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Seasonal abundance of *Halomonas meridiana*, *Halomonas subglaciescola*, *Flavobacterium gondwanense* and *Flavobacterium salegens* in four Antarctic lakes

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Abstract: Indirect immunofluorescence was used to quantify the seasonal variation of four halophilic aerobic Antarctic bacteria in Antarctic saline lakes from July 1990 to January 1991. Antibodies were raised against type strains from the Australian Collection of Antarctic Microorganisms. During summer, all four serogroups were identified in the aerobic waters of lakes with total dissolved salts above 61‰. Maximal abundances of *Halomonas meridiana*, *H. subglaciescola* and *Flavobacterium gondwanense* serogroups were observed at discrete depths within the water column in the two most hypersaline lakes at about midsummer, coincident with the time of maximum sunlight and the commencement of the summer thaw. At this time the *Halomonas* spp. serogroups comprised up to 40% of the total bacteria and the *F. gondwanense* serogroup up to 10% of the total bacteria. The *F. salegens* serogroup was in low numbers (>2% of total bacteria) in some aerobic waters. Up to 2% of the total bacterial populations in the lakes were autofluorescent or stained non-specifically. Dissolved organic carbon values were measured throughout the sampling period and correlated well with total bacterial numbers but not with changes in species composition. Change in species abundance, as indicated by immunofluorescence, was not reflected in the total bacterial count, indicating compositional change of the total bacterial population.

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Introduction

Since 1981 a number of biogeochemical processes occurring in the lakes of the Vestfold Hills, Antarctica, have been examined in detail. These include sulphur cycling (Hand & Burton 1981, Franzmann *et al.* 1987a, Franzmann *et al.* 1988a, Gibson *et al.* 1991), bacterial photosynthesis (Burke & Burton 1988a, Burke & Burton 1988b), methane generation (Franzmann *et al.* 1991a) and the distribution and abundance of volatile organic molecules (N.J. Roberts, personal communication 1991). Volkman *et al.* (1988) and Mancuso *et al.* (1990) examined microbial community structure by analysis of microbial pigments and lipids.

Descriptions of the changing microbial environment and the associated changes in bacterial number and state have limited use unless many of the species involved can be recognized and enumerated; an understanding of the microbial ecology of an environment requires studies of temporal and spatial changes in species composition (Brock 1987, Tabor & Neihof 1982). Microorganisms involved in these processes, including several new species (Dobson *et al.* 1991, Franzmann & Rohde 1991, Franzmann *et al.* 1988b, Franzmann *et al.* 1987b, Franzmann *et al.* 1991b, James *et al.* 1990, McGuire *et al.* 1987), have been isolated and characterized from the lakes of the Vestfold Hills and the local marine areas.

Only four bacterial species have been consistently isolated from the oxylimnion of the hypersaline lakes of the Vestfold Hills. Though this reflects the limitation of cultural techniques rather than these being the only bacteria present, in this study we used immunofluorescence microscopy to enumerate the spatial and temporal change of these target species in Antarctic environments. The technique has been used extensively in medical microbiology (Herbert 1990), for the detection of microbial pathogens (Kaspar & Tartera 1990) and to some extent in the field of microbial ecology (Herbert 1990). The seasonal abundance of *Halomonas meridiana*, *H. subglaciescola*, *Flavobacterium gondwanense* and *F. salegens* (Table I) were quantified throughout the austral spring and summer in the meromictic Organic, Ekho, Fletcher, and Ace lakes and a local seawater site (Table II).

Materials and method

Preparation of antibacterial antibodies

The following protocol for antibacterial antisera production was developed from the methods of Kawamura & Aoyama (1983) and Gerencser (1979).

Bacterial cells were harvested from half strength artificial Organic Lake peptone yeast (1/2AOLPY) (Franzmann *et al.* 1987 a) agar plates into 1/2AOLPY broth. Cells were collected

by centrifugation then the cell pellet was weighed, resuspended in 1/2AOLPY broth with 6% formaldehyde (w/v) and incubated for 1 h at room temperature. Cells were collected and resuspended in 1/2AOLPY broth to a density of *c.* 1×10^{10} cells ml^{-1}. Formaldehyde was added to a final concentration of 0.25% (w/v). Ten New Zealand white rabbits were immunized to these cell suspensions during a 50 day period. Suspensions were combined either in equal proportions with Freund's incomplete adjuvant for intramuscular and subcutaneous injections or used alone for intravenous injections.

