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Bacterial and viral abundance in Ross Sea summer pack ice communities

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ABSTRACT: Abundance of single, non-attached bacteria and viruses <110 nm (likely to have infected bacteria) were determined for surface, interior and bottom ice microhabitats between 66 and 75° S in the Ross Sea during the austral summer of 1999. Emphasis was on sites of ice algal blooms, and bacterial abundance was examined with respect to physical characteristics, chlorophyll *a*, phaeophytin and, in particular, microbial communities. Bacterial abundance ranged from 1.5×10^5 to 6.7×10^6 ml^{-1} melted sample and viral abundance from 6.3×10^6 to 1.19×10^8 ml^{-1} melted sample over all the microhabitats. Neither bacterial nor viral abundance differed among microhabitats, and bacterial abundance was not related to physical characteristics of the habitats. Although bacterial abundance was positively correlated with chlorophyll *a* and phaeophytin concentrations, only chlorophyll *a* was significant in explaining a small (28%) degree of the variability in bacterial abundance. Abundance of diatoms, heterotrophic dinoflagellates and other flagellates, however, explained 85% of the variability in bacterial abundance; these groups were positively correlated with bacterial abundance. Neither viral lysis nor grazing by bacteriovores appeared to be strong controls of bacterial abundance. Community analysis showed that samples were >90% similar with respect to abundance of bacteria, viruses, and microeukaryote groups. Distinct clusters could be attributed to different algal bloom stages, with relationships to *Phaeocystis* spp. blooms being particularly apparent, indicating the strength of algal blooms as processes structuring microbial communities. Microbial communities in Ross Sea summer ice microhabitats could furthermore be viewed within the same general successional sequence characteristic of algal blooms in polar and temperate marine waters.

KEY WORDS: Bacteria · Viruses · Pack ice · Algal blooms · Ross Sea

INTRODUCTION

Bacteria and viruses are significant members of the microbial loop and can reach their greatest abundance and have their greatest roles in association with algal blooms. Although most examples are for diatom blooms in lower latitude waters and mesocosms (e.g. Bratbak et al. 1990, Smith et al. 1995, Riemann et al. 2000), the few studies to date suggest that polar regions are no different (Maranger et al. 1994, Yager et al 2001). Increases in bacterial abundance, biomass or production during the course of algal blooms occur in Antarctic ice (e.g. Grossi et al. 1984, Kottmeier et al. 1987). High bacterial numbers, biomass or production often occur where ice algal biomass is high (Sullivan & Palmisano 1984, Kottmeier & Sullivan 1990, Gleitz et al. 1996, Grossmann et al. 1996).

Ice algal blooms, dominant features of the austral summer pack ice, are noteworthy in Antarctic marine productivity because of timing and distribution (re-

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viewed by Lizotte 2001). In contrast to the water column, where the extent of a bloom can be more easily determined, blooms in pack ice are patchy and can occur in surface, interior and bottom microhabitats (Garrison 1991, Palmisano & Garrison 1993). Biomass in most ice microhabitats is concentrated into a small volume within brine inclusions. Biomass and abundance of several groups of organisms often equals or greatly exceeds that of similar volumes of the underlying water column (e.g. Sullivan & Palmisano 1984, Levasseur et al. 1994, Maranger et al. 1994). Therefore, ice microhabitats should be good places to examine microbial interactions during blooms. Knowledge of the viruses and bacteria in different ice microhabitats is needed for an understanding of the Southern Ocean marine ecosystem (Brierley & Thomas 2002). In addition, the unique Antarctic environment may allow viral studies there to address broad microbial ecological questions (Pearce & Wilson 2003).

In this study we (1) determine the abundance and distribution of 1 component of the bacterial community, the single unattached bacteria, and of the viruses <110 nm likely to have infected bacteria; (2) determine which physical and biological factors affected bacterial abundance; (3) examine abundances of bacteria, viruses and microeukaryotes using community analysis. Sites of summer algal blooms were emphasized, and sampling included a variety of ice microhabitats over a spatial area encompassing ~10° of latitude along 3 longitudinal transects. This is the first study to consider bacteria in relation to viruses and microeukaryotes of the ice microbial community. Abundance was addressed because the interactions, viral infection of bacteria, algal exudation of compounds affecting bacteria, and grazing by bacteriovores are all density-dependent.

MATERIALS AND METHODS

Sample collection. We sampled consolidated pack ice and surface slush (ice crystals plus seawater) between 65 and 75° S along 3 longitudinal transects and at 1 off-transect site in the Ross Sea during Cruise NBP99-1 of the RV 'Nathaniel B. Palmer' (Fig. 1). Sampling was from 1 January to 2 February 1999, and stations were at approximately every degree of latitude. One site was sampled on small floes (Stns 001 to 004, 019 to 026). On larger floes, a 150 m transect representing the floe's surface topographic variability was laid out, and 2 ice cores for physics studies were taken at 3 unridged sites along the transect (e.g. Jeffries et al. 2001). If the cores for ice physics (IP cores) were visibly colored at a site, a bacteria–virus (BV) core and an ice community (IC) core were taken there; otherwise the latter cores were taken from the site closest to the ship. BV and IC cores (7.5 cm diameter) were measured and split into 10 or 20 cm sections using a saw. IC core sections were placed in plastic jars in an insulated box to minimize temperature changes during transport to the ship. BV core sections were immediately split longitudinally into subsamples, placed in plastic bags and transported to the ship in an insulated box. Temperature was measured at 10 cm intervals in the field on 1 of the IP cores; the second IP core was placed in an insulated box for shipboard crystal texture and stratigraphy analysis. The BV, IC and IP cores were taken within 50 cm of each other and were assumed to be replicates.

