Rapid adaptation of prokaryotes to changing substrate pools in epipelagic waters of an Antarctic polynya during austral summer 2003

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Abstract

Antarctic polynyas are among the most productive areas in the ocean. They are subjected to strong temporal variations, having profound effects on the local ecosystems. We tested the changes occurring in several biogeochemical features as well as in biological dynamics at the transition between late spring and summer in the Terra Nova Bay polynya, in the Ross Sea. Samplings were performed from January 17 to January 20 and after an interval of 5 days, from January 25 to January 26. Hydrological casts and water sampling were performed in the upper 125 m at 14 stations. Chlorophyll *a* (Chl *a*), phaeopigment, dissolved and particulate organic carbon (DOC and POC) concentrations, prokaryotic and nanoplanktonic standing stocks, heterotrophic carbon production (^{3}[H] – thymidine incorporation) and extracellular enzymatic activities were measured at selected depths. The first sampling period was characterized by spring conditions still maintained by the presence in the area of two mega-icebergs (B15 and C19), which caused a delay in the ice melting that started during the second survey. Chlorophyll *a* concentrations were highest during the first period whereas phaeopigments/Chl *a* ratio increased during the following sampling time suggesting a release of senescent phytoplankton from the melting ice, and a change in organic matter (OM) quality. No significant differences in DOC and POC concentration were detected between the two periods together with non varied abundances of prokaryotes and small protists. The changes in OM over time were evidenced also by changing exoenzymatic activity patterns. Notwithstanding the general variations occurring during the surveys, heterotrophic production and prokaryotic growth were not substantially modified, suggesting a rapid (timescales of days) adaptation of prokaryotes to changing organic matter quality for the maintenance of their metabolic requirements.

Key words: Bacteria, Exoenzymatic activity, DOC, POC, Ross Sea

1. Introduction

Dissolved Organic Carbon (DOC) represents the most important substrate on which heterotrophic marine bacterioplankton (Bacteria and Archaea) rely (Azam and Malfatti, 2007). Its temporal dynamics are strongly related to seasonal phytoplankton growth (source) and microbial mineralization (sink) both in coastal and in offshore marine systems (e.g. Hansell et al. 2009). Prokaryotes transform, utilize and produce DOC; under strong predation, mainly exerted by heterotrophic nanoflagellates, their biomass is transferred to higher trophic levels. When grazing pressure is limited, their growth is mainly controlled by viral lysis and DOC availability (Azam et al., 1983). Since DOC is composed by a plethora of molecules with a wide spectrum of liability (Hansell, 2013), prokaryotes need to reshape its pool in order to obtain the compounds they need to sustain their metabolism (Arnosti, 2011). This transformation is typically performed by extracellular enzymes that make bioactive compounds (monosaccharides, amino acids, nucleotides, esters, etc.) available through the hydrolysis of more complex substrates (Hoppe, 1983). Extracellular hydrolytic enzymes can be constitutive or subjected to the specific cell metabolic state which can up- or down-regulate their production. Besides this, enzyme activity is inherently dependent on many environmental factors such as temperature, pH, substrate concentration and structure (Pomeroy and Wiebe, 2001; Arnosti, 2011).

Strong seasonal increase of DOC concentration after phytoplankton blooms are typical of systems that receive high inputs of nutrients over the winter period and polar environments are not an exception. Many Antarctic coastal zones are characterized by the occurrence of polynyas, areas of seasonally recurring open water surrounded by sea-ice (Williams et al., 2007). Due to the combined effects of several factors (e.g. light availability, nutrients supply from the deepest layers and from the melting sea-ice), polynyas are among the most productive marine ecosystems (Sedwick and DiTuillo, 1997; Smith and Gordon, 1997; Arrigo and van Dijken, 2003b). The Ross Sea polynya, for example, experiences one of the widest spring phytoplankton blooms among the Southern Ocean (Sullivan et al., 1993; Arrigo and McClain, 1994) although the interannual variability in the timing of the bloom maximum varies year to year (Arrigo et al., 1998), being mainly related to sea ice dynamics and iron concentration (Smith et al., 2014). Carlson et al. (2000) report DOC increases of 15-30 μM where *Phaeocystis* and diatom (the dominant phytoplankton taxa in the Ross Sea; Smith and Asper, 2001) blooms are particularly intense. Where the blooms are small because of various controls on algal growth, DOC concentrations remain low (Sweeney et al., 2000). When compared to other systems with similar primary production rates, the Ross Sea DOC concentrations are generally modest (Carlson et al., 2000). On the contrary, it has been estimated that particulate

organic carbon (POC) accumulation during austral summer can be as high as 90% of the total organic carbon pool (Carlson et al., 2000).

The downward export of accumulated organic carbon can follow two main pathways, according to the biological or the physical pump (Chisholm, 2000). In the latter case, newly formed water masses can carry to the ocean interior both DOC and POC. In the former case, the sinking of particulate organic material occurs by means of marine snow, faecal pellets, dead organisms, etc. (see Catalano et al., 2009 for a review about C budgets in the Ross Sea). The biological pathway is tightly related to mineralization processes occurring in the euphotic layer i.e. respiration (following production) and degradation processes.

The dynamics of planktonic microbes in the coastal Southern Ocean are known to display marked seasonal patterns (Smith et al., 2014, Pearce et al., 2007); it has also been shown that important variations occur on shorter timescales, as a consequence of defined environmental changes such as sea-ice melting and phytoplankton bloom development and break-down (Monticelli et al., 2003; Celussi et al., 2009b; Hyun et al., in press).

