Distribution of major bacterioplankton groups in the Atlantic Ocean

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Summary

The Atlantic Ocean is comprised of several ecological provinces with characteristic properties that constitute the habitat of prokaryotic marine picoplankton. As part of the Atlantic Meridional Transect (AMT) Programme the AMT16 cruise (May – June 2005) crossed the Atlantic Ocean in a south-north transect and enabled the sampling of ecological productive provinces like the Benguela Current Coastal Province (BENG), the Western Tropical Atlantic Province (WTRA), and the North Atlantic Drift Province (NADR). But also the oligotrophic South Atlantic Gyral Province (SATL) and the North Atlantic Gyral Province (NAG) were sampled along the AMT16 transect. The VISION cruise (September 2006) expanded this investigation by adding another seasonal sampling in the NAG, NADR and additionally the productive Atlantic Arctic Province (ARCT).

The focus of this PhD thesis was to explore in unprecedented resolution the distribution of individual microbial groups in the upper 500 m of these different ecological provinces by catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) with rRNA-targeted oligonucleotide probes targeting major bacterial and archaeal clades. Automated microscopic analysis of samples from the two transects across the Atlantic Ocean – AMT16 Cape Town to Falmouth and VISION Reykjavik to Lisbon – showed specific distribution patterns for the individual microbial groups. A similar prokaryotic community composition could be found in the photic zone of the entire Atlantic Ocean, but this community was largely different from the community in the aphotic zone.

In the photic zone the SAR11 clade (*Alphaproteobacteria*) dominated the bacterioplankton community in all provinces with an apparent higher abundance during AMT16. The photoautotrophic cyanobacteria of the genus *Prochlorococcus* were often the second most abundant group in the tropical and subtropical provinces SATL, WTRA and NAG. The *Bacteroidetes* and *Gammaproteobacteria* showed both a clear preference for temperate (NADR) and, in case of the *Bacteroidetes,* additionally the subpolar province (ARCT). The marine group I *Crenarchaeota* and the SAR202 clade prevailed in the aphotic zone. However, the distribution of these two microbial groups had an

opposing trend with the *Crenarchaeota* being more abundant in the productive provinces (BENG, WTRA, NADR) while SAR202 preferentially occurred in the oligotrophic gyres (SATL, NAG).

A second investigation focused on the characterization of flow cytometrically defined fractions of bacterioplankton. Typically a fraction with a high nucleic acid (HNA) and one with a low nucleic acid (LNA) content can be distinguished in marine water samples. In surface waters of the North Atlantic Ocean both fractions were comprised of different bacterial groups. HNA samples from the productive ARCT were dominated by *Bacteroidetes* while *Prochlorococcus* obviously dominated the oligotrophic NAG. Other groups present in the diverse HNA fraction were *Alpha*- and *Gammaproteobacteria* including their respective subgroups *Roseobacter* and SAR86. In contrast, about two-thirds of the LNA fraction was comprised of the single clade SAR11 while only small proportions could be assigned to the *Beta*- and *Gammaproteobacteria*. Available genome data for some of the phylogenetic groups suggests an average genome size of approximately 4 Mbp for the large HNA and less than 1.6 for the LNA.

Zusammenfassung

Der Atlantische Ozean umfasst mehrere Provinzen mit charakteristischen ökologischen Eigenschaften. Gleichzeitig bildet der Atlantische Ozean den Lebensraum für das marine prokaryotische Picoplankton. Im Rahmen des AMT (Atlantic Meridional Transect) Programms durchquerte die AMT16 Cruise während Mai – Juni 2005 den Atlantischen Ozean in einem Süd-Nord-Transekt, auf dem folgende ökologische Provinzen beprobt wurden: nährstoffreiche Provinzen wie die Benguela Current Coastal Provinz (BENG), die Western Tropical Atlantic Provinz (WTRA), und die North Atlantic Drift Provinz (NADR) sowie nährstoffarme Gewässer wie die South Atlantic Gyral Provinz (SATL) und die North Atlantic Gyral Provinz (NAG). Zusätzlich ermöglichte die VISION Cruise die Beprobung der nördlichen Provinzen NAG und NADR sowie der Atlantic Arctic Provinz (ARCT) während September 2006 in einem Nord-Süd-Transekt.

Fokus dieser Doktorarbeit war es, die Verteilung individueller mikrobieller Gruppen in den oberen 500 m Wassersäule dieser Provinzen mittels Fluoreszenz *in situ* Hybridisierung mit enzymmarkierten Oligonukleotidsonden und Tyramid-Signalverstärkung zu bestimmen. Die automatisierte Analyse der Proben von den zwei Transekten – der AMT16 von Kapstadt nach Falmouth und der VISION Cruise von Reykjavik nach Lissabon – zeigte spezifische Verteilungsmuster der individuellen mikrobiellen Gruppen in den verschiedenen Provinzen. In der photischen Zone des Atlantischen Ozeans erwies sich die Zusammensetzung der prokaryotischen Gemeinschaft als sehr ähnlich, sie unterschied sich jedoch weitgehend von der prokaryotischen Gemeinschaft in der aphotischen Zone.

Die bakterielle Gemeinschaft in der photischen Zone wurde eindeutig von der SAR11 Gruppe dominiert, welche während der AMT16 deutlich höhere Zellzahlen erreichte. Ebenfalls als sehr abundant erwies sich der photoautotrophe Genus *Prochlorococcus* (Cyanobakterien) in den tropischen und subtropischen Provinzen SATL, WTRA und NAG. *Bacteroidetes* und *Gammaproteobacteria* zeigten beide eine Präferenz für die temperierte Provinz NADR und, im Falle der *Bacteroidetes*, auch für die subpolare Provinz ARCT. Die marine Gruppe I *Crenarchaeota* und die SAR202 Gruppe wurden

überwiegend in der aphotischen Zone detektiert. Das Verteilungsmuster dieser beiden mikrobiellen Gruppen erwies sich jedoch als gegenläufig. Der Anteil der *Crenarchaeota* war höher in den nährstoffreicheren Provinzen (BENG, WTRA, NADR), während SAR202 eher die nährstoffärmeren Provinzen SATL und NAG zu bevorzugen schien.

In einem zweiten Projekt dieser Doktorarbeit wurden durchflusszytometrische Fraktionen des Bakterioplanktons untersucht. Per Durchflusszytometrie werden gewöhnlich zwei Fraktionen des marinen Bakterioplanktons differenziert, eine Fraktion mit hohem Nukleinsäuregehalt (HNA) und eine Fraktion mit niedrigerem Nukleinsäuregehalt (LNA). Im Oberflächenwasser des Nordatlantischen Ozeans erwies sich die bakterielle Gemeinschaft dieser beiden Fraktionen als unterschiedlich. Die HNA Fraktion von Proben aus der ARCT Provinz war dominiert von den *Bacteroidetes* , während *Prochlorococcus* in der HNA Fraktion von Proben aus der NAG Provinz den größten Anteil ausmachten. Desweiteren wurden *Alpha*- und *Gammaproteobacteria* samt ihrer respektiven Untergruppen *Roseobacter* und SAR86 in der HNA Fraktion gefunden. Zellen der LNA Fraktion bestanden zu zwei Dritteln aus Angehörigen der SAR11 Gruppe. Lediglich ein geringer Anteil der LNA Fraktion konnte den *Beta*- und *Gammaproteobacteria* zugeordnet werden. Verfügbare Genomdaten dieser phylogenetischen Gruppen lassen den Schluss zu, dass die durchschnittliche Genomgröße der bakteriellen Zellen in der HNA Fraktion bei etwa 4 Mbp liegt und für Zellen der LNA Fraktion bei weniger als 1.6 Mbp.

List of abbreviations

°C	degree Celsius
°N	degree North
°S	degree South
°W	degree West
AMT	Atlantic Meridional Transect Programme
ARCT	Atlantic Arctic Province
BATS	Bermuda Atlantic Time Series
BENG	Benguela Current Coastal Province
C	carbon
C1	compounds containing one carbon atom
CARD	catalyzed reporter deposition
Chl *a*	chlorophyll *a*
CNRY	Canary Coastal Province
CZCS	Coastal Zone Color Scanner
DAPI	4',6-diamidino-2-phenylindole
DCM	deep chlorophyll maximum layer
DIN	dissolved inorganic nitrogen
DMSP	3-dimethylsulfoniopropionate
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
DOM	dissolved organic matter
DON	dissolved organic nitrogen
et al.	and co-worker
ETRA	Eastern Tropical Atlantic Province
FCM	flow cytometry
FISH	fluorescence *in situ* hybridization
HNA	high nucleic acid content cells
HOTS	Hawaiian Ocean Time Series
HRP	horseradish peroxidase
ISI	Institute for Scientific Information (now Thomson Scientific)
LNA	low nucleic acid content cells
MAR	Mid-Atlantic Ridge

Mbp	mega base pairs = 10^6 base pairs
N	nitrogen
NADR	North Atlantic Drift province
NAG	North Atlantic Gyral Province
NAST	North Atlantic Subtropical Gyral Province (West W, East E)
RNA	North Atlantic Tropical Gyral Province (=NATL)
NAC	North Atlantic Current
NEC	North Equatorial Current
NECC	North Equatorial Counter Current
NERC	British National Environmental Research Council
OMZ	oxygen minimum zone
P_0	phosphorus/phosphate plankton production
P	plankton production
PCR	polymerase chain reaction
PON	particulate organic nitrogen
PP	primary production
R	respiration
rDNA	gene of ribosomal RNA
RNA	ribonucleic acid
rRNA	ribosomal RNA
RDP	ribosomal database project
SATL	South Atlantic Gyral Province
SeaWIFS	Sea-viewing Wide Field-of-view Sensor
SEC	South Equatorial Current
spp.	several species
SSTC	South Subtropical Convergence
WTRA	Western Tropical Atlantic province

Part I: Combined presentation of results

A Introduction

1 The marine pelagic zone

Approximately 71% of the Earth's surface is covered by oceans that can be subdivided into a number of marine environments (Fig. 1). The pelagic zone comprises all living space within the water column, from surface waters (epipelagic region) to the deepest zone, the hadalpelagic region.

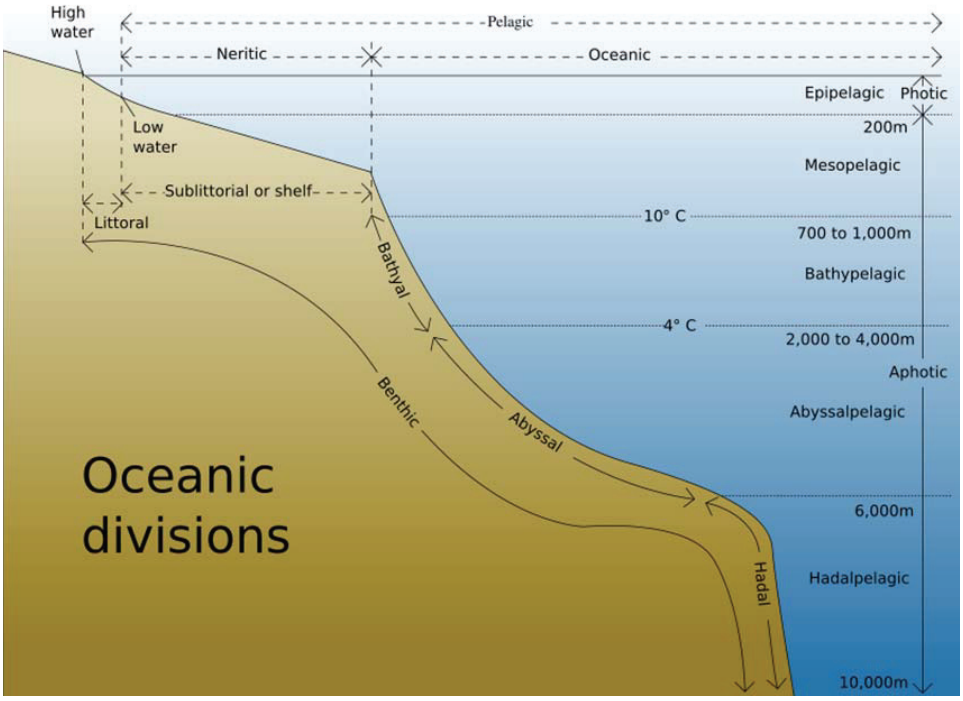


Fig. 1: The basic ecological divisions of the ocean. The neritic (or inshore) pelagic zone is separated from the oceanic (or offshore) pelagic zone by the edge of the continental shelf (modified from (Lalli and Parsons, 1997b).

Most of these marine waters belong to the oceanic zone beyond the shelf break defined by water of depths >1000 m. Only 8% of the pelagic area comprises the neritic zone, which is closer to the continental shores and relatively shallow in depth. Nevertheless, the neritic zone plays an important role in the cycling of elements, because primary productivity in the neritic environment is higher than in the oceanic zone (Lalli and Parsons, 1997a). Consequently, about 20% of the marine pelagic primary productivity derives from the neritic and 80% from the oceanic zone (Smith and Hollibaugh, 1993). Large areas of the open ocean are characterized by low nutrient levels as they are barely influenced by terrestrial sources (e.g. continental river run-off and tidal currents) and rather rely on ocean circulation and atmospheric input (Kennish, 2001).

Two basic types of marine organisms can be found in the pelagic environment. One type, the nekton, comprises free-swimming animals, such as fish, squid and marine mammals that can swim against the ambient flow of the water environment and control their position. The remaining inhabitants of the pelagic environment form the plankton. Those organisms are incapable of independent movement and thus are passively transported by ocean currents. Consequently, the plankton is exposed to large scale as well as small scale changes of environmental parameters such as temperature, turbulence, and nutrient concentrations and need to be able to cope with this heterogeneity.

There are two ways to distinguish between different classes within the plankton. One way is to divide them according to size, defining groups such as pico-, nano-, and microplankton. They range from 0.2 – 2.0 µm, 2.0 – 20 µm, and 20 – 200 µm, respectively, in size. Another approach distinguishes between classes such as zooplankton, phytoplankton, and bacterioplankton according to their affiliation to animals, plants or prokaryotes (Lalli and Parsons, 1997b).

1.1 Prokaryotic marine picoplankton

Prokaryotic organisms in the ocean are morphologically simple: microscopic rods, spheres, and filaments generally less than 1 – 2 µm in size. However, as revealed by both culture-dependent and culture-independent studies, prokaryotes are highly diverse, and contain representatives of virtually all of the roughly 40 divisions of bacteria and the major archaeal groups found so far in the biosphere (Hugenholtz et al., 1998). The diversity of marine bacterioplankton is estimated to comprise 10^6 “species” (Curtis et al., 2002). However, it seems that only around 20 phylogenetic clades play numerically an important role in the water column (Giovannoni and Stingl, 2005) (Fig. 2).

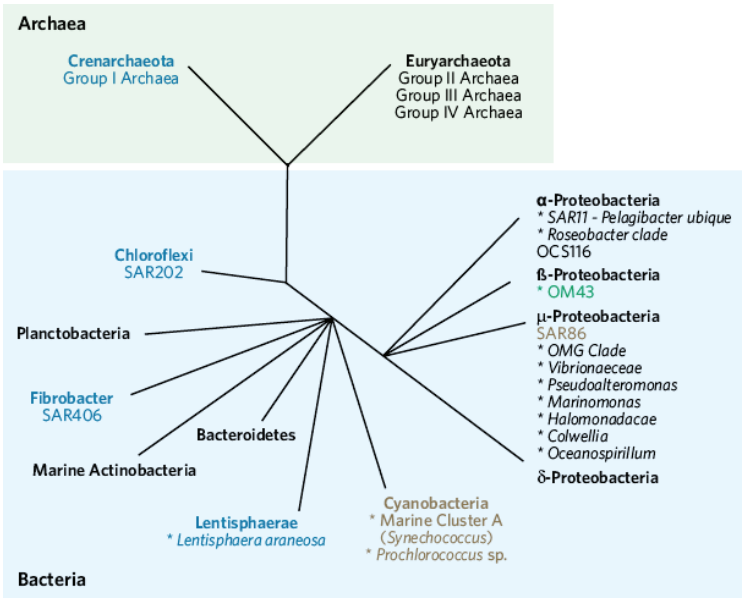


Fig. 2: Schematic illustration of the phylogeny of the major plankton clades. Black letters indicate microbial groups that seem to be ubiquitous in seawater. Grey indicates groups found in the photic zone. Blue indicates groups confined to the mesopelagic and surface waters during polar winters. Green indicates microbial groups associated with coastal ocean ecosystems (from Giovannoni and Stingl, 2005).

Pedros-Alio proposed that the taxa retrieved by PCR are considered to form the more abundant part of total diversity of an ecosystem (Pedros-Alio, 2006). Thus, most bacteria that are relevant and active in carbon, energy and nutrient flows would fall within the diversity retrievable by molecular techniques, and would not be from, what might be known as the functionally less important “rare biosphere”.

Autotrophic and heterotrophic *Bacteria* dominate the prokaryotic biomass in surface waters, as *Archaea*, in particular the marine group I *Crenarchaeota*, appear to be abundant mainly in the plankton of the deep oceans (Karner et al., 2001). Among the heterotrophic bacteria, the two most abundant groups are often the *Proteobacteria* and the *Bacteroidetes* cluster.

1.1.1 SAR11-clade

The alphaproteobacterial clade SAR11 is the most abundant group of heterotrophic bacteria in marine pelagic waters worldwide (Fuhrman and Ouverney, 1998; Morris et al., 2002) (Fig. 2). It was first discovered by cloning and sequencing of the 16S rRNA gene from Sargasso Sea samples (Giovannoni et al., 1990). Since then, SAR11 was found all around the globe in

over 50 studies of marine bacterioplankton microbial diversity and is consistently dominating 16S rRNA gene clone libraries (Morris et al., 2002). Morris *et al.* reported that the SAR11 clade accounts on average for one third of the picoplankton present in surface waters (e.g. in the Sargasso Sea), and can even exceed 50% in other regions. Studies of one isolate from this group (*Candidatus* Pelagibacter ubique) revealed a streamlined, small genome of approximately 1.3 Mbp (Rappé et al., 2002; Giovannoni et al., 2005b). This points to a perfect adaptation to oligotrophic oceanic regions (Sowell et al., 2008; Tripp et al., 2008). Additionally, these studies indicated niche partitioning of SAR11 subgroups over depth (Field et al., 1997).

1.1.2 *Synechococcus* and *Prochlorococcus*

Another important marine clade of the domain *Bacteria* are the *Cyanobacteria*. They are represented in the oceans by the phylogenetically closely related genera *Synechococcus* and *Prochlorococcus* (Urbach et al., 1998) (Fig. 2). Most of the coccoid cyanobacteria are in the genus *Synechococcus* and occur in abundance (10^2 – 10^5 cells mL^{-1}) in the euphotic zone of both coastal and open ocean waters. The only parts of the ocean in which these cyanobacteria do not appear to be of great importance are polar seas (Waterbury et al., 1986; Zwirglmaier et al., 2008). In oligotrophic open ocean gyres, phototrophs smaller than 1 – 3 µm have been estimated to contribute 60 - 80% of water column primary productivity (Takahashi and Bienfang, 1983). The pelagic prochlorophytes of the genus *Prochlorococcus* are photoautotrophic cells slightly smaller than *Synechococcus* (Chisholm et al., 1992). Flow cytometric enumeration of prochlorophytes in seawater indicates that this group of prokaryotic autotrophs is usually more abundant than *Synechococcus* (in the order of 10^4 to $>10^5$ *Prochlorococcus* cells mL^{-1}) (Chisholm et al., 1992; Campbell and Vaulot, 1993). Several studies have shown that *Prochlorococcus* is a genetically diverse group with several distinct clades, which have SSU rRNA gene sequences that differ by less than 3% (Urbach and Chisholm, 1998; Rocap et al., 2002; Rocap et al., 2003). Other studies on cultured strains of *Prochlorococcus* showed differences in pigmentation, maximum growth rates, metal tolerances, nutrient utilization, and photophysiological characteristics leading to the establishment of distinct ecotypes (Moore and Chisholm, 1999;

West and Scanlan, 1999; Mann et al., 2002; Moore et al., 2002; Johnson et al., 2006). Most of the work on marine pelagic prochlorophytes has been done in open ocean systems (Chisholm et al., 1992; West et al., 2001; Zubkov and Tarran, 2005; Bouman et al., 2006; Johnson et al., 2006; Rabouille et al., 2007; Zwirglmaier et al., 2008). Prochlorophytes appear to represent an important part of the biomass and to be responsible for a large fraction of the productivity of phytoplankton in such regions. *Prochlorococcus* has indeed been observed to account for 21 to 43% of the photosynthetic biomass in oligotrophic oceans and 13 to 48% of the net primary production (Vaulot et al., 1995; Campbell et al., 1997; DuRand et al., 2001). Both *Prochlorococcus* spp. and *Synechococcus* spp. can take up amino acids thereby saving energy compared to uptake of inorganic nitrogen such as ammonium (Zubkov et al., 2003). However, the ecological significance for each of the cyanobacterial genera differs, as *Prochlorococcus* is able to consume approximately one third of the amino acid pool which is 10 times more than *Synechococcus* was observed to consume.

1.1.3 *Bacteroidetes*

The abundant, but diverse group of the *Bacteroidetes* (also often referred to as Cytophaga-Flavobacteria-Bacteroides (CFB) group) has been found in a variety of both benthic and pelagic marine environments including coastal and offshore waters (Eilers et al., 2001; Kirchman et al., 2003; DeLong et al., 2006), but also in polar regions (Brinkmeyer et al., 2003; Abell and Bowman, 2005a) (Fig. 2, p. 10). In coastal pelagic habitats *Bacteroidetes* often constitute the most abundant group of bacteria as measured by fluorescence *in situ* hybridization (FISH) (Cottrell and Kirchman, 2000a; Eilers et al., 2001). Clone library and FISH studies have indicated that *Bacteroidetes* are either free-living or attached to organic aggregates (DeLong et al., 1993; Eilers et al., 2001; Abell and Bowman, 2005b) and that they can be associated with marine phytoplankton or animals (Webster et al., 2001; Grossart et al., 2005). Due to their high abundance during phytoplankton blooms and their occurrence as primary colonizers of marine phytoplankton it has been suggested that *Bacteroidetes* play a role as consumers of algae-derived metabolites (Simon et al., 1999; Riemann et al., 2000; O'Sullivan et al., 2004; Pinhassi et al., 2004). Most of the

marine isolates are obligatory aerobic or facultatively anaerobic chemoorganotrophs and able to degrade high molecular weight organic substrates. Molecular analyses with microautoradiography indicate that not-cultivated members of this group also participate actively in the degradation of polymeric organic material in aquatic ecosystems (Cottrell and Kirchman, 2000b; Kirchman, 2002).

1.1.4 *Gammaproteobacteria*

Various clades of the *Gammaproteobacteria* have been found in different marine habitats, often with a preference for coastal environments (Rappé et al., 1997). In oligotrophic oceans *Gammaproteobacteria* seem to represent only a minor fraction of the bacterioplankton as indicated by FISH counts (Glöckner et al., 1999). One yet-uncultured member of the *Gammaproteobacteria*, the marine SAR86 clade (Fig. 2, p. 10), formed 7% of total cell numbers in transects across the German Bight. A fraction of 3 to 6% of all bacterial 16S rRNA genes in Monterey Bay surface waters were affiliated with this group during an upwelling event (Suzuki et al., 2001). This clade was also the first described to have genes encoding proteorhodopsin, a light-harvesting pigment which yields energy to drive bacterial membrane proton pumps. Recent findings suggest that this feature might be widespread in the marine bacterioplankton (Venter et al., 2004). Since many representatives of *Gammaproteobacteria* are comparatively easily cultivated, it comes as a surprise that the *in situ* abundance of most species is often rather low – inspite of the growth potential that they display in isolation attempts and cultivation experiments. Possibly, this is another observation matching the phenomenon of "the great plate count anomaly". This term was coined by Staley and Konopka in 1985 (Staley and Konopka, 1985) in order to describe the difference in orders of magnitude between the numbers of cells from natural environments that form colonies on agar media and the numbers actually counted by microscopic examination (Jannasch and Jones, 1959). However, one example of *Gammaproteobacteria* which is able to reach rather high *in situ* abundance is the NOR5 cluster KT71. The abundance of this strain was observed to reach 1.4×10^5 mL^{-1} cells in the North Sea (Eilers et al., 2001; Yan, in press).

1.1.5 Other bacterial groups

Since their initial detection at the Bermuda Atlantic Time Series (BATS) station at 250 m depth (Giovannoni et al., 1996), members of the green nonsulfur bacteria SAR202 have been found in several marine environments, e.g. the Atlantic and Pacific Ocean (Wright et al., 1997; Giovannoni and Rappé, 2000; Bano and Hollibaugh, 2002; DeLong et al., 2006). Recently, members of the *Chloroflexi*-type SAR202 cluster were enumerated using a cluster-specific oligonucleotide probe and FISH at the BATS site in the Atlantic and the Hawaiian Ocean Time Series (HOTS) site in the Pacific (Morris et al., 2004). Direct cell counts from different oceans sites showed that cells of the SAR202 cluster were most abundant below the deep chlorophyll maximum (Morris et al., 2004; Mincer et al., 2007) (Fig. 2, p. 10). Their predominance in the ocean's lower surface layer was one of the first pieces of evidence to suggest that marine bacterioplankton communities occur stratified throughout the water column (Morris et al., 2004). In addition, one recent study by Varela *et al.* (Varela et al., 2008a) observed a high abundance of SAR202 cells in the meso- and bathypelagic waters of the Atlantic Ocean. Uptake measurements indicated that members of the thus far uncultured SAR202 cluster might play an important role in the deep-ocean biogeochemistry.

The occurrence of other bacterial groups of planktonic prokaryotes is less well documented. The alphaproteobacterial family of *Rhodobacteraceae* is often observed in coastal areas or shelf regions in the aftermath of phytoplankton blooms (Eilers et al., 2001; Selje et al., 2004; Perez et al., 2006; West et al., 2008). *Betaproteobacteria* seem to play only a minor role in the marine systems, but their abundance can increase rapidly in low salt concentrations, e.g. estuaries (Pinhassi et al., 1997; Bouvier and del Giorgio, 2002) or in the coastal North Sea (Sekar et al., 2004). A number of other, mainly yet-uncultured microbial lineages have also been found in the marine picoplankton, e.g. the uncultured *Firmicutes* clade SAR406 (Gordon and Giovannoni, 1996), the deltaproteobacterial clade SAR324 (Fuhrman and Davis, 1997), *Planctomycetes* (DeLong et al., 1993) and the marine *Actinobacteria* (Rappé et al., 2000; Agogué et al., 2005) (Fig. 2, p. 10).

1.1.6 *Archaea*

It was the big discovery of the early 1990s that members of the domain *Archaea* are also major members of marine bacterioplankton (DeLong, 1992; Fuhrman et al., 1992). *Archaea* are divided into the classes *Euryarchaeota* and *Crenarchaeota* (Fig. 2, p. 10). Members of the *Euryarchaeota* – often referred to as marine group II – seasonally formed about 30% of all cells in the surface picoplankton of the North Sea (Pernthaler et al., 2002b). Seasonal blooms of *Euryarchaeota* were also observed during a long-term study in surface waters of the upper Santa Barbara Channel (Murray et al., 1999). Generally, *Euryarchaeota* have been found to be more abundant in surface waters than *Crenarchaeota* (Massana et al., 1998; Massana et al., 2000; DeLong et al., 2006). They may be also present in some deep-water masses of the North Pacific as metagenomic data indicate (DeLong et al., 2006).

In contrast, the *Crenarchaeota* (marine group I) seem to be more abundant in deep ocean waters (Karner et al., 2001). Quantitative studies with FISH have revealed a high abundance of *Crenarchaeota* in meso- and bathypelagic waters of the subtropical Pacific Gyre (Karner et al., 2001), the northern North Atlantic (Teira et al., 2004; Herndl et al., 2005), and Antarctic waters (Church et al., 2003). A higher contribution of *Crenarchaeota* to total prokaryotic plankton was also found in the mesopelagic waters of the Arctic Ocean as compared to surface waters (Kirchman et al., 2007). All these studies indicate that *Archaea* and *Crenarchaeota* in particular, might be ubiquitously distributed in the deep ocean.

1.2 The concept of high nucleic acid and low nucleic acid populations

The flow cytometric analysis of bacterioplankton stained by nucleic acid dyes (Fig. 3) often shows two distinct populations based on differences in the individual cell fluorescence and in side and forward light scatter signals (Li et al., 1995; Gasol et al., 1999; Lebaron et al., 2001; Zubkov et al., 2001b; Zubkov et al., 2001a). Li *et al.* (Li et al., 1995) were the first to describe this clustering of cells based on DNA content with the terms high nucleic acid content (HNA) and low nucleic acid content (LNA).

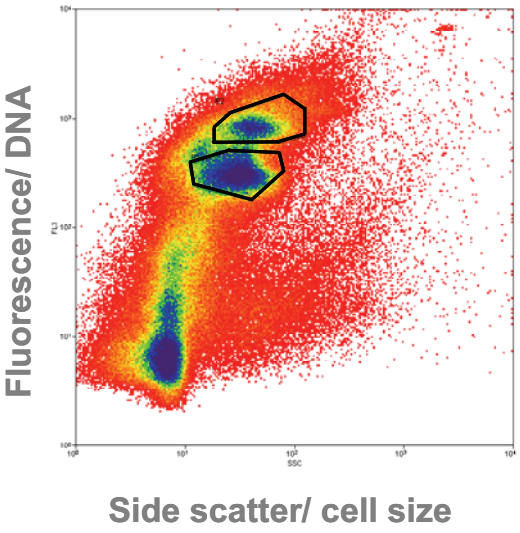


Fig. 3: Flow cytometric dotplot showing differentiation of HNA (blue cluster on top) and LNA cells (blue cluster below).

HNA and LNA populations have since been detected in a wide range of aquatic ecosystems covering large environmental gradients, e.g. from fresh to salt water, from eutrophic to oligotrophic environments (Gasol and del Giorgio, 2000; Jochem, 2001; Lebaron et al., 2002; Jochem et al., 2004; Bouvier et al., 2007a). The co-occurrence of these two populations has thus been assumed to be a general feature of bacterioplankton communities. However, most of the earlier studies focused mainly on the abundance of these cytometrically distinct populations and their relation to total bacterial abundance in which changes in total bacterial abundance were often associated with changes in the HNA fraction. Furthermore, these studies indicated that HNA cells are larger and also more active, with higher specific metabolic and growth rates than LNA cells (Troussellier et al., 1999; Gasol and del Giorgio, 2000; Lebaron et al., 2002; Servais et al., 2003). The role of LNA cells has remained controversial, as different studies described them either as inactive, dead or fragmented (Lebaron et al., 2001), or as viable cells which were as active as other members of the community (Zubkov et al., 2001a; Jochem et al., 2004; Longnecker et al., 2005). Beside distinct flow cytometric patterns of fluorescence that could be assigned to the cyanobacteria *Prochlorococcus* and *Synechococcus* (Chisholm et al., 1988; Li, 1994; Vaulot et al., 1995), phylogenetic analysis of HNA and LNA populations led to conflicting observations. Some approaches resulted in the conclusion that these two fractions were not phylogenetically distinct (Bernard et al., 2000b; Servais et al., 2003; Fuchs et al., 2005; Longnecker et al., 2005) while on the contrary, others suggested that the composition differered between fractions (Eilers et al., 2000; Fuchs et al., 2000; Zubkov et al., 2001a). FISH studies showed that HNA bacterioplankton was commonly dominated by *Alphaproteobacteria*, in particular *Roseobacter*, *Gammaproteobacteria* and *Bacteroidetes* (Zubkov et al., 2001a; Zubkov et al., 2002b; Zubkov et al.,

2002a; Zubkov et al., 2004b) whereas LNA bacterioplankton appeared to be dominated by the alphaproteobacterial SAR11 clade (Mary et al., 2006b).

2 The Atlantic Ocean

2.1 General features of the Atlantic Ocean

Named after Atlas, one of the Titans of Greek mythology, the Atlantic Ocean is the second largest of the Earth's four oceans: the Pacific, Atlantic, Southern and Indian.

The Atlantic Ocean is divided into two nominal sections: The part which is north of the equator is called the North Atlantic Ocean; the part which is south of the equator, the South Atlantic Ocean. The Atlantic Ocean is basically an S-shaped north-south channel, extending from the Arctic Ocean in the north to the Antarctic continent in the south and situated between the eastern coast of the American continents and the western coasts of Europe and Africa (Fig. 4).

