Abundances of crenarchaeal amoA genes and transcripts in the Pacific Ocean

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Summary

Planktonic Crenarchaea are thought to play a key role in chemolithotrophic ammonia oxidation, a critical step of the marine nitrogen (N) cycle. In this study, we examined the spatial distributions of ammonia-oxidizing Crenarchaea across a large (~5200 km) region of the central Pacific Ocean. Examination of crenarchaeal 16S rRNA, ammonia monooxygenase subunit A (amoA) genes, and amoA transcript abundances provided insight into their spatial distributions and activities. Crenarchaeal gene abundances increased three to four orders of magnitude with depth between the upper ocean waters and dimly lit waters of the mesopelagic zone. The resulting median value of the crenarchaeal amoA: 16S rRNA gene ratio was 1.3, suggesting the majority of Crenarchaea in the epi- and mesopelagic regions of the Pacific Ocean have the metabolic machinery for ammonia oxidation. Crenarchaeal amoA transcript abundances typically increased one to two orders of magnitude in the transitional zone separating the epipelagic waters from the mesopelagic (100–200 m), before decreasing into the interior of the mesopelagic zone. The resulting gene copy normalized transcript abundances revealed elevated amoA expression in the upper ocean waters (0–100 m) where crenarchaeal abundances were low, with transcripts decreasing into the mesopelagic zone as crenarchaeal gene abundances increased. These results suggest ammonia-oxidizing Crenarchaea are active contributors to the N cycle throughout the epi- and mesopelagic waters of the Pacific Ocean.

Introduction

The physiological activities of diverse groups of planktonic microorganisms form major controls on the transformation and availability of nitrogen (N) containing compounds in the sea. Nitrification is the aerobic oxidation of ammonia (NH3) to nitrate (NO3−). The process occurs through two independent steps initiated by the oxidation of ammonia to nitrite (ammonia oxidation), followed by oxidation of nitrite to nitrate (nitrite oxidation). The two steps of the complete nitrification reaction are facilitated by different groups of microorganisms. Recent cultivation-dependent and independent approaches have revealed that members of the marine Crenarchaea appear to play an important role in ammonia oxidation, while bacterial genera, including Nitrospina may mediate nitrite oxidation (Francis et al., 2005; Konneke et al., 2005; Wuchter et al., 2006; Mincer et al., 2007). Over the past two decades, non-thermophilic archaea have increasingly become recognized as abundant, ubiquitous, and dynamic components of the ocean plankton (Delong, 1992; Fuhrman et al., 1992; Karner et al., 2001). This recognition has stemmed in large part from advances in geochemical and molecular-based approaches to study these microorganisms. 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., 2006a; 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) have provided insight into marine crenarchaeal metabolism, indicating these microorganisms may rely on metabolic strategies that include chemolithoautotrophy and assimilation of organic matter. Isolation of the ammonia-oxidizing Crenarchaeon Nitrosopumilus maritimus (Konneke et al., 2005) revealed that chemolithoautotrophic growth on ammonia resulted in stoichiometric production of nitrite. There is also evidence that marine Crenarchaea are capable of assimilating organic matter (Ouverney and Fuhrman, 2000; Herndl et al., 2005; Kirchman et al., 2007; Varela et al., 2008). The relative contribution of different carbon sources and modes of 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), with chemoorganoheterotrophy postulated to predominate in the cold, dark waters of the ocean’s bathypelagic interior (Agogue et al., 2008).

Despite the potential importance of Crenarchaeota to the marine N cycle, there is only limited information available on the distributions and physiological activities of these microorganisms. In this study, we examined the transcriptional activities and distributions of presumptive nitrifying marine Crenarchaeota sampled throughout the euphotic zone and into the mesopelagic regions (< 1000 m) across a ~5200 km region of the Pacific Ocean. Quantitative PCR-derived estimates of crenarchaeal gene abundances indicate that Crenarchaeota are ubiquitous components of the mesopelagic microbial assemblage. Moreover, reverse transcriptase quantitative PCR (RT-QPCR) amplification of amoA transcripts revealed active gene expression throughout both the epi- and mesopelagic waters of the Pacific Ocean. These data provide additional support that Crenarchaeota actively contribute to ammonia oxidation in many different oceanic provinces, and further suggest that despite their lower abundance in the upper ocean, these microorganisms may be active in N cycling.

