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Bacteria-algae relationships in Antarctic sea ice

F.J. STEWART* and C.H. FRITSEN

Division of Earth and Ecosystem Sciences, Desert Research Institute, 2215 Raggio Parkway, Reno, NV 89512, USA
**fstewart@fas.harvard.edu*

Abstract: Energy transfer in microbial food webs is partly quantified by the relationship between bacterial and algal biomass. Tight spatial relationships suggest active bacterial assimilation of dissolved photosynthate in temperate marine and fresh waters. However, studies in the Antarctic suggest that bacterial biomass generation from algal-derived dissolved organic matter is highly variable across seasons and habitats. Regression analysis was used to measure how bacteria covaried with algae in sea ice and water column habitats at three sites around Antarctica. Bacteria and algae were positively related in sea ice of the Weddell Sea during early winter 1992 (r^2 = 0.16, slope = 0.24) and across sea ice and upper water column habitats of the Ross Sea during summer 1999 (r^2 = 0.52, slope = 0.50). Conversely, bacteria and algae exhibited no discernible relationship in the water column and first year ice habitats of the Western Antarctic Peninsula region in winter 2001 (r^2 = 0.003, slope = -0.04). Low algal production and residual biomass probably limited bacterial production and facilitated bacteria-algae uncoupling in winter sea ice of the Western Antarctic Peninsula. Winter sea ice algal biomass was probably limited by a relatively late date of initial ice formation, reduced multi-year ice coverage, and a lack of radiant energy in the winter ice pack.

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Key words: austral winter, date of sea ice formation, microbial loop, Ross Sea, Weddell Sea, Western Antarctic Peninsula

Introduction

Microorganisms in sea ice function ecologically in the Antarctic marine ecosystem where pelagic and sea ice habitats are intrinsically coupled (Laws 1985, Garrison 1991, Eicken 1992, Knox 1994, Garrison & Mathot 1996, Arrigo *et al*. 1997, Brierley & Thomas 2002). For instance, sea ice algae - primarily diatoms and phototrophic flagellates (e.g. Garrison 1991) - are inocula for ice-edge phytoplankton blooms during ice melt in the spring and summer (Smith & Nelson 1985, Giesenhagen *et al*. 1999). They are a vital food source for crustaceous zooplankton, such as the Antarctic krill *Euphasia superba* Dana, that graze on assemblages at the under-surface of sea ice floes, or on cells released from the interior of the pack via brine drainage, ice melting, or ice break-up (Garrison & Buck 1989 and references therein, Daly 1990, Brierley *et al.* 2002, Hegseth & Von Quillfeldt 2002, Meyer *et al* 2002).

Grazing on sea ice microorganisms varies seasonally and may be particularly important in winter when reduced sunlight limits the abundance of pelagic phytoplankton, the primary prey for crustaceous zooplankton (Ross & Quetin 1996). In particular, in the continental shelf waters west of the Antarctic Peninsula, abundance and winter survival of *E. superba* may depend, in part, on whether larval krill supplement their diet by grazing on sea ice microbes during winter (Daly 1990, Legendre *et al*. 1992, Ross & Quetin 1996, Meyer *et al* 2002). Determining if and when sea ice microbial assemblages represent a viable food source for krill and other zooplankton requires an understanding of how energy and material cycle through different members of the sea ice assemblage as well as how physical factors, such as ice type and date of ice formation, impact these processes over seasonal time-scales.

Of particular relevance to energy and material cycling in marine habitats is the trophic relationship between algae and heterotrophic bacteria. This relationship develops primarily when bacteria assimilate dissolved organic matter (DOM) that is generated from algae via processes including rupture and degradation of cells during grazing, viral lysis, direct extracellular release of dissolved photosynthate, and algal production of exopolymeric substances (Nagata 2000, Krembs *et al*. 2002, Thomas & Dieckmann 2002). Bacterial assimilation of DOM is a core process in a microbial loop that, in the presence of bacterivory (Strom 2000), cycles dissolved products of photosynthesis back to the planktonic food web (Pomeroy 1974, Azam *et al*. 1983). Prior studies in mid- to low-latitude marine waters document microbial loop dynamics, in part, by a spatial relationship between bacterial and algal biomass or production that results from bacterial uptake of DOM (Pomeroy 1974, Bird & Kalff 1984, Cole *et al*. 1988). However, in Antarctic waters the nature of the coupling between phytoplankton and bacteria is equivocal (Carlson *et al*. 1998, Bird & Karl 1999, Ducklow *et al.* 2001, Pedrós-Alió *et al.* 2002, Moran *et al*. 2002).

Significant positive relationships between pelagic bacterial abundance and chlorophyll *a* (Chl *a*) concentration (a surrogate for phytoplankton biomass) have been found in regions of the Southern Ocean (Cota *et al*. 1990, Lochte *et al*. 1997). Conversely, instances when bacterial biomass

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or production does not increase with increasing Chl *a* or primary production have also been noted (Cota *et al*. 1990, Fiala & Delille 1992, Bird & Karl 1999, Pedrós-Alió *et al.* 2002, Moran *et al*. 2002). Similar variability in the bacteria-algae relationship has been documented for Antarctic sea ice.

Positive relationships between bacterial and algal biomass or production have been found in sea ice during spring to summer in several locations around the continent (Grossi *et al*. 1984, Kottmeier *et al*. 1987, Kottmeier & Sullivan 1990, Helmke & Weyland 1995, Grossmann *et al*. 1996, Fritsen & Sullivan 1999). Conversely, no significant relationship between bacteria and algae was found in late winter sea ice west of the Antarctic Peninsula (Kottmeier & Sullivan 1987). Such variability suggests that a complete assessment of the role of the microbial loop in Southern Ocean carbon cycling requires additional quantification of autotrophic and heterotrophic biomass and production over seasonal time scales and in both pelagic and sea ice habitats. Given the potentially significant role of sea ice microorganisms in sustaining pelagic zooplankton, especially larval krill during the winter (Daly 1990, Meyer *et al*. 2002), increased scientific inquiry should be directed towards determining the effect of large- and small-scale physical factors on bacterial-algal linkages in Antarctic sea ice.

The goal of this study was to use regression analysis to assess the relationship between bacterial and algal biomass in Antarctic sea ice at various seasonal stages. Prior studies documenting links between bacterial and algal biomass or production have focused primarily on pack ice or land-fast ice during spring to autumn (Sullivan & Palmisano 1981, Grossi *et al*. 1984, Kottmeier *et al*. 1985, Kottmeier & Sullivan 1987, 1988, 1990, Fritsen & Sullivan 1999). This study combines data on both pack ice and pelagic bacterial and algal biomass collected during summer, autumn, and winter from three regions of the western Antarctic marine ecosystem. The regression relationship between bacterial and algal biomass, the relative amount of biomass, and the size structure of the bacterial community were used to assess the impact of large-scale physical factors (i.e. ice type, season) on the Antarctic sea ice microbial community. The data show that algal biomass is a poor indicator of bacterial biomass in Antarctic sea ice during winter in years when ice algal biomass is limited. Yet algal biomass and bacterial properties are linked at other times of the year. These results provide insight into when and the extent to which the algal-bacterial food web contributes to total production in the coupled ice-water ecosystem of the Southern Ocean.

