

Uncoupling of bacteria and phytoplankton during the austral spring bloom in Gerlache Strait, Antarctic Peninsula

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ABSTRACT: The response of the bacterial (*Bacteria* and *Archaea*) community to vernal phytoplankton blooms was examined over a grid of stations in Gerlache Strait, Antarctic Peninsula, during the RACER II program (29 October to 26 November 1989). Total bacterial production (0.13 to 10.6 mg C m⁻³ d⁻¹), based on the incorporation of ³H-leucine into protein, increased with increasing chlorophyll *a* (chl *a*) concentration. Bacterial cell-specific growth rate also increased with increasing primary production among stations. Nevertheless, bacterial cell abundance was greatest at the sites that had the lowest chl *a* concentrations, and declined wherever phytoplankton bloomed. Early bloom communities had few nanoprotozoan grazers; grazing was undetectable by the Landry-Hassett dilution method during this period. Fully developed bloom communities (chl *a* > 10 mg m⁻³) had a profusion of nanoprotozoan grazers (median 3000 cells ml⁻¹). Despite relatively low ingestion rates per individual (0.9 bacteria cell⁻¹ h⁻¹), the abundant grazing community kept bacterial biomass very low in Gerlache Strait, to the point that the metabolism of the pelagic bacterial surface community was only a minor fraction of total ecosystem metabolism. Grazing was the apparent cause, although biomass limitation of the bacteria due to lack of resources (e.g. bioavailable dissolved organic matter) may be the ultimate cause of the uncoupling of bacterial and phytoplanktonic communities in these habitats.

KEY WORDS: Bacteria · Antarctic Peninsula · Gerlache Strait · Spring bloom · Heterotrophic nanoflagellates · Microbial food web · Grazing · Heterotrophic dinoflagellates

INTRODUCTION

In temperate and tropical oceans, a well-developed and well-documented microbial food web is responsible for a large part, if not the majority, of planktonic respiration. In these warmer waters, it has been shown that the greater the development of photosynthetic biomass, the greater is the abundance and activity of the bacterial (*Bacteria* and *Archaea*) community (Bird & Kalf 1984, Cole et al. 1988). Bacteria may form a substantial, or even dominant, fraction of total planktonic biomass (Cho & Azam 1990, Simon et al. 1992). The quantitative importance of bacteria within coastal

waters of Antarctica, however, remains uncertain. In Antarctica, large coastal diatom blooms accumulate more planktonic biomass than any other unpolluted site in the world ocean, yet the biomass response of the bacteria is very often weak (Karl et al. 1991) or even nonexistent (Rivkin et al. 1991). Bacterial biomass here may be an order of magnitude or more lower than predicted on the basis of relations at lower latitudes.

Opinions diverge on the importance of bacteria and their consumers in the Southern Ocean food web and the degree of their interaction with the photoautotrophs (see Karl 1993, Karl et al. 1996). Von Bröckel (1981) demonstrated that nanoplankton can dominate summer pelagic communities. Hewes et al. (1985) found that bacteria and protistan grazers formed a large part of a diverse summer food web in the Wed-

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dell Sea, and emphasized the importance of the microbial loop in the Antarctic. On the other hand, Kottmeier et al. (1987) had difficulty finding a link between annual sea ice microalgae and bacteria. Over the course of the spring-summer bloom, bacterial growth increased but final biomass was less than 1% of microalgal biomass. Sea ice and low temperatures also seem to prevent the development of an active bacterioplankton community in the Arctic Ocean (Pomeroy et al. 1990). Lochte et al. (1997) found strong correlations between bacterial numbers and chlorophyll *a* (chl *a*) in a transect across the Atlantic sector of the Southern Ocean. Nevertheless, they acknowledged the relatively low production of bacteria relative to phytoplankton, which they attributed to an unexplained time lag of bacterial response. Bacterial community growth is slow in the spring, although it may be much stronger by autumn (see also Karl 1993).

The objective of this study was to establish the effect of the spring bloom on bacterial abundance, biomass and growth rate in Gerlache Strait. Gerlache Strait has been identified as a 'hot spot' of production within the Southern Ocean. The waters of the Strait are isolated from the turbulence of the Bransfield Strait (Holm-Hansen & Mitchell 1991) and enriched by iron eroded from the Antarctic Peninsula shelf (Martin et al. 1990, Westerlund & Öhman 1991). These characteristics foster the development of massive blooms that draw down nutrients (Kocmur et al. 1990) and surface pCO₂ (Karl et al. 1991) and enhance reproduction and survival of krill and Antarctic fishes (Huntley & Brinton 1991, Laman & Loeb 1994). The specific null hypotheses established during the RACER I field experiment (Karl & Bird 1993) that we set out to test were that bacteria would not respond to additions of organic nutrients, and that predation on bacteria by protistan grazers was not responsible for uncoupling of bacterial and algal biomass.

METHODS

Study design. Studies were done as part of the second Research on Antarctic Coastal Ecosystem Rates (RACER II) program (Huntley et al. 1990). The cruise was planned to coincide with the pre-bloom and spring bloom period in the Gerlache Strait, where a bloom had been sampled in 1987 during RACER I (Karl 1991). A grid of 32 to 37 stations (Fig. 1) was sampled rapidly, over 3 to 4 d, 4 times over the period from 2 to 26 November 1989: these are called 'fast grids' 1 through 4. Samples at fast grid stations were taken at the surface. Between these synoptic surveys more in-depth, process-oriented studies were conducted at a centrally located station, Station Andersson (Stn A; Fig. 1),

named after Gunnar Andersson, one of the first Antarctic research scientists. These are indicated in the text as Stn A1 through A4. At this station, samples were taken from 10 depths between 0 and 200 m.

Community biomass and production. Heterotrophs: Sample filtrations were 30 ml. Bacteria were counted using 0.5 µg ml⁻¹ DAPI on 1% formaldehyde fixed samples (Coleman 1980, Porter & Feig 1980). Bacteria were sized at selected sites by digital scanning (Nikon slide scanner LS-1000) of black-and-white negatives of microphotographs (Leitz DMRB microscope). Cell volume was determined as in Massana et al. (1997) using Adobe Photoshop 3.0 and UTHSCSA ImageTool. Bacterial carbon was estimated using a conversion factor of 580 fg C µm⁻³ (Bratbak 1985), which compensated for the nucleoid-only staining of DAPI (Bird & Karl 1991, Sherr & Sherr 1993). Biochemistry was used to size bacteria by estimation of total particulate LPS concentration (pLPS) using *Limulus*-amebocyte lysate and spectrophotometry (see Watson et al. 1977, Karl & Dobbs 1998). Total bacterial community carbon was then estimated by multiplication of pLPS by 6.35 (Watson et al. 1977), and carbon per cell was calculated by division of this total by bacterial abundance. This calculation assumes that most, if not all, bacteria are Gram-negative (Karl & Dobbs 1998).

Nano- and microprotists were counted using DAPI or proflavine (Haas 1982) on 1% formaldehyde or 1% glutaraldehyde fixed samples. There was no difference in counts or ability to distinguish heterotrophs from autotrophs, neither between the 2 stains nor the 2 fixatives. The main focus was on heterotrophic nanoflagellates—larger protists were counted with precision at only a few stations.

