RESEARCH ARTICLE

Patterns of marine bacterioplankton biodiversity in the surface waters of the Scotia Arc, Southern Ocean

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Received 7 April 2011; revised 6 January 2012; accepted 12 January 2012.
Final version published online 19 March 2012.

DOI: 10.1111/j.1574-6941.2012.01313.x

Editor: Riks Laanbroek

Keywords
bacteria; Antarctic; marine; patchiness; distribution; Southern Ocean.

Abstract

Spatial patchiness in marine surface bacterioplankton populations was investigated in the Southern Ocean, where the Antarctic Circumpolar Current meets the islands of the Scotia Arc and is subjected to terrestrial input, upwelling of nutrients and seasonal phytoplankton blooms. Total bacterioplankton population density, group-specific taxonomic distribution and six of eight dominant members of the bacterioplankton community were found to be consistent across 18 nearshore sites at eight locations around the Scotia Arc. Results from seven independent 16S rRNA gene clone libraries (1223 sequences in total) and fluorescent *in situ* hybridization suggested that microbial assemblages were predominantly homogeneous between Scotia Arc sites, where the *Alphaproteobacteria*, *Gammaproteobacteria* and the *Cytophaga–Flavobacterium–Bacteroidetes* cluster were the dominant bacterial groups. Of the 1223 useable sequences generated, 1087 (89%) shared $\geq$ 97% similarity with marine microorganisms and 331 (27%) matched published sequences previously detected in permanently cold Arctic and Antarctic marine environments. Taken together, results suggest that the dominant bacterioplankton groups are consistent between locations, but significant differences may be detected across the rare biodiversity.

Introduction

To date, there have been a variety of studies of bacterioplankton populations in marine surface waters (Zubkov *et al.*, 2002; Venter *et al.*, 2004; Pinhassi *et al.*, 2006; Malmstrom *et al.*, 2007; Riemann *et al.*, 2008), including the Southern Ocean (Murray *et al.*, 1999; Simon *et al.*, 1999; Church *et al.*, 2003; Selje *et al.*, 2004; Abell & Bowman, 2005; Corzo *et al.*, 2005; Gentile *et al.*, 2006; Grzymski *et al.*, 2006; Moreira *et al.*, 2006; Topping *et al.*, 2006; Murray & Grzymski, 2007). These studies show that spatial and temporal differences can be detected in the structure and composition of marine bacterioplankton communities. However, results are conflicting; in some cases, no significant correlation was found between physicochemical parameters and this bacterioplankton distribution (Zubkov *et al.*, 2002), while others have shown differences that have been attributed to specific environmental factors (Giovannoni & Stingl, 2005). These include nutrient availability, chlorophyll-*a* concentration (Abell & Bowman, 2005; Corzo *et al.*, 2005; Pinhassi *et al.*, 2006), island effects (Delille, 2003), overlying sea ice (Delille & Rosiers, 1996), algal blooms (Gentile *et al.*, 2006; Topping *et al.*, 2006) and latitude (Weitz *et al.*, 2010). In addition, while some biogeographical studies have suggested that marine bacteria have a global distribution (Pommier *et al.*, 2005), others have linked specific distributions with particular regions (Malmstrom *et al.*, 2007; Murray & Grzymski, 2007; Pommier *et al.*, 2007; Weitz *et al.*, 2010). In Antarctic waters, a number of studies have investigated small-scale biogeographical differences in bacterial distribution; in the Ross Sea (Gentile *et al.*, 2006), between the east and west regions of the Scotia Sea (Topping *et al.*, 2006) and between the polar front and ice edge (Simon *et al.*, 1999). In this study, total bacterioplankton population density and species diversity were estimated, dominant bacterioplankton groups were identified, and species richness was determined, to compare bacterioplankton populations at 18 sites from eight key locations along a transect of the

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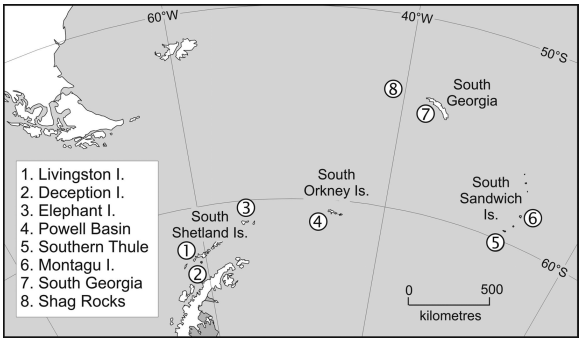


Fig. 1. Map of the Scotia Sea showing sampling locations.

Scotia Sea (Fig. 1), where the Antarctic Circumpolar Current (ACC) crosses the islands of the Scotia Arc.

Materials and methods

Sampling sites and regime

Surface seawater samples were obtained from 18 locations along a transect of the Scotia Arc during the austral summer 2006. Sample sites were close to Livingston Island (LI or LV) (62.2°S 61.3°W), Deception Island (DI) Caldera (62.5°S 60.3°W), Elephant Island (EI) (61.2°S 55.1°W), Powell Basin (PB) (61.0°S 46.5°W), the South Thule (ST) Caldera (59.5° S 27.3° W), Montagu Island (MI) (58.2°S 26.2°W) and South Georgia (SG) (53.3°S 46.9°W). Some data were also obtained for a single Shag Rocks (SR) station. Although part of a transect, the sample sites could be broadly grouped into three distinct zones. LI, DI, EI and PB are all within the South Shetland Islands, close to the Antarctic Peninsula. As such, they are South of the Southern Antarctic Circumpolar Current Boundary, and all but PB are to the North of the Weddell Front (Murphy *et al.*, 2007). These islands are not within the region controlled predominantly by upwelling and mixing of micronutrient rich deep waters (Hendry *et al.*, 2011). They are within the region subjected to seasonal sea ice, and hence, as this sea ice recedes, phytoplankton bloom and succession follow. The islands are also within reach of terrestrial runoff, particularly during the melt season. The second zone consists of ST and MI, both in the centre of the Scotia Arc and within the path of the Antarctic Circumpolar Current. There is a smaller land area for terrestrial runoff, and they are approximately at the limit of the sea ice extent at its height in October and benefit moderately from upwelling owing to topography (Sloyan, 2005). The third zone comprises SG and SR. These are further north than the sea ice can reach, north of the Southern Antarctic Circumpolar Front but South of the Polar Front, and there is a large annual phytoplankton bloom to the north of the islands as a result of nutrient upwelling (Moore & Abbott, 2002).

Seawater was collected from above the pycnocline, to target the chlorophyll maximum at 30 m using a conductivity–temperature–density (CTD) profiler (Sea-Bird Electronics Inc.). Four litres from each location was transferred into sterile, chilled, prerinsed Nalgene sampling bottles. Bacteria were harvested from samples by vacuum filtration (< 30 kPa) onto Whatman 3 M nitrocellulose membranes using a sterile filtration unit (Sartorius). Cells were resuspended in 10 mL prefiltered (0.2 μm) sterile seawater. For each analysis, 1 mL of cell suspension was drawn through a 0.2-μm polycarbonate filter (Poretics, Osmotics Inc.) and fixed with 2 mL paraformaldehyde (4% wt/v) for 30 min. Excess fixative was removed with 5 mL sterile seawater and 5 mL phosphate-buffered saline (10mM Na_2HPO_4, 10 mM NaH_2PO_4 and 130 mM NaCl adjusted to pH 7.2), and the filter paper was air-dried and stored at −20 °C.

Bacterioplankton population density

Bacterioplankton population density was determined by DAPI 4′-6-diamidino-2-phenylindole (Sigma) staining and enumerated using an Olympus epifluorescence microscope fitted with a 50-W, high-pressure mercury lamp (Porter & Feig, 1980). A minimum of 2500 cells were counted per sample from > 50 randomly selected fields of view across each slide.