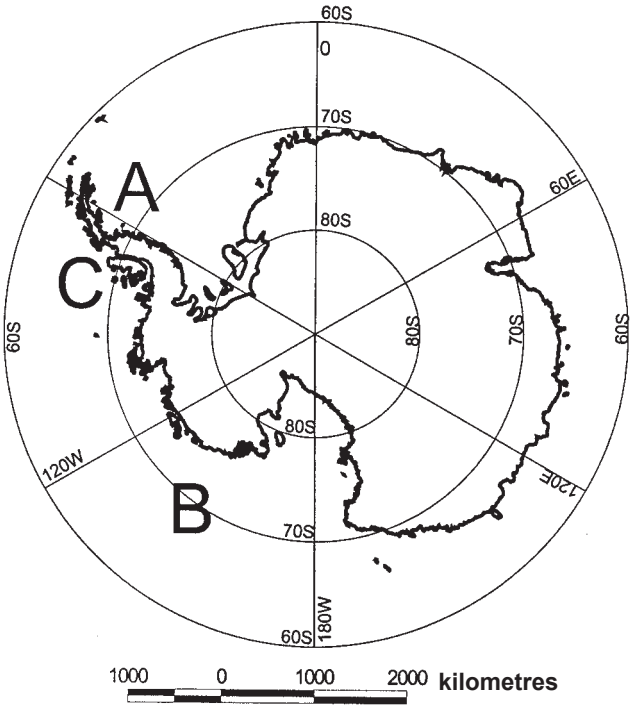


Fig. 1. Approximate locations of sample sites in **a.** the north-western Weddell Sea, 1992, **b.** in the eastern Ross Sea, 1999, and **c.** in the Western Antarctic Peninsula (WAP) region, 2001.

Methods and materials

Sample sites

Sea ice microbial assemblages were collected from three sites around the Antarctic continent: the north-western Weddell Sea, the eastern Ross Sea, and the Marguerite Bay region of the Western Antarctic Peninsula (WAP, Fig. 1). Weddell Sea samples were collected in early winter 1992 from 10 sites visited by the RV/IB *Nathaniel B. Palmer* (NBP) during the Ice Station Weddell Recovery Cruise (ISWRC, 10–22 June, Fritsen & Sullivan 1999). Ross Sea samples were collected during summer 1999 from 33 sites along three longitudinal transects (135°W, 150°W, 165°W) traversed by the NBP (NBP9901 Ice Cruise, 1–31 January). In support of the first field season of the Southern Ocean Global Ocean Ecosystem Dynamics (SO GLOBEC) program, WAP samples were collected in autumn (29 April–26 May) and winter (27 July–25 August) 2001 during cruises by the RV/IB *Laurence M. Gould* (LMG) and the NBP.

Ice conditions varied between sample sites. Sea ice at the Weddell Sea site in 1992 was composed of consolidated multi-year floes (floes that had survived at least one seasonal cycle of formation and melting) or older (> 2 months) first year floes ranging in thickness from 0.68–2.33 m. Similarly, the Ross Sea sites were primarily covered by consolidated pack ice ranging in thickness from 0.42–2.41 m. A latitudinal transition from old (3–6 months) first year ice to a region containing larger floes and more multi-year ice occurred at approximately 70°S (Morris & Jeffries 2001). In contrast to the Weddell and Ross Sea sites, sea ice in the WAP, when present, consisted of young

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Table I. Collection parameters and methods relevant to the determination of bacterial biomass for individual cruises. Abbreviations are identified in the text.

parameter	ISWRC	NBP9901	LMG0104	LMG0106	NBP0104
date	10–22 June 1992	1–31 January 1999	29 April–26 May 2001	27 July–25 August 2001	27 July–25 August 2001
storage time prior to staining	~2 months	~24–48 hrs	~24–48 hrs	~24–48 hrs	~1–2.5 months
counting protocol	Hobbie *et al*. 1977	Kepner & Pratt 1994	Kepner & Pratt 1994	Kepner & Pratt 1994	Kepner & Pratt 1994
bacteria nucleic acid stain	AO	DAPI	DAPI	DAPI	SYBR® Gold
sizing technique	enlarged images on whiteboard	digital imagery	digital imagery	digital imagery	digital imagery
carbon per volume conversion factor	Simon & Azam 1989	Loferer-Krößbacher *et al*. 1998	Loferer-Krößbacher *et al*. 1998	Loferer-Krößbacher *et al*. 1998	Simon & Azam 1989

(< 2 months) first year floes. During autumn 2001 (29 April–26 May, cruise LMG0104) sea ice was absent from most locations in the WAP, excluding patches of pancake and young grey ice in Dog Leg fjord in the north-east sector of Marguerite Bay and in Laserev Bay and King George VI Sound in the southern sector of the sampling area; total sea ice coverage in the study area was < 5% at the end of May. However, extensive sea ice formation occurred in the WAP in the interim between autumn and winter cruises. When the region was sampled again during July to August (cruises LMG0106, NBP0104), consolidated first year floes ranging in size from small (< 20 m diameter) to vast (> 2000 m diameter) were present at > 90% of the sampling sites; floe thickness ranged from 0.16 to 1.73 m.

Sample collection

Samples were taken from distinct habitats including the consolidated first and multi-year sea ice, the loosely consolidated new (hours to < 1 week old) ice (frazil ice, grease ice, pancake ice, nilas), the layers of slush (snow + sea water) overlying the consolidated sea ice, the interstitial pore water from the interior of floes (brine), the water that flooded core holes after drilling through to the bottom of a floe (corewater), and the upper (< 100 m depth) water column. Not all sample types were collected at each site.

Consolidated sea ice cores were collected using SIPRE or Kovacs core augers (7–10 cm inner diameter) and sectioned at regular (10–20 cm) intervals, or at natural breakage points in the ice. Individual core sections were placed in 0.2 µm filtered seawater (FSW) at a ratio of ~3:1 FSW to ice volume in order to minimize osmotic shock due to salinity changes during melting (Palmisano *et al*. 1985). New ice samples were collected in acid-washed plastic buckets. Bulk slush samples were collected in acid-washed Nalgene jars; slush interstitial waters were collected with 60 ml sterile syringes. Brine samples were collected by drilling a series of small (7–10 cm diameter) holes in a honeycomb array within a ~1.0 m^2 area. Brine holes did not penetrate the bottom of the floe and were generally less than 30 cm in depth. Brine was allowed to percolate into the holes for 10–60 min. Brines from individual holes were collected with 60 ml syringes and then integrated to form composite samples. FSW-diluted core samples and undiluted new ice, slush, and brine samples were allowed to melt in the dark at -1.8º to 4ºC until processing (within 6–12 hours after collection). Corewater samples were collected from the interior of core holes using acid-washed plastic bottles attached to a meter stick. Upper water column samples were collected with 10 l Niskin bottles deployed on a Rossette. Corewater and water column samples were processed immediately after collection.

Sample processing

Melted samples were immediately subsampled for Chl *a* and bacterial biomass (BB). Methodological differences in sample processing among sites are outlined in Table I.

Chlorophyll a

Subsamples for Chl *a* (mg m^{-3}) were filtered onto GF/F filters (Whatman®). Filters were stored frozen (-20ºC) until overnight extraction in 90% acetone and fluorometric determination of Chl *a* according to Parsons *et al*. (1984).

Bacterial biomass

Bacteria abundances (BA, cells m^{-3}) were determined from epifluorescent direct counts of non-filamentous and filamentous (cells > 10 µm in length) cells stained with either 4',6-diamidino-2-phenylindole (DAPI, 0.1 to 0.3% final concentration), Acridine Orange (AO, 0.04% final concentration), or SYBR® Gold (Molecular Probes®, 0.01% final concentration) according to Hobbie *et al*. (1977) and Kepner & Pratt (1994, see Table I). During counting, bacteria length and width were measured using digital images or enlarged images projected onto a white board. Bacterial cell biovolume (BCV, μm^3 $cell^{-1}$) was determined based on mean length and width and the assumption that cells were either cylinders with a hemispherical cap (rods) or spheres (cocci, Simon & Azam

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Table II. Mean (± s.e.) Chl *a*, BA, BB, % total BB occupied by filamentous bacteria, and BCV for sea ice and water column habitats during early winter in the Weddell Sea (ISWRC), summer in the eastern Ross Sea (NBP9901), and autumn (LMG0104) and winter (LMG0106, NBP0104) in the Western Antarctic Peninsula region. ND = not determined.