We collected 2 surface slush samples from different areas on the ice floes than those used as core sites; they were chosen to be representative of surface topography such as level areas or ridges or hummocks. Surface snow and consolidated ice (if present) were removed from a 50 × 50 cm area, and slush was scooped into a 4 l plastic jar and placed in an insulated box for transport to the ship (Fritsen et al. 2001).

Sample processing and examination. Methods for shipboard processing of cores have been described in detail by Gowing et al. (2002) and those for slush by Gowing (2003). For cores, a 'fast melt plus preservative' regime was used to prevent bacteria and viruses

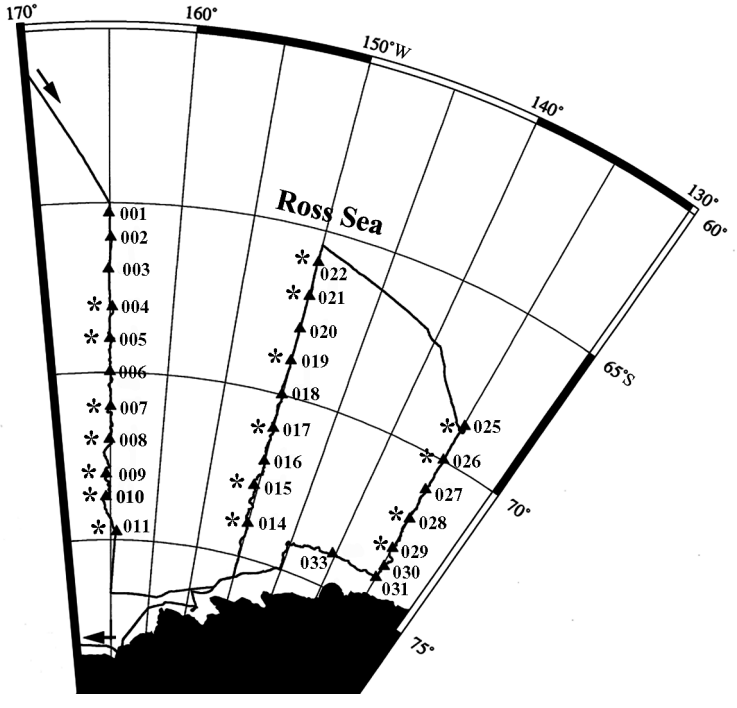

Fig. 1. Cruise track and stations sampled. Station numbers are day of the year, with Stn 001 sampled 1 January 1999 and Stn 033 sampled 2 February 1999. Asterisks indicate stations for which ice community analysis of some samples was made. Image modified from that of C. Masters and T. Schunk

being grazed by heterotrophs. Core subsections were placed into 200 ml of 0.02 µm-filtered seawater with borate-buffered paraformaldehyde (~1% final concentration) and allowed to melt overnight at room temperature. The total volume was measured to determine the dilution; dilution factors ranged from 0.4 to 1.0. Aliquots for bacterial and viral counts were removed to sterile centrifuge tubes and refrigerated. Slush was allowed to melt at ~4°C in the dark before aliquots were fixed with 0.02 µm-filtered paraformaldehyde fixative. Abundances have been corrected for dilution and are all presented as number ml^{-1} melted sample.

Filter preparations for bacterial and viral counts using the SYBR Green I light microscopy protocol of Noble & Fuhrman (1998) were made aboard ship. For each sample, 2 to 4 replicate filters with 0.2 to 2.0 ml of sample were prepared. If less than 2.0 ml of sample were used, the difference was made up with 0.02 µm-filtered seawater. Solution blanks were prepared at the beginning and end of each batch of samples processed. Filters were mounted in ProLong™ Antifade medium (Molecular Probes) on slides, frozen at −80°C and air-shipped to the home laboratory. Filters were thawed before counting, and a minimum of 200 bacteria and 200 virus-like particles were counted in 20 fields of view at 1000× using an Olympus BX-60 epifluorescence microscope equipped with an Acridine Orange filter set.

In addition to single and small clusters of bacteria, some samples contained large aggregations of bacteria and/or bacteria attached to diatoms or frustules. Our methods were inadequate for determining the contribution of the latter 2 bacterial populations. Bacteria in large aggregates could not be counted because we did not sonicate sample aliquots to disperse the aggregates for filter preparation at the time of collection. Attached bacteria could not be counted because appropriate amounts of sample for this were not filtered at the time of collection. We recorded the presence of epiphytic bacteria in the samples; this is considered in the 'Discussion'. Our counts are therefore of predominantly single, unattached bacteria, and are an underestimate of the total bacteria.

