



# Microzooplankton grazing along the Western Antarctic Peninsula

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**ABSTRACT:** The significance of microzooplankton as grazers in pelagic ecosystems has been established, yet relatively few studies of microzooplankton grazing, compared to that of macrozooplankton, have been conducted in the Southern Ocean. We report phytoplankton and bacterial growth and grazing mortality rates along the Western Antarctic Peninsula (WAP), a region of rapid climate change. Growth and grazing rates were determined by dilution experiments at select stations along the WAP in January of 2009 to 2011 and in the nearshore waters near Palmer Station in February and March 2011. Microzooplankton exerted higher grazing pressure on bacteria compared to phytoplankton along the WAP and also selectively grazed on smaller phytoplankton (picoautotrophs and nanophytoplankton) and on the more actively growing (high nucleic acid) bacterial cells. Among all phytoplankton size classes, growth rates ranged from undetectable (i.e. not significant; NS) to  $0.99\text{ d}^{-1}$ , grazing mortality rates were NS to  $0.56\text{ d}^{-1}$ , and microzooplankton removed  $<100\%$  of daily phytoplankton production in all but one experiment. For high and low nucleic acid content bacteria, growth rates were NS to  $0.95\text{ d}^{-1}$ , and grazing mortality rates were NS to  $0.43\text{ d}^{-1}$ ; microzooplankton often removed  $>100\%$  of daily bacterial production. There was a significant (albeit weak) exponential relationship between temperature and phytoplankton mortality, although the range of experimental temperatures was small. The present study provides a reference point of microzooplankton grazing impact along the WAP in the summer and contributes valuable information to studies modeling the flow of carbon through the WAP food web, improving our ability to predict climate-induced changes in the WAP ecosystem.

**KEY WORDS:** Microzooplankton · Protozoa · Grazing · Western Antarctic Peninsula · Southern Ocean · Climate

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## INTRODUCTION

Microzooplankton are significant grazers on phytoplankton and bacteria, can consume a wide range of prey types and sizes (Sherr & Sherr 2002), and influence phytoplankton and bacterial assemblages due to selective feeding (Stoecker 1988, Banse 1992, Sherr & Sherr 1994). Although the importance of microzooplankton grazers in the Southern Ocean has been established (von Bröckel 1981, Buck & Garrison 1983, Hewes et al. 1985, Heinbokel & Coats 1986), there

have been relatively few studies of microzooplankton, compared to meso- and macrozooplankton (i.e.  $>200\text{ }\mu\text{m}$ ). This is due in part to the historical focus on the role of krill as grazers, along with the inherent difficulty of studying microzooplankton and measuring their grazing rates (Landry & Hassett 1982, Dolan et al. 2000, Calbet & Landry 2004, Dolan & McKeon 2004), especially in an extreme environment.

Studies that quantify microzooplankton grazing impact on primary producers in the Southern Ocean report extremely variable phytoplankton mortality

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rates (Garrison 1991), from low to undetectable grazing (maximum grazing mortality =  $0.26 \text{ d}^{-1}$ , Caron et al. 2000) to removal of >100% of primary production (maximum grazing mortality =  $2.36 \text{ d}^{-1}$ , Pearce et al. 2010). Pearce et al. (2008) reported that micrograzers removed up to 762% of primary production (grazing mortality =  $1.13 \text{ d}^{-1}$ ) in the marginal ice zone near Davis Station, East Antarctica, and concluded that microzooplankton are key to controlling and ending phytoplankton blooms at the end of summer in coastal Antarctic waters. In contrast, Caron et al. (2000) assessed microzooplankton grazing in the Ross Sea and found grazing rates statistically greater than zero in only 9 of 34 experiments. Of those 9, all the rates were low ( $<0.26 \text{ d}^{-1}$ ), and they concluded that much of the phytoplankton bloom was not grazed but removed by aggregation and sinking. In the Ross Sea, these low to negligible grazing rates may be due to very low water temperatures that constrain microzooplankton activity and to the presence of large colonies of *Phaeocystis antarctica* and possibly large, unpalatable diatoms that potentially deter microzooplankton grazing (Caron et al. 2000).

In a meta-analysis of the role of temperature on growth rates of aquatic protists, Rose & Caron (2007) proposed that temperature differentially affects heterotrophic protist and phytoplankton growth rates, which could lead to imbalances between phytoplankton growth and mortality in this system. Colder temperatures constrain the growth of heterotrophic protists to a greater degree than phytoplankton, potentially causing low microzooplankton grazing rates at very low temperatures (as seen by Caron et al. 2000). This release from microzooplankton grazing pressure could allow for the large phytoplankton blooms often observed in the Southern Ocean (Rose & Caron 2007).

In addition to their importance as herbivores, microzooplankton are key consumers of bacterioplankton. Flagellate populations can graze from 25% to >100% of the measured daily production of bacterioplankton (Sherr & Sherr 1994) and can considerably alter bacterial assemblages by selective feeding (Sherr et al. 1992, Sherr & Sherr 1994). Bacteria in coastal Antarctic waters ultimately depend on phytoplankton production for organic matter and therefore should be coupled to the phytoplankton dynamics. In a recent time series analysis (2003 to 2011) of bacterial production along the Western Antarctic Peninsula (WAP) during austral summer, bacterial production was positively correlated with phytoplankton biomass (Ducklow et al. 2012b). However, Bird & Karl (1999) reported that bacteria were not correlated

with chlorophyll *a* (chl *a*) during a spring bloom in the northern Antarctic Peninsula and concluded that the bacterial response to the phytoplankton bloom was likely suppressed by grazing by heterotrophic nano-flagellates. Summer bacterial abundances in the WAP are relatively constant (Ducklow et al. 2012a), which could be explained by microzooplankton grazing pressure.

The WAP is a region undergoing rapid warming, with  $1^\circ\text{C}$  increases in average winter air temperature each decade since 1950 (Smith et al. 1996, Vaughan et al. 2003, Ducklow et al. 2012a). The waters along the WAP are seasonally productive and support large populations of zooplankton (e.g. krill) and top predators, such as penguins, seals, and whales (Ducklow et al. 2007, Ross et al. 2008, Vernet et al. 2008, Steinberg et al. 2012). Many components of the food web in this region have been studied extensively (Ducklow et al. 2012a), while microzooplankton have been largely overlooked until recently (Garzio & Steinberg 2013). A previous study that assessed microzooplankton grazing rates near our sampling area (to the north, near the tip of the WAP) showed that although there was significant variability in phytoplankton growth ( $0$  to  $1.16 \text{ d}^{-1}$ ) and mortality ( $0$  to  $0.29 \text{ d}^{-1}$ ), a balance between phytoplankton growth and mortality was observed in half of the experiments (Tsuda & Kawaguchi 1997).

In the present study, we report the first comprehensive analysis of microzooplankton grazing rates along the WAP. We present phytoplankton and bacterial growth and mortality rates as measured by the dilution method (Landry & Hassett 1982) at select locations along the WAP as well as in the nearshore waters near Palmer Station. We investigate selective feeding by microzooplankton on different phytoplankton size classes and bacterial types, in addition to temperature effects on microzooplankton grazing rates. These measurements of microzooplankton grazing rates on phytoplankton and bacteria will help us better understand microbial food web dynamics in a region of rapid climate change and will provide a reference point for future studies.

## MATERIALS AND METHODS

### Study site

As part of the Palmer Antarctica Long-Term Ecological Research (PAL LTER) project (Ducklow et al. 2012a), phytoplankton and bacterial growth and mortality rates were calculated using the dilution method

(Landry & Hassett 1982) on research cruises aboard the ARSV 'Laurence M. Gould' in January (austral summer) 2009, 2010, and 2011 (a total of 12 experiments) in continental shelf waters along the WAP. Experiments were also conducted using near-shore water collected from small boats near Palmer Station, Antarctica (sampling location: 64.78° S, 64.04° W) in February through March 2011 (7 experiments) (Fig. 1).

### Experimental set-up

On the annual January research cruises, water samples for each experiment were collected within the mixed layer (10 to 50 m) using 12 l Niskin bottles mounted on a CTD rosette. At Palmer Station, water samples were collected at a depth of 5 or 10 m using 5 l Go-Flo bottles. All experimental bottles, silicone tubing, and other materials were acid-washed (10% HCl)

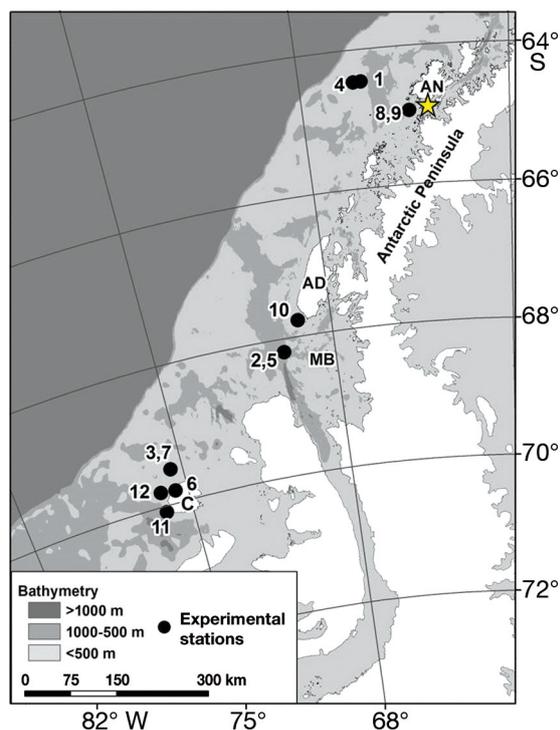


Fig. 1. Palmer Antarctica Long-Term Ecological Research study region where dilution experiments were conducted in January 2009 (Expts 1 to 3), 2010 (Expts 4 to 7), and 2011 (Expts 8 to 12). Stations are labeled with Experiment #, multiple labels (e.g. 8, 9) indicate >1 experiment conducted at that site. The star marks the location where dilution experiments were conducted near Palmer Station in February and March 2011. AN: Anvers Island, AD: Adelaide Island, MB: Marguerite Bay, C: Charcot Island. The continental shelf is ~200 km wide and averages 430 m in depth. Detailed bathymetry is indicated. The light/dark gray interface indicates the shelf break to waters ~3000 m deep (Ducklow et al. 2012a)

and rinsed with Milli-Q water prior to use and between experiments. Plastic nitrile gloves were worn throughout all sampling and experimental manipulations. Filtered seawater (FSW) for experiments was generated by gentle gravity filtration using cartridge filters (0.2  $\mu\text{m}$  pore size), and 'whole' seawater was collected by gentle, reverse-flow filtration through 200  $\mu\text{m}$  mesh to exclude mesozooplankton. According to Caron & Dennett (1999), gentle gravity filtration using cartridge filters does not cause detectable changes in dissolved organic carbon or inorganic nutrients.

