Limnol. Oceanogr., 57(6), 2012, 1826–1837

doi:10.4319/lo.2012.57.6.1826

Viral lysis and microzooplankton grazing of phytoplankton throughout the Southern Ocean

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Abstract

We investigated microzooplankton grazing and viral lysis of the pico- and nanophytoplankton community in the Southern Ocean during the "Sensitivity of Sub-Antarctic Zone Waters to Global Change" cruise to the Australian sector (January–February 2007) and the Antarctic–XXIV/3 (February–March 2008) expedition to the Greenwich Meridian and the Drake Passage. A dilution assay was employed in concert with flow cytometry at stations that spanned the sub-Antarctic, Polar Frontal, and Antarctic Zones and in the Weddell Gyre. Both viral lysis and microzooplankton grazing played a significant role in controlling cyanobacterial populations, except in the sub-Antarctic west of Tasmania, where their growth was regulated by grazing alone. Three main groups of eukaryotic algae were detected, and these were calculated to have average cell diameters of 0.8, 2.1, and 3.7 μm. Grazing was significant in the control of their growth at all locations, whereas viral lysis of eukaryotic algae was comparatively minor, as it was detected in only 40% of the experiments. Throughout the surveyed sites no pattern between geographical location and growth rates and/or mortality rates could be established for any of the phytoplankton groups. Estimates of iron regeneration indicated that as a result of its widespread occurrence, microzooplankton grazing is a more consistent source of regenerated iron to the Southern Ocean but that viral lysis can be responsible for significant pulses of iron into the dissolved pool.

Nano- and pico-sized phytoplankton are ubiquitous throughout the Southern Ocean and can comprise a significant fraction of the biomass and primary productivity. Haptophytes, prasinophytes, dinoflagellates, small diatoms, and, at the lower latitudes, cyanobacteria are among the dominant components of this small-sized phytoplankton (Marchant et al. 1987; El-Sayed and Fryxell 1993). The growth of smaller cells may be favored by the low iron concentrations and light limitation predominant throughout the open waters and, in the sub-Antarctic zone (SAZ), by low silicate concentrations that preclude large diatoms. Despite their small size, their prevalence makes nano- and pico-sized phytoplankton important contributors to the Southern Ocean's ecology and biogeochemistry.

Grazing is a major pathway controlling the fate of pico- and nanophytoplankton production in the Southern Ocean (Smetacek et al. 2004), but rates of grazing have not always been found to balance rates of production (Safi et al. 2007), indicating the potential importance of alternative loss mechanisms. In other marine environments viral lysis has been found to be significant in the control of phytoplankton populations (Evans et al. 2003; Baudoux et al. 2006). To our knowledge, only one study (Brussaard et al. 2008) has been conducted to examine viral lysis of small phytoplankton cells in the Southern Ocean, finding it to be a minor loss factor compared to grazing in the naturally iron-fertilized waters over the Kerguelen Plateau. However, the significance of viruses to phytoplankton mortality in the high-nutrient, low-chlorophyll (HNLC) conditions that predominant throughout the Southern Ocean or in the coastal seas is still unknown.

The Southern Ocean is a major sink for atmospheric CO_2 and is iron limited (De Baar et al. 1995), making factors that affect carbon and iron cycling throughout its waters of particular interest. Grazing leads to the transfer of biomass to higher trophic levels, the release of dissolved organic matter, nutrient regeneration, and the loss of matter and energy from the euphotic zone via sedimentation (Calbet and Landry 2004). The viral shunt also converts particulate to dissolved organic matter, thereby generating substrates for heterotrophic primary producers, increasing respiration, and intensifying nutrient regeneration (Suttle 2007 [and references therein]). However, viral lysis is thought to short-circuit the transfer of matter and energy to higher trophic levels and to promote the retention of nutrients and carbon within the euphotic zone (Suttle 2007). Studies have shown that both microzooplankton grazing (Bowie et al. 2001; Strzepek et al. 2005) and viral lysis (Gobler et al. 1997; Poorvin et al. 2004) of phytoplankton lead to the transfer of iron to the dissolved fraction, although rate estimates vary.

The Southern Ocean is composed of a number of distinct zones. The continental seas adjacent to Antarctica are characterized by intense seasonal blooms that support a highly productive food web. The Antarctic Circumpolar Current (ACC) flows around the coastal zone; it is the largest iron-limited HNLC area (De Baar et al. 1995). The innermost part of the ACC is the Antarctic Zone (AAZ) and its outermost part is the Polar Frontal Zone (PFZ). The SAZ forms the mostly northerly portion of the Southern Ocean and has higher temperatures and salinities than the ACC, may also be iron limited, and is characterized by low levels of silicate and sometimes nitrate. We aimed to investigate the growth, microzooplankton grazing, and viral lysis rates of the pico- and nanophytoplankton at sites throughout the major zones of the Southern Ocean. Modified dilution experiments (Evans et al. 2003) were conducted in the SAZ and the ACC of the

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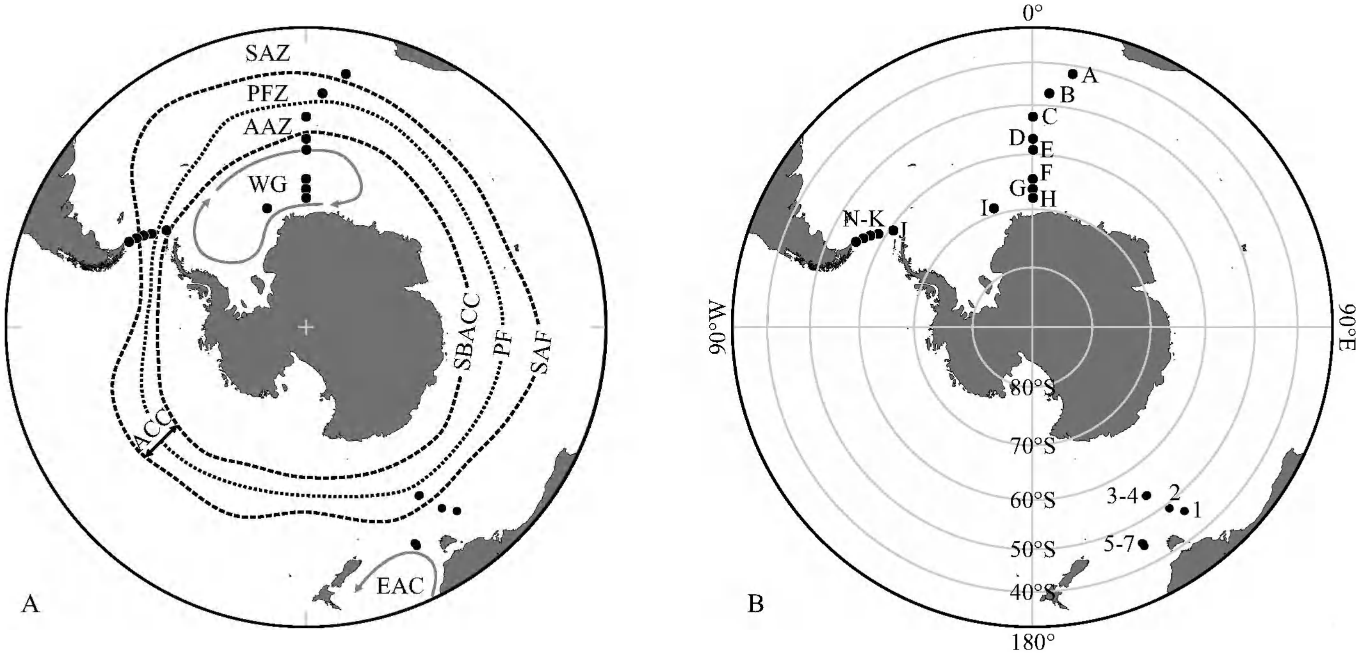

