

**TEMPORAL AND SPATIAL VARIABILITY OF NITRIFYING ARCHAEA  
IN THE PACIFIC OCEAN**

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I certify that we have read this thesis and that, in my opinion, it is  
satisfactory in scope and quality as a thesis for the degree of Bachelor of  
Science in Global Environmental Science

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## Abstract

*Crenarchaea* are putative ammonia oxidizers in the marine environment. The nitrifying role that these microorganisms play is an integral component of the marine nitrogen (N) cycle. Here we present two separate, but very similar studies to assess both the temporal and spatial dynamics of ammonia oxidizing *Crenarchaea*. One study looked at the seasonal variability in the abundances and distributions of ammonia oxidizing *Crenarchaea* over a four-year time scale at the same location in the North Pacific Ocean (Station ALOHA). We found total crenarchaeal gene abundances typically increased three to four orders of magnitude between the near-surface (~5m) ocean and the epi- mesopelagic boundary (200 m), decreasing about an order of magnitude throughout the rest of the mesopelagic zone, and staying relatively constant in the bathypelagic water. Annual occurrences of 10,000 fold increases in crenarchaeal abundances in near-surface waters appeared linked to winter mixing, while during summer months, a predominately upper ocean dwelling *Crenarchaea* increased in abundance in upper mesopelagic waters coincident with periods of increased particulate nitrogen flux to the deep sea. Our other study examined meridional distributions of crenarchaeal ammonia monooxygenase (*amoA*) genes and transcripts across a vast (~5200 km) region of the central Pacific Ocean. Throughout the transect, crenarchaeal *amoA* genes showed a nearly identical depth-dependent distribution when compared to estimates at Station ALOHA. Crenarchaeal *amoA* transcripts typically increased one to two orders of magnitude between 100 m and the epi- mesopelagic boundary (200 m), before decreasing throughout the mesopelagic zone. When normalized to gene abundances, *amoA*

transcripts revealed elevated expression in the upper ocean waters (0-100m), where crenarchaeal abundances were low and transcript abundances decreased throughout the mesopelagic zone as crenarchaeal gene abundances increased. Both studies suggest that throughout the entire water column, ammonia oxidizing *Crenarchaea* are dynamic contributors to the marine nitrogen cycle in the Pacific Ocean.

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## **Chapter 1: Introduction**

Nitrogen is an essential element for life, required as an integral component of amino acids, proteins, nucleic acids, and other complex organic molecules (Mackenzie, 2003); as a result, the nitrogen cycle has been studied extensively in both terrestrial and marine environments. In the marine environment, the movement of nitrogen between inorganic and organic forms is controlled by diverse groups of microorganisms. Nitrogen has eight redox states, with nitrogen compounds ranging from oxidized inorganic forms such as  $\text{NO}_3^-$  to reduced forms that include organic matter and ammonia. As a result of this complex redox chemistry, organisms can assimilate nitrogen as an essential nutrient, or nitrogen substrates may be utilized as sources of energy or they may serve as electron acceptors in dissimilatory metabolisms (Gruber, 2008).

### ***Nitrogen biogeochemistry and nitrification***

The marine nitrogen cycle is wholly mediated by the metabolic activities of diverse groups of microorganisms. Microbes take advantage of redox gradients in the nitrogen cycle, resulting in transformations of nitrogen into various forms (Figure 1). Transforming nitrogen from oxidized to reduced states requires energy, while utilization of reduced nitrogen forms provide energy for cellular biosynthesis. Among the key biogeochemical transformations in the marine nitrogen cycle are the dissimilatory processes denitrification and nitrification. Denitrification describes the use of oxidized nitrogen substrates, notably including nitrate, as a terminal

electron acceptor during heterotrophic respiratory metabolism. This process occurs predominately in low oxygen or anaerobic regions of the sea (Devol, 2008).

Nitrification describes the aerobic utilization of ammonia ( $\text{NH}_3$ ) as an energy source, producing nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ). Complete nitrification is defined as the aerobic oxidation of ammonia to nitrate (Ward, 2008). The first step of nitrification occurs when ammonia is oxidized to nitrite ( $\text{NO}_2^-$ ), which is then independently followed by nitrite oxidation to nitrate. The two steps of complete nitrification are made possible by different groups of microbes, including obligate and facultative chemolithotrophic *Bacteria* and *Archaea*. This thesis addresses an important aspect of the process of nitrification, ammonia oxidation, with specific focus on identifying distributions of ammonia oxidizing microorganisms in the open ocean.

Historically, various groups of *Bacteria* were considered to be the predominant ammonia oxidizing microorganisms in the ocean (Ward, 2008). More recently however, cultivation-dependent and independent approaches have revealed that members of the marine *Crenarchaea* play a crucial role in stoichiometric conversion of ammonia to nitrite (Konneke *et al.*, 2005). Ammonia oxidizing *Archaea* may possibly be more abundant than ammonia oxidizing *Bacteria* (Wuchter *et al.*, 2006). The protein responsible for oxidizing ammonia is ammonia monooxygenase, encoded in part by the *amoA* gene. The oxidation of nitrite to nitrate appears primarily governed by *Bacteria*, including members of the genus *Nitrospina* (Francis *et al.*, 2005; Konneke *et al.*, 2005; Wuchter *et al.*, 2006; Mincer *et*

*al.*, 2007). Although nitrite oxidation has a smaller energy yield than ammonia oxidation, it nonetheless still has redox potential and is also found in low concentrations in the marine environment (Table 1).

### ***Importance of ammonia oxidizing Crenarchaea***

Research into non-thermophilic *Archaea* has revealed that these microorganisms are ubiquitous, abundant, and dynamic components of the ocean picoplankton (DeLong, 1992; Fuhrman *et al.*, 1992; Karner *et al.*, 2001). This recent recognition of the marine *Archaea* stemmed in large part from advances in geochemical and molecular biological approaches to study these microorganisms. For example, by targeting archaeal *amoA* genes, numerous studies have examined the presence and diversity of ammonia oxidizing *Crenarchaea*. Development of quantitative polymerase chain reaction (QPCR) based approaches to amplify *amoA* genes has provided insight into the abundances and distributions of *Crenarchaea* in the sea (Jenkins and Zehr, 2008). Moreover, quantification of *amoA* gene expression has provided insight into the physiological capabilities of these microorganisms (Frias-Lopez *et al.*, 2008; Church *et al.*, 2009; Santoro *et al.*, 2010).

Previous work has shown that pelagic *Crenarchaea* represent one of the oceans single most abundant cell types (Karner *et al.*, 2001). There are an estimated  $10^{28}$  individual *Crenarchaea* comprising some 20% of the total picoplankton in the world's oceans (Karner *et al.*, 2001). *Crenarchaeal* abundances vary strongly with depth, often increasing three to four orders of magnitude between the near-surface ocean and the lower mesopelagic zone (DeLong *et al.* 1999, Karner *et al.* 2001,

Mincer *et al.* 2007, Church *et al.*, 2009). Crenarchaeal abundances in the upper epipelagic often range  $\sim 10^2$  to  $10^3$  cells  $l^{-1}$ , typically increasing through the lower euphotic zone, and ranging from  $\sim 10^5$  to  $\sim 10^7$  cells  $l^{-1}$  in the meso- and bathypelagic regions of the ocean's interior (Karner *et al.* 2001).

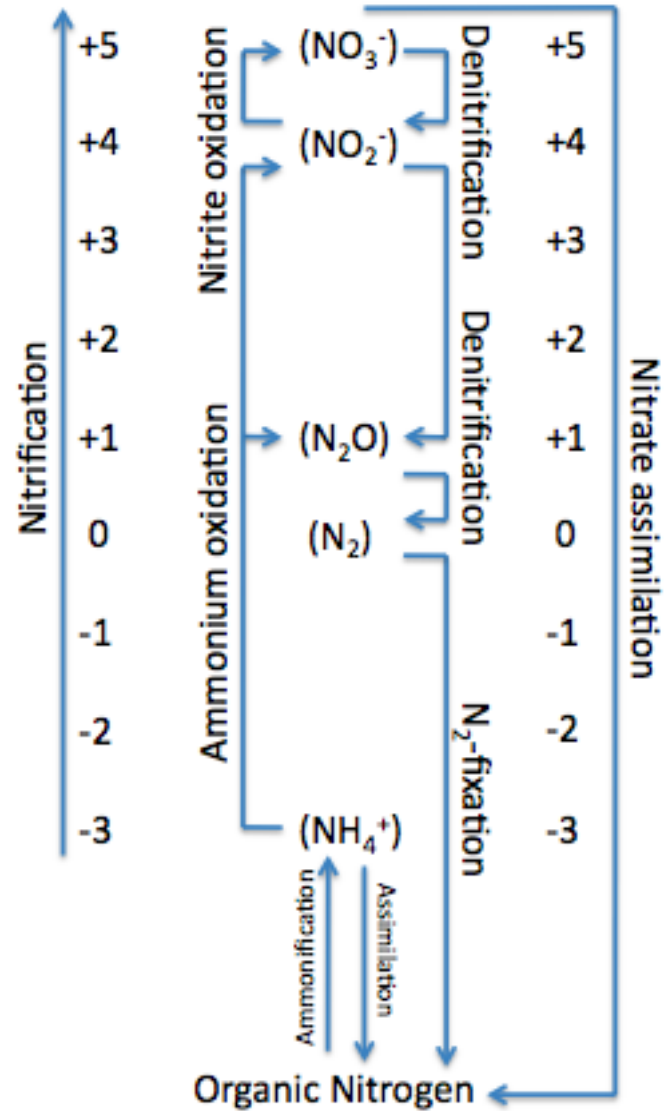
Studies on ocean nitrification indicate rates of ammonia oxidation often peak near the base of the euphotic zone. To date it remains unclear what processes limit the abundances and activities of ammonia oxidizing microbes in the sunlit regions of the ocean. Limited information on the distributions and physiological activities of marine *Crenarchaea* are available, so we have attempted to address that issue by examining the transcriptional activities and distributions of these putative nitrifying microorganisms. In this thesis, I explore patterns of crenarchaeal distributions and gene expression from samples collected during the C-MORE BULA (Biogeochemistry of the Upper ocean: Latitudinal Assessment) cruise transect from Fiji to Hawaii. This transect covered  $\sim 5200$  km region of the Pacific Ocean, and included sampling of the euphotic and mesopelagic waters ( $< 1000$ m). Reverse Transcriptase PCR (RT-QPCR) amplification of *amoA* transcripts demonstrated active gene expression throughout the epi- and mesopelagic waters of the Pacific Ocean. Examination of crenarchaeal *amoA* gene expression indicated that relative to *amoA* gene abundances, crenarchaeal *amoA* gene transcripts were less variable with depth. In the near-surface ocean, *amoA* transcripts ranged  $\sim 10^4$  and  $\sim 10^5$  transcripts  $l^{-1}$ , typically increasing one to two orders of magnitude in the dimly lit waters of the

lower half of the euphotic zone, and declining into the deep ocean (Church *et al.* 2009).