A dilution series of 20, 40, 60, 80, and 100% whole seawater was prepared in 9 l clear polycarbonate carboys by first adding the appropriate volume of FSW to each carboy and then gently siphoning whole water directly from the Niskin bottles into the carboys (with outflow carefully maintained under the FSW level to avoid cell breakage). Macronutrients were not added to the experimental carboys on the ARSV 'Laurence M. Gould' cruises because phytoplankton growth in the Southern Ocean is generally not limited by macronutrients (silicate, nitrate, and phosphate), which occur at high concentrations (Hayes et al. 1984, Martin 1990, Ducklow et al. 2012a). All treatments on the 2011 research cruise were enriched with  $\text{FeCl}_3$  to a final iron concentration of 5 nM to avoid iron limitation. A control without iron enrichment was also prepared. Subsequent studies indicate iron limitation only at the offshore stations along the WAP (R. Sherrell & M. Séguret pers. comm.); therefore, iron additions in our experiments, which were conducted at more coastal stations with no evidence of iron limitation, were likely unnecessary but would not have altered the results.

In 2 experiments at Palmer Station (15 and 16 February), carboys were enriched with inorganic nutrients and trace metals (with final concentrations of 10  $\mu\text{M}$  nitrogen as  $\text{NH}_4\text{Cl}$ , 1  $\mu\text{M}$  phosphorus as  $\text{Na}_2\text{HPO}_4$ , 5 nM iron as  $\text{FeCl}_2$ , and 0.1 nM magnesium as  $\text{MgSO}_4$ ) to assess the effects of nutrient addition on the dilution experiments. An additional 100% treatment carboy was prepared without nutrient additions as a control. Experiments in which the difference between the 100% treatment and Fe/macronutrient control were significantly different (Student's *t*-test,  $p < 0.05$ ) were noted, but data were not corrected.

Water from each carboy/dilution treatment was apportioned into triplicate 1.2 l clear polycarbonate bottles. The experimental bottles were placed in an outside incubator with running seawater to maintain ambient temperatures. Two layers of gray window screening were used to reduce light to 20 to 25% of surface irradiance; the bottles were incubated for 72 h (Caron et al. 2000).

## Sample analyses

### Fluorometric chl *a* analysis

Seawater samples (200 to 1000 ml) were taken at initial and final time points (from the carboys and incubation bottles, respectively) and filtered in the dark onto 25 mm Whatman GF/F glass fiber filters (nominal pore size 0.7  $\mu\text{m}$ ; effective pore size is smaller, Chavez et al. 1995) using a vacuum of <200 mm Hg. Filtrate from the 100% treatments were analyzed for initial and final nutrient concentrations. Filters were frozen at  $-80^{\circ}\text{C}$  and stored until analysis at Palmer Station. After removing filters from the freezer, the pigments were extracted in 90% acetone for 24 h in the dark at  $-20^{\circ}\text{C}$ , and chl *a* concentrations were determined fluorometrically using a Turner Designs 10AU digital fluorometer (Knap et al. 1997). These samples are hereafter referred to as the 'Total phytoplankton' size fraction.

### Bacteria and phytoplankton enumeration

SYBR Green1-stained (bacteria) and autofluorescent particles (phytoplankton, <20  $\mu\text{m}$ ) were counted live immediately after initial or final samples were taken using an Accuri<sup>®</sup> C6 flow cytometer equipped with a blue laser beam (50 mW, 488 nm) and CFlow Plus Software. Fluorescent microspheres (1  $\mu\text{m}$ , Polysciences) were added to all samples as standards and were calibrated daily using a SureCount Particle Count Standard (10  $\mu\text{m}$ , Polysciences) bead solution. Particle size was determined by forward scatter (FSC-H) vs. side scatter (SSC-H) comparison with Polyscience Beads of varying size (0.5 to 20  $\mu\text{m}$ ). Flow cytometry was not used for the dilution experiments conducted in 2009.

The prokaryote assemblage along the WAP is a variable mixture of bacterial and archaeal cells. Archaea abundance is relatively low in summer in surface waters where our experiments were conducted (1 to 2%, Church et al. 2003). Our experiments likely included only a small proportion of planktonic archaea; thus, hereafter, we term the assemblage bacterial for convenience. Bacteria were enumerated by staining 0.5 ml water samples with SYBR Green I (final conc. 5  $\mu\text{M}$ ) for 30 to 60 min in the dark. Samples were run for 1 min at a slow flow rate (14  $\mu\text{l min}^{-1}$ , core diameter 10  $\mu\text{m}$ ). High and low nucleic acid content bacteria (HNA and LNA, respectively) (Gasol et al. 1999) were differentiated by plotting red fluorescence (FL3-H) against green fluores-

cence (FL1-H) (Fig. 2a,b). Autotrophic particles were enumerated by running 0.5 ml water samples for 3 min at a fast flow rate (66  $\mu\text{l min}^{-1}$ , core diameter: 22  $\mu\text{m}$ ). Different autotrophic assemblages were separated by plotting SSC-H against FL3-H, and hereafter referred to as the following size fractions: Nano = nanophytoplankton (ca. 2 to 20  $\mu\text{m}$ ), Pico = picoeukaryotes (ca. 1 to 2  $\mu\text{m}$ ) (Fig. 2c,d). SYBR Green counts were not corrected for small numbers of autofluorescent picoplankton (<1% of total).

### Microzooplankton enumeration

Microzooplankton (single-celled protists, 20 to 200  $\mu\text{m}$ ) were enumerated by preserving 250 to 500 ml water samples from the 100% whole water treatments at the initial and final time points with acidic Lugol's (final conc. 6 to 8%). All samples were processed in our laboratory at the Virginia Institute of Marine Science. Microzooplankton were identified to major taxa, but some abundant or conspicuous forms were identified to genus or species level. Samples were first concentrated by settling in the collection jars for at least 1 wk and then removing the top half of the supernatant. Aloricate ciliates, tintinnids, and the silicoflagellate *Dictyocha speculum* were enumerated by settling subsamples of the remaining half in 50 ml Utermöhl chambers, and dinoflagellates were enumerated by settling subsamples in either 10 ml or 50 ml Utermöhl chambers. The entire contents (containing at least 100, but typically 200 to 500 cells of the most abundant taxa) were counted using an inverted microscope after a minimum 24 h settling period (Utermöhl 1931). The silicoflagellate *Dictyocha speculum* was included in the analysis because live silicoflagellate cells have been observed to have pseudopodia extending from their spines, indicating heterotrophy (Martini 1977). Dinoflagellates were counted separately after clearing the sample with several drops of 5% sodium thiosulfate. Heterotrophic nanoflagellates (<20  $\mu\text{m}$ ) were not enumerated as the Utermöhl method severely underestimates their abundance (Davis & Sieburth 1982) (but these organisms are included in grazing measurements as determined by the dilution method, see 'Growth and grazing calculations').

Heterotrophic dinoflagellates were distinguished from autotrophic dinoflagellates using epifluorescence microscopy, both by spot-checking live samples shipboard and by filtration and DAPI staining for analysis back in our home laboratory. Samples (20 to 50 ml) were preserved with glutaraldehyde (final

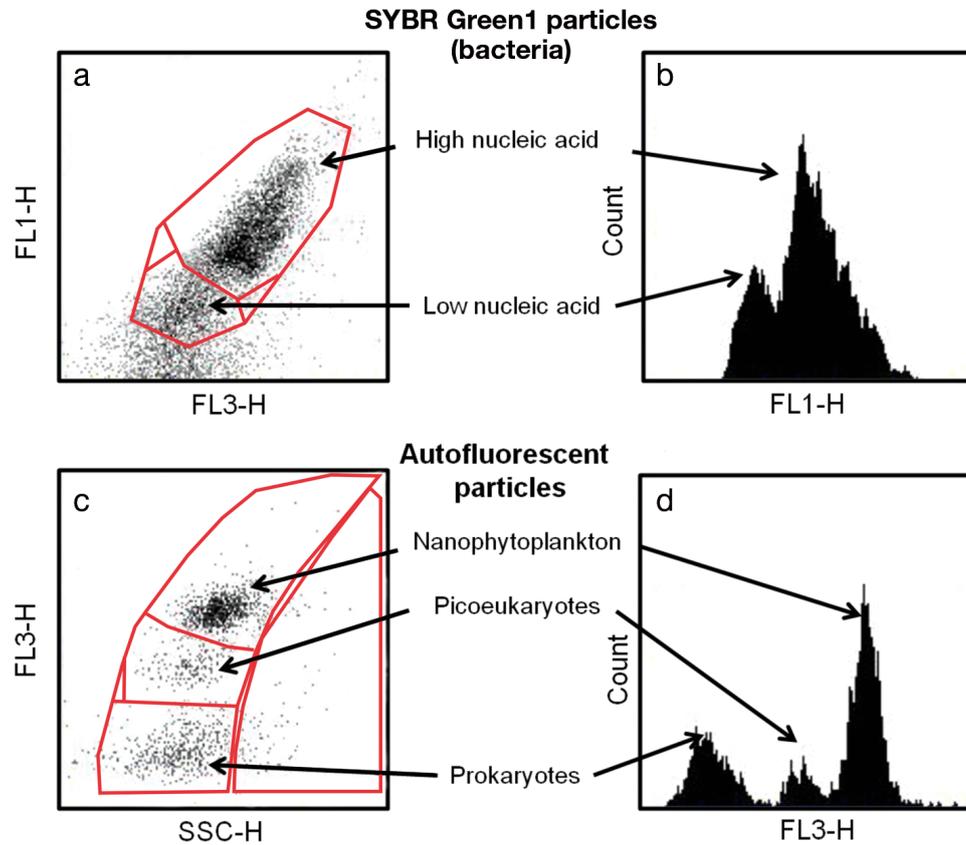


Fig. 2. Examples of (a,c) flow cytometric cytograms and (b,d) histograms generated with CFlow Plus Software to distinguish between different populations of (a,b) SYBR Green1-stained bacteria and (c,d) autofluorescent particles. (a) Red fluorescence (FL3) vs. green fluorescence (FL1) scatter plot, (b) green fluorescence histogram, (c) side scatter (SSC) vs. FL3 red fluorescence scatter plot, and (d) red fluorescence histogram. Size fractions: nanophytoplankton (ca. 2 to 20  $\mu\text{m}$ ), picoeukaryotes (ca. 1 to 2  $\mu\text{m}$ ). Autofluorescent prokaryotes were not analyzed in these experiments

