RESEARCH ARTICLE

Seasonal succession and UV sensitivity of marine bacterioplankton at an Antarctic coastal site

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Abstract

Despite extensive microbial biodiversity studies around the globe, studies focusing on diversity and community composition of Bacteria in Antarctic coastal regions are still scarce. Here, we studied the diversity and development of bacterioplankton communities from Prydz Bay (Eastern Antarctic) during spring and early summer 2002–2003. Additionally, we investigated the possible shaping effects of solar UV radiation (UV-R: 280–400 nm) on bacterioplankton communities incubated for 13–14 days in 650-L minicosm tanks. Ribosomal DNA sequence analysis of the natural bacterioplankton communities revealed an initial springtime community composed of three evenly abundant bacterial classes: *Cytophaga–Flavobacteria–Bacteroidetes* (CFB), *Gammaproteobacteria* and *Alphaproteobacteria*. At the end of spring, a shift occurred toward a CFB-dominated community, most likely a response to the onset of a springtime phytoplankton bloom. The tail end of Prydz Bay clone library diversity revealed sequences related to *Deltaproteobacteria*, *Verrucomicrobiales*, *Planctomycetes*, *Gemmatimonadetes* and an unclassified bacterium (ANT4E12). Minicosm experiments showed that incubation time was the principal determinant of bacterial community composition and that UV-R treatment significantly changed the composition in only two of the four experiments. Thus, the successional maturity of the microbial community in our minicosm studies appears to be a greater determinant of bacterial community composition rather than the nonprofound and subtle effects of UV-R.

Introduction

Bacterioplankton play a key role in the global carbon cycle. The main carbon pools in marine systems – particulate organic carbon and dissolved organic carbon – are largely produced by phytoplankton, while heterotrophic bacteria are largely responsible for the degradation and recycling of organic carbon and essential nutrients, thereby fuelling marine productivity through the microbial loop (Azam, 2001).

Initially, studies conducted on Antarctic bacterioplankton focused on their role in the carbon cycle and studied the bacterial carbon uptake, by measuring productivity and activity in relation to phytoplankton abundance. Thereafter, scientific interest shifted toward determining the species composition of Antarctic bacterioplankton communities. Species identification was first implemented by cultivation and later by culture-independent approaches, provided by the development of molecular tools. Analysis of the 16S rRNA gene from various oceanic regions revealed an unexpected diversity and led to the discovery of numerous novel bacterial and archaeal groups (Giovannoni *et al.*, 1990; Fuhrman *et al.*, 1992; DeLong, 2001; Venter *et al.*, 2004). In recent years, the number of studies devoted to analyzing the composition of marine bacterial communities has increased considerably, providing a huge and valuable database of rRNA sequences. Yet, our knowledge of Antarctic bacterioplankton diversity and dynamics is still limited (Murray & Grzymski, 2007). Most studies have been performed in the Antarctic Peninsula and Ross Sea regions (Gentile *et al.*, 2006; Grzymski *et al.*, 2006), while bacterioplankton communities from other regions, such as the Eastern Antarctic,

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are still scarcely studied. Furthermore, little is known about the possible shaping effect of environmental conditions on marine bacterial communities in this area. Natural UV radiation (UV-R: 280–400 nm), whether or not enhanced by springtime stratospheric ozone depletion, is thought to affect Antarctic marine organisms significantly, but primarily bacterioplankton through the induction of DNA damage (Visser *et al.*, 1999; Davidson & van der Heijden, 2000; Buma *et al.*, 2001). Therefore, it can be hypothesized that prolonged natural UV-R exposure significantly shapes bacterial community composition, as occasionally demonstrated for other areas (Arrieta *et al.*, 2000; Winter *et al.*, 2001; Alonso-Sáez *et al.*, 2006).

In the present study, the bacterial community composition of subsurface samples collected from late spring to early summer from Prydz Bay (Eastern Antarctic) was studied by analyzing clone libraries of partial 16S rRNA gene fragments. The time span of the sampling period coincided with the transition from sea ice covered, sea ice melting toward the peak of the summer phytoplankton bloom, providing an insight into the natural succession of the bacterial community. Additionally, four series of UV-R incubation experiments were conducted using natural microbial communities harvested from Prydz Bay during the season. These experiments were performed for up to 2 weeks in 650-L containers covered with six different spectral UV cut-off filters. Community shifts were assessed over time by generating molecular fingerprints of partial 16S rRNA gene fragments by denaturing gradient gel electrophoresis (DGGE). Samples exposed to the lowest and the highest UV-R conditions were selected for cloning and sequencing.

Materials and methods

Sample collection and experimental setup

Prydz Bay subsurface seawater (2 m depth) was sampled 60 m offshore from Davis Station (68°35′S, 77°58′E), Antarctica, on November 11 and 26, December 14 and January 6, 2002/2003. Samples were collected using a Teflon diaphragm pump to minimize both contamination of the seawater and damage to the microbial community. The intake was covered with a 200-μm mesh to exclude mesozooplankton. In November and December, samples were obtained through a hole drilled in the sea ice, while later in summer (January), when the sea ice had disappeared, a buoy and anchor were deployed to pump water from the same location and depth. Samples (2 L) were immediately transported to the laboratory for further processing.

Natural Prydz Bay marine microbial communities ($< 200\,\mu m$) were used for the four UV-R incubation experiments. Briefly, six 650-L minicosm tanks, housed in a refrigerated shipping container, were filled simultaneously and directly from the sampling site with the 200-μm prefiltered seawater samples. Each minicosm was gently mixed by a paddle set at 3 r.p.m. to prevent cell sedimentation and to ensure vertical mixing of the community over the entire water column. The marine microbial communities were incubated for 13–14 days at ambient temperatures ($\pm 0.84\,^{\circ}C$) under six distinct solar irradiation conditions: PAR (P), one PAR+UVA (A) and four PAR+UVA+UVB (B_1–B_4). More details on the experimental setup and UV-irradiation conditions are provided in Piquet *et al.* (2008) and Thomson *et al.* (2008). The initial sample (T_0), obtained from the pump while filling the minicosm tanks, reflected the natural community in Prydz Bay. Teflon sample lines fitted to the tanks were used to obtain samples from each of the six tanks after 7 and 14 days (T_7 and T_{14}, respectively).

Sample handling and DNA isolation

Each sample (2 L) was prefiltered over 2-μm pore-size filters, then collected onto 0.2-μm pore-size polycarbonate filters (Millipore). The samples were stored in 1.5-mL sterile lysis buffer (EDTA 40 mM; Tris-HCl 50 mM, pH 8.5; sucrose 0.75 M) at $-80\,^{\circ}C$ until further processing. DNA isolation was performed by application of mechanical, chemical and enzymatic DNA extraction procedures as described previously (Piquet *et al.*, 2008). The DNA pellet was resuspended in sterile MilliQ and purified using the Wizard DNA Clean-Up kit (Promega Benelux B.V., Leiden, the Netherlands).

16S rRNA gene amplification, cloning and sequencing

A 1400-bp fragment of the 16S rRNA gene was amplified using the bacterial-specific primer set: B8F (5′-AGAGTTTGATCMTGGCTCAG-3′) forward primer (Edwards *et al.*, 1989) and universal U1406R (5′-ACGGGCGGTGTGTRC-3′) reverse primer (Lane, 1991). Twenty-five microliters of amplification mixtures consisted of a dNTP mix in a final concentration of 200 μM, 200 nM primers, 1 × PCR buffer (GE Healthcare, Diegem, Belgium), 2.3 mM $MgCl_2$, 2% dimethyl sulfoxide, $0.2\,mg\,mL^{-1}$ bovine serum albumin (Roche, Woerden, the Netherlands) and 1 U Taq DNA polymerase (GE Healthcare). The reaction was run on a thermal cycler (GeneAmp®, PCR system 9700, Perkin-Elmer, Applied Biosystems, Nieuwerkerk a/d IJsel, the Netherlands) using the following program: 94 °C for 130 s; 35 cycles of 94 °C for 30 s, 56 °C for 45 s, 72 °C for 130 s; followed by a final elongation step of 72 °C for 7 min. PCR products were separated by DNA gel electrophoresis on a 1% agarose gel, stained with ethidium bromide and visualized with an Image Master (Amersham Biosciences, Pharma Biotech, Roosendaal, the Netherlands). Amplicon size and

yield was estimated by comparison with a DNA Smart Ladder (Eurogentec, Maastricht, the Netherlands).