Fig. 4: Relief of the Atlantic Ocean floor. (http://orange-smile.com/ru/foto/oceans/atlantic-ocean.jpg)

The Atlantic Ocean has a surface area of about 82 million km^2 (Pandolfo, 2005). Including its marginal seas - the Gulf of Mexico, the

Caribbean Sea, the Arctic Ocean, and the North, Baltic, Mediterranean, and Black seas - the total area is about 106,190,000 km^2, which represents about 23% of the total world ocean area. The boundary between the North Atlantic and the Arctic Ocean is arbitrarily designated as lying along a system of submarine ridges that extend between the land masses of Baffin Island, Greenland, and Scotland. More clearly defined is the boundary between the Atlantic Ocean and the Mediterranean Sea at the Strait of Gibraltar and with the Caribbean Sea along the arc of the Antilles. The South Atlantic is arbitrarily separated from the Indian Ocean on the east by the 20° east meridian and from the Pacific Ocean on the west along the line of shallowest depth between Cape Horn and the Antarctic Peninsula. The Atlantic Ocean has an average depth of 3926 m. At its deepest point, in the Puerto Rico Trench, the sea floor is 8742 m below the surface. The Mid-Atlantic Ridge (MAR) runs along the length of the North and South Atlantic in a sinuous path midway between the continents and divides the Atlantic Ocean into two elongated depressions, the Eastern Atlantic trough and the Western Atlantic trough. Roughly 1500 km wide, the ridge has a more rugged topography than any mountain range on land, and is a frequent site of volcanic eruptions and earthquakes due to plate tectonics. The ridge itself ranges from about 1 to 3 km above the ocean bottom.

The circulatory system of the surface waters of the Atlantic Ocean are best depicted as two large gyres, or circular current systems, one in the North Atlantic Ocean and the other in the South Atlantic Ocean (Fig. 5). These currents are primarily wind driven, but are also affected by the rotation of the Earth. The currents of the North Atlantic Ocean, which include the North Equatorial Current, the Canaries Current, and the Gulf Stream, flow in a clockwise direction. The currents in the South Atlantic Ocean, among which are the Brazil, Benguela, and South Equatorial currents, travel in a counterclockwise direction. Each gyre extends from near the equator to about a latitude of 45°; closer to the poles are the less concisely defined counter-rotating gyres, one rotating counterclockwise in the Arctic regions of the North Atlantic Ocean (Fig. 5) and one rotating clockwise near Antarctica in the South Atlantic Ocean.

The Atlantic Ocean receives the waters of many of the principal rivers of the world, among them are the Saint Lawrence, Mississippi, Orinoco, Amazon, Paraná, Congo, Niger, and Loire, and the rivers emptying into the North, Baltic, and Mediterranean seas. Nevertheless, primarily because of the high salinity of outflow from the Mediterranean Sea, the Atlantic Ocean is slightly more saline than the Pacific or Indian Oceans. Within the ocean gyres, there is an increase in salinity from high latitudes to the subtropics. This raise in salinity is closely coupled with temperature, and is a manifestation of the increase in evaporation associated with higher temperatures and lower latitudes. This relationship, however, breaks down in the tropics, at about 25°N to 15°S, because of the increase in precipitation within these latitudes.

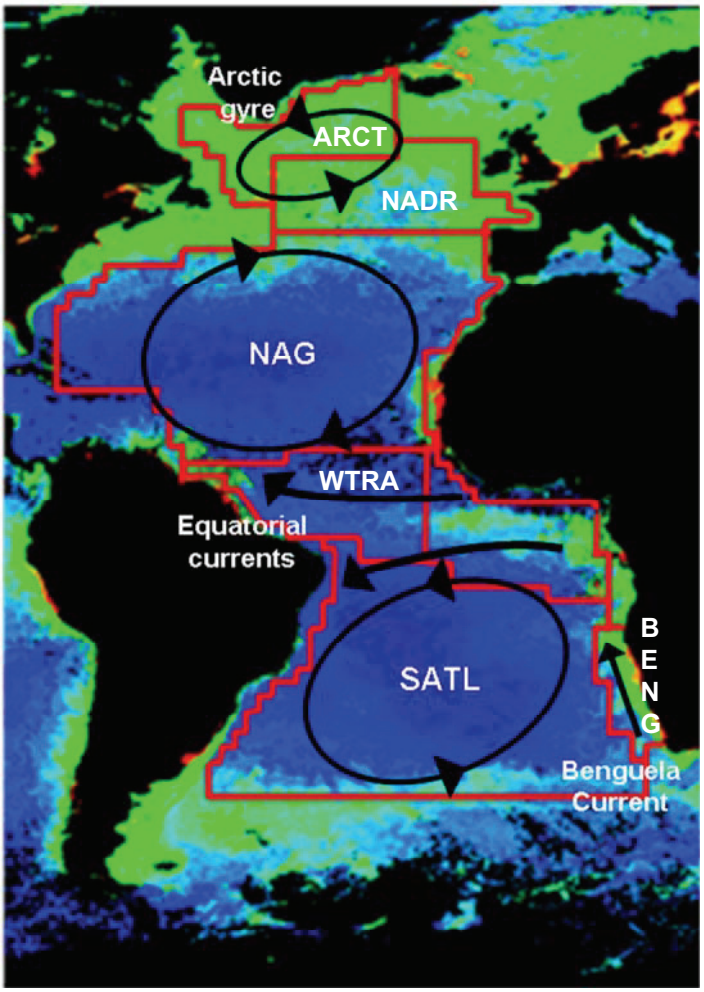


Fig. 5: SeaWiFS composite image showing chlorophyll *a* concentration in the surface layer of the Atlantic Ocean during boreal spring 2005.
The approximate boundaries of the ecological provinces (after (Longhurst, 1998) and the major current systems are schematically added. (http://oceancolor.gsfc.nasa.gov/)

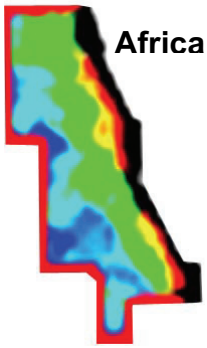
Within the context of the global thermohaline circulation, warm and saline upper waters flow northwards to the subpolar and polar regions where they increase in density by cooling, sink down and return southwards as deep and bottom waters. This is a very simplistic manner to explain the relevance of the North Atlantic Ocean within the global conveyor belt circulation introduced by Broecker (Broecker, 1987). As a consequence of this deep water formation

the North Atlantic Ocean is the “youngest” ocean and also presents the highest oxygen and lowest nutrient concentrations.

2.2 Oceanic provinces

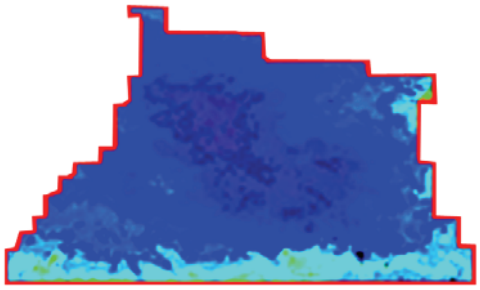
Traditionally, oceanographers have partitioned the oceans on the basis of physical and biological characteristics: e.g. topography, geostrophic flows (currents resulting from the balance between gravitational forces and the Coriolis effect), wind-driven circulation, gyres, fronts, upwelling zones and patterns of seasonal stratification, biological productivity, as well as phytoplankton and zooplankton assemblages and community structure. Taken together, this biophysical partitioning provides the descriptors of regional ecosystems or biogeochemical provinces with discrete boundaries and distinct flora and fauna (Fig. 5).

The concept of biogeochemical provinces has been promoted by Longhurst, Sathyendranath, Platt and Caverhill (Longhurst et al., 1995) as a means of evaluating basin-scale productivity from remotely sensed measurements of ocean color, making use of province-specific physical and biological parameterizations derived from climatological values of key variables such as temperature and wind force. The culmination of Longhurst was a comprehensive worldwide partitioning of pelagic production and consumption based on an exhaustive analysis of a variety of global data sets (Longhurst, 1998). In the following pages, those provinces investigated during the course of this thesis will be introduced in accordance with Longhurst (Longhurst, 1998).

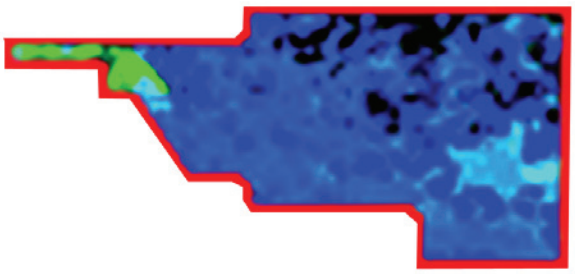


The **Benguela Current Coastal Province** (BENG) extends along the coastline of Africa from the Cape of Good Hope (34°S) north to Cape Frio (18°S) including the offshore eddy field of the Benguela Current. Excluded is the retroflection region of the Agulhas Current south and southwest of the Cape of Good Hope. There is significant deposition of aerosol particulates at the sea caused by southwestern African eolian

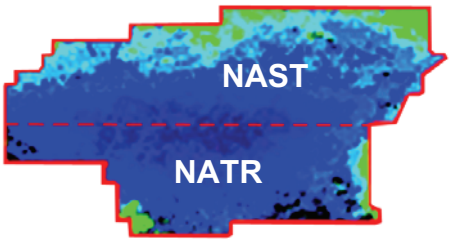
dust events. South of 15°S winds are perennially favorable to upwelling in the Benguela Current with corresponding effects on primary production.



The **South Atlantic Gyral Province** (SATL) comprises the anticyclonic circulation of the South Atlantic Ocean, excluding the coastal boundary currents (Brazil & Benguela). The east and west boundaries of the province are therefore within the eddy fields seawards of these two currents. The northern boundary is taken as the westward flow of the South Equatorial Current (SEC) from Africa to the coast of South America, and the southern boundary is the border of the increase in primary production associated with the Subtropical Convergence Province (around 40°S). An effect of the anticyclonic circulation of the subtropical gyre is the upwards-slope of the isopleths for nutrients toward the edges of the gyre. Therefore, surface chlorophyll values are minimal in the central regions of the open subtropical gyre where the mixed layer is very deep.



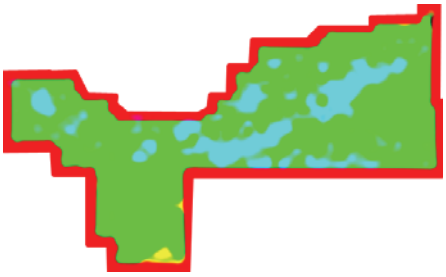
The **Western Tropical Atlantic Province** (WTRA) comprises the tropical Atlantic Ocean west of 20°W and south of the seasonally varying North Equatorial Counter Current (NECC) at about 8 – 10°N. In the south, the northern edge of the seasonally varying flow of the Southern Equatorial Current (SEC) toward the northwest marks the limit of this province. The SEC itself is referred to as the South Atlantic Province (SATL). A zone of high chlorophyll values between 20° - 40°W of the WTRA is associated with a strong vertical Ekman flux (upwelling) creating divergence along the axis of the NECC. Additionally, the strongly meandering flow of the NECC itself induces vertical motion and thus a source of nutrient flux to surface waters (Dadou et al., 1996).



The **North Atlantic Gyral Province** (NAG) encompasses two provinces spanning tropical and subtropical latitudes. One province, the **North Atlantic Tropical Gyral Province** (NATR), comprises the oceanic flow around the southern half of the anticyclonic gyre of the North Atlantic Ocean south of the Subtropical Convergence Zone which runs across the ocean at about 30°N. The southern boundary of the NATR is the North Equatorial Current (NEC)/ North Equatorial Counter Current (NECC) conjunction at 10 – 12°N. The western boundary is taken to be the edge of the coastal boundary biome seawards of the Antilles and the Bahama Islands. It includes the continuation of the offshore Canary Current south of the Canaries and the flow into the NEC and then to the western limb of the gyre. The NATR has a consistently low and uniform surface chlorophyll field with the lowest values in the North Atlantic Ocean – well seen as a big blue hole in the seasonal ocean color images (e.g. SeaWIFS, CZCS). Likewise, nitrate values in the mixed layer appear to be uniformly low (0.2 µM) during all seasons (Wroblewski, 1989). Continuing northward, the **North Atlantic Subtropical Gyral Province** (NAST) is bounded to the west and northwest by the eddy field of the Gulf Stream and to the northeast by the bifurcation of flow between the Azores Current and the North Atlantic Drift at about 40 – 42°N. To the south, the boundary is the Subtropical Convergence. In the east, the equatorward limb of the gyre is formed by the offshore Canary Current.

The **North Atlantic Drift Province** (NADR) comprises part of the westwind drift region of the North Atlantic Ocean in the sense of Dietrich (1964). To the north it is bounded by the Atlantic Arctic Province and the Subarctic Front above the Iceland-Faeroer Ridge (55 – 56°N). Southward, a separation lies across the ocean at about 42°N between the northeasterly flow of the North Atlantic Current (NAC) and the associated westwind drift and the southeasterly flow of the Azores Current into the northern limb of the anticyclonic subtropical gyre (Krauss, 1986). In the east, the edge of the European continental shelf is

taken as the boundary of the province. Regarding surface chlorophyll, the eastern part (at approximately 20°W) of the NADR is consistently lower in concentration throughout the spring and summer. Conversely, in the western part of the province, surface chlorophyll tends to be rather high during the summer as observed in Coastal Zone Color Scanner (CZCS) chlorophyll imagery. The most probable mechanism seems to be a conjunction between the baroclinic upsloping of nitrate isopleths towards the edges of the anticyclonic gyre and the eddy fields associated with the separation of the Gulf Stream/NAC from the continental edge. The NADR shows a pronounced seasonality with the start of a spring bloom, transition to summer oligotrophy, and a late summer situation of a late chlorophyll maximum within the seasonal pycnocline (Strass, 1988; Strass and Woods, 1991).



The **Atlantic Arctic Province** (ARCT) is located between the edge of the Greenland coastal currents and the Oceanic Polar Front (Dietrich, 1964), the latter of which crosses the ocean diagonally from Flemish Cup to the Faeroes. The limits of the province are rather variable, and its southern boundary often cannot be traced by satellite imagery. The surface circulation north of the Oceanic Polar Front is dominated by cyclonicity in comparison with the anticyclonic, subtropical gyre to the south.

3 The Atlantic Meridional Transect Programme

The Atlantic Meridional Transect (AMT) Programme funded by the British National Environmental Research Council (NERC) (Aiken and Bale, 2000; Aiken et al., 2000) is a time series of oceanographic stations along a 13,500 km north–south transect in the Atlantic Ocean. This transect crosses a range of ecosystems from sub-polar to tropical and from euphotic shelf seas and upwelling systems to oligotrophic mid-ocean gyres (Fig. 6).

The programme began in 1995, and the scientific aims were to assess mesoscale to basin scale phytoplankton processes, the functional interpretation of bio-optical signatures and a description of the seasonal, regional and latitudinal variations in mesozooplankton dynamics. The programme provided a platform for international scientific collaborations, including the calibration and validation of Sea-viewing Wide Field-of-view Sensor (SeaWiFS) measurements and products (McClain et al., 2004). The measurements of hydrographic and bio-optical properties, plankton community structure and primary production completed on the first 12 transects (1995-2000) represent the most coherent set of repeated biogeochemical observations over ocean basin scales. This unique dataset has led to several important discoveries concerning the identification of oceanic provinces (Hooker et al., 2000), the validation of ocean color algorithms (Hooker and McClain, 2000), the distribution of picoplankton (Zubkov et al., 2000a), the identification of new regional sinks of pCO_2 (Lefevre et al., 1998), and the variability in rates of primary production (Marañón et al., 2000) and respiration (Robinson et al., 2002).

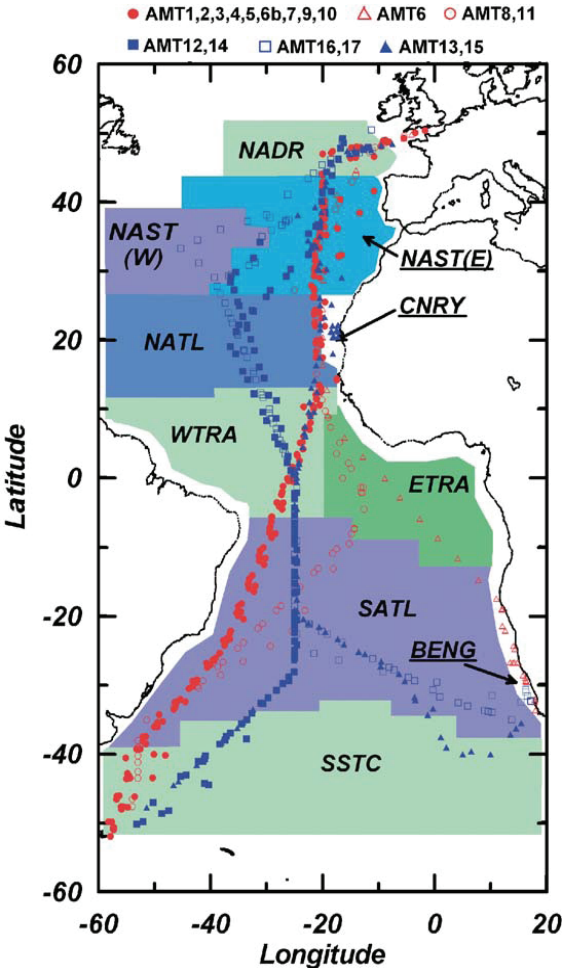


Fig. 6: AMT1–17 cruise tracks overlain on province boundaries of the Atlantic Ocean.

North Atlantic Drift (NADR), North Atlantic Subtropical Gyral (West) (NAST(W)), North Atlantic Subtropical Gyral (East) (NAST(E), North Atlantic Tropical Gyral (NATL), Canary Coastal (CNRY), Western Tropical Atlantic (WTRA), Eastern Tropical Atlantic (ETRA), South Atlantic Gyral (SATL), Benguela Coastal Current (BENG), and South Subtropical Convergence (SSTC) (from (Longhurst, 1998).

In 2002, the programme was restarted (2002-2006) and broadened in order to address a suite of cross-disciplinary questions concerning ocean plankton ecology and biogeochemistry and their links to atmospheric

processes. The objectives included the determination of how 1) the structure, functional properties and trophic status of the major planktonic ecosystems vary in space and time; 2) physical processes control the rates of nutrient supply to the planktonic ecosystem and 3) atmosphere-ocean exchange and photodegradation influence the formation and fate of organic matter. The data collected will be distributed for use in the development of models to describe the interactions between the global climate system and ocean biogeochemistry. Between 1995 and 2005, the AMT Programme has included 18 research cruises and contributed to 130 ISI (Institute for Scientific Information) refereed publications (http://web.pml.ac.uk/amt/publications/amt_publications.html). This unique spatially extensive decadal dataset continues to be deposited and made available to the wider community through the British Oceanographic Data Centre (http://www.bodc.ac.uk/projects/uk/amt/).

4 Methodological approaches

Approaches from different microbiological disciplines are necessary to describe microbial communities. Classic techniques such as enrichment, isolation and cultivation of microorganisms need to be combined with molecular biological methods, in particular the different components of the full cycle 16S rRNA approach (Olsen et al., 1986; Pace et al., 1986; Amann et al., 1995) in order to identify and quantify bacterial and archaeal populations.

4.1 The rRNA approach

Characterization of microbial communities from environmental samples can be achieved by the application of the individual steps of the full cycle 16S rRNA approach (Fig. 7). The 16S rRNA gene as phylogenetic marker allows identification without cultivation and laborious characterization of isolates (Woese, 1987) of abundant microbial taxa in the sea (Giovannoni et al., 1990;

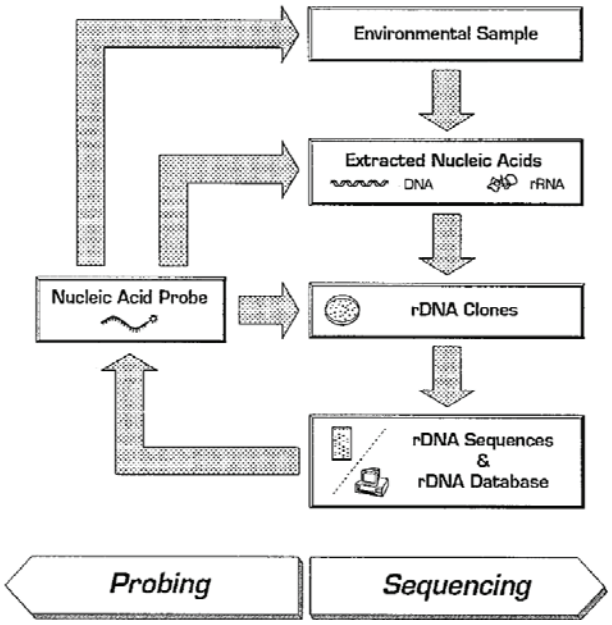


Fig. 7: Principle phases of the full cycle 16S rRNA approach for cultivation-independent analysis of microbial communities. Nucleic acids are isolated directly from environmental (or experimental) samples to be amplified and cloned. The genes of the 16S rRNA are sequenced in order to compare the new genotypes against existing databases. A comprehensive dataset can be the basis to design specific probes for fluorescence *in situ* hybridization (FISH) in order to detect and quantify the microorganisms of interest in the environment (from Amann et al., 1995).

Giovannoni and Rappé, 2000). A 16S rRNA gene sequence library serves as a first inventory of the members of prokaryotic communities.

However, 16S rRNA gene clone libraries do not accurately reflect the abundances of microbes from particular phylogenetic clades due to the so-called PCR bias which is introduced during analysis (Reysenbach et al., 1992; Farrelly et al., 1995; Suzuki and Giovannoni, 1996; Wintzingerode et al., 1997). Hybridization techniques, in particular the fluorescence in situ hybridization (FISH) with rRNA-targeted oligo- or polynucleotide probes (Amann et al., 1990) (Giovannoni et al., 1988; DeLong et al., 1989; Amann et al., 1995; Pernthaler et al., 2001b; Pernthaler et al., 2004), allow the identification and quantification of different microbial taxa in environmental samples. Comprehensive 16S rRNA gene sequences databases such as RDP (Cole et al., 2007) or Silva (Pruesse et al., 2007), allow the design of specific oligonucleotide probes in software environments such as ARB (Ludwig et al., 2004). These probes are used for direct microscopic visualization and quantification of populations of interest, e.g., by catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) (Pernthaler et al., 2004). CARD-FISH combines *in situ* hybridization with horseradish peroxidase labeled oligonucleotide probes and enzymatic signal amplification with fluorescently labeled tyramides (Bobrow et al., 1989). Thus, CARD-FISH allows the quantitative detection of marine pelagic microbes even if the ribosomal content was so low that they were insufficiently visualized by fluorescently monolabeled probes (Pernthaler et al., 2002a; Sekar et al., 2003).

Total cell counts and relative abundances of different microbes within aquatic samples can be evaluated in high-throughput, applying e.g. flow cytometry (Marie et al., 1997) or automated microscopy (Pernthaler et al., 2003).

4.2 Metagenomics

A new approach for the characterization of natural microbial assemblages is the cultivation-independent genomic survey (Handelsman, 2004; DeLong, 2005). Shotgun sequencing and whole genome assembly has been reported for a number of marine environments (Tyson et al., 2004; Venter et al., 2004). In the famous Sargasso Sea survey Venter *et al.* (Venter et al., 2004) obtained more than 1 billion bases and 1.2 million protein-coding genes, revealing a remarkable microbial diversity between 1,800 and 40,000 species comprised mostly of *Proteobacteria*, *Cyanobacteria*, *Firmicutes*, and *Bacteroidetes*. However, the assembly of a few nearly complete bacterial genomes - one *Burkholderia* and two *Shewanella* species – was assessed mostly as contaminants rather than as true residents of the Sargasso Sea (DeLong, 2005). Yet the information of this and other studies helps to assess the abundance and diversity of microbes in the world's oceans.

4.3 Flow cytometry

Flow cytometry (FCM) has become one of the most powerful tools to study natural microbial communities (Gasol and del Giorgio, 2000; Legendre et al., 2001). Because of its technical advantages and the development of sensitive fluorescent nucleic acid stains with very high quantum yields and an excitation which is compatible with relatively inexpensive blue lasers (Porter and Pickup, 2000; Steen, 2002), FCM is now routinely used to enumerate and determine cell-specific properties of aquatic microbes, notably planktonic bacteria (Bouvier et al., 2007a).

Flow cytometry allows the physical sorting of particular microbial populations of interest for further analyses (Bernard et al., 2000b; Fuchs et al.,

2000). Generally, microbial cells from bacterioplankton samples are sorted by phenotypic features, e.g. cell volume or cellular DNA or protein content (Servais et al., 1999; Zubkov et al., 1999; Fuchs et al., 2000; Lebaron et al., 2001) (see Fig. 3, p. 16). Sorted bacteria can be analyzed by molecular methods (Zubkov et al., 2001b) and for radiotracer incorporation (Lebaron et al., 2002; Zubkov et al., 2003). Direct sorting of microbial cells after FISH staining has first been shown in highly productive wastewater treatment systems (Snaidr et al., 1999) and has also been used for bacterial cells from coastal marine bacterioplankton, taking advantage of the superior signal intensities of CARD-FISH staining (Sekar et al., 2004). Potentially, such a combination of cell identification and flow sorting offers the ability to quantitatively investigate substrate uptake of single populations in natural samples (Zubkov et al., 2003). Moreover, it might eventually provide a means of obtaining functional genes or larger genome fragments from phylogenetically coherent groups of microbes directly sorted from environmental samples.

4.4 Automated microscopy

Automated microscopy systems for the counting of multiple samples of double-stained microbial cells represent another high-throughput technique for the analysis of bacterioplankton (besides flow cytometry).

Fig. 8: Automated counting system developed by Pernthaler et al. (Pernthaler et al., 2003)

One of theses systems developed by Pernthaler *et al.* (Pernthaler et al., 2003) combines an epifluorescence microscope equipped with motorized *z*-axis drive, shutters, and filter wheels with a scanning stage, a digital camera, and image analysis software (Fig. 8). The acquisition of microscopic image pairs from numerous positions on several slides at two different wavelengths runs automatically. Microbial cells stained with both a general DNA dye and labeled by FISH/CARD-FISH are counted after object edge detection and setting of the signal-to-background ratio threshold. Microscopic fields that are inappropriate

for cell counting (e.g. with poor hybridization quality) are automatically excluded at this stage. The system is guided in two nested walkpaths across a series of triangular samples until a user-defined number of total cells has been analyzed per sample (default ≥2000 DAPI counts). Automated refocusing between individual samples is achieved by a backup autofocusing routine at incident light to reestablish the focal plane after fatal focusing errors at epifluorescence illumination (for details see Pernthaler et al., 2003). The system requires high sample quality, and recalibration for sample sets from different habitats. Prior to analyzing bacterioplankton samples the system has to be calibrated in order to produce counts that are comparable to results obtained by manual evaluation.

The main advantage of automated microscopy is the fast evaluation of high numbers of samples without operator interference. So far, the amount of time spent on enumerating cells was the main factor limiting large scale studies e.g. those analyzing numerous microbial populations in horizontal transects and depth profiles from open ocean cruises. However, with this automated counting system at hand, comprehensive comparative FISH analyses have become feasible. These may eventually become as valuable for the study of the heterotrophic marine picoplankton as flow cytometry has been for the autotrophic picoplankton (Chisholm et al., 1988; Partensky et al., 1999a). The development of this automated microscopy system, in particular, facilitated this study.

5 Aims

Although only a few phylogenetic groups are able to form large populations (Mullins et al., 1995; Giovannoni and Stingl, 2005), little information is available on the actual depth distribution of these bacterioplankton populations at the scale of whole oceanic basins, as most basin scale studies focus mainly on cell numbers of total bacterioplankton and cyanobacteria (Li, 1998; Zubkov et al., 1998; Ducklow et al., 2001). Therefore, the main objective of the present thesis was to determine the vertical and horizontal distribution of these phylogenetic

groups on a South – North transect in the Atlantic Ocean by CARD-FISH and automated cell counting. It was hypothesized that (i) the abundance of specific microbial groups would change with latitude across different ecological provinces and (ii) that the composition of the microbial communities in surface waters would be substantially different from that in the deep chlorophyll maximum and mesopelagic water layers. Additionally, it was investigated whether the microbial groups differ in their cell sizes and their contribution to total bacterioplankton biomass. The results of this investigation, which are based on samples obtained on two cruises (AMT16 and VISION), are presented and discussed in Publication I.

The second project of this thesis aimed at an understanding of the phylogenetic composition of bacterioplankton groups containing low (LNA) and high (HNA) amounts of nucleic acid. Samples from contrasting waters of the North Atlantic Ocean were sorted by flow cytometry and identified by an improved protocol for fluorescence *in situ* hybridization (Pernthaler et al., 2004). The results of this investigation are presented and discussed in Publication II.

B Results & Discussion

1 Distribution of major microbial groups in the Atlantic Ocean

For a long time it has not been possible to separate routinely the most basic groups (e.g. SAR11) within the taxonomically diverse (Field et al., 1997) heterotrophic bacteria of the oceanic picoplankton. Thus, these ubiquitous prokaryotes were often considered as a single category in studies of the marine picoplankton. New methods pointed at a vast total diversity of *Bacteria* and *Archaea* in marine waters (Curtis et al., 2002; Pedros-Alio, 2006; Sogin et al., 2006). Interestingly, only approximately 10 – 20 phylogenetic groups seem to be able to form large populations of >5% relative abundance (Mullins et al., 1995; Giovannoni and Stingl, 2005). Moreover, with only a few exceptions, like for cyanobacteria (Li, 1998; Zubkov et al., 1998; Ducklow et al., 2001), little information is available on the depth distribution of picoplankton in an oceanic-basin scale.

Parameters influencing the distribution of marine pelagic prokaryotes are not only the physical ones like temperature (Partensky et al., 1999b), but also the chemical ones like the availability of dissolved organic matter (DOM) (Zubkov et al., 2000c). Heterotrophic bacteria dominate the mineralization of the largely phytoplankton-derived DOM, and bacterial production usually represents a substantial fraction of about 20 – 40% (Ducklow et al., 1993) of the primary production (Azam and Hodson, 1977). It is well established that DOM dynamics are not constant throughout the year but vary systematically and seasonally with the hydrodynamic regime and primary productivity (Williams, 1995; Teira et al., 2001). Also linked to the hydrodynamic regime is the annual cycling of elements since for example vertical mixing brings nutrients into the euphotic zone during winter (Steinberg et al., 2001). Typically, the annual upward nutrient flux supports a short spring-bloom period of higher primary production rates, enhanced concentrations of chlorophyll *a* (Chl *a*), and higher concentrations of suspended particulate organic carbon (POC) and nitrogen (PON). During thermal stratification in summer, nutrient levels are low within the euphotic zone, primary production rates are low and a subsurface chlorophyll maximum is present (Steinberg et al., 2001).

Along the AMT and the VISION cruise track we passed six biogeochemical provinces according to Longhurst's classification (Longhurst, 1998; Li and Harrison, 2001) (Fig. 5, p. 19). The Benguela Current Coastal Province (BENG), the Western Tropical Atlantic (WTRA), the Northern Atlantic Drift (NADR) and the Atlantic Arctic Province (ARCT) were considered as productive oceanic areas (defined as Chl *a* ≥0.25 mg m^{-3}), whereas the North Atlantic Gyral Province (NAG) (comprising North Atlantic Tropical Province NATR and North Atlantic Subtropical Province NAST) and the South Atlantic Gyral province (SATL) as oligotrophic oceanic areas (Chl *a* <0.25 mg m^{-3}). The differences in availability of nutrients in these provinces should also influence the distribution of prokaryotes.

In my first study, I was indeed able to show by CARD-FISH that the distribution of *Bacteria* in the Atlantic Ocean was vertically and horizontally stratified. *Bacteria* were dominant in the photic zone, approximately the upper 200 m of the water column. In the northern hemisphere, the abundance of *Bacteria*, especially in the upper 50 m, was higher during the austral autumn/boreal spring season (AMT16) but decreased slightly during the boreal autumn (VISION). The community composition in the photic zone appeared to be quite homogeneous from surface to the deep chlorophyll maximum layer (DCM) which may be explained by the rather even distribution of primary production over the entire photic zone (Poulton et al., 2006). However, we can not exclude that within the bacterial clades significant differences may occur between the communities of the surface and DCM layer.

