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Experimental test of bacteria–phytoplankton coupling in the Southern Ocean

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Abstract

A set of eight large (20 m^3) mesocosms were moored in Johnson's Dock (62°39.576′S, 60°22.408′W, Livingston Island, Antarctica) to experimentally generate a gradient of phytoplankton biomass and production in order to test the extent of coupling between bacteria (heterotrophic *Bacteria* and *Archaea*) and phytoplankton, as well as the role of bacterial losses to protist grazers. This was achieved by imposing four light levels (100%, 50%, 25%, and 10%) in the presence or absence of nutrient additions (0.1 mol NH_4Cl, 0.1 mol F_6Na_2Si, and 0.01 mol KH_2PO_4 per day per mesocosm). The experimental treatments resulted in a broad range of chlorophyll *a* (Chl *a*) (0.31–93.5 μg Chl *a* L^{-1}) and average primary production rates, while bacteria responded in a much narrower range of biomass (3–447 μg C L^{-1}) and production (0.21–15.71 μg C L^{-1} d^{-1}). Results confirm that bacteria–chlorophyll and bacterial production–primary production relationships in the Southern Ocean differ from the typical relationships applicable to aquatic ecosystems elsewhere. Bacteria respond to phytoplankton blooms, but they respond so weakly that bacterial production represents a small percentage of primary production (1–10%). Although other mechanisms might also contribute to the weak bacterial response to phytoplankton blooms, we demonstrate that the reason for it is likely the tight control of bacterial populations by their predators. Protist grazers are able to sustain faster growth rates in the cold waters of the Southern Ocean than are bacteria, thereby preventing bacteria from responding to phytoplankton blooms more forcibly.

The close dependence of pelagic marine bacteria (heterotrophic *Bacteria* and *Archaea*) on phytoplankton has often been demonstrated through the existence of robust, general relationships between the abundance and production of bacteria and phytoplankton (Bird and Kalff 1984; Cole et al. 1988). The confirmation, in subsequent studies, of these relationships has led to the consideration of the link between bacterial and phytoplankton abundance as an undisputed tenet of aquatic microbial ecology (cf. Gasol and Duarte 2000). Yet the universality of these relationships has been questioned by the finding of weak relationships between the abundance and production of bacteria and phytoplankton in

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the Southern Ocean, suggesting a weak coupling between bacteria and phytoplankton therein (e.g., Karl et al. 1991; Fiala and Delille 1992; Bird and Karl 1999). Failure to demonstrate a relationship between bacteria and phytoplankton in the Southern Ocean may derive from the confounding effects of the multiple sources of error (e.g., sampling and measurement error; time lags and the associated spatial shift in the responses; predator control), typically resulting in order-of-magnitude uncertainty associated with comparative correlative analyses (Gasol and Duarte 2000). If bacteria in the Southern Ocean are indeed less reactive to phytoplankton development than they are elsewhere, as suggested in the past for cold waters (Gasol and Duarte 2000; Pomeroy and Wiebe 2001), then such order-of-magnitude uncertainty may mask bacteria–phytoplankton relationships. It is therefore unlikely that further correlative analyses will help elucidate the nature of bacteria–phytoplankton relationships in the Southern Ocean.

The power of comparative analysis is indeed limited because the independent variables (e.g., chlorophyll *a* [Chl *a*] concentration or primary production [PP]) are not under the control of the experimentalist, permitting confounding factors to affect the underlying relationships (Gasol and Duarte 2000). Mesoscale iron-addition experiments have also shown a much weaker response of bacteria relative to phytoplankton (Hall and Safi 2001; Arrieta et al. 2004; Oliver et al. 2004). However, bacteria–phytoplankton relationships derived from such individual experiments (e.g., Oliver et al. 2004) are confounded because the data are not independent, as they represent a time series. We thus opted to test this relationship by experimentally controlling phytoplankton development. We did so on the basis of the experimental treatment of the resources for phytoplankton growth (light and nutrients) in large (20 m^3) mesocosms. The experimental treatments aimed at generating a gradient of phytoplankton abundance and PP. The treatments applied, therefore, both stimulated (nutrient additions) and suppressed (shading) phytoplankton. The broad gradient of phytoplankton biomass and production generated allowed us to resolve the existence of even a weak coupling between phytoplankton and bacteria in the Southern Ocean.

Methods

Experimental site, design, and sampling—The ESEPAC experiment was conducted in Johnson's Dock (62°39.576′S, 60°22.408′W, Livingston Island, Antarctica), a sheltered bay receiving glacial melt water (Agustí and Duarte 2000). A floating platform holding eight mesocosms, consisting of large (14 m tall, 2.3 m in diameter) ultraviolet-stabilized polyethylene bags, was moored in the deepest sector of the bay (about 25 m) on 21 January 2000, with the top meter of the mesocosms extending above the water level so as to avoid accidental intrusions of water by breaking waves. The mesocosms were filled with ambient, unscreened water (from a depth range of 0.5–3 m) on 23 January, which resulted in an average volume of 20 m^3 in the mesocosms. The natural communities enclosed included grazers (mainly copepods, salps, and amphipods) at concentrations comparable to those in the ambient waters. The outer surface and top of duplicate mesocosms were covered with neutral screens nominally transmitting 50%, 25%, and 10% of the natural irradiance by divers, and two additional mesocosms received the full ambient irradiance. The actual shading imposed, which deviated somewhat from the nominal values due to shading, possible folds in the screening material and the position of each mesocosm in the platform, was verified through casts inside the mesocosms of a Seabird CTD (conductivity–temperature–depth instrument) fitted with a PAR (photosynthetically active radiation) sensor. Four mesocosms were amended with Si, P, and N in the form of ammonia, while the other four were not. On the basis of results obtained in a pilot experiment (Agustí and Duarte 2000), nutrient-amended mesocosms received a daily addition of 0.1 mol N (as NH_4Cl), 0.1 mol Si (as F_6Na_2Si), and 0.01 mol P (as KH_2PO_4) per mesocosm, using previously described procedures (Agustí and Duarte 2000). Nutrient additions were discontinued when ammonium concentrations approached 10 μmol L^{-1}, to avoid potentially toxic levels, and were resumed when concentrations declined below this threshold. The experiment was conducted for 25 d, which was sufficient to allow the response of the planktonic community to the experimental conditions (Agustí and Duarte 2000). Additional details on the experimental procedures and the responses of the phytoplankton community are reported in Agawin et al. (2002).

Integrated (0–13 m) water samples were collected from each mesocosm, as well as from the unenclosed ambient waters, at 2-d intervals (09:00 h local time) prior to nutrient additions. These samples were used to determine bacteria and phytoplankton abundance. Bacteria and phytoplankton abundance and production, as well as grazer abundance and grazing rates, were also determined from these samples at 3–6-d intervals.