**Results**

**Biogeochemical and hydrographic variability**

Sampling for this study included a wide range of spatially distinct oceanic environments that included well-lit, epipelagic waters to the cold, dimly lit regions of the mesopelagic zone. The most prominent 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. Upward doming of the thermocline in the equatorial region carried cold, nutrient-enriched, O2-depleted waters closer to the ocean’s surface. The region of most intense upwelling (as reflected in both uplift of isotherms and nutrient distributions) occurred in the northern equatorial waters (8.6°N; Fig. 1). In contrast, upper ocean nutrient concentrations in the northern and southern subtropical gyres were very low (e.g. average N+K concentrations in the upper 100 m in these regions were generally < 100 nmol l⁻¹). Among the most prominent meridional patterns observed in the waters of the mesopelagic zone (> 300 m) were changes in concentrations of N+K and dissolved O2, with N+K concentrations generally increasing concomitant with decreases in dissolved O2 concentrations from south to north (Fig. 1).

**Distributions of crenarchaeal amoA and 16S rRNA genes**

Vertical profiles of QPCR-derived crenarchaeal amoA gene abundances varied strongly with depth. Gene abundances typically increased three to four orders of magnitude between the near-surface ocean and the lower mesopelagic zone (Figs 2 and 3). Crenarchaeal amoA gene abundances in the near surface ocean ranged from...
$1 \times 10^2$ to $7 \times 10^3$ copies l$^{-1}$, with concentrations increasing through the lower euphotic zone, and ranging from $2 \times 10^2$ to $2 \times 10^7$ copies l$^{-1}$ through the mesopelagic waters (300–1000 m; Fig. 3).

Despite distinct differences in biogeochemical properties, with one exception, there were no notable differences in amoA gene abundances among the various stations sampled (Fig. 3). The one exception occurred within the mesopelagic waters of the station sampled closest to the equator (1.7°N). Below the euphotic zone at this station, amoA gene abundances were approximately an order of magnitude greater (exceeding $10^7$ amoA copies l$^{-1}$) than those sampled at any other station in this study (Fig. 3; Table 1).

We also examined the vertical and meridional distributions of Crenarchaeota based on QPCR amplification of Marine Group I (MGI) and psL12 phylotype crenarchaeal 16S rRNA genes. Similar to the distributions observed in amoA genes, the MGI 16S rRNA gene abundances were relatively low ($10^2$–$10^3$ copies l$^{-1}$) in the upper ocean (< 100 m) and increased three to four orders of magnitude below the epipelagic layer (Fig. 3). Similar to patterns observed in amoA gene distributions, there were no clear regional differences in MGI crenarchaeal 16S rRNA genes, except for the large increase in gene abundances in the mesopelagic waters near the equator (1.7°N) (Fig. 3; Table 1).

Gene abundances of the psL12 crenarchaeal phylotype were consistently lower than those of the MGI, with psL12 gene abundances ranging from below detection (less than ~20 copies l$^{-1}$) in the upper ocean, to a maximum of $1 \times 10^5$ copies l$^{-1}$ in the mesopelagic waters (Fig. 2). Similar to the distributions of the other crenarchaeal genes, the psL12 16S rRNA abundances generally increased with depth with peak gene abundances ($10^8$ to $10^9$ copies l$^{-1}$) often occurring between 125 and 300 m depth (Fig. 3). As observed with the MGI and amoA gene abundances, concentrations of the psL12 phylotype were greatest at the station near the equator (1.7°N). Unlike the other phylotypes measured in this study, the psL12 gene
absolutely measured at this station were elevated throughout the upper ocean rather than the mesopelagic waters (Fig. 3).

Comparing the amoA gene abundances to the sum of the crenarchaeal 16S rRNA gene abundances \((\text{MGI} + \text{psL12})\) revealed that at most depths and stations, amoA gene abundances were very similar to 16S rRNA gene abundances (Fig. 3; Table 1). The resulting crenarchaeal amoA : 16S rRNA gene ratios did not vary significantly with depth (one-way analysis of variance, \(P = 0.06\)), although at several stations, the ratio was elevated in the upper 150 m of the ocean and declined

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**Table 1.** Variability in depth-averaged concentrations of nitrate + nitrite \((\text{N-N})\), dissolved \(\text{O}_2\), crenarchaeal genes \((\text{amoA} 16\text{~S} \text{rRNA})\), and crenarchaeal amoA gene transcripts at stations sampled for this study.