Bacterioplankton species richness

Bacterioplankton species richness was determined by fluorescence *in situ* hybridization (FISH). Twelve 5′ CY-3-labelled, oligonucleotide probes (VH Bio Ltd, Gateshead, UK) were used: EUB338 Eubacteria; Bacterial 16S rRNA gene (338–355) 5′-GCT GCC TCC CGT AGG AGT-3′; (Amann *et al.*, 1990); NON338 negative control 5′-ACT CCT ACG GGA GGC AGC-3′ (Wallner *et al.*, 1993); ARCH915 Archaea; Archaeal 16S rRNA gene (915–934) 5′-GTG CTC CCC CGC CAA TTC CT-3′ (Stahl & Amann, 1991); ALF968 most *Alphaproteobacteria* 16S rRNA gene (968–986); most *Pelobacter*/*Geobacter* spp. 5′-GGT AAG GTT CTG CGC GTT-3′ (Manz *et al.*, 1992); GAM42a *Gammaproteobacteria* 5′-GCC TTC CCA CAT CGT T-3′ (Glöckner *et al.*, 1999); BET42a *Betaproteobacteria* 23S rRNA gene (1027–1043) 5′-GCC TTC CCA CTT CGT TT-3′ (Manz *et al.*, 1992); CF319 *Cytophaga*/*Flavobacterium* cluster of the *Bacteroidetes* phylum 16S rRNA gene (319–336) 5′-TGG TCC GTG TCT CAG TAC-3′ (Manz *et al.*, 1996); PLA46 *Planctomycetales* 5′-GCC TTG CGA CCA TAC TCC C-3′ (Neef *et al.*, 1998); SRB385 sulphate-reducing *Deltaproteobacteria* 16S

rRNA gene (385–402) 5′-CGG CGT CGC TGC GTC AGG-3′ (Amann *et al.*, 1990); ANME-1-350 for archaeal methanogens group ANME-1 5′-AGT TTT CGC GCC TGA TGC-3′ (Boetius *et al.*, 2000); HGC236 *Actinobacteria* 16S, 235–253 Gram-positive high G+C content 5′-AAC AAG CTG ATA GGC CGC-3′ (Glöckner *et al.*, 2000) and LGC354 *Firmicutes* 16S rRNA gene (354–371) and Gram-positive low G+C content 5′-TGG AAG ATT CCC TAC TGC-3′ (Meier *et al.*, 1999). Each probe was added at a final concentration of 50 ng mL^{-1} to 250 μL hybridization solution (0.9 M NaCl, 20 mM Tris–HCl (pH 7.4), 0.01% SDS) containing 10% v/v formamide (for EUB338, NON338), 20% (for ALF968, HGC236, LGC354) and 35% v/v formamide (for GAM42a, BET42, PLA42, CF319, ARCH915, ANME-1) with 20 μL hybridization solution. Cells were incubated in a humid atmosphere (46 °C, 90 min) and rinsed twice with 5 mL warmed wash buffer (20 mM Tris–HCl, 5 mM EDTA, 0.01% SDS and 70 mM NaCl). Air-dried cells were mounted in Vectashield (Vector Labs) and enumerated by epifluorescence microscopy.

Dominant bacterioplankton groups

Dominant bacterioplankton groups were determined by DGGE. Aliquots of 250 μL concentrated bacterial cell suspension were subjected to freeze–thaw cycles for PCR amplification (Øvreås *et al.*, 1997). A volume of 2 μL was used in a 25-μL reaction mix containing 1 μL of each DGGE primer 338F and 518R, 20 μL ReddyMix™ (Abgene) and 1 μL BSA (10 mg μL^{-1}; Sigma). The reaction was carried out on a Techne thermocycler under the following conditions: initial denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C extension for 1 min 15 s and a final elongation step of 72 °C for 15 min. PCR products (180 bp length) from replicate amplifications were combined, and 50 μL was loaded per lane. Gel casting and running were undertaken according to the study of Helms (1990) using the INGENYphorU-2 System (Ingeny International BV) according to the manufacturer's instructions. A 30–80% denaturing gel containing 6.5% (v/v) acrylamide/bis–acrylamide was cast using 50 μL ammonium persulphate (20%) and 5 μL TEMED (Sigma) to initiate polymerization. The gel was run overnight at (120 V, 18 h) and post-stained with SYBR Gold nucleic acid stain (Molecular Probes Inc.). The DGGE bands with highest intensities common to all sites were selected for sequencing and taxonomic identification from the banding pattern of sample LV-1. Small clone libraries were constructed for each of eight gel bands and de-replicated by RFLP prior to sequencing, to avoid the possibility of co-migration of bands (Jackson *et al.*, 2000; Sekiguchi *et al.*, 2001; Gafan & Spratt, 2005). Gel bands were excised into 30 μL TE buffer (0.1×; 10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0) using sterile, disposable scalpels (Swann-Morton) and incubated overnight at 4 °C to allow diffusion of the DNA into solution. DNA was reamplified with primers 338F and 518R as described previously. PCR products were cleaned using Illustra GFX™ PCR DNA Purification Kits (GE Healthcare UK Ltd) and eluted into 30 μL filtered nuclease-free water (Sigma). Ligation of DNA fragments was performed using the pGEM-T Easy Vector system (Promega UK Ltd) according to manufacturer's instructions. Ligated products were transformed into ultraefficient XL-2 blue MRF competent cells (Stratagene) with the addition of 200 mM IPTG (isopropyl-beta-D-thiogalactopyranoside) and X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside, Bioline Ltd) and plated onto LB agar containing ampicillin (100 μg mL^{-1}). Approximately 200 white/positive colonies per site were picked into 30 μL sterile water and subjected to two freeze–thaw cycles. Between 2 and 5 μL of plasmid DNA was used in a PCR with primers M13F (5′-CGC CAG GGT TTT CCC AGT CAC GAC-3′) and M13R (5′-GAG CGG ATA ACA ATT TCA CAC AGG-3′) using reagents as previously described with the following cycle conditions: 95 °C for 5 min followed by 29 cycles of 95 °C for 30 s, 58.5 °C for 30 s and 72 °C for 1 min 10 s. Amplifications were checked by electrophoresis, and products were cleaned with purification columns. Sequencing reactions were performed with BigDye® Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) using the M13F primer, and products were run on a Mega Bace300 DNA Sequencing System (Molecular Dynamics). Sequence files were checked for base-calling accuracy, and vector was removed manually. Cloned sequences were submitted as BLASTn searches against the EBI–EMBL nucleotide sequence database to identify the closest matching relative. Alignment of sequences was performed using EBI ClustalW multiple sequence alignment program and BioEdit version 7.0.9 (Hall, 1999). Cloned sequences were compared to sequences in the EMBL nucleotide sequence database by BLAST search and deposited in the EMBL database under accession numbers AM920826–AM921620. The banding patterns for each DGGE profile were processed, normalized and statistically analysed using Gel ComparII software version 3.5 (Applied Maths). A similarity matrix based on band positions and contribution of the optical intensity of each band to the total intensity of each DGGE profile (densitometric curve) was generated and used for cluster analysis (Pearson's correlation; UPGMA) of the profiles.

Bacterioplankton species diversity

Bacterioplankton species diversity was determined through the construction and analysis of seven indepen-

dent 16S rRNA gene clone libraries. Ribosomal 16S RNA genes were amplified by PCR using primers 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-TAC GG(C/T) TAC CTT GTT ACG ACT T-3′) in a reaction containing 5 ng DNA, 1 μL each primer (10 mM), 10 μL 10× NH_4-based reaction buffer, 2.5 μL $MgCl_2$ (50 mM), dNTPs (20 nmol each), 0.3 μL BIOTAQ polymerase 0.05 U Biotaq^{TM} DNA polymerase (Bioline Ltd), 0.8 μL BSA (10 mg μL^{-1}; Sigma) adjusted to a final volume of 30 μL using sterile water. PCR amplification was performed under the following conditions: initial denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min 15 s, 72 °C for 1 min and a final elongation step at 72 °C for 10 min. PCR products were cleaned using the Illustra GFX PCR DNA purification kit (GE Healthcare UK Ltd). Ligation, transformation, cloning and sequencing of approximately half the full length 16S fragment were undertaken as described above for DGGE.