Clone libraries were generated for the selection of 12 samples: the inoculum samples (T_0) and T_{14} samples incubated under the lowest (P) and highest (B4, B3 for experiment 2) irradiation conditions. PCR products were cloned in the pGEM-t Easy vector system (Promega Benelux B.V.) and transformed to *Escherichia coli* strain JM109 according to the manufacturer's protocol. Positive inserts were amplified by colony PCR using the pGEM-t-specific primers T7 (5′-TAATACGACTCACTCTAGGG-3′) and SP6 (5′-GATTTAGGTGACACTATAG-3′). PCR mixtures were identical to the above description, while the amplification conditions were: 94 °C for 5 min, 30 cycles of 94 °C for 60 s, 48 °C for 30 s, 72 °C for 4 min, followed by a final elongation step of 72 °C for 7 min. Amplicons were cleaned by polyethylene glycol 8000 (Sigma-Aldrich Chemie B.V., Zwijndrecht, the Netherlands) precipitation and insert size was checked on agarose gels. All clones with inserts of the proper size were selected for sequencing. Positive amplicons were selected for partial 16S rRNA gene sequencing using the Big Dye chemistry (Applied Biosystems, Nieuwerkerk a/d IJsel, the Netherlands) and the reverse primer U1406R as the sequencing primer. Sequence products were cleaned by standard isopropanol precipitation and analyzed on an automated ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA).

Sequence analysis

The partial sequences obtained were manually checked with CHROMAS v.2.3.1. Suspected chimeric sequences were checked online using the Ribosomal Database Project II 8.1 CHIMERA CHECK program. Chimeric sequences were excluded from the dataset. MOLECULAR EVOLUTIONARY GENETICS ANALYSIS (MEGA) version 4.0 (Tamura *et al.*, 2007) and its add-in CLUSTALW was used to align the DNA sequences and to create neighbor-joining trees based on the maximum composite likelihood algorithm (Hartl *et al.*, 1994; Zhu & Bustamante, 2005) with 1000 bootstrap permutations (Felsenstein, 1985). In the phylogenetic tree, sequences with 99% identity were grouped into operational taxonomic units (OTUs). OTUs were classified at the taxonomic phylum and class level as deduced from their closest match obtained with NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST), which were included in the phylogenetic analysis.

Shannon–Weaver indices of diversity and richness estimators Chao and Ace were calculated for each clone library and the entire sequence dataset using the DOTUR program (http://www.plantpath.wisc.edu/fac/joh/dotur.html) on the distribution of bacterial OTUs defined at the 99% identity level using average neighbor sequence assignment (Schloss & Handelsman, 2005). For comparison purposes, the bacterioplankton community composition was simplified and sequences (excluding 16S-like chloroplast sequences) were redistributed into OTUs at the 97% identity level and presented as pie charts to depict changes in community composition among clone libraries. The similarity between the bacterial communities of the samples selected for clone libraries was determined using the Morisita–Horn index of similarity, which is not affected by differences in sample size (Wolda, 1981). Comparison of the bacterial communities was performed with PAST (Hammer *et al.*, 2001) by cluster analysis and presented in the form of a dendrogram.

DGGE

In order to maximize the similarity between the bacterioplankton community analyzed through cloning sequencing and community fingerprinting: a nested PCR was performed on the products of the B8F-U1406R amplification, using the primer set 968GC-1401R that amplifies an ~430-bp fragment of the variable V6 region of the 16S rRNA bacterial gene (Nübel *et al.*, 1996). The forward primer 968 was extended with a GC-rich clamp at the 5′-end for DGGE application (Muyzer *et al.*, 1993; Nübel *et al.*, 1996). DGGE was run on the PhorU system (Ingeny, Goes, the Netherlands) as described previously (Muyzer *et al.*, 1993, 2004). The best separation was obtained on a 6% polyacrylamide gel using a 40–70% urea-formamide DNA denaturing gradient, with 100% urea-formamide being defined as 7 M urea (Bio-Rad, Veenendal, the Netherlands) and 40% deionized formamide (Sigma-Aldrich Chemie B.V.). We ran 200 ng of PCR product for each sample, supplemented with loading buffer (0.05% w/v bromophenol blue, 40% sucrose, 0.1 M EDTA pH 8.0, 0.5% sodium lauryl sulfate), for 16 h at 100 V in 1 × TAE buffer. Gels were stained with Sybr®GOLD (Invitrogen, Molecular Probes, Breda, the Netherlands) and visualized with UV-R using the Image Master (Amersham Biosciences). A marker sample was added onto each gel for references and analytical purposes.

DGGE pattern analysis was performed using BioNumerics® version 3.5 (Applied Maths N.V., Sint-Martens-Latem, Belgium) as described in Piquet *et al.* (2008). In brief, the gels were digitized and normalized using the flanking marker bands. BIONUMERICS translated band patterns into a presence/absence matrix. Ordination analysis was run on the presence and absence of bands, from each experiment, using CANOCO version 4.5.2 (Ter Braak & Šmilauer, 1998). Both time and cumulated UV-irradiance variables were used in the redundancy analysis (Van den Wollenberg, 2007). A Monte–Carlo's unrestricted permutation test (1000 permutations) was run to determine the significance of the variables in explaining the observed variation.

Nucleotide sequence accession numbers

Sequences reported in this paper have been deposited in GenBank under accession numbers GU191464–GU191546.

Results

Bacterial community composition

A total of 671 partial 16S rRNA gene sequences (~750 bp) were recovered in this study. Phylogenetic analysis revealed that these sequences included members of the *Cytophaga–Flavobacteria–Bacteroidetes* (CFB), *Gammaproteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria*, *Planctomycetes*, *Gemmatimonadetes*, *Verrucomicrobia* and an unclassified bacterium class (Fig. 1). Additionally, 90 sequences were identified to be related to phytoplankton 16S rRNA gene-like chloroplast/plastids (16S-like, Fig. 1). Phylogenetic analysis unveiled 83 OTUs, including 41 unique sequences (phylotypes). An overview of all identified OTUs is given in Table 1, with the nearest uncultivated clone, the nearest cultivated BLAST match or both, if available.

Overall, members of the CFB class dominated with 284 sequences and included the most abundant OTU '120-52' related to the uncultured *Polaribacter* Arctic 96B-11 clone (AF354261). The *Gammaproteobacteria* was the most diverse class with 31 OTUs, comprising 15 phylotypes. Within the sequence dataset, four phylotypes had 93% or less identity to any sequence from the NCBI database, suggesting that these have derived from yet undiscovered species: two CFB (156-46 and 120-51), one *Gemmatimonadetes* (141-63) and one alphaproteobacterium (42-64).

Prydz Bay bacterial community succession

Samples collected on November 11 ($T_0$1), November 26 ($T_0$2), December 14 ($T_0$3) 2002 and January 6 ($T_0$4) 2003 provide an insight into the seasonal changes of natural Prydz Bay bacterial communities from mid-spring to early summer. Clones with at least 97% sequence identity were grouped into another set of OTUs (51 in total) and were named according to taxonomic affiliation. For example, 'uGprot' stands for 'uncultivated *Gammaproteobacteria*'; 'uPolarib' for uncultivated *Polaribacter*; and 'Roseob2' for cluster 2 of *Roseobacter*-related sequences. Their proportion within each clone library is presented in Fig. 2. *Gammaproteobacteria* (shades of blue) constituted an abundant fraction of the bacterial community comprising 41%, 32%, 43% and 35% of the sequences at the start of experiments 1–4 ($T_0$1–$T_0$4), respectively. In mid-spring ($T_0$1 and $T_0$2), *Alphaproteobacteria* (shades of red to yellow) codominated the bacterial community, but they were gradually replaced by members of the CFB class, along with the appearance of some 'rare sequences' related to *Deltaproteobacteria*, *Planctomycetes* and *Gemmatimonadetes*. In the last two samples ($T_0$3 and $T_0$4), collected in late-spring and early summer, the CFBs dominated the Prydz Bay bacterial community, forming 45% and 52% of the community. Sequences related to the *Polaribacter* genus, which includes the 'uPolarib' OTU (clone '120-52' related to clone Arctic 96B-11, AF354621) and 'Polarib' OTU (clone '120-39' related to *Polaribacter irgensii*, AY771712), formed the most abundant group, comprising 30% and 40% of the sequences.

The samples collected in spring before the sea-ice break-up had the highest microbial diversity, with Shannon–Weaver indices (H') of 2.6, 2.7 and 2.6. After the sea-ice break-up, the diversity index decreased to 2.1 (Table 2). The first sample had the highest richness, with a predicted total of 81 and 89 OTUs using Chao and Ace's indices, respectively.

Community shifts during incubation

Samples collected from the Prydz Bay community were used in the minicosm experiments and incubated under six distinct irradiation conditions. The average downwelling UV-R dose in the middle of each minicosm tank was cumulated over time for each tank. After 14 days, the communities incubated under PAR irradiation had experienced an average 83.1 ($\pm$ 4.2) J m^{-2} UV-R erythemal dose, while under the highest UV-R condition (B_4), the average cumulated irradiation was 1892.4 ($\pm$ 74.7) J m^{-2} (see Table 1 in Piquet *et al.*, 2008).