| Property                | 16.0°S | 7.5°S | 3.0°S | 1.7°N | 5.1°N | 8.6°N | 13.5°N | 16.9°N | 22.75°N |
|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|---------|
| MLD (m)                 | 47    | 68    | 90    | 110   | 102   | 81    | 66    | 29    | 55      |
| 1% PAR (m)              | ND    | ND    | ND    | 104   | 116   | 107   | 163   | 127   | 115     |
| 0–100 m                 |       |       |       |       |       |       |       |       |         |
| N-N (\(\mu\text{mol} \text{~N~m}^{-1}\)) | \(3 \times 10^{-2}\) | \(1.4 \times 10^{-1}\) | 3.9    | 4.9    | 2.8   | 1.8   | \(6 \times 10^{-3}\) | \(5 \times 10^{-3}\) | \(4 \times 10^{-3}\) |
| \(\text{O}_2\) (\(\mu\text{mol} \text{~O}_2 \text{~m}^{-1}\)) | 209   | 202   | 203   | 196   | 202   | 200   | 213   | 215   | 213     |
| amoA genes (genes \(\text{m}^{-1}\)) | \(2.5 \times 10^3\) | \(2.3 \times 10^3\) | \(1.0 \times 10^4\) | \(2.4 \times 10^4\) | \(6.7 \times 10^2\) | \(7.5 \times 10^2\) | ND     | \(8.5 \times 10^2\) | \(8.2 \times 10^2\) |
| MGI + psL12 rRNA genes (genes \(\text{m}^{-1}\)) | \(1.8 \times 10^5\) | \(1.4 \times 10^5\) | \(1.6 \times 10^5\) | \(1.0 \times 10^5\) | \(3.9 \times 10^3\) | \(8.4 \times 10^2\) | ND     | \(5.4 \times 10^2\) | \(5.2 \times 10^2\) |
| amoA transcripts (transcripts \(\text{m}^{-1}\)) | \(8.1 \times 10^4\) | \(4.0 \times 10^4\) | \(1.3 \times 10^5\) | \(3.5 \times 10^5\) | \(2.5 \times 10^4\) | \(6.4 \times 10^3\) | ND     | \(4.8 \times 10^3\) | \(2.5 \times 10^3\) |
| amoA transcripts per amoA gene | \(3.2 \times 10^7\) | \(1.8 \times 10^7\) | \(1.3 \times 10^8\) | \(1.5 \times 10^7\) | \(3.7 \times 10^6\) | \(8.5 \times 10^5\) | ND     | \(5.6 \times 10^5\) | \(3.1 \times 10^5\) |
| 100–300 m               |       |       |       |       |       |       |       |       |         |
| N-N (\(\mu\text{mol} \text{~N~m}^{-1}\)) | 3.4    | 8.9    | 20    | 21    | 23    | 28    | 18    | 8.9   | 3.4     |
| \(\text{O}_2\) (\(\mu\text{mol} \text{~O}_2 \text{~m}^{-1}\)) | 214   | 193   | 170   | 173   | 151   | 119   | 173   | 243   | 207     |
| amoA genes (genes \(\text{m}^{-1}\)) | \(8.6 \times 10^5\) | \(1.6 \times 10^6\) | \(2.3 \times 10^5\) | \(1.8 \times 10^5\) | \(1.4 \times 10^6\) | \(1.6 \times 10^5\) | ND     | \(4.9 \times 10^5\) | \(6.8 \times 10^5\) |
| MGI + psL12 rRNA genes (genes \(\text{m}^{-1}\)) | \(9.9 \times 10^5\) | \(3.3 \times 10^6\) | \(2.5 \times 10^5\) | \(1.7 \times 10^5\) | \(1.7 \times 10^5\) | \(5.6 \times 10^4\) | ND     | \(4.1 \times 10^5\) | \(1.9 \times 10^5\) |
| amoA transcripts (transcripts \(\text{m}^{-1}\)) | \(7.1 \times 10^4\) | \(2.2 \times 10^5\) | \(3.9 \times 10^5\) | \(5.3 \times 10^5\) | \(2.1 \times 10^5\) | \(5.0 \times 10^4\) | ND     | \(2.1 \times 10^5\) | \(3.1 \times 10^5\) |
| amoA transcripts per amoA gene | \(8.3 \times 10^4\) | \(1.4 \times 10^5\) | \(1.7 \times 10^5\) | \(2.8 \times 10^5\) | \(1.5 \times 10^4\) | \(3.1 \times 10^4\) | ND     | \(4.3 \times 10^4\) | \(4.5 \times 10^4\) |
| 300–1000 m              |       |       |       |       |       |       |       |       |         |
| N-N (\(\mu\text{mol} \text{~N~m}^{-1}\)) | 28    | 33    | 41    | 39    | 43    | 43    | 43    | 39    | 34      |
| \(\text{O}_2\) (\(\mu\text{mol} \text{~O}_2 \text{~m}^{-1}\)) | 182   | 129   | 77    | 94    | 66    | 47    | 29    | 83    | 119     |
| amoA genes (genes \(\text{m}^{-1}\)) | \(1.2 \times 10^6\) | \(2.5 \times 10^6\) | \(2.9 \times 10^6\) | \(2.1 \times 10^7\) | \(1.8 \times 10^6\) | \(2.9 \times 10^5\) | \(2.3 \times 10^5\) | \(7.4 \times 10^5\) | \(2.9 \times 10^5\) |
| MGI + psL12 rRNA genes (genes \(\text{m}^{-1}\)) | \(1.2 \times 10^6\) | \(3.8 \times 10^6\) | \(3.7 \times 10^6\) | \(1.6 \times 10^7\) | \(1.9 \times 10^6\) | \(7.6 \times 10^5\) | \(6.9 \times 10^5\) | \(6.2 \times 10^5\) | \(3.0 \times 10^5\) |
| amoA transcripts (transcripts \(\text{m}^{-1}\)) | \(1.8 \times 10^4\) | \(7.9 \times 10^4\) | \(1.7 \times 10^5\) | \(9.0 \times 10^4\) | \(6.1 \times 10^4\) | \(1.3 \times 10^5\) | \(2.4 \times 10^4\) | \(7.9 \times 10^4\) | \(1.5 \times 10^5\) |
| amoA transcripts per amoA gene | \(1.5 \times 10^5\) | \(3.2 \times 10^5\) | \(6.0 \times 10^5\) | \(4.3 \times 10^4\) | \(3.3 \times 10^5\) | \(4.4 \times 10^4\) | \(1.0 \times 10^5\) | \(1.1 \times 10^5\) | \(5.2 \times 10^4\) |