1.1 The oligotrophic provinces

The gyral provinces SATL and NAG are characterized by oligotrophic conditions (nominally defined as where NO_3^- is <0.05 µmol L^{-1}). Several studies of the AMT Programme have shown that the inorganic nutrient concentrations are typically low (<0.03 µM e.g. Rees et al., 2006) in surface waters of the SATL and NAG. Especially, the concentration of particulate organic nitrogen (PON) is much reduced, typically 0.1 – 0.2 µM, while dissolved organic nitrogen (DON) is typically 6 – 8 µM (Roussenov et al., 2006). Some DON components

can be consumed directly by bacteria (Keil and Kirchman, 1999), and, at the same time, bacteria remineralize DON through the release of ammonium and urea (Berman et al., 1999). With respect to phosphate, low concentrations (2.2 ± 1.2 nM) of bioavailable material were repeatedly determined during spring and autumn research cruises in surface waters of the North Atlantic oligotrophic gyre (Zubkov et al., 2007) as well as in oligotrophic regions of the North Pacific Ocean (Karl et al., 1997), the western North Atlantic Ocean (Wu et al., 2000) and the Mediterranean Sea (Thingstad et al., 2005). These low phosphate concentrations have been interpreted as growth limiting to phytoplankton species (Sanudo-Wilhelmy et al., 2001; Mills et al., 2004). Thus, phosphorus (P_0) has been identified as another potentially productivity limiting nutrient beside the availability of mineral nitrogen (N). In the North Atlantic oligotrophic gyre, P_0 is also essential for the bacterioplankton as the main consumers of phosphate (Zubkov et al., 2007). Low rates of primary production are commonly observed in the oligotrophic gyres (0.2 and 1 g C m^{-2} d^{-1}) (Tilstone et al., in press; Marañón et al., 2003; Teira et al., 2005). However, the large areal extent of the subtropical gyres and the absence of strong seasonality in the lower latitudes are believed to make even these low production rates important for the export of carbon from the upper ocean to deeper waters in a global sense.

Primary production rates within the subtropical Atlantic Ocean vary between years by at least a factor of three (Marañón et al., 2000; Poulton et al., 2006). For most time of the year, the nutrient depleted surface waters of both gyres are separated from the nitracline by well developed thermoclines; only when stratification breaks down, nutrient-limited blooms occur (Longhurst, 1998). Studies assessing plankton production (P) and respiration (R) in the euphotic zone imply that the NAG is more heterotrophic than the SATL (R>P) displaying respiration rates of 69 ± 22 and 62 ± 24 mmol O_2 m^{-2} d^{-1} for the NAG and SATL, respectively (Gist et al., in press; López-Urrutia et al., 2006). For the SATL, Gist *et al.* (Gist et al., in press) could show a pronounced seasonality with higher nutrient limitation during autumn when the nitracline was lying below the mixed layer. P in the SATL was significantly lower during autumn than during spring, and autumn P was lower here than in the NAG during the

same season. Gist *et al.* related this seasonality of P and P/R to the changes in the relative depths of the thermocline and nitracline (Gist et al., in press).

In the photic zone of these oligotrophic provinces solely members of the SAR11 clade and the cyanobacterial genus *Prochlorococcus* were found in substantial numbers. This is likely due to the fact that both populations are well adapted to oligotrophic environments. SAR11 cells are generally small in size (Rappé et al., 2002) which is in accordance with the model proposed by Button (Button, 1991) for natural selection optimizing the surface-to-volume ratios in oligotrophic cells in order to match the capacity of the cytoplasm to process substrates with steady-state membrane transport rates (Giovannoni et al., 2005b). *Candidatus* Pelagibacter ubique was the first cultured member of the SAR11 clade (Rappé et al., 2002) and has the smallest genome which encodes the smallest number of predicted open reading frames known for a free-living microorganism, containing only the most fundamental cellular systems (Giovannoni et al., 2005b). SAR11 cells grow by assimilating organic compounds from the ocean's DOC reservoir, and generate metabolic energy not only by respiration (Rappé et al., 2002) but also by a light-driven proteorhodopsin proton pump (Giovannoni et al., 2005a). Additionally, incubation experiments demonstrated high uptake activity for amino acids (Mary et al., 2008a) and 3-dimethylsulfoniopropionate (DMSP) (Malmstrom et al., 2004). SAR11 utilizes transporters with broad substrate ranges and a number of specialized substrate targets like nitrogenous compounds and osmolytes and is thus able to efficiently replicate even under oligotrophic conditions (Giovannoni et al., 2005b). In the present investigation, SAR11 indeed dominated the impoverished provinces SATL and NAG with relative abundances of 27% ± 5% and 41% ± 5%, respectively, in surface water (Fig. 9, left panel). The abundance of SAR11 in surface water of NAG decreased about half (25% ± 4%) on the VISION cruise during late summer (Fig. 9, right panel).

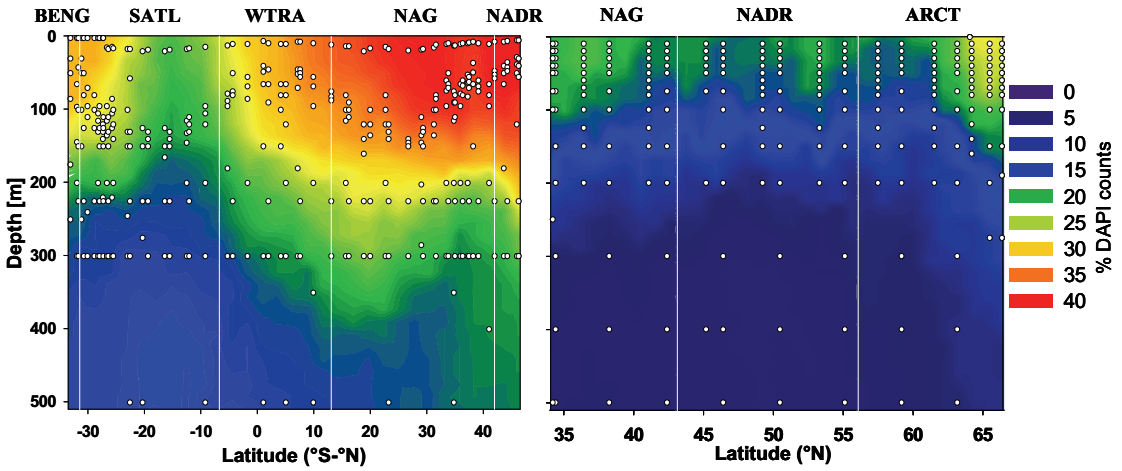


Fig. 9: Latitudinal depth contours of SAR11 as relative abundance (% of total cell number). The left panel shows the distribution of SAR11 during austral autumn/ boreal spring, right panel shows the distribution during boreal late summer in distinct ecological provinces of the Atlantic Ocean (see Fig. 5, p. 19). Dots represent sampling locations.

The abundant marine cyanobacteria of the genus *Prochlorococcus* are typical of the more oligotrophic regions of the oceans. As expected for a photoautotroph they are particularly abundant in the euphotic zone (Campbell and Vaulot, 1993; Heywood et al., 2006). *Prochlorococcus* spp. are the most prominent phytoplankton in large parts of the subtropical and tropical ocean basins (Zubkov et al., 1998; West and Scanlan, 1999; Dufresne et al., 2003) where their contribution to total phytoplankton production can be as high as 50% (Li, 1994; Veldhuis et al., 1997). *Prochlorococcus* is in general limited by temperatures below approximately 17°C (Olson et al., 1990) restricting its distribution to tropical and subtropical latitudes (40°S – 40°N). Comparing data from AMT14 with AMT cruises conducted in the late 1990s (AMT3, 4 and 6), Heywood *et al.* (Heywood et al., 2006) reported no significant variation in *Prochlorococcus* spp. During AMT16 (2005) a very similar distribution of *Prochlorococcus* along the temperature gradient mentioned above was found again, with *Prochlorococcus* being on average the second most abundant organism after SAR11 in the photic zone. In the oligotrophic southern gyre (SATL) *Prochlorococcus* spp. reached abundances of up to 16% in the surface water layer (Fig. 10). In the NAG, the number of *Prochlorococcus* spp. in the surface waters reached its maximum mostly in the DCM (11% ± 7%) which confirms earlier distribution data obtained by flow cytometry (Partensky et al., 1999a) (Fig. 10).

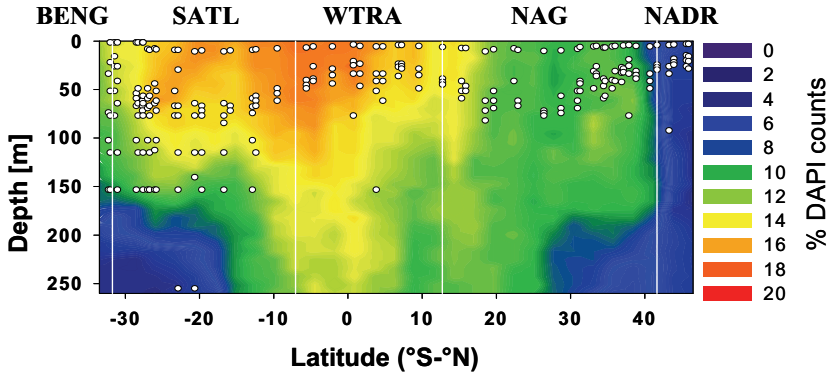


Fig. 10: Latitudinal depth contours of *Prochlorococcus* spp. as relative abundance (% of total cell number).The panel shows the distribution of *Prochlorococcus* spp. during austral autumn/ boreal spring (AMT16) in distinct ecological provinces of the Atlantic Ocean (see Fig. 5, p. 19). Dots represent sampling locations.

As suggested by Moore (Moore et al., 1998), the ecological success of *Prochlorococcus* may partly be based on the occurrence of several different species or ecotypes along the vertical light gradient (Moore et al., 1998; West et al., 2001) specifically adapted to different light regimes (Bibby et al., 2003). Two major ecotypes have been distinguished that exhibit contrasting physiological and genomic adaptation to light, and hence also feature distinct depth preferences (Campbell and Vaulot, 1993; Moran et al., 2004). The vertical distribution of *Prochlorococcus* often exceeds the boundaries of the euphotic zone. These organisms seem to be able to sustain their populations over an irradiance range spanning over three orders of magnitude of light intensity. Moreover, distinct genotypes of *Prochlorococcus* have been found to inhabit different oceanic regions (Johnson et al., 2006).

Another reason for the success of *Prochlorococcus* ecotypes could be their partial heterotrophy. Generally, *Prochlorococcus* has the competitive advantage in nutrient uptake of a higher surface area to volume ratio as compared to eukaryotic algae (Chisholm, 1992). This will be far more important in nutrient poor subtropical gyres where nutrient regeneration within the microbial loop (Azam et al., 1983) predominates over nitrate influx. Physiological experiments on cultivated *Prochlorococcus* strains (Moore et al., 2002) and genomic analysis (Rocap et al., 2003; Garcia-Fernandez et al., 2004) showed that they predominantly use reduced forms of inorganic nitrogen (ammonium) and seem to be unable to assimilate the oxidized forms (nitrate, nitrite). While some strains can assimilate nitrite, nitrate seemed to be unable to support growth, but the latter was recently contested by field data (Casey et al.,

2007). It was that Casey *et al.* reported a significant (5 – 10%) assimilation of nitrate by *Prochlorococcus* populations from the Sargasso Sea. They suggested that the observed population-specific nitrate uptake rates support the net seasonal *Prochlorococcus* population growth (Casey et al., 2007). In yet another investigation Zubkov et al. (Zubkov et al., 2003) estimated that uptake of dissolved amino acids satisfies about 10% of *Prochlorococcus*' requirements for nitrogen, while ammonium may satisfy the remainder of their nitrogen requirements. The observed additional uptake of nitrate by some *Prochlorococcus* populations would remove an important source of organic nitrogen for the heterotrophic bacteria from the water. They would be required to use inorganic nitrogen at a higher energetic cost.

The observed distribution of *Prochlorococcus* spp. is well in accordance with the NH_4^+ distribution reported by Clark *et al.* (Clark et al., 2008). NH_4^+ generally was the dominant component of the dissolved inorganic nitrogen (DIN) pool with lower concentrations of ~20 nM in the northern provinces NADR and NAG as opposed to more variable concentrations of 20 – 100 nM in the southern gyre (SATL). The highest NH_4^+ concentration of ~100 nM was found at the equator where we also detected the highest abundance of *Prochlorococcus* spp. during AMT16 (see Fig. 10). Clark *et al.* also showed that nitrification and NH_4^+ regeneration rates were sufficient to completely replace the dissolved inorganic nitrogen (DIN) pool within 1 d (Clark et al., 2008).

In addition to ammonium, it has been shown before that environmental *Prochlorococcus* populations incorporated significant amounts of DIN in the form of amino acids (Zubkov et al., 2003; Mary et al., 2008b). *Prochlorococcus* likely exert competitive pressure on other bacterioplankton populations by controlling the pool of labile organic nutrients. This partial heterotrophy would give *Prochlorococcus* very considerable ecological advantages in oligotrophic oceanic conditions. By directly consuming amino acids, *Prochlorococcus* deprive other bacteria of an important source of organic nitrogen, forcing the latter to use inorganic nitrogen at a higher energetic cost. Comparing the amino acid uptake of the high-light and low-light adapted ecotypes of *Prochlorococcus*, Zubkov et al (Zubkov et al., 2004a) estimated that the first was consuming about 25% of the total dissolved amino acid pool in the surface mixed layer whereas the latter controlled the amino acid pool by consuming

50%. Therefore, the amino acid pool seems to be an important source of nutrients for the low-light adapted ecotypes of *Prochlorococcus.*

1.2 The productive provinces

The larger part of the Atlantic Ocean is characterized by productive conditions. Ecological provinces like the Benguela Current Coastal Province (BENG), the Western Tropical Atlantic (WTRA), the Northern Atlantic Drift (NADR) and the Atlantic Arctic Province (ARCT) are generally rich in nutrients and therefore more productive (see Fig. 5, p. 19). Comparing several provinces Li *et al.* (Li and Harrison, 2001) confirmed the general progression of chlorophyll to significantly increase (almost 10-fold) from provinces at low latitudes (NAG) to high latitudes (BENG, NADR, ARCT) (see also Fig. 5, p. 19; http://oceancolor.gsfc.nasa.gov). Within the AMT Programme high Chl *a* concentrations were also routinely measured in surface waters of the WTRA with a typical shallow subsurface maximum (Perez et al., 2005; Robinson et al., 2006). This equatorial region is clearly a very complex and dynamic one and strongly affected by upwelling off the African coast that results in shallowing of the thermocline and intensified nutrient flux.

Phytoplankton in the Atlantic Ocean show considerable latitudinal variation in abundance and C biomass with higher abundance in areas with detectable nutrients (e.g. temperate and upwelling regions). This general pattern of distribution remains the same at different times of the year (May – June, September – October) (Tarran et al., 2006). The time-series by Tilstone *et al.* (Tilstone et al., in press) showed a generally higher primary production (PP) in the eutrophic provinces with mean PP in the BENG ranging from 0.5 to 3 g C m^{-2} d^{-1}, similar to values in the WTRA where PP reached 3 g C m^{-2} d^{-1} in July and 0.5 to 1 g C m^{-2} d^{-1} throughout the other months (Marañón et al., 2000). In the NADR primary production was >2 g C m^{-2} d^{-1} during May which decreased to 0.2 g C m^{-2} d^{-1} in September. In nutrient-rich provinces with high phytoplankton abundance, the release of DOM, either directly from primary producers or as a consequence of trophic processes (Nagata, 2000; Bronk, 2002), plays an integral part in the microbial food web. DOM constitutes one of

the main sources of labile organic substances for bacteria (Norrman et al., 1995), and, therefore, they are largely reliant on phytoplankton for their principal energy supply. In an equilibrium situation between DOM production and consumption, it would be reasonable to expect a covariation between DON release rates and bacterioplankton activity. Standing stocks of DON and PON in eutrophic waters are generally around 5 µM and 5 – 10 µM respectively (Roussenov et al., 2006).

The SAR11 clade and, to a certain extent, *Prochlorococcus* were also detected in the productive provinces along the AMT16 and VISION transects (Fig. 9, 10; p. 36, 37). During boreal spring, SAR11 comprised abundances of >30% in the provinces along AMT16 (BENG 44%, WTRA 32%, NADR 45%), and even 31% in the ARCT on the VISION cruise during late summer (see Fig. 9, p. 36). The occurrence of SAR11 in these productive provinces may indicate that even the productive waters of the Atlantic Ocean are rather "oligotrophic" for heterotrophic bacteria. An alternative explanation would be a strong dependence on reduced sulfur compounds like dimethylsulfoniopropionate (DMSP) which is the prime algal osmolyte (Tripp et al., 2008). This stands in line with the significant co-occurrence of SAR11 and nano- and picoeukaryotic algae along the AMT16 transect (r^2 = 0.56 and r^2 = 0.51, respectively, p <0.001, Tarran G., unpublished data) and the co-occurrence of SAR11 and picoeukaryotic algae along the VISION transect (r^2 = 0.56, p <0.001, Fuchs B., unpublished data). In contrast to SAR11, *Prochlorococcus* spp. were only abundant (22%, see Fig. 10, p. 37) in the surface water layer of the WTRA on AMT16 confirming the distribution pattern found by Heywood *et al.* (Heywood et al., 2006) (see Fig. 10, p. 37).

Microbial clades different from SAR11 or *Prochlorococcus* were only abundant in the nutrient-rich provinces BENG, WTRA, NADR or ARCT. The *Bacteroidetes* phylum is a highly diversified group in the marine pelagic environment (Alonso et al., 2007) that can reach high abundances both in coastal and offshore waters (Pernthaler et al., 2001a; Kirchman et al., 2003; Bauer et al., 2006; DeLong et al., 2006; González et al., 2008). They have been shown to be also abundant in polar regimes (Simon et al., 1999). On the

AMT16 transect highest abundances of *Bacteroidetes* were found in the nutrient-rich surface waters of the coastal influenced BENG (9%) as well as in the offshore NADR (13%) during boreal spring (Fig. 11, left panel). Comparable abundances of *Bacteroidetes* (12%) were detected in the NADR during late summer (VISION transect), while a slight increase (16%) was observed towards the ARCT province (Fig. 11, right panel).

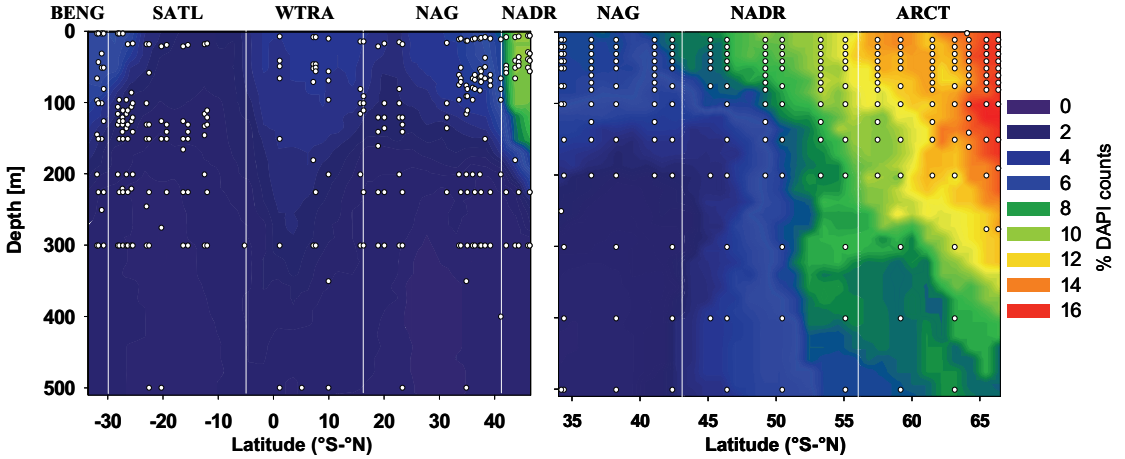


Fig. 11: Latitudinal depth contours of *Bacteroidetes* as relative abundance (% of total cell number). The left panel shows the distribution of *Bacteroidetes* during austral autumn/ boreal spring, right panel shows the distribution during boreal late summer in distinct ecological provinces of the Atlantic Ocean (see Fig. 5, p. 19). Dots represent sampling locations.

Each of those provinces had comparably high chlorophyll concentrations indicating a high abundance of phytoplankton. Indeed, members of the *Bacteroidetes* are linked to algal blooms. As consumers of algae-derived metabolites (Simon et al., 1999; Riemann et al., 2000; O'Sullivan et al., 2004; Pinhassi et al., 2004; Grossart et al., 2005) they are able to degrade polymers such as polysaccharides and proteins (Simon et al., 1999; Cottrell and Kirchman, 2000b; Eilers et al., 2001). This is in accordance with the relation found for the AMT16 transect where the abundance of *Bacteroidetes* was highly correlated with nanoeukaryotic phytoplankton (r^2 = 0.66, Tarran G., unpublished data). Additionally, a significant correlation of Chl *a* fluorescence with relative abundance of *Bacteroidetes* was shown for BENG (r^2 = 0.70, $p < 0.01$), which is what also Abell and coworker found for marine *Flavobacteria*, the largest marine class of *Bacteroidetes* (Abell and Bowman, 2005a). Although the *Bacteroidetes* occurred in all water layers examined, they were preferentially observed in the photic zone. Recent genome analyses of a range of marine *Bacteroidetes* isolates showed that some strains possess

proteorhodopsins. It has been shown that their biomass increases faster with light (Gomez-Consarnau et al., 2007).

Gammaproteobacteria have been commonly found in coastal picoplankton (Rappé et al., 1997) where they may even form pronounced blooms (Alonso-Saez et al., 2007). Coastal bacterial communities are often exposed to a wide variety of changing environmental conditions. Environmental parameters which seem critical at selecting particular populations include temperature, as imposed by seasonality, substrate and nutrient availability, and grazing (Shiah and Ducklow, 1994; Eilers et al., 2001; Pernthaler, 2005; Mary et al., 2006a). In our samples of AMT16, the abundance of *Gammaproteobacteria* did not increase in the coastal influenced BENG province but remained rather stable (2% - 4%) across the southern part of the AMT16 transect as well as in all deeper water layers (Fig. 12). On the contrary, *Gammaproteobacteria* increased in abundance from the NAG northward to 9% ± 3% in the surface layer of the NADR (Fig. 12).

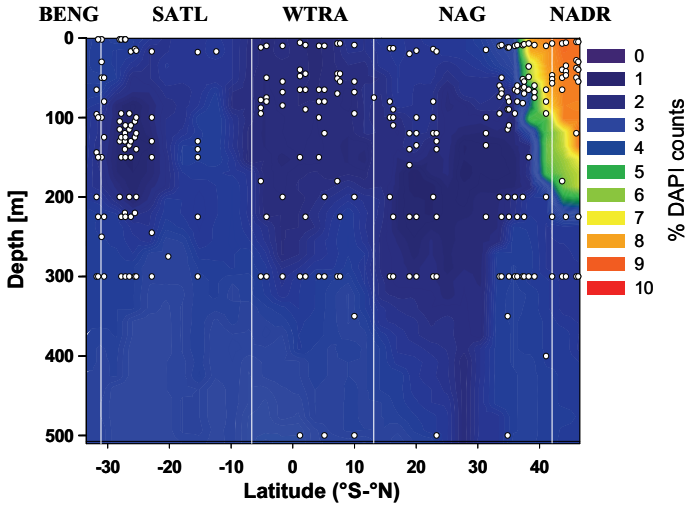


Fig. 12: Latitudinal depth contours of *Gammaproteobacteria* as relative abundance (% of total cell number). The panel shows the distribution of *Gammaproteobacteria* during austral autumn/ boreal spring (AMT16) in distinct ecological provinces of the Atlantic Ocean (see Fig. 5, p. 19). Dots represent sampling locations.

Only surface water samples of the VISION cruise in late summer were analyzed for the abundance of *Gammaproteobacteria*. Compared to boreal spring (AMT16), the abundance of *Gammaproteobacteria* decreased by half (4% ± 1%) in the surface layer of NAG and NADR and was slightly higher in the ARCT (6% ± 2%). This distribution pattern of *Gammaproteobacteria* appears

indeed to be influenced by seasonality. The southern provinces of the AMT16 transect were sampled during austral autumn whereas the provinces in the northern hemisphere depict spring conditions. The decrease in the relative abundance of *Gammaproteobacteria* in the NAG and NADR province during the VISION transect may be also explained by the late summer conditions during sampling.

The distribution of *Gammaproteobacteria* showed one distinct discontinuity. There was a bloom in the NADR at stations 60 – 62 of AMT16 with a maximum relative abundance of *Gammaproteobacteria* of >50% in the surface water of station 61. CARD-FISH results with probes specific for the gammaproteobacterial genera *Alteromonas*, *Pseudoalteromonas* and *Oceanospirillum* indicated the presence of these culturable genera in significant amounts (2% - 5%). *Gammaproteobacteria* have the potential to form blooms in response to sudden nutrient pulses released from phytoplankton (Cottrell and Kirchman, 2000b). Members of these genera are well known to rapidly form blooms upon excess nutrient supply (Bano and Hollibaugh, 2002; Beardsley et al., 2003; Allers et al., 2007; Allers et al., 2008). Based on satellite images from the NADR area that indicate the end of the spring phytoplankton bloom (declining chlorophyll concentration in the surface waters, data source: http://neo.sci.gsfc.nasa.gov) we can speculate that decaying phytoplankton had fueled massive growth of *Gammaproteobacteria* in that region.

1.3 The aphotic zone

The environmental parameters in the water column change while moving from the sea surface towards the bottom. In the photic zone steep gradients of light quality and intensity, temperature, and macronutrient and trace-metal concentrations are influencing the distribution of prokaryotes (Hochachka and Somero, 1984). In contrast, low temperature, increasing hydrostatic pressure, the disappearance of light, and dwindling energy supplies largely determine the vertical stratification of oceanic biota of the aphotic zone. In general, nutrient concentrations of NO_3^- and PO_4^{3-} tend to be significantly higher in the deeper waters than those measured in the photic zone (Mahaffey et al., 2004;

Roussenov et al., 2006) therefore indicating less nutrient limitation for prokaryotes. The export of sinking of particulate material (faecal pellets, marine snow) via the biological pump transfers for example, particulate organic nitrogen (PON) to deeper waters (Checkley and Miller, 1989), which is remineralized below the photic zone into nitrate. The pathways of remineralization are complex and involve at least two processes: ammonification, the breakdown of organic N to ammonium (Berman et al., 1999), and nitrification, a two-step process involving ammonium oxidation and nitrite oxidation by microorganisms (Bothe et al., 2000). However, though samples were taken at a maximum depth of 1000 m during AMT cruises, most investigations (e.g. on primary production and phytoplankton community, trace gas measurements) concentrated only on the upper 200 – 300 m (Robinson et al., 2006). Therefore only little information is available about those mesopelagic waters of the Atlantic Ocean.

As expected from literature data (Karner et al., 2001; Giovannoni and Stingl, 2005; DeLong et al., 2006) the composition of marine picoplankton assemblages shifted with increasing depth. In contrast to the photic zone of the Atlantic Ocean, the microbial community in the mesopelagic layer was dominated by members of the marine group I *Crenarchaeota*, during both the AMT16 (15% - 20%) (Fig. 13, left panel) as well as the VISION transect (14% ± 4%) (Fig. 13, right panel). *Crenarchaeota* typically occur in the deep water layers below the photic zone and can comprise 50% or more of the total microbial community (Karner et al., 2001; Morris et al., 2002; Teira et al., 2004). During boreal spring (AMT16) we detected a striking spatial distribution of *Crenarchaeota* in the aphotic zone along the south – north transect (Fig. 13, left panel).

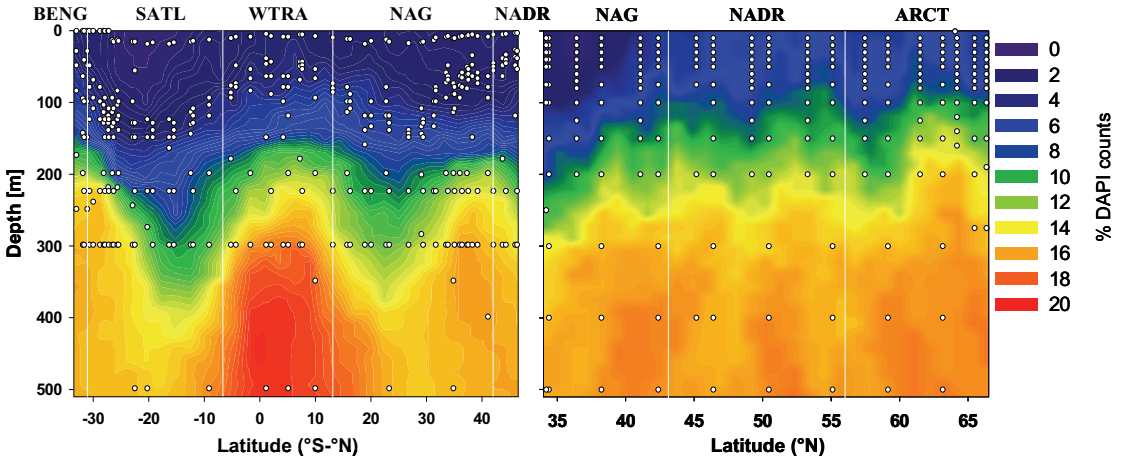


Fig. 13: Latitudinal depth contours of *Crenarchaeota* as relative abundance (% of total cell number). The left panel shows the distribution of *Crenarchaeota* during austral autumn/ boreal spring, right panel shows the distribution during boreal late summer in distinct ecological provinces of the Atlantic Ocean (see Fig. 5, p. 19). Dots represent sampling locations.

Their relative abundance was generally higher in the productive provinces of BENG, WTRA and NADR (17% – 25%) than in the oligotrophic SATL and NAG (14% – 15%, Fig. 13, left panel) of AMT16. In the deep layers of all those productive provinces relatively high concentrations of nitrate were measured (BENG 12 µM, WTRA 26 µM, and NADR 15 µM). Generally nitrate concentrations correlated well with the abundance of *Crenarchaeota* (r^2 = 0.58 – 0.83, p <0.001). The abundance of *Crenarchaeota* was particularly high at high nitrate and low oxygen concentrations in the WTRA (r^2 = 0.81 and r^2 = 0.68, p <0.001, respectively). Previous studies have already shown an association of *Crenarchaeota* with low oxygen environments such as the Black Sea (Lam et al., 2007). Oxygen minimum zones (OMZ) are also known as sites of ammonia oxidation (Kuypers et al., 2005). One organism able to oxidize ammonia in such locations is the recently isolated non-thermophilic Crenarchaeum "*Nitrosopumilus maritimus*" (Koenneke et al., 2005). We can only speculate that the co-occurrence of high nitrate and low oxygen concentrations in mesopelagic waters of the WTRA is due to ammonia oxidation by *Crenarchaeota* (Wuchter et al., 2006). The presence of *Crenarchaeota* in the two other eutrophic provinces BENG and NADR during AMT16 coincided with relatively high levels of oxygen (>200 µmol kg^{-1}). These *Crenarchaeota* obviously cope well with the aerobic conditions in these oceanic regions. Similar percentages of *Crenarchaeota* as in the BENG and NADR (17% – 25%) have been found by Fuchs B. (personal note) in oxygenated

waters of the Namibian upwelling. It needs to be explored further if these types of *Crenarchaeota* comprise more subclades than those found in the low-oxygen depths of the WTRA. In contrast to the distribution pattern of AMT16 during boreal spring, *Crenarchaeota* abundance was rather stable at 14% - 15% in the aphotic zone of the North Atlantic Ocean during the VISION cruise in late summer (Fig. 13, right panel). *Crenarchaeota* correlated negatively with oxygen saturation (r^2 = 0.58, p <0.001) and positively with nitrate concentration (r^2 = 0.63, p <0.001).

A second clade was abundant in the mesopelagic zone of the Atlantic Ocean, namely the uncultured *Chloroflexi*-related clade SAR202 (Fig. 14).