Samples $T_{14}B_4$_Exp2 and T_7B1_Exp4 were lacking in the analysis: DNA extraction failed for samples $T_{14}B_4$_Exp2, while T_7B1_Exp4 could not be sampled due to water shortage during the experiment. Samples collected from each minicosm after 7 and 14 days of incubation were run alongside on a DGGE (Fig. 3).

Overall, the duration of incubation was the principal factor that affected the composition of the bacterial community, while UV-R had a significant effect on half of the experiments performed. In experiment 1 (Fig. 3a), the initial community fingerprint (T_0) was conserved in all tanks after 7 days of incubation, but changed after 14 days of incubation, with communities exposed to the highest UVB irradiances (B_3 and B_4), showing a decrease in band numbers and intensity. UV-R-induced changes in species composition were confirmed by a Monte–Carlo permutation test (Table 3), where 15.7% of the variation among band patterns was explained by UV-R. However, the variable incubation time significantly explained 27.8% of the variance. Similarly, UV-R significantly explained 15.4% of the variance in band patterns for experiment 3 (Fig. 3c), while the incubation time significantly explained 30.3% of the variance.

In contrast, only the incubation time significantly explained the variance among the banding patterns in

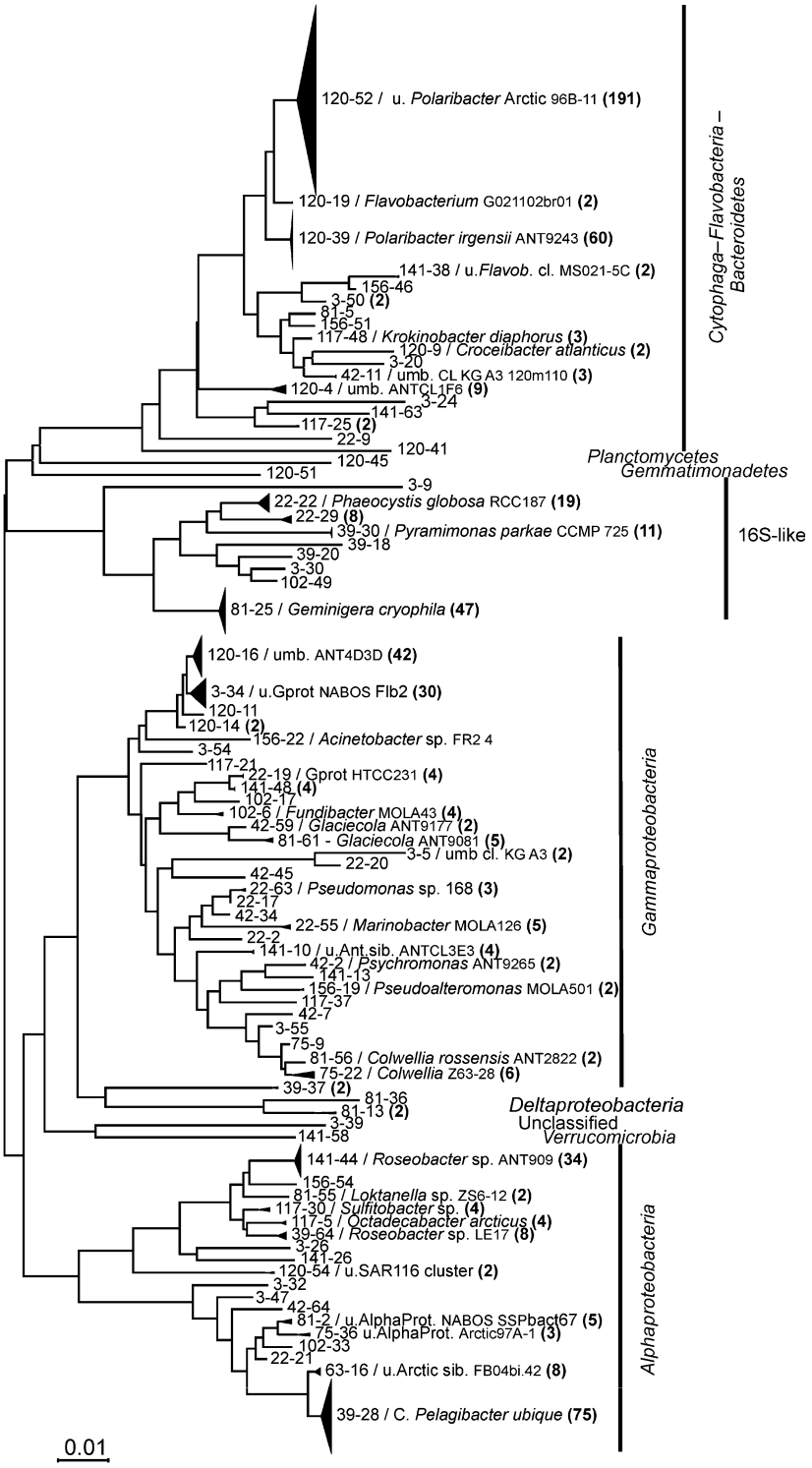

Fig. 1. Neighbor-joining phylogenetic tree of partial 16S rRNA gene sequences.

experiments 2 and 4 (Fig. 3b and d). In experiment 2 (Fig. 3b), banding patterns differed little between incubation time and light treatments; however, incubation time explained a significant 20% of the variance. In experiment 4 (Fig. 3d), banding patterns were also similar among incubation time and light treatments, except in sample T_7A_1, which was

Table 1. Description of OTUs shown in the phylogenetic tree and their respective NCBI BLAST of best uncultivated and cultivated matches

	OTUs	Accession no.	Nearest uncultivated BLAST match	Accession no.	Similarity (%)	Nearest cultivated BLAST match	Accession no.	Similarity (%)
CFB	120-52	GU191532	U. *Polaribacter* Arctic 96B-11	AF354621	99	*Polaribacter irgensii*	AY771712	99
	120-19	GU191527	*Flavobacteriaceae* Bac. G021102br01	AY353812	99	*Polaribacter franzmanii*	NR025959	99
	120-39	GU191528	U. Mar. Bac. Cl. ANT13D9	DQ925855	99	*Polaribacter irgensii* strain ANT9243	AR167325	99
	141-38	GU191537	U. Bac. ARCTIC37_F_06	EU795244	99	NA		
	156-46	GU191544	U. Bac. Cl. 131768	AY922235	93	*Flavobacteriaceae* Bac. 14III/A01/012	AY576695	93
	3-50	GU191474	U. Bac. ARCTIC26_B_07	EU795086	100	NA		
	81-5	GU191505	Arctic sea ice Bac. ARK10217	AF468427	99	*Flavobacteriaceae* Bac. G512M1	AY285948	100
	156-51	GU191545	U. Mar. Bac. Cl. KG_A3_120m29	EU005733	100	*Bizionia* sp. J69	EU143366	96
	117-48	GU191522	NA			*Krokinobacter diaphorus*	AB198089	97
	120-9	GU191524	NA			*Croceibacter atlanticus* strain HTCC2559T	AY163576	99
	3-20	GU191466	NA			*Flavobacteriaceae* Bac. G812M2	AY298788	95
	42-11	GU191495	U. Mar. Bac. Cl. KG_A3_120m110	EU005814	100	Mar. Bac. CON-A2	EF100858	97
	120-4	GU191523	U. Mar. Bac. Cl. AntCL1F6	DQ906732	98	*Flexibacter aggregans* ssp. catalaticus	AB078042	92
	3-24	GU191467	U. *Bacteroidetes* Bac. Cl. NABOS_SSPbact85	EU544840	99	*Flavobacterium indicum* strain GPTSA100-9	AY904351	83
	141-63	GU191541	U. CFB group Bac. MERTZ_0CM_154	AF424351	90	*Owenweeksia hongkongensis*	AB125062	88
	117-25	GU191519	U. *Bacteroidetes* Bac.	AB189346	97	*Owenweeksia hongkongensis*	AB125062	91
	22-9	GU191478	U. Bac. Cl. S23_1585	EF573486	99	*Microscilla* sp. DG946	AY258124	94
	120-41	GU191529	U. *Bacteroidetes* Bac. Cl. 131719	AY922223	99	*Bacteroidetes* Bac. MOLA 103	AM990877	98
P.	120-45	GU191530	U. Bac. Cl. FS266-28B-03	DQ513078	98	*Planctomycetes* A-2	AM056027	83
G.	120-51	GU191531	U. Bac. Cl. P9X2b2H06	EU491266	89	Bac. Ellin5220	AY234571	81
16S-like	3-9	GU191465	NA			*Mantoniella squamata*	X90641	98
	22-22	GU191483	U. Bac. Cl. b78	EU919793	99	*Phaeocystis globosa* strain RCC187	AY702143	99
	22-29	GU191484	U. Bac. Cl. S25	EF574491	98	*Dinophysis mitra* chloroplast	AB199883	96
	39-30	GU191490	U. Prasinophyte Cl. MC597	EF051850	96	*Pyramimonas parkeae*	AF393608	91
	39-18	GU191487	NA			*Ankylochrysis lutea* strain RCC286	AY702146	97
	39-20	GU191488	U. Bac. Cl. A072	FJ456835	96	*Bolidomonas mediterranea* strain RCC238	AY702144	96
	3-30	GU191469	NA			*Thalassiosira antarctica* isolate C128	FJ002200	99
	102-49	GU191516	NA			*Asterionellopsis glacialis* isolate C42	FJ002233	99
	81-25	GU191507	U. Bac. Cl. s116	EU919855	98	*Geminigera cryophila* plastid	AB07311	98
Gammaproteobacteria	120-16	GU191513	U. Bac. Cl. S28	EU919834	99	*Gammaproteobacteria* IMCC2047	EF468718	92
	3-34	GU191471	U. Mar. Bac. Cl. FB04bw.22	EU837024	99	*Gammaproteobacteria* IMCC2047	EF468718	93
	120-11	GU191525	U. Mar. Bac. Cl. FB04bw.22	EU837024	95	*Glaciecola* sp. NF1-37	FJ196025	91
	120-14	GU191526	U. Mar. Bac. Cl. AntCL1C7	DQ906718	99	*Gammaproteobacteria* IMCC2047	EF468718	93
	156-22	GU191543	NA			*Acinetobacter* sp. BF-5	FJ592170	99
	3-54	GU191475	NA			*Pseudomonas stutzeri*	AM905854	95
	117-21	GU191518	U. Bac. Cl. B78-116	EU287080	99	Mar. Bac. MSC35	EU753145	93
	22-19	GU191480	U. Mar. Bac. Cl. KG_A3_120m32	EU919848	99	*Pseudomonadaceae* Bac. MOLA 455	AM990927	99
	141-48	GU191539	U. Bac. ARCTIC09_G_07	EU795095	99	*Pseudomonadaceae* Bac. MOLA 455	AM990927	99
	102-17	GU191514	NA			*Alcanivorax* sp. PA7	EU647560	99
	102-6	GU191512	NA			*Fundibacter* sp. MOLA 43	AM990818	99
	42-59	GU191498	NA			*Glaciecola* sp. ANT9177	FJ196025	100
	81-61	GU191511	NA			Arctic sea ice Bac. ARK10130	AF468395	96

