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Prokaryotic plankton biomass and heterotrophic production in western Antarctic waters during the 1995–1996 Austral summer

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Abstract

We examined prokaryotic biomass (PB) and prokaryotic heterotrophic production (PHP) during spring and summer in three Antarctic areas: Bellingshausen Sea, Bransfield Strait and Gerlache Strait in December 1995 and in January–February 1996. The data gathered allowed comparisons among areas, between seasons, and with the RACER study, carried out 10 years earlier in the same zone (Deep-Sea Res. 38 (1991) 1029). Prokaryotic numbers ranged from 0.67 to 13.3×10^5 cells ml^{-1}, with a mean of 3.86×10^5 cells ml^{-1}.

In order to obtain accurate estimates of PHP, the effect of temperature on leucine incorporation was studied and conversion factors (CF) were determined empirically. Several experiments were carried out to determine the influence of temperature on leucine incorporation rate. In 5 out of 7 experiments, leucine incorporation increased on increasing temperature from -2 to $4^\circ C$, but remained constant at higher temperatures (up to $11^\circ C$). In one experiment no response of leucine incorporation rate to temperature was found. CF were determined in filtration-dilution cultures. Parallel cultures from different areas were incubated with different organic matter amendments and at different temperatures. These treatments did not affect the conversion factor except for the incubation at $-1.5^\circ C$ in one experiment. The remaining cultures produced CF that were not significantly different. The average value was 0.81 kgC $(mol\ leucine)^{-1}$.

The PHP was higher in December (between 25.2 mgC $m^{-2}\,d^{-1}$ in Bransfield Strait and 47.7 mgC $m^{-2}\,d^{-1}$ in Gerlache Strait) than in January (between 8.0 mgC $m^{-2}\,d^{-1}$ in Bransfield Strait and 22.9 mgC $m^{-2}\,d^{-1}$ in Gerlache Strait) in all the areas studied. As a result, doubling times in Bellingshausen Sea and Gerlache Strait increased from 14–17 days in December to 32–75 days in January. In Bransfield Strait doubling times were always around 45 days. In Gerlache Strait this decrease in activity between December and January occurred despite a doubling of the chlorophyll concentration. Prokaryotic abundance was lower than that expected from chlorophyll concentrations and general relationships between chlorophyll and prokaryotic numbers. The prokaryotic plankton was not capable of processing all the organic matter provided by phytoplankton and was, therefore, not limited by resource supply. Lysis due to viruses, on the other hand, was shown to be an important loss factor for prokaryotes (Guixa-Boixereu et al., Deep-Sea Res. II (2002) 827–845). Temperature and predation by viruses might be the two main factors controlling the activity of prokaryotes in these waters.

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1. Introduction

Antarctic planktonic food webs are extremely variable in both time and space. Away from the coastal zone, four main types of habitats have been identified: open-oceanic areas, frontal systems, the ice-edge zone, and microhabitats within the sea-ice (Lancelot et al., 1991). A series of events tied to the annual phytoplankton bloom takes place superimposed on this spatial heterogeneity. This time sequence has been summarized by Karl (1993) in four succesive phases: In early spring, phytoplankton increases due to water column stratification and increased light levels. During this phase, most primary production sinks to deeper layers. In the second phase, zooplankton consume and balance the phytoplankton production. Export of POM is through fecal pellets. In the third phase, small phytoplankton species substitute the larger diatoms of the first two phases, and their production is channeled through the microbial food web. There is little export to deeper layers since most organic matter is recycled within the upper layers. During this phase algal biomass decreases and prokaryotic biomass increases. Finally, in the fourth phase, winter light levels do not allow significant primary production. Production and biomass are minimal and only chemotrophic prokaryotes maintain some activity. In principle, these four phases would correspond to early spring, late spring–early summer, late summer and winter.

The relationships between phytoplankton and heterotrophic prokaryotes along this succession in time and in such different areas in space must necessarily be very different. Yet, these models of spatial and temporal distribution of microbial biomass and activities are based on very few studies. Particularly detailed studies were carried out in Drake Passage (Hanson and Lowery, 1985; Hanson et al., 1983), the AMERIEZ project in the Weddell Sea (Sullivan et al., 1990; Cota et al., 1990; Kottmeier and Sullivan, 1987), in the Scotia-Weddell confluence (Lancelot et al., 1991; Bjørnsen and Kuparinen, 1991; Kuparinen and Bjørnsen, 1992), and the RACER program around the western Bransfield Strait (Karl et al., 1991; Bird and Karl, 1991, 1999). There is also considerable information on other areas of the Southern Ocean such as Pridz Bay (Billen and Becquevort, 1991; Leakey et al., 1996) or the Ross Sea (Carlson et al., 1998, 1999; Ducklow et al., 1999). Gathering new data is especially relevant to the question of whether the degree of coupling between phytoplankton and heterotrophic prokaryotic plankton is looser in Antarctic waters than in temperate waters. Assessing this question requires simultaneous, good quality, data on phytoplankton and prokaryotes in different Antarctic ecosystems and at different times.

The purpose of the present study was to determine values of prokaryotic abundance (PN) and prokaryotic heterotrophic production (PHP) in order to compare them to phytoplankton biomass and primary production estimates gathered during the same cruises (Varela and Fernández, 2002; Moran and Estrada, 2002). We made a special effort to obtain accurate estimates of PHP in these waters. This implied a careful analysis of the influence of temperature on leucine incorporation and an effort to determine conversion factors (CF) from leucine incorporation to PHP suitable for these waters.

The FRUELA cruises were designed to provide estimates of carbon fluxes between the atmosphere and surface waters and between such waters and deep sediments. This purpose is very similar to that of the RACER program (Huntley et al. 1991). The RACER study comprised four cruises in December, January, February and March 1987, in approximately the same area as the FRUELA cruises. A second part of the project included a cruise in Gerlache Strait in 1989 (Tupas et al., 1994; Bird and Karl, 1999). RACER therefore provided an excellent baseline against which to compare our data collected almost ten years later. The emphasis in RACER was on the temporal variations and, thus, only surface samples were taken at most stations. Our study was more limited in time, but the whole upper mixed layer was studied in all stations. The physical (Gomis et al., 2002; García et al., 2002), chemical (Castro et al., 2002; Doval et al., 2002) and phytoplankton (Rodríguez et al., 2002; Moran and Estrada, 2002; Varela and Fernández, 2002) fields

during the FRUELA cruises have been described in other papers.

2. Methods

Archaea have been found to make up a substantial fraction of the prokaryotic cells normally counted as bacteria in Antarctic waters (DeLong et al., 1994; Massana et al., 1998). The metabolic activities of these microorganisms remain unknown. Archaea could be either taking up leucine as bacteria do, or not taking up leucine despite being active. Indeed, it has been recently shown that about 60% of the marine archaea take up aminoacids in two samples from the northwestern Mediterranean and Monterrey Bay (Ouverney and Fuhrman, 2000). In the first case, when we talk about bacterial heterotrophic production we should more properly talk about PHP. In the second case we would be underestimating prokaryotic production and overestimating doubling times. However, this is the same situation as when thymidine is used to estimate production, since many bacteria are known not to take up thymidine despite being active. The only solution is to carry out autoradiography with tritiated leucine or thymidine and count how many DAPI stained cells are labeled by the leucine (Pedrós-Alió and Newell, 1989). But this is time consuming and impractical in a cruise. Of course all these considerations do not affect the comparisons between FRUELA and RACER, or among different areas during the cruise. For the purpose of recognizing the potential importance of Archaea we will use the terms heterotrophic prokaryotic plankton, prokaryotic biomass and PHP in this paper.

