway et al., 2004), and almost all of it must undergo nitrification at least once (Karl, 2002). Yet, ammonia-oxidizing bacteria (AOB) often comprise only 0.1% of bacterial

assemblages in pelagic and benthic oceanic environments (Ward, 2000). In 2004, a unique ammonia monooxygenase (*amoA*) gene was discovered on an archaeal-associated genomic scaffold, suggesting that some archaea may be capable of performing ammonia oxidation (Venter et. al., 2004). A year later *amoA* genes were found in thaumarchaea (then referred to as mesophilic crenarchaea) (Treusch et al., 2005), and an autotrophic ammonia oxidizer, *Nitrosopumilus maritimus* (a marine group I archeon of CGI.1a), was isolated from a marine aquarium (Könneke et. al., 2005). It has been suggested that ammonia-oxidizing archaea (AOA) are of particular importance in low-oxygen environments such as sediments and oxygen minimum zones (Moin et al. 2009; Park et al., 2010). They may also be well-adapted to oligotrophic environments where the amount of available nitrogen is comparatively low (Martens-Habbana et al., 2009).

Because of the role that marine thaumarchaea may play in ammonia oxidation, most studies concerning their metabolism have focused on autotrophy and the uptake of inorganic carbon (bicarbonate), as ammonia oxidation is generally linked to some form of carbon fixation. Bicarbonate uptake by archaea has been traced via the incorporation of $^{13}C$ into lipids (Wuchter et. al., 2003). Ingalls et al., using a $^{14}C$ isotopic mass balance model to analyze archaeal membrane lipids, determined that 83% of the carbon uptake by deep ocean archaea is inorganic. It is uncertain, however, if this suggests a community of both heterotrophs and autotrophs, or a uniform community of mixotrophs (Ingalls et. al., 2006). Indeed, genomic sequencing results indicate that thaumarchaea may be capable of both strict autotrophy and mixotrophy; analysis of fosmid sequences from an uncultivated marine thaumarchaeon revealed the presence of genes associated with a modified 3-

hydroxypropionate cycle of autotrophic carbon assimilation, as well as an oxidative tricarboxylic acid cycle (Hallam et al., 2006). Subsequent metagenomic research on open ocean thaumarchaea also resulted in the detection of genes for 3-hydroxypropionate carbon fixation and oligopeptide transport, suggesting that this group takes up amino acids as a carbon source in addition to fixing inorganic carbon (Martin-Cuadrado et al., 2008). It has also been suggested that while thaumarchaea may express the *amoA* gene, they may not all be oxidizing ammonia or fixing inorganic carbon (Mußmann et al., 2011).

However, not all metabolic studies of marine archaea have focused on autotrophy and ammonia oxidation. Ouverney and Fuhrman (2000) used a microradiographic method combined with fluorescent *in situ* hybridization (MARFISH) to show that up to 60% of the archaea in the deep Mediterranean and Pacific accumulate amino acids, and the same technique was employed in the Arctic Ocean to demonstrate that archaea readily take up amino acids, glucose, proteins, and diatom extracellular polymers (Kirchman et al., 2007). Likewise, a study by Biddle et al. (2006) indicated that marine sedimentary crenarchaea and bathyarchaea are mostly heterotrophic, based on stable isotope signatures of available carbon and crenarchaeol in membrane lipids. Metagenomic studies of bathyarchaea have suggested that they may be important in the degradation of aromatic compounds (Meng et al., 2014).

Current research suggests that thaumarchaea are more likely to be found at depth in the open ocean, while euryarchaea tend to dominate at the surface (Massana et. al., 1997; Murray et. al., 1999; Karner et al., 2001). The thaumarchaea appear to be the most

abundant in the mesopelagic (Kirchman et al., 2007; De Corte et al., 2008), especially in oxygen minimum zones (Francis et al., 2005), but there is also evidence of a complex, genetically distinct, numerically dominant archaeal community at 4,000 m in the Pacific Ocean (Konstantinidis and DeLong, 2008). The uptake of inorganic carbon (Herndl et al., 2005) and amino acids (Varela et al., 2008) by archaea both appear to increase with depth; in deep waters the percentage of archaea taking up aspartic acid is generally either the same as or higher than the bacterial percentage (Varela et al., 2008). Genetic variability also seems to be more dependent on depth than latitude or geographic location, with "deep" and "shallow" clades often being observed (Herndl et al., 2005; De Corte et al., 2008; Hu et al., 2011b). Ratios of archaeal amoA gene copy numbers to 16S rRNA gene copy numbers also appear to decrease drastically with depth (De Corte, et al., 2008; Agogué et al., 2008; Yakimov et al., 2011; Hu et al., 2011a). It may be that archaeal metabolism strategies vary significantly with depth, but not with geographic location.

It is clear that marine and estuarine archaea exhibit variations in population composition, distribution, and metabolic capabilities, yet the factors controlling these variations are as yet unknown. Marine archaea may respond to seasonal cues (Murray et al., 1998), redox conditions (Nelson et al., 2009), or some other variable or combination of variables. The nature of the relationship between these archaea and the bacteria that occupy the same habitat is likewise poorly understood.

As prokaryotes are responsible for nutrient recycling and remineralization in ecosystems, it can only be assumed that archaea play some role (or many roles) in these processes. One would also surmise that if the archaea and the bacteria were both living in

the same environment and using the same sources of carbon and energy, competitive exclusion would cause one group to dominate over the other. Yet we find both living together in the same environment, at sometimes astonishing levels of diversity (e.g., McInerney et al., 2001; Varela et al., 2008; Chen et al., 2009; Hu et al., 2011). G.E. Hutchinson addressed a similar conundrum in his paper "The Paradox of the Plankton" (Hutchinson, 1961). The phytoplankton life strategy creates intense competition for the same basic resources (sunlight, inorganic carbon, etc.) and leaves limited room for niche specialization. Yet, tens or hundreds of thousands of phytoplankton species can be found within a small volume of water (Hutchinson, 1961; Scheffer and Carpenter, 2003). Hutchinson suggested that because environmental conditions can change very rapidly in aquatic systems, competing species can never reach a true equilibrium. No one species has the advantage long enough to competitively exclude the others (Hutchinson, 1961; Richardson et. al., 1970). In addition to the presence of rapidly opening and closing niches, there may also be equalizing and stabilizing forces that reduce intra- and inter-specific competition within a community (Chesson and Huntly, 1997; Chesson, 2000). These forces may include temporal or seasonal separation of activity, resource partitioning, immigration and emigration, differential responses to predation, phage lysis, and dormancy. One or a combination of these processes could reduce the competitive factors between the archaea and bacteria in a community, allowing them to stably coexist.

**Experimental Design**

This PhD work examined the competitive relationship between the archaea and the bacteria in both salt marsh sediments and the pelagic Atlantic Ocean. Additionally, the role of predators controlling both archaea and bacteria populations was examined in the deep ocean. These studies aimed to determine the mechanisms by which marine archaea and bacteria are able to coexist. The metabolic capabilities of archaea belonging to the TACK superphylum were demonstrated using stable isotope probing. For the purposes of this Ph.D. work, the Bathyarchaea are included in the TACK superphylum, as until recently they were classified as a subphylum of the Crenarchaeota (referred to as the Miscellaneous Crenarchaeal Group).

*Specific Objectives*

**1. To determine if TACK archaea take up organic carbon, and are able to utilize organics at low concentrations.**

For the second chapter of this dissertation, SIP studies were performed in the tidal levee of Hooks Creek, a brackish tidal creek located in Cheesequake State Park, Middlesex County, New Jersey. 5-mL core samples of sediment were taken from the tidal levee and incubated with $^{13}$C-labeled substrates including three simple organic carbon compounds- sodium acetate, glycine, and urea- and three complex mixtures of organic polymers- whole extract of algal lipids and pigments, extract of algal proteins, and growth medium (ISOGRO, Sigma-Aldrich). These were added at two concentrations: 30 or

150 M for the simple organics, and 2 or 10 g/mL for the complex mixtures. After a 5-day incubation, SIP analyses indicated that salt marsh TACK archaea are strictly heterotrophic, double in 2-3 days, and compete with heterotrophic bacteria for most organic carbon substrates. Resource partitioning of whole proteins and low concentrations of urea was also observed. Lower concentrations of substrate yielded more complex active populations; microcosms amended with high concentrations of substrate were dominated by a few active OTUs.

**2. To determine if thaumarchaeal heterotrophy can be observed in a pelagic environment.**

For the third chapter of this dissertation, SIP studies were performed along a transect across the North Atlantic from French Guiana to the Grand Banks. Samples were collected in the euphotic zone, a local oxygen minimum (215-835 m), the bathypelagic zone, and the benthypelagic at 16 stations along 52ºW longitude. SIP microcosms were established using: $^{13}$C acetate (20-30 μM), $^{13}$C urea (20-30 μM), or $^{13}$C sodium bicarbonate (5 mM) at 6 of these stations, and incubated for 48 hours in the dark. The results demonstrated thaumarchaea incorporate both inorganic and organic carbon substrates into newly synthesized DNA. No thaumarchaeal OTUs detected were strictly autotrophic, but half were exclusively heterotrophic. The dominant peaks detected by SIP exhibited mixotrophy. 16S rRNA was amplified and sequenced using the Illumina MiSeq platform at all stations,

and archaeal abundance was confirmed using qPCR. Archaeal diversity and abundance correlated to both depth and oxygen concentration.

3. **To determine if archaeal or bacterial specific predation can be observed by SIP methods.**

The fourth chapter of the dissertation is an extension of the former study in the North Atlantic. SIP analyses of microcosms amended with $^{13}$C-urea showed that archaea generally outcompeted bacteria for urea in the mesopelagic and bathypelagic. These results indicate there is resource partitioning between archaea and bacteria for organic nitrogen compounds in the deep sea. Additionally, there were two microcosms taken from the bathypelagic in which bacteria outcompeted archaea for the uptake of acetate. The selective uptake of urea resulted in microcosms where only the archaea or only the bacteria were labeled with $^{13}$C. This $^{13}$C label was subsequently incorporated into protists grazing on the prokaryotic community in each microcosm. In this final study, SIP analyses were performed using 18S rRNA primers to investigate the uptake of archaea and bacteria by *in situ* predators.

## Conclusion

This Ph.D. project to understand the mechanisms of coexistence between archaea and bacteria in marine environments provides a framework for studying microbial interactions in general and sheds light on the processes promoting diversity in the microbiota. There are currently very few SIP studies in the literature for salt marsh or

open ocean archaea, and our knowledge of this important group of microorganisms is very limited and largely speculative. Understanding the mechanisms that contribute to their success *in situ* may help us isolate them in a laboratory setting, where classical microbiology techniques can then be utilized to further improve our understanding of marine archaeal physiology and ecology.

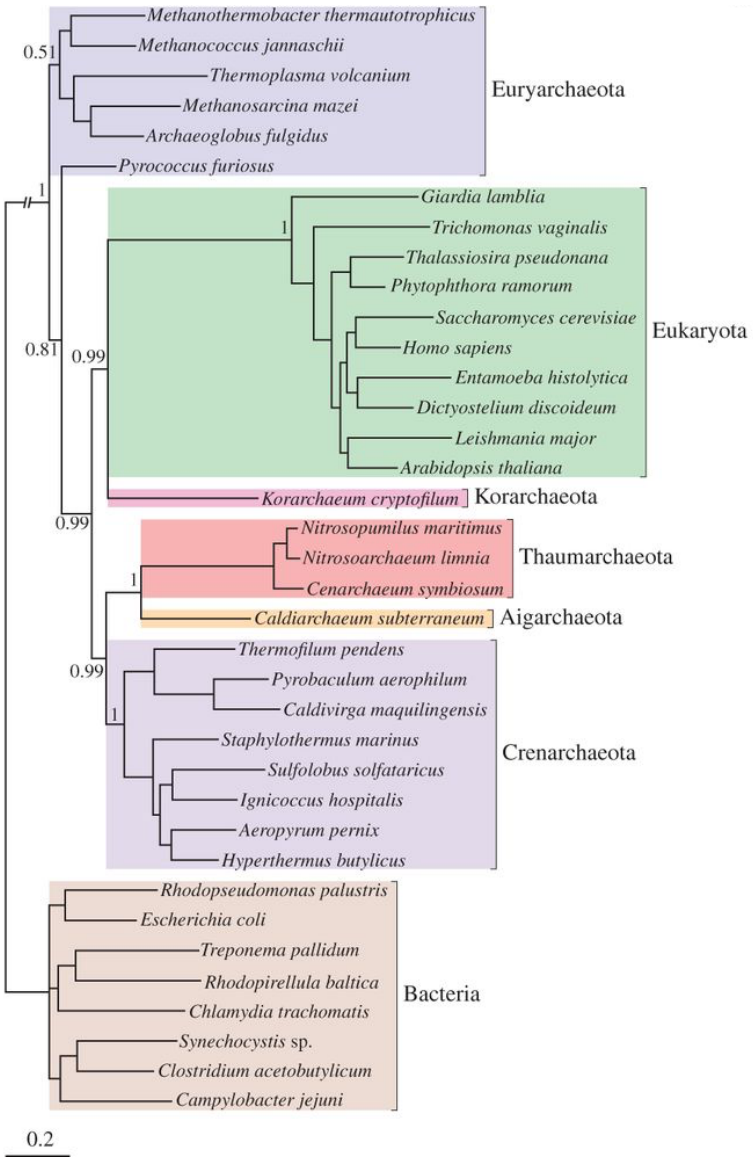



Figure 1.1. Phylogenetic tree representing some of the major archaeal phyla, and the placement of the eukarya within the archaeal domain (from Williams et al., 2012).

## References


Abreu, C., Jurgens, G., De Marco, P., Saana, A. and Bordalo, A.A. (2001) Crenarchaeota and Euryarchaeota in temperate estuarine sediments. J of Appl Microbio 90: 713-718.

Agogué, H., Brink, M., Dinasquet, J. and Herndl, G.J. (2008) Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic. Nature 456: 788-791.

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

Avaniss-Aghajani, E., Jones, K., Chapman, D. and Brunk, C. (1994) A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences. Biotechniques 17: 144-146.

Babcock, D.A., Wawrik, B., Paul, J.H., McGuinness, L. and Kerkhof, L.J. (2007) Rapid screening of a large insert BAC library for specific 16S rRNA genes using TRFLP. J. Microbiol.Methods 71: 156-61.

Barns, S.M., Delwiche, C.F., Palmer, J.D. and Pace, N.R. (1996) Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. PNAS 93(17): 9188-9193.

Beman, J.M. and Francis, C.A. (2006) Diversity of ammonia-oxidizing archaea and bacteria in the sediments of a hypernutrified subtropical estuary: Bahía del Tóbari, Mexico. Appl Env Microbiol 72: 7767-7777.

Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørenson, K.B., Anderson, R., et al., (2006)Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. PNAS 103: 3846-3851.

Biddle, J.F, Fitz-Gibbon, S., Schuster, S.C., Brenchley, J.E. and House, C.H. (2008) Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. PNAS 105: 10583-10588.

Birtrim, S.B., Donohue, T.J., Handelsman, J., Roberts, G.P. and Goodman, R.M. (1997) Molecular phylogeny of Archaea from soil. PNAS 94: 277-282.

Boschker, H.T.S., Nold, S.C., Wellsbury, P., Bos, D., de Graaf, W., Pel, R., et al. (1998) Direct linking of microbial populations to specific biogeochemical processes by $^{13}$C-labeling of biomarkers. Nature 392: 801-805.

Brochier-Armanet, C., Boussau, B., Gribaldo, S. and Forterre, F. (2008) Mesophilic crenarchaeota: Proposal for a third archaeal phylum, the Thaumarchaeota. Nature Rev Microbiol 6(3): 245-252.

Chen, Y., Wu, L., Boden, R., Hillebrand, A., Kumaresan, D., Moussard, H., et al. (2009) Life without light: microbial diversity and evidence of sulfur- and ammonium-based chemolithotrophy in Movile Cave. ISME 3: 1093-1104.

Chesson, P. and Huntley, N. (1997) The roles of harsh conditions in the dynamics of ecological communities. Amer Nat 150: 519-553.

Chesson, P. (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31: 343-366.

Corredor, J.E., and Wawrik, B., Paul, J.H., Tran, H., Kerkhof, L., López, J.M., et al. (2004) Geochemical rate-RNA integration study: Ribulose-1,5-bisphosphate carboxylase/oxygenase gene transcription and photosynthetic capacity of planktonic photoautotrophs. Appl Env Microbiol 70: 5459-5468.

De Corte, D., Yokokawa, T., Varela, M.M., Agogué, H. and Herndl, G.J. (2009) Spatial distribution of Bacteria and Archaea and amoA gene copy numbers throughout the water column of the Eastern Mediterranean Sea. ISME 3: 147-158.

DeLong. E.F. (1992) Archaea in coastal marine environments. PNAS 89: 5685-5689.

DeLong, E.F., Wu, K.Y., Prézelin, B.B. and Jovine, R.V.M. (1994) High abundance of Archaea in Antarctic marine picoplankton. Nature 37:, 695-697.

Dubnau, D. and Losick, R. (2006) Bistability in bacteria. Mol Microbiol 61: 564-572.

Forterre, P. and Philippe, H. (1999) Where is the root of the universal tree of life? Bioessays 21: 871-879.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E. and Oakley B.B. (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102: 14683-14688.

Fuhrman, J.A., McCallum, K. and Davis, A.A. (1992) Novel major archaebacterial group from marine plankton. Nature 356: 148-149.

Gallagher, E., McGuinness, L., Phelps, C., Young, L.Y., and Kerkhof, L.J. (2005) 13C carrier DNA shortens the incubation time needed to detect benzoate-utilizing denitrifying bacteria by stable-isotope probing. Appl Environ Microbiol 71: 5192-5196.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, et. al. (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70: 153-226.

Gonzalez, J.M., Portilo, M.C. and Saiz-Jimenez, C. (2006) Metabolically active Crenarchaeota in Altamira Cave. Naturwissenschaften 93: 42-45.
Grabowski, B. and Kelman, Z. (2003) Archaeal DNA replication: Eukaryal proteins in a bacterial context. Ann Rev Microbiol 57: 487-516.
Gribaldo, S. and Philippe, H. (2002) Ancient phylogenetic relationships. Theor Popul Biol 61: 391-408.
Gribaldo, S., and Brochier-Armanet, C. (2006) The origin and evolution of Archaea: A state of the art. Philos. Trans. R Soc Lond B Biol Sci 361: 1007-1022.
Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M. and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. PLoS Biol 4: 520-536.
Hamberger, A., Horn, M.A., Dumont, M.G., Murrell, J.C. and Drake, H.L. (2008) Anaerobic consumers of monosaccharides in a moderately acidic fen. Appl Env Microbiol 74: 3112-3120.
Head, I. M., Saunders J.R. and Pickup, R.W. (1998) Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. Microbiol Ecol 35: 1-21.
Herndl, G.J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A. and Pernthaler, J. (2005) Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. Appl Env Microbiol 71: 2303-2309.
Hu, A., Jiao, N. and Zhang, C. (2011a) Community structure and function of planktonic Crenarchaeota: changes with depth in the South China Sea. Microb Ecol 62: 549-563.
Hu, A., Jiao, N, Zhang, R. and Yang, Z. (2011b) Crenarchaeota in the euphotic and upper mesopelagic zones of the East China Sea. Appl Environ Microbiol 77: 7469-7478.
Huber, H., Hohn, M.J., Rachel, R., Fuchs, T., Wimmer, V.C. and Stetter, K.O. (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. Nature 417: 63-67.
Hug L.A., Castelle, C.J., Wrighton, K.C., Thomas, B.C., Sharon, I., Frischkorn, K.R. et al. (2013) Community genomic analyses constrain the distribution of metabolic traits across the Chloroflexi phylum and indicate roles in sediment carbon cycling. Microbiome 1:22 http://www.microbiomejournal.com/content/1/1/22.
Hugenholtz, P., Goebel, B.M. and Pace, N.R. (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. Jour Bacteriol 180: 4765-4774.
Hutchinson, G.E. (1961) The paradox of the plankton. Amer Nat 45: 137-145.
Ichinotsuka, D., Katano, T., Takeoka, H. and Nakano, S. (2010) Effects of nutrient supplies on the growth rates of planktonic bacteria in Uchiumi Bay, Japan. Aquatic Biol 9: 123-130.
Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., et al. (2006) Biographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. PNAS 103: 2815-2820.
Ingalls, A.E., Shah, S.R., Hansman, R.L., Aluwihare, L.I., Santos, G.M., Druffel, E.R.M. and Pearson, A. (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. PNAS 103: 6442-6447.
Iverson, V., Morris, R.M., Frazar, C.D., Berthiaume, C.T., Morales, R.L. and Armbrust, E.V. (2012) Untangling genomes from metagenomes: revealing an uncultured class of marine Euryarchaeota. Science 335(6068): 587-590.
Jannasch, H.W. and Jones, G.E. (1959) Bacterial populations in seawater as determined by different methods of enumeration. Limnology and Oceanography 4: 128-139.
Karl, D.M. (2002) Nutrient dynamics in the deep blue sea. Trends Microbiol 10(9): 410-418.
Karner, M.B., DeLong, E.F. and Karl, D.M. (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409(6819): 507-510.
Kerkhof, L. (1992) A comparison of substrates for quantifying the signal from a non-radiolabeled DNA probe. Anal Biochem 205: 359-364.
Kerkhof, L. and Ward, B.B. (1993) Comparison of nucleic acid hybridization and fluorometry for measurement of the relationship between RNA/DNA ratio and growth rate in a marine bacterium. Appl Environ Microbiol 59: 1303-1309.
Kerkhof, L. and Kemp, P. (1999) Small ribosomal RNA content in marine bacteria during non-steady state growth. FEMS Micro Ecol 30: 253-260.
Kerkhof, L., Santoro, M., and Garland, J. (2000) Response of soybean rhizosphere communities to human hygiene water addition as determined by community-level physiological profiling (CLPP) and terminal restriction fragment length polymorphism (TRFLP). FEMS Microbiol Letters 184: 95-101.
Kerkhof, L.J., Williams, K.H., Long, P.E. and McGuinness, L.R. (2011) Phase preference by active,

acetate-utilizing bacteria at the Rifle, CO Integrated Field Research Challenge Site. Environ Sci Technol 45: 1250-1256.
Kirchman, D.L., Elifantz, H., Dittel, A.I., Malmstrom, R.R. and Cottrell, M.T. (2007) Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean. Limnol Oceanogr 52: 495-507.
Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543-546.
Konstantinidis, K.T. and DeLong, E.F. (2008) Genomic patterns of recombination, clonal divergence and environment in marine microbial populations. ISME 2: 1052-1065.
Liu, W. T., Marsh, T.L., Cheng, H. and Forney, L.J. (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Env Microbio 63: 4516-4522.
López-García, P. and Moreira, D. (1999) Metabolic symbiosis at the origin of eukaryotes. Trends Biochem Sci 24: 88-93.
Marsh, T.L. (1999) Terminal restriction fragment length polymorphism (T-RFLP): An emerging method for characterizing diversity among homologous populations of amplification products. Current Opinion in Microbiol 2: 323-327.
Martens-Habbena, W., Berube, P.M., Urakawa, H., de la Torre, J.R. and Stahl, D.A. (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461: 976-979.
Martin-Cuadrado, A.B., Rodriguez-Valera, F., Moreira, D., Alba, J.C., Ivars-Martínez, E., Henn, et al. (2008) Hindsight in the relative abundance, metabolic potential and genome dynamics of uncultivated marine archaea from comparative metagenomic analyses of bathypelagic plankton of different oceanic regions. ISME 2: 865-886.
Massana, R., Murray, A.E., Preston, C.M. and DeLong, E.F. (1997) Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. Appl Env.Microbiol 63: 50-56.
Massana R., DeLong, E.F. and Pedrós-Alió, C. (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Env Microbiol 66, 1777-1787.
McInerney, J.O., Mullarkey, M., Wernecke, M.E. and Powell, R. (2001) Bacteria and Archaea: Molecular techniques reveal astonishing diversity. Biodiversity 3: 3-10.
Meng, J., Xu, J., Qin, D., He, Y., Xiao, X. and Wang, F. (2014) Genetic and functional properties of uncultivated MCG archaea assessed by metagenome and gene expression analyses. ISME. 8(3): 650-659.
Meselson, M. and Stahl, F. (1958) The replication of DNA in Escherichia coli. PNAS 44: 671-682.
Moin, N.S., Nelson, K.A., Bush, A. and Bernhard, A.E. (2009) Distribution and diversity of archaeal and bacterial ammonia oxidizers in salt marsh sediments. Appl Env Microbiol 75: 7461-7468.
Mosier, A.C. and Francis, C.A. (2008) Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. Env Microbiol 10: 3002-3016.
Murray, A.E., Preston, C.M., Massana, R., Taylor, L.T., Blakis, A., Wu, K. and DeLong, E.F. (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Islans, Antarctica. Appl Env Microbiol 64: 2585-2595.
Murray, A.E., Blakis, A., Massana, R., Strawzewski, S., Passow, U., Alldredge, A. and DeLong E.F. (1999) A time series assessment of planktonic archaeal variability in the Santa Barbara Channel. Aquat Microb Ecol 20: 129-145.
Mußmann, M., Brito, I., Pitcher, A., Sinninghe Damsté, J.S., Hatzenpichler, R., Richter A. et al. (2011) Thaumarchaeotes abundant in refinery nitrifying sludges express amoA but are not obligate autotrophic ammonia oxidizers. PNAS 108(40): 16771-16776.
Myllykallio, H., Lopez, P., López-García, P., Heilig, R., Saurin, W., Zivanovic, Y., et al. (2001) Bacterial mode of replication with eukaryotic-like machinery in a hyperthermophilic archaeon. Science 288: 2212-2215.
Nelson, K.A., Moin, N.S. and Bernhard, A.E. (2009) Archaeal diversity and the prevalence of Crenarchaeota in salt marsh sediments. Appl Env Microbiol 75: 4211-4215.
Nunoura, T., Takaki, Y., Kakuta, J., Nishi, S., Sugahara, J., Kazama, H. et al. (2011) Insights into the evolution of Archaea and eukaryotic protein modifier systems revealed by the genome of a novel archaeal group. Nucleic acids research 39(8): 3204-3223.
Olsen, G.J. and Woese, C.R. (1993) Ribosomal RNA: a key to phylogeny. FASEB J. 7: 113-123.
Olsen, G.J., Matsuda, H., Hagstrom, R. and Overbeek, R. (1994) fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Comput Appl Biosci 10:

41-48.
Ouverney, C.C. and Fuhrman, J.A. (2000) Marine planktonic archaea take up amino acids. Appl Env Microbiol 66: 4829-4833.
Pace, N.R. (1997) A molecular view of microbial diversity and the biosphere. Science 276: 734-740.
Park, B.J., Park, S.J., Yoon, D.N., Schouten, S., Sinninghe Damsté, J.S. and Rhee, S.K. (2010) Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in coculture with sulfur-oxidizing bacteria. Appl Environ Microbiol 76(22): 7575-7587.
Perry, J.J., Staley, J.T. and Lory, S. (2002) Microbial Life. Sunderland: Sinauer Associates, Inc.
Phelps C.D., Kerkhof, L.J. and Young, L.Y. (1998) Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. FEMS Microbio Ecol 27: 269-279.
Preston, C.M., Wu, K.Y., Molinski, T.F. and DeLong, E.F. (1996) A psychrophilic crenarchaeon inhabits a marine sponge: Cenarchaeum symbiosum gen. nov., sp. nov. PNAS 93: 6241-6246.
Qin, W., Amin, S.A., Martens-Habbena, W., Walker, C.B., Urakawa, H., Devol, A.H., et al. (2014) Marine ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation. PNAS 111: 12504-12509.
Radajewski, S., Ineson, P., Parekh, N.R. and Murrell, J.C. (1999) Stable-isotope probing as a tool in microbial ecology. Nature 403: 646-649.
Rew, R. and Davis, G. (1990) NetCDF: an interface for scientific data access. IEEE Computer Graphics and
Applications. 10: 76-82.
Richardson, P., Armstrong, R. and Goldman, C.R. (1970) Contemporaneous disequilibrium, a new hypothesis to explain the "Paradox of the Plankton." PNAS 67: 1710-1714.
Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N.N., Anderson, I.J., Cheng, J.F. et al., (2013) Insights into the phylogeny and coding potential of microbial dark matter. Nature 499: 431-437.
Rivera, M.C. and Lake, J.A. (2004) The ring of life provides evidence for a genome fusion origin of eukaryotes. Nature 431: 152-155.
Scala, D.J. and Kerkhof, L.J. (2000) Horizontal heterogeneity of denitrifying bacterial communities in marine sediments by terminal restriction fragment length polymorphism analysis. Appl Env Microbiol 66: 1980-1986.
Scheffer, M. and Carpenter, S.R. (2003) Catastrophic regime shifts in ecosystems: linking theory to observation. Trends Ecol Evo 18: 648-656.
Schopf, J.W. and Packer, B.M. (1987) Early Archaean (3.3-billion to 3.5-billion-year-old microfossils from Warrawoona Group, Australia. Science 237: 70-73.
Seitzinger, S.P. (1988) Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Limnol Oceanogr 33: 702-724.
Smits, W.K., Kuipers, O.P. and Veening, J.W. (2006) Phenotypic variation in bacteria: the role of feedback regulation. Nat Rev Microbiol 4: 259-271.
Søresnson, K.B. and Teske, A. (2006) Stratified communities of active archaea in deep marine subsurface sediments. Appl Env Microbiol 72: 4596-4603.
Spang, A., Saw, J.W., Jørgensen, S.L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A.E. et al. (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521: 173-179.
Stein, J.L. and Simon, M.I. (1996) Archaeal ubiquity. PNAS 93: (13), 6228-6230.
Teske, A., Hinrichs, K-U., Edgcomb, V., Gomez, A.D.V., Kysela, D., Sylva, S.P., et al. (2002) Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. Appl Env Microbiol 68: 1994-2007.
Torvisk, V., Øvreås, L. and Thingstad, T.F. (2002) Prokaryotic diversity- magnitude, dynamics, and controlling factors. Science 296: 1064-1066.
Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P. and Schleper, C. (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environ Microbiol 7: 1985-1995.
Varela, M.M., van Aken, H.M., Sintes, E. and Herndl, G.J. (2008) Latitudinal trends of Crenarchaeota and Bacteria in the meso- and bathypelagic water masses of the Eastern North Atlantic. Env Microbiol 10: 110-124.
Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., et. al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66-74.
Versalovik, J., Kapur, V., Koeuth, V., Mazurek, G.H., Whittam, T.S., Musser, J.M. and Lupski, J.R. (1995) DNA fingerprinting of pathogenic bacteria by fluorophore-enhanced repetitive sequenced-based polymerase chain reaction. Pathol Lab Med Arch 119: 23-29.

