Antarctic Science 15 (1): 85–93 (2003) © Antarctic Science Ltd Printed in the UK DOI: 10.1017/S0954102003001081

Dynamics of bacterioplankton activities after a summer phytoplankton bloom period in Terra Nova Bay

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Abstract: A late summer study of marine bacteria activities, and the interrelationships with the microbial loop and the microbial food chain was carried out from 22 January to 10 February 2000 in a coastal area of Terra Nova Bay (Ross Sea). The objective was to investigate the transition from the end of a phytoplanktonic bloom to the start of winter. Intense bacterial activities, comparable to those of temperate marine environments, were observed. The carbon potentially mobilized from proteinaceous matter was quantitatively the most important source of carbon for the bacterioplankton. The leucine aminopeptidase activity was higher in January samples and decreased towards 10 February whereas an opposite trend was observed for alkaline phosphatase and ß-glucosidase activities. The bacterial production was supported by *c.* 0.2% of the amounts of dissolved organic carbon mobilised by hydrolytic activities and by 7% of inorganic phosphate mobilised by alkaline phosphatase activity. A sharp reduction in the bacterial biomass, possibly due to zooplankton grazing or viral lysis, was observed for the first time in Terra Nova Bay.

Received 1 March 2002, accepted 23 September 2002

Key words: Antarctic, bacterial biomass, bacterial production, ectoenzymatic activities, Ross Sea

Introduction

Antarctic coastal ecosystems have perhaps the most extreme seasonality observed anywhere in the world oceans (Karl 1993). Seasonal sea ice, *c*. 1.5 m thick, covers Terra Nova Bay (Ross Sea) during winter and melts each summer. From late spring to midsummer very major changes in the microbial biomasses has been observed, often comparable to those reported for temperate environments (El-Sayed & Frywell 1993, Karl 1993, Christian & Karl 1995, Ducklow *et al.* 2001).

In Terra Nova Bay previous studies on micro-organisms in the seawater column have focused on estimating population characteristics (distribution, taxonomy, biomass, etc) with little attention to understanding marine bacterial activities (Crisafi *et al.* 2000, Bruni *et al.* 1995, La Ferla *et al.* 1995). This is also true for the whole Antarctic marine environment where, despite the recognized importance of the microbial processes, the energy flux and biogeochemical cycling efficiency has yet to be fully explored (Azam *et al.* 1983, Vincent 1988, Karl 1993, Marchant & Murphy 1994).

The results obtained during three summer investigations in Terra Nova Bay (1987–88, 1989–90 and 1994–95) showed that phytoplankton biomass was generally very high, and during the summer two blooms developed (Innamorati *et al.* 2000). Phytoplankton shows a patchy distribution, with some areas of bloom dominated by diatoms (*Fragilariopsis*, *Nitzchia*) and *Phaeocystis* sp, and others, mainly dominated by dinoflagellates and other flagellates (Nuccio *et al.* 2000).

An important fraction of the organic matter produced from phytoplankton (proteins, polysaccharides, DNA, RNA, etc) is present in polymeric form, requiring hydrolysis before uptake by bacteria (Azam *et al.* 1995). Therefore, ectoenzymatic hydrolysis of dissolved and particulate organic matter (DOM, POM) for the production of dissolved free amino acids, monomeric carbohydrates and inorganic nutrients is a critical step in the bacterial cycle. These enzymes are mainly associated with the bacterial surfaces and only a small fraction is dissolved in the water (Hoppe 1983*)*. However, the proportion of dissolved free enzymes depends on the type of enzyme and on the trophic processes that have been developed in the environment (Bochdansky *et al.* 1995, Hoppe *et al.* 1998).

During the XV Antarctic Expedition (Italian National Program for Antarctic Research (PNRA)), a study was carried out in a coastal area of Terra Nova Bay. The main aim of the study of bacterial activities was to assess the interrelationships, especially with the microbial loop and the microbial food chain, during the transitional period from the end of a phytoplanktonic bloom to the start of the winter. In particular, the C, N and P mobilisation rates by ectoenzymatic activities (leucine aminopeptidase, ß-glucosidase and alkaline phosphatase), bacterial production, bacterial standing stocks and the amount of C, and other main elements, that have flowed from monomers and inorganic salts to bacterial biomass, were determined.