Phytoplankton and PP—Chl *a* concentrations were determined fluorometrically (Parsons et al. 1984) from integrated samples withdrawn at 2-d intervals. PP, based on ^{14}C incorporation rates of whole seawater, was determined every 3–6 d, depending on the mesocosm. Twelve 125-mL polycarbonate Nalgene bottles were each filled with 120 mL of water from the mesocosm units. Additional samples were filled with water taken from outside the mesocosms (Johnson Dock). Duplicate bottles were suspended at different depths inside the mesocosm units to expose them to irradiances corresponding to 13%, 21%, 47%, 68%, and 100% (surface) of the incident light levels. Duplicate dark bottles were also suspended inside and outside the mesocosm units. One milliliter of a $^{14}CO_3H^-$ solution (corresponding to 370–740 kBq) was added to each bottle, which was then incubated in situ for 3 h. After this time, the samples were filtered through 0.45-μm Millipore filters, and the filters were fumed over concentrated HCl to remove traces of inorganic C. Radioactivity on the filters was measured with a liquid scintillation counter with correction for quenching. All materials were acid cleaned prior to use. PP estimates were converted to daily rates by multiplying by the average daylight hours during each experimental day (average = 14.9 h).

Microheterotroph abundance and activity—Samples of 1.2 mL were fixed immediately with 1% paraformaldehyde plus 0.05% glutaraldehyde (final concentration), incubated for 10 min at room temperature, and then stored frozen in liquid nitrogen. To count heterotrophic bacteria, 200-μL samples were stained with a dimethyl sulfide–diluted SYTO-13 (Molecular Probes) stock (10 : 1) to a final concentration of 2.5 μmol L^{-1}. To complete staining, samples were maintained for ~10 min in the dark and were then run in a flow cytometer. We used a Becton Dickinson FACScalibur bench cytometer as described in Gasol and del Giorgio (2000). Bacterial cell size was estimated using the relationship between average bacterial size (obtained by image analysis of DAPI (4,6-diamidino-2-phenylindole) preparations following common procedures) and average fluorescence of the SYTO-13–stained sample relative to beads (Gasol and del Giorgio 2000). Bacterial biomass was calculated by the carbon : volume relationship derived by Norland (1993).

For nanoflagellate counts, samples of 100 mL were preserved with glutaraldehyde (1% final concentration). Duplicate subsamples of 20 mL were filtered throughout 0.6-μm polycarbonate filters and stained with DAPI at a final concentration of 5 μg mL^{-1} (Sieracki et al. 1985). The abundance of these microorganisms was determined by epifluorescence (Nikon Optiphot) microscopy. Nanoflagellates showing red-orange fluorescence (when illuminated with blue light) and/or plastidic structures were considered phototrophic forms (PNFs), while colorless nanoflagellates were counted as heterotrophic forms (HNFs). Mixotrophic nanoflagellates could not be reliably identified. Nanoflagellate size was determined by measuring the lengths and widths of 50–150 cells (HNF + PNF) per sample under the epifluorescence microscope with a calibrated ocular micrometer. HNFs were grouped into four size classes: $\leq$2 μm, 2–5 μm, 5–10 μm, and 10–20 μm. Cell volumes were estimated using the nearest geometrical figure, and the carbon content was estimated using a volume : carbon ratio of 0.22 pg C μm^{-3} (Børsheim and Bratbak 1987).

Ciliates and large dinoflagellates such as *Gyrodinium* sp. were counted in 1-liter samples preserved in a 1% final concentration of acidic Lugol solution. A 100-mL subsample was placed in 100-mL sedimentation chambers for 48 h before counting under $\times$400 magnification using an inverted microscope (Zeiss). Ciliate and dinoflagellate average cell sizes were determined after measuring the lengths and widths of 30–100 cells and were used to calculate their volumes by approximations to the nearest geometric form. Carbon content of ciliates and dinoflagellates was estimated using the equations described in Menden-Deuer and Lessard (2000).

We estimated bacterial activity as the rate of radioactive leucine incorporation by bacteria, by the method described by Kirchman (1993), using a conversion factor from leucine to carbon incorporation (83 mol C mol^{-1} leucine) obtained during a previous cruise in the same area (Pedrós-Alió et al. 2002). From these estimates of production, specific growth rates (μ) were calculated as:

$$\mu = \ln(1 + \mathrm{BP}/\mathrm{BB})/t \quad (1)$$

BB is bacterioplankton biomass, and t is the time over which production is considered.

Estimates of grazing on bacteria by protists (HNFs, ciliates, and dinoflagellates) were obtained by following the disappearance of a fluorescently labeled bacteria (*Brevundimonas diminuta*—formerly *Pseudomonas diminuta*—strain provided by the Spanish Type Culture Collection, Burjassot, Valencia, Spain) over time using the procedure of Vazquez-Dominguez et al. (1999). Grazing on bacteria was determined four times in the mesocosms receiving 50% and 10% light and in those receiving 50% and 10% light plus nutrients (27 January, 2 February, 8 February, and 14 February), and grazing on bacteria was also determined four times in the mesocosms receiving 25% and 100% light and 25% and 100% light with nutrients (24 January, 30 January, 5 February, and 11 February). Bacteria-specific growth rate (k) was calculated as the sum of specific net growth rate (a) and specific grazing rate (g) (k, $d^{-1} = a + g$); and gross bacterial production (BP, μg C L^{-1} d^{-1}) was calculated as the sum of total grazing (G) and net BP (NBP). These calculations are based on the conservative assumption that all losses of bacteria during the incubations were due to grazing by protists.

The relationships between bacteria and phytoplankton abundance and biomass were described using least-squares linear regression analysis, with log-log transformation when comparing average values across mesocosms. The presence of lagged responses between bacteria and phytoplankton was tested using cross-correlation analysis. For those mesocosm units in which significant lagged responses were observed, the relationship between bacteria and phytoplankton biomass was derived after shifting one of the variables over time by the time interval yielding the highest cross-correlation value.