Carbon isotope analyses of archaeal lipids (Pearson *et al.*, 2001; Ingalls *et al.*, 2006), incubation-growth experiments (Ouverney and Fuhrman, 2000; Herndl *et al.*, 2005; Teira *et al.*, 2006; Wuchter *et al.*, 2006; Kirchman *et al.*, 2007), and reconstructions of archaeal metagenomes (DeLong *et al.*, 2006; Hallam *et al.*, 2006a,b; Martin-Cuadrado *et al.*, 2008) all suggest that marine crenarchaeal metabolism may rely on strategies that include chemolithoautotrophy and assimilation of organic matter (Ouverney and Fuhrman, 2000; Herndl *et al.*, 2005; Kirchman *et al.*, 2007; Varela *et al.*, 2008). Recent findings suggest that *Archaea* in the Tyrrhenian Sea are simultaneously expressing ammonia-oxidizing and lipid-building genes (Yamikov, 2009), which would have implications for the ocean's carbon budget. The relative contribution of different carbon sources and modes of crenarchaeal metabolism may vary with depth; chemolithoautotrophic growth has been hypothesized to prevail in the upper ocean and mid depth waters of the mesopelagic (Hansman *et al.*, 2009), while chemoorganoheterotrophy predominantly occurring in the ocean's cold, dark bathypelagic interior (Agogue *et al.*, 2008).

**Table 1.** Nitrogen inventories and ranges in concentrations of various pools of nitrogen; Tg is  $10^{12}$  grams.

Species	Oceanic Inventory (Tg N)	Upper Euphotic Station ALOHA (25m) ( $\mu\text{mol l}^{-1}$ )	Upper Mesopelagic Station ALOHA (200m) ( $\mu\text{mol l}^{-1}$ )
Nitrate ( $\text{NO}_3^-$ )	$5.8 \times 10^5$	0.0003-0.04	0.9-7
Nitrite ( $\text{NO}_2^-$ )	160	0.0002-0.009	0.004-0.08
Ammonia ( $\text{NH}_4^+$ )	340	<0.03	<0.03
DON	$7.7 \times 10^4$	3.8-7.7	3.6-5.7



**Figure 1.** Diagram showing various redox states of nitrogen and their transformation pathways. The oxidation of ammonia via nitrification is an energy yielding process.

*Crenarchaea* are potentially very important to the marine nitrogen cycle, yet only partial information of the temporal distributions of these microorganisms is available. In this study, we examined the variation in abundance of ammonia oxidizing *Crenarchaea* over a four-year period in the oligotrophic North Pacific Ocean from the sea surface into the deep bathypelagic waters. We conducted our study at Station ALOHA (A Long Term Oligotrophic Habitat Assessment), located 100km north of Oahu, the research station for the HOT (Hawaii Ocean Time-series) program. By placing our study in the context of this on-going ocean time-series program, we gained insight into nitrogen cycling in an environment relatively isolated from human impact. Moreover, this arena provided an ideal location for studying temporal fluctuations in microorganisms involved in the marine nitrogen cycle. Based on QPCR-derived estimates of crenarchaeal ammonia-oxidizing gene abundances, our study indicated that *Crenarchaea* represent ubiquitous components of microbial assemblage and that both physical and biological processes regulate their distributions in the open sea.

## **Chapter 2: Methods**

### ***Seawater collection at Station ALOHA***

Seawater samples from Station ALOHA (22°45'N, 158°W) were collected on an approximately monthly basis from the near-surface (5 to 10 m) ocean into the bathypelagic region (4000 m) for ensuing biogeochemical and molecular biological analyses. Using 24 10 l polyvinyl chloride sampling bottles attached to a Conductivity-Temperature-Depth rosette sampling system, samples were collected from 16 discrete depths per cruise, with most intensive sample collections in the epipelagic region (0-200 m). Although the HOT program has been running since 1988, collection of samples used for this study began in 2005.

### ***Nucleic acid collection and extraction***

Seawater was subsampled from the CTD rosette into acid-rinsed polycarbonate carboys and between 2 to 4 l was filtered using a peristaltic pump onto 25 mm diameter 0.2 µm pore size Supor® filters. After filtration, the filters were removed from the apparatus and placed in 2 ml microcentrifuge tubes. Filters for eventual RNA extraction were immersed in 500 µl RLT buffer (Qiagen RNeasy) containing 1% B-mercaptoethanol. Filters for subsequent DNA extraction were immersed in 500 µl lysis buffer (20 mM Tris-HCl, pH 8.0; 2mM EDTA, pH 8.0; 1.2% Triton X and 20 mg ml<sup>-1</sup> lysozyme). DNA and RNA samples were immediately flash frozen in liquid nitrogen then stored at -80°C until processed in the shore-based laboratory.

In the laboratory, microcentrifuge tubes containing the sample filters for DNA extraction were placed in a water bath at 37°C for 1 h, after which 42 µl of proteinase K and 334 µl of lysis buffer AL (Qiagen DNeasy) were added to each sample. Samples were vortexed and placed in a hybridization oven at 70°C for 30 minutes. Following this incubation, 334 µl of 100% ethanol was added to each sample, and the microcentrifuge tubes were vortexed and transferred to Qiagen DNeasy spin columns. DNA was purified following the manufacturer's recommended protocols.

RNA extraction involved both physical and chemical techniques (Church *et al.*, 2005; Church *et al.*, 2009). Approximately 0.2g of 0.1 mm zircon beads were added to microcentrifuge tubes containing sample filters. Tubes were placed in a Fast Prep machine (Bio 101, Carlsbad, CA, USA) and agitated for a total time of 1.5 minutes. After bead-beating, tubes were centrifuged at 8500 *g* for 30 s. An equal volume of 70% ethanol was added to each microcentrifuge tube. Supernatants were transferred to Qiagen RNeasy® Mini columns (Qiagen, Valencia, CA, USA) and total RNA was purified and eluted following the manufacturer's protocol. RNA extracts were treated with DNase I following the Qiagen On-Column DNase I® RNA extraction protocol. RNA was eluted from each spin column with 30 µl RNase-free water and stored at -80°C for later molecular analyses. RNA concentrations were determined fluorometrically using the Quant-iT® RNA assay kit (Invitrogen, Carlsbad, CA, USA) and a Turner TD-700 fluorometer (Turner Designs, Sunnyvale, CA, USA).

*AmoA* genes were reverse transcribed from the mRNA extracts. For these reactions, total RNA samples were reverse transcribed using SuperScript® III first strand cDNA synthesis kit (Invitrogen) following the manufacturer's recommended protocol. Reactions for cDNA synthesis were composed of 2-4 ng total RNA, 1 mmol l<sup>-1</sup> dNTPs, 1 × RT buffer, 5 mmol l<sup>-1</sup> MgCl<sub>2</sub>, 10 mmol l<sup>-1</sup> DTT, 40 U RNaseOUT (Invitrogen), 200 U SuperScript III RT, and 0.5 μmol l<sup>-1</sup> of the gene-specific reverse primer (*CrenAmoAModR*; Mincer *et al.*, 2007). Shortly after cDNA synthesis, samples were diluted to 50 μl total volume with nuclease-free water, and stored at -20°C until analyzed by QPCR assays. An identical set of no reverse transcriptase (no SuperScript III RT) samples were used to demonstrate controls for examining potential contributions of carryover genomic DNA during RT-QPCR amplification of the cDNA.

### ***QPCR components and thermocycling conditions***

Crenarchaeal *amoA* gene copy abundances were examined using previously described QPCR protocols (Mincer *et al.*, 2007). The QPCR assays consisted of duplicate 25 μl containing: 12.5 μl 2X SYBR® Green Master Mix (Applied Biosystems, Foster City, CA, USA), 8 μl of nuclease-free water, 2 μl of environmental DNA, and 0.5 μM final concentration of both forward and reverse primers. PCR primer sequences are as follows: *CrenAmoAQ\_F* (5'-GCARGTMGGWAARTTCTAYAA-3') and *CrenAmoAModR* (5'-AAGCGCCATCCATCTGTA-3'). Primers used for detection of an upper ocean clade of *Crenarchaea* were as follows: Arch-amoAFA (5'-ACACCAGTTTGGYTACCWTCDGC-3') and *CrenAmoAModR* (as above; Beman *et al.*,

2008). Quantitative PCR reactions were analyzed using an Applied Biosystems 7300, following these thermal cycling reaction conditions: 94°C for 15 min; followed by 50 cycles of 94°C for 15 s, 52°C for 30 s, 72°C for 30 s, with a final 78°C for 1 s at the end of each cycle to assure product detection. Standards for QPCR reactions consisted of serial 10-fold dilutions of plasmids containing amplified fragments of the targeted *amoA* genes of interest. QPCR efficiency for Mincer et al. (2007) primers averaged 100% ( $\pm 0.06$ ), while QPCR efficiency for Beman et al. (2008) upper ocean clade primers averaged 99% ( $\pm 0.03$ ).

#### ***Other biogeochemical parameters measured***

Seawater for subsequent determination of nutrient concentrations was collected without filtration into clean, sample-rinsed high-density polyethylene (HDPE) bottles (Karl *et al.*, 2001). Samples were frozen in the upright position and stored at -20°C in the dark until analyzed at shore (Dore *et al.*, 1996).

Temperature (°C) was measured using a SeaBird CTD system affixed to the CTD sampling rosette. Concentrations of chlorophyll *a* ( $\mu\text{g Chl l}^{-1}$ ) were determined from High Performance Liquid Chromatography (HPLC) separation of algal pigments as described in Bidigare *et al.* (2005). Dissolved oxygen ( $\mu\text{mol O}_2 \text{l}^{-1}$ ) was measured based on Winkler Titrations as described in Carpenter (1965). Nitrate + nitrite ( $\mu\text{mol N l}^{-1}$ ) was measured using a 4-channel continuous flow Bran+Luebbe Autoanalyzer III at a shore-based laboratory. Because surface concentrations of N are below detection limits of the standard autoanalyzer procedures, a high-

sensitivity chemiluminescence method was employed for upper ocean (<200 m) nitrate + nitrite concentrations (Karl *et al.*, 2001).

## Chapter 3: Results

For the purposes of our study, we were interested in understanding spatial and temporal distributions of archaeal nitrifiers. We utilized the *amoA* gene as a molecular biological marker to identify the abundances, activities and distributions of ammonia oxidizing *Crenarchaea* for two separate studies: a ~5200 km transect from Fiji to Hawaii, and a time series study in the oligotrophic waters at Station ALOHA. Our intent was to better understand the role that archaeal nitrifiers play in the marine nitrogen cycle, assess habitat characteristics of marine the marine *Crenarchaea*, and understand processes influencing the spatial and temporal distributions of *Crenarchaea* in the sea.

