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Contribution of *Archaea* to Total Prokaryotic Production in the Deep Atlantic Ocean

Gerhard J. Herndl,[1]* Thomas Reinthaler,[1] Eva Teira,[1] Hendrik van Aken,[2] Cornelius Veth,[2] Annelie Pernthaler,[3] and Jakob Pernthaler[3]

Department of Biological Oceanography[1] and Department of Physical Oceanography,[2] Royal Netherlands Institute for Sea Research, 1790 AB Den Burg, The Netherlands, and Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359 Bremen, Germany[3]

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Fluorescence in situ hybridization (FISH) in combination with polynucleotide probes revealed that the two major groups of planktonic *Archaea* (*Crenarchaeota* and *Euryarchaeota*) exhibit a different distribution pattern in the water column of the Pacific subtropical gyre and in the Antarctic Circumpolar Current system. While *Euryarchaeota* were found to be more dominant in nearsurface waters, *Crenarchaeota* were relatively more abundant in the mesopelagic and bathypelagic waters. We determined the abundance of archaea in the mesopelagic and bathypelagic North Atlantic along a south-north transect of more than 4,000 km. Using an improved catalyzed reporter deposition-FISH (CARD-FISH) method and specific oligonucleotide probes, we found that archaea were consistently more abundant than bacteria below a 100-m depth. Combining microautoradiography with CARD-FISH revealed a high fraction of metabolically active cells in the deep ocean. Even at a 3,000-m depth, about 16% of the bacteria were taking up leucine. The percentage of *Euryarchaeota* and *Crenarchaeaota* taking up leucine did not follow a specific trend, with depths ranging from 6 to 35% and 3 to 18%, respectively. The fraction of *Crenarchaeota* taking up inorganic carbon increased with depth, while *Euryarchaeota* taking up inorganic carbon decreased from 200 m to 3,000 m in depth. The ability of archaea to take up inorganic carbon was used as a proxy to estimate archaeal cell production and to compare this archaeal production with total prokaryotic production measured via leucine incorporation. We estimate that archaeal production in the mesopelagic and bathypelagic North Atlantic contributes between 13 to 27% to the total prokaryotic production in the oxygen minimum layer and 41 to 84% in the Labrador Sea Water, declining to 10 to 20% in the North Atlantic Deep Water. Thus, planktonic archaea are actively growing in the dark ocean although at lower growth rates than bacteria and might play a significant role in the oceanic carbon cycle.

Prokaryotic plankton are the major driver of biogeochemical cycles in the ocean. While the importance of bacteria in the oceanic biogeochemical cyles is well established, particularly for surface waters, our knowledge of the metabolically active fraction of the prokaryotic community in the mesopelagic and bathypelagic realms of the ocean, comprising about 70% of the ocean's volume, is rather rudimentary. Using fluorescence in situ hybridization (FISH) and polynucleotide probes, it has been shown recently that planktonic *Archaea*, consisting of two major groups, the *Crenarchaeota* and *Euryarchaeota*, might account for about one-third of all prokaryotic cells in the global ocean (15). In the Antarctic Circumpolar Current, however, the abundance of archaea was found to be generally lower than at the Hawaiian Ocean Time-Series (HOT) station (4). In the deep mesopelagic and bathypelagic realm of the HOT station in the Pacific gyre, the abundance of *Crenarchaeota* is about equal to that of bacteria (15). In this paper, we present the distribution of bacteria, *Crenarchaeota*, and *Euryarchaeota* in the mesopelagic and bathypelagic waters of the North Atlantic, following the path of the North Atlantic Deep Water (NADW) over a transect of more than 4,000 km using a modified catalyzed reporter deposition-FISH (CARD-FISH) method and specific oligonucleotide probes (33).

Up to now, planktonic archaea have resisted all attempts at culture. Consequently, nothing is known about their metabolic requirements. Compound specific stable isotope analysis on membrane lipids specific for *Crenarchaeota* indicates that they might be chemoautotrophic, fixing inorganic carbon (25, 37). However, it was also shown that planktonic archaea might take up amino acids (22).

There are only a few estimates of bulk prokaryotic activity in deep waters, none of them at a single-cell level. Thus, the fraction of metabolically active cells among the deepwater prokaryotic community taking up specific compounds remains unknown. We used the CARD-FISH method combined with microautoradiography (MICRO-CARD-FISH) to determine the fraction of the bacteria, *Crenarchaeota*, and *Euryarchaeota* metabolically active in the deep waters of the North Atlantic.