Results

The experimental treatments resulted, as intended, in a broad range of Chl *a* (Fig. 1) and PP (Table 1), encompassing the range of values previously reported for the Southern Ocean (Table 2). Phytoplankton abundance, as Chl *a* concentration, started to deviate greatly among mesocosms in response to the experimental treatments after only 5 d (Fig. 1, insert). The largest phytoplankton blooms were dominated by large diatoms (*Thalassiosira antarctica*), whereas pico- and nanoautotrophs dominated at low algal biomass. Further details on the response of the autotrophic community to the experimental treatments can be found in Agustí et al. (unpubl. data). In contrast, bacterial abundance followed a similar pattern across mesocosms for almost 2 weeks (Fig. 1). The average size of the bacteria in the experiment was 0.067 μm^3. Cross-correlation analysis revealed that lagged responses of bacterial biomass to increasing Chl *a* ranged from no lag response time to a maximum of 4 d across mesocosms (Table 1). Bacterial biomass increased steadily over time in all mesocosms (Fig. 1), with the average net growth rates ranging twofold, from 0.056 to 0.12 d^{-1}, among treatments (Table 1). The bacterial community in the ambient waters also showed a steady increase at a net rate similar to that of the unamended mesocosm exposed to full irradiance (net growth rate = 0.079 $\pm$ 0.021 d^{-1}, R^2 = 0.58, p = 0.006).

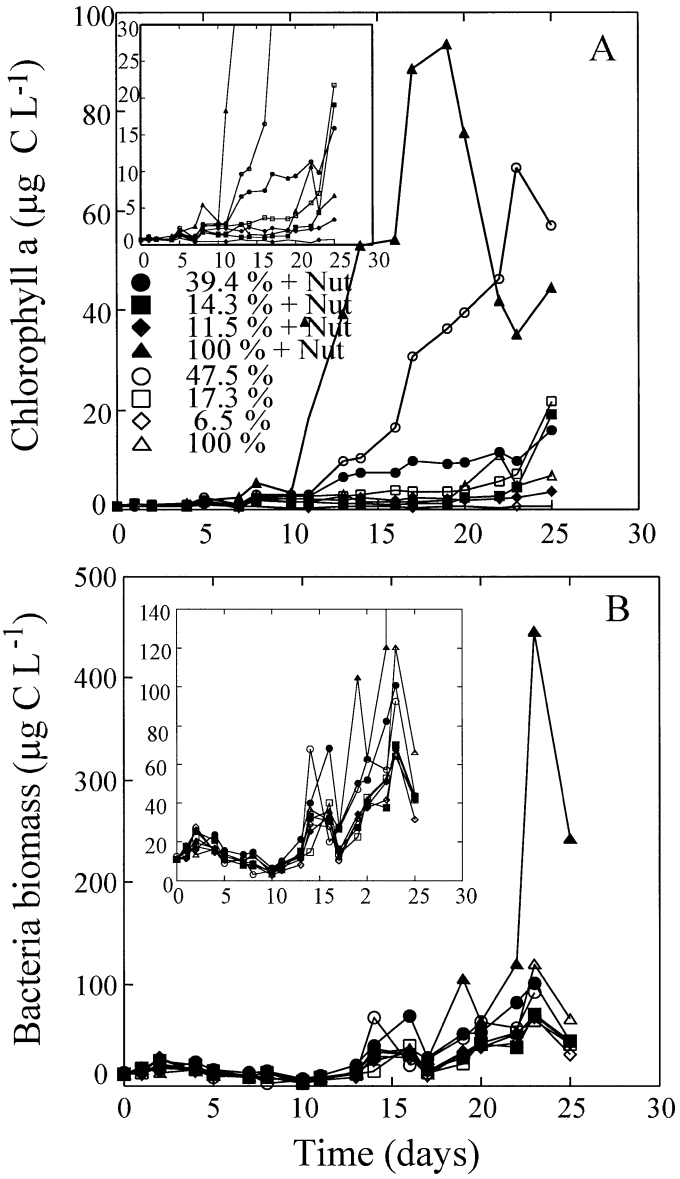

Fig. 1. Time course of Chl *a* concentration and bacterial biomass in the mesocosms subject to different shading conditions and nutrient additions (Table 1). Inserts highlight the initial time courses.

Analysis of covariance showed that growth rates increased linearly with increasing incident irradiance (*t*-test, $p = 0.025$). The growth rate increase was much faster for nutrient-enriched mesocosms than for unamended mesocosms (*t*-test, $p = 0.003$), accounting for 97% of the variance in bacterial net growth rates among mesocosms (Fig. 2).

Bacteria–phytoplankton biomass relationships, corrected for the time lags detected (Table 1), were significant for all mesocosms, with the exception of the 11.5% irradiance mesocosm receiving no nutrient additions (Fig. 3). The strength of the relationships, expressed as the percentage of the variance in bacterial biomass explained by the relationship with Chl *a* concentration (R^2), declined with declining irradiance, from asymptotic values of about 70% of the variance in bacterial biomass, explained by the relationship with Chl *a* for

Table 1. Experimental treatments and mean (±SE) bacterial biomass (BB), net growth (BG) and production (BP); chlorophyll *a* concentration (Chl *a*); and primary production (PP) in the mesocosm units during the experiment. Also shown is the lag time, as the time shift between time series to achieve the maximal cross-correlations between BB and Chl *a* (cf. "Methods").

% irradiance	Treatment	Lag (d)	BB (μg C L^{-1})	BG (d^{-1})	BP (μg C L^{-1} d^{-1})	Chl *a* (μg L^{-1})	PP (μg C L^{-1} d^{-1})
100	—	1	29±7.44	0.075±0.018	1.61±0.18	2.72±0.14	30.87±7.70
39.4	—	0	30±7.07	0.066±0.023	1.64±0.12	18.33±1.23	255.60±82.50
14.3	—	0	24±4.65	0.058±0.018	1.38±0.18	3.64±0.27	58.53±23.40
11.5	—	0	22±7.44	0.057±0.021	0.98±0.11	0.56±0.01	6.24±2.10
100	+ nutrients	3	70±28.30	0.120±0.024	3.01±0.17	31.13±1.78	432.81±141.70
47.5	+ nutrients	1	35±7.47	0.076±0.015	1.72±0.13	5.51±0.26	92.74±16.50
17.3	+ nutrients	1	24±4.43	0.057±0.018	1.51±0.11	2.39±0.12	58.71±42.20
6.5	+ nutrients	4	24±4.70	0.065±0.017	1.25±0.13	1.75±0.11	12.38±1.80

Table 2. Slopes of the relationships between bacterial biomass and chlorophyll and bacterial production–primary production in studies of the Southern Ocean compared to studies elsewhere. *N* stands for number of individual points in the regression.

