UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA



SUFICIENCIA INVESTIGADORA

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Abstract

The distribution of Crenarchaeota, Euryarchaeota and some major groups of Bacteria (SAR 11, Roseobacter, Gamma-proteobacteria and Bacteroidetes) was investigated in the North Atlantic water column (surface-2000 m) along a transect from the coastal waters of the NW Africa upwelling to the offshore waters of the Canary Coastal Transition Zone (CTZ). Catalyzed reported deposition fluorescence in situ hybridization (CARD-FISH) was used to describe the composition of the prokaryotic assemblages. Additionally bulk picoplankton abundance and leucine incorporation was determined Pronounced changes in the composition of the prokaryotic assemblages were observed from the coastal region to the open ocean and at the Deep Chlorophyll Maximum (DCM) accompanied with decreasing bulk heterotrophic activity. All bacterial groups decreased in absolute abundances from the coast to the open ocean, whereas both archaeal groups increased towards the open ocean. SAR 11, was the most abundant prokaryotic group in the DCM and probably responsible of major changes in bulk heterotrophic production. Prokaryotic abundance and activity decreased two and three orders of magnitude, respectively, from the surface to 2000 m depth. Prokaryotic growth rates were, however, high in the mesopelagic zone (about 0.13 d^{-1}), compared to other reports from the central North Atlantic. Throughout the water column, the contribution of SAR11 to total picoplankton abundance decreased from 42% in the DCM to 4% at 2000 m depth, while Crenarchaeota increased from 1% in the DCM to 39% in the Oxygen Minimum (OM) layer. A clear influence of the different intermediate water masses was observed on the bulk picoplankton heterotrophic activity with lower leucine incorporation rates corresponding to layers where patches of Antarctic Intermediate Water were detected. Our results show that coastal-ocean and surface-depth gradients in bulk prokaryotic abundance and production, as well as in assemblage composition, were comparable to changes observed in basin-scale studies, pointing the coastal transition zones as regions of strong variability in microbial diversity and metabolism.

Introduction

Over the past few decades it became clear that prokaryotes are an important component of marine plankton communities, playing a key role in mediating a range of biogeochemical cycles (e.g. Azam et al., 1983). Prokaryotic plankton have been enumerated in a "black box" approach, ignoring the fact that changes in community structure could lead to concomitant changes in activity. With the advent of molecular biology techniques, marine microbiologists have been able to analyze the prokaryotic communities from a "Gleasonian" point of view (Gleason, 1926), focusing on distinct prokaryotic groups that can be reliably identified and quantified in marine assemblages (Pernthaler et al., 2005).

Small-subunit ribosomal (RNA) genes, analyzed by molecular techniques, have become the universal phylogenetic markers and the main criteria by which microbial plankton groups are identified (Giovannoni & Rappé, 2000). Most of these groups were first identified by sequencing 16S rRNA genes cloned from seawater (Britschgi & Giovannoni, 1991; Fuhrman et al., 1992; 1993; Giovannoni et al., 1990; Schmidt et al., 1991). It became soon apparent that less than 20 microbial clades accounted for most of the genes recovered (Mullins et al., 1995). Nevertheless, 16S rRNA gene clone libraries do not accurately reflect the abundance of microbes from particular phylogenetic clades in the environment. Such analyses need to be complemented by other strategies to study single populations in situ, like hybridization techniques such as the catalyzed reported deposition fluorescence in situ hybridization (CARD-FISH; Pernthaler et al., 2002). Indeed, some groups of marine bacteria had been known for years from their 16S rRNA gene sequences before their abundances could be determined. This is the case for bacteria related to the marine SAR11 (Morris et al., 2002), SAR86 (Pernthaler et al., 2002), SAR116 (Fuchs et al., 2005), SAR202 (Morris et al., 2004), or SAR406 (Fuchs et al., 2005) clades.

The presence of planktonic prokaryotes in a given oceanic habitat is thought to be determined by local environmental conditions and not by restricted dispersal (Pedrós-Alió 2006; Finlay 2002). Members of the SAR11 clade are among the most common prokaryotes in the marine plankton. They can contribute >50% to the total picoplankton abundance in the surface waters of the northwestern Sargasso Sea, and 25% of mesopelagic picoplankton assemblages (Morris et al., 2002). Bacteria related to *Roseobacter* sp., also referred to as the SAR83 cluster (Rappe et al., 2000), are another common component of coastal and offshore picoplankton assemblages constituting up to