MATERIALS AND METHODS

Sample collection. Sampling of mesopelagic and bathypelagic waters was carried out along a >4,000-km-long transect from Bermuda to east of Greenland, following the western branch of the NADW to its origin during the TRANSAT-2 cruise with R/V *Pelagia* in May-June 2003 (Fig. 1). Samples were taken from the 100-m layer, the oxygen minimum, the Labrador Seawater (LSW), the NADW, and, if present, the Denmark Strait Overflow Water (DSOW). For the depth range and the physical characteristics of the sampled water masses, see Table 1. Additionally, samples were collected in the southern Atlantic Ocean at the Polar Front between depths of 200 and 3,000 m during the Eifex cruise on board R/V

* Corresponding author. Mailing address: Department of Biological Oceanography, Royal Netherlands Institute for Sea Research, 1790 AB Den Burg, The Netherlands. Phone: 31 222 369 507. Fax: 31 222 319 674. E-mail: herdl@nioz.nl.

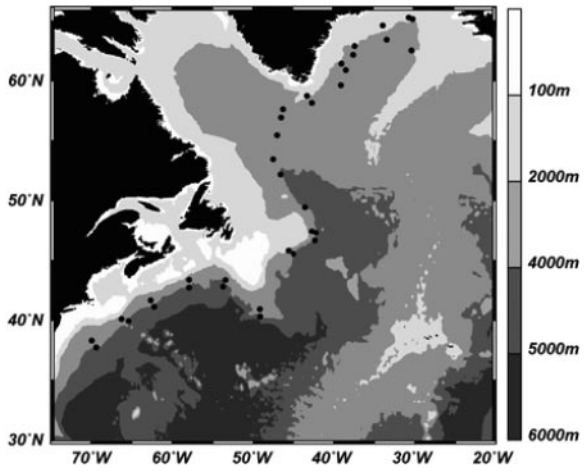

FIG. 1. Map of stations occupied during the TRANSAT-2 cruise (indicated by dots) in May-June 2003 in the North Atlantic following the NADW.

Polarstern between January and March 2004. Along the transect in the North Atlantic, water masses were identified based on their salinity-temperature characteristics (35, 36). Water from the distinct water masses was collected with Neox bottles mounted on a conductivity-temperature-depth (CTD) frame during the TRANSAT-2 cruise and with Niskin bottles during the Eifex cruise. Samples were taken for the enumeration of total picoplankton, bacteria, and archaea and fixed instantly with formaldehyde (2% final concentration).

Enumeration of bacteria and archaea. Heterotrophic picoplankton were enumerated after DAPI (4′,6′-diamidino-2-phenylindole) staining (28), and bacteria and archaea were enumerated by CARD-FISH (26) under the epifluorescence microscope. For enumeration of bacteria, the oligonucleotide probe Eub338 (1) was used. Specific probes were designed: for marine *Crenarchaeota* group I, Cren537 (5′-TGACCACTTGAGGTGCTG-3′); for marine *Euryarchaeota* group II, Eury806 (5′-CACAGCGTTTACACCTAG-3′). All the probes were tested for their specificity prior to the study. The probes target the same cells as the polynucleotide probes used in previous studies (4, 6, 15). Cell walls were permeabilized for Eub338 (1) with lysozyme (10 mg ml^{-1} in 0.05 M EDTA, 0.1 Tris-HCl [pH 8]; Sigma) or with proteinase K for Eury806 and Cren537 (1,844 U mg^{-1}; 10.9 mg ml^{-1} [Sigma]) at a concentration of 0.2 μl ml^{-1} in 0.05 EDTA–0.1 Tris-HCl (pH 8) at 37°C for 1 h (33). Probe working solution (50 ng μl^{-1}) was added at a final concentration of 2.5 ng μl^{-1}. Hybridization was done at 35°C for 8 to 12 h. Negative control counts (hybridization with HRP-Non338) averaged 1.5%.

MICRO-CARD-FISH. To 30- to 40-ml samples, [^{3}H]leucine (specific activity [SA], 157 Ci $mmol^{-1}$; Amersham) at a final concentration of 20 nM or [^{14}C]bicarbonate (100 μCi; SA, 54.0 mCi $mmol^{-1}$; Amersham) was added, and the samples were incubated in the dark at in situ temperatures for 8 to 10 h and 60 to 72 h, respectively. Controls were fixed with paraformaldehyde, 2% final concentration. Incubations were terminated by the addition of paraformaldehyde (2% final concentration), and the samples were stored in the dark at 4°C for 12 to 18 h. The autoradiographic development was conducted by transferring previously hybridized filter sections onto slides coated with photographic emulsion (type NTB-2, melted at 43°C for 1 h). Subsequently, the slides were placed in a dark box with a drying agent and exposed at 4°C for 36 to 48 h. The slides were developed and fixed using Kodak specifications (Dektol developer [1:1 dilution with Milli-Q water] for 2 min, a rinse with Milli-Q water for 10 s, and in fixer for 5 min, followed by a Milli-Q water rinse for 2 min). Cells were counterstained with a DAPI mixture (5.5 parts of Citifluor, 1 part of Vectashield, and 0.5 parts of phosphate-buffered saline amended with DAPI at a final concentration of 1 μg ml^{-1}). The silver grains in the autoradiographic emulsion were detected by switching to the transmission mode of the microscope. More than 700 DAPI-stained cells were counted per sample.