Abundances of viruses (<110 nm capsid diameter) likely to infect bacteria were determined by subtracting the abundance of extracellular large (≥110 nm capsid size) viruses from the abundance of total (all sizes) viruses determined from the light microscope filter counts. The abundance of larger viruses (assumed to have infected eukaryotes) had been determined from sample aliquots counted using transmission electron microscopy (TEM) as described by Gowing et al. (2002).

Ice communities and environmental characteristics. Sea ice microbial communities were analyzed microscopically from aliquots of a subset (29 samples) of the IC core sections and slush samples as described for slush samples by Fritsen et al. (2001). Chlorophyll *a* and phaeophytin were determined from aliquots of most core sections and slush samples as described by Fritsen et al. (2001) for slush samples. Salinity of slush samples was measured with a refractometer after samples had melted. Brine salinity of cores was calculated from *in situ* temperature according to Assur (1958). Ice crystal texture and stratigraphy were determined in the walk-in freezer aboard ship for an IP core from each site according to the methods of Jeffries & Adolphs (1997). Granular frazil ice and granular infiltration ice (snow ice) were distinguished by stable oxygen isotope analysis as described by Jeffries et al. (2001). Multi-year ice was distinguished from first-year ice mainly by a higher freeboard and rougher surfaces, and in some cases by the presence of superimposed ice 'buried' below the ice surface. In a few instances, abundance of bacteria and viruses was determined for the bottom core section of an IP core after the structural analysis was complete.

Rationale for sample collection and analysis. Sampling was not strictly random because the focus of this project was bacteria, whose abundance was expected to increase during blooms of ice algae. More samples were collected than could be processed at sea. Samples with visible coloration indicative of high algal biomass were given priority for analysis, and other samples were processed as time permitted. Complete cores were not analyzed, and variable numbers (0 to 6 core sections) of interior habitats were analyzed at a station. We use the term 'virus', although infectivity was not demonstrated. Strictly defined, these are 'virus-like particles'.

Community analysis. For community analysis we used procedures similar to those described by Field et al. (1982) on the samples with ice community data. Log-transformed abundances were used for 10 groups (bacteria, viruses, diatoms, autotrophic dinoflagellates, *Phaeocystis* spp. non-motile [colony-associated] cells, *Phaeocystis* spp. motile cells, other autotrophic flagellates, heterotrophic dinoflagellates, other heterotrophic flagellates, and non-plastidic ciliates). Dissimilarities were calculated between paired samples using the Bray-Curtis measure (Similarity = 1−Bray-Curtis measure). Single-linkage cluster analysis and multidimensional scaling (MDS) were used to analyze the resulting sample-by-sample matrix (see Field et al. 1982 for details of the procedures).

RESULTS

Summer pack ice environment

Ice thickness ranged from 39 to 258 cm. Stns 008, 017, and 028 were multi-year floes; the others were

Table 1. Range of pigment and physical characteristics of the ice microhabitats. FY: first-year ice; MY: multi-year ice; S: snow ice; SI: superimposed ice; F: frazil ice; C: congelation ice; P: platelet ice. Number of samples or core sections are shown in parentheses. Slush samples ranged from 4 to 51 cm depth; surface core samples ranged from top 5 to 22 cm; interior habitat samples were generally 20 cm long and ranged from starting depths of 8 to 221 cm; bottom habitat samples, also generally 20 cm long, ranged from starting depths of 28 to 241 cm. Some of these samples were also used for a study of large viruses, and ranges of characteristics for those samples are given in Gowing (2003)

Habitat	Chlorophyll *a* ($\mu g\ l^{-1}$)	Phaeophytin ($\mu g\ l^{-1}$)	Temperature (°C)	Brine salinity (psu)	Ice type
Slush	0.15–120.26 (35)	0.03–72.10 (35)	–1.8 to –1.0 (28)	11.00–32.19 (31)	
Surface					
FY	2.18–28.34 (5)	0.44–13.18 (5)	–1.6 to –1.4 (5)	25.22–28.72 (5)	S (2), F (1), F +S (1), S + SI (1)
MY	4.16 (1)	0.34 (1)	–1.4 (1)	25.22 (1)	S + SI (1)
Interior					
FY	1.81–41.88 (25)	0.16–7.85 (25)	–1.9 to –1.3 (24)	20.04–33.92 (20)	F (16), C + F (5), C (3)
MY	4.33–84.0 (9)	0.37–91.08 (9)	–2.1 to –1.6 (9)	28.72–37.36 (9)	F + P (2), F (4), C (3), C + F (2)
Bottom					
FY	2.08–71.31 (17)	0.23–43.40 (17)	–1.8 to –1.3 (19)	23.46–30.46 (14)	F (11), C (7), C + F (3)
MY	13.72 (1)	4.2 (1)	–1.6 (1)	28.72 (1)	F (1)

first-year ice. Ice temperatures ranged from –2.1 to –1.0°C, and brine salinity ranged from 11.00 to 37.36 psu within the different ice microhabitats (Table 1). Several ice types were present in the core sections, and chlorophyll *a* and phaeophytin concentrations ranged over 1 to several orders of magnitude within the microhabitats (Table 1).

