Virus and bacteria abundances in the Drake Passage during January and August 1991

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High abundances of viruses have recently been discovered in various oceanic regimes (Torella and Morita 1979; Bergh et al. 1989; Proctor and Fuhrman 1990; Paul et al. 1991) and this has led to the suggestion that viruses may have a profound effect on food-web dynamics. If so, then current views on biogeochemical cycles in the ocean will need to be modified. Whether viruses are present in the southern ocean and, if so, whether they are as abundant as in other areas has not previously been addressed. We used transmission electron microscopy (TEM) to examine water samples from transects in both the winter and the summer in order to determine the spatial and temporal distribution of viruses in the southern ocean and its relationship to the distribution of bacteria.

Surface water was collected from the uncontaminated seawater intake on the R/V *Polar Duke* during two transects from Palmer Station, Antarctica, to Punta Arenas, Chile (January and August 1991). In addition, water was collected with Niskin bottles from five stations during the August 1991 transect to examine the depth distribution of viruses. Water samples were fixed with either glutaraldehyde (electron microscopy grade, 2.5 percent final concentration) for virus counts or formalin (borate buffered, 0.2 micrometer filtered, 2 percent final concentration) for bacteria counts and stored at 4 °C. Ten milliliter water samples were centrifuged (207,000 × force of gravity, for four hours) directly onto TEM grids for virus enumeration (Nomizu and Mizuike 1986). The TEM grids (copper with carbon-stabilized formvar film) were negatively charged prior to centrifugation to prevent clumping of viruses (Hayat and Miller 1990). Samples from the January 1991 transect were diluted 10- to 50-fold prior to centrifugation to achieve a workable dilution for enumeration. After centrifugation, the grids were stained with uranyl acetate (0.5 percent final concentration) and rinsed with Milli-Q water. Viruses were counted using TEM at 40,000 × magnification (Hitachi H-500) (figure 1). Samples for bacteria counts (2-10 milliliters) were stained with 4', 6 diamidino-2-phenylindole (DAPI, 1 microgram per milliliter final concentration) and enumerated using an Olympus BH-2 microscope with an epifluorescence attachment (Porter and Feig 1980).

A seasonal variation of over one order of magnitude was found in virus abundance (see table). January virus abundances ($1.2\text{-}5.4 \times 10^6$ milliliter^{-1}) were significantly greater (T-test; $p < 0.001$) than August virus abundances ($0.67\text{-}4.9 \times 10^5$ milliliter^{-1}).

The depth distribution of viruses from the August 1991 cruise (figure 2) showed only minor variations with depth or latitude. This is in spite of the strong thermocline present at approximately 100 meters (temperature profiles not shown).