Fig. 1. Map of the Southern Ocean depicting with black dots the location of stations sampled for dilution experiments during the SAZ Sense cruise in the Australian Sector (experiments 1 to 7) and the ANT-XXIV/3 expedition in the Atlantic Sector (experiments A to N). (A) Station positions relative to the water masses and frontal systems. Cirulation and location of the Weddell Gyre (WG) and Eastern Australian Current (EAC) are also indicated. (B) Station designations and their positions relative to longitude and latitude. Additional abbreviations in alphabetical order: AAZ: Antarctic Zone; ACC: Antarctic Circumpolar Current; PF: Polar Front; PFZ: Polar Frontal Zone; SAF: Sub-Antarctic Front; SAZ: Sub-Antarctic Zone; SB ACC: Southern Boundary of the ACC.

Australian and Atlantic sectors as well as in the coastal Weddell Sea.

Methods

Study site and sampling procedure—The study was conducted during the "Sensitivity of Sub-Antarctic Zone Waters to Global Change" (SAZ Sense [20 January–16 February 2007]) and the Antarctic (ANT)–XXIV/3 (11 February–13 April 2008) expeditions to the Southern Ocean aboard R/Vs *Aurora Australis* and *Polarstern*, respectively. Samples were collected at stations to the southwest, south, and southeast of Tasmania on the SAZ Sense cruise and along transects of the Greenwich Meridian and the Drake Passage during ANT-XXIV/3 (Fig. 1).

Seawater was collected by General Oceanics Niskin bottles mounted on a rosette frame equipped with a SBE-9 plus conductivity, temperature, and depth (CTD) sensor or a Seabird 911+ CTD. Dissolved inorganic phosphate, silicate, nitrate, and nitrite were analyzed onboard using a TrAAcs 800 autoanalyzer from Bran and Luebbe. Seawater for experiments was collected at a depth of 10 m during the SAZ Sense cruise and at the chlorophyll maximum (20–80 m) during the ANT-XXIV/3 cruise.

Flow cytometry—The abundances of pico- and nanoeukaryotic algae (< 20 μm) and prokaryotic cyanobacteria were determined on fresh samples. All samples were maintained at ambient seawater temperatures prior to processing, which was completed within 4 h of collection. The photoautotrophic organisms were enumerated using benchtop flow cytometry (FCM) with a Becton Dickinson Fluorescence Activated Cell Sorter Calibur during the SAZ Sense and a Beckman Coulter XL-Multi Carousel Loader during the ANT-XXIV/3. Both FCMs were equipped with a 488 nm argon laser, and the photoautotrophic cells were discriminated from other particles using their autofluorescence on bivariate scatter plots of orange and red fluorescence and side scatter.

Average cell sizes of the eukaryotic phytoplankton clusters identified by flow cytometry were calculated according to the method of Veldhuis and Kraay (2000 [and references therein]). Briefly, seawater was size-fractionated by sequentially filtering 10 mL whole water samples through 25 mm–diameter polycarbonate filters of pore sizes 8, 5, 3, 2, 1, 0.8, and 0.4 μm, using a disposable sterile plastic syringe combined with a Swinnex filtration unit (Millipore). For each pore size an individual sample was generated and filtered directly into a sterile flow cytometry tube immediately prior to analysis. A logistic regression was performed on the number of cells retained after each filtration step plotted against filter pore size. The equivalent spherical diameter for each cell cluster was determined as the size displayed by the median (50%) number of cells retained for that cluster. Assuming cells were spherical, cell biomass was calculated using the carbon-to-volume relationships of 0.36 pg C μm^{-3} for a cell volume of < 10 μm^3 and 0.24 pg C μm^{-3} for cell volumes of 10–100 μm^3. For cyanobacteria a carbon content of 200 fg C $cell^{-1}$ (Caron et al. 1991) was applied.

Viral lysis and microzooplankton grazing—Growth rates and coefficients of viral lysis and microzooplankton grazing of phytoplankton were determined using the parallel dilution method of Evans et al. (2003). Whole seawater samples were prefiltered through a 200 μm mesh to remove mesozooplankton and combined with either 0.45 μm filtered water or 30 kDa filtered seawater over a dilution series of 20%, 40%, 70%, and 100% to create gradients in microzooplankton grazing mortality and grazing plus viral-induced mortality, respectively. All treatments were set up in triplicate in clear polycarbonate bottles of either 500 mL on the SAZ Sense or 1.2 liters on the ANT-XXIV/3. Preparation of the 0.45 μm filtered water was performed on 200 μm prefiltered whole water by gentle peristaltic filtration through a 0.45 μm, 145 mm polycarbonate membrane filter on the SAZ Sense cruise, and on the ANT-XXIV/3 the water was prepared by gravity filtration through a 0.45 μm Sartopore capsule filter complete with 0.8 μm prefilter. On both cruises 30 kDa seawater was generated by tangential flow filtration using a polyethersulfone membrane (Vivaflow 200, Vivascience). After preparation of the parallel dilution series a 3 mL subsample was removed from all bottles for immediate enumeration of phytoplankton. The bottles were then incubated for 24 h. On the SAZ Sense cruise incubations were performed on a rotating (0.5 revolutions per minute) plankton wheel under in situ temperature and light irradiance. On the ANT-XXIV/3 cruise incubations took place on deck in a flow-through seawater incubator under typical overcast conditions. After the 24 h incubation period a second phytoplankton count was made and the growth rate was calculated for each bottle. Coefficients of growth and of grazing and viral-induced mortality of phytoplankton were then calculated according to the method of Evans et al. (2003). Growth rates were plotted against levels of dilution, and a model 1 regression analysis was performed on both dilution series. Where the growth rate at the 20% level of dilution equaled or fell below that at the 40% level, the former was removed from the regression. The coefficient of microzooplankton grazing (m_g) was derived from the slope of the 0.45 μm filtered seawater dilution series. The coefficient of both microzooplankton grazing and viral-induced mortality ($m_g + m_v$) was derived from the slope of the 30 kDa filtered seawater dilution series. To calculate the coefficient of viral-induced mortality (m_v) the microzooplankton grazing coefficient (m_g) was subtracted from the microzooplankton grazing plus the viral-induced mortality coefficient ($m_g + m_v$). Phytoplankton growth rate (μ) in the absence of both mortality factors was derived from the y intercept of the 30 kDa dilution series regression. All seawater handling was performed at in situ temperature and under dim light conditions using Nitrile gloves.