Vetriani, C., Jannasch, H.W., MacGregor, B.J., Stahl, D.A. and Reysenbach, A.L. (1999) Population structure and phylogenetic characterization of marine benthic archaea in deep-sea sediments. Appl. Env Microbiol 65: 4375-4384.

Ward, B. (2002) How many species of prokaryotes are there? PNAS 99: 10234-10236.

Williams, T.A., Foster, P.G., Nye, T.M.W., Cox, C.J., and Embley, T.M. (2012) A congruent phylogenomic signal places eukaryotes within the Archaea. Proc R Soc B 279: 4870-4879.

Williams, T.A., Foster, P.G., Cox, C.J., and Embley, T.M. (2013) An archaeal origin of eukaryotes supports only two primary domains of life. Nature 504: 231-236.

Woese, C.R. (1987) Bacterial evolution. Microbiol Rev 51: 221-271.

Woese, C.R., Kandler, O., and Wheelis, M.L. (1990) Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. PNAS 87: 4576-4579.

Wuchter, C., Schouten, S., Boschker, H.T.S. and Damsté J.S.S. (2003) Bicarbonate uptake by marine Crenarchaeota. FEMS Microbiol Letters 219,: 203-207.

Yakimov, M.M., La Cono, V., Smedile, F., DeLuca, T.H., Juárez, S., Ciordia, S., et al. (2011) Contribution of crenarchaeal autotrophic ammonia oxidizers to the dark primary production in Tyrrhenian deep waters (Central Mediterranean Sea). ISME 5: 945-961.

# Chapter 2. Crenarchaeal heterotrophy in salt marsh sediments

(Published in ISME 8: 1534–1543)

**Abstract**

Mesophilic Crenarchaeota (also known as Thaumarchaeota) are ubiquitous and abundant in marine habitats. However, very little is known about their metabolic function *in situ*. In this study, salt marsh sediments from New Jersey were screened by stable isotope probing (SIP) using 3 simple $^{13}$C-labeled organic compounds (acetate, glycine, and urea), 3 complex $^{13}$C-polymers (lipids, proteins, and growth medium (ISOGRO), and $^{13}$C-bicarbonate. Incubations were done from 3-14 days at low and high concentrations (30-150 $\mu$M; 2-10 mg/ml) and $^{13}$C-labeled DNA was analyzed by terminal restriction fragment length polymorphism (TRFLP), using the 16S rRNA gene. A library of clones matching many of the $^{13}$C-TRFLP peaks contained 7 sequences from the Miscellaneous Crenarchaeal Group and 7 sequences from the Marine Group 1.a Crenarchaeota. Our findings indicate salt marsh crenarchaea are exclusively heterotrophic, double within 2-3 days, and often compete with heterotrophic bacteria for the same organic substrates. Some crenarchaeal OTUs displayed a preference for certain carbon sources, while others incorporated nearly every substrate provided. The data suggest salt marshes may be an excellent model system for studying crenarchaeal metabolic capabilities and can provide information on the competition between crenarchaea and other microbial groups to improve our understanding of microbial ecology.

**Introduction**

For many years it was assumed that archaea were either methanogens or extremophiles, descendants of a more extreme primordial world. However, in the last few decades, small ribosomal-RNA subunit and gene analysis of environmental samples revealed the presence of archaea in aerobic, marine environments at moderate temperatures (DeLong, 1992; Fuhrman et al., 1992). These archaea (mostly belonging to the subdomain Crenarchaeota) have been found in soils (Birtrim et al., 1997), polar seas (Murray et al., 1998), estuaries (Abreu et al., 2001), caves (Gonzalez et al., 2006), a wide variety of oceanic settings (DeLong et al., 1994; Stein and Simon, 1996; Massana et al., 1997; Massana et al., 2000; Karner et al., 2001), deep-sea sediments (Vetriani et al., 1999; Teske et al., 2002; Sørenson and Teske, 2006; Biddle et al., 2008) and salt marshes (Nelson et al., 2009). Although the crenarchaeota are globally ubiquitous and frequently abundant in various environments, relatively little is known about their metabolic capabilities.

One thought is that crenarchaea play a role in ammonia oxidation. In 2004, an ammonia monooxygenase gene (*amoA*) was discovered on an archaeal-associated genomic scaffold, suggesting that some archaea may be capable of performing ammonia oxidation (Venter et. al., 2004). Subsequently, archaeal associated *amoA* genes were found in mesophilic crenarchaeota genome fragments (Treusch et. al., 2005; Schleper et al., 2005) and an autotrophic ammonia oxidizer, *Nitrosopumilus maritimus* (a marine group I archeon of CGI.1a), was isolated from a marine aquarium (Könneke et. al., 2005). It has been proposed that AOA are of particular importance in low-oxygen environments

such as sediments and oxygen minimum zones (Moin et. al. 2009; Park et. al., 2010) and may be well-adapted to oligotrophic environments where the amount of available nitrogen is comparatively low (Martens-Habbana et al., 2009).

Because of the role that marine crenarchaea may play in ammonia oxidation, most studies concerning their metabolism have focused on uptake of inorganic carbon. For example, $^{13}$C-bicarbonate incorporation into crenarchaeal lipids was observed in waters of the North Sea, the deep waters of the North Atlantic, and the Pacific Gyre (Wuchter et al., 2003; Herndl et al., 2005; Ingalls et al., 2006). Additionally, genomic sequencing results indicate an uncultivated marine crenarchaeote contained genes associated with a modified 3-hydroxypropionate cycle for autotrophic carbon assimilation (Hallam et al., 2006). In contrast, there is evidence of archaeal heterotrophy as described by Ouverney and Fuhrman (2000) where up to 60% of the crenarchaeota in the deep Mediterranean and Pacific accumulate amino acids as measured by STARFISH (MICRO-FISH) methods. Likewise, a study by Biddle et al. (2006) determined that deep sea sedimentary crenarchaeota are mostly heterotrophic based on stable isotope signatures of carbon in archaeal membrane lipids. Finally, metagenomic research on open ocean Group IA crenarchaeota also found genes for 3-hydroxypropionate carbon fixation and oligopeptide transport, suggesting amino acid uptake in addition to fixing inorganic carbon for cellular needs (Martin-Cuadrado et al., 2008). Unfortunately, most bulk approaches or metagenomic studies cannot elucidate the particular substrates are used by specific crenarchaea under a given condition, nor can they provide information on the doubling time of crenarchaea *in situ*. A method is needed, such as SIP (Radajewski et al., 2000)

which directly links carbon utilization with specific members of the mesothermal crenarchaea community.

In this report, SIP experiments are presented which show $^{13}C$-incorporation of various organic substrates by salt marsh sediment-associated members of the Miscellaneous Crenarchaeota Group (Inagaki et al., 2003) and Marine Group I (Delong, 1992). Our SIP results demonstrated salt marsh crenarchaeota are capable of assimilating a wide array of organic carbon substrates which are also utilized by bacterial populations *in situ*, suggesting competition between the two domains. However, there is also evidence of resource partitioning for urea and whole proteins between the domains. Interestingly, a minimum incubation of 5 days was required to obtain unambiguous SIP signal for the crenarchaea, suggesting a relatively long (2-2.5 day) doubling time compared to *in situ* bacteria. These findings suggest both top-down and bottom-up mechanisms are allowing for the stable co-existence of crenarchaea and bacteria in salt marsh settings.

**Materials and Methods**

Surficial sediment was collected in a salt marsh in Cheesequake State Park, a 635-hectare state park located in Middlesex County, New Jersey. 5-mL core samples of sediment were taken from the tidal levee at Hooks Creek using a cut-off, sterile syringe. The sediment was placed in 170-mL glass bottles which had been filled to the top with sterile-filtered (0.2 μm Supor, Pall) site water as in Kerkhof et al., 2011. To determine heterotrophic activity one of the following $^{13}C$-labeled substrates was added: a simple

organic carbon compound (acetate, glycine, or urea), or a complex mixture of organic polymers (methanol extract of algal lipids/pigments, extract of algal proteins, or growth medium (ISOGRO®, Sigma-Aldrich)). $^{13}$C-labeled bicarbonate was added to a separate set of microcosms to assess carbon fixation. Each organic substrate was added at two concentrations: 30 or 150 μM for the simple organics, and 2 or 10 mg/ml for the complex mixtures. Bicarbonate was added at 5 mM (2.5 times that of seawater), raising the pH of the site water from 7.6 to 8.3, which is within the range normally observed in seawater (Strickland and Parsons, 1972). 30 μM $^{12}$C-labeled urea, 5 mM $^{12}$C-labeled bicarbonate, and no-amendment microcosms were set up as controls. Each SIP microcosm was placed in serum vials, sealed with rubber stoppers, incubated for 3-14 days in the dark, and gently turned once per day to ensure exposure of substrate to the sediment. After the incubation, the sediment was collected and centrifuged to remove any liquid, then immediately frozen in liquid nitrogen. DNA was extracted from each sample by phenol-chloroform methods, and fractionated using isopycnic cesium chloride gradient ultracentrifugation with $^{13}$C-labeled *H. salinarum* when detecting bacterial DNA or *E. coli* DNA when targeting archaeal DNA, as a carrier (Gallagher et. al., 2005).

After ultracentrifugation at 200,000 x g for 36 h, the $^{12}$C (upper) and $^{13}$C (lower)-labeled bands were collected by pipette and amplified by PCR with 5'-fluorescently-labeled, archaea-specific (21f [5′-TCCGGTTGATCCYGCCGG]/958r [5′-YCCGGCGTT GAMTCCAATT) and crenarchaeota-specific forward/reverse primers (7f [5'-TTCCGGT TGATCCYGCCGGACC]/518r [5'-GCTGGTWTTACCGCGGCGGCTGA]) or bacteria-specific forward/reverse primers (27f [5'AGAGTTTGATCMTGGCTC AG]/1100r [5'-

GGGTTGCGCTCGTTG]) targeting the 16S rRNA gene. The amplicons were digested with *MnlI* in 20 $\mu$l volumes for 6 h at 37 $^{o}$C. Sodium acetate and glycogen were added and 37 $\mu$l of 95 % ethanol was used to precipitate the DNA (McGuinness *et al*., 2006). The samples were dried and re-suspended in 19.7 $\mu$l de-ionized formamide with 0.3 $\mu$l ROX 500 size standard (Applied Biosystems). TRFLP fingerprinting (Avaniss-Aghajani et al., 1994) was carried out on an ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA) using Genescan software. Peak detection was set at 25 arbitrary fluorescent units.

An archaeal amplicon clonal library was also constructed using the Topo TA cloning kit, as per the manufacturer's instruction (Invitrogen, CA). To determine the phylogenetic affiliation of the various $^{13}$C-crenarchaeal peaks observed in the SIP study, 100 recombinant clones from the $^{13}$C-labeled bands were screened in a multiplex format as in Babcock *et al*. (2007), to determine the TRF of the recombinant amplicons. The 16S rRNA genes that matched TRFs of interest (24 total) were sequenced via Sanger methods using M13 primers (Genewiz, Inc. NJ), producing 14 unique sequences (<99% similarity to each other). . Unique clonal sequences were compared to known sequences by BLAST. A maximum likelihood phylogenetic tree was re-constructed using 433 unambiguously aligned bases among 44 taxa with Geneious analysis software (Guindon and Gascuel, 2003; Drummond *et al*., 2009).

**Results**

SIP experiments were predicated on detecting a PCR signal in the $^{13}C$ carrier band when $^{13}C$ substrates were added, and not detecting a PCR signal in the $^{13}C$ carrier when no substrate or $^{12}C$ substrates were added. As it was unclear how long it would take to detect a crenarchaeal signal in these experiments, all SIP incubations were done for up to 14 days. A minimum incubation time of 5 days was required to observe unambiguous $^{13}C$ uptake by crenarchaea in the carrier band, with clean controls from the $^{12}C$ amendment. Therefore, all data reported represents the 5-day incubations; the 14 day incubations were not processed for fear of archaeal and bacterial cross feeding.

An example of a successful SIP experiment is shown in figure 2.1A. In this incubation, crenarchaeal amplicons are only detected in the $^{13}C$-carrier band when $^{13}C$-lipids/pigments (lanes 1A-6 and 7) or $^{13}C$-ISOGRO media at the higher concentration (lane 1A-11) has been added to the incubations. With carrier DNA alone (lane 1A-4), when no substrate has been added to the incubations (lane 1A-5), or interestingly when $^{13}C$- whole proteins are added (lanes 1A-8 and 9), no crenarchaeal PCR signal was observed. In our incubations, crenarchaeal $^{13}C$-incorporation was detected at both high and low concentrations for $^{13}C$-acetate, glycine, urea, and the algal lipid/pigment extract. Incorporation was only detected at higher concentrations for $^{13}C$-ISOGRO. Three quarters of the SIP experiments demonstrated crenarchaeal DNA synthesis from the $^{13}C$ amendments. A summary of crenarchaeal $^{13}C$ carbon incorporation for all SIP treatments is presented in Table 1.

Bacterial $^{13}C$ carbon incorporation was also measured in the SIP experiments. In 83% of the microcosms, bacterial $^{13}C$-DNA synthesis was detected, except for the low

concentrations of urea and ISOGRO. However, the long SIP incubation time (5 days) most likely allowed for extensive cross-feeding between the bacteria and the generation of $^{13}C$-$CO_2$ (data not shown). To test whether the crenarchaea were taking up $^{13}CO_2$ rather than our $^{13}C$-labeled substrates during our SIP experiment, a $^{13}C$-bicarbonate incubation was also conducted. The results are presented in figure 2.1B. The $^{13}C$ bicarbonate treatment did not yield any amplicon (lane 1B- 9) and a test for PCR inhibitors by spiking a positive DNA sample with $^{13}C$ bicarbonate DNA indicated no PCR suppression from this extract (land 1B-11). These results demonstrate the SIP crenarchaeal signal resulted from the incorporation of $^{13}C$-organics, not $^{13}C$-labeled bicarbonate produced by bacterial respiration.

TRFLP analysis of the 16S rRNA gene amplicons was performed to ascertain which members of the crenarchaeal community were actively synthesizing DNA from the $^{13}C$ carbon sources. A small number of crenarchaeal OTUs (7-10) were found to dominate the total community profiles, representing ~80% of the total community peak area (Figure 2.2) for the $^{12}C$ and the $^{13}C$ bands in the cesium gradients. A compilation of all fingerprints from our crenarchaeal SIP experiments is provided in Figure 2.3. Some of the major OTUs in the $^{12}C$ fraction were suppressed in the $^{13}C$ fraction (such as the 124 or 253 bp TRF) while other TRFs were enriched in the $^{13}C$ fraction compared to the $^{12}C$ fraction (such as the 89 bp TRF). Many of these $^{13}C$-TRFs for a given carbon source were also observed in the other $^{13}C$ carbon SIP treatments. For example, the 89-bp TRF and a 116-bp TRF appeared in many treatments that yielded $^{13}C$-crenarchaeal signal, with the exception of the 30 μM glycine, urea, and acetate treatments. In contrast, there were

other crenarchaeal TRFs that were only detected in the $^{13}C$-labeled community under specific treatments. For example, a 223-bp TRF was found to represent 9% of the active crenarchaeal community (based on total community area) in the 30 μM acetate-amended microcosms (Supplemental figure 2.1), but did not appear to be active in any other treatments. This 223 bp TRF could be observed in the $^{12}C$ band in every SIP microcosm, accounting for up to 12.4% of the total crenarchaeal community. Similarly, a 124-bp TRF accounted for 12.6%, 1%, and 2% of the $^{13}C$ uptake in the crenarchaeal communities in the 30 μM acetate, 30 μM urea, and 150 μM urea microcosms, respectively, but did not take up the labeled substrate in any other treatment, though it comprised up to 23.5% of the $^{12}C$-crenarchaeal community (Supplemental figures 2.1, 2.3, 2.4). Overall, the crenarchaeota present in the salt marsh sediment appear to be much less diverse (in terms of operational taxonomic units; OTUs) than the bacteria. The average number of peaks in each bacterial total community profile was 42±14 (Table 2.2); the archaeal total community was nearly half that number (23±5.0-Table 1).

Clonal library screening accounted for 7 of the observed TRFLP peaks within our SIP profiles which are highlighted in grey in Figure 2.2. Three clones (2 unique sequences) matched the 101 bp peak, 5 clones (3 unique sequences) matched the 116 bp peak, 4 clones (2 unique sequences) matched the 124 bp peak, 1 clone matched the 159 bp peak, 4 clones (2 unique sequences) matched the 223 bp peak, 6 clones (3 unique sequences) matched the 251 bp peak, and 1 clone matched the 253 bp peak. These cloned crenarchaeal 16S rRNA gene sequences were aligned and a phylogenetic tree was re-constructed using maximum likelihood methods (Figure 2.4). Seven of these

sequenced clones align with uncultured crenarchaeota from the deep biosphere belonging to the Miscellaneous Crenarchaeotal Group (MCG-6 and MCG-8; Kubo et al., 2012). The other 7 clones aligned with members of the Marine Group 1.a Crenarchaeota. Several of the peaks in our TRFLP profiles yielded more than one clone, suggesting that archaeal diversity may be more extensive than our TRFLP profiles indicate, and additional enzyme digests are required for higher OTU resolution.

**Discussion**

SIP methodology has only recently been used to elucidate the metabolic potential of archaea. Most of these studies have focused on autotrophy and ammonia oxidation in various environments, such as rice paddies (Lu and Conrad, 2005), a variety of soils (Adair and Schwartz, 2011; Pratscher et al., 2011; Lu et al., 2013), and in freshwater sediment (Wu et al., 2013). However, there have also been a few heterotrophic reports from an acidic fen (Hamberger et al., 2008) and an estuarine setting in the UK (Webster et al., 2010). The fen study utilized soil samples suspended in a minimal salts media, pre-incubated for 8 days, and supplemented with $^{13}C$ xylose or glucose. After 13 days, most archaeal clones associated with the heavy fraction were methanogens, while 25% of the colonies screened (n=16) were identified as being crenarchaea. Likewise, in the SIP study in the Severn Estuary, various sediments were added to a minimal salt media and amended with $^{13}C$-glucose, $^{13}C$- acetate, and $^{13}CO_2$ for up to 14 days (Webster et al., 2010). There was no archaeal $CO_2$ uptake observed in sediment taken from the methanogenic zone, and after $^{13}C$ glucose amendment the aerobic zones were highly

similar in the $^{12}C$ and the $^{13}C$ fractions. However, in sediments from the sulfate reducing zone amended with $^{13}C$ acetate, active members of the Miscellaneous Crenarchaeal Group were detected which were clearly enriched when compared with the $^{12}C$ fraction.

In this report, SIP analysis utilized filter sterilized site water and incubations at only 5 days. The crenarchaeota of the Cheesequake salt marsh were found to be exclusively heterotrophic, with no $^{13}C$ uptake from bicarbonate at 5 mM. Certain crenarchaeal OTUs were detected in the $^{13}C$ fraction from nearly every microcosm amended with organic $^{13}C$- carbon, regardless of the type of substrate provided. This suggests these particular crenarchaea are able to assimilate a wide variety of organic substrates: simple metabolic intermediates (acetate), amino acids (glycine), organic nitrogen compounds (urea), lipids and pigments, and complex mixtures of biopolymers (ISOGRO). Other OTUs, however, were only detected in the active fraction of the microcosms amended with a specific substrate (acetate or urea) and may have more restricted metabolic capabilities.

Interestingly, bacterial and archaeal populations seem to be competing for the same resources in our SIP incubations. Members from both domains were active on acetate, glycine, lipids, and high levels of urea and ISOGRO (Table 2.1, Table 2.2). The only resource partitioning for active microbes was observed in microcosms amended with whole proteins (used exclusively by bacteria) and at low concentrations of urea (used exclusively by crenarchaea). Presumably, these crenarchaea in our samples are running the ornithine cycle backwards to incorporate the organic carbon from urea. Since our $^{13}C$-bicarbonate incubations would also produce $^{13}CO_2$ via equilibrium reactions, this study

has shown that crenarchaea are not fixing urea breakdown products ($^{13}CO_2$) directly into biomass. The lack of crenarchaeal protein uptake was surprising, considering recent single-cell genome sequencing of an MCG from marine sediment discovered several genes for extracellular cysteine peptidases (Lloyd et al., 2013). This finding underscores the need for direct incubations (such as SIP) to determine metabolic capabilities, in addition to assessing genetic potential by sequence analysis at the genome level.

Finally, the concentration of $^{13}C$-substrate had a dramatic effect on the profiles of the active crenarchaeal community. Specifically, low concentrations of urea produced twice as many active OTUs compared to microcosms amended with high concentrations of urea (Figure 2.5). Similarly, 30 μM acetate and glycine yielded more diverse active archaeal communities in terms of number of OTUs present (3-6 fold increase in total active OTUs, respectively). Higher concentrations of acetate (150 μM) produced communities that were dominated by 3 OTUs (101, 116, and 158 bp) comprising 68% of the community profile. High concentrations of glycine (150 μM) yielded an active community in which 2 of the same OTUs (116 and 158 bp) accounted for 80% of the community profile, while 150 μM urea yielded a 89-bp and 116-bp peak which also dominated the community profile (80%). There are several possible explanations for this result. One is that these particular crenarchaeal OTUs are very successful at competing for substrates at high concentration and their transport systems internally mobilize virtually all of the substrate. This prevents other microorganisms from accessing the $^{13}C$-organic carbon and synthesizing DNA. If this is the case, there must be a tipping point between low concentrations (tens of micromolar) and higher concentrations (hundreds of

micromolar) which triggers this hoarding response by certain crenarchaea. Another possibility is that minor OTUs in the $^{13}C$ carrier band are having their signal suppressed by the dominant OTU in the high concentration SIP experiment. Although our fingerprinting methods are highly reproducible (McGuinness et al; 2006; Tuorto et al., 2013), there is a suppression of smaller peaks in the TRFLP profile when screening clone libraries (Babcock et al., 2007) or samples where one OTU is >10% of the community. This same mechanism may be inhibiting the other active crenarchaea which are not synthesizing sufficient DNA in our incubations. Finally, low concentrations of some carbon compounds such as urea may not be bioavailable to the bacteria. Our low concentration ISOGRO additions did not produce any $^{13}C$-archaeal or $^{13}C$-bacterial signal. However, we know that 2 mg/ml of ISOGRO in liquid culture will allow for bacterial growth. It is possible that the salt marsh sediments are preventing the bioremineralization of the proteins and other biomolecules at low ISOGRO concentrations, as has been reported by Keil et al., 1994. However, uptake at low concentrations may be observed given a longer incubation time.

In conclusion, we have discerned a portion of the heterotrophic capability of crenarchaea in salt marsh sediments. From our data, it is possible to estimate crenarchaeal growth parameters *in situ*. Assuming two doubling events are required for complete $^{13}C$ labeling of DNA and detection in our 100% labeled $^{13}C$ carrier band, this suggests salt marsh crenarchaea have a doubling time on the order of 2-2.5 days. Prior studies have measured the growth rate of cultured ammonia-oxidizing crenarchaea (Könneke et al., 2005) and/or inferred doubling times by determining copy numbers of

16S rRNA (Park et al., 2010). These studies observed a maximum growth rate of AOA in liquid medium at 25-28˚C between 0.57 and 0.78 $day^{-1}$ (or a doubling rate between 1.28 and 1.75 days). Our findings are consistent with prior observations of slightly slower growth rates in sediment (0.2 $day^{-1}$, Mosier et al., 2012). Finally, this information on metabolic capability and activity from SIP studies will not only provide insight into the competitive relationships between crenarchaea and bacteria, but may help in determining conditions for isolating heterotrophic crenarchaea in a laboratory setting, where traditional microbiological techniques can then be utilized to improve our understanding of their physiological capabilities.

**Acknowledgements**

The authors wish to thank Dr. S.K. Rhee for his preliminary work on culturing Crenarchaeota in our lab, and the Cheesequake State Park rangers for their cooperation during this project. This research was partially supported by funds from Rutgers University and from the National Science Foundation.

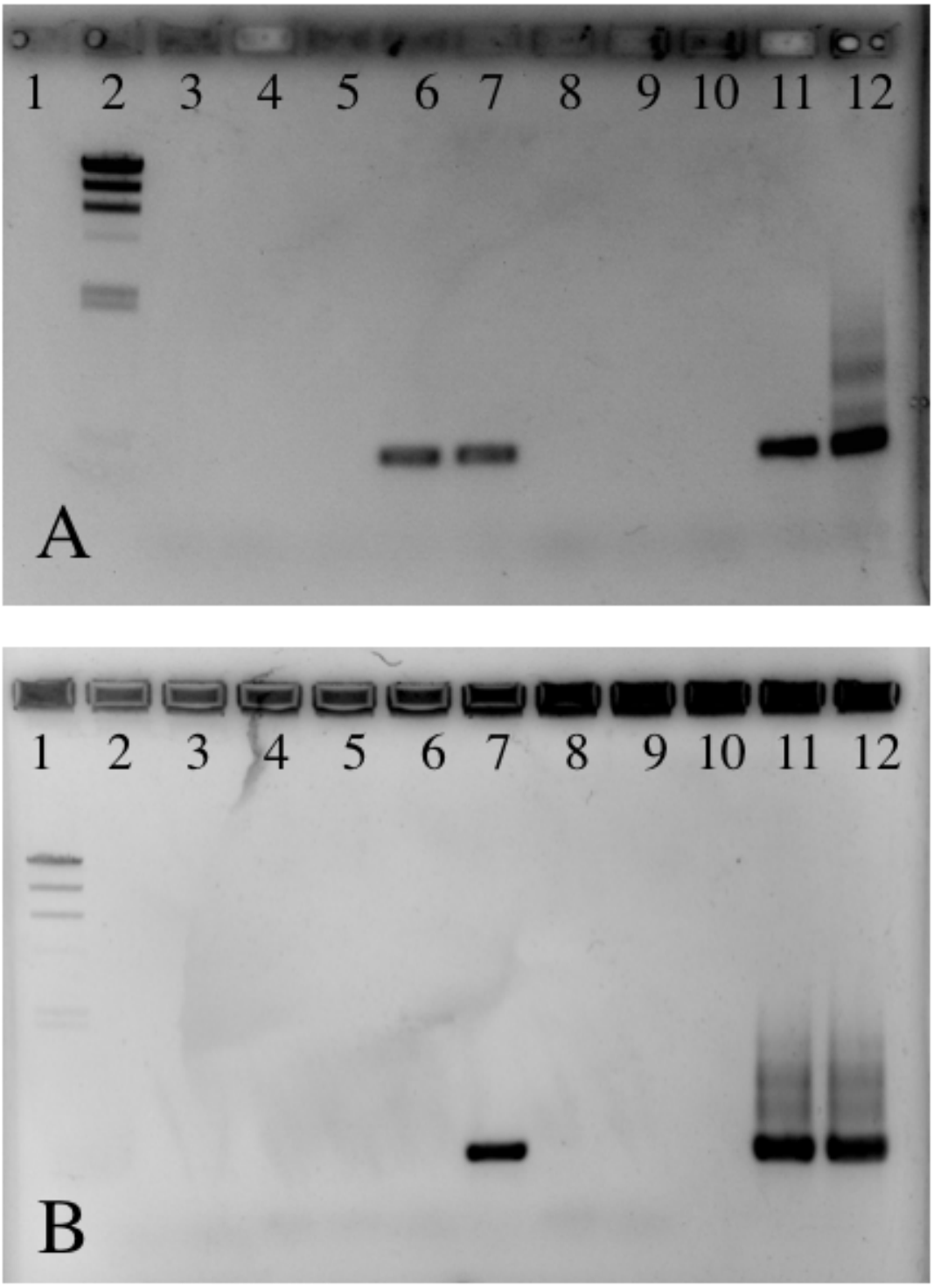



Figure 2.1. Agarose gel of amplification of bottom bands. A1) empty; A2) Lambda DNA; A3) negative; A4) carrier; A5) no substrate; A6) $^{13}C$ 2 mg/ml lipid; A7) $^{13}C$ 10 mg/ml lipid; A8) $^{13}C$ 2 mg/ml protein; A9) $^{13}C$ 10 mg/ml protein; A10) $^{13}C$ 2 mg/ml ISOGRO; A11) $^{13}C$ 10 mg/ml ISOGRO; A12) positive control. B1) Lambda DNA; B2) negative; B3) carrier; B4) T=0; B5) no substrate; B6) $^{12}C$ urea; B7) $^{13}C$ urea; B8) $^{12}C$ bicarbonate; B9) $^{13}C$ bicarbonate; B10) empty; B11) inhibitor test (positive+sample); B12) positive control.