2.1. Sampling

Samples were collected during two cruises of the BIO *Hespérides* in December 1995 and January 1996 (FRUELA 95) and from January to February 1996 (FRUELA 96). This period of time corresponded to late spring and summer. The area studied and the sampling sites are presented in Fig. 1. This area includes several representative

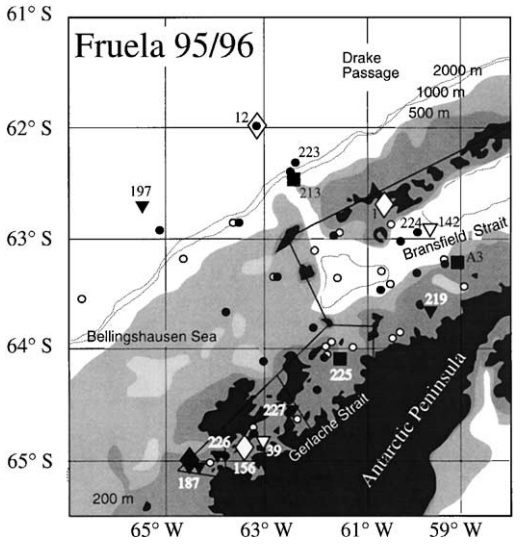

Fig. 1. Map of the zone studied, with indication of the different hydrographical zones identified: Bellingshausen Sea, Bransfield Strait and Gerlache Strait. The latter was further divided into three zones (Gerlache-0, 1, and 2, from the southwest to the northeast). Stations where experiments were carried out (triangles for temperature effects on leucine, squares for conversion factor experiments and diamonds for saturation curves) or where vertical profiles of prokaryotic abundance and production were collected (circles) also are shown. Empty symbols correspond to FRUELA95 and filled symbols to FRUELA96. Only a few station numbers are shown for clarity.

Antarctic waters: (1) two different deep-water zones (the western basin of Bransfield Strait and the Bellingshausen Sea); and (2) the eutrophic and mesotrophic coastal region of Gerlache Strait (Huntley et al., 1991). The latter was subdivided into three zones (Gerlache 0–2) on the basis of the dominant phytoplankton communities during our two cruises. Gerlache-0 was very much influenced by the Bellingshausen Sea waters; Gerlache-1 was dominated by diatoms; and Gerlache-2 showed a bloom of *Cryptomonas* sp. (see Rodríguez et al., 2002; Varela and Fernández, 2002). Seawater samples were collected with 12-l Niskin bottles mounted on a rosette with a General Oceanics MkIIIC WOCE probe provided with extra oxygen, fluorescence and light transmission sensors.

Subsamples were collected from the Niskin bottles and either immediately preserved for

prokaryotic counts with glutaraldehyde (1% final concentration) or kept in polypropylene bottles in an opaque plastic box, with surface water to keep ambient temperature, until used for leucine uptake measurements (less than 15 min).

2.2. Prokaryotic abundance and biovolume measurements

Samples for determination of prokaryotic abundance (10–20 ml) were filtered through 0.2 μm pore diameter black polycarbonate filters and stained with DAPI (1 μg ml^{-1} final concentration) for 5 min before sucking the filters dry (Porter and Feig, 1980). Filters were then mounted on microscope slides with non-fluorescent oil (R.P. Cargille Lab., Inc.) and stored frozen until counted. Filters were counted by epifluorescence microscopy with a Nikon Diaphot microscope. About 200–400 prokaryotic cells were counted per sample. Cell volumes were determined with an image analysis system measuring at least 200 cells per sample. A Hamamatsu C2400-08 video camera was used to examine microscopic preparations. Images were captured in a PC with the software MIP (from Microm España SA). The video images were downloaded to a Macintosh computer and analyzed with the shareware program NIH Image. Objects occupying less than 7 pixels (equivalent to a sphere with diameter less than 0.2 μm) were discarded. The remaining objects were measured and the volume calculated from area and perimeter measurements with the formula of Fry (1990). The system was calibrated with fluorescent latex beads and with natural prokaryotic plankton samples measured simultaneously by phase contrast microscopy and by epifluorescence (Massana et al., 1997). Biomass was calculated by using the carbon to volume relationship derived by Norland (1993) from the data of Simon and Azam (1989):

$$\text{pg C cell}^{-1} = 0.12 * (\mu\text{m}^3\ \text{cell}^{-1})^{0.7.}$$

2.3. Prokaryotic heterotrophic production measurement

PHP was determined by ^{3}H-leucine incorporation (Kirchman et al., 1985) as modified for micro-centrifugation by Smith and Azam (1992). Aliquots of 1.2 ml were dispensed into 2-ml microcentrifuge tubes with a step pipette. Control tubes received 133 μl of 50% TCA and were vortexed. Next, 48 μl of a 1 μM solution of ^{3}H-leucine was added to the tubes providing a final concentration of 40 nM (which was found to be saturating in these waters). At least four replicates and two killed controls were incubated per sample. After vortexing, tubes were placed in whirl-pack plastic bags and these were incubated in the dark in a water bath, at temperatures close to those in situ, for 2–4 h. Incubations were stopped with 133 μl of 50% TCA and vortexing. Next, tubes were spun in a microcentrifuge for 10 min at 16,000*g*. Liquid was aspirated with a Pasteur pipette connected to a vacuum pump, taking care not to leave any droplets, especially around the cap. Pellets were rinsed with 1.5 ml of 5% TCA, vortexed, and spun again. Supernatant was sucked again and 0.5 ml of scintillation cocktail was added. The tubes were counted within standard 20-ml scintillation vials in a Beckman scintillation counter on board. Counts were repeated after 48 h of adding cocktail. These second sets of counts were less variable and had lower blanks than the initial counts. DPM were calculated by the instrument using the H number.

The PHP was calculated from leucine (Leu) incorporation according to the equation

$$\text{PHP} = \text{Leu} * \text{CF},$$

where CF is a conversion factor expressed in kg C mol^{-1}. These CF were empirically derived for different samples (see below). From these estimates of production and those of prokaryotic biomass, specific growth rates (μ) were calculated as

$$\mu = [\text{Ln}(1 + \text{PHP}/\text{PB})]/t,$$

where PB is the prokaryotic biomass, and t is the time over which the PHP is considered.

Several experiments were carried out to determine the effect of temperature on leucine incorporation rates. Samples were collected either from the surface with a bucket or from 5 m depth with the Niskin bottles in the rosette. Care was taken to keep sample bottles in an opaque container with surface water to maintain temperature

stable until samples were inoculated with ^{3}H-leucine. Aliquots were incubated at different temperatures immediately after sampling. The protocol was the same as that described above. The temperatures used in each particular experiment can be seen in Fig. 3.

Several experiments were used to calculate CF. To determine empirical CF (eCF), three experiments (TOM1 to TOM3) were carried out. 81 of water was filtered through Nuclepore 0.8 μm pore filters and 1-l aliquots were distributed into 8 sterile polyethylene bottles. A randomized block design was used with three levels of organic matter (0, 5 and 10 μmol glucose plus acetate) and two or three temperatures (−1.5 and 3 for TOM1, and −1, 0.5 and 3 for TOM2 and TOM3). Two replicates were carried out for each treatment (a total of 18 bottles per experiment). Each day samples were withdrawn for prokaryotic counts and leucine incorporation experiments. Leucine incorporation was determined at the temperature of incubation of each bottle. Finally, the eCF were calculated with both the integrative and the cumulative methods (Riemann et al., 1987; Bjørnsen and Kuparinen, 1991). Since results were not significantly different, only the results of the integrative method are shown in this paper.

"Semitheoretical" CF (sCF) were calculated according to the equation

$$\mathrm{sCF} = \mathrm{PM} * (1/L_\mathrm{p}) * C_\mathrm{cp} * \mathrm{DI},$$

where PM is the molecular weight of leucine (0.1312 kg mol^{-1}), L_p is the leucine content of cellular protein (0.073), Ccp is the cellular carbon in kg equivalent to 1 kg of protein (0.86), and DI is the isotope dilution as determined below. Using a DI of 2, Simon and Azam (1989) calculated the different factors of this equation and obtained a "theoretical" CF of 3.1 kgC l^{-1}. We used all their factors and substituted our estimates of DI in the equation to obtain the sCF. Saturation curves (SC-I to SC-IV) were obtained with the protocol described above and adding concentrations of radioactive leucine between 0 and 100 nmol. Assuming no dilution at saturation, the actual dilution (DI) of leucine in the samples is calculated as

$$\mathrm{DI} = V_\mathrm{max}/V_\mathrm{r},$$

where V_max is the maximal incorporation rate, and V_r is the incorporation rate at the leucine concentration used in the routine assays (van Looij and Riemann, 1993). V_max was estimated by fitting a hyperbolic curve to the data $V_i = (V_\mathrm{max} * A)/((K + S) + A)$, where V_i are the incorporation rates at the different tritiated leucine concentrations used, A the known concentrations of leucine used, and S the unknown diluting concentration of leucine. V_r was obtained from the fitted curve and the known concentration of leucine used.

Integrated values were computed from the surface to 100 m, which was the approximate depth of the photic layer.