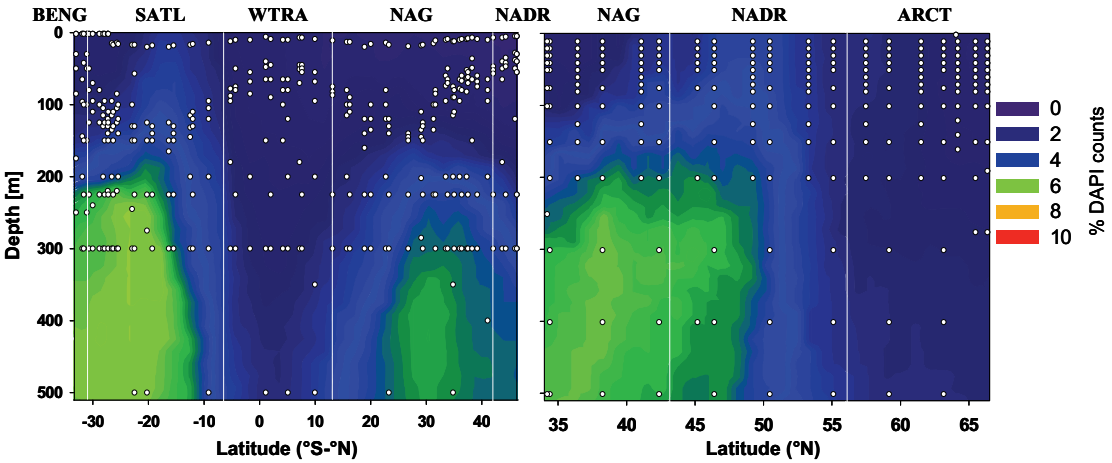


Fig. 14: Latitudinal depth contours of SAR202 as relative abundance (% of total cell number). The left panel shows the distribution of SAR202 during austral autumn/ boreal spring, right panel shows the distribution during boreal late summer in distinct ecological provinces of the Atlantic Ocean (see Fig. 5, p. 19). Dots represent sampling locations.

It had been observed that the highest abundance of the SAR202 group coincides with oxygen concentrations >190 µM (Giovannoni et al., 1996), but in the present study (AMT16, Publ. I) we could not detect any significant correlation with oxygen concentrations or any other physico-chemical parameter measured. A similar observation was reported by Giovannoni (Giovannoni et al., 1996) who suggested that the stratification of SAR202 in vertical profiles is most likely a consequence of differential growth and mortality since no physical factors were able to account for the concentration of theses cells at these depths. The general distribution of SAR202 depicts an increase in abundance below the DCM (>100 m) accounting for approximately 6% of total picoplankton in the mesopelagic zone during boreal spring (AMT16, Fig. 14, left

panel) and approximately 4% during late summer (VISION). An interesting result was the distribution of the SAR202 clade on a basin-scale along AMT16. Representatives of the SAR202 clade were three times more abundant in the mesopelagic zone of both gyres (>5% in SATL, NAG) than in the one of the WTRA (<1.5%, Fig. 14, left panel). A similar observation has been reported by Varela *et al.* (Varela et al., 2008a). The abundance of SAR202 cells in the mesopelagic layer decreased from north (25°N - 15°N) towards the equator. Indeed, this decrease coincided with a local oxygen minimum (<100 µmol kg^{-1}) close to the equator (~10°N) reflecting a similar situation as found in our study where oxygen concentrations were also particularly low in the WTRA (<100 µmol kg^{-1}). The distribution of SAR202 during the VISION cruise in late summer (Fig. 14, right panel) showed a high abundance in the mesopelagic zone of NAG (5% ± 2%) but decreased drastically towards the ARCT (2% ± 1%).

Currently, there is only very limited information on the physiology of members of the SAR202 clade. It has been shown that the fraction of *Bacteria* and *Crenarchaeota* utilizing D- aspartic acids (Asp) remained rather stable with depth while the fraction of *Bacteria* utilizing L-Asp showed a pronounced decrease (Pérez et al., 2003; Teira et al., 2006; Varela et al., 2008b). However, the SAR202 clade efficiently takes up L-Asp at all depths (Varela et al., 2008a). Thus, the SAR202 cluster seemed to be well adapted to exploit the available DOM source in the bathypelagic realm. However, this metabolic adaptation appears to give SAR202 no advantage over *Crenarchaeota* in the WTRA as seen by the virtual absence of SAR202 cells in this province (Fig. 14, left panel). Based on the strong indications for autotrophic ammonium oxidation for members of the marine group I *Crenarchaeota* (Koenneke et al., 2005; Wuchter et al., 2006; Lam et al., 2007), we can only speculate that the *Crenarchaeota* have the metabolic advantage and better growth conditions in oceanic regions with higher ammonium concentrations like the BENG, WTRA and NADR (Rees et al., 2006).

In summary, the main objective of this study was to determine the vertical and horizontal distribution of distinct phylogenetic groups across six ecological provinces in the Atlantic Ocean.

The vertical composition of the microbial communities in surface waters and the deep chlorophyll maximum (DCM) was rather similar along both transects (AMT16, VISION). Changes in heterotrophic bacterioplankton composition between these two layers were only small although they were detected for *Prochlorococcus.* However, confirming current knowledge, the bacterioplankton communities of the photic zone were fundamentally different from those in the mesopelagic water layers along the entire transect. SAR11 was by far the most abundant bacterioplankton clade in the photic zone and *Prochlorococcus* was on average the second most abundant organism (Fig. 9, 10, p. 36, 37). Below the DCM the microbial community composition changed entirely to the marine group I *Crenarchaeota* dominating the bacterioplankton in the mesopelagic layer (Fig. 13, p. 45). Additionally, the uncultured clade SAR202 was also abundant at the mesopelagic sites (Fig. 14, p. 46).

Horizontally, the distribution of distinct microbial groups differed between the ecological provinces and appeared to be influenced by their respective properties. In the photic zone of the two oligotrophic provinces, SATL and NAG, only the SAR11 clade and the photoautotrophic genus *Prochlorococcus* were found in substantial numbers (Fig. 9, 10, p. 36, 37). High abundances of *Bacteroidetes* were generally found in the more productive provinces BENG, NADR and the ARCT (Fig. 11, p. 41). Similarly, the *Gammaproteobacteria* preferentially occurred in the northern provinces NADR and ARCT (Fig. 12, p. 42).

The vertical distribution showed a clear preference of the marine group I *Crenarchaeota* for the mesopelagic layer of the productive provinces BENG, WTRA and NADR along the AMT16. However, during the VISION cruise *Crenarchaeota* appeared rather constantly in all provinces (NAG – ARCT) (Fig. 13, p. 45). In contrast, the uncultured SAR202 clade was found to be more abundant in the oligotrophic provinces SATL and NAG (Fig. 14, p. 46).

2 Bacterioplankton with high and low nucleic acid content

In the second part of this thesis I analyzed the community composition of the flow cytometric populations HNA and LNA with additional focus on the genome size within those populations.

2.1 Phylogenetic composition and distribution

As mentioned before (see A 1.2), the differentiation of cells in two distinct fractions based on their individual DNA content by flow cytometry is a common feature of bacterioplankton analysis (Li et al., 1995; Gasol et al., 1999; Lebaron et al., 2001; Sherr et al., 2006) (see Fig. 3, p. 16). Phylogenetic studies by FISH (Fuchs et al., 2000; Zubkov et al., 2001a; Zubkov et al., 2004b) or fingerprinting techniques (Longnecker et al., 2005) showed that the high nucleic acid populations (HNA) comprise generally *Bacteroidetes*, *Alphaproteobacteria*, in particular the genus *Roseobacter*, and *Gammaproteobacteria*. The phylogenetic affiliation of the low nucleic acid (LNA) population is less well studied, only the gammaproteobacterial clade SAR86 was commonly found within the LNA bacterioplankton of shelf waters (Zubkov et al., 2001a; Zubkov et al., 2002b; Zubkov et al., 2002a). In addition, Mary *et al.* recently determined that the LNA bacterioplankton in the open ocean was predominately comprised of the alphaproteobacterial SAR11 clade (Mary et al., 2006b).

In this study, which analyzed the surface water sampled during the VISION cruise, the majority of cells in both cytometric fractions (HNA 90% ± 3%, LNA 78% ± 7%) were identified as *Bacteria* (Fig. 15, p. 51). Based on the distribution results as seen in the analysis of the VISION transect (Fig. 13, p. 45) with the marine *Crenarchaeota* specific probe Cren554, we assumed archaeal abundance to be negligible. Members of the SAR11 clade comprised about two thirds of all *Bacteria* in the LNA fraction (Fig. 15, p. 51), thus confirming the dominating status of this clade in the open ocean (Mary et al., 2006b). The reason to detect SAR11 exclusively in the LNA bacterioplankton is

likely due to their small genome size. Genome analysis of one cultured representative of SAR11 showed a size of 1.3 Mbp (Rappé et al., 2002; Giovannoni et al., 2005b). A small genome results in a small fluorescence signal with a DNA-specific dye, and in consequence these cells fall into the LNA fraction of flow cytometry. In this study, the SAR11 cluster represented quite a stable assemblage that was similar in different parts of the North Atlantic Ocean. A slightly higher abundance was detected in the ARCT (67% ± 7%) than in the oligotrophic NAG (60% ± 15%), but this difference was statistically not significant.

Of the remaining LNA cells, only small fractions could be assigned to *Beta*- and *Gammaproteobacteria* with 3% ± 1% and 1% ± 1%, respectively (Fig. 15). *Betaproteobacteria* are a main component of freshwater systems (Methé et al., 1998; Glöckner et al., 1999), and are rather rare in the marine environment (Glöckner et al., 1999). Yet, newer studies indicated that some betaproteobacterial lineages do occur in various marine environments, including the water column and sediments (Phillips et al., 1999; Bano and Hollibaugh, 2000; Hollibaugh et al., 2002; Sekar et al., 2004; DeLong et al., 2006; Giovannoni et al., 2008). One example of a marine betaproteobacterial clade is the uncultured OM43 group that is commonly found in highly productive coastal ecosystems, but rarely detected in ocean gyres (Rappé et al., 1997; Giovannoni et al., 2008). This fits well with the detection of OM43 in ARCT (about 1%), and the positive correlation of OM43 identification with characteristic high coastal parameters like phosphate (r^2 = 0.91; p <0.003), nitrite and nitrate (r^2 = 0.99; p <0.001), the picoeukaryotic phytoplankton concentration (r^2 = 0.94; p <0.001) and Chl *a* (r^2 = 0.97; p <0.001).

The low abundance of *Gammaproteobacteria* in the LNA fraction was unexpected with respect to previous studies. Zubkov *et al.* showed that the LNA bacterioplankton comprised in particular of the clade SAR86, accounting for >10% (Zubkov et al., 2001a; Zubkov et al., 2002a). In contrast to the findings of Zubkov *et al.* (Zubkov et al., 2001a; Zubkov et al., 2002a) no LNA cells could be assigned to the SAR86 clade in the present study (Fig. 15, Publ. II).

The HNA bacterioplankton was much more diverse, confirming results of previous studies (Zubkov et al., 2002a; Zubkov et al., 2004b). However, the composition within HNA was not stable when analyzing surface samples from two opposed provinces, namely NAG and ARCT. We observed a clear shift in the community composition of HNA (Fig. 15). In general, abundances of the bacterial clades within the HNA fraction were similar to their distribution pattern as seen in the latitudinal depth analysis of the VISION transect (Figs. 9 – 12, p. 36 – 42).

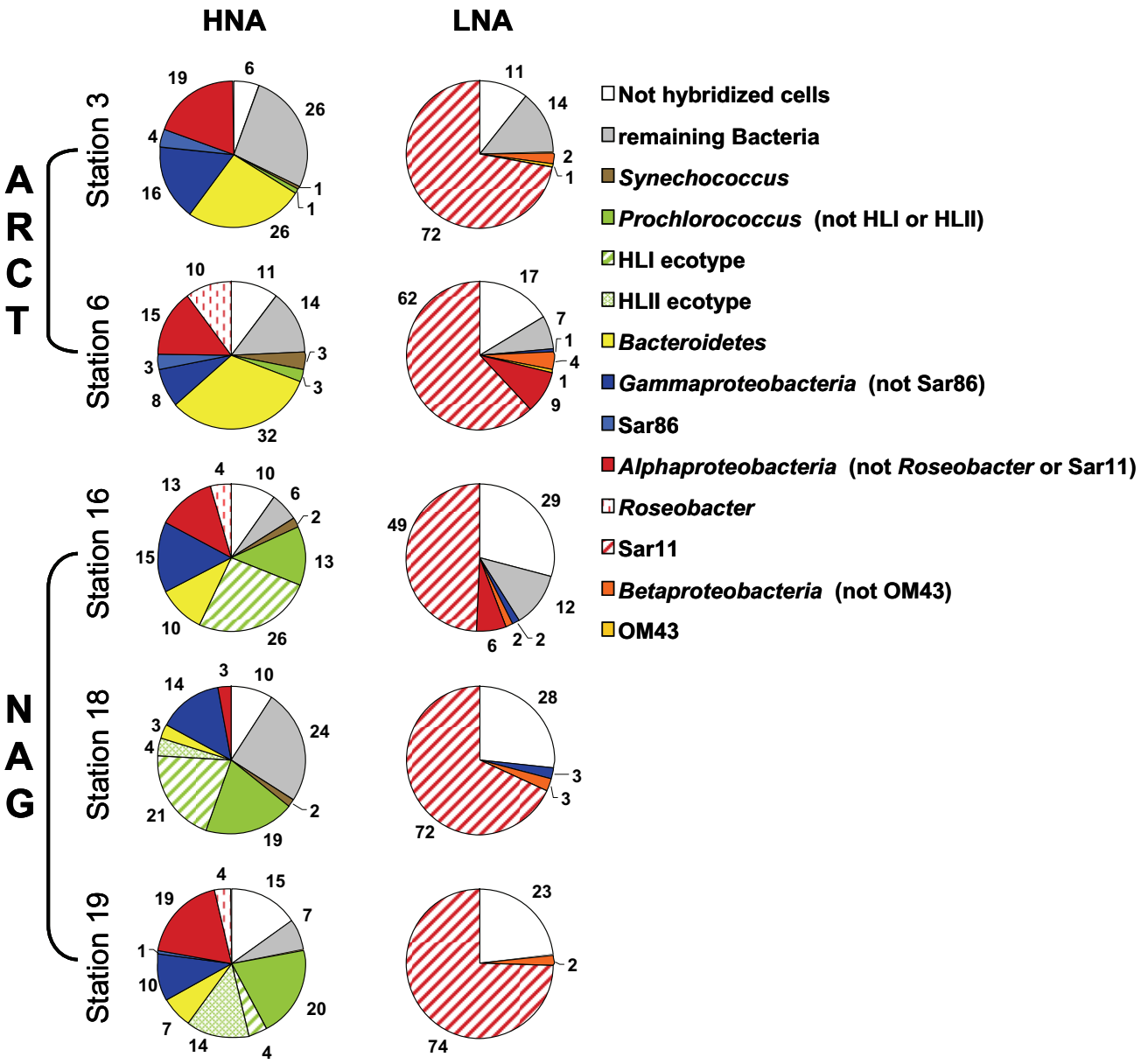


Fig. 15: Contribution of major bacterial groups to the flow-sorted HNA and LNA populations in two oceanic provinces along the VISION transect.

More precisely, *Bacteroidetes* and *Alphaproteobacteria* dominated the community of HNA cells in the ARCT province with 29% ± 4% and 22% ± 4%, respectively (Fig. 15).

Bacteroidetes (O'Sullivan et al., 2004; Pinhassi et al., 2004; Grossart et al., 2005) and some subclusters of the *Alphaproteobacteria* (Eilers et al., 2001; Pinhassi et al., 2004; Pinhassi et al., 2005; West et al., 2008) are often associated with systems of high nutrient or phytoplankton concentrations. However, we observed that only *Bacteroidetes* correlate significantly with nutrients like phosphate (r^2 = 0.95; p <0.001), picoeukaryotic phytoplankton concentration (r^2 = 0.92; p <0.003) or Chl *a* (r^2 = 0.89; p <0.006). In the oligotrophic NAG the abundance of *Bacteroidetes* was only one fourth (7% ± 3%) of that in the ARCT, emphasizing the preference of marine representatives of this phylum for more nutrient-rich environments.

Roseobacter spp. and relatives are an important marine clade of the *Alphaproteobacteria*. They represent a phylogenetically diverse and physiologically versatile group of bacteria (Buchan et al., 2005). Members of this clade are commonly found associated with phytoplankton blooms where they benefit from algal exudates (Eilers et al., 2001; Pinhassi et al., 2004; Pinhassi et al., 2005; West et al., 2008). A high abundance of *Roseobacter* spp. was particularly observed in the productive ARCT province where *Roseobacter* spp. represented approximately half of the *Alphaproteobacteria*, and 10% of all bacterioplankton at station 6 (Fig. 15). This finding stands in line with the higher chlorophyll content of ARCT. The abundance of *Roseobacter* spp. decreased in the oligotrophic NAG to 4% ± 1% despite only a slight decrease in *Alphaproteobacteria* abundance (from 22% ± 4% in ARCT to 17% ± 11% in NAG), suggestive of another alphaproteobacterial subcluster in the oligotrophic environment in the NAG.

The numbers of *Gammaproteobacteria* in HNA remained stable at approximately 14% ± 2% throughout the whole transect (Fig. 15). Within the *Gammaproteobacteria,* members of the SAR86 clade were preferentially detected in the productive ARCT (~4%, Fig. 15). The positive correlations to oxygen (r^2 = 0.83; p = 0.011) and phosphate concentration (r^2 = 0.79; p = 0.017) affirmed the preference of SAR86 for highly productive environmental sites (Eilers et al., 2001; Suzuki et al., 2001). As mentioned earlier, the presence of

the gammaproteobacterial clade SAR86 in the LNA population, as reported previously by Zubkov et al 2002 (Zubkov et al., 2002a), could not be confirmed in this study (Fig. 15, p. 51, Publ. II).

Previous studies regarding the phylogenetic diversity of HNA bacterioplankton generally excluded the autotrophic *Cyanobacteria*, and sorted this group separately based on the chlorophyll autofluorescence per cell (Chisholm et al., 1988; Vaulot et al., 1995; Zubkov et al., 2001a; Zubkov et al., 2004b). We investigated the cyanobacterial abundance and diversity as part of the bacterioplankton community. *Cyanobacteria* contain the phylogenetically closely related genera *Synechococcus* (Urbach et al., 1998) and *Prochlorococcus* (Chisholm et al., 1992). The distribution of both genera is well documented: (i) where they co-occur *Prochlorococcus* cell abundance is approximately one order of magnitude higher than the abundance of *Synechococcus* cells, (ii) *Prochlorococcus* is more abundant in oligotrophic waters, whereas *Synechococcus* dominates in coastal and temperate open ocean waters (Partensky et al., 1999b), and (iii) standing stocks of *Synechococcus* and *Prochlorococcus* are relatively stable in the Atlantic Ocean (Heywood et al., 2006). In this study the abundance of *Synechococcus* was stable over the whole transect with approximately 2% ± 1%, with this representing half of the *Cyanobacteria* abundance in the ARCT. The distribution of *Prochlorococcus* corresponded to the temperature limited distribution (>15°C) (Zubkov et al., 1998; Partensky et al., 1999a) with low abundance in the ARCT (2% ± 2%) and high abundance in the NAG (38% ± 4%).

Temperature has not only been identified as a major determinant of the spatial distribution of *Prochlorococcus* but also as a key ecological determinant dictating community structure of *Prochlorococcus* (Bouman et al., 2006; Johnson et al., 2006; Zwirglmaier et al., 2007). The high abundance of *Prochlorococcus* in the NAG could be partly further resolved by using a very specific probe set to discriminate between two high-light (HLI and HLII) and a low-light (LL) adapted ecotypes (Rocap et al., 2002; Ahlgren et al., 2006) (Fig. 15, p. 51). The HLII ecotype was found to be distributed over a temperature range of 23 – 30°C, while the HLI and LL ecotypes are generally found at 14 – 24°C and 14 – 25°C respectively (Zwirglmaier et al., 2008). A general spatial

partitioning analysis of HL ecotypes revealed that the HLI ecotype dominated temperate latitudes (35° – 48°N and 35° – 40°S), whereas HLII was restricted to subtropical and tropical regions (30°N – 30°S), although with a certain overlap at the transition between temperate and subtropical zones (Zwirglmaier et al., 2008). In this study the ecotype HLI comprised up to 67% of all *Prochlorococcus* found in the HNA bacterioplankton in the NAG (34° – 38°N, St. 16-18). However, we also found up to 10% of the ecotype HLII (37% of *Prochlorococcus*) at one station in the NAG, where it even exceeded the abundance of HLI (Fig. 15, p. 51). West *et al.* reported HLII as absent from the eastern North Atlantic Ocean (PRIME Cruise, 36.82°N, 19.24°W (West and Scanlan, 1999; West et al., 2001) in contrast to phylogenetic analysis of 16S rRNA sequences by Moore *et al.* that placed HLII in the Sargasso Sea (Moore et al., 1998). West *et al.* suggested geographical influences or the source of the *Prochlorococcus* seed population as means which separate the different HL strains in the eastern and western North Atlantic Ocean (West and Scanlan, 1999; West et al., 2001). However, our results are in accordance with the temperature impact on the distribution of HLI and HLII as found by Zwirglmeier *et al.* (Zwirglmaier et al., 2008). The temperature ranged between 22 – 23.6°C at the stations with high HLI abundance (21% ± 4%), reflecting the higher end of the tolerance range for this ecotype. The highest abundance of HLII (14%) was found at >24°C (optima 23 – 30°C) while the abundance of HLI decreased drastically (<4%, Fig. 15, p. 51). Aside from the horizontal spatial separation of HLI and HLII ecotypes, several studies also reported a vertical separation between HL and LL ecotypes (Partensky et al., 1999a; West and Scanlan, 1999; Johnson et al., 2006; Garczarek et al., 2007; Zwirglmaier et al., 2007). However, due to the depth range in the present study (10 m), our focus was limited to the detection of HL ecotypes. The LL ecotypes generally occur in water depths below 40 m (West et al., 2001) which may explain the virtual absence of this ecotype from the present dataset (Publ. II).

Any relations between HNA and LNA cells are still controversially discussed. Nevertheless, Bouvier *et al.* specified four scenarios that may adequately describe interactions between the HNA and LNA fractions (Fig. 16; for details see (Bouvier et al., 2007a).

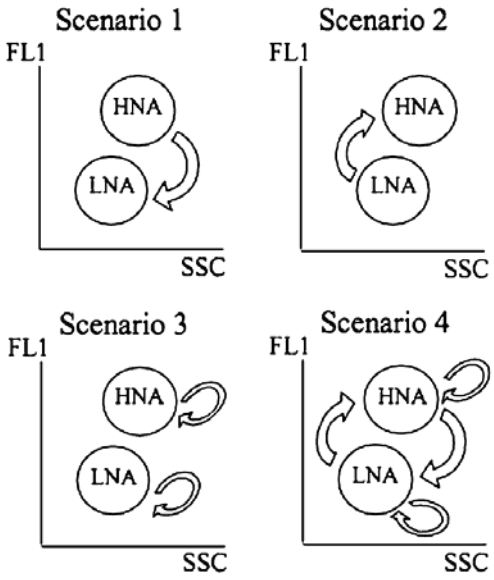


Fig. 16: Illustrated outline of the four potential scenarios that may be envisioned concerning the nature of the HNA and LNA groups of bacterioplankton cells as discussed in the text (Bouvier et al., 2007a).

(1) the HNA fraction comprises active and growing cells, while the LNA fraction is composed of inactive, dormant or dead cells that originate in the HNA fraction.

(2) the LNA fraction is composed of a wide range of physiological states with a similar range of DNA content. HNA cells simply originate of active LNA cells that are undergoing fast cell division and have multiple genome copies.

(3) HNA and LNA are entirely different communities with their own intrinsic characteristics that are independent of one another, with little or no interaction between them.

(4) HNA and LNA are communities with their own intrinsic characteristics but they are also interacting with each other.

Thus, if LNA cells originate from the inactivation or degradation of HNA cells (scenario 1), one would expect to find the same phylogenetic groups in HNA as well as in LNA and to have a continuous transition between the fractions, rather than a clear separation between both of them. In the inverse case (scenario 2) the composition of both fractions would again have to be similar and the average distance between the HNA and LNA bacterioplankton would be within

what could be expected from either the replication of the DNA or the presence of several genome copies.

Our cytometric or phylogenetic data support neither of these two scenarios as most of the analyzed phylogenetic groups were limited to one of the two fractions, e.g. SAR11 was detected exclusively in LNA (Fig. 15, p.51). In addition, studies have shown that SAR11 represents a metabolically active microorganism that is actively taking up amino acids or other substrates (Mary et al., 2006b; Tripp et al., 2008). From cytometric data both HNA and LNA fractions were clearly separated (Fig. 3, p. 16). To derive the average genome size for HNA cells we used the established genome size of the SAR11 strain *Candidatus* Pelagibacter ubique (1.3 Mbp) as proxy for the LNA cells (Table 1).

Table 1: Cytometric fluorescence data of LNA and HNA and their derived average genome size by using *Candidatus* Pelagibacter ubique as proxy (1.3 Mbp).

	Fluorescence		**average genome size (Mbp)**	
Station	**LNA**	**HNA**	**LNA**	**HNA**
3	82.1 ± 21.5	293.1 ± 120.3	~1.3	4.4 ± 0.7
6	59.4 ± 15.6	204.9 ± 84.6	~1.3	4.3 ± 0.7
16	63.0 ± 18.9	214.3 ± 70.9	~1.3	4.4 ± 0.1
17	59.3 ± 19.8	211.4 ± 70.3	~1.3	4.6 ± 0.0
18	61.3 ± 17.4	200.0 ± 76.5	~1.3	4.1 ± 0.5
19	55.3 ± 15.7	208.7 ± 72.4	~1.3	4.8 ± 0.3

Converting the cytometric fluorescence data, we obtained an estimated genome size of HNA cells between 3.7 – 5.2 Mbp. This fits rather well with other studies that could show that the average distance between the HNA and LNA fractions, in terms of average DNA content, is >4x and therefore exceeds what could be expected on the basis of DNA replication within natural aquatic bacteria (Marie et al., 1997; Button and Robertson, 2001).

Scenario 3 implies that intrinsic differences in composition between HNA and LNA cells explain the bimodal distribution of the bacterial community. Phylogenetic studies using molecular fingerprinting analysis or FISH are still inconsistent. They either concluded that the composition of LNA is considerably similar to the HNA (e.g. Bernard et al., 2000a; Servais et al., 2003; Longnecker et al., 2005), or that each fraction has unique or dominant phylogenetic groups

or clades (e.g. SAR11 in LNA and *Roseobacter*, *Gammaproteobacteria*, *Bacteroidetes* in HNA) (Eilers et al., 2000; Fuchs et al., 2000; Zubkov et al., 2001a; Zubkov et al., 2002a; Fuchs et al., 2005; Mary et al., 2006b). Our results appear to support the second conclusion of distinct bacterial clades within each cytometric fraction. However, the unassigned percentages (11% - 22%) are large enough that we can not exclude to have missed the detection of bacterial groups that are present in both HNA and LNA.

The last scenario (scenario 4) was the one Bouvier *et al.* concluded to be most likely. Cells are transferred from one fraction to the other, and certain bacterial groups are characteristic of either HNA or LNA fractions (Zubkov et al., 2001a; Jochem et al., 2004). Our results support this scenario. SAR11 and *Bacteroidetes* are indeed intrinsic to the LNA or HNA bacterioplankton, respectively (Fig. 15, p. 51, Publ. II). Other groups like the SAR86 clade occur in both fractions (Zubkov et al., 2001a; Zubkov et al., 2002a) (Publ. II), indicating that metabolism and growth may influence the link between the HNA and LNA fractions. In general, our results do not support the hypothesis of two distinct and independent communities, but rather suggest a scenario in which there are intrinsic components to each fraction, as well as passage of cells from one to the other fraction.

2.2 Genome size and copy numbers

Although the existence of HNA and LNA fractions is widely accepted, there is still no consensus as to their ecological significance. The bimodal distribution of DNA content does not appear to be restricted to aquatic bacterioplankton, and has also been reported for soil bacterial communities (Christensen et al., 1993; Christensen et al., 1995). This distribution may reflect general adaptive processes among prokaryotes. In addition, comparing the genome sizes of 641 different prokaryotes Islas *et al.* (Islas et al., 2004) reported a bimodal distribution for free-living prokaryotes with two distinct peaks, one at approximately 2 Mbp and a second one at 4 to 5 Mbp. The evolutionary significance of this bimodal distribution of prokaryotic genomes is not understood, but may correspond to the observed discrimination of planktonic

cells into HNA and LNA fractions, which in turn may mirror a particular differentiation of ecological niches and functions (Bouvier et al., 2007b). Thus, we speculate that the HNA bacterioplankton should represent the genomes with 4 to 5 Mbp, the LNA bacterioplankton comprised of cells with ~ 2 Mbp genomes.

However, despite a genome size range of 1.6 – 2.4 Mbp (Rocap et al., 2003) *Prochlorococcus* spp. is exclusively found in the HNA fraction (Zubkov et al., 2000a, Publ. II), and would theoretically fall into the 2 Mbp-peak. An increase of the cytometric fluorescence due to the autofluorescence of *Prochlorococcus* cells is unlikely. The red autofluorescence of *Prochlorococcus* is clearly separable from the green emission of the SYBR Green DNA staining used in this study. Furthermore the cellular content of Chl *a* is sharply reduced in *Prochlorococcus* cells that inhabit the surface waters (Partensky et al., 1996; Campbell et al., 1997) and staining DNA with SYBR Green I is a common method to enumerate the concentration of *Prochlorococcus* in these waters. As a common observation SYBR Green I stained *Prochlorococcus* cells have generally a slightly higher green fluorescence (more DNA) than heterotrophic bacteria (Zubkov et al., 2000a; Zubkov et al., 2000b).

Another explanation may be in the method of flow cytometry itself. It uses the DNA content of the individual cells to discriminate between HNA and LNA bacterioplankton. A high cellular DNA content can be based on different cellular settings: either having a single large genome or having several genome equivalents or both. There is evidence that some marine prokaryotes indeed contain a range of genome copies (Binder and Chisholm, 1995; Button et al., 1998). The cell cycle of *Prochlorococcus* populations is highly synchronized by the light-dark cycle (Vaulot et al., 1995) and is separated into individual stages as observed in lab experiments (Mary et al., 2008b). Based on Vaulot *et al.* (Vaulot and Partensky, 1992) the stages consist of G_1 and G_2 cells, containing one and two genome copies, respectively, separated by a trough of DNA-synthesizing cells (S cells). From dawn to midday the majority of *Prochlorococcus* cells was in the G_1 stage containing only a single chromosome copy according to SYBR Green I DNA staining (Marie et al., 1997). At dusk most of the cells entered the S stage with the synthesis of the second chromosome copy, followed by the G_2 stage (about 10PM local time)

when two chromosome copies could be detected in cells, before cells divided during the night, returning to the G_1 stage and completing the cell growth cycle (Vaulot et al., 1995; Zubkov et al., 2000a; Mary et al., 2008b). The samples analyzed in this study (Publ. II) were taken either in the morning (8AM local time) or early evening (4 – 7PM local time), thus the majority of the *Prochlorococcus* cells was in the G_1 containing either a single genome or in the early phase of the S stage (Zubkov et al., 2000a). This may explain why our data appear to be inconsistent with the genome distribution by Islas *et al.* (Islas et al., 2004). In addition, the data by Islas *et al.* did not reflect the actual levels of prokaryotic diversity in an accurate way due to overrepresentation of pathogens and parasites (Islas et al., 2004).

The differentiation of bacterioplankton cells based on their nucleic acid content into two distinct populations (HNA and LNA) implies a certain classification of genome size (Button and Robertson, 2001). Phylogenetic community studies of the HNA and LNA fractions provide information about the abundance of major phyla and subgroups (Fuchs et al., 2000; Zubkov et al., 2001a; Zubkov et al., 2004b; Longnecker et al., 2005). By combining phylogenetic information with available genome sequence data the HNA population appears to comprise mainly a genome size range of 3 – 5 Mbp (Table 2). However, the cyanobacteria *Synechococcus* spp. and *Prochlorococcus* spp. have to be included in the HNA bacterioplankton. Consequently, a conservative estimation places the upper threshold for the LNA population at a genome size of approximately 1.6 Mbp.