While every effort was made to prevent contamination, this assay could not be executed under trace metal–clean conditions (e.g., all types of ultrafiltration cartridges tested showed a minor enrichment of iron [C. P. D. Brussaard unpubl.]). Previous studies in the Southern Ocean have indicated that iron enrichment does not appreciably alter phytoplankton growth rates within the 24 h time frame of our experiments and, furthermore, that any changes are far more pronounced in species of large diatoms (Timmermans et al. 2001). We therefore assume that our results were unaffected by any potential iron contamination.

Results

Study areas—Experiments 1 and 2 were conducted on waters from the SAZ to the southwest of Tasmania, which exhibited surface salinities of $\geq$ 34 and temperatures of $\geq$ 10°C with relatively low macronutrient concentrations ($\leq$ 11.4, 0.8, and 0.6 $\mu mol\ L^{-1}$ NO_x, PO_4, and SiO_4, respectively; Table 1). Experiments 3 and 4 were in the PFZ, as indicated by salinities of 33.8, temperatures of $\leq$ 2.4°C at depths below 100 m, and elevated macronutrient levels (24.7, 1.6, and 0.8 $\mu mol\ L^{-1}$ NO_x, PO_4, and SiO_4, respectively). Experiments 5 to 7 to the southeast of Tasmania were also in SAZ waters, but they were influenced by the input of subtropical waters due to penetration of the Eastern Australian Current, and as a result they exhibited slightly higher salinities and temperatures, as well as slightly lower NO_x concentrations, than were noted in the southwestern SAZ. Iron concentrations were low over the entire cruise track, with the exception of stations southeast of Tasmania, and ranged from 0.1 to 0.7 nmol L^{-1} (Bowie et al. 2009).

Experiments along the Greenwich Meridian (designated A to I) began at approximately 42°S in the warm, salty ($\geq$ 10°C and $\geq$ 34 at surface, respectively), and comparatively macronutrient depleted ($\geq$ 10, 1, and 10 $\mu mol\ L^{-1}$ NO_x [$NO_2 + NO_3$], PO_4, and SiO_4, respectively) waters of the SAZ. At approximately 45°S the SAF was encountered, marking the shift into the PFZ, which was characterized by decreased temperatures and salinities ($\leq$ 8°C and $\leq$ 34 at surface, respectively) and higher macronutrient concentrations ($\geq$ 18, 1.2, and 12 $\mu mol\ L^{-1}$ NO_x, PO_4, and SiO_4, respectively). The Polar Front was located at 49°S, beyond which point the colder ($\leq$ 5°C) macronutrient-replete waters ($\geq$ 20, 1.5, and 25 $\mu mol\ L^{-1}$ NO_x, PO_4, and SiO_4, respectively) of the AAZ were encountered. South of the AAC in the Weddell Gyre (WG) temperatures fell to below 0°C, and salinity increased to over 34. Nutrient concentrations in the WG were similar to those in the AAZ, with the exception of elevated silicate ($\geq$ 50 $\mu mol\ L^{-1}$). Dissolved free iron concentrations were low over the entire transect, with concentrations never exceeding 0.5 nmol L^{-1} (Klunder et al. 2011).

Experiments J to N were conducted on the south-to-north transect over the Drake Passage, where the PF and SAF were located at approximately 58°S and 56°S, respectively. The characteristics of the AAZ, PFZ, and SAZ in this region were largely similar to the north-to-south transect of the Greenwich Meridian, with the exception that the physiochemical gradients occurred over a much shorter geographical distance. Additionally, throughout the upper water column at the southern extent of the transect and in the surface waters to the north, iron

Table 1. Location, dates, and physicochemical characteristics of the water masses sampled in the Southern Ocean for dilution experiments.

Experiment No.	Water mass	Latitude (°S)	Longitude (°E)	Date	Depth (m)	Temperature (°C)	Salinity	NO_x (μmol L^{-1})	PO_4 (μmol L^{-1})	SiO_4 (μmol L^{-1})
SAZ Sense—Australian sector										
1	W-SAZ	46.43	140.52	23 Jan 07	10	12.9	34.7	4.6	0.3	0.0
2	W-SAZ	49.00	143.00	29 Jan 07	10	10.3	34.4	11.4	0.8	0.6
3	PFZ	54.00	145.87	01 Feb 07	10	5.4	33.8	25.0	1.6	0.5
4	PFZ	54.02	146.13	03 Feb 07	10	5.4	33.8	24.7	1.6	0.8
5	E-SAZ	45.55	153.18	11 Feb 07	10	13.5	34.9	3.0	0.4	0.6
6	E-SAZ	44.75	153.00	13 Feb 07	10	14.8	35.2	0.1	0.2	0.3
7	E-SAZ	45.27	153.01	16 Feb 07	10	14.6	35.1	1.6	0.2	0.3
ANT-XXIV/3—Greenwich Meridian and Weddell Gyre										
A	SAZ	42.34	8.99	14 Feb 08	50	10.4	34.2	11.4	0.8	1.2
B	PFZ	47.38	4.16	17 Feb 08	50	6.5	33.7	20.4	1.4	5.7
C	AAZ	52.60	0.00	20 Feb 08	50	1.2	33.8	26.1	1.8	35.8
D	WG	57.00	0.00	23 Feb 08	50	0.4	34.1	26.4	1.8	67.1
E	WG	59.00	0.00	25 Feb 08	50	0.1	34.0	25.7	1.8	62.4
F	WG	64.60	0.00	29 Feb 08	40	−0.2	34.0	25.5	1.7	64.9
G	WG	66.50	0.00	08 Mar 08	40	−0.5	34.5	28.9	2.0	75.9
H	WG	68.00	0.00	10 Mar 08	20	−0.7	34.0	25.9	1.7	63.6
I	WG	68.80	−17.95	15 Mar 08	10	−1.8	33.8	26.6	1.9	63.3
ANT-XXIV/3—Drake Passage										
J	AAZ	60.63	−55.17	02 Apr 08	50	0.5	34.3	29.6	2.1	75.0
K	PFZ	58.58	−58.83	05 Apr 08	70	2.3	33.9	27.5	1.9	43.4
L	PFZ	57.38	−60.54	07 Apr 08	80	2.3	33.8	26.4	1.9	14.9
M	SAZ	56.51	−62.29	09 Apr 08	60	5.4	33.9	22.6	1.6	4.2
N	SAZ	55.45	−64.23	11 Apr 08	60	5.1	33.9	23.6	1.6	4.2