A pre-immunization bleed of 10 ml on day 1 was followed by immunization with a 2 ml subcutaneous injection of the cell suspension and a 3 ml intramuscular injection of the cell suspension. On day 28, the rabbits received a 0.5 ml intravenous injection of the cell suspension. On day 35, the rabbits received a 1 ml intravenous injection of the cell suspension. Finally, on day 42, the rabbits were bled from the marginal ear vein.

Serum antibody titre was checked by slide agglutination. One drop of the appropriate cell suspension was added to one drop of either normal (pre-immunization bleed) serum or trial bleed serum diluted with 3% NaCl (w/v) to 1/10, 1/100, 1/1000 and 1/10000 concentrations. Cell/serum combinations showing agglutination after incubation for 1 h at 37°C at serum dilutions of 1/1000 or greater were considered acceptable.

Rabbits showing acceptable titres were bled at four day intervals. In most cases 30 ml of serum was obtained. Other rabbits were intravenously injected with 1 ml of the cell suspension on days 44 and 46. Then the trial bleed was repeated on day 50.

Serum globulins were precipitated with anhydrous sodium sulfate and purified using a Biogel P-6DG column. Sodium azide at a final concentration of 0.01% (w/v) was added to all purified antisera as a preservative. Antisera were rapidly frozen in liquid nitrogen then stored at 20°C.

The four bacterial strains used to raise antibodies were obtained from the Australian Collection of Antarctic Microorganisms (ACAM). Slide agglutination was used to determine specificity of these antisera against different strains of the test species, as well as other species within the family Halomonadaceae. Indirect immunofluorescence was used to confirm these results. No other species of the genus *Flavobacterium* were tested for antisera reactivity as the two Antarctic *Flavobacterium* spp. are the first halophilic species of the genus *Flavobacterium* to be described (Dobson *et al.* 1991). Antibody specificity was tested on bacterial strains grown under different temperature, salinity, light and nutrient levels and on 1/2AOLPY agar and broth.

Bacterial strains used in cross reactivity testing were; *Deleya halophila* (CCM 3662), *D. aesta* (NCMB 1980), *D. aquamarina* (DSM 30161), *D. pacifica* (DSM 4742), *D. cupida* (DSM 4740), *D. venusta* (DSM 4743), *D. marina* (DSM 4741), *Halomonas halmophila* (NCMB 1971) and *H. elongata* (ATCC

Table I. A list of *Halomonas* and *Flavobacterium* strains used to raise antibacterial antibodies, their temperature and total dissolved salts growth range and growth optima as determined by growth in artificial broth.

Organism	Strain	Temperature (°C)		TDS (‰)	
		range	optimum	range	optimum
H.meridiana	ACAM 246[a]	0 to 45	28 to 40	0 to 250	10 to 30
H. subglaciescola	ACAM 12[b]	-3 to 32	24	4 to 290	80 to 100
F. gondwanense	ACAM 44[c]	-6 to ≥25[d]	≃18	0 to ≥150e	50
F. salegens	ACAM 48[c]	-5 to ≥25[d]	≃18	0 to ≥200[e]	50

[a] Also - ATCC 49692, NCIMB 13119; data from James *et al.* (1990).
[b] Also - ATCC 43668, DSM 4683; data from McMeekin & Franzmann (1988) and Dobson (1988).
[c] Temperature data from closely related strains; data from Dobson (1988).
[d] Cultures grew well at 25°C but were not tested at higher temperatures.
[e] Cultures grew well at 150 and 200 °/oo respectively but were not tested at higher salinities.

Table II. Location and characteristics of lake and seawater sites sampled during 1990–1991.

Site		TDS range[a] (‰)	Temperature range[b] (°C)	Depth (m)	Oxycline (m)
Organic Lake	(68°27.2'S, 78°12.3'E)	66 to 215	-10 to + 8	7	4
Ekho Lake	(68°31.2'S, 78°16.0'E)	35 to 166	-3 to + 19	42	20
Fletcher Lake	(68°27.2'S, 78°15.2'E)	25 to 126	-5 to + 8	12	8
Ace Lake	(68°28.4'S, 78°11.1'E)	10 to 51	-3 to + 11	23	11
Local sea water	(68°34.1'S, 77°59.0'E)	34	-2 to 1	3	-

[a] Lake TDS range from a summer time surface melt-water minimum to constant maximum levels in the anaerobic bottom waters.
[b] Lake temperatures range from a winter upper waters minimum to summer maximum in the middle waters.