During the XVIII Italian Expedition to Antarctica a set of 14 stations was sampled in Terra Nova Bay (Ross Sea) at the transition between late spring and summer. Samplings were performed in order to provide insights on the short-term dynamics of marine microbial plankton and biogeochemical features of surface polynya waters with a focus on the response of prokaryotes to fast environmental changes (sea-ice melting).

2. Material and methods

2.1 Sampling

Sampling was carried out onboard the R/V Italica in the Ross Sea (Antarctica) as a part of the Italian National Program for Antarctic Research (PNRA) in the framework of the CLIMA Project (Climatic Long-term Interactions of the Mass balance in Antarctica). Stations were located in the Terra Nova Bay polynya and were sampled from January 18th to 26th 2003 (Fig.1). Hydrological casts and water samplings were performed using a SBE 9/11 Plus CTD equipped with different sensors (double temperature and conductivity sensors, oxygen, light transmission, fluorescence, pH) coupled with a SBE 32 Carousel sampler, carrying 24 12-L Niskin bottles. Temperature and conductivity sensors were calibrated before and after the cruise at the SACLANTCENTRE of La Spezia (Italy). The major sensors used have the following ranges and accuracy: pressure 0/10,000, 1.56 psi; temperature -5/+35, 0.002 °C; conductivity 0/7, 0.0003 S m^{-1}.

Samples for chemical and biological analyses were collected at selected depths (from 2 to 5) in the upper 120 m. Detailed information about sampled parameters, stations and depth is provided in Supplementary Table 1.

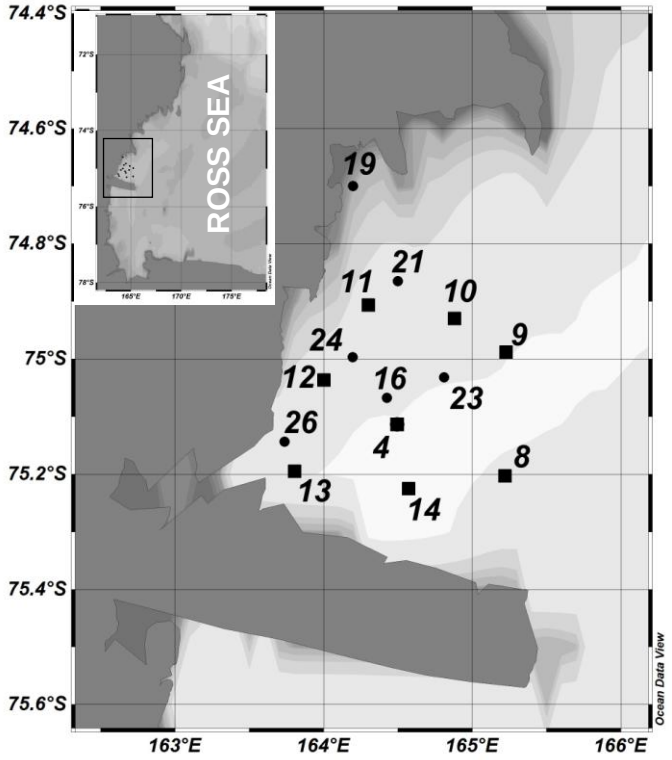

Fig. 1. Sampling stations in Terra Nova Bay (Ross Sea, Southern Ocean). Stations indicated with a full circle (stations 4 to 14) were sampled during the first period (18-21 January 2003); stations indicated with a full square (station 16 to 26) were sampled during the second period (25-27 January 2003). Image drawn by means of the ODV software (Schlitzer, 2014)

2.2 Biogeochemical analyses

The analysis of chlorophyll *a* (Chl *a*) and phaeopigments (Phaeo) was carried out on board. Samples (1 L) were filtered onto glass fibre filters (Whatman GF/F) and stored at –20°C. Pigments were extracted overnight in the dark at 4°C with 90% acetone from the homogenate filter and determined spectrofluorometrically according to Lorenzen and Jeffrey (1980). The measurements of Chl *a* were performed before and after acidification with 2 drops of HCl 1 N by means of a Shimdzu RF-1501 spectrofluorometer at 450 nm excitation and 665 nm emission wavelengths. Calibration was made with pure Chl *a* standards from spinach (Sigma).

Samples for DOC analyses were filtered immediately after collection through precombusted (4h at 480°C) and acidified (HCl 1 - 2 N) Whatman GF/F and stored frozen (- 20 °C) in 20 ml glass vials (previously treated with chromic acid for 24 h, washed with Milli-Q water and precombusted 4h at 480°C). All vials were rinsed three times with the respective sample water before filling to the appropriate volume and immediately closed with teflon-lined screw caps. Before analysis, the

samples were acidified to pH < 2 using 100 µL of a HCl 6 N solution and purged for 8 min using high-purity oxygen bubbling (150 mL min^{-1}). DOC analyses were made with the HTCO method using a Shimadzu TOC 5000A with a quartz combustion column in the vertical position filled with 1.2 % Pt on silica pillows with an approximate diameter of 3 mm (Cauwet, 1994). Details on the procedure are reported by Celussi et al. (2009a).