Materials and methods

Between 22 January–10 February 2000 water samples were taken eight times at two hydrographical stations (Stn

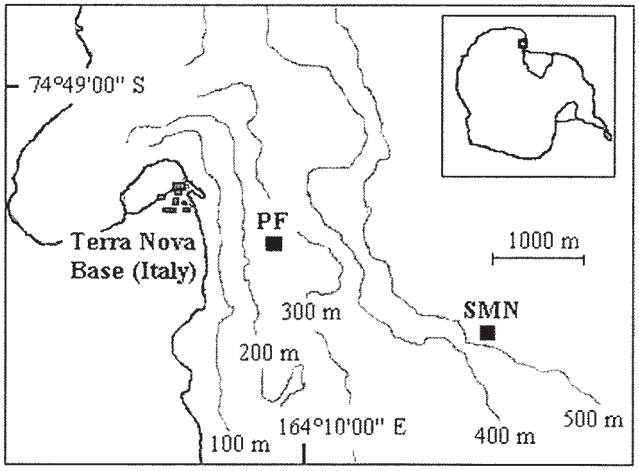

Fig. 1. Area studied and sampling stations.

PF: 74°42.18'S, 164°09.97'E and Stn SMN: 74°43.07'S, 164°16.85'E) at different depths between the surface and 200 m (Fig. 1). Samples were taken using a CTD profiler (conductivity, temperature, depth) equipped with Niskin bottles and a multiparametric probe for fluorescence and oxygen). Samples were collected between 09h00 and 10h00 to minimise the daily variations in the microbiological parameters (Monticelli & La Ferla 2000).

Net bacterial production was estimated using the [^{3}H]-leucine uptake (Smith & Azam 1992) and 1.5 kg C mol^{-1} as the leucine conversion factor (Ducklow 1999). Triplicate samples and blanks were incubated in the dark, for 1 hr at 0.5 ± 0.5°C. Biomass duplication time (in days) of the bacterial assemblage were calculated by dividing bacterial biomass (as µg C l^{-1}) by bacterial production (µg C l^{-1} d^{-1}).

Ectoenzymatic activities (leucine aminopeptidase (LA), alkaline phosphatase (AP) and ß-glucosidase (ßG)) were measured using the fluorogenic substrate analogues l-leucine-4-methylcoumarinyl-7-amide, 4-methylum belliferone-phosphate and 4-methylumbelliferone-ß-D-glucopyranoside, according to Hoppe (1983, 1993). LA and ßG were expressed as amounts of carbon (taking into consideration the number of C atoms of leucine and ß-glucoside) potentially liberated from protein and carbohydrates respectively. AP was expressed as phosphorus potentially liberated from organic phosphorus compounds. All chemicals were purchased from Sigma Co. V_{max} and K_m were calculated from each sample using the double reciprocal transformation of the Michaelis-Menten equation. Triplicate samples and blanks were incubated, in the dark, for two hours at 0.5 ± 0.5°C.

Bacterioplankton (*Eubacteria* + *Archaea*) direct counts (BDC) were determined by epifluorescence microscopy. Water samples were fixed with 2% (final concentration) of neutralized formalin, stored at 0°C for 40 days, filtered through 0.2 µm Nuclepore Corp black filters and stained with DAPI solution for 5 min (Porter & Feig 1980). Bacterial standing stock (as C) and the amounts of N, P and S were determined using conversion factors: 20 fgC per cell (Fuhrman & Azam 1980, Lee & Fuhrman 1987, Ducklow & Carlson 1992) and C:N:P molar ratios (50:13:1) (Fagerbakke *et al.* 1996).

Heterotrophic Culturable Bacteria were estimated on Marine Agar (Difco) plates, spread plated and incubated in the dark, at 2°C ± 2°C for 30 ± 2 days. Results were expressed as colony forming units (CFU) ml^{-1}. Bacterial production, ectoenzymatic activities and culturable bacteria were carried out within two hours of the sampling. Respiration rates (R) and metabolic production of CO_2 of microplanktonic assemblage (0.5–200 µm sized) were obtained by electron transport systems determination according to La Ferla & Azzaro (2001).

A Pearson linear correlation algorithm was used to develop a correlation matrix between bacterial parameters.

Results

Environmental characteristics

During the sampling period there was still some fragments of sea ice around the sampling points. The meteorological and marine conditions of both stations were different. At PF it was possible to carry out the entire sampling programme whereas at station SMN, 2 nm distant and more exposed to intense winds from the south, the original sampling program was drastically reduced. Consequently, the analysed data are mainly from PF.