Table 2: Size range of whole genome sequences of major bacterioplankton groups found in the HNA fraction (data from http://www.megx.net)

Phylogenetic group	Genome size range(Mbp)	Representative	Genome size(Mbp)
Bacteroidetes	--	*Gramella forsetii*	3.8
Alphaproteobacteria	3.4 – 4.6	*Silicibacter pomeroyi* DSS-3	4.6
		Roseobacter denitrificans OCh 114	4.3
Gammaproteobacteria	3.1 – 5.9	*Alteromonas macleodii* Deep ecotype, DSM 17117	4.4
		Shewanella baltica OS185	5.3
		Congregibacter litoralis	4.4
Synechococcus	2.2 – 2.7	*Synechococcus elongatus* PCC 7942	2.7
		Synechococcus sp. CC9902	2.2
Prochlorococcus	1.6 – 2.7	*Prochlorococcus marinus* MIT 9301	1.6
		Prochlorococcus marinus NATL1A	1.9
		Prochlorococcus marinus MIT 9303	2.7

In this study the majority of LNA cells consisted of SAR11 including members of the OM43 clade. With a genome size of 1.31 Mbp (1,308,759 base pairs) a member of the SAR11 clade, *Candidatus* Pelagibacter ubique, has one of the smallest genomes for a free-living heterotrophic prokaryote (Rappé et al., 2002; Giovannoni et al., 2005b). In addition, Giovannoni and colleagues reported that the first axenic isolate of the OM43 clade, strain HTCC2181 had an even smaller genome with 1.3 Mbp (1,304,428 base pairs) (Giovannoni et al., 2008). They suggested these microorganisms as evidence for genome streamlining. One explanation for the relatively small genomes of some marine bacterioplankton is supposedly the elimination of redundant DNA from genomes due to selection. Another explanation possibly is the metabolic cost of replicating DNA for maintenance. Selection is particularly effective in large populations and habitats with N and P limitation. Genome streamlining therefore may lead to specialization of organisms like the OM43 clade which grows solely on oxidized C1 compounds (Giovannoni et al., 2008). Transporters with broad substrate ranges (Button et al., 2004) and high affinity

to specific substrate targets (Giovannoni et al., 2005b) make *Candidatus* Pelagibacter ubique another example for specialization.

In the HNA population many marine bacterioplankton organisms containing “standard genome sizes” (Moran et al., 2004; Fuchs et al., 2007) harbor nonetheless a wide range of physiological diversity. Kunin *et al.* (Kunin et al., 2008) proposed that an increase in bacterial genome size reflects an amplified functional complexity essential for life under constantly changing conditions. Moreover, by estimating the average effective genome size a rough measure of functional potential per organism can be made (Raes et al., 2007). Marine *Bacteroidetes* for instance degrade complex organic matter such as polysaccharides, proteins (Bauer et al., 2006; González et al., 2008) and other high-molecular-weight organic matter (Cottrell and Kirchman, 2000a). This versatile life style requires a substantial number of genes for attachment to surfaces or particles. Also genes for gliding motility and an extensive collection of enzyme systems for polymer degradation lead to large genomes in *Bacteroidetes*.

3 Outlook

Identification and quantification of microbial groups in the bacterioplankton

Assuming that only around 20 phylogenetic clades play numerically an important role in the water column (Giovannoni and Stingl, 2005) we were able to identify with the probe set used in the present study six microbial groups that can reach abundance >5% in the marine pelagic zone. However, as our hybridization data suggests there is still room for additional populations like marine *Actinobacteria*, *Roseobacter*, *Lentispherae* or OM43. These populations were not targeted during the first project of the present study and previous studies suggest that they most likely do not account for the whole missing percentages. Based on the high diversity found by Venter *et al.* (Venter et al., 2004) during the Sargasso Sea survey we hypothesize that most of the missing fraction are likely populations only present in the per mill range. New strategies

are required for the quantification of such rare populations by FISH in the future. The development of such new protocols would not only facilitate the correct enumeration of small populations, but also complete our knowledge of the quantitative composition of marine bacterioplankton.

The role of cytometric fractions

The differentiation into the two distinct cytometric groups HNA and LNA appears to be a general feature of bacterioplankton (Li et al., 1995; Gasol et al., 1999; Lebaron et al., 2001; Sherr et al., 2006). However, the interactions between these fractions are still controversially discussed. In the present study we suggested that both fractions contain phylogenetic groups intrinsic for each fraction but also groups which can move between HNA and LNA. It would be interesting to analyze the influence of different habitats like e.g. open ocean and shelf seas on such interactions between HNA and LNA and their respective community composition.

In addition, we could show that genome size appears to play a role for bacterioplankton cells to fall either into the HNA or LNA fraction. However, our threshold of approximately 1.6 Mbp is an estimation based on the detection of *Prochlorococcus* spp. exclusively in the HNA fraction and known genomes of this genus. An investigation with focus on the role of *Prochlorococcus* could provide more information about the meaning of genome size for the cytometric differentiation of HNA and LNA. The availability of more fully sequenced genomes, especially from the marine environment, could help to further investigate the connection between the differentiation of HNA and LNA by flow cytometry and the already observed bimodal distribution of genome sizes in free-living prokaryotes (Islas et al., 2004).

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Part II: Publications

A List of publications

I. **Martha Schattenhofer, Bernhard M. Fuchs, Rudolf Amann, Mikhail V. Zubkov, Glen Tarran, Jakob Pernthaler.** 2008. Latitudinal Distribution of Bacterioplankton Populations in the Atlantic Ocean. *Accepted.*

Sampling for CARD-FISH, sample analysis and data evaluation; writing of a first draft of the manuscript and of the first subsequent update.

II. **Martha Schattenhofer, Rudolf Amann, Mikhail V. Zubkov, Bernhard M. Fuchs.** 2008. Phylogenetic Affiliation of Bacterioplankton with High and Low Nucleic Acid Content in the North Atlantic Ocean. *Manuscript in preparation.*

Sample analysis and data evaluation; writing of a first draft of the manuscript.

Not presented in this thesis:

Bernhard M. Fuchs, Stefan Spring, Hanno Teeling, Christian Quast, Jörg Wulf, Martha Schattenhofer, Shi Yan, Steve Ferriera, Justin Johnson, Frank Oliver Glöckner, and Rudolf Amann. 2007. Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 2891-2896

Member of the annotation team.

B Publications

Publication I

Latitudinal Distribution of Bacterioplankton Populations in the Atlantic Ocean

Martha Schattenhofer, Bernhard M. Fuchs, Rudolf Amann, Mikhail V. Zubkov, Glen Tarran, Jakob Pernthaler.

Accepted.

Latitudinal Distribution of Bacterioplankton Populations in the Atlantic Ocean

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Abstract

Members of the prokaryotic picoplankton are the main drivers of the biogeochemical cycles over large areas of the world's oceans. In order to ascertain changes in picoplankton composition in the euphotic and twilight zones at an ocean basin scale we determined the distribution of 11 marine bacterial and archaeal phyla in three different water layers along a transect across the Atlantic Ocean from South Africa (32.9°S) to the United Kingdom (46.4°N) during boreal spring. Depth profiles down to 500 m at 65 stations were analyzed by catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) and automated epifluorescence microscopy. There was no obvious overall difference in microbial community composition between the surface water layer and the deep chlorophyll maximum (DCM) layer. There were, however, significant differences between the two photic water layers and the mesopelagic zone. SAR11 (35 ± 9%) and *Prochlorococcus* (12 ± 8%) together dominated the surface waters, whereas SAR11 and *Crenarchaeota* of the marine group I formed equal proportions of the picoplankton community below the DCM (both ~15%). However, due to their small cell sizes *Crenarchaeota* contributed distinctly less to total microbial biomass than SAR11 in this mesopelagic water layer. Bacteria from the uncultured *Chloroflexi*-related clade SAR202 occurred preferentially below the DCM (4-6%). Distinct latitudinal distribution patterns were found both in the photic zone and in the mesopelagic waters: In the photic zone, SAR11 was more abundant in the Northern Atlantic Ocean (up to 45%) than in the Southern Atlantic gyre (~25%), the biomass of *Prochlorococcus* peaked in the tropical Atlantic Ocean, and *Bacteroidetes* and *Gammaproteobacteria* bloomed in the nutrient rich northern temperate waters and in the Benguela upwelling. In mesopelagic waters, higher proportions of SAR202 were present in both central gyre regions, whereas *Crenarchaeota* were clearly more abundant in the upwelling regions and in higher latitudes. Other phylogenetic groups such as the *Planctomycetes*, marine group II *Euryarchaeota* and the uncultured clades SAR406, SAR324, and SAR86 rarely exceeded more than 5% of relative abundance.

Introduction

Recent progress in methodology and conceptual understanding points to a vast total diversity of Bacteria and Archaea in marine waters (Pedros-Alio, 2006; Sogin et al., 2006). On the other hand, there are indications that only approximately 10 phylogenetic groups are able to form large populations of >5% relative abundance (Giovannoni and Stingl, 2005). It was the primary goal of this study to quantify these groups along a south – north transect across the Atlantic Ocean.

Two lineages appear to be of particular importance: The cyanobacterial *Prochlorococcus* and the alphaproteobacterial SAR11 clade. Members of *Prochlorococcus* are the most prominent primary producers in large parts of the subtropical and tropical ocean basins (Dufresne et al., 2003). Two major ecotypes have been distinguished that exhibit contrasting physiological and genomic adaptation to light and hence also feature distinct depth preferences (West et al., 2001; Johnson et al., 2006). Moreover, distinct genotypes of *Prochlorococcus* have been found to inhabit different oceanic regions (Johnson et al., 2006). The alphaproteobacterial SAR11 clade - first described from the Sargasso Sea (Giovannoni et al., 1990) - likely represents the most abundant heterotrophic bacterial group in pelagic marine environments (Morris et al., 2002). SAR11 bacteria may comprise between 24 and 55% of prokaryotic cells at the Bermuda Atlantic Time Series (BATS) station (Morris et al., 2002), and similar proportions were found in the Atlantic Ocean (Malmstrom et al., 2004; Mary et al., 2006), the Mediterranean Sea (Alonso-Saez et al., 2007) and the coastal North Sea (Alonso and Pernthaler, 2006).

The occurrence of other groups of planktonic prokaryotes is less well documented. *Rhodobacteriaceae* (*Alphaproteobacteria*) are often observed in costal areas or shelf regions in the aftermath of phytoplankton blooms (West et al., 2008). *Gammaproteobacteria* have also been mostly detected in coastal picoplankton (Rappé et al., 1997) where they may even form pronounced blooms (Alonso-Saez et al., 2007). The gammaproteobacterial SAR86 clade represented approximately 5% of total cell numbers (or of bacterial 16S rRNA genes) in some

coastal waters (Pernthaler et al., 2002a), but was rare in others (Alonso-Saez et al., 2007). The *Bacteroidetes* phylum is a highly diversified group in the marine pelagic environment (Alonso et al., 2007) that may be present in high abundance both in coastal and offshore waters (Kirchman et al., 2003; DeLong et al., 2006). A number of other, mainly uncultured microbial lineages have also been found in the marine picoplankton, e.g. SAR406 (Gordon and Giovannoni, 1996), SAR324 (Fuhrman and Davis, 1997), *Planctomycetes* (DeLong et al., 1993), marine Group II *Euryarchaeota* (Pernthaler et al., 2002b) and the *Chloroflexi*-related SAR202 lineage (Morris et al., 2004).

The composition of marine picoplankton assemblages may dramatically shift with depth. Members of the marine group I *Crenarchaeota* typically comprise 50% and more of the total microbial community in the deep water layers below the photic zone (e.g (Karner et al., 2001; Teira et al., 2004). Other microbial groups also appear to show a marked preference for a particular water layer. SAR324 and SAR406 have been mostly found in the lower photic zone below the deep chlorophyll maximum (DCM) (Gordon and Giovannoni, 1996; Wright et al., 1997), whereas members of the SAR202 clade were predominantly present in the meso- and bathypelagic realms (Varela et al., 2008a). Our current knowledge about the depth distribution of picoplankton populations is mainly derived from time series at a few oceanic observatories, e.g., BATS (Morris et al., 2005), station ALOHA (Damste et al., 2002) or the SPOTS site (Fuhrman et al., 2006). Many studies have been in coastal waters (Glöckner et al., 1999; Eilers et al., 2001) or report only cell numbers of total picoplankton and cyanobacteria (Li, 1998). Few studies examined the composition of prokaryotic picoplankton at a basin scale especially in the open ocean. In order to follow the population distribution of non-pigmented heterotrophic picoplankton populations, molecular biological tools such as RT-PCR or FISH are required. A modified FISH staining protocol specifically developed for marine picoplankton (Pernthaler et al., 2002a) allows for the reliable identification and quantification of microbial cells at various levels of phylogenetic resolution in oligotrophic marine waters. This has, for

example, been demonstrated for group I *Crenarchaeota* and SAR202 in the bathypelagic North Atlantic Ocean (Teira et al., 2006b; Varela et al., 2008a).
We studied the horizontal and vertical distributions of 11 microbial populations in the top 500 m along a transatlantic transect leading from Cape Town, South Africa, to Falmouth, UK. Samples were taken within the surface water layer, defined by 55% surface irradiance (Agustí and Duarte, 1999), the deep chlorophyll maximum (DCM) layer corresponding to 33, 1 and 0.1% of surface irradiance, and additionally 2 - 3 depths below the DCM down to 500 m representing the mesopelagic (upper aphotic) water layers. Specifically we wanted to assess (i) if the composition of the microbial communities in surface waters is substantially different from that in the DCM and mesopelagic water layers, (ii) if microbial groups differ in their horizontal distribution in the North and South Atlantic Ocean, and (iii) if the microbial groups differ in their cell sizes and their contribution to total picoplankton biomass. These questions were addressed by >3600 FISH analyses in combination with high-throughput automated epifluorescence microscopy (Pernthaler et al., 2003).

Results

Hydrography of oceanic provinces. According to the latitudinal distribution of the thermohaline properties and in agreement with the classification suggested by Longhurst et al. (Longhurst, 1998) five major oceanographic provinces could be distinguished along the transect (Fig. 1, 2a): the Benguela Current Coastal Province (BENG), the South Atlantic Gyral province (SATL), the Western Tropical Atlantic (WTRA), the North Atlantic Gyral Province (NAG) and the Northern Atlantic Drift (NADR) (Fig. 1, 2a). From the BENG northward the halocline (163 m, Fig. 2a) deepened while approaching the SATL. From station 4 to station 5 salinity increased abruptly to >36, reflecting a frontal system associated with the transition from the BENG to the SATL. A similar increase in salinity also occurred at stations 35 – 37, marking the transition from the WTRA to the NAG at ~ 16°N. Salinity declined from the SATL towards the WTRA to <35.7 between stations 25 and 26, and also from the NAG

towards the NADR which started from station 62. These provinces were also separated by the depth of the deep chlorophyll maximum (DCM) (Fig. 2b). The DCM layer deepened from the BENG (107 m) towards the SATL (156 m), before it went up again in the WTRA to 112 m. From the WTRA northward the DCM layer deepened again to 153 m in the NAG, before it reached 122 m in the NADR. Highest chlorophyll *a* levels of up to 0.5 mg m^{-3} were found in the DCM of the temperate and upwelling provinces BENG, WTRA and NADR (Fig. 2b, from (Robinson et al., 2006).

Microbial cell numbers. Absolute cell numbers of prokaryotic picoplankton were generally between 0.5 – 1.5 x 10^6 mL^{-1} in the euphotic zone which refers to the surface and DCM layers (Fig. 2c). Highest cell numbers were measured in the NADR surface water with up to 1.9 x 10^6 mL^{-1}. In both gyres the average cell numbers in surface waters were 0.7 – 0.9 x 10^6 mL^{-1} lower than in the adjacent provinces (1.1 – 1.5 x 10^6 mL^{-1}), marking the oligotrophic status of these oceanic provinces. Cell numbers decreased strongly with depth to around 7.8 x 10^4 mL^{-1} at 500 m (Fig. 2c).

Bacteria and Archaea. Bacteria, as detected by the probe mix of Eub338, Eub338-II and Eub338-III (Table 1), dominated the microbial community throughout the water column (62 ± 14% of DAPI) (Suppl. Table 1). The relative abundance of Bacteria was higher in surface waters, where they comprised 77 ± 6% of the prokaryotic picoplankton. In the mesopelagic waters below the DCM layer Bacteria made up only 46 ± 8% of DAPI counts. Relative abundance of bacteria in surface waters increased along the transect from 70 ± 8% in the BENG to 87 ± 8% in the NADR, whereas the absolute numbers in the surface waters of these two provinces remained stable at 1.0 x 10^6 ml^{-1} (Suppl. Table 1). In contrast to the Bacteria the abundance of the *Crenarchaeota* identified with probe Cren554, targeting the marine group I Archaea, increased with depth (Fig. 3d). In the DCM layer the abundance of *Crenarchaeota* were 2 - 3% in all provinces (except for the BENG with 7%) and exceeded 10% only in the mesopelagic waters (Fig 3d). In the BENG, the WTRA and the NADR province the relative abundance increased up to 17 - 25%, whereas in the gyre regions

the relative abundance of marine group I was around 15%. Marine group II *Euryarchaeota* made up <0.5% of the total picoplankton community throughout the AMT 16 transect. Exceptions were the photic water layer of the BENG (0 – 6.2%, n=16) where a single maximum of 6.2% at station 1 (30 m) was observed, and the DCM layer in the provinces northern NAG (>30°N) and NADR (0.6 ± 0.6%, n=49; Suppl. Fig 1a).

SAR11. The most abundant bacterial group in the Atlantic Ocean is the alphaproteobacterial group SAR11. Members of this clade accounted, on average for 26 ± 12% of picoplankton (Fig. 3a) throughout all stations and depths. They were clearly more abundant in surface waters (35 ± 9%) and the DCM layer (32 ± 9%) than in mesopelagic waters (15 ± 5%). Maxima exceeded 50% at 6 stations (Suppl. Table 1) with the highest relative abundance of SAR11 along the transect in the upper 100 – 150 m of the BENG (44 ± 15%) and from the NAG northward (40 ± 5%). SAR11 counts in surface waters and the DCM were 25 ± 10% in the SATL, significantly lower compared to the same water layers of the NAG (38 ± 5%, $p<0.001$).

***Prochlorococcus*.** Members of the cyanobacterial genus *Prochlorococcus* as detected by probe Pro405 dominated the picophytoplankton in surface waters in most of the provinces. Our results were highly correlated with parallel flow cytometry counts ($r^2=0.90$, $p<0.0001$, slope 1.1) and the results were in line with previous on-board flow cytometric analyses during former AMT cruises (e.g. Zubkov et al., 2000). The highest abundance measured on the transect was in the WTRA with peak relative abundance of 28.6% at station 35 (10.0°N, Fig. 3b). On average the relative abundances of *Prochlorococcus* exceeded 15% in the photic layer of all provinces, except for the NADR where abundances dropped sharply to <5%. *Prochlorococcus* rapidly decreased below the DCM and was virtually absent below 200 m water depth.

***Gammaproteobacteria*.** The abundance of *Gammaproteobacteria* ranged from 2 - 4% in all water layers in the southern part of the transect, and remained in this range in all deeper water layers throughout the transect (Suppl. Table 1). However, it increased from the NAG province northward to a maximum of 9 ± 3%

in the surface layer of the NADR. In this province we found a single conspicuous maximum of 49% in the surface layer at station 61 (7 m; -25.07°E, 42.11°N). An attempt was made to resolve this high abundance of *Gammaproteobacteria* into subgroups by CARD-FISH with more specific probes. Members of the genera *Alteromonas/Colwellia* and *Pseudoalteromonas* accounted for 2 - 5% each (n=6), *Vibrio* for about 1% (n=4) and *Oceanospirillum* for up to 4% (n=6) (Suppl. Table 1). For the uncultured clade SAR86 a maximum of 0.5% could be detected at station 8 in the DCM layer of the SATL, but most of the time the abundance was below 0.5% (n=38) or SAR86 was not detected (n=48) (Suppl. Table 1).

***Bacteroidetes*.** Probe CF319a was used to identify most marine members of the class *Flavobacteria* within the phylum *Bacteroidetes*. On average counts with this probe were in the range between 2 - 4% (Suppl. Table 1). Throughout the transect the relative abundances were distinctly higher in the photic water layers (4.5 ± 3.9 %) compared to the mesopelagic waters (0 - 9.9%). Higher abundance in *Bacteroidetes* was found in surface waters (9 ± 3.5 %) and in the mesopelagic (5.3% ± 3.6%) of the BENG, but highest counts were found in the surface waters of the temperate province NADR (13.5% ± 10%).

SAR202. The relative abundance of SAR202 increased with depth at every station on AMT16 (Fig. 3c). The highest relative abundance of SAR202 cells was found in the deeper waters of the gyres SATL (5.7 ± 3%) and NAG (4.4 ± 1.7%). In the same water layers of the BENG and NADR provinces the SAR202 group constituted only 2.5 ± 2 % and was even lower in the WTRA (1.5 ± 0.5%). The absolute cell numbers and biomass values (Suppl. Fig. 2) did not support a preference of the SAR202 clade for mesopelagic waters: While the counts increased with depth in the NAG and NADR, they remained fairly stable in SATL and WTRA.

Groups of low abundance: SAR406, SAR324, *Planctomycetales*. The relative abundance of members of the uncultured *Firmicutes* clade SAR406 was low (0 - 3.4%) throughout the entire transect (Suppl. Fig. 1c). The proportions of these bacteria were lower in the surface (0 - 1.1%) than in the DCM (0 - 2.9%) and in mesopelagic waters (0 - 3.4%).

Probe SAR324-1412 was used to measure the relative abundance of the deltaproteobacterial clade SAR324. Their relative abundance was found to be low with 0 - 2.2% throughout the whole transect (Suppl. Fig. 1b). A relative maximum of 0.9 ± 0.4% was detected in the DCM layer of the WTRA, with a local maximum of 2.2% at station 29 (25.56°W, 1.17°N) in 47 m depth, and SAR324 counts were also elevated in the adjacent depths and stations (to around 1%).

A subset of 62 samples distributed over the whole cruise track was analyzed with probe Pla46 specific for *Planctomycetales*. Their relative abundance was highest at depths >150 m (0 - 1.2%), with a relative maximum of 1.2% in 300 m depth at station 22 (25.00°W, 15.40°S) in the SATL (Suppl. Table 1). In surface waters counts with probe Pla46 were close to the detection limit (0 - 0.3%).

Additional information for average relative abundance and locations of minimum and maximum of the individual populations analyzed in this study are provided in the supplement (Suppl. Table 2).

Cell volumes and biomass. Cell volumes were only determined for the four most abundant and ubiquitous phylogenetic groups SAR11, *Prochlorococcus*, marine group I *Crenarchaeota* and SAR202, because these groups occurred along the entire transect (Fig. 4, Suppl. Table 3). The cellular volumes for SAR11 cells ranged broadly between 0.030 to 0.120 μm^3 (n=367). The average on the transect was 0.067 ± 0.017 μm^3. Generally, SAR11 cell volumes gradually increased with depth (Fig. 4a). The largest cells were found in the DCM layer and deeper waters of the BENG (0.073 – 0.082 μm^3) and in all samples from the SATL (0.075 μm^3). SAR11 cells were smaller in the surface and DCM layers of the remaining provinces (0.048 – 0.056 μm^3). SAR11 biomass was highest in the photic zone of the nutrient-rich provinces WTRA (5.2 ± 1.5 mg C m^{-3}) and NADR (6.0 ± 1.6 mg C m^{-3}) (Suppl. Fig. 2a) and was in the range of 1.8 - 4.3 in the other provinces. SAR11 biomasses were low in all mesopelagic waters (0.40 – 0.83 mg C m^{-3}).

Cell volumes of *Prochlorococcus* cells varied between 0.033 and 0.086 μm^3 (n=187) along the transect (Fig. 4b). Average values for the surface and

DCM layers were almost identical for the surface (0.055 ± 0.012 μm^3, n=46) and for the DCM (0.053 ± 0.012 μm^3, n=138). Largest volumes were found in the surface layer of the southern NAG (0.064 ± 0.012 μm^3, n=6) and the DCM of the NADR (0.069 ± 0.009 μm^3, n=6). Cell volumes in the WTRA and the northern NAG never exceeded 0.052 μm^3 in the analyzed depths. The *Prochlorococcus* biomass was almost double in the WTRA surface waters (3.0 ± 0.6 mg C m^{-3}) compared to the other provinces and water layers (0.5 – 1.6 mg C m^{-3}) (Suppl. Fig. 2b).

SAR202 cell volumes were similar to the values determined for *Prochlorococcus* (0.050 ± 0.010 μm^3 (n=358) (Fig. 4d) and varied only in a narrow range between 0.046 – 0.051μm^3) along the transect. Only in the NADR surface waters were the SAR202 cell volumes higher, at 0.064 μm^3, but this value was only based on two sampling points. The biomass of SAR202 was highest in the mesopelagic waters (0.10 – 0.12 mg C m^{-3}), with the exception of the WTRA (0.04 mg C m^{-3}), and the photic zone of the BENG (0.18 – 0.19 mg C m^{-3}) (Suppl. Fig. 2d).

The smallest cells measured belonged to the marine group I *Crenarchaeota* (0.040 ± 0.010 μm^3, n=203) (Fig. 4c). *Crenarchaeota* were relatively big in the DCM layers of BENG (0.049 ± 0.012 μm^3, n=8) and NADR (0.058 μm^3, n=1). In all other provinces cell volumes were slightly smaller in mesopelagic waters (0.033 – 0.044 μm^3) compared to the DCM waters (0.037 – 0.058 μm^3) and were as low as 0.033 μm^3 in the WTRA. The crenarchaeotal biomass peaked in the mesopelagic water layer and ranged between 0.21 – 0.56 mg C m^{-3}. Lowest values were determined for the SATL province with 0.18 mg C m^{-3} and 0.21 mg C m^{-3} for the DCM and mesopelagic water, respectively (Suppl. Fig. 2c).

Discussion

In this study we present counts of 11 prokayotic picoplankton clades from 400 samples taken on a transect with 65 stations resulting in more than 3,600

data points. On average, more than two-third of the picoplankton cells hybridized with one or more of our oligonucleotide probes. Of the 11 picoplankton populations monitored, only six had an average relative abundance >1%, whereas five were <1% throughout the AMT16 cruise. In the following we refer to the former as the “major” and the latter as the “minor” populations.

We can not decide whether cells detected by DAPI but not hybridizing with any of the domain- and group-specific probes (white areas in Fig. 5) are (i) large viruses, (ii) Bacteria or Archaea with very low ribosome content (dead/dormant cells) or (iii) impermeable cells of Bacteria/Archaea or (iv) Bacteria/Archaea with deviations in the respective probe target sites. The grey areas in Fig. 5 in contrast refer to cells that are detected by the bacterial probe mix, but not with the group-specific probes used to classify the Bacteria. Hence, these bacterial cells are permeable for probes, have sufficient ribosomes for probe-based classification and might be from bacterial populations for which we had no probes, including members of the “rare biosphere” (Sogin et al., 2006). Our discussion will focus on the main findings, because a detailed consideration of all data is beyond the scope of a single manuscript.

Similar community composition of surface and DCM water layers. Changes in heterotrophic picoplankton composition between the surface layer and the DCM were small whereas there were significant changes for photoautotrophic cyanobacteria of the genus *Prochlorococcus* (Suppl. Table 1). It was previously assumed that most of the primary production in the oligotrophic ocean gyres occurs near or in the DCM (Herbland and Voituriez, 1979). Hence, the heterotrophic picoplankton should be influenced by freshly produced organic material. Recently, it has been shown that primary production is rather evenly distributed over the entire photic zone (Poulton et al., 2006). Therefore, it is not surprising to see that heterotrophic populations show similar abundance in the surface waters and the DCM along the transect. However, we cannot exclude that within the clades monitored by FISH probes, significant differences may occur between these two water layers. One example along these lines is the well known depth distribution of high-light adapted and low-light adapted ecotypes of

cyanobacteria which was not examined in this study (Johnson et al., 2006; Zwirglmaier et al., 2007). Most probes used in this study are fairly “broad” and do therefore not yield information on distinct functions of the detected populations. Studying phylum level distributions is however a necessary first step before functionally coherent populations can be detected with much more “narrower” probes.

Depth distribution. Confirming current knowledge, the prokayotic picoplankton communities of the photic zone were fundamentally different from those in the mesopelagic water layers along the entire transect (Giovannoni and Stingl, 2005). While the former was clearly dominated by Sar11 followed by *Prochlorococcus* as the second most abundant organism, below the DCM the marine group I *Crenarchaeota* co-dominated with SAR11 the prokayotic picoplankton community (Figs. 3a, 3d, 5, Suppl. Table 1). These data are well within previously reported abundances of *Crenarchaeota* (Karner et al., 2001) and agree with observations by Morris and co-workers in the Northwestern Atlantic Ocean where SAR11 was also present in high numbers in the mesopelagic (Morris et al., 2002). In terms of biomass SAR11 was the largest single clade in the mesopelagic and may therefore play a more important ecological role in these waters than previously thought. Confirming the reports by Varela et al. (Varela et al., 2008a) a relatively high abundance of the uncultured clade SAR202 was found in the mesopelagic of this study (Figs. 3c, 5). Previously, it had been noted that the highest abundance of the SAR202 group coincides with oxygen concentrations >190 µM (Giovannoni et al., 1996), but in our study we could not detect any significant correlation with oxygen concentrations or any other physico-chemical parameter measured with the exception of depth (r^2=0.74, p<0.001, Suppl. Table 5).

Interestingly, although the *Bacteroidetes* occurred in all water layers examined, they were observed mostly in the photic zone. Their abundance were highly correlated with nanoeukaryotic phytoplankton (r^2=0.73, p<0.001, Tarran G., unpublished data, Suppl. Table 5) along the transect. This fits well to previous findings. Recent genome analyses of a range of marine *Bacteroidetes* isolates

showed that they possess proteorhodopsins and grow better with light (Gomez-Consarnau et al., 2007). They also indicate a broad potential for degradation of polysaccharides and proteins (Bauer et al., 2006; González et al., 2008). *Bacteroidetes* are well known as consumers of algae-derived metabolites (Grossart et al., 2005). It is likely that different populations of this quite diverse group of picoplankton might have different ecological strategies that are realized in the photic zone (Alonso et al., 2007).

Horizontal distribution of major prokayotic picoplankton populations. One of the main goals of this study was the comparison of five oceanic provinces and we did find distinct regional differences in clade abundance along the transect. In general, SAR11 comprised a higher abundance in the photic zone of the two northern Atlantic provinces as compare to the SATL (Fig. 5). Evidence that the lower abundance in the Southern gyre seems to be a rather stable feature comes from a cruise conducted in October 2003 during austral spring (AMT13). There, only ~22% of all picoplankton cells were SAR11 (Suppl. Fig. 3) (Mary et al., 2006). Two reasons might be responsible for a lower abundance of SAR11 in the SATL: seasonality and low nutrient content. At the BATS site in the northwestern Sargasso Sea SAR11 abundance fluctuated over a period of 3.5 years in a similar range with a trend to higher abundance in the summer (Morris et al., 2002). Correlation analyses of SAR11 abundance with yet unpublished results on nanoeukaryotic and piceukaryotic algae abundances indicate significant co-occurrence along the AMT16 transect (r^2=0.8 and r^2=0.72, respectively, $p<0.001$, Tarran G., unpublished results, Suppl. Table 5). Additionally recent experiments with cultures of the SAR11 clade demonstrated a strong dependence of SAR11 on reduced sulfur compounds like DMSP which is the prime algal osmolyte (Tripp et al., 2008). The Southern Atlantic gyre is quite isolated from the surrounding nutrient rich ocean regions and concomitantly nutrient concentrations are lower compared to e.g. the Northern Atlantic Gyre (Mather et al., 2008). Consequently, a lower abundance of nutrient-limited primary producers could result directly in a low abundance of SAR11 in the SATL. Nevertheless, SAR11 is highly competitive for the remaining substrates

and showed high uptake rates for methionine in the southern gyre (Mary et al., 2006).

In line with earlier observations we detected the highest relative abundance of *Prochlorococcus* in the surface water layer of the nutrient-rich WTRA followed by the Southern Gyre (Fig. 3b). In the oligotrophic Northern Gyre counts of *Prochlorococcus* doubled from the surface waters to the DCM layer, which also confirms data obtained by flow cytometry (Partensky et al., 1999). Such distributions seem to be typical for the Northern Gyre (Zubkov et al., 2000). It is remarkable that our direct FISH counts agree very well with the estimations done by qPCR (Johnson et al., 2006) and dot blot hybridization (Zwirglmaier et al., 2007) and the flow cytometric data based on chlorophyll autofluorescence (Zubkov et al., 2000).

Horizontal differences were also found in the mesopelagic. The relative abundance of marine group I *Crenarchaeota* and SAR202 showed a conspicuous distribution pattern. A correlation analysis of the relative abundance at a depth of 300 m showed a significant negative correlation of both populations along the transect (r^2=-0.51, $p<0.001$). There were, however, no significant correlations for absolute numbers of SAR202 and marine group I *Crenarchaeota*. Recent publications reported strong indications for autotrophic ammonium oxidation for members of the marine group I *Crenarchaeota* (Koenneke et al., 2005). One can speculate that the *Crenarchaeota* find better growth conditions in oceanic regions with higher ammonium concentrations like the BENG, WTRA and NADR (Rees et al., 2006). For the *Chloroflexi*-like SAR202 there is only very limited information on the physiology of members of the SAR202 clade. Substrate incubations with D- and L- amino acids showed that in contrast to the decreasing fraction of *Bacteria* utilizing L-Asp (Teira et al., 2006a; Varela et al., 2008b) the SAR202 clade efficiently takes up L-Asp at all depths (Varela et al., 2008a). Thus, the SAR202 cluster seemed to be well adapted to exploit the available DOM source in the bathypelagic realm. However, without further knowledge on the physiology of clade SAR202 the factors influencing its distribution remain largely elusive.