Table 2. Abundances of cyanobacteria and eukaryotic algae detected by flow cytometry and their daily turnover rates as a result of microzooplankton grazing and viral lysis. Experiment designations given in bold type indicate experiments for which the 20% growth rates were subtracted from the regression analysis (*see* main text), and turnover rates given in italic type represent the summed daily turnover due to both grazing and viral lysis.

Experiment No.	Water mass	Abundance (cells L^{-1})				Turnover due to microzooplankton grazing (% d^{-1})				Turnover due to viral lysis (% d^{-1})			
			Eukaryotes				Eukaryotes				Eukaryotes		
		Cyanobacteria	Group 1	Group 2	Group 3	Cyanobacteria	Group 1	Group 2	Group 3	Cyanobacteria	Group 1	Group 2	Group 3
1	W-SAZ	4.97×10^{7}	2.41×10^{6}	5.37×10^{6}	2.34×10^{6}	8	15	11	12	0	0	10	6
2	W-SAZ	9.66×10^{6}	5.28×10^{6}	3.66×10^{6}	9.87×10^{5}	3	19	13	3	0	0	0	3
3	PFZ	0	2.34×10^{6}	6.15×10^{6}	7.64×10^{5}		5	1	18		8	3	0
4	PFZ	0	2.82×10^{6}	6.23×10^{6}	5.85×10^{5}		36	23	58		0	0	0
5	E-SAZ	2.94×10^{8}	7.28×10^{6}	9.02×10^{6}	0	0	17	14		16	0	0	*46*
6	E-SAZ	2.71×10^{8}	5.96×10^{6}	8.43×10^{6}	3.41×10^{6}	27	57	30	11	10	0	0	0
7	E-SAZ	3.84×10^{8}	6.11×10^{6}	6.88×10^{6}	3.26×10^{6}	12	4	29	2	20	13	0	0
A	SAZ	1.39×10^{7}	9.11×10^{6}	5.44×10^{5}	3.40×10^{6}	10	3	4	9	12	1	0	0
B	PFZ	9.09×10^{5}	2.10×10^{6}	4.07×10^{6}	1.65×10^{6}	3	62	53	13	38	0	0	0
C	AAZ	0	1.45×10^{6}	0	5.91×10^{5}		2		30		11		10
D	WG	0	1.13×10^{6}	0	0		61				0		
E	WG	0	5.28×10^{5}	2.11×10^{6}	2.57×10^{5}		7	13			0	20	
F	WG	0	2.80×10^{5}	3.32×10^{5}	1.32×10^{6}		18	0	0		9	64	68
G	WG	0	0	1.11×106	0			29	20			0	0
H	WG	0	9.47×10^{5}	1.37×10^{6}	9.83×10^{5}		13	17	9		13	12	0
I	WG	0	8.58×10^{5}	7.83×10^{5}	7.94×10^{5}		34	34	59		4	0	0
K	PFZ	3.53×10^{6}	2.82×10^{6}	4.95×10^{5}	3.31×10^{6}	8	5	9	25	8	8	1	32
L	PFZ	0	1.82×10^{6}	4.60×10^{5}	0		133	42			0	0	
M	SAZ	0	9.42×10^{5}	1.93×10^{6}	3.39×10^{5}		125	71	44		45	0	78
N	SAZ	3.83×10^{5}	6.10×10^{5}	2.92×10^{6}	0	74	2	75		11	0	12	*175*

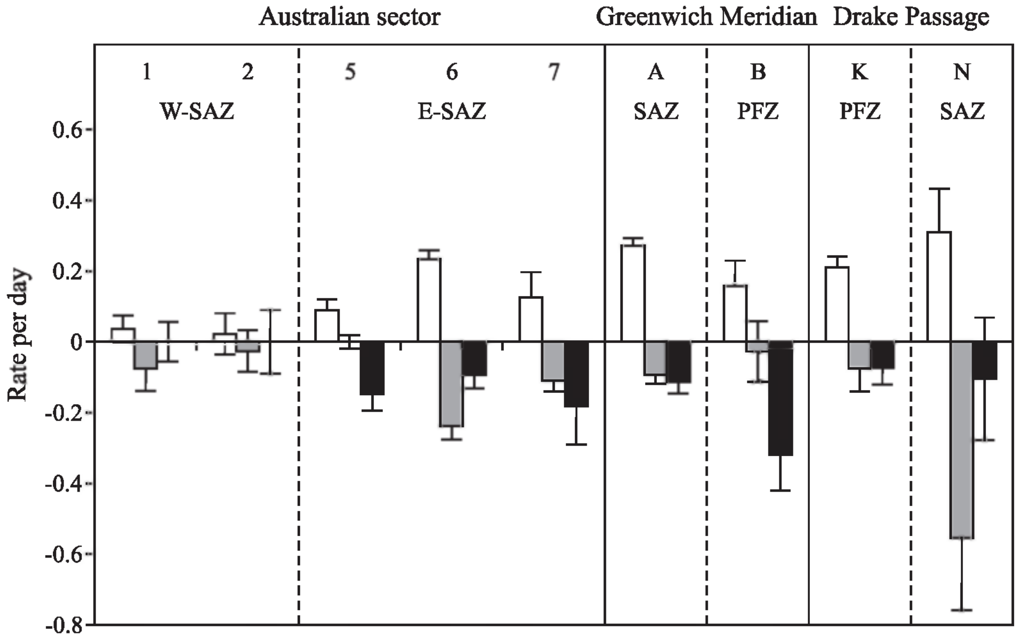

Fig. 2. Rates of growth (white bars), microzooplankton grazing (gray bars), and viral lysis (black bars) for the cyanobacteria at sites surveyed in the Southern Ocean. Experiment designation, water mass, and region are indicated, and the data represent the average and standard deviation from triplicate incubations.

concentrations were elevated (≥ 1 nmol L^{-1}), likely as a result of inputs from the Antarctic Peninsular and South America (M. Klunder pers. comm.).