conc. 1%) and stained with 4'6'-diamidino-2-phenylindole (DAPI), then subsequently filtered on 5  $\mu\text{m}$  Nuclepore polycarbonate black membrane filters under gentle vacuum filtration (<200 mm Hg) shipboard. The filter was mounted on a slide and stored at  $-20^{\circ}\text{C}$  until examination with epifluorescence microscopy in our home laboratory (Sherr & Sherr 1993, Sherr et al. 1993). However, because mixotrophy is common among protists (Fenchel 2008), creating divisions between heterotrophs and autotrophs can be misleading. Dinoflagellates were binned into size classes rather than identified by genus or species, and some autotrophs/mixotrophs were most likely included in these analyses.

### Growth and grazing calculations

Dilution experiment results reflect the grazing of the entire microzooplankton community (20 to

200  $\mu\text{m}$ , protozoans and tiny metazoans) as well as all protozoans <20  $\mu\text{m}$  (e.g. heterotrophic nanoflagellates). The dilution method is routinely used to estimate rates of herbivory by microzooplankton, and the assumptions of this method have been tested and proven valid for estimating rates of bacterivory as well (Tremaine & Mills 1987). Numerous studies have calculated bacterial growth and mortality rates using this method (Ducklow et al. 1992, Rivkin et al. 1999, Putland 2000, Anderson & Rivkin 2001, Tjeldens et al. 2008, Pearce et al. 2010, 2011, Dupuy et al. 2011); therefore, rates of bacterivory were also estimated. Growth and mortality (the latter assumed to be equivalent to microzooplankton grazing, hereafter referred to as grazing mortality) rates of phytoplankton and bacteria were estimated using the exponential model developed by Landry & Hassett (1982):

$$P_t = P_0 e^{(\mu - g)t} \quad (1)$$

where  $P_t$  and  $P_0$  are the prey (phytoplankton or bac-

teria) concentrations at time  $t$  and time 0, and  $\mu$  and  $g$  are the instantaneous coefficients of phytoplankton (bacterial) growth and mortality, respectively. The apparent (net) growth rate for each dilution was calculated according to the following equation (Landry & Hassett 1982):

$$\mu = 1/t \ln(P_t/P_0) \quad (2)$$

The basic assumption of the dilution technique is that the observed rate of change of phytoplankton (bacterial) density is a linear function of the dilution factor; in this model, the negative slope of the relationship is the mortality coefficient  $g$ , and the  $y$ -intercept is the phytoplankton (bacterial) growth rate  $\mu$ . The coefficients were determined by least-squares regression analysis of changing phytoplankton (bacterial) concentration vs. dilution factor at the  $\alpha = 0.05$  level of significance. When the slope of the regression was not significant ( $p > 0.05$ , NS), rates were categorized as (1) zero grazing (flat line, no significant difference among growth rates at all dilution treatments determined by ANOVA,  $p > 0.05$ ) or (2) undetectable (scattered points, significant difference in growth rates among  $\geq 1$  dilution treatments determined by ANOVA,  $p < 0.05$ ) (see Tables S1 to S5 in the Supplement at [www.int-res.com/articles/suppl/a070p215\\_supp.pdf](http://www.int-res.com/articles/suppl/a070p215_supp.pdf)). Nonlinear functional responses would indicate saturated grazing in treatments with larger fractions of whole water (Evans & Paranjape 1992); however, this was not apparent in our experiments.

For mortality rates statistically greater than zero, microzooplankton grazing pressure on initial phytoplankton and bacterial stock ( $P_i$  and  $B_i$ , respectively) and potential grazing pressure on primary and bacterial production ( $P_p$  and  $B_p$ , respectively) were calculated according to the following equations (Li et al. 2001):

$$P_i \text{ or } B_i = 1 - e^g \times 100 \quad (3)$$

$$P_p \text{ or } B_p = (e^\mu - e^{(\mu-g)}) / (e^\mu - 1) \times 100 \quad (4)$$

## RESULTS

### Phytoplankton and microzooplankton abundance

Chl  $a$  concentrations at the beginning of the experiments ranged from 0.48 to 12.7  $\mu\text{g l}^{-1}$  on the cruises in 2009 to 2011 (Table S1) and from 0.65 to 4.33  $\mu\text{g l}^{-1}$  at Palmer Station (Table S2). Chl  $a$  concentrations generally increased to the south in all 3 years (Table S1). Expt 10, with a chl  $a$  concentration of

7.40  $\mu\text{g l}^{-1}$  (Table S1), was located in Marguerite Bay, historically a phytoplankton productivity hot-spot (Ducklow et al. 2012a,b). Phytoplankton assemblages in the northern part of the Peninsula were dominated by small autotrophs, while those in the southern part were dominated by large diatoms (L. M. Garzio pers. obs.). Chl  $a$  concentrations in experiments conducted across 2 mo at the same location near Palmer Station (0.65 to 4.33  $\mu\text{g l}^{-1}$ ) did not vary as widely as those observed along the Peninsula. At Palmer Station, the higher chl  $a$  concentrations in experiments in early February (Expts P1 and P2, Table S2) are indicative of the latter part of the summer phytoplankton bloom, followed by decreased chl  $a$  concentrations and then a small secondary bloom in March (Fig. 3).

On average, microzooplankton assemblages in the 100% whole water treatments were numerically dominated by athecate dinoflagellates and aloricate ciliates (Fig. 4), although on the LTER cruises, tintinnids were as abundant as the former 2 groups (Fig. 4a). Tintinnid abundance was dominated by *Salpingella* spp., a genus comprised of generally small tintinnids, while larger tintinnids (i.e. *Laackmaniella* spp.) were much less abundant.

Microzooplankton abundance often changed over the course of the experiments. In each experiment in which microzooplankton were enumerated (all dilution experiments except those performed in 2009), at least 1 taxonomic group either increased or decreased

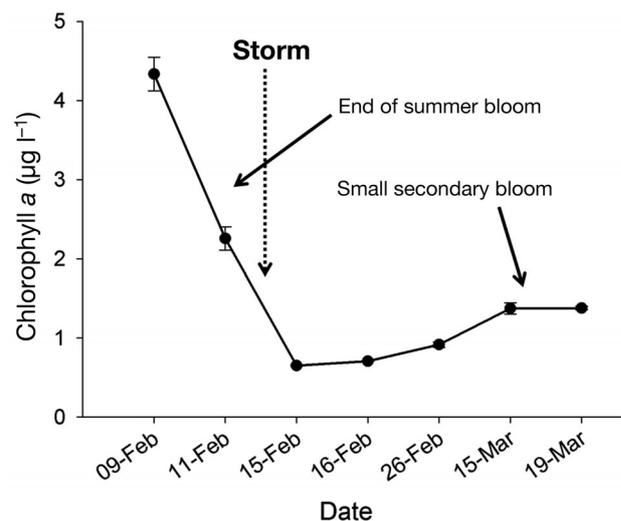


Fig. 3. Chlorophyll  $a$  concentrations from whole water treatments (water screened through 200  $\mu\text{m}$  mesh) at the initial time points from dilution experiments conducted in coastal waters near Palmer Station (64.78° S, 64.04° W) in 2011. Water was collected at 5 or 10 m. A strong storm with wind gusts up to 60 knots occurred between the experiments on 11 February and 15 February. Error bars are standard errors,  $n = 2$