The high abundance of *Bacteroidetes* in the nutrient-rich surface waters of the NADR and the BENG (Fig. 5, Suppl. Table 1) suggests a relation to their physiology. *Bacteroidetes* are known to be abundant in coastal or polar regimes and, as mentioned before, are apparently linked to algal blooms and involved in the degradation of polymers such as polysaccharides and proteins (Cottrell and Kirchman, 2000; Eilers et al., 2001). Moreover, for the BENG a significant correlation of chlorophyll *a* fluorescence with relative abundance of *Bacteroidetes* could be shown (r^2=0.70, p<0.01), which is what also Abell and coworker found for marine *Flavobacteria* (Abell and Bowman, 2005). In our study we could also detect relative abundances of 10% and more of *Bacteroidetes* in the oligotrophic gyre provinces (Suppl. Table 1). These *Bacteroidetes* obviously cope well with the limiting conditions in these oceanic regions. It needs to be explored further if these types of *Bacteroidetes* comprise different subclades than those found in more nutrient-rich environments.

Cell biovolume and biomass. With this study we are for the first time able to assign cell volume and biomass values to distinct major phylogenetic clades (Fig. 4, Suppl. Table 2). This is an important step towards a better understanding of the contribution of the individual picoplankton populations to the flux of elements through the marine water column. The average cell volume measured was remarkably stable along the transect (0.054 ± 0.016 μm^3, n=1180) and was very close to previously reported values (~0.05 μm^3; Malmstrom et al., 2005). Whereas the cell sizes of the SAR202 clade and *Prochlorococcus* were constant and in the range of an average picoplankton cell, distinct differences were detected for the other major populations examined, SAR11 and marine group I *Crenarchaeota*. SAR11 cells spanned the largest range in cell size along the entire transect. In surface waters of the northern NAG and the NADR we found the smallest SAR11 cells (0.03 – 0.04 μm^3). The cell volume values were in the range of previously reported data from surface samples of the Gulf of Maine, the Sargasso Sea, and from the North Carolina coast (Malmstrom et al., 2004). Larger cell sizes of SAR11 were found in the SATL and almost all mesopelagic waters (~0.07 μm^3). These agree well with volumes recently

reported from lab grown cultures of SAR11 strains (Tripp et al., 2008). Interestingly, these large celled variants of SAR11 coincide with low relative abundance of SAR11 of <25% (Fig. 3a, 4a). Future research needs to address the question whether these large cells either belong to a distinct subclade (Field et al., 1997) or one of the multiple ecotypes within the SAR11 clade that showed a pronounced vertical and seasonal pattern in the distribution (Carlson et al., 2008). SAR11 was not only numerically most abundant, but also dominated the picoplankton biomass in the photic water layer sampled on AMT16, and also in the mesopelagic (average 0.56 mg C m^{-3}). In the mesopelagic, SAR11 attained the largest cell size of all measured clades. Further studies are required to test whether the dominance of SAR11 biomass continues down to the bathypelagic waters and how active these SAR11 are in the deeper water layers. In contrast, the equally abundant marine group I *Crenarchaeota* had the smallest cell size and hence formed only two thirds of the biomass of SAR11 in the mesopelagic (average 0.38 mg C m^{-3}) (Suppl. Fig. 2a, 2c). However, compared to data reported from the western Arctic Ocean (Kirchman et al., 2007) our observed size range of marine group I Crenarchaota was nearly 50 % smaller which placed this archaeal group at the lower size range of all analyzed groups in this study.

Bloom events. *Gammaproteobacteria* and *Bacteroidetes* have the potential to form blooms in response to sudden nutrient pulses released from phytoplankton (Cottrell and Kirchman, 2000; Eilers et al., 2001). We observed that both groups had on average higher population densities outside the oligotrophic regions in the temperate and upwelling provinces. Highest abundance for *Bacteroidetes* occurred in the photic water layer at the northern most station 65 in the NADR (26%). Similarly, *Gammaproteobacteria* bloomed at station 61 accounting for up to 50% of all picoplankton cells in the surface water (Suppl. Table 1). Preliminary FISH data with probes specific for the gammaproteobacterial genera *Alteromonas*, *Pseudoalteromonas* and *Oceanospirillum* indicated the presence of these culturable genera in significant amounts (Suppl. Table 1). Members of these genera are well known to rapidly form blooms upon excess nutrient supply (Allers et al., 2007). Satellite images

from this area in the NADR suggested the end of the spring phytoplankton bloom as indicated by declining chlorophyll values in the surface waters from May to June 2005 (data source: http://neo.sci.gsfc.nasa.gov). We speculate that the decaying phytoplankton supported massive growth of *Bacteroidetes* and *Gammaproteobacteria* in this region.

Minor populations. Populations below 1% relative abundance might still be responsible for most of the turnover of important nutrients (Musat et al., 2008). For example, the rare Anammox bacteria accounted for almost 100% of the nitrogen loss in the Namibian upwelling region (Kuypers et al., 2005). Some of the populations which were minor on AMT16 had been reported to be more frequent in previous studies. For the marine group II *Euryarchaeota* our data from 1000 m depth (<0.5%, Suppl. Fig. 1a) were very different from those in the deep water study of Teira et al. (Teira et al., 2006b) who reported relative abundance of up to 20% below 500 m. Also, the yet uncultured deltaproteobacterial clade SAR324 had been quantified by rRNA dot blot hybridisation analysis in the Sargasso Sea with relative abundance of up to 18% at a water depth of 170 m (Wright et al., 1997). With our newly developed probe SAR324-1412, we counted on average below 0.5% throughout the transect at all depths (Suppl. Fig. 1b). The SAR406 clade has been described as vertically stratified in the water column of both the Atlantic and Pacific Ocean, with peak abundance below the deep chlorophyll maximum (Gordon and Giovannoni, 1996). We observed a rather patchy distribution of SAR406 along the AMT16 transect, but we could not determine a statistical significant peak in abundance of SAR406 in the water column along the transect (Suppl. Fig 1c). Possible reasons for such discrepancies might be (i) natural variation due to e.g. seasonality (Fuhrman et al., 2006; Carlson et al., 2008) or (ii) methodological aspects e.g. dot blot hybridizations versus CARD-FISH or differences in the specificity of the probes used.

Global extrapolations. We transferred our present data set to similar ecological provinces (as defined by Longhurst (Longhurst, 1998) and extrapolated cell abundance in the photic and upper aphotic zone of the

latitudinal bands of the southern (sub)tropical, the equatorial, the northern (sub)tropical and temperate regions (Table 2), and calculated global abundances. The photic zone (0 – 200 m) of the global ocean contains 3.2 x 10^{28} prokaryotic cells (Bacteria and Archaea) and the upper aphotic zone (200 – 500 m) has 2.2 x 10^{28} prokaryotes. This is in good agreement with the estimation of 3.6 x 10^{28} cells in the photic zone by Whitman *et al.* (Whitman et al., 1998). Our data show that in the top 500 m Bacteria dominate with approximately 4.7 x 10^{28} cells over Archaea (0.6 x 10^{28} cells).

SAR11 and *Prochlorococcus* are the most abundant bacteria (Table 2). Our calculations yield approximately 2.0 x 10^{28} SAR11 cells in the upper 500 m of which 1.4 x 10^{28} are located in the photic zone. Globally we estimate there are 2.2 x 10^{28} SAR11 cells based on average relative abundance of 10% at depths >500 m (Suppl. Table 1). Similar numbers were reported by Morris *et al.* (Morris et al., 2002) who estimated a global abundance of 2.4 x 10^{28} SAR11 cells in the ocean, half of which are located in the photic zone. Third most abundant was the marine group I *Crenarchaeota* with 6.0 x 10^{27} cells in the upper 500 m of the global ocean of which 88% were present in the aphotic zone. This extrapolation represents approximately half of the global estimation of 1.3 x 10^{28} crenarchaeal cells by Karner *et al.* (Karner et al., 2001). Their estimation is based on measurements at the Hawaii Ocean Timeseries station ALOHA site in combination with continental shelf data (DeLong et al., 1999) and ranged down to a depth of 4750 m. By using the estimation of crenarchaeal cell abundances by Herndl et al. (Herndl et al., 2005) we could extend our data set to a comparable depth. As a result our extrapolation suggests that there might be as much as 2.7 x 10^{28} *Crenarchaeota* in the world ocean.

The extrapolation of biomass shows that the global ocean contains 0.26 Gt of C of SAR11 and 0.07 Gt of C of *Prochlorococcus* in the top 500 m (Table 3). Taking into account that both groups together represent approximately 55% of total bacteria, the top 500 m of the global ocean contains nearly 0.6 Gt of C in bacterial biomass which corresponds to 27% of global prokaryotic biomass in the oceanic habitat as determined by Whitman et al. (2.2 Gt of C) or 20% of the

phytoplankton biomass of 3 Gt of C (Mackenzie, 1997). Additional information for global extrapolation of biovolume of particular archaeal and bacterial groups for latitudinal bands is provided in the supplement (Suppl. Table 4).

Concluding remarks. Bookkeeping of our hybridization data suggests that there is still room for additional populations. For about 20% of the Bacteria detected on the transect with the general bacterial probes, we did not obtain any signals with any of the clade specific probes applied. These bacteria might belong to other well known picoplankton clades like marine *Actinobacteria*, *Roseobacter*, *Lentispherae* and OM43, which were not targeted during this study. Previous studies suggest that these clades most likely do not account for the missing 20%. We hypothesize that a part of the missing fraction is likely to belong to the "rare biosphere", present at very low concentrations. New quantification strategies are required for the quantification of such rare populations by FISH in the future.

This study demonstrates that the "picoplankton" black box can be dissected in a quantitative way at global scales. With the help of automated counting systems massively parallel FISH analyses have become feasible. Marine microbiologists are now able to produce data sets at an unprecedented resolution similar to those from well established marine research areas such as physical and chemical oceanography.

Experimental procedures

Sampling. Samples were taken onboard *RRS Discovery* from 20th May to 29th June 2005 during the Atlantic Meridional Transect cruise 16 (AMT16) from Cape Town (South Africa) to Falmouth (UK, Fig. 1). Vertical profiles of conductivity, temperature, depth, and chlorophyll fluorescence were recorded by a Sea-Bird 9/11 *plus* instrument at 65 stations. Samples were taken with Niskin bottles at defined depths. At 10 stations water was sampled down to 1000 m. All samples with exact dates and geo-references are listed in Suppl. Table 1.

Fixation and CARD-FISH. Water samples were fixed within 30 min with particle-free formaldehyde solution (37% w/v, Fluka, Taufkirchen, Germany; final concentration, 1% v/v) for 2 h at room temperature. Picoplankton from the fixed samples were then collected onto polycarbonate filters (type GTTP; pore size, 0.2 µm; diameter, 47 mm; Millipore, Eschborn, Germany). The sample volume applied to each polycarbonate filter varied with depth from 30 mL in surface waters to 250 mL in mesopelagic water in order to achieve a relatively even cell density per filter. Filters were stored and transported at -20 °C.

Prior to CARD-FISH filters were embedded in agarose (low gelling point agarose ≥1200 g cm^{-2}, Biozym, Oldendorf, Germany), and cut in sections (Pernthaler et al., 2002a). For permeabilization of bacterial cell walls, filter sections were incubated in lysozyme (10 mg mL^{-1}; Fluka, Taufkirchen, Germany) for 1 h at 37°C. The permeabilization protocol was modified for identification of Archaea. *Crenarchaeota* were permeabilized with 0.1 M HCl for 1 minute at room temperature, for *Euryarchaeota* the above lysozyme treatment was followed by a proteinase K incubation (from Tritirachium album, 600 mAnson U mL^{-1}, Merck, Darmstadt, Germany, final concentration, 1.5 µg mL^{-1}) for 45 minutes at 37°C.

Endogenous peroxidases were inactivated by using 0.01 M HCl at room temperature for 20 min. Hybridization with HRP-labeled oligonucleotide probes (Table 1; Biomers.net, Germany) and tyramide signal amplification was done according to the protocol by Pernthaler et al. (Pernthaler et al., 2002a) with the following modification: hybridization was done overnight, and tyramide signal amplification was done for 45 min, both at 46°C. After amplification filter sections were washed twice in 96% ethanol before drying. Filter sections were arranged on microscope slides, and embedded in antifading reagent (VECTASHIELD® Mounting Medium H-1000, Vector Laboratories, Inc., Burlingame, CA, USA and Citifluor Ltd., London, U.K.) containing DAPI (4´,6´-diamidino-2-phenylindole) at a final concentration of 1 µg mL^{-1}. Slides were stored at -20°C until further analysis. All probes were checked against the Silva 16S rRNA sequence database for specificity (Pruesse et al., 2007).

Calibration of automated microscopic analysis. Thresholds for automated counting of CARD-FISH signals were set as described previously (Pernthaler et al., 2003). In short, this was based on 17 filter sections on which at least 1,000 DAPI-stained cells were manually scored for CARD-FISH signals. The same sections were subsequently counted with an automated image analysis and counting system coupled to a Zeiss epifluorescence microscope (Axioplan II Imaging, Carl Zeiss, Jena, Germany). The results were compared using regression analysis and parameters adjusted accordingly. In addition, the detection level of false-positive cells at each threshold level was determined on 48 samples hybridized with the antisense probe Non338 and subtracted from the sample counts (0.8% ± 0.7%). DAPI stained viruses were excluded as false positive cells by using a size threshold. At least 2,000 DAPI stained cells were counted per sample preparation (3,600 hybridizations, >7 x 10^6 cells).

Total cell numbers. Numbers of DAPI stained cells before and after CARD-FISH processing were not significantly different, indicating that the fraction of cells lost during processing was negligible (see (Teira et al., 2004). The total cell counts reported in this study are therefore DAPI signals in the size range of picoplankton (i.e. without virus signals) obtained during automated microscopic counting of filters after CARD-FISH. Total cell counts were also determined by flow cytometry and were highly correlated with microscopic counts (r^2=0.91, $p<0.001$, n=235).

Biovolume and biomass determinations. Cell dimensions (area, perimeter) were measured on images of DAPI stained cells identified by CARD-FISH and length and width were derived assuming a coccoid or rod-shaped object. Biovolumes were calculated using a geometrical approximation of cell shape as either spherical or cylindrical and capped by semispheres. Only the cell volumes of SAR11, SAR202, *Prochlorococcus* and marine group I *Crenarchaeota* were determined. Biomass (in mg C m^{-3}) was calculated from cell volume data by using an allometric conversion factor specifically developed for DAPI-stained cells by Loferer-Krößbacher et al (Loferer-Krossbacher et al., 1998).

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Tables

Table 1: Overview of the probes and hybridization conditions applied for CARD-FISH.

probe	target organisms	sequence (5' → 3')	FA[1] [%]	reference
Cren554	*Crenarchaeota m*arine group I	TTAGGCCCAATAATCMTCCT	0	(50)
Eury806	*Euryarchaeota* marine group II	CACAGCGTTTACACCTAG	0	(77)
Eub338	Bacteria	GCTGCCTCCCGTAGGAGT	35	(7)
Eub338-II	supplement to Eub338	GCAGCCACCCGTAGGTGT	35	(15)
Eub338-III	supplement to Eub338	GCTGCCACCCGTAGGTGT	35	(15)
Non338	control	ACTCCTACGGGAGGCAGC	35	(80)
Gam42a[2]	γ-subgroup of *Proteobacteria*	GCCTTCCCACATCGTTT	35	(48)
SAR86-1245	SAR86-clade	TTAGCGTCCGTCTGTAT	35[3]	(86)
CF319a	*Bacteroidetes*	TGGTCCGTGTCTCAGTAC	35	(47)
SAR11-441	SAR11-clade	GGACCTTCTTATTCGGGT	25[3]	(53)
Pro405	*Prochlorococcus*	AGAGGCCTTCGTCCCTCA	40	(83)
SAR406-97	SAR406-clade	CACCCGTTCGCCAGTTTA	40[3]	(24)
SAR202-312R	SAR202-clade	TGTCTCAGTCCCCCTCTG	40[3]	(54)
SAR324-1412	SAR324-clade	GCCCCTGTCAACTCCCAT	35[3]	this study
Pla46	*Planctomycetes*	GACTTGCATGCCTAATCC	30	(58)
Alt1413	*Alteromonas / Colwellia*	TTTGCATCCCACTCCCAT	40	(21)
Psa184	*Pseudoalteromonas*	CCCCTTTGGTCCGTAGAC	30	(21)
GV (841)	*Vibrio*	AGGCCACAACCTCCAAGTAG	30	(30)
Oce232	*Oceanospirillum*	AGCTAATCTCACGCAGGC	40	(21)

Abbreviations: FA: formamide (v/v);

[1]: formamide concentration in CARD-FISH hybridisation buffer;
[2]: including an unlabeled competitor probe Bet42a (5'-GCCTTCCCACTTCGTTT-3'), see (48)) for details;
[3]: formamide concentration determined or reevaluated by Clone-FISH (72) for this study;

Table 2: Extrapolation of total cell abundances (x 10^{26}) of particular archaeal and bacterial groups for latitudinal bands.

		Crenarchaeota	*Euryarchaeota*	*Bacteria*	SAR11	*Prochlorococcus* sp.	SAR202	*Bacteroidetes*	*Gammaproteobacteria*	SAR406	SAR324
South. (sub)tropics	p	1.1 ± 1.6	0.3 ± 0.4	58.4 ± 25.5	23.2 ± 11.2	13.4 ± 8.2	1.7 ± 1.2	2.7 ± 2.5	2.0 ± 1.9	0.6 ± 0.6	0.4 ± 0.3
	a	6.3 ± 2.2	0.1 ± 0.1	24.4 ± 14.5	7.2 ± 5.2	1.2 ± 1.7	2.7 ± 1.6	0.7 ± 0.9	1.5 ± 1.2	0.5 ± 0.4	0.3 ± 0.2
Equator	p	1.4 ± 1.6	0.2 ± 0.2	50.8 ± 17.6	21.9 ± 7.4	12.4 ± 7.6	0.6 ± 0.6	3.2 ± 1.1	1.4 ± 0.9	0.5 ± 0.3	0.6 ± 0.4
	a	10.9 ± 2.7	0.1 ± 0.1	25.5 ± 6.7	7.6 ± 3.3	0.4 ± 0.2	0.7 ± 0.3	0.7 ± 0.4	1.4 ± 0.7	0.4 ± 0.5	0.2 ± 0.1
North. (sub)tropics	p	1.4 ± 1.6	0.4 ± 0.4	47.9 ± 20.4	25.3 ± 10.8	7.2 ± 5.6	0.4 ± 0.4	3.2 ± 2.7	3.4 ± 6.7	0.3 ± 0.3	0.2 ± 0.2
	a	9.1 ± 2.5	0.1 ± 0.02	30.9 ± 1.8	11.3 ± 4.5	0.8 ± 0.0	1.5 ± 0.9	0.7 ± 0.7	0.8 ± 0.5	0.3 ± 0.2	0.1 ± 0.02
North. temperate	p	0.2 ± 0.3	0.1 ± 0.1	14.4 ± 5.9	7.9 ± 3.0	0.3 ± 0.2	0.02 ± 0.03	2.1 ± 1.4	1.0 ± 1.1	0.02 ± 0.01	0.01 ± 0.003
	a	3.0 ± 0.9	0.02 ± 0.01	10.4 ± 0.6	3.8 ± 1.5	0.3 ± nd	0.5 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.03 ± 0.01
Global	p	**0.7 ± 0.9 x 10^{27}**	**1.7 ± 2.0 x 10^{26}**	**3.1 ± 1.3 x 10^{28}**	**1.4 ± 0.6 x 10^{28}**	**6.0 ± 3.9 x 10^{27}**	**4.8 ± 3.9 x 10^{26}**	**2.0 ± 1.4 x 10^{27}**	**1.4 ± 1.9 x 10^{27}**	**2.6 ± 2.2 x 10^{26}**	**2.4 ± 1.6 x 10^{26}**
	a	**5.3 ± 1.5 x 10^{27}**	**0.6 ± 0.5 x 10^{26}**	**1.6 ± 0.4 x 10^{28}**	**0.5 ± 0.3 x 10^{28}**	**0.5 ± 0.4 x 10^{27}**	**9.6 ± 5.3 x 10^{26}**	**0.4 ± 0.4 x 10^{27}**	**0.7 ± 0.5 x 10^{27}**	**2.2 ± 2.1 x 10^{26}**	**1.0 ± 0.5 x 10^{26}**

Abbreviations:
p: photic zone (0 – 200 m);
a: upper aphotic zone (200 – 500 m)
nd: not determined due to single measurement

Table 3: Biomass extrapolations (Tg of C) of particular archaeal and bacterial groups for latitudinal bands.

		Crenarchaeota	SAR11	*Prochlorococcus* sp.	SAR202
South. (sub)tropics	p	2.8 ± 1.6	35 ± 6.4	15.8 ± 10.0	1.6 ± 1.3
	a	5 ± 1.6	9.6 ± 6.4	1 ± nd	2.4 ± 1.2
Equator	p	3.4 ± 1.6	31 ± 10.2	13.7 ± 8.1	0.6 ± 0.6
	a	8.8 ± 2.8	124 ± 8.1	nd	0.8 ± 0.5
North. (sub)tropics	p	2.7 ± 2.3	32.7 ± 12.9	9.2 ± 7.2	0.5 ± 0.6
	a	6.2 ± 3.1	99.6 ± 4.5	nd	2 ± 0.8
North. temperate	p	1.6 ± nd	8.5 ± 3.1	0.6 ± 0.2	0.03 ± 0.04
	a	3.2 ± 1.0	4.8 ± 2.5	nd	0.6 ± 0.4
Global (GT of C)	p	**0.02 ± 0.01**	**0.2 ± 0.06**	**0.07 ± 0.05**	**0.005 ± 0.005**
	a	**0.04 ± 0.02**	**0.07 ± 0.04**	**0.001 ± nd**	**0.01 ± 0.005**

Abbreviations:
p: photic zone (0 – 200 m);
a: upper aphotic zone (200 – 500 m)
nd: not determined due to single measurement

Figure legend

Fig. 1: AMT 16 Cruise track from Cape Town, South Africa to Falmouth, UK during May – June 2005. Abbreviations: Benguela Current Coastal Province (BENG), South Atlantic Gyral province (SATL), Western Tropical Atlantic (WTRA), North Atlantic Gyral Province (NAG), Northern Atlantic Drift (NADR).

Fig. 2: Hydrographic parameters with classification of oceanic provinces; A: Salinity (psu); B: Chlorophyll *a* concentration (mg m^{-3}) (from (Robinson et al., 2006) C: Total cell counts of Picoplankton; dots represent the sampling depths.

Fig. 3: Latitudinal-depth contours of specific microbial groups as relative abundance (% DAPI counts).

Fig. 4: Latitudinal-depth contours of average cellular volumes of individual picoplankton populations.

Fig. 5: Contribution of major groups to the picoplankton along the AMT16 transect.

Supplementary material

Suppl. Table 1: Total cell numbers and values of all hybridizations obtained in this study.

Suppl. Table 2: Average relative abundance and locations of minimum and maximum of the individual populations analyzed in this study.

Suppl. Table 3: Average cell size of particular groups in different ecological provinces

Suppl. Table 4: Global budget of biovolume (x 10^{20} μm^3) of particular archaeal and bacterial groups for latitudinal bands. p: photic zone (0-200 m); a: aphotic zone (200 – 500 m)

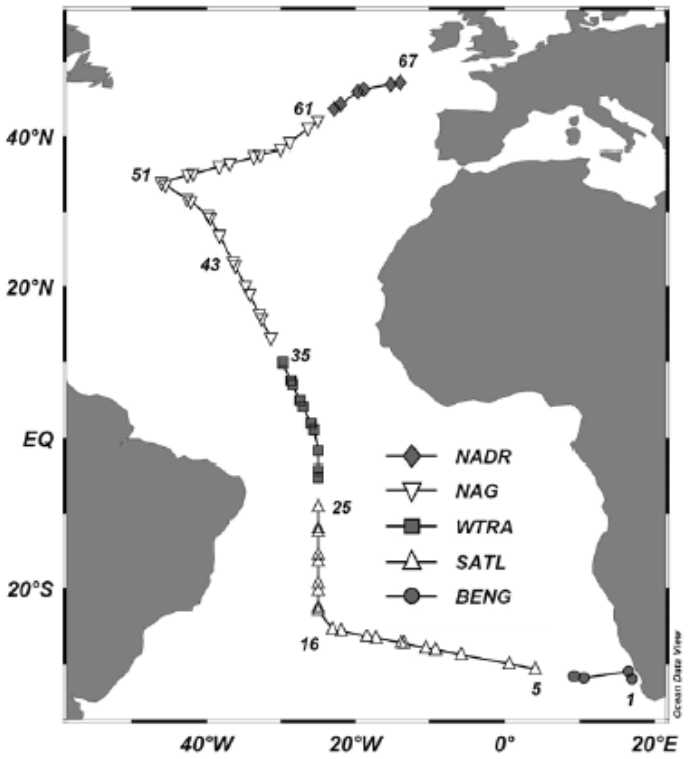
Suppl. Table 5: Correlation between physical parameters, nutrient concentration and relative abundance obtained in this study.

Suppl. Fig. 1: Bubble plot of minor picoplankton populations. The size of a bubble represents the relative abundance of the respective population.

Suppl. Fig. 2: Latitudinal-depth contours of biomass of individual picoplankton populations.

Suppl. Fig. 3: Distribution of SAR11 (% DAPI counts) along the southern section of the AMT13 transect during September-October 2003. Sampling locations are indicated by circles.

Fig. 1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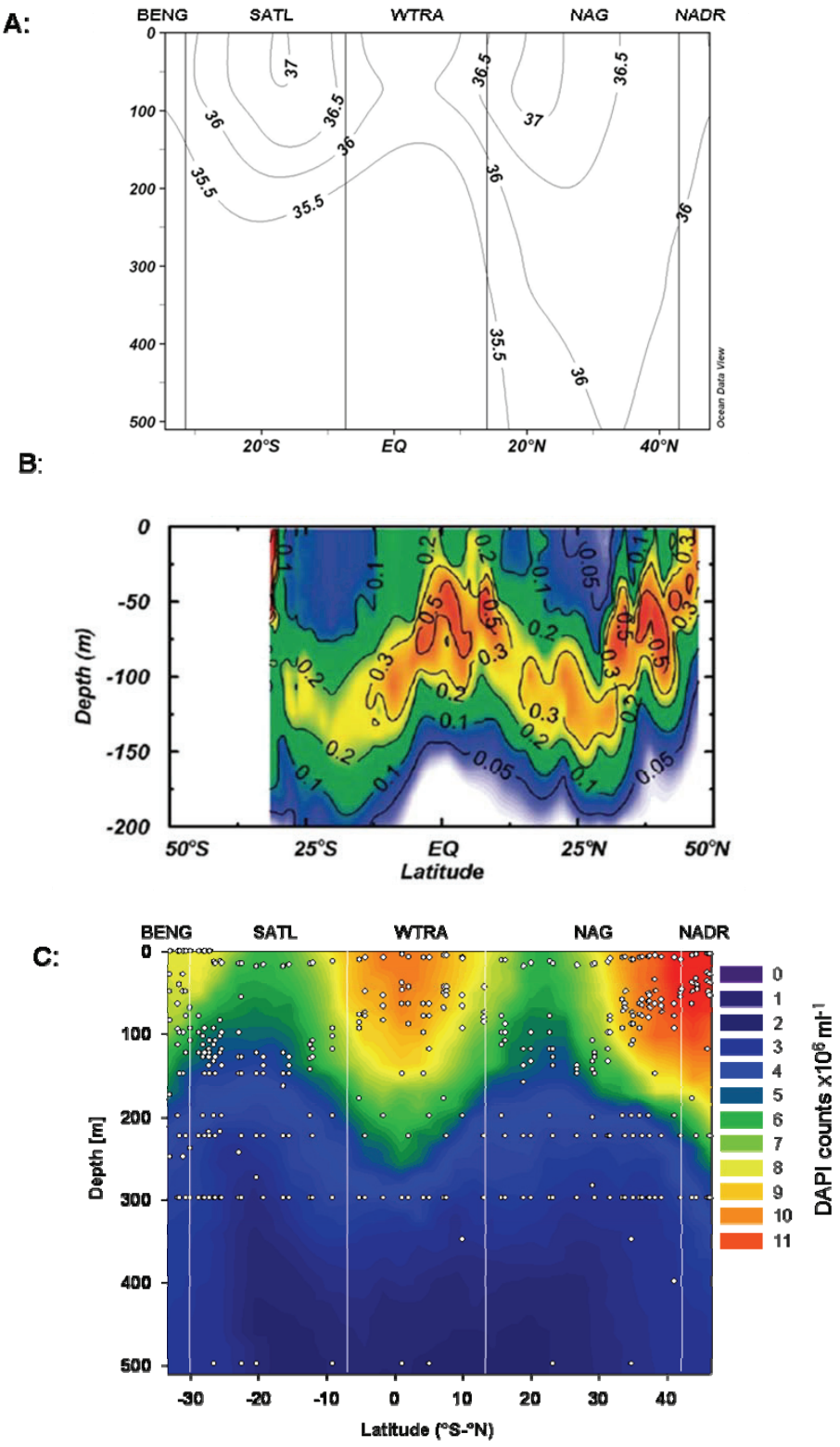
Ocean Data View

Fig. 2: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B:
Depth (m)
0
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-100
-150
-200
50°S
25°S
EQ
25°N
50°N
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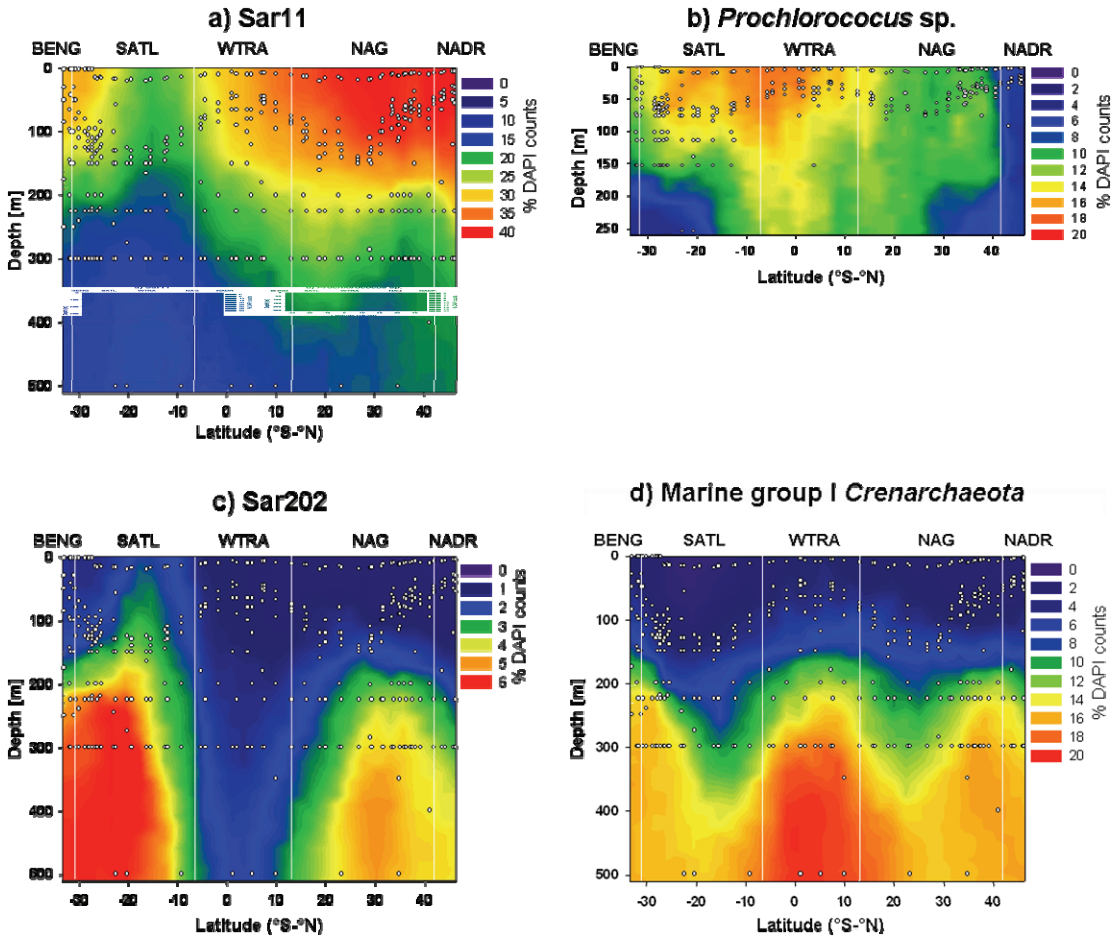
Latitude (°S-°N)
DAPI counts x10⁶ ml⁻¹