3. Results

3.1. Prokaryotic abundance and production

Examples of the vertical distribution of prokaryotic abundance and activity are shown in Fig. 2. The four stations chosen represent four different hydrographical areas (Fig. 1). Prokaryotic numbers ranged from 0.67 to 13.3×10^5 cells ml^{-1}, with a mean of 3.86×10^5 cells ml^{-1}. Prokaryotic cell volumes, in turn, ranged between 0.46 and 0.105 μm^3. Both prokaryotic abundance and PHP were higher at the surface or slightly below the surface and decreased down to about 200 m. Prokaryotic abundance remained essentially constant below this depth. This pattern was always the same despite different vertical distributions of salinity, temperature and fluorescence. PHP, however, showed a second and smaller maximum of activity between 200 and 300 m.

3.2. Temperature effects on PHP

In FRUELA 95 we incubated all samples at 0°C. In FRUELA 96 we chose two or three temperatures and incubated samples from different depths at the closest of the chosen temperatures. The average difference between incubation and in

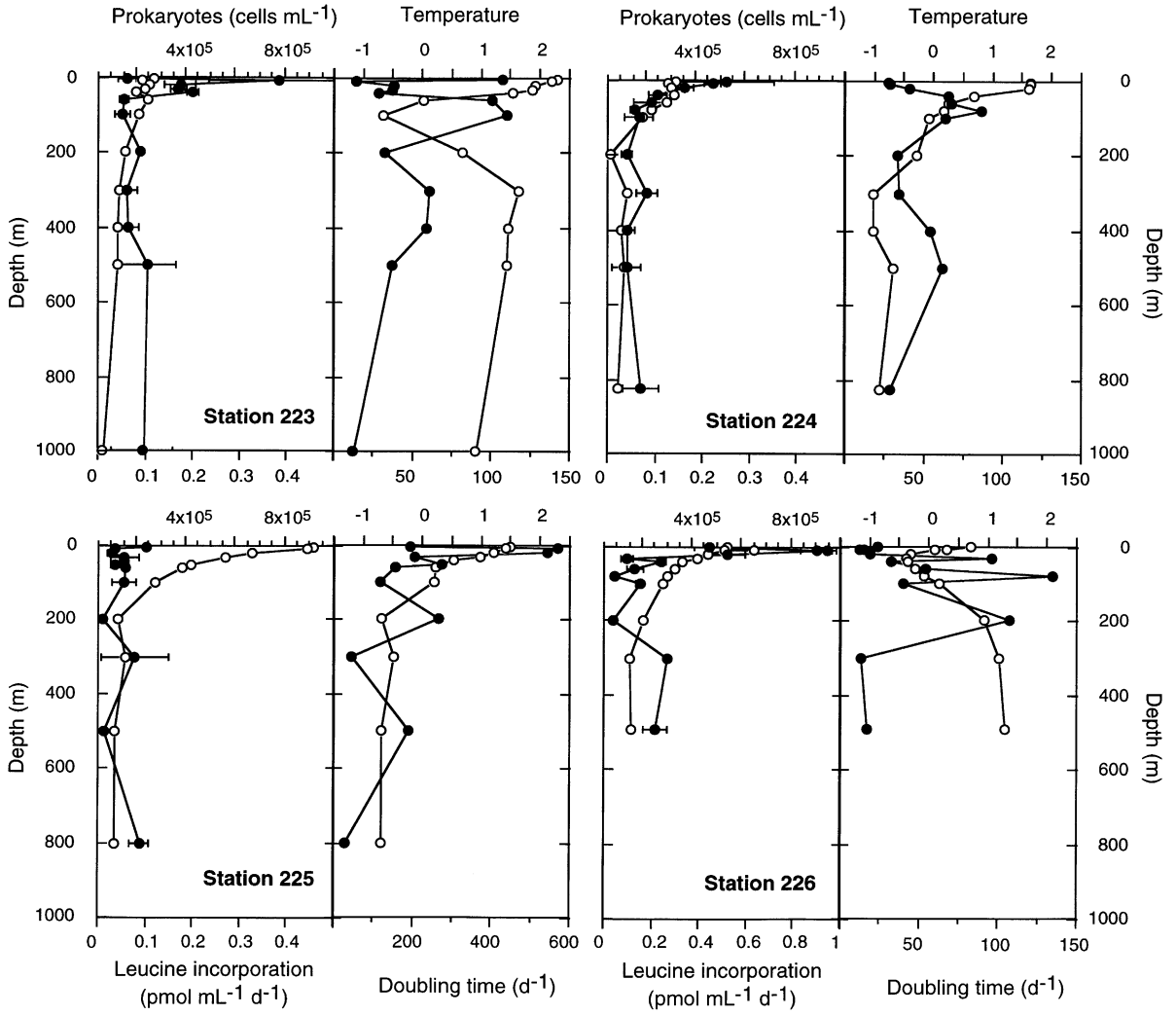

Fig. 2. Vertical distribution of temperature (empty symbols), prokaryotic abundance (empty symbols), leucine incorporation rates (filled symbols), and doubling times (filled symbols) at four representative stations, where profiles of the whole water column were studied.

situ temperatures was 0.6°C in FRUELA 95 and 0.3°C in FRUELA 96 (the maximal differences were 1.4 and 1.7°C, respectively). Only 5% of the individual samples were incubated at temperatures which differed from the in situ temperature by more than 1°C. However, in order to correct leucine incorporation rates for these small temperature differences, we conducted two experiments in FRUELA 95 and 5 in FRUELA 96 to examine the effects of temperature on leucine incorporation and to calculate Q_{10} values (Fig. 3). The equation used to correct leucine (Leu) incorporation was

$$\mathrm{Leu}T_2 = \mathrm{Leu}T_1\, e^{(\ln(Q_{10})*(T_1-T_2)*0.1)},$$

where T_2 is the in situ temperature, and T_1 the incubation temperature.