Data analysis

One-way ANOVAs were conducted in Minitab 14. All DAPI and FISH data were analysed untransformed, as an examination of the residuals and normality tests showed that they did not break the assumptions of ANOVA. FISH data from replicate stations were combined and averaged for three of the four geographical sites analysed, LV, EI and PB. Data from DI were excluded from the analysis, because the availability of only one replicate reduced the ability of the tests to detect differences between sites. If a one-way ANOVA detected a significant difference between sites (at $P < 0.05$), then *post hoc* Tukey's tests were conducted to detect which pairs of means were significantly different. A comparative analysis of bacterial community structure as determined by FISH was conducted by multivariate statistical analysis using PRIMER 5 software (Clarke & Warwick, 2001). For each station, nontransformed FISH data incorporating all probes except EUB338 and NON338 were clustered based on group averages following generation of a Bray–Curtis similarity matrix. Differences between the four geographical sites LV, DI, EI and PB were also examined at assemblage level by a one-way analysis of similarity (ANOSIM), using the bacterial group–specific probes: GAM42a, ALF968, CF319a, PLA46, HCG236 and LGC354b. A SIMPER analysis was conducted to determine which bacteria were most responsible for the observed differences, and results were plotted as a multidimensional scaling (MDS) ordination based on a Bray–Curtis similarity matrix. Multivariate statistical analysis was also conducted to examine differences in clone library data between sites. For sequence analyses, an operational taxonomic unit (OTU) was defined as any sequence showing $\geq$ 97% identity to a 16S rRNA gene sequence in the EMBL database. Maximum likelihood distance matrices for the seven clone libraries were visualized by MDS ordination.

Results

Bacterioplankton population density

The bacterioplankton population densities of surface water ranged between 1.13 (± 0.16) and 2.13 (± 0.21) $\times 10^5$ cell mL^{-1} (Table 1). Population densities were consistent across geographical sites and between samples within sites, with no significant differences detected ($F = 2.88$, d.f. = 2, $P > 0.05$). However, the highest mean densities were recorded to the north of the Scotia Arc near SR and SG, while lowest mean densities were observed around EI.

Bacterioplankton species richness

Eubacterial numbers as determined by fluorescence *in situ* hybridization were also consistent across all sites and ranged between 1.1 and 1.3 $\times 10^5$ cells mL^{-1} (Table 2). The sum of the group-specific probes combined was between 40% and 79% of total eubacterial hybridizations. The *Gammaproteobacteria* and *Cytophaga–Flavobacterium–Bacteroidetes* groups were the most frequently detected at all sites, comprising 28% and 21% of eubacterial counts, respectively. The *Alphaproteobacteria* and *Planctomycetes* were next followed by sulphate-reducing, high GC content and low GC content bacteria. The abundance of *Beta-proteobacteria* was very low. The single DI sample supported higher numbers of sulphur-reducing bacteria (SRB) and methanogenic archaea (ANME-1) than any other sample, comprising 23% and 7% of the total cell count, respectively. Elsewhere, the greatest contribution of SRB and ANME-1 hybridizations to the total count was observed at PB; PB-3 (10%) and PB-2 (6%), respectively. The number of Archeal probe hybridizations was greatest at DI, where the Archaea contributed ~11% of DAPI-stained cells. Hybridizations to the Actinobacterial probes were 9-4% of the total DAPI count for HCG236 and 9-3% for LGC354b.

Cluster analysis of FISH data showed that the group composition was > 85% similar across all sites examined (Fig. 2a). However, replicate communities analysed for Livingston Island (LI or LV) LV-1 to LV-4 clustered together, suggesting a detectable difference in richness between LI communities and those elsewhere. The DI bacterioplankton community structure was similar to EI and PB communities and most dissimilar to the LV-4 community. However, it must be noted that FISH data for

Table 1. Physical parameters and bacterial population density in surface water at 18 Scotia Arc stations

Date	Station	Latitude	Longitude	Bottom water depth (m)	Temperature (°C)	Salinity	Chlorophyll-*a** (mg m^{-3})	Population density (cells × 10^5 mL^{-1} seawater and No. fields of view)
3 March 2006	LV-1	62°16′S	61°35′W	1433	2.01	34.01	0.1957	1.57 (± 0.26) 56
3 March 2006	LV-2	62°26′S	61°38′W	789	1.96	34.01	0.2298	1.59 (± 0.28) 53
4 March 2006	LV-3	62°23′S	61°46′W	495	1.95	34.05	0.2602	1.56 (± 0.30) 52
4 March 2006	LV-4	62°31′S	61°49′W	191	1.88	34.09	0.3177	1.75 (± 0.23) 56
6 March 2006	D-1	62°57′S	60°38′W	161	2.06	33.88	0.7991	1.47 (± 0.19) 71
11 March 2006	EI-1	61°36′S	55°13′W	1518	1.28	34.13	0.3197	1.43 (± 0.17) 71
12 March 2006	EI-2	61°34′S	55°12′W	1019	1.43	34.08	0.3376	1.60 (± 0.13) 74
12 March 2006	EI-3	61°23′S	55°12′W	498	1.42	34.08	0.2252	1.54 (± 0.12) 74
13 March 2006	EI-4	61°20′S	55°11′W	244	1.33	34.13	0.3376	1.57 (± 0.13) 67
17 March 2006	PB-1	60°58′S	47°01′W	2763	0.61	33.70	0.7572	1.73 (± 0.12) 86
17 March 2006	PB-2	61°01′S	46°51′W	1006	0.71	33.63	0.7572	1.69 (± 0.14) 86
18 March 2006	PB-3	60°59′S	46°49′W	513	0.71	33.66	0.7572	1.64 (± 0.18) 79
29 March 2006	SG-1	53°33′S	46°49′W	1508	3.98	33.70	0.3662	1.53 (± 0.16) 64
29 March 2006	SG-2	53°34′S	37°51′W	1023	4.01	33.70	0.3662	2.11 (± 0.19) 64
29 March 2006	SG-3	53°36′S	39°53′W	223	3.68	33.74	18.4583	1.45 (± 0.19) 66
5 April 2006	SR-1	53°37′S	40°54′W	1507	4.76	33.77	0.1390	2.13 (± 0.21) 73
6 April 2006	SR-2	53°35′S	40°76′W	1029	4.82	33.75	0.1390	1.61 (± 0.18) 57
6 April 2006	SR-3	53°36′S	40°62′W	522	4.72	33.76	0.1330	1.89 (± 0.18) 72

Data are averages of replicate counts ± SD.

*Surface chlorophyll-*a* data from satellite images on sample dates March–April 2006.

DI-1 was derived from a single sample. Bacterioplankton community structures at station DI-1 and LV-4 showed the greatest separation. Significant differences were detected by ANOVA and *post hoc* Tukey's test between locations LV and EI, and LI and PB, for probes GAM42a ($F = 8.89$, d.f. = 2, $P = 0.009$), ALF968 ($F = 27.06$, d.f. = 2, $P = 0.009$) and PLA46 ($F = 32.9$, d.f. = 2, $P = 0.000$) and between all three stations for probe HCG236 ($F = 49.37$, d.f. = 2, $P = 0.000$).

MDS ordination of community structures (Fig. 2b) showed that the LI community was most dissimilar to all other sites. A one-way ANOSIM (999 permutations) showed that in general, assemblage structure differed strongly and significantly between sites (global ANOSIM $R = 0.66$, $P = 0.002$). Pairwise comparisons showed that the global difference between sites was attributable to high dissimilarity between LI and EI (ANOSIM $R = 0.96$, $P = 0.03$) and LI and PB (ANOSIM $R = 0.96$, $P = 0.03$). SIMPER analysis conducted on these paired sites showed that the *Alphaproteobacteria* (ALF968) were the principal contributor (between 23% and 30%) to the observed dissimilarity.