Fig. 3:


a) Sar11
b) Prochlorococcus sp.
c) Sar202
d) Marine group I Crenarchaeota
BENG
SATL
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NAG
NADR
Depth [m]
Latitude (°S-°N)
% DAPI counts

Fig. 4:

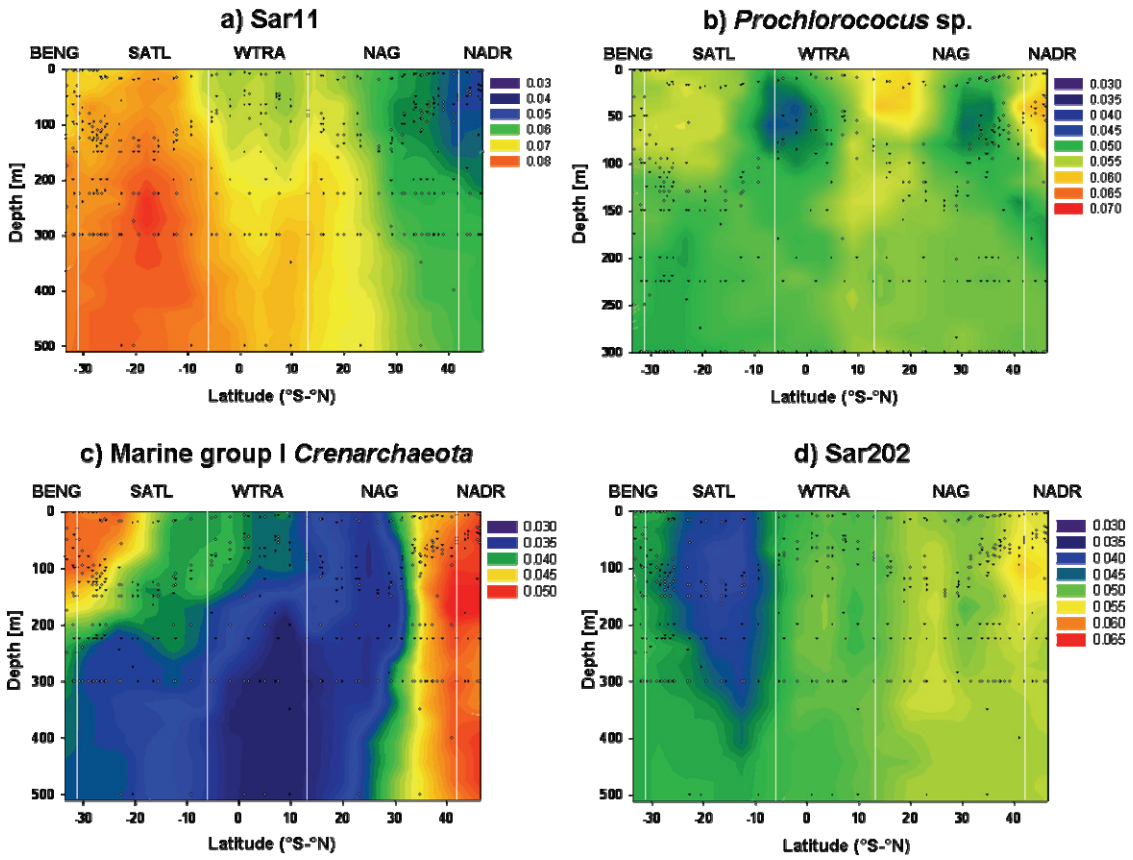
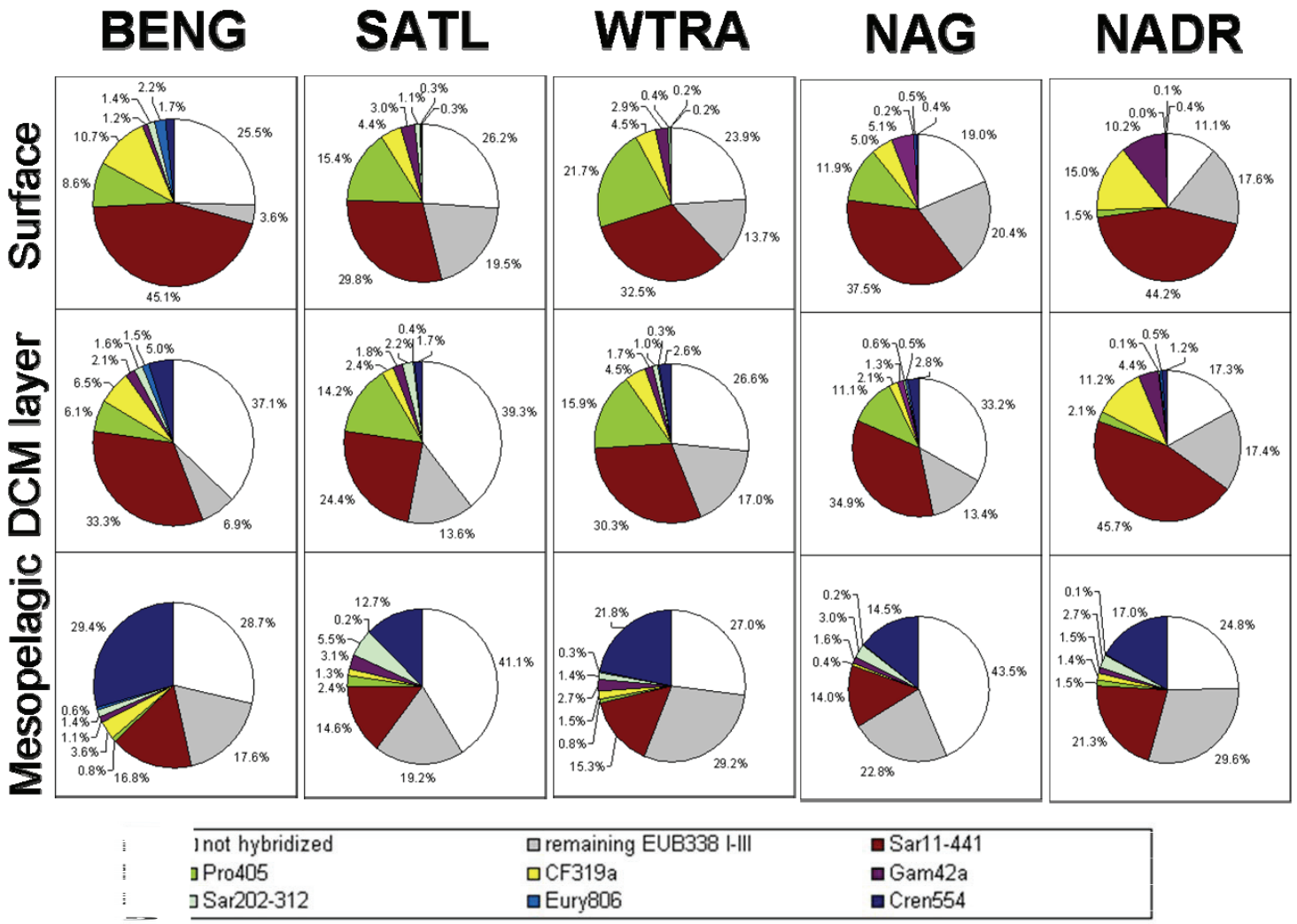
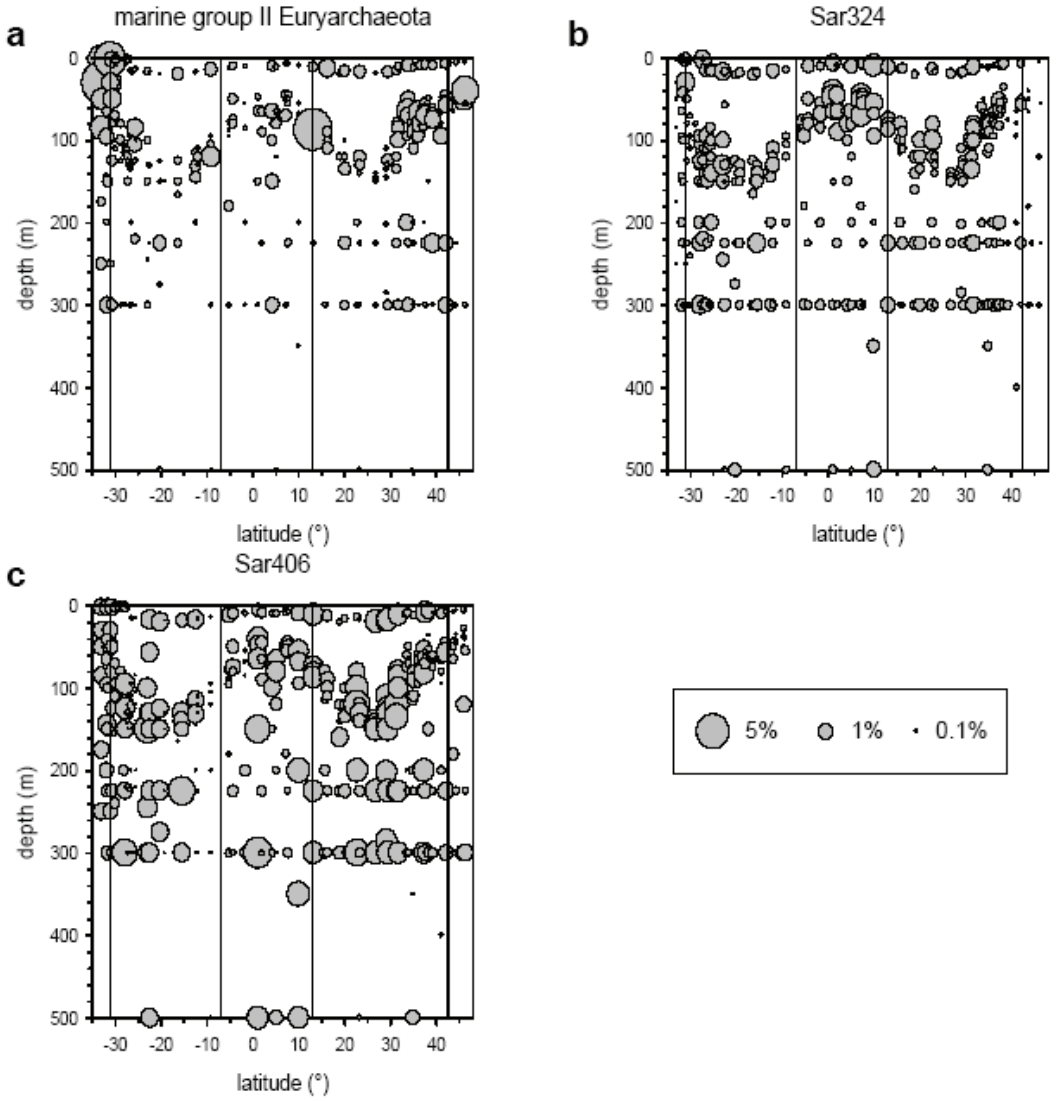

a) Sar11
BENG
SATL
WTRA
NAG
NADR
Depth [m]
Latitude (°S-°N)
b) Prochlorococcus sp.
c) Marine group I Crenarchaeota
d) Sar202

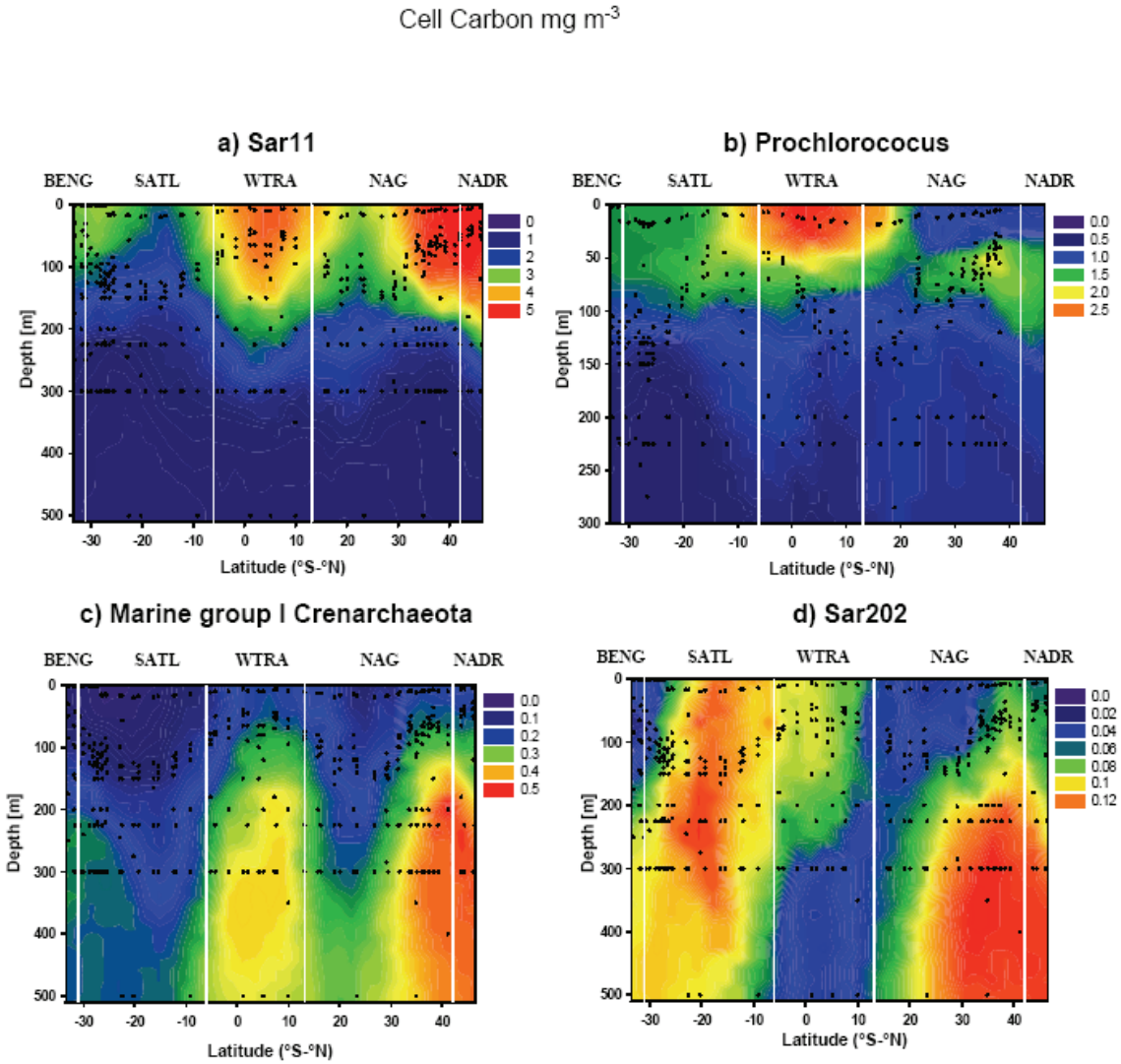
Fig. 5:


BENG
SATL
WTRA
NAG
NADR
Surface
DCM layer
Mesopelagic
not hybridized
remaining EUB338 I-III
Sar11-441
Pro405
CF319a
Gam42a
Sar202-312
Eury806
Cren554

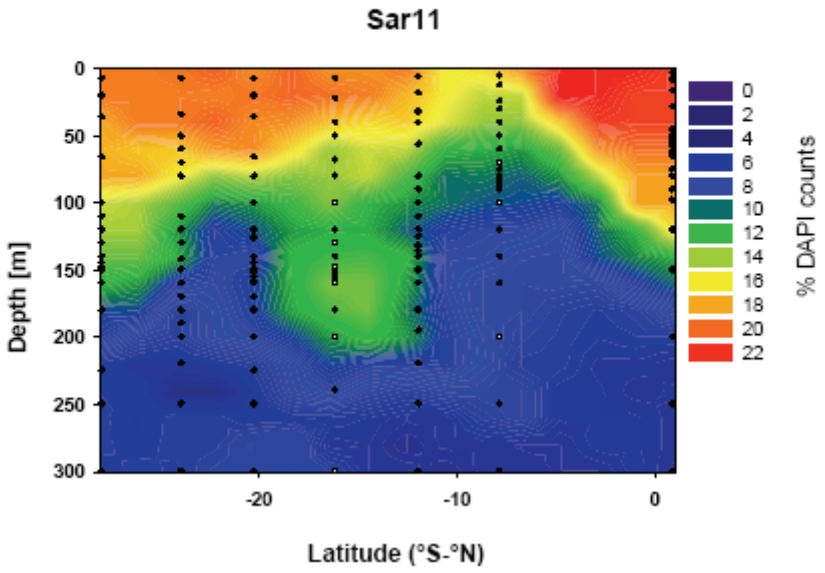
Suppl Fig. 1: Minor Bacterioplankton groups



Suppl Fig. 2: Latitudinal-depth contours of biomass of individual bacterioplankton populations



Suppl Fig. 3: SAR11 distribution during AMT13



Publication II

Phylogenetic Affiliation of Bacterioplankton with High and Low Nucleic Acid Content in the North Atlantic Ocean

Martha Schattenhofer, Rudolf Amann, Mikhail V. Zubkov, Bernhard M. Fuchs

Manuscript in preparation.

Phylogenetic Affiliation of Bacterioplankton with High and Low Nucleic Acid Content in the North Atlantic Ocean

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Abstract

We have analyzed surface water samples from 6 stations of two different oceanic provinces in the North Atlantic Ocean, obtained during a transect from Iceland to the Azores. Bacterioplankton populations with high (HNA) and low nucleid acid content (LNA) were sorted by flow cytometry and phylogenetically characterized. HNA and LNA bacterioplankton accounted each for 50% ± 4% throughout the transect. Fluorescence *in situ* hybridization analysis revealed distinct phylogenetic composition for the LNA and HNA populations, which have virtually no overlap. The LNA population comprised the dominating SAR11-clade (63%) and the rare betaproteobacterial OM43 clade (1%). Both clades harbor strains with small genomes of around 1 Mb in size. Smaller percentages (<5%) of *Gammaproteobacteria* were also found in the LNA population. Similar composition and abundances of these phylogenetic clades were found in the Arctic Atlantic and North Atlantic Gyre provinces. The HNA bacterioplankton contained a wider variety of different clades and the abundance of some of the bacterial clades varied considerably in both oceanic provinces. The phylogenetic composition of the HNA population was comprised of *Cyanobacteria* (25% ± 17%), *Alphaproteobacteria* (19% ± 8%), *Gammaproteobacteria* (14% ± 2%), *Bacteroidetes* (14% ± 11%), and members of the SAR406-clade (4% ± 3%). Additionally, with subcluster-specific probes we were able to determine the abundance of certain subgroups (3% ± 1% of SAR86, 5% ± 3% of *Roseobacter*) and different ecotypes of *Prochlorococcus* in the North Atlantic Ocean.

Thus, differentiation of bacterioplankton cells into HNA and LNA populations may indicate distinct phylogenetic groups harboring different ecological niches and functions and provide us further with references to genome size and lifestyles of cells in their respective population.

Introduction

Flow cytometric analyses of marine water samples frequently show a characteristic pattern. In a scatter versus DNA dot plot diagramm two populations are discernable (18, 28, 30, 61, 62): one with an apparently high (HNA) and one with a low nucleid acid (LNA) content. HNA cells have been proposed to be active cells whereas the role of LNA cells has remained controversial, with different studies describing them either as inactive, dead or fragmented (28), or as viable cells which are as active as other members of the community (61).

The biological nature and ecological function of these cytometrically defined populations remains poorly understood. It was shown that certain flow cytometric pattern could be assigned to the cyanobacteria *Prochlorococcus* and *Synechococcus* (11, 29, 55), In samples from the Mediterranean Sea, however no differences in phylogenetic composition could be detected for the HNA and LNA populations (51). In North Sea bacterioplankton each flow cytometrically defined population was dominated by a different phylogenetic group of bacteria (16, 17, 62). The HNA population was commonly dominated by *Alphaproteobacteria*, in particular *Roseobacter*, *Gammaproteobacteria* and *Bacteroidetes* (60-63). The LNA population though was dominated by the alphaproteobacterial SAR11 clade (35).

The aim of the present study was to unravel the phylogenetic composition of HNA and LNA cells in the North Atlantic by using an improved FISH protocol. We hypothesized that (1) the phylogenetic diversity in the HNA is higher than in the LNA population and (2) that the phylogenetic composition of the LNA and HNA populations is similar in the different station investigated. We tested our hypotheses on samples from 6 stations obtained during a transect in the North Atlantic between Greenland and the Azores (VISION cruise).

Materials and Methods

Sampling. Samples were taken onboard *MS Merian* during 20th to 29th September 2006 during the VISION cruise from Reykjavik (Iceland) to Lisbon (Portugal, Fig. 1). Seawater samples were collected from a depth of 20 – 500 m with a sampling rosette of 20-litre Niskin bottles mounted on a conductivity-temperature-depth (CTD) profiler at stations identified in Fig. 1. At 2 stations water was sampled down to 3000 m. All samples with exact dates, time and geo-references are listed in Suppl. Table 1.

Fixation and CARD-FISH. Water samples were fixed within 30 min with particle-free formaldehyde solution (37% w/v, Fluka, Taufkirchen, Germany; final concentration, 1% v/v) for 2 h at room temperature. Bacterioplankton from the fixed samples were then collected onto polycarbonate filters (type GTTP; pore size, 0.2 µm; diameter, 47 mm; Millipore, Eschborn, Germany). The sample volume applied to each polycarbonate filter varied with depth from 30 ml in surface waters to 250 ml in mesopelagic water in order to achieve a relatively even cell density per filter. Filters were stored and transported at -20 °C.

Prior to CARD-FISH filters were embedded in agarose (low gelling point agarose ≥1200 g cm^{-2}, Biozym, Oldendorf, Germany), and cut in sections (42). For permeabilization of bacterial cell walls, filter sections were incubated in lysozyme (10 mg mL^{-1}; Fluka, Taufkirchen, Germany) for 1 h at 37°C. The permeabilization protocol was modified for identification of Archaea. *Crenarchaeota* were permeabilized with 0.1 M HCl for 1 minute at room temperature, for marine *Actinobacteria* the above lysozyme treatment was followed by a achromopeptidase incubation (Sigma, Taufkirchen, Germany, final concentration 60 U mL^{-1}) for 30 minutes at 37°C. Endogenous peroxidases were inactivated by using 0.01 M HCl at room temperature for 20 min. Hybridization with HRP-labeled oligonucleotide probes (Table 1; Biomers.net, Germany) and tyramide signal amplification was done according to the protocol by Pernthaler et al. (42) with the following modification: hybridization was done overnight, and tyramide signal amplification was done for 45 min, both at 46°C. After amplification filter sections were washed twice in 96% ethanol before drying.

Filter sections were arranged on microscope slides, and embedded in antifading reagent (VECTASHIELD® Mounting Medium H-1000, Vector Laboratories, Inc., Burlingame, CA, USA and Citifluor Ltd., London, U.K.) containing DAPI (4´,6´-diamidino-2-phenylindole) at a final concentration of 1 µg ml^{-1}. Slides were stored at -20°C until further analysis.

Calibration of automated microscopic analysis. Thresholds for automated counting of CARD-FISH signals were set as described previously (43). In short, this was based on sixteen filter sections on which at least 1,000 DAPI-stained cells were manually scored for CARD-FISH signals. The same sections were subsequently counted with an automated image analysis and counting system coupled to a Zeiss epifluorescence microscope (Axioplan II Imaging, Carl Zeiss, Jena, Deutschland). The results were compared using regression analysis and parameters adjusted accordingly. In addition, the detection level of false-positive cells at each threshold level was determined on 14 samples hybridized with the antisense probe NON338 and subtracted from the sample counts. Additionally, DAPI stained viruses were excluded as false positive cells by adjusting the size threshold. At least 2,000 DAPI stained cells were counted per sample preparation.

Total cell numbers. Numbers of DAPI stained cells before and after CARD-FISH processing were not significantly different, indicating that the fraction of cells lost during processing was negligible (see (54)). The total cell counts reported in this study are therefore DAPI signals in the size range of bacterioplankton (i.e. without virus signals) obtained during automated microscopic counting of filters after CARD-FISH.

Flow cytometry sorting of bacterioplankton cells for catalyzed reporter deposition (CARD)-FISH Analysis

Sampling. The composition of total bacterioplankton was assessed using a set of probes (Table 1) in surface samples (10 m) of six stations on the VISION cruise (Fig. 1). Replicated 1.6 mL seawater subsamples were fixed with 1% PFA at 2°C for 12 h and stored frozen at −80°C.

Sorting The PFA-fixed cells were stained with SYBR Green (conc. 1 in 1000) 30 min prior to flow sorting with a MoFlo flow cytometer (Beckman Coulter). For excitation, an Argon ion laser (Innova-A300) was tuned to 488 nm with an output power of 500 mW. SSC was analysed through a 488 ± 10 nm bandpass filter, green fluorescence (FL1) of SYBR Green-stained cells was measured through a 530 ± 20 nm bandpass filter. Online analysis was done on a bivariate dot plot diagram using the Summit software V3.1 (DakoCytomation). The dotplot diagrams were used for defining sorting gates (Suppl fig. 1). Particle-free and autoclaved (<0.1 µm) 0.1% NaCl (w/v) solution was used as a sheath fluid for sorting. The sort mode 'single one drop' was selected to get the highest sorting purity. The performance was evaluated by sorting a known number of beads onto microscopic slides which were subsequently enumerated under an epifluorescence microscope.

CARD-FISH. Approximately 1×10^4 cells were sorted and subsequently filtered onto 0.2 µm pore-size polycarbonate filters for CARD-FISH analysis as described above. Cells were manually counted under an Axioplan II microscope (Carl Zeiss, Jena, Germany) equipped with an HBO 100-W Hg vapor lam, appropriate filter sets for FITC and DAPI fluorescence (21), and a 100x Plan Apochromat objective. At least 300 cells were counted per sample and probe-positive cells were presented as percentages of DAPI stained cells.

Results

Hydrography of oceanic provinces. After the classification suggested by Longhurst et al. (31) three major oceanographic provinces could be distinguished along the transect (Fig. 1): the Atlantic Arctic Province (ARCT), the Northern Atlantic Drift (NADR) and the North Atlantic Gyral Province (NAG). Water masses in the ARCT were characteristically low in temperature (<13 °C) and salinity (<35 psu) but high in oxygen concentrations (>250 µmol). Within the NADR temperature and salinity steadily increased towards the south up to 20°C and >36.0 psu in surface waters. In contrast, oxygen concentrations dropped to <230 µmol and continued decreasing in the NAG (max 216 µmol in surface water). The NAG had the highest temperatures (> 23°C) and salinity (>36.5 psu) along the VISION transect.

Quantification of major microbial groups along the transect

Bacteria, as detected by the probe mix of EUB338, EUB338-II and EUB338-III (Table 1), dominated the bacterioplankton at all depths (56% ± 14% of DAPI). Highest relative abundance of *Bacteria* was found in surface waters down to 150 m with approximately 61% ± 12%. In contrast, the abundance of the *Crenarchaeota* identified with probe CREN554, targeting the marine group I Archaea, increased with depth at every station. Below 150 m the average was 16% ± 4 (Fig. 2a).

Within the *Bacteria* members of the SAR11-clade comprised the most abundant fraction with average 14% ± 11%. Highest values were found in the upper 150 m where SAR11 reached values of >30% relative abundance, particular at latitudes north of 60°N (Fig. 2b). Members of the uncultured SAR202 clade were more abundant (5% ± 2%) south of 50°N than in the north (1.5% ± 0.7%) at depths below 150 m (Fig. 2c). In contrast to the SAR202 distribution, members of the *Bacteroidetes* increased with latitude reaching the highest relative abundance (14% ± 6%) in the upper 200 m north of 60°N. South of 50°N *Bacteroidetes* made up 5% ± 4% in the water column, decreasing with depth (Fig. 2d). Marine *Actinobacteria* comprised only a minor fraction of the Bacteria (average 1% ± 1%). Their fraction of total bacterioplankton remained quite constant (1.5% ± 1%)

in the upper 70 m along the transect with the exception of stations 5 – 8 where the abundance in surface waters partly dropped below 1%. Below 200 m depth marine *Actinobacteria* were virtually absent.

Phylogenetic affiliation of flow cytometrically sorted groups of bacterioplankton

The composition of the bacterioplankton community was flow cytometrically characterized on the basis of the nucleic acid content of each individual cell. On density plots two main groups were clearly observed in samples from all stations: cells with a high nucleic acid content (HNA population) and cells with a low nucleic acid content (LNA population) (Suppl. fig. 1). Cells from surface water of six selected stations were flow cytomerically analyzed for and sorted into distinct DNA-content groups (Fig. 3). The sorted cells were phylogenetically characterized using CARD-FISH (Table 1) and abundances were determined in relation to DAPI counts.

The HNA fraction was dominated by *Bacteria* as detected by CARD-FISH (probes EUB I-III) in all samples, making up 90% ± 3%. Members of the genus *Prochlorococcus* accounted for almost all *Cyanobacteria* (26%) and the HNA group was predominantly composed of this genus. About 14% each of all HNA cells belonged to the *Bacteroidetes* or the *Gammaproteobacteria*. *Alphaproteobacteria* accounted for 19% of the HNA group.

In the LNA group the majority of cells were also *Bacteria* with 78% ± 7% of the sorted cells. In contrast to their absence in the HNA group on average 63% ± 13% of LNA cells hybridized with a probe specific for the SAR11 cluster and accounted for almost all *Alphaproteobacteria* there.

Smaller percentages (<5%) were detected with probes specific to *Gammaproteobacteria* and *Betaproteobacteria*.

The community composition of the LNA and the HNA groups were similar at the two northern stations 3 and 6 in high latitudes (59 – 65°N, $r^2 = 0.99$) as well as at the four southern stations 16 -19 in the lower latitudes (34 – 38°N, $r^2 = 0.87$-0.97) (Fig. 1). Therefore the results of the northern and southern stations were

pooled together, respectively. The quantification efficiency of the HNA cells ranged from 72% - 100% of EUB compared to 81% - 96% of EUB in the LNA group. However, there was a significant shift in the relative abundance of phylogenetic groups from northern to the southern stations.

In the HNA group the compositional shift was most pronounced. The *Cyanobacteria* increased nine fold between the northern and southern stations from 3% ± 2% to 37% ± 7%. Within the *Cyanobacteria Prochlorococcus* showed the same trend, increasing from the northern stations with 2% ± 2% up to 38% ± 4% (st.16-19). The abundance of *Prochlorococcus* at the four southern stations could be partly resolved by using three subcluster-specific probes (Table 3). With the probe 645HLI, specific for the high-light adapted *Prochlorococcus* clade I, an abundance of 17% - 26% was detected at station 16 -18, dropping rapidly at station 19 to 4%. However, probe 645II, specific for the high-light adapted *Prochlorococcus* clade II, showed low counts (< 4%) between st. 16 -18 with an increase up to 14% (station 19). With the third probe 645LL, specific for the low-light adapted *Prochlorococcus* clade, counts >1% were rarely found (Table 3). The abundance of *Synechococcus*, another major group of *Cyanobacteria*, was stable over the whole transect with approximately 2% ± 1%. *Synechococcus* made up around half of the *Cyanobacteria* abundance in the northern stations but only a small part in the southern stations.

Members of the *Bacteroidetes* showed a similar pattern but in the opposite direction. Their abundance decreased rapidly from 29% ± 4% at the northern stations to 7% ± 3% at the four southern stations. *Alphaproteobacteria* were less abundant in the northern part and made up 22% ± 4%. Towards the gyre they decreased slightly to an abundance of 17% ±11%. Members of the *Roseobacter* cluster represented approximately half (10%) of the *Alphaproteobacteria* at station 6 but were absent at the northern station 3. Between stations 16 to 19 relative abundance of *Roseobacter* was relatively stable with 3% - 4% of all HNA cells. The numbers of *Gammaproteobacteria* remained stable at approximately 13 - 14% over the whole transect. The uncultured clade SAR86 was abundant in the HNA fraction at the northern stations 3 - 6 with 4% and 3% respectively. At

the southern stations SAR86 contributed at most 2% to the *Gammaproteobacteria* abundance. The abundance of the uncultured *Firmicutes* clade SAR406 was constant in both regions with 3% ± 1% (north) and 4% ± 4% (south) respectively.