Virus abundance correlated well with bacteria abundance

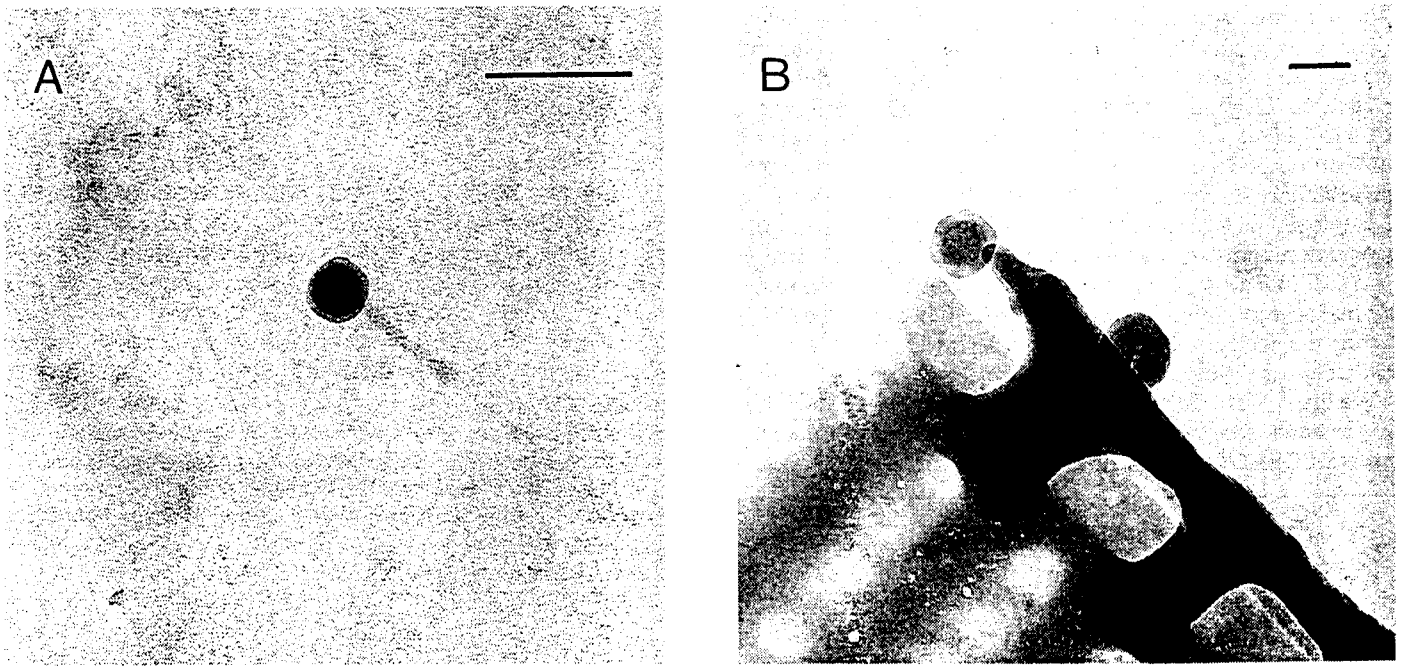

Figure 1. *(A)* Tailed bacteriophage (Bradley group A) from a surface sample collected 1 January 1991; *(B)* Two viruses on a diatom frustule from a surface sample collected 19 August 1991. Scale bar = 200 nanometers.

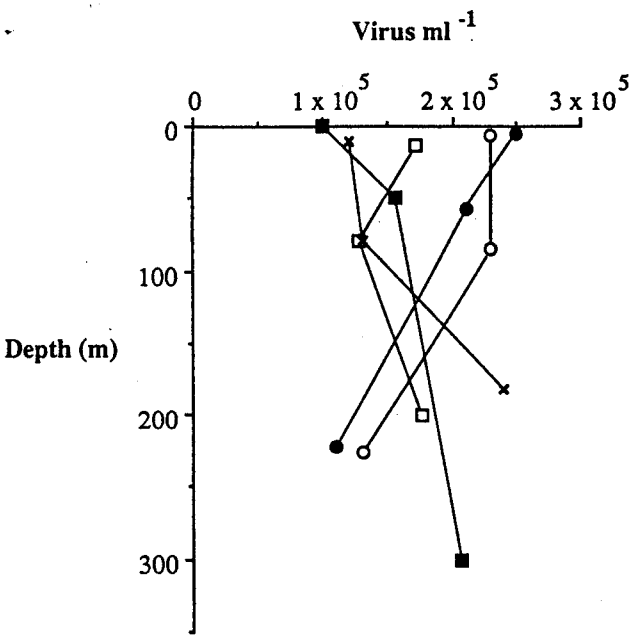

Figure 2. Depth profiles of virus abundance in samples collected with Niskin bottles on 19 August 1991: Station 1 (■) 60°40' S 64°48' W; Station 2 (□) 62°05' S 64°08' W; Station 3 (○) 62°59' S 64°35' W; and on 20 August 1991 Station 4 (●) 64°00' S 64°48' W; and Station 5 (X) 64°50' S 64°05' W.

during the August 1991 transect (0.875; Spearman rank correlation coefficient) but not during the January transect (0.100). This may have been fortuitous; the samples were stored at 4 °C for 9-17 months prior to counting, and it has since been shown that long-term storage of formalin-fixed samples results in underestimation (39 percent on average) of bacteria abundance (Turley and Hughes 1992). Whether these correlations are real need to be addressed by examing freshly collected samples.

Virus abundances in the southern ocean during the austral summer are comparable to those reported for samples from lower latitudes (Bergh et al. 1989; Proctor and Fuhrman 1990; Paul et al. 1991; Heldal and Bratbak 1991). The austral winter values, while significantly lower, are similar to those found in the Barents Sea in January (Bergh et al. 1989). Since virus decay rates can be high (Heldal and Bratbak 1991), the presence of viruses in the water column during the winter suggests that virus production occurs during this period as well as in the more productive summer season. Alternatively, virus degradation may be lower during the winter which would result in the persistence of residual viruses produced during the previous summer.

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List of Field Personnel

Name	Deployment dates
Azam, Farooq	13 Nov 90–6 Jan 91
Hollibaugh, James T.	28 Sep 90–13 Dec 90
Smith, David C.	13 Nov 90–6 Jan 91
Steward, Grieg F.	13 Nov 90–20 Jan 91

Bacteria and virus abundances in surface samples from the Drake Passage collected 1-2 January 1991 and 23-26 August 1991

Date	Location	Bacteria ($\times 10^5$ ml^{-1})	Virus ($\times 10^5$ ml^{-1})
1 Jan 91	60° 28' S 63° 07' W	2.82	54.1
	59° 41' S 63° 22' W	2.22	24.6
	59° 17' S 63° 22' W	1.03	12.0
2 Jan 91	58° 44' S 63° 42' W	1.83	41.6
	57° 59' S 63° 59' W	1.94	41.7
	56° 08' S 64° 38' W	1.11	39.4
	55° 27' S 64° 46' W	1.53	35.6
	54° 56' S 64° 55' W	2.66	17.3
	54° 26' S 65° 20' W	6.45	12.5
23 Aug 91	64° 53' S 64° 25' W	0.41	2.32
24 Aug 91	62° 52' S 64° 54' W	0.39	4.90
	62° 06' S 64° 59' W	0.23	0.67
	60° 00' S 65° 05' W	0.34	2.60
25 Aug 91	58° 13' S 65° 02' W	0.23	1.01
	56° 13' S 64° 59' W	0.27	2.22
26 Aug 91	53° 51' S 66° 35' W	0.28	2.22
	52° 58' S 70° 30' W	1.87	4.77