Table 1. Continued.

	OTUs	Accession no.	Nearest uncultivated BLAST match	Accession no.	Similarity (%)	Nearest cultivated BLAST match	Accession no.	Similarity (%)
	3-5	GU191464	U. Mar. Bac. Cl. KG_A3_120m16	EU005720	99	NA		
	22-20	GU191481	U. Bac. ARCTIC10_F_05	EU795251	99	NA		
	42-45	GU191497	U. Bac. ARCTIC31_H_08	EU795096	100	Thiotrophic endosymbiont	EU683304	95
	22-63	GU191486	NA			*Pseudomonas* sp. 168	AM396934	99
	22-17	GU191479	NA			*Pseudomonas* sp. NUST03	AY293865	99
	42-34	GU191496	NA			*Pseudomonas* sp. 168	AM396934	96
	22-55	GU191485	NA			*Marinobacter* sp. MOLA 126	AM990900	99
	22-2	GU191477	NA			*Cycloclasticus spirillensus*	DQ659429	99
	141-10	GU191534	U. Antarctic sea ice Bac. Cl. AntCL3E3	DQ906761	99	*Gammaproteobacteria* UMB6E	AF505730	98
	42-2	GU191493	NA			*Psychromonas* sp. ANT9265	AY167326	99
	141-13	GU191535	NA			*Moritella* sp. JML-2005	DQ087416	100
	156-19	GU191542	NA			*Pseudoalteromonas* sp. Mn13	FJ594949	100
	117-37	GU191521	NA			*Marinomonas* sp. NF3-30	FJ196055	99
	42-7	GU191494	NA			*Alteromonadaceae* Bac. SN-1009	AB097610	97
	3-55	GU191476	U. Bac. ARCTIC03_F_03	EU795247	99	*Colwellia* sp. ANT9381	AY167329	97
	75-9	GU191501	NA			*Colwellia* sp. ANT9183a	DQ906771	99
	81-56	GU191510	NA			*Colwellia rossensis* strain ANT9279	AY167311	99
	75-22	GU191502	NA			*Colwellia* sp. ZS3-28	FJ196031	99
	39-37	GU191491	U. Bac. Cl. s2	EU919823	99	NA		
DP.	81-36	GU191508	U. Bac. Cl. B8S-8	EU652615	98	*Bacteriovorax* sp. NZAH13	DQ631790	94
	81-13	GU191506	U. *Deltaproteobacteria* Sva0447	AJ240999	99	*Bacteriovorax* sp. EPC3	AY294222	90
U.	3-39	GU191472	U. Mar. Bac. Ant4E12	DQ295238	100	NA		
V.	141-58	GU191540	U. *Verrucomicrobiales* cl.	DQ351768	96	*Fucophilus fucoidanolyticus*	AB073078	96
Alphaproteobacteria	141-44	GU191538	NA			*Roseobacter* sp. ANT909	AY167254	100
	156-54	GU191546	NA			*Roseobacter* sp. ANT9274	AY167261	99
	81-55	GU191509	NA			*Loktanella* sp. ZS6-12	FJ196061	99
	117-30	GU191520	U. Bac. Cl. EPR4055-N3-Bc60	EU491925	99	*Sulfitobacter dubius* strain LMG20555	DQ915635	99
	117-5	GU191517	NA			*Octadecabacter arcticus* strain CIP106732	DQ915618	99
	39-64	GU191492	U. Mar. Bac. Cl. KG_C11_100m8	EU005899	99	NA		
	3-26	GU191468	U. *Alphaproteobacteria* Cl. MS190-2A	EF508147	99	NA		
	141-26	GU191536	NA			*Hyphomonas* sp. MOLA 116	AM990890	99
	120-54	GU191533	U. Mar. Bac. Cl. KG_C11_100m97	EU005965	99	NA		
	3-32	GU191470	U. Bac. Cl. 1C226611	EU799056	95	NA		
	3-47	GU191473	U. Bac. Cl. 4C230385	EU802984	96	NA		
	42-64	GU191499	U. *Alphaproteobacteria* Cl. NABOS_SSPbact67	EU544837	91	NA		
	81-2	GU191504	U. *Alphaproteobacteria* Cl. NABOS_SSPbact67	EU544837	99	NA		
	75-36	GU191503	U. Bac. Cl. 1C227590	EU799917	99	NA		
	102-33	GU191515	U. Mar. Bac. Cl. AntCL1F11	DQ906730	99	NA		
	22-21	GU191482	U. Bac. Cl. b107	EU919806	98	NA		
	63-16	GU191500	U. Bac. Cl. 2C228982	EU800782	99	*Pelagibacter ubique* strain HTCC1062	AF510191	99
	39-28	GU191489	NA			*Pelagibacter ubique* strain HTCC1062	EU836995	99

P., *Planctomycetes*; G., *Gemmatimonadetes*; DP., *Deltaproteobacteria*; U., uncultivated; V., *Verrucomicrobia*; Bac., bacterium; Mar., marine; Cl., clone; NA, not available.