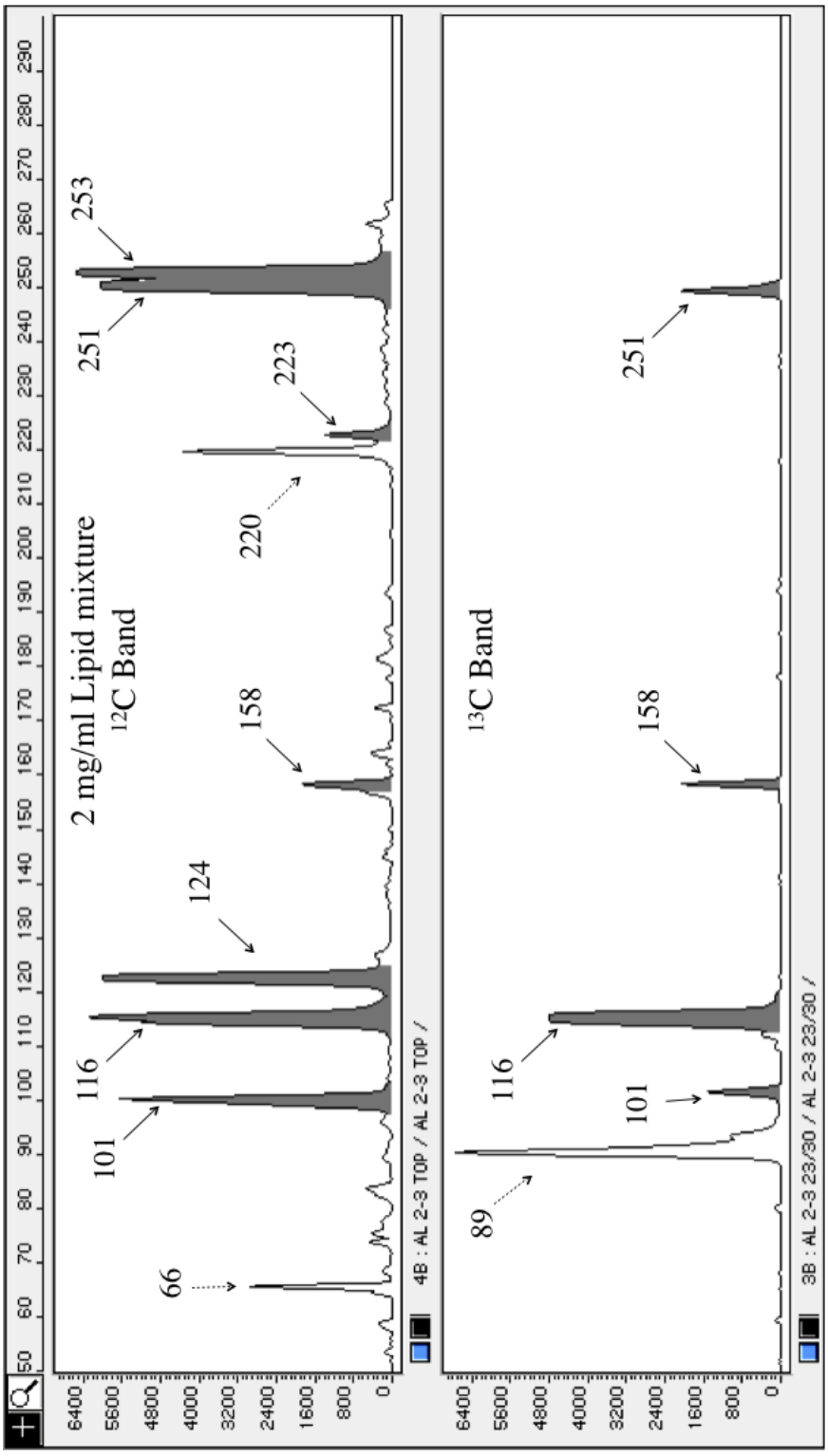



Figure 2.2. Example of TRFLP profiles of crenarchaeal 16S rRNA genes in $^{12}C$ and $^{13}C$ bands from lipid amendment. Highlighted peaks (grey) are represented in the clonal library.

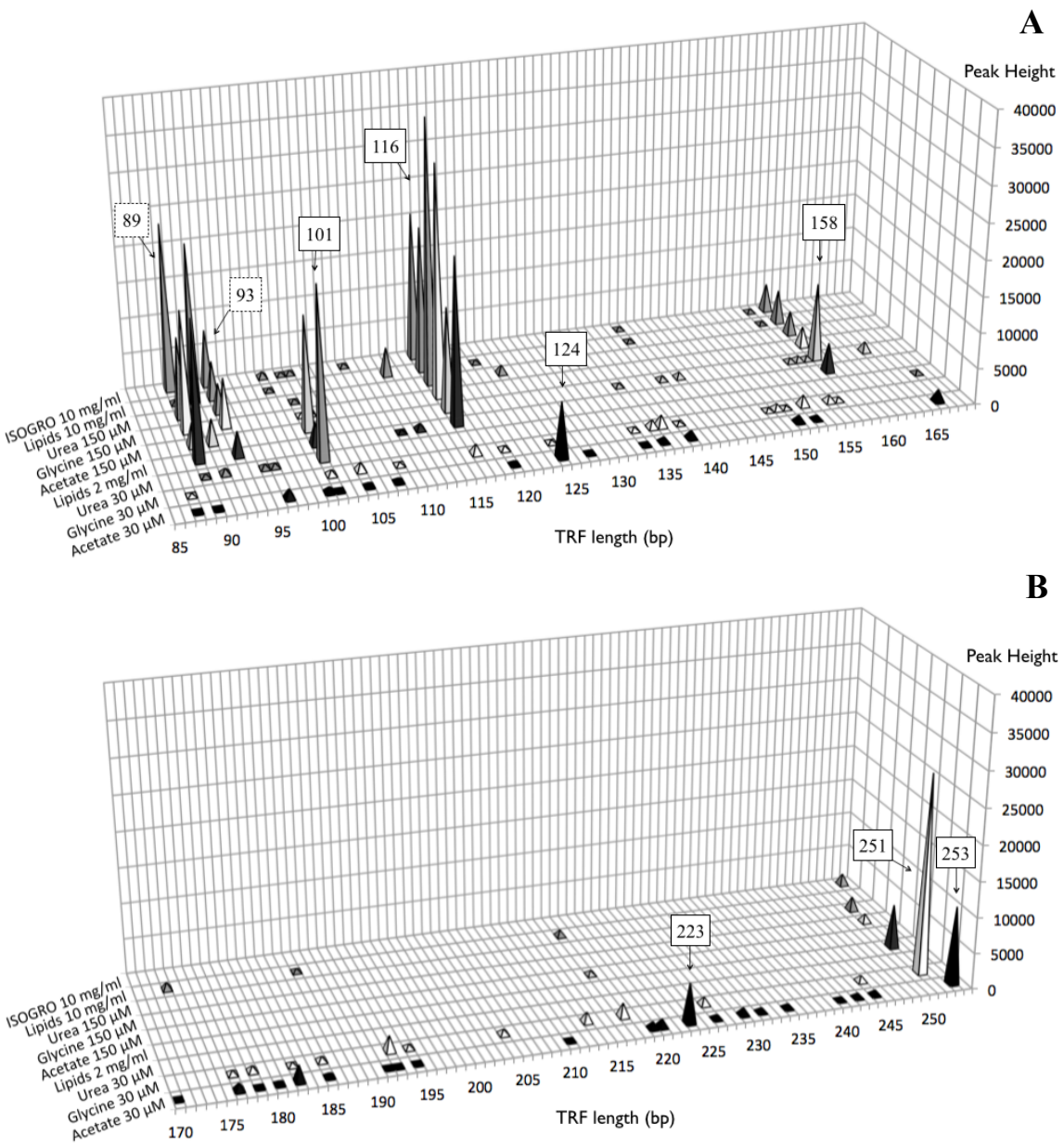



Figure 2.3. A compilation of crenarchaeal fingerprints with TRFs from 85 to 169 bp (A) and 170 to 254 bp (B) in length. Major peak sizes are indicated by boxes (solid-detected in the clone library; dashed-not detected). Light shades indicate higher concentration amendments; dark shades indicate lower concentration amendments.

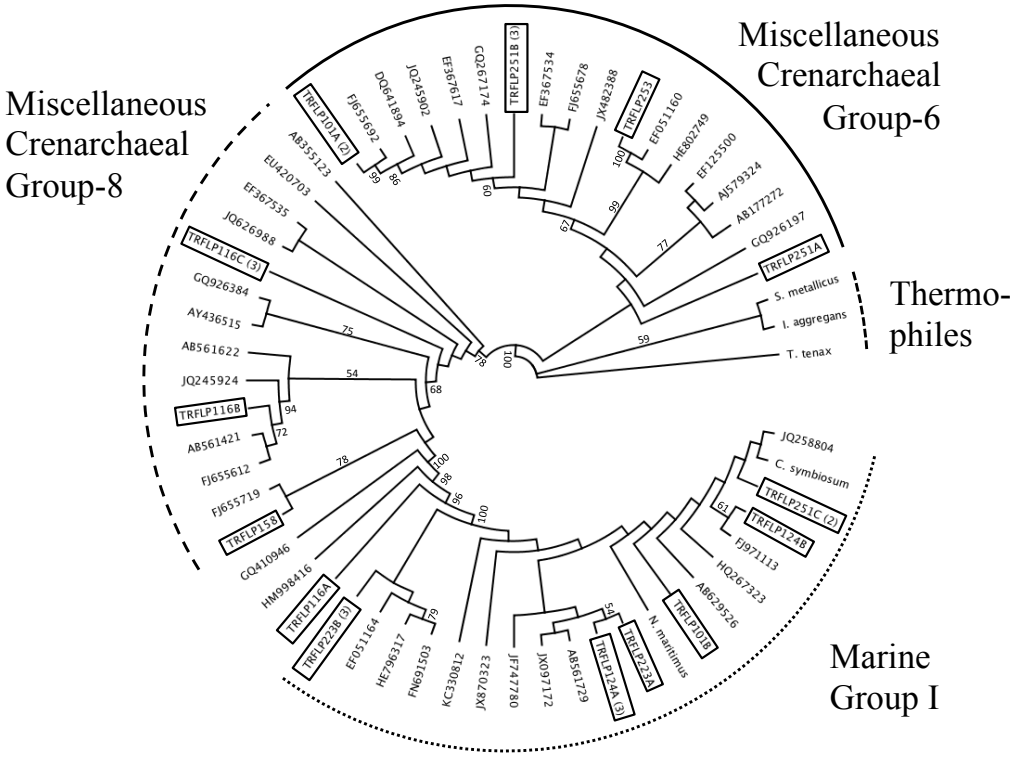



Figure 2.4. PhyML maximum-likelihood tree with support from 100 bootstrap runs indicated. The clones in this study are indicated with TRF size and GenBank accession numbers. Black dots represent sequences found in coastal or estuarine sediments. Open dots signify sequences from the marine deep biosphere.

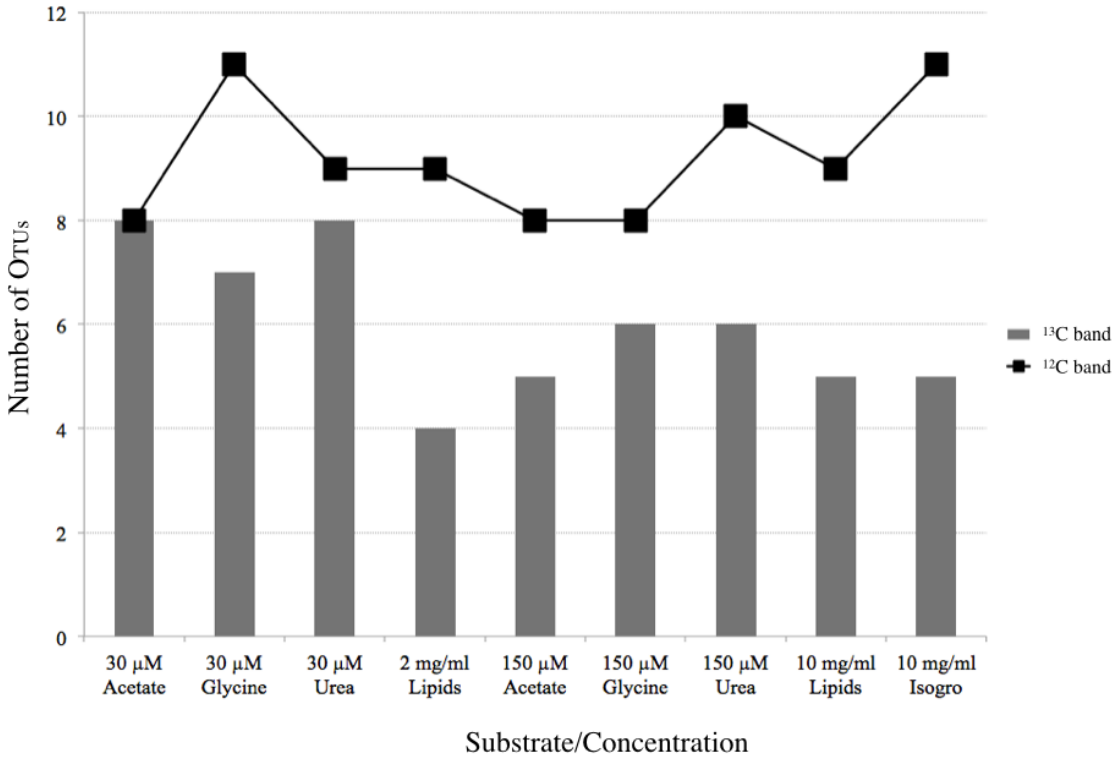
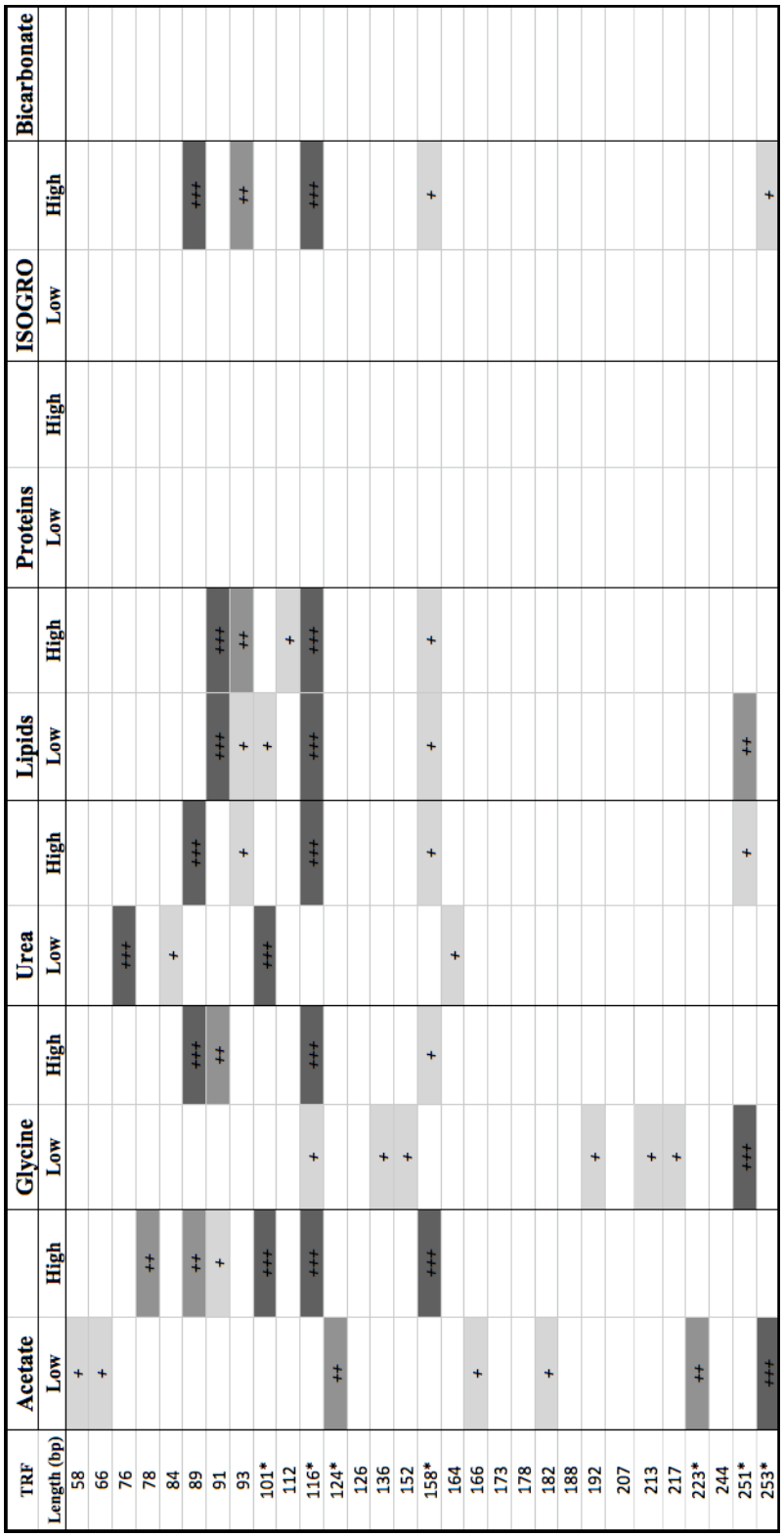



Figure 2.5. Total number of TRFs in the $^{12}C$ band (line) and in the $^{13}C$ band (gray bars) for peaks representing more than 2% of the community profile.



| TRF | Acetate | | Glycine | | Urea | | Lipids | | Proteins | | ISOGRO | | Bicarbonate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length (bp) | Low | High | Low | High | Low | High | Low | High | Low | High | Low | High | |
| 58 | + | | | | | | | | | | | | |
| 66 | + | | | | | | | | | | | | |
| 76 | | | | | +++ | | | | | | | | |
| 78 | | ++ | | | | | | | | | | | |
| 84 | | | | | + | | | | | | | | |
| 89 | | ++ | | +++ | | +++ | | | | | | +++ | |
| 91 | | + | | ++ | | | +++ | +++ | | | | | |
| 93 | | | | | | + | + | ++ | | | | ++ | |
| 101* | | +++ | | | +++ | | + | | | | | | |
| 112 | | | | | | | | + | | | | | |
| 116* | | +++ | + | +++ | | +++ | +++ | +++ | | | | +++ | |
| 124* | ++ | | | | | | | | | | | | |
| 126 | | | | | | | | | | | | | |
| 136 | | | + | | | | | | | | | | |
| 152 | | | + | | | | | | | | | | |
| 158* | | +++ | | + | | + | + | + | | | | + | |
| 164 | | | | | + | | | | | | | | |
| 166 | + | | | | | | | | | | | | |
| 173 | | | | | | | | | | | | | |
| 178 | | | | | | | | | | | | | |
| 182 | + | | | | | | | | | | | | |
| 188 | | | | | | | | | | | | | |
| 192 | | | + | | | | | | | | | | |
| 207 | | | | | | | | | | | | | |
| 213 | | | + | | | | | | | | | | |
| 217 | | | + | | | | | | | | | | |
| 223* | ++ | | | | | | | | | | | | |
| 244 | | | | | | | | | | | | | |
| 251* | | | +++ | | | | ++ | | | | | | |
| 253* | +++ | | | | | + | | | | | | + | |

Table 2.1. Summary of crenarchaeal TRFs representing at least 2% of the total community profile on the various 13C-carbon amendments. The plus signs indicate: (+) 250-5,000 arbitrary fluorescence units; (++) 5,000-10,000 afu; (+++) > 10,000 afu. The asterisk (*) are TRFs represented in the clonal library.

| TRF | Acetate | | Glycine | | Urea | | Lipids | | Proteins | | ISOGRO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length (bp) | Low | High | Low | High | Low | High | Low | High | Low | High | Low | High |
| 63 | | | | | | | | + | + | | | |
| 66 | | | | | | | + | | | | | |
| 89 | | | | ++ | | | | | | | | |
| 94 | | | | | | | + | | | | | |
| 101 | | | | + | | | | | | | | |
| 103 | | | | + | | | | | | + | | |
| 106 | | | | | | | | ++ | | | | |
| 108 | | | | + | | | | | | | | |
| 113 | | | | | | | | | | + | | |
| 117 | | | | | | | + | | | | | |
| 119 | + | | | | | | + | | ++ | | | + |
| 126 | + | ++ | | + | | | + | | | | | |
| 128 | | | | | | | | | | | | + |
| 130 | + | | | + | | | | | | + | | |
| 134 | + | | | + | | | + | + | + | | | |
| 138 | | | | | | | | + | | | | |
| 142 | | | | | | | | | + | | | |
| 145 | | | | | | | + | | | | | |
| 148 | | | | + | | | + | | | - | | - |
| 150 | + | | | | | | + | + | | | | |
| 153 | | | | | | | | | | + | | + |
| 162 | + | | | + | | | | | | | | |
| 164 | + | | +++ | | | ++ | | + | +++ | | | |
| 167 | | | + | | | ++ | | + | | + | | |
| 169 | + | | | | | | | + | | | | |
| 172 | | | | | | | + | | | | | |
| 177 | | + | | | | | | | | | | |
| 180 | + | + | | + | | | + | | | | | |
| 182 | | + | | + | | | + | | | | | + |
| 186 | + | + | | + | | | | ++ | | + | | |
| 189 | | | | | | | + | ++ | | | | |
| 192 | | | +++ | + | | | | | | | | +++ |
| 198 | | | +++ | | | | + | | | | | |
| 200 | | +++ | | + | | | | + | | | | |
| 204 | | +++ | + | | | + | | | + | | | |
| 206 | + | | | | | | | | + | | | |
| 208 | | | | | | | | ++ | | + | | + |
| 210 | + | | | + | | | + | | | + | | + |
| 213 | + | | | + | | | + | | | + | | + |
| 215 | | | | | | | | + | | | | |
| 216 | + | | | | | + | + | + | | + | | + |
| 219 | + | | | | | | | | | + | | |
| 225 | | | | + | | | | | | + | | + |
| 227 | | | | | | | | + | | | | |
| 235 | | | | | | ++ | | | | | | |
| 237 | | | | | | | | | | | | + |
| 240 | + | | + | + | | | | + | + | | | + |
| 246 | | | | | | +++ | | | | | | |
| 248 | + | | | | | | | + | | | | |
| 250 | + | | | ++ | | | + | | +++ | ++ | | +++ |

Table 2.2. Summary of bacterial TRFs representing at least 2% of the total community profile on the various 13C-carbon amendments. The plus signs indicate: (+) 250-5,000 arbitrary fluorescence units; (+ +) 5,000-10,000 afu; (+++) > 10,000 afu.

## References


Abreu, C., Jurgens, G., De Marco, P., Saana, A. and Bordalo, A.A. (2001) Crenarchaeota and Euryarchaeota in temperate estuarine sediments J of Appl Microbiol 90: 713-718.

Adair, K.L. and Schwartz, E. (2011) Stable isotope probing with 18O-water to investigate growth and mortality of ammonia oxidizing bacteria and archaea in soil. In: Klotz M.G. (ed). Methods in enzymology: research on nitrification and related processes. Academic Press: San Diego, pp 155–169.

Avaniss-Aghajani, E., Jones, K., Chapman, D. and Brunk, C. (1994) A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences. Biotechniques 17: 144-146.

Babcock, D.A, Wawrik, B., Paul, J.H., McGuinness, L.R., Kerkhof, L.J. (2007) Rapid screening of a large insert BAC library for specific 16S rRNA genes using TRFLP. J Micro Methods 71: 156–161.

Biddle, J.F., Lipp, J.S,. Lever, M.A,. Lloyd, K.G., Sørenson, K.B., Anderson, R. et al. (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. PNAS 103: 3846-3851.

Biddle, J.F., Fitz-Gibbon, S., Schuster, S.C., Brenchley, J.E. and House, C.H. (2008) Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. PNAS 105: 10583-10588.

Birtrim, S.B., Donohue, T.J., Handelsman, J., Roberts, G.P. and Goodman, R.M. (1997) Molecular phylogeny of Archaea from soil. PNAS 94: 277-282.

DeLong, E.F. (1992) Archaea in coastal marine environments. PNAS 89: 5685-5689.

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

Drummond, A.J., Ashton, B., Cheung, M., Heled, J., Kearse, M., Moir, R. et al. (2009) Geneious v3.8 and v4.6, available from http://www.geneious.com.

Fuhrman, J.A., McCallum, K. and Davis, A.A. (1992) Novel major archaebacterial group from marine plankton. Nature 356: 148-149.

Gallagher, E., McGuinness, L., Phelps, C., Young, L.Y. and Kerkhof, L.J. (2005) $^{13}C$ carrier DNA shortens the incubation time needed to detect benzoate-utilizing denitrifying bacteria by stable-isotope probing. Appl Environ Microbiol 71: 5192-5196.

Gonzalez, J.M., Portilo, M.C. and Saiz-Jimenez, C. (2006) Metabolically active Crenarchaeota in Altamira Cave. Naturwissenschaften 93: 42-45.

Guindon, S. and Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52: 696-704.

Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M. and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. PLoS Biol 4: 520-536.

Hamberger, A., Horn, M.A., Dumont, M.G., Murrell, J.C. and Drake, H.L. (2008) Anaerobic consumers of monosaccharides in a moderately acidic fen. Appl Env Microbiol 74: 3112-3120.

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

Inagaki, F., Suzuki, M., Takai, K., Oida, H., Sakamoto, T., Aoki, K. et al. (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. Appl Env Microbiol 69: 7224-7235.

Ingalls, A.E., Shah, S.R., Hansman, R.L., Aluwihare, L.I., Santos, G.M., Druffel, E.R.M., Pearson, A. (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. PNAS 103: 6442-6447.

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

Keil, R.G., Montluçon, D.B., Prahl, F.G. and Hedges JI. (1994) Sorptive preservation of labile organic matter in marine sediments. Nature 370: 549-552.

Kerkhof, L.J., Williams, K.H., Long, P.E. and McGuinness, L.R. (2011) Phase preference by active, acetate-utilizing bacteria at the Rifle, CO integrated field research challenge site. Environ Sci Tech 45: 1250–1256.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543-546.
Kubo, K., Lloyd, K.G., Biddle, J.F., Amann, R., Teske, A. and Knittel, K. (2012) Archaea of the Miscellaneous Crenarchaeotal Group are abundant, diverse and widespread in marine sediments. ISME 6: 1949-1965.
Lloyd, K.G., Schreiber, L., Petersen, D.G., Kjeldsen, K.U., Lever, M.A., Steen, A.D. et al. (2013) Predominant archaea in marine sediments degrade detrital proteins. Nature 496: 215-218.
Lu, Y. and Conrad, R. (2005) In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. Science 309: 1088-1090.
Lu ,L. and Jia, Z. (2013) Urease gene-containing Archaea dominate autotrophic ammonia oxidation in two acid soils. Environ Microbiol 15: 1795-1809.
Martens-Habbena, W., Berube, P.M., Urakawa, H., de la Torre, J.R. and Stahl, D.A. (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461: 976-979.
Martin-Cuadrado, A.B., Rodriguez-Valera, F., Moreira, D., Alba, J.C., Ivars-Martínez, E., Henn, M.R. et al. (2008). Hindsight in the relative abundance, metabolic potential and genome dynamics of uncultivated marine archaea from comparative metagenomic analyses of bathypelagic plankton of different oceanic regions. ISME 2: 865-886.
Massana, R., Murray, A.E., Preston, C.M. and DeLong, E.F. (1997) Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. Appl Env Microbiol 63: 50-56.
Massana, R., DeLong, E.F. and Pedrós-Alió, C. (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Env Microbiol 66: 1777-1787.
McGuinness, L.M., Salganik, M., Vega, L., Pickering, K.D. and Kerkhof, L.J. (2006) Replicability of bacterial communities in denitrifying bioreactors as measured by PCR/T-RFLP analysis. Environ Sci Technol 40: 509-515.
Moin, N.S., Nelson, K.A., Bush, A. and Bernhard, A.E. (2009) Distribution and diversity of archaeal and bacterial ammonia oxidizers in salt marsh sediments. Appl Env Microbiol 75: 7461-7468.
Mosier, A.C., Lund, M.B. and Francis, C.A. (2012) Ecophysiology of an ammonia-oxidizing archaeon adapted to low-salinity habitats. Microb Ecol 64: 955-963.
Murray, A.E., Preston, C.M., Massana, R., Taylor, L.T., Blakis, A., Wu, K. et al. (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl Env Microbiol 64: 2585-2595.
Nelson, K.A., Moin, N.S., Bernhard, A.E. (2009) Archaeal diversity and the prevalence of Crenarchaeota in salt marsh sediments. Appl Env Microbiol 75: 4211-4215.
Ouverney, C.C. and Fuhrman, J.A. (2000) Marine planktonic archaea take up amino acids. Appl Env Microbiol 66: 4829-4833.
Park, B.J., Park, S.J., Yoon, D.N., Schouten, S., Sinninghe Damsté, J.S. and Rhee, S.K. (2010) Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in coculture with sulfur-oxidizing bacteria. Appl Environ Microbiol 76: 7575-7587.
Pratscher, J., Dumont, M.G. and Conrad, R. (2011) Ammonia oxidation coupled to CO2 fixation by archaea and bacteria in an agricultural soil. PNAS 108: 4170-4175.
Radajewski, S., Ineson, P., Parekh, N.R. and Murrell, J.C. (2000) Stable-isotope probing as a tool in microbial ecology. Nature 403: 646-649.
Schleper, C., Jurgens, G. and Jonuscheit, M. (2005) Genomic Studies of Uncultivated Archaea. Nature Rev Microbiol 3: 479-488.
Søresnson, K.B. and Teske, A. (2006) Stratified communities of active archaea in deep marine subsurface sediments. Appl Env Microbiol 72: 4596-4603.
Stein, J.L. and Simon, M.I. (1996) Archaeal ubiquity. PNAS 93: 6228-6230.
Strickland, J.D.H. and Parsons, T.R. (1972) A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada: Ottawa.
Teske, A., Hinrichs, K.U., Edgcomb, V., Gomez, A.D.V., Kysela, D., Sylva, S.P. et al. (2002) Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. Appl Env Microbiol 68: 1994-2007.
Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P. and Schleper, C. (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environ Microbiol 7: 1985-1995.
Tuorto, S.J., Darias, P., McGuinness, L.R., Panikov, N., Zhang, T., Häggblom, M.M. and Kerkhof, L.J. (2013) Bacterial genome replication at subzero temperatures in permafrost. ISME 8(1): 139-149.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A. et al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66-74.