	Area	Slope	R^2	*n*	Range of "X" value	Reference
A) Bacterial biomass to chlorophyll						
All oceans	All planktonic systems	0.844	0.90	40	0.05–120	Bird and Kalff (1984)
	Marine systems	0.736	0.79	19	0.05–9	Bird and Kalff (1984)
	All planktonic systems	0.524	0.75	35	0.3–180	Cole et al. (1988)
	All planktonic systems	0.660	0.79	101	0.05–33	Maranger and Bird (1995)
	Oligotrophic oceans	0.360	0.73	47	0.04–1.2	Buck et al. (1996)
	Average slope of all equations (all)	0.470				Gasol and Duarte (2000)
	Average slope of all equations (Antarctic data excluded)	0.515				Gasol and Duarte (2000)
Southern Ocean	Bransfield—cruise ECOANTAR	0.174	0.05	146	0.1–3	Vaqué et al. (2002)
	Weddell Sea—cruise DOVETAIL	0.266	0.10	19	0.1–1.4	Morán et al. (2001)
	Weddell Sea—cruise DHARMA	−0.026	0.00	144	0.1–18	Gasol and Pedrós-Alio unpubl. data
	Bransfield, Gerlache, Weddell—cruises FRUELA	0.153	0.16	110	0.04–25	Pedrós-Alió et al. (2002)
	All above	0.067	0.01	419	0.04–25	
	Antarctic Peninsula—cruises RACER	0.264	0.28	127	0.4–29	Karl and Tien (1991)
	Weddell Sea	0.150	0.10	483	0.02–16	Cota et al. (1990)
	All Southern Ocean	0.410	0.26	620	0.003–105	Stewart and Fritsen (2004)
	Water column	0.100	0.01	205	0.003–2	Stewart and Fritsen (2004)
	Sea ice	0.620	0.29	415	0.01–105	Stewart and Fritsen (2004)
	Bransfield Strait	0.160	0.18			Karl et al. (1991)
	Southern Ocean—surface water	0.570	0.45	103	0.2–3	Lochte et al. (1997)
	Southern Ocean—deep water	0.130	0.19	54	0.02–0.8	Lochte et al. (1997)
	Weddell–Scotia*	−0.231	0.02	45	5–24	Mordy et al. (1995)
	Median slope†	0.156				
	Median slope only significant)‡	0.265				
This work	Mesocosm experiments (averages)	0.230	0.57	8	0.3–93	Fig. 5
B) Bacterial production to primary production						
All oceans	All planktonic systems	0.814	0.59	54	3–300	Cole et al. 1988
	Marine systems	0.860	0.77	30	3–280	Cole et al. 1988
	All planktonic systems*	0.746	0.56	36	180–2,600	Cole et al. 1988
	Average slope of all equations (all)	0.670				Gasol and Duarte 2000
	Average slope of all equations (Antarctic data excluded)	0.776				Gasol and Duarte 2000
Southern Ocean	Weddell Sea–DOVETAIL	0.411	0.14	21	1.5–27	Morán et al. 2001
	Bransfield, Gerlache, Weddell—FRUELA	0.262	0.16	94	0.2–569	Pedrós-Alió et al. 2002
	All above	0.289	0.17	115	0.2–569	
	Weddell Sea	0.250	0.07	404	0.2–60	Cota et al. 1990
	Gerlache Strait*	0.693	0.29	122	225–2,800	Bird and Karl 1991
	Southern Ocean—deep water*	0.706	0.63	33	30–3,000	Lochte et al. 1997
	Gerlache Strait	0.486	0.50	11	0.14–6.4	Kelley et al. 1999
	Weddell–Scotia confluence*	0.439	0.36	19	57–1,400	Kuparinen and Bjørnsen 1992
	Polar front region*	0.776	0.62	5	40–1,120	Simon et al. 2004
	SOIREE—Southern Ocean	0.830	0.61	18	5–29	Hall and Safi 2001
	SOFEX—all	0.403	0.75	16	3.5–29	Oliver et al. 2004
	SOFEX—inside the patch	0.437	0.64	10	7–29	Oliver et al. 2004
	SOFEX—outside the patch	0.148	0.15	6	3.5–8.5	Oliver et al. 2004
	Median slope†	0.411				
	Median slope (only significant)‡	0.437				
This work	Mesocosm experiments (averages)	0.200	0.71	8	7–300	Fig. 5

* Aerial values.
† Median slope of the presented equations plus others in the original reference.
‡ Median slope of only the significant regressions.

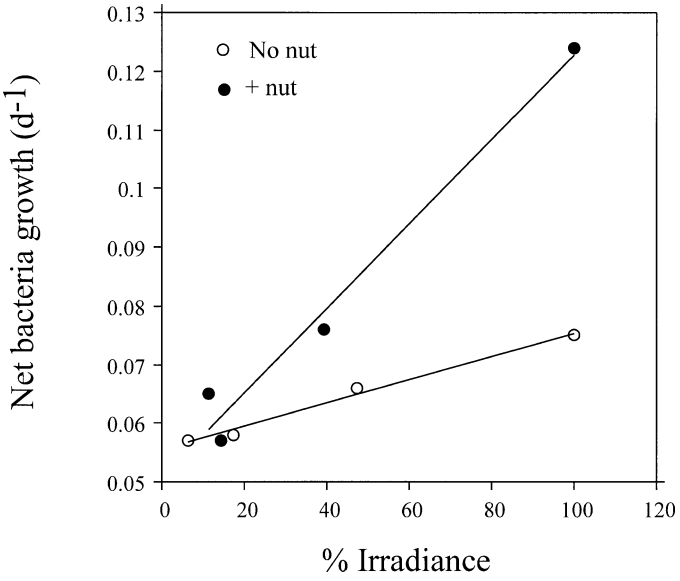

Fig. 2. The relationship between the average net growth rate of bacteria in each mesocosm across the duration of the experiment and the percentage of irradiance received. The solid lines represent the fitted linear regression equations (both $R^2 > 0.98$).

mesocosms receiving >40% of the incident irradiance, to very low values of <20% of the variance for highly shaded communities (Fig. 4).

The average bacterial biomass in each mesocosm was closely correlated with the average Chl *a* concentration ($R^2 = 0.57$, $p = 0.018$) (Fig. 5). The fitted linear regression equation showed that bacterial biomass increases as the one-fourth power of Chl *a* concentration (Fig. 5). The average heterotrophic BP was also closely correlated with the average PP ($R^2 = 0.71$, $p = 0.005$) (Fig. 5). The fitted linear regression equation showed that BP increases as the one-fifth power of PP (Fig. 5). The very low allometric exponent of the relationship between BP and PP indicates that BP must decline relative to PP as PP increases. There was a strong, inverse relationship between the average relative BP (as % PP) and the average PP across mesocosms (Fig. 6). The relative magnitude of BP declined from accounting for about 15% at lowest PP to about 0.6% of PP at high PP (Fig. 6).