Bacterial abundance

Abundance of single, unattached bacteria ranged from 1.5×10^5 ml^{-1} in slush to 6.72×10^6 ml^{-1} in a bottom habitat sample (Table 2, Fig. 2). Abundance of bacteria did not differ between surface core samples and slush (Mann-Whitney *U*-test, $p > 0.05$) or among surface (cores and slush combined), interior and bottom habitats (Kruskal-Wallis test, $p > 0.05$). The mean coefficient of variation for replicate filters for bacterial counts was 21% for surface cores, 23% for surface slush, 36% for interior habitats, and 17% for bottom habitats.

Table 2. Range of abundance of unattached, non-aggregated bacteria and viruses <110 nm (likely to have infected bacteria) in the different habitats. N: number of samples

Habitat	Nos. ml^{-1} × 10^6 Min.	Max.	N
Surface habitat (cores)			
Bacteria	0.18	1.68	6
Viruses <110 nm	4.01	18.37	6
Surface habitat (slush)			
Bacteria	0.15	5.94	38
Viruses <110 nm	1.25	119.00	34
Interior habitat (cores)			
Bacteria	0.20	5.81	36
Viruses <110 nm	0.91	53.50	34
Bottom habitat (cores)			
Bacteria	0.18	6.72	24
Viruses <110 nm	0.63	15.15	22

Viral abundance

Abundance of viruses <110 nm ranged from 6.3×10^5 ml^{-1} in a bottom habitat to 1.19×10^8 ml^{-1} in surface slush (Table 2, Fig. 2). Viruses were commonly an order of magnitude more abundant than bacteria. Viral abundance did not differ between surface core samples and slush (Mann-Whitney *U*-test, $p > 0.05$) or among habitats (Kruskal-Wallis test, $p > 0.05$). Viruses <110 nm made up an average of 97% or more of the total viral abundance in different ice microhabitats (data not shown). The mean coefficient of variation for replicate filters for total viral counts (from which the abundance of viruses <110 nm was determined) was 29% for surface cores, 29% for surface slush, 23% for interior habitats and 26% for bottom habitats. Viral abundance was significantly positively correlated with bacterial abundance (Spearman's rank correlation coefficient 0.44, $p < 0.001$; see Fig. 2). The virus to bacteria ratio (VBR) ranged from 0.7 to 119 over the samples.

Relationship of bacterial abundance to various parameters

Bacterial abundance showed no relationship to temperature or salinity; bacterial abundance did not differ among the ice types for any habitat, and bacterial abundance did not differ among first-year ice and multi-year ice in interior habitats. Bacterial abundance

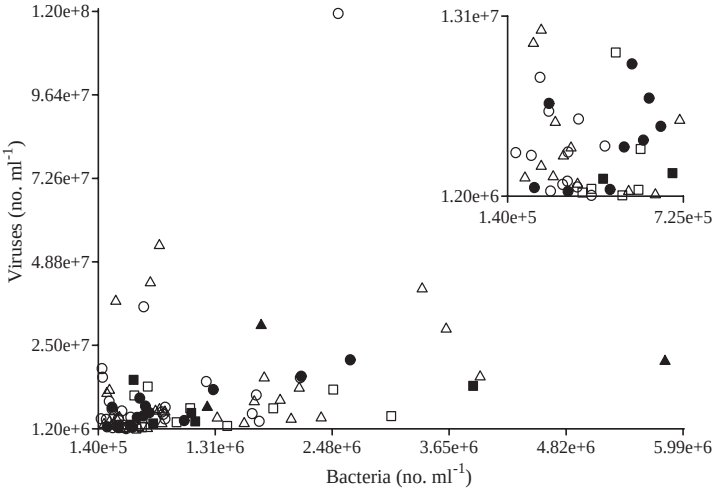

Fig. 2. Bacterial and viral abundance. Note different scales. Inset is expansion of distribution near the lower values. (○, ●) Surface habitats; (△, ▲) interior habitats; (□, ■) bottom habitats; black symbols indicate stations for which ice community data were recorded. Total sample number = 103

was significantly positively correlated with chlorophyll *a* and phaeophytin concentrations (Spearman's rank correlation coefficients 0.56 and 0.48, respectively, $p < 0.001$, for both). A stepwise multiple regression using bacterial abundance as the dependent variable and chlorophyll *a* and phaeophytin as independent variables showed that chlorophyll *a* explained 28% of the variability in bacterial abundance and was the only significant predictor ($p < 0.001$).

Among the eukaryote groups (diatoms, autotrophic dinoflagellates, non-*Phaeocystis* autotrophic flagellates, *Phaeocystis* spp. motile cells, *Phaeocystis* spp. non-motile [colonial] cells, heterotrophic dinoflagellates, other heterotrophic flagellates, and non-plastidic ciliates) in the 29 samples with ice community data, diatoms (Spearman's rank correlation coefficient 0.51, $p < 0.01$), motile *Phaeocystis* spp. cells (Spearman rank correlation coefficient –0.39, $p < 0.05$), and heterotrophic dinoflagellates (Spearman's rank correlation coefficient 0.42, $p < 0.05$) were significantly correlated with bacterial abundance. Using 28 of these samples with complete group data, a stepwise multiple regression was performed using bacterial abundance as the dependent variable and abundances of viruses and the aforementioned eukaryote groups as the independent variables. The resultant model that explained 85% of the variability in bacterial numbers included diatoms, heterotrophic flagellates and heterotrophic dinoflagellates. Diatoms explained 65% of the variability, with heterotrophic flagellates adding an additional 13% and heterotrophic dinoflagellates an additional 7%.