Dataset	Chl *a* (mg m^{-3})	BA (10^{11} cells m^{-3})	BB (mg C m^{-3})	% filament BB	BCV (μm^3 $cell^{-1}$)
All cruises	4.13 ± 0.36	3.46 ± 0.27	10.11 ± 0.98	2.17 ± 0.26	0.06 ± 0.001
	(*n* = 1051)	(*n* =666)	(*n* =666)	(*n* = 575)	(*n* = 9038)
ISWRC	6.71 ± 1.59	11.1 ± 1.16	41.54 ± 4.16	ND	0.11 (cocci)
	(*n* = 62)	(*n* = 91)	(*n* = 91)		0.39 (rods)
NBP9901	11.55 ± 1.08	5.84 ± 0.83	18.88 ± 2.97	8.30 ± 0.96	0.10 ± 0.003
	(*n* = 299)	(*n* = 130)	(*n* = 130)	(*n* = 130)	(*n* = 2493)
ice core	12.10 ± 1.36	7.84 ± 2.02	23.24 ± 6.90	4.49 ± 0.76	0.10 ± 0.004
	(*n* = 150)	(*n* = 45)	(*n* = 45)	(*n* = 45)	(*n* = 899)
slush	12.22 ± 1.92	5.96 ± 0.96	22.27 ± 3.74	15.47 ± 1.91	0.13 ± 0.005
	(*n* = 119)	(*n* = 49)	(*n* = 49)	(*n* = 49)	(*n* = 1074)
water column	0.30 ± 0.04	1.57 ± 0.13	1.60 ± 0.14	1.42 ± 0.68	0.03 ± 0.001
	(*n* = 26)	(*n* = 26)	(*n* = 26)	(*n* = 26)	(*n* = 520)
LMG0104	0.16 ± 0.01	1.85 ± 0.21	1.41 ± 0.17	0.08 ± 0.06	0.02 ± 0.001
	(*n* = 208)	(*n* = 87)	(*n* = 87)	(*n* = 87)	(*n* = 1220)
new ice	0.36 ± 0.06	1.20 ± 1.82	1.22 ± 0.23	0.47 ± 0.35	0.03 ± 0.001
	(*n* = 20)	(*n* = 15)	(*n* = 15)	(*n* = 15)	(*n* = 340)
water column	0.14 ± 0.01	1.98 ± 0.25	1.43 ± 0.19	0	0.02 ± 0.0004
	(*n* = 188)	(*n* = 72)	(*n* = 72)	(*n* = 72)	(*n* = 880)
LMG0106	0.77 ± 0.06	1.08 ± 0.05	1.14 ± 0.05	0.69 ± 0.20	0.04 ± 0.001
	(*n* = 380)	(*n* = 278)	(*n* = 278)	(*n* = 278)	(*n* = 3141)
brine	0.81 ± 0.19	1.81 ± 0.21	1.79 ± 0.21	1.40 ± 1.15	0.03 ± 0.001
	(*n* = 20)	(*n* = 20)	(*n* = 20)	(*n* = 20)	(*n* = 399)
ice core	1.09 ± 0.10	0.69 ± 0.05	0.86 ± 0.06	0.92 ± 0.33	0.05 ± 0.001
	(*n* = 237)	(*n* = 145)	(*n* = 145)	(*n* = 145)	(*n* = 1534)
new ice	0.14 ± 0.02	1.88 ± 0.30	1.76 ± 0.35	0	0.03 ± 0.001
	(*n* = 9)	(*n* = 9)	(*n* = 9)	(*n* = 9)	(*n* = 200)
slush	0.16 ± 0.03	0.91 ± 0.18	0.78 ± 0.20	2.38 ± 1.53	0.03 ± 0.002
	(*n* = 15)	(*n* = 13)	(*n* = 13)	(*n* = 13)	(*n* = 240)
water column	0.02 ± 0.001	1.47 ± 0.08	1.40 ± 0.09	0	0.03 ± 0.001
	(*n* = 99)	(*n* = 91)	(*n* = 91)	(*n* = 91)	(*n* = 768)
NBP0104	1.60 ± 0.40	0.69 ± 0.11	0.83 ± 0.10	0.63 ± 0.22	0.05 ± 0.001
	(*n* = 102)	(*n* = 80)	(*n* = 80)	(*n* = 80)	(*n* = 2184)
ice core	2.15 ± 0.54	0.76 ± 0.15	0.91 ± 0.14	0.85 ± 0.29	0.06 ± 0.002
	(*n* = 74)	(*n* = 59)	(*n* = 59)	(*n* = 59)	(*n* = 1599)
corewater	0.14 ± 0.06	0.50 ± 0.07	0.56 ± 0.08	0	0.03 ± 0.002
	(*n* = 28)	(*n* = 21)	(*n* = 21)	(*n* = 21)	(*n* = 585)

1989). BB (mg C m^{-3}) was determined using BA values, BCV values, and allometric conversion factors for bacterial carbon per volume (Simon & Azam 1989, Loferer-Krößbacher *et al.* 1998, Table I).

Stain-specific carbon per volume conversion factors were used to correct for differential staining of cellular material by AO and DAPI (Suzuki *et al*. 1993). To our knowledge, a conversion factor specific for cells stained with SYBR® Gold is not available. However, dual staining of the same sample with both DAPI and SYBR® Gold showed that the mean BCV of DAPI-stained cells was 70% of the mean BCV of SYBR® Gold-stained cells (n = 400 cells per stain across 11 samples, data not shown). This difference between stains was significant ($P < 0.001$, ANOVA) and was similar to differences recorded in comparisons between DAPI- and AO-stained bacteria (Suzuki *et al*. 1993, Posch *et al*. 2001). Given that both AO and SYBR® Gold appear to stain bacteria similarly relative to DAPI (Fritsen & Stewart, unpublished data), an AO-specific conversion factor was used in calculating BB in SYBR® Gold-stained samples (NBP0104 samples). SYBR® Gold-stained samples represent ~12% of the total number of bacteria samples analysed in this study. See Posch *et al*. (2001) for a detailed discussion of varying carbon per volume conversion factors for use in determining bacterial biomass.

Most subsamples were stained for counting within 24 to 48 hours following collection; however, due to logistical constraints, preserved Weddell Sea and WAP NBP0104 subsamples were stored for 1–2.5 months prior to staining. Decreases in BA with storage time have been documented for aquatic samples (Nishino 1986, Turley & Hughes 1992, Gundersen *et al.* 1996); BA in seawater samples preserved with gluteraldehyde may decline by up to 69% over a 6-month period (Nishino 1986). Unfortunately, the relative impact of storage time (~2 months) on the loss of bacteria was not determined for our preserved samples; therefore,

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BA and BB in Weddell Sea and WAP NBP0104 sea ice may be underestimated.

Statistical Analysis

All data were log-transformed (base 10) to obtain a normal distribution prior to analysis of variance (ANOVA) and least squares linear regression analysis. Single-factor ANOVA was used to test for significant differences in mean Chl *a*, BA, BB, and BCV across habitats. A Tamhane test was then applied *post hoc* to detect significant differences among multiple pairs of means (Tamhane 1979). Least squares linear regressions of log-transformed data were used to assess the relationships between Chl *a* and BA and Chl *a* and BB within and across habitats. Significant differences among multiple pairs of correlation coefficients (*r*) and regression coefficients (*b*, slopes) were detected using Tukey-type tests as described in Zar (1999). Pairwise comparisons of *r* and *b* values were done only between pairs for which the slopes of the regression lines were first determined via ANOVA to vary significantly from zero. Statistical analyses were performed using MS Excel and SPSS 11.0.

Additional abbreviations used in the text include: BR = brine, CR = ice core, NI = new ice (brash, grease, pancake, or nilas ice), SL = slush, WC = water column.