Dominant bacterioplankton groups

DGGE band patterns were largely consistent across stations and between samples from the same geographical site. DGGE analysis revealed between 9 and 13 easily resolvable bands per profile. These patterns were highly reproducible between gels. Profiles from EI and PB sites had the most bands, and LI the least. Eight bands common to most sites were cloned and sequenced from banding pattern LV-1 (Table S1, Supporting information). All sequences shared $\geq$ 97% identity with sequences isolated from marine or estuarine environments. With the exception of *Polaribacter glomeratus* S3-30, most sequences matched uncultured clones (uncultured *Bacteroidetes* bacterium clone NABOS_SSPb-act59, uncultured marine *Gammaproteobacterium* clone KG_A3_120m53, uncultured marine *Flavobacteriaceae* bacterium clone KG_C11_100m45, uncultured *Alphaproteobacterium* clones NABOS_FLbact4, NABOS_FLbact21 and BHSS17 and an uncultured *Cyanobacterium* clone 16B_215). Sequences matching six of the eight bands analysed were previously isolated from polar regions. Sequence affiliations to *Flavobacteraceae* and *Alphaproteobacteria* were dominant in this profile. Cluster analysis of the banding patterns resulted in a dendrogram divided by two groupings of samples that were 38.9% similar (Fig. 2c). Within each group, the banding patterns shared at least 74% similarity. In the first group, all three SG replicates clustered with one PB replicate where SG-2 and PB-3 shared the highest similarity (98%). The second grouping was divided in two, the first subset comprised 12 stations exhibiting no more than 26% dif-

Table 2. Abundance data ($\times 10^3$ cells mL^{-1}) for bacterial groups analysed by FISH

Probe	LV-1	LV-2	LV-3	LV-4	D-1	EI-1	EI-2	EI-3	EI-4	PB-1	PB-2	PB-3
EUB338	122 ± 1	124 ± 18	131 ± 13	118 ± 12	123 ± 13	122 ± 13	119 ± 14	122 ± 14	130 ± 14	129 ± 14	134 ± 13	130 ± 13
GAM42A	22 ± 8	26 ± 9	21 ± 9	30 ± 10	32 ± 6	32 ± 8	35 ± 7	35 ± 9	29 ± 7	36 ± 9	33 ± 7	33 ± 8
CF319a	15 ± 10	14 ± 10	13 ± 7	24 ± 10	22 ± 6	21 ± 7	20 ± 6	20 ± 6	19 ± 7	19 ± 7	21 ± 5	22 ± 7
ALF968	8 ± 6	9 ± 7	7 ± 5	13 ± 7	19 ± 6	19 ± 8	21 ± 6	18 ± 5	16 ± 5	22 ± 6	19 ± 6	25 ± 7
ARCH915	11 ± 6	7 ± 5	9 ± 7	6 ± 5	16 ± 10	8 ± 6	11 ± 6	6 ± 5	7 ± 5	12 ± 6	11 ± 6	14 ± 5
LGC354b	11 ± 6	8 ± 5	9 ± 6	4 ± 4	14 ± 5	12 ± 6	13 ± 4	12 ± 6	11 ± 6	14 ± 6	15 ± 6	9 ± 6
PLA46	8 ± 6	7 ± 5	7 ± 6	8 ± 6	15 ± 5	15 ± 6	16 ± 6	16 ± 6	12 5	13 ± 5	15 ± 6	14 ± 5
HCG236	7 ± 6	7 ± 5	6 ± 5	7 ± 5	6 ± 5	13 ± 6	15 ± 6	13 ± 5	15 ± 5	12 ± 6	9 ± 5	10 ± 5
SRB385	2 ± 3	1 ± 3	1 ± 2	1 ± 2	10 ± 7	1 ± 3	1 ± 3	1 ± 3	2 ± 3	6 ± 5	9 ± 6	3 ± 4
ANME-1	2 ± 4	0.8 ± 2	0.8 ± 2	0.9 ± 2	33 ± 11	0.5 ± 2	1 ± 3	1 ± 2	2 ± 3	2 ± 3	4 ± 4	15 ± 5
BET42	0.7 ± 3	0.9 ± 2	0.6 ± 2	0.7 ± 2	1 ± 2	0.3 ± 1	0.3 ± 1	0.5 ± 1	0.3 ± 1	0.6 ± 2	0.5 ± 1	0.5 ± 1
NON338	1 ± 2	0.4 ± 2	0.9 ± 2	1 ± 2	1 ± 3	0.5 ± 1	1 ± 2	0.7 ± 2	0.9 ± 2	1 ± 3	2 ± 3	0.9 ± 2

Data are mean replicate counts ± SD.

ference between banding patterns and the second comprising two SR replicate samples. Greater variation between banding patterns was observed among the replicate samples from SG (SG-1, SG-2 and SG-3) and from SR (SR-1, SR-2 and SR-3) than among replicates from any other sites. Overall, variability in banding patterns between replicates was greatest at SR and PB stations. This was exemplified by a single sample at PB having high affinity with all samples from SG despite considerable geographical separation.

Bacterioplankton species diversity

A total of 1484 clones generated 1223 useable sequences across seven independent clone libraries (Table S2a). A total of 1087 gave matches of $\geq$ 97% similarity to 445 different existing 16S rRNA gene sequences. Between 94 and 184 sequences were generated from each surface water library (Pearce, 2008) corresponding to 42–75 OTUs per library. An OTU was defined as any sequence showing $\geq$ 97% identity to a known 16S rRNA gene sequence. The 136 sequences that could not be matched at this level represented 11% of the total, suggesting that a relatively low number of clones were completely new observations. Diversity was estimated using the Simpson diversity index and species richness using the nonparametric Chao index (Chao, 1984). The Simpson diversity index suggested a good spread within each library among different sequences (from 0.043 at ST to 0.113 at DI). The Chao index gave values of between 106 and 253 where SG exhibited the highest richness and ST the lowest. Coverage of each clone library was obtained using Good's coverage estimate (Good, 1953) and gave an average of 58.3% (this ranged from 50% at ST to 64% at LI). The majority of sequences analysed from each library showed $\geq$ 97% similarity with uncultured marine clones. Of the OTUs identified to group level, the *Alphaproteobacteria* were the dominant bacterial group in all seven clone libraries comprising between 79% and 55% of OTUs (Fig. 3). The *Gammaproteobacteria* were the next most abundant in six of the seven clone libraries comprising up to 20.9%, followed by the *Cytophaga–Flavobacterium–Bacteroidetes* (CFB) cluster and the *Betaproteobacteria*. In the LI sample, the CFB constituted a slightly larger portion of the clone library than the *Gammaproteobacteria*, and this site therefore did not follow the trend in dominant groupings. Sequences related to the *Verrumicrobia* and comprising up to 1.5% of the total OTUs were identified in the LI, PB and MI clone libraries. MI was also the only clone library to contain *Planctomycete*-related sequences. The only Actinobacterial sequences were identified in the EI library. These results differ slightly from the relative abundances observed for the FISH data where the *Gammaproteobacteria* and CFB

