# Bacteria-algae associations in the sea ice and upper water column of the Ross Sea in the late austral summer

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The ecological role of heterotrophic bacteria in the microbial food web of the Southern Ocean is unresolved. A coupling between phytoplankton and bacterial production is well documented in mid-to low-latitude oceans (e.g., Bird and Kalff 1984; Cole, Findlay and Pace1988) and is thought to result from the heterotrophic uptake of dissolved organic carbon (DOC) released by the primary producers (i.e. the "microbial loop;" Pomeroy 1974; Bjørnsen 1988). In Antarctic waters, however, the extent to which bacteria rely on phytoplankton production, and consequently contribute to total ecosystem production, is disputed. A positive correlation between algal and bacterial biomass has been observed in regions of the Southern Ocean (e.g., Cota *et al*. 1990; Lochte *et al*. 1997). Conversely, variability in the strength of this correlation, and even an uncoupling of algae and bacteria, has also been documented (e.g. Cota *et al*. 1990; Bird and Karl 1999). Accurate characterization of the microbial loop in the Southern Ocean requires quantification of algal and bacterial biomass and activity over a seasonal time scale and in the diversity of marine habitats that surround Antarctica. This necessitates that bacteria-algae associations in the pelagic environment should not be studied apart from similar associations in the sea ice that is a prominent feature of most antarctic waters.

Between 1 and 31 January 1999, we collected 125 bacteria samples from 33 sites along three longitudinal transects (135W, 150W, 165W) made by the U.S. Antarctic Program research ship *Nathaniel B. Palmer* in the eastern Ross Sea (figure 1). We primarily sampled three distinct habitats—the consolidated sea ice, the layers of slush (snow + seawater) overlying the consolidated sea ice, and the top of the water column. Core samples were collected with a SIPRE or Kovacs core barrel (inner diameter of 7 cm), sectioned, placed in 0.2 μm filtered seawater at a ratio of 3:1 seawater to ice-volume, and melted for 8-12 hours at 0-4°C. Bulk slush samples were collected using acid-washed Nalgene jars and melted at 0-4°C for 4-6 hours; slush interstitial waters were collected with sterile syringes. Water-column samples were collected with 10L Niskin bottles deployed on a Rossette (equipped with a CTD, a Fluorometer, and a PAR sensor). Samples were fixed aboard ship with phosphate-buffered glutaraldehyde (GTA) at a final concentration of 0.5%. Sub-samples ranging in volume from 0.5-60 mls were then stained with 4',6-diamidino-2-phenylindole (DAPI), filtered through 0.2 μm black polycarbonate membrane filters, and mounted onto glass microscope slides. Slides were frozen until processing at home institutions.

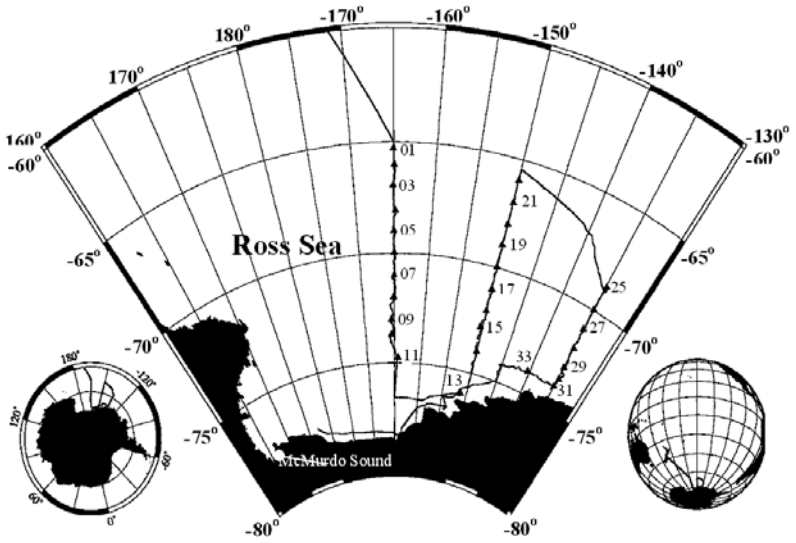


***Figure 1. Track of the icebreaking research ship* Nathaniel B. Palmer *showing the 33 sites in the eastern Ross Sea at which sea ice and water column algae and bacteria were collected in January 1999.***

Non-filamentous and filamentous bacteria were enumerated using epifluorescence direct count techniques (Kepner and Pratt 1994). Direct counts of bacteria from each sample were used to determine bacteria cell concentration (cells $m^{-3}$). Digital images were taken during counting using the imaging program Image Pro Plus and were used to size non-filamentous and filamentous cells. Total bacterial biomass (non-filamentous and filamentous bacteria; mg C $m^{-3}$) was determined using cell concentration values, cell-size estimates, and a conversion factor for bacterial carbon per volume that is based on data over a range of cell sizes (Simon and Azam 1989). Chlorophyll-*a* concentrations ($\mu g\ l^{-1}$) were determined fluorometrically. Linear regressions of log-transformed data sets were used to test for a correlation between algal biomass (expressed as chl *a* concentration) and bacterial biomass.