At PF surface water temperature varied from 0.45°C (22 January) to 0.11°C (10 February) during which period the -1.0°C isotherm rose from 61 m depth to 21 m. In addition, an increase of the water column salinity was observed with a gradual elevation of the isohaline of 34.50 from 47 m to 17 m depth. Below 50 m depth no remarkable variations were observed. The strong stratification observed in the upper layers supported an intense biological activity related to photoautotrophic organisms. *In situ* measures of water chlorophyll (Chl) showed higher values (3–4 mg m^{-3}) in the upper 50 m depth with peaks at *c.* 14 m depth. At the end of the study low Chl values (< 0.6 mg.m^{-3}) were observed. Similar environmental conditions were observed at SMN.

From hereon, unless specifically indicated, all data refer to Stn PF.

Ectoenzymatic activities

Activity profiles of the three ectoenzymes showed similar patterns with higher values in the upper 50 m with decreasing trends from surface towards the bottom.

Leucine aminopeptidase (LA) activity ranged from 0.02 to 113.75 µg C l^{-1} h^{-1} with a mean value of 17.07 ± 24.20 (SD) µg C l^{-1} h^{-1} (n = 48). Integrated values 1–200 m (Fig. 2) vary between 11.39 and 87.71 g C m^{-2} d^{-1} with a mean value of 35.08 ± 26.21(SD) g C m^{-2} d^{-1} (n = 8). An increasing

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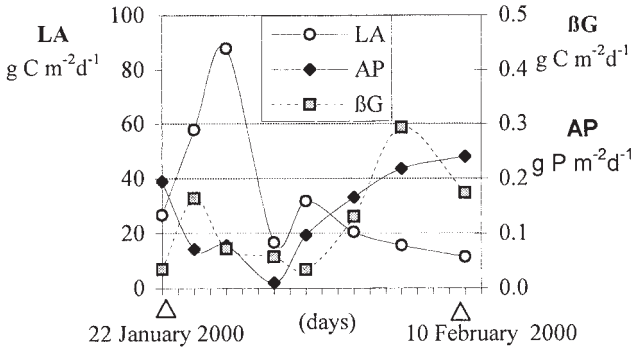

Fig.2. Station PF integrated values (1–200 m) of ectoenzymatic activities: Leucine aminopeptidase (LA), ß-Glucosidase (ßG) and alkaline phosphatase(AP).

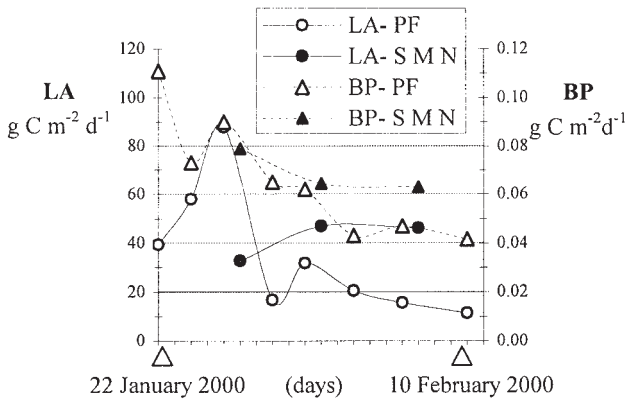

Fig. 3. Stations PF and SMN. Integrated values (1–200 m) of leucine aminopeptidase activity (LA) and bacterial production (BP).

trend was observed from 22–26 January when it reach its maximum value and later, a decreasing trend, reaching the minimal value at the end of the period. In Fig. 3 integrated values in the two stations are presented. At Stn SMN a similar trend was observed with a value *c*. 250% higher than Stn PF at the end of the study.

ß-Glucosidase (ßG) activity ranged from 0 (< detection limit) to 0.13 µg C l^{-1} h^{-1} with a mean value of 0.03 ± 0.03 µg C l^{-1} h^{-1} (n = 48). Integrated values 1–200 m (Fig 3) ranged from 0.03 to 0.28 g C m^{-2} d^{-1}, low values were observed during January and high ones during February; a contrary trend was observed for LA values.

LA activities were always higher than ßG activities. The LA/ßG ratios, calculated from integrated values, ranged from 55 to 1218 (Table I). Higher values were observed in 22 and 26 January, when some single samples reached values of 1626 and 3237. Lower ratios were observed on February samples when some samples reached values of 14 and 24. From each single water column, higher LA/ßG ratios always were observed in the superficial layer (1–25 m depth).

Alkaline phosphatase (AP) activity ranged from 0 (< detection limit) and 0.20 µg P l^{-1} h^{-1} with a mean value of 0.03 ± 0.04 (SD) µg P l^{-1} h^{-1} (n = 42). Integrated values (1–200 m) (Fig. 2) varied form 0.01 to 0.24 g P m^{-2} d^{-1} and showed a similar trend to ßG activities. Molar relations from amounts of C and P potentially mobilized by EEA are indicated in Table I.