Community analysis

Single-linkage cluster analysis of the samples showed that all samples entered clusters at >90% similarity (dendrogram not shown). Clustering of the samples was consistent using both single-linkage and multidimensional scaling (MDS); samples could be grouped into 3 distinct clusters and 1 outlier (Fig. 3). Samples did not cluster by habitat or by spatial distribution. Table 3 lists the mean abundances of organisms and concentrations of pigments for each cluster as well as the corresponding values for the outlier sample (slush at Stn 011). In general, Cluster 1 was lowest in mean pigments and organisms and Cluster 3 was highest.

DISCUSSION

Bacterial abundance

Bacterial abundance in summer Ross Sea ice microhabitats was within ranges reported for similar types of ice sampled at other Antarctic locations during spring and summer (Table 4). Although Grossmann et al. (1996) found a higher maximum abundance of bacteria

Table 3. Mean abundance (nos. l^{-1}) of the various groups and mean pigment concentrations (μg l^{-1}) in the 3 clusters and values for outlier slush sample. Value of 0 indicates that abundance was below detection limits on filters

	Cluster 1 (N = 17)	Cluster 2 (N = 7)	Cluster 3 (N = 3)	Slush (Stn 011)
Groups				
Bacteria	8.7×10^8	1.8×10^9	3.2×10^9	1.3×10^9
Viruses	6.8×10^9	8.3×10^9	1.9×10^{10}	1.2×10^{10}
Diatoms	5.1×10^6	7.1×10^6	1.4×10^7	1.2×10^7
Autotrophic flagellates	6.2×10^5	3.9×10^6	1.1×10^7	2.8×10^4
Phaeocystis spp. motile	1.7×10^5	0	0	0
Phaeocystis spp. non-motile	3.5×10^6	1.1×10^6	0	9.6×10^7
Autotrophic dinoflagellates	1.9×10^4	2.9×10^4	9.7×10^4	8.4×10^4
Heterotophic flagellates	1.2×10^6	3.2×10^6	7.7×10^6	2.8×10^4
Heterotophic dinoflagellates	5.1×10^3	1.1×10^4	1.2×10^4	2.4×10^4
Ciliates	2.4×10^3	4.7×10^3	2.1×10^3	2.8×10^3
Pigments				
Chlorophyll *a*	7.87	23.15	38.93	120.26
Phaeophytin	1.10	16.41	58.79	23.25

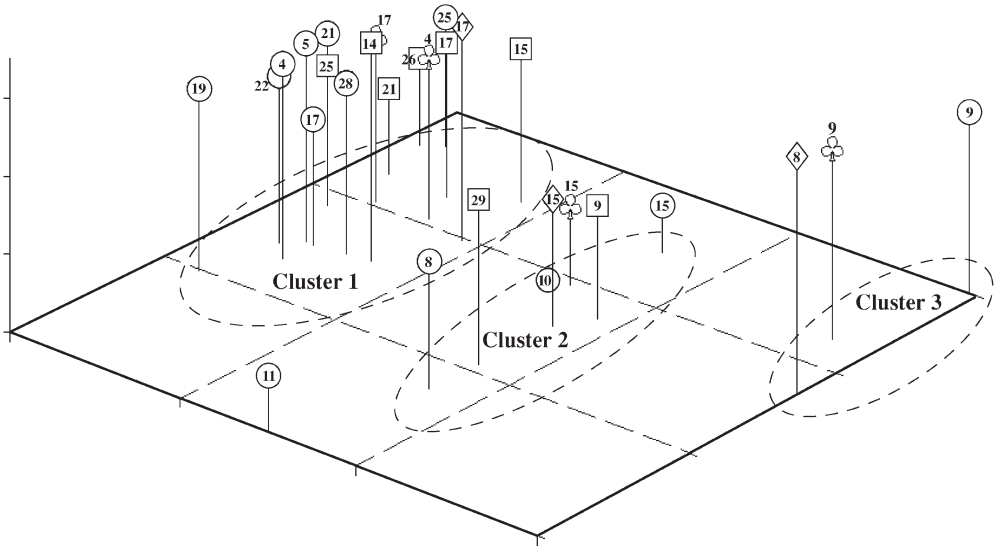

Fig. 3. Similarity plot using 3-dimensional representation of multidimensional scaling (MDS). Axes are arbitrary distance, and distances between samples are proportional to similarity (1 minus Bray-Curtis measure) between the assemblages. In this rotational view most of variability is shown in *x*–*y* axis view. (○) surface habitats (slush); (♧) = surface habitats (cores); (◇) interior habitats; (□) bottom habitats; numbers are station numbers in Fig. 1 with initial zeros removed; dashed lines delineate clusters

in platelet ice, our bottom microhabitats did not include this specialized ice type, which is not commonly found distant from ice shelves. The overall similar range of abundances suggests that the ice bacterial assemblage has responded in a similar way to algal blooms in a variety of locations during many years.