In the Ross Sea, the mean total bacterial biomass in the upper water column was significantly lower than that in the consolidated sea ice and slush—1.68 mg C $m^{-3}$ in the water column versus 19.17 and 24.13 mg C $m^{-3}$ in the sea ice and slush, respectively ($p<0.01$, Student's t-test). Bacteria cell concentration and total bacterial biomass corresponded positively and significantly with algal biomass in the sea ice and slush ($p<0.05$ for least squares regressions; table). The inverse was true at the surface of the water column below the ice where algal biomass was negatively correlated with both bacteria cell concentration and total bacterial biomass ($p<0.02$ and 0.13, respectively, for least squares regressions; table). However, analysis of the combined data for the water column, sea ice, and slush revealed a significant positive relationship between algal and bacterial biomass across the range of sample types ($P<0.00001$ for least squares regression; table; figure 2).

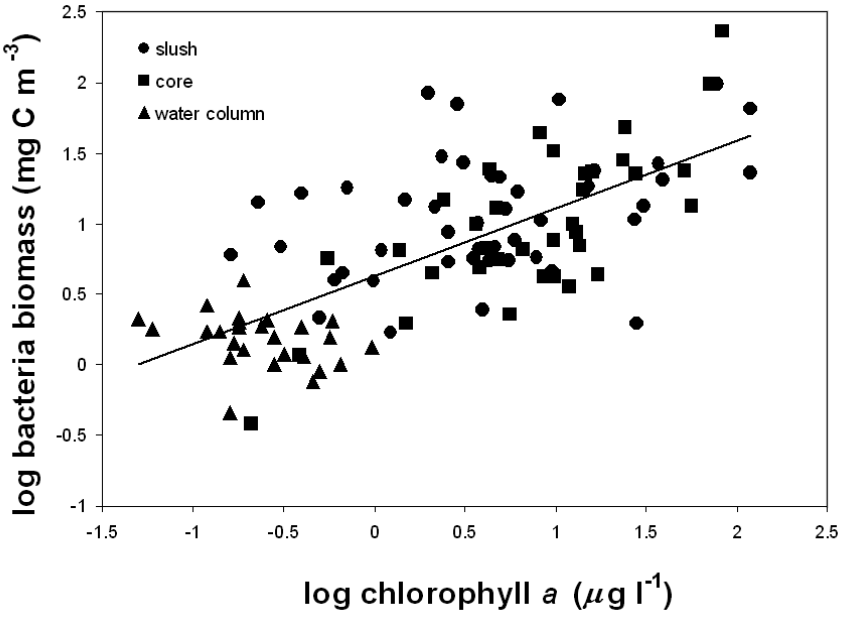


***Figure 2. Results of a least squares linear regression of log-transformed total bacterial biomass and chlorophyll a concentration [chl a] amongst habitats sampled in the Ross Sea. The linear regression equation is log [biomass] = 0.4805 (log[chl a]) + 0.6298, $r^2$ = 0.5051***

***Regression statistics for $log_{10}$ transformed data for bacterial biomass (BB, mg C $m^{-3}$) vs. chlorophyll a (CHLA, $\mu g\ l^{-1}$) and bacterial cell concentration (BC, cells $m^{-3}$) vs. CHLA for slush, ice core, water column, and combined (slush, core, and water column) data sets. Symmetrical 95% confidence limits (CL) are given for geometric mean (GM) and least squares (LS) slopes. P-values (p), y-intercepts (intercept), and $R^2$ values ($r^2$) are for LS regressions.***

| Habitat | y, x | N | GM slope(CL) | LS slope CL) | p | intercept | $r^2$ |
|---|---|---|---|---|---|---|---|
| **slush** | BB, CHLA | 45 | 0.6251 (0.18) | 0.24 (0.18) | 0.009 | 0.91 | 0.147 |
| | BC, CHLA | 45 | 0.5855 (0.17) | 0.19 (0.17) | 0.030 | 11.47 | 0.104 |
| **ice core** | BB, CHLA | 35 | 0.8948 (0.21) | 0.66 (0.21) | 0.00001 | 0.41 | 0.554 |
| | BC, CHLA | 35 | 0.9149 (0.20) | 0.72 (0.20) | 0.00001 | 11.00 | 0.620 |
| **water column** | BB, CHLA | 26 | -0.6122 (0.25) | -0.19 (0.25) | 0.127 | 0.06 | 0.094 |
| | BC, CHLA | 26 | -0.6311 (0.24) | -0.29 (0.24) | 0.016 | 10.97 | 0.218 |
| **combined** | BB, CHLA | 106 | 0.6761 (0.09) | 0.48 (0.04) | 0.00001 | 0.63 | 0.505 |
| | BC, CHLA | 106 | 0.5554 (0.09) | 0.33 (0.04) | 0.00001 | 11.36 | 0.357 |

The lack of correlations between algae and bacteria in the water column of the eastern Ross Sea suggests an uncoupling similar to that observed by Bird and Karl (1999) during springtime phytoplankton blooms in the Gerlache Straight. The authors attribute suppression of bacterial biomass and breakdown of the microbial loop to top-down control by unusually large

numbers of protistan bacterivores and to a lack of DOC exudation by phytoplankton. Similar mechanisms may exist in the upper water column of the eastern Ross Sea in late austral summer to early fall.

Conversely, the positive relationship between algae and bacteria in the sea ice suggests that the microbial loop is functioning in the Ross Sea ecosystem. The algae-bacteria association in the sea ice is considerably stronger (1.9-6.6 fold) than that observed by Cota *et al*. (1990) for bacterioplankton in the Weddell Sea. The association is more similar to that shown by Bird and Kalff (1984) for sub-polar fresh and marine waters where bacteria and algae are thought to be strongly coupled. The tight linkage between algae and bacteria in the eastern Ross Sea sea ice and the geographic and seasonal variability in the strength of this linkage in other ecosystems invite reexamination of the factors thought to drive the microbial loop in aquatic habitats.

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