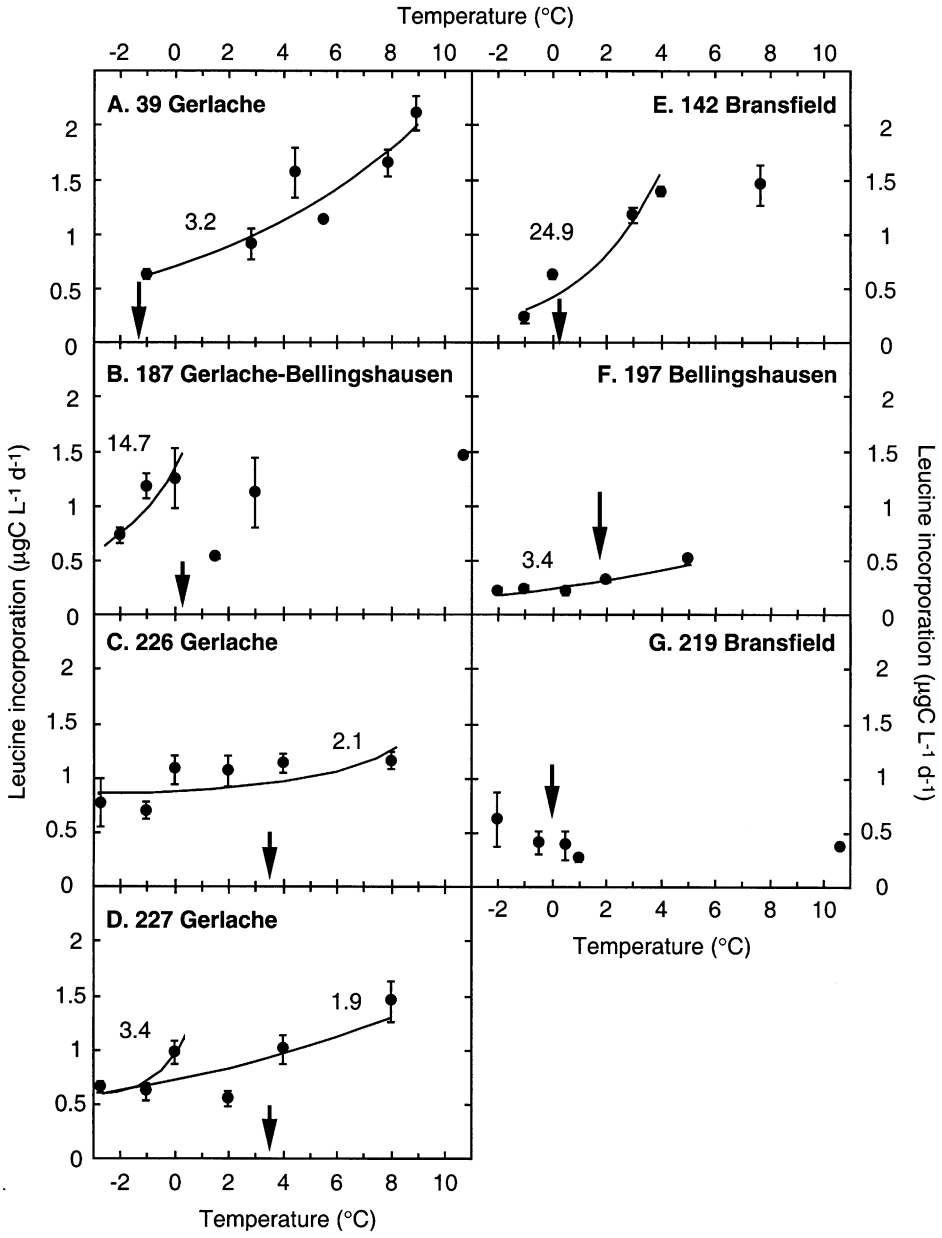

Fig. 3. Effect of incubation temperature on leucine incorporation rates in different experiments. The vertical arrow indicates the in situ temperature in each case. Error bars indicate standard error of four (FRUELA 95) or six (FRUELA 96) replicates. When error bars are not visible, error is smaller than the symbol. Curves were fitted as indicated in the text. Points outside of the line were excluded from the calculation. The number is the Q_{10} value calculated with the fitted curve.

The values obtained are shown in Table 1 together with the statistics of their calculation. In one experiment (Fig. 3G) we could not detect a significant influence of temperature on leucine incorporation, and results were quite variable in the other experiments. The sampled area was divided into hydrographically and biologically coherent zones (Fig. 1) and for each station the

Table 1
Q_{10} values calculated in different experiments[a]

Experiment	Zone	Station	a	b	r^2	n	Q_{10}
FRUELA95							
TGRZ-1	Gerlache	39	0.71	0.12	0.85	6	3.22
TGRZ-2	Bransfield	142	0.43	0.32	0.93	4	24.86
FRUELA96							
LvsT-1	Bellingshausen–Gerlache	187	1.34	0.27	0.79	3	14.72
LvsT-2	Bellingshausen	197	0.26	0.12	0.93	5	3.40
LvsT-3	Bransfield	219				0	—
LvsT-4	Gerlache	226	0.55	0.07	0.84	6	2.09
LvsT-5	Gerlache	227	0.51	0.06	0.61	6	1.88

[a] The coefficients a and b are the fitted coefficients for the equation leucine incorporation rate $= a e^{(b \text{ temperature})}$. The number of points used in the calculations is also shown (n). Q_{10} values were calculated as $e^{b_{10}}$.

Q_{10} value determined within the zone and closest in time was chosen to correct leucine incorporation values (Table 2).

3.3. Conversion factors

The three experiments used to determine eCF resulted in very similar CF, despite differences in incubation temperature or organic matter additions (Table 3, Fig. 4). Moreover, the three experiments were carried out with water from three different areas, Bellingshausen Sea, Bransfield and Gerlache straits (Fig. 1), and yet no significant differences could be found among the CF (Table 3). The only significant difference that could be detected was that between incubations at -1.5°C in TOM1 and all the other incubations (Fig. 4). The average conversion factor was 0.81 kgC (mol leucine)$^{-1}$ (or 3.21×10^4 cells pmol^{-1}), and this value was used to calculate PHP from leucine incorporation for the two cruises.

Isotope dilution was determined from saturation curves, and these values were used to calculate sCF. The four experiments available provided very similar values of dilution: between 1.0 (no dilution) and 1.4 (Table 3). As a consequence, the resultant sCF were also very similar, between 1.6 and 2.2 kgC mol^{-1} or between 8.6 and 11.3×10^4 cells pmol^{-1}.

3.4. PHP and growth rates in different hydrographical areas

Stations were assigned to one of five different hydrographical areas identified during the two cruises (see materials and methods). Average integrated values for chlorophyll *a* (chl *a*), prokaryotic abundance and production, and prokaryotic growth rate were then computed for each area and each cruise (Table 4). The three Gerlache areas showed higher chl *a* and prokaryotic numbers in January than in December (Fig. 5). PHP, however, was much higher in the first cruise than in the second. As a result of these opposite trends, prokaryotic growth rates were between two and three times faster in December than in January. Average doubling times were between 13 and 23 days in December and between 32 and 75 days in January (Table 4).

PHP was also higher in December than in January in the remaining two areas (Fig. 5). But changes in biomass and growth rates were different in each one. In Bellingshausen Sea chl *a* decreased with the season, prokaryotic abundance remained constant, and prokaryotic growth rate decreased significantly (Fig. 5). In Bransfield Strait chl *a* and prokaryotic abundance decreased slightly, while prokaryotic growth rate remained essentially the same (Table 4).

Table 2
Q_{10} values used for correction of leucine incorporation rates at each station

Stations	Zone	Q_{10}	Experiment	Station of experiment
FRUELA95				
1–8	Bransfield	24.86	TGRZ-2	142
9–33	Bellingshausen	8.36	Average[a]	
34	Bellingshausen–Gerlache	3.22	TGRZ-1	39
35–47	Gerlache	3.22	TGRZ-1	39
72	Gerlache	3.22	TGRZ-1	39
79–142	Bransfield	24.86	TGRZ-2	142
156–164	Gerlache			
166–168	Bransfield	24.86	TGRZ-2	142
169	Gerlache	3.22	TGRZ-1	39
170–184	Gerlache	3.22	TGRZ-1	39
FRUELA96				
185	Bransfield	—[b]	LvsT3	219
186	Gerlache	2.09	LvsT4	226
187–189	Bellingshausen–Gerlache	14.72	LvsT1	187
190–196	Gerlache	2.09	LvsT4	226
197–213	Bellingshausen	3.40	LvsT2	197
214–222	Bransfield	—	LvsT3	219
223	Bellingshausen	3.40	LvsT2	197
224	Bransfield	—	LvsT3	219
225	Gerlache	2.09	LvsT4	226
226	Bellingshausen–Gerlache	2.09	LvsT4	226
227	Gerlache	1.88/3.44	LvsT5	227

[a] Average of all Q_{10} values obtained during both cruises, average 8.36, standard deviation 9.43, $n = 6$.
[b] No effect of temperature on leucine incorporation and, thus, no correction for temperature.

Table 3
Experiments used to calculate conversion factors

Experiment	Zone	Station	Method	N	Dilution[a]	CF	
						kgC/mol	cells/pmol ($\times 10^4$)
FRUELA95							
CS-I	Bransfield	1	SemiTheor	1	1.4	2.1	11.3
CS-II	Bellingshausen	12	SemiTheor	1	1.4	2.2	11.8
CS-III	Gerlache-1	156.7	SemiTheor	1	1.0	1.6	8.6
FRUELA96							
CS-IV	Gerlache-0	187	SemiTheor	1	1.3	2.1	11.0
TOM1	Bransfield	A3	Empirical	12		0.57	2.86
TOM2	Bellingshausen	213	Empirical	18		0.89	3.42
TOM3	Gerlache-2	225	Empirical	18			3.07

[a] Isotope dilution calculated from saturation curves (see Materials and Methods).