33173). A number of different strains of the four test species were also examined including; *H. meridiana* (ACAM 246) (type strain), *H. meridiana* (ACAM 233), *H. meridiana* (ACAM 235), *H. subglaciescola* (ACAM 12) (type strain), *H. subglaciescola* (ACAM 227), *H. subglaciescola* (ACAM 222), *H. subglaciescola* (ACAM 251), *H. subglaciescola* (ACAM 243), *H. subglaciescola* (ACAM 230), *H. subglaciescola* (ACAM 255), *H. subglaciescola* (ACAM 247), *F. gondwanense* (ACAM 44) (type strain), *F. gondwanense* (ACAM 49), *F. gondwanense* (ACAM 62), *F. gondwanense* (ACAM 46), *F. gondwanense* (ACAM 56), *F. salegens* (ACAM 48) (type strain), *F. salegens* (ACAM 52) and *F. salegens* (ACAM 54).

Site description

The surface of all five of the study sites remained frozen after the winter, maintaining an ice cover of *c.* 1.5 m thick until midsummer. With the thaw, the upper 4–5 m of each of the lakes mixed and became less saline. All the study lakes were meromictic; permanently stratified with lighter, less salty water overlying a more saline layer (Table II). This stratification leads to anoxia in the lower layer through bacterial action over long periods of time. In all the study lakes the boundary layer (oxycline) between aerobic and anaerobic lake water masses remained stable during the year (Table II). pH was 7.0–8.6 at all sites. All the lakes sampled supported microalgal populations, most commonly *Tetraselmis* sp. (Hirsch & Siebert 1991). Soil run-off and melt waters associated with the summer thaw provide additional nutrient sources to the lake (Burton 1981).

Sample collection

From July 1990 to January 1991, water samples were collected from deep water sites in Organic, Ekho, Fletcher and Ace lakes from the aerobic zone, the oxycline and the anaerobic bottom waters (Table II) every two months. Samples were also taken from a local sea water site about 10 m off-shore.

In situ temperature and density (*in situ* density adjusted to water density at 20°C) was measured using a profiling DMA 35 density meter (Anton PAAR, Austria). Densities were converted to total dissolved salts (TDS) by the formula of Stark (S.C. Stark, personal communication 1991). Samples were taken using a 2 l Kammerer bottle. During the summer thaw (from late December), sampling and *in situ* measurement was from the side of a boat. Samples were stored in sterile clean 6 l plastic autoclave bags tied at the top and transported in insulated drums. Sample pH was determined with a Metrohm model E604 pH meter.

Dissolved Organic Carbon (DOC) levels were measured using a Skalar DOC Analyser (Analytical Technologies Australia, New South Wales). This unit digested DOC by acidification, chemical degradation (using persulphate) and strong ultra-violet light, quantitatively oxidizing the DOC to carbon dioxide which was then reduced to methane and measured by flame ionization detection (Hine & Bursill 1985). Samples were analysed following storage for up to 12 days in the dark at 1°C. Samples were filtered through Nuclepore polycarbonate filters (0.2 μm; 25mm) just prior to analysis. Each sample set was processed against a glucose standard containing 10 mg of carbon per litre (mg C l^{-1}) made up in double distilled water passed through a Millipore 'Norganic' organic scavenger resin cartridge. When necessary, the sample was diluted before analysis to reduce the DOC level to within the range 0–10 mg C l^{-1}. Dilution was required for most of the samples from anaerobic waters.

Total bacterial count

Samples used for microscopy were fixed with 0.5% glutaraldehyde at the sample site, stored in the dark at 1°C and analysed within 12 days. Samples showed only slightly reduced counts after storage for 60 days. The sample was incubated in the dark at room temperature with 0.1 μg ml^{-1} 4,6-diamidino-2-phenylindole (DAPI; a DNA specific stain for total counts) for 10 min. Sufficient sample, typically 0.2–2.0 ml, was then filtered through a Nuclepore polycarbonate filter (0.2 μm; 25mm) to give *c.* 100 bacteria per field of view. The filter was placed on a slide with one drop of phosphate buffered saline salts in glycerol instead of water (PBS-glycerol) and examined microscopically using a Leitz Laborlux 12 epifluorescence microscope with filter block A. Sufficient fields of view, typically 5–8, were counted to give a running average count of ± 10%.