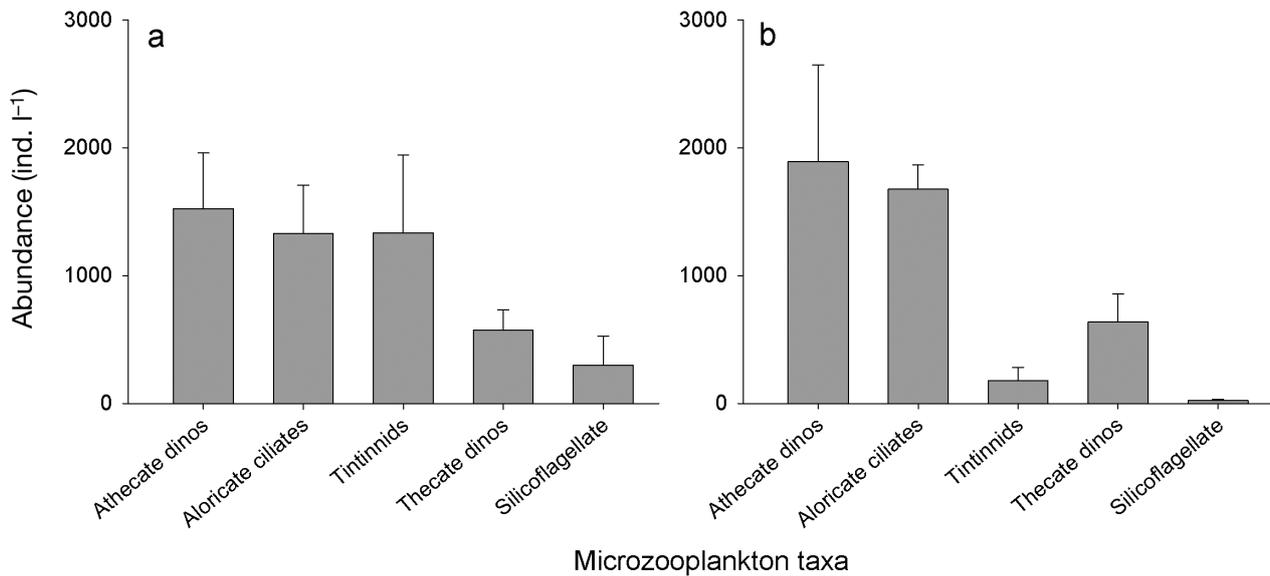


Fig. 4. Initial microzooplankton abundance in dilution experiments. Data from microscope counts of samples taken from the initial time points of dilution experiments done (a) in January 2010 and 2011 ( $n = 8$ ) and (b) at Palmer Station in February and March 2011 ( $n = 7$ ). Error bars are standard errors. For Palmer Station (b), 1 outlier was omitted from the aloricate ciliate average:  $39\,080 \text{ ind. l}^{-1}$  in Expt P5

by at least 25%. For example, in Expts 8 and 9, all groups of microzooplankton more than doubled their abundances by the end of the experiments. In contrast, in Expt P5 at Palmer Station on 26 February, all microzooplankton except the silicoflagellate decreased in abundance by more than half. In fact, a large ciliate bloom at the beginning of this experiment ( $39.1 \times 10^3 \text{ ind. l}^{-1}$ ) was reduced by 1 order of magnitude (final conc.  $3.62 \times 10^3 \text{ ind. l}^{-1}$ ) by the end of the experiment (Table S3).

### Phytoplankton growth and mortality

On the annual LTER cruises along the WAP in 2009 to 2011, phytoplankton mortality rates were significantly different from zero in only 3 of 12 experiments (linear regression,  $p < 0.05$ , Table S1). In these 3 experiments, pico-autotrophs and total phytoplankton growth rates ranged from  $0.13$  to  $0.50 \text{ d}^{-1}$ , and microzooplankton removed 62 to 71% of pico-autotrophic production and 89 to 116% of total phytoplankton production (Table S1). In the nearshore waters close to Palmer Station in February to March 2011, phytoplankton grazing mortality rates were significantly different from zero in 4 of 7 experiments (Table S2). In these 4 experiments, total phytoplankton growth rates were  $0.33$  to  $0.55 \text{ d}^{-1}$  and grazing mortality rates were  $0.10$  to  $0.31 \text{ d}^{-1}$  (Fig. 5a, Table S2). These rates were significantly lower than growth and

grazing mortality of the smaller phytoplankton size classes (Pico + Nano) ( $0.52$  to  $0.99 \text{ d}^{-1}$  and  $0.34$  to  $0.56 \text{ d}^{-1}$ , respectively) (Fig. 5a, Table S2, Student's  $t$ -test,  $p < 0.01$ ). Phytoplankton growth rates were always higher than grazing mortality rates, and microzooplankton removed 32 to 71% of phytoplankton production (Fig. 5b, Table S2). Microzooplankton removed significantly more small phytoplankton (Pico + Nano) production (61 to 71%) compared to total phytoplankton production (32 to 63%) (Student's  $t$ -test,  $p < 0.05$ ).

A strong storm with wind gusts up to 60 knots occurred between experiments conducted on 11 and 15 February 2011. Before the storm, chl *a* concentrations and phytoplankton growth rates were high, and there was relatively high grazing mortality on all 3 size classes. Immediately after the storm (experiments conducted on 15 and 16 February), phytoplankton growth and mortality rates were low or negligible, and phytoplankton biomass was low (Figs. 3 & 5).

### Bacterial growth and mortality

On the LTER cruises in 2010 and 2011, microzooplankton grazing rates on HNA, LNA, or the total bacterial assemblage were significantly different from zero in all but one experiment (Table S4). Growth and grazing mortality rates for the total bac-

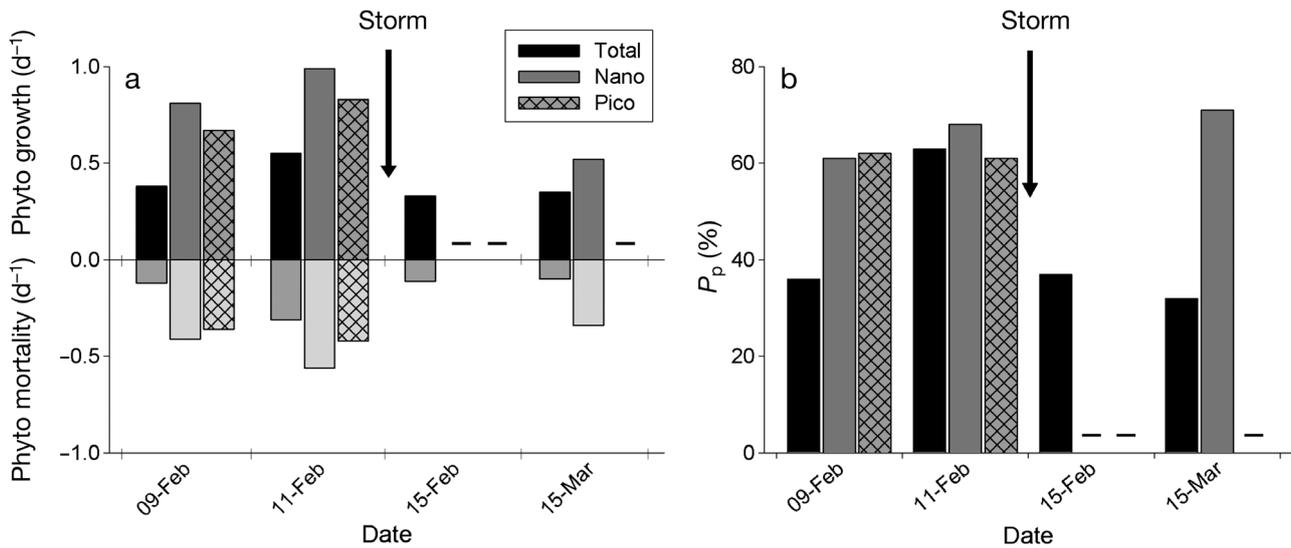


Fig. 5. (a) Phytoplankton growth and grazing mortality rates and (b) the percent of primary production ( $P_p$ ) removed in dilution experiments conducted at Palmer Station in February and March 2011. Size fractions: Total: chl *a* on GF/F filters, nominal pore size  $0.7 \mu\text{m}$ ; Nano: nanophytoplankton (ca. 2 to  $20 \mu\text{m}$ ); Pico: picoeukaryotes (ca. 1 to  $2 \mu\text{m}$ ). Nano and Pico size fractions determined using flow cytometry. One experiment per date. Experiments with no grazing/mortality rates on any phytoplankton size fraction significantly different from zero were omitted (see Table S2 in the Supplement for all results). Arrow indicates occurrence of a strong storm with wind gusts up to 60 knots

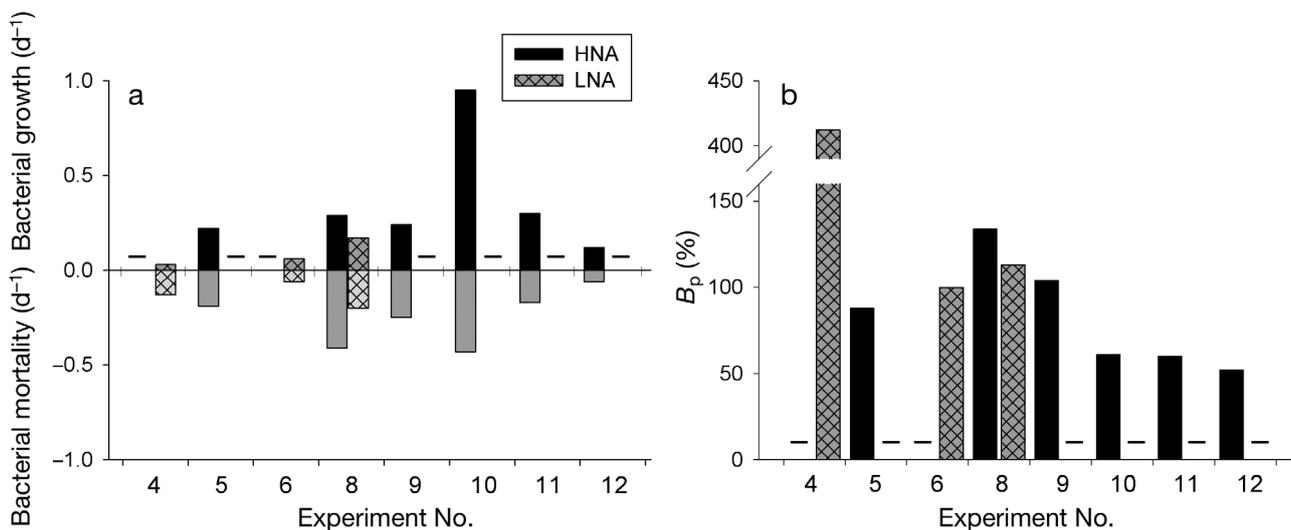


Fig. 6. (a) Bacterial growth and grazing mortality rates and (b) the percent of bacterial production removed in dilution experiments conducted in January 2010 (Expts 4 to 6) and 2011 (Expts 8 to 12). HNA: high nucleic acid content bacteria; LNA: low nucleic acid content bacteria. For information on 'Total' bacteria, see Table S4 in the Supplement. Experiments with no grazing/mortality rates on any bacterial type significantly different than zero were omitted (see Table S4 for all results). See Fig. 1 and Table S1 in the Supplement for experiment locations

terial assemblage ranged from  $0.04$  to  $0.82 \text{ d}^{-1}$  and from  $0.08$  to  $0.38 \text{ d}^{-1}$ , respectively (Table S4). In both years, average growth and grazing mortality rates for HNA bacteria were higher than those for LNA bacteria (Student's *t*-test,  $p = 0.07$  and  $0.1$ , respectively). Growth and grazing mortality rates of HNA were  $0.17$  to  $0.95 \text{ d}^{-1}$  and  $0.08$  to  $0.43 \text{ d}^{-1}$ , respectively; growth and grazing mortality rates of LNA were  $0.03$

to  $0.17 \text{ d}^{-1}$  and  $0.06$  to  $0.20 \text{ d}^{-1}$ , respectively (Fig. 6a, Table S4). Although growth and grazing mortality were higher for HNA bacteria, the calculated proportion of HNA or LNA production removed by grazing did not necessarily follow the same pattern, as microzooplankton removed 52 to 134% of HNA production and 100 to 412% of LNA production (Fig. 6, Table S4). Therefore, when growth and grazing of LNA bacteria

were detectable (in fewer than half of experiments), microzooplankton had a large grazing impact on LNA bacterial production. For example, in Expt 8, LNA bacterial growth and grazing mortality rates were about half of the HNA rates, but microzooplankton removed a comparable amount of HNA and LNA production (Fig. 6, Table S4).