### ***CMORE BULA transect habitat characteristics***

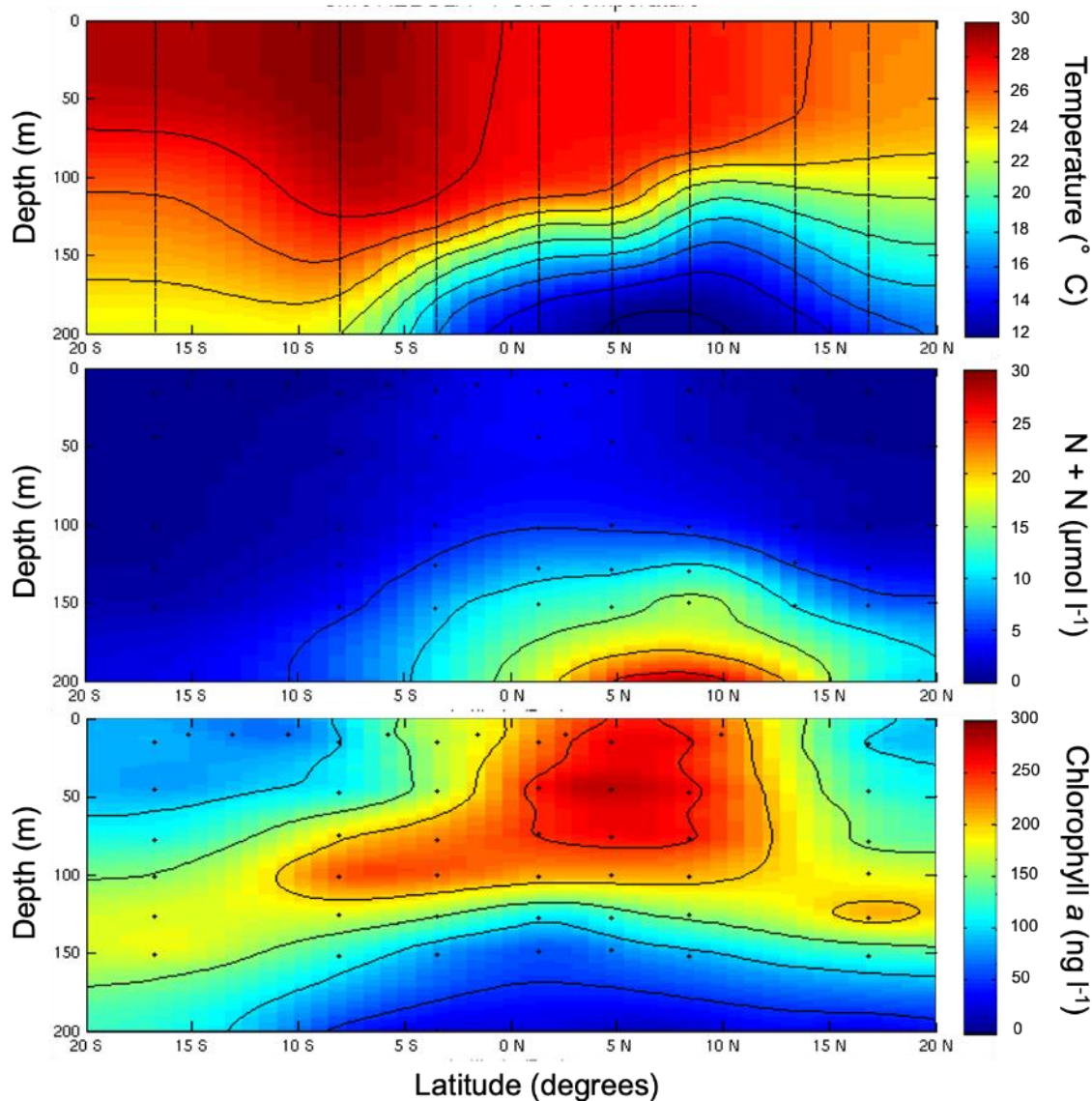
Sampling for this study included a wide range of spatially distinct oceanic environments that included near-surface epipelagic waters, and the cold, dimly lit waters of the mesopelagic. Moreover, our sampling of distinct oceanic regions along the CMORE BULA transect provided insight into latitudinal variations in both the ocean habitat and microorganism abundances and activities. The most striking meridional changes in physical and biogeochemical structure of the upper ocean occurred in the transitional region separating the oligotrophic subtropical gyres from the relatively nutrient-enriched regions of the equatorial waters. Doming of the thermocline in the equatorial region carried cold, nutrient-enriched, O<sub>2</sub>-depleted waters closer to the ocean's surface. Most intense upwelling, as reflected in both

uplift of isotherms and nutrient concentrations, occurred in the northern equatorial waters (8.6°N) (Figure 2).

Concentrations of nitrate + nitrite (N+N), phosphate, and silicic acid all increased sharply in the near-equatorial waters. In contrast, upper ocean nutrient concentrations in the northern and southern subtropical gyres were very low (*e.g.* average N+N concentrations in the upper 100 m in these regions were generally < 100 nmol l<sup>-1</sup>). Changes in concentrations of N+N and dissolved O<sub>2</sub> were the most prominent in the waters of the mesopelagic zone (below 200 m), with N+N concentrations generally increasing with decreases in dissolved O<sub>2</sub> concentrations from south to north. Chlorophyll *a* concentrations peaked in the 250 to 300 ng l<sup>-1</sup> range, shifting slightly north of the equator, centered around 5°N at approximately 50 m deep (Figure 2).

### ***Crenarchaeal amoA genes and gene transcripts across transect***

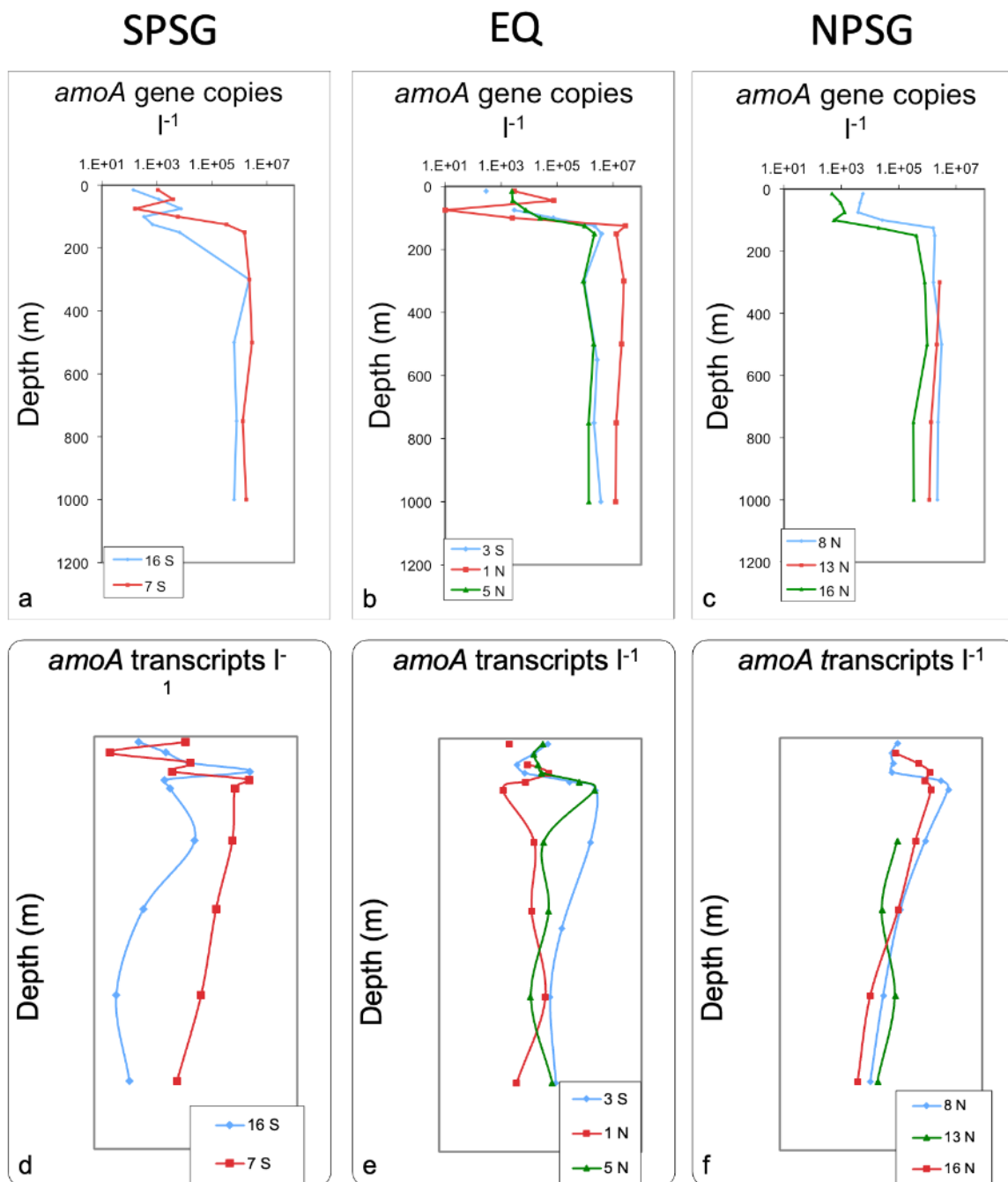
Vertical profiles of QPCR-derived crenarchaeal *amoA* gene copy abundances demonstrated pronounced depth-dependent changes throughout our 5200 km transect from Fiji to Hawaii. Gene abundances generally increased three to four orders of magnitude between the near-surface ocean and the lower mesopelagic (Figure 3). Crenarchaeal gene abundances in the upper epipelagic ranged between  $1 \times 10^2$  and  $7 \times 10^3$  copies l<sup>-1</sup>, with abundances increasing through the lower euphotic zone, and ranging from  $2 \times 10^5$  to  $2 \times 10^7$  copies l<sup>-1</sup> through the mesopelagic (Figure 3).



**Figure 2.** Contour plots depicting vertical and meridional distributions in ocean temperature (top panel), nitrate + nitrite (middle panel), and chlorophyll *a* (bottom panel). Color bars are temperature (°C), Nitrate + Nitrite (μmol l<sup>-1</sup>) and chlorophyll *a* (ng l<sup>-1</sup>), respectively. Dots indicate areas of discrete sampling.

Although there were distinct differences in biogeochemical properties throughout the transect, there were no extreme differences in *amoA* gene abundances except for one station sampled close to the equator (1.7°N). Within the mesopelagic region of this near-equatorial station, *amoA* gene abundances were approximately an order of magnitude greater than any other station across this Fiji to Hawaii transect (Figure 3).

We also examined vertical and meridional patterns of crenarchaeal *amoA* gene expression based on RT-QPCR amplification of *amoA* mRNA transcripts throughout our 5200 km transect. Crenarchaeal *amoA* transcripts were less variable with depth when compared to *amoA* gene abundances. In the near-surface ocean, *amoA* transcripts ranged between  $9 \times 10^3$  and  $2 \times 10^5$  transcripts  $l^{-1}$ , typically increasing one to two orders of magnitude into the lower half of the epipelagic (Figure 3). Below the depth of maximum *amoA* expression, transcript abundances declined into the interior of the mesopelagic zone (Figure 3). Among the various regions throughout the 5200km transect, transcript abundances were greatest in the northern equatorial water (8.6°N) where cold, N+N enriched and O<sub>2</sub> depleted waters upwelled into the upper ocean. Near this upwelling region (Figure 2), *amoA* transcript concentrations were approximately fivefold greater ( $> 1 \times 10^6$  transcripts  $l^{-1}$ ) than *amoA* expression measured at any other region of the study site (Figure 3). In contrast, the lowest transcript abundances ( $\sim 1 \times 10^3$  transcripts  $l^{-1}$ ) were measured at the near-equatorial station, where *amoA* gene abundances were maximal.