TABLE 1. Characteristics of the main water masses sampled during TRANSAT-2[a]

Water mass	Depth (m)	Temp (°C)	Salinity (psu)	Oxygen (μmol/kg)
Surface	88–103	4.1–18.8	34.6–36.6	206–305
O_2 minimum	200–626	4.3–10.1	34.8–35.3	144–257
LSW	809–2,060	3.1–3.7	34.87–34.90	274–291
NADW	2,435–2,790	2.6–3.2	34.90–34.95	265–282
DSOW	1,216–3,869	0.8–2.4	34.81–34.90	271–309

[a] The data show the range of the individual parameter in each water mass where samples were collected. LSW, Labrador Seawater; NADW, North Atlantic Deep Water; DSOW, Denmark Strait Overflow Water.

Bulk prokaryotic and archaeal production measurements. Bulk prokaryotic activity was measured by incubating 30 ml of water in duplicate and one formaldehyde-killed blank with 10 nM [^{3}H]leucine (final concentration, SA 157 Ci $mmol^{-1}$; Amersham) in the dark at in situ (±1°C) temperatures for 4 to 7 h. Thereafter, the incubation was terminated by the addition of formaldehyde (2% final concentration) to the samples; the samples were then filtered through 0.22-μm cellulose nitrate filters (25-mm filter diameter; Millipore). Subsequently, the filters were rinsed with 5% ice-cold trichloroacetic acid, placed in scintillation vials, and stored at −20°C until being counted with a liquid scintillation counter. The disintegrations per minute (DPM) of the formalin-fixed blank were subtracted from the samples, and the resulting DPM were converted into leucine incorporation rates. These incorporation rates were further converted into bacterial carbon production values using the theoretical conversion factor with and without the intracellular dilution factor (×2) (30) and the empirically determined factors as described below.

Archaeal production was estimated via the incorporation of [^{14}C]bicarbonate (100 μCi; SA, 54.0 mCi $mmol^{-1}$; Amersham) in 40-ml samples in duplicate and one formaldehyde-fixed blank incubated in the dark at in situ temperatures for 60 to 72 h. Subsequently, the incubations were terminated by the addition of formaldehyde (2% final concentration) to the samples, filtered onto 0.22-μm filters (Millipore, cellulose nitrate), and rinsed three times with 10 ml of ultrafiltered seawater (30-kDa molecular mass cutoff); the filters were exposed to a fume of concentrated HCl for 12 h. Then, the filters were placed in scintillation vials and stored in the dark at −20°C until the radioactivity of the filters was counted. The resulting DPM of the samples were corrected for the DPM of the abiotic control and converted into organic carbon produced over time in three different ways. The most conservative estimate of archaeal production assumes that inorganic carbon is the sole carbon source. We also determined empirical conversion factors by following the increase in archaeal abundance and the corresponding increase in carbon fixation in unamended seawater batch cultures as described below.

Seawater batch cultures to empirically determine conversion factors. On specific locations, water from the NADW was filtered through rinsed 0.8-μm polycarbonate filters to remove potential grazers. Two-liter seawater batch cultures were established in duplicate and incubated at in situ water temperatures (3.1°C) in the dark for 6 to 8 days. At irregular intervals, samples were taken to determine picoplankton, bacterial, and archaeal abundance and prokaryotic and archaeal production as described above. Empirical conversion factors were determined from the observed increase in cell abundance and the respective incorporation rates. For total prokaryotes, the increase in DAPI-stainable cells was related to the [^{3}H]leucine incorporation rates by integrative (18) and modified derivative (8) approaches. For archaea, the increase in archaeal cells over time (*Crenarchaeota* and *Euryarchaeota*) was related to the radiolabeled inorganic carbon incorporated by integrative (18) and cumulative (3) approaches.

Prior to terminating the batch culture incubations, samples were taken to determine the number of cells taking up leucine and bicarbonate by using MICRO-CARD-FISH as described above.

For estimating C biomass production, the calculated prokaryotic and archaeal cell production was converted into C production by assuming a C content of 10 fg C $cell^{-1}$ (7).