Bacterial heterotrophic production was measured using ³H-leucine (Kirchman et al. 1985, Simon & Azam 1989, Kirchman 1993), except that only protein-fraction labelling was used in the calculation of production. Samples were prescreened through 200 µm Nitex net to remove macrozooplankton, then incubated in 25 ml portions in high-density polyethylene bottles. Labelled leucine (53 Ci mmol⁻¹, 7.6 nM final conc.) was added to replicate samples. Incubations lasted 6 h; macromolecules were separated into nucleic acid (RNA, DNA) and protein fractions (Karl 1982). The protein fraction averaged 87% (SD ± 5%) of total incorporated tritium, which did not vary among fast grids. Exponential growth rate (µ) was calculated as:

$$\mu = \log_e[(BB + BP)/BB] \quad (1)$$

where BB is bacterial community carbon biomass (g C m⁻³) and BP is heterotrophic production (g C m⁻³ d⁻¹).

If bacteria are strictly grazer-limited, the addition of organic carbon should not change the community growth rate. This was tested on 2 occasions. Plankton

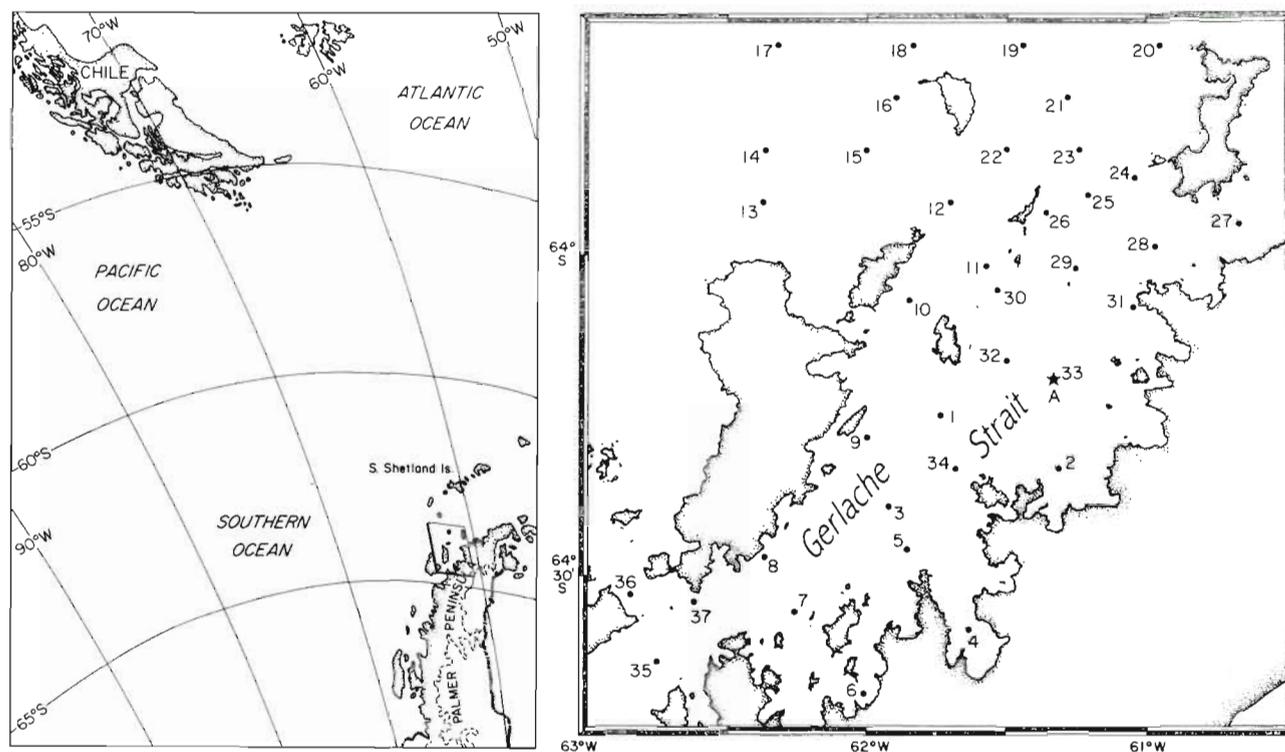


Fig. 1. Grid of stations sampled during RACER II, covering 4000 km² of northern Gerlache Strait and western Bransfield Strait. Stn A and Stn 33 are co-located

samples were filtered through 1.0 μm Nuclepore filters to remove grazers and phytoplankton, sampled for bacterial enumeration, and then incubated at surface water temperature, either unamended or following addition of 5 μM (final) concentrations of 17 amino acids plus ammonium (Sigma cat. no. AA-S-18). Incubations were terminated after 2 to 5 d and exponential growth rate was determined by microscopical enumeration. These incubations also allowed us to verify the accuracy of the leucine incorporation-to-growth rate conversion factor.

Phytoplankton: Chl *a* was measured by fluorometry (Holm-Hansen & Riemann 1978) and phytoplankton carbon biomass was estimated using a carbon-to-chl *a* ratio of 50 (Hewes et al. 1990). Primary production was estimated using the model of Platt et al. (1990), which is an analytic solution to areal production that incorporates the daily cycle of irradiance at high latitudes, as well as the photosynthetic coefficients α and P_{max} from several *in situ* production experiments (Holm-Hansen unpubl.). Percent transmission (660 nm, 25 cm transmissometer) is used in some figures to represent total plankton biomass accumulation (Amos et al. 1990).

Grazing experiments. Dilution experiments: Two dilution experiments were done. The first had 3 objectives: (1) to see if surface bacterial growth rate differed

depending on the source of water used to dilute the community (surface vs 200 m), (2) to determine bacterial removal rate (Landry & Hassett 1982), and (3) to compare the community grazing rate using this method to the fluorescently labelled bacteria (FLB) method (Sherr & Sherr 1993). Unfiltered surface water (Stn A2, November 7) was diluted with <0.2 μm membrane filtered water (Li & Dickie 1985), at final percentages of unfiltered water of 100, 66, 25 and 10%. The diluent water came from surface water in one series and from 200 m in another. Bacteria were counted at the start of the experiment and again after a 1.9 d incubation experiment. An FLB-uptake experiment (see next section) was done using the same water sample.

The second experiment was similar to the first except that only surface water was used. An additional objective was to examine the effect of bottle size on growth rate. Unfiltered surface water (Stn A3, November 15) was diluted with filtered water at final percentages of 100, 50, 25, and 12.5%, and then incubated in 50 ml polypropylene tubes or 500 ml polycarbonate bottles. Incubation time was 2.0 d. As for the first, an FLB-uptake experiment was carried out using the same sample water.

FLB experiments: Bacteria were fluorescently labelled and fed to protistan grazers in timed incubations

tions (Sherr & Sherr 1993). Colorants used were DTAF (Sherr & Sherr 1993) or lucifer yellow VS (Stewart 1981). Prey bacteria were either the cultured Antarctic *Vibrio* strain ANT-300 (starved to reduce cell size by suspending in filtered North Pacific gyre water for 27 d before staining; Moyer & Morita 1989), or else a concentrated sample of the native bacterial community produced and stained in the field. Lucifer yellow was used to stain the community in order to maintain cell viability—we did not verify stained cell viability however. Final FLB concentrations varied from 30 to 900% of the *in situ* community; most experiments used FLB concentrations that were approximately equal to *in situ* concentrations.

Sampling of incubations was done at variable points of time, from 0.2 to 13 h, and samples were fixed with 20% glutaraldehyde (2% final). Note that both 37% formaldehyde and 50% glutaraldehyde had been diluted with 0.2 μm filtered seawater to the 20% working strength. Ingested cells were counted by microscopy of 2 to 6 ml proflavine or DAPI-stained samples. Time course incubations of feeding, and loss rates following dilution with native bacteria, were used to determine digestion time (Sherr et al. 1988). DAPI-stained preparations permitted us to also count ingested natural bacteria in heterotrophic cells at the same time as FLB. The ratio of natural bacteria to FLB inside flagellates after equilibrium was reached (one digestion time) was then used to detect selectivity of predators towards labelled prey.