Although bacterial abundance was positively correlated with chlorophyll *a*, a common proxy for algal biomass, chlorophyll *a* only explained 28% of the variability in bacterial abundance. This may be a result of 2 factors. First, ice communities comprise many different algae that may or may not be affecting bacteria directly, although all would contribute to chlorophyll *a* concentration. Second, bacterial abundance is that of the non-epibacterial component of the bacterial assemblage, whereas chlorophyll *a* concentration is that of the total microalgal assemblage. This may be less likely to have influenced the relationship for the following reasons. Epiphytic bacteria, generally on *Amphiprora*-like diatoms, were noted in 4 slush samples, 8 interior habitat samples, and 11 samples from bottom habitats. Although the contribution to total bacterial numbers in the data set is unknown, it is unlikely that the epiphytic bacteria were a significant component of the total bacterial assemblage because they did not occur on a numerically dominant alga. Where ice community data were available, *Amphiprora* spp. did not dominate, and where epibacteria were observed, they were generally not on every *Amphiprora* cell. Our pack ice samples, therefore, differed from the diatom assemblages with dominant epibacteria described at McMurdo Sound (Grossi et al. 1984, Sullivan & Palmisano 1984) and at Davis Station (Archer et al. 1996). Future work should, however, assess epiphytic as well as unattached bacteria for a complete understanding of bacterial abundances.

High concentrations of phaeopigments are indicative of senescent or grazed algae. Bacterial abundances would be expected to be correspondingly high if bacteria were obtaining nutrients exuded by senescent algae or nutrients released as algae were grazed. Bacterial abundance was positively correlated with phaeophytin concentration, but phaeophytin was not significant in the multiple regression for pigments. We have no data on algal senescence during the study. Bacterial abundance was positively correlated with abundance of *Cryothecomonas* spp. and *Telonema* spp., grazers on scaled algae (Gowing 2003) (Spearman's rank correlation coefficient 0.41, $p < 0.05$). Other algal grazers were present in all samples, and grazing on diatoms also occurred (M. Gowing pers. obs.). The bulk pigment concentrations, although suggestive of what might have caused variability in bacterial abundance, were not as useful as the abundance of major groups of organisms for interpreting variability in bacterial abundance in the summer pack ice.

Among the autotrophic groups, diatoms were most important in relation to bacterial abundance. Diatoms produce a variety of extracellular polymeric substances (EPS) that appear to influence bacteria, but the exact role of extracellular polymeric substances in mediating diatom–bacteria interactions is unknown (Hoagland et al. 1993). In the ocean, TEP (transparent exopolymeric particles), a type of EPS, often peaks in association with diatom (and *Phaeocystis* spp.) blooms (reviewed by Passow 2002). Bacteria can utilize TEP, but the degree and importance of such utilization appears to vary (reviewed by Passow 2002). In Arctic winter sea ice, EPS 'correlated positively with bacteria abundance (although no functional relationship could be deduced)' (Krembs et al. 2002, p. 2163). Additional

Table 4. Abundance of bacteria in ice in Antarctic marine regions in spring and summer. Designations of bottom habitats are not identical because different investigators sampled different core-section lengths

Habitat	Abundance (nos. ml^{-1})	Location, season	Source
Ice habitat			
Land fast ice (bottom)	1.02×10^6 (max.)	McMurdo, spring	Sullivan & Palmisano (1984)
Land fast ice (upper)	$0.2–3.6 \times 10^5$	McMurdo, spring	Sullivan & Palmisano (1984)
Pack ice (cores)	$1–30 \times 10^5$	Weddell/Scotia Seas, spring	Miller et al. (1984)
Land fast ice (bottom)	$2–8.5 \times 10^5$	McMurdo, spring	Grossi et al. (1984)
Land fast (bottom)	$1–5 \times 10^5$	McMurdo, spring and summer	Kottmeier et al. (1987)
Land fast (platelet)	$0.05–0.4 \times 10^6$	McMurdo, spring and summer	Kottmeier et al. (1987)
Pack ice (cores)	8.82×10^5 (avg.)	Weddell Sea, spring	Kottmeier & Sullivan (1990)
Land fast ice (surface)	$0.3–1.5 \times 10^6$	Adelie Land, spring	Delille & Rosiers (1995)
Land fast ice (bottom)	$0.3–0.4 \times 10^6$	Adelie Land, spring	Delille & Rosiers (1995)
Pack ice (platelets)	$1.5–2 \times 10^7$ (max.)	Weddell Sea, late summer	Grossmann et al. (1996)
Pack ice (infiltration layer)	2.32×10^5, 15.4×10^5	Weddell Sea, summer	Gleitz et al. (1996)

indirect evidence that ice diatoms could stimulate bacterial growth has come from observations that growth of epiphytic bacteria appeared to have been enhanced by association with the diatoms (Grossi et al. 1984, Smith et al. 1989, Archer et al. 1996). Diatoms were abundant and diverse in the Ross Sea summer ice samples (A. H. Gibson et al. unpubl.); laboratory experiments using dominant species might elucidate the effects of diatoms on bacteria.