25% of the marine picoplankton (Eilers et al., 2001; González et al., 1997; Suzuki et al., 2001). The seasonal dynamics in abundance of Roseobacter sp. are following closely the development of phytoplankton biomass in the North Sea (Eilers et al., 2001). The Bacteroidetes cluster has been associated with upwelling events (Fandino et al 2001, Alonso-Sáez et al., 2007b), their members showing a high ability to degrade highmolecular weight compounds (Cottrell and Kirchman 2000, Kirchman 2002). Member of the SAR86 lineage, related to the gamma-Proteobacteria, are characterized by high growth rates probably using efficiently nutrient pulses (Eilers et al., 2000, Fuchs et al. 2000, Yokokawa et al., 2004). Archaea are widespread among marine prokaryotic plankton (De Long et al. 1994; Massana et al. 1998; Crump and Baross 2000). Quantitative studies with fluorescence in situ hybridization (FISH) have shown the dominance of Crenarchaeota in meso- and bathypelagic waters, whereas Euryarchaeota were thought to comprise <5-8% of total picoplankton abundance in deep waters of the Pacific and the Antarctic Oceans (Karner et al. 2001; Church et al. 2003). Using CARD-FISH, in combination with the proteinase-K permeabilization of the archaeal cell wall, Teira et al. (2004, 2006) and Herndl et al. (2005) found higher (10-20%) abundances of Euryarchaeota in deep waters of the North Atlantic Ocean. All these studies support the view that diverse prokaryotic groups are linked to different environmental conditions and hence, changes in assemblage structure are likely to be more pronounced in regions with strong oceanographic gradients.

Most of the studies describing variability in the composition of picoplankton have been carried out in surface oceanic waters (e.g. Alonso-Sáez et al. 2007b, Winter et al. 2005, Reinthaler et al. 2005, Riemann & Middelboe 2002, Acinas et al. 1997), whereas only a few have quantified the importance of the different groups of prokaryotes in the dark ocean. Among these, some used PCR-based fingerprinting techniques (Hewson et al. 2006) as a tool for identifying the community components, while others applied PCRindependent group-level FISH. The later, however, used two or three prokaryotic probes targeting Bacteria, Crenarchaeota and Euryarchaeota (Karner et al. 2001, Teira et al. 2004, 2006, Herndl et al. 2005).

Likewise, information regarding the activity of deep-water prokaryotic communities is rather scarce compared to that of surface water communities (Dufour and Torretón 1996, Nagata et al. 2000, Tanaka and Rassoulzadegan 2004, Reinthaler et al., 2006). Measurements on de-pressurized samples indicate a one to two orders of magnitude decrease in picoplankton production from the euphotic zone to the bathypelagic waters (Patching and Eardly 1997, Nagata et al. 2000, Hansell and Ducklow 2003, Reinthaler et al. 2006), declining with depth slightly more than picoplankton abundance .

Variations in bulk prokaryotic activity may be related to changes in the abundance of specific groups. Some studies have shown, a clear link between the distribution of the major prokaryotic groups to bulk (Massana et al. 1998, Murray et al. 1999), and group-specific, prokaryotic activity in the dark ocean (Ouverney and Fuhrman 2000, Teira et al. 2004, 2006, Herndl et al. 2005). This relationship could be more evident in regions with strong environmental gradients and high picoplankton activity. Arístegui et al. (2005) measured high prokaryotic activity in the mesopelagic waters of the subtropical northeast Atlantic, a region characterized by strong hydrological variability, with most pronounced gradients close to the NW African coastal upwelling (Arístegui et al. 2003).

In this study we report the distribution of both the bulk prokaryotic activity and the abundance of major prokaryotic groups (Crenarchaeota, Euryarchaeota, SAR 11, Roseobacter, gamma-proteobacteria and Bacteroidetes) along a transect from the coastal waters of the Northwest African upwelling to the offshore waters of the Canary region. We used the catalyzed reported deposition FISH (CARD-FISH) method to assess the composition of the prokaryotic community and relate it to bulk leucine incorporation. We hypothesized that the contribution of Crenarachaeota and Euryarchaeota to total picoplankton abundance increases from along the coastal-ocean transect in the mesopelagic waters and, overall, that the surface-depth patterns of abundance and metabolism of Bacteria and Archaea are related to local hydrographic conditions.

Material and Methods

Location and sampling

A zonal section of 10 stations was sampled from the coastal waters of the NW Africa upwelling (27.9°N, 13.1°W) to the offshore waters of the Canary Coastal Transition Zone (CTZ) region (27.5° N, 15.3° W) (Fig. 1) during the CONAFRICA cruise (22 March-7 April, 2006), on board the R/V Hespérides. At each station, temperature, salinity and fluorescence were recorded down to 2000 m depth using a SeaBird 9-11+ CTD system, mounted on a General Oceanics rosette sampler, equipped with twenty-four 12 l Niskin bottles. Samples for prokaryotic heterotrophic production and abundance were collected at each station from 5 depths ranging from 5 m to 2000 m including the Deep Chlorophyll Maximum (DCM; between 30-82 m), 200 m and the Oxygen Minimum Zone (OMZ; 740-



<u>Fig. 1.</u> (A) Near sea-surface temperature and (B) SeaWiFS chlorophyll image from 7 April 2006. Station positions (4-70) overlaid on maps. Notice the upwelling filament extending offshore from the coast.