Phytoplankton distribution and rates of growth, microzooplankton grazing, and viral lysis—Throughout the study area cyanobacteria were only detected in the SAZ and occasionally in the PFZ (Table 2). The highest concentrations were found in the eastern Australian SAZ ($\geq 2.7 \times 10^8$ mL^{-1}) at stations within a cyanobacterial bloom. At the other locations their abundances ranged from 0.4 to 5.0×10^7 mL^{-1}, with the lowest abundances found in the autumnal Drake Passage. Cyanobacterial growth rates were lowest in the western Australian SAZ (average 0.03 d^{-1}) and, surprisingly, highest in the Drake Passage SAZ (average 0.31 d^{-1}) (Fig. 2). With the exception of the western Australian SAZ, where growth was regulated by microzooplankton grazing alone (average 0.06 d^{-1}), both viral lysis (0.09–0.32 d^{-1}) and microzooplankton grazing (0–0.55 d^{-1}) played a significant role in controlling cyanobacterial populations, with viral lysis being the dominant mortality source in more than half of the experiments.

Three groups of eukaryotic algae were detected, and these were calculated to have average cell volumes of 0.2, 4.9, and 26.5 μm^3. Generally, all three groups were distributed throughout both cruise tracks, and, where present, their concentrations ranged from 0.3 to 9.1×10^6 mL^{-1}, 0.3 to 9.0×10^6 mL^{-1}, and 0.3 to 3.4×10^6 mL^{-1}, respectively. Highest abundances were typically found in the SAZ, particularly to the southeast of Tasmania, and along the Greenwich Meridian, with concentrations generally falling with progression southward (Table 2). However, in the Drake Passage during autumn this low-to-high distribution from south to north was not observed. Rates of growth were similar for the eukaryotic algal groups, ranging from -0.12 to 0.68 d^{-1} for group 1, from -0.46 to 0.60 d^{-1} for group 2, and from 0.00 to 0.66 d^{-1} for group 3 (Fig. 3).

Throughout the surveyed sites no pattern between geographical location and growth rates and/or mortality rates could be established for any of the three groups. At all locations rates of growth were frequently matched or exceeded by rates of grazing (-0.02 to -0.85 d^{-1} for group 1; 0 to -0.56 d^{-1} for group 2; and 0 to -0.46 d^{-1} for group 3), particularly in the autumnal Drake Passage, where growth rates were lower than mortality rates. Viral lysis was a comparatively minor source of eukaryotic algal mortality and was detected in approximately 40% of the experiments. Where detected, viral lysis rates were -0.01 to -0.38 d^{-1} for group 1; -0.01 to -0.50 d^{-1} for group 2; and -0.03 to -0.58 d^{-1} for group 3.

Discussion

Evaluation of experimental approach—The modified dilution technique employed in our study is presently the only method capable of simultaneously estimating rates of phytoplankton growth and mortality due to microzooplankton grazing and viral infection. When used in concert with flow cytometry, its power of resolution is extended to individual phytoplankton populations distinguishable within the water column. Viral mortality has been successfully determined by this method in productive environments (Evans et al. 2003; Baudoux et al. 2006) and in the oligotrophic open ocean (Baudoux et al. 2007). The Southern Ocean is a HNLC area, and algal abundances were low, particularly in the Weddell Sea and at stations in the Drake Passage. Low host densities have been proposed