The grazer community was composed of a diverse assemblage, including heterotrophic nanoflagellates, ciliates, and phagotrophic dinoflagellates (*Gyrodinium* sp.). At the onset of the experiment, the biomass of the grazer assemblage (12.86 μg C L^{-1}), dominated by small (2–5 μm) heterotrophic nanoflagellates, was comparable to that of bacteria (11.21 μg C L^{-1}). The grazer community grew steadily during the experiments in most treatments (Fig. 7). The average exponential growth rates were up to 0.11 ± 0.02 and 0.27 ± 0.08 d^{-1} during the experiment for HNFs and ciliates plus dinoflagellates, respectively, in the nutrient-amended experimental mesocosm receiving the full ambient irradiance (Table 3). The grazer community responded to the increase in Chl *a* before the heterotrophic prokaryotes did (Figs. 1, 7). The average growth rate of HNFs remained below that of bacteria across most mesocosms. The growth rate of ciliates

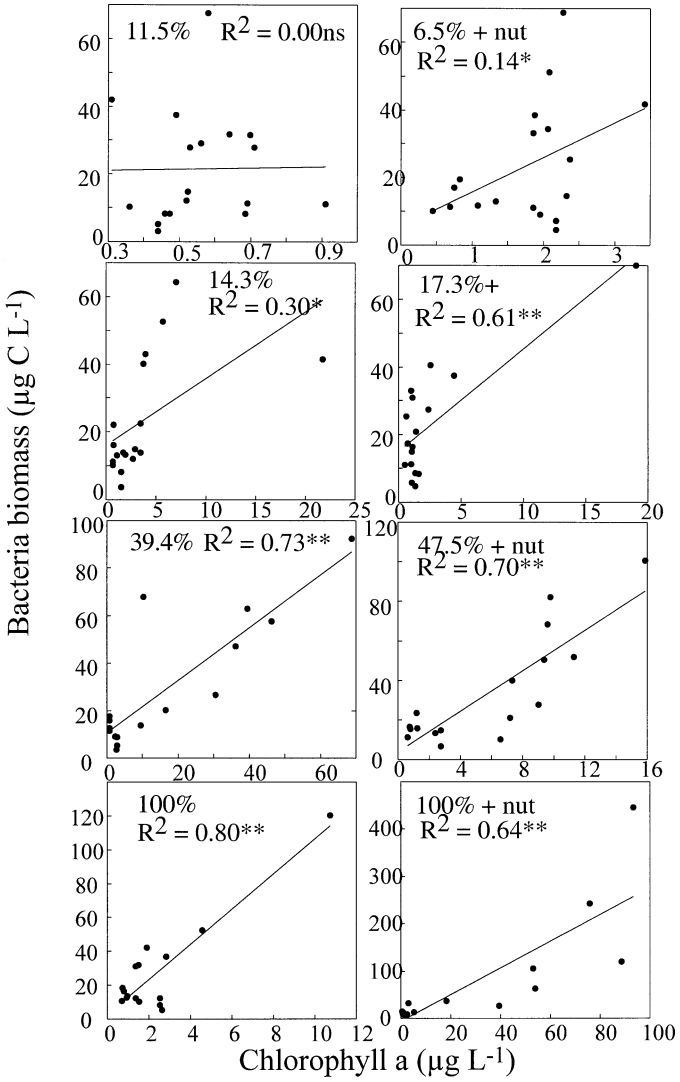

Fig. 3. The relationship between bacteria biomass and Chl *a* concentrations for mesocosms subject to different shading conditions and nutrient additions (Table 1). Solid lines represent the fitted regression equations, adjusted for the time lags observed for each mesocosm (Table 1). The coefficient of determination (R^2) and significance (ns, not significant; * $p < 0.05$; ** $p < 0.001$) of the linear regression equations are shown in inserts.

was, on average, 2.45 ± 0.75 times greater than that of bacteria and increased with increasing bacterial growth rates across mesocosms (Fig. 8). Hence, grazer growth rates exceeded bacterial growth rates (Table 3), so that the average biomass of protist grazers increased with increasing average bacterial biomass ($R^2 = 0.56$, $p = 0.02$) (Fig. 9; Table 3). Average grazer biomass exceeded that of bacteria at moderate-to-high average bacteria biomass (Fig. 9). Grazers removed daily, on average, 102% ± 18% of the BP across treatments (Table 3), independently of the treatments.

Discussion

The results presented show that bacteria are responsive to enhanced phytoplankton biomass and production but that

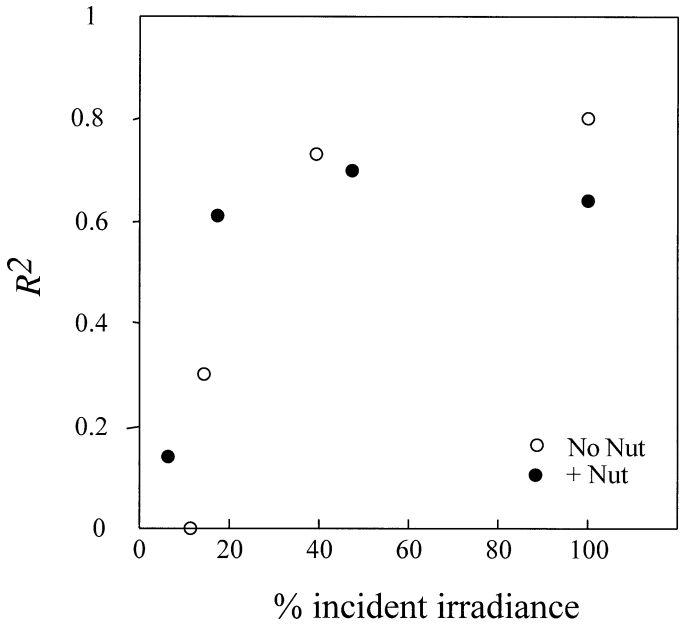

Fig. 4. The relationship between the percent incident irradiance in the mesocosms and the percentage of the variance in bacterial biomass explained by the relationship with Chl *a* concentration (from Fig. 3, R^2). Closed symbols: nutrient amended; open symbols: unamended.