Indirect immunofluorescence count

The sample was incubated in the dark at room temperature for 20 min with 1.25×10^{-3} ml of antisera per ml of sample. Sufficient sample, typically 0.2–2.0 ml, was then filtered through a Nuclepore polycarbonate filter (0.2 μm; 25mm) to give *c.* 100 bacteria per field of view and the filter rinsed with phosphate buffered saline (PBS). The filter was re-incubated in the dark at room temperature for 30 min with 2 ml of fluorescein isothiocyanate (FITC) conjugated 'anti-rabbit' antibody solution (Silenus; sheep raised anti-rabbit immunogloblin code RF) diluted to 1:1600 with PBS. The filter was then rinsed with PBS, placed on a slide with one drop of PBS-glycerol and examined microscopically using a Leitz Laborlux 12 epifluorescence microscope with filter block H2.

Results and discussion

Specificity of the antisera

Antibodies raised against the type strains of *H. subglaciescola* and *F. gondwanense* were strain specific. Antibodies raised against the type strain of *H. meridiana* were species specific. The antibodies raised against the type strain of *F. salegens* cross-reacted outside the species.

No reactivity was demonstrated between the antisera and

other non-target bacterial species with the exception of the marine bacterium, *D. venusta* (DSM 4743) which showed strong agglutination reaction with antisera raised against the type strain of *F. salegens*. In a detailed chemotaxonomic study of the family *Halomonadaceae*, Franzmann & Tindall (1990) demonstrated that the fatty acid composition (and thus the membrane structure) of *D. venusta*, "....did not conform well to the general pattern of fatty acids possessed by other members of the family *Halomonadaceae*.". Franzmann & Tindall went on to suggest that *D. venusta* is a peripheral member of the family. Though this does not account for the strong agglutination reaction to antibodies raised against bacteria of a different genus, it does offer some explanation why *D. venusta* alone demonstrated this reaction. Despite the close chemotaxonomic relationship reported between *H. meridiana* and *D. aesta* (Franzmann & Tindall 1990) no cross-reactivity was observed between either of the *Halomonas* spp. antisera and any of the *Deleya* spp. tested, including *D. aesta*.

Background to lake biota

The Antarctic bacteria were originally isolated from the aerobic waters of hypersaline Organic Lake and Ekho Lake during the summers of 1986 and 1988, on nutrient supplemented lake water media incubated aerobically at 10°C (Franzmann *et al.* 1987b, Garrick & Gibson 1988). Since then, Hirsch & Siebert (1991) and the authors have isolated more bacteria from the aerobic waters of Organic and Ekho lakes on a variety of media. A small number of isolates probably belong to five genera: *Planctomyces* (six isolates), *Pirellula* (four isolates), *Gemmata* (two isolates), *Hyphomicrobium* (two isolates) and *Blastobacter* (two isolates). Some others are likely to belong to the genera: *Bacillus*, *Vibrio* and *Spirillum* (Hirsch & Siebert 1991). However the majority of isolates belong to the genera *Halomonas* and *Flavobacterium* (unpublished data). This reduced diversity, characteristic of harsh environments (Alexander 1976), is probably due to the high salinities and low temperatures of both these lakes.

Presence of test species

Up to 2% of the total bacterial population of the lakes were autofluorescent or stained non-specifically. Background counts were accounted for in immunofluorescence counts. Background fluorescence was easily distinguishable from DAPI stained bacteria. Known quantities of the four Antarctic bacteria used to raise the antisera (suspended in PBS) were used as internal standards in the immunofluorescence counts. At cell concentrations above 2×10^3 cells ml^{-1}, recovery was > 95%. When analysing samples no attempt was made to count fluorescent cells at concentrations below 2×10^3 cells ml^{-1}; these data were recorded as zero cells ml^{-1}.