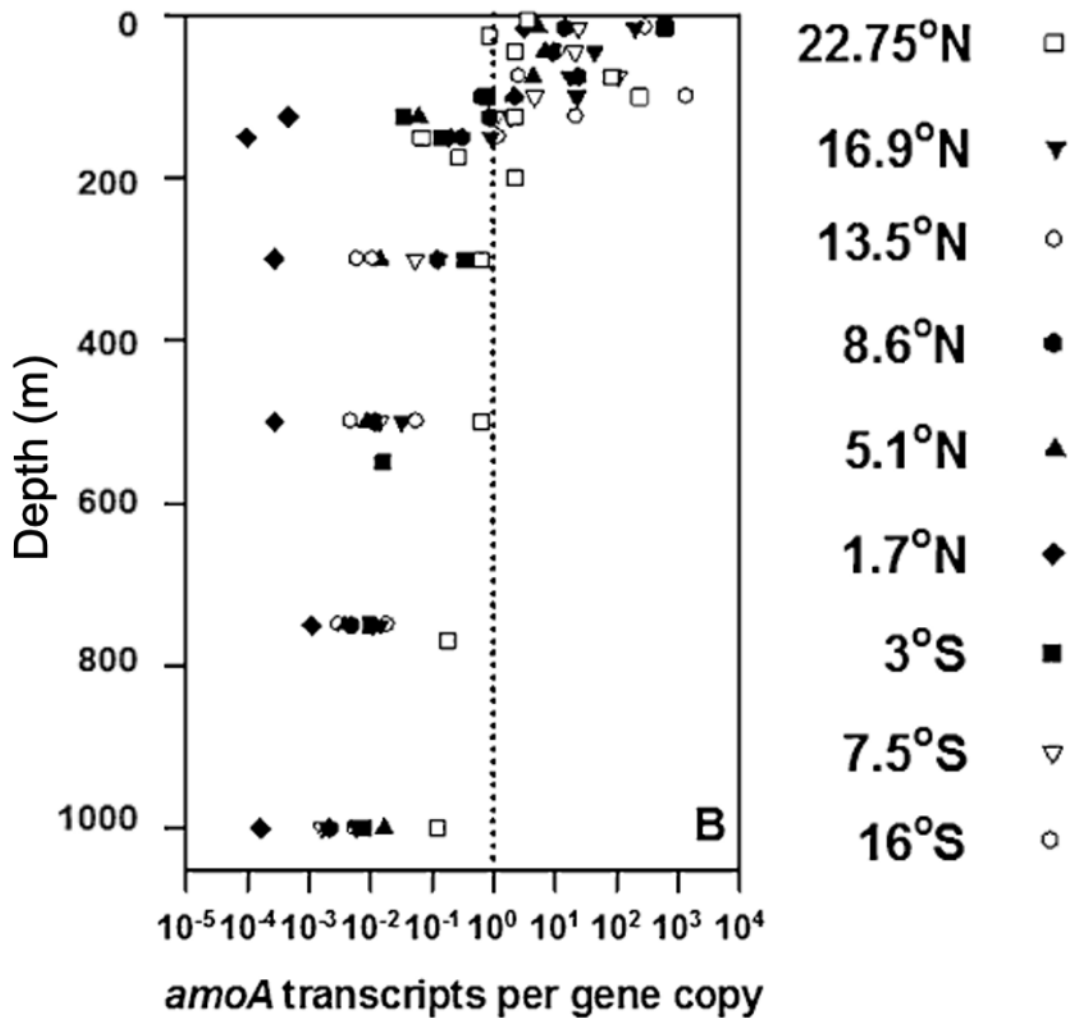


**Figure 3.** Depth profiles of *amoA* gene copy abundances and *amoA* gene expression at various stations sampled along the CMORE BULA transect. Y-axis is depth in meters from the surface through the mesopelagic, the X-axes are *amoA* genes per liter (a-c) or *amoA* transcripts per liter (d-f) plotted on a logarithmic scale.

The most surprising difference between the distributions of crenarchaeal *amoA* transcripts and *amoA* gene abundances occurred in the upper ocean (<100 m) waters where archaeal *amoA* gene abundance was low, but *amoA* transcripts were relatively high. The gene-copy normalized *amoA* transcript abundances averaged ~80 transcripts per gene copy in the well-lit region of the ocean (Figure 4). The ratio of *amoA* genes to transcripts approached 1:1 near the transitional region separating the epipelagic and mesopelagic zones, eventually decreasing with depth into the lower mesopelagic (Figure 4). Throughout the mesopelagic, *amoA* gene abundances increased concurrently with decreases in gene expression resulting in low and spatially variable (ranging  $1 \times 10^{-4}$  and  $6 \times 10^{-1}$ ) transcripts per gene copy (Figure 4).

#### ***Station ALOHA habitat characteristics***

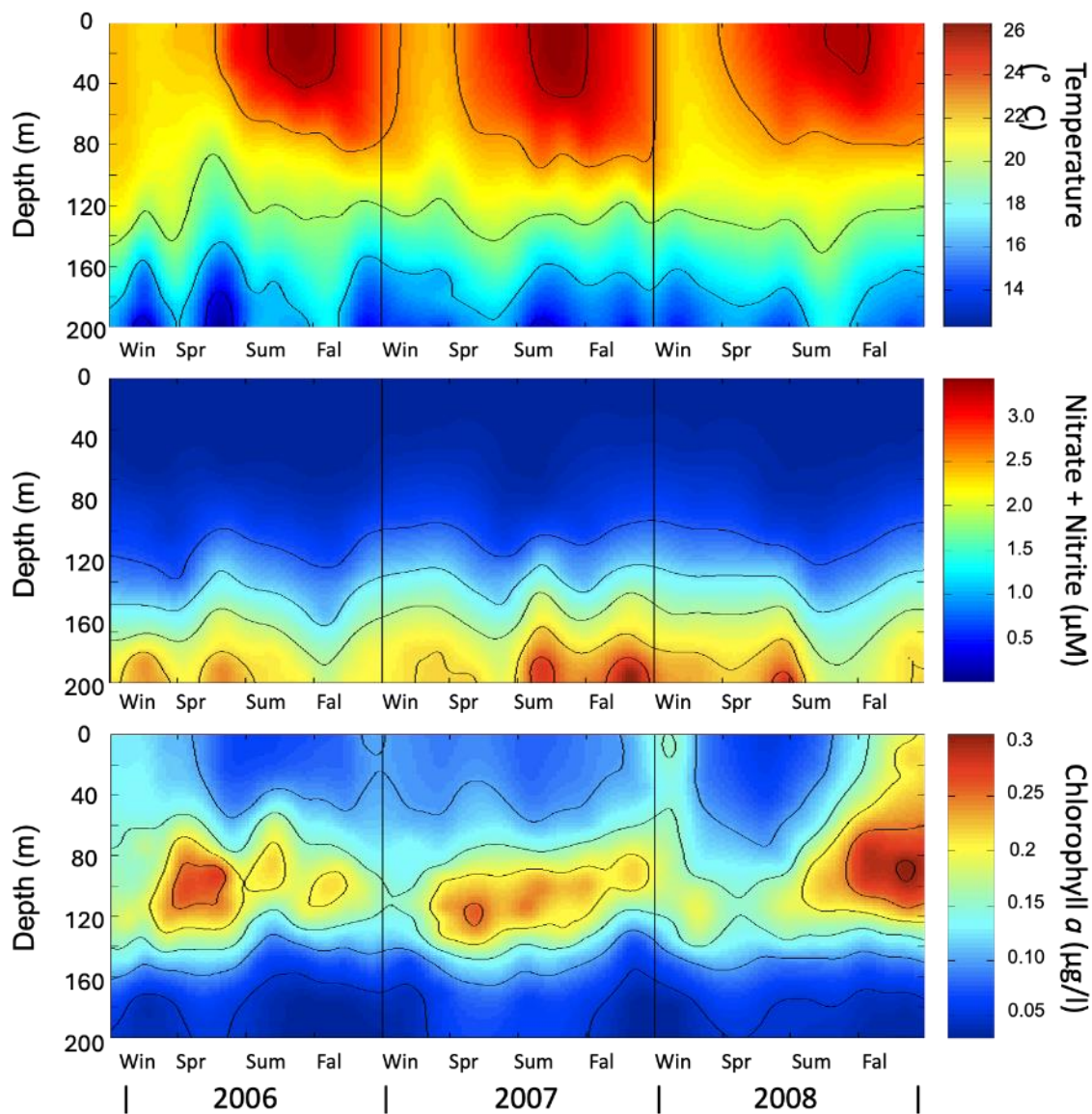
By focusing our sampling efforts at a single location in the North Pacific Subtropical Gyre, Station ALOHA, we were able to better understand the vertical and temporal relationships between nitrifying microorganisms and physical and biogeochemical dynamics in the open ocean. Samples were collected from the top of the epipelagic region (5-10 m) to the depths of the bathypelagic zone (4000 m) from near-monthly HOT cruises to Station ALOHA between 2005 and 2009. During the period of this study, near-surface ocean temperatures varied ~4°C, undergoing clear seasonal fluctuations, with elevated temperatures (25.9°C – 26.5°C) in the late summer/early fall, and lower temperatures (23.1°C – 24.9°C) in the winter and early spring (Figure 5).



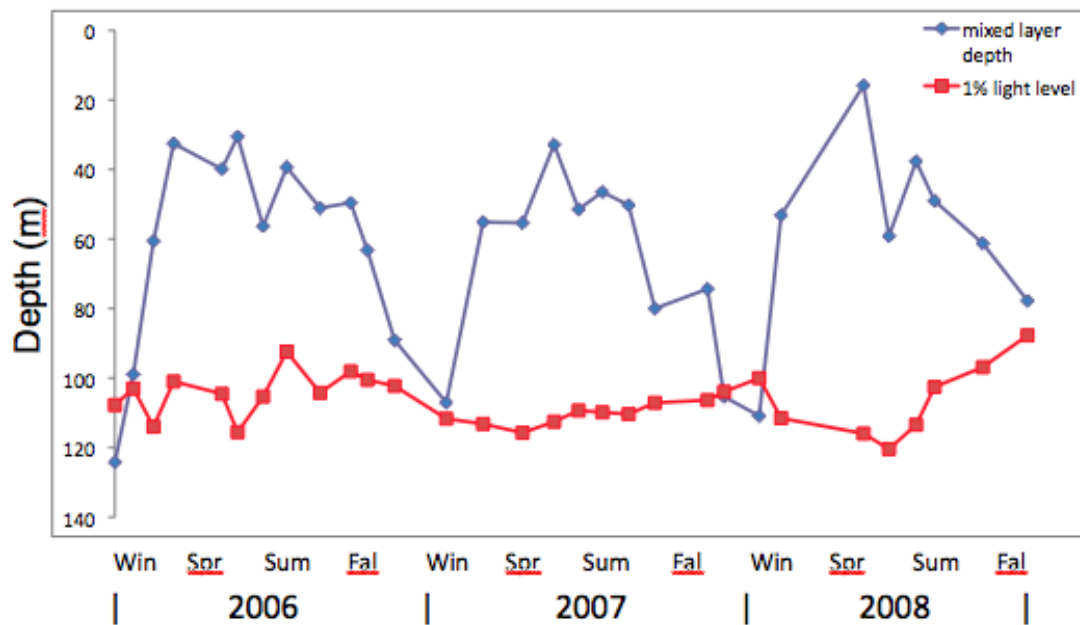
**Figure 4.** Depth profile of normalized *amoA* transcripts per *amoA* gene copy during the BULA transect. Y-axis is depth in meters. X-axis is the ratio of *amoA* transcripts to genes on a logarithmic scale. Dotted line represents 1:1 ratio.

These seasonal periods of lower temperature (winter months) reduced the density gradient between the nutrient-depleted surface waters and the more nutrient-enriched, dimly lit lower euphotic zone. As a result, the depth of the upper wind-driven mixed layer increased in the winter (53 – 125 m) and shoaled through the spring and summer (31 – 59 m) (Figure 6). The depth of penetration of sunlight through the water was much more seasonally invariant. The depth of the 1% surface isopleth (defined here as the base of the euphotic zone) was deeper than the mixed layer throughout most of the year (Figure 6). However, on selected occasions during winter months, the mixed layer penetrated below the euphotic zone (Figure 6). The resulting breakdown in stratification observed in the winter introduced N+N to the nutrient-depleted surface waters (Figure 5).

On average, inventories of N+N integrated through the upper 150 m increased from 46 mmol N m<sup>-2</sup> in the summer to 91 mmol N m<sup>-2</sup> in the winter. In contrast, the flux of particulate nitrogen out of the upper 150 m increased from 51 mmol N m<sup>-2</sup> day<sup>-1</sup> in the winter to 73 mmol N m<sup>-2</sup> day<sup>-1</sup> in the summer (Table 2). In the near surface waters, concentrations of chlorophyll *a* increased during the winter months (Figure 5); however, in the dimly lit regions of the lower euphotic zone, chlorophyll *a* concentrations decreased in the winter and increased in the spring and summer (Figure 5).