RESULTS AND DISCUSSION

Bacterial and archaeal abundance. Bacterial and archaeal abundance lacked latitudinal trends in cell abundance along the transect in the North Atlantic; therefore, averages for the individual water masses are given in Fig. 2. Generally, the contribution of bacteria to picoplankton abundance was rather constant throughout the water column (at a depth of 100 m, 32% of DAPI counts; in the NADW, 30% of DAPI counts), as was that of the *Euryarcheota* (≈17% of picoplankton abun-

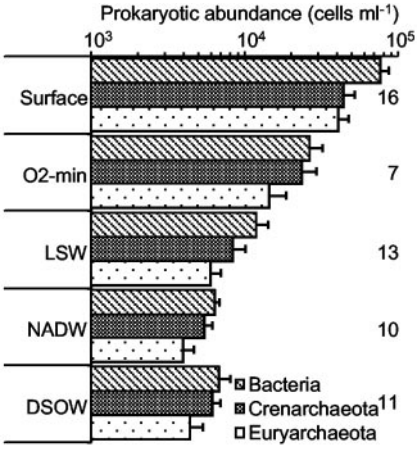

FIG. 2. Abundance of bacteria and archaea (*Crenarchaeota* and *Euryarchaeota*) enumerated with CARD-FISH in the different water masses of the North Atlantic (O2-min, O_2 minimum). Horizontal bars indicate errors of the mean. Numbers on the right indicate the number of samples.

dance). The contribution of *Crenarchaeota*, however, increased with depth from 18.5% in the 100-m layer to 26.4% of the picoplankton abundance in the NADW. A similar distribution pattern was obtained from depth profiles (down to a depth of 3,000 m) in the Antarctic Circumpolar Current (Fig. 3). However, while archaea comprised >50% of the prokaryotic abundance below a depth of 100 m in the North Atlantic, archaea never exceeded bacterial abundance in the Antarctic Circumpolar Current (Fig. 3). Church et al. (4) also reported a lower abundance of archaea than bacteria in the Antarctic Circumpolar Current with rRNA-targeted polynucleotide probes as also used in another study at the HOT station (15). Thus, our data confirm previous observations that archaea are apparently less abundant in the Antarctic Circumpolar Current waters than in both the open Pacific and the Atlantic. Generally, euryarchaeotal abundance reported by Church et al. (4) was <10% of the total picoplankton abundance, while *Euryarchaeota* in this study comprised 10 to 20% of picoplankton abundance between 500 m and 3,000 m (Fig. 3). Thus, our estimated euryarchaeotal abundance is about twice as high as reported previously (4, 15), probably due to the improved permeabilization procedure of the archaeal cell wall applied here. We used proteinase K instead of lysozyme, allowing a more efficient penetration of the oligonucleotide probe into the archaeal cell, as shown recently (33). An archaeal dominance of the picoplankton, as we found throughout the aphotic layer of the North Atlantic (Fig. 2), was also found in coastal waters and the Mediterranean Sea (9, 19, 22).

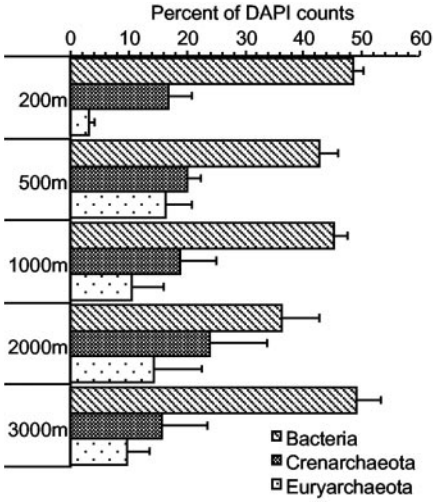

FIG. 3. Distribution of bacteria, *Crenarchaeota*, and *Euryarchaeota* in the water column of the Antarctic Circumpolar Current (at 49°08′ to 49°24′S, 1°52′ to 2°15′E) as percentages of DAPI counts. Columns indicate means, and bars indicate SDs of three depth profiles.

The sum of the abundance of bacteria, *Crenarchaeota*, and *Euryarchaeota* ranged from 67 to 76% of the total picoplankton for the different water masses. This detection yield obtained by CARD-FISH (26) with rRNA-targeted oligonucleotide probes is similar to that obtained with polynucleotide probes (27). Commonly, a fraction of DAPI-stained cells (20 to 30%) shows clear signs of degraded intracellular structures and can therefore be considered dead cells (12). Thus, our recovery efficiency of 67 to 76% of DAPI-stained cells indicates that essentially all the prokaryotic cells containing ribosomes, i.e., all the living prokaryotic cells, were detected by the modified CARD-FISH method and rRNA-targeted oligonucleotide probes. Recovery efficiencies of bacteria plus archaea with FISH of close to 100% of DAPI-stained cells should be treated therefore with caution, due to the unspecific DAPI staining of cells (38).