RESULTS

Stability and temperature

Mixed-layer depth, determined as the surface layer of uniform density (change in $\sigma\theta < 0.01$), was shallowest on the western side of Gerlache Strait (Stns 2 to 7

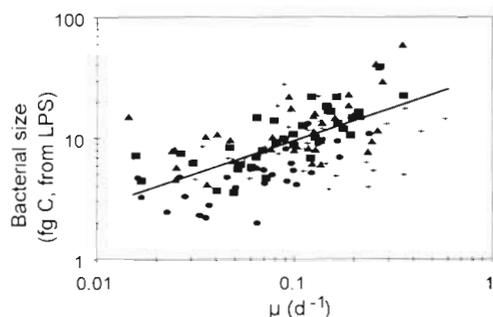


Fig. 2. Relation between bacterial cell size (fg C cell^{-1}), determined using particulate lipopolysaccharide concentration, and bacterial growth rate (d^{-1}). (●) 2 to 6 Nov, (■) 10 to 13 Nov, (▲) 16 to 19 Nov, (♦) 22 to 25 Nov

and 31) and in a gyre centered on Stn A (Niiler et al. 1991). Average mixed-layer depth was 24, 12, 18 and 15 m over the 4 fast grids. Mean surface temperature increased from -0.49 , to 0.09 , to 0.31 and to 0.80°C during the same period.

Community biomass

Dense surface phytoplankton blooms developed where the depth of the mixed layer did not exceed 12 m, repeating the pattern of coastal spring blooms described by Mitchell & Holm-Hansen (1991). Surface chl *a* concentration ranged from 0.5 to $29 \text{ mg chl a m}^{-3}$. Stations tended to fall into one or the other of 2 distinctive communities, either cryptophyte or diatom-dominated (Ferrario 1990, see also Mura et al. 1995).

Striking increases in the apparent size of the bacteria, from the deep samples ($2.9 \text{ fg C cell}^{-1}$, 200 m, Stn A3) to surface samples ($11 \text{ fg C cell}^{-1}$, 10 m, Stn A3), and over time and space, indicated that the bacteria of surface waters were responding positively to the net accumulation of planktonic community biomass during the spring bloom (Fig. 2).

Bacterial abundance among stations ranged almost an order of magnitude, from 0.9 to $7.5 \times 10^5 \text{ cells ml}^{-1}$. However, contrary to expectation, community abundance declined wherever phytoplankton were abundant (Spearman's $r_s = -0.28$, $n = 123$, $p = 0.0007$; Fig. 3). This negative correlation is the inverse of relations observed in other marine, non-polar ecosystems (Simon et al. 1992). Bacterial biomass was negligible at the richest bloom sites. Where chl *a* exceeded 5 mg m^{-3} (48 stations), bacteria did not form more than 2% of the total biomass (sum of phytoplankton and bacteria); the bacterial fraction was sometimes as low as 0.3%.

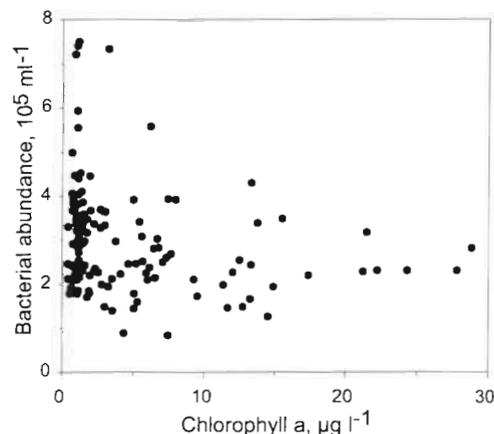


Fig. 3. Relationship between bacterial abundance (10^5 ml^{-1}) and surface chl *a* ($\mu\text{g l}^{-1}$) in Gerlache Strait during the spring bloom period was negative

Community production

The grazer-free incubations demonstrated, first, that bacteria are capable of reasonably rapid growth in cold Antarctic water, showing increases of 0.14 and 0.33 d⁻¹ in unamended controls (Fig. 4). Second, these growth rates were roughly doubled by the addition of amino acids, to 0.33 and 0.64 d⁻¹, respectively. The directly observed growth rates corresponded with leucine uptake estimates only if biomass per cell was assumed to be low, corresponding to 5 to 12 fg C cell⁻¹.

Despite fewer bacteria at more eutrophic sites, there was a strong positive relationship between bacterial community production and both surface temperature (Spearman's $r_s = 0.61$, $p < 0.0001$) and chl *a* concentration ($r_s = 0.55$, $p < 0.0001$). The strongest community growth occurred when water temperature exceeded 0.2°C. Higher temperatures occurred in stable water masses where phytoplankton could bloom, so that separation of these 2 influences was difficult. Multiple regression showed that phytoplankton biomass, bacterial community abundance, predator abundance and water temperature were all important in predicting bacterial production:

$$\log BP = -7.3 + 0.26T + 0.42\log CHLA + 0.89\log BA - 0.20\log HNF \quad R^2 = 0.51$$

where BP is bacterial production (mg C m⁻³ d⁻¹), *T* is temperature (°C), CHLA is chl *a* concentration (mg m⁻³), BA is bacterial abundance (cells ml⁻¹) and HNF is heterotrophic nanoflagellate abundance (cells ml⁻¹). The simple correlation of bacterial production with chl *a* was stronger when production was expressed as a cell-specific rate ($r_s = 0.67$; Fig. 5).

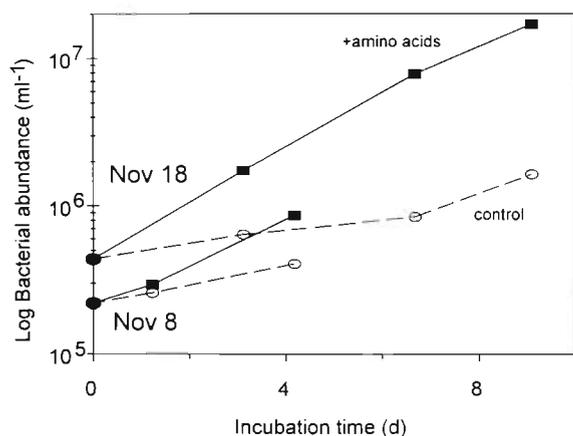


Fig. 4. Growth of bacteria in grazer-free incubations, at Stns A2 (lower starting point) and A3 (upper starting point). Dashed lines and open circles represent unamended controls, solid lines and squares represent batch cultures supplemented with a mixture of 17 amino acids, at 5 μ M each final concentration

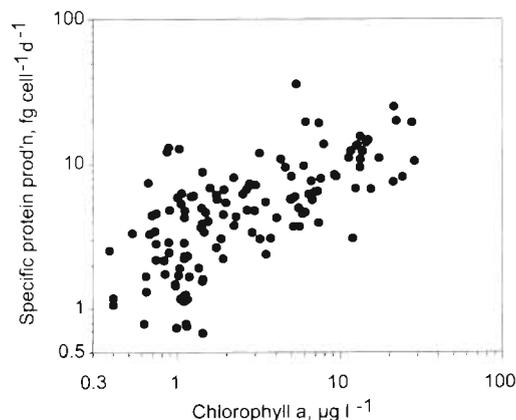


Fig. 5. Cell-specific bacterial production rate (fg protein cell⁻¹ d⁻¹), which is an expression for growth rate, was moderately positively correlated to chl *a* concentration (mg m⁻³)

Although there was a positive correlation of bacterial and phytoplanktonic growth rates, bacterial production was a small fraction of primary production (mean 2.9%, median 2.2%) at most sites (Fig. 6). This is 10-fold less than the mean found by Cole et al. (1988).