**Figure 5.** Contour plots depicting vertical and temporal distributions in ocean temperature (top panel), nitrate + nitrite, and chlorophyll *a* (bottom panel) at Station ALOHA from 2006 through 2008. Color bars represent temperature ( $^{\circ}\text{C}$ ), Nitrate + Nitrite ( $\mu\text{M}$ ) and chlorophyll *a* ( $\mu\text{g l}^{-1}$ ), respectively.



**Figure 6.** Temporal variation in mixed layer depth (diamonds) and 1% light level (squares) at Station ALOHA from 2006 to 2008.

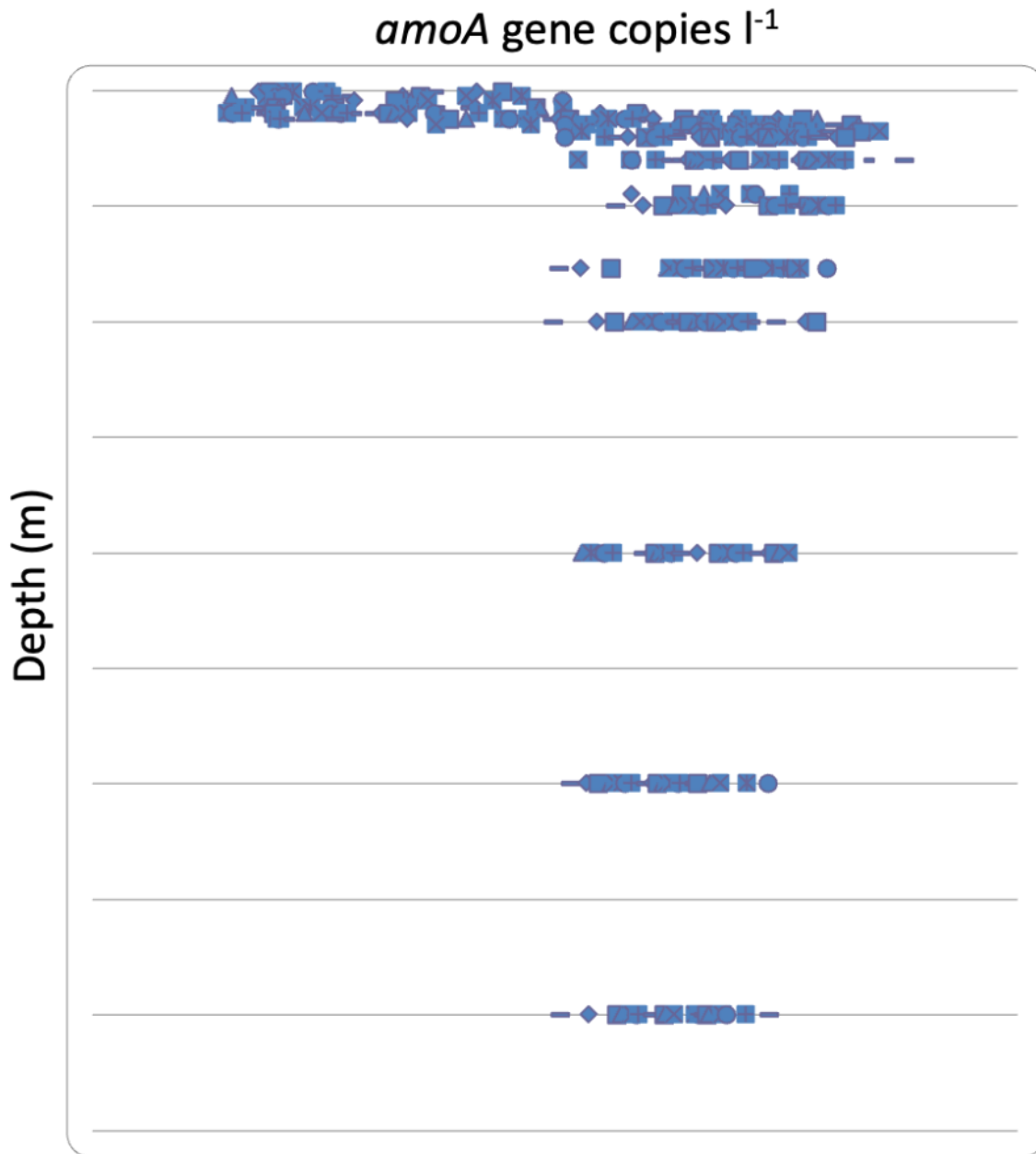
### ***Temporal variations in Crenarchaeal nitrifiers***

The four-year time-series study (2005-2009) on the distributions of ammonia oxidizing *Crenarchaea* demonstrated that the abundances of these organisms varied both vertically and in time. Total *amoA* gene abundances typically increased 3 to 4 orders of magnitude between the near-surface ocean and the depths of the bathypelagic (Figure 7). QPCR-based crenarchaeal *amoA* gene abundances in the near-surface ocean ranged from 0 to  $10^4$  copies  $l^{-1}$ , with abundances increasing through the epipelagic to an average of  $6 \times 10^5$  gene copies  $l^{-1}$  at the epi- mesopelagic boundary (200 m) (Figure 7). Abundances of crenarchaeal *amoA* genes in the upper mesopelagic zone (200 – 300 m) ranged between  $10^4$  to  $10^7$  copies  $l^{-1}$ , averaging  $1 \times 10^6$  copies  $l^{-1}$ , before decreasing  $\sim 10$ -fold with increasing depth in the lower mesopelagic zone. Crenarchaeal *amoA* gene concentrations in the bathypelagic waters were less variable than observed in the mesopelagic zone, varying between  $10^4$  to  $10^6$  copies  $l^{-1}$ , and averaging  $\sim 2 \times 10^5$  copies  $l^{-1}$  (Figure 7).

Crenarchaeal *amoA* gene abundances in the epipelagic waters demonstrated moderate seasonal variability. Throughout most of the year, *amoA* gene copy abundances in the near surface waters were either very low or below the limits of detection ( $\sim 10$  copies  $l^{-1}$ ). However, during periods of deeper winter mixing, gene abundances in the epipelagic increased to upwards of  $10^4$  copies  $l^{-1}$  in near-surface waters (Figure 8).

**Table 2.** Seasonally binned depth integrated (0-150 m) inventories of nitrate + nitrite and particulate nitrogen; particulate nitrogen fluxes, and depth integrated (0-150 m) *amoA* gene abundances at Station ALOHA. Both mean and ranges for the seasonally binned inventories and fluxes are provided.

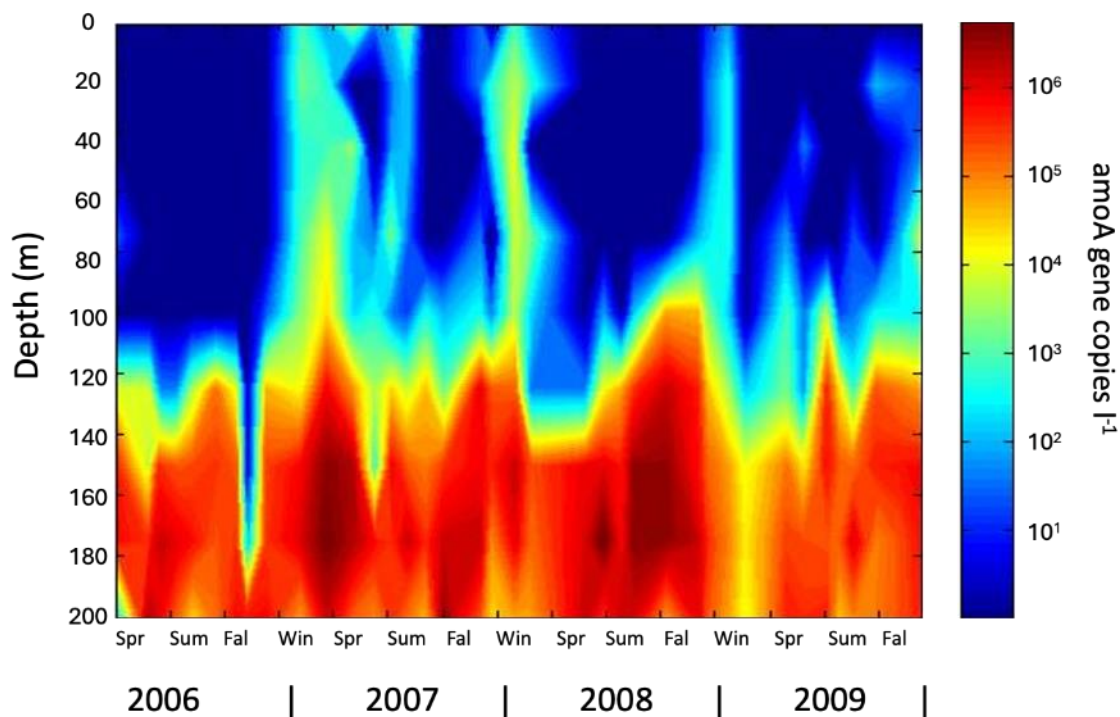
Seasons (0-150m)	Nitrate + nitrite (mmol N m <sup>-2</sup> )	Particulate N (mmol N m <sup>-2</sup> )	Nitrogen Flux (mmol N m <sup>-2</sup> day <sup>-1</sup> )	Total <i>amoA</i> genes (copies m <sup>-2</sup> )	Upper ocean clade <i>amoA</i> genes (copies m <sup>-2</sup> )
Winter	91 (13-283)	42 (36-49)	51 (31-80)	1.9×10 <sup>10</sup> (1.6×10 <sup>8</sup> - 7.5×10 <sup>10</sup> )	1.3×10 <sup>10</sup> (1.2×10 <sup>9</sup> - 3.9×10 <sup>10</sup> )
Spring	59 (11-154)	45 (44-48)	65 (51-76)	7.7×10 <sup>9</sup> (1.3×10 <sup>8</sup> - 3.5×10 <sup>10</sup> )	7.1×10 <sup>9</sup> (1.8×10 <sup>8</sup> - 2.0×10 <sup>10</sup> )
Summer	46 (20-149)	45 (38-51)	73 (46- 101)	1.0×10 <sup>10</sup> (1.6×10 <sup>9</sup> - 5.5×10 <sup>10</sup> )	1.8×10 <sup>10</sup> (1.8×10 <sup>9</sup> - 3.6×10 <sup>10</sup> )
Fall	54 (26-86)	43 (38-50)	43 (39-51)	2.2×10 <sup>10</sup> (1.4×10 <sup>8</sup> - 9.3×10 <sup>10</sup> )	3.1×10 <sup>10</sup> (3.5×10 <sup>9</sup> - 1.5×10 <sup>11</sup> )