Particulate Organic Carbon (POC) concentrations were measured on samples (1 L) filtered onto precombusted (450 °C for 4h) Whatman GF/F. Filters were placed in precombusted glass tubes and dried at 60°C. POC was determined by high temperature oxidation using a CHNS 2400 Perkin Elmer Elemental Analyzer after acidification with 1 N HCl to remove the inorganic carbon (UNESCO, 1994).

2.3 Microbial abundance and prokaryotic activities

Prokaryote and nanoplankton (heterotrophic nanoflagellates –HNF– and *Phaeocystis* cells) abundances were determined by epifluorescence microscopy after staining the cells with 4,6-diamidino-2-phenylindole (DAPI, Sigma) at 1 µg mL^{-1} final concentration (Porter and Feig, 1980). For prokaryotes, samples (10 mL) were fixed with 2% final concentration of borate-buffered formalin (prefiltered through a 0.2 µm Acrodisc filter). Subsamples were filtered in triplicate onto 0.2 µm black polycarbonate filters (Nuclepore). For nanoplankton, 250 mL were preserved with glutaraldehyde (1% final concentration). Subsamples (30-40 mL) were filtered onto 0.8 µm black polycarbonate filters (Nuclepore). Filters were mounted on microscope slides, between layers of non-fluorescent immersion oil (Olympus), and counted within a few hours using an Olympus BX 60 F5 epifluorescence microscope at X 1000 under a UV filter set (BP 330–385 nm, BA 420 nm). A minimum of 300 cells were counted for each filter.

Heterotrophic C Production (HCP) was assayed by the incorporation of [^{3}H]-thymidine according to Fuhrman and Azam (1982) and Smith and Azam (1992). Triplicate (1.7 mL) samples and one killed control (5% trichloracetic acid, f. c.) were amended with 20 nM of [^{3}H]-thymidine and incubated for 5 h at *in situ* temperature. Incubations were stopped with 100% TCA (5% f. c.).The extraction was carried out with sequential washing with 5% TCA and 80% ethanol. The incorporated radioactivity was counted using a liquid scintillation counter (Packard Tri-Carb 2900 TR). One millilitre of scintillation cocktail (Ultima Gold MV; Packard) and a 3 min counting time were used. Thymidine incorporation was converted into prokaryotic heterotrophic production using conversion factors of Ducklow et al. (1999) established for the Ross Sea bacterial communities (8.6×10^{17} cells mol^{-1}). Carbon content of bacterial cells was taken as 1.87×10^{8} µg C per cell (Lochte et al., 1997)

Extracellular enzymatic activities were assayed on board using fluorogenic substrate analogues (Hoppe, 1993) derived from 7-amino-4-methylcoumarin (AMC) and 4-methylumbelliferone (MUF). Leucine aminopeptidase activity (LAP) was assayed as the hydrolysis rate of leucine-AMC. ß-D-glucosidase (BGLU), lipase (LIP) and alkaline phosphatase activities (AP) were assayed using MUF-ß-D-glucoside, MUF-oleate and MUF-phosphate, respectively. Enzyme activities were expressed in terms of the rate of MUF or AMC production. The substrates were added to 2.5 ml samples at 50-200 µM final concentration (as detailed in Celussi et al., 2009a) and incubated for 3-6 h in the dark at *in situ* temperature. All samples were run in triplicate with 0.2 µm filtered and boiled seawater as controls. Increase of fluorescence due to MUF and AMC hydrolysed from the model substrates was measured using a Shimadtzu RF-1501 spectrofluorometer (MUF = 365 nm excitation and 455 nm emission; AMC = 380 nm excitation and 440 nm emission). Standard solutions of MUF and AMC were used to perform calibration curves. Results are expressed in terms of maximum velocity of hydrolysis (Vmax).

2.4 Statistical analysis

In order to highlight significant differences in the measured parameters between the two periods, data were integrated along the water column (in order to minimize depth-related variations) by means of the trapezoid method and then the nonparametric Mann-Whitney test was performed (STATISTICA, StatSoft).

In order to highlight the main spatial and temporal pattern in organic matter degradation a cluster analysis was performed by using LIP, AP, LAP and BGLU as variables (Celussi and Del Negro, 2012). The analysis was performed by creating a correlation-based distance matrix for all pairs of sampling. The dendrogram was then built by using the unweighted pair-group average method (STATISTICA, StatSoft).

3. Results

Potential temperature / salinity profiles (Fig. 2) highlighted two distinct situations: the first one is a typical spring situation, with a linear mixing in the surface and subsurface layers down to 100 m depth and almost isotherm to the bottom. Surface water did not present the characteristics of real surface water, but seemed the result of modified circumpolar deep water and in situ water below the sea-ice: sea ice in fact is not yet melt and large amount of fast ice is still present. During the second sampling, after the 5 days gap, the situation was quite different. In the intermediate and bottom layers the thermohaline field was unchanged as in the surface layer below $\gamma^n = 28.27$ kg m^{-3}, from 20-30 m depth down to 100. At the surface ice melting was occurring with a strong decrease of

salinity (range from 34.5 to 34.25) and a consequent small decrease of potential temperature. This is particularly evident in the upper 20 m.