At Palmer Station, microzooplankton grazing rates on bacteria were significantly different from zero in all but one experiment. Growth and grazing mortality rates for the total bacterial assemblage ranged from 0.11 to 0.31  $\text{d}^{-1}$  and from 0.05 to 0.26  $\text{d}^{-1}$ , respectively (Table S5). Growth and grazing mortality rates of HNA cells were 0.18 to 0.36  $\text{d}^{-1}$  and 0.09 to 0.34  $\text{d}^{-1}$ , respectively; growth and grazing mortality rates of LNA cells were 0.07 to 0.23  $\text{d}^{-1}$  and 0.09 to 0.15  $\text{d}^{-1}$ , respectively (Fig. 7a, Table S5). The HNA bacteria often had higher growth rates compared to LNA bacteria. Microzooplankton removed 42 to 158% of HNA production and 51 to 141% of LNA production (Fig. 7b, Table S5).

Although not as dramatic as the effect on phytoplankton growth and grazing rates, the storm also impacted the bacterial dynamics in the microbial food web. HNA bacterial mortality was significantly higher in the experiments directly after the storm compared to before the storm (Student's *t*-test,  $p = 0.02$ ). This is in contrast to phytoplankton growth and mortality, which were lower after the storm.

## Nutrient limitation

Nutrient analyses of water from the initial and final time points from the 100% whole water treatments in experiments indicate that dissolved inorganic nutrients (silicate, phosphate, nitrate + nitrite) were not limiting to phytoplankton growth in experiments in 2010 (Expts 4 to 7) and the experiments conducted in coastal waters at Palmer Station, as nutrients were not depleted in any of the experimental bottles. Also, the N:P ratios at the beginning and end of the experiments were not substantially different from the canonical Redfield ratio of N:P = 16:1 (Table 1; Redfield et al. 1963). Si:P ratios at the beginning and end of the experiments were always well-above the typical ratio of 15:1 documented in other regions of the Southern Ocean (Le Jehan & Tréguer 1983). Nutrients could have been limiting in several experiments along the WAP in 2011, as phosphate was almost depleted in Expts 10 to 12 (Table 1). Although low phosphate concentrations could be explained by the internal storage of phosphate by diatoms (Lund 1950, Tilman & Kilham 1976), any values under 0.1  $\mu\text{M PO}_4$  were considered to be potentially limiting in these experiments. In the few experiments (P3 and P4) with nutrient amendments, phytoplankton growth rates were not significantly different from those treatments without amendments (Student's *t*-test,  $p > 0.05$ ). A few bacterial growth rates were significantly higher

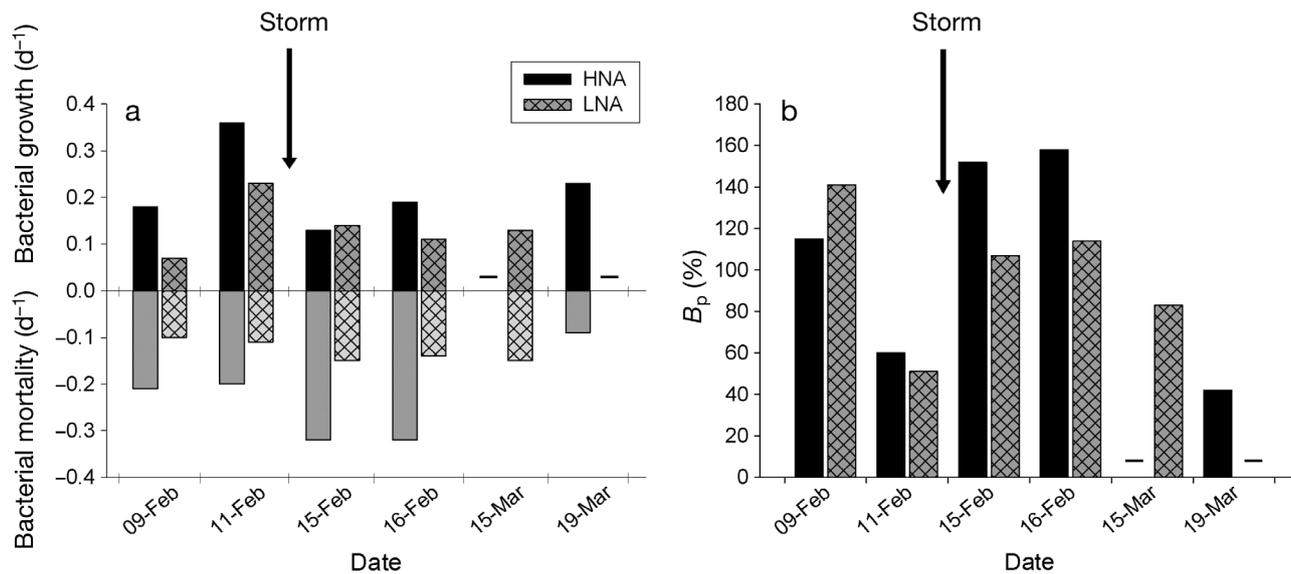


Fig. 7. (a) Bacterial growth and grazing mortality rates and (b) the percent of bacterial production ( $B_p$ ) removed in dilution experiments performed at Palmer Station in February and March 2011. HNA: high nucleic acid content bacteria; LNA: low nucleic acid content bacteria. For information on 'Total' bacteria, see Table S5. One experiment per date. Experiments with no grazing/mortality rates on any bacterial type significantly different than zero were omitted (see Table S5 for all results). Arrow indicates occurrence of a strong storm with wind gusts up to 60 knots

Table 1. Nutrient concentrations and elemental ratios in control treatments at the beginning and end of 72 h dilution experiments. Calculated from nutrient analyses of initial and final time points of 100% whole water treatments in each dilution experiment done on the annual LTER cruises in January 2010 and 2011 and at Palmer Station in February and March 2011. Si:  $\mu\text{M}$  silicate ( $\text{SiO}_4$ ); N:  $\mu\text{M}$  nitrate ( $\text{NO}_3$ ) + nitrite ( $\text{NO}_2$ ); P:  $\mu\text{M}$  phosphate ( $\text{PO}_4$ ). Nutrient concentrations reported as mean  $\pm$  SE. Initial time point,  $n = 2$ . Final time point,  $n = 3$ . No SE recorded,  $n = 1$ . **Bold:** possible nutrient limitation (values under  $0.1 \mu\text{M}$   $\text{PO}_4$  considered to be potentially limiting). Dates reported as dd-mo-yy. NS: non-significant nutrient uptake rate. Note: nutrients were not measured in Expts 1–3