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Measurement of bacterioplankton production in antarctic coastal waters: Comparison of thymidine and L-leucine methods and verification of labeling patterns

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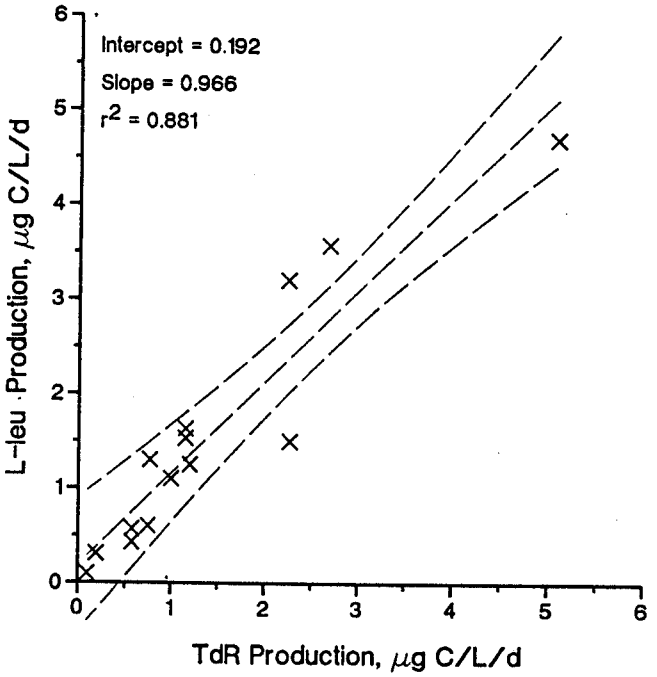
Bacterioplankton are important components of marine pelagic ecosystems (Azam et al. 1983) and bacterioplankton production is an important sink for organic material in most of the ecosystems studied to date (Cole et al. 1988). Recently, questions have been raised about the quantitative significance of bacterioplankton production in carbon fluxes in polar waters (Pomeroy and Deibel 1986; Pomeroy et al. 1991; Huntley et al. 1991). To investigate the significance of, and factors controlling, bacterioplankton production in antarctic coastal waters, we measured bacterioplankton production using the ^{3}H thymidine (Fuhrman and Azam 1982) and ^{3}H L-leucine methods (Kirchman et al. 1985; Simon and Azam 1989). In view of the potential for metabolism of exogenous thymidine to complicate interpretation of incorporation data (Hollibaugh 1988), we performed experiments to verify the expected macromolecular labeling patterns. The results of those experiments, and a comparison of bacterial carbon production estimates calculated from thymidine and L-leucine incorporation rates are presented.

Experiments were performed with surface water collected at a station in the vicinity of Spume Island, about 3 kilometers from Palmer Station, Antarctica (64° 48' S 64° 06' W). This area was sampled from 15 October 1990 through 15 January 1991. Another sample was collected in the vicinity (64° 50.2' S 64° 5.7' W) on 20 August 1991. Near-surface water was collected in clean glass or plastic containers and immediately returned to the laboratory where it was held in a dark incubator at -1.0 °C.

Routine incorporation-rate measurements were begun by adding methyl-[^{3}H]-thymidine (TdR, 81.0 uCi/nmol) or L-4, 5-[^{3}H]-leucine (L-leu, 53 uCinmol) to triplicate 10.0 mL samples contained in sterile polystyrene culture tubes to final concentrations of 10 nanomoles. A subsample [5.0 mL] was removed from each tube immediately and filtered through 0.45 micrometers pore size Millipore filters (HAMF) as a blank. Filters were rinsed with seawater with chilled 5 percent (w/v) trichloroacetic acid (TCA). The remaining sample was incubated at -1.0 °C for 6-8 hours (time courses were linear over this interval) then filtered through HAMF. Filters were placed in scintillation vials, dissolved with ethyl acetate, and radioassayed using an LKB Rackbeta Model 1217 liquid scintillation spectrometer with Aquasol II as the scintillation cocktail. Incorporation rates were converted to bacterial biomass production rates using the factors given in Fuhrman and Azam (1982), Lee and Fuhrman (1987), and Simon and Azam (1989).



Comparison of bacterioplankton productivity measurements based on methyl-[^{3}H]-thymidine incorporation vs. productivity measured in the same sample by L-4, 5-[^{3}H]-leucine incorporation. The model 1 least squares linear regression line for the data and 95 percent confidence belts are also shown.