Grazer biomass

There was a strong and immediate response of the heterotrophic nanoflagellate (HNF) community to spring bloom conditions. Mean HNF abundance increased from 530 to 1150 to 2460 to 2610 cells ml⁻¹ in the 4 fast grids, respectively, which represents a sus-

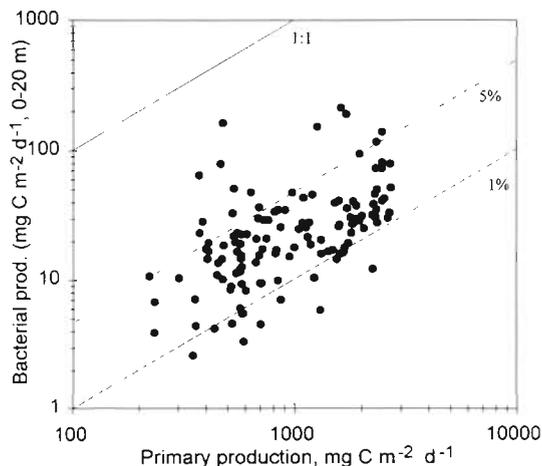


Fig. 6. Bacterial community production (mg C m⁻² d⁻¹), integrated over the top 20 m of the water column, was weakly correlated to areal primary production (mg C m⁻² d⁻¹) (PP). Lines are shown to indicate a 1:1, a 5% and a 1% BP:PP relationship. Most values of BP fell between 1 and 5% of PP

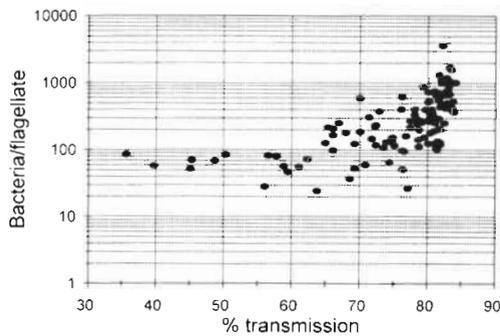


Fig. 7 Scatter plot of the logarithm of the ratio of bacterial abundance to heterotrophic nanoflagellate abundance versus transmission (%; 25 cm). Low transmission indicating bloom conditions was associated with very low bacteria/HNF ratios

tained net growth rate of roughly 0.1 d^{-1} during the first 14 d of the study. Whereas HNF made up 10 to 30% of the abundance of the small flagellate community (i.e. not including cryptophytes, prasinophytes or larger dinoflagellates) at the start of November, they were 20 to 50% 1 wk later, and by the third week of the month they were often 60 to 90%. Once again this environment produced observations outside the limits of planktonic community composition elsewhere, exceeding the normal abundances of flagellates relative to bacterial prey (see 'Discussion'). HNF biomass equalled or exceeded bacterial biomass at 17 of the 20 stations where chl *a* was greater than 10 mg m^{-3} . Nevertheless, HNF abundance was only weakly correlated with chl *a* ($r^2 = 0.20$, logarithms). The multiple regression equation describing the response of HNF is:

$$\log \text{HNF} = -0.49 + 0.32T + 0.57 \log \text{BA} + 0.20 \log \text{CHLA} + 0.33 \log \text{SIZE} \quad R^2 = 0.53$$

where HNF is heterotrophic nanoflagellate abundance (cells ml^{-1}), *T* is temperature ($^{\circ}\text{C}$), BA is bacterial abundance (cells ml^{-1}), CHLA is chl *a* concentration (mg m^{-3}), and SIZE is bacterial cell size (fg C cell^{-1}), in order of statistical importance.

The HNF community was not homogeneous, but varied markedly over time and space. Early November communities were sometimes made up largely of tiny cells a few microns in diameters in which it was rare to see signs of ingested bacteria; others contained large cells that were ingesting both bacteria and autotrophic nanoflagellates. At most stations, choanoflagellates were minor constituents, although they became locally abundant, reaching $2015 \text{ cells ml}^{-1}$ (60% of the HNF community abundance) at Stn 6 in Wilhelmina Bay (Fig. 1), for example. The choanoflagellates were small (mean $23 \mu\text{m}^3$), roughly 50% of the volume of the other free HNF at that station. On the other hand, there was a variable number of much larger (means 80, 150 and

$180 \mu\text{m}^3$ for 3 types), more active colonial and epiphytic cells, which rapidly increased in number during the study period. Mean colony size was 70 cells in coastal sites, where there were 2 to 5 colonies ml^{-1} . We note in passing that a variable fraction of the plastidic flagellates were mixotrophic and ingested FLB during the feeding experiments. Mixotrophic flagellates were not included in HNF counts.

It follows from the increase of HNF at richer, more hydrodynamically stable sites and the bacterial decline at those sites that the ratio of bacteria to HNF showed a strong decrease with increasing total plankton community size (Fig. 7).

Larger protistan grazers (heterotrophic dinoflagellates [HDF] and ciliates) were also locally abundant. Bloom initiation assemblages had up to 30 HDF ml^{-1} . By the fourth week of November, coastal bloom stations 2 and 34 had up to 250 HDF ml^{-1} , most of them small (10 to $15 \mu\text{m}$ diameter). These cells were largely herbivorous, the small ones often containing *Cryptomonas*. At Stn A on 7 November, there were 35 HDF ml^{-1} ; 40% of these contained a centric diatom ($150 \text{ pg C cell}^{-1}$ —probably *Thalassiosira*) even though there were only 121 solitary cells of this diatom species present per ml. At the end of November, there were still 5 to 30 HDF ml^{-1} in the top 75 m at Stn A. *Gyrodinium* was particularly conspicuous by its size (mean $244\,000 \mu\text{m}^3$, SD $212\,000$) and abundance (up to 3 ml^{-1}). Ciliates were much rarer than HDF, and none seemed to be specialized exclusively for bacterial prey. Nonetheless, at bloom stations they were in much greater abundance than is generally reported in Antarctic samples, attaining concentrations greater than 50 ml^{-1} . It is probable that these large protists were responsible for the majority of phytoplankton grazing (Vernet & Karl 1990). Because their cell-specific ingestion rates of bacteria were roughly equal to the rate of the colonial nanoflagellates, their contribution to grazing was not considered further.

Grazing studies

Early in the study period, grazing was undetectably low by the dilution technique (Fig. 8a). There was no difference in growth rate due to the source of the dilution water, i.e. between surface and deep water dilutions, suggesting that surface water was not a better growth medium than deep water. Mean growth rate (μ) across dilutions was high, 0.5 to 0.8 d^{-1} . The grazing rate measured by direct microscopy at the same station where there were 330 HNF ml^{-1} , was -0.03 d^{-1} , a value that was indistinguishable from zero by dilution experiment, whose limit of detection was 0.18 d^{-1} in this case.