Table I. Station PF. Molar ratios calculated from integrated values of ectoenzymatic activities. Total C mobilized as amounts of C from LA + ßG activities and P mobilized by AP activity.

Sampling date	LA/ßG	C/P (LA+ßG/AP)
22 January	1199	523
24 January	353	2093
26 January	1218	2873
29 January	288	4135
31 January	931	843
3 February	100	-
6 February	55	188
10 February	65	125

Bacterial biomass

Integrated values (1–200 m) of bacterial standing stock decreased from 5.63 x 10^{13} (22 January) to 5.80 x 10^{11} cells m^{-2} (10 February) (Fig. 4). From each water column lower single values of BB were always observed below 50 m depth. Integrated values of BB expressed as C, N and P are indicated in Table II**.** The biomass doubling time of the bacterial assemblage varied from mean values of 12.8 days (22 January) to 0.4 days (10 February).

Bacterial production

Absolute values of net production ranged from 0.005 to 0.283 mg C m^{-3} h^{-1} with a mean of 0.059 ± 0.063 (SD) mg C m^{-3} h^{-1} (n = 48). At Stn PF, integrated values (1–200 m) varied from 3.596 (10 February) to 9.596 mg C m^{-3} h^{-1} (22 January). A marked decreasing trend towards 10 February

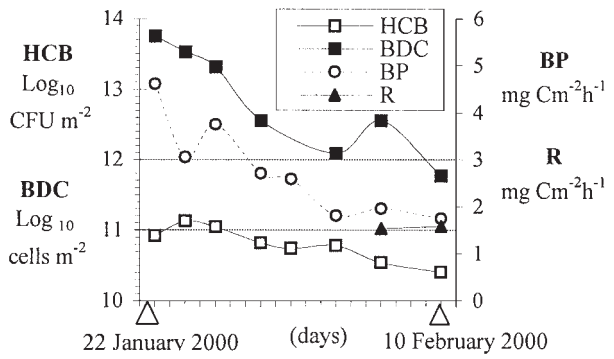

Fig. 4. Station PF. Integrates values (1–200 m) of bacterial production (BP), bacterial direct counts (BDC), heterotrophic culturable bacteria (HCB) and respiration (R).

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Table II. Station PF. Integrated values (1–200 m) of bacterial standing stock and bacterial production as C, N and P.

Sampling date	Bacterial standing stock			Bacterial production		
	mg C m^{-2}	mg N m^{-2}	mg P m^{-2}	mg C m^{-2} d^{-1}	mg N m^{-2} d^{-1}	mg P m^{-2} d^{-1}
22 January	1126.2	341.2	56.3	110.7	33.5	5.5
24 January	671.8	203.6	33.6	73.2	22.2	3.7
26 January	415.2	125.8	20.8	90.0	27.3	4.5
29 January	71.8	21.8	3.6	64.9	19.7	3.2
31 January	7.4	2.3	0.4	62.0	18.8	3.1
3 February	24.3	7.4	1.2	43.2	13.1	2.2
6 February	71.8	21.8	3.6	46.8	14.2	2.3
10 February	11.6	3.5	0.6	41.8	12.7	2.1

was observed (Fig. 4). Considering that new bacterial biomass incorporates elements other than carbon, it is possible to evaluate the amounts of N and P incorporated (Table II).

At Stn SMN, absolute values of production ranged from 0.001 to 0.259 mg C m^{-3} h^{-1} whereas integrated values (1–200 m) ranged from 4.417 to 6.792 mg C m^{-2} h^{-1}. At 1 February and 7 February integrated values were *c.* 104 % and *c.* 134% higher than Stn PF values (Fig. 3).

Respiration

The respiration data are from 6 and 10 February only. Absolutes values of metabolic production of CO_2 ranged from 1.82 and 3.76 µg C m^{-3} h^{-1} (mean 2.37 ± 0.66 (SD) µg C m^{-3} h^{-1} (n = 11) whereas integrated values (1–200 m) were 0.46 mg C m^{-2} h^{-1} (6 February) and 0.47 mg C m^{-2} h^{-1} (10 February)(Fig. 4).

Heterotrophic culturable bacteria

Integrated values as CFU m^{-2}, are shown in Fig. 4. Mean culturable bacteria comprised 1.1% of bacterial direct counts, with a minimum of 0.02% (22 January) and a maximum of 4.4% (10 February). From single data, a negative relationship between culturable bacteria fraction and direct counts was observed (r = -0.35, $P < 0.05$).