Expt. #	Date	Time point	Si ( $\mu\text{M}$ )	N ( $\mu\text{M}$ )	P ( $\mu\text{M}$ )	N:P	$\Delta\text{N}:\Delta\text{P}$	
4	08-Jan-10	Initial	64.9	27.0	1.8	15:1		
		Final	43.1 $\pm$ 7.6	18.7 $\pm$ 4.5	1.2 $\pm$ 0.2	16:1	13:1	
5	18-Jan-10	Initial	71.5	23.3	1.4	16:1		
		Final	66.9 $\pm$ 6.8	20.8 $\pm$ 2.2	1.3 $\pm$ 0.1	16:1	22:1	
6	26-Jan-10	Initial	81.1	28.6	1.9	15:1		
		Final	78.2 $\pm$ 0.9	28.0 $\pm$ 0.3	1.7 $\pm$ 0.01	16:1	6:1	
7	27-Jan-10	Initial	49.7	11.1	0.7	15:1		
		Final	55.6 $\pm$ 0.9	7.3 $\pm$ 0.3	0.5 $\pm$ 0.0	15:1	16:1	
8	11-Jan-11	Initial	sample lost					
		Final	52.3 $\pm$ 0.3	21.0 $\pm$ 0.3	1.2 $\pm$ 0.01	18:1	–	
9	12-Jan-11	Initial	62.1 $\pm$ 1.2	22.7 $\pm$ 0.9	1.3 $\pm$ 0.0	17:1		
		Final	61.0 $\pm$ 1.0	21.0 $\pm$ 0.4	1.2 $\pm$ 0.01	18:1	NS	
10	18-Jan-11	Initial	28.6 $\pm$ 3.7	4.3 $\pm$ 1.0	0.2 $\pm$ 0.0	23:1		
		Final	<b>28.1 <math>\pm</math> 3.7</b>	<b>1.7 <math>\pm</math> 0.3</b>	<b>0.07 <math>\pm</math> 0.0</b>	<b>25:1</b>	<b>23:1</b>	
11	29-Jan-11	Initial	34.0 $\pm$ 1.8	4.6 $\pm$ 0.5	0.2 $\pm$ 0.0	26:1		
		Final	<b>37.7 <math>\pm</math> 1.6</b>	<b>2.5 <math>\pm</math> 0.5</b>	<b>0.04 <math>\pm</math> 0.0</b>	<b>40:1</b>	<b>15:1</b>	
12	29-Jan-11	Initial	38.8 $\pm$ 2.0	7.5 $\pm$ 0.1	0.2 $\pm$ 0.0	38:1		
		Final	<b>38.9 <math>\pm</math> 1.3</b>	<b>1.5 <math>\pm</math> 0.3</b>	<b>0.03 <math>\pm</math> 0.0</b>	<b>59:1</b>	<b>35:1</b>	
P1	09-Feb-11	Initial	39.7 $\pm$ 1.6	15.0 $\pm$ 0.3	0.7 $\pm$ 0.0	22:1		
		Final	35.9 $\pm$ 2.3	11.2 $\pm$ 0.5	0.5 $\pm$ 0.01	24:1	16:1	
P2	11-Feb-11	Initial	43.8 $\pm$ 2.3	17.2 $\pm$ 0.7	1.0 $\pm$ 0.01	18:1		
		Final	41.5 $\pm$ 2.7	16.1 $\pm$ 0.5	0.8 $\pm$ 0.01	20:1	NS	
P3	15-Feb-11	Initial	37.4 $\pm$ 3.0	17.2 $\pm$ 0.5	1.8 $\pm$ 0.1	9:1		
		Final	39.5 $\pm$ 4.2	16.3 $\pm$ 0.1	1.7 $\pm$ 0.01	9:1	NS	
P4	16-Feb-11	Initial	38.9	17.4	1.8	9:1		
		Final	40.7 $\pm$ 3.6	18.4 $\pm$ 0.1	1.8 $\pm$ 0.1	10:1	NS	
P5	26-Feb-11	Initial	28.9 $\pm$ 7.5	13.3 $\pm$ 0.9	0.7 $\pm$ 0.1	18:1		
		Final	44.8 $\pm$ 1.3	17.5 $\pm$ 0.1	0.9 $\pm$ 0.0	20:1	28:1	
P6	15-Mar-11	Initial	36.9 $\pm$ 2.3	15.8 $\pm$ 0.5	1.0 $\pm$ 0.0	16:1		
		Final	55.7 $\pm$ 0.3	22.5 $\pm$ 0.2	1.3 $\pm$ 0.01	18:1	26:1	
P7	19-Mar-11	Initial	36.7 $\pm$ 3.3	14.2 $\pm$ 0.4	0.9 $\pm$ 0.0	15:1		
		Final	39.3 $\pm$ 1.4	16.4 $\pm$ 0.6	0.9 $\pm$ 0.01	19:1	NS	

with inorganic nutrient enrichments compared to those without amendments (see Tables S4 & S5).

#### Relationship between growth and grazing, and the effect of temperature on grazing

There was a significant positive linear relationship between phytoplankton growth and phytoplankton grazing mortality (Fig. 8a), but growth and mortality rates were not balanced (slope  $\neq 1$ ) because phytoplankton growth rates were usually higher than mortality rates (Tables S1 & S2). There was also a signifi-

cant positive linear relationship between bacterial growth and bacterial grazing mortality (Fig. 8b), although not as strong as the phytoplankton growth vs. mortality relationship. Bacterial growth and mortality rates were also not balanced (slope  $\neq 1$ ).

We also considered the effect of temperature on microzooplankton grazing rates on phytoplankton and bacteria. Phytoplankton growth and grazing mortality rates significantly exponentially increased at higher temperatures ( $p = 0.03$  and  $0.03$ , respectively), although water temperature was a poor indicator of phytoplankton growth and grazing mortality ( $r^2 = 0.21$  and  $0.22$ , respectively) (Fig. 9a). We found a trend of increasing bacterial growth and grazing mortality with increasing temperatures, but these were not statistically significant ( $p > 0.05$ ) (Fig. 9b).

## DISCUSSION

### Phytoplankton growth and grazing mortality

Average phytoplankton grazing mortality rates along the WAP and in the nearshore waters near Palmer Station ( $0.24 \text{ d}^{-1}$  and  $0.30 \text{ d}^{-1}$ , respectively; includes Pico-, Nano-, and Total in Tables S1 & S2) were generally lower than average phytoplankton grazing mortality rates reported in tropical and temperate habitats ( $0.72$  and  $0.69 \text{ d}^{-1}$ , respectively; Calbet & Landry 2004) but near the average for polar waters

( $0.44 \text{ d}^{-1}$ , Calbet & Landry 2004). While there are no published phytoplankton mortality rates using the dilution method in the LTER study region along the WAP, phytoplankton grazing mortality in the Bellinghausen Sea (south of our study area) (Burkill et al. 1995) and near King George Island (north of Palmer Station) (Tsuda & Kawaguchi 1997) are within the same range as our study (Table 2). Other studies in the Southern Ocean show wide ranges in phytoplankton growth (NS to  $2.6 \text{ d}^{-1}$ ) and grazing mortality rates (NS to  $2.36 \text{ d}^{-1}$ ), and most of them report mortality rates that are not significantly different from zero (Table 2).

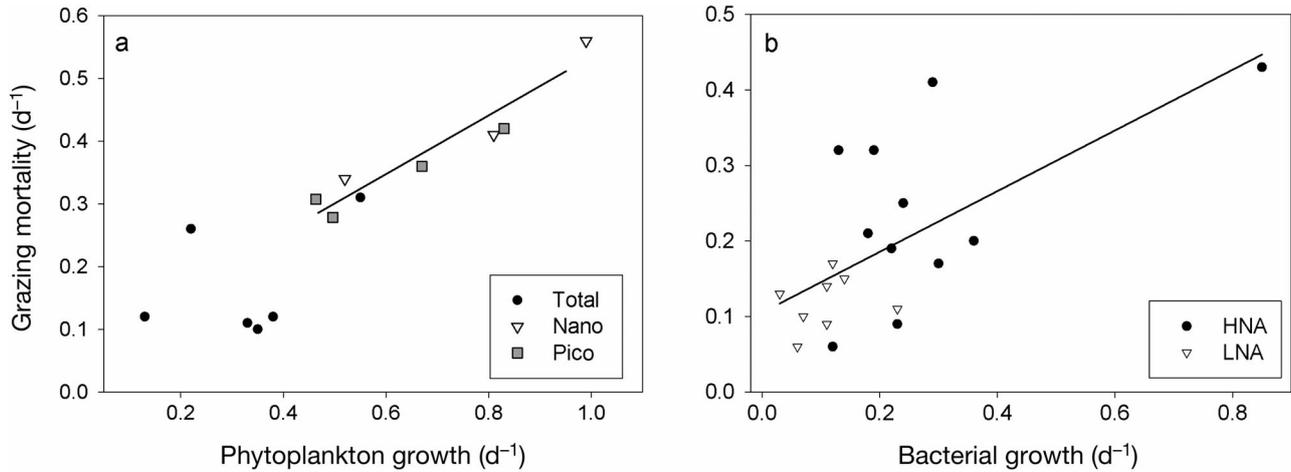


Fig. 8. Relationship between (a) phytoplankton growth and grazing mortality and (b) bacterial growth and grazing mortality. Data pooled from dilution experiments done on the annual LTER cruises in January 2009, 2010, and 2011 and at Palmer Station in February and March 2011. Each point represents growth and mortality rates significantly different from zero on different (a) phytoplankton size fractions and (b) bacterial types. Linear regression equations: (a)  $y = 0.44x + 0.08$  ( $p = 0.001$ ,  $r^2 = 0.88$ ; note: the regression excludes the Total size fraction as Nano and Pico constitute most of Total with the exception of large diatoms) and (b)  $y = 0.40x + 0.11$  ( $p = 0.003$ ,  $r^2 = 0.42$ ). See Figs. 5 & 6 for abbreviations

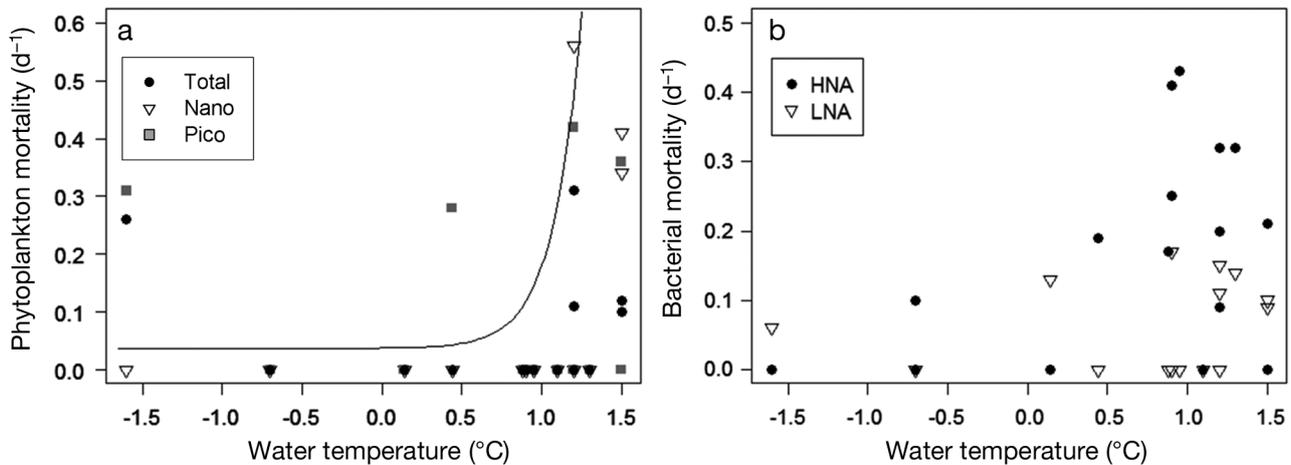


Fig. 9. (a) Phytoplankton grazing mortality and (b) bacterial grazing mortality as a function of water temperature. Data pooled from dilution experiments performed in January 2010 and 2011 and at Palmer Station in February and March 2011. Each point represents grazing mortality rates (non-significant rates = 0) on different (a) phytoplankton size fractions and (b) bacterial types. Each experiment has (a) 3 or (b) 2 corresponding mortality rates. Water temperature was measured by CTD at the location where the water was taken for each experiment. Best-fit equation: (a)  $y = 0.03 + 0.0005e^{5.6x}$  ( $r^2 = 0.21$ ,  $p = 0.03$ ; note: curve excludes the Total size fraction as Nano and Pico constitute most of Total with the exception of large diatoms). Best-fit equation (data not shown) for phytoplankton growth vs. temperature:  $y = 0.006 + 0.0002e^{5.2x}$  ( $r^2 = 0.22$ ,  $p = 0.03$ ). See Tables S1, S2, S4, & S5 (in the Supplement) for values and Figs. 5 & 6 for abbreviations