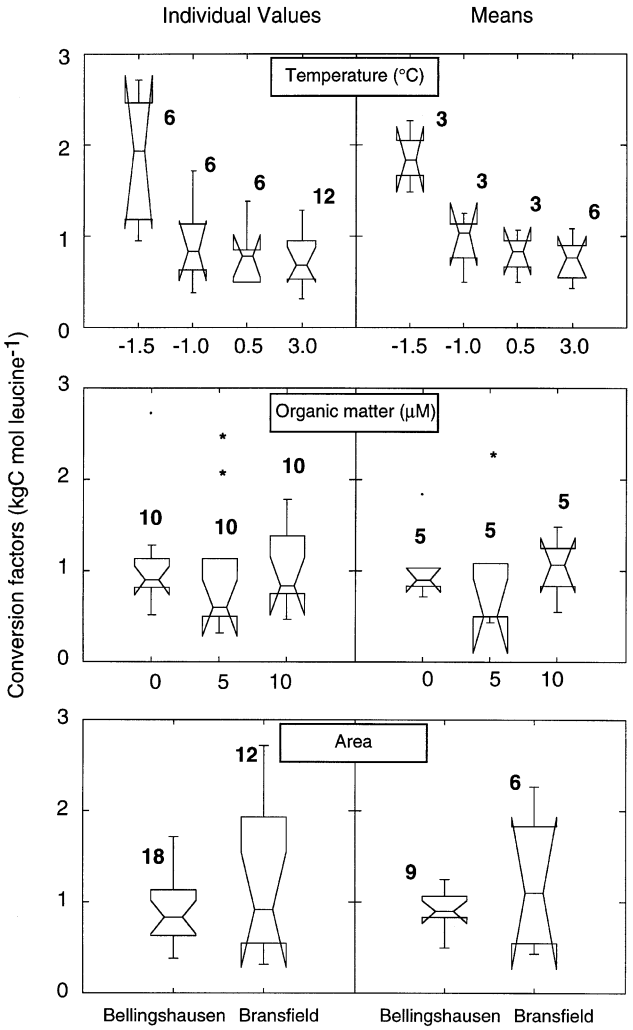

Fig. 4. Box and whiskers plots of the CF calculated under different conditions of temperature, organic matter additions and in different hydrographical areas. Values from all experiments have been pooled according to incubation temperature (upper panels), organic matter added (middle panels) or area where the experiment was carried out (lower panels). The boldface numbers indicate the number of values used to calculate the set of statistics. The left-hand panels show the variability of results considering each replicate separately. In the right-hand panels, the two replicate numbers for each treatment have been averaged before calculating the variability shown in the figure. The central horizontal line in each box represents the median of the distribution, whereas the other two horizontal lines contain 50% of the values between them. The endpoints of the inclined straight lines indicate 95% confidence intervals for the median, whereas the whiskers indicate the total range of values without outliers (asterisks; defined as those values greater than 1.5 times the 95% confidence interval for the median).

3.5. General relationships

Neither chl *a* nor prokaryotic production, biomass or specific activity (leucine incorporation per cell, Fig. 6) were significantly related to temperature. The relationships between prokaryotic abundance and chl *a* were not significant when calculated for the whole data set or for the FRUELA95 data alone. However, the FRUELA96 data set showed a significant relationship (Table 5, Fig. 7). When the regression was calculated for each area separately, only Gerlache

Table 4
Average values, and standard errors in parentheses, integrated to 100 m, for each zone during FRUELA95 and FRUELA96

Zone	Cruise	PN (10^{13} cells m^{-2})	PHP (mgC m^{-2} d^{-1})	Chl *a* (mg m^{-2})	μ (d^{-1})	Dt (d)	Stations
Bransfield	95	4.24 (0.32)	25.2 (4.7)	75.0 (8.7)	0.022 (0.004)	46.6 (12.4)	11 (5,8,79,81, 94,97,123,138, 140,142,168)
	96	3.03 (0.19)	8.0 (0.6)	52.8 (7.8)	0.016 (0.001)	45.0 (3.9)	8 (185,215,217, 219,220,221,224)
Bellingshausen	95	2.86 (0.38)	37.2 (7.9)	183.1 (38.05)	0.053 (0.011)	17.4 (5.8)	5 (12,15,17,24,29)
	96	3.01 (0.17)	9.6 (0.9)	28.3 (4.6)	0.019 (0.001)	38.3 (2.9)	11 (197,198,201,203, 204,206,208, 210, 211,213,223)
Gerlache 0	95	2.57 (0.55)	42.1 (23.5)	107.0	0.055 (0.020)	15.6 (4.2)	3 (34,36,37)
	96	5.83 (1.14)	22.9 (5.2)	263.8 (91.4)	0.025 (0.006)	32.0 (8.3)	3 (187,189,226)
Gerlache 1	95	3.41 (0.72)	47.7 (12.3)	219.9 (43.0)	0.054 (0.005)	13.6 (1.6)	5 (39,40,169, 177,156)
	96	5.83 (0.12)	14.2 (2.5)	294.7 (74.6)	0.015 (0.003)	48.9 (9.4)	2 (191,193)
Gerlache 2	95	4.62 (0.60)	45.5 (6.9)	72.0 (17.0)	0.038 (0.009)	22.7 (6.5)	5 (47,72,164, 178,184)
	96	4.93 (0.36)	9.6 (2.8)	192.5 (89.7)	0.011 (0.003)	75.1 (27.4)	3 (186,195,225)

1 and 2 showed significant relationships (Table 5). The result was similar for the relationship between prokaryotic biomass and PHP (Fig. 7). Again, only the FRUELA96 data set showed a significant relationship. In this case, none of the studied areas individually showed any significant relationships.

4. Discussion

4.1. Methodological considerations

4.1.1. Corrections for temperature

Although the range of temperatures found in the area was relatively small (from -1.7°C to $+3$°C), prokaryotic activity can be greatly affected by just a small change in temperature (Fig. 3). Thus, we took precautions to avoid heating of the samples during processing by keeping them in an icebox with surface water and processing the samples as fast as possible. Since it is impractical to incubate each sample at its in situ temperature (often this would require one incubator per depth sampled), corrections had to be applied for the difference between in situ and incubation temperatures. In FRUELA95 we incubated all samples at 0°C. In FRUELA96 the surface layers were a few degrees warmer while the bottom waters were as cold as during FRUELA95. Thus, for each station, we chose two or three temperatures and incubated samples from different depths at the closest of the chosen temperatures. Then we carried out experiments to detemine the influence of temperature on leucine incorporation and calculated Q_{10} values from them.

Calculation of Q_{10} assumes an exponential relationship between the activity and temperature. When deviations from such behavior appeared in the experiments, we used the data points for the relevant range of temperatures alone (Fig. 3). This sometimes resulted in extremely high values of Q_{10}. However, one must bear in mind two things: first, these values do not correspond to a simple enzymatic reaction but to a mixed assemblage of prokaryotic populations, and second, these values