Uptake of specific compounds by bacteria and archaea assessed by MICRO-CARD-FISH. MICRO-CARD-FISH of samples collected throughout the water column of the Antarctic Circumpolar Current revealed that the percentage of bacteria taking up leucine decreased from 42% at a depth of 200 m to 16% at a depth of 3,000 m (Fig. 4A). However, the percentage of *Euryarchaeota* and *Crenarchaeaota* taking up leucine did not follow a specific trend with depth, ranging from 6 to 35% and 3 to 18%, respectively (Fig. 4A). Bacteria taking up bicarbonate ranged from 0 to 2% at depths of 500 to 1,000 m; at a depth of 200 m, 4% of the bacteria took up bicarbonate (Fig. 4B). The percentage of *Euryarchaeota* and *Crenarchaeota* incorporating bicarbonate ranged from 7 to 23% and 6 to 18%, respectively (Fig. 4B). While the percentage of *Crenarchaeota* taking up inorganic carbon was rather constant between depths of 200 and 2,000 m and varied only between 6 to 8%, at a depth of 3,000 m about 18% of the *Crenarchaeota* took up bicarbonate. In contrast, the percentage of the *Euryarchaeota* taking up inorganic carbon decreased with depth (Fig. 4B).

As indicated by MICRO-CARD-FISH, a considerable fraction of the mesopelagic and bathypelagic bacterial and archaeal community appears to be metabolically active (Fig. 4). Remarkably, the percentage of prokaryotic cells scoring as metabolically active in the deep waters is similar to the range for prokaryotes in surface waters by microautoradiography and with the use of simple organic substrates such as glucose, thymidine, or leucine (5, 10, 13, 14, 24, 34). No comparable data are available for oceanic deep waters. The relatively high percentage of deepwater prokaryotic cells scoring metabolically active indicates the presence of a community adapted to the specific conditions, despite the general decrease in pro-

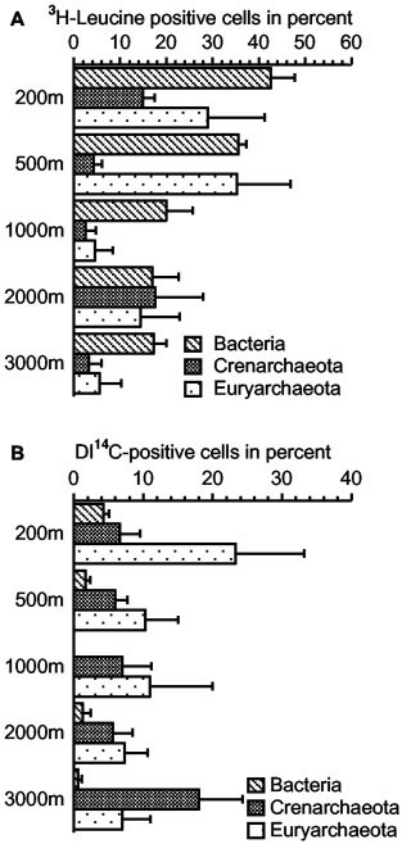

FIG. 4. Percentage of bacteria, *Crenarchaeota*, and *Euryarchaeota* taking up leucine (A) and bicarbonate (B) at different depth horizons of the Antarctic Circumpolar Current as determined by MICRO-CARD-FISH. Horizontal bars indicate the errors of the mean of three depth profiles.

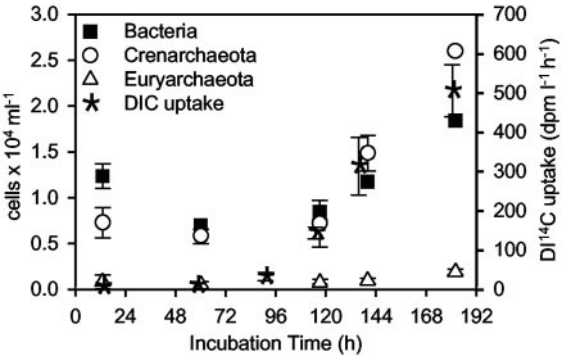

FIG. 5. Development of bacterial and archaeal abundance and DI^{14}C uptake in unamended seawater batch cultures processed through a 0.8-μm filter. Water was collected from the NADW at 47.62°W, 53.45°N. Symbols represent means of duplicate cultures. Bars indicate the range; if not visible, the range was smaller than the symbol. The increase in archaea was almost exclusively due to *Crenarchaeota*.

karyotic abundance with depth by about 1 order of magnitude (Fig. 2).

Bacterial uptake of inorganic carbon could indicate the presence of chemoautotrophic bacteria or the anaplerotic metabolism of bacteria responsible for the uptake of CO_2. It has been shown in laboratory studies that the anaplerotic uptake of CO_2 might be responsible for up to 5% of the cellular carbon in bacteria if easily metabolizable substrate is available (2, 29, 32). Whether anaplerotic metabolism of bacteria and/or archaea could be responsible for the observed bicarbonate uptake was tested in batch cultures with NADW processed through a 0.8-μm filter (Fig. 5).