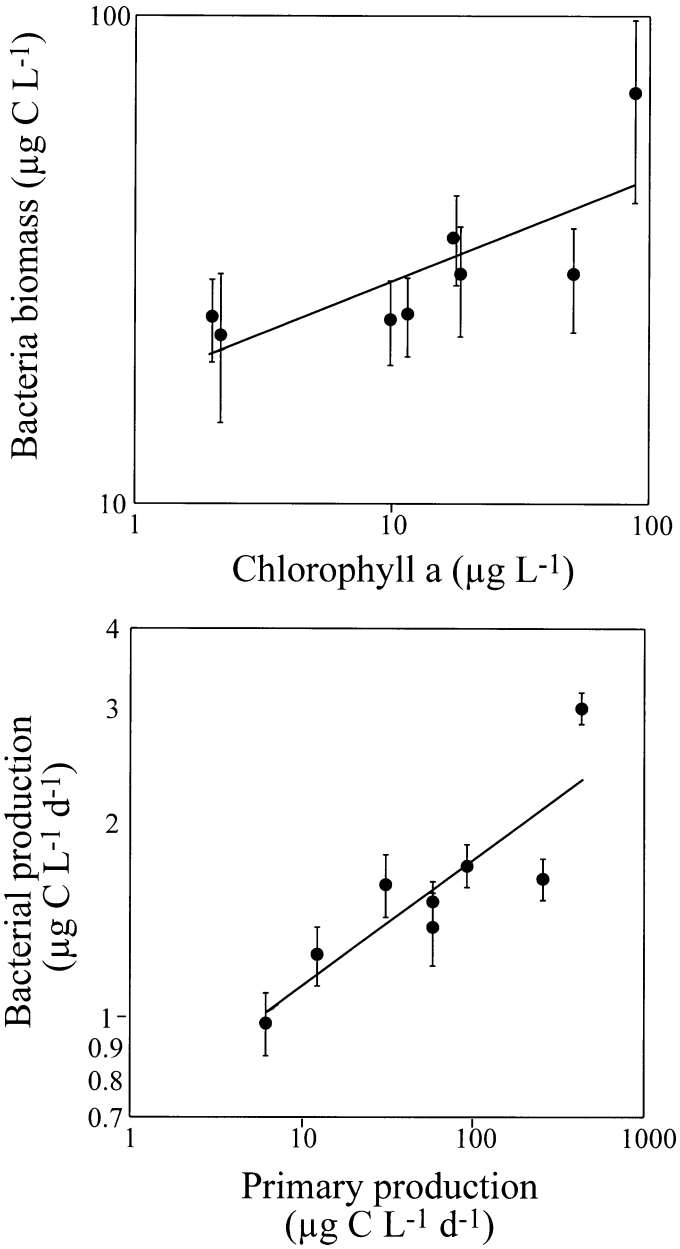

Fig. 5. The relationship between the average bacterial biomass and production and the average Chl *a* concentration and PP in the mesocosms. Solid lines showed the fitted regression lines: log BB = 1.33 + 0.23 (±0.07)log Chl *a* (μg L^{-1}) and log BP (μg C L^{-1} d^{-1}) = −0.15 + 0.20 (±0.04) log PP (μg C L^{-1} d^{-1}). Error bars represent ±1 SE.

their response is weak compared to that of phytoplankton. Bacterial biomass showed an overall increasing trend throughout the experiment at an average rate of ≥0.05 d^{-1}, regardless of the experimental treatments. This rate matched the rate of increase in the ambient, unenclosed waters. This finding is consistent with previous reports of a significant, steady net increase in bacterial biomass between spring and summer in the Southern Ocean (Ducklow et al. 2001), suggesting a strong seasonal behavior of the bacterial community. The response lag in the experiment, however, was shorter than that reported for the Ross Sea (1 month) (Ducklow et al. 2001). The reason for this may be that the phytoplankton community in the Ross Sea was dominated by *Phaeocystis.* The contrasting patterns of temporal development of bacterial biomass and Chl *a* concentration do not simply reflect the occurrence of time lags, but are also indicative of a weak response of bacteria to phytoplankton blooms.

The relationships between bacterial biomass and production and Chl *a* concentration and PP derived experimentally in this study show evidence of a coupling between phytoplankton and bacteria in the Southern Ocean in areas of >39% irradiance (Fig. 3). Although the relationships were strong, they provided evidence of a weak reactivity of bacteria to phytoplankton growth, as bacterial biomass and production increased only as the one-fourth and one-fifth powers of Chl *a* and PP, respectively. These low power scalings indicate that Chl *a* concentration and PP must increase by 22,000- and 100,000-fold, respectively, to induce a 10-fold increase in bacterial biomass and production (cf. range for variables in Table 1). The slopes describing the relationships between bacterial biomass and production and Chl *a* concentration and PP in the experimental mesocosms are indeed very low compared with those reported in the literature, with the average power scaling of bacterial biomass to Chl *a* (0.23 ± 0.07) observed being toward the low end of the range of published relationships (mean = 0.47, range = 0.2–0.8) (Gasol and Duarte 2000) and the power scaling of BP to PP (0.20 ± 0.04) being below the range of published relationships (mean = 0.67, range = 0.3–0.9) (Gasol and Duarte 2000). This suggests that whereas bacterial communities in the Southern Ocean are reactive to changes in phytoplankton abundance and PP, they are far less responsive to autotrophs

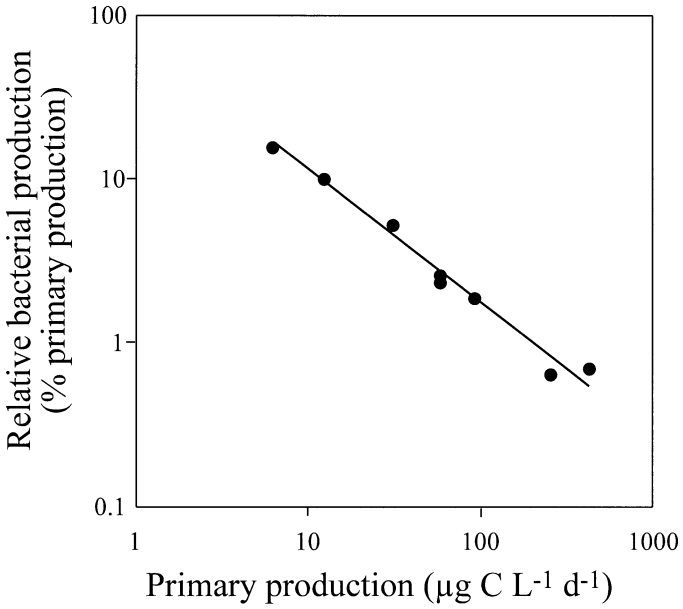

Fig. 6. The relationship between the average percentage of PP represented by BP and the average PP in the mesocosms subject to different shading conditions and nutrient additions. Solid line represents the fitted linear regression equation log % BP = 1.84 − 0.80 log PP (μg C L^{-1} d^{-1}) (R^2 = 0.98, $p < 0.0001$).