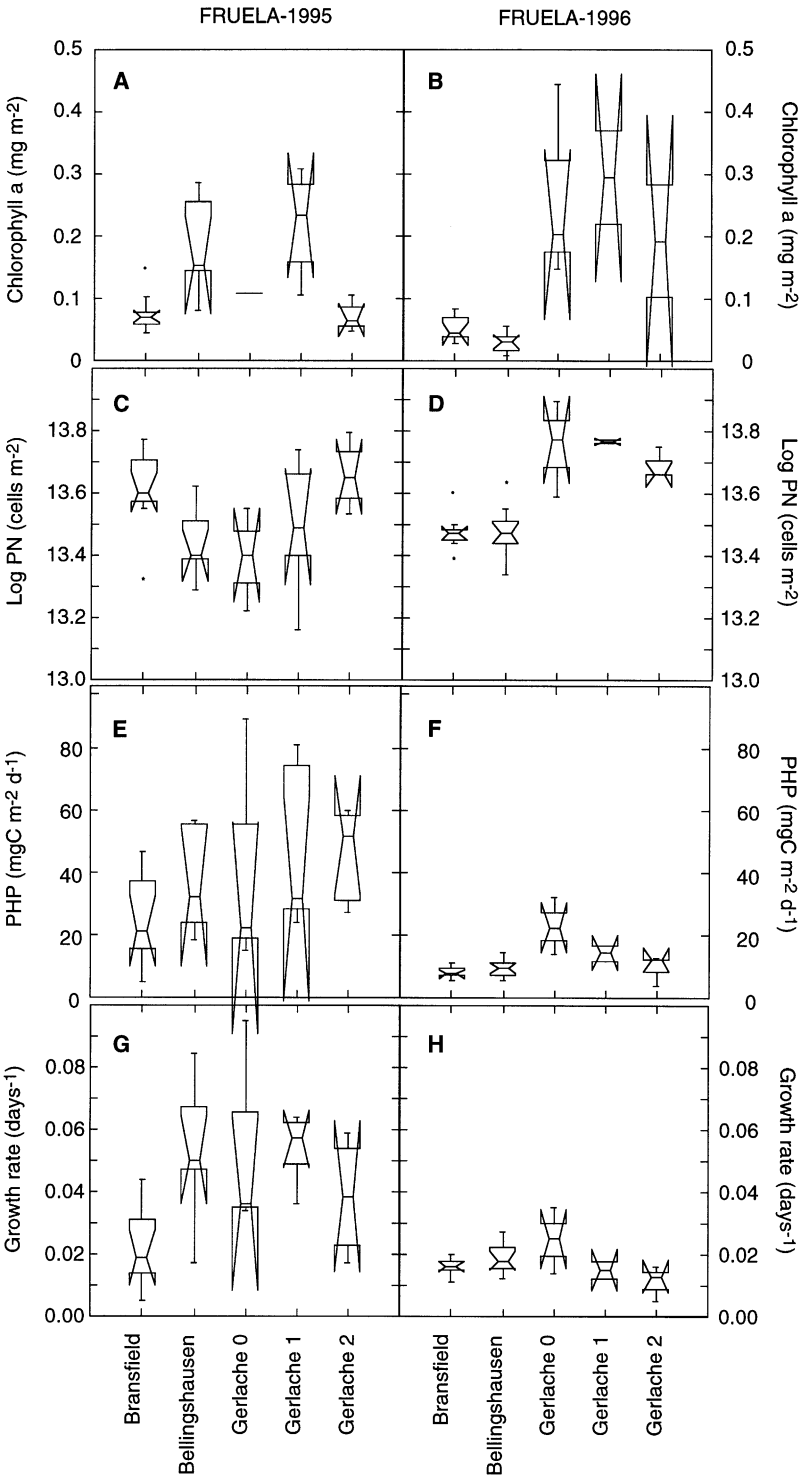

Fig. 5. Box and whiskers plots of integrated average values of chl *a* (A, B), prokaryotic abundance (C, D), PHP (E, F), and prokaryotic growth rates (G, H) in the two cruises FRUELA95 and FRUELA96. The five different areas correspond to those indicated in Fig. 1.

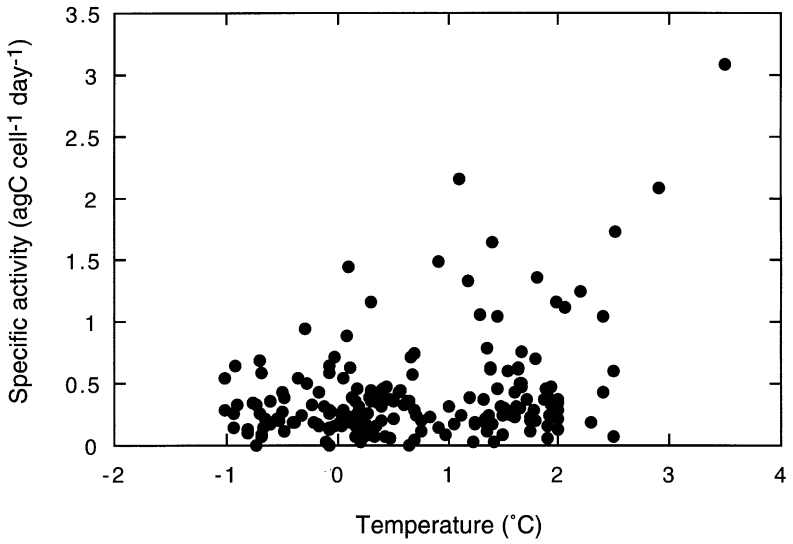

Fig. 6. Plot of prokaryotic specific activity (leucine incorporation per cell) versus temperature for the whole data set. No significant relationship could be found.

Table 5
Statistics for the linear regressions between prokaryotic abundance (PN) and chlorophyll *a* (Chl *a*), and between prokaryotic biomass (PB) and prokaryotic heterotrophic production (PHP)[a]

Zone	PN/Chl *a*			PB/PHP		
	Slope	Intercept	*r*	Slope	Intercept	*r*
By zone						
Gerlache 1	1.043	11.07	0.70		ns	
Gerlache 2	0.453	12.78	0.79		ns	
By cruise						
FRUELA96	0.280	13.11	0.604	0.538	2.24	0.726
Global		ns			ns	

[a] Regressions for the zones and cruises not appearing in this table were all nonsignificant.

are only applicable to the extremely narrow temperature range around 0°C. Thus, corrections remain small despite these high Q_{10} values. These Q_{10} values are not valid outside the narrow temperature range for which they were calculated (shown in Fig. 3). Most corrected values were only slightly different from experimental values due to the small temperature difference, despite the large Q_{10} values. In conclusion, we believe our corrected values are a good estimate of the leucine incorporation activity in situ.

4.1.2. Calculation of PHP

The only significant difference that could be detected in the experiments to calculate CF was that between incubations at −1.5°C in TOM1 and all the other incubations (Fig. 4). Since the absolute majority of waters sampled had temperatures between −1°C and +3°C, we decided to average all the eCF determined at these temperatures (a total of 24 independent cultures) and use a single eCF for the two cruises. This factor was 0.81 kgC (mol leucine)$^{-1}$, which is about 4 times

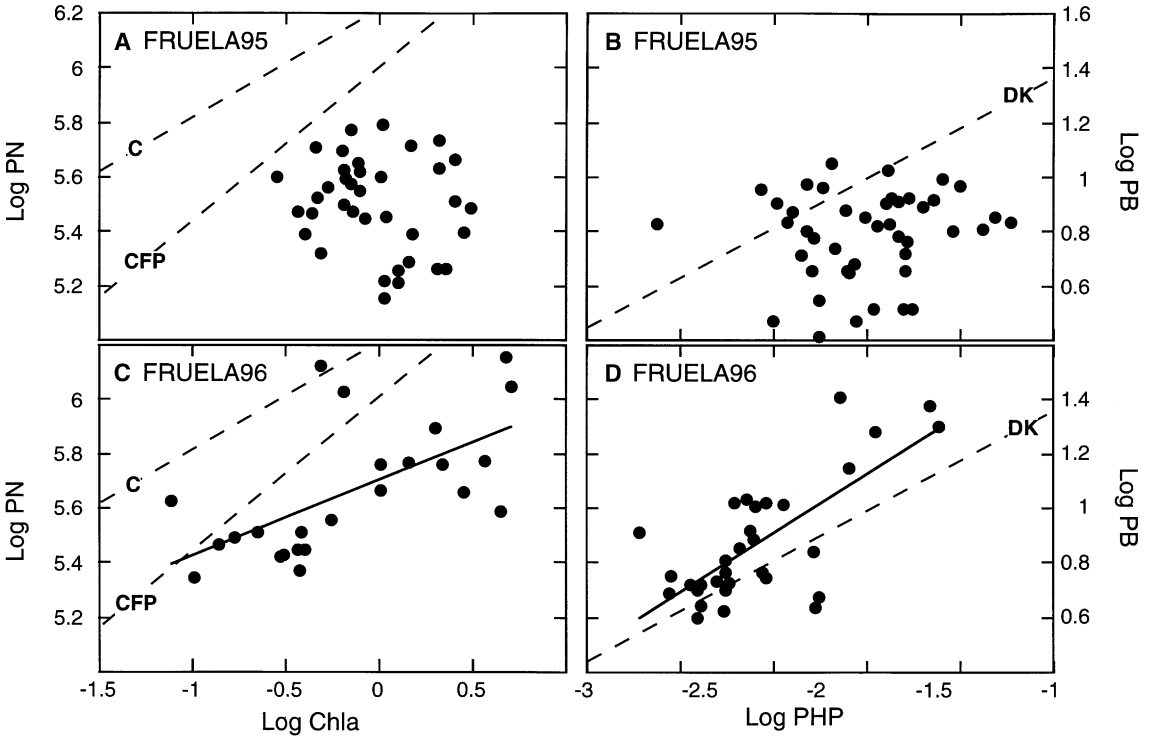

Fig. 7. Relationships between prokaryotic abundance and chlorophyll (A, C) and between prokaryotic biomass and PHP (B, D) for FRUELA95 (A, B) and FRUELA96 (C, D). Only the relationships for the second cruise were significant. The discontinuous lines are those calculated by Cole et al. (1988, CFP), Pedrós-Alió et al. (2000, C), and Ducklow (1992, DK). The units are cells ml^{-1} for prokaryotic numbers, $\mu g l^{-1}$ for chl *a* concentration, $\mu gC l^{-1}$ for prokaryotic biomass, and $\mu gC l^{-1} h^{-1}$ for PHP.