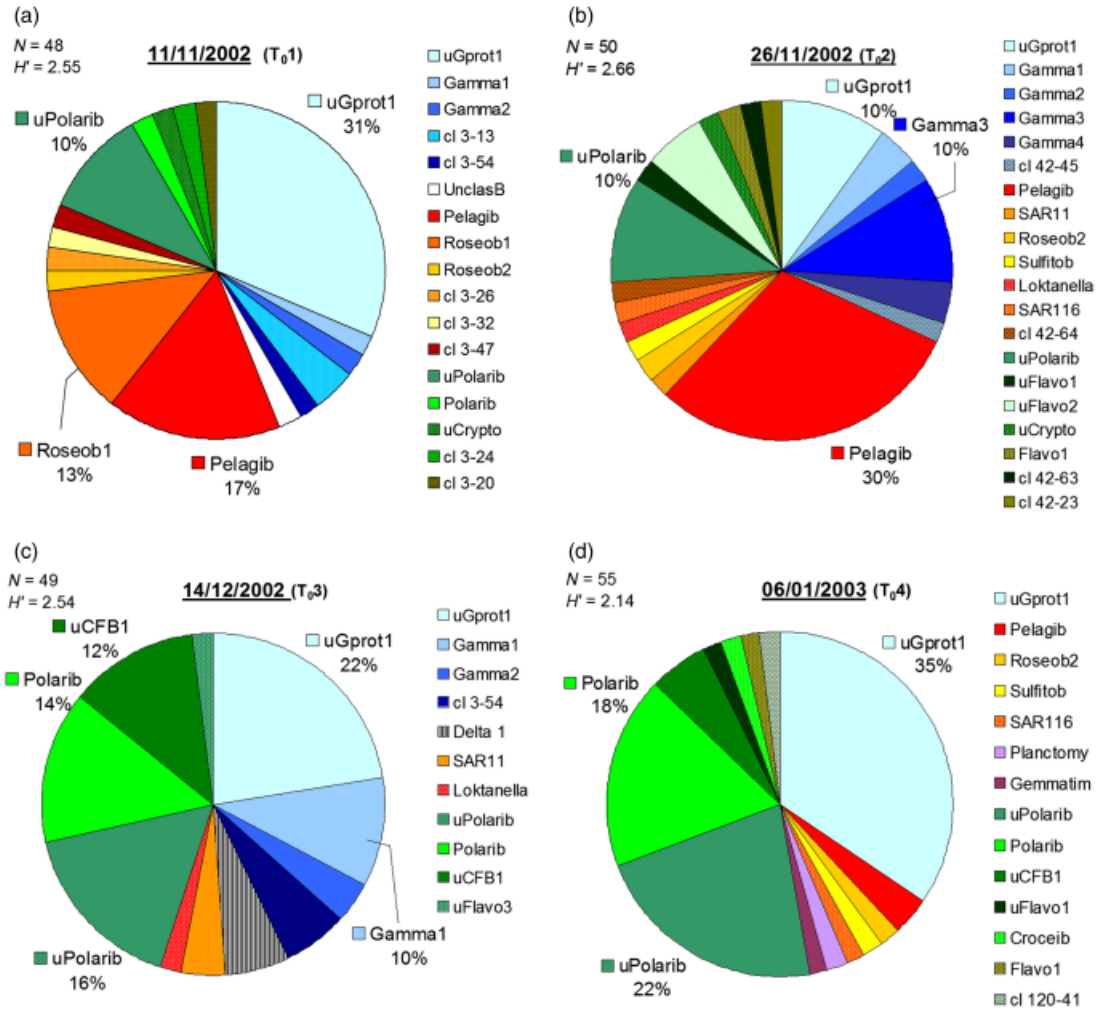

Fig. 2. Prydz Bay bacterioplankton community composition on November 11, 2002 (a), November 26, 2002 (b), December 14, 2002 (c) and January 6, 2003 (d), serving as inocula (T_0) for incubation experiments 1–4. Sequences were classified into OTUs at the 97% similarity level. OTUs representing at least 10% of the community are specified next to the chart. *N* is the number of analyzed sequences and *H'* is Shannon's diversity index. Color codes used: shades of blue, *Gammaproteobacteria*; red to yellow, *Alphaproteobacteria*; shades of green, CFB; white, unclassified bacteria; gray, *Deltaproteobacteria*; mauve, *Planctomycetes*; purple, *Gemmatimonadetes*.

probably an artifact of prefiltration. Here, incubation time significantly explained 18.9% of the variance among the band patterns. Thus, changes in the bacterial community composition were not rapid or dramatic. Incubation time was either the sole or the dominant factor effecting the composition of the bacterial community, while UV-R commonly exerted little effect.

Minicosm incubation and bacterial community composition

As done previously for the Prydz Bay (T_0) samples, the sequences were clustered into OTUs at the 97% identity level (Fig. 4). This revealed that the OTU composition and relative abundance of clones of communities exposed to PAR and the highest UV-R (B_4, B_3 for experiment 2) for 14 days differed from the initial (T_0) community composition.

The composition of clone libraries generated from experiment 1 communities exposed to PAR (P) and high UVB (B4) irradiation revealed that the proportion of CFBs increased, while *Gammaproteobacteria* decreased. *Alphaproteobacteria* reacted differently to the irradiation treatments: after PAR-only irradiation, their proportion was unchanged, while incubation under high UVB reduced the proportion of *Alphaproteobacteria* clones. Incubation also affected the community composition within classes as revealed by

Table 2. Clone library sequence details including number of analyzed clones, number of OTUs and diversity (Shannon–Weaver) and estimated community richness (Chao and Ace), diversity and richness estimates were calculated with DOTUR

	Experiment 1			Experiment 2			Experiment 3			Experiment 4		
	11/11/2002	T14P	T14B4	11/26/2002	T14P	T14B3	12/14/2002	T14P	T14B4	1/6/2003	T14P	T14B4
Clones surveyed	55	60	50	52	60	60	54	52	60	56	54	58
16S-like sequences	7	17	18	2	4	7	5	8	21	1	0	0
Bacterial clones	48	43	32	50	56	53	49	44	39	55	54	58
OTUs (99% similarity)	21	16	8	23	5	8	19	10	14	17	15	16
Shannon–Weaver index	2.550	2.109	1.55578	2.658	1.079	1.141	2.538	1.649	2.235	2.148	2.237	2.107
(±SD)	(±0.32)	(±0.36)	(±0.37)	(±0.32)	(±0.21)	(±0.33)	(±0.28)	(±0.33)	(±0.32)	(±0.3)	(±0.26)	(±0.3)
Chao	81 (38–237)	49 (24–147)	9 (8–19)	49 (30–115)	6 (5–19)	11 (8–31)	52 (27–150)	15 (11–42)	32 (18–95)	35 (21–92)	29 (18–82)	39 (21–113)
Ace	87 (40–250)	77 (29–294)	10 (8–24)	51 (32–111)	9 (5–48)	14 (9–45)	43 (26–104)	19 (12–56)	33 (19–89)	68 (32–185)	30 (19–77)	41 (23–109)

changes in dominant OTU. Initially, the 'uGprot1' OTU (cluster of clones '120-16, 3-34, 120-11, 120-14', with clone 120-16 sharing 99% identity with clone ANT4D3) dominated among the *Gammaproteobacteria*, 'Pelagib' (clones '39-28, 141-58') and 'Roseob1' (clone '141-44' related to ANT909) OTUs among the *Alphaproteobacteria*, and 'uPolarib' dominated within the CFBs. After incubation under PAR, the 'uGprot1' OTU no longer dominated the *Gammaproteobacteria*, OTU 'Pelagib' overtook the *Alphaproteobacteria*, while the relative abundance of 'uPolarib' increased among the CFBs. In the B_4-community, 'uPolarib' constituted half of all sequences, 'Pelagib' dominated within the *Alphaproteobacteria* and 'uGprot1' dominated within the *Gammaproteobacteria*. In experiment 1, the bacterial diversity decreased under both irradiation conditions from $H' = 2.6$ to $H' = 2.1$ (P) and $H' = 1.6$ (B_4) (Table 2).

The community composition of the clone libraries generated from experiment 2 revealed similar changes for the PAR-only and high UVB (B3) communities: members of the CFB class overtook the entire community after 14 days of incubation, representing ~80% of all sequences. Within this class, 'uPolarib' OTU dominated, with 59% and 68% of all sequences. Consequently, the diversity decreased from $H' = 2.7$ to $H' = 1.1$. The proportion of *Alphaproteobacteria* and *Gammaproteobacteria* decreased strongly: the latter only subsisted as a tiny proportion of the community.

Experiment 3 had a different initial community and incubation appeared to have a different effect on the 'P' and 'B' community composition. The proportion of CFB sequences remained stable, representing ~50% of all sequences; *Gammaproteobacteria* decreased from 43% to 25% and 10%, for the P and B_4 communities, respectively. The proportion of *Alphaproteobacteria*-related sequences increased from 6% in the inoculum to 18% and 38% in the P and B_4 communities.

Experiment 4 clone libraries of the incubated communities also revealed a strong increase in the proportion of *Alphaproteobacteria*, with 'Roeseob1'-related sequences dominating both the 'P' and the 'B' communities. The proportion of CFB sequences decreased slightly, and as in the other experiments, the proportion of *Gammaproteobacteria* strongly decreased, especially in the community exposed to high UVB.