Peak percentages of the four serogroups in Organic, Ekho and Fletcher lakes as well as sample date, depth, total bacteria, DOC, TDS and temperature are shown in Table III. The abundances of the three main serogroups in Organic and Ekho lakes are represented graphically (Figs 1 & 2) to illustrate localized blooms during the height of summer. Fletcher Lake contained < 4% of all four serogroups and showed no seasonal pattern. All Ace Lake and local marine samples contained < 0.1% of the four serogroups and showed no seasonal pattern.

All four bacterial species, originally isolated from the oxylimnion of Vestfold Hills meromictic lakes with ≥147‰ TDS (Garrick & Gibson 1988, Dobson *et al.* 1991), were shown by this study to occur in lakes where the TDS was between 61‰ (Fletcher Lake) and 165‰ (Organic Lake) and temperature

Table III. Peak percentage of total bacteria of the four bacterial serogroups at the three most saline sites.

Serogroup	Site	Peak % of total	Sample date	Site data specific to sample depth				
				Depth (m)	Total bacteria (cells ml^{-1})	DOC (mg C l^{-1})	TDS (‰)	Temp. (°C)
H. meridiana	Organic L.[a]	2	Oct. 1990	4	2×10^7	39.0[d]	165	-5.6
	Ekho L.[b]	23	Dec. 1990	16	8×10^5	4.9	83	15.6
	Fletcher L.[c]	3	Oct. 1990	2	3×10^6	1.0	61	-3.1
H. subglaciescola	Organic L.	3	Jan. 1991	3	1×10^7	30.1	160	5.8
	Ekho L.	16	Dec. 1990	16	8×10^5	4.9	83	15.6
	Fletcher L.	2	Oct. 1990	4	3×10^6	0.9	62	-3.1
F. gondwanense	Organic L.	10	Nov. 1990	2	6×10^6	24.5	146	-8.4
	Ekho L.	9	Dec. 1990	6	1×10^6	2.0	65	11
	Fletcher L.	2	Aug. 1990	2	4×10^5	1.0	56	-2.0
F. salegens	Organic L.	<1	Oct. 1990	3	6×10^6	24.5[d]	163	-10.1
	Ekho L.	2	Sept. 1990	14	2×10^5	4.1	83	15.9
	Fletcher L.	1	Nov. 1990	6	8×10^6	2.5	74	2.7

[a] Organic lake was 7 m deep with an oxycline at 4 m. [c] Fletcher lake was 12 m deep with an oxycline at 8 m and is subject to periodic seawater incursions.
[b] Ekho lake was 42 m deep with an oxycline at 22 m. [d] DOC levels were not determined during October 1990 in Organic Lake, (results from November 1990).