Results

Chlorophyll a

Mean Chl *a* concentrations ranged over three orders of magnitude and differed significantly among habitats and seasons ($P < 0.001$, ANOVA, Table II). Highest concentrations were found in sea ice habitats of the eastern Ross Sea during summer 1999 (Table II), with means ranging from 4 mg m^{-3} in surface slush layers over first year floes north of 70°S to 44 mg m^{-3} in new ice (nilas). Peak

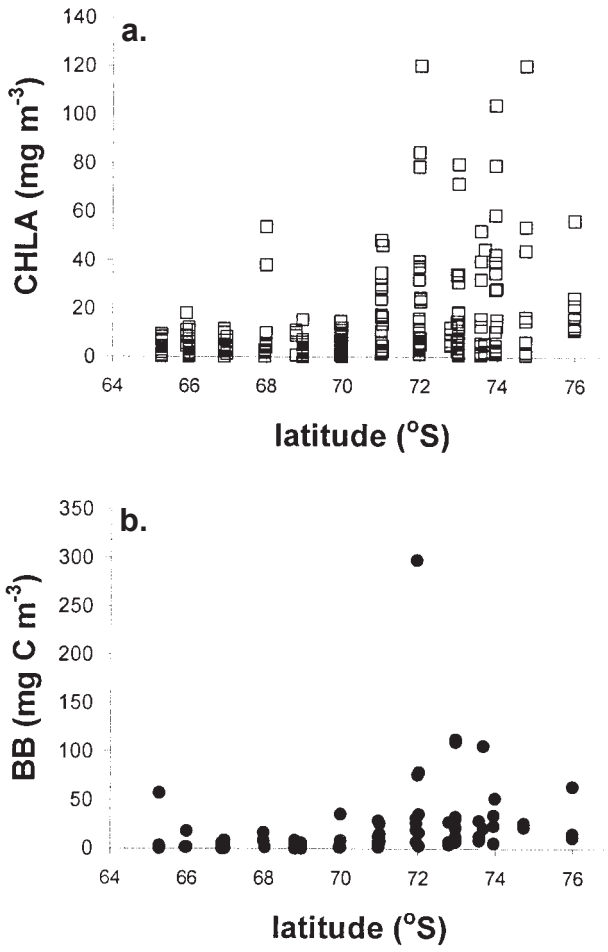


Fig. 2. Latitudinal increase in **a.** Chl *a*, and **b.** bacterial biomass in the Ross Sea during summer 1999. A north to south transition from first year to multi-year ice occurred at ~70°S.

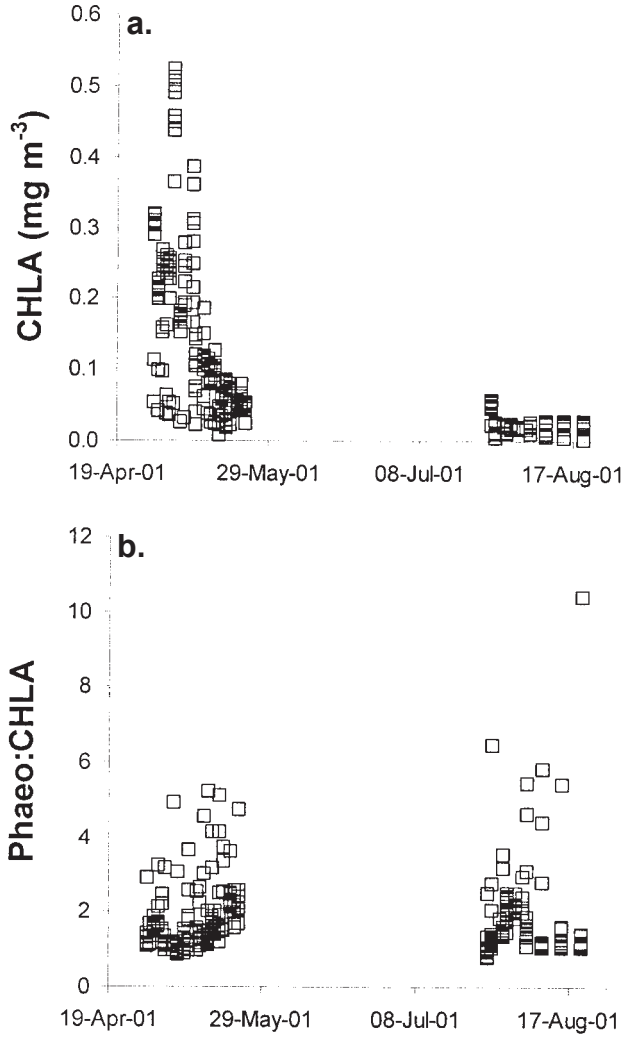


Fig. 3. Temporal change in water column **a.** Chl *a*, and **b.** phaeopigment:Chl *a*, in the WAP from late April to late August 2001. The absence of data during June and July indicates the gap between cruises.

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values over 100 mg m^{-3} were observed in multi-year ice south of 70°S and were consistent with a latitudinal trend of increasing Chl *a* with proximity to the continent (Fig. 2a). In marked contrast, mean Chl *a* in Western Antarctic Peninsula (WAP) sea ice, though above the level of detection, ranged from 0.14 mg m^{-3} in new ice (nilas, pancake ice) to 2.15 mg m^{-3} in consolidated first year floes during winter 2001. Mean Chl *a* in older first year or multi-year ice from the Weddell Sea (early winter 1992) was intermediate in concentration relative to WAP and Ross Sea sea ice (Table II). Chl *a* in all Weddell Sea and Ross Sea sea ice habitats, excluding Ross Sea new ice due to low sample size (n = 5), was significantly elevated relative to Chl *a* in ice habitats of the WAP region during autumn and winter 2001 ($P < 0.05$, Tamhane test). Chl *a* was not significantly related to ice thickness at any of the sampling sites (data not shown).

In both the Ross Sea and the WAP, mean water column Chl *a* was significantly lower than Chl *a* in overlying ice habitats ($P < 0.05$, Tamhane test, Table II); water column samples were not collected from the Weddell Sea. In the Ross Sea, water column Chl *a* remained relatively constant throughout the sampling period (January). However, in the WAP region, water column Chl *a* declined from a peak value of ~0.5 mg m^{-3} in late April to a low of ~0.02 mg m^{-3} during August after the autumn phytoplankton bloom (Fig. 3a).

The distribution of phaeopigments, the degradation products of Chl *a* (e.g. SooHoo & Kiefer 1982), was analysed for WAP sea ice and water column habitats. WAP phaeopigments averaged 0.17 mg m^{-3} in sea ice (range: 0.003 to 6.43 mg m^{-3}, n = 426) and 0.13 mg m^{-3} in the water column (range: 0.01 to 0.66 mg m^{-3}, n = 294); as anticipated, phaeopigments closely paralleled Chl *a* in both the sea ice (r^2 = 0.75) and the water column (r^2 = 0.92). An increase in the ratio of phaeopigment to Chl *a* during April to May (LMG0104) suggested an increase in algal cell degradation as winter approached (Fig. 3b) but ratios decreased slightly two months later during the winter cruise (LMG0106, Fig. 3b). Water column phaeopigment:Chl *a* averaged 1.79 (range: 0.63 to 10.38, n = 294) across both cruises. In contrast to the water column, sea ice phaeopigment:Chl *a* was significantly lower (mean: 0.34, range: 0.02 to 2.98, n = 427, $P < 0.05$, ANOVA) during autumn and winter 2001.