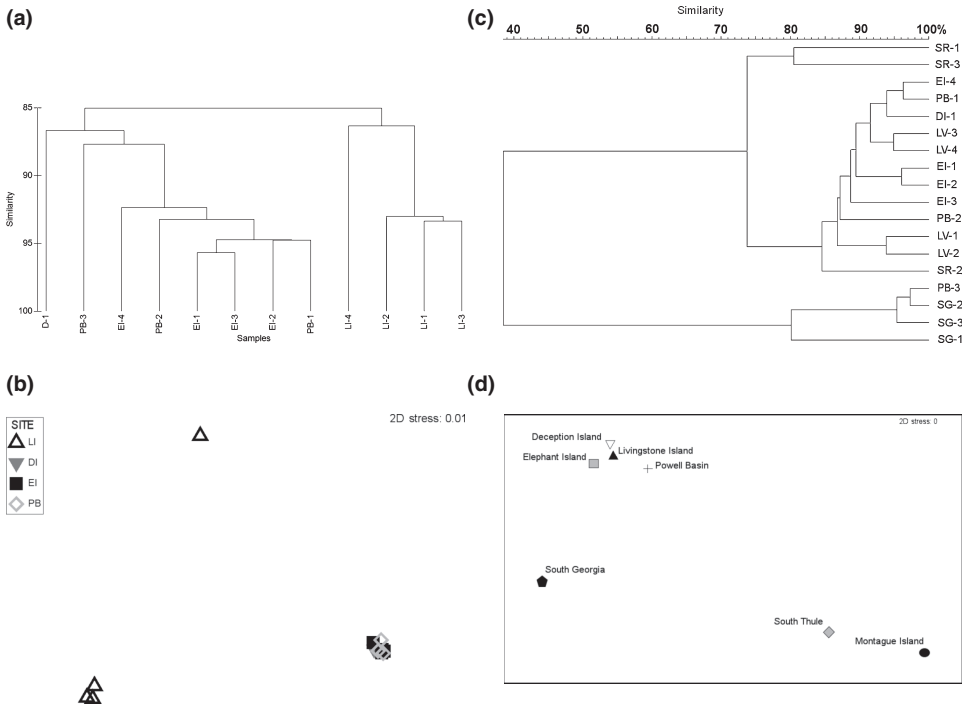


Fig. 2. (a) Cluster analysis dendrogram showing percentage similarity of 12 stations based on Bray–Curtis resemblance of the relative abundance of bacterial groups obtained by FISH. (b) MDS ordination of bacterioplankton community structure at each Scotia Arc station, based on a Bray–Curtis similarity matrix using FISH data for GAM42a, ALF968, CF319a, PLA46, HCG236 and LGC354b, (c) Cluster analysis dendrogram showing similarity of DGGE banding patterns between stations based on Pearson's correlation, (d) MDS ordination showing similarity between clone library composition based on Bray–Curtis resemblance.

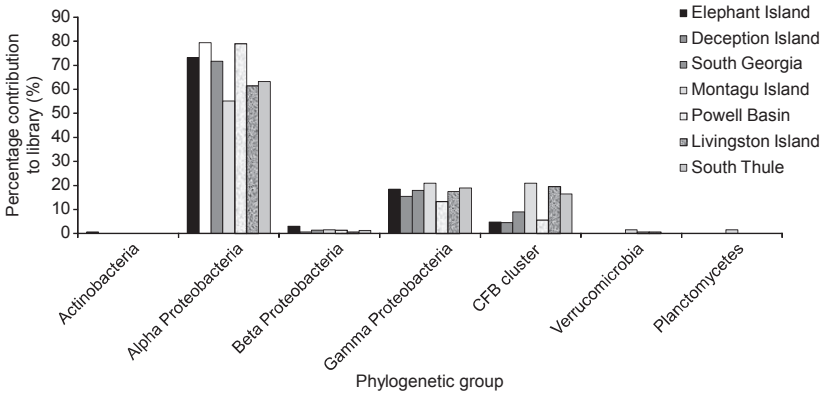


Fig. 3. Percentage contribution of main phylogenetic groups to Scotia Arc clone libraries.

cluster were slightly more abundant than the *Alphaproteobacteria*.

Of 445 OTUs across all clone libraries, 58 OTUs shared matches with cultured bacterial strains. At least eight strains including *Octadecabacter antarcticus* 307, *Aequorivita antarctica* isolate S4-8 and *Polaribacter irgensii* strain ANT9210 were previously isolated from Antarctic environments. The dominant OTUs in five of the seven clone libraries were *Pelagibacter ubique* contributing to up to 13% and 29%, respectively, of total OTUs. High numbers of clones matched uncultured marine bacterium clones isolated from Antarctic surface water and shelf

sediments (Bowman & McCuaig, 2003; Murray & Grzymski, 2007) and Arctic surface water (Bano & Hollibaugh, 2002).

Comparing percentage similarity in species composition using Sorensen's Index, three patterns emerge; the sites to the South (LI, DI and EI) are very similar to each other, their similarities ranging from 43% to 33% and in line with the relative geographical distances between them. If the two sites in the centre of the Scotia Arc, containing MI and ST, are added, they are less similar, but are as similar to each other as the southern stations and continue the trend of decreasing similarity with distance (i.e. ST is more similar to LI, DI and EI than MI). PB is an anomaly in that it is a basin and therefore much further from land than any of the other sites. Although it is between 22% and 30% similar to the two sites either side of it, it is very dissimilar (3–12%) to everything else. SG, to the north, is also unusual in that it matches well the southern stations. So location seems to be the predominant factor in the comparison, with SG potentially receiving colonists from around the northern tip of the Antarctic Peninsula via the ACC and able to exploit upwelling nutrient at SG (Table S2b).

Highest similarities were identified between the LI and DI libraries and between the LI and EI libraries. The least similarity was observed between the MI library and all others, with the exception of the ST library. Interestingly, the least similarity was observed between the MI and DI libraries. An MDS ordination of all seven clone libraries (representing the rare diversity) showed geographical differences in community composition within the Scotia Arc (Fig. 2d). The community compositions of MI, ST and, to some extent, SG were distinctly different from the other libraries, which clustered together. When aligned against known Antarctic sequences (Fig. 4a–c), sequences were evenly distributed among known polar sequences.

Discussion

Trends in microbial assemblages across sites

Cluster analysis of data from FISH, DGGE and clone library analysis, showed strong structural and compositional similarities between the communities at sites around the Scotia Arc, suggesting that communities around the Arc share the same dominant community members. However, some differences in the composition of assemblages could be detected between the southern sites (LI, DI, EI and PB) and those in the northern regions (ST/MI and SG/SR). Bacterioplankton communities located above PB, including ST, MI and SG, also exhibited greater variation in community structure and composition compared to communities of LI, DI and EI, closer to the Antarctic Peninsula.

Similarities between sites

Bacterioplankton population density

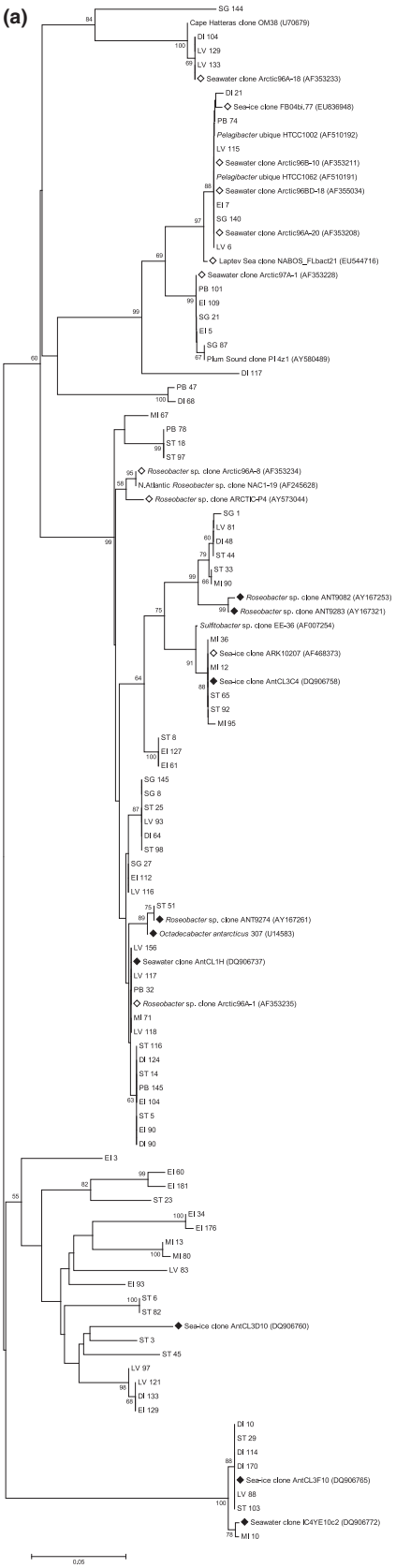
This was between 1.13 (±0.16) and 2.13 (±0.16) × 10^5 cells mL^{-1} and therefore consistent with other values reported for Antarctic surface waters. Marginally higher densities were noted at sites to the north of the Scotia Arc, which could reflect the patchiness of blooms in the area. In addition, population densities of the Scotia Arc did not differ greatly from those published for other latitudes (Zubkov *et al.*, 2001; Bano & Hollibaugh, 2002; Riemann *et al.*, 2008). Similar densities have also been observed in surface water during phytoplankton blooms in the Southern Ocean (West *et al.*, 2008), although higher bacterial densities of up to 3×10^6 cell mL^{-1} have been reported during a bloom event in the Ross Sea (Ducklow *et al.*, 2001). Elsewhere, a slight increase in bacterial population density from north to south has been reported in Antarctic waters near the polar front (Brown & Landry, 2001).