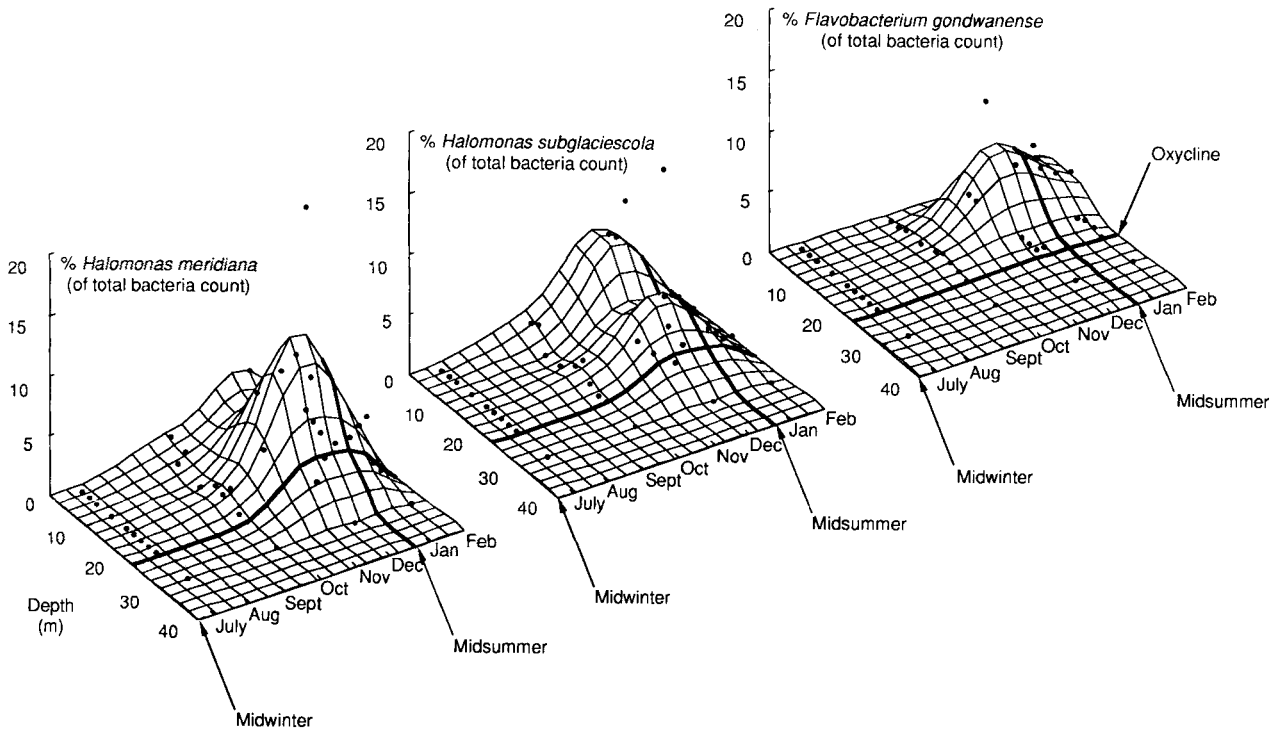

Fig. 1. Surface plots (smoothed by weighted least squares method) of percentage serogroups of total bacteria in Ekho Lake, over time and depth. Serogroup percentages as determined by immunofluorescent staining. Ten depths were sampled at intervals of two months.

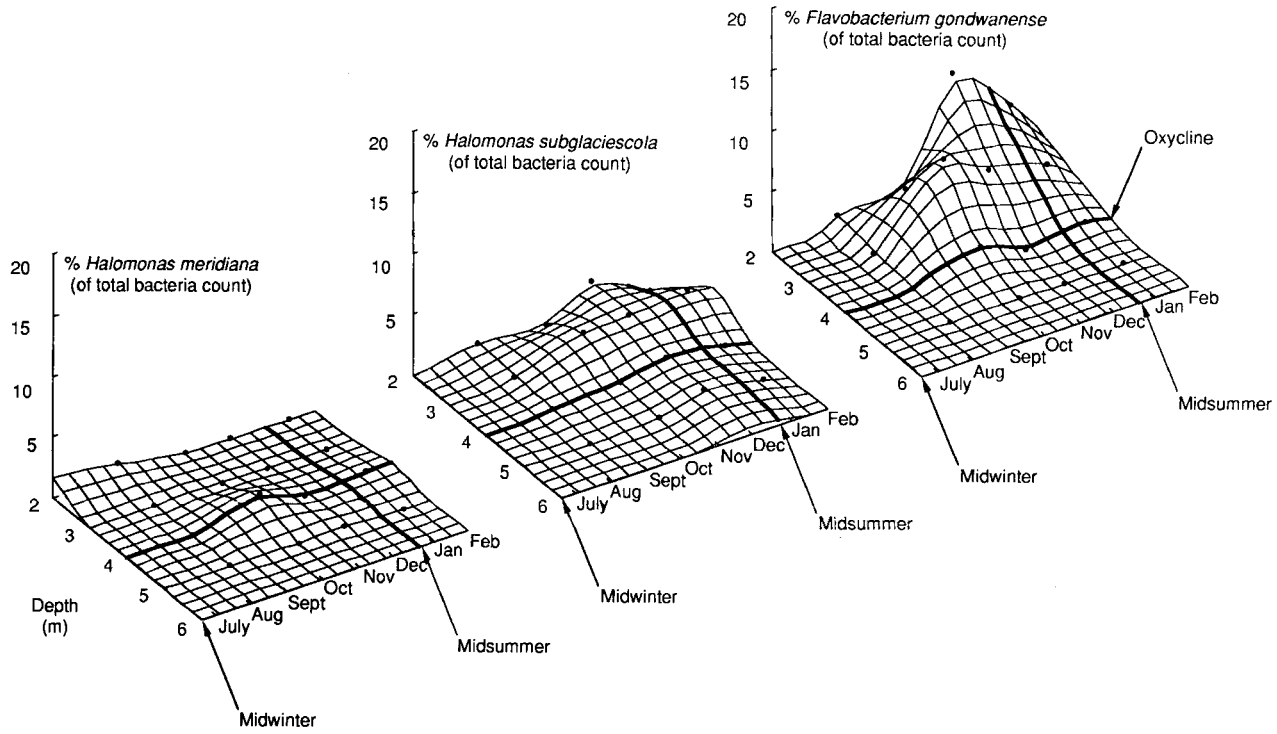

Fig. 2. Surface plots (smoothed by weighted least squares method) of percentage serogroups of total bacteria in Organic Lake, over time and depth. Serogroup percentages as determined by immunofluorescent staining. Four depths were sampled at intervals of two months.