Vetriani, C., Jannasch, H., MacGregor, B., Stahl, D. and Reysenbach, A. (1999) Population structure and phylogenetic characterization of marine benthic Archaea in deep-sea sediments. Appl Environ Microbiol 65: 4375-4384.

Webster, G., Rinna, J., Roussel, E.G., Fry, J.C., Weightman, A.J. and Parkes, R.J. (2010) Prokaryotic functional diversity in different biochemical depth zones in tidal sediments of the Severn Estuary, UK, revealed by stable-isotope probing. FEMS Microbiol Ecol 72: 179-197.

Wuchter, C., Schouten, S., Boschker, H.T.S. and Damsté, J.S.S. (2003) Bicarbonate uptake by marine Crenarchaeota. FEMS Microbiol Letters 219: 203-207.

Wu, Y., Ke, X., Hernandez, M., Wang, B., Dumont, M.G., Jia, Z. et al. (2013) Autotrophic growth of bacterial and archaeal ammonia oxidizers in freshwater sediment microcosm incubated at different temperatures. Appl Environ Microbiol 79: 3076-3084.

# Chapter 3. Discerning marine archaeal heterotrophy in the North Atlantic by stable isotope probing

(To be submitted to ISME Journal)

**Abstract**

In this study, stable isotope probing (SIP) was used to track carbon uptake by archaea belonging to the TACK superphylum, along a meridional transect (Long. 52ºW) across the North Atlantic from French Guiana to the Grand Banks. Additionally, 0.5-L samples were collected at the start of the SIP incubations for 16 rRNA amplicon sequencing. Methods: At 6 stations, 1-L water SIP incubations were established from 3 depths: the euphotic zone (60-115 m), local oxygen minimum (215-835 m), and bathypelagic zone 2085-2835 m). Microcosms were amended with $^{13}$C sodium acetate (20-30 μM), $^{13}$C urea (20-30 μM), and $^{13}$C sodium bicarbonate (5 mM). Marine TACK archaea incorporated both inorganic and organic carbon substrates into newly synthesized DNA within 48 hours of amendment. The most abundant archaeal operational taxonomic units (OTUs) readily incorporated either DIC or DOC; others only incorporated organic carbon. 16S amplicon sequence analysis indicated that archaea were most abundant in the mesopelagic and benthopelagic zones in the deep ocean, and in the euphotic zone over the continental shelf and slope. There were more members of the phylum Euryarchaeota in the euphotic zone, while Thaumarchaeota dominated at depth. Analysis of similarities (ANOSIM) confirmed a significant correlation between archaeal beta diversity and ecological zone, and between archaeal beta diversity and dissolved oxygen concentration.

## Introduction

Archaea are ubiquitous and abundant in the deep ocean (Fuhrman et al., 1992; Delong, 1994; DeLong et al., 1994; Stein and Simon, 1996; Karner et. al., 2001). However, the modes of nutrition of marine archaea remain mostly unknown. It is well established that thaumarchaea play an important role in ammonia oxidation, the first step of nitrification (Kendall, 1998), in marine environments (Francis et al., 2007; Martens-Habbana et al., 2009). Metagenomic analyses have demonstrated that many thaumarchaea possess an ammonium monooxygenase (*amoA*) gene (Venter et. al., 2004; Treusch et. al., 2005) suggesting ammonia-oxidation partly drives the observed patterns of archaeal diversity in the water column (Francis et al., 2005). Additionally, ammonium-oxidizing archaea (AOA) are thought to be important microorganisms in suboxic environments (Francis et al., 2005; Molina et al., 2010; Stewart et al., 2012) or well-adapted to oligotrophic environments where the amount of available nitrogen is comparatively low (Martens-Habbana et al., 2009).

Because ammonia oxidation can drive chemoautotrophic carbon fixation, most studies concerning marine archaeal metabolism have also focused on the uptake of inorganic carbon (bicarbonate). Carbon fixation by marine archaea has been traced via the incorporation of $^{13}$C-bicarbonate into lipids (Wuchter et. al., 2003). Additionally, an isotopic mass balance model analysis of archaeal membrane lipids determined that 83% of the carbon uptake by deep ocean archaea is inorganic (Ingalls et. al., 2006). However, it is uncertain if this result suggests an archaeal community of both heterotrophs and autotrophs, or a uniform community of mixotrophs. Interestingly, genomic sequencing

results also indicate that thaumarchaea may be capable of both strict autotrophy and mixotrophy. For example, analysis of fosmid sequences from the sponge symbiont *Cenarchaeum symbiosum* revealed the presence of genes associated with a modified 3-hydroxypropionate cycle of autotrophic carbon assimilation, as well as an oxidative tricarboxylic acid cycle (Hallam et al., 2006). Thaumarchaea contain genes for 3-hydroxypropionate carbon fixation and oligopeptide transport, suggesting that this group takes up amino acids as a carbon source in addition to fixing inorganic carbon (Martin-Cuadrado et al., 2008). Likewise, Ouverney and Fuhrman (2000), using a microradiographic/fluorescent *in situ* hybridization method (MARFISH), demonstrated that up to 60% of the archaea in the deep Mediterranean and Pacific accumulate amino acids. Uptake of D- and L-aspartic acid has also been observed in archaea in deep waters of the North Atlantic (Teira et al., 2006). Recently, researchers obtained pure cultures of two new *Nitrosopumilus* strains, HCA1 and PS0, both of which were found to be obligate mixotrophs requiring the addition of α-ketoglutaric acid to grow in culture (Qin et al, 2014).

Each of these reports provides evidence of single carbon source utilization (e.g. genetic potential, etc.) for marine archaea at a limited phylogenetic resolution. In this study, stable isotope probing (SIP) coupled to 16S rRNA gene analysis was employed to directly explore heterotrophy and autotrophy along a north-south transect in the North Atlantic Basin, in members of the TACK archaeal superphylum (signifying Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota, Guy and Ettema,

2011). Archaeal abundance was confirmed by quantitative PCR. The results indicate most thaumarchaea in the deep ocean are mixotrophic.

**Materials and Methods**

In April of 2012, samples were taken onboard the UNOLS ships R/V Atlantis, as part of the US CLIVAR/$CO_2$ Repeat Hydrography program. The cruise was a repeat of World Ocean Circulation Experiment (WOCE) line A20 (longitude 52ºW), previously conducted in 2003 (Figure 3.1). 10-L water samples were collected at a total of 83 stations at varying depth intervals, using a 36-Niskin bottle rosette. At each station, water was collected from three distinct zones in the water column: the mixed layer (the euphotic zone, 60-115 m), the local oxygen minimum (in the mesopelagic zone, 215-835 m), and the bathypelagic zone (2085-2835 m). At 5 stations, samples were also drawn from 10 m above the seafloor. For analysis of TACK communities, 0.5-L subsamples were collected at 15 stations along the transect and biomass was collected on a 0.2-μm filter using vacuum filtration.

SIP microcosms were established at 6 stations (indicated by yellow dots in Figure 3.1). 1-L samples of water from each depth were amended with one or more of the following stable isotope-labeled substrates: $^{13}$C sodium acetate (20-30 μM), $^{13}$C urea (20-30 μM), and $^{13}$C sodium bicarbonate (5 mM). Additionally, $^{12}$C sodium acetate, $^{12}$C urea, and $^{12}$C sodium bicarbonate were amendments as controls. Bicarbonate was only employed in SIP incubations from the epipelagic and mesopelagic zones, since previous studies found that archaeal ammonia monooxygenase [*amoA*] gene copy numbers

decrease markedly with depth, suggesting that most bathypelagic archaea are heterotrophic (e.g., Agogué et al., 2008). These SIP microcosms were incubated in covered containers on deck for 48 hours, with a constant inflow of surface sea water to maintain stable temperature (on average, 22.3˚C). Biomass was collected on a 0.2-µm filter using vacuum filtration.

DNA extractions for next-generation sequencing were performed on a total of 55 filtered samples along the Atlantic transect using the PowerWater kit from Mol Bio, as per the Earth Microbiome Project protocol (http://www.earthmicrobiome.org/emp-standard-protocols/dna-extraction-protocol/). 16S rRNA amplicon sequencing was performed using the Illumina MiSeq platform on each sample using 515F (5'-AATGATA CGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTA A) and 806R barcoded (5'CAAGCAGAAGACGGCATACGAGATXXXXXXXXXXXX AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT) primers (Caporaso et al., 2010a). The sequencing data from 2 samples was discarded via single rarefaction. Data from the remaining 53 samples was run through beta diversity analysis, and taxa were summarized using QIIME (Caporaso et al., 2010b).

Quantitative-PCR was performed on a subset (22 total) of these amplicon-sequenced samples, to determine if the relative abundance of archaea and bacteria as reported by amplicon sequencing was accurate. Agilent Brilliant II SYBR Green qPCR Low ROX Master Mix was used following the manufacturer's protocol, using archaeal 16S A915F (5'-AGGAATTGGCGGGGGAGCAC, DeLong, 1992)/A1059R (5'GCCAT GCACCWCCTCT, Yu et al., 2005) and bacterial 16S 1114F (5'-CGGCAACGAGCGCA

ACCC)/1200R (5'-CCATTGTAGCACGTGTGTAGCC) (Reysenbach and Pace, 1995) primers on a Stratagene MX3500P machine. Positive controls were created for use as a standard, using 16S rDNA from cultured representatives cloned into a vector and purified using a Zymo Research Zyppy™ Plasmid Prep Kit. Because the bacterial 16S primers amplified both the bacterial and archaeal positive controls, these primers were treated as universal prokaryote primers during analysis of the qPCR data.

DNA extractions for SIP were performed using phenol-chloroform methods (McGuinness et al., 2006). The $^{12}C$ and $^{13}C$ DNA was fractionated by isopycnic cesium chloride gradient ultracentrifugation at 200,000 x g for 36 h, using $^{13}C$-labeled *E. coli* DNA as a carrier (Gallagher et. al., 2005). The $^{12}C$ (upper) and $^{13}C$ (lower) bands were collected by pipette and amplified by PCR with 5'-fluorescently-labeled, 16S rRNA archaea-specific (21F/958R) and crenarchaeota-specific forward/reverse primers (7F/518R). Amplicons were digested with *Mnl I* in 20 $\mu$l volumes for 6 h at 37 $^{o}$C, then precipitated using sodium acetate and 95% ethanol (McGuinness *et al*., 2006). Precipitated DNA was dried and re-suspended in 19.7 $\mu$l de-ionized formamide with 0.3 $\mu$l ROX 500 size standard (Applied Biosystems). TRFLP fingerprinting (Avaniss-Aghajani et al., 1994) was carried out on an ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA) using Genescan software. Peak detection was set at 25 arbitrary fluorescent units.

**Results**

Depth profiles in the Western Atlantic indicated bacteria comprised the majority of the total prokaryotic community, averaging 94% with a range of 83-99%. Archaea ranged between 0.5% and 16% of the total community, averaging 5%. Higher percentages of archaea were most often found in the mesopelagic (200-1,000 m depths) (Figure 3.2). However, relatively high percentages of archaea were also observed in the euphotic zone at specific sampling stations on the continental slope or shelf, averaging 10% (range 8-12). Archaeal populations were mostly members of phylum Euryarchaeota in the euphotic zone and TACK at depth, consistent with previous studies (Karner et al., 2001; Herndl et al., 2005). Parvarchaeota were found in relatively low numbers (between 0.1 and 5%). Additionally, a small percentage of the archaeal OTUs (between 0.1 and 8%) were not classified as Euryarchaeota, TACK, or Parvarchaeota ("Other"). Both the Parvarchaeota and the unclassified archaea were found in the greatest numbers in the bathypelagic and benthopelagic zones. NCBI's BLAST tool aligned many of these OTUs with uncultured crenarchaea found in the marine deep subsurface.

Beta diversity analysis suggested a correlation between observed microbial communities and the water mass where the sample was collected (Figure 3.3). Analysis of similarity (ANOSIM) of the basin samples (excluding samples taken on the slope or shelf) confirmed a strong correlation between beta diversity and sampling zone ($R = 0.81$, p-value = 0.01). This correlation was slighter stronger for the bacteria ($R = 0.81$, p-value = 0.01) than the archaea ($R = 0.73$, p-value = 0.01) (Figure 3.4). Many of the OTUs associated with this correlation were cyanobacteria, chloroplasts, or other OTUs

associated with photosynthesis, as determined by analysis of variance (ANOVA) and a G-test of independence. This would suggest that the correlation to depth is related to light penetration in the water column. To test the depth dependence of prokaryotes below the euphotic zone, a separate ANOSIM analysis was conducted on only the samples taken from the mesopelagic, bathypelagic, and benthopelagic zones. Surprisingly, within this subset there was an even stronger correlation between prokaryotic beta diversity and ecological zone ($R = 0.83$, p-value = 0.01). This correlation was greater for the bacteria ($R = 0.83$, p-value = 0.01) than for the archaea ($R = 0.60$, p-value = 0.01). No significant correlation was detected between microbial beta-diversity and latitude, exact depth in meters, or temperature. An ANOVA test was used to compare OTU frequencies between sampling zones and determine which archaea were differentially represented in one sampling zone over another (Figure 3.5).

Prior research has also suggested that AOAs are of particular importance in areas of low oxygen concentration (Francis et al., 2005; Molina et al., 2010; Stewart et al., 2012). ANOSIM analysis of our Atlantic transect confirmed this correlation between observed archaeal beta diversity and dissolved oxygen concentration ($R = 0.73$, p-value = 0.01). A Pearson correlation test was also performed to determine the archaeal OTUs whose relative abundance was most strongly correlated with oxygen concentration. Most of these OTUs were also strongly correlated to sampling depth (in Figure 3.6, these OTUs are indicated with stars). Bacterial beta diversity displayed a similar correlation to dissolved $O_2$ ($R = 0.75$, p-value = 0.01).

Overall, prokaryotic beta diversity was more related to phosphate concentration ($R = 0.72$, p-value = 0.01) than to nitrate concentration ($R = 0.56$, p-value = 0.01). Bacterial beta diversity exhibited a greater correlation to dissolved phosphate concentrations ($R = 0.74$, p-value = 0.01) than the archaea ($R = 0.45$, p-value = 0.01). The same was true for nitrate, with the bacteria displaying a higher correlation ($R = 0.58$, p-value = 0.01) than the archaea ($R = 0.30$, p-value = 0.05).

Interestingly, the qPCR results indicate that the 16S amplicon sequencing may under represent the abundance of archaea. This may be due to primer mismatch, a change in the average ribosomal copy number for bacteria, or the inability of the technique to accurately measure abundance. In nearly every sample, qPCR gave a higher percentage of archaea as a total of prokaryotic abundance than yielded by QIIME analysis of the sequence data (Table 3.1). In many cases, the archaeal percentage was 2-3x greater than the sequencing data, with one sample more than an order of magnitude higher. Table 1 shows a comparison of the average archaeal relative abundances in the North Atlantic Basin, as determined by both amplicon sequencing and qPCR, compared to the average concentration of dissolved oxygen throughout the water column. In the amplicon sequencing data, the greatest relative archaeal abundance is observed in the middle of the oxycline, which may be indicative of the expected primer bias (Figure 3.7). In contrast, the qPCR data shows the greatest relative archaeal abundance is found deeper in the water column, at the local oxygen minimum.

Depth-dependent archaeal OTUs were identified using analysis of variance (ANOVA). A figure showing the relative abundance of these OTUs (excluding those that

comprised less than 1% of the total archaeal community) in each depth regime shows how certain OTUs may dominate in one depth regime while being absent in others (Figure 3.6). 60% of the archaeal OTUs that were most strongly correlated to sampling zone (those with a Bonferroni correction value of $< 0.05$) were members of phylum Thaumarchaeota (formerly known as Marine Group I). This is consistent with prior studies describing distinct communities of ammonia-oxidizing archaea associated with different zones in the water column (Francis et al., 2005, Hu et al., 2011a; Hu et al., 2011b). The remaining 40% were members of Marine Groups II and III (Euryarchaeota), one member of Marine Benthic Group A (MBGA, within the phylum Crenarchaeota), and a member of the Parvarchaeota. The maximum-likelihood tree of these depth-dependent archaea shows some phylogenetic clustering based on sampling depth, mostly by OTUs found in the mesopelagic (Figure 3.6). The cultured members of the thaumarchaea cluster closely together. OTUs that represent greater than 1% of the total archaeal community in at least one depth regime are indicated with an open circle. OTUs that also correlated closely to oxygen concentration are indicated with a star.

In order to determine the type of organic and/or inorganic carbon utilized by the archaeal populations, SIP microcosms were established along the transect. TRFLP analysis of the active archaeal community ($^{13}$C-labeled) at six stations is shown in Table 2. A total of 17 $^{13}$C-archaeal peaks/OTUs were identified with less than half of all observed OTUs (8 total) incorporating both inorganic (bicarbonate) and organic (acetate/ urea) carbon. Of these mixotrophic OTUs, 6 peaks (66, 83, 116, 123, 223, and 265) tended to be the most dominant peaks in the TRFLP profiles in which they appeared (data

not shown). Peaks 83 and 223 were detected in almost every sampling location, with the exception of the epipelagic in the North Equatorial Current. The greatest amount of diversity and metabolic flexibility was generally found in the mesopelagic, except in the Sargasso Sea stations, where only organic carbon was incorporated at this depth. No OTU appeared to be exclusively autotrophic (although the archaeal community in the epipelagic at Station 60 exclusively incorporated bicarbonate, these same OTUs incorporated organic carbon at greater depths). However, more than half of all OTUs (peaks 64, 76, 80, 93, 95, 158, 182, 195, and 213) only incorporated acetate and/or urea and not bicarbonate. Only 2 of these exclusively heterotrophic OTUs (93 and 195) appeared at more than one station/depth.

**Discussion**

Previous studies examining archaeal distribution throughout the water column in the open ocean have shown that the relative abundance of archaea tends to increase with depth, reaching peak numbers in the mesopelagic and remaining high throughout the bathypelagic to the bottom (e.g., Karner et al., 2001; Teira et al., 2006; Varela et al., 2008; Agogué et al., 2008). Quantitative-PCR results agree with these studies, and indicate an archaeal relative abundance in the Mid-Atlantic that peaks around the local oxygen minimum and remains relatively stable throughout the lower water column. However, amplicon sequencing results suggest that archaeal relative abundance in the North Atlantic basin peaked in the mesopelagic decreased in the bathypelagic (Figure 3.2). Similarly, the qPCR findings suggest that archaeal relative abundance may be higher on

the shelf and slope than the sequencing data indicates (Table 3.1). In terms of actual abundance (using qPCR as a proxy for cell count), archaeal and total prokaryotic abundance follows the classic open ocean distribution for prokaryotes, with high numbers just below the surface, decreasing with depth and displaying a slight spike in abundance just above the seafloor. This disparity between amplicon sequencing and qPCR relative abundance data for the archaea (Figure 8), particularly at depths where thaumarchaea/ crenarchaea have been reported in relatively high numbers (De Corte et al., 2008; Konstantinidis and DeLong, 2008), suggests further optimization of the primer set used in this study may be required.

The separation of marine archaea into depth-dependent clades in this study is consistent with prior reports in the global ocean. For example, genetic variability in archaea tends to be more dependent on depth than latitude, with “deep” and “shallow” clades often being observed (Herndl et. al., 2005; De Corte et al., 2008; Hu et al., 2011b). Ratios of archaeal amoA gene copy numbers to 16S rRNA gene copy numbers appear to decrease drastically with depth (De Corte, et al., 2008; Agogué et al., 2008; Yakimov et al., 2011; Hu et al., 2011a). It may be that archaeal metabolism strategies vary significantly with depth, but not with geographic location; however, latitudinal trends in archaeal relative abundance have been previously reported in the North Atlantic (Teira et al., 2006; Varela et al., 2008). 16S amplicon sequencing data in this study supports the distinction of depth-dependent clades of archaea (Figures 3.3 and 3.4). Sampling depth accounted for over 36% of archaeal variation between samples (e.g., euphotic zone vs mesopelagic zone). It is probable that the observed correlation between beta diversity and

depth regime reflects the difference in microbial communities in different deep water masses, as has been previously explored in the North Atlantic (Teira et al., 2006). While euryarchaea tended to dominate at the surface, as has been previously reported (Massana et. al., 1997; Murray et. al., 1999; Karner et al., 2001), sequence data also indicated a strong euryarchaeal presence in the mesopelagic, nearly as high as that of the TACK superphylum (Figure 3.2). The qPCR data supports the previously observed abundance of archaea in the mesopelagic (De Corte et al., 2008), but there is also evidence of a genetically distinct, numerically dominant archaeal community at depth (as observed in the Pacific in Konstantinidis and DeLong, 2008).

The correlation between archaeal abundance/diversity and dissolved oxygen concentration has been explored in previous studies, though these mostly focused on ammonia-oxidizing archaea (AOA) and their contribution to nitrite production in marine environments (Francis et al., 2005; Beman et al., 2008; Molina et al., 2010; Pitcher et al., 2011; Stewart et al., 2012). However, total archaeal diversity and distribution within North Atlantic deep waters (Teira et al., 2006; Varela et al., 2008), oxygen minimum zones (Belmar et al., 2011) and seasonally hypoxic waters (Parsons et al., 2014) has also been reported. In general, archaea (specifically thaumarchaea) tend to be found in greater relative numbers (compared to bacteria) in areas of low oxygen concentration.

The SIP results indicate the North Atlantic pelagic archaeal community is dominated by a select group of ubiquitous mixotrophs. Previous studies have suggested that a significant number of marine members of the TACK superphylum (in particular, the thaumarchaea) may be mixotrophic (Ingalls et al., 2006; Hallam et al., 2006; Martin-

Cuadrado et al., 2008). Indeed, two currently cultured members of genus *Nitrosopumilus* are obligate mixotrophs that require the addition of organic carbon to grow and reproduce (Qin et al., 2014). Our SIP results indicate nearly half of observed OTUs of the TACK superphylum readily incorporated both organic and inorganic carbon over a two-day incubation period (Table 3.2). In general, these OTUs represented the largest and most frequently observed peaks in TRFLP analysis. However, there also appears to be a subset of rare OTUs that exclusively incorporate organic substrates. Strictly heterotrophic thaumarchaea have also been observed in New Jersey salt marsh sediment (Seyler et al., 2014).

In conclusion, while much of the recent research focus on marine thaumarchaea has concentrated on life strategies revolving around ammonia-oxidation and autotrophy, our SIP results demonstrate that ammonium oxidation only represents a subset of the full metabolic capability of the TACK superphylum as a whole. Additionally, these SIP methods recover archaeal ribosomal RNA sequences distantly related to the currently cultured thaumarchaea cluster, indicating that we are only beginning to discover the total diversity of marine archaea in the deep sea.

**Acknowledgements**

I wish to thank Dr. Michael McCartney for allowing me to participate as a student researcher onboard the R/V Atlantis during CLIVAR leg A20. This research was made possible through the support of the International Global Ocean Carbon and Repeat Hydrography Program, and the Earth Microbiome Project, and the generous support of

Justin A. Bean, Theresa O'Toole Bean, James and Kristy Bell, Joseph R. Gates, Meredith Gelfand, Thomas Joyce, Devon Martinez, Spenser Thomas Paige, Scott Strenger, MD, MMM, and Kimberly Strenger, MLS.

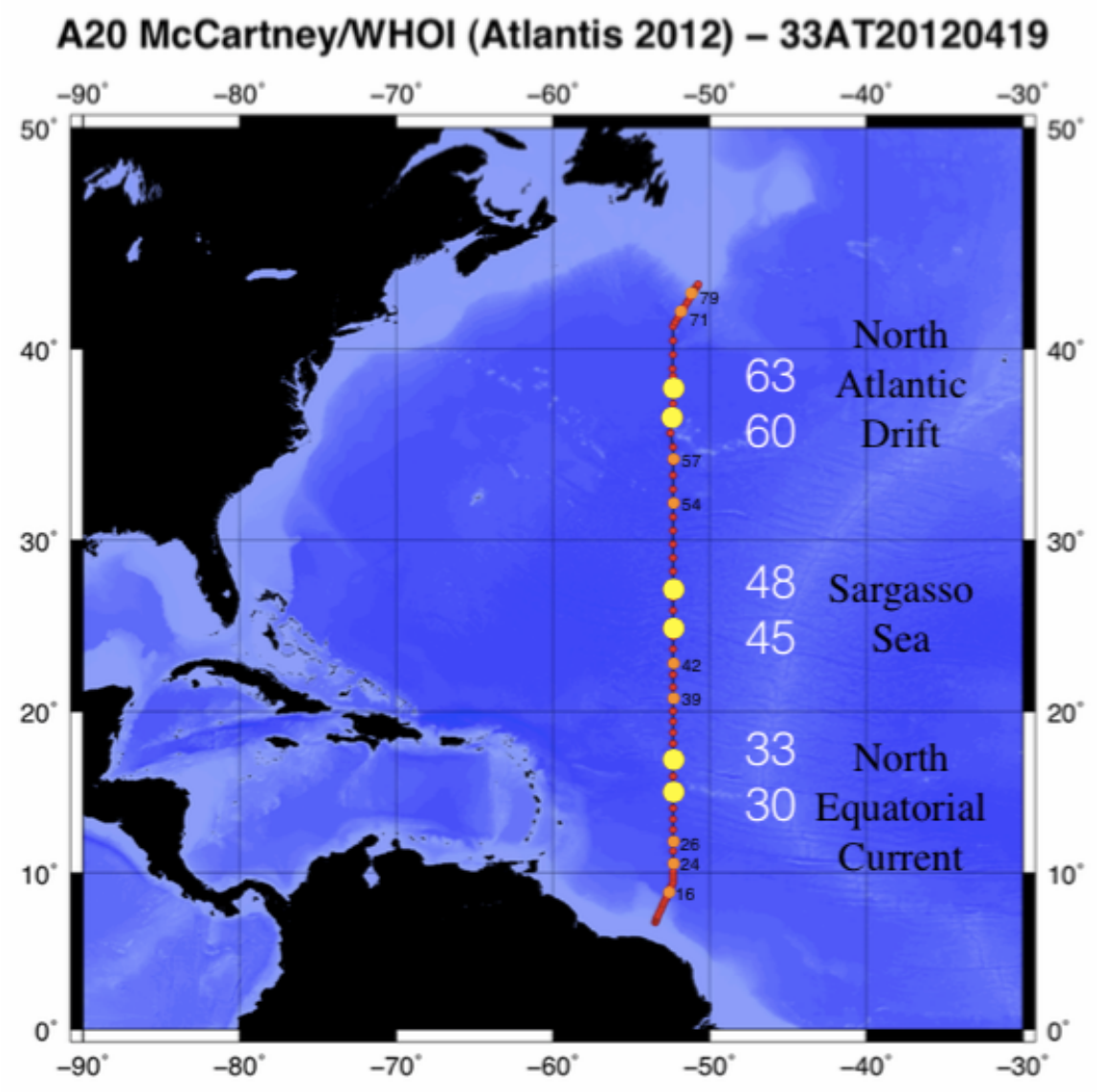



Figure 3.1. Map of the CLIVAR A20 cruise track, with each CLIVAR sampling station indicated by a red dot. Orange dots represent stations where biomass was taken for 16S amplicon sequencing; yellow dots indicate stations where SIP microcosms were established.

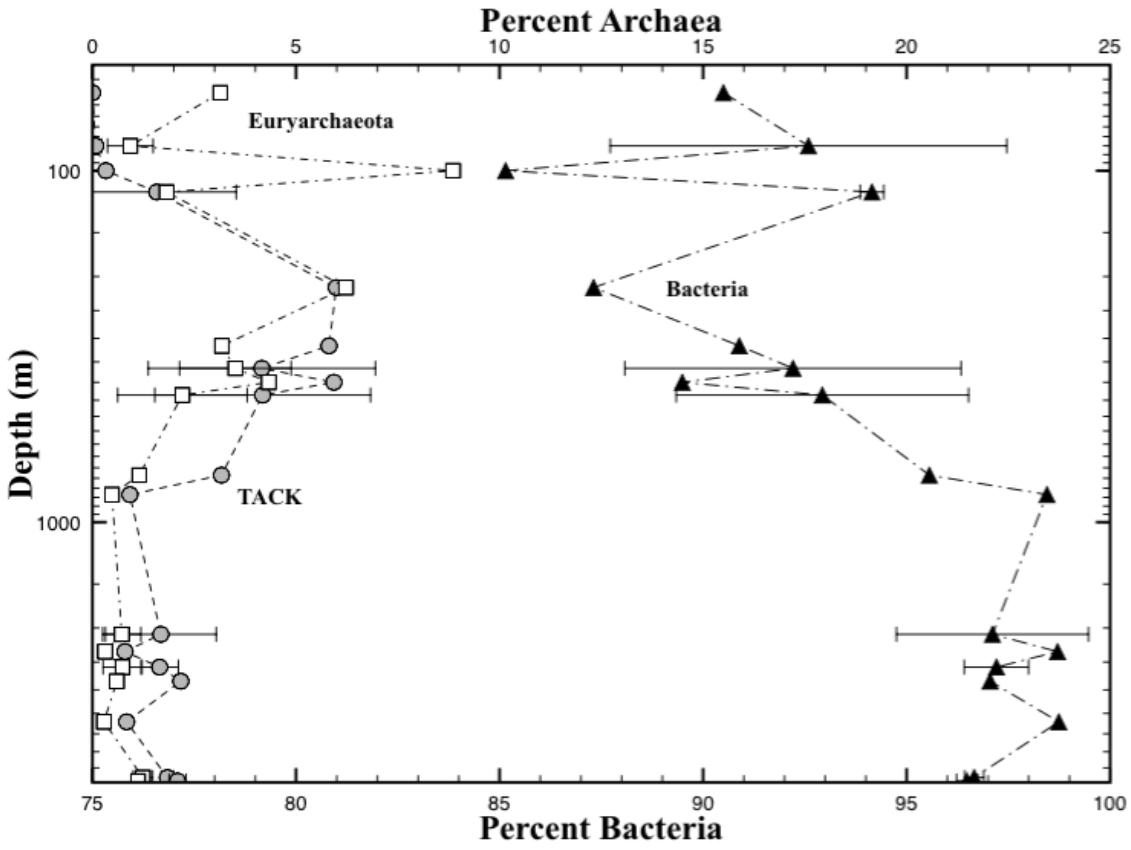



Figure 3.2. Average prokaryote population composition versus depth in the North Atlantic basin along longitude 52º W, based on 16S amplicon sequencing data.