760 m). Samples for CARD-FISH were collected at 6 selected stations and at 3 depths (DCM, OMZ and 2000 m) only.

Prokaryotic abundance and biomass

Prokaryotic abundance was determined by flow cytometry using a FACScalibur (Becton Dickinson) with a laser emitting at 488 nm wavelength. Samples (1.5 ml) were fixed with paraformaldehyde (1% final concentration), incubated at 4°C for 15-30 min and then stored frozen in liquid nitrogen until analyzed. Prior to counting the cells in the flow cytometer, 200 µl of sample was stained with a DMS-diluted SYTO-13 (Molecular Probes Inc.) stock (10:1) at 2.5 µM final concentration. Prokaryotes were identified by their signatures in a plot of side scatter (SSC) versus green fluorescence (FL1). High and low nucleic acid cells (L-NA, H1-NA, H2-NA) were separated in the scatter plot (Gasol et al. 1999). Picocyanobacteria were discriminated in a plot of FL1 versus FL3 (red fluorescence). When the fluorescence signal of the prochlorophytes was very low, they were first enumerated in an unstained sample and their abundance subtracted from the total prokaryotic abundance determined by SYTO-13 staining. Prokaryotic biomass was estimated from prokaryotic abundance assuming a conversion factor of 12 fg C cell⁻¹ (Fukuda et al. 1998). A suspension of yellow-green 1 μ m latex beads (10⁵ beads ml⁻¹ for phytoplankton and 10^6 beads ml⁻¹ for prokaryotes) was added as an internal standard (Polysciences, Inc.). The concentration of beads was determined under an epifluorescence microscope.

Prokaryotic heterotrophic production

Prokaryotic heterotrophic production was estimated from the rates of protein synthesis determined by the incorporation of tritiated leucine using the centrifugation method (Simon and Azam 1992). ³H-Leucine (Leu, Amersham, SA 171 Ci mmol⁻¹) was added at saturating concentration (40 nmol l⁻¹) to four replicate subsamples of 1.2 ml. Duplicate controls were established with the addition of 120 µl of 50% trichloroacetic acid (TCA) before the isotope addition. The Eppendorf tubes were incubated at temperatures as close as possible to the *in situ* in temperature-controlled chambers for 2 to 7 h. The incorporation was stopped by adding 120 µl ice-cold 50% TCA to the quadruplicate subsamples. Subsequently, the subsamples and the controls were kept at -20° C until centrifugation (at ca. 12000 rpm) for 20 min followed by aspiration of the water. Finally, 1 ml of scintillation cocktail was added to the Eppendorf tubes before determining the incorporated radioactivity after 24-48 h on a Wallac scintillation counter with quenching correction using an external standard. Leucine incorporated into prokaryotic biomass was

converted to carbon production using the theoretical conversion factor of 1.5 kg C mol⁻¹ Leu assuming no isotope dilution (Simon and Azam 1989). Since both Bacteria and Archaea incorporate leucine, it is more appropriate to refer to the term "prokaryotic heterotrophic production" instead of the commonly used "bacterial production".

Catalyzed reporter deposition-FISH (CARD-FISH)

Immediately after collecting the samples from the Niskin bottles, 10-40 ml subsamples were fixed with paraformaldehyde (2% final concentration) and stored at 4°C in the dark for 12–18 h. The sample was then filtered through a 0.2-µm polycarbonate filter (Millipore, GTTP, 25-mm filter diameter) supported by a cellulose nitrate filter (Millipore, HAWP, 0.45 mm), washed twice with Milli-Q water, dried and stored in a microfuge vial at -20°C until further processing in the home laboratory. Filters for CARD-FISH were embedded in low-gelling-point agarose and incubated either with lysozyme for the Bacteria probes Eub338-III (mixture of probes Eub338, Eub II, and Eub III, Amann et al. 1990, Daims et al. 1999), for Roseobacter using Ros537 (Eilers et al. 2001), for Gamma-proteobacteria Gam42a (Manz et al. 1992), for Bacteroidetes CF319a (Amann et al. 1990) and SAR11 using SAR11-441R (Morris et al. 2002), or proteinase-K for the Euryarchaeota probe Eury806 and for the Crenarchaeota probe Cren537 (Teira et al. 2004). Filters were cut in sections and hybridized with horseradish peroxidase (HRP)-labeled oligonucleotide probes and tyramide-Alexa488 for signal amplification, following the protocol described in Teira et al. (2004). Cells were counterstained with a DAPI-mix (5.5 parts of Citifluor [Citifluor, Ltd.], 1 part of Vectashield [Vector Laboratories, Inc.] and 0.5 parts of phosphate-buffered saline (PBS) with DAPI [final concentration 1 µg mL⁻¹]). The slides were examined under a Zeiss Axioplan 2 microscope equipped with a 100-W Hg lamp and appropriate filter sets for DAPI and Alexa488. More than 800 DAPI-stained cells were counted per sample. For each microscope field, two different categories were enumerated: (1) total DAPI-stained cells, (2) cells stained with the specific probe. The counting error, expressed as the percentage of standard error between replicates, was 2% for DAPI counts.