Bacterioplankton species richness

Eubacterial numbers were consistent across all sites and ranged between 1.1 and 1.3×10^5 cells mL^{-1}. Analysis of the Scotia Sea surface waters by FISH detected consistent dominant bacterial groups: *Gammaproteobacteria*, CFB, *Alphaproteobacteria*, *Planctomycetes*, sulphate-reducing, high GC and low GC bacteria. Clustering of FISH data showed that the group composition was > 85% similar across all sites examined.

Dominant bacterioplankton groups

Analysis of the Scotia Sea surface waters by DGGE detected the same dominant bacterial groups. DGGE analysis revealed between 9 and 13 easily resolvable bands per profile. DGGE band patterns were largely consistent across stations and between samples from the same geographical site. Six of the eight sequences obtained from DGGE bands were homologous (100% identical) to sequences obtained in the clone libraries. The other two sequences shared > 98% similarity with clone library sequences. Six of the eight bands identified also represented sequences identified from polar regions. However, this similarity could still be further improved, as a range of primers are now available for DGGE analysis of marine bacterioplankton profiles. Sanchez *et al.* (2007) used five different primer sets in an oligotrophic coastal system and recommend 357fGC-907rM as the most suitable for the routine use of PCR-DGGE analyses in this environment.



Bacterioplankton species diversity

From clone library analysis, each Scotia Arc community was dominated by sequences related to the *Alphaproteobacteria*, *Gammaproteobacteria* and CFB bacterial groups. This pattern of dominance has already been reported in the eastern Scotia Sea (Topping *et al.*, 2006) although west of the Antarctic Peninsula, the *Gammaproteobacteria* have been found to be the dominant group followed by the *Alphaproteobacteria* and the CFB (Murray & Grzymski, 2007). In the Ross sea, members of the *Gammaproteobacteria* and CFB groups, usually associated with sea ice, dominated bacterioplankton clone libraries (Gentile *et al.*, 2006). The *Roseobacter* clade comprised between 1.4% and 8% of the total bacteria in each library, which is within the same range as those detected by FISH elsewhere (Topping *et al.*, 2006). In a global distribution study, the highest abundance of an uncultured *Roseobacter* clade comprised up to 20% of the prokaryotic community in Weddell Sea surface waters (Selje *et al.*, 2004). *Roseobacter* are often associated with phytoplankton blooms and primary production (Zubkov *et al.*, 2001; Alonso & Pernthaler, 2006) and showed the highest frequencies in the ST and MI communities.

The dominance of *Alphaproteobacteria* in the clone library results was not reflected by the FISH data where members of the *Gammaproteobacteria* and CFB cluster were slightly more abundant. In addition, a higher proportion of both *Planctomycetes* and *Actinobacteria* were detected by FISH than those that appeared in the clone libraries. The overrepresentation of *Alphaproteobacteria* within marine clone libraries when compared to FISH analyses has been reported in other studies (Cottrell & Kirchman, 2000; Alonso-Sáez *et al.*, 2007). The discrepancies between these techniques might be a result of differential PCR amplification efficiencies (Dutton *et al.*, 1993), and some bias towards the *Proteobacteria* has been observed using universal primers, to the detriment of other groups such as the CFB cluster (O'Sullivan *et al.*, 2002). It has also been suggested that microorganisms with a low G+C content such as some *Alphaproteobacteria* may increase in proportion during amplification as a result of PCR bias (Alonso-Sáez *et al.*, 2007). In addition, universal primers do not encompass all *Planctomycetes*, owing to the considerable phylogenetic depth of this group (Vergin *et al.*, 1998). However, although FISH do have some technical limitations, notable consistencies emerge. The *Gammaproteobacteria* and the CFB cluster were about the same frequency, with

Fig. 4. Phylogenetic trees showing the distribution of clones found among known Antarctic marine sequences for (a) the *Alphaproteobacteria* (b) the *Gammaproteobacteria* and (c) the *Cytophaga–Flavobacterium–Bacteroidetes* group.