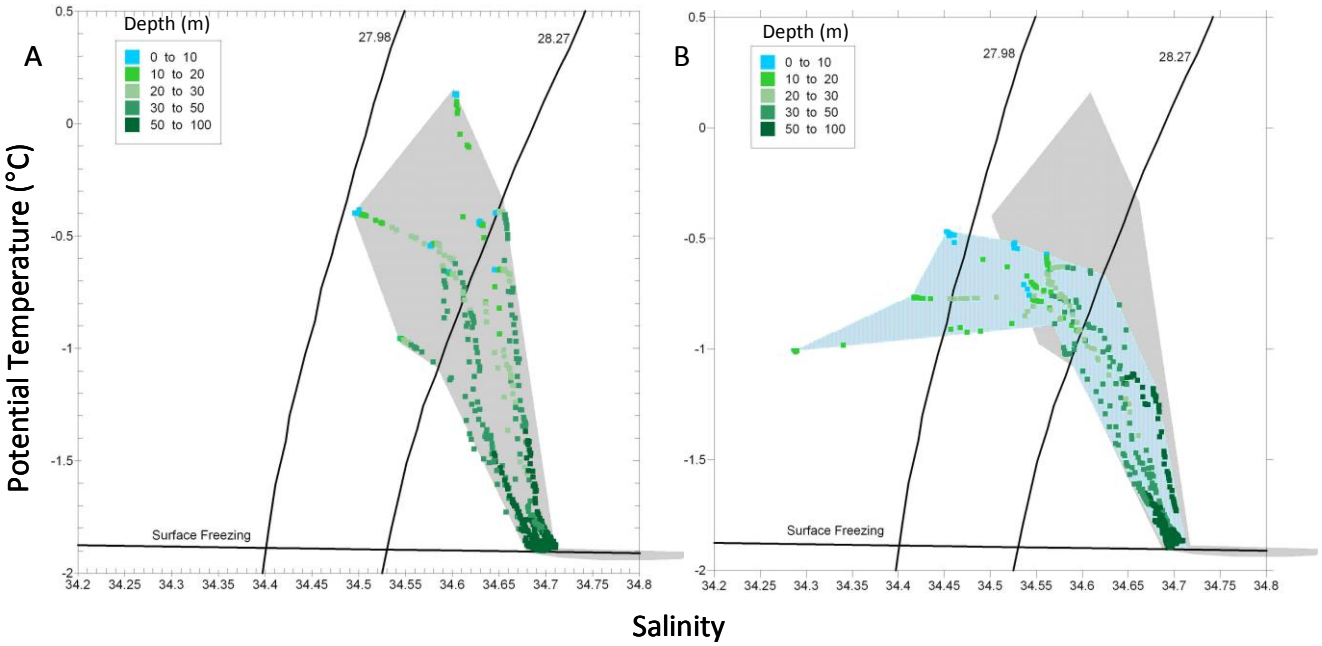

Fig. 2: Θ-S profiles of the upper 120 m during the first (A) and the second (B) sampling period. The plots also show the neutral density anomaly of two characteristic water masses, the γ^n = 27.98 kg m^{-3}, that usually serves as bound between AASW (Antarctic Surface Water) and MCDW (Modified Circumpolar Deep Water), and γ^n = 28.27 kg m^{-3} used to separate LCDW (Lower Circumpolar Deep Water) and the Ross Sea Shelf Water.

Chlorophyll *a* concentrations were maximal during the first period, with values reaching 5.1 µg L^{-1} (median 1.58 µg L^{-1}) (Fig. 3). Concentrations at depths deeper than 50 m were lower, with minima varying between 0.04 µg L^{-1} and 0.30 µg L^{-1} at the bottom of the photic layer (100 - 120 m). In the second period Chl *a* concentrations decreased to a median concentration of 0.62 µg L^{-1}, deeper values ranged between 0.05 µgl^{-1} and 0.30 µgl^{-1}. Median depth integrated Chl *a* values decreased from 1.36 to 0.79 µg L^{-1} in the 2 considered periods (Table 1), being significantly different (Mann-Whitney test: Z = 2.2; p = 0.03; N = 8). As the phytoplankton bloom progressed, depth integrated phaeopigments/chlorophyll *a* ratio significantly differed (Mann-Whitney test: Z = -1.9 ; p = 0.05; N = 8) increasing from 1.16 to 1.50 (median values of the 2 periods, Table 1).

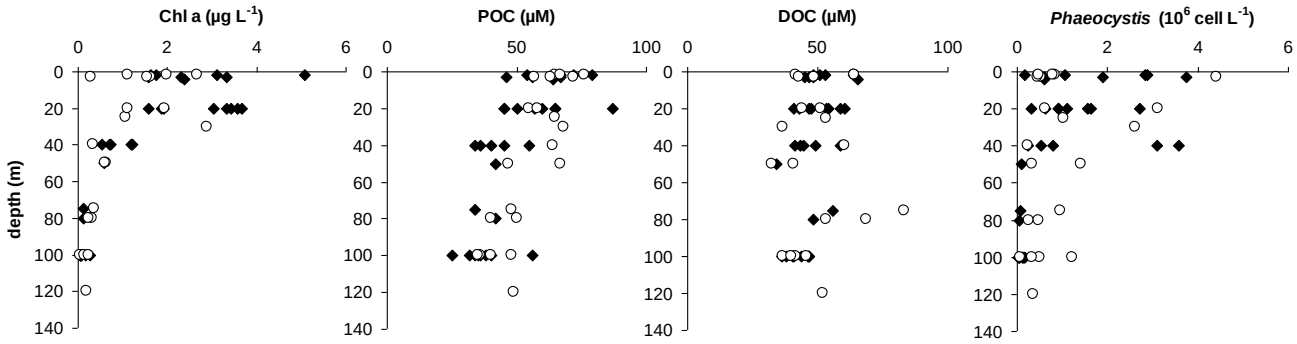

Fig. 3: Depth profiles of Chlorophyll *a* (Chl *a*), Particulate Organic Carbon (POC), Dissolved Organic Carbon (DOC) concentration and *Phaeocystis* abundance in the upper 120 m during the first (full diamonds) and the second (open circles) sampling period.