Results

Regional oceanographic settings

The stations were placed along a coastal – ocean transect crossing an upwelling filament and an offshore anticyclonic eddy centered at station 57 (Fig. 1A). Filament-eddy systems have been described in previous studies for the same region (Barton et al. 1988, 2004). The eddy entrained filament-water with higher content in chlorophyll in a meandering fashion, giving rise to sharp fronts in chlorophyll and temperature. Fig. 1B (from 7 April 2006) illustrates the advection of high chlorophyll-containing water along the filament, coinciding with a low surface temperature field (Fig. 1A) recorded during the time of study (22 March to 7 April 2006). Fig. 2A shows the vertical cross-section of density and chlorophyll concentrations. The DCM was between 20-40m near the upwelling region and deepened slightly towards the open ocean with the most pronounced deepening of the DCM at the eddy center at St. 57). The sharpest gradients in density and chlorophyll were found between Sts. 11 & 20 (upwelling front), Sts. 57 & 64 (eddy-filament front) and Sts. 64 & 70 (eddy-open ocean water front).

The North Atlantic Central Water (NACW) occupied the main thermocline (Fig. 2B). Below the NACW, two intermediate water masses dominated the 600-1500 m depth range, the Antarctic Intermediate Water (AAIW), detected by its fresh anomaly values (salinity values <35.3), and the warm, high salinity Mediterranean Sea Outflow Water (MSOW). The AAIW was manifested as tongues of lower salinity water, centered at 750-900 m, in the eastern part of the section (Sts. 20, 36 & 43; Fig. 2B). The AAIW is transported northward by a current wider than the usual along-shore poleward undercurrent (Hernández-Guerra et al. 2001). All stations from the western part of the section (Sts. 70, 64, 57, 51), as well as St. 27 were dominated by MSOW. The water masses below the intermediate waters represented a mixture between North Atlantic Deep Water (NADW) and intermediate waters. As described below, the presence of the different hydrographic structures or water masses clearly influenced the distribution and activity of the prokaryotic community.



<u>Fig. 2.</u> Contour plots of (A) potential density (kg m⁻³, grey lines) and chlorophyll fluorescence (relative units, color palette) and (B) salinity. AAIW: Antarctic Intermediate Water; NACW: North Atlantic Central Water.

Prokaryotic abundance and activity

Generally, prokaryotic abundance in the surface waters and the DCM decreased from the upwelling region to the open ocean while for the 200 m depth horizon, the oxygen minimum zone (OMZ) and the 2000 m depth horizon no lateral trend was detectable (Fig. 3A). Prokaryotic abundance in the surface and the DCM ranged from 1.5 to 8.3×10^5 cells ml⁻¹, decreasing exponentially (by 2 orders of magnitude) to $8.1 \times 10^3 - 3.7 \times 10^4$ cells ml⁻¹ at 2000 m depth (Fig. 3A). As for prokaryotic abundance, leucine incorporation rates were highest in the surface waters and the DCM in the coastal upwelling region (398 pmol Leu l⁻¹ h⁻¹) decreasing more than 20 fold towards the oceanic stations (16.7 pmol Leu l⁻¹ h⁻¹) and by 3 orders of magnitude with depth (Fig. 3B). The highest values of cell-specific production were also found at the stations close to upwelling region in the surface and the DCM (3.8 and 3.6 fmol C cell⁻¹ d⁻¹) (Fig. 3C). Interestingly, leucine incorporation and



Fig.3. (A) Prokaryotic abundance (cells ml⁻¹), (B) bulk leucine incorporation rates (pmol Leu l⁻¹ h⁻¹) and (C) Cell-specific heterotrophic production (fmol C cell⁻¹ d⁻¹) along the coastal-offshore transect in the surface waters (5 m), DCM (200 m), oxygen minimum zone (OMZ) and 2000 m depth. Standard errors are not plotted since they are smaller than the symbols. Notice that the coastal stations have not deep samples.

cell-specific production followed a similar pattern in the OMZ and at 2000 m as in the epipelagic zone (Fig. 3B. C), although lower in magnitude. Mean cell-specific production was about one order of magnitude higher in the surface layer and the DCM than in deeper waters (Table 1, Fig. 3C). Cell-specific leu incorporation between the 200 m layer and the OMZ were not significantly different (Student's *t*-test; p = 0.24, n = 8) (Table 1) but were only about half of that determined for the 200 m layer at 2000 m depth (Table 1; Fig. 3C). The prokaryotic biomass turnover time increased with depth (from <2 d in the surface waters to >30 d at 2000 m, Table 1). The lowest values were observed at St. 70, where prokaryotic activity was very low at all depths but cell numbers increased in the deeper layers compared to the other stations, coinciding with the presence of large prokaryotic populations with H-NA content (Fig. 4). In this station a sudden decrease in the % of L-NA cells and in one of the groups of the H-NA (High-1) was observed, reaching the maximum relative abundance of the other H-NA group (High-2).