Bacterial biomass

Like Chl *a*, bacterial biomass (BB) also varied significantly among habitats and seasons ($P < 0.001$, ANOVA, Table II). Coincident with the distribution of Chl *a*, mean BB was highest in the first and multi-year sea ice of the eastern Ross Sea and the Weddell Sea, ranging from 11.7 mg C m^{-3} in Ross Sea first year ice north of 70°S to 41.5 mg C m^{-3} in older first year or multi-year ice of the Weddell Sea. A peak

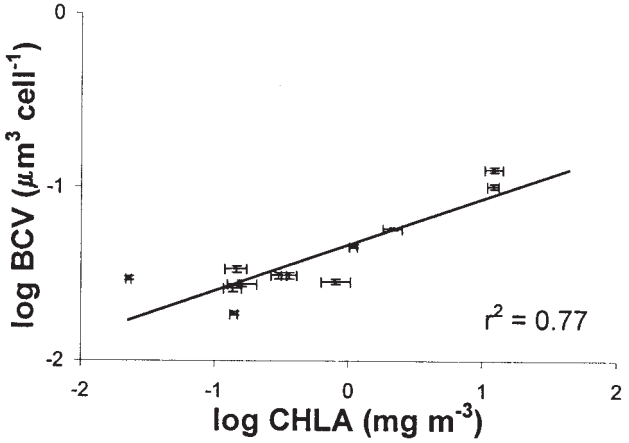


Fig. 4. Relationship between mean log Chl *a* and mean log bacterial cell volume (BCV) across sea ice (brine, ice core, new ice, and slush) and water column habitats in the Ross Sea during summer 1999 and in the WAP during autumn and winter 2001. The linear regression equation is log BCV = - 1.33 + 0.27 log Chl *a*, $P < 0.001$. Error bars are ± 1 s.e.

concentration exceeding 200 mg C m^{-3} was observed in sections of sea ice in both the Ross and Weddell Seas. In the Ross Sea, where a southward transition from first year to multi-year ice was observed, BB increased slightly with proximity to the continent (Fig. 2b).

Mean BB in WAP and Ross Sea water columns and in WAP sea ice ranged from 0.56 to 1.79 mg C m^{-3} and was significantly lower than BB in the Ross Sea and Weddell Sea ice habitats, excluding Ross Sea new ice ($P < 0.05$, Tamhane test, Table II). In contrast to BB in the Ross Sea water column and to Chl *a* in both the Ross Sea and the WAP, BB in the WAP water column was not significantly different than BB in overlying ice habitats during autumn and winter ($P > 0.05$, Tamhane test).

As expected bacterial abundance (BA) was highly correlated with BB across habitats (r^2 = 0.89, n = 666, $P < 0.001$, ANOVA). Mean BA ranged from 4.99 x 10^{10} cells m^{-3} in the under-ice water column (corewater) of the WAP during winter to 1.13 x 10^{12} cells m^{-3} in multi-year winter pack ice of the Weddell Sea (Table II). In some instances, BA and BB in habitats of the Western Antarctic Peninsula were just above the level of detection (~10^3 cells ml^{-1}) based on the volumes of samples available.

The mean bacterial cell volume (BCV) of Weddell Sea sea ice bacteria ranged from 0.11 µm^3 $cell^{-1}$ for cocci cells to 0.39 µm^3 $cell^{-1}$ for rod-shaped cells (Table II). Mean BCV differed significantly among Ross Sea and WAP habitats ($P < 0.001$, ANOVA), ranging from 0.02 µm^3 $cell^{-1}$ for bacterioplankton in the WAP during autumn to 0.13 µm^3 $cell^{-1}$ for bacteria in summertime consolidated pack ice of the Ross Sea (Table II). This increase in mean cell size corresponded to an increase in mean Chl *a* among habitats (r^2 = 0.77, Fig. 4). The fraction of total BB occupied by filaments also differed among habitats. Filaments were

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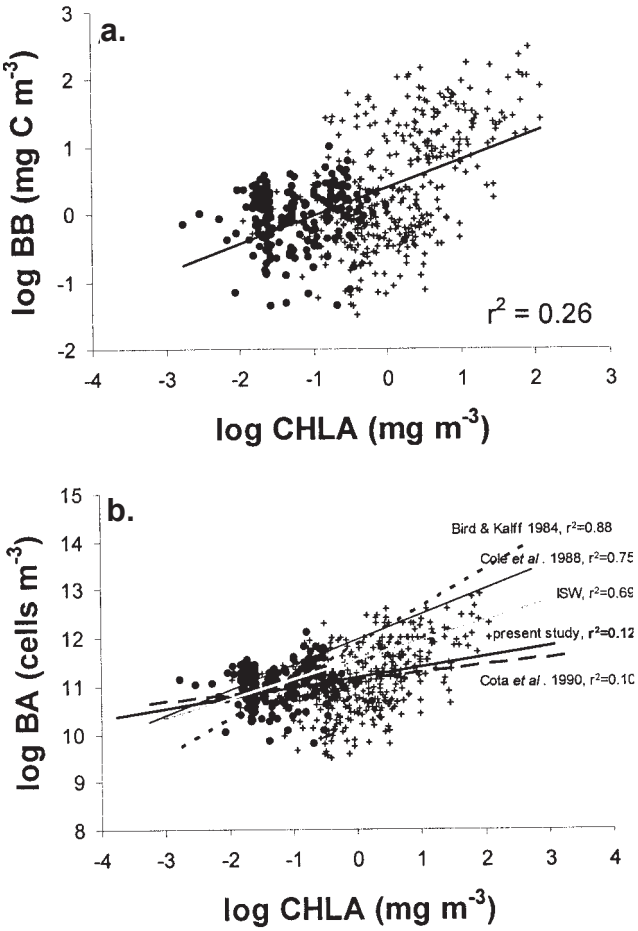


Fig. 5. a. The relationship between log Chl *a* and log bacterial biomass (BB) across all sea ice (+) and water column (•) habitats sampled in this study. **b.** The relationship between log Chl *a* and log bacterial abundance (BA) observed in the present study in comparison to relationships for temperate freshwater and marine euphotic zones across seasons (Bird & Kalff 1984, Cole *et al*. 1988), for upper water column and sea ice habitats of the Weddell Sea during the summer-to-winter transition (Ice Station Weddell, Fritsen & Sullivan, unpublished data), and for the water column during autumn in the marginal ice zone of the Weddell Sea (Cota *et al.* 1990).

abundant in Ross Sea sea ice but constituted a smaller fraction of total BB in WAP sea ice during autumn and winter (Table II). Filamentous biomass was not documented for Weddell Sea samples.

Consistent with Chl *a*, neither BB nor BA was significantly related to ice thickness at any of the sampling sites (data not shown).

Relationships between chlorophyll and bacteria

A significant, positive relationship between BB and Chl *a* existed across the combined (sea ice + water column habitats, all cruises) dataset (Table III, Fig. 5a). The overall relationship is:

Table III. Regression statistics for log BB versus log Chl *a* for sea ice and water column habitats during early winter in the Weddell Sea (ISWRC), summer in the eastern Ross Sea (NBP9901), and autumn (LMG0104) and winter (LMG0106, NBP0104) in the WAP. Standard errors (s.e.) and F-values (F) are given for each slope.

dataset	*n*	slope	s.e.	Y-int	r^2	F
all cruises	620	0.41	± 0.03*	0.39	0.26	213.8
all ice data	415	0.62	± 0.05*	0.32	0.29	170.8
all WC data	205	0.10	± 0.06	0.10	0.01	2.8
ISWRC	62	0.24	± 0.07*	1.42	0.16	11.6
NBP9901	122	0.50	± 0.04*	0.66	0.52	131.6
ice core	43	0.69	± 0.10*	0.46	0.55	50.9
slush	48	0.24	± 0.08*	0.97	0.15	8.2
water column	26	-0.17	± 0.12	0.06	0.08	2.0
LMG0104	85	0.34	± 0.13*	0.22	0.07	6.7
new ice	14	-0.45	± 0.46	-0.29	0.07	0.9
water column	71	0.53	± 0.15*	0.43	0.15	11.8
LMG0106	274	-0.10	± 0.03*	-0.17	0.04	11.0
brine	20	-0.02	± 0.17	0.16	0.001	0.02
ice core	145	0.02	± 0.07	-0.21	0.001	0.1
new ice	9	-0.20	± 0.42	0.003	0.03	0.2
slush	13	-0.35	± 0.20	0.56	0.22	3.0
water column	87	0.25	± 0.18	0.45	0.02	1.9
NBP0104	77	0.08	± 0.06	-0.23	0.02	1.6
ice core	56	0.05	± 0.10	-0.23	0.01	0.3
corewater	21	0.08	± 0.13	-0.23	0.02	0.4

*indicates slopes are significant at $P < 0.05$. NBP9901 new ice data ($n = 5$) not shown.

$$\log BB = 0.39 + 0.41 \log Chl\ a \qquad r^2 = 0.26,\ n = 620,\ P < 0.001, \text{ANOVA} \quad (1)$$

However, the relationship is weakened slightly when BA is used to measure bacterial abundance (Fig. 5b):

$$\log BA = 11.18 + 0.22 \log Chl\ a \qquad r^2 = 0.12,\ n = 620,\ P < 0.001, \text{ANOVA} \quad (2)$$

Except when explicitly used in comparison with published literature values, bacteria-algae relationships discussed in the remainder of this text refer to BB-Chl *a* comparisons.