Neither motile nor non-motile (colony-associated) *Phaeocystis* spp. cells were significant in explaining variability in bacterial numbers. This was unexpected in the light of literature on bacteriocidal activity of *Phaeocystis* spp. (Davidson & Marchant 1992, Montfort et al. 2000; see also discussion in Putt et al. 1994), associations between bacteria and *Phaeocystis* sp. in McMurdo Sound (Putt et al. 1994), and recent studies suggesting that extracellular polymers of *P. antarctica* (isolated from the Ross Sea) could be utilized by bacteria (Solomon et al. 2003). As most authors have noted, the differences in bacteriocidal properties of *Phaeocystis* spp. may be due to differences in strains. More than 1 species of *Phaeocystis* may have been present in our samples: undescribed species of *Phaeocystis*, whose motile cells differ but whose colonies are indistinguishable, occur in Antarctic sea ice (Marchant & Thomsen 1994). Most studies of *Phaeocystis* spp. and bacteria have focused on *Phaeocystis* spp. colonies. Our finding of a negative correlation between motile *Phaeocystis* spp. cells and bacterial abundance suggests that research on effects of *Phaeocystis* spp. should also consider solitary motile cells.

Heterotrophic dinoflagellates and other flagellates were significant (although less so than diatoms) in explaining variability in bacterial abundance. As discussed above, algal grazers would be expected to have a positive effect on bacterial abundance. Relationships between bacteriovores and their prey, however, are likely to be a function of threshold concentrations of bacteria (e.g. Davis & Sieburth 1984) and to cycle (e.g. Fenchel 1982). Seasonal bacteria–flagellate abundance cycles have been demonstrated, for example, for Arctic coastal lagoon ice (Sime-Ngando et al. 1997). Shorter-term cycles also probably occur, and our data could reflect these. Bacterial abundance was not significantly correlated with abundance of choanoflagellates, grazers on bacteria and smaller particles. The positive relation between bacterial abundance and that of the heterotrophic dinoflagellates and flagellates suggested that, overall, bacteriovores were not controlling bacterial abundance at the time of our sampling.

Role of viruses

Virus abundance in Ross Sea ice was positively correlated with bacterial abundance, as would be expected, because high bacterial abundance is necessary for transfer of viruses. Virus abundance, however, was not a significant parameter in the multiple regression. The simplest conclusion is that viral abundance was indeed less important than that of the other groups of organisms (diatoms and all heterotrophic flagellates) in explaining variability in bacterial abundance. Other factors that may have contributed to the result should also be noted: (1) Although marine bacteriophages are generally <110 nm (Weinbauer & Peduzzi 1994), not all viruses <110 nm are bacteriophages (e.g. Van Etten et al. 1991, Lawrence et al. 2001). However, if significant numbers of small viruses were infecting major algal groups in any habitat, a positive correlation with chlorophyll *a* would be expected; this did not occur (Spearman's rank-order correlation test, $p > 0.05$ for all habitats). Furthermore, thin sections of >30 000 algal cells in these samples were examined in a study of viruses infecting microeukaryotes (Gowing 2003), and

no algal cells infected with viruses <110 nm were observed. Thin sections of thousands of microheterotrophs were also examined, and only 2 cells were infected with viruses <110 nm, so it is unlikely that large numbers of the small viruses in the samples came from infected eukaryotes. (2) Another consideration related to the viruses counted is the finding by Montanié et al. (2002) that in a temperate coastal pond, dynamics of tailed phages were better linked with bacterial dynamics than was the total viral assemblage (of which generally >90% were <105 nm). Whether this is true in Antarctic ice is unknown. (3) We studied unattached, single bacteria, whereas bacteria in dense clusters as well as epibacteria could also be infected with viruses. On the other hand, because viruses must contact bacteria for infection, bacteria in dense clusters might be protected from infection. Epiphytic bacteria have an exopolymeric layer that penetrates the puncta of diatoms (Sullivan & Palmisano 1984). This substance might protect the epibacteria from viruses; however, some unattached bacteria also have a surface texture suggesting exopolymeric substances (Sullivan & Palmisano 1984).

Other factors are related to viral dynamics. Viral abundances may be more related to bacterial production than to bacterial abundance (Maranger et al. 1994, Steward et al. 1996, Marchant et al. 2000), and bacterial production in the samples probably differed. Loss rates of viruses (reviewed by Wommack & Colwell 2000) in the different samples might differ. We have data only on consumption, and thin sections through >4000 microheterotrophs revealed no viruses <110 nm in food vacuoles (Gowing 2003). Finally, viruses may 'punish' the dominant community members (Thingstad et al. 1993, but see Schwalbach et al. 2004), with high abundances of viruses resulting from lysis of abundant bacteria. Thus the relationship between viral and bacterial abundance would undoubtedly vary over time, and our samples comprised different successional stages of the ice communities (see next subsection).