Phytoplankton growth rates often exceeded microzooplankton grazing rates in this productive season. Microzooplankton grazed 62 to 116% (average = 85%) of primary production in the offshore region in January 2009 to 2011 and 32 to 71% (average = 55%) nearshore by Palmer Station in February to March 2011. Phytoplankton growth and grazing mortality were significantly positively correlated but were not balanced because phytoplankton growth rates were

usually higher than mortality rates. In fact, mortality rates only exceeded growth rates in 1 experiment (Expt 6, Total phytoplankton size class, Table S1). While microzooplankton exert considerable grazing pressure on phytoplankton at certain times and locations in the Southern Ocean (Pearce et al. 2008, 2010), in our study, phytoplankton production exceeded microzooplankton grazing, which could partially explain the large phytoplankton blooms that

Table 2. Summary of published phytoplankton growth and grazing mortality rates calculated from dilution experiments in the Southern Ocean.  $\mu$ : phytoplankton growth rate;  $g$ : grazing mortality rate;  $P_i$  and  $P_p$  are the potential microzooplankton grazing pressure on initial phytoplankton stock and primary production, respectively. NR: not reported; NS: not significant. Values presented for the present study include the pico-, nano-, and total phytoplankton groups

Location	Sampling month	$\mu$ ( $d^{-1}$ )	$g$ ( $d^{-1}$ )	$P_i$ (%)	$P_p$ (%)	Reference
Western Antarctic Peninsula	Jan–Mar	NS–0.99	NS–0.56	NS–43	NS–116	Present study
Bellingshausen Sea	Nov–Dec	NR	0.03–0.52	3–40	NR	Burkill et al. (1995)
Southern Ocean, 0° longitude	Jan–Feb	0.24–1.68	0.04–0.25	4–22	9–46	Froneman & Perissinotto (1996a)
Southern Ocean, 0° longitude	Jun–Jul	0.45–1.48	0.28–0.72	25–51	56–83	Froneman & Perissinotto (1996a)
Southern Ocean, 0° longitude	Jun–Jul	0.17–1.87	0.02–0.58	2–44	9–61	Froneman & Perissinotto (1996b)
King George Island, Antarctic Peninsula	Dec–Feb	NS–1.16	NS–0.29	NR	0–333	Tsuda & Kawaguchi (1997)
Indo-Pacific sector of Southern Ocean	Dec–Jan	NS–0.66	0.01–0.69	NR	0–105	Tsuda & Kawaguchi (1997)
Ross Sea	Oct–Dec	NR	NS–0.26	NR	NR	Caron et al. (2000)
Ross Sea	Jan–Feb	NR	NS–0.11	NR	NR	Caron et al. (2000)
Ross Sea	Apr	NR	NS	NR	NR	Caron et al. (2000)
Prydz Bay	Dec–Jan	0.11–2.60	0.11–1.06	10–65	34–100	Li et al. (2001)
Southern Ocean, 6° E	Dec–Jan	NR	0.04–0.28	5–24	11–35	Froneman (2004)
Southern Ocean, 140° E	Nov–Dec	NR	0.19–1.70	NR	NR	Safi et al. (2007)
Near Davis Station, East Antarctica	Feb–Mar	NS–0.81	NS–0.55	NS–42	NS–40	Pearce et al. (2008)
Near Davis Station, East Antarctica	Apr–Sep	NS–0.44	NS–1.54	NS–79	NS–762	Pearce et al. (2008)
East Antarctica, 30–80° E	Jan–Feb	0.28–1.81	0.31–2.36	30–87	16–223	Pearce et al. (2010)
Sub-Antarctic zone, near Tasmania	Jan–Feb	NS–1.02	NS–1.39	NS–60	NS–118	Pearce et al. (2011)
Kerguelen Islands	Jan–Feb	NR	NS–1.78	NR	NR	Brussaard et al. (2008)

occur in the WAP. During the study period at Palmer Station, chl *a* did not increase, even though growth was greater than grazing. This suggests that other removal processes, such as mesozooplankton grazing, sinking, viral lysis, and advection, may have balanced growth.

Phytoplankton mortality rates were significantly different from zero in 25% (3 of 12) of dilution experiments offshore and in 57% (4 of 7) of dilution experiments nearshore by Palmer Station. Of the non-significant regressions, more than half indicated zero grazing (Tables S1 & S2). The low grazing mortality rates (compared to temperate and tropical habitats) in addition to the fraction of experiments in which grazing was not statistically significant (zero or undetectable) in our study are consistent with the results of Caron et al. (2000). They attributed low ( $<0.26 d^{-1}$ ) and low proportion of (25%; 9 of 34 experiments) significant grazing mortality rates partially to extremely low temperatures, which likely limited microzooplankton herbivory, and to phytoplankton community composition, which can influence microzooplankton grazing.

We compared microzooplankton to macrozooplankton grazing impact in the nearshore and shelf regions of the WAP and found that microzooplankton grazing pressure on phytoplankton, when statistically significant grazing was measured, was higher than macrozooplankton grazing. Grazing rates of the 5 dominant macrozooplankton species in the WAP

(krill *Euphausia superba*, *Euphausia crystallorophias*, and *Thysanöessa macrura*; the pteropod *Limacina helicina*; and the salp *Salpa thompsoni*) were determined separately on the same cruises in 2009 and 2010. In nearshore and shelf waters (where dilution experiments were conducted), the dominant macrozooplankton removed 0 to 3% of primary productivity (Bernard et al. 2012), considerably lower than that removed by microzooplankton (NS to 116%, usually between 30 and 70%). The highest grazing pressure by macrozooplankton (up to 641% of primary productivity) was associated with large salp blooms, which were usually located offshore near the shelf break (Bernard et al. 2012). Although no dilution experiments were conducted off shelf in oceanic waters, macrozooplankton grazing pressure on phytoplankton could have exceeded that of microzooplankton in this region where salps form dense blooms. In the nearshore and shelf waters of the WAP, macrozooplankton plus microzooplankton grazing was not sufficient to control phytoplankton growth.

#### Environmental and experimental considerations for measurement of phytoplankton growth and microzooplankton grazing

A number of factors can affect phytoplankton growth and microzooplankton grazing rates, includ-

ing phytoplankton and microzooplankton assemblage composition, nutrient limitation, and predation by larger zooplankton (Landry & Hassett 1982, Weisse et al. 1994, First et al. 2007). *Phaeocystis antarctica*, a colonial prymnesiophyte that is widely distributed in the Southern Ocean, may deter protozoan grazers by forming large colonies (Weisse et al. 1994, Brussaard et al. 1996). Single-celled *P. antarctica* occur in the coastal regions of the WAP (Garibotti et al. 2003), but colonies were not present in our study area and thus would not have reduced microzooplankton grazing rates. Large diatoms, however, could have reduced microzooplankton grazing rates on phytoplankton in these experiments. Large diatoms are abundant, especially in the southern part of our study region (Montes-Hugo et al. 2009). Diatoms can be too large and have spines or other grazing deterrents that prevent their consumption by herbivorous ciliates (Verity & Villareal 1986), although due to their varied feeding strategies (Hansen & Calado 1999, Jeong 1999, Stoecker 1999), dinoflagellates may consume large diatoms. With few exceptions, ciliates and dinoflagellates were relatively small (<30  $\mu\text{m}$  and <60  $\mu\text{m}$ , respectively) in our study and would have targeted small autotrophs and bacteria rather than large diatoms. Furthermore, the lowest grazing rates were on the Total phytoplankton size class, which would include large diatoms (see 'Selective feeding' below).

Macronutrients (silicate, nitrate + nitrite, phosphate) were not added to our experiments as phytoplankton growth is generally not limited by macronutrients in the Southern Ocean (Martin 1990, Ducklow et al. 2012a). Macronutrients were not depleted and N:P ratios in experiments were not substantially different from the Redfield ratio in most experiments. In a few experiments in productive areas near Marguerite Bay (Expt 10) and in the south near Charcot Island (Expts 11 and 12), macronutrients could have been limiting, but nutrient limitation likely did not confound results of other experiments (Table 1).

Although grazer dynamics are an inherent part of dilution experiments, substantial changes in the grazer assemblage could affect grazing mortality rates (First et al. 2007). Even though larger zooplankton predators were removed from our experiments, microzooplankton can consume other microzooplanktonic grazers (Dolan 1991), complicating interpretation of dilution experiment results. While one taxonomic group either increased or decreased by at least 25% in each experiment, substantial changes in the entire grazer assemblage (e.g. all taxa increasing or decreasing by more than half) were apparent in

only a few of our experiments. For example, in Expt P5 on 26 February, all microzooplankton except the silicoflagellate decreased in abundance by more than half. There was a very large ciliate bloom at the beginning of the experiment (39 080 ind.  $\text{l}^{-1}$ ) that was reduced by 1 order of magnitude by the end of the experiment. This decrease in microzooplankton grazers in this experiment (Table S3) could indicate complicated trophic dynamics and might have caused phytoplankton mortality rates in this experiment to be zero or undetectable. Other studies have reported varying microzooplankton growth in different dilution treatments (Dolan et al. 2000), which could result in nonlinear feeding responses and non-significant results.