Discussion

A decrease in bacterial cell abundance was observed between 22–31 January (98.2% of BDC) and 6–10 February (84% of BDC) (Fig. 4). From 22 January–10 February the bacterial biomass doubling time (BBDT) decreased from 12.8 to 0.4 days. Bacterial generation times of 3.5 to 12 days were observed by Grossmann & Dieckmann (1994) in Antarctic waters. Only BDC from 22–26 January reached mean values (10^{11}–10^{12} cells m^{-3}) in a range commonly observed in Terra Nova Bay waters and other Antarctic marine environments (Bruni *et al.* 1992, 1997, Heinanen *et al.* 1997, Ducklow 1999), at the end of the study BDC had declined to between 10^{8} and 10^{9} cells m^{-3}. This decreasing trend had never been observed in previous studies in Terra Nova Bay (Bruni *et al.* 1992, 1997).

Several factors can control the bacterioplankton standing stock, e.g. availability of dissolved organic matter, inorganic nutrients, abundance and activity of viruses and bacteriovorous microbial assemblages, temperature, UV radiation, etc. In late summer, phosphate concentration in the upper mixed layer varied from 0.21 to 2.48 µM (Grotti & Catalano 1997), but concentration *c.* 0.1–0.2 µM are not a limiting factor for bacterial growth. The moderate alkaline phosphatase activity observed from 22 January–10 February furnished an additional flux of dissolved inorganic phosphate in the upper mixed layer (Fig. 2). Particulate organic carbon (POC) and dissolved organic carbon (DOC) were abundant during a post bloom period (Poutanen & Morris 1983, Fabiano & Pusceddu 1998, Doval *et al.* 2001) which can support an increase in some bacterial ectoenzymatic activities, as observed in Fig. 2. Antarctic bacterial assemblages are mainly composed of psychrophilic and psychrotrophic organisms that display a good adaptation to the prevailing low temperatures (Heinanen *et al.* 1997). Consequently, a variation of *c.* 0.5°C, as observed in surface waters from 22 January–10 February, would produce only very slight variations in bacterial activities. From all controlling factors cited above, only bacterivory by protists and viral lysis can produce severe losses of bacteria cells. In a polar environment, viral lysis is comparable to estimated grazing by flagellates as a source of bacterial mortality (Steward *et al.* 1996). The heterotrophic nanoflagellate community appears to graze substantial bacterial production in Antarctic coastal waters during summer (late January–early February) (Leakey *et al.* 1996). Bird & Karl (1999) observed an average of 3000 cells ml^{-1} of nanoprotist grazers during the spring phytoplankton bloom in Antarctic Peninsula that kept bacterial biomass very low. Also, during late summer, Anderson & Rivkin (2001) noted that microzooplankton consumed nearly all of the local bacterial production in McMurdo Sound (Antarctica). In agreement with these results, it is possible to assume an intense viral lysis and/ or bacteriovory by protists at the PF station during February 2000. Therefore, changes in biomass doubling time could be underestimated because of the simultaneous removal of bacteria by predators (Landry & Hassett 1982)

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The bacterial standing stock (BSS) can be considered as the amount of particulate organic matter, possessing high nutritional quality, available to the planktonic food web. BSS reach a maximum carbon content of 1.12 g C m^{-2} on 22 January and decreased to 0.01 g C m^{-2} (10 February) (Table II) but, as those values depend on carbon conversion factors, the choice of 8.3 fg C $cell^{-1}$ (Fuhrman & Azam 1980) or 11 fg C $cell^{-1}$ (Crisafi *et al.* 2000) against the 20 fgC $cell^{-1}$ used here might reduce by *c.* 50% the amount of carbon indicated in Table II. From 22–31 January a simplistic calculation of the amount of carbon transferred from bacterial biomass to protists biomass may thus be *c.* 1.119 g C m^{-2}, as the difference between initial and final values (1.126–0.007 g C m^{-2}) in nine days, or considered as protein 1.639 g protein m^{-2} in nine days (Jeffrey *et al.* 1996).

Organic detritus, produced in the photic layer, flows to the deep layers and constitutes the main organic nutriment for bottom heterotrophic organisms. Mediated by ectoenzymatic activities bacteria reduce this particulate matter forming monomeric soluble molecules available for the bacterial production. Thus, BSS of free-living cells (not attached to particles) may be considered, also, as the amount of biogenic particulate matter (C, P, N etc) suspended in the water column that can be used by the planktonic community.