Depth	$CSHP (fmol \ C \ cell^1 \ d^1)$) $PGR(d^{-1})$	Turnover time (d)	% High-NA
	Average $\pm SE$	Average $\pm SE$	Average $\pm SE$	Average $\pm SE$
5	1.71 ± 0.30	1.02 ± 0.18	1.4 ± 0.4	23 ± 3
	(10)	(10)	(10)	(10)
DCM	1.45 ± 0.29	1.34 ± 0.27	1.1 ± 0.3	27.8 ± 4
	(8)	(8)	(8)	(8)
200	0.15 ± 0.02	0.14 ± 0.02	10.5 ± 4	36.6 ± 4
	(8)	(8)	(8)	(8)
OMZ	0.13 ± 0.03	0.12 ± 0.03	24.9 ± 15	66.5 ± 17
	(8)	(8)	(8)	(8)
2000	0.08 ± 0.02	0.07 ± 0.02	30.2 ± 12	67.6 ± 19
	(8)	(8)	(8)	(8)

<u>Table 1.-</u> mean prokaryotic activity (±standard error, SE) along the coastal-offshore transect in surface waters (5 m depth), DCM, 200 m depth, OMZ and 2000 m depth. CSHP: cell-specific heterotrophic production. PGR: Prokaryotic growth rate. High-NA – high nucleic acid content prokaryotes; number of measurements given in parenthesis.



<u>Fig.4.</u> Percentage of low and high nucleic-acid (NA) content cells to total prokaryotic abundance, at (A) the OMZ and (B) at 2000 m depth.

Prokaryotic assemblage structure

The recovery efficiency of the prokaryotic community using CARD-FISH, i.e., the sum of the relative abundance of Bacteria and Crenarchaeota and Euryarchaeota was $77 \pm 3\%$ (n=14) of total DAPI-stainable cells. Bacteria accounted from $54 \pm 2\%$ (n=6) in the DCM to $45 \pm 2\%$ (n=8) at 2000 m depth of DAPI-stained cells averaged over the entire water column $49 \pm 3\%$ (n=14).

Generally, Bacteria were relatively more abundant in the upwelling region and decreased in their contribution to total prokaryotic abundance towards the oceanic stations, particularly in the DCM and the OMZ, while the archaeal contribution increased towards the open ocean (Fig. 5). The most abundant prokaryotic group in the DCM was SAR11 with a relative contribution to total DAPI-stainable cells ranging from 36% to 42%. In the OMZ, the relative contribution of Crenarchaeota and Bacteroidetes increased from the upwelling region towards the open ocean, while the contribution of SAR11 decreased and Euryarchaeota and Roseobacter sp. remained almost invariant along the transect. The abundance of SAR11 declined steeply with depth, reaching the lowest relative abundance at 2000 m depth (Fig. 5). Crenarchaeota showed a contrasting trend with a low relative contribution in the DCM and the highest relative abundance in deep waters, where they accounted for up to 39.5% of the DAPI-stained cells. The highest abundance of Crenarchaeota, Bacteroidetes (with the exception of St. 20) and gamma-proteobacteria were found in the OMZ. Euryarchaeota contributed up to 13% and 20% to the DAPIstained cells in the DCM and 2000 m depth layer, respectively, without a clear vertical distribution pattern. Roseobacter sp., however, exhibited highest relative abundance in the DCM, particularly in the upwelling region, and lowest in the deep waters, except at St. 20.



<u>Fig. 5.</u> Relative abundances of bacterial groups detected by HRP-oligonucleotide probes and CARD-FISH scaled to DAPI counts at the DCM, the OMZ and at 2000 m depth. C: Crenarchaeota, E: Euryarchaeota, S11: SAR11, CF: Bacteroidetes, R: Roseobacter, g: Gamma-proteobacteria.