In contrast, within the LNA group a compositional shift in the relative abundance of phylogenetic groups was less distinctive. *Alphaproteobacteria* clearly dominated the community of these cells and showed only a slight decline from the northern (70% ± 2%) towards the southern stations (60% ± 5%) with SAR11 representing 67% ± 7% and 60% ± 15% of the community respectively. Only a minor fraction of all LNA cells belonged to the *Betaproteobacteria*, 4% ± 1% in the North dropping to 2% ± 0.5% in the gyral region. The betaproteobacterial clade OM43 was found at the northern stations 3 - 6 with 1% (Table 3). The abundance of *Gammaproteobacteria* increased from 0.5% ± 0.2% southward to 2% ± 1%.

To identify possible relationships between bacterioplankton groups as well as phytoplankton and physical parameters, linear regression coefficients were computed for all parameters measured and more significant coefficients between bacterioplankton groups were summarized in table 2. *Bacteroidetes* and the betaproteobacterial clade OM43 were both significantly positive related (r^2 = 0.91 – 0.99; $p<0.003$) with nutrient concentrations of phosphate and the sum of nitrite and nitrate. Furthermore both *Bacteroidetes* and the OM43 clade also showed a positive relation to the picoeukaryote phytoplankton concentration with r^2 = 0.92; $p<0.003$ and r^2 = 0.94; $p<0.001$ respectively. Relation to chlorophyll a concentrations were positive for *Bacteroidetes* (r^2 = 0.89; $p<0.006$) and OM43 (r^2 = 0.97; $p<0.001$) as well. *Prochlorococcus* showed a significant relation to the parameters oxygen concentration (r^2 = 0.94; $p<0.002$) and phosphate (r^2 = 0.97; $p<0.001$).

Discussion

Phylogenetic composition and distribution

In a recent study, Zubkov et al. found a stable community composition of cytometric fractions HNA and LNA in the northern North Sea (63). The composition of bacterioplankton within the fractions remained constant while the proportion of the bacterial groups varied considerably at the 10 to 100 km scale. This compositional constancy was also observed in the LNA population of this study.

In both sampling areas the LNA population was dominated by the SAR11 clade as previously reported by Mary et al. (35) (Fig. 4). From genome analysis of cultured members of the SAR11 clade it is well known that they posses small genomes (20, 46). This is likely the reason why they are exclusively detected in the LNA fraction.

Besides we could detect only low abundances of *Beta*- and *Gammaproteobacteria* in the LNA fraction (Fig. 3). Generally *Gammaproteobacteria* constitute only a minor part of the bacterioplankton, particularly in oligotrophic areas (21). Though previous analyses suggested that *Betaproteobacteria* are rare in marine surface waters but are a main component of freshwaters (21, 36) molecular evidence is accumulating that some betaproteobacterial lineages do occur in various marine environments, including the water column and sediments (4, 26, 44, 47). In our study a low abundance of about 1% could be assigned to members of the uncultured betaproteobacterial OM43. This clade is commonly found in highly productive coastal ecosystems and some freshwater environments but only rarely detected in ocean gyres (19, 47, 50). We observed OM43 exclusively in samples from the productive northern region St. 3 and 6 (Table 3). However, the abundance of *Beta*- and *Gammaproteobacteria* does not account for unassigned/ undetected fraction within the LNA group. Further identification of the remaining cells within the LNA group could not be achieved as we could not detect any other bacterial groups.

The diversity in the LNA fraction is lower than in the HNA fraction. Indeed, 58% to nearly 100% of bacteria in the LNA group could be assigned to the

SAR11 cluster, representing quite a stable SAR11 clade-dominated assemblage that was similar in different parts of the North Atlantic Ocean.

The diversity in the HNA population has already been analyzed in previous studies by FISH (17, 60, 62) or fingerprint techniques (32). However, those previous studies did not include the autotrophic *Cyanobacteria* but rather sorted this group separately by means of their chlorophyll cell content. We investigated the cyanobacterial abundance and diversity as part of the bacterioplankton community. With two exceptions (Table 3) *Cyanobacteria* were only present in the HNA group and comprised by *Prochlorococcus* and *Synechococcus*. Within the *Prochlorococcus* three subclusters could be differentiated (HLI, HLII, LL). Additionally, we could also show the presence of *Bacteroidetes*, *Alpha*- and *Gammaproteobacteria* in the HNA fraction, and the presence of SAR406 in substantial numbers (Table 3, Fig. 3) in the HNA group.

The community composition of the HNA population varied in both sampling regions. In the southern gyral stations *Cyanobacteria* were the most abundant group, particularly *Prochlorococcus* (Fig. 3). The distribution of members of the genus *Prochlorococcus* (10) within the 40°N - 40°S latitudinal band (41) stands in line with the results of this study as the abundance of *Prochlorococcus* drops drastically towards the stations north of 40°N. In addition to the general *Prochlorococcus* probe Pro405 we used a probe set to discriminate between high light (645HLI & 645HLII) and low light (645LL) ecotypes (59) (Table 3). The subcluster HLI comprised up to 67% of all *Prochlorococcus,* the second type HLII (37% of *Prochlorococcus*) was only more abundant at station 19 with 10%. West et al. reported HLII as absent in the North Atlantic (PRIME Cruise, 36.82°N, 19.24°W (59), (57), while this genotype was found in substantial numbers of the HNA group at three southern Atlantic stations. Furthermore West et al. suggested a dominance of the HLI genotype in the Mediterranean Sea and HLII in the Sargasso Sea suggesting a relation to features of the water column like physical properties, ocean circulation or the source of the *Prochlorococcus* seed population (59). Changes in those parameters may explain the detection of HLII in this study. The virtual absence of

low light adapted members of the *Prochlorococcus* (with probe 645LL) may be explained by the water depth analyzed in this study (10 m) as they generally occur in water depths below 40 m (59) (Table 3). *Bacteroidetes* were detected in all water layers. They showed a clear preference for the photic zone of the ARCT (Fig. 2c). We observed a similar distribution of *Bacteroidetes* in the HNA population where they comprised the majority of bacterioplankton at the northern stations (Fig. 3). It was shown that isolates of *Bacteroidetes* possess proteorhodopsins that enables them to grow better with light (22). *Bacteroidetes* are consumers of algae-derived metabolites and highly abundant in areas of primary production, e.g. nutrient rich surface waters and upwelling regimes (52), (48), (25, 40, 45). A positive correlation with high seawater chlorophyll a was shown for the marine *Flavobacteria* (1) and was also observed in this study (r^2 = 0.89; $p<0.005$). *Bacteroidetes* abundance can be further related to picoeukaryotic phytoplankton concentrations (r^2 = 0.92; $p<0.003$). Like *Bacteroidetes* also the *Alphaproteobacteria* were more abundant at the northern stations, in particular *Roseobacter* at station 6 (Table 3). The marine *Roseobacter* clade comprises phylogenetically diverse and physiologically versatile bacterial species (7). Members of *Rhodobacteriaceae* have been found frequently to be abundant in costal areas or shelf regions in the aftermath of phytoplankton blooms (14, 58, 61).

No latitudinal shift was observed with the *Gammaproteobacteria* and members of the *Firmicutes* related SAR406 clade (Fig. 3). The abundance of both bacterial clusters remained stable along the transect. Quantitative measurements of the SAR406 clade have indicated that they are vertically stratified in the water column of both the Atlantic and Pacific Ocean, with peak abundance occurring below the deep chlorophyll maximum (24). Additionally, there are regular seasonal oscillations in the relative abundance of this group in surface waters of the western Sargasso Sea, with a strong positive correlation to surface chlorophyll *a* values (24). Such a correlation with chlorophyll was not found in this study.

Within the *Gammaproteobacteria* a higher percentage of SAR86 cells were detected at the northern stations. The SAR86 cluster is the only numerically abundant *Gammaproteobacteria* SSU rRNA gene type identified in marine plankton clone libraries and has been found in the Pacific and the Sargasso Sea. The positive relation to oxygen (r^2 = 0.83; p = 0.011) and phosphate concentrations (r^2 = 0.79; p = 0.017) affirmed the preference of the SAR86 cluster for highly productive environmental sites like upwelling events (53), nutrient-rich habitats like the North Sea (14) or as shown in this study the Atlantic Arctic province. The presence of the gammaproteobacterial clade SAR86 in the LNA population, as reported previously by Zubkov et al 2002 (63), could not be confirmed in this study (Table 3).

Genome size and copy number

Differentiation of bacterioplankton cells based on their nucleic acid content into two distinct populations (HNA and LNA) has been observed in a wide range of aquatic ecosystems covering large environmental gradients (6) and implies a certain classification of genome size (9). In addition, based on DNA contents of 641 different prokaryotes Islas and colleagues (27) have shown that the genome sizes of free-living prokaryotes are also distributed bimodally, with two distinct peaks at 2 Mb and 4-5 Mb. Bouvier (6) hypothesized that this bimodal distribution of genomes may be linked to the generalized separation of planktonic cells into HNA and LNA and in turn may be indicative of different ecological niches and functions. Previous studies of phylogenetic community compositions of flow sorted bacterioplankton populations provided information about the abundance of major phyla and subgroups (17, 32, 60, 62). The combination of phylogenetic data with available genome sizes suggests that most genomes found in the HNA population are average in size, like the *Roseobacter* clade (3.2 – 4.1 Mbp), members of the *Gammaproteobacteria* (*Congregibacter litoralis*/ OM60 4.4 MBp) or of the cyanobacteria (*Synechococcus* spp. 2.4 – 3.0 Mbp, *Prochlorococcus* spp. 1.6 – 2.4 Mbp). In conclusion, cells of the LNA population would have smaller genomes than found in the HNA population (< 1.6 Mbp). In this study the

majority of LNA cells were comprised of SAR11 together with a low abundance of the OM43 clade. With a genome size of 1.31 Mbp (1,308,759 base pairs) a member of the SAR11 clade, *Candidatus* Pelagibacter ubique, has one of the smallest genomes for a free-living heterotrophic prokaryote (20, 46). In addition recently Giovannoni and colleagues reported that the first axenic isolate of the OM43 clade, strain HTCC2181 had an even smaller genome size with 1.3 Mbp (1,304,428 base pairs) (19). Giovannoni et al. suggested those microorganisms as evidence of streamlining selection. Cause for the small genomes of some marine bacterioplankton is supposedly the elimination of unnecessary DNA from genomes due to selection and the metabolic burden of replicating DNA with no adaptive value. Selection is particularly effective in large sized population and in habitats that are frequently limited in the macronutrients N and P, which are stochiometrically high in nucleid acids. Genome streamlining therefore may lead to specialization of organisms like the OM43 clade. Despite being abundant in productive coastal habitats OM43 is related to Type I methylotrophs of the family of *Methylophilaceae* and specialized for growth solely on oxidized C1 compounds (e.g. methanol) as carbon source (19). Similarly *Candidatus* Pelagibacter ubique achieved its efficiency in a low-nutrient system by using transporters with broad substrate ranges (8) and a number of specialized substrate targets, in particular, nitrogenous compounds and osmolytes (20).

In the HNA population many marine bacterioplankton organisms, albeit those with smaller population sizes and with standard genome sizes (15, 37) harbor a wide range of physiological diversity. For instance marine *Bacteroidetes* are successful in the degradation of particulate organic matter like polysaccharides, proteins (5, 23) and other high-molecular-weight organic matter (12). This versatile life style requires a substantial number of genes for attachment to surfaces or particles, for gliding motility (present in many representatives) and an extensive collection of enzyme systems for polymer degradation, thus leading to large genomes or multiple copies of their genome in *Bacteroidetes*. It seems likely that different populations of the quite diverse *Bacteroidetes* group might have different ecological strategies (2).

Also the genomic and physiological diversity of the *Roseobacter* lineage is considerable. Generally present in the *Roseobacter* spp. are genes for motility, the degradation of aromatic compounds and genes to demethylate dimethylsulphoniopropionate. Additionally, some members of the *Roseobacter* clade possess strain-specific genes for aerobic anoxygenic phototrophy, lithotrophy by the oxidation of carbon monoxide or reduced sulphur compounds, or for phosphonate degradation and nitrite reduction.

Conclusion

It is interesting to find bacteria with standard genome sizes comprising the comparably high diversity of bacterioplankton in the HNA population. Available genome data indicate a trend to find generalist bacteria in the HNA fraction as compared to the LNA fraction. The diversity within the LNA population is restricted to certain bacterial clades (SAR11, OM43), that are specialists in their respective environment. Thus, the classification into HNA and LNA population may not only provide us with hints about the respective genome size but also which lifestyles the cells in their respective population pursue.

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Figure legend

Fig. 1 VISION Cruise track from Rejkiavik, Iceland to Lisbon, Portugal during September – October 2006. Abbreviations: Atlantic Arctic province (ARCT), Northern Atlantic Drift (NADR), North Atlantic Gyral Province (NAG). Flow cytometrically sorted stations are highlighted in black.

Fig. 2 Latitudinal-depth contours of specific microbial groups as relative abundance (% DAPI counts).

Fig. 3 Representative dot plot for flow-sorting of HNA/ LNA populations

Fig. 4 Contribution of major groups to the flow-sorted HNA and LNA populations in two oceanic provinces along the VISION transect.

Tables:

Table 1 Overview of the probes and hybridization conditions applied for CARD-FISH

Table2 Correlation between bacterial groups in flow cytometrical populations and selected parameter. Values of $r^2 < 0.8$ were dismissed as not highly significant and therefore not shown.

Table 3 Composition of the bacterioplankton community in the flow cytometrically sorted groups HNA & LNA as relative abundance (% DAPI counts)

Tables

Table 1: Overview of the probes and hybridization conditions applied for CARD-FISH.

probe	target organisms	sequence (5' → 3')	FA[1] %	reference
Eub338	Bacteria	GCTGCCTCCCGTAGGAGT	35	(3)
Eub338-II	supplement to EUB338	GCAGCCACCCGTAGGTGT	35	(13)
Eub338-III	supplement to EUB338	GCTGCCACCCGTAGGTGT	35	(13)
Non338	control	ACTCCTACGGGAGGCAGC	35	(56)
Cya664	*Cyanobacteria*	GGAATTCCCTCTGCCCC	35	Schönhuber
Pro405	*Prochlorococcus*	AGAGGCCTTCGTCCCTCA	40	(59)
Syn405	*Synechococcus*	AGAGGCCTTCATCCCTCA	40	(59)
645HLI	High-light *Prochlorococcus* group 1	ACCATACTCAAGCCGATC	35	(59)
645HLII	High-light *Prochlorococcus* group 2	ACCATACTCAAGCCTTTC	30	(59)
645LL	Low-light *Prochlorococcus* group	ACCATACTCAAGCCAATC	30	(59)
Alf968	α-subgroup of *Proteobacteria*	GGTAAGGTTCTGCGCGTT		
SAR11-441	SAR11-clade	GGACCTTCTTATTCGGGT	25[3]	(39)
Gam42a[2]	γ-subgroup of *Proteobacteria*	GCCTTCCCACATCGTTT	35	(34)
SAR86-1245	SAR86-clade	TTAGCGTCCGTCTGTAT	35[3]	(62)
Bet42a[2]	β-subgroup of *Proteobacteria*	GCCTTCCCACTTCGTTT	35	(34)
OM43 (62R)	OM43-clade	CTCTCTATGCTGCCGTTCGA	35	(38)
CF319a	*Bacteroidetes*	TGGTCCGTGTCTCAGTAC	35	(33)
SAR406-97	SAR406-clade	CACCCGTTCGCCAGTTTA	40[3]	(16)
SAR324-1412	SAR324-clade	GCCCCTGTCAACTCCCAT	35[3]	Study submitted
Ac1m36[2]	*Actinobacteria* marine cluster	CATAAGGCGCACCGCCAG	30[3]	this study

Abbreviations: FA: formamide (v/v);

[1]: formamide concentration in CARD-FISH hybridisation buffer;
[2]: including an unlabeled competitor probe Bet42a (5'-GCCTTCCCACTTCGTTT-3') or Gam42a (5'- GCCTTCCCACATCGTTT -3') respectively, see (34)) for details; Ac1m36comp (5'-CATTAGGCGCACCGCCAG-3')
[3]: formamide concentration determined or reevaluated by Clone-FISH (49) for this study; detailed information in supplement

Table 2: Correlation between bacterial groups in distinct flow cytometrical populations and selected parameters

	HNA				LNA		
r^2	EUB	CYA664	Pro	CF	EUB	Beta	OM43
Temp			**0.88**		**0.82**		
p			*0.005*		*0.013*		
Ox calib		**0.88**	**0.94**	**0.82**	**0.85**		
p		*0.005*	*0.001*	*0.013*	*0.009*		
Sal			**0.81**		**0.8**		
p			*0.014*		0.016		
PO4		**0.89**	**0.97**	**0.95**		0.57	**0.91**
p		*0.004*	*<0.001*	*<0.001*		*0.081*	*0.003*
NO2+3			**0.81**	**0.92**		**0.81**	**0.99**
p			*0.014*	*0.002*		*0.015*	*<0.001*
NH4				0.41		**0.82**	0.606
p				*0.171*		*0.012*	*0.068*
Pico			**0.81**	**0.92**		**0.74**	**0.94**
p			*0.013*	*0.003*		*0.026*	*<0.001*

Table 3: Composition of the bacterioplankton community in the flow cytometrically sorted groups HNA & LNA as relative abundance (% DAPI counts)

Probes	HNA						LNA					
	St. 3	St.6	St.16	St.17	St.18	St.19	St.3	St.6	St.16	St.17	St.18	St.19
EUBI-III	94.3	89.3	89.9	88.2	90.5	84.8	89.3	83.3	70.9	78.4	71.7	76.6
CYA664	1.1	4.5	44.7	29.1	40.8	32.7	n.d.	n.d.	0.4	n.d.	n.d.	n.d.
405Syn	0.9	3.4	2.0	2.3	2.1	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
405Pro	0.8	2.9	38.6	33.9	43.8	37.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
645HLI	n.d.	n.d.	25.9	17.4	20.6	3.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
645HLII	0.4	n.d.	n.d.	1.2	3.9	13.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
645LL	n.d.	n.d.	n.d.	1.9	n.d.	0.8	n.d.	n.d.	n.d.	0.3	n.d.	n.d.
CF319a	26.3	32.4	10.3	7.4	3.3	7.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gam42	16.5	11.8	15.3	13.4	14.2	10.4	0.4	0.7	1.9	2.3	2.7	0.2
SAR86-1245	4.1	3.5	n.d.	2.1	n.d.	0.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alf968	19.3	24.9	17.3	26.4	2.0	22.6	68.3	71.0	55.8	54.9	62.6	65.0
Ros537	n.d.	10.3	4.5	4.5	2.7	3.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SAR11-441R	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	72.3	61.9	49.5	45.4	72.1	74.4
SAR406-97	2.8	4.1	8.6	8.6	n.d.	1.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SAR324-1412	n.d.	n.d.	0.5	n.d.	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bet42a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.6	4.6	1.6	2.0	2.8	1.8
OM43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	0.9	n.d.	n.d.	n.d.	n.d.

Abbreviations: n.d. not detected

Fig. 1

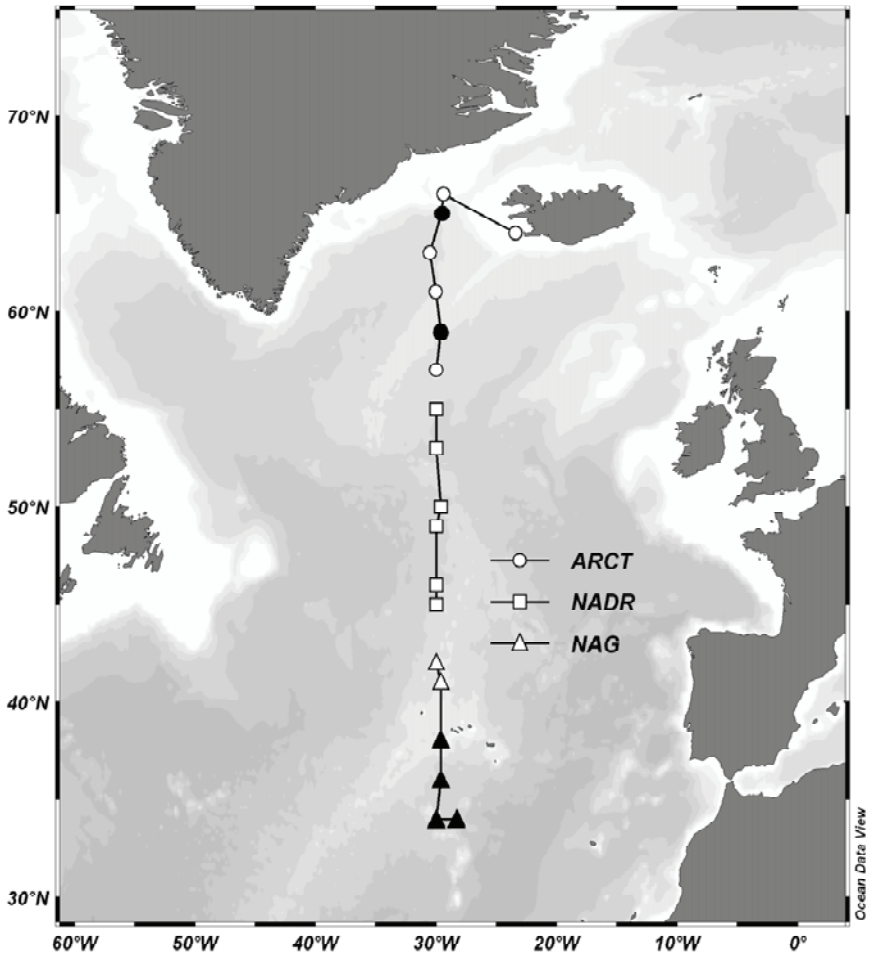

70°N
60°N
50°N
40°N
30°N
60°W
50°W
40°W
30°W
20°W
10°W
0°
ARCT
NADR
NAG
Ocean Data View

Fig. 2

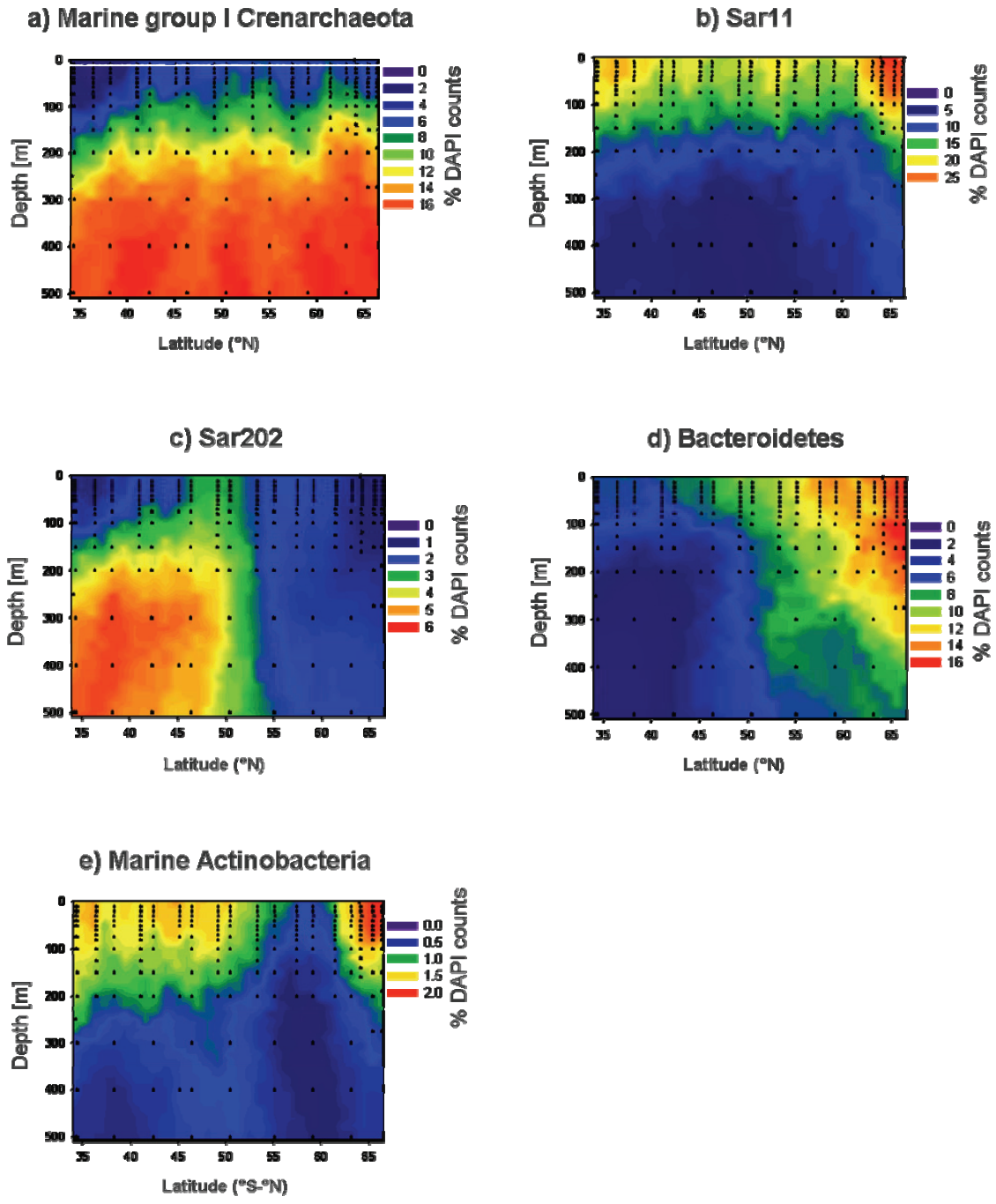

a) Marine group I Crenarchaeota
Depth [m]
Latitude (°N)
% DAPI counts
b) Sar11
Depth [m]
Latitude (°N)
% DAPI counts
c) Sar202
Depth [m]
Latitude (°N)
% DAPI counts
d) Bacteroidetes
Depth [m]
Latitude (°N)
% DAPI counts
e) Marine Actinobacteria
Depth [m]
Latitude (°S-°N)
% DAPI counts

Fig. 3

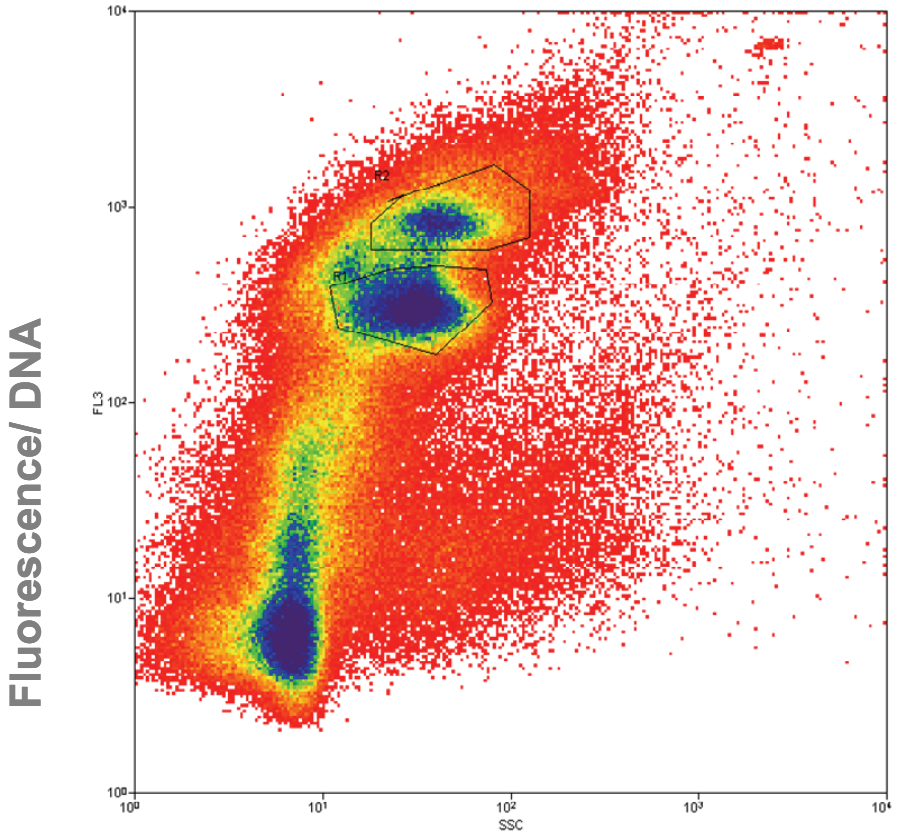
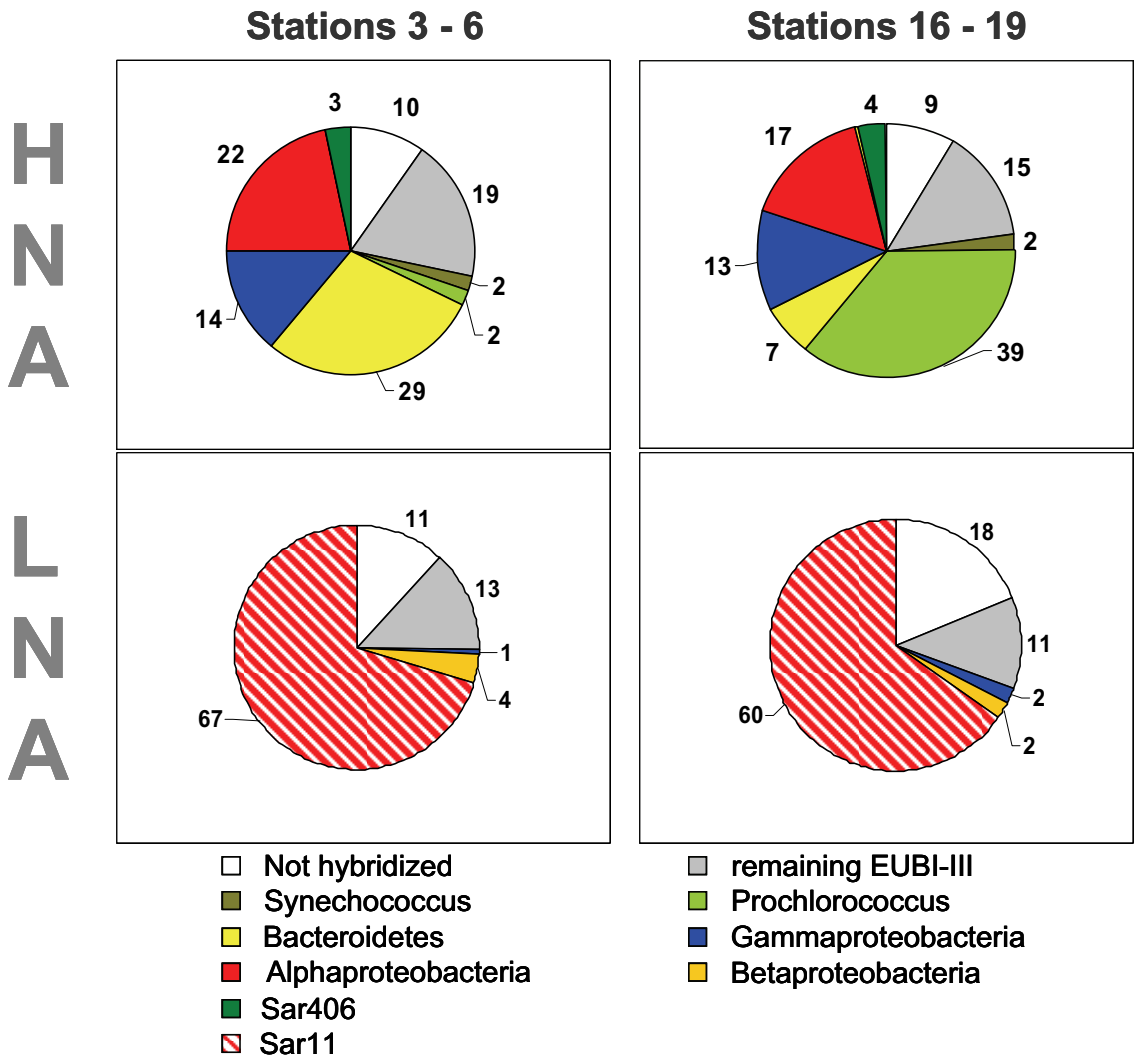


Fig. 4


Stations 3 - 6
Stations 16 - 19
HNA
LNA
3
10
22
19
2
14
2
29
4
9
17
15
2
13
7
39
11
13
1
4
67
18
11
2
60
2
Not hybridized
Synechococcus
Bacteroidetes
Alphaproteobacteria
Sar406
Sar11
remaining EUBI-III
Prochlorococcus
Gammaproteobacteria
Betaproteobacteria

Das Dankeschön

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