		Chl *a* ($\mu g\ L^{-1}$)	Ph/Chl *a*	DOC (μM)	POC (μM)	*Phaeocystis* (10^6 cell L^{-1})	Prokaryotes (10^9 cell L^{-1})	HCP (ngC L^{-1} h^{-1})	μ (d^{-1})	HNF (10^6 cell L^{-1})	BGLU (nM h^{-1})	LIP (nM h^{-1})	AP (nM h^{-1})	LAP (nM h^{-1})
Period I	median	**1.36**	**1.16**	46.97	46.00	0.65	0.27	29.83	0.13	0.22	0.11	0.31	**0.19**	2.68
	st.dev	**0.37**	**0.33**	4.26	8.16	0.71	0.06	29.84	0.18	0.14	0.19	0.23	**0.92**	4.48
	N	**8**	**8**	8	8	8	8	4	4	8	4	4	**4**	4
Period II	median	**0.79**	**1.50**	50.95	53.53	0.65	0.26	30.60	0.15	0.34	0.38	4.05	**3.02**	1.62
	st.dev	**0.29**	**0.27**	10.19	10.39	0.65	0.10	8.53	0.05	0.24	0.47	3.40	**1.81**	1.05
	N	**6**	**6**	6	6	6	6	4	4	6	4	4	**4**	4

Table 1: Median, standard deviation and number of observations of depth integrated values obtained during the first (January 17th to 20th) and the second (January 25th and 26th) period. Text in bold denotes significant differences between periods ($p < 0.05$) according to the Mann-Whitney test. Chl *a* = chlorophyll *a* concentration; Ph/Chl *a* = phaeopigments/chlorophyll *a* concentration ratio; DOC = Dissolved Organic Carbon; POC = Particulate Organic Carbon; HCP = Heterotrophic Carbon Production; μ = specific growth rate; HNF = Heterotrophic NanoFlagellates; BGLU = β-glucosidase activity; LIP = Lipase activity; AP = Alkaline Phosphatase activity; LAP = Leucine AminoPeptidase activity.

POC concentrations varied between 25 µM and 87 µM in the first period with a median concentration of 46 µM (Fig. 3); in the second sampling time frame the median value increased to 57 µM, with concentrations ranging from 35 µM to 76 µM. Values generally decreased with depth, reaching minima at the bottom of the photic layer. No statistical difference was found between period I and II.

Concentrations of DOC were heterogeneously distributed throughout the photic layer of the water column. In the first days, values varied between 34 µM and 65 µM, with a median of 47 µM (Fig. 3); afterward concentrations generally increased showing slightly higher values (although not significantly different, on an integrated basis), reaching a maximum of 83 µM (median 49 µM) (Fig. 3).

Abundances of *Phaeocystis* showed a general decreasing trend during both periods and did not vary along with time (Fig. 3, Table 1). The maximum, equal to 4.4×10^6 cell L^{-1}, was found at the surface of station 26.

Prokaryotic abundance was rather homogeneously distributed throughout the study period, averaging 3×10^8 cell L^{-1} (Fig. 4). During the first period the maximum abundance ($4.5 \pm 0.4 \times 10^8$ cell L^{-1}) was observed at the surface at station 11, showing a general depth-related decrease, to a minimum of $1.0 \pm 0.1 \times 10^8$ cell L^{-1} (station 11, 100 m). A similar trend was observed in the last days, with values ranging from 1.4 ± 0.1 (station 21, 100 m) to $5.1 \pm 0.3 \times 10^8$ cell L^{-1} (station 19, surface).

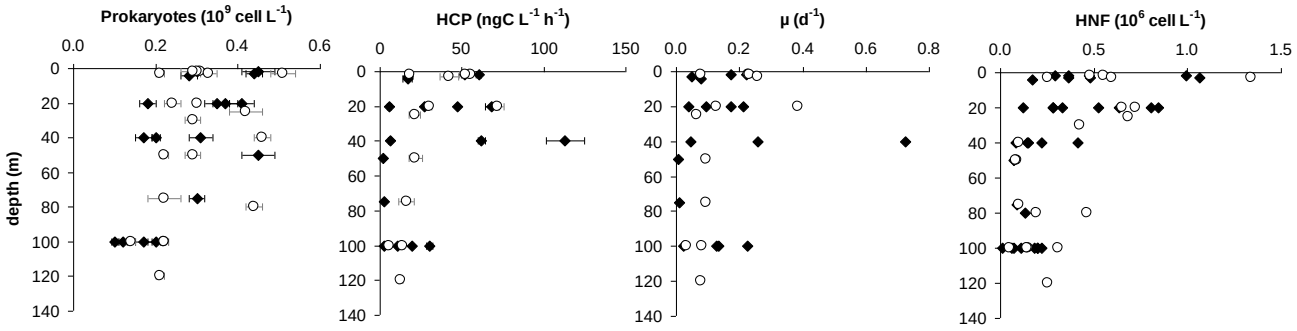

Fig. 4: Depth profiles of Prokaryote abundance, Heterotrophic Carbon Production (HCP) rates, prokaryotic specific growth rates (µ) and Heterotrophic Nanoflagellate (HNF) abundance in the upper 120 m during the first (full diamonds) and the second (open circles) sampling period. Horizontal bars indicate the standard deviation.