BB-Chl *a* relationships varied substantially among individual habitats (Table III). BB most strongly varied with Chl *a* in the consolidated sea ice of the eastern Ross Sea during summer ($r^2 = 0.55$). Chl *a* was a much weaker, but still significant, indicator of BB in the slush layers overlying consolidated Ross Sea sea ice, in the consolidated multi-year ice of the Weddell Sea during winter 1992, and in the water column of the WAP during autumn 2001 (Table III). BB did not vary significantly with Chl *a* in Ross Sea new ice (data not shown), in WAP autumn new ice or in any of the sea ice or water column habitats sampled during winter in the WAP (Table III).

Regressions of combined datasets for individual cruises showed a strong positive relationship across the sea ice and water column of the Ross Sea (Table III, Fig. 6a):

$$\log BB = 0.66 + 0.50 \log Chl\ a \qquad r^2 = 0.52,\ n = 122,\ P < 0.001, \text{ANOVA} \quad (3)$$

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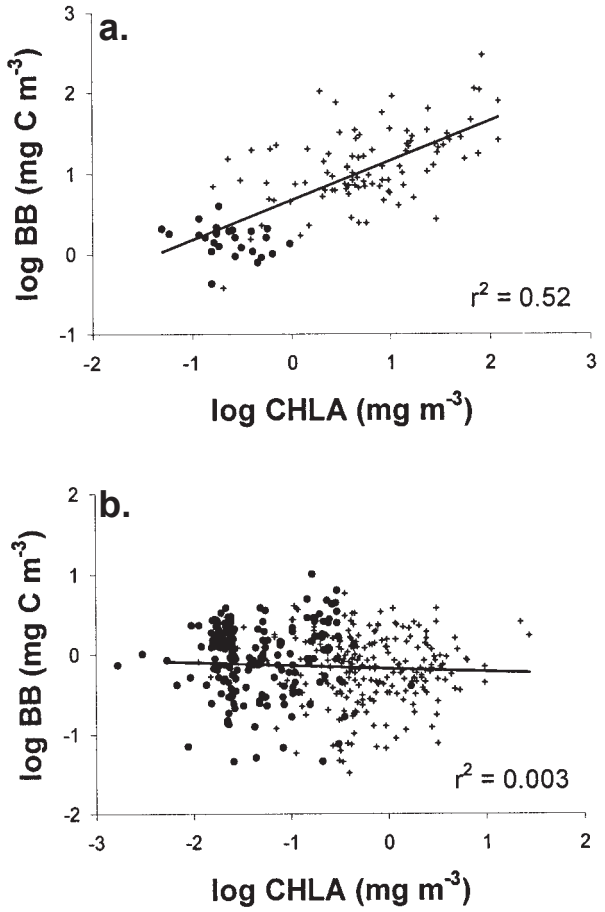


Fig. 6. a. The relationships between log Chl *a* and log bacterial biomass (BB) across sea ice (+) and water column (•) habitats in the Ross Sea during summer 1999, and **b.** in the WAP region during autumn and winter 2001 (LMG0104, LMG0106, NBP0104 combined).

In contrast, no cross-habitat (ice + water column) relationship was observed in the WAP region during autumn or winter (Table III, Fig. 6b); the regression slope and the correlation coefficient for the WAP relationship were significantly lower than those for the combined Ross Sea dataset ($P < 0.05$, Tukey-type tests). However, at low bacteria concentrations, such as those recorded in the WAP (Table II), bacteria counts must be very accurate in order to detect small-scale differences in BB among samples. It is possible that manual direct counting was not able to resolve such variation in WAP samples. Consequently, a relatively high likelihood for high variance relative to total abundance may have reduced the ability to detect significant regression relationships in WAP samples.

Discussion

Bacteria-algae coupling

In this study coupling refers explicitly to a concurrent temporal and spatial relationship between bacterial and algal biomass that probably results, in part, from assimilation of algal-derived DOM by bacteria. Regression statistics, including the coefficient of determination (r^2) and the slope (b) of the regression equation, are used to describe how the bacterial assemblage responds to variation in algal biomass (Bird & Kalff 1984, Cole *et al*. 1988, Bird & Karl 1999, Moran *et al*. 2002). Although the ratio of bacterial production to primary production may be low (< 10%) in many Antarctic habitats (Brierley & Thomas 2002 and references therein), it is still possible for bacteria and algae to be tightly coupled spatially (high r^2). However, the overall bacteria-algae relationship (Eqs 1 & 2) for this study resembles that observed by Cota *et al*. (1990) for the water column of the Weddell Sea during autumn when both bacterial abundance and algal biomass were relatively low (Fig. 5b). As in Cota *et al*. (1990), the relationship shown here contrasts with empirical relationships demonstrating that in other marine and fresh waters (e.g. Bird & Kalff 1984, Cole *et al* 1988) bacterial properties tend to increase predictably along gradients of algal biomass and production (Fig. 5b).

However, our results and those from prior studies in the Southern Ocean (Cota *et al.* 1990, Fiala & Delille 1992, Bird & Karl 1999, Ducklow *et al*. 2001, Pedrós-Alió *et al*. 2002, Moran *et al*. 2002) suggest that bacterial biomass generation from algal-derived DOM in Antarctic waters is highly variable across seasons and habitats. Regression statistics for datasets combining biomass measurements from different Antarctic habitats and seasons (e.g. Eqs 1 & 2) may therefore over or underestimate the degree to which heterotrophic bacteria are spatially and energetically coupled with algae via the uptake of dissolved photosynthate.

Bacteria-algae coupling in the Ross Sea

Variation in Chl *a* concentration explained 52% of the variation in bacterial biomass across the upper water column and sea ice habitats of the Ross Sea (Table III, Fig. 6a). A similar cross-habitat relationship ($r^2 = 0.69$, slope = 0.41) has been defined for bacteria abundance and algal biomass across upper water column and multi-year ice habitats of the Weddell Sea during the summer-to-winter transition (Ice Station Weddell, Fritsen & Sullivan unpublished data, Fig. 5b). These relatively strong cross-habitat relationships are due partly to large variations in microbial abundance among and within habitats and to a tight spatial coupling of bacterial and algal biomass in sea ice. For instance, in the Ross Sea during summer 1999, sea ice algal biomass spanned up to three orders of magnitude (Fig. 6a) and was, on average, 13 to 70 times higher than in the water column (Table II). High algal biomass was likely due to both physical mechanisms that concentrate organisms in various sea ice microhabitats over time (e.g. congelation or frazil ice growth, flood and freeze cycles,

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and ridging; Garrison *et al*. 1989, Ackley & Sullivan 1994, Fritsen *et al*. 1994, 1998, Arrigo *et al*. 1997, Melnikov 1998, Fritsen & Sullivan 1999) and to *in situ* algal growth stimulated by seasonal increases in irradiance and temperature (Grossi *et al*. 1984, 1987, Kottmeier *et al*. 1985, 1987, Palmisano *et al*. 1985, 1987, Kottmeier & Sullivan 1988, McMinn *et al*. 2000, Rysgaard *et al*. 2001). A tight coupling of bacterial and algal biomass might therefore be expected in Antarctic sea ice during spring to autumn when sufficient light is available to support pockets of high algal biomass and, consequently, high levels of DOM for bacterial consumption. Prior reports corroborate this hypothesis (Grossi *et al.* 1984, Kottmeier *et al*. 1987, Kottmeier & Sullivan 1988, 1990, Grossmann *et al*. 1996, Fritsen & Sullivan 1999).