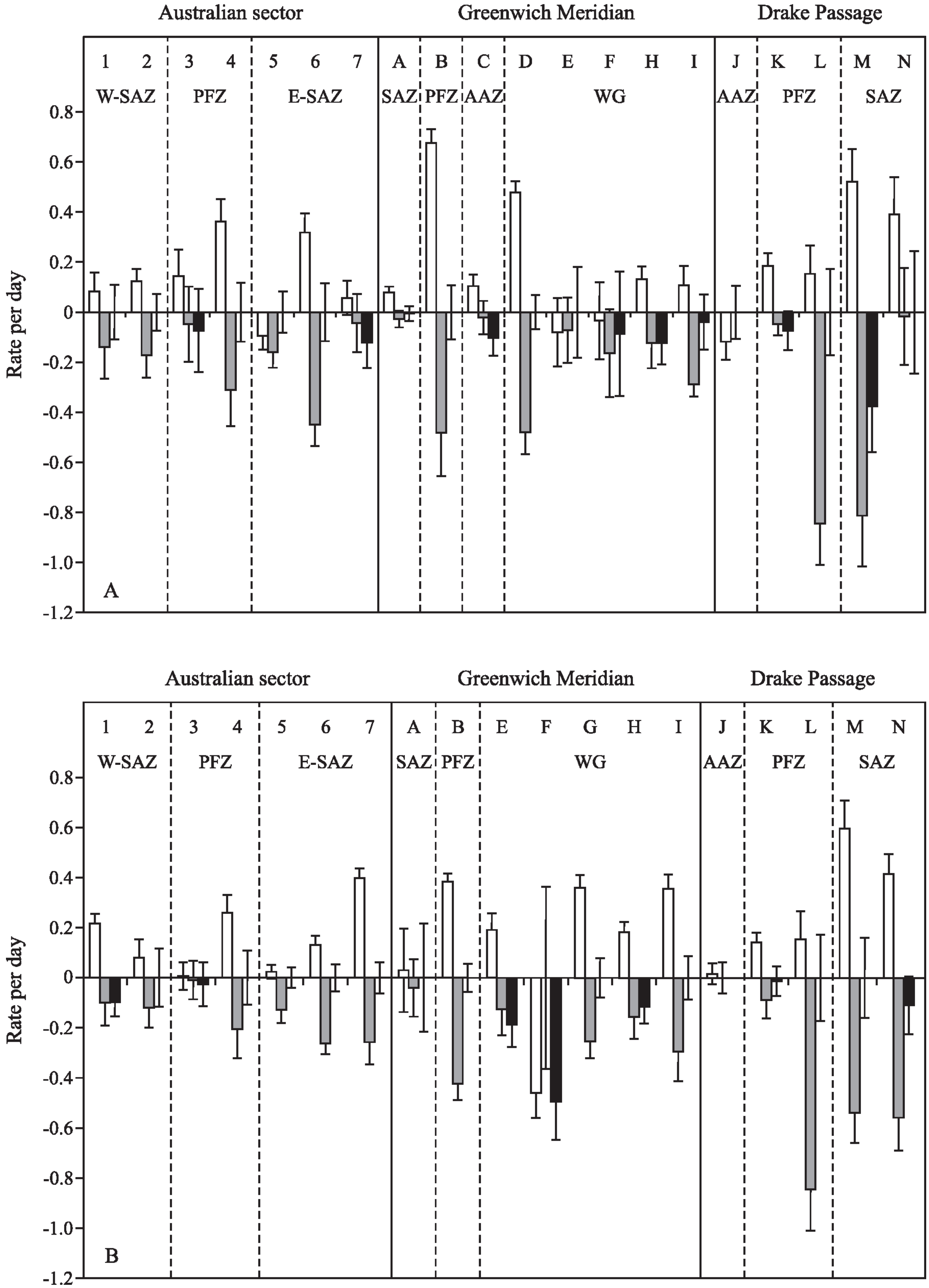

Fig. 3. Rates of growth (white bars), microzooplankton grazing (gray bars), viral lysis (black bars), and microzooplankton grazing plus viral lysis (hashed bars) for the eukaryotic algal groups 1, 2, and 3 at sites surveyed in the Southern Ocean. Experiment designation, water mass, and region are indicated, and the data represent the average and standard deviation from triplicate incubations.

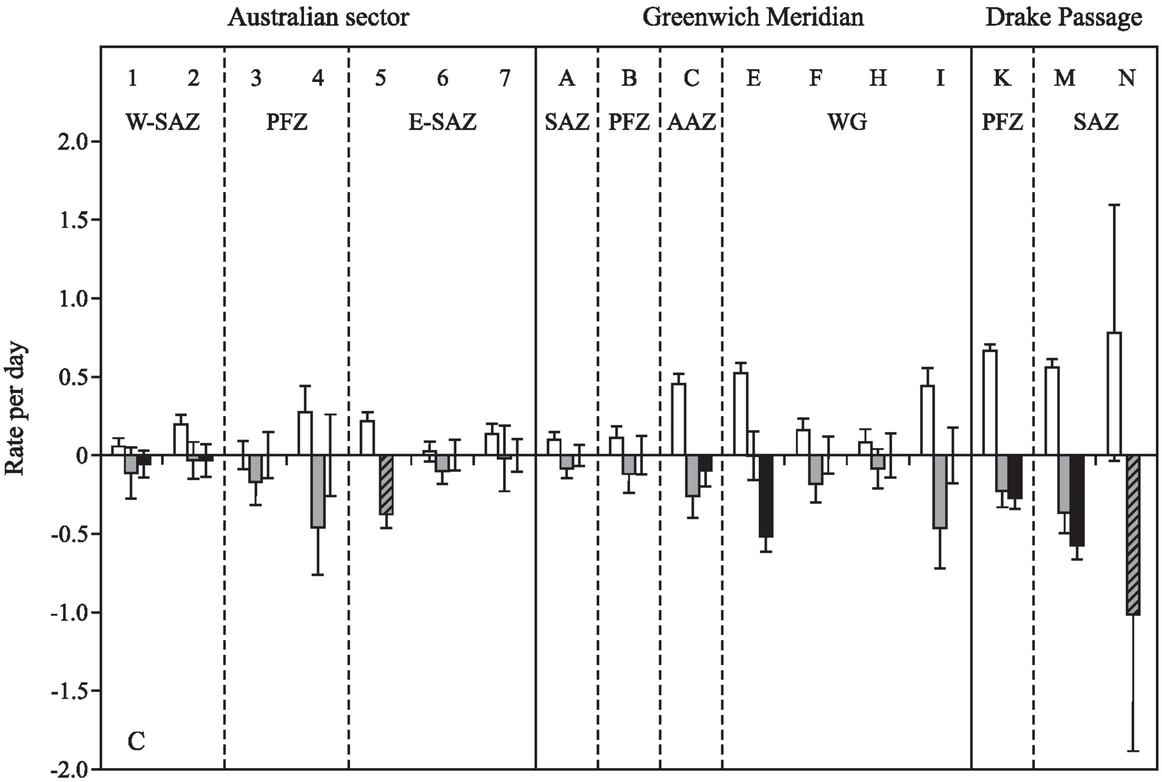

Fig. 3. Continued.

as an impediment to the successful application of the dilution technique, as there may be threshold prey or host levels at which both grazing and viral production occur (Kimmance et al. 2007). However, nonlinear responses (in which growth rate did not increase relative to dilution), potentially caused by reducing the cell density below these threshold levels, occurred on only four occasions. This indicates that low host density did not preclude the estimation of mortality rates in our experiments. Secondly, to accurately measure viral-induced mortality, the time from infection to host cell lysis must be shorter than the incubation period but longer than half of the incubation period, in order to prevent multiple infection rounds. To our knowledge, no characterizations of viruses infectious to marine algal species from the Southern Ocean are available in the literature, so the typical period between their infecting and lysing of a host is unknown. If the period to full cell lysis of the host was longer than the 24 h incubation period, then lysis rates may have been underestimated by our study, which could explain why viral lysis was not commonly detected.

Viral lysis is a source of organic matter to marine microbes, and, thus, reduced viral abundance in the 30 kDa dilution series could cause artificial nutrient limitation in the more dilute incubations. Recently, Weinbauer et al. (2011) suggested this artifact to preclude the modified dilution technique's use for *Synechococcus* populations after they found that virus-free water inhibited growth of the cyanobacteria in the Gulf of Mexico and the Mediterranean Sea. Conversely, Baudoux et al. (2007) found *Synechococcus* growth rates to be unaffected by dilution with virus-free water in the northeastern Atlantic and the North Seas, indicating this is likely to be an environment-specific effect. Out of the nine experiments we conducted at sites with cyanobacteria present, seven yielded rates of viral-induced mortality, and only one experiment showed reduced growth rates in the highest level of dilution, indicative of possible nutrient limitation. In the SAZ we generally found high viral production rates (10^{10}–10^{11} particles L^{-1} d^{-1}; Evans et al. 2009; Evans and Brussaard 2012), which indicates that rates of viral-induced nutrient regeneration were enough to sustain cyanobacterial growth in our experiments, even at 20% of the ambient concentration.