During late summer high values of leucine aminopeptidase activity (LA) were observed in both stations (Figs 2 & 3). LA activity detected in Terra Nova Bay was comparable to values observed in both Antarctic waters and temperate environments (Christian & Karl 1995, Hoppe *et al.* 1998, La Ferla *et al.* 2001). During post bloom periods in coastal waters of Terra Nova Bay the particulate organic matter, derived from large phytoplankton cells or phytodetritus, is rich in proteins (57% as biopolymeric particulate carbon) and carbohydrates (25%) (Fabiano & Pusceddu 1998). Thus, the high values of LA observed were expected since they reflect the biochemical nature of particulate matter present in the water. In addition, the dual importance of the LA enzyme, not only in the C cycle but also in the N cycle, should not be forgotten. LA activity was more closely associated with the bacterial cells rather than as dissolved enzyme. This observation was supported by a significant positive correlation between LA activity and bacterial direct counts, bacterial production and culturable bacteria (Table III).

The high LA values indicate a high availability of polypeptides both in the soluble as well as the particulate phase. Such availability is reflected in the high LA/βG ratios observed mainly in surface waters (1–50 m depth). Along the water column a decreasing trend of LA/ ßG ratios were always observed from surface to bottom layers and from 22 January–10 February with the lowest value (LA/ßG = 14) in a 200 m sample at the end of the sampling period. The decreasing trend of LA/ßG from 22 January–10 February depended both on the gradual increase in βG as well as the gradual decrease in LA activities (Fig. 2). The low LA/ßG ratios observed in depth samples were of similar magnitude to the LA/ßG ratios observed in sediments of the Ross Sea by Fabiano & Danovaro (1998). These decreasing trends are indicative of a shift in both availability and nutritional quality of degradable organic matter. Thus, an important fraction of the proteins produced during the phytoplanktonic bloom remain in the water column as dissolved monomers whereas the particulate organic matter, enriched in polymeric carbohydrates fraction, reaches the sediment surface. In any case, senescent diatom cells sink faster than actively growing cells as well as detritus derived from skeletal-less micro-organisms (Mann & Lazier 1996**).** Consequently, particulate matter, rich in proteins, can "rain" on the coastal sediment. Absolute values of ß-glucosidase activities appeared 1–2 orders higher than values observed by Christian & Karl (1995) in Antarctic waters and comparable with values found by Hoppe *at al*. (1998) in Schlei Fjord (northern Germany).

Table III. Station PF. Correlation matrix between ectoenzymatic activities (leucine amino-peptidase (LA), ß-Glucosidase (ßG) and alkaline phosphatase (AP)), bacterial direct counts (BDC), bacterial production (BP) and heterotrophic culturable bacteria (HCB)

Parameter	LA	ßG	AP	BDC	HCB
LA (48)					
ßG (42)	0.44[c]				
AP (42)	0.14	0.19			
BDC (42)	0.70[c]	0.43[c]	0.38[b]		
HCB (48)	0.92[c]	0.55[c]	0.13	0.70[c]	
BP (48)	0.90[c]	0.50[c]	0.32[a]	0.70[c]	0.87[c]

[a]$P < 0.05$, [b]$P < 0.02$, [c]$P < 0.01$

A lower positive correlation observed between ßG activity and bacterial direct count, bacterial production and culturable bacteria (Table III) could suggest that a higher proportion of dissolved (not cell bound) ß-glucosidase, with respect to dissolved LA, was present during the study. An enhancement in the excretion of extracellular enzymes into the surrounding water during the initial phase of bacterial starvation was observed by Albertson *et al.* (1990). In addition, higher amounts of dissolved ßG than dissolved LA, were observed in diverse marine environments (Bochdansky *et al.* 1995, Hoppe *et al.* 1998**).**

Integrated values (Fig. 2) of ßG activities present an increasing trend in February, as does alkaline AP, in opposition to a decreasing trend of LA activity, bacterial production and culturable bacteria (Fig. 4). During the post bloom period an intense bacterial mortality by grazing was suggested. During the zooplankton grazing a considerable amount of dissolved enzymes, mainly α and ß glucosidases, are produced (Bochdansky *et al.* 1995). The increased ßG activity observed during February may be due to grazing activity coupled with a shift in the composition of the polymeric organic matter. Moreover, a possible repression of ßG activity by dissolved monosaccharides could be