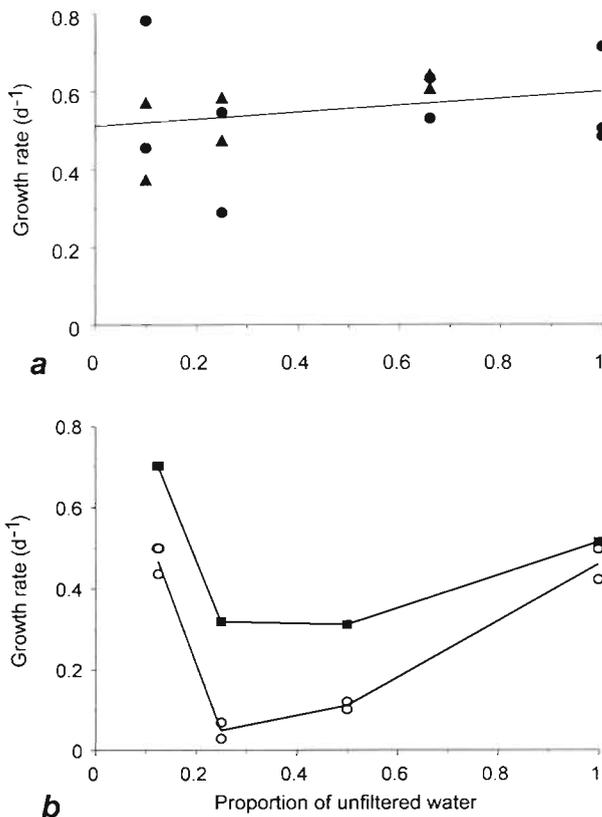


Fig. 8. Dilution experiments for the measurement of bacterial grazing rate, bacterial growth rate, and the effect of dilution with deep water on surface bacterial growth. (a) Experiment done at Stn A2, 7 to 9 Nov 1989. At this time, grazing rate was negligible relative to growth rate. (●) Deep water; (▲) surface water diluent. (b) Experiment done at Stn A3, 15 to 17 Nov 1989. Nonlinear effect of dilution can be explained as an effect of food-saturation among the nanoprotoists. (■) 500 ml incubations; (○) 50 ml incubations

The results of the second dilution experiment were strikingly different from the first. FLB-uptake measurements indicated that the grazing rate by nanoprotoists was now -0.15 d^{-1} . The response of the bacterial community to dilution was now markedly nonlinear (Fig. 8b). Growth rate was similar to the earlier results in undiluted water, then dropped strongly by about 50% in the 2 intermediate dilutions, and then reestablished itself at the original level or increased to a higher level in the the most strongly diluted water. The differences were highly significant by ANOVA (H_0 : no bottle size effect, $p = 0.001$; H_0 : no dilution effect, $p < 0.001$; H_0 : no interaction between dilution and bottle size, $p = 0.10$).

The 10 different FLB grazing experiments produced an unexpected result. There was a highly significant, negative linear relationship between the concentration of FLB added and the apparent clearance rate. When

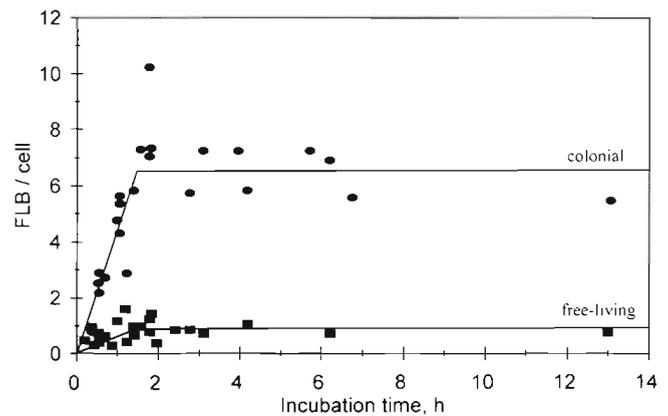


Fig. 9. Time course of FLB consumption by free-living and colonial heterotrophic nanoflagellates. Individual experiments were combined when it was discovered that prey abundance had little effect on ingestion rate

the number of bacteria ingested per HNF cell was plotted against time of incubation, there was little difference among experiments (Fig. 9). This means that flagellate grazers were saturated with prey despite the low abundance of bacteria, and that ingestion rate, not clearance rate, is an appropriate expression of grazing potential. Therefore the results were combined to derive a single measure of ingestion for free and for colonial cells: **0.4 and 4.6 bacteria h⁻¹, respectively**. Digestion time was estimated to be 1.75 h (SE 0.22 h, $n = 4$) in different experiments (Fig. 10); free and colonial cells did not differ. Selectivity for or against FLB varied from no preference to a 2-to-1 preference for FLB. The former would indicate that total grazing rates were greater than observed, which would be consistent with the average number of ingested bacteria in nanoflagellates from DAPI-stained preparations. For

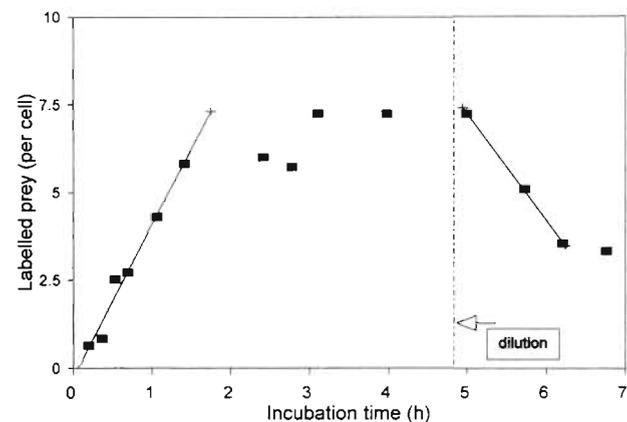


Fig. 10. Example of a digestion rate experiment. Colonial flagellates from Stn A3 on 16 Nov 1989 required 1.75 (uptake) to 2.4 h (loss following dilution) to digest FLB

example, the mean apparent bacterial content of flagellates at the surface of Stn A at the midpoint of the study period was 1.4, 2.2 and 4.4 bacteria cell⁻¹ for small and large free cells and choanoflagellates, respectively, suggesting grazing rates (adjusted for digestion time) of 0.8, 1.3 and 2.6 bacteria h⁻¹. Because preferences were not consistent, however, and to err on the side of underestimation if errors were unavoidable, we did not adjust the ingestion rate estimates.

Community grazing rate, calculated by multiplying flagellate community abundance by the grazing rate per cell, was -0.05 , -0.14 , -0.25 and -0.19 d⁻¹ on average during the 4 fast grids. When this community rate is compared to the exponential growth rate, growth exceeded predation losses at most stations during early November (fast grid 1, 2 to 6 November), but grazing was similar (fast grids 2 and 4, 10 to 13 and 22 to 25 November) or greater than growth later in the month (fast grid 3, 16 to 19 November; Fig. 11). The median ratios of growth to grazing rate were 1.65 ($p = 0.002$), 1.28 ($p = 0.11$), 0.60 ($p = 0.005$) and 0.99 ($p = 0.95$) for the 4 fast grids, where p is the probability of equality between growth and removal (paired t -test). Even where growth rate exceeded grazing losses, those losses were sufficient to significantly delay a bacterial response to any environmental stimulation.

DISCUSSION

These results introduce a new perspective on the role of the microbial loop in the cold ocean. The Pomeroy hypothesis had cast doubt on the efficacy of microbial use of phytoplankton production in cold temperate waters (Pomeroy & Deibel 1986). Although the physiological basis of the 'Pomeroy hypothesis' is disputed (Li & Dickie 1987, Kottmeier & Sullivan 1988, Robinson & Williams 1993, Rivkin et al. 1996), the evidence remains that blooms in water near 0°C often elicit little response from microheterotrophs, whereas an annual bacterial biomass peak occurs in summer when chl *a* is in decline. In this study, the bacterial community was also only marginally involved in ecosystem metabolism, at least during this period, but this was due to predatory suppression by a great abundance of nanoflagellate grazers. The result was that in Gerlache Strait during the spring bloom period, a negative relation developed between bacterial and algal biomass that strongly diverged from empirical relationships established in marine environments elsewhere.