smaller than the "standard" CF provided by Simon and Azam (1989), but similar to those determined in open sea samples in the Mediterranean (Pedrós-Alió et al., 1999), the Sargasso Sea (Carlson et al., 1998) or the subarctic Pacific (Kirchman, 1992). In terms of cells produced per mol of leucine incorporated, we could compare our results to a larger data base of eCF (Table 3). Our overall average was 3.21×10^4 cells $pmol^{-1}$. This value falls within the range we found in the Weddell Sea during an earlier cruise (Calderón-Paz, 1997), where 20 independent cultures were studied and resultant values varied between 2.3 and 6.4×10^4 cells $pmol^{-1}$. Again, these values are similar to those reported for open sea waters, but lower than those commonly used in coastal or estuarine waters. Only a few studies have determined eCF for leucine in the Southern Ocean: Ducklow et al. (1999) determined leucine CF in the Ross Sea and found values slightly higher than ours (average 8×10^4 cells $pmol^{-1}$). Bjørnsen and Kuparinen (1991) found a factor of 2.87 kgC mol leucine^{-1} in the Scotia-Weddell confluence, and Lochte et al. (1997) calculated an eCF of 3 kgC mol leucine^{-1} in the Polar Frontal zone around the 3°W meridian. These latter values are similar to the coastal values and to the factor calculated by Simon and Azam (1989), but about four times higher than our values. There is no sufficient information to explain these differences. The only common thing among the two latter studies, which is different from our studies in the Weddell Sea (Calderón-Paz, 1997) and here, is the proximity of the retreating ice-edge. In our studies the ice edge was far away, while both Bjørnsen and Kuparinen (1991) and Lochte et al.

(1997) carried out their sampling along transects that penetrated into the ice zone.

Saturation curves provide an indication of the dilution of leucine taking place in the samples (van Looij and Riemann, 1993) and allow calculation of a semitheoretical conversion factor. The four experiments available provided very similar values of dilution: between 1.0 (no dilution) and 1.4. As a consequence, the resultant sCF were also very similar, between 1.6 and 2.2 kgC mol^{-1} or between 8.6 and 11.3×10^4 cells $pmol^{-1}$. These values are closer to the standard values from coastal waters than the eCF. Likewise, in the Weddell Sea, sCF were also larger than eCF (8.8–43.8×10^4 and 2.3–6.4×10^4 cells $pmol^{-1}$, respectively; Calderón-Paz, 1997). We also found this difference between sCF and eCF in the Mediterranean Sea (Pedrós-Alió et al., 1999). However, since sCF are subject to many assumptions, we think the use of the eCF will result in more accurate estimates of PHP. Since these factors are lower than sCF, our estimates of PHP may be conservative.

4.2. Comparison with the RACER program

The RACER program included an extensive study of the area considered here with the aim "to observe physical, chemical and biological structure in the upper ocean before, during and after the annual phytoplankton bloom" (Huntley et al. 1991). The distribution of microbial abundance and activity during these cruises were presented by Karl et al. (1991) and Bird and Karl (1991). Our two cruises were carried out in essentially the same area ten years later. Thus, the two data sets offer an excellent opportunity to distinguish constant and variable features of the microbial assemblage distribution. The area chosen is especially attractive because it offers contrasting Antarctic ecosystems within a relatively short distance.

There are some basic differences between the two studies. The main differences and a comparison of results for the different areas are summarized in Table 6. Ranges of values for chl *a* and prokaryotic numbers overlapped extensively between studies. In the FRUELA program minimal values were lower than in RACER, while maximal values were quite similar. Since biomass of both phototrophic and heterotrophic microorganisms was obtained from these variables multiplying by a factor, the similarities between chl *a* and prokaryotic numbers suggest that the proportions in biomass of phototrophic and heterotrophic microorganisms were similar in both studies. This similarity holds when values are compared separately for each one of the three common study areas (Table 6b). The situation, however, is completely different for the activity measurements. Our PHP estimates were between 10 and 50 times lower than those of Karl et al. (1991). Values from the RACER study were calculated from adenine uptake, and thus they integrate the activity of both autotrophic and heterotrophic microorganisms, while our values were calculated from leucine incorporation experiments and, therefore, include only PHP. Bird and Karl (1999) and Tupas et al. (1994), using leucine incorporation, found a range of values similar to ours. If the situation were similar among the two studies, this comparison suggests that most of the microbial activity in the RACER study was due to the autotrophic microorganisms.

Karl et al. (1991) also determined rates of incorporation of ^{3}H-thymidine. For a number of reasons they decided that these values could not be converted to estimates of PHP. However, if we do the exercise using their most conservative conversion factor (2×10^{18} cells mol thymidine^{-1}) and their experimental thymidine incorporation rates, average PHP values during RACER would be 0.058 gC m^{-2} d^{-1} in December and 0.116 gC m^{-2} d^{-1} in January. In general, thymidine incorporation was more or less uniform throughout the studied area in December. In January, peak values around 0.5 gC m^{-2} d^{-1} were found in Gerlache Strait and lower values around 0.035 gC m^{-2} d^{-1} were measured in Bellingshausen Sea. Bransfield Strait showed intermediate values around 0.12 gC m^{-2} d^{-1}. Considering all the crude transformation involved in these calculations, the resultant values are not very different from our leucine-based estimates during the FRUELA cruises. The only clear difference was found for the Gerlache Strait values in January, our estimates being more than ten times lower than those from the RACER study. Thus, the conclusion that most of the total microbial activity calculated by Karl

Table 6

	RACER	FRUELA
(a) *Comparison of sampling design and methods between the RACER[a] and FRUELA studies*		
Dates	December 1986 January 1987 February 1987 March 1987	December 1995 January 1996
Areas	Bellingshausen Sea Western Bransfield Strait Northern Gerlache Strait	Bellingshausen Sea Western Bransfield Strait Northern Gerlache Strait Central and Southern Gerlache
Stations	Fast grid only surface water 20 stations with depth profiles	56 stations with depth profiles 10 diel stations
Microbial activity	Adenine incorporation Thymidine incorporation	Leucine incorporation
	RACER[a]	FRUELA
(b) *Comparison of results between the RACER[a] and FRUELA studies*		
Chlorophyll **(mg C m^{-2})**		
December	291	131 (Gerlache 133)
January	176	167 (Gerlache 251)
February	58	
March	50	
Prokaryotic biomass **(μgC l^{-1})**		
December		
Bellingshausen		2.2–11
Bransfield		4.8–12
Gerlache		5.6–16
January		
Bellingshausen	8–12	3.2–12
Bransfield	12–20	1.2–8.2
Gerlache	20–24	2.1–15.9
PHP **(gC m^{-2} d^{-1})**		
December		
	(from adenine)	(from leucine)
Bellingshausen	0.35–1.83	0.037
Bransfield	0.43–3.07	0.025
Gerlache	0.50–1.49	0.045
January		
Bellingshausen	0.82–2.74	0.010
Bransfield	3.73–11.20	0.008
Gerlache	3.80–12.04	0.010–0.023
Doubling times **(days)**		
December		
Bellingshausen	2.1–2.6[b]	17.4[c]
Bransfield	1.7–2.4	46.6
Gerlache	3.0–3.9	18.2
January		
Bellingshausen	1.4–2.0	45.0
Bransfield	0.9–1.0	46.6
Gerlache	1.1–1.3	62.0

[a] RACER data from Karl et al. (1991), Bird and Karl (1991) and Holm-Hansen and Mitchell (1991).
[b] From adenine incorporation and ATP/C (whole microbial assemblage).
[c] From leucine incorporation and prokaryotic biomass (heterotrophic prokaryotes only).

et al. (1991) from adenine incorporation was due to autotrophs and not to heterotrophic prokaryotes is consistent with their thymidine incorporation rates.

Our direct estimates of PHP, as opposed to the total microbial activity calculated in RACER, allowed determination of growth rates and doubling times of heterotrophic prokaryotes. As shown in Tables 4 and 6, doubling times were rather long in all cases. During December, doubling times ranged from around 15 days in Gerlache Strait and Bellingshausen Sea to 46 days in Bransfield Strait. In January, doubling times were longer, between 32 and 75 days in all areas. This is opposite to the sequence found in the RACER study, where a peak of autotrophic activity in December was followed by a peak in prokaryotic heterotrophic activity in January. This sequence was particularly marked in Gerlache Strait. In the FRUELA study, on the other hand, PHP was always higher in December than in January (Table 4), while prokaryotic abundance and chlorophyll concentrations were higher in December in some areas (for example Bransfield Strait) and in January in other areas (Gerlache Strait). In Bellingshausen Sea prokaryotic abundance did not change significantly, while chl *a* concentration decreased four times between December and January. This suggests that the spring phytoplankton bloom event, and the ensuing heterotrophic prokaryotic plankton activity peak followed separate courses in the different areas studied. Comparing FRUELA and RACER, these results also indicate that the sequence may occur at different times in different years. Thus, the model of microbial succession proposed by Karl (1993) will accelerate or decelerate depending on the particular area, local weather and year analyzed. On the other hand, the FRUELA data confirm the lack of a closely coupled response shown by heterotrophic prokaryotes to changes in phytoplankton abundance and activities in several Antarctic ecosystems.