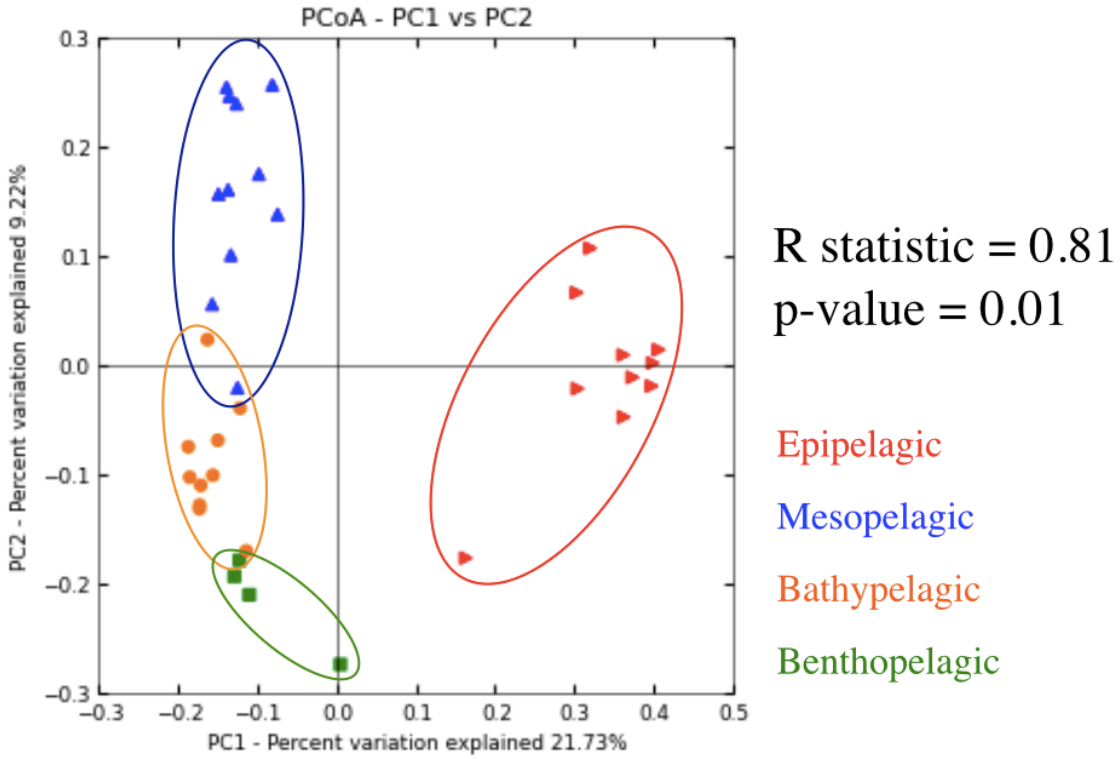



Figure 3.3. Analysis of similarity (ANOSIM) of all 16S amplicon sequencing data from basin samples, based on depth regime.

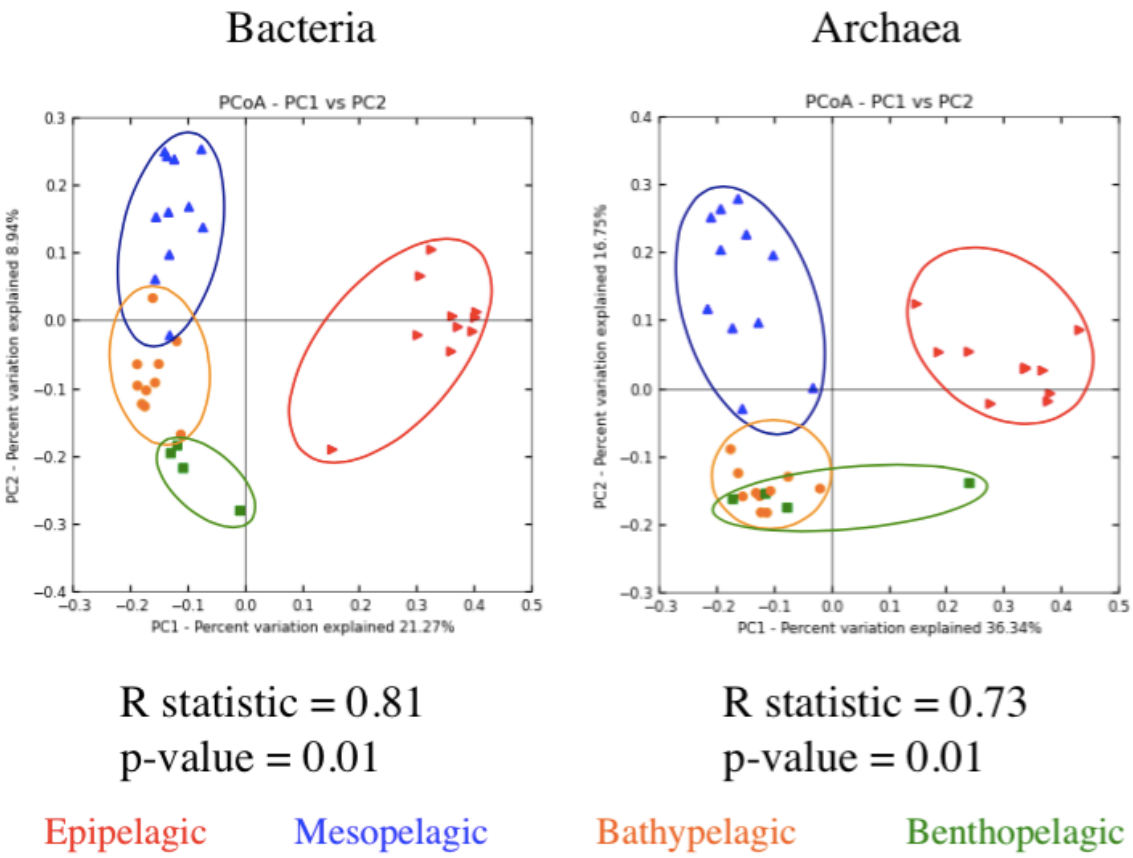



Figure 3.4. Analysis of similarity (ANOSIM) of bacterial and archaeal 16S amplicon sequencing data from basin samples, based on depth regime.

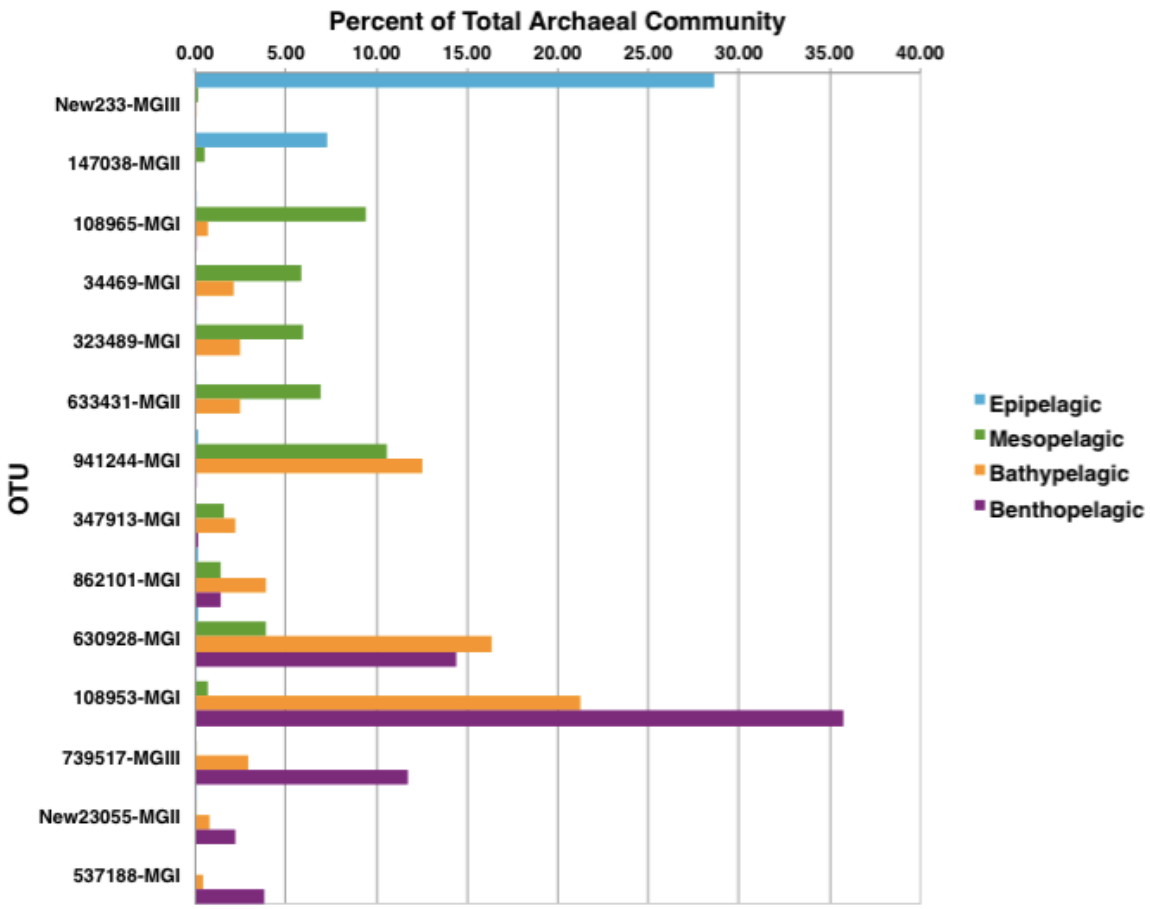



Figure 3.5. Depth-dependent archaeal OTUs, identified using analysis of variance (ANOVA), with a Bonferroni correction value of $< 0.05$. Only OTUs that comprised at least 1% of the total archaeal population at at least one depth are depicted. Each OTU is identified by its accession number in the Greengenes database, with two OTUs not found in the database indicated by the word "New" at the beginning of the accession number.

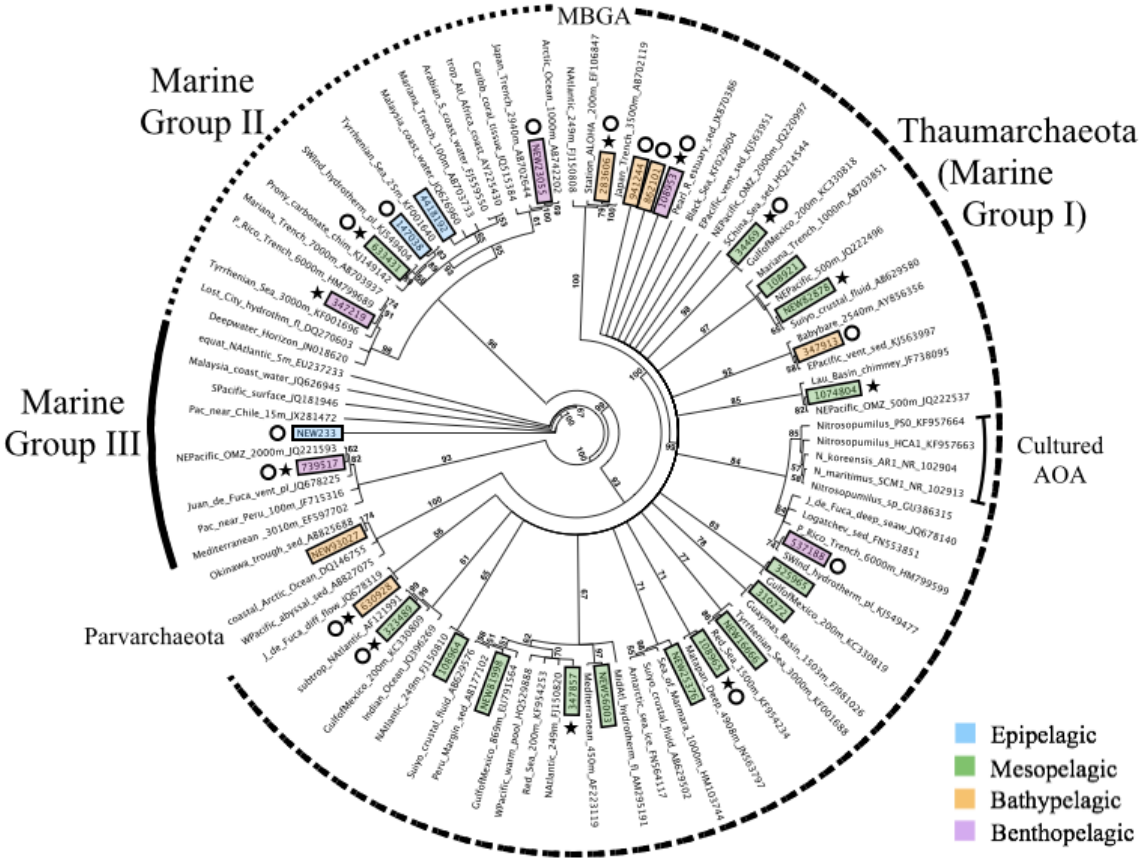



Figure 3.6. Maximum likelihood tree of depth-dependent archaeal OTUs, identified using analysis of variance (ANOVA), with a Bonferroni correction value of < 0.05. OTUs that represent greater than 1% of the total archaeal community in at least one depth regime are indicated with an open circle. OTUs that also correlated to oxygen concentration (determined using a Pearson correlation test) are indicated with stars. Each OTU is identified by its accession number in the Greengenes database, with two OTUs not found in the database indicated by the word "New" at the beginning of the accession number.

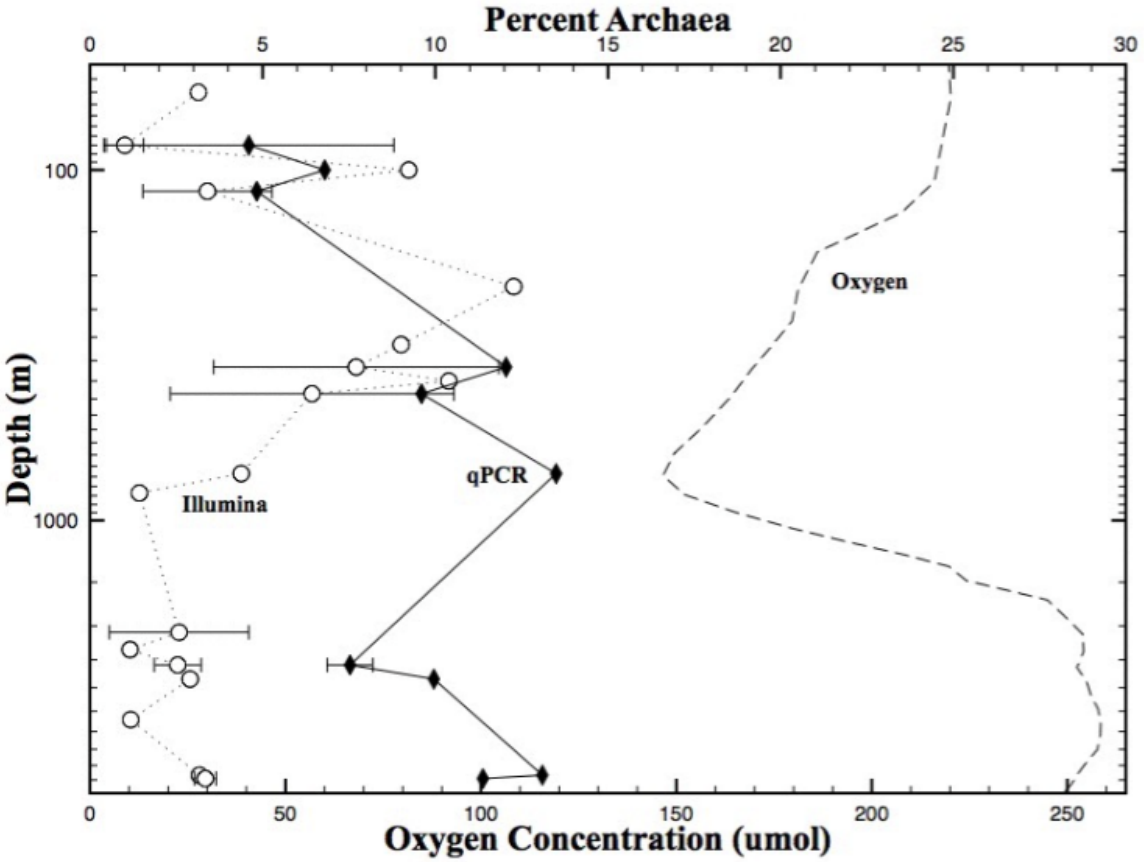



Figure 3.7. Quantitative-PCR and 16S amplicon sequencing results for basin archaeal numbers as a percentage of the total prokaryote population, and the average basin $O_2$ concentration, versus depth.

| Sample Name | Latitude | Longitude | Depth (m) | Archaea qPCR Count | Prokaryote qPCR Count | Percent Archaea (qPCR) | Precent Archaea (Illumina) |
|---|---|---|---|---|---|---|---|
| S16D4611 | 8 46 N | 52 38 W | 4611 | 1.42E+04 | 4.50E+04 | 24.02% | 12.14% |
| S16D2165 | 8 46 N | 52 38 W | 2165 | 4.76E+02 | 2.83E+03 | 14.40% | 4.88% |
| S16D40 | 8 46 N | 52 38 W | 40 | 4.86E+04 | 3.81E+05 | 11.31% | 8.02% |
| S24D365 | 10 45 N | 52 20 W | 365 | 1.44E+03 | 2.97E+03 | 32.61% | 10.08% |
| S24D85 | 10 45 N | 52 20 W | 85 | 1.78E+04 | 1.04E+05 | 14.68% | 11.48% |
| S24D35 | 10 45 N | 52 20 W | 35 | 5.29E+04 | 5.86E+05 | 8.28% | 6.01% |
| S26D75-100 | 12 5 N | 52 20 W | 75-100 | 1.67E+04 | 2.28E+05 | 6.80% | **9.23%** |
| S33D2585 | 16 44 N | 52 20 W | 2585 | 3.85E+02 | 4.43E+03 | 8.00% | 1.92% |
| S33D365 | 16 44 N | 52 20 W | 365 | 4.92E+03 | 3.59E+04 | 12.05% | 4.79% |
| S33D60-85 | 16 44 N | 52 20 W | 60-85 | 8.05E+03 | 5.69E+05 | 1.39% | *1.39%* |
| S39D5329_2 | 20 44 N | 52 20 W | 5329 | 1.28E+02 | 8.51E+02 | 13.10% | 3.17% |
| S39D2585 | 20 44 N | 52 20 W | 2585 | 1.01E+03 | 1.34E+04 | 7.06% | 1.93% |
| S39D435 | 20 44 N | 52 20 W | 435 | 2.90E+03 | 2.73E+04 | 9.60% | 2.66% |
| S42D735 | 22 59 N | 52 20 W | 735 | 2.32E+03 | 1.49E+04 | 13.50% | 4.38% |
| S45D115 | 25 14 N | 52 20 W | 115 | 6.43E+03 | 1.27E+05 | 4.83% | 2.09% |
| S54D60-85 | 32 00 N | 52 20 W | 60-85 | 4.61E+03 | 5.39E+04 | 3.05% | 1.47% |
| S57D3700 | 34 15 N | 52 20 W | 3700 | 1.43E+03 | 1.83E+05 | 2.46% | 1.18% |
| S60D5450 | 36 30 N | 52 20 W | 5450 | 1.43E+03 | 1.12E+04 | 11.38% | 3.12% |
| S60D2835 | 36 30 N | 52 20 W | 2835 | 6.07E+03 | 5.48E+04 | 9.96% | 2.90% |
| S63D60-85 | 38 20 N | 52 20 W | 60-85 | 3.77E+03 | 3.65E+04 | 9.35% | 0.83% |
| S71D62 | 41 40 N | 52 53 W | 62 | 1.23E+05 | 5.74E+05 | 17.64% | 12.23% |
| S79D5 | 42 53 N | 50 53 W | 5 | 3.37E+03 | 1.49E+05 | 2.21% | 0.34% |

Table 3.1. Quantitative-PCR and 16S amplicon sequencing results for archaeal numbers as a percentage of the total prokaryote population.

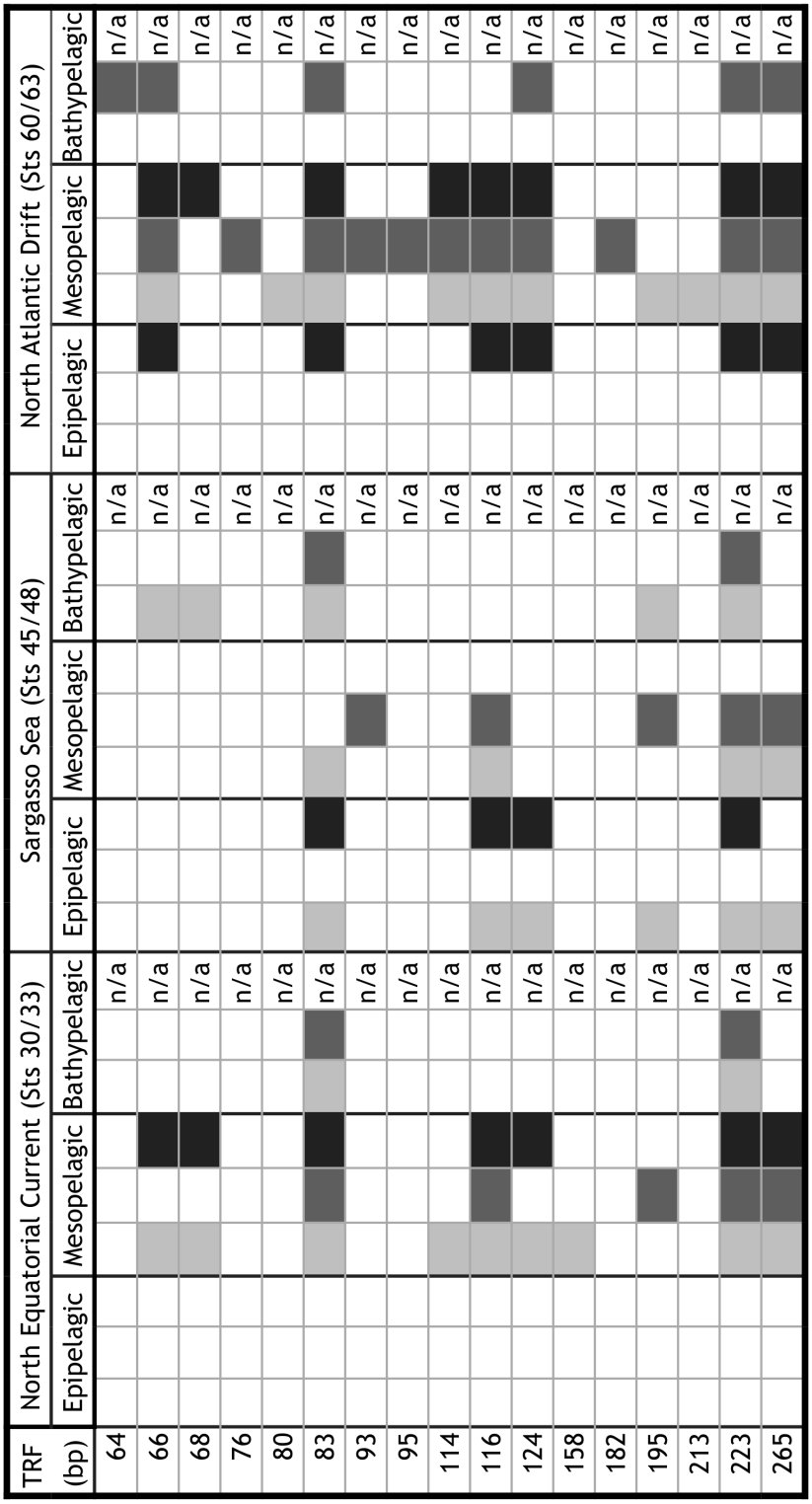



Table 3.2. Incorporation of $^{13}$C-labeled acetate, urea, and bicarbonate as determined using SIP analysis. Bicarbonate was not tested in microcosms established from water taken from the bathypelagic zone.

## References


Agogué, H., Brink, M., Dinasquet, J. and Herndl, G.J. (2008) Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic. Nature 456: 788-791.

Avaniss-Aghajani, E., Jones, K., Chapman, D. and Brunk, C. (1994) A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences. Biotechniques 17: 144-146.

Beman, J.M., Popp, B.N. and Francis, C.A. (2008) Molecular and biogeochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of Mexico. ISME J 2: 429-441.

Berg, C., Listmann., L., Vandieken, V., Vogts, A. and Jürgens, K. (2015) Chemoautotrophic growth of ammonia-oxidizing Thaumarchaeota enriched from a pelagic redox gradient in the Baltic Sea. Front Microbial 5:786 doi: 10.3389/fmicb.2014.00786.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J. et al. (2010a) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108: 4516-4522.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K. et al. (2010b) QIIME allows analysis of high-throughput community sequencing data. Nature Methods doi: 10.1038/nmeth.f.3

De Corte, D., Yokokawa, T., Varela, M.M., Agogué, H. and Herndl, G.J. (2009) Spatial distribution of Bacteria and Archaea and amoA gene copy numbers throughout the water column of the Eastern Mediterranean Sea. ISME J 3: 147-158.

DeLong, E.F. (1992) Archaea in coastal marine environments. PNAS 89, 5685-5689.

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

Drummond, A.J., Ashton, B., Cheung, M., Heled, J. and Kearse, M. Geneious v4. 8. (2009) Biomatters Ltd., Auckland, New Zealand.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E. and Oakley, B.B. (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102: 14683-14688.

Francis, C.A., Beman, J.M. and Kuypers, M.M.M. (2007) New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME 1: 19-27.

Fuhrman, J.A., McCallum, K. and Davis, A.A. (1992) Novel major archaebacterial group from marine plankton. Nature 356, 148-149.

Gallagher, E., McGuinness, L., Phelps, C., Young, L.Y. and Kerkhof, L.J. (2005) $^{13}C$ carrier DNA shortens the incubation time needed to detect benzoate-utilizing denitrifying bacteria by stable-isotope probing. Appl Environ Microbiol 71: 5192-5196.

Guindon, S. and Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52: 696-704.

Guy, L. and Ettema, T.J.G. (2011) The archaeal 'TACK' superphylum and the origin of eukaryotes. Trends microbiol 19: 580–587.

Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M. and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. PLoS Biol 4: 520-536.

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

Hu, A., Jiao, N. and Zhang, C. (2011a) Community structure and function of planktonic Crenarchaeota: changes with depth in the South China Sea. Microb Ecol 62: 549-563.

Hu, A., Jiao, N., Zhang, R. and Yang, Z. (2011b) Niche partitioning of Marine Group I Crenarchaeota in the euphotic and upper mesopelagic zones of the East China Sea. Appl Env Microbio 77: 7469-7478.

Ingalls, A.E., Shah, S.R., Hansman, R.L., Aluwihare, L.I., Santos, G.M., Druffel, E.R.M. and Pearson, A. (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. PNAS 103: 6442-6447.

Iverson, V., Morris, R.M., Frazar, C.D., Berthiaume, C.T., Morales, R.L. and Armbrust, E.V. (2012) Untangling genomes from metagenomes: revealing an uncultured class of marine Euryarchaeota. Science 335: 587-590.