Rates of HCP did not significantly differ between the two periods (Table 1, Fig. 4), ranging from 1.8 ± 0.0 to 113.1 ± 11.8 ngC L^{-1} h^{-1} during the first days and from 5.8 ± 0.4 to 71.9 ± 3.5 ngC L^{-1} h^{-1} during the last ones. Specific growth rates followed the same patterns as HCP, with a mean value of 0.15 d^{-1} during both sampling times.

Mean HNF abundance changed from 3.2×10^5 cell L^{-1}, in the first period, to 5.7×10^5 cell L^{-1} in the second period (Fig. 4). Notwithstanding these diverse mean values, depth-integrated cell abundance did not change significantly over time (Table 1).

The fastest exoenzymatic activity was LAP (Fig. 5, Fig. S1) with values as high as 27.2 ± 0.5 nM h^{-1} (station 4, 20 m). Lipase activity was relatively fast during the second sampling period, reaching 10.7 ± 0.9 nM h^{-1} at the surface at station 24. The hydrolysis of polysaccharides, by means of the enzyme β-glucosidase, was the lowest, being < 1 nM h^{-1} with the only exception of station 26, where the highest value equal to 2.8 ± 0.15 nM h^{-1} was measured at 20 m depth. The only hydrolytic activity that displayed significant differences between the two periods was alkaline phosphatase activity (Table 1). Its median integrated value during the first period was 0.19 (±0.92) nM h^{-1} and increased to 3.02 (±1.81) nM h^{-1} during the last days.

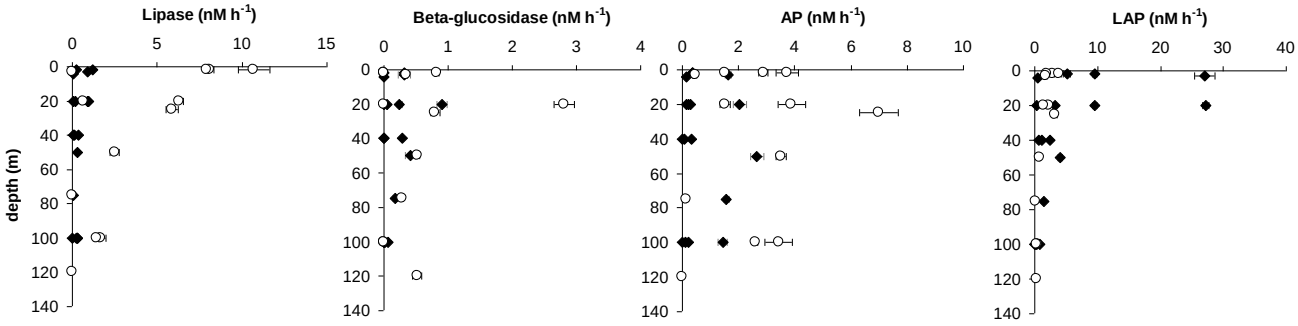

Fig. 5: Depth profiles of lipase, beta-glucosidase, alkaline phosphatase (AP) and leucine aminopeptidase (LAP) activity in the upper 120 m during the first (full diamonds) and the second (open circles) sampling period. Horizontal bars indicate the standard deviation.

The cluster analysis applied to the enzymatic dataset (29 cases) highlighted three main clusters (Fig. 6). The clustering grouped together samples analysed during the first period, those analysed during the second period (except station 26) and those relative to station 26, the latter highly diverse from the first two. The three groups were characterised by a prevailing (in one case by a co-dominance of two) hydrolytic activity. In particular, during the first days, LAP was higher than the other activities, fastest lipase and alkaline phosphatase activities characterised the last days and at station 26 beta-glucosidase was the main degradative activity (surface excluded).