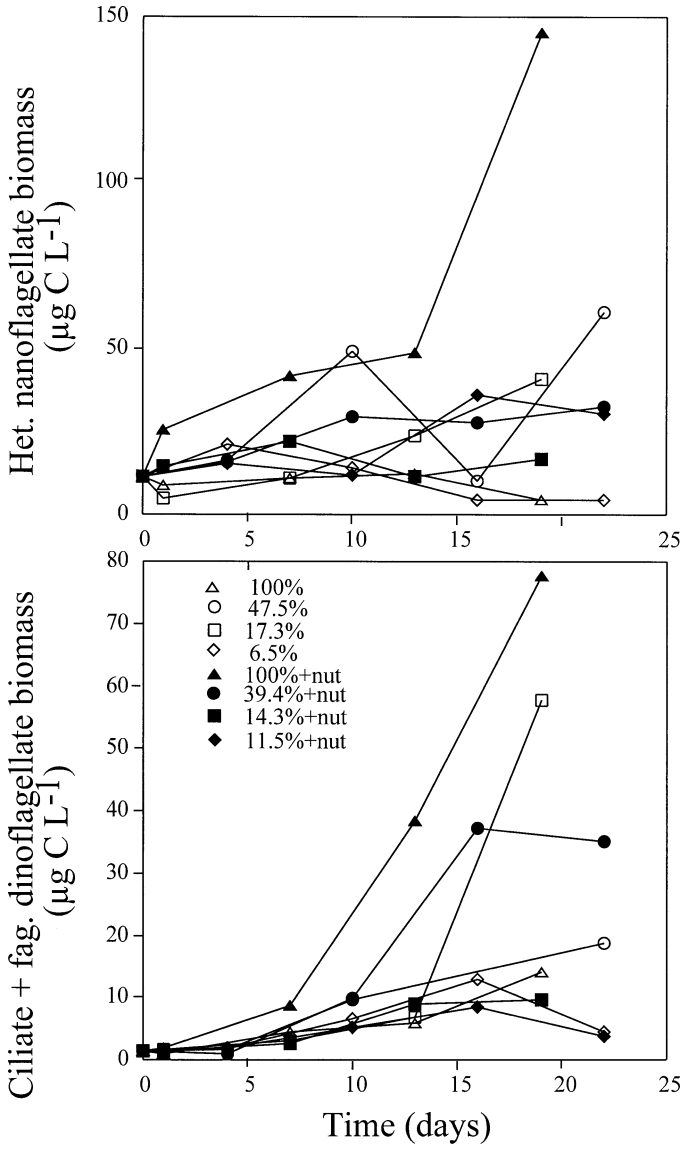

Fig. 7. Time course of the biomass of heterotrophic nanoflagellates and that of heterotrophic ciliates and phagotrophic dinoflagellates in the mesocosms subject to different shading conditions and nutrient additions (Table 1).

than are bacteria communities elsewhere (Gasol and Duarte 2000). Indeed, BP ranged, on average, from <1% to 15% of particulate PP, consistent with previous reports in the Southern Ocean (Ducklow et al. 2001; Oliver et al. 2004), with this proportion decreasing with increasing PP.

The very modest fraction of BP relative to PP explains the modest response of bacteria to phytoplankton blooms in the experiment. As a result, a relatively large fraction of the organic carbon produced by phytoplankton blooms in the Southern Ocean, such as those resulting from nutrient additions, escapes remineralization and either flows up the food web or sinks to result in high CO_2 sequestration efficiency. The finding of a modest response of bacteria to phytoplankton blooms in the Southern Ocean explains why highly productive communities are net autotrophic (Agustí et al. 2004; Agustí and Duarte 2005).

Our results appear to be typical for Southern Ocean com-

Table 3. Experimental treatments and mean (±SE) heterotrophic nanoflagellate (Het. nanno.) and heterotrophic ciliate plus phagotrophic dinflagellate (Cil.+dinof.) biomass and the absolute and relative (% BP) grazing on bacteria in the mesocosm units during the experiment, as well as the initial biomass of heterotrophic nanoflagellate and heterotrophic ciliates plus phagotrophic dinflagellates.

% irradiance	Treatment	Het. nanno. (μg C L^{-1} d^{-1})	Cil.+dinof. (μg C L^{-1} d^{-1})	Grazing rate (μg C L^{-1} d^{-1})	BP grazed (%)
Initial	(t=0)	11.46	1.37		
100	—	9.58±0.79	5.35±1.20	1.10±0.26	84.15±7.58
39.4	—	34.14±12.43	9.91±4.26	1.35±0.36	87.46±11.24
14.3	—	20.11±8.00	17.31±13.52	0.74±0.23	70.76±11.29
11.5	—	11.03±4.03	6.44±2.34	1.24±0.34	115.54±19.80
100	+ nutrients	65.29±27.13	31.50±17.33	2.37±1.73	114.74±17.91
47.5	+ nutrients	26.40±3.65	20.80±9.08	2.17±0.73	156.83±36.03
17.3	+ nutrients	16.13±2.22	5.62±2.12	0.39±0.17	29.84±4.14
6.5	+ nutrients	23.40±5.85	4.77±1.42	1.89±0.52	162.43±23.96

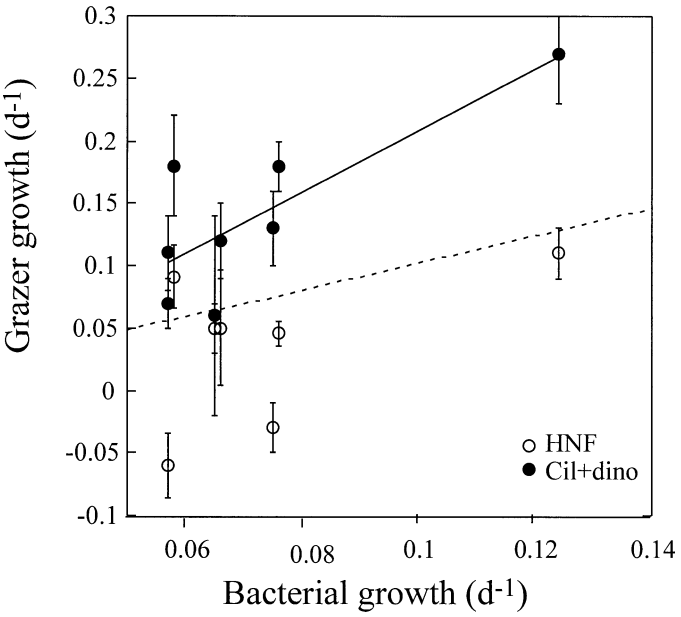

Fig. 8. The relationship between the average net exponential growth rate of heterotrophic nanoflagellates and that of heterotrophic ciliates and phagotrophic dinoflagellates and the average net exponential growth rate of bacteria. The solid line shows the fitted regression equation relating ciliate plus dinoflagellate growth and bacterial growth ($R^2 = 0.57$, $p = 0.017$). The broken line shows the 1 : 1 line. Error bars are ±1 SE.