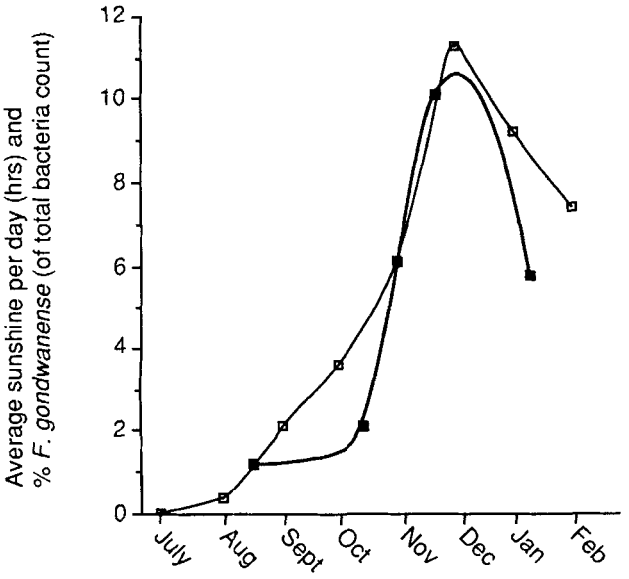

Fig. 3. Average hours of sunshine per day (□) (from monthly meteorological reports) and % *Flavobacterium gondwanense* of total bacterial count (■), from Organic Lake at 2 m depth from July 1990 to February 1991.

between -10°C (Organic Lake) and +18°C (Ekho Lake).

H. subglaciescola was originally isolated and characterized by Franzmann *et al.* (1987b) from Organic Lake. This species was also isolated from a number of Vestfold Hills saline lakes by Garrick & Gibson (1988). Franzmann *et al.* (1987b) characterized 29 strains of *H. subglaciescola* from Organic Lake and James *et al.* (1990) characterized a further 20 strains. As indicated by immunofluorescence, the single strain ACAM 12 comprised a considerable proportion of the total bacterial population of Organic, Ekho and Fletcher lakes. It is probable that the species *H. subglaciescola* comprised a much larger part of the bacterial biota of these lakes. Similarly, the antiserum used to detect *F. gondwanense* was strain specific and only indicative of the presence of one of a number of strains of *F. gondwanense*. Dobson *et al.* (1991) characterized 10 other strains of the same species from Organic Lake alone. It is probable that *F. gondwanense* comprised a larger part of the bacterial population of Organic, Ekho and Fletcher lakes than indicated by immunofluorescence.

F. salegens was originally isolated from Organic Lake and characterized by Dobson *et al.* (1991). From immunofluorescent cross-reactivity data it was seen that the *F. salegens* serogroup included *D. venusta*. The bacterial biota of the saline lakes of the Vestfold Hills is probably of marine origin (James *et al.* 1990) so it is conceivable that *D. venusta*, a halophilic marine bacterium, should be present. Thus cell counts for the *F. salegens* serogroup were less useful than for the other three serogroups. The *F. salegens* serogroup represented only a small proportion of the total count in all lakes and was always less than 2% of the total bacterial count (Table III).

Though species composition changed markedly over time, total bacterial numbers in the aerobic layers of the lakes remained relatively constant but decreased slightly over season. Bacterial numbers were generally 2–5 times greater around the oxycline and in the anaerobic waters of the lakes than bacterial numbers in the aerobic waters. Total bacterial counts increased with increasing salinity with the notable exception of Ekho Lake where the warmer waters supported increased bacterial activity and lower bacterial numbers (Table III).