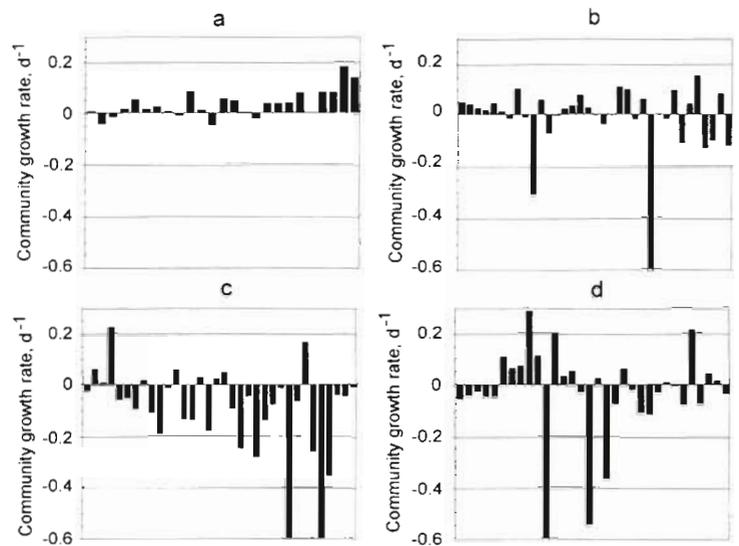


Fig. 11. Bacterial community net growth rate in the springtime Gerlache Strait, calculated as the difference between exponential growth rate based on leucine uptake, and daily grazing loss rate by nanoflagellate predators. Stations are ordered, in each panel, from left to right, by increasing chl *a* concentration. (a) 2 to 6 Nov, (b) 10 to 13 Nov, (c) 16 to 19 Nov, and (d) 22 to 25 Nov. Grazing rate estimates were similar to or exceeded growth rates in later November, especially sampling grid 3

This unequivocal demonstration of an uncoupling of biomass inventories of phytoplankton and bacteria, of the weak connection between their growth rates, and of a positive relationship between the protistan grazers and chl *a* concentrations (see also Garrison & Buck 1989, Kivi & Kuosa 1994), should lead to a closer examination of links between, and perhaps special qualities of, Antarctic planktonic organisms. The first reports of a dynamic, substantial microbial food web in Antarctica were part of the enthusiastic period of discovery of the existence and importance of nano- and picoplanktonic communities worldwide. They were usually based on observations made in austral summer (von Bröckel 1981, Hewes et al. 1983, 1990) or autumn (Cota et al. 1990) or the special conditions of the ice-edge (Garrison et al. 1984), and may be most applicable in those same circumstances.

As pointed out by Pomeroy et al. (1990), polar seas are as diverse as temperate marine systems, or more so. In other words, the importance of geographical and seasonal differences among sampling campaigns are greater than may be evident in the general conclusions about the Southern Ocean drawn in any particular study. In any selected local ecosystem, in order to establish that the microbial loop is equally as important as it is at lower latitudes, it should be shown that bacterial community production is not just correlated with primary production, but is a substantial fraction of it (mean 30%: Cole et al. 1988; note however that spring

time, cold water observations were also outliers in that paper); that the biomass response of the bacteria to the presence of active phytoplankton fits models developed elsewhere (Bird & Kalff 1984, Simon et al. 1992); and that the quantitative response of microbial consumers (both community biomass and consumption rate) is parallel to relations at temperate latitudes (Gasol & Vaqué 1993, Sanders et al. 1992). We have shown here that springtime relationships within the Gerlache Strait differ significantly on all these counts. The 'microbial loop' in this case is more accurately described as an 'Antarctic microbial droop.'

It is no longer informative to affirm that microbial food webs are present in oxygenated aquatic habitats; current focus has turned toward the recognition of factors which control or contribute to seasonal and geographic variability in coupling (e.g. Cho et al. 1994). Carlson et al. (1996) showed that bacterial production, and the bacterial biomass peak, was out of synchronisation with the seasonal cycle of primary production at the Bermuda time-series site. They attributed the time-shifts in growth rate and cell size to delayed processing of polymers (Billen 1990), and to increased grazing during the bloom, respectively. Short (1 to 2 wk) delays in response of the bacteria were also seen during the North Atlantic Bloom Experiment (Kirchman et al. 1994) and the spring bloom in the Menai Strait (North Wales) (Blight et al. 1995).

Grazing is the proximate, observable cause in this study of the lack of response of bacterial biomass to bloom conditions. In this regard, these results are highly unusual since the consensus from other work is that bacterial biomass is limited by resources (Ducklow & Carlson 1992). This indicates that some feature of the Antarctic marine environment permits the grazers to dominate. Some alternatives to consider are, first, that the grazers are in some way favored, either by unusually high growth efficiency, or by lack of top-down control themselves. Second, that the bacteria are in fact resource limited, to a greater extent than in warmer waters. We will consider each of these in turn.

An interesting possibility is that a cold-water existence favors flagellate grazers. We do not have a measure of growth efficiency but 2 aspects of flagellate grazing potential should be considered. The first of these is that the average growth rate (about 0.1 d^{-1}) achieved by the HNF across the sampling area between fast grids exceeded that of any other food web component we studied in the RACER program including phytoplankton and bacterial production, and exceeded the rate of increase in total biomass measured as particulate ATP. In fact, it was greater than the theoretical maximum growth rate calculated on the basis of estimated bacterial consumption (free-living: 0.015 to 0.025 d^{-1} , colonial 0.08 d^{-1}), suggesting that

either the grazing rate was underestimated here or that 'aplastidic' flagellate counts included some autotrophs. Note that our community grazing rates are independent of our ability to distinguish auto- and heterotrophs. The specific grazing rates we measured are in the same range as other recent estimates. Becquevort (1997) measured maximum grazing potentials, in the Atlantic sector of the Southern Ocean, of $1.1 \text{ bacteria h}^{-1}$ for HNF $< 5 \mu\text{m}$ and $7.3 \text{ bacteria h}^{-1}$ for HNF $> 5 \mu\text{m}$ in diameter, but these rates were not achieved until more than $4 \times 10^6 \text{ bacteria ml}^{-1}$ were available. Heterotrophic nanoflagellates in maritime Antarctic lakes Heywood and Sombre, on Signy Island, consumed 0.83 and $0.51 \text{ bacteria h}^{-1}$ respectively (Laybourn-Parry et al. 1996). In the more oligotrophic Crooked Lake, hourly grazing was $0.2 \text{ bacteria HNF}^{-1} \text{ h}^{-1}$ (Laybourn-Parry et al. 1995). Cell-specific grazing rates estimated for the Arctic Ocean (Sherr et al. 1998) ranged from 0.16 to $1 \text{ bacteria h}^{-1}$. Our own community rate, including the colonial flagellates, was $0.9 \text{ bacteria HNF}^{-1} \text{ h}^{-1}$. The predictive equation of Peters (1994; his Eq. 5) does very well here, predicting 0.8 to $1.9 \text{ bacteria h}^{-1}$ for free flagellates (depending on predator and prey abundances) and 4 to $6 \text{ bacteria h}^{-1}$ for colonial cells, although the equation does not explicitly accommodate the situation seen here where grazers are at a saturation limit.