4.3. Comparison with phytoplankton biomass and production

Using a carbon:Chl *a* factor of 50 and the cited prokaryotic carbon content, prokaryotic biomass was on average 16% of phytoplankton biomass. The maximal value was 50% and most values were under 25%. This ratio around 20% is similar to that in many coastal and eutrophic environments, and much lower than that in oligotrophic oceans (see discussion in Pedrós-Alió et al. 1999).

PHP and primary production (Varela and Fernández, 2002) were determined simultaneously in 18 stations. PHP was generally a small fraction of primary production, ranging between 0.4% and 28%. Only in one station was PHP larger than primary production (133%) in a case where primary production was extremely small. This percentage decreased from December to January five times in Bransfield Strait (from 20% to 4%) and about 4 times in Gerlache Strait (from 9% to 2.6%). In Bellingshausen Sea this comparison could be carried out only in January. Excluding the station with a 133%, the average was 21%. Altogether, heterotrophic prokaryotic plankton made up a modest percent of both phytoplankton biomass and primary production.

4.4. Comparison with general models of the microbial food web in aquatic systems

In this section we analyze how the relationships between heterotrophic prokaryotes and phytoplankton parameters found above compare to the empirical models from the literature.

4.4.1. Prokaryotic abundance (PN) vs chlorophyll a (Chl a)

A relationship between prokaryotic abundance (PN) and Chl *a* concentration has been found across planktonic systems (Bird and Kalff, 1984; Cole et al., 1988). This relationship is assumed to be a consequence of the trophic relationship between primary producers and heterotrophic prokaryotes. Individual data sets can then be compared with the general relationship, and this may reflect differences between the particular environment studied and the "standard" situation derived from a large data set in the literature (Pedrós-Alió et al., 2000). In Fig. 7 we compare data from the two FRUELA cruises to the general relationship found by Cole et al. (1988), which has been generally used as a standard. Since the latter

study was completed, the available data have increased considerably. Of special significance, data from oligotrophic open-sea systems have increased significantly. As a consequence, new regression relationships, such as those calculated by Li et al. (1992) and Buck et al. (1996), have a lower slope and higher intercept than the early ones. In Fig. 7 we have plotted the regressions of Cole et al. (1988) and a regression line calculated by us with a larger data set (Pedrós-Alió et al., 2000) for comparison with the Antarctic data (Fig. 7A and C). The first observation is the lack of a significant relationship in 1995 (Fig. 7A) and the presence of such a relationship in 1996 (Fig. 7C). In the second place, both data sets seem to show less prokaryotic abundance than expected from the amount of Chl *a*. This is especially apparent if data are compared with our regression line or with those from Li et al. (1992) or Buck et al. (1996).

Karl et al. (1991) also found this lower than expected abundance of prokaryotes during RACER. These authors, though, only found this discrepancy to be significant at chlorophyll concentrations above $2.5\,\mu g\,l^{-1}$. Almost all our Chl *a* values were lower than this, and yet the same tendency could be seen. This difference between RACER and FRUELA is exclusively due to the way in which the relationships were calculated: while surface values were used in RACER, we used photic zone weighted average values. These values are necessarily lower than surface ones for chlorophyll, since most of the phytoplankton accumulates close to the surface in this area, especially in Gerlache Strait, where 90% of the chlorophyll may be found, at times, in the upper 10 m. In contrast, our weighted average values for prokaryotes, if anything, should be slightly higher than surface values. Thus, in relation to the RACER numbers we should have higher prokaryotic numbers and lower chlorophyll values. Despite this, we still find lower than expected values of prokaryotes with respect to chlorophyll. If the data are compared to our general relationship, the discrepancy is even more apparent. The tendency for lower than expected values of prokaryotes, therefore, is robust. This tendency is consistent with an inefficient transfer of carbon between primary producers and prokaryotes.

4.4.2. *Prokaryotic biomass (PB) vs prokaryotic heterotrophic production (PHP)*

Billen et al. (1990) proposed that the relationship between PB and PHP could be used to examine the relative importance of resource supply (bottom-up control) vs predation (top-down-control) in determining the actual biomass of prokaryotes in any given system. This approach was expanded by Ducklow (1992) using data from very different areas of the world ocean. According to his conclusions, when PB is expressed in $\mu gC\,l^{-1}$ and PHP in $\mu gC\,l^{-1}\,h^{-1}$, slopes lower than 0.4 indicate weak control by resource supply; slopes between 0.4 and 0.6 indicate a moderate degree of resource limitation, while slopes higher than 0.6 indicate strong control by resource supply. In the FRUELA cruises we had estimates of all the factors involved including bacterivory (Vaqué et al., 2002), viral infection (Guixa-Boixereu et al., 2002), and supply of photosynthate by phytoplankton (Moran and Estrada, 2002). Therefore, we had an excellent opportunity to compare the slopes of the regression lines for the different times and areas with the actual measurements of bottom-up and top-down factors. During FRUELA95, Moran and Estrada (2002) compared phytoplankton release of DOC with PHP. Assuming a range of prokaryotic efficiencies in converting DOC to production, DOC release was higher than prokaryotic requirements in 19 out of 20 experiments (Moran and Estrada, 2002). This strongly indicates that prokaryotes were not limited by carbon supply. The fact that all the relationships between PB and PHP calculated for FRUELA95 were not significant is consistent with this lack of resource limitation (Fig. 7B). During FRUELA96, DOC release experiments were not carried out. The slope of the regression line (0.54, Table 5) would suggest moderate limitation by resource supply.

In the Ross Sea, Carlson et al. (1998) found that increases in prokaryotic biomass and carbon demand along a *Phaeocystis* bloom were small compared to changes in phytoplankton biomass and productivity. Since they did not find an accumulation of DOM along the same time period, these authors concluded that DOM release by phytoplankton must have been the limiting

factor for prokaryotic growth and production. However, Carlson et al. (1998) did not determine DOM release directly. Their results, however, are in contrast to those from FRUELA. Perhaps the difference is due to the different composition of the phytoplankton: whereas the community studied by Carlson et al. (1998) was dominated by *Phaeocystis*, the communities during FRUELA had a more diverse composition, including diatoms, cryptophytes, small flagellates and dinoflagellates in addition to some *Phaeocystis* (Varela and Fernández, 2002). Obviously, this question deserves further study, since it is relevant for the long-standing discussion on what limits prokaryotic growth in Antarctic waters and whether the coupling between phytoplankton and bacterioplankton is tight or loose (Billen and Becquevort, 1991; Bird and Karl, 1999; Karl, 1993; Carlson et al., 1998).

If substrate limitation were non-existent (FRUELA95) or moderate (FRUELA96), control of prokaryotic plankton abundance would need to be exerted by top-down factors, and these factors should be stronger in 1995 than in 1996. Bacterivory by protists did not seem to be particularly important or higher in the first cruise than in the second (Vaqué et al., 2002). The impact of viruses, however, was very high (Guixa-Boixereu et al., 2002). In 4 out of 5 experiments, viral infection caused losses equivalent to 100% of PHP or more and to 60–100% of prokaryotic biomass during FRUELA95. During FRUELA96 none of the methods attempted allowed determination of viral impact, perhaps indicating a lower impact (below the detection level of the methods available). If low temperatures were more inhibitory to protists than to prokaryotes, the role usually played by protists in controlling prokaryotic abundance in temperate waters, could be taken over by viruses in cold waters. Since viruses use the prokaryotic cell machinery for reproduction, there would be no differential effect of temperature on prokaryotes and viruses. Although these estimates of viral impact have to be considered very cautiously (see discussion in Guixa-Boixereu et al., 2002), it is attractive to think that top-down control could be exerted, at least partially, by viruses in Antarctic waters.

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