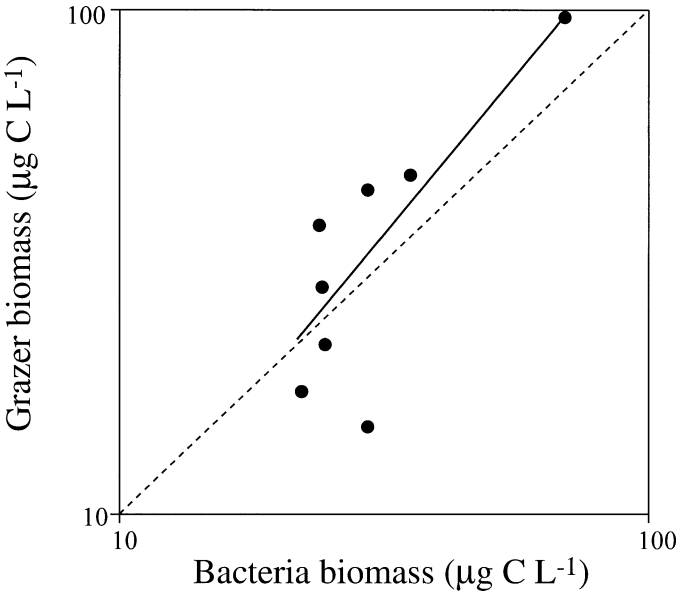

Fig. 9. The relationship between the average biomass of grazers and that of bacteria in the different mesocosms. The solid line shows the fitted regression equation log Grazer biomass = −0.40 + 1.29 (±0.41)log BB, and the broken line shows the 1 : 1 line.

munities, since a compilation of published relationships between Chl *a* and bacterial biomass show that, in most cases (Table 2), a good relationship between these two variables is not obtained and that, when the relationship is significant, the slope is smaller than the slopes for communities in other oceans. The median slope for Southern Ocean communities was 0.27, compared to 0.52 for communities elsewhere (Table 2). Similarly, the median slope for the relationship between primary production and BP was 0.44 for Southern Ocean communities compared to 0.78 for communities elsewhere.

These results are consistent with observations of bacterial development in response to phytoplankton blooms induced by experimental iron additions in the Southern Ocean (Hall and Safi 2001; Arrieta et al. 2004; Oliver et al. 2004), which show weak bacterial responses to the phytoplankton blooms induced. As the responses are weak in such large-scale experiments, the likelihood of detecting empirically a coupling between bacteria and phytoplankton in unmanipulated Southern Ocean waters is low. The very weak responses of bacterial communities to autotrophs in the Southern Ocean make it difficult to detect these responses, which are easily confounded by the noise and variability derived from lagged responses and spatial shifts (cf. Gasol and Duarte 2000). This may explain earlier failures to demonstrate a coupling from comparative analyses of bacterial and autotrophic communities across the Southern Ocean (e.g., Fiala and Delille 1992; *see also* the low R^2 of the equations compiled in Table 2).

Our results show a difference in the response of heterotrophic prokaryotes and that of heterotrophic eukaryotes to the development of the algal bloom. Together with the biomass ratios between bacteria and protists and their growth rates (Figs. 8, 9), they clearly portray bacteria communities as strongly top-down controlled. Due to the comparatively high biomass and net growth rate of the grazer assemblage, particularly that of ciliates, they were able to maintain a high grazing pressure, removing close to all BP along the range of PP. This high ciliate biomass is presumably the result of the consumption on prokaryotes, but mostly on phytoplankton and on nanoflagellates. HNFs exerted important pressure on prokaryotes before ciliates started to grow rapidly during the algal bloom (Figs. 1, 7). The capacity of some prokaryote grazers, especially ciliates, to consume a wide diversity of prey, including phytoplankton, allowed them to increase in abundance before prokaryotes did, thereby maintaining a high pressure on prokaryotes throughout. The capacity of prokaryote grazers to directly respond to phytoplankton blooms, thereby preceding prokaryote response, is likely responsible for the weak coupling between bacteria and phytoplankton biomass and production in the Southern Ocean.

Hence, grazing pressure kept bacterial communities relatively sparse, such that bacteria were able to express only a weak response to increased phytoplankton production. This finding is consistent with recent reports in the Southern Ocean that suggest that bacteria are closely controlled by predators (Bird and Karl 1999; Hall and Safi 2001; Vaqué et al. 2004). This accounts for the weak bacteria response to phytoplankton blooms. Bacteria can only escape the close predatorial control if their growth rates increase well above those of their predators. However, there is evidence of low maximum bacterial growth rates at low temperatures, such as the low ambient temperatures in the Southern Ocean (e.g., Rivkin et al. 1996). The capacity to confine bacteria to a tight top-down control is enhanced by the capacity of some

components of the grazing community to predate on phytoplankton, which allows grazer abundance to increase before bacterial abundance does, thereby increasing grazing pressure at the onset of the bacteria response.

Weak reactivity of bacteria to phytoplankton blooms in the Southern Ocean has been explained on the grounds that low temperature limits bacterial response by reducing their growth and enhancing their substrate requirements (Wiebe et al. 1993; Pomeroy and Wiebe 2001). In addition, the unsuitability of the macromolecular nature of the organic matter derived from the blooms of large Antarctic diatoms to be readily used by bacteria has been proposed to explain this weak reactivity (Fiala and Delille 1992). Low temperature alters the structure of bacterial membranes, which become gel-like and more resistant to diffusive flow of substrates (Nedwell 1999). Our data, however, suggest that bacterial response to the phytoplankton bloom is mainly attributable to grazing pressure (e.g., Bird and Karl 1999). Furthermore, if the maximum growth rates are restricted by temperature or limited by the relatively low production of usable organic matter by the phytoplankton (e.g., Ducklow 2003), the response of the heterotrophic bacteria to the algal bloom will be smaller than expected and lower than what occurs elsewhere in the ocean. Note that in areas where a low lability of the phytoplankton-derived dissolved organic carbon has been invoked as the reason for the low response of bacteria to algal blooms, bacteria predators in the Southern Ocean were present at a lower concentration than in other areas (average bacteria per HNF in the Ross sea area were 500 and, in the Gerlache area, they were 85) (Ducklow 2003). Our results also suggest that predation by protists is the most parsimonious explanation to account for the limited bacterioplankton response to algal blooms in the Southern Ocean and is, once the response lag is accounted for, mainly what limits bacterioplankton and that there is no need to invoke other factors to account for the observed results.

In conclusion, the results presented confirm the observation that bacteria–Chl *a* and BP–PP relationships in the Southern Ocean differ from the typical relationships found in fresh and marine waters (Table 2). Southern Ocean bacterial communities are able to respond to phytoplankton blooms; however, BP represents a small percentage of PP. The reason for this weak response is the tight control of bacterial populations by their predators, which are able to sustain higher growth rates than bacteria in the cold waters of the Southern Ocean, thereby precluding bacteria from showing a stronger reaction to phytoplankton blooms.

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