Archaeal growth in seawater batch cultures with NADW. In seawater batch cultures with NADW processed through a 0.8-μm filter and incubated at in situ temperature in the dark, *Crenarchaeota* and bacteria started to increase in cell numbers after 4 to 5 days (Fig. 5). Based on the increase in cell numbers after this lag phase, growth rates for *Crenarchaeota* and bacteria were calculated. In both seawater batch cultures, each performed in duplicate, *Crenarchaeota* exhibited higher growth rates than bacteria (0.23 and 0.47 day^{-1} for *Crenarchaeota* versus 0.13 and 0.29 day^{-1} for bacteria). *Euryarchaeota* did not increase in abundance over the incubation period (Fig. 5), reflecting the general abundance pattern in the deep ocean, with *Crenarchaeota* dominating over *Euryarchaeota*. Remarkably, *Crenarchaeota* are apparently capable of growing at higher rates than bacteria in the NADW. Also, Church et al. (4) concluded from the dynamics in abundance at different stations in the Antarctic Circumpolar Current that *Crenarchaeota* are apparently capable of growing at high rates in the mesopelagic and bathypelagic waters.

Based on the increase in archaeal abundance over time in the seawater batch cultures and the bicarbonate incorporated, we can calculate the minimum carbon content of newly produced archaeal cells. Based on the assumption that inorganic carbon is the sole carbon source for archaea, we arrive at an average C content of 8.39 fg C archaeon^{-1} (standard deviation [SD] = 2.01; n = 5). This is at the lower end of the C content of similar-sized open ocean bacteria (≈10 fg C bacterium^{-1}) (7) but provides evidence that it is unlikely that the incorporation of inorganic carbon as observed by MICRO-CARD-FISH is due to anaplerotic metabolism. Rather, it provides evidence that a substantial fraction of the carbon requirements of planktonic archaea might be derived from the uptake of inorganic carbon, supporting previous reports based on compound-specific stable isotope analysis (25, 37). As shown by microautoradiography combined with FISH in this work (Fig. 4A) and a previous study (22), archaea also take up amino acids. Therefore, bicarbonate might not be the only carbon source for archaea. As shown in Fig. 4B, dark inorganic carbon uptake by the picoplankton is largely restricted to archaea particularly in layers below 500 m; they might therefore be considered as, at least partially, chemoautotrophs (20, 25). As archaea numerically dominate the prokaryotic community in the North Atlantic from a depth of 100 m to the NADW (Fig. 2), archaea might represent a hitherto-unrecognized sink for inorganic carbon in the largest oceanic subsystem, the mesopelagic and bathypelagic realm.

Estimating bulk prokaryotic and archaeal production. Our results obtained by MICRO-CARD-FISH suggest that the commonly used leucine incorporation method to estimate bacterial biomass production (17, 30) might actually be considered, at least to some extent, an estimate of total prokaryotic (bacteria plus archaea) biomass production. As bicarbonate

TABLE 2. Prokaryotic and archaeal production in the different water masses using different conversion factors[a]

WM	*n*	Prokaryotic production[b] (μmol C m^{-3} day^{-1})					Archaeal production[b] (μmol C m^{-3} day^{-1})		
		P1	P2	P3	P4	P5	A1	A2	A3
O_2 minimum	10	2.57 (2.34)	5.30 (4.82)	9.42 (8.58)	19.84 (18.07)	33.31 (30.34)	0.54 (0.50)	0.71 (0.65)	1.23 (1.13)
LSW	17	0.60 (0.44)	1.24 (0.90)	2.21 (1.60)	4.65 (3.36)	7.80 (5.65)	0.11 (0.14)	0.15 (0.18)	0.26 (0.31)
NADW	13	0.58 (0.32)	1.19 (0.66)	2.12 (1.18)	4.47 (2.48)	7.51 (4.16)	0.09 (0.11)	0.12 (0.14)	0.20 (0.25)
DSOW	8	0.81 (0.47)	1.67 (0.97)	2.97 (1.73)	6.26 (3.64)	10.52 (6.11)	0.08 (0.10)	0.10 (0.14)	0.17 (0.24)

[a] Mean values are given, and SDs are in parentheses. *n*, pairs of data; WM, water masses. For abbreviations of water masses, see Table 1.

[b] The following conversion factors were used: P1, 1.55 kg C mol^{-1} leucine (30); P2, 3.1 kg C mol^{-1} leucine (30); P3, empirical conversion factor (0.55×10^{18} cells mol^{-1} leucine) determined by Ducklow (8) by the modified derivative method; P4, conversion factor (1.94×10^{18} cells mol^{-1} leucine) determined from batch culture incubations with NADW by the derivative method (8); P5, conversion factor (1.16×10^{18} cells mol^{-1} leucine) determined by the integrative approach (18); A1, calculated based on the assumption that archaea only take up bicarbonate or CO_2; A2, conversion factor (1.01×10^3 archaeons DPM^{-1}) derived from the batch culture calculated by the integrative approach (18); A3, by the cumulative approach (3) (1.85×10^3 archaeons DPM^{-1}).

uptake is largely restricted to archaea (Fig. 4B), it might serve as a proxy for archaeal biomass production.