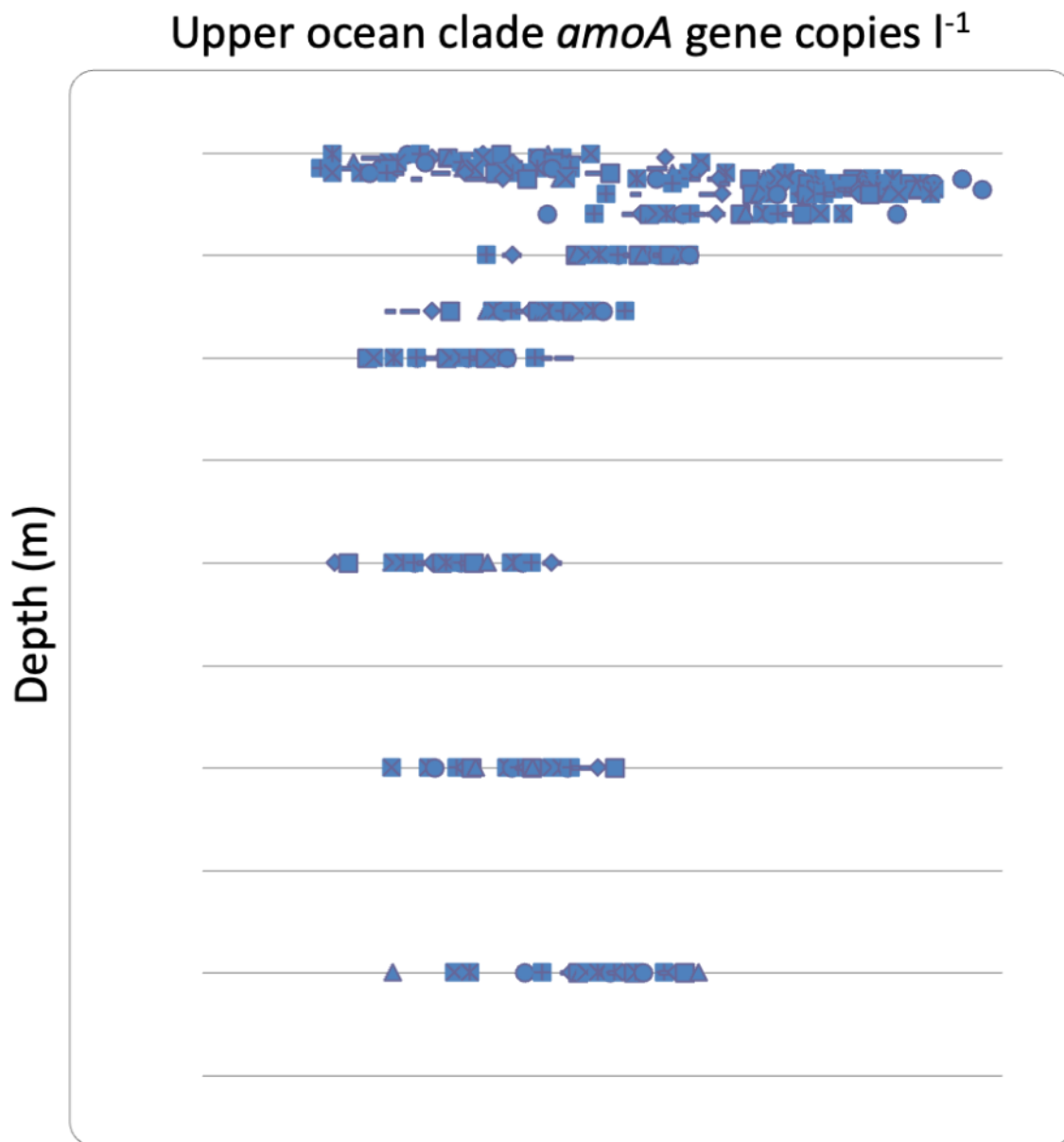
**Figure 7.** Depth profiles of *amoA* genes at Station ALOHA from 2006 through 2009. The X-axis is *amoA* gene copies per liter on a logarithmic scale, Y-axis is depth in meters.

By utilizing gene primers that selectively targeted a clade of *Crenarchaea* known to predominate in the upper mesopelagic zone (Beman *et al.*, 2008), we were able to quantitatively track distributions of ammonia-oxidizing *Crenarchaea* over time. Abundances of the upper ocean *amoA*-containing *Crenarchaea* averaged  $\sim 4 \times 10^2$  copies  $l^{-1}$  in near-surface waters, increasing  $\sim 4$  orders of magnitude into the upper mesopelagic zone (varying between  $3.5 \times 10^3 - 2.4 \times 10^6$  copies  $l^{-1}$  at 200 m depth). The gene abundances of this clade then decreased through the lower mesopelagic (Figure 9), averaging  $2.9 \times 10^2$  copies  $l^{-1}$  at 1000 m. Surprisingly, *amoA* genes associated with this upper ocean clade increased in abundance deeper in the bathypelagic waters, averaging nearly  $6 \times 10^3$  copies  $l^{-1}$  between 2000 and 4000 m depths (Figure 9).

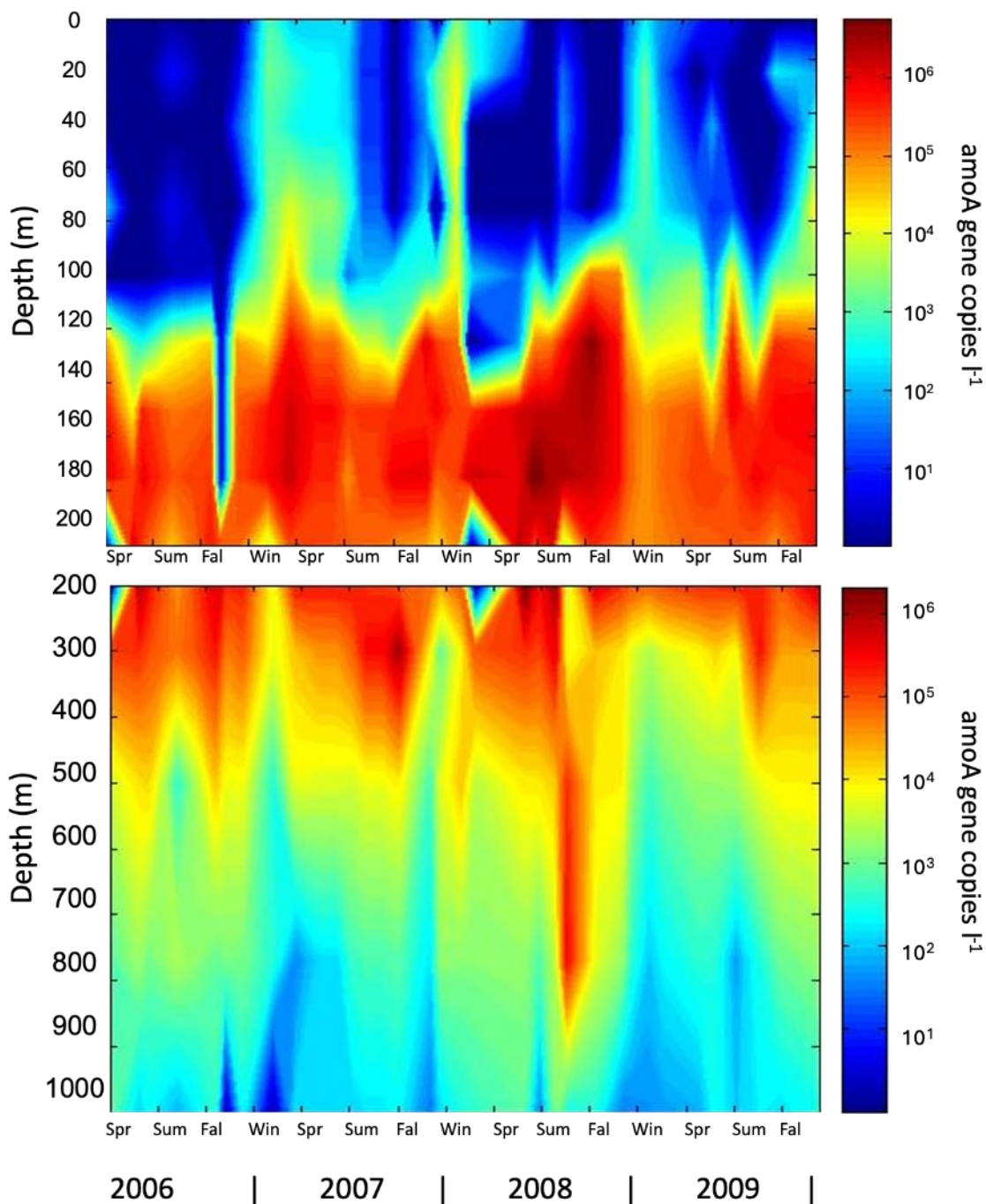
Comparison of the time-series upper ocean clade *amoA*-containing *Crenarchaea* to total archaeal *amoA* estimates were similar in the euphotic zone and upper waters of the mesopelagic zone. The upper clade *amoA* copy abundances peaked near the epi- mesopelagic boundary, averaging  $4 \times 10^5$  copies  $l^{-1}$ . Along this boundary, concentrations of upper clade *amoA* gene copies were relatively constant when compared to regions around the epi- mesopelagic boundary (Figure 10). Strikingly, *amoA* gene abundances above and below the epi- mesopelagic boundary appeared most variable in the winter, when abundances in the upper epipelagic increased by upwards of 3 orders of magnitude, while abundances decreased  $\sim 10$ -fold below this mid-depth boundary into the mesopelagic (Figure 10).



**Figure 8.** Contour plot of total *amoA* genes per liter seawater at Station ALOHA from 2006 through 2009. The Y-axis is depth in meters through the epipelagic. Seasons and years are plotted on the X-axis. The color bar represents *amoA* gene copies per liter on a logarithmic scale.



**Figure 9.** Depth profiles of upper ocean *amoA* clade gene copies at Station ALOHA from 2006 through 2009.



**Figure 10.** Contour plots of upper ocean clade *amoA* genes per liter seawater in the epipelagic (top panel) and mesopelagic (bottom panel) at Station ALOHA from 2006 through 2009. Seasons are plotted on the X-axis. Color bars represent upper ocean clade *amoA* gene copies per liter on a logarithmic scale.

## Chapter 4: Discussion

### *Crenarchaeal amoA genes and transcripts during the CMORE BULA transect*

Amplification of crenarchaeal *amoA* genes and RT-QPCR amplification of *amoA* mRNA transcripts across a 5200km transect in the Pacific Ocean provided new insights into the distributions and activities of *Crenarchaea* in the open ocean. Among the notable findings from this oceanic survey, we observed that *amoA* gene abundances typically increased three to four orders of magnitude between the well-lit near-surface waters and the lower mesopelagic zone. In contrast, crenarchaeal *amoA* transcripts tended to be elevated near the epi- mesopelagic boundary (slightly above 200 m). Normalizing *amoA* transcripts to *amoA* gene copies revealed active *amoA* gene expression in the well-lit regions of the ocean.

Using the *amoA* gene as proxy, we examined the abundances of ammonia oxidizing *Crenarchaea* and found their distributions to vary throughout the water column. Previous studies have shown that there are depth-dependent patterns in the abundances of *Archaea* in the ocean, with phylogenetically diverse groups of *Crenarchaea* inhabiting different depths in the ocean (Massana *et al.*, 2000; Francis *et al.*, 2005; Hallam *et al.*, 2006b; Mincer *et al.*, 2007; Beman *et al.*, 2008). Based on depth distributions of crenarchaeal *amoA* genes and <sup>15</sup>N-based measurements of ammonia oxidation in the Gulf of California, two phylogenetically distinct groups of *Crenarchaea* appear to be vertically separated throughout the water column (Beman *et al.*, 2008). The QPCR primers used throughout the BULA transect study were

designed to target both upper ocean and deep-sea archaea indiscriminately (Francis *et al.*, 2005). Although biogeochemical conditions varied strongly along this transect, we did not observe extreme differences in *amoA* gene abundances. The only exception was a near equatorial station (1.7°N), where *amoA* gene abundances in the mesopelagic were an order of magnitude higher than any other station (Figure 3b).