Karner, M.B., DeLong, E.F. and Karl, D.M. (2001) Archaeal dominance in the mesopelagic zone of the

Pacific Ocean. Nature 409: 507-510.
Kendall, C. (1998) “Tracing nitrogen sources and cycling in catchments,” in Isotope Tracers in Catchment Hydrology, eds C. Kendall and J. J. McDonnell (Amsterdam: Elsevier Science), 519–576. doi: 10.1016/B978-0-444-81546-0.50023-9.
Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543-546.
Konstantinidis, K.T. and DeLong, E.F. (2008) Genomic patterns of recombination, clonal divergence and environment in marine microbial populations. ISME 2: 1052-1065.
Martin-Cuadrado, A.B., Rodriguez-Valera, F., Moreira, D., Alba, J.C., Ivars-Martínez, E., Henn, M.R. et al. (2008) Hindsight in the relative abundance, metabolic potential and genome dynamics of uncultivated marine archaea from comparative metagenomic analyses of bathypelagic plankton of different oceanic regions. ISME 2: 865-886.
Martens-Habbena, W., Berube, P.M., Urakawa, H., de la Torre, J.R. and Stahl, D.A. (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461: 976-979.
Massana, R., Murray, A.E., Preston, C.M. and DeLong, E.F. (1997) Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. Appl Env Microbiol 63: 50-56.
McGuinness, L.M., Salganik, M., Vega, L., Pickering, K.D. and Kerkhof, L.J. (2006) Replicability of bacterial communities in denitrifying bioreactors as measured by PCR/T-RFLP analysis. Environ Sci Technol 40: 509-515.
Molina, V., Belmar, L., and Ulloa, O. (2010) High diversity of ammonia-oxidizing archaea in permanent and seasonal oxygen-deficient waters of the eastern South Pacific. Env Microbiol 12: 2450-2465.
Murray, A.E., Blakis, A., Massana, R., Strawzewski, S., Passow, U., Alldredge, A. and DeLong, E.F. (1999) A time series assessment of planktonic archaeal variability in the Santa Barbara Channel. Aquat Microb Ecol 20: 129-145.
Ouverney, C.C. and Fuhrman, J.A. (2000) Marine planktonic archaea take up amino acids. Appl Env Microbiol 66: 4829-4833.
Park, S.J., Kim, J.G., Jung, M.Y., Kim, S.J., Cha, I.T., Kwon, K. et al. (2012) Draft genome sequence of an ammonia-oxidizing archaeon, “Candidatus Nitrosopumilus koreensis” AR1, from marine sediment. J Bacteriol 194: 6940-6941.
Pitcher, A., Villanueva, L., Hopmans, E.C., Schouten, S., Reichart, G.J. and Sinninghe Damsté, J.S. (2011) Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabia Sea oxygen minimum zone. ISME J 5: 1896-1904.
Qin, W., Amin, S.A., Martens-Habbena, W., Walker, C.B., Urakawa, H., Devol, A.H. et al. (2014) Marine ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation. PNAS 111: 12504-12509.
Reysenbach, A.L. and Pace, N.R. (1995) Reliable amplification of hyperthermophilic Archaeal 16S rRNA genes by polymerase chain reaction. In Archaea, A Laboratory Manual. Robb, F.T., Place, A.R., Sowers, K.R., Schreier H.J., DassarmaS., FleischmannE.M. (eds) New York: Cold Spring Harbor Press, pp. 101–106.
Stein, J.L. and Simon, M.I. (1996) Archaeal ubiquity. PNAS 93: 6228-6230.
Stewart, F.J., Ulloa, O. and DeLong, E.F. (2012) Microbial metatranscriptomics in a permanent marine oxygen minimum zone. Env Microbio 14: 23-40.
Teria, E., Lebaron, P., van Aken, H. and Herndl, G.J. (2006) Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. Limnol Oceanogr 51: 2131-2144.
Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P. and Schleper, C. (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Env Microbio 7: 1985-1995.
Varela, M.M., van Aken, H.M., Sintes, E. and Herndl, G.J. (2008) Latitudinal trends of Crenarchaeota and Bacteria in the meso- and bathypelagic water masses of the Eastern North Atlantic. Env Microbiol 10: 110-124.
Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A. et al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66-74.
Wanninkhof, R., Feely, R., Dickson, A., Hansell, D., Key, R., Swift J. et al. (2013) Carbon dioxide, hydrographic, and chemical data obtained during the R/V Atlantis cruise in the Atlantic Ocean on CLIVAR Repeat Hydrography Section A20 (Apr. 19 - May 15, 2012). http://cdiac.ornl.gov/ftp/oceans/CLIVAR/A20_2012/. Carbon Dioxide Information Analysis Center, Oak Ridge National

Laboratory, US Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.CLIVAR_A20_2012

Wuchter, C., Schouten, S., Boschker, H.T.S. and Damsté J.S.S. (2003) Bicarbonate uptake by marine Crenarchaeota. FEMS Microbiol Letters 219: 203-207.

Yakimov, M.M., La Cono, V., Smedile, F., DeLuca, T.H., Juárez, S., Ciordia, S. et al. (2011) Contribution of crenarchaeal autotrophic ammonia oxidizers to the dark primary production in Tyrrhenian deep waters (Central Mediterranean Sea). ISME 5: 945-961.

# Chapter 4. Resource partitioning and predation effect thaumarchaeal community structure in the North Atlantic Basin

(To be submitted to Nature Microbiology)


## Abstract

Stable isotope probing (SIP) was used to track prokaryotic and eukaryotic carbon uptake along a meridional transect (Long. 52ºW) across the North Atlantic to assess if resource partitioning between bacteria and archaea could be observed and if the $^{13}$C-labeled DNA from eukaryotes could be detected. SIP microcosms amended with $^{13}$C-acetate or $^{13}$C-urea indicated archaea often outcompeted bacteria for $^{13}$C-urea while both archaea and bacteria could incorporate $^{13}$C-acetate. This $^{13}$C label could also be tracked into eukaryotic microbes during the incubation. The largest number of $^{13}$C-labeled eukaryotic OTUs were observed in the absence of either $^{13}$C-archaeal or $^{13}$C-bacterial signal, suggesting an osmotrophic lifestyle. However, other specific $^{13}$C-eukaryotic OTUs were exclusively associated with either $^{13}$C-archaeal or $^{13}$C-bacterial OTUs or both. These archaeal-specific and bacterial-specific eukaryotic OTUs are often related to known bactivorous predators. Our findings suggests both resource partitioning and selective predation can account for a portion of the archaeal/bacterial coexistence in the deep-sea environment.

**Introduction**

G.E. Hutchinson confronted a major contradiction between ecological theory and the natural world in "The Paradox of the Plankton" (Hutchinson, 1961). According to the competitive exclusion principle, two species that are competing for the same resources cannot stably coexist. However, hundreds or thousands of phytoplankton species can be found within a small volume of water, despite the limited room for niche specialization and intense competition for the same basic resources (Hutchinson, 1961; Scheffer and Carpenter, 2003). One of the major reasons for this apparent dilemma, Hutchinson surmised, is that aquatic environments may not be a habitat where competing species are at equilibrium, since environmental conditions can change very rapidly in aquatic systems. These dynamic environments ensure that no one species has the advantage long enough to promote the exclusion of others (Hutchinson, 1961; Richardson et al., 1970). More recently, this notion of rapidly opening/closing niches leading to coexistence has been updated by postulating that equalizing and stabilizing forces exist that reduce intra- and inter-specific competition (Chesson and Huntly, 1997; Chesson 2000). Multiple mechanisms have been proposed to account for the co-occurrence of highly similar species within the same location, such as temporal separation of activity, resource partitioning, immigration/emigration in structuring communities, differential response to predation, and dormancy.

Archaea and bacteria are thought to have existed on Earth for nearly 3.5 billion years (Schopf and Packer, 1987). What is not known are the mechanisms that can account for this coexistence. For a long time, all archaea were believed to be extremophiles,

growing under conditions of high temperatures, salinity, or extreme anaerobiosis. Because archaea occurred in habitats that would not support the growth of eukaryotes or prokaryotes, no apparent conflict was perceived between these ancient life forms. However, the concept of archaea being obligate extremophiles changed when marine archaea were discovered in 1992 (Fuhrman et al., 1992; DeLong, 1992). These mesothermal or low-temperature archaea were inhabiting environments teeming with bacterial competitors. Since this discovery, multiple studies have focused on determining archaeal metabolic capabilities and their potential roles in the environment (e.g., Ouverney and Fuhrman, 2000; Hallam et al., 2006; Ingalls et. al., 2006; Varela et al., 2008; Seyler et al., 2014). For example, thaumarchaea are thought to be involved in ammonia oxidation, in conjunction with ammonia-oxidizing bacteria (Francis et al., 2005), directly competing for similar resources. Additionally, members of the thaumarchaea and bathyarchaea (formerly the Miscellaneous Crenarchaeal Group) were found to compete with bacteria for various organic substrates in salt marsh sediments (Seyler et al., 2014). Currently,the thaumarchaea have proved extremely resistant to culturing, with only four thaumarchaea currently available as a pure culture, all of them ammonia oxidizers (Könneke et al., 2005; Park et al., 2012; Qin et al., 2014).

In this study, the mechanisms that may allow for archaea to stably coexist with bacteria in the deep ocean were investigated using stable-isotope probing techniques (SIP; Radajewski et al., 1999). The first step in the investigation was to determine if resource partitioning between archaea and bacteria could be observed utilizing two model organics: urea and acetate. Prior research had demonstrated that archaea could

successfully out compete bacteria for theses organic carbon substrates in coastal sediment systems and deep ocean samples (Seyler et al. 2014; Seyler et al. [Chapter 3]). The second step of the study was to ascertain if $^{13}C$-eukaryotic DNA within the SIP incubations could be detected as suggested by Gallagher et al. (2005). The premise is that if there is resource partitioning, only archaeal or bacterial populations would become labeled by the specific $^{13}C$ substrates. This $^{13}C$ label will become detectable in the eukaryotic DNA when these $^{13}C$-labeled microbes are consumed by their respective predators. Our results indicate roughly 1/3 of the $^{13}C$-eukaryotic OTUs are observed when both archaea and bacteria are labeled with $^{13}C$, 1/3 of the eukaryotic signal is only associated with archaeal $^{13}C$ uptake, and the remaining 1/3 is only observed when there is bacterial $^{13}C$ uptake. These findings suggests both resource partitioning and selective predation can account for archaeal/bacterial coexistence. Furthermore, this SIP approach can be used to map predator/prey interactions in the microbial loop to better estimate carbon flow among these compartments in the marine environment.

**Materials and Methods**

In April of 2012, samples were collected onboard the R/V Atlantis as part of the US CLIVAR/$CO_2$ Repeat Hydrography program, along World Ocean Circulation Experiment (WOCE) line A20 (longitude 52ºW) (Figure 4.1). SIP microcosms were established at 3 stations (indicated by yellow dots in Figure 4.1), from three distinct zones in the water column: the mixed layer (the euphotic zone, 65-115 m), the local oxygen minimum (in the mesopelagic zone, 365-835 m), and the bathypelagic zone (2335-2585 m). Duplicate 1-L samples of water from each depth were amended with either $^{13}C$

sodium acetate or $^{13}C$ urea (20 μM final concentration). $^{12}C$ sodium acetate and $^{12}C$ urea were used as controls. The SIP microcosms were incubated in covered containers on deck for 48 hours, with a constant inflow of surface sea water to maintain stable temperature. Biomass was collected on a 0.2-μm filter using vacuum filtration.

DNA from the SIP incubations was purified using phenol-chloroform methods (McGuinness et al., 2006) and the $^{12}C$ and $^{13}C$ DNA was separated by isopycnic cesium chloride gradient ultracentrifugation at 200,000 x g for 36 h, using $^{13}C$-labeled *H. salinarum* or *E. coli* DNA as a carrier (Gallagher et. al., 2005). The $^{12}C$ (upper) and $^{13}C$ (lower) bands were collected by pipette and amplified by PCR with 5'-fluorescently-labeled, 16S rRNA archaea-specific (21F/958R) and crenarchaeota-specific forward/ reverse primers (7F/518R), bacteria-specific forward/reverse primers (27F/1100R), and 18S rRNA universal eukaryote forward/reverse primers (328f [5'-ACCTGGTTGATCCT GCCAG] and 516r [5'-ACCAGACTTGCCCTCC]). Amplicons were digested with *Mnl I* or *HaeIII* in 20 $\mu$l volumes for 6 h at 37 ˚C, then precipitated using sodium acetate and 95% ethanol (McGuinness *et al*., 2006). Precipitated DNA was dried and re-suspended in 19.7 $\mu$l de-ionized formamide with 0.3 $\mu$l ROX 500 size standard (Applied Biosystems). TRFLP fingerprinting (Avaniss-Aghajani et al., 1994) was carried out on an ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA) using Genescan software. Peak detection was set at 25 arbitrary fluorescent units.

An 18S amplicon clonal library was also constructed using the Topo TA cloning kit, as per the manufacturer's instruction (Invitrogen, CA). To determine the phylogenetic affiliation of the various $^{13}C$-eukaryotic peaks observed, 192 recombinant clones from the

$^{13}$C-labeled bands were screened in a multiplex format as in McGuinness *et al*. (2006), to determine the TRF of the recombinant amplicons. Thirteen 18S rRNA genes that matched TRFs of interest were sequenced via Sanger methods using M13 primers (Genewiz, Inc. NJ), producing 9 unique sequences (<99% similarity to each other). Unique clonal sequences were compared to known sequences by BLAST (Table 4.3).

**Results**

Previous SIP results from estuarine thaumarchaea/crenarchaea demonstrated that low concentrations of urea will only be incorporated by archaea in coastal sediments (Seyler et al., 2014). In this oceanic study, 18 samples were amended with $^{13}$C urea and the microbial groups taking up the $^{13}$C were determined by SIP methods. In 8 of the SIP microcosms (mostly epipelagic samples), no uptake by archaea or bacterial $^{13}$C signal could be detected. In 7 microcosms from mesopelagic or bathypelagic samples, archaea were found to have outcompeted bacteria for $^{13}$C urea. In 1 microcosm, taken from the bathypelagic at Station 63, the bacteria outcompeted archaea for $^{13}$C-urea. Three SIP microcosms had both archaea and bacteria $^{13}$C signals resulting from the $^{13}$C-urea amendments. Acetate was taken up by both archaea and bacteria in all microcosms from the mesopelagic and the bathypelagic, with the exception of duplicate microcosms taken from the bathypelagic at Station 63, where the bacteria outcompeted the archaea for the uptake of acetate (Table 4.1).

Amplification of the 18S rRNA gene within the $^{13}$C band from the SIP microcosms indicated 37 eukaryotic OTUs could be detected (Table 4.2). The eukaryotic

OTUs are separated based on whether the $^{13}C$ signal could be detected in the archaea and bacteria, in the archaea alone, in the bacterial alone, or if the 18S OTUs were detected without a signal from either prokaryotic group (presumably representing osmotrophy). Six eukaryotic OTUs were detected in SIP microcosms with both bacterial and archaeal $^{13}C$ uptake. Ten eukaryotic OTUs appeared associated exclusively with archaeal $^{13}C$ uptake, and 8 eukaryotic OTUs were only observed when bacteria incorporated the $^{13}C$-urea. A total of 14 eukaryotic OTUs were identified in samples in which both archaea and bacteria incorporated the $^{13}C$ substrate provided or in which neither prokaryotic group could be observed. One may assume that these eukaryotic members either took up the $^{13}C$ urea directly, or preyed upon a group of prokaryotes which were not detected in our SIP incubations.

The total number of observed archaeal-specific predator OTUs was higher than that of bacterial-specific predators. Using the total adjusted TRFLP peak area as a proxy for relative abundance, the bacterial predators tended to constitute a greater percentage of the total eukaryotic community profile. Archaea-specific predators only accounted for an average of 13% of the eukaryotic community in microcosms where archaea incorporated $^{13}C$-labeled substrate, whereas bacteria-specific predators made up an average of 22% of bacteria-dominated microcosms. In microcosms where both archaea and bacteria actively incorporated $^{13}C$-labeled substrate, bacteria-specific predators accounted for an average of 20% of the total eukaryotic community; archaea-specific predators accounted for only 9% on average. Overall, eukaryotic OTUs that preyed upon both archaea and bacteria comprised roughly 25% of the community profile in microcosms where archaea

incorporated $^{13}$C-labeled substrate, and 50% of the eukaryotic community in microcosms where bacteria incorporated $^{13}$C-labeled substrate. Osmotrophic or eukaryotic OTUs accounted for 62% of the total eukaryotic community profile, on average. In contrast, these OTUs only accounted for an average of 30% of the total eukaryotic community in microcosms where bacteria and not archaea incorporated $^{13}$C-labeled substrate.

Nine TRF peaks were identified in the 18S clonal library (Table 4.3). Of these, three were found exclusively in microcosms where bacteria incorporated the $^{13}$C signal, three were found only in microcosms where archaea incorporated $^{13}$C signal, two were found in both, and one was found in the microcosm where neither bacteria nor archaea incorporated $^{13}$C signal. Bacterial uptake-associated eukaryotes included two apusozoans (known bacterivorous protists) and a parasitic dinoflagellate. A radiolarian, a dinoflagellate, and a mixotrophic species of algae were found in the archaea uptake-associated microcosms. *Cafeteria sp.* (a bacterivorous bicosoecid) and a marine fungus were found in both bacteria and archaea uptake-associated microcosms. The clone from the microcosm in which neither bacteria nor archaea incorporated $^{13}$C signal was identified as *Caecitellus paraparvulus*, another bicosoecid.

**Discussion**

Currently very little is known about how marine archaea compete with bacteria that may be occupying similar niches. The question of competition is particularly compelling. One would surmise that if the archaea and the bacteria are living in the same environment, using similar carbon and energy sources, there should be competitive

exclusion and one group would eventually out-compete the other. Yet we find both living together in the same environment.

In this study, we have attempted to identify some of the mechanisms leading to this coexistence by archaea and bacteria in deep ocean samples. Our results are focused on resource partitioning and specific predation and suggest both processes are occurring in the bathypelagic. The data suggest archaea-specific predators are more diverse but represent a smaller percentage of the overall eukaryotic community compared to bacteria-specific and eukaryotic predators for both microbial groups. The non-specific prokaryotic predators seem to prefer bacteria over archaea, as indicated by their relative percentage in archaea-dominated versus bacteria-dominated microcosms. This finding is consistent with the overall abundance of archaea and bacteria in marine samples (e.g. Karner et al., 2001; Seyler et al. [Chapter 3]). While we did not test for euryarchaeal signal in these microcosms, it would be interesting to see if euryarchaea also outcompete bacteria for uptake of urea in the epipelagic and potentially have their own set of distinct predators.

In our SIP studies, we demonstrate that certain $^{13}$C-eukaryotic OTUs are associated with either $^{13}$C-archaeal or $^{13}$C-bacterial signals. This transfer of $^{13}$C-carbon from prokaryote to eukaryote could result from either predation or by exudation/ episymbiosis. Often ciliates or flagellates can have surface associations with bacteria for detoxification or defense (Petroni et al., 2000; Edgcomb et al., 2011). Although we did not test for the presence of archaea or bacteria in food vacuoles or on the external surfaces of deep-sea eukaryotes by microscopy, an assessment of whether predation or symbiosis is the predominant mechanism for carbon transfer can be made using the SIP

results. On a first order, if predation is the dominant mechanism, we can presume that in many microcosms, the $^{13}$C-carbon will pass directly from substrate to prokaryote to eukaryote and a much higher eukaryotic signal compared to a prokaryotic signal will be observed in the PCR product from the $^{13}$C-labeled DNA. If growth of the eukaryote is predicated on growth of an epibiont, there should always be a comparable PCR signal from both prokaryote and eukaryote within the $^{13}$C-band. All of our SIP microcosms had significantly higher eukaryotic signal than prokaryotic signal in the $^{13}$C band (data not shown), suggesting a tight coupling between these groups that is likely a predation signal.

If differential predation does indeed influence the stable coexistence of archaea and bacteria, the mechanisms used by predators to discern between the archaea and bacteria are, as of this writing, unknown. It has been observed that most phagotrophic protists are able to recognize various traits in their prey, allowing them to be selective consumers (Jürgens and Matz, 2002). Size is an important factor in prey differentiation in planktonic systems (Lampert, 1987). Archaea have been demonstrated to have a >1.6-fold greater cell volume on average than bacteria in the upper water column of the western Arctic Ocean; however, below 500 m, there is no significant difference in cell size between the two domains (Kirchman et al., 2007). If this trend holds for the North Atlantic, then size is likely not a determining factor in this study, as the samples utilized here were collected at depths between 365 and 2585 m. Aggregation on detrital particles may provide refuge from predation (Langenheder and Jürgens, 2001; Šimek et al., 2001), as well as a host of biochemical interactions, exopolymers, and toxicity (Jürgens and

Matz, 2002). Further studies will be necessary to explore these mechanisms of resistance in deep ocean archaea.

Our SIP findings have direct implications on the efficiency of carbon transfer in microbial systems. It is widely believed that the structure of the marine microbial food web is a major determinant in the rate of oceanic carbon sequestration and the type/ amount of higher consumers in the ecosystem (Limardo and Worden, 2015). Although autotrophic and heterotrophic prokaryotes can mobilize dissolved carbon into biomass, it is the heterotrophic strategies of the small eukaryotic consumers, that will determine whether the net flow of OM will ultimately end up as a trophic "link" (i.e. trophic transfer to top predators and export) versus a trophic "sink" (i.e. be remineralized as DOM or CO2). The archaeal contribution to the microbial loop is still poorly understood and, as a consequence, usually ignored. SIP studies, such as described here, can map out these trophic interaction between microbial predators and their prey to better predict the fate of dissolve and particulate organic carbon in marine ecosystems.

**Acknowledgements**

I wish to thank Dr. Michael McCartney for allowing me to participate as a student researcher onboard the R/V Atlantis during CLIVAR leg A20. This research was made possible through the support of the International Global Ocean Carbon and Repeat Hydrography Program.

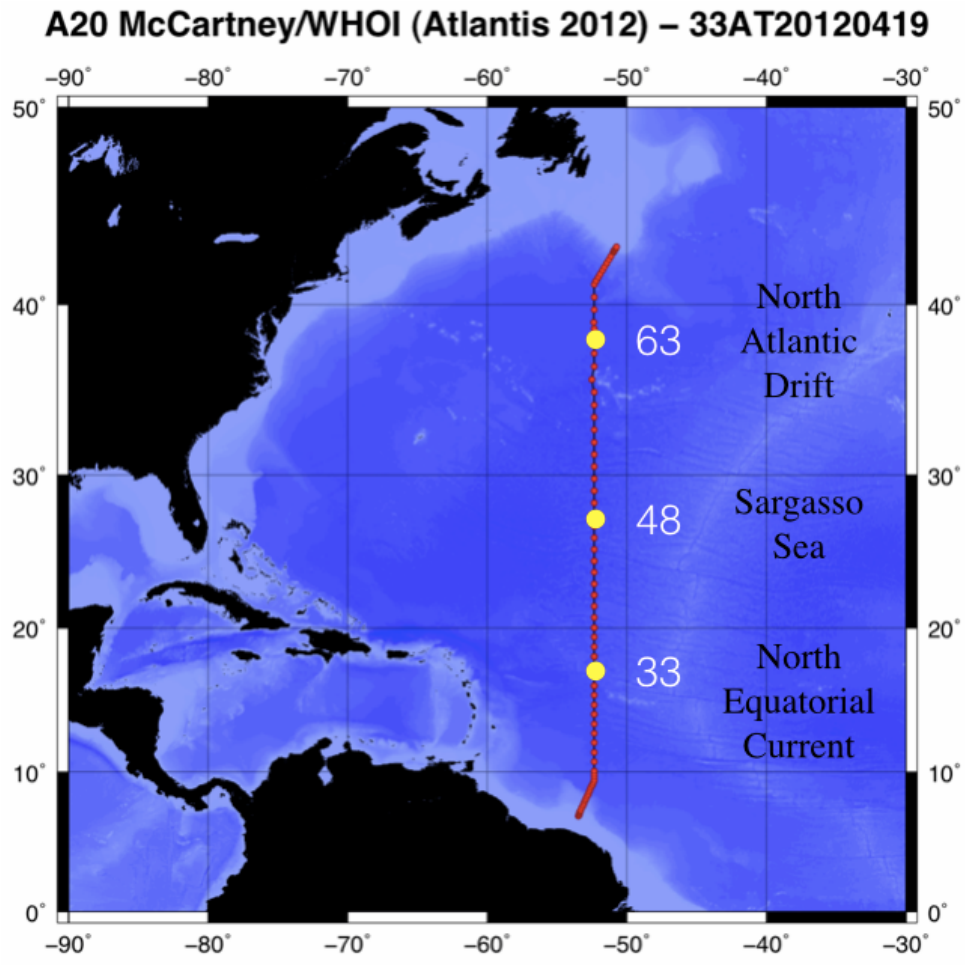
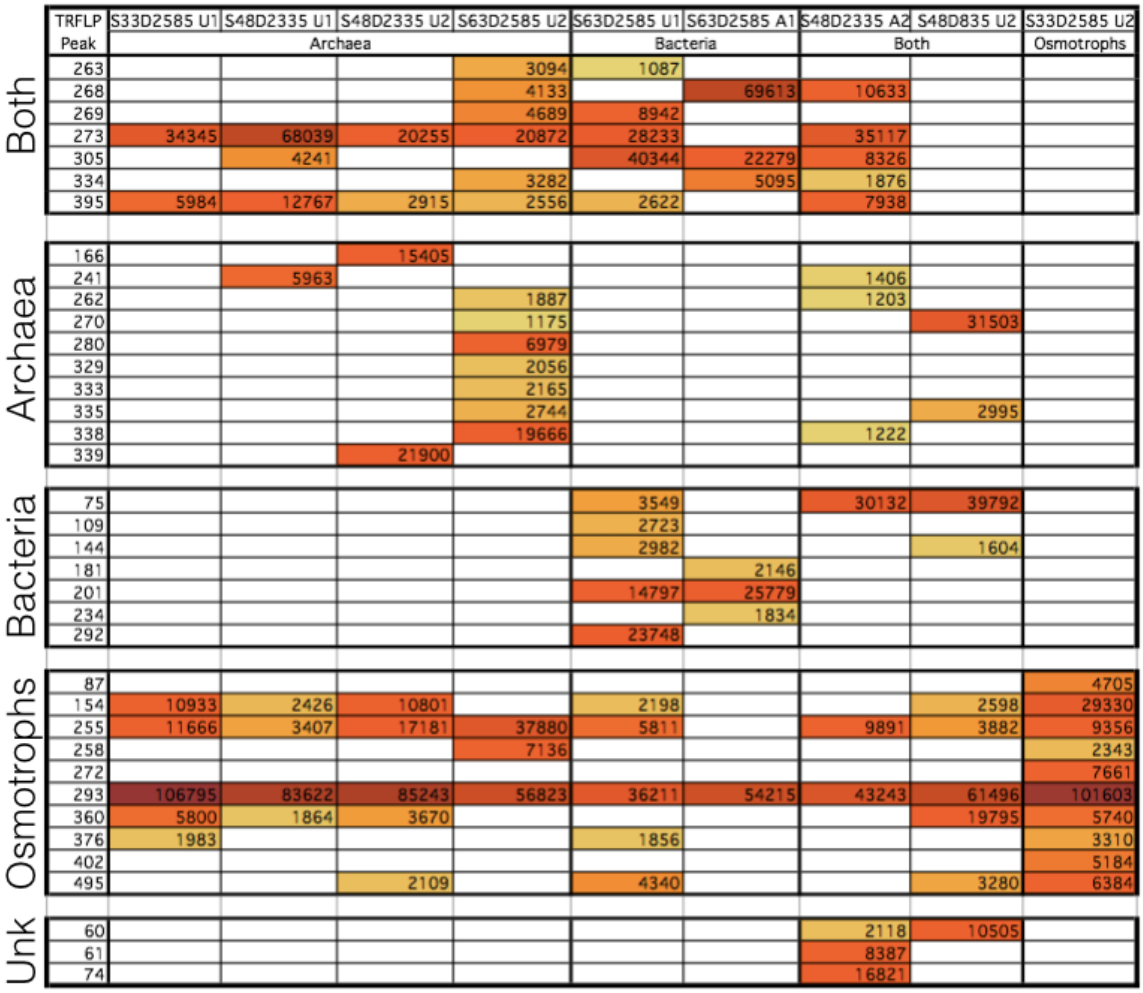



Figure 4.1. Map of the CLIVAR A20 cruise track, with each CLIVAR sampling station indicated by a red dot. Yellow dots indicate stations where SIP microcosms were established.

| Urea | Station 33 | | Station 48 | | Station 63 | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 |
| **Mesopelagic** | A B | A — | A B | A B | A — | A — |
| **Bathypelagic** | A — | —— | A — | A— | — B | A — |

| Acetate | Station 33 | | Station 48 | | Station 63 | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 |
| **Mesopelagic** | A B | A B | A B | A B | A B | A B |
| **Bathypelagic** | A B | A B | A B | A B | — B | — B |

Table 4.1. Incorporation of $^{13}C$ urea in SIP microcosms by crenarchaea (A) and bacteria (B). No incorporation of urea was detected in any microcosms established with water sampled from the epipelagic zone.



| | TRFLP Peak | S33D2585 U1 | S48D2335 U1 | S48D2335 U2 | S63D2585 U2 | S63D2585 U1 | S63D2585 A1 | S48D2335 A2 | S48D835 U2 | S33D2585 U2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Archaea | | | | Bacteria | | Both | | Osmotrophs |
| Both | 263 | | | | 3094 | 1087 | | | | |
| | 268 | | | | 4133 | | 69613 | 10633 | | |
| | 269 | | | | 4689 | 8942 | | | | |
| | 273 | 34345 | 68039 | 20255 | 20872 | 28233 | | 35117 | | |
| | 305 | | 4241 | | | 40344 | 22279 | 8326 | | |
| | 334 | | | | 3282 | | 5095 | 1876 | | |
| | 395 | 5984 | 12767 | 2915 | 2556 | 2622 | | 7938 | | |
| Archaea | 166 | | | 15405 | | | | | | |
| | 241 | | 5963 | | | | | 1406 | | |
| | 262 | | | | 1887 | | | 1203 | | |
| | 270 | | | | 1175 | | | | 31503 | |
| | 280 | | | | 6979 | | | | | |
| | 329 | | | | 2056 | | | | | |
| | 333 | | | | 2165 | | | | | |
| | 335 | | | | 2744 | | | | 2995 | |
| | 338 | | | | 19666 | | | 1222 | | |
| | 339 | | | 21900 | | | | | | |
| Bacteria | 75 | | | | | 3549 | | 30132 | 39792 | |
| | 109 | | | | | 2723 | | | | |
| | 144 | | | | | 2982 | | | 1604 | |
| | 181 | | | | | | 2146 | | | |
| | 201 | | | | | 14797 | 25779 | | | |
| | 234 | | | | | | 1834 | | | |
| | 292 | | | | | 23748 | | | | |
| Osmotrophs | 87 | | | | | | | | | 4705 |
| | 154 | 10933 | 2426 | 10801 | | 2198 | | | 2598 | 29330 |
| | 255 | 11666 | 3407 | 17181 | 37880 | 5811 | | 9891 | 3882 | 9356 |
| | 258 | | | | 7136 | | | | | 2343 |
| | 272 | | | | | | | | | 7661 |
| | 293 | 106795 | 83622 | 85243 | 56823 | 36211 | 54215 | 43243 | 61496 | 101603 |
| | 360 | 5800 | 1864 | 3670 | | | | | 19795 | 5740 |
| | 376 | 1983 | | | | 1856 | | | | 3310 |
| | 402 | | | | | | | | | 5184 |
| | 495 | | | 2109 | | 4340 | | | 3280 | 6384 |
| Unk | 60 | | | | | | | 2118 | 10505 | |
| | 61 | | | | | | | 8387 | | |
| | 74 | | | | | | | 16821 | | |

Table 4.2. Summary of 18S TRFLP profiles from a subset of microcosms, in which $^{13}$C-labeled urea was incorporated by only the archaea, only the bacteria, both the archaea and the bacteria, and neither the archaea nor the bacteria. TRF peaks are separated based on predator specificity, from top to bottom: non-specific predators, archaea-specific predators, bacteria-specific predators, and eukaryotes that preyed on neither the archaea nor the bacteria. TRFs at the bottom of the table incorporated $^{13}$C-labeled substrate in microcosms where both archaea and bacteria actively incorporated $^{13}$C-labeled substrate, but didn't appear in any other microcosms. Intensity of cell shading corresponds to the adjusted TRF peak area, with darker cells having a higher adjusted peak area than lighter cells. TRF peak area roughly corresponds to relative abundance of OTUs.

| TRF peak | Microcosm | Classification |
|---|---|---|
| 182 | Bacteria | Amoebophrya sp. (parasitic dinoflagellate) |
| 201 | Bacteria | Amastigomonas sp. (apusozoan) |
| 269 | Bacteria | Ancyromonas sigmoides (apusozoan) |
| 273 | Both | Paraphysoderma sedebokerense (marine fungus) |
| 293 | Osmotroph | Caecitellus paraparvulus (bicosoecid) |
| 305 | Both | Cafeteria sp. (bacterivorous bicosoecid) |
| 329 | Archaea | Sticholonche sp. (radiolarian) |
| 333 | Archaea | Pyramimonas sp. (mixotrophic algae) |
| 339 | Archaea | Pentapharsodinium tyrrhenicum (dinoflagellate) |

Table 4.3. Eukaryote sequences cloned from $^{13}C$-labeled DNA, the prokaryotes that incorporated $^{13}C$ signal in the microcosms in which these clones were detected, and the closest match to each clone using NCBI's BLAST search tool.