Another measure that may be related to growth efficiency is the digestion time. There is a strong positive exponential relationship between prey digestion rate and temperature (Sherr et al. 1988). The Antarctic flagellates show evidence of adaptation to the cold by digesting prey more rapidly than predicted (Fig. 12). It would be surprising, however, if this were reflected in greater gross growth efficiency; this efficiency has been shown to be independent of temperature for a temperate flagellate (Caron et al. 1986). Because the rate is relatively high, slowed digestion cannot be used to explain the observed HNF feeding satiability.

Although this satiability is difficult to explain from any perspective, it has consequences for nanoflagellate ecology and for the understanding of our experimental results. First, the nanoprotist grazers of Gerlache Strait are not food-limited despite the relative sparseness of bacterial prey. If this conclusion is correct, it is contrary to results from warmer waters, where indications are that grazers clear a constant amount of water per unit time almost independently of prey concentration, suggesting food limitation (Bird & Kalff 1993). Second, predator saturation is the only way we see to explain the nonlinearity of the second dilution experiment. The decline at intermediate dilutions would be observed if predators continued to consume the same or similar absolute numbers of bacteria (i.e. by increasing clearance rate), before finally reduc-

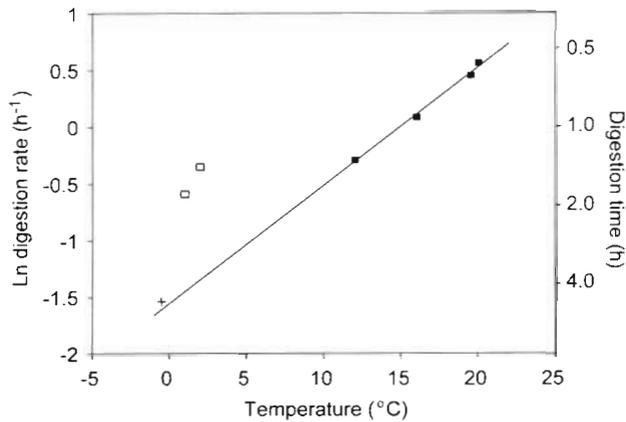


Fig. 12. Antarctic heterotrophic nanoflagellates digested bacterial prey at faster rates than expected based on predictions from temperate flagellates. (■) Values from Georgia shelf (Sherr & Sherr 1993), (+) from Halifax Harbour, Nova Scotia, June 1990 (unpubl.), (□) measured in Gerlache Strait during RACER II. Line of best fit: Digestion rate (h^{-1}) = $0.18 e^{0.106 \times \text{Temperature}}$, where temperature is measured in $^{\circ}\text{C}$

ing their consumption at the greatest dilution. Dilution experiments depend on the assumptions that predators are food-limited, and that there are no upper or lower thresholds where clearance rate changes (Landry & Hassett 1982, Gifford 1988, Gallegos 1989). The hypothetical equation to describe the present situation of exponential prey growth but saturable ingestion rate is:

$$B_t = B_0 \exp(\mu t) - I F t \quad (2)$$

where B_t = final bacterial concentration, B_0 = initial concentration, μ is the bacterial exponential growth rate (units of t), t = time of incubation, I is the flagellate ingestion rate (cells per unit t), and F the number of flagellate grazers. I is hypothesized to be constant down to low levels of B , where at some point it must eventually decline. The results of the dilution experiment described in Fig. 10b may then be modelled approximately, based on Eq. (2), using the observed $B_0 = 3 \times 10^5$ cells ml^{-1} (whole water), $I = 0.9$ cells h^{-1} and $F = 2100$ HNF ml^{-1} , to yield, first, $\mu = 0.6$ to 0.7 d^{-1} . The apparent 'exponential' grazing loss rate at the different levels of dilution would then be 0.15 d^{-1} (100%), 0.30 to 0.50 (50%), 0.30 to 0.50 (25%) and 0 to 0.1 (12.5%). The latter occurred at a bacterial concentration of about 0.4×10^5 bacteria ml^{-1} , which is roughly half of the lowest concentration seen during the grazing maximum that occurred during fast grid 3. The true underlying grazing rates would be $I = 0.9$ cell h^{-1} (100%), $I = 0.9$ (50%), $I = 0.5$ to 0.9 (25%) and $I < 0.1$ or is 0 , presumably due to cell death (12.5%).

The second aspect of flagellate grazing that should be considered is the possibility that during our study the HNF somehow escaped top-down control. In most

oligotrophic marine waters, the prey:predator ratio of bacteria to HNF is between 400 and 1000; the greater ratios occur where the HNF are themselves under strong predation pressure (Sanders et al. 1992, Gasol & Vaqué 1993; Fig. 13). This ratio increases in more eutrophic systems. The prey:predator ratio at the richest sites in Gerlache Strait was significantly lower than observed elsewhere (median 85, $p < 0.001$, Wilcoxon rank-sum test; Fig. 9). In fact, the HNF abundance exceeded the 'Maximum Attainable Abundance' based on bacterial resources, suggesting that bacterial communities at the richest sites must be declining (Gasol 1994). Low ratios do not appear to be unusual for the Southern Ocean, however—an even more extreme response of flagellate grazers relative to bacteria was seen in the springtime Ross Sea, where the median bacteria-to-HNF ratio was 22 (Putt et al. 1991). Similarly, the spring-summer ratio in Prydz Bay was 142 bacteria per HNF (Leakey et al. 1996). The unusually low bacterium-to-flagellate ratio might arise in several circumstances. First, flagellate abundance depends on the total biomass of susceptible prey that may include organisms other than bacteria. In particular, by the evidence of microscopy, there is a fraction of the nanoprotoists that consumes other flagellates in addition to prokaryotes. Second, perhaps the grazing pressure on nanoprotoists that suppresses their abundance in other environments (Gasol & Vaqué 1993) is relieved in Antarctica during this period. Tranvik & Hansson (1997) found that the trophic cascade from copepods to flagellates to bacteria in sub-Antarctic lakes was dependent on the species of copepod present as well as their abundances. When the larger *Pseudoboeckella poppei* was present, even if abundant, it had no impact on any of the microbial web components.

Given the possibility of taxonomic differences and unique adaptations in the perpetually cold waters of Antarctica, the discovery of results that fall outside the limits set in past work in temperate habitats suggests the necessity of a careful examination of the applicability to local conditions, of methods developed in warmer waters, before the conclusions can be accepted. As mentioned above, we cannot be certain that the grazing rates we (and others) have measured are not underestimates because the realized HNF growth rates exceeded the theoretical maximum calculated from an ingestion-assimilation carbon budget. Fortunately this uncertainty does not compromise the conclusion that grazing was critical to the lack of bacterial biomass development. Another possibility recently raised (Rivkin et al. 1996) is that the standard factor used to convert radioisotope incorporation measurements into estimates of bacterial biomass production increases radically in cold water. If such were the case here, it would mean that grazing was indeed

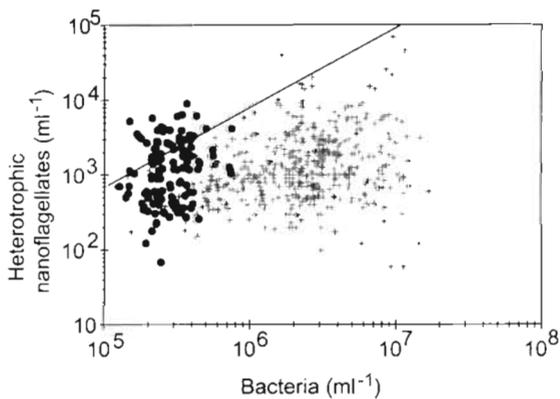


Fig. 13. Relation between log heterotrophic nanoflagellate abundance (ml^{-1}) and log bacterial prey (ml^{-1}). HNF fall both lower and higher than observed values from the literature. The line describes the 'Maximum Attainable Biomass' based on resources, from the model of Gasol (1994). (+) From non-polar marine environments (Gasol & Vaqué 1993), (●) from RACER II (this study)

greater, because no biomass response was seen in the bacteria whatever their true growth rate might be.