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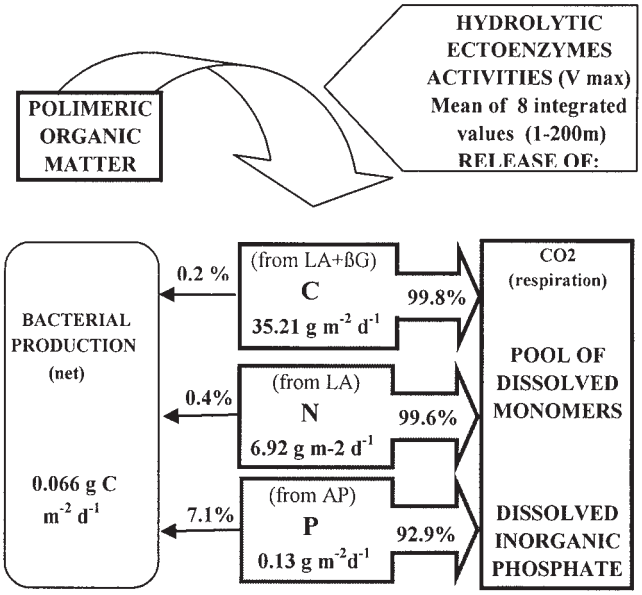

Fig. 5. Station PF. C, N and P fluxes from polymeric organic matter to bacterial production and pool of dissolved monomers and inorganic phosphate.

considered during the first step of the phytoplanktonic post bloom (Middelboe *et al.* 1995).

The aquatic cycling of phosphorus (P) is essentially the conversion of P from organic to inorganic state and vice versa, with micro-organisms playing a key role in both transformations (Jones 1997). Alkaline phosphatase (AP) may be considered both as an indicator of P limitation and an indicator of the potential conversion of organic P into inorganic P. During late summer no limiting concentrations of inorganic phosphate for the bacterial and phytoplankton growth have been previously observed (Grotti & Catalano 1997). Thus, the moderate values of AP activity observed might be partly explained by a sufficient supply of inorganic phosphate for the microbial growth processes. Alkaline phosphatase activity is not specific to bacteria and has been demonstrated in association with zooplankton and phytoplankton as well (Chrost 1991). Therefore, an important proportion of AP activity during later summer may not have a bacterial origin. This was supported by the low positive correlations observed between AP activity and bacterial direct counts and bacterial production, and the lack of relationship with heterotrophic culturable bacteria and the other hydrolytic enzymes. The enzymatic regeneration of inorganic phosphate is coupled with the regeneration of organic carbon. In mesopelagic waters with high limitation of organic matter for bacterial growth, bacterial phosphatase activity was focused mainly on the recovery of organic carbon compounds, which were contemporaneously produced, rather than on the liberated inorganic phosphate (Hoppe & Ullrich 1999). However, the C/P molar ratios observed (Table I) are higher than ratios indicating a possible carbon limitation. In addition, in the post phytoplanktonic bloom period, considerable amounts of dissolved and particulate organic matter, and ectoenzymatic activities, as observed here, were always present, suggesting it is not an environment with limited carbon for bacterial growth. In late summer, alkaline phosphatase activity, as an indicator for the potential conversion of organic into inorganic phosphate, gave a mean value of 0.13 g P m^{-2} d^{-1} (from integrated values 1–200 m depth) (Fig. 5) remaining mainly (*c.* 93%) as dissolved inorganic phosphate. Dissolved organic phosphate compounds are produced by phytoplankton and bacterioplankton (Paul *et al.* 1987, DeFlaun *et al.* 1987), so bacteria with the enzymatic capabilities of hydrolysing and mineralising these molecules could be founded in any environment. A fraction of the AP activity observed in Terra Nova Bay may be due to this origin.

A summary of C, N and P fluxes mediated by hydrolytic activities, from polymeric organic matter to dissolved monomers and bacterial production, is indicated in Fig. 5**.** These values were calculated from potential enzymatic determinations (as V_{max}) assayed in laboratory under standard conditions utilizing fluorogenic substrate analogues. At substrate saturation the rate of hydrolysis may be significantly higher than *in situ* rates as observed by Christian & Karl (1995) for LA activity. Thus, *in situ* rates of hydrolysis of natural polymeric organic matter could be considerably lower.