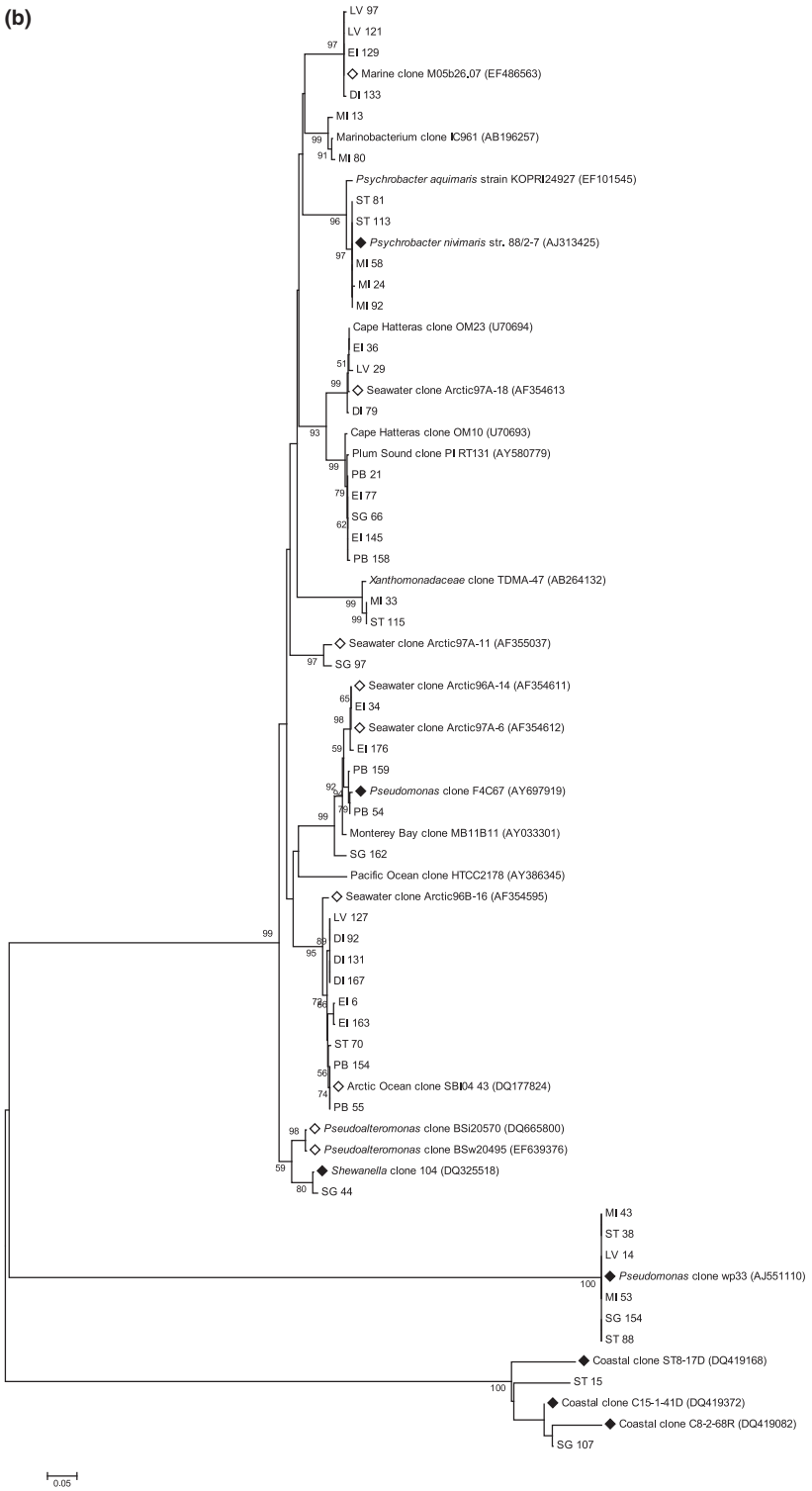


Fig. 4. Continued.

Gammaproteobacteria being slightly more abundant at all sites, and the *Betaproteobacteria* were below the frequency of the NON probe (as expected for marine environments). So most importantly, the order of abundance of each group of bacteria was consistent across each of the different study sites for each independent technique.

The majority of clones identified in the Scotia Sea nearshore communities were related to sequences isolated from

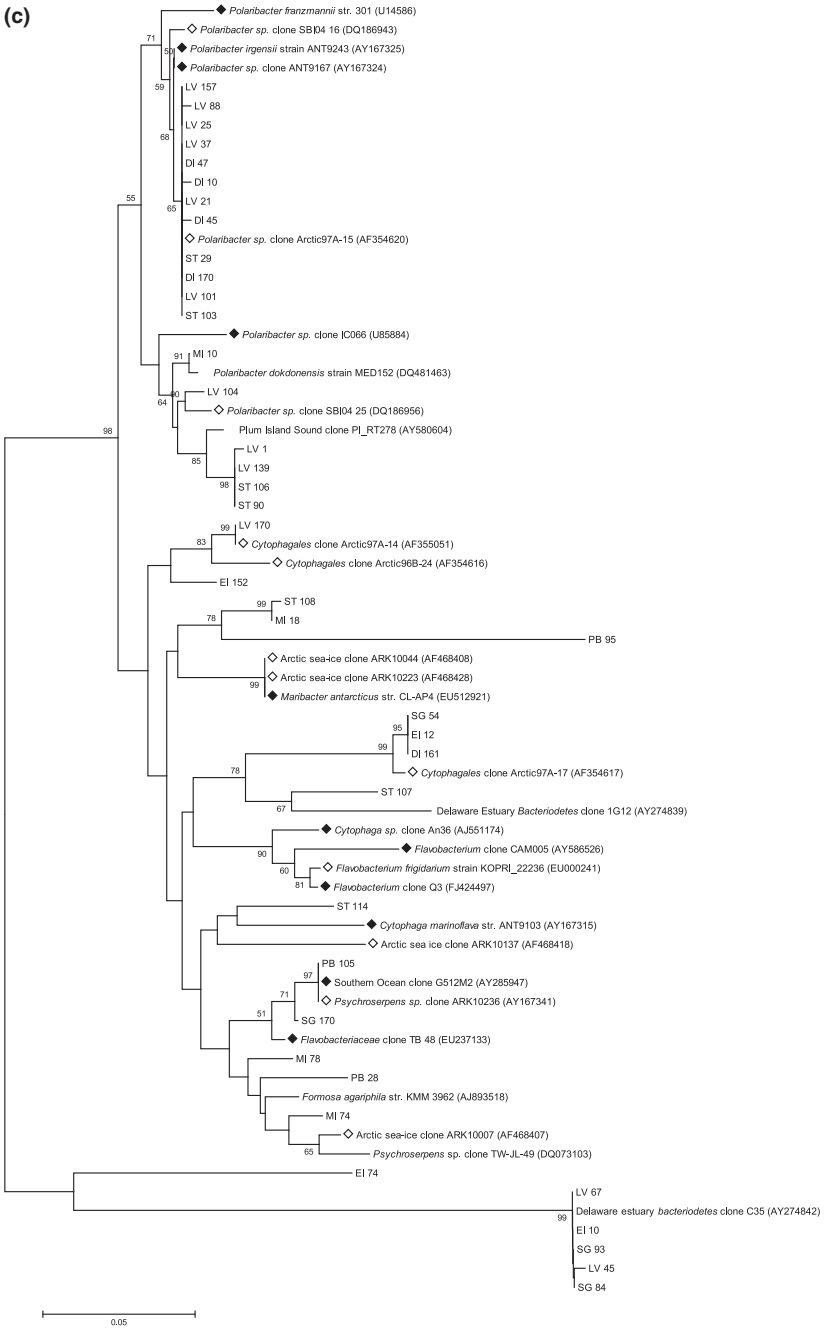


Fig. 4. Continued.

permanently cold environments, and six OTUs represented *Psychrobacter*, *Psychroserpens* and *Psychromonas* spp. OTUs related to the *Betaproteobacteria* were found in all libraries but at levels of <3%. A small number of *Actinobacteria*- and *Planctomycete*-related OTUs were identified solely in EI and MI libraries, respectively. However, the presence of the uncultured *Gammaproteobacteria* OM10 and *Pelagibacter ubique* strains in Scotia Arc nearshore libraries, indicated that these bacterioplankton communities also contained cosmopolitan bacteria belonging to the SAR86 and SAR11 clades (Giovannoni *et al.*, 2005; Pommier *et al.*, 2005). Sequences related to the widespread *Alcanivorax* sp. (Liu & Shao, 2005) were identified in addition to phylogenetic groups identified in polar regions such as *Polaribacter* sp. and *Roseobacter* sp. (ANT9274 and ANT9283), Antarctic bacterium IC4YE10c2 and numerous *Alphaproteobacte-*

ria clones from the Arctic96A and B clusters. Two phylotypes were also identified in the ST library (*Octadecabacter antarcticus* 307 and *Octadecabacter* sp. ANT9035), related to genus *Octadecobacter*, which exhibit a bipolar distribution (Staley & Gosink, 1999).

Differences between sites

Livingston Island

The structural differences observed between the LI community (samples LV-1 to LV-4) and those elsewhere were attributed to lower *Alphaproteobacteria* and *Planctomycete* numbers and to a lower species richness (Chao 1 value). This is interesting given that the *Alphaproteobacteria* were identified in higher abundances in all other locations and have been previously shown to dominate bacterioplankton communities in the eastern region of the Scotia Sea (Topping *et al.*, 2006). It is possible that the comparatively low surface chlorophyll-*a* levels measured at LI (0.1957–0.3177 mg m^{-3}) might contribute to the observed differences in community structure between this and other sites. The differences observed between the LV and other communities might reflect the increasing surface chlorophyll-*a* gradient along the south-to-north transect that peaks at SG, the site of a seasonal phytoplankton bloom. The greater abundance of *Planctomycetes* observed at EI and PB sites, situated north-east of LI, might reflect a productivity gradient because algae or their decomposition products have been reported to enrich *Planctomycetes* populations (Fuerst, 1995), *Planctomycetes* have been previously associated with surface chlorophyll-*a* levels in phytoplankton bloom areas (Morris *et al.*, 2006) and *Planctomycetes* have been shown to be involved in the mineralization of algal biomass and the removal of nitrogen (Pizzetti *et al.*, 2011a, b).