## References


Babcock, D.A., Wawrik, B., Paul, J.H., McGuinness, L. and Kerkhof, L.J. (2007) Rapid screening of a large insert BAC library for specific 16S rRNA genes using TRFLP. J. Microbiol.Methods 71: 156-61.

Chesson, P. and Huntley, N. (1997) The roles of harsh conditions in the dynamics of ecological communities. Amer Nat 150: 519-553.

Chesson, P. (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31: 343-366.

DeLong. E.F. (1992) Archaea in coastal marine environments. PNAS 89: 5685-5689.

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

Edgcomb, V.P., Breglia, S.A., Yubuki, N., Beaudoin, D., Patterson, D.J., Leander, B.S. and Bernhard, J.M. (2011) Identity of epibiotic bacteria on symbiontid euglenozoans in $O_2$-depleted marine sediments: evidence for symbiont and host co-evolution. ISME 5: 231-243.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., and Oakley, B.B. (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102: 14683-14688.

Fuhrman, J.A., McCallum, K. and Davis, A.A. (1992) Novel major archaebacterial group from marine plankton. Nature 356: 148-149.

Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M. and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. PLoS Biol 4: 520-536.

Hutchinson, G.E. (1961) The paradox of the plankton. Amer Nat 45: 137-145.

Ingalls, A.E., Shah, S.R., Hansman, R.L., Aluwihare, L.I., Santos, G.M., Druffel, E.R.M. and Pearson, A. (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. PNAS 103: 6442-6447.

Karner, M.B., DeLong, E.F. and Karl, D.M. (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409: (6819), 507-510.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543-546.

Lampert, W. (1987) Predictability in lake ecosystems: the role of biotic interactions. In: Schultz, E.D. and Zwölfer, H. (eds) Potential and Limitations of Ecosystem Analysis. Ecological Studies 61 (pp 333-346). Springer-Verlag, Berlin.

Langenheder, S. and Jürgens, K. (2001) Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol Oceanogr 46: 121-134.

Limardo, A.J. and Worden A.Z. (2015). Microbiology: Exclusive networks in the sea. Nature doi:10.1038/nature14530.

McGuinness, L.M., Salganik, M., Vega, L., Pickering, K.D., and Kerkhof, L.J. (2006) Replicability of bacterial communities in denitrifying bioreactors as measured by PCR/T-RFLP analysis. Environ. Sci. Technol. 40: 509-515.

Moin, N.S., Nelson, K.A., Bush, A. and Bernhard, A.E. (2009) Distribution and diversity of archaeal and bacterial ammonia oxidizers in salt marsh sediments. Appl Env Microbiol. 75: 7461-7468.

Mosier, A.C. and Francis, C.A. (2008) Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. Env. Microbiol. 10: 3002-3016.

Murray, A.E., Blakis, A., Massana, R., Strawzewski, S., Passow, U., Alldredge, A. and DeLong, E.F. (1999) A time series assessment of planktonic archaeal variability in the Santa Barbara Channel. Aquat Microb Ecol 20: 129-145.

Ouverney, C.C. and Fuhrman, J.A. (2000) Marine planktonic archaea take up amino acids. Appl. Env Microbiol 66: 4829-4833.

Park, B.J., Park, S.J., Yoon, D.N., Schouten, S., Sinninghe Damsté, J.S. and Rhee, S.K. (2010) Cultivation of autotrophic ammonia-oxidizing archaea from marine sediments in coculture with sulfur-oxidizing bacteria. Appl. Environ. Microbiol. 76(22): 7575-7587.

Park, S.J., Kim, J.G., Jung, M.Y., Kim, S.J., Cha, I.T., Kwon, K. et al. (2012) Draft genome sequence of an ammonia-oxidizing archaeon, “Candidatus Nitrosopumilus koreensis” AR1, from marine sediment. J Bacteriol 194: 6940-6941.

Petroni, G., Spring, S., Schleifer, K.H., Verni, F. and Roasati, G. (2000) Defensive extrusive ectosymbionts of Euplotidium (Ciliophora) that contain microtubule-like structures are bacteria related to Verrucomicrobia. PNAS 97(4): 1813-1817.

Qin, W., Amin, S.A., Martens-Habbena, W., Walker, C.B., Urakawa, H., Devol, A.H. et al. (2014) Marine ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation. PNAS 111: 12504-12509.

Radajewski, S., Ineson, P., Parekh, N.R. and Murrell, J.C. (1999) Stable-isotope probing as a tool in microbial ecology. Nature 403: 646-649.

Richardson, P., Armstrong, R. and Goldman, C.R. (1970) Contemporaneous disequilibrium, a new hypothesis to explain the "Paradox of the Plankton." PNAS 67: 1710-1714.

Scheffer, M. and Carpenter, S.R. (2003) Catastrophic regime shifts in ecosystems: linking theory to observation. Trends Ecol Evo 18: 648-656.

Seyler, L.M., McGuinness, L.M., and Kerkhof, L.J. (2014) Crenarchaeal heterotrophy in salt marsh sediments. ISME 8: 1534-1543.

Šimek, K., Pernthaler, J., Weinbauer, M.G., Hornak, K., Dolan, J.R., Nedoma, J. et al. (2001) Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. Appa Environ Microbial 67: 2723-2733.

Stein, J.L. and Simon, M.I. (1996) Archaeal ubiquity. Proc Natl Acad Sci USA 93: 6228-6230.

Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P. and Schleper, C. (2005). Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Env Microbio 7: 1985-1995.

Varela, M.M., van Aken, H.M., Sintes, E. and Herndl, G.J. (2008) Latitudinal trends of Crenarchaeota and Bacteria in the meso- and bathypelagic water masses of the Eastern North Atlantic. Env Microbiol 10: 110-124.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A. et al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66-74.

# Chapter 5. Conclusions and future directions

## Summary of Completed Work

In the first portion of this Ph.D. research, stable isotope probing techniques were used to test organic carbon uptake in a New Jersey salt marsh. Thaumarchaea (as well as members of the Miscellaneous Crenarchaeotal Group, also referred to as Bathyarchaea) in this environment were found to be exclusively heterotrophic, and competing with bacteria for the same organic compounds. The concentration of substrate provided had a profound effect on community structure, with lower concentrations of $^{13}$C-labeled substrate resulting in greater observed diversity in the active archaeal community. Resource partitioning between the archaea and the bacteria was also observed, with archaea exclusively incorporating urea at low concentrations, and bacteria outcompeting archaea for the uptake of whole proteins. While autotrophy or mixotrophy may be available life strategies for thaumarchaea in the salt marsh, the relatively high availability of organic carbon in this setting may make carbon fixation an unnecessary energy expense.

In the North Atlantic pelagic ocean, mixotrophy was shown to be the dominant life strategy for the most abundant archaeal OTUs, though some members of the rare biosphere appeared to be exclusively heterotrophic in this environment as well. Doubling times were faster in the open ocean (~1 day) compared to the estuary (~2-2.5 days). 16S amplicon sequencing revealed a diverse archaeal community, the structure of which was

strongly influenced by depth and oxygen concentration. The highest numbers of archaea were observed at the local oxygen minimum and just above the seafloor.

Resource partitioning was also observed in the pelagic North Atlantic. Archaea often outcompeted bacteria for the uptake of urea in the deep ocean, particularly in the bathypelagic. Differential uptake of $^{13}$C-labeled substrate made it possible to track the consumption of archaea and bacteria by eukaryotic predators. Archaea-specific predators were more diverse than bacteria-specific predators in the deep ocean, but made up a smaller percentage of the total eukaryotic community. A significant percentage of the eukaryotic community took up neither archaea nor bacteria, and therefore either directly incorporated the provided $^{13}$C-labeled substrate, or consumed prey that was not detected in this study.

## Future Directions

Several SIP experiments were initiated during the course of my Ph.D. work which also address additional mechanisms of coexistence, such as spatial or seasonal differences in archaeal activity. The biomass from these SIP experiments is currently being stored at -80°C and could be processed in the future to provide additional answers to a number of questions concerning marine archaeal ecology.

### *Salt Marsh Zonation*

In addition to the tidal levee sediment samples analyzed for the first portion of the

Ph.D. work, SIP microcosms were established using sediment from two other vegetal zones in the same location: the low marsh (populated by *Spartina spp.* grasses) and transition communities (characterized by sandy soil and low scrub). The water from these and the tidal levee microcosms, filter-sterilized prior to incubation, was also filtered for biomass post-incubation. (A full list of these samples can be found in Supplemental Table 5.1.) SIP and 16S data from these samples would reveal spatial and organic carbon uptake patterns in salt marsh archaea, as well as enable the comparison of particle-associated and free-living archaea in an estuarine setting.

*Time Course*

In February 2011, samples were taken from the tidal levee in Hooks Creek and used to establish a series of SIP microcosms, amended with 30 μM $^{13}$C urea and incubated at 4 different temperatures (4°C, 18°C, 25°C, and 37°C) and destructively sampled at 4 different time points (3, 5, 7, and 14 days). The purpose of this time course was to establish the effects of temperature on archaeal growth, and to monitor changes in the archaeal community as $^{13}$C-labeled substrate is incorporated by various OTUs and oxygen is depleted from the microcosm via respiration. The longer time points could also be used to examine eukaryotic predation, as performed in Chapter 4. A complete list of these samples is available in Supplemental Table 5.2.

*Complex Biopolymers in the Open Ocean*

In June 2014, I participated in a chief scientist training cruise sponsored by the University-National Oceanographic Laboratory System (UNOLS), sampling biomass and

establishing SIP microcosms (using the same methods employed in Chapters 3 and 4) along a track from Barbados to Bermuda. Microcosms were amended with either $^{13}$C-labeled mixed fatty acids or $^{13}$C-labeled Bioexpress medium, at 25 μg/L and 250 μg/L concentrations. These samples will be processed in order to examine the uptake of complex biopolymers by archaeal communities in the open ocean (compared to the simple organics utilized in Chapters 3 and 4) and to determine if substrate concentration influences archaeal community structure as it did in the salt marsh in Chapter 2. Additionally, resource partitioning of these complex biopolymers between the archaea and bacteria may also be observed in these samples. (A complete list of these samples is summarized in Supplemental Table 5.3.)

*Tara Oceans*

Planktonic archaea may be one of the most abundant groups of organisms on the planet. They can comprise up to 50% of the marine microbial community, particularly at depths below 1,000 meters, which represent the majority of the ocean's volume (Karner et al., 2001). The relationship between these organisms and the rest of the planktonic ecosystem, however, is still very poorly understood. There is currently very little in the scientific literature concerning the relationship between archaea and protists in the open ocean. It is reasonable to assume that because archaea are both abundant and ubiquitous in open oceanic ecosystems, they must interact with the protists as food, symbionts, competitors, or a combination of roles.

From October 2014 through March 2015, I worked as a guest student researcher in l'Equipe Evolution des Protistes et Ecosystèmes Pélagiques (Evolution of Protists and Pelagic Ecosystems Group) at the Station Biologique de Roscoff, under the direction of Dr. Colomban de Vargas, through the award of a Fulbright fellowship. Dr. de Vargas is one of the coordinators of the Tara-Oceans international expedition, a 3-year circumglobal navigation that lasted from 2009-2012 and sampled the world's plankton thoroughly from viruses to jellyfish. He is now the coordinator of the research program Oceanomics, whose goal is to analyze the ±30,000 samples taken from 153 sites all over the globe using a combination of high-throughput eco-morpho-genetic analyses. This constitutes the largest modern-day collection of plankton sampled "end-to-end" around the world. The purpose of my project as part of the Oceanomics program was to explore potential symbiotic relationships between marine archaea and protists, by identifying archaeal 16S tag sequences in the protist-sized fractions (20-180 μm and 180-2000 μm) from the Tara Oceans expedition.

Archaeal diversity in all size fractions from the Tara Oceans collection was assessed using a two-pronged approach. Archaeal 16S rRNA sequences were identified in V9 sequence data obtained using universal eukaryotic primers. While these primers only amplified a fraction of the total archaeal community (mostly euryarchaea, members of Marine Group II), the data provided valuable information concerning the global distribution of marine archaea. Using the V9 data, archaeal "hotspots" were located for three different depth regimes: the surface, the deep chlorophyll maximum (DCM), and the mesopelagic (Figure 5.1). These areas of high archaeal biomass tended to be located

along coasts, in upwelling zones, and in oxygen minimum zones (OMZs). These results are consistent with the findings in Chapter 3, in which high archaeal biomass correlated to low oxygen concentration in the water column.

Additionally, a subset of surface samples from Tara Oceans were 16S amplicon sequenced on the Illumina MiSeq platform using archaea-specific primers (519F/1041R), concentrating on the smallest (0.2-3 μm) and two largest (20-180 μm and 180-2000 μm) size fractions (Figure 5.2a,b,c). These samples were collected in the pelagic North Atlantic, Indian, and South Pacific Oceans, and around a group of islands in the equatorial Pacific (referred to as Steffi). The primers used for sequencing amplified a significant amount of other organisms, most notably bacteria (in all size fractions sequenced) and metazoans (likely larvae, chiefly in the largest size fraction). The largest numbers of archaea were detected in the 0.2-3 μm size fractions taken from the North Atlantic (51%), Steffi (22%), and Indian Ocean (14%), and the 20-180 μm fractions taken from the South Pacific (59%), Indian (25%), and North Atlantic (22%). Some archaea were detected in the 180-2000 μm fraction in the North Atlantic (9%) and South Pacific (9%). The number of archaea detected in the 20-180 μm fraction in Steffi, and the 180-2000 μm fractions in Steffi and the Indian Ocean, were negligible (0.3%, 1%, and 0.7%, respectively).

Thaumarchaea dominated the archaeal community in all size fractions taken from the North Atlantic, while euryarchaea dominated in all size fractions from the Indian Ocean and South Pacific. At Steffi, euryarchaea dominated the 0.2-3 μm and 180-2000

μm size fractions, but thaumarchaea dominated the 20-180 μm size fraction; however, archaeal numbers in general were negligible in all but the smallest size fraction in these samples.

Future steps for this project include further 16S amplicon sequencing, targeting the archaeal hotspots identified in the V9 data. Additionally, protistan beta-diversity will be correlated to archaeal beta-diversity, to determine if there is potential for symbiotic relationships between certain protists and archaea. A recent paper (Orsi et al., 2015) describing particle-associated archaea in the euphotic zone of the central California Current System found that while euryarchaea tended to be associated with particular organic matter (POM), these archaea displayed no association with living protists. Thaumarchaea in this environment were apparently free-living and unassociated with POM. Data from the Tara Oceans expedition does not completely agree with these findings; rather, thaumarchaea and euryarchaea were both found associated with larger size fractions, in multiple locations worldwide. However, it has yet to be determined if these archaea are colonizing POM, or existing in a symbiotic relationship with particular protist species.

**Significance of this Work**

The aim of this Ph.D. research was to further illuminate the metabolic capabilities and potential environmental roles of marine archaea. The current focus in most research concerning coastal and open ocean archaea is on ammonia oxidation and, by extension, autotrophy, while heterotrophy is more extensively explored in deeply buried marine

sediment. However, early work on marine archaea prior to and immediately after the discovery of the *amoA* gene in archaea (Venter et al., 2004; Treusch et al., 2005) and the subsequent culturing of an ammonia-oxidizing archaeon (Könneke et al. 2005) provided ample evidence of both heterotrophic and mixotrophic lifestyles (Ouverney and Fuhrman, 2000; Hallam et al., 2006; Martin-Cuadrado et al., 2008; Varela et al., 2008). Indeed, the most recent successful culturing of two AOA strains revealed that both required the addition of organic carbon for growth (Qin et al., 2014). The decline in the ratio of archaeal *amoA* to 16S rRNA copy numbers with depth in the open ocean (De Corte, et al., 2008; Agogué et al., 2008; Yakimov et al., 2011; Hu et al., 2011) would imply that deep ocean archaea are employing other life strategies apart from ammonia oxidation. Additionally, the currently cultured representatives of the thaumarchaea are all closely related to each other, compared to the wealth of phylogenetic diversity currently available in ribosomal RNA databases (e.g., NCBI, Silva). The extent of this phylum, and the conflicting evidence concerning carbon uptake in the literature, would suggest that the genetic and metabolic potential of the thaumarchaea encompasses more than ammonia oxidation. The results of this Ph.D. work indicate that the thaumarchaea are significant consumers of organic carbon in both estuarine and pelagic environments, that they compete with bacteria for organic substrates and exhibit resource partitioning of particular carbon sources (urea and whole proteins), and that the competition and coexistence between marine thaumarchaea and bacteria is at least to some extent influenced not only by organic substrate availability (“bottom-up” control) but also by predation (“top-down” control).

## Conclusion

While much work has been performed to describe the ecological role and metabolic capabilities of marine archaea since their discovery in 1992, many questions remain. This Ph.D. work challenges current paradigms concerning the activity of thaumarchaea in marine environments, and, thanks to the cooperation of the International Global Ocean Carbon and Repeat Hydrography Program, the Earth Microbiome Project, and the Oceanomics Project, has explored archaeal distribution and diversity on an unprecedented scale. Future studies of marine archaea will likely uncover a wealth of diversity and metabolic potential, as well as myriad complex relationships with other prokaryotes and higher organisms. The marine archaea truly represent an ocean of possibilities.

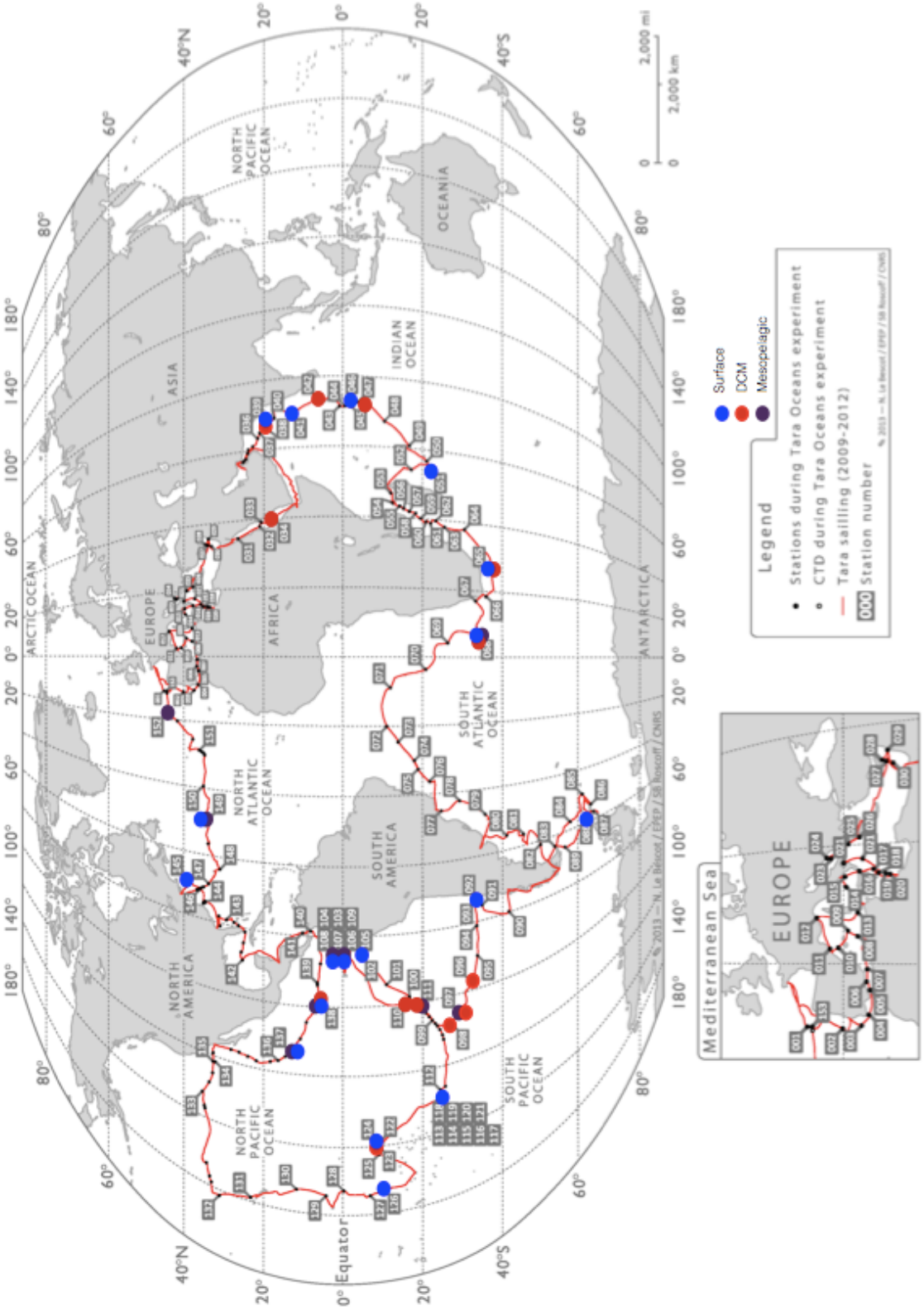



Figure 5.1. Map of the Tara Oceans cruise track. Archaea "hotspots," determined using V9 amplicon sequence data, are indicated by colored dots.

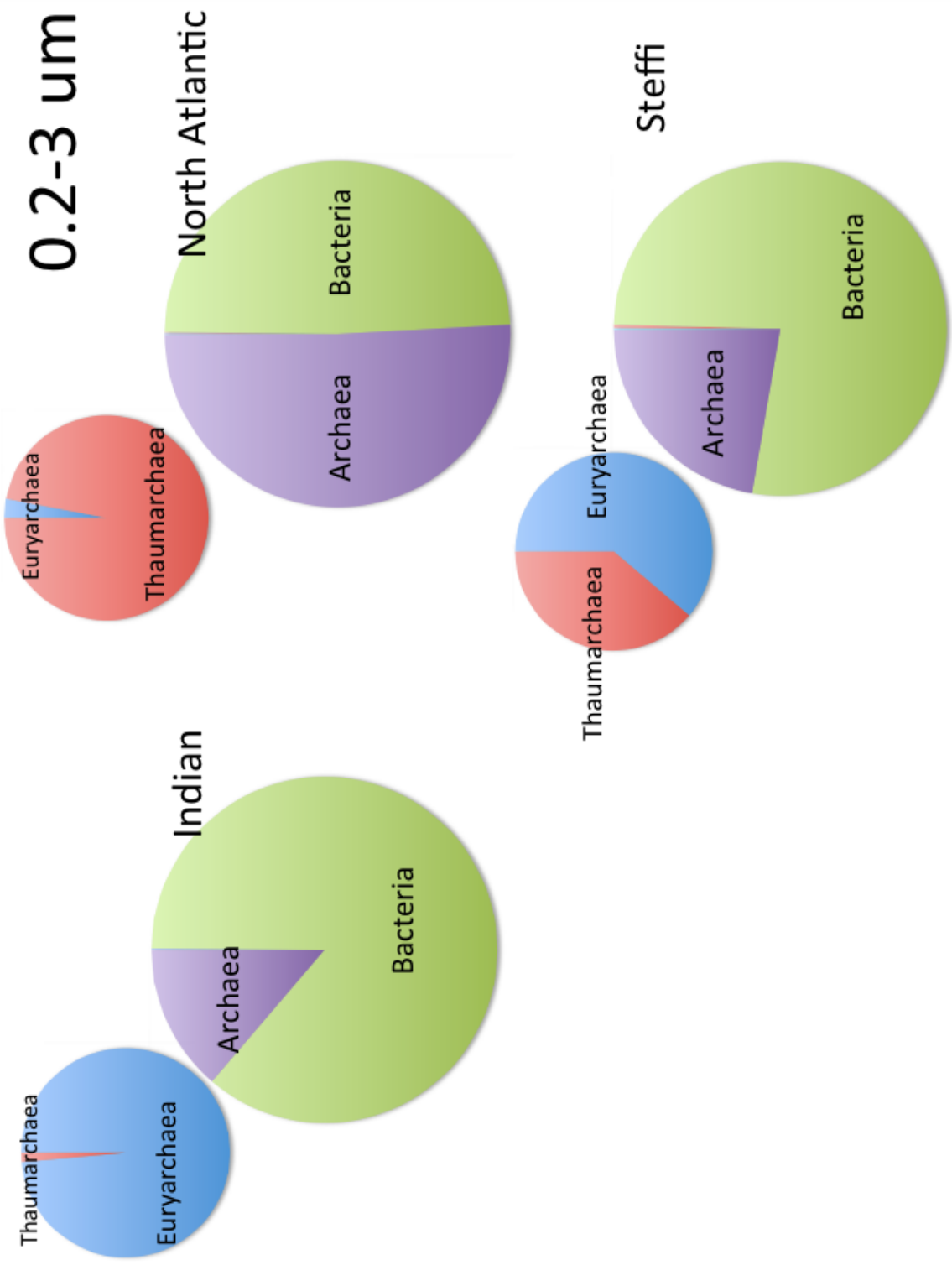



Figure 5.2a. Results of 16S amplicon sequencing of select Tara Oceans samples from the Indian and North Atlantic Ocean, and a series of islands in the equatorial Pacific (Steffi) (0.2-3 µm size fraction).

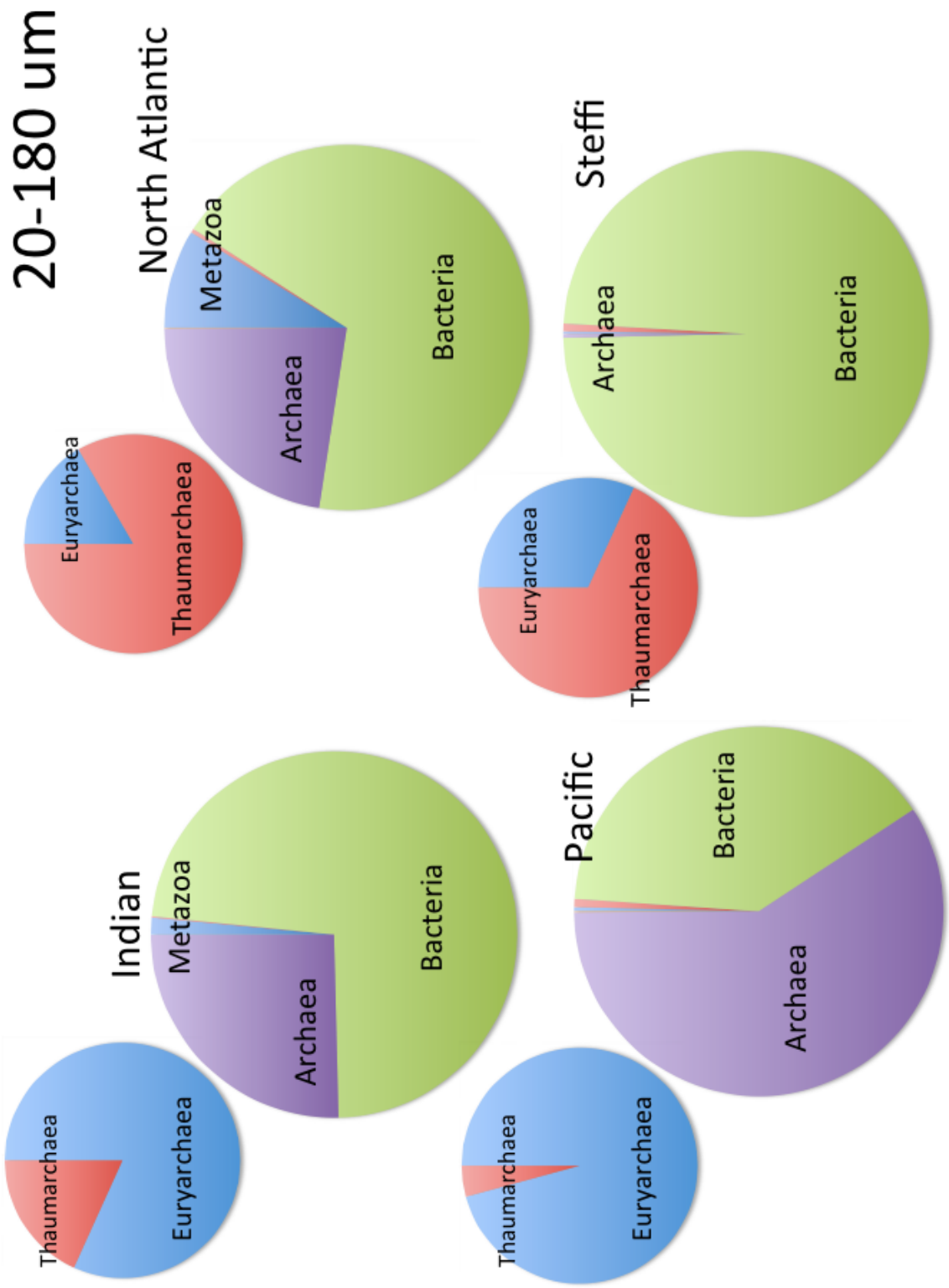



Figure 5.2b. Results of 16S amplicon sequencing of select Tara Oceans samples from the Indian, North Atlantic, and South Pacific Ocean, and a series of islands in the equatorial Pacific (Steffi) (20-180 µm size fraction).