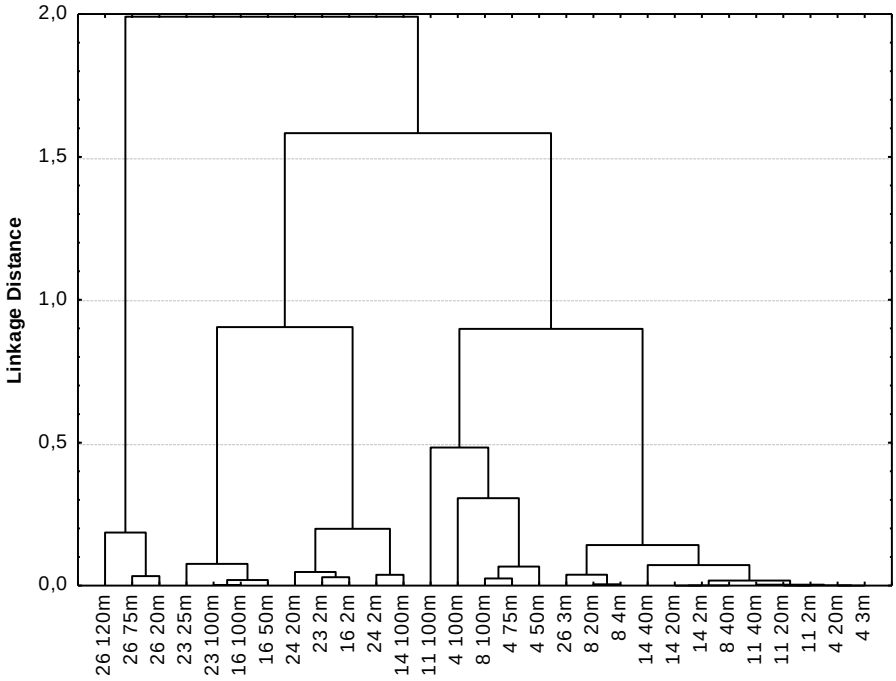

Fig. 6. Cluster analysis dendrogram of degradation patterns performed by using BGLU, LIP, AP and LAP as variables. The dendrogram was built from a correlation-based distance matrix for all pairs of sampling and clusters were formed using the unweighted pair-group average method. Labels indicate station numbers and depths.

4. Discussion

4.1 Short-term changes in water column chemistry and pigment dynamics

During our survey the austral spring-summer dynamics were strongly influenced by the presence of two mega-icebergs (B15 and C19) that prevented the retreat of pack ice, causing a reduction of melting processes. In January 2003 in Terra Nova Bay, and in general all along Victoria Land, sea ice was not melt yet and we observed the presence of a widespread amount of fast ice (Arrigo and van Dijken, 2003a). The climate-induced changes in sea ice coverage have a marked impact on the timing and magnitude of the phytoplankton blooms in the highly productive Ross Sea. Phytoplankton blooms that develop later in the year (beginning in December-January) in the SW Ross Sea, are generally sustained by a high degree of stratification due to the melting of large amounts of sea ice. Not surprisingly, years with heavy sea ice covering are characterized by a delay in phytoplankton blooming and lower annual production than years with lighter sea ice cover (Arrigo and van Dijken, 2003a).

During our first sampling period the water column photic layer showed a typical spring condition; surface and sub-surface layers seem to be the result of mixing between the modified circumpolar deep water with local Antarctic Surface Winter Water below the sea-ice (see Fig. 2). This condition is supported by very limited percentage of meltwater in the surface layers (< 0.4%; Rivaro et al., 2011). At the end of January the sea-ice melting processes started: salinity and potential

318 temperature decreased. The melting was not so intense (meltwater > 0.5%; < 1.5%, calculated
319 according to Rivaro et al., 2011) due to the persistent wide distribution of sea-ice outside Terra
320 Nova Bay, thus we were able to observe the potential temperature decreasing phase, due to the high
321 melting latent heat. During the transition between the two periods the concentration of Chl *a*
322 decreased from 1.36 to 0.79 µg L^{-1} and the Phaeo/Chl *a* ratio increased from 1.16 to 1.50 (median
323 integrated values). Such values are rather low (even considering the maximum = 5.1 µg L^{-1}; Fig. 3)
324 if compared to phytoplankton bloom data in the Ross Sea (up to 11 µg L^{-1}; Arrigo and van Dijken,
325 2004; Mangoni et al., 2004). However, it must be taken into account that ice coverage limits the
326 penetration of light in the water column, thus representing a limiting factor for primary production
327 (Arrigo and van Dijken, 2004). The presence of high concentration of phaeopigments in the second
328 part of the survey indicated a senescent stage of phytoplankton cells, or, most likely, it should be
329 related to phytoplankton derived material released by ice melting processes. It has been previously
330 observed that chlorophyll degradation products are usually more abundant in ice-edge zones where
331 phytoplankton biomass is concentrated and, due to intense predation, phaeopigments are copiously
332 found in faecal pellets of crustaceans (Mangoni et al., 2004). Furthermore, the non significant
333 changes in *Phaeocystis* abundance (Fig. 3, Table 1) coupled with the significant ones in chlorophyll
334 *a* concentration indicate that during the first period a higher relative abundance of diatoms was
335 present (approximately 10-fold higher in abundance than in the second period, M. Cabrini,
336 unpublished data).
337 DOC concentrations measured during our study (median value 46 and 57 µM in the first and second
338 period, respectively) are similar to those reported by Carlson et al. (1998, 2000) for the Southern
339 Ross Sea and the lack of changes between the two periods indicate a relatively small rate of
340 exudation by phytoplankton.
341 We estimated POC/Chl *a* ratio with a Model II Regression (geometric mean) as in DiTullio and
342 Smith (1996). POC/Chl *a* was = 128 in the first period [POC (µg L^{-1}) = 128 Chl *a* (µg L^{-1}) + 401]
343 and 168 in the second period [POC (µg L^{-1}) = 168 Chl *a* (µg L^{-1}) + 512], within the ranges
344 previously reported in the same area (Fonda Umani et al., 2002). Cifuentes et al. (1988) estimated
345 that a POC/Chl *a* ratio value lower than 200 is characteristic of a predominance of newly produced
346 phytoplankton, and that a value higher than 200 is characteristic of detrital or degraded material. In
347 the second period the increased of both slope and intercept suggests a stronger presence of carbon
348 detritus. POC/Chl ratio by DiTullio and Smith (1996) in a diatom-dominated system in the SW
349 Ross Sea showed a POC/Chl *a* ratio relatively higher (210) than ours. Nevertheless, in the SE Ross
350 Sea the same authors found in a *Phaeocystis*-dominated system a value substantially lower (92) than
351 the ratios observed in the diatom-dominated waters. The intermediated values of ratio in our study

are probably correlated to the presence of both *Phaeocystis* and diatoms. Garrison et al. (2003) showed that the development of phytoplankton assemblage in the Ross Sea is not always a simple progression from a *Phaeocystis* bloom in the spring to diatom-dominated conditions in the summer. In our study the evidence of ice melting during the second period strongly suggests that the proliferation of *Phaeocystis*-diatom phytoplankton assemblage was related to seeding from the melting ice.