In contrast to the sea ice, a negative relationship between bacterioplankton and phytoplankton biomass occurred at the top of the water column immediately below summertime Ross Sea sea ice (Table III). Such an inverse relationship might develop if bacterial production lags primary production, causing bacterial biomass to accumulate rapidly while phytoplankton biomass declines. Ducklow *et al*. (2001) suggest that a one-month lag between phytoplankton and bacterioplankton blooms in the central Ross Sea resulted primarily from delayed production and utilization of algal-derived dissolved organic carbon (DOC) and also from direct exoenzymatic hydrolysis of senescent phytoplankton cells by bacteria (as described in Smith *et al*. 1992). Similarly, Vaqué *et al*. (2002) note a separation in time between increases in phytoplankton and heterotrophic (bacteria and nanoflagellates) abundance along a north–south transect in the Bransfield Strait (fig. 4). Such a temporal uncoupling of primary and bacterial production may account for the inverse bacteria-algae biomass relationship during January 1999 in the eastern Ross Sea. For the Southern Ocean in general, variation in this water column relationship is likely high in response to the seasonally and spatially variable nature of phytoplankton blooms in the region (e.g. Knox 1994).

Bacteria-algae coupling in the Western Antarctic Peninsula

Chlorophyll *a* concentrations were poor indicators of bacterial biomass ($r^2 = 0.003$) across water column and sea ice habitats of the Western Antarctic Peninsula (WAP) region during autumn and winter 2001 (Fig. 6b). Undoubtedly, the cross-habitat relationship observed was weakened in part by weak ($r^2 < 0.22$, $P > 0.05$) bacteria-algae correlations in most individual habitats (Table III). A significant ($P < 0.001$) bacteria-algae relationship ($r^2 = 0.15$) was observed only in the water column during the autumn-to-winter transition. During this period elevated irradiance and the absence of sea ice contributed to high phytoplankton biomass relative to winter concentrations recorded two months later when *in situ* photosynthesis was suppressed by seasonally lower light levels and by the presence of light-shielding sea ice (Fig. 3a). Low phytoplankton biomass, and therefore a potentially low DOM supply, may have facilitated a trophic uncoupling of bacteria and phytoplankton, resulting in the spatial uncoupling of bacterial and algal biomass observed in the WAP water column during winter 2001. Low DOM fluxes also may have contributed to an uncoupling of pelagic bacteria and phytoplankton production during spring in Gerlache Strait (Bird & Karl 1999) and a lag in bacterial response to a bloom of the colonial haptophyte *Phaeocystis antarctica* during summer to autumn in the central Ross Sea (Ducklow *et al*. 2001).

We propose that low ice algal biomass, and a resulting condition of substrate limitation for bacteria, may also have facilitated the uncoupling of WAP sea ice bacteria and algae during winter 2001. Substrate limitation of bacterial growth may have been exacerbated by the low temperatures characteristic of Antarctic sea ice during winter.

Substrate limitation in sea ice

The interactive effects of substrate (DOM) concentration and temperature exert particularly strong controls on bacterial growth and biomass accumulation in Antarctic sea ice (Kottmeier & Sullivan 1988, Helmke & Weyland 1995, Pomeroy & Wiebe 2001). Sea ice bacterial assemblages are composed primarily of cold-adapted psychrotolerant and psychrophilic strains capable of growth at or below 0°C (Staley & Gosink 1999). However, bacterial growth in sea ice is usually suboptimal at temperatures encountered *in situ* (= -1.8°C, Kottmeier & Sullivan 1988, Helmke & Weyland 1995, Staley & Gosink 1999). At low temperatures the ability of the bacteria cell to assimilate organic substrate is hindered by a decrease in both the activity of membrane-associated active transport proteins and the reaction rates of extracellular hydrolytic enzymes (Nedwell 1999, Huston *et al*. 2000). Although sea ice bacteria may possess adaptive mechanisms to help combat these physiological effects (e.g. high levels of unsaturated membrane lipids to increase membrane fluidity, increased enzyme production, synthesis of cold-adapted enzymes with low activation energies; Reichardt 1988, Nedwell 1999, Huston *et al*. 2000), there is strong evidence that bacterial growth at temperatures characteristic of sea ice also requires enhanced concentrations of labile substrate (DOM, Pomeroy *et al*. 1991, Wiebe *et al*. 1992, Helmke & Weyland 1995, Nedwell 1999, Pomeroy & Wiebe 2001). An inverse relationship between substrate dependence and temperature suggests that WAP sea ice bacteria may be especially susceptible to substrate limitation during the winter when mean monthly atmospheric temperatures decline to < -10°C (Smith *et al*. 1996).

In newly formed winter ice that may have little residual DOM from past biological activity or from physical

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concentration over time (Thomas *et al*. 2001) DOM flux to bacteria may be influenced primarily by processes that degrade the accumulated algal stock (e.g. direct bacterial catabolism, algal lysis, or grazing). Low algal abundance in WAP sea ice during winter 2001 therefore suggests the potential for substrate limitation. Chl *a* in WAP sea ice (mean: 0.14 to 2.15 µg l^{-1} across ice habitats, range: < 0.001 to 28.0 µg l^{-1}) was markedly lower than concentrations reported for other sea ice habitats where coupling of bacteria and algae was detected, including Ross Sea and Weddell Sea sea ice examined in this study (e.g. Gradinger & Zhang 1997, Grossman *et al*. 1996, Fritsen & Sullivan 1999, Monfort *et al*. 2000). Likewise, a small number of DOC measurements from WAP winter brine also suggest substrate limitation. Mean DOC in WAP brine was 93 µM (n = 4, unpublished data), up to 40 times lower than concentrations reported for spring and summer pack ice off the coast of Japan and in the Canadian Arctic (Smith *et al*. 1997) and up to 250 times lower than concentrations reported for autumn pack ice of the south-eastern Weddell Sea (Thomas *et al*. 2001). Low WAP DOC concentrations are consistent with the low phaeopigment to Chl *a* ratios (mean = 0.34) observed in WAP sea ice. These low ratios suggest that pathways of algal degradation that provide DOM for bacterial production (e.g. zooplankton grazing; SooHoo & Kiefer 1982) were potentially shut down in WAP sea ice. We also cannot exclude the possibility that low DOC in WAP sea ice may have resulted from decreased rates of DOM production via alternative mechanisms, including the production of exopolymeric substances by ice algae (Krembs *et al*. 2002).

While low DOC may also be interpreted as evidence for a tight coupling between DOM production and assimilation by bacteria, the small volumes measured for WAP sea ice bacteria suggest a state of substrate limitation. The cell volume of psychrophilic heterotrophic bacteria has been shown to vary with both substrate quality and quantity (Wiebe *et al*. 1992). A similar trend of increasing cell size with Chl *a* concentration was apparent in this study (Fig. 4). WAP sea ice bacteria were up to five times smaller than bacteria in Ross Sea sea ice where mean Chl *a* was one to two orders of magnitude higher than Chl *a* in WAP ice habitats (Table II). WAP cell volumes were similar to those measured for Antarctic pelagic bacterioplankton during potentially substrate-limiting conditions (Ducklow *et al*. 2001). Similarly, WAP sea ice contained considerably smaller percentages of large (> 10 µm) filamentous bacteria relative to Ross Sea sea ice. Despite qualitative observations of dividing cells in epifluorescent counts of WAP bacteria, our data suggest that during the sampling period *in situ* bacterial growth contributed little to carbon cycling and was potentially limited by energy supply from algal-derived DOM. Low levels of sea ice microbial activity and biomass may have been directly influenced by biological and physical controls including grazing, date of initial ice formation, percentage multi-year ice, and differential scavenging of bacteria and algae during ice formation.