Discussion

Variability in bulk prokaryotic abundance and heterotrophic production

The range of variability in leucine incorporation rates (16.7-398 pmol Leu $l^{-1} h^{-1}$) measured in the surface waters along the coastal-ocean transect (13-15.3°W) was similar to other studies covering much larger spatial scales. For instance, Alonso-Sáez et al. (2007a) found leucine incorporation rates from 15-331 pmol Leu l⁻¹ h⁻¹ in the eastern boundary region of the North Atlantic Ocean (14.5-26°W) and Hoppe et al. (2006) reported a range of 2-141 pmol Leu l⁻¹ h⁻¹ along a meridional transect across the Central Atlantic Ocean (53°N-65°S). The highest prokaryotic activities in our study were measured in the upwelling stations (Fig. 3B), while the lowest activities were measured in the most oceanic station. Leucine incorporation at the 200 m depth horizon was highest in the upwelling region and decreased to St. 20 and remarkably, increased thereafter towards St. 64 (Fig. 3B), probably reflecting the accumulation of organic matter in the core of the anticyclonic eddy. Arístegui et al. (2003) observed large accumulations of DOC in the center of anticyclonic eddies in this region. The most pronounced changes in the incorporation rate of leucine and cell-specific production was observed form St. 64 to St. 70 (Fig. 3B, C). In contrast, prokaryotic abundance did not decrease significantly (in the DCM) or even increased (OMZ and 2000 m depth) (Fig. 3A). As indicated in Fig 1A & B, St. 64 is within the offshore extension of the filament while St. 70 represents open ocean waters. Aristegui et al. (2004) and Arístegui and Montero (2005), reported high concentrations of prokaryotes in the epipelagic zone at frontal regions between filaments and eddies, although no heterotrophic metabolic activities were measured. In their study, an increase in prokaryotic abundance was noticed at the 200 m depth horizon and deeper, but not in shallower waters. The boundary between eddy systems may extend through the thermocline. In our study, this pattern was not observed, since no stations were sampled west of St. 70, but other studies have described excursions of isopycnals down to 800 m depth in eddy-pair fronts (e.g. Arístegui et al. 2003). This would explain the accumulation of bacteria at St. 70, although surprisingly the increase is observed even at 2000 m depth. None of the prokaryotes assemblages, among those identified by CARD-FISH, seemed to contribute specifically to this increase in prokaryotic abundance (see below). However, it is evident that large cells with high-NA content contributed to the increase in prokaryotic abundance in the OMZ and 2000 m depth (Fig. 4). This could result from a decrease in grazing pressure, allowing cells to grow larger in size, since cell-specific production was

significantly lower than at the other stations. At intermediate waters such as the OMZ, prokaryotic activity was lower in the AAIW than in the MSOW, excepting at St. 70, which exhibited the lowest prokaryotic activity in almost all depth layers. The lower activity in the AAIW is presumably the result of lower (or more refractory) organic carbon in this old water mass. At 2000 m depth, the prokaryotic activity pattern paralleled that of the OMZ, suggesting mixing of the NADW with the intermediate waters.

Cell-specific prokaryotic production in surface waters was about one order of magnitude higher than in deep waters (Table 1) resulting in an average prokaryotic growth rate of 1.0 ± 0.2 d⁻¹ for surface waters. Our growth rates are substantially higher than the 0.4±0.1 d⁻¹ reported by Arístegui et al. (2005) for the Canary Current region although their stations were more oceanic. For St. 70, the most oceanic station, prokaryotic growth rate in surface waters was 0.26 d⁻¹, comparable to those reported by Aristegui et al. (2005). The average cell-specific prokaryotic production in the mesopelagic (~ 0.14 fmol C cell⁻¹ d⁻¹) and bathypelagic (0.08 fmol C cell⁻¹ d^{-1}) zone is considerably higher than the range of 0.01-0.06 fmol C cell⁻¹ d⁻¹ obtained by Reinthaler et al. (2006) for the meso- and bathypelagic realms of the eastern and western North Atlantic basin. Consequently, their estimated turnover times of 34-54 d are higher than the turnover times of 24 and 30 d obtained in our study for the OMZ and 2000 m depth, respectively (Table 1). Moreover, Reinthaler et al. (2006) used a conversion factor of 3.1 kg C mol⁻¹ Leu (assuming a twofold isotopic dilution), whereas we used 1.5 kg C mol⁻¹ Leu (assuming no isotopic dilution). If we recalculate their data with 1.5 kg C mol⁻¹ Leu, the cell-specific prokaryotic production would be even lower and consequently, the turnover times higher, enhancing the differences to our results. Hence, the variability in the metabolic rates must rely on other causes, aside of the carbon to leucine conversion factors used when calculating prokaryotic production. Differences in prokaryotic activity between the deep waters of the central North Atlantic and the eastern boundary region of the subtropical North Atlantic might be caused by a higher supply in organic matter to prokaryotic communities closer to the continental margin. Arístegui et al. (2005) estimated an average prokaryotic growth rate of 0.13 ± 0.02 d⁻¹ for the mesopelagic zone of the Canary Current, which is in agreement with the growth rate obtained in our study for the 200 m horizon $(0.14\pm0.02 \text{ d}^{-1})$ and the OMZ $(0.12\pm0.03 \text{ d}^{-1})$. These authors concluded from their study, after analyzing a set of metabolic indices, that prokaryotic communities in the mesopelagic zone were very active, acting as major sinks for organic carbon in the subtropical NE Atlantic Ocean.