Grazing vs. viral lysis of eukaryotic phytoplankton—While our understanding of bacteriophage ecology in the Southern Ocean is growing (Evans and Brussaard 2012), our knowledge of viral-induced mortality of phytoplankton remains limited by a paucity of studies. Pioneering work by Gowing (2003) found that cells of the bloom-forming species *Pyramimonas tychotreta* from Ross Sea summer pack ice contained virus particles, but no attempt was made to quantify the potential effect of infection. To our knowledge, only the study of Brussaard et al. (2008) has determined viral-induced mortality of phytoplankton in the Southern Ocean and placed this into the context of host growth rates and grazing. They found that rates of viral lysis of pico- and nanoeukaryotic phytoplankton were low or undetectable (0–0.11 d^{-1}) in the majority of experiments conducted in a naturally iron-fertilized phytoplankton bloom, whereas the highest rates (0.18 d^{-1}) occurred at a site outside of the bloom, more typical of a HNLC area. Although the majority of stations we surveyed were typical of HNLC conditions, our results agree with the general findings of Brussaard et al. (2008) in that viral-induced mortality of eukaryotes could only sporadically be detected. Detection of viral infection did not appear to be related to water mass, season, phytoplankton population, or cell size. Thus, while viral control does factor in the ecology of small eukaryotic species of the Southern Ocean, it appears to play a reduced role as a loss factor in comparison with grazing. It is possible that conditions in the Southern Ocean select against viral infection of algae, as the typically low phytoplankton abundances would decrease the likelihood of the passive process of virus–host contact and favor active prey acquisition by grazers. Additionally, free viruses may be subject to strong decay pressures due to the increased ultraviolet B at higher latitude, further reducing virally induced lysis rates, although further investigation is needed to confirm this.

Grazing is widely accepted as a significant factor of phytoplankton control in the Southern Ocean (Smetacek et al. 2004; Safi et al. 2007). In the majority of water masses grazing rates were close to, or exceeded, rates of eukaryotic phytoplankton growth, consistent with observations that microzooplankton grazing is vigorous and responds rapidly to variations in primary production (Safi et al. 2007). However, in some regions a reduced significance of grazing is indicated. For example, on a transect from Australia to Antarctica Safi et al. (2007) found a weaker coupling between growth and grazing in the south of the PF and in the ACC. We found the lowest coupling between growth and grazing for group 1 in the SAZ and ACC of the Greenwich Meridian (average 43% ± 26% of new production grazed) and for group 3 in the Drake Passage (average 49% ± 31% of new production grazed). For all of the other eukaryotic algal groups at the other sites, on average $\geq$ 76% ± 9% of new production was found to be grazed, and we could identify no clear relationship with region.

Distribution, growth, and mortality of cyanobacteria—Cyanobacteria were only detected in the SAZ and at two stations located in the PFZ, consistent with previous observations that they do not occur at high latitudes. This has been attributed to low temperatures (Moore et al. 1995), although marine cyanobacteria have been observed at temperatures $\leq$ 5°C (Marchant et al. 1987), and a potential link to concentrations of trace metals, such as cobalt, has also been proposed (Ellwood and van den Berg 2001). Maximal abundances were found at a cyanobacterial bloom to the southeast of Tasmania, which was thought to have been stimulated by the mixing of subtropical with sub-Antarctic waters, creating stable surface layers (Evans et al. 2011). Cyanobacterial growth rates at this site were higher (0.09–0.23 d^{-1}) than at a nonblooming site to the southwest of Tasmania (0.02–0.04 d^{-1}), although they were similar to those of other nonblooming regions in the Greenwich Meridian and Drake Passage (0.16–0.27 d^{-1} and 0.21–0.31 d^{-1}, respectively). In combination with the fact that cyanobacterial production was tightly controlled by mortality (average 63% ± 55% of new production grazed and 117% ± 67% lysed by viruses) to the southeast of Tasmania, this could indicate that the bloom was in a declining phase. In fact, as with the eukaryotic phytoplankton, cyanobacterial growth was found to be tightly controlled by mortality at all sites assessed, but with the critical difference that both microzooplankton grazing and viral lysis were integral to this control. Viral lysis was detected in seven of the nine experiments conducted where cyanobacteria were present, indicating it is a widespread phenomenon throughout Southern Ocean cyanobacterial populations. This is interesting, as while viral control of marine cyanobacteria has been indicated (Proctor and Fuhrman 1990), previous attempts to quantify it using the dilution technique in the northeastern Atlantic, the English Channel, and the North Sea largely inferred a comparatively minor role of viral lysis when compared to grazing (Baudoux et al. 2007; Kimmance et al. 2007). Collectively, these studies may indicate that the ecology of Southern Ocean cyanophages differs from that of cyanophages in the temperate regions previously surveyed, although additional studies are recommended to confirm this hypothesis.

Phytoplankton mortality and iron regeneration—In HNLC regions new sources of iron have been suggested (Hutchins et al. 1995) to support only 20% or less of the primary productivity, and, thus, iron regeneration is likely to be a key parameter in ecosystem productivity (Landry et al. 1997). It has been calculated that regenerated iron could supply as much as 90% of the demand in limited areas (Poorvin et al. 2004), and both microzooplankton grazing (Hutchins and Bruland 1994) and viral lysis (Gobler et al. 1997; Poorvin et al. 2004) of phytoplankton have been shown to be sources of regenerated iron. Using mortality coefficients generated by our experiments and rates taken from the literature, we estimated iron regeneration as a consequence of phytoplankton mortality throughout the Southern Ocean (Table 3). A range in microzooplankton regeneration rates of 25% to 60% was taken from the studies of Strzepek et al. (2005) and Bowie et al. (2001), both of whom used ^{55}Fe-labeled plankton incubation experiments to examine grazing in the Southern Ocean. Rates of iron regeneration by viral lysis were taken from laboratory experiments, although unfortunately the only experiment to examine iron dynamics during viral lysis of a eukaryotic alga was conducted in the presence of bacteria (Gobler et al. 1997). Therefore, we used the 5% regeneration rate derived from this study as a lower estimate, as much of the iron released during the experiment was observed to be rapidly assimilated by the prokaryotes present. More recently, Poorvin et al. (2004) examined viral lysis of *Synechococcus* and calculated an iron regeneration rate of 82%. While this rate is based on a prokaryotic phytoplankter, we applied it as an upper estimate of iron regeneration during viral lysis of eukaryotes, as to our knowledge no other estimates are currently available for eukaryotic phytoplankton.