Depth-averaged concentrations calculated based on depth-integrated inventories divided by depth of integration for each property. No data (ND).
with increasing depth (Fig. 3). The resulting amoA to crenarchaeal 16S rRNA gene abundance ratio ranged between 0.4 and 13 throughout the water column, with a median value of 1.3. Variability in this ratio is likely influenced by the wide range of absolute abundances of Crenarchaea, such that small variance where abundances were low could have large effects on the resulting amoA : 16S rRNA ratio.

Crenarchaeal amoA transcript abundances

In addition to evaluating archaeal gene copy abundances, we also examined vertical and meridional patterns of crenarchaeal amoA gene expression based on RT-QPCR amplification of mRNA transcripts. Relative to amoA gene abundances, crenarchaeal amoA transcripts were less variable with depth. For example, in the near surface ocean, amoA transcripts ranged between $9 \times 10^3$ and $2 \times 10^5$ transcripts l$^{-1}$, often increasing one to two orders of magnitude in the dimly lit waters of the lower euphotic zone (100–200 m; Fig. 2). Below this zone of peak amoA expression, transcript abundances declined into the interior of the mesopelagic zone (Fig. 4). Among the various regions sampled, transcript abundances were greatest in the northern equatorial waters (8.6°N) where cold, N+N- enriched and O$_2$ depleted waters upwelled into the upper ocean. At this station, amoA transcript abundances were approximately fivefold greater ($> 1 \times 10^6$ transcripts l$^{-1}$) than those measured in other regions of the study site. In contrast, the lowest amoA transcript abundances ($\sim 1 \times 10^3$ transcripts l$^{-1}$) were measured at the near-equatorial station (1.7°N), where amoA and 16S rRNA gene abundances were maximal.

One of the most notable differences between the distributions of crenarchaeal amoA transcripts and gene abundances occurred in the upper ocean (< 100 m) waters where the abundance of archaeal amoA genes was relatively low, but transcripts were relatively abundant. In this well-lit region of the ocean, the gene-copy normalized amoA transcript abundances averaged $\sim$80 transcripts per gene copy (Fig. 4). The copy normalized transcript abundances approached unity in the transitional region separating the epipelagic from mesopelagic zone (125–200 m), before decreasing with depth into the lower mesopelagic zone (Fig. 4). In the mesopelagic waters amoA gene abundances increased coincident 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 (Fig. 4, Table 1).

Discussion

Quantitative PCR amplification of crenarchaeal amoA and 16S rRNA genes together with RT-QPCR amplification of amoA mRNA transcripts provided insight into the distributions and abundances of crenarchaeal amoA genes and transcripts across ~5200 km of the Pacific Ocean. At all stations sampled, amoA gene abundances increased three to four orders of magnitude between the well-lit near-surface waters and the lower mesopelagic zone. In contrast, amoA transcripts tended to be elevated in the transitional waters (100–200 m) separating the epipelagic...
region from the mesopelagic zone. Strikingly, when normalized to gene copies, amoA transcription was greatest throughout the well-lit portions of the upper ocean.