Despite the appearance of community limitation via grazing, it is quite possible that Antarctic bacteria are ultimately resource-limited. First, the fascinating prospect that bacterial biomass in the Gerlache Strait is controlled by iron availability was raised recently (Pakulski et al. 1996). If iron limitation had been important during RACER II, however, it would be very difficult to explain the extreme biomass levels achieved by the diatom blooms. A second possibility is that dissolved organic carbon (DOC) is unusually limiting to bacteria because very little is being transferred from the primary producers. Karl et al. (1996) reported that the DOC concentration in coastal regions of the Antarctic Peninsula, even at the height of blooms when chl *a* is $> 20 \text{ mg m}^{-3}$, is lower than concentrations typical of most surface oceans including the oligotrophic North Pacific gyre. Bacterial biomass accumulation in Polar Front and Antarctic Circumpolar Current water is strongly dependent on the concentration of DOC, and the springtime DOC concentrations are poorer there than in other oceans as well (Kähler et al. 1997). The recent results of Carlson et al. (1998) provide definitive confirmation: despite 4- to 5-fold greater carbon fixation during the Ross Sea spring bloom, almost 10-fold less DOC accumulated there than was found in the Sargasso Sea. Careful elimination of alternatives led to the conclusion that bacteria were limited by the low supply of organic substrate. This poverty of DOC within the riches of Antarctic production is yet to be explained. Recent results from the Arctic Ocean provide an interesting suggestion that this uncoupling may not be unique to Antarctica. A mid-summer trans-

arctic cruise found elevated concentrations of DOC and high bacterial production:primary production ratios at slope and basin stations (Rich et al. 1997) although BP:PP ratios were low on the Chukchi shelf where DOC was also very low (Wheeler et al. 1998).

Jumars et al. (1989) stressed the necessity of crustacean grazing for the generation of DOC to fuel the microbial loop. Zooplankton-mediated DOC production has been shown to increase bacterial biomass and production rate during the decline of the spring diatom bloom in a Danish lake (Hygum et al. 1997). Highest DOC, lowest phytoplankton biomass and highest BP:PP ratios were associated with maximum mesozooplankton biomass in the ice-covered Arctic Ocean (Gosselin et al. 1998, Rich et al. 1997). Interestingly, the microbial food web was inactive during the spring bloom in the North Sea when copepod grazers were absent (Nielsen & Richardson 1989). It is possible that stochastic variations in crustacean grazing impact, which may be common in the Southern Ocean (Heywood et al. 1985, Brinton 1991), are at least partly behind the variable importance of microbial activity seen among studies. The utilisation of phytoplankton by crustacean zooplankton is negligible during Antarctic blooms (Biggs 1982) but may be substantial in low biomass, open waters of the Antarctic Peninsula (Schnack et al. 1985). This pattern was also revealed during RACER II: the closed bays of the southeastern Strait where chl *a* was greatest had the lowest larval abundances of the dominant euphausiid *Thysanoessa macrura* (Nordhausen 1994); Antarctic krill *Euphausia superba* were also most abundant in the low-biomass northern and eastern stations (Huntley et al. 1990); the copepods were probably the most important crustacean grazers, yet they too removed insignificant fractions of daily primary production—many populations of copepods stayed below the mixed layer (Lopez & Huntley 1995). A reduced level of macrozooplanktonic grazing was indicated in the budget of chl *a* and phaeopigments (Vernet & Karl 1990). Lack of crustacean grazing impact is also consistent with the superabundance of HNF. If lack of DOC production due to limited macrozooplanktonic grazing during the bloom is responsible for the weak microbial response, it would be an ironical demonstration of the necessity of the classical food chain to the microbial loop, after 2 decades of work aimed at showing the necessity of the microbial activities to growth of organisms at higher trophic levels.

The controls on bacterial activity clearly vary according to the spatial and temporal scales of observation. On the local (weekly, 8 to 50 km) scale seen during this study of coastal blooms, phytoplankton, bacteria and heterotrophic nanoflagellates were out of equilibrium. We can see the dynamics of top-down control of the

flagellates on bacteria, but the only response of the bacteria to the bloom is in terms of increased growth rate and cell size, and not yet of biomass. These responses were different from those acting on the seasonal and mesoscale (monthly, 20 to 250 km) found during RACER I (Bird & Karl 1991, Karl et al. 1991). At that scale, a slow shift to heterotrophy from autotrophy was more evident, and we could see evidence of long delays in biomass response to both production and temperature increases which would be invisible at the scale of this study. Of the hypotheses put forward in RACER I (Bird & Karl 1991) one in particular has been supported here: that bacterial grazers might increase disproportionately where phytoplankton bloom near the Antarctic Peninsula, helping to disrupt the normal correlation seen between trophic levels. On the ocean basin to global scale, the local uncoupling of primary and secondary production shown here is consistent with an important role for the coastal Southern Ocean in maintaining a positive carbon balance, in being a source of fixed carbon for pelagic and deep-sea metabolism.

The recent discovery of the quantitative importance of *Archaea* within the coastal Antarctic bacterioplankton may be important to understand some of our results. DeLong et al. (1994) showed that 21 to 34 % of prokaryotic rRNA in late winter near Arthur Harbor was made up of novel types of *Archaea*. This was a greater fraction than had been found in other planktonic marine environments. More recently, Murray et al. (1998) and Massana et al. (1998) have shown that *Archaea* are most abundant in deeper water and in late winter, and that they seem to be outcompeted during the early spring bloom. There was a negative relation of their abundance with chl *a* concentration over the year. For this reason we have not considered in detail the extraordinary positive relationship of LPS-based cell size with chl *a* concentration found here, because these estimates would be biased to an unknown extent by changes in taxonomy. In particular, a decrease in the fraction of *Archaea* would appear as an increase in cell size. This effect cannot explain all of the observed change in size, however, for 2 reasons. First, the image analysis results corroborated the direction of cell-size change, and second, the apparent size increase was up to 5-fold along the chl *a* gradient, whereas *Archaea* would have varied only between 33 % and 0 % of the abundance, which is insufficient to account for this increase.

To sum up, it has been demonstrated that the uncoupling of the microbial loop in Gerlache Strait during the spring bloom period was the direct result of protistan grazing. Factors contributing to the top-down dominance of the bacteria by HNF may have included lack of DOC release from the primary producers (directly or

indirectly), unusual efficiency of bacterial grazers, and lack of top-down control on the grazers in their turn. The point was made that this uncoupling need not hold in other Antarctic environments, or in later seasons, as has been shown in other studies. Hydrological studies have shown the Gerlache Strait to be a potentially important source of fixed carbon for a large area of the Bransfield Strait (Niiler et al. 1991); springtime delays in POC metabolism in the Gerlache and other coastal hot spots may thus contribute to uncoupling, and consequently to a lack of quantitative relationships between the microbial and crustacean communities throughout the summer in a wide area of the Antarctic Peninsula.

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