Absolute values of bacterial production fell to the ranges previously observed in Antarctic coastal waters from spring to summer seasons (Bird & Karl 1999, Ducklow & Carlson 1992). During a phytoplanktonic spring bloom in the Antarctic Peninsula Bird & Karl (1999) noted an increasing trend in BP values directly associated with Chl *a* concentrations. An opposite situation was observed in Terra Nova Bay during late summer with a decreasing trend of BP values coupled with a decreasing trend of Chl concentration. BP was positively correlated to the bacterial direct counts ($P < 0.01$) and to culturable heterotrophic bacteria ($P < 0.01$) (Table III). This suggested that BP was controlled by the same factors controlling bacterial population. The suggested presence of an abundant community of nanoprotist grazers was the apparent cause of that bacterial reduction. This supposition was also supported by the imbalance observed between bacterial standing stock and bacterial production from 31 January–10 February (Table II). From these values it is possible to make an approximate estimate of the grazed or lysed bacterial biomass.

On 6 and 10 February very low rates of respiration were detected, almost one order lower than those observed in the Ross Sea during ice-melting processes (Crisafi *et al.* 2000), and comparable to those noted by La Ferla & Azzaro (2001), in oligotrophic deep waters of the Levantine Sea (Mediterranean). On the same dates the lowest values of

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bacterial production, heterotrophic culturable bacteria counts, bacterial direct counts and LA activity were also observed. This correspondence of low values suggests a strong reduction of the bacterial population rather than a large proportion of ETS-inactive bacterial cells (Choi *et al.* 1999).

The bacterial contribute to whole community respiration varies widely according to the environmental trophic conditions. Since 1984 it has been well known that heterotrophic organisms < 100 µm in size account for most planktonic respiratory activity in several marine environments (Williams 1984). The few studies performed on size fractionated respiratory rates estimated that 40–90% of the total community respiration is due to bacterial activity (Pomeroy & Johannes 1966, Biddanda *et al.* 1994, Griffith *et al.* 1990, Jahnke & Craven 1995, Toolan 2001). However, the prefiltration method adopted for sizing could reduce the bacterial cell count because of those which remain attached on detritus or trapped within the filters, with a consequent underestimation of the actual activity rates. Other authors (Ducklow *et al.* 2000, Robinson *et al.* 1999, Karl *et al.* 1996, Carlson *et al.* 1999) have suggested that in the Antarctic environments bacterial respiration contributes less than in other marine systems (20–10%). They assumed the proportion of bacterial respiration in the total microbial community respiration was equivalent to the biomass weight because direct measurements were lacking. Notwithstanding that we have a direct estimate of the respiratory activity measured with a well-known technique (Packard 1971, Packard *et al.* 1988), we cannot easily extrapolate the actual bacterial contribution to total respiration owing to the paucity of ETS data and to the lack of size-fractionated measurements. However, assuming that a conservative percentage of 50% of the community respiration rate was from bacterial origin, we can estimate the bacterial growth efficiency (BGE) from the following equation: BGE = BP/BCD (bacterial carbon demand), where BCD = BP + R (Del Giorgio & Cole 1998). The mean value of BGE was 0.60 ± 0.22 (SD) (n = 11), comparable with values observed in coastal areas (Del Giorgio & Cole 1998) and seawater cultures (Carlson *et al.* 1999), as well as from polar oceans by Rivkin & Legendre (2001), who stated that BGE is an inverse function of temperature. Since BGE is also regulated by the BP the use of a leucine conversion factor of 3.1 C mol^{-1} (Kirchman 1993, Ducklow 1999) can produce an important change in BGE value.

In conclusion, during late summer intense bacterial activities, comparable to those of temperate marine environments, were observed. The carbon mobilized from proteinaceous matter was quantitatively the most important source of carbon for the bacterioplankton and for the pool of dissolved monomers. The leucine aminopeptidase activity was higher during January and decreased toward 10 February. An opposite trend was observed for alkaline phosphatase and ß-glucosidase activities. The bacterial production was supported by *c.* 0.6% of the amounts of dissolved organic carbon and dissolved organic nitrogen mobilised by hydrolytic activities and by a 7.1% of inorganic phosphate mobilized by alkaline phosphatase activity. Consequently, most of the hydrolysed organic matter remains available for other trophic levels or chemical processes in the form of monomers and inorganic phosphates.

The sharp reduction in the number of bacterial cells, observed for the first time in Terra Nova Bay was attributed to zooplankton grazing or viral lysis.

Acknowledgements

This study was supported by the National Programme for Antarctic Research (PNRA) funded by the Ministero dell'Università e Ricerca Scientifica e Tecnologica of Italy (Project 8.5/ 1999–2001). We gratefully acknowledge the help with sampling from the crew of the RV *Malippo* and from the technical assistants at the Terra Nova station. Two anonymous reviewers are thanked for comments and constructive criticism of the manuscript.

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