To date, there is limited information on processes regulating the transcriptional activities of ammonia-oxidizing microorganisms in the sea. The observed depth-dependent patterns of gene copy normalized amoA expression likely reflect vertical modification of crenarchaeal growth rates deriving from changes in substrate availability, temperature, and perhaps sunlight. Laboratory studies with cultivated marine ammonia-oxidizing bacteria and natural assemblages of soil-dwelling Crenarchaea indicate expression of the amo operon is upregulated by external concentrations of ammonia (Treu sch et al., 2005; Berube et al., 2007; El Sheikh and Klotz, 2008). Although we did not measure ammonia concentrations as part of this study, concentrations in the open ocean typically range in the 10 to 100 nmol l\(^{-1}\) (Lipschultz, 2001; Woodward and Rees, 2001; Rees et al., 2006), with peak concentrations often measured in the mid to lower euphotic zone, above the depth of the primary nitrite maxima (Gruber, 2008). Our finding that amoA gene transcripts often peaked in the transitional region separating the euphotic zone from mesopelagic waters could be consistent with the presumed depth-dependent changes in ammonia concentrations. Temperature has also been shown to play a role in regulating crenarchaeal amoA transcription in terrestrial soils (Tourna et al., 2008). In the present study, we observed elevated gene-copy normalized amoA transcript abundances throughout the warm upper ocean waters; however, we did not observe a significant relationship (least-squares linear regression, \(P > 0.05\)) between amoA transcripts per gene copy and temperature in our study.

Prior studies evaluating crenarchaeal amoA transcription in the sea have largely focused on regions where low concentrations of dissolved oxygen play an important role in shaping N cycling processes. For example, in the Black Sea, Lam and colleagues (2007) observed most active amoA transcription coincided with the vertical transition from oxic to suboxic waters. Within this depth stratum, crenarchaeal amoA transcripts were \(~10^4\) transcripts l\(^{-1}\) (Lam et al., 2007). In the waters of the Peruvian upwelling region, crenarchaeal amoA transcript abundances were somewhat greater than measured in the Black Sea, ranging from \(10^5\) to \(10^6\) transcripts l\(^{-1}\). Peak transcript abundances in the Peruvian upwelling waters occurred coincident with the top of the nitracline, where dissolved O\(_2\) concentrations decreased to < 10 \(\mu\)mol l\(^{-1}\) and ammonium concentrations exceeded \(~1\) \(\mu\)mol l\(^{-1}\) (Lam et al., 2009). In the present study, transcript abundances ranged between \(10^2\) and \(10^4\) transcripts l\(^{-1}\), with peak transcript abundances occurring near the top of the nitracline in the O\(_2\) depleted, upwelled waters north of the equator (8.6°N). However, although dissolved O\(_2\) concentrations of these upwelled waters were lower than those measured at comparable depths along our transect, the O\(_2\) content of this water was upwards of 65 \(\mu\)mol l\(^{-1}\), suggesting the availability of O\(_2\) likely did not play a major role in structuring N cycle processes in these waters.

The observation that total amoA transcript abundances often peaked in the dimly lit region separating the epipelagic from the mesopelagic waters appears consistent with previously reported depth-structure in rates of marine ammonia oxidation (Wada and Hattori, 1971; Olson, 1981a; Ward, 1987; Yoshida et al., 1989; Dore and Karl, 1996a). In the northern regions of the North Pacific Subtropical Gyre (NPSG), Olson (1981a) found that rates of ammonia oxidation ranged between 2.2 and 7.3 nmol N l\(^{-1}\) day\(^{-1}\), with peak rates coinciding with the vertical position of the primary NO\(_2\) maxima of the lower euphotic zone (100–175 m). Similarly, at Station ALOHA in the central NPSG, Dore and Karl (1996a) found that rates of ammonia oxidation ranged from 1 to 134 nmol l\(^{-1}\) day\(^{-1}\) in the upper 200 m of the water, with peak rates typically occurring in the low light regions (150–175 m) of the primary nitrite maxima.