Prokaryotic assemblages variability

One of the main goals of our study was to decipher patterns in the distribution in bulk prokaryotic properties and assemblage structure linked to water mass characteristics and hydrographical regimes, particularly in the deep ocean. Previous studies using PCRindependent methods have identified and quantified the dominant bacterial groups in surface waters (Glöckner et al. 1999, Eilers et al. 2001, Fuchs et al. 2005), however, there is only rather rudimentary information available on the abundance of the major bacterial groups in the meso- and bathypelagic zones using FISH (see Introduction). Some of these authors found minor differences in the proportion of major phylogenetic groups, but larger differences in the proportion of more specific groups such as SAR86, SAR11, SAR116 (Fuchs et al. 2005) and SAR202 (Morris et al. 2004). Alonso-Sáez et al. (2007b), using CARD-FISH, found that bacterial assemblage structure in surface waters of the subtropical NE Atlantic was highly influenced by environmental factors, showing a distinct community in the upwelling region different from the more oceanic waters. In our study, we observed a large variability in the prokaryotic assemblage along the coastal-ocean transect. At the DCM, the relative contribution of SAR11, Euryarchaeota and Crenarchaeota to DAPI-stained cells increased towards the open ocean, while gammaproteobacteria, Roseobacter sp. and Bacteroidetes were more abundant towards the upwelling region (Fig. 5), where bulk prokaryotic activity was generally higher (Fig. 3B, C). This is in agreement with other studies which also reported high contributions of the Bacteroidetes cluster to total prokaryotic abundance in upwelling systems (Fandino et al. 2001, Alonso-Sáez et al. 2007b). Roseobacter sp. was also related previously to regimes with high phytoplankton biomass (Eilers et al. 2001,; González et al. 1997, Suzuki et al. 2001), while the gamma-proteobacteria were associated with nutrient pulses (Eilers et al. 2000, Fuchs et al. 2000, Yokokawa et al. 2004, Alonso-Sáez et al. 2007b). SAR11 was the most abundant group in the DCM, as described in previous studies (Morris et al. 2002, Alonso-Sáez et al 2007a). This cosmopolitan group has been reported to represent >50% of the prokaryotic abundance in the surface waters of the northwestern Sargasso Sea and 25% of the subeuphotic microbial assemblages (Morris et al. 2002), declining in its contribution to prokaryotic abundance with depth (Morris et al. 2004).

In the OMZ, the SAR11 clade showed a contrasting distribution pattern of than in surface waters, being more abundant at St. 20 affected by upwelling AAIW. Crenarchaeota and Bacteroidetes increased in their relative abundance towards the open ocean with Sts.

51 to 70 (affected by MSOW) exhibiting very similar community composition (Fig. 5). At 2000 m depth, no clear gradient in prokaryotic community composition was discernable, although Bacteroidetes and the Crenarchaeota were more abundant in the more oceanic waters (Fig. 5). St. 20 showed a marked increase in the relative abundance of Roseobacter sp. (7% of the DAPI-stained cells) and SAR11 at 2000 m depth. Sequences of Roseobacter sp. have been retrieved from deep-sea sites, including sediments (Li et al. 1999, Gallagher et al. 2004). Since Roseobacter is generally associated with phytoplankton in the euphotic layer, it is likely that Roseobacter is transported via sedimenting phytoplankton into the deep sea where it has been found in sedimented fluff at the deep-sea floor. In accordance to that, St. 20 is situated over the continental slope and the 2000 m depth horizon was just a few meters above the bottom. Hence, it is feasible that the peaks in Roseobacter and SAR11 abundances could be caused by resuspended detrital material.

The estimated relative abundance of Euryarchaeota (4% to 13% in the DCM and 6% to 20% in deep waters) are in agreement with the observations reported by Teira et al. (2006) for the North Atlantic Ocean, where Euryarchaeota ranged from <10% to >30% in subsurface waters and <15% of the total picoplankton community at the 100 m layer. While SAR11 decreases in relative abundance with depth, Crenarchaeota increase with depth (Fig. 5). The highest relative abundance of Crenarchaeota, Bacteroidetes and gamma-proteobacteria were found in the OMZ, except at St. 20, the latter influenced by AAIW. Teira et al. (2006) also found the highest absolute abundance of Crenarchaeota (accounting for >40% of DAPI-stained cells) in the OMZ of the North Atlantic . The increase of Crenarchaeota and gamma-proteobacteria in the OMZ might be related to nitrification processes in the OMZ. Marine nitrification is mediated by three different groups of prokaryotes belonging to the beta- and gamma-proteobacteria (Ward 2002), although recently there is robust evidence to believe that Crenarchaeota also contributes to marine nitrification (Wuchter et al., 2006). It is noteworthy, however, that Wuchter et al. (2006) did not find any gamma-proteobacteria harboring the amoA gene, a gene indicative for the potential to oxidize ammonia. Only beta-proteobacterial and crenarchaeotal sequences were retrieved by amoA gene analyses (Wuchter et al. 2006). Noteworthy, crenarchaeal amoA gene copy numbers determined by q-PCR dominated over betaproteobacterial *amoA* gene copy numbers throughout the North Atlantic mesopelagic (Wuchter et al. 2006). A similar pattern in the distribution of amoA gene copy numbers has been reported for soil prokaryotic communities (Leininger et al, 2006). If Crenarchaeota oxidize ammonia aerobically as Wuchter et al. (2006) observed, the Crenarchaeota could play an important, previously ignored, role in the nitrogen biogeochemical cycle of the ocean.