The measured leucine incorporation was converted into prokaryotic production using different conversion factors (Table 2) (8, 30). To convert bicarbonate incorporation into archaeal biomass production, it was assumed that inorganic carbon is the sole carbon source of archaea and that the carbon dioxide uptake by bacteria via their anaplerotic pathway is insignificant, due to the low percentage of bacteria taking up bicarbonate (Fig. 4B) and their lower relative abundance compared to archaea below a depth of 100 m (Fig. 2). Additionally, the seawater batch culture-derived relation between the increase in archaeal abundance versus DPM of the radiolabeled bicarbonate incorporated (Fig. 5) was used to calculate the conversion factors by two approaches (3, 18). The five different conversion factors used to estimate prokaryotic production in the different water layers of the mesopelagic and bathypelagic North Atlantic resulted in prokaryotic production estimates differing by up to 1 order of magnitude (Table 2). The conservative estimates of prokaryotic production using the theoretical conversion factor with and without the intracellular isotope dilution taken into account (30) agree well with the few prokaryotic production data available from mesopelagic and bathypelagic realms of the global ocean (11, 21, 23).

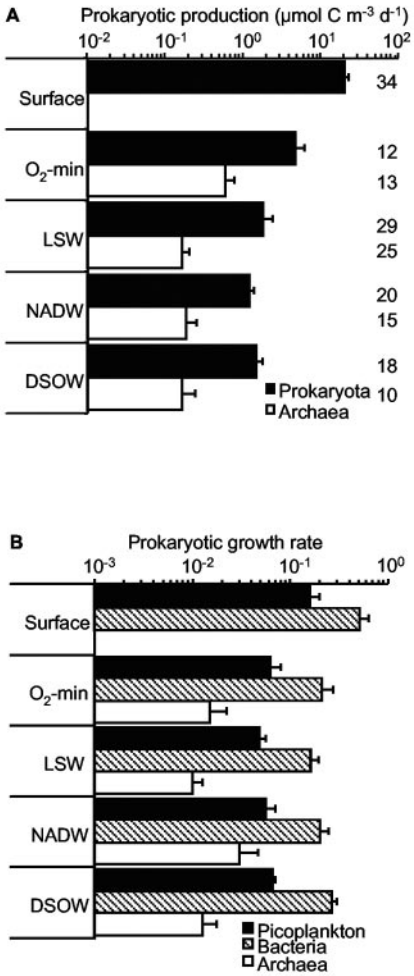

FIG. 6. Prokaryotic and archaeal production (A) and turnover rate (production/biomass day^{-1}) (B) in the different water masses of the North Atlantic. For abbreviations, see Table 1. In panel A, numbers on the right indicate the number of samples. Prokaryotic production is based on the theoretical conversion factor with an intracellular isotope dilution factors of 2 (30) (i.e., 3.1 kg C mol^{-1} leucine incorporated); archaeal production was calculated with the empirical conversion factor obtained from the batch culture incubations using the integrative approach (18).

Generally, prokaryotic production declined from the 100-m layer to the NADW by 1 order of magnitude regardless of the underlying conversion factor used to convert leucine incorporation into prokaryotic production rates (Table 2). The lower limit of archaeal production is set by the conversion of radiolabeled inorganic carbon into organic intracellular compounds of archaea, assuming that natural inorganic carbon is taken up at the same rate. This conservative estimate is only about 20 to 27% lower than the estimate based on our empirically determined conversion factor by the integrative approach (18) but only about half of the archaeal production estimated with the conversion factor determined by the cumulative approach (3) (Table 2). Thus, archaeal production appears to be better constrained than prokaryotic production.

Similar to bacterial and archaeal abundance, prokaryotic and archaeal production in the mesopelagic and bathypelagic water column of the North Atlantic did not show any latitudinal trends. Thus, the data were pooled according to the water masses. The most likely estimate of the actual prokaryotic and archaeal production of the main mesopelagic and bathypelagic water masses of the North Atlantic is shown in Fig. 6A. Mean prokaryotic production declined from the 100-m layer to the oxygen minimum layer at a depth of around 400 m from 20 μmol C m^{-3} day^{-1} to about 5 μmol C m^{-3} day^{-1}, declining further to 1.2 μmol C m^{-3} day^{-1} in the NADW. Archaeal production generally followed the same decreasing trend with

TABLE 3. Percentage of archaeal to prokaryotic production in water masses[a]

WM	*n*	Ratio of archaeal versus prokaryotic production (%)					
		A1:P1	A1:P2	A2:P1	A2:P2	A2:P3	A2:P5
O_2 minimum	10	20.7 (12.5)	10.1 (6.1)	27.2 (16.4)	13.2 (7.9)	7.4 (4.5)	2.1 (1.3)
LSW	17	64.1 (140.1)	31.1 (68.0)	84.0 (183.7)	40.8 (89.1)	22.9 (50.1)	6.5 (14.2)
NADW	13	15.5 (12.9)	7.5 (6.3)	20.3 (16.9)	9.8 (8.2)	5.5 (4.6)	1.6 (1.3)
DSOW	8	8.1 (7.5)	3.9 (3.7)	10.6 (9.9)	5.2 (4.8)	2.9 (2.7)	0.8 (0.8)