Previous studies have demonstrated inhibitory influences of sunlight on ammonia oxidation and growth of ammonia-oxidizing bacteria (Olson, 1981b; Ward, 1987; Horrigan and Springer, 1990; Guerrero and Jones, 1996). Although the exact mechanisms underlying this photoinhibition remain unknown, laboratory studies have found evidence for reversible photooxidative damage to the ammonia monooxygenase protein under short wavelength (< 410 nm) radiation (Hooper and Terry, 1974; Hyman and Arp, 1992). The influences of light on crenarchaeal ammonia oxidation activities or transcriptional responses have not yet been reported. However, the observation that the gene copy normalized amoA transcript abundances were elevated in the upper ocean where light intensities were upwards of 80–90% of the incident flux raises the possibility that marine Crenarchaea might be less sensitive to photoinhibition than their more well-studied bacterial counterparts. Alternatively, the observed depth-related changes in amoA transcription may not reflect changes in rates of ammonia oxidation by these microorganisms, but rather reflects post-transcriptional or translational modification of the transcribed gene product. As such, the elevated copy normalized transcriptional activities observed throughout the upper ocean could reflect increased turnover of the ammonia monooxygenase protein (perhaps compensating for photooxidative damage) rather than increases in the per cell ammonia oxidation activities. Future investigations focused specifically on examining the role of sunlight on crenarchaeal ammonia oxidation, gene
transcription, as well as proteomic analyses, should provide insight to these transcriptional patterns.

Numerous studies have examined depth-dependent structure in the abundance of Archaea in the ocean; with several of these studies providing evidence that phylogenetically distinct populations of Crenarchaea vertically segregate within the epi-, meso-, and bathypelagic waters (Massana et al., 2000; Francis et al., 2005; Hallam et al., 2006b; Mincer et al., 2007; Beman et al., 2008). In a recent study in the Gulf of California, Beman and colleagues (2008) measured depth distributions of crenarchaeal amoA genes together with ^15N-based measurements of ammonia oxidation. These authors identified two vertically segregated groups of phylogenetically distinct crenarchaeal clades with rates of ammonia oxidation greatest in those regions where the upper ocean clade was most abundant (Beman et al., 2008). Although the QPCR primers utilized in the present study did not allow discrimination of the transcriptional activities of these vertically separated amoA-containing crenarchaeal groups, we observed gene copy normalized amoA transcript abundances were greatest in the epipelagic waters, decreasing several orders of magnitude with increasing depth. This depth-dependent decrease in gene copy normalized amoA transcript abundances would be consistent with the presumed reduced input of ammonia accompanying vertical attenuation of particle flux into the mesopelagic zone (Karl et al., 1984). However, the observation that transcriptional activity appears greatest in the upper ocean opens the possibility that despite low abundances, Crenarchaea might play a role in ammonia oxidation in the epipelagic layers of the ocean. Such results might shed insight into microorganisms responsible for upper ocean nitrification (Dore et al., 1998; Yool et al., 2007).

Over a wide range of epi- and mesopelagic habitat conditions, we generally observed close correspondence between crenarchaeal amoA gene abundances and total (MGI + psL12) 16S rRNA gene abundances. The median value of the amoA : total crenarchaeal 16S gene ratios measured in our study was 1.3, similar to previously published estimates (Wuchter et al., 2006; Mincer et al., 2007; Beman et al., 2008). This ratio did not vary significantly with depth, although the ratio was as great as 13 at one site in the upper ocean (< 100 m), and as low as 0.4 in the lower regions of the euphotic zone (150–175 m) at another station. A recent study found that crenarchaeal amoA : MGI 16S rRNA gene ratios in the mesopelagic waters of the subtropical Atlantic Ocean were often < 10^{-2} (Agogue et al., 2008), a finding these authors’ attribute to an increasing archaeal reliance on chemooorganoheterotrophy rather than chemolithoautotrophy. In the present study, across a large region of the subtropical and tropical Pacific Ocean, we found no clear evidence of depth-dependent or regional changes in the crenarchaeal amoA : 16S rRNA ratios between the near-surface ocean and the mesopelagic waters of the central Pacific Ocean.

Additional constraint on amoA to 16S rRNA gene ratios can be obtained based on genomic and metagenomic sequences. The genome sequence of Nitrosopumilus maritimus, a MGI Cenarchaeum, contains a single copy of the amoA gene. Similarly, the Cenarchaeum symposium genome also contains a single gene copy of the amoA gene (Hallam et al., 2006a). Moreover, metagenomic reconstruction of natural assemblages of Crenarchaeae in the bathypelagic waters of the Pacific Ocean indicates that when normalized to gene length, the ratio of amoA to 16S rRNA genes approaches unity (Konstantinidis et al., 2009).

Although the observation that crenarchaeal amoA : 16S rRNA gene ratios approached 1 appears consistent with genome reconstructions and metagenomic surveys, such results appear contrary to the study by Agogue and colleagues (2008) in the Atlantic Ocean. Differences in these studies may stem from the selection of QPCR primers. We examined the specificity of the QPCR primers utilized by Agogue and colleagues (2008) against a database containing > 200 publicly available crenarchaeal amoA gene sequences derived from both PCR clone libraries and ocean metagenomes. Consistent with the results of Konstantinidis and colleagues (2009), we found several nucleotide mismatches in these primers to the dominant amoA phylotypes reported from the meso- and bathypelagic waters of the Pacific Ocean.