Studies describing the depth-structure in rates of ammonia oxidation seem consistent with our findings of peak *amoA* expression occurring above the dimly lit epi- mesopelagic boundary. In the North Pacific Subtropical Gyre (NPSG), rates of ammonia oxidation ranged between 2.2 and 7.3 nmol N l<sup>-1</sup> day<sup>-1</sup> with peak rates occurring around the primary nitrite maxima (100-175 m)(Olson 1981a). At Station ALOHA, rates of ammonia oxidation ranged between 1 to 134 nmol l<sup>-1</sup> day<sup>-1</sup> in the epipelagic with peak rates occurring optimally near the low light regions of the primary nitrite maximum (150-175 m)(Dore and Karl, 1996).

The processes regulating the physiological activities of ammonia oxidizing microorganisms in the ocean continues to be an active area of research. Changes in substrate availability (i.e. fluxes of ammonia), temperature, and possibly sunlight have all been considered as possible candidates to explain the observed depth-dependent patterns of *Crenarchaea* in the sea. Cultivation-based laboratory studies using ammonia oxidizing bacteria and natural populations of soil-dwelling *Crenarchaea* revealed that expression of the *amo* operon is regulated by external concentrations of ammonia (Treush *et al.*, 2005; Berube *et al.*, 2007; El Sheikh and

Klotz, 2008). In the open ocean, ammonia concentrations are typically in the 10 - 100 nmol l<sup>-1</sup> range (Lipschultz, 2001; Woodward and Rees, 2001; Rees *et al.*, 2006), and peak concentrations of ammonia often occur in the mid to lower euphotic zone (Gruber, 2008). These depth dependent changes in ammonia concentrations seem to be consistent with our results that demonstrated peak *amoA* transcripts occurring slightly above the epi-mesopelagic boundary (Figure 3). Our observations that when normalized to the number of gene copies, *amoA* transcription was greatest in the well-lit regions of the upper ocean may suggest that, despite low abundances (Figure 3), *Crenarchaea* could possibly have an important role in ammonia oxidation in the euphotic zone.

Rates of nitrification and expression of the *amoA* gene have also been shown to demonstrate a positive relationship with temperature (Tourna *et al.*, 2008). One of the most notable vertical gradients we sampled during the BULA transect was in temperature. In our study, upper ocean temperatures were 12°C to 16°C warmer than waters of the mesopelagic zone, so temperature may have played a role in controlling rates of ammonia oxidation, as reflected in elevated *amoA* transcripts in the upper ocean. However, despite observing elevated *amoA* gene transcription throughout warm upper ocean waters, least-squares linear regression analyses ( $P > 0.05$ ) revealed no significant temperature dependence of *amoA* transcription.

Sunlight has also been shown to have inhibitory effects on ammonia oxidation and the growth of nitrifying microorganisms (Olson, 1981b; Ward, 1987; Horrigan and Springer, 1990; Guerrero and Jones, 1996). To date, the exact

mechanisms causing ammonia monooxygenase photoinhibition remain unclear. Previous studies have shown that short wavelength radiation (< 410 nm) may have reversible photooxidative effects on the membrane-bound ammonia monooxygenase protein (Hooper and Terry, 1974; Hyman and Arp, 1992). The effects of sunlight on *amoA* expression by *Crenarchaea* remain unclear, but there are a few possibilities for explaining our observed increased *amoA* transcription occurring in the well-lit regions of the ocean. One important caveat regarding our observations that crenarchaeal *amoA* expression was elevated in well-lit regions in the ocean is that *amoA* transcriptional activities may or may not be indicative of actual ammonia oxidation rates. *Crenarchaea* may be actively transcribing *amoA* genes in well-lit regions of the ocean as a consequence of increased turnover rate of the ammonia monooxygenase protein, perhaps due to photooxidative damage. Future directions in interrogating the activities of ammonia-oxidizing *Crenarchaea* would ideally involve the examination of the effects of sunlight on the membrane-bound ammonia monooxygenase protein and rates of *amoA* expression. The information gained from a study like this will provide us with new ideas for understanding microbially mediated upper ocean nitrification.

### ***Crenarchaeal amoA genes and temporal fluctuations***

In addition to examining spatial changes in the distributions and transcriptional activities of ammonia oxidizing *Crenarchaea* we also evaluated temporal variability in crenarchaeal populations at Station ALOHA. For these studies, we utilized two sets of QPCR *amoA* primers designed to target all ammonia

oxidizing *Crenarchaea* (Francis *et al.*, 2005) and a clade of upper ocean dwelling ammonia oxidizing *Crenarchaea* (Beman *et al.*, 2008). We examined nearly four years of vertical profiles (5m to 4000 m) collected at approximately monthly intervals as part of the HOT program cruises. The purpose of this study was to see if there were spatial and temporal fluctuations in the abundance of nitrifying *Crenarchaea* and possibly relate any observed variability to other biogeochemical and physical parameters measured at Station ALOHA. Our intent was to gain insight into the factors that control temporal and spatial variability in ammonia oxidizing *Crenarchaea* distributions.

One of the most striking temporal changes we observed occurred in the well-lit regions of the euphotic zone. Station ALOHA undergoes moderate seasonality in biogeochemical and physical conditions, and this seasonality appeared to influence the *Crenarchaea*. During the wintertime, ocean surface temperatures decrease and destratify the upper regions of the euphotic zone. During this period, wind driven mixing deepens convective mixing, and on occasion, the depth of the mixed layer penetrated below the base of the euphotic zone. This deeper mixing appeared to transport waters enriched in *Crenarchaea* into the well-lit regions of the euphotic zone. With warming of the upper ocean in the spring and summer, the mixed layer depth shoaled well above the euphotic zone depth. During these well-stratified periods, abundances of *Crenarchaea* remained low or undetectable through the euphotic zone.

Prior studies on the abundances and depth-distributions of *Crenarchaea* show similar vertical patterns that we observed (Karner *et al.*, 2001; Mincer *et al.*, 2007). The most comparable study at Station ALOHA showed very similar depth distributions of *Crenarchaea*, with relative abundances typically very low (<10% total picoplankton) in the top 100 m of the epipelagic and increasing to account for upwards of half of the total picoplankton biomass below the epipelagic (Karner *et al.*, 2001). In our study of temporal and spatial variability of ammonia oxidizing *Crenarchaea*, we found similar changes in crenarchaeal abundances with depth, with *amoA* genes typically increasing 3 to 4 orders of magnitude between the near-surface ocean and the bathypelagic. The high abundances of *amoA* genes in the deep sea relative to concentrations in surface waters could be attributed to faster growth rates of *Crenarchaea* (Konneke *et al.*, 2005) compared to *Bacteria*, or might be a function of differential removal of these two cell populations.

Throughout most of the year, *amoA* gene abundances near the base of the euphotic zone (~100 m) are roughly four orders of magnitude higher than concentrations found in near-surface waters. During periods of winter mixing to depths below 100 m (Figure 6), we observed upwards of 10,000- fold increases in the abundance of *amoA* genes in near-surface waters. This striking increase in abundance in near-surface waters appeared linked to winter mixing. Our use of QPCR primers that differentiate a clade of upper ocean *amoA* genes revealed that the time-variability associated in crenarchaeal abundances in the upper ocean was almost exclusively driven by changes to these upper ocean clades.

Our study highlights apparent seasonal variability in crenarchaeal abundances in the upper ocean waters. This seasonality appeared linked to deepening of the upper mixed layer and subsequent entrainment of *Crenarchaea* into the upper ocean. However, there may also be moderate seasonality in *Archaea* in the mesopelagic zone. Our data suggest a moderate increase in archaeal abundances in the mesopelagic waters in the summer and late fall. The mechanisms underlying the apparent summer-fall increases in *amoA* gene abundances in the mesopelagic zone remain unknown. However, the 22+ year time series at Station ALOHA reveals that particulate nitrogen flux out of the euphotic zone increases approximately two-fold in the summer months (Table 2; Karl *et al.* 1996). The increase in crenarchaeal abundance in mesopelagic might be attributed to this enhanced nitrogen flux, thereby reflecting a response to increased substrate (ammonia) availability.

Below approximately 500 m depth, the upper ocean *amoA* clade abundances decreased as much as 3 orders of magnitude, comprising <1% of the total crenarchaeal *amoA* gene abundances in the deeper regions of the mesopelagic. The decreasing contribution of this upper-ocean dwelling clade of *Crenarchaea* could be linked to the vertical attenuation of particle fluxes through the mesopelagic zone (Karl *et al.*, 1984). If true, we would anticipate that the kinetics of substrate utilization might differ among the upper ocean and deep-sea clades of *Crenarchaea*, with the upper ocean clade better adapted to utilizing the seasonally elevated concentrations of ammonia that coincide with particle fluxes. Such results reinforce

the importance of continued effort to cultivate these different groups of microorganisms for laboratory-based hypothesis testing.

Based on the results of this study, it remains unclear what processes control population dynamics of *Crenarchaea* growing in the dimly lit regions of the deep sea. Although fluxes of ammonia to the deep sea are likely low and infrequent, *Crenarchaea* may potentially utilize other metabolic pathways for sustaining growth. While our study highlights that numerous and diverse groups of *Crenarchaea* in the Pacific Ocean are genetically capable of oxidizing ammonia, other modes of obtaining energy such as through chemoorganoheterotrophy are certainly possible. Various other studies have shown that *Crenarchaea* can assimilate organic and inorganic carbon substrates during growth (Teira *et al.*, 2006; Kirchman *et al.*, 2007; Varela *et al.*, 2008; Yamikov *et al.*, 2009). Moreover, metagenomic analyses indicate that these microbes may use other reduced forms of nitrogen, such as urea, as sources of energy (Hallam *et al.*, 2006b). Future studies directed toward the understanding the metabolism of *Crenarchaea* would be valuable in helping to understand controls on population ecology of these abundant members of ocean plankton.

## Chapter 5: Conclusion

*Crenarchaea* appear to be important regulators of the marine nitrogen cycle. We conducted two separate, but closely related studies of temporal and meridional crenarchaeal distributions to shed insight on factors controlling nitrifying *Crenarchaea*. QPCR amplification of crenarchaeal *amoA* genes provided us with information on the spatial distributions of these nitrifying microorganisms, while RT-QPCR amplification of *amoA* gene transcripts provided us with insight on crenarchaeal activities. During both studies, crenarchaeal abundances displayed strong vertical structure with greatest abundances occurring near the dimly lit epimesopelagic boundary. During our time series study at Station ALOHA, we observed a  $\sim 10^4$  increase in crenarchaeal abundances in the well-lit upper ocean coincident with increases in winter mixing; these dynamics appear to have introduced mesopelagic *Crenarchaea* into the epipelagic waters. Ecologically speaking, the presence of *Archaea* in near-surface waters during winter months may have an effect on the occurrence of nitrification happening in this region of the water column. Using QPCR primers that are meant to specifically target an upper ocean dwelling clade of *Crenarchaea*, we observed seasonal changes occurring throughout the mesopelagic zone. During summer months, increased particulate nitrogen flux to the deep sea may sustain elevated archaeal population abundances. This may influence carbon cycling dynamics below the photic zone, as ammonia oxidizers may use the energy gained from ammonia oxidation to assimilate inorganic carbon. Based on patterns of *amoA* gene expression across the Pacific Ocean, we found that

transcript abundances were less variable with depth, when compared to *amoA* gene abundances. Normalizing *amoA* transcripts to gene copy abundances revealed that, despite low abundances, *Crenarchaea* may be physiologically active in ammonia oxidation throughout the euphotic zone.