Table 3. The effect of microzooplankton grazing and viral lysis on iron (Fe) regeneration. Biogenic Fe was estimated as the sum total of cellular Fe, using the Fe quotas of 2.73 μmol (mol C)$^{-1}$ for eukaryotic phytoplankton (Bowie et al. 2001) and 19 μmol (mol C)$^{-1}$ for cyanobacteria (Brand 1991). Steady state Fe uptake rates were calculated as the product of Fe content and growth rate derived from the dilution experiments and summed for the phytoplankton groups. Fe regeneration rates were estimated by applying the lower and upper microzooplankton regeneration rates of 25% (Strzepek et al. 2005) and 60% (Bowie et al. 2001), respectively. For viral lysis of eukaryotic phytoplankton the lower and upper Fe regeneration estimates of 5% and 82% were used, based on the laboratory experiments of Gobler et al. (1997) and Poorvin et al. (2004), respectively. For viral lysis of cyanobacteria Fe regeneration was calculated solely according to the 82% estimate of Poorvin et al. (2004).

Experiment No.	Water mass	Biomass (μmol C L^{-1})	Biogenic Fe (pmol Fe L^{-1})	Steady-state Fe uptake (pmol L^{-1} d^{-1})	Microzooplankton grazing Fe regeneration (pmol L^{-1} d^{-1})		Viral lysis Fe regeneration (pmol L^{-1} d^{-1})	
1	W-SAZ	2.82	21.16	1.23	0.45	1.07	0.02	0.32
2	W-SAZ	1.23	5.98	0.47	0.08	0.19	0.00	0.04
3	PFZ	1.30	3.55	0.03	0.05	0.12	0.00	0.05
4	PFZ	1.22	3.34	0.88	0.22	0.54	0.00	0.00
5	E-SAZ	6.26	96.74	8.18	0.12	0.29	11.21	11.21
6	E-SAZ	7.52	94.07	20.68	5.46	13.11	6.62	6.62
7	E-SAZ	9.10	128.92	16.90	3.51	8.43	18.14	18.15
A	SAZ	2.11	9.52	1.68	0.21	0.50	0.41	0.41
B	PFZ	1.47	4.25	0.95	0.25	0.59	0.08	0.08
C	AAZ	0.31	0.85	0.38	0.05	0.13	0.00	0.07
D	WG	0.01	0.02	0.01	0.00	0.01	0.00	0.00
E	WG	0.44	1.21	0.35	0.03	0.06	0.02	0.28
F	WG	0.72	1.98	0.23	0.08	0.20	0.00	0.05
G	WG	0.16	0.44	0.16	0.03	0.07	0.00	0.00
H	WG	0.71	1.93	0.21	0.05	0.12	0.00	0.05
I	WG	0.53	1.43	0.60	0.15	0.37	0.00	0.00
K	PFZ	1.84	5.98	3.34	0.29	0.69	0.13	1.11
L	PFZ	0.08	0.22	0.03	0.02	0.06	0.00	0.00
M	SAZ	0.46	1.26	0.73	0.15	0.36	0.01	0.23
N	SAZ	0.43	1.29	0.52	0.18	0.43	0.02	0.11

During our study we elected to use iron quotas for eukaryotic phytoplankton and cyanobacteria that have been previously employed in the construction of Southern Ocean microbial iron budgets (Bowie et al. 2001; Strzepek et al. 2005). However, it should be noted that phytoplankton iron quotas are known to change appreciably with iron status (Strzepek et al. 2011), which may have varied over the study site in response to iron availability (dissolved free iron < 0.05 to > 1 nmol L^{-1}; Bowie et al. 2009; Klunder et al. 2011; M. Klunder pers. comm.). Indeed, on the basis of the maximum quantum yield of PSII (Fv : Fm), Doblin et al. (2011) suggested that phytoplankton in the western Australian SAZ (experiments 1 and 2) were iron limited, whereas those in the Australian PFZ and eastern SAZ (experiments 3 to 7) were iron sufficient. Over the Greenwich meridian Fv : Fm was consistently below suboptimal levels (Alderkamp et al. 2010), potentially indicating iron limitation in experiments A to I, and although Fv : Fm was not assessed in the Drake Passage transect, the maximal iron concentrations (≥ 1 nmol L^{-1}; M. Klunder pers. comm.) encountered there indicated a stronger likelihood of iron sufficiency.

As a result of its widespread occurrence, microzooplankton grazing was found to be a more consistent source of regenerated iron than was viral lysis. However, upper estimates of iron regeneration due to viruses exceeded those for grazing, and on average the two processes were calculated to contribute similar amounts to the dissolved iron pool (1.3 ± 3.3 pmol L^{-1} d^{-1} for grazing and 1.9 ± 4.7 pmol L^{-1} d^{-1} for viral lysis). On closer inspection, the average viral regeneration rate was found to be strongly skewed by the high values generated by experiments 5 to 7, which were conducted in a predominantly virally controlled cyanobacterial bloom. After the removal of these results, the average upper estimate of iron regeneration by viruses (0.2 ± 0.3 pmol L^{-1} d^{-1}) was found to be significantly less than that for grazing. In the Australian sub-Antarctic, Strzepek et al. (2005) also found that, although higher in rate (16.5–18.4 pmol L^{-1} d^{-1}), grazing was a less variable source of regenerated iron, but that maximal rates were derived from viral lysis. Our results on grazing-mediated regeneration of iron from phytoplankton show good agreement with those reported for the Australian AAC (Bowie et al. 2001) and Kerguelen Plateau (Brussaard et al. 2008).

To our knowledge, this study is the first to attempt to estimate levels of iron regeneration due to phytoplankton mortality over spatial scales within the Southern Ocean. Owing to the presence of cyanobacteria and generally higher numbers of eukaryotes, maximal levels of iron regeneration were calculated to occur in the SAZ. This may help to explain the higher levels of production observed in this zone relative to higher latitudes, as we calculated that regeneration could supply over 100% of the iron demand of pico- and nanophytoplankton in the SAZ. In the ACC and the WG, iron regeneration was calculated to supply 61% and 77% of the iron demand, respectively, which, while lower than in the SAZ, still supports the findings of Poorvin et al. (2004) that regenerated iron represents a significant source to the marine food web of HNLC regions.

Acknowledgments

We thank the captains and crews of R/Vs *Aurora Australis* and *Polarstern* and the chief scientists Brian Griffiths and Eberhard Fahrbach. We are grateful to Jan van Ooijen, Mark Rosenberg, and Neale Johnston for physical and nutrient data. We thank Erwin Frijling for help with analysis of samples onboard R/V *Polarstern* and Joost Brandsma for the preparation of figures. We gratefully acknowledge the comments of two anonymous reviewers, which improved this manuscript. The investigations were supported by the Division for Earth and Life Sciences, with financial aid from the Netherlands Organization for Scientific Research.

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Associate editor: Thomas Kiørboe

Received: 20 January 2012
Accepted: 10 August 2012
Amended: 14 August 2012