While our results suggest the majority of Crenarchaeae in the epi- and mesopelagic waters of the Pacific contain amoA genes, and thus likely possess the genetic capacity to oxidize ammonia for energy, such results do not preclude the possibility that marine Crenarchaeae utilize diverse metabolic pathways, including chemooorganoheterotrophy, to sustain their growth. Numerous studies indicate MGI Crenarchaeae are capable of assimilating both inorganic and organic carbon substrates during growth (Teira et al., 2006b; Kirchman et al., 2007; Varela et al., 2008), and metagenomic analyses suggest these microorganisms have the capacity to utilize other forms of reduced N, including urea as potential sources of energy (Hallam et al., 2006b). Whether or not MGI Crenarchaeae can derive energy from any of these alternative substrates remains an open question.

In conclusion, QPCR amplification of crenarchaeal genes and RT-QPCR amplification of amoA gene transcripts provided information on the spatial distributions and transcriptional activities of these microorganisms in the Pacific Ocean. Crenarchaeal abundances demonstrated strong vertical structure with greatest abundances observed in the dimly lit waters of the mesopelagic zone. In contrast, crenarchaeal amoA gene expression was less variable with depth, and generally decreased below the
euphotic zone. When normalized to gene copy abundances, the resulting patterns of transcription suggest that despite low abundances, Crenarchaeaea may be physiologically active in ammonia oxidation throughout the euphotic zone. Future studies focused on defining specific processes controlling crenarchaeal physiology (e.g. light, ammonia, temperature, dissolved oxygen) and abundance (e.g. grazing and viral infection), should provide important new insights into controls on ammonia oxidation in the sea.

**Experimental procedures**

**Biogeochemical analyses**

Seawater samples for subsequent biogeochemical analyses were collected from discrete depths in the upper ocean using 24 10 l polyvinyl chloride (PVC) sampling bottles attached to a Conductivity-Temperature-Depth (CTD) rosette sampling system. In total, 8–12 discrete depths were sampled from vertical profiles (0–1000 m) at nine locations between −16°S, 170°W and 22.75°N, 158°W in the Pacific Ocean (Fig. 1). Two stations were sampled in the warm, low chlorophyll northern waters of the South Pacific, including a station in the subtropical gyre (16°S, 170°W) and a station in the eastern portion of Western Pacific Warm Pool (7.5°S, 167°W). Samples were also collected from within the near-equatorial waters of both the North and South Pacific (3.0°S, 166°W; 1.7°N, 163°W; 5.1°N, 161°W; 8.6°N, 161°W), and at three stations in the NPSG (13.5°N, 159°W; 16.9°N, 159°W; 22.75°N, 158°W).

High resolution vertical scale (1 m) hydrographic data in the upper ocean were obtained using a Sea Bird CTD equipped with dissolved O2 and fluorescence sensors. The O2 and fluorescence sensors were calibrated against discrete measurements of dissolved O2 (Carritt and Carpenter, 1966) and chloropigment (chlorophyll + phaeopigment) active fluorescence (Letelier et al., 1996). Seawater for determination of nutrient concentrations was subsampled into acid washed 125 ml or 500 ml polyethylene bottles and the bottles were capped and frozen upright. At the shore-based laboratory, high sensitivity measurements of NO3-+ NO2- (N-N) were determined based on the chemiluminescence methodology developed by Garside (1982) as modified by Dore and Karl (1996b).

**Nucleic acid sampling**

Seawater samples for subsequent extraction of planktonic nucleic acids were collected from 10 discrete depths spanning the epipelagic and mesopelagic zones (15 m to 1000 m) at eight stations located between 16°S, to 16°N during the CMORE-BULA (Center for Microbial Oceanography: Research and Education-Biogeochemistry Underwater; Lati
dudinal Assessment) research cruise (15–26 April 2007). Additional samples were collected from 12 discrete depths (15–1000 m) during a Hawaii Ocean Time-series program cruise (3–7 May 2007) to Station ALOHA (22.75°N, 158°W) in the NPSG. Seawater samples were collected using 10 l PVC bottles attached to a CTD rosette sampler. Water was subsampled from the CTD rosette into 10 l, acid-rinsed polyethylene carboys and between 2 and 4 l was immediately filtered using a peristaltic pump onto 25 mm diameter 0.2 μm pore size Supor® filters ( Pall Ge
lman). Upon completion of filtration, the filters were removed from the filter holders and placed in 2 ml microcentrifuge tubes. Filters for subsequent RNA extraction were immersed in 500 μl buffer (Qiagen RNaseasy) containing 1% β-mercaptoethanol. Filters for subsequent DNA extraction were immersed in 500 μl of lysis buffer (20 mM Tris-HCL, pH 8.0; 2 mM EDTA, pH 8.0; 1.2% Triton X and 20 mg ml−1 lysozyme). Samples were immedi
dately flash frozen in liquid nitrogen then stored at −80°C until processed in the shore-based laboratory.