Controls on sea ice algal biomass and substrate availability

Biological control of microbial biomass involves grazing of bacteria or algae by protists or microzooplankton. Although rates of bacterivory were not determined in this study, protistan grazing, while highly variable over gradients of protist abundance (Vaqué *et al*. 2002), has been shown to significantly influence bacterioplankton abundance in the Southern Ocean (Bird & Karl 1999, Anderson & Rivkin 2001, Vaqué *et al*. 2002) and may play a similar role in structuring sea ice microbial assemblages (Kottmeier *et al*. 1987, Kivi & Kuosa 1994, Laurion *et al*. 1995, Scott *et al*. 2001). In addition, many studies report that krill graze on sea ice algae at the ice-water interface and in the interstices of pack ice (Garrison & Buck 1989 and references therein, Daly 1990, Brierley *et al*. 2002). During winter 2001 larval krill were encountered within sea ice brine channels on several occasions. Unfortunately, the impact of krill grazing on WAP sea ice algae was not directly quantified. However, low phaeopigment to Chl *a* ratios indirectly suggest that algal degradation via zooplankton grazing was limited in winter ice. While krill or bacteriovore grazing cannot be dismissed as a factor limiting sea ice microbial activity and biomass, it is more likely that the weak relationship between bacterial and algal biomass in WAP winter pack ice was due to physical, rather than biological, controls.

Ice effects - date of formation and percent older ice

Both a relatively late date of initial pack ice formation and a low abundance of multi-year ice influenced the concentration of algae and the activity of the bacterial community in WAP pack ice during winter. Algal biomass in newly formed winter ice is controlled primarily by the date of ice formation (Hoshiai 1977, Fritsen & Sullivan 1999). In the WAP the date of ice formation is subject to high interannual variability (Stammerjohn & Smith 1996). In 2001 substantial sea ice formation over the WAP study area did not take place until early to mid-June, following a sharp decrease in phytoplankton abundance (Fig. 3a) and approximately three weeks later than the mean historic date of ice formation for the region (Stammerjohn & Smith 1996). Consequently, both the amount of phytoplankton available for physical incorporation into newly formed ice and the period of time during which sufficient light is available to support *in situ* primary production may have been reduced relative to years when substantial ice growth occurred several weeks prior. A late forming ice cover may therefore have limited the quantity of algal biomass available to support bacterial growth in the months subsequent to ice formation.

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The relative percentage of multi-year ice in the WAP (< 10%) may also have limited algal abundance and bacterial growth in WAP sea ice. Variability in the age of sea ice directly impacts the ice carbon pool by influencing the amount of time for *in situ* algal and bacterial production and degradation and the incorporation of algal cells via physical mechanisms (Ackley & Sullivan 1994, Fritsen *et al*. 1994, Arrigo *et al*. 1997, Melnikov 1998, Fritsen & Sullivan 1999). For instance, Arrigo *et al*. (in press) document higher Chl *a* concentrations in thick multi-year or older first year ice at the interior of the western Ross Sea ice pack relative to concentrations in younger and thinner ice to the north and south. Similar variability is evidenced in this study. In the eastern Ross Sea, Chl *a* in multi-year ice to the south of 70°S was significantly higher than Chl *a* in younger first year ice to the north ($P < 0.05$, Tamhane test, Fig. 2a). In addition, Chl *a* in winter multi-year ice of the Weddell Sea was significantly higher than Chl *a* in winter first year ice of the WAP ($P < 0.05$, Tamhane test). Chl *a* in Weddell Sea multi-year ice might have been sufficient to support the weak, but still significant, bacteria-algae association observed during early winter 1992 (Table III). Conversely, low Chl *a* resulting from low multi-year ice coverage may have contributed to the uncoupling observed in WAP sea ice during winter 2001. In years with higher concentrations of multi-year ice, the abundance of ice algae - and potentially the strength of the bacteria-algae association - in WAP sea ice may vary considerably relative to 2001.

In addition, the relative age of sea ice may influence the ability of the bacterial community to grow under adverse conditions. Bacterial assemblages in new ice can be dominated by water column bacteria that are poorly adapted for growth at sea ice temperatures (Grossmann & Gleitz 1993, Grossmann 1994). When ice ages, however, the relative proportion of psychrophilic bacteria increases as cold-adapted strains are selected (Helmke & Weyland 1995). Consequently, bacterial communities in newly formed winter ice may be less able to cope with the interactive effects of temperature and substrate limitation than communities in older first year or multi-year ice. Testing this hypothesis will require molecular phylogenetic analysis and physiological assessments of the bacterial community during and following ice formation.

Ice effects - scavenging

Differential incorporation of bacteria and algae during ice formation may also have influenced the bacteria-algae association in WAP pack ice. Several studies indicate that planktonic algae are physically concentrated by the formation of frazil ice either by serving as nucleation sites for ice crystal growth or by adhering to frazil ice crystals as they rise through the water column (Garrison *et al*. 1982, 1983, 1989, reviewed in Brierley & Thomas 2002). These mechanisms result in Chl *a* concentrations in newly formed ice up to 50 times higher than concentrations in the underlying water column (Garrison *et al*. 1983). Evidence of physical enrichment of algae is provided in this study. Chl *a* concentrations in WAP new ice (nilas, pancake ice, brash ice), consolidated ice cores, brine, and slush were significantly elevated relative to water column concentrations during autumn and winter when biomass accumulation by *in situ* production is expected to be minor ($P < 0.05$, Tamhane test). Conversely, bacterial biomass was not significantly different among water column and sea ice habitats ($P > 0.05$, Tamhane test), suggesting that ice formation selectively incorporates algal cells relative to bacteria cells. Grossman & Dieckmann (1994) note a similar lack of bacterial enrichment in newly-formed grease ice. The authors suggest that, while algal cells may be above a minimum size threshold, many bacterioplankton are too small to be directly scavenged by ice crystals. In addition, they suggest that bacterial enrichment via attachment to larger algal cells (Grossman & Gleitz 1993, Grossman 1994) may be limited at times when phytoplankton concentrations are low (e.g. during winter; Grossman & Dieckmann 1994). Such selective harvesting may therefore result in a physically induced uncoupling of bacteria and algae in newly formed sea ice. In such instances a coupling between bacterial and algal biomass may only develop after a sufficient lag period during which time bacterial production varies with algal biomass and bacteria accumulate *in situ*.

Conclusions

Our study and the results of prior studies suggest that general conclusions about the importance of the microbial loop in Southern Ocean carbon cycling can be drawn when taking into account the general seasonal controls on the bacteria-algae relationship (Bird & Karl 1999, Pedrós-Alió *et al*. 2002). The strength of the bacteria-algae relationship and the extent to which primary production is cycled through the microbial loop in first year winter sea ice may be highly impacted by the synergistic effects of substrate and temperature limitation. Substrate limitation of bacterial growth is particularly likely in years when ice formation occurs relatively late in the season or in years when the ice cover is dominated primarily by newly formed first year ice. In contrast, substrate concentrations may become non-limiting during spring and summer when light availability and warmer ice temperatures stimulate algal production, hydrolytic DOM breakdown and utilization by bacteria, and the subsequent development of a functional microbial loop.

All evidence suggests that a functioning microbial coupling occurs during the seasonal development of sea ice communities. Such coupling occurs despite low temperatures but may be weaker than that observed in temperate systems. Variability of the biota in time and space, imparted by this relatively weak coupling, may make

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the detection of statistically significant associations difficult and only possible during instances when production is high and ice habitats are stable for extended periods of time (> 2 weeks). In such instances an uncoupling of pelagic bacteria and algae may occur while coupling of sea ice biota is the normal state of affairs.

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