Overall, under PAR irradiation and after 14 days of incubation, two patterns emerged: either a strong dominance of CFBs or an even contribution by CFBs, *Gamma*- and *Alphaproteobacteria*. Under high UV-R irradiation ('B'), the proportion of *Gammaproteobacteria* sequences strongly decreased, while sequences belonging to the CFBs formed at least 50% of the community. *Alphaproteobacteria* showed two responses: their contribution to the community composition decreased during incubation in the first two

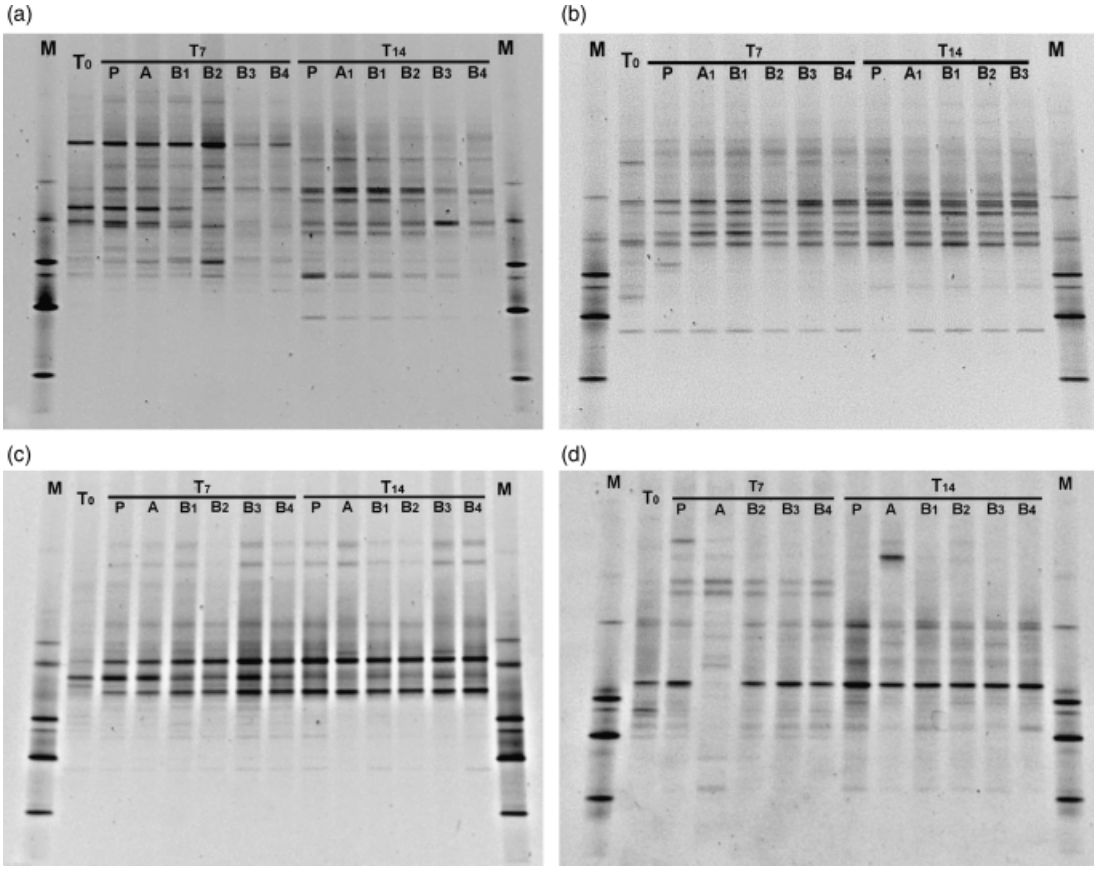

Fig. 3. DGGE of the bacterial communities from experiments 1 (a), 2 (b), 3 (c) and 4 (d). Codes used are: M, marker; T_0, inoculum; T_7, day 7 and T_{14}, day 14 of the incubation, irradiation conditions P = PAR, A = PAR+UVA, B_1–B_4 = low (1) to highest (4) PAR+UVA+UVB irradiation treatment.

experiments, but increased in the last two, reaching at least a third of the community.

Cluster analysis of the bacterioplankton community composition, calculated from the relative abundance of sequences clustered within OTUs at the 97% identity level, is presented as a dendrogram (Fig. 5). All the initial (T_0) bacterioplankton communities, except for the second experiment, appeared as grouped. T_0 for the second experiment was grouped separately with Exp1 T_{14} P. All the remaining incubated communities clustered together. Among the incubated communities, samples from experiments 2 and 4 were highly similar, reflecting a consistent community composition irrespective of the irradiance treatment to which they were exposed.

Discussion

Prydz Bay bacterioplankton

The data presented in this study provide a first insight into the composition of coastal Eastern Antarctic marine bacteria using culture-independent molecular methods. So far, only a handful of studies have characterized marine bacterial communities from the Antarctic, while a few more have explored the composition of archaeal communities (see the review by Hollibaugh *et al.*, 2007; Murray & Grzymski, 2007). Analysis of late spring – early summer bacterioplankton communities from Prydz Bay revealed the presence and relative dominance of bacterial classes that are expected for polar marine systems: the CFB (48.9%), *Alpha-* (26.3%) and *Gammaproteobacteria* (23.6%) classes that were also reported in other bacterioplankton studies conducted in the Arctic and Antarctic (Bano & Hollibaugh, 2002; Hollibaugh *et al.*, 2007). Murray & Grzymski (2007) studied the springtime bacterioplankton in offshore waters collected near Anvers Island, Antarctic Peninsula, and observed a similar springtime bacterial community composition with an equal distribution of clones over those three classes (CFB, *Alpha-* and *Gammaproteobacteria*).

Most *Alphaproteobacteria* sequences collected from Prydz Bay revealed a close relationship with *Pelagibacter ubique*, a cosmopolitan marine bacterium and a member of the

Table 3. Redundancy analysis and Monte–Carlo's permutation test results of species variance and environmental variables time (T) and UV-R (UV) explaining a part of the variation in the bacterial communities

	Experiment 1			Experiment 2			Experiment 3			Experiment 4		
	T and UV	T	UV	T and UV	T	UV	T and UV	T	UV	T and UV	T	UV
$\delta^{\dagger}$	35.2	27.8	15.7	31.8	20.5	13	36.7	30.3	15.4	27.4	18.9	12.3
$P^{\ddagger}$	0.002**	0.001**	0.031*	0.004**	0.001**	NS	0.002**	0.001**	0.037*	0.019*	0.004**	NS

†Part of the variation (in percentage).
‡*P*-value, significance level:
* < 0.05,
** < 0.005.

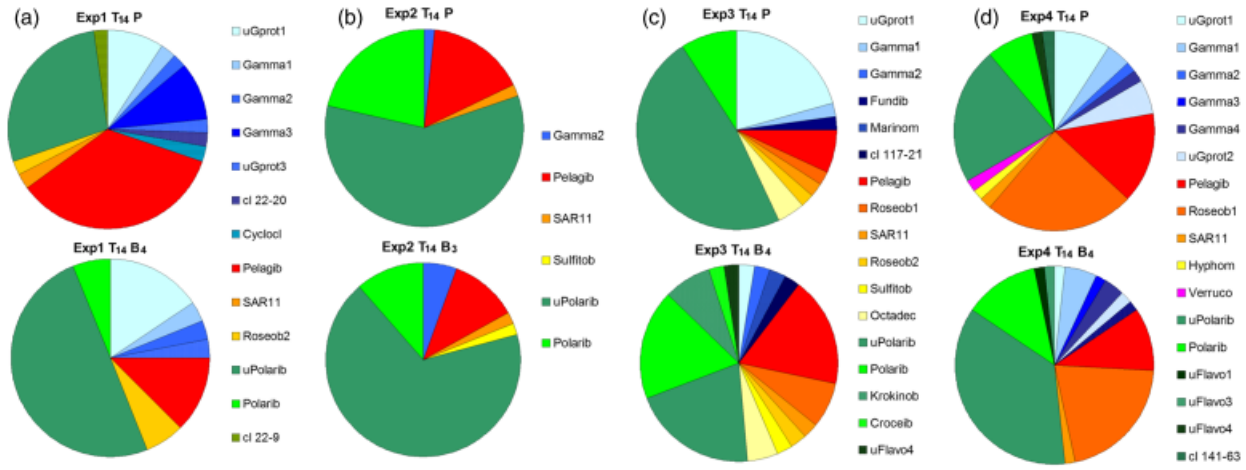

Fig. 4. Pie chart of the relative abundance of OTUs revealing shifts in community composition after 14 days of incubation under PAR (P) or high UV-R (B). For color codes, see Fig. 2.

SAR11 cluster (Morris *et al.*, 2002; Giovannoni *et al.*, 2005). Within the SAR11 members of Prydz Bay, we also recovered sequences closely related to Arctic SAR11 sequences: Arctic 97A-1 (Bano & Hollibaugh, 2002). The second most dominant group belonged to the *Roseobacter*, which included sequences related to the *Roseobacter* spp., *Sulfitobacter* spp. and *Octadecabacter* spp. The latter is a common inhabitant of sea ice and underlying seawater (Brinkmeyer *et al.*, 2003).