**Nucleic acid extraction, QPCR and RT-QPCR**

Planktonic RNA was extracted following the protocol described in Church and colleagues (2005). Briefly, 0.2 g of 0.1 mm glass beads was added to microcentrifuge tubes containing the sample filters and the tubes were placed inside a Fast Prep machine (Bio 101, Carlsbad, CA, USA) and agitated for 1.5 min. Following this bead beating step, tubes were centrifuged at 8500 g for 30 s, and the supernatants transferred to clean 2 ml microcentrifuge tubes with an equal volume of 70% ethanol. Samples were applied to Qiagen RNeasy® Mini columns (Qiagen, Valencia, CA, USA) and total RNA was purified and eluted following the manufacturer’s specifications. RNA extracts were treated with DNase I following the Qiagen On-Column DNase I® RNA extraction protocol; RNA was eluted from spin column with 30 μl RNase-free water and stored frozen at −80°C. 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).

Microcentrifuge tubes containing the sample filters for DNA extraction were placed in a water bath at 37°C for 1 h, after which 84 μl of proteinase K and 600 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 min. Following this incubation, 668 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. DNA concentrations were determined using the Quant-it® DNA assay kit and quantified fluorometrically.

Total RNA was reverse transcribed using SuperScript® III first strand cDNA synthesis kit (Invitrogen) following the manufacturer’s specifications. cDNA reactions consisted of 2–4 ng total RNA, 1 mmol l−1 dNTPs, 1× RT buffer, 5 mmol l−1 MgCl2, 10 mmol l−1 DTT, 40 U RNaseOUT (Invitrogen), 200 U SuperScript III RT, and 0.5 μmol l−1 of the antisense gene specific primer (CrenAmo4ModR; Mincer et al., 2007). Upon completion of the cDNA synthesis, 1 U RNase H was added to each reaction. The resulting cDNA was diluted to 50 μl total volume with nuclease-free water, and stored at −20°C until analysed by QPCR assays. An identical set of reactions minus the reverse-transcriptase (no-RT reactions) were performed for each RNA extract; these reactions served as
controls to examine the potential contributions of carryover genomic DNA on the RT-QPCR amplification of the cDNA.

Crenarchaeal amoA transcript abundances and copy abundances of crenarchaeal amoA and 16S rRNA genes were examined using previously described QPCR protocols (Mincer et al., 2007). The QPCR assays consisted of duplicate 25 μl reactions containing: 12.5 μl 2x SyberGreen Master Mix (Applied Biosystems, Foster City, CA, USA), 8 μl of nuclelease-free water, 2 μl of environmental DNA or cDNA reaction mixes (including no-RT reactions), and 0.5 μM final concentration of both forward and reverse primers. Quantitative PCR reactions were analysed using an Applied Biosystems ABI 7300, following the thermal cycling reaction conditions described in Mincer and colleagues (2007). Standards for the QPCR and RT-QPCR reactions for the amoA and MGI 16S rRNA consisted of serial 10-fold dilutions of plasmids containing amplified fragments of the targeted genes of interest. QPCR amplification efficiencies averaged 99%; 98%, 94% and 104% for the crenarchaeal amoA transcripts and gene copies of amoA, 16S, rRNA, and psL12 phenotypes respectively.

Acknowledgements

We are grateful to the numerous scientists and staff that contributed to the success of the CMORE-BULA cruise; of particular note, Karin Björkman, Claire Mahaffey, Susan Curless, Donn Viviani, Blake Watkins, Lance Fujieki and Brett Updyke all assisted with sample collections, analyses, and data management. We acknowledge the support of the RV Kilo Moana captain and crew. The comments of three anonymous reviewers were useful in improving this manuscript. Brenner Wai was supported by the C-MORE Scholars program. This project was supported by grants from the National Science Foundation: OCE 0425363 to M.J.C., EF 0425363 to M.J.C., and EF 0424599 (C-MORE) to D.M.K, and additional support from the Gordon and Betty Moore Foundation Marine Microbiology Initiative (to D.M.K. and E.F.D.).

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