### Bacterial growth and mortality

Microzooplankton exerted substantial grazing pressure on bacteria, often removing >100% of bacterial production. This is in contrast to grazing control on phytoplankton, as microzooplankton removed >100% of phytoplankton production in only 1 experiment. There are limited terrestrial sources of dissolved organic matter (DOM) in Antarctic waters; therefore, bacterioplankton in coastal waters depend on phytoplankton production for organic matter and should be coupled with phytoplankton populations. In a recent analysis (2003 to 2011), WAP bacterial production during summer (measured on January LTER cruises) was correlated with chl *a* and, on average, chl *a* explained ~50% of the variation in bacterial production as measured by  $^3\text{H}$ -leucine incorporation rates (Ducklow et al. 2012b). Although bacterioplankton are more abundant in summer compared to winter in the WAP, they do not form as conspicuous of an annual bloom as they do in the Ross Sea (Ducklow et al. 2001); thus, WAP bacterial abundances are comparatively constant (Ducklow et al. 2012a). The high grazing pressure on bacteria determined in our study could effectively crop bacterial production, helping explain the relatively constant bacterial abundances in this region and why bacterial production is not more tightly coupled with chl *a*. Our calculations of bacterial growth and grazing mortality and of high grazing pressure on bacterial production are similar to previous studies in the Southern Ocean (Table 3).

Using data compiled from the Arctic and Antarctica, Anderson & Rivkin (2001) found a significant positive correlation between bacterial growth and grazing mortality and that grazing losses generally equaled

Table 3. Summary of published bacterial growth and grazing mortality rates in the Southern Ocean.  $\mu$ : bacterial growth rate;  $g$ : grazing mortality rate;  $B_i$  and  $B_p$  are the potential microzooplankton grazing pressure on initial bacterial stock and bacterial production, respectively. NR: not reported; NS: not significant. Values presented for the present study are total bacteria to allow comparison to previous studies

Location	Austral season	$\mu$ (d <sup>-1</sup> )	$g$ (d <sup>-1</sup> )	$B_i$ (%)	$B_p$ (%)	Source
Western Antarctic Peninsula	Jan–Mar	NS–0.82	NS–0.38	NS–32	NS–208	Present study <sup>a</sup>
McMurdo Sound	Sep–Jan	0.14–0.68	0.04–0.58	NR	28–86	Anderson & Rivkin (2001) <sup>a</sup>
East Antarctica, 30–80° E	Jan–Feb	0.3–2.3	0.40–2.6	33–93	10–163	Pearce et al. (2010) <sup>a</sup>
Sub-Antarctic zone, near Tasmania	Jan–Feb	0.14–0.87	0.20–1.03	13–58	35–215	Pearce et al. (2011) <sup>a</sup>
Gerlache Strait, Antarctic Peninsula	Oct–Nov	NS–0.8	NS	NR	NR	Bird & Karl (1999) <sup>a</sup>
Prydz Bay	Dec–Feb	0.22–0.69	0.03–0.13	NR	10–35	Leakey et al. (1996)
Antarctic Confluence	Nov–Dec	0.44	0.22–0.44	NR	50–100	Kuparinen & Bjørnsen (1992)
McMurdo Sound	Nov–Jan	NS–0.26	NS–0.06	NR	<0.5–5	Moisan et al. (1991), Putt et al. (1991)

<sup>a</sup>Rates calculated from dilution experiments

bacterial growth. We also found a significant positive correlation between bacterial growth and mortality, but these rates were not balanced. Grazing mortality was often greater than growth, resulting in removal of >100% of bacterial production. Our results suggest that microzooplankton can exert high grazing pressure on bacteria along the WAP during summer, leaving open the role of other sources of mortality including viral lysis. In an earlier study conducted in the WAP, Guixa-Boixereu et al. (2002) found that viral lysis could account for the majority of bacterial mortality when both grazing and viral lysis were measured. Brum et al. (2012) observed that viral assemblages at Palmer Station were dynamic and responded to seasonal shifts in bacterial production rates, removing an important fraction of the bacteria.

The use of the dilution method to calculate bacterial mortality has certain limitations, and our results should be interpreted within this context. As the dilution method was designed to measure community-level growth and mortality, bacterial mortality rates presented here may also include mortality due to viral lysis. In addition, bacterial growth is partly dependent on DOM supplied by grazers (Nagata 2000). Therefore, the availability of DOM can be dependent on the dilution series (e.g. more DOM supply in 100% whole seawater). However, in a companion study of the effect of krill excretia on natural bacterial assemblages, bacterial growth rates began to rapidly increase only after 4 d of incubation (D. K. Steinberg and H. W. Ducklow unpubl. data). Our dilution experiments incubated for 3 d, and bacterial growth rates were not likely to have been more rapidly stimulated by DOM excreted by microzooplankton. We present our results of bacterivory with these

methodological limitations in mind and anticipate these data will be useful in future methodological comparisons.

### Selective feeding

Selective feeding by microzooplankton is common and widespread (Stoecker et al. 1981, Tillmann 2004, Strom et al. 2007), and selective grazing on fast-growing phytoplankton taxa has been documented in the North Atlantic (Gaul & Antia 2001). At Palmer Station, grazing mortality and the fraction of primary production removed were significantly greater for the smaller phytoplankton size classes (Pico + Nano, which also had significantly higher growth rates) compared to the Total phytoplankton size class, which included large diatoms. This could indicate selective feeding on smaller phytoplankton and avoidance of large diatom prey and/or preferential grazing on the more actively growing phytoplankton size classes. An alternative explanation is that the Total phytoplankton size class had lower growth rates compared to the smaller phytoplankton (Pico and Nano), and microzooplankton inherently have lower grazing rates when phytoplankton growth rates are lower (Fig. 8a).

In addition to selective feeding on phytoplankton, the grazers in these experiments also appeared to selectively graze the HNA bacteria compared to the LNA bacteria. In more temperate waters, HNA bacteria are often the larger, more actively dividing cells in the population (Gasol et al. 1999; Morán et al. 2011), and selective grazing by protists on these larger, motile, actively growing cells has been docu-

mented (Sherr et al. 1992, Gonzalez et al. 1993, del Giorgio et al. 1996). In our experiments, HNA bacteria had higher growth rates than LNA bacteria. Grazing mortality rates were also higher compared to LNA bacteria. In all but 1 experiment on the LTER cruises in 2011 (Expt 8), growth and grazing mortality rates were not statistically significant for LNA bacteria. Collectively, these results suggest that microzooplankton selectively grazed HNA bacteria, the more actively growing bacterial cells in these experiments (Sherr et al. 1992).

### Physical forcing and the effect of temperature on microbial food web dynamics

The wind storm that occurred between Expts P2 and P3 at Palmer Station might have altered microbial food web dynamics. Chl *a* concentrations and phytoplankton growth rates decreased after the storm, affecting microzooplankton grazing capacity on phytoplankton. The effect of storms on phytoplankton dynamics and microzooplankton grazing in lower latitude systems (Wetz et al. 2006, Zhou et al. 2011) and decreases in microzooplankton biomass in coastal systems after strong storms (Zhang & Wang 2000, Lawrence et al. 2004) have been documented. There was no evidence of significant changes in microzooplankton biomass after the storm in our study (Table S3). It is more likely that the post-storm decrease in phytoplankton biomass and growth rates affected microzooplankton grazing, as microzooplankton selectively grazed the faster growing phytoplankton cells in the assemblage (see discussion above). In contrast, microzooplankton exerted higher grazing pressure on HNA bacteria after the storm. Microzooplankton communities are able to shift between herbivory and bacterivory (Anderson & Rivkin 2001); although based on only 1 storm event, our results suggest that microzooplankton may have shifted their major prey item from phytoplankton (before the storm) to bacteria (after the storm).

Temperature affects microzooplankton physiological rates (Sherr et al. 1988, Choi & Peters 1992, Sherr & Sherr 1994, Rose et al. 2008), and several studies suggest that low water temperatures constrain microzooplankton growth and grazing rates, accounting for the often low phytoplankton mortality rates reported in the Southern Ocean (Caron et al. 2000, Rose & Caron 2007). We found a significant ( $p < 0.05$ ) exponential relationship between temperature and phytoplankton growth and mortality rates, although only 22% of phytoplankton growth and 21% of

phytoplankton mortality could be explained by temperature. The relationship between temperature and bacterial mortality rates was not statistically significant. Thus, temperature was in general not a strong predictor of phytoplankton and bacterial growth and mortality, although we caution this result may be due to the narrow range of experimental temperatures ( $<4^{\circ}\text{C}$ ) in our study. Other studies in polar waters have found a positive correlation between phytoplankton growth and temperature (Tsuda & Kawaguchi 1997) but no effect of temperature on rates of microzooplankton grazing on phytoplankton (Froneman & Perissinotto 1996b, Tsuda & Kawaguchi 1997, Pearce et al. 2010).

### CONCLUSIONS

The present comprehensive analysis of microzooplankton grazing rates on phytoplankton and bacteria in the WAP, a region of rapid climate change, illustrates the key and complex role microzooplankton play in microbial food webs. Phytoplankton grazing mortality rates were comparable to the average reported for polar waters (Calbet & Landry 2004) but rarely exceeded phytoplankton growth rates. This could partially explain the large phytoplankton blooms that occur along the WAP in this productive season. In contrast, microzooplankton exerted substantial grazing control on bacterioplankton, which could explain the relatively constant bacterial abundances during the summer in the WAP (Ducklow et al. 2012a,b). Microzooplankton preferentially grazed on the smaller, faster-growing phytoplankton cells as well as the HNA bacteria, which were the more actively dividing cells in the bacterial assemblages. While the present study provides a reference point for microzooplankton grazing impact along the WAP in summer, further research is needed to clarify their trophic role during different seasons and to better define the specific effect of temperature on microzooplankton grazing rates. Incorporating these results into food web and biogeochemical models (Sailley et al. in press) will substantially improve our ability to predict changes in the WAP ecosystem with changing climate.

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