The dominant fraction of the *Gammaproteobacteria* sequences clustered within the 'uGprot1' OTU, which is related to clone ANT4D3 from Grzymski *et al.* (2006), a member of the oligotrophic marine *Gammaproteobacteria* HTTC2180 (Cho & Giovannoni, 2004). A fraction of the Prydz Bay bacterioplankton clustered within the proteorhodopsin containing the SAR92 clade, which had a strong identity (98%) to the cultivated HTCC231 strain (Stingl *et al.*, 2007). The *Gammaproteobacteria* sequences revealed a high level of microdiversity with 15 single sequence phylotypes, making it by far the most diverse class within the Prydz Bay bacterioplankton community.

The major fraction of CFB-related sequences had the highest identity to the cultivated *P. irgensii* ANT4243 strain (Brinkmeyer *et al.*, 2003) and an uncultivated *Polaribacter* Arctic 96B-11 sequence (Bano & Hollibaugh, 2002). The psychrophilic genus *Polaribacter* forms a dominant fraction of the CFB group and is known to have a bipolar distribution (Staley & Gosink, 1999; Brinkmeyer *et al.*, 2003; Abell & Bowman, 2005).

The tail end of our Prydz Bay clone library (1.1%) comprised 'rare' bacterial classes with sequences related to the *Deltaproteobacteria*, *Verrucomicrobiales*, *Planctomycetes*, *Gemmatimonadetes* and an uncultured bacterium (ANT4E12, Grzymski *et al.*, 2006). The clone library generated from Prydz Bay seawater is the first study reporting on the presence of these five 'rare' classes within a single sample set, once again supporting the endless potential of marine samples in discovering novel and rare sequences (Pedrós-Alió, 2006). Previous reports of these five classes from Antarctic waters exist, but, as in our study, showed that they were minor components of the community.

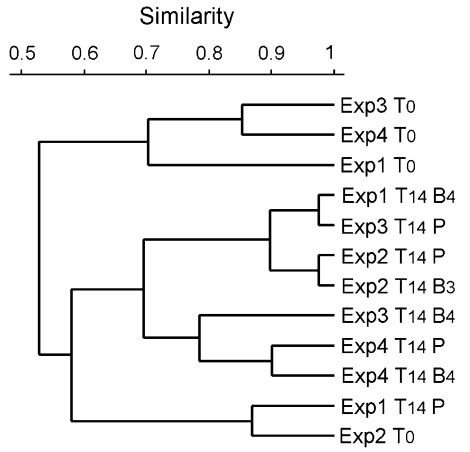

Fig. 5. Cluster analysis of Morisita–Horn similarities between natural and incubated bacterioplankton communities. Similarity was calculated using the relative abundance of sequences within 97% OTUs.

Natural springtime bacterioplankton succession

Previously, the Prydz Bay microeukaryotic communities were analyzed using the same samples as those presented in this study (Piquet *et al.*, 2008; Thomson *et al.*, 2008). Those studies revealed three microeukaryotic communities: a mixed postwinter community ($T_0$1: November 11), a flagellate-dominated community ($T_0$2 and $T_0$3: November 26 and December 14) and a diatom-dominated community ($T_0$4: January 6). The temporal shifts in the bacterioplankton community composition presented in this study did not mirror those observed for microeukaryotic communities. In contrast, Morisita similarity analysis of the four natural (T_0) communities calculated from the relative abundance of bacterial classes (data not shown) revealed two clusters: the November 11 and 26 ($T_0$1 and $T_0$2) samples were 97.5% similar, while the December 14 ($T_0$3) and January 6 ($T_0$4) samples shared 98% similarity. Previous studies have shown that bacterioplankton community composition and productivity change in response to phytoplankton blooms and species succession (Pinhassi & Hagström, 2000; Alonso-Sáez *et al.*, 2007), albeit with a delay of several days (Billen & Becquevort, 1991; Fuhrman & Steele, 2008). Differences in the bacterioplankton response to phytoplankton blooms can be attributed to the size and composition of the available DOM pool released by the phytoplankton after the bloom. Horner-Devine *et al.* (2003) conducted mesocosm experiments and observed a variable response time to increased primary productivity between bacterial classes, with CFBs responding most rapidly. Especially CFB bacteria are capable of rapidly degrading high-molecular-weight organic matter produced by phytoplankton (Glöckner *et al.*, 1999). The apparent mismatch between microeukaryotic and bacterial community shifts observed in the Prydz Bay might have been caused by a similar lag in the bacterioplankton response. However, the shift observed in $T_0$3 occurred before the microeukaryotic community composition shift. Here, the bacterioplankton community composition might have been shaped by organic material released from the surrounding decaying sea ice, often enriched in sea-ice microalgae. The observed similarity of the bacterial communities composition agrees with changes in the abundance of eukaryotes as phytoplankton abundance was low in experiments 1 and 2, but had more than doubled in T_0 samples used in experiments 3 and 4 (Thomson *et al.*, 2008). Thus, our results could indicate that the composition of the bacterial community is principally determined by eukaryote abundance rather than species composition.

Incubation-driven community composition shifts

Several studies performing incubation experiments have successfully used DGGE to reveal shifts in prokaryotic and eukaryotic communities (Schäfer *et al.*, 2000, 2001; Massana *et al.*, 2001; Casamayor *et al.*, 2002; Vázquez-Domínguez *et al.*, 2005; Piquet *et al.*, 2008). Winter *et al.* (2001) used DGGE to assess the impact of distinct irradiation regimes on natural North Sea bacterioplankton communities. Small, but consistent, shifts in the DGGE patterns were revealed after exposure to UVB and to a lesser extent UVA radiation (315–400 nm). Although DGGE cannot unveil quantitative shifts, its efficiency and applicability in revealing changes in microbial community composition following incubation experiments is supported by the publications cited above.

Studying the response of natural microbial communities to specific variables, like for example irradiance, remains a major challenge. Incubation itself can affect the eukaryotic as well as the bacterial fraction of the community (as discussed in Piquet *et al.*, 2008). The simulated system should be large enough in order to prevent enclosure artifacts and yet not too large for logistic constraints. The experiments carried out in Prydz Bay had relatively large volumes (650 L). The community was 200 μm prefiltered in order to avoid zooplankton grazing-mediated shifts and it was mixed to ensure even irradiance regimes. It is also evident that different initial communities will respond differently to irradiance exposure. The DGGE patterns generated for heterotrophic bacteria sampled along an eastern Pacific Ocean transect revealed biogeographic clustering according to latitude. Light incubation experiments conducted with these bacterioplankton communities revealed a latitudinal trend in the incorporation rates of leucine and thymidine matching the observed bacterial community clusters (Pakulski *et al.*, 2007).

Exposure of the Prydz Bay bacterioplankton community to distinct UV-R conditions in minicosms for 13–14 days

did not induce a clear response in community composition. Two out of the four experiments were significantly shaped by UV irradiation (experiments 1 and 3). This contrasts with previous studies of bacterioplankton sensitivity to UV-R. Analyses of natural Antarctic and tropical bacterioplankton have reported the accumulation of UV-induced DNA damage in the smallest size fraction (Visser *et al.*, 1999; Buma *et al.*, 2001) and diel patterns were also observed in the expression of dark repair mechanisms (RecA protein) (Booth *et al.*, 2001). Analysis of the sensitivity of coastal marine bacterial isolates to UVB revealed large interspecific differences between isolates (Joux *et al.*, 1999; Arrieta *et al.*, 2000). Dilution cultures of North Sea bacterioplankton exposed to UV-R analyzed by community fingerprints with DGGE revealed minor shifts in the banding pattern, with interspecific responses for CFB-related OTUs (Winter *et al.*, 2001). Furthermore, in Mediterranean waters, Alonso-Sáez *et al.* (2006) found that the heterotrophic activity of *Alphaproteobacteria* was inhibited, while CFB and *Gammaproteobacteria* remained unaffected. In our incubation experiments, the only class-related effect was observed for the relative abundance of *Gammaproteobacteria*, in particular the 'uGprot1' OTU, which decreased as a result of UVB exposure.

In conclusion, changes in the Prydz Bay bacterioplankton community were mostly affected by the incubation itself as well as the incubation duration. Furthermore, our study has shown that the impact of UV-R can be significant, affecting the composition of natural Antarctic bacterioplankton communities. However, these changes are not profound and are frequently subtle.

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References

Abell GCJ & Bowman JP (2005) Ecological and biogeographic relationships of class *Flavobacteria* in the Southern Ocean. *FEMS Microbiol Ecol* **51**: 265–277.

Alonso-Sáez L, Gasol JM, Lefort T, Hofer J, Sommer U & Sommaruga R (2006) Effects of natural sunlight on bacterial activity and differential sensitivity of natural bacterioplankton groups in northwestern Mediterranean coastal waters. *Appl Environ Microb* **72**: 5806–5813.

Alonso-Sáez L, Arístegui J, Pinhassi J, Gómez-Consarnau L, González JM, Vaqué D, Aqustí S & Gasol JM (2007) Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic Ocean. *Aquat Microb Ecol* **46**: 45–53.