The VBR (virus-to-bacteria ratio) indicated a varying degree of viral influence from minimal to high. This ratio, generally used to assess the probability of viral control of bacterial populations, changes over time and space (Maranger et al. 1994, Marchant et al. 2000, Laybourn-Parry et al. 2001). Where blooms have been followed in polar areas, the VBR has increased and then decreased for a fast-ice bottom habitat (Maranger et al. 1994) and for surface waters (Yager et al. 2001). Hypotheses for causes of changes in VBR include proliferation of bacteria that are resistant to phage later in the season, reduction of active bacterial cells, or viral infection of algae (Maranger et al. 1994, Yager et al. 2001). In the Ross Sea summer samples, the first 2 possibilities might have contributed to the VBR variability. Additionally, restriction of entry of new viruses through brine channels and changes from lysogenic to lytic cycles in bacteria triggered by external factors such as ultraviolet light might affect the VBR in different ice microhabitats.

Although viral particles have been observed in glacial and accretion ice from the Vostok core (J. Priscu pers. comm.), there is only 1 other published study of predominantly small viruses in ice. Maranger et al. (1994) found viruses in the bottom 4 cm of Arctic land fast ice ranging from 9×10^6 to 1.5×10^8 ml^{-1} during the course of the Arctic spring diatom bloom. Minimum viral abundances in Ross Sea ice microhabitats were all lower than the value at the start of the Arctic bloom, and maxima were also lower than the Arctic maximum, although the maximum in surface slush was only slightly lower. The differences could reflect the differences in data from 1 site over time versus data from many sites sampled over 1 mo as well as differences in microbial community composition and dynamics between land fast ice and pack ice. In the Arctic bottom microhabitat, 5 species of pennate diatoms comprised 91% of algal cell numbers (Levasseur et al. 1994). In contrast, the Antarctic samples comprised microhabitats with diverse algal assemblages with many diatom and non-diatom species at bloom levels (this study; A. H. Gibson unpubl.). This fundamental difference in autotrophs probably would directly and indirectly affect bacterial dynamics and related viral dynamics.

Ice microbial communities

Despite the wide spatial coverage, the different microhabitats examined, and the month's temporal coverage, the samples were highly (>90%) similar, based on the abundance of major groups of microorganisms. This suggests that summer algal blooms are processes that structure pack-ice microbial communities. If summer algal blooms in Antarctic pack ice follow the same general course as blooms in other marine environments, different bloom stages should affect bacterial abundance, as shown in Table 5. Reality will be more complex, because different groups and species of algae will bloom at different times, causing the attendant processes and characteristics to overlap, as well as periodic injections of nutrients if viral lysis terminates the blooms (e.g. Castberg et al. 2001). The clusters can be considered within the general successional framework. Cluster 1 appears to represent stages before peaks of algal blooms: average chlorophyll *a* and phaeophytin concentrations were lowest; average bacterial, viral, diatom, autotrophic flagellate, autotrophic dinoflagellate, heterotrophic dinoflagellate and heterotrophic flagellate abundances were lowest; both motile and non-motile *Phaeocystis* spp. cells were pre-

Table 5. Changes in factors influencing bacterial abundance during course of an algal bloom. Table combines results from time-series studies by Davidson & Marchant (1992), Maranger et al. (1994), Putt et al. (1994), Sime-Ngando et al. (1997), Castberg et al. (2001), Yager et al. (2001) and Monticelli et al. (2003)

Bloom stage	Algal biomass or abundance	Bacterial abundance	Dominant characteristics/ processes with respect to bacteria
Early	Low	Low	Low substrate
Mid	Moderate	Moderate	Increasing substrate, low mortality
Peak	High	High	High substrate, high algal effects, predation increasing, viral lysis beginning
Post-peak	High	Highest	High substrate, high algal effects, high predation, high viral lysis
Late	Declining	Declining	High substrate, predation declining, viral lysis declining

sent, a condition that occurs before the peak of water column blooms of *P. antarctica* in the Ross Sea (Smith et al. 2003). Cluster 2 can be interpreted as closest to the peak of a bloom of *Phaeocystis* spp., with a high mean abundance of non-motile cells and no detectable motile cells; mean concentrations of pigments and mean abundances of bacteria, viruses and some autotroph and heterotroph groups are intermediate. Cluster 3 lacked any detectable *Phaeocystis* spp. cells, which could be a result of patchiness in bloom occurrence and the fact that the cluster comprised only 3 samples; Cluster 3 appeared to be a late stage of diatom and autotrophic flagellate blooms because of the high mean phaeophytin concentration coupled with the high mean abundances of diatoms, autotrophic flagellates, heterotrophic flagellates, bacteria, and viruses. The outlier slush sample can be interpreted as close to or an actual bloom stage of *Phaeocystis* spp. because of its abundant non-motile cells; diatoms were also blooming, and chlorophyll *a* concentration was highest in this data set (Table 3). Bacterial and viral abundance was high, and the phaeophytin concentration indicates grazing and/or some algal senescence. The community analysis and abundance relationships suggest that bacterial and viral dynamics in ice microhabitats during the austral summer may be similar to the complex interactions during algal blooms in marine temperate and polar water columns.

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