Deception Island

Higher mean abundance of Archaea, methanogenic Archaea and SRB at DI compared with other stations might reflect the chemical composition of the water at this site (within a volcanic caldera), as it has been previously demonstrated that volcanism in this region can affect local sea water chemistry (Elderfield, 1972).

South Thule

The community at ST (also inside a caldera) showed the highest diversity of all sites and least similarity to DI. The higher diversity at ST may reflect both its close proximity to higher concentrations of chlorophyll-*a* at SG and its situation within a volcanic caldera.

Differences within sites

Livingston Island

An intersite difference in community structure was observed between sample LV-4, and samples LV-1, LV-2 and LV-3 (Fig. 2a and b). The community structure of LV-4 differed from other LV replicates in that it comprised greater numbers of *Alphaproteobacteria*, *Gammaproteobacteria* and CFB cluster members and far fewer Archaea. Sample LV-4 were collected closest to the shoreline (bottom water depth 191 m); thus, differences observed between this and other replicate samples may have resulted from microscale patchiness in the water chemistry in these areas caused by ice-melt-induced stratification of the water column (Topping *et al.*, 2006), terrestrial runoff, geological activity of the islands and/or coastal upwelling which enriches surrounding waters (Zdanowski & Figuerias, 1997; Delille, 2003).

Environmental factors

Latitude

Observed differences in community structure (predominantly between LI, DI and PB) coincided with changes in position/latitude between the sites (as observed by Fuhrman *et al.*, 2008; Weitz *et al.*, 2010).

Temperature

SR and SG are much warmer than the other sites at > 3.5 °C vs. < 2 °C. Extensive matches with psychrophilic marine bacteria strongly suggest that temperature could be an important selection pressure. A number of microorganisms show adaptation to their atypically hot or cold niches and therefore show distribution patterns confined to particular regions, for example temperate and polar (Selje *et al.*, 2004). These differences are linked to optimum temperature and genotypic and biochemical adaptations that control the functional capability of bacterial proteins (Grzymski *et al.*, 2006) such as cold-adaptive enzymic activity (Zhang & Zeng, 2007).

Salinity

The difference in salinity observed (0.35 PSU on average) between southerly (LI, DI, EI) and more northerly sites (SG, SR) may be partially responsible for compositional variation observed among subdominant members of these communities. Biogeographical differences in bacterial community composition have been linked to salinity gradients in the past (Crump *et al.*, 2004), although within

oceanic ranges of salinity, only weak relationships between bacterioplankton richness and salinity have been observed (Fuhrman *et al.*, 2008).

Maximum depth/distance to shore

Overall results indicate that distance from the shore and increased surface water productivity from island runoff may be partially responsible for microscale variations in water composition that can affect bacterial biomass (Delille, 2003) and cell-specific bacterial activity (Longnecker *et al.*, 2005). Effects of such variation on Scotia Sea bacterioplankton community composition may be responsible for any differences between same-site replicates but were weak given the overarching similarity between all communities analysed. EI, LI and DI samples vary enormously in depth below the sample (191–1433 m), and where this is a proxy for distance to a coast and consequent terrestrial runoff, it could well account for intrasite variation. For example, EI 4 was above 244 m and distinct from EI 1, EI 2 and EI 3, which were taken over deeper water.

Ice melt and terrestrial runoff

Island runoff, ice-melt stratification of the water column and upwelling of nutrients within the Antarctic Circumpolar Current have all been implicated as responsible factors for differences observed in bacterioplankton composition between east and west regions of the Scotia Sea in 2006 (Topping *et al.*, 2006). It is possible that such factors may have contributed to the minor differences observed between communities in this study too, although samples were collected during late austral summer and thus the impact of overlying sea ice, ice-melt stratification and island runoff effects would have most likely to been reduced at this time of year.

Chlorophyll concentrations

It is possible that variable chlorophyll-*a* measurements along the north-to-south transect might contribute to the minor structural and compositional differences between communities located towards the north or south of the Scotia Arc. The elevated chlorophyll-*a* concentration (18 mg m^{-3}) shown at the SG station may contribute to the 60% difference in community structure observed (Fig. 2c), compared to a 15% difference between all other communities that share similar, lower chlorophyll-*a* levels (0.19–0.79 mg m^{-3}). These results are likely to reflect the tail end of the seasonal phytoplankton bloom that develops to the west of SG as a result of iron enrichment of the surface waters (Korb *et al.*, 2005). Structural and compositional differences in water column bacterial communities inside and outside of bloom regions (Topping *et al.*, 2006; West *et al.*, 2008) have been reported, as well as compositional changes during bloom events (Fandino *et al.*, 2001; Pinhassi *et al.*, 2006), although absence of any differences has also been documented (Arrieta *et al.*, 2004).

Bacterial phylotypes specifically associated with phytoplankton blooms have also been identified (Gonzalez *et al.*, 2000). Two uncultured marine clones (clones KG_A3_120m53 and KG_C11_100m45) detected by DGGE analysis from the Scotia Arc profiles were previously isolated from inside and outside a phytoplankton bloom at Kerguelen in the Southern Ocean, suggesting that the presence of these phylotypes in the Scotia Arc might be linked to water conditions defined by the elevated chlorophyll-*a* levels (West *et al.*, 2008). In addition, members of the CFB cluster found to be dominant in all communities across the Scotia Arc are aggregate-forming bacteria (Delong *et al.*, 1993), often associated with the dissolved organic matter from bloom events (Kirchman, 2001), and may play a role in the degradation of organic matter from SG bloom events.

Biogeography

Within the *Alphaproteobacteria*, the *Gammaproteobacteria* and the CFB cluster, new sequences derived within this study were spread evenly throughout existing sequences deposited in the databases, including both identical and closely related sequences. There did not appear to be any novel groups in the region (although their existence may be possible within the 11% sequences without any matches). The sequence matches, however, are predominantly to marine environments amenable to the growth of psychrophiles, suggesting that these may be the key selection pressures. The dominant groups appear to be consistent, and this is in agreement with Pommier *et al.* (2005), who cite consistent community structures. It is possible, however, to determine significant differences between the rare groups ($P < 0.05$), and these differences can be detected using both amplification-dependent and amplification-independent techniques. This is in agreement with observations by Malmstrom *et al.* (2007), Murray & Grzymski (2007), Pommier *et al.* (2007) and Weitz *et al.* (2010).

Conclusions

Seven spatially separated communities shared similar population densities and were dominated by the same species and by similar relative abundances of the main phylogenetic groups: *Alphaproteobacteria*, *Gammaproteobacteria* and CFB groups. Sequences related to phylotypes

isolated from permanently cold marine environments dominated Scotia Arc clone libraries, although cosmopolitan microorganisms were also present. However, significant differences ($P < 0.05$) could still be detected in communities located above PB, to the north of the island chain, such that the sites could be split into three distinct groups: a northern group (SG and SR), an eastern group (MI and ST) and a southern group (LI, DI, EI and PB). This roughly north–south gradient of differences in subdominant or rare diversity is likely a result of a combination of environmental factors related to the extent of nutrient enrichment from terrestrial runoff, upwelling and seasonal phytoplankton blooms, where the Antarctic Circumpolar Current crosses the islands of the Scotia Arc.

Acknowledgements

This work was supported by the Natural Environment Research Council, through the Crozet consortium Grant No, NER/A/S/2003/00574.2004 and through BAS Global Science in the Antarctic Context – Long Term Monitoring and Survey Programme (LTMSB task 7). We would also like to thank the Master and crew of the James Clark Ross for their help and support on cruise JR144 and two anonymous reviewers for improving the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Closest phylogenetic affiliations to DGGE band sequences from EMBL-EBI database and the percentage similarity of the match.

Table S2. (a) Matrix showing percentage similarity (Sorensen's Index) in species composition between seven Scotia Arc libraries. (b) Clone library diversity calculated for seven Scotia Arc clone libraries. *Coverage calculated according to Good (1953).

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