Bacteria versus Archaea

Recent reports on the distribution of Bacteria and Archaea in the oceans indicate that planktonic Crenarchaeota increase in their relative contribution to prokaryotic abundance with depth reaching cell numbers similar to or higher than those of Bacteria in the mesopelagic zone (Karner et al. 2001, Teira et al. 2006). Euryarchaeota, the other major archaeal group are, however, considered more abundant in surface than deep waters (Massana et al. 2000, Karner et al. 2001, Church et al. 2003), comprising up to 10-30% of picoplankton abundance in subsurface waters (Herndl et al. 2005, Teira et al. 2006).



<u>Fig. 6.</u> Abundance (cells ml⁻¹) of total Bacteria and Archaea (Crenarchaeota and Euryarchaeota) detected by CARD-FISH and specific probes in the DCM.

Euryarchaeota in surface waters have been shown to harbor proteorhodopsin, a lightharvesting pigment, providing the potential to use light as an additional energy source while deep water Euryarchaeaota lack this proteorhodopsin (Frigaard et al. 2006). The fact that Crenarchaeota increase in their relative abundance with depth while bacterial abundance decreases (Fuhrman et al. 1992, Massana et al. 1997, Ouverney et al. 2000, Herndl et al. 2005, Teira et al. 2006), suggests that they are occupying different ecological niches, likely caused by environmental conditions and different substrate requirements. Our results show a relative increase with depth (Fig. 5) of both Crenarchaeota and Euryarchaeota, although absolute archaeal abundances were always higher at the DCM. In all cases the ratio of total Archaea/Bacteria was ≤ 1 (average±SD: 0.24 ±0.08 for the DCM, 0.82 ±0.11 for the OMZ and 0.68 ±0.17 for the 2000 m depth layer.

An even more pronounced trend in the distribution of Bacteria vs Archaea than over depth was observed laterally along the coastal-offshore gradient in the DCM (Fig. 6). Although Archaea were always 2-7 times lower in abundance than Bacteria, the decrease in bacterial abundance from the upwelling region towards the open ocean coincided with a concomitant increase in Archaea as described by the equation: Bacteria (cells ml⁻¹) = 0.61Archaea (cells ml⁻¹) - 60255 (R²= 0.98, p<0.00001). A similar, although less pronounced contrasting abundance pattern of Archaea and Bacteria was found in the OMZ (data not shown). The Crenarchaeota constituted 79% of the total abundance of Archaea in the OMZ, while in the DCM both archaeal groups contributed roughly equally. Lateral gradients in the relative abundance of Bacteria vs Archaea have not been reported thus far. The decrease in bulk leucine uptake along the coastal-ocean gradient was positively related (R² = 0.72, n=6) to the decrease in the bacterial abundance in the DCM, and negatively related (R² = -0.76, n=6) to the increase in archaeal abundance, suggesting that most of the bulk prokaryotic heterotrophic production, as estimated by leucine incorporation, was due to bacterial activity.

SAR11 was the most abundant prokaryotic group in the DCM, decreasing two fold from Sts. 4 to 70, although increasing in the relative contribution to prokaryotic abundance towards the oceanic stations. Thus, this group is probably the major contributor to the bulk prokaryotic metabolism in the open ocean's euphotic layer as indicated earlier.

Conclusions

A high variability was observed in bulk prokaryotic abundance and metabolism, as well as in changes in community structure, along the surface waters from the NW Africa upwelling region towards the open ocean. This variability was comparable to the

variability reported for ocean-basin studies. Bacteria (SAR11, Roseobacter, gammaproteobacteria and Bacteroidetes) were more abundant in coastal than in oceanic regions, in contrast to the distribution of Euryarchaeota and Crenarchaeota. SAR11 was the main responsible of surface-water variability in bulk prokaryotic abundance and presumably, also in bulk production, increasing its relative contribution with respect to other bacterial groups towards the ocean. Prokaryotic abundance decreased about 2 orders of magnitude from surface to 2000 m deep waters, whereas prokaryotic production decreased by 3 orders of magnitude. This, together with the presence of deep waters populations with higher NA content and larger size than surface water cells (data not shown) suggest that grazing pressure could be lower at depth, allowing cells grow larger in size. Both archaeal groups, Crenarchaeota and Eurychaeota, contributed up to a 50% to the total prokaryotic abundance in the dark ocean. AAIW exhibits lower prokaryotic activity than MSOW, presumably due to lower organic matter content of the AAIW. Prokaryotic growth rates in the deep ocean were considerably higher than those reported for the Central Atlantic, but comparable to previous studies from the same region. This supports the view that prokaryotic communities in the deep waters of the subtropical NE Atlantic maintain higher metabolic rates than in the central ocean, due to lateral transport of organic matter from the continental margins.

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