Future studies directed towards the factors that control crenarchaeal physiology (*e.g.* ammonia, light, temperature, dissolved oxygen) and abundance (*e.g.* grazers and viral lysis), will help provide a better understanding for factors that control ammonia oxidation in the sea. A likely avenue for us to pursue will be the examination of time-dynamics associated with crenarchaeal *amoA* gene expression at Station ALOHA and its possible relationship to the above-mentioned environmental parameters. With this information, we would gain additional insight into the dynamics associated with microbes mediating a crucial process in the marine nitrogen cycle.

## References

- Agogue, 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**: 788-792.
- 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 California. *International Society for Microbial Ecology Journal* **2**: 429-441.
- Berube, P.M., Samudrala, R., and Stahl, D.A. (2007) Transcription of all *amoC* copies is associated with recovery of *Nitrosomonas europaea* from ammonia starvation. *Journal of Bacteriology* **189**: 3935-3944.
- Bidigare, R.R., Van Heukelem, L., Trees, C.C., (2005) Analysis of algal pigments by high performance liquid chromatography. In *Algal Culturing Techniques*. Anderson R.A. Academic Press, New York, pp. 327-345.
- Carpenter, J.H. (1965) The accuracy of the Winkler method for dissolved oxygen analysis. *Limnology and Oceanography* **10**: 135-140.

- Church, M.J., Wai, B.R.K., Karl, D.M., DeLong, E.F. (2009) Abundances of crenarchaeal *amoA* genes and transcripts in the Pacific Ocean. *Environmental Microbiology* **12**: 679-688.
- DeLong, E.F. (1992) Archaea in coastal marine environments. *Proceedings of the National Academy of Sciences USA* **89**: 5685-5689.
- DeLong, E.F., Taylor, L.T., Marsh, T.L., Preston, C.M. (1999) Visualization and enumeration of marine planktonic archaea and bacteria by using polynucleotide probes and fluorescent in situ hybridization. *Applied and Environmental Microbiology* **65**: 5554-5563.
- DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N.U., *et al.* (2006) Community genomics among stratified microbial assemblages in the ocean's interior. *Science* **311**: 496-503.
- Devol, A.H. c2008 Denitrification including Anammox. In *Nitrogen in the Marine Environment*. Capone, D.G., Bronk, D.A., Mulholland, M.R., and Carpenter, E.J. (eds). Amsterdam, The Netherlands: Elsevier, pp. 263-264.

Dore, J.E., Houlihan, T., Hebel, D.V., Tien, G., Tupas, I., Karl, D.M. (1996) Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater. *Marine Chemistry* **53**: 173-185.

Dore, J.E., and Karl, D.M. (1996a) Nitrification in the euphotic zone as a source for nitrite, nitrate, and nitrous oxide at Station ALOHA. *Limnology and Oceanography* **41**: 1619-1628.

Ducklow, H. (2000) Bacterial Production and Biomass in the Oceans. In *Microbial Ecology of the oceans*. Kirchman, D.L. Wiley, New York, pp. 85-120.

El Sheikh, A.F., and Klotz, M.G. (2008) Ammonia-dependent differential regulation of the gene cluster that encodes ammonia monooxygenase in *Nitrosococcus oceani* ATCC 19707. *Environmental Microbiology* **10**: 3026-3035.

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. *Proceedings of the National Academy of Sciences USA* **102**: 14683-14688.

Friaz-Lopez, J., Shi, Y., Tyson, G.W., Coleman, M.L., Schuster, S.C., Chisholm, S.W., *et al.*

(2008) Microbial community gene expression in ocean surface waters. *Proceedings of the National Academy of Sciences USA* **105**: 3805-3810.

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

Gruber N. c2008 The marine nitrogen cycle: overview and challenges. In *Nitrogen in the Marine Environment*. Capone, D.G., Bronk, D.A., Mulholland, M.R., and Carpenter, E.J. (eds). Amsterdam, The Netherlands: Elsevier, pp. 1-4.

Guerrero, M.A., and Jones, R.D. (1996) Photoinhibition of marine nitrifying bacteria. 1. Wavelength-dependent response. *Marine Ecology Progress Series* **141**: 183-192.

Hallam, S.J., Konstantinidis, K.T., Putnam, N., Schleper, C., Watanabe, Y., Sugahara, J., *et al.* (2006a) Genomic analysis of the uncultivated marine crenarchaeote *Cenarchaeum symbiosum*. *Proceedings of the National Academy of Sciences USA* **103**: 18296-18301.

Hallam S.J., Mincer, T.J., Schleper, C., Preston, C.M., Roberts, K., Richardson, P.M. *et al.*

(2006b) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. *Public Library of Science Biology* **4**: 520-536.

Hansman, R.L., Griffin, S., Watson, J., Druffel, E.R.M., Ingalls, A.E., and Aluwihare, L.I.

(2009) The radiocarbon signature of microorganisms in the mesopelagic ocean. *Proceedings of the National Academy of Sciences USA* **106**: 6513-6518.

Herndl, G.J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., *et al.* (2005)

Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. *Applied and Environmental Microbiology* **71**: 2303-2309.

Hooper, A.B., and Terry, K.R. (1974) Photoinactivation of ammonia oxidation in

*Nitrosomonas*. *Journal of Bacteriology* **119**: 899-906.

Horrigan, S.G., and Springer, A.L. (1990) Oceanic and estuarine ammonium oxidation

– effects of light. *Limnology and Oceanography* **35**: 479-482.

Hyman, M.R., and Arp, D.J. (1992)  $^{14}\text{C}_2\text{H}_2$  labeling and  $^{14}\text{C}\text{O}_2$  labeling studies of

the *de novo* synthesis of polypeptides by *Nitrosomonas europaea* during recovery from acetylene and light inactivation of ammonia monooxygenase.

*Journal of Biological Chemistry* **267**: 1534-1545.

Ingalls, A.E., Shah, S.R., Hansman, R.L., Aluwihare, L.I., Santos, G.M., Druffel, E.R.M., *et al.* (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. *Proceedings of the National Academy of Sciences USA* **103**: 6442-6447.

Jenkins, B.D., Zehr J.P. c2008 Molecular approaches to the nitrogen cycle. In *Nitrogen in the Marine Environment*. Capone, D.G., Bronk, D.A., Mulholland, M.R., and Carpenter, E.J. (eds). Amsterdam, The Netherlands: Elsevier, pp. 1328-1329.

Karl, D.M., Bjorkman, K.M., Dore, J.E., Fujieki, L., Hebel, D.V., Houlihan, T., Letelier, R.M., Tupas, L.M. (2001) Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. *Deep Sea Research II* **48**: 1529-1566.

Karl, D.M., Christian, J.R., Dore, J.E., Hebel, D.V., Letelier, R.M., Tupas, L.M., Winn, C.D. (1996) Seasonal and interannual variability in primary production and particle flux at Station ALOHA. *Deep Sea Research II* **43**: 539-568.

Karl, D.M., Knauer, G.A., Martin, J.H., and Ward, B.B. (1984) Bacterial chemolithotrophy in the ocean is associated with sinking particles. *Nature* **309**: 54-56.

- 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.
- 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, *Limnology and Oceanography* **52**: 495-507.
- Konneke, 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.
- Lipshultz, F. (2001) A time-series assessment of the nitrogen cycle at BATS. *Deep Sea Research II* **48**: 1897-1924.
- Mackenzie, F.T. c2003. Our Changing Planet. Upper Saddle River, New Jersey: Pearson Education, Inc. An Introduction to Earth System Science and Global Environmental Change, 3<sup>rd</sup> ed. pp. 186-187, 189-190, 317-321, 333.
- Martin-Cuadrado, A.B., Rodriguez-Valera, F., Moriera, D., Alba, J.C., Ivars-Martinez, 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. *International Society for Microbial Ecology Journal* **2**: 865-886.

Massana, R., DeLong, E.F., and Pedros-Alio, C. (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. *Applied and Environmental Microbiology* **66**: 1777-1787.

Mincer, T.J., Church, M.J., Taylor, L.T., Preston, C., Karl, D.M., and DeLong, E.F. (2007) Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. *Environmental Microbiology* **9**: 1162-1175.

Olson, R.J. (1981a) <sup>15</sup>N tracer studies of the primary nitrite maximum. *Journal of Marine Research* **39**: 203-226.

Olson, R.J. (1981b) Differential photoinhibition of marine nitrifying bacteria – a possible mechanism for the formation of the primary nitrite maximum. *Journal of Marine Research* **39**: 227-238.

Ouverney, C.C., and Fuhrman, J.A. (2000) Marine planktonic Archaea take up amino acids. *Applied and Environmental Microbiology* **66**: 4829-4833.

- Pearson, A., McNichol, A.P., Benitez-Nelson, B.C., Hayes, J.M., and Eglinton, T.I. (2001) Origins of lipid biomarkers in Santa Monica Basin surface sediment: a case study using compound specific delta <sup>14</sup>C analysis. *Geochimica Cosmochimica Acta* **65**: 3123-3137.
- Poretsky, R.S., Bano, N., Buchan, A., Leclair, G., Kleikemper, J., Pickering, M., *et al.* (2005) Analysis of microbial gene transcripts in environmental samples. *Appl Environmental Microbiology* **66**: 5368-5382.
- Rees, A.P., Woodward, E.M.S., and Joint, I. (2006) Concentrations and uptake of nitrate and ammonium in the Atlantic Ocean between 60 degrees N and 50 degrees S. *Deep Sea Research II* **53**: 1649-1665.
- Teira, E., van Aken, H., Veth, C., and Herndl, G.J. (2006) Archaeal uptake of enantiomeric amino acids in the deep water masses of the North Atlantic. *Limnology and Oceanography* **51**: 2131-2144.
- Tourna, M., Freitag, T.E., Nichol, G.W., and Prosser, J.I. (2008) Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environmental Microbiology* **10**: 1357-1364.
- 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.

*Environmental Microbiology* **7**: 1985-1995.

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

Wada, E., and Hattori, A. (1971) Nitrite metabolism in the euphotic layer of the central North Pacific Ocean. *Limnology and Oceanography* **16**: 766-772.

Ward, B.B. (1987) Nitrogen transformations in the Southern California Bight.

*Deep Sea Research I* **34**: 785-805.

Ward, B.B. c2008 Nitrification in Marine Systems. In *Nitrogen in the Marine Environment*. Capone, D.G., Bronk, D.A., Mulholland, M.R., and Carpenter, E.J. (eds). Amsterdam, The Netherlands: Elsevier, pp. 200, **1328-1329**.

Woodward, E.M.S., and Rees, A.P. (2001) Nutrient distributions in an anticyclonic eddy in the northeast Atlantic Ocean, with reference to nanomolar ammonium concentrations. *Deep Sea Research II* **48**: 775-793.

- Wuchter, C., Abbas, B., Coolen, M.J.L., Hertfort, L., van Bleijswijk, J., Timmers, P., *et al.* (2006) Archaeal nitrification in the ocean. *Proceedings of the National Academy of Sciences USA* **103**: 12317-12322.
- Yamikov, M.M., La Cono, V., Denaro, R. (2009) A first insight into the occurrence and expression of functional amoA and accA genes of autotrophic and ammonia-oxidizing bathypelagic *Crenarchaeota* of Tyrrhenian Sea. *Deep-Sea Research II* **56**: 748-754.
- Yoshida, N., Morimoto, H., Hirano, M., Koike, I., Matsuo, S., Wada, E., *et al.* (1989) Nitrification rates and <sup>15</sup>N abundances of N<sub>2</sub>O and NO<sub>3</sub><sup>-</sup> in the Western North Pacific. *Nature* **342**: 895-897.