[a] Mean values are given, and SDs are in parentheses. *n*, pairs of data; WM, water masses. For abbreviations of water masses, see Table 1. For conversion factors (A1, A2, P1 to P3, and P5), see Table 2, footnote *b*.

depth as prokaryotic production (Fig. 6A) and comprised, depending on calculation of the production rates, from 13 to 27% of total prokaryotic production in the oxygen minimum layer, from 41 to 84% in the Labrador Seawater, from 10 to 20% in the NADW, and from 12 to 24% in the Denmark Strait Overflow Water (Table 3).

Based on the estimates of prokaryotic and archaeal production and the picoplankton and archaeal abundance, we can compare the growth rate (production/biomass) of the total picoplankton community with that of archaea (*Crenarchaeota* plus *Euryarchaeota*). Prokaryotic growth rate declined by a factor of 3 from 0.16 day^{-1} in the 100-m layer to 0.06 day^{-1} in the NADW, taking intracellular isotope dilution into account for calculating prokaryotic production (Fig. 6B). Archaeal growth rates were always significantly lower (Mann-Whitney; $P < 0.05$) than that of the total prokaryotic community (0.01 to 0.03 day^{-1}). In the seawater batch cultures (Fig. 5), however, *Euryarchaeota* barely increased in abundance. This could indicate for the above-given growth rates for archaea that crenarchaeotal growth rates are higher and those of *Euryarchaeota* are lower than overall estimated archaeal growth rates (Fig. 6B).

As all our production measurements were made under surface pressure conditions, decompression of the cells could have influenced the production measurements. It might be that the contrasting patterns in the growth rates between archaea and bacteria observed in the short-term incubations (archaea exhibiting lower growth rates than bacteria) and in the batch cultures where archaea grew at higher rates than bacteria reflect differences in sensitivity to changing pressure conditions (Fig. 5). Clearly, more work has to be done on the influence of pressure on deep-sea prokaryotes.

Organic versus inorganic carbon utilization by planktonic archaea. We presented evidence that archaea are actively growing in the oxygenated water column using, at least to some extent, bicarbonate or CO_2 as a carbon source as described previously for some *Crenarchaeota* (20, 25). Archaea numerically dominate the prokaryotic community in the North Atlantic below a depth of 100 m to the NADW. Therefore, archaea might represent a hitherto-unrecognized sink for inorganic carbon in the largest oceanic subsystem, the mesopelagic and bathypelagic realm. Combining the depth-integrated mean C fixation rate (derived from the conservative estimate that inorganic carbon is the sole carbon source for archaea) of 0.014 fmol C $archaeon^{-1}$ day^{-1} and assuming that approximately 1.3×10^{28} archaeal cells inhabit the global ocean (15), we arrive at a global inorganic carbon fixation in the dark ocean by archaea of 6.55×10^{13} mol C $year^{-1}$. Current estimates of oceanic methane formation, as a major sink of CO_2 in the dark ocean, amount to about 8×10^{11} mol C $year^{-1}$ (16). Thus, archaeal dark C fixation might be 2 orders of magnitude higher than oceanic methane formation and is in the same range as global methane formation (approximately 4.5×10^{13} mol C $year^{-1}$) (16).

The metabolic requirements of planktonic archaea remain a major unsolved issue. CO_2 and O_2 and nitrate, as potential electron acceptors, are generally readily available throughout the dark ocean. Archaeal and bacterial growth rates remained rather constant throughout the water column from the oxygen minimum layer to the DSOW (Fig. 6B), reflecting the similar decrease in prokaryotic abundance and production with depth. Commonly, the availability of the energy source for prokaryotes is assumed to decrease with water column depth. While this is certainly true for heterotrophic bacteria, the energy source for planktonic archaea is unknown. A previous study suggested that archaea might use hydrogen as electron donor to reduce nitrate (31). Besides hydrogen, all the known electron donors for chemolithotrophic metabolism are only available at trace concentrations in the ocean's interior. A fraction of the required energy might also be derived from the uptake of dissolved organic matter (DOM), or some archaea utilize DOM while others live on an autotrophic mode. The uptake of amino acids as indicated by microautoradiography in this study could be an indication of the utilization of DOM as an energy source. Since archaea exhibited lower growth rates than bacteria in the short-term incubations, it might well be that some archaea are utilizing different fractions of the generally refractory DOM in the ocean's interior than bacteria. However, the question about the nature of the electron donor responsible for fuelling cell growth and CO_2 fixation in pelagic archaea remains to be answered.

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