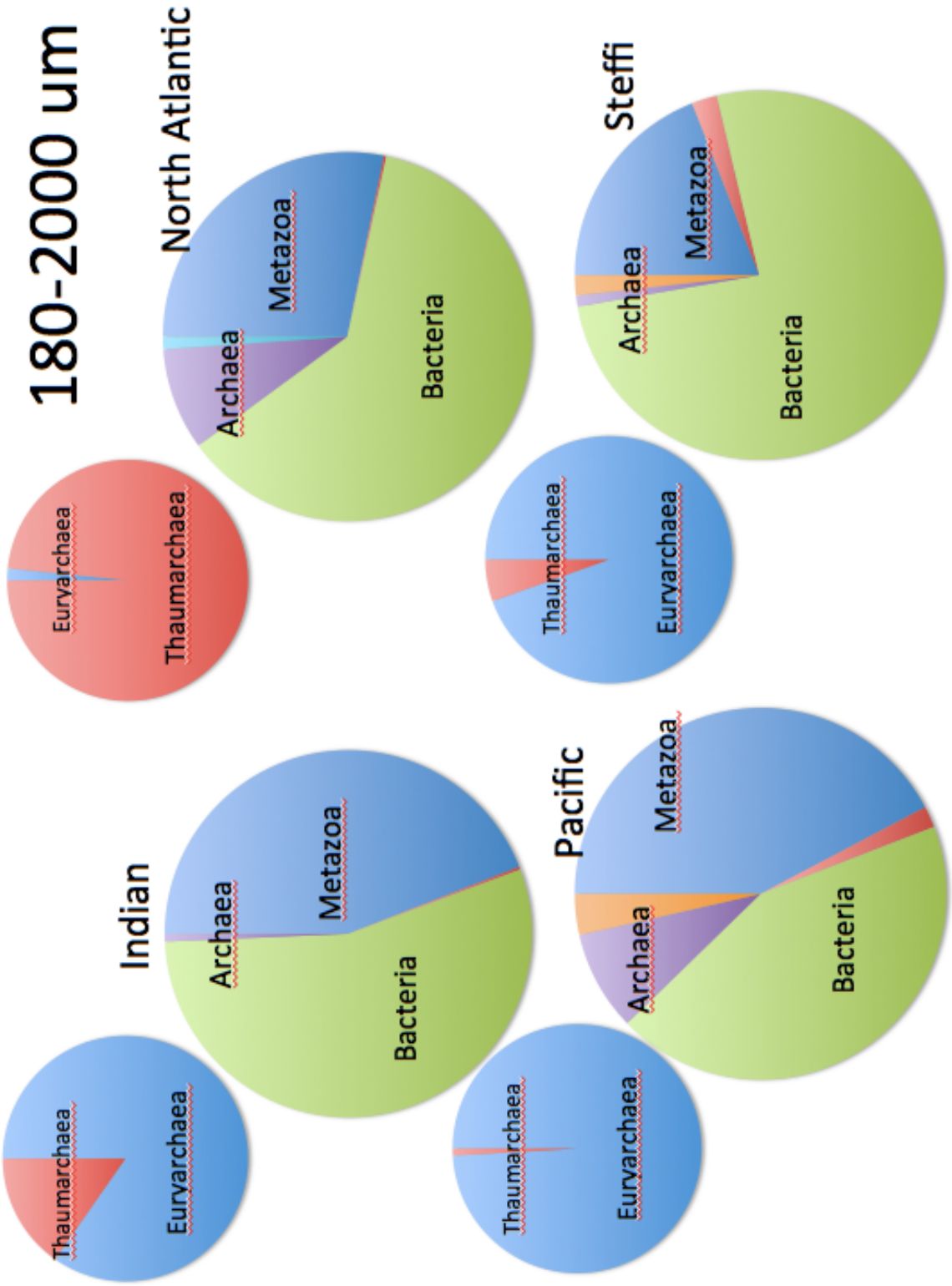



Figure 5.2c. Results of 16S amplicon sequencing of select Tara Oceans samples from the Indian, North Atlantic, and South Pacific Ocean, and a series of islands in the equatorial Pacific (Steffi) (180-2000 µm size fraction).

## References


Agogué, H., Brink, M., Dinasquet, J. and Herndl, G.J. (2008) Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic. Nature 456: 788-791.

De Corte, D., Yokokawa, T., Varela, M.M., Agogué, H. and Herndl, G.J. (2009) Spatial distribution of Bacteria and Archaea and amoA gene copy numbers throughout the water column of the Eastern Mediterranean Sea. ISME 3: 147-158.

Hallam, S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M. and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. PLoS Biol 4: 520-536.

Hu, A., Jiao, N. and Zhang, C. (2011) Community structure and function of planktonic Crenarchaeota: changes with depth in the South China Sea. Microb Ecol 62: 549-563.

Karner, M.B., DeLong, E.F. and Karl, D.M. (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409: (6819), 507-510.

Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437: 543-546.

Martin-Cuadrado, A.B., Rodriguez-Valera, F., Moreira, D., Alba, J.C., Ivars-Martínez, E., Henn, et al. (2008) Hindsight in the relative abundance, metabolic potential and genome dynamics of uncultivated marine archaea from comparative metagenomic analyses of bathypelagic plankton of different oceanic regions. ISME 2: 865-886.

Orsi, W.D., Smith, J.M., Wilcox, H.M., Swalwell, J.E., Carini, P., Worden, A.Z. and Santoro, A.E. (2015) Ecophysiology of uncultivated marine euryarchaea is linked to particular organic matter. ISME doi:10.1038/ismej.2014.260.

Ouverney, C.C. and Fuhrman, J.A. (2000). Marine planktonic archaea take up amino acids. Appl Env Microbiol. 66: 4829-4833.

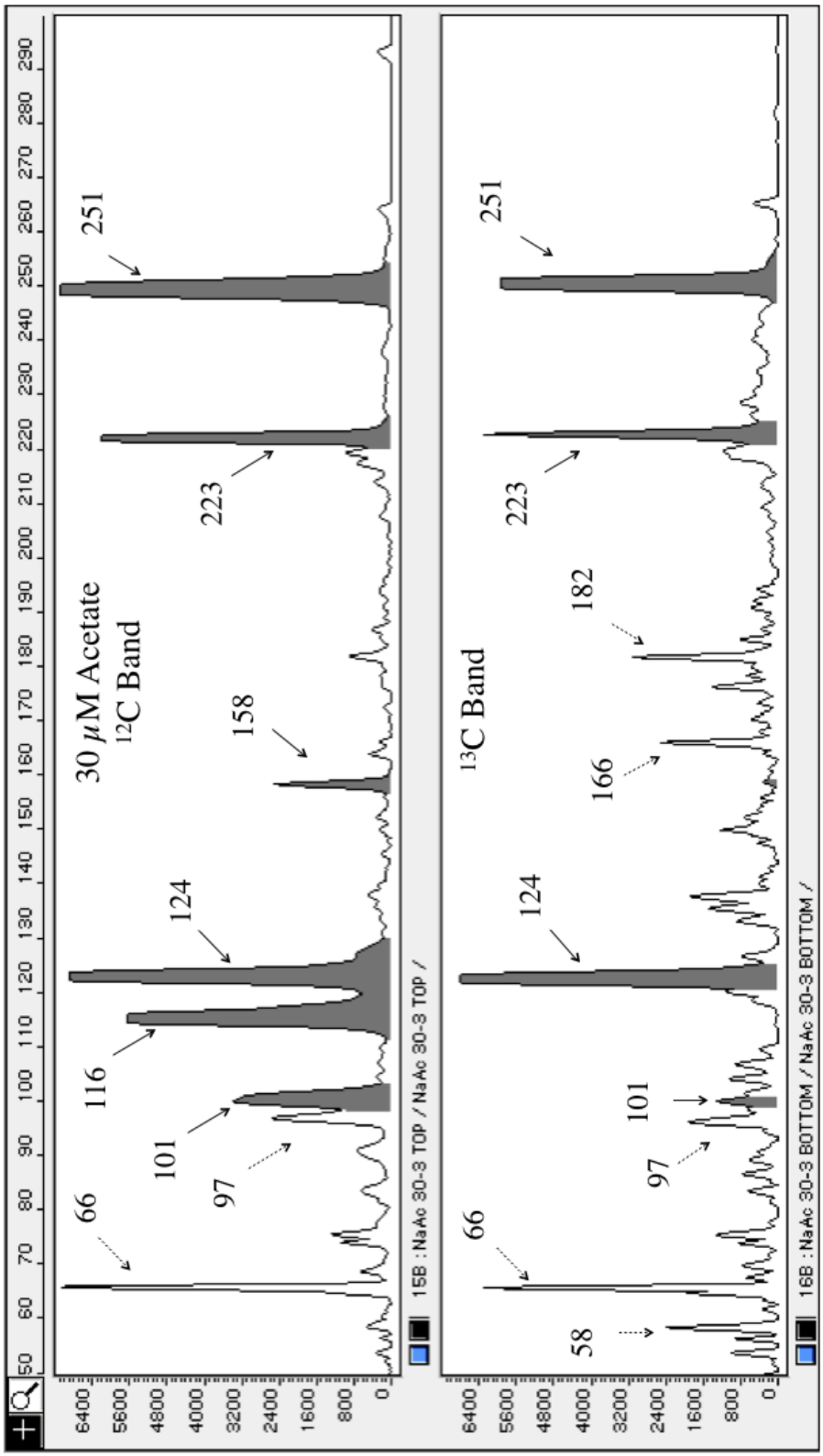
Qin, W., Amin, S.A., Martens-Habbena, W., Walker, C.B., Urakawa, H., Devol, A.H. et al. (2014) Marine ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation. PNAS
111: 12504-12509.

Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P. and Schleper, C. (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environ Microbiol 7: 1985-1995.

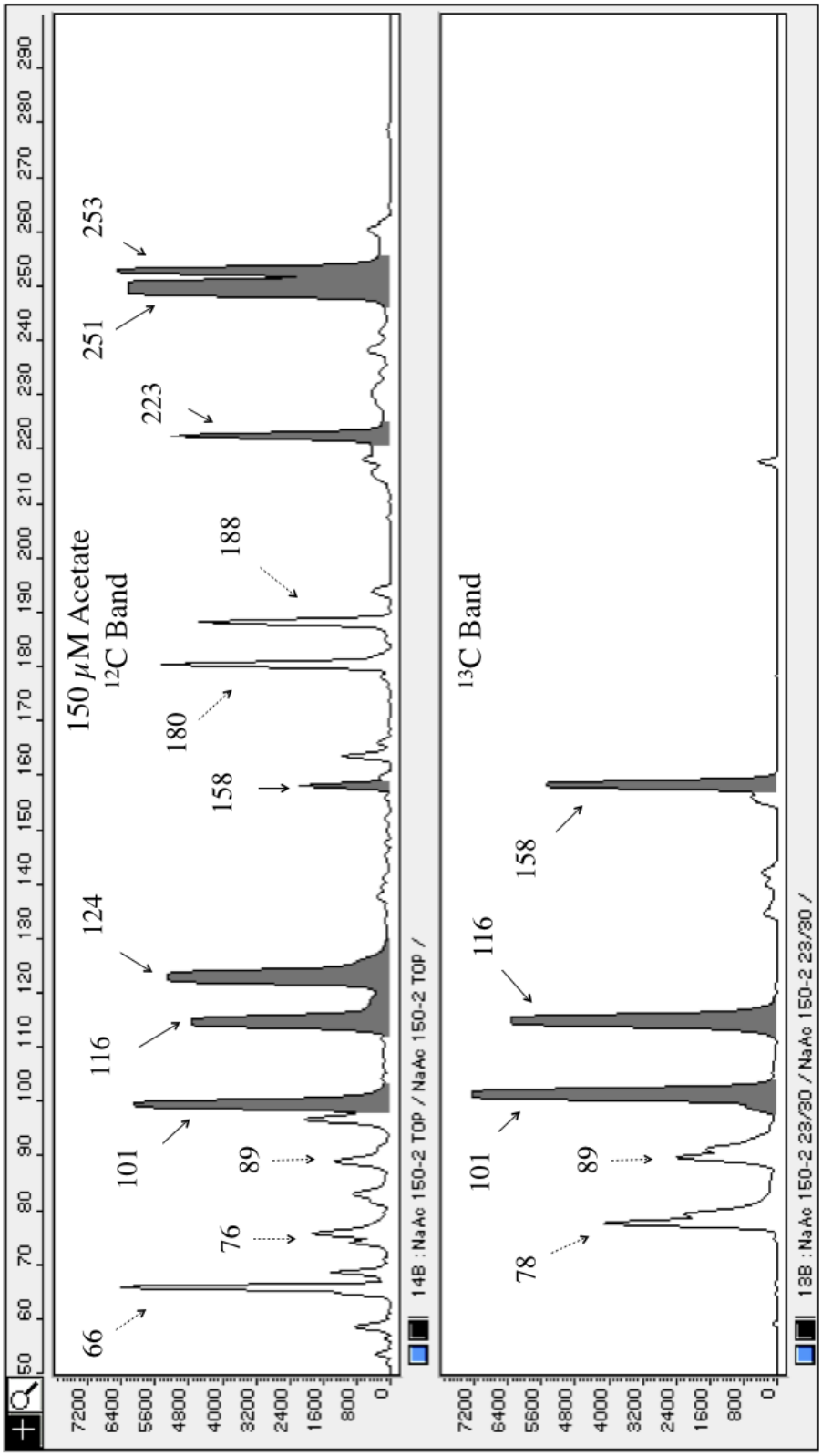
Varela, M.M., van Aken, H.M., Sintes, E. and Herndl, G.J. (2008) Latitudinal trends of Crenarchaeota and Bacteria in the meso- and bathypelagic water masses of the Eastern North Atlantic. Env Microbiol 10: 110-124.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A. et. al. (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304: 66-74.

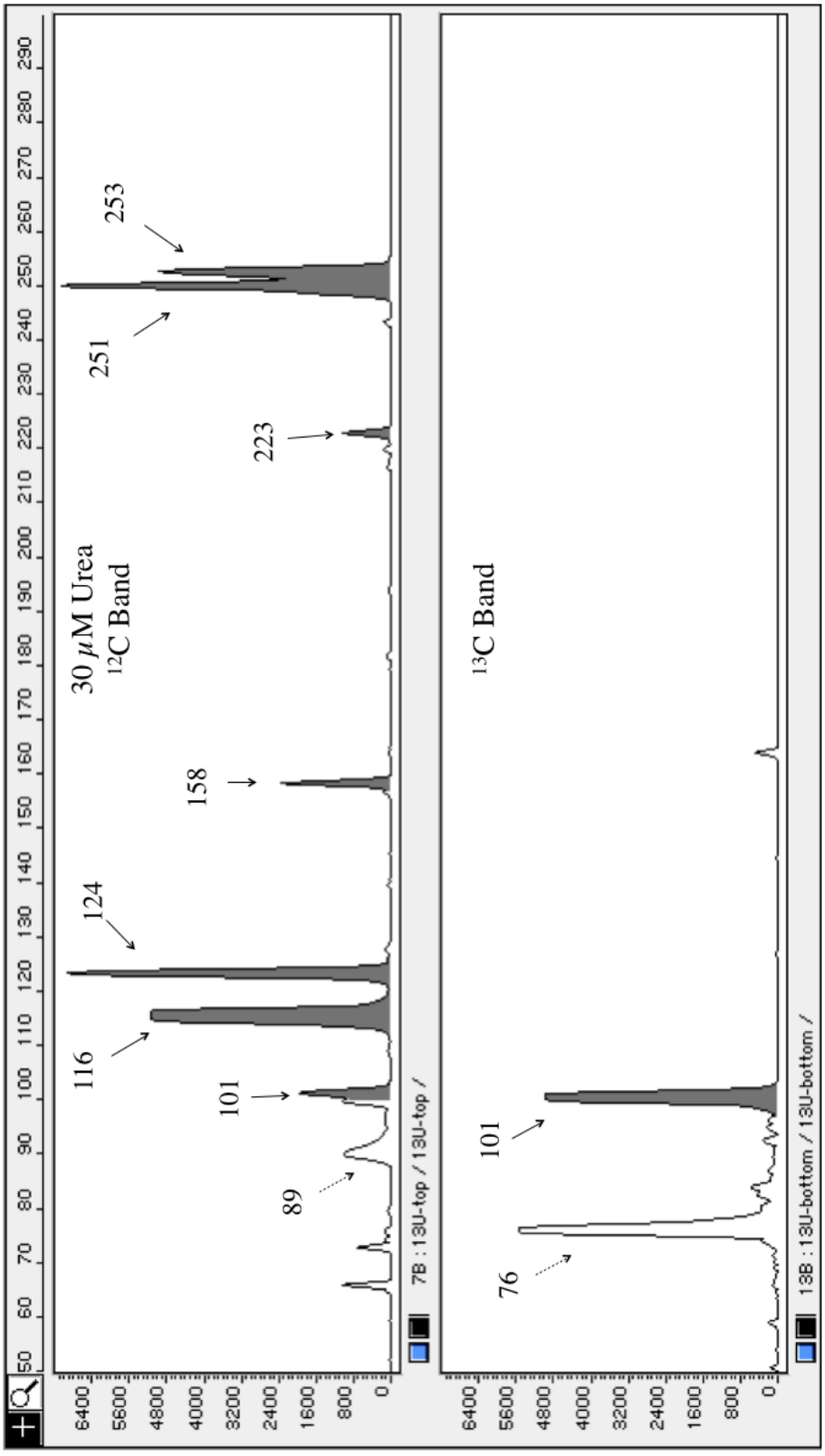
Yakimov, M.M., La Cono, V., Smedile, F., DeLuca, T.H., Juárez, S., Ciordia, S. et al. (2011) Contribution of crenarchaeal autotrophic ammonia oxidizers to the dark primary production in Tyrrhenian deep waters (Central Mediterranean Sea). ISME 5: 945-961.



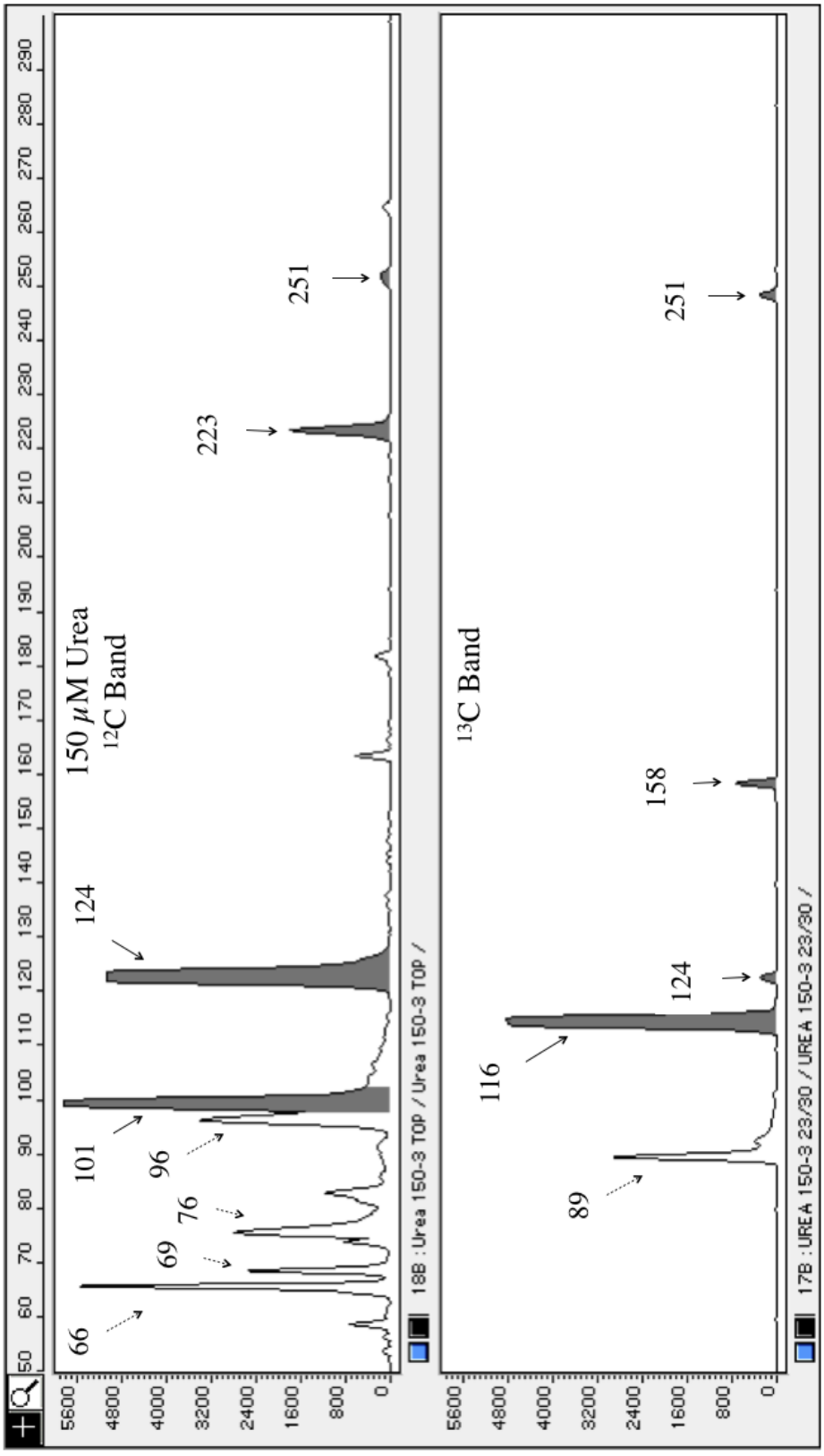

Supplemental Figure 2.1. TRFLP profiles of Crenarchaeal 16S rRNA genes in salt marsh sediments from the 30 $\mu$M acetate amendment. Peaks represented in the clonal library are highlighted in gray.




Supplemental Figure 2.2. TRFLP profiles of Crenarchaeal 16S rRNA genes in salt marsh sediments from 150 $\mu$M acetate amendment. Peaks represented in the clonal library are highlighted in gray.




Supplemental Figure 2.3. TRFLP profiles of Crenarchaeal 16S rRNA genes in salt marsh sediments from 30 $\mu$M urea amendment. Peaks represented in the clonal library are highlighted in gray.




Supplemental Figure 2.4. TRFLP profiles of Crenarchaeal 16S rRNA genes in salt marsh sediments from 150 $\mu$M urea amendment. Peaks represented in the clonal library are highlighted in gray.

| Salt Marsh Zone | Substrate | Concentration |
|---|---|---|
| Low Marsh | $^{13}$C acetate | 30 μM |
| Low Marsh | $^{13}$C acetate | 150 μM |
| Low Marsh | $^{13}$C glycine | 30 μM |
| Low Marsh | $^{13}$C glycine | 150 μM |
| Low Marsh | $^{13}$C urea | 30 μM |
| Low Marsh | $^{13}$C urea | 150 μM |
| Low Marsh | $^{13}$C lipids | 2 mg/mL |
| Low Marsh | $^{13}$C lipids | 10 mg/mL |
| Low Marsh | $^{13}$C proteins | 2 mg/mL |
| Low Marsh | $^{13}$C proteins | 10 mg/mL |
| Low Marsh | $^{13}$C ISOGRO | 2 mg/mL |
| Low Marsh | $^{13}$C ISOGRO | 10 mg/mL |
| Low Marsh | no amendment | N/A |
| Transition | $^{13}$C acetate | 30 μM |
| Transition | $^{13}$C acetate | 150 μM |
| Transition | $^{13}$C glycine | 30 μM |
| Transition | $^{13}$C glycine | 150 μM |
| Transition | $^{13}$C urea | 30 μM |
| Transition | $^{13}$C urea | 150 μM |
| Transition | $^{13}$C lipids | 2 mg/mL |
| Transition | $^{13}$C lipids | 10 mg/mL |
| Transition | $^{13}$C proteins | 2 mg/mL |
| Transition | $^{13}$C proteins | 10 mg/mL |
| Transition | $^{13}$C ISOGRO | 2 mg/mL |
| Transition | $^{13}$C ISOGRO | 10 mg/mL |
| Transition | no amendment | N/A |

Supplemental Table 5.1. Complete list of SIP microcosms established from salt marsh sediment in low marsh and transition zones.

| No of Samples | Substrate | Temperature (ºC) | Incubation Time (days) |
|---|---|---|---|
| 3 | 30 µM $^{13}$C urea | 4 | 3 |
| 3 | 30 µM $^{13}$C urea | 18 | 3 |
| 3 | 30 µM $^{13}$C urea | 25 | 3 |
| 3 | 30 µM $^{13}$C urea | 37 | 3 |
| 3 | 30 µM $^{13}$C urea | 4 | 5 |
| 3 | 30 µM $^{13}$C urea | 18 | 5 |
| 3 | 30 µM $^{13}$C urea | 25 | 5 |
| 3 | 30 µM $^{13}$C urea | 37 | 5 |
| 3 | 30 µM $^{13}$C urea | 4 | 7 |
| 3 | 30 µM $^{13}$C urea | 18 | 7 |
| 3 | 30 µM $^{13}$C urea | 25 | 7 |
| 3 | 30 µM $^{13}$C urea | 37 | 7 |
| 3 | 30 µM $^{13}$C urea | 4 | 14 |
| 3 | 30 µM $^{13}$C urea | 18 | 14 |
| 3 | 30 µM $^{13}$C urea | 25 | 14 |
| 3 | 30 µM $^{13}$C urea | 37 | 14 |
| 3 | 30 µM $^{12}$C urea | 4 | 14 |
| 3 | 30 µM $^{12}$C urea | 18 | 14 |
| 3 | 30 µM $^{12}$C urea | 25 | 14 |
| 3 | 30 µM $^{12}$C urea | 37 | 14 |

Supplemental Table 5.2. Complete list of SIP microcosms established from tidal levee sediment for time course study.

| Station | Latitude | Longitude | Depth (m) | No of Samples | Vol (L) | Substrate | Incubation Time (h) |
|---|---|---|---|---|---|---|---|
| 3 | 16° 27.62' N | 60° 02.33' W | 400 | 3 | 1 | 250 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 3 | 1 | 25 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 1 | 1 | 250 µg/L $^{12}$C Bioexpress | 48 |
| | | | | 3 | 1 | 250 µg/L $^{13}$C fatty acids | 48 |
| | | | | 3 | 1 | 25 µg/L $^{13}$C fatty acids | 48 |
| | | | | 1 | 1 | 100 µl/L ethanol | 48 |
| | | | 800 | 3 | 1 | 250 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 3 | 1 | 25 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 1 | 1 | 250 µg/L $^{12}$C Bioexpress | 48 |
| | | | | 3 | 1 | 250 µg/L $^{13}$C fatty acids | 48 |
| | | | | 2 | 1 | 25 µg/L $^{13}$C fatty acids | 48 |
| | | | | 1 | 1 | 100 µl/L ethanol | 48 |
| | | | 2400 | 3 | 1 | 250 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 3 | 1 | 25 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 1 | 1 | 250 µg/L $^{12}$C Bioexpress | 48 |
| | | | | 3 | 1 | 250 µg/L $^{13}$C fatty acids | 48 |
| | | | | 3 | 1 | 25 µg/L $^{13}$C fatty acids | 48 |
| | | | | 1 | 1 | 100 µl/L ethanol | 48 |
| 5 | 25° 29.99' N | 62° 36.49' W | 400 | 3 | 1 | 250 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 3 | 1 | 25 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 2 | 1 | 250 µg/L $^{12}$C Bioexpress | 48 |
| | | | | 3 | 1 | 250 µg/L $^{13}$C fatty acids | 48 |
| | | | | 2 | 1 | no amendment | 48 |
| | | | 800 | 3 | 1 | 250 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 3 | 1 | 25 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 2 | 1 | 250 µg/L $^{12}$C Bioexpress | 48 |
| | | | | 3 | 1 | 250 µg/L $^{13}$C fatty acids | 48 |
| | | | | 2 | 1 | no amendment | 48 |
| | | | 2400 | 3 | 1 | 250 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 3 | 1 | 25 µg/L $^{13}$C Bioexpress | 48 |
| | | | | 2 | 1 | 250 µg/L $^{12}$C Bioexpress | 48 |
| | | | | 3 | 1 | 250 µg/L $^{13}$C fatty acids | 48 |
| | | | | 2 | 1 | no amendment | 48 |
| 6 | 27° 12.54' N | 67° 58.29' W | 400 | 3 | 1 | 25 µg/L $^{13}$C fatty acids | 48 |
| | | | 800 | 3 | 1 | 25 µg/L $^{13}$C fatty acids | 48 |
| | | | 2400 | 3 | 1 | 25 µg/L $^{13}$C fatty acids | 48 |

Supplemental Table 5.3. Complete list of SIP microcosms established on chief scientist training cruise, June 2014.