Arrieta JM, Weinbauer MG & Herndl GJ (2000) Interspecific variability in sensitivity to UV-radiation and subsequent recovery in selected isolates of marine bacteria. *Appl Environ Microb* **66**: 1468–1473.

Azam F (2001) Microbial control of oceanic carbon flux: the plot thickens. *Science* **280**: 694–697.

Bano N & Hollibaugh JT (2002) Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean. *Appl Environ Microb* **68**: 505–518.

Billen G & Becquevort S (1991) Phytoplankton–bacteria relationship in the Antarctic marine ecosystem. *Polar Res* **10**: 245–253.

Booth MG, Hutchinson L, Brumstead M *et al.* (2001) Quantification of *rec*A gene expression as an indicator of repair potential in marine bacterioplankton communities of Antarctica. *Aquat Microb Ecol* **24**: 51–59.

Brinkmeyer R, Knittel K, Jürgens J, Weyland H, Amann RI & Helmke E (2003) Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Appl Environ Microb* **69**: 6610–6619.

Buma AGJ, de Boer MK & Boelen P (2001) Depth distribution of DNA damage in Antarctic marine phyto- and bacterioplankton exposed to summertime UV radiation. *J Phycol* **37**: 200–208.

Casamayor EO, Massana R, Benlloch S, Øvreås L, Díez B, Goddard VJ, Gasol JM, Joint I, Rodríguez-Valera F & Pedrós-Alió C (2002) Changes in archaeal, bacterial and eukaryal assemblages along a salinity gradient by comparison of genetic fingerprinting methods in a multipond solar saltern. *Environ Microbiol* **4**: 338–348.

Cho J-C & Giovannoni SJ (2004) Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. *Appl Environ Microb* **70**: 432–440.

Davidson AT & van der Heijden A (2000) Exposure of natural Antarctic marine microbial assemblages to ambient UV radiation: effects on bacterioplankton. *Aquat Microb Ecol* **21**: 257–264.

DeLong EF (2001) Microbial seascapes revisited. *Curr Opin Microbiol* **4**: 290–295.

Edwards U, Rogall T, Blöcker H, Emde M & Böttger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* **17**: 7843–7853.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Fuhrman JA & Steele JA (2008) Community structure of marine bacterioplankton: patterns, networks, and

relationships to function. *Aquat Microb Ecol* **53**: 69–81.

Fuhrman JA, McCallum K & Davis AA (1992) Novel major archaebacterial group from marine plankton. *Nature* **356**: 148–149.

Gentile G, Giuliano L, D'Aurelia G, Smedile F, Azzaro M, Domenico MD & Yakimov MM (2006) Study of bacterial communities in Antarctic coastal waters by a combination of 16S rRNA and 16S rDNA sequencing. *Environ Microbiol* **8**: 2150–2161.

Giovannoni SJ, Britschgi TB, Moyer CL & Field KG (1990) Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345**: 60–63.

Giovannoni SJ, Tripp HJ, Givan S *et al.* (2005) Genome streamlining in a cosmopolitan oceanic bacterium. *Science* **309**: 1242–1245.

Glöckner FO, Fuchs BM & Amann RI (1999) Bacterioplankton composition of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Appl Environ Microb* **65**: 3721–3726.

Grzymski JJ, Carter BJ, DeLong EF, Feldman RA, Ghadiri A & Murray AE (2006) Comparative genomics of DNA fragments from six Antarctic marine planktonic bacteria. *Appl Environ Microb* **72**: 1532–1541.

Hammer Ø, Harper DAT & Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontol Electron* **4**: 38–47.

Hartl DL, Moriyama EN & Sawyer SA (1994) Selection intensity for codon bias. *Genetics* **138**: 227–234.

Hollibaugh JT, Lovejoy C & Murray AE (2007) Microbiology in polar oceans. *Oceanography* **20**: 140–145.

Horner-Devine M, Leibold MA, Smith VH & Bohannan BJM (2003) Bacterial diversity patterns along a gradient of primary productivity. *Ecol Lett* **6**: 613–622.

Joux F, Jeffrey WH, Lebaron P & Mitchell BG (1999) Marine bacterial isolates display diverse responses to UV-B radiation. *Appl Environ Microb* **65**: 3820–3827.

Lane DJ (1991) 16S/23S rRNA sequencing. *Nucleic Acid Techniques in Bacterial Systematics* (Stackebrandt E & Goodfellow M, eds), pp. 115–175. John Wiley and Sons, New York.

Massana R, Pedrós-Alió C, Casamayor EO & Gasol JP (2001) Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. *Limnol Oceanogr* **46**: 1181–1188.

Morris RM, Rappé MS, Connon SA, Vergin KL, Siebold WA, Carlson CA & Giovannoni SJ (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420**: 806–810.

Murray AE & Grzymski JJ (2007) Diversity and genomics of Antarctic marine micro-organisms. *Philos T R Soc B* **362**: 2259–2271.

Muyzer G, de Waal EC & Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rDNA. *Appl Environ Microb* **59**: 695–700.

Muyzer G, Brinkhoff T, Nübel U, Santegoeds C, Schäfer H & Wawer C (2004) Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. *Molecular Microbial Ecology Manual* (Kowalchuk GA, de Bruin FJ, Head IM, Akkermans DL & van Elsas JD, eds), pp. 743–770. Kluwer Academic Publishers, Dordrecht.

Nübel U, Engelen B, Felske A, Snaidr J, Wieshuber A, Amann RI, Ludwig W & Backhaus H (1996) Sequence heterogeneities of genes encoding 16S rRNA in *Paenibacillus polymyxa* detected by TGGE. *J Bacteriol* **178**: 5636–5643.

Pakulski JD, Baldwin AJ, Dean AL *et al.* (2007) Response of heterotrophic bacteria to solar irradiance in the eastern Pacific Ocean. *Aquat Microb Ecol* **47**: 153–162.

Pedrós-Alió C (2006) Marine microbial diversity: can it be determined? *Trends Microbiol* **14**: 257–263.

Pinhassi J & Hagström Å (2000) Seasonal succession of marine bacterioplankton. *Aquat Microb Ecol* **21**: 245–256.

Piquet AM-T, Bolhuis H, Davidson AT, Thomson PG & Buma AGJ (2008) Diversity and dynamics of Antarctic marine microbial eukaryotes under manipulated environmental UV radiation. *FEMS Microbiol Ecol* **66**: 352–366.

Schäfer H, Servais P & Muyzer G (2000) Successional changes in the genetic diversity of a marine bacterial assemblage during confinement. *Arch Microbiol* **173**: 138–145.

Schäfer H, Bernard L, Courties C, Lebaron P, Servais P, Pukall R, Vives-Rego J & Muyzer G (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in genetic diversity of bacterial populations. *FEMS Microbiol Ecol* **34**: 243–253.

Schloss PD & Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimation species richness. *Appl Environ Microb* **71**: 1501–1506.

Staley JT & Gosink JJ (1999) Poles apart: biodiversity and biogeography of sea ice bacteria. *Annu Rev Microbiol* **53**: 189–215.

Stingl U, Desiderio RA, Cho J-C, Vergin KL & Giovannoni SJ (2007) The SAR92 clade: an abundant coastal clade of culturable marine bacteria possessing proteorhodopsin. *Appl Environ Microb* **73**: 2290–2296.

Tamura K, Dudley J, Nei M & Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**: 1596–1599.

Ter Braak CJF & Šmilauer P (1998) *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4.5.2)*. Microcomputer Power, Ithaca, New York.

Thomson PG, Davidson AT & Cadman N (2008) Temporal changes in effects of ambient UV radiation on natural communities of Antarctic marine protists. *Aquat Microb Ecol* **52**: 131–147.

Van den Wollenberg AL (2007) Redundancy analysis. An alternative for canonical correlation analysis. *Psychometrika* **42**: 207–219.

Vázquez-Domínguez E, Casamayor EO, Català P & Lebaron P (2005) Different marine heterotrophic nanoflagellates affect differentially the composition of enriched bacterial communities. *Microb Ecol* **49**: 474–485.

Venter JC, Remington K, Heidelberg JF *et al.* (2004) Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304**: 66–74.

Visser PM, Snelder E, Kop AJ, Boelen P, Buma AGJ & van Duyl FC (1999) Effects of UV radiation on DNA photodamage and production in bacterioplankton in the coastal Caribbean Sea. *Aquat Microb Ecol* **20**: 49–58.

Winter C, Moeseneder MM & Herndl GJ (2001) Impact of UV radiation on bacterioplankton community composition. *Appl Environ Microb* **67**: 665–672.

Wolda H (1981) Similarity indices, sample size and diversity. *Oecologia* **50**: 296–302.

Zhu L & Bustamante CD (2005) A composite-likelihood method for detecting directional selection from DNA sequence data. *Genetics* **170**: 1411–1421.