DOC in the aerobic layers remained relatively constant, between 2 and 6 mg C l^{-1}, (decreasing slightly over the season) except in Organic Lake, the coldest, most saline of the study sites, where the upper waters had 23–38 mg C l^{-1} indicating substantially reduced bacterial activity. Local seawater contained 0.3–1.6 mg C l^{-1}. Around the oxycline and in the anaerobic waters of the lakes DOC levels were generally greater. DOC levels were not associated with changes in species composition but were closely associated with peaks in total bacteria numbers in lower waters. Bacterial numbers and DOC level peaked below the oxycline in all lakes in August with the return of sunlight to these lakes. Burke & Burton (1988a) noted a similar effect in Burton Lake (TDS 44‰) where they identified a bloom of the photosynthetic *Chlorobium* spp. which grew in response to the return of low light levels. Recent work using flow cytometry has identified *Chlorobium* spp. in Ace and Fletcher Lakes and unidentified naturally fluorescing populations in Ace, Fletcher, Ekho and Organic Lakes (L. Rankin, personal communication 1993).

Distribution of test species

No standard statistical techniques may be applied to these data due to the inevitable interdependency of results. Observational interpretation has confirmed that bacterial populations do vary with season, depth and lake (Table III; Figs 1 & 2). From August 1990 all four serogroups were observed in Organic and Ekho lakes and to a small extent in Fletcher Lake but not in Ace Lake or the local seawater. Significantly, the *H. meridiana*, *H. subglaciescola* and *F. gondwanense* serogroups occurred in discrete locations within the water column. In Organic Lake, *F. gondwanense* was found mainly at 2 m depth and *H. subglaciescola* at 3 m depth from October 1990 until the end of the sampling period (Fig. 2). The correlation of the Antarctic summer with maximum serogroup abundance was particularly apparent with *F. gondwanense* (Fig. 3). In Ekho Lake, serogroup abundances peaked in December 1990, falling off by January 1991. The *H. subglaciescola* serogroup was found mainly from 2–6 m depth, *F. gondwanense* from 6–14 m depth and *H. meridiana* from 10–18 m (Fig. 1). Small populations of the three main serogroups occurred up to 2 m below the oxycline in Organic and Ekho lakes (Figs 1 & 2). As all bacteria shown to react to the four antibodies were aerobes (Dobson *et al.* 1991, Franzmann *et al.* 1987b, James *et al.* 1990), these populations were very probably comprised of cells settled from the upper waters.

Though lake conditions associated with the presence of the four serogroups were within, or marginally exceeded (*F. gondwanense*), the experimentally determined temperature and salinity ranges for the four original strains (Table I), all but *H. subglaciescola* were present only at salinities above their experimentally determined optimum (Tables I & III). This could indicate selective pressures, perhaps competition, in the other more optimal environments. During the winter sample period (from July 1990 until August 1990) none of the four serogroups were observed in numbers greater than 0.1% of the total bacterial count though lake conditions were often within the experimentally determined temperature and salinity ranges for the four original strains (Table I). Both *Halomonas* spp. and *Flavobacterium* spp. have been isolated from Organic, Ekho and Fletcher lakes. Though these four serogroups comprised the majority of isolates on nutrient supplemented lake water media, there exists larger populations of unidentified, uncultured bacteria that dominate the aerobic waters of these lakes for the most part of the year.

Conclusion

The harsh conditions in Antarctic hypersaline lakes and the consequent reduced species diversity make immunofluorescent techniques particularly useful for characterizing species composition. Total bacterial counts, DOC and pH remained relatively constant throughout the aerobic waters, all decreasing slightly over the season. Changes in species composition as indicated by immunofluorescent counts were not reflected in the total bacterial count, indicating compositional change of the bacterial population. Temperature, light and salinity changed with depth, season and lake. As reported by previous workers (Burke & Burton 1988a, Garrick & Gibson 1988, Hirsch & Siebert 1991) and noted by the authors, the warming and dilution of aerobic lake waters associated with the Antarctic summer were generally correlated with changes in bacterial species composition. The *H. meridiana*, *H. subglaciescola* and *F. gondwanense* serogroups were in very high proportions and discrete populations in aerobic waters amongst the summer biota of Organic and Ekho lakes.

The four groups of Antarctic bacteria, able to grow on a wide variety of substrates and over a large range of temperature and salinity, were opportunistically growing in the sub-optimum conditions associated with early summer in Organic and Ekho lakes. The seasonality and discrete locations of these populations is now the subject of study using species specific fluorescent 16S RNA probes.

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