4.2 Prokaryotic response to short-term environmental changes

In the different conditions that characterised the two sampling periods prokaryotic abundance did not change in a significant way. Although some studies describe a prompt response of prokaryotes to increased primary production (Obernosterer et al., 2008; Hyun et al., in press), it is generally reported that bacterioplankton blooms in Antarctic waters are not coupled to phytoplankton proliferation, but, more frequently, a time gap separates the growth of these groups (Bird and Karl, 1999; Ducklow et al., 1999). The reasons behind this phenomenon are still controversial, indicating a high grazing pressure of bacterivores (Duarte et al., 2005) and/or a combined effect of low temperature and low labile DOC availability (Kirchman et al., 2009) as potential causes. Therefore, it is not surprising that prokaryote abundance did not decrease in the second sampling period following the chlorophyll *a* trend. However, it must be pointed out that the changes we observed in chlorophyll *a* concentrations were not dramatic (~ -0.5 μg L^{-1}), yet significant (Table 1). Variations in environmental conditions, such as during the developmental stages of a phytoplankton bloom, are often linked to changes in prokaryotic community structure (Fandino et al., 2001; Teeling et al., 2012), because algal-derived substrate availability provides a series of ecological niches in which specialized populations can bloom. Since we did not analyse prokaryotic community structure we cannot rule out the possibility that assemblages shifts occurred, still keeping the standing stock unvaried. The succession of different taxa is usually paralleled by variations in functional traits as evidence by Teeling et al. (2012) through metaproteomic analysis. In our dataset heterotrophic production and prokaryotic specific growth rates remained rather constant during the 10-day sampling, indicating that the potential substrate changes (in terms of organic matter quality; POC and DOC did not change in concentration) inferred by the varied phaeopigments over chlorophyll *a* ratio was not *per se* enough to significantly alter secondary production (HCP), as observed elsewhere (Sjöstedt et al., 2013).

Among the prokaryotic activities we have tested, only alkaline phosphatase displayed significant activity changes over time (Table 1). However, the synoptical analysis of several hydrolytic activities can shed light on organic matter quality (Misic et al., 2002; Sala and Güde, 2004; Celussi

and Del Negro, 2012) and therefore we performed a multivariate analysis in order to verify if the changing conditions that were observed between the two sampling periods were reflected into different degradation patterns. The outcome of the analysis clearly highlighted the diverse conditions (Fig. 6). The effect of melting sea ice on organic matter degradation patterns has been previously observed both in the Arctic Ocean and in the Ross Sea (Sala et al., 2010; Celussi et al., 2009b). During our first sampling period, when chlorophyll *a* concentrations were higher, leucine aminopeptidase was the fastest among the tested enzymes (Fig. S1). This is in agreement with what reported by Misic et al. (2002) who displayed a direct correlation between primary production and proteolysis/glycolysis in an extended offshore area in the Ross Sea. The dominance of proteinaceous material in particles is typical of highly productive areas (Pusceddu et al., 1999) and therefore this substrate availability would enhance proteolysis. During the second period, a co-dominance of lipase and alkaline-phosphatase activities was detected. The strong interdependence between these two enzymatic activities has already been observed in a temperate systems (Celussi and Del Negro, 2012) and it has been hypothesised that, since phospholipids represent a large fraction of the dissolved and particulate organic P pool, they both represent a tool for acquiring phosphorus (in the organic and inorganic forms). Since we infer that in our study organic matter changes over time are mainly due to the seeding of material from the melting sea ice, and there is a large body of knowledge on the enhanced lipid production in sympagic communities (Arrigo and Thomas, 2004), the enhanced lipid degradation of the second period could be attributable to a more abundant substrate availability compared to the first sampling days. The large amount of orthophosphates in Ross Sea (see Rivaro et al., 2011 for P-PO4 during the same cruise) prevents planktonic organisms from being P-limited and thus AP is kept to relatively low levels (see Celussi and Del Negro, 2012 for a compilation of literature AP data in P-limited systems). This rapid succession of a prevalent proteolysis-based system followed by a glycolytic/dephosphorilating one had been observed few years before our survey in the same area at 2 fixed stations (Monticelli et al., 2003).

We conclude that, during our survey in Terra Nova Bay, prokaryotic communities rapidly (timescales of days) modified their overall degradation patterns responding to the changing quality of organic matter in order to acquire the needed substrates to keep their metabolic state (unchanged HCP and μ). In such conditions, unvaried prokaryotic standing stocks and production had no effect also on bacterivores that did not display significant modifications in term of abundance over time and this could be possibly extended to the transfer of C to higher trophic levels, highlighting the pivotal role of prokaryotic short-term adaptations within the whole food web.

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