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Large viruses in Ross Sea late autumn pack ice habitats

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ABSTRACT: Large (>110 to 424 nm capsid diameter) extracellular viruses occurred in Ross Sea first year ice samples at 33 stations from 66 to 78° S during the period from 9 May to 11 June 1998. As the viruses were of a size and morphology likely to infect algae and protozoans, infected eukaryotes were present in ice habitats as winter approached. Abundance ranged from below detection limits to 2.5×10^6 ml^{-1} brine (the liquid component of sea ice). It was positively correlated with chlorophyll *a* (chl *a*) and phaeophytin and did not differ among vertical location in cores or with ice type. Transmission electron microscopy (TEM) of thin sections of more than 9000 diatoms revealed no viruses; it is therefore unlikely that diatoms were the source of the viruses in the samples. Despite examination of over 2000 other algal cells and heterotrophs, we only observed 5 infected unidentified heterotrophs. They were from bottom communities at 2 stations with chl *a* ranging from 16 to 73 µg l^{-1} and where several taxa were present as 10^4 to 10^5 cells ml^{-1} melted sample. Although viruses of all sizes occurred in fecal pellets from ice habitats and in food vacuoles of phaeodarian radiolarians from water directly below the ice, we did not observe viruses in food vacuoles of ice community microheterotrophs. Viruses were therefore not a major food source in late autumn. Abundance of total (all sizes) viruses (10^8 to 10^9 ml^{-1} brine, equivalent to 10^6 to 10^8 ml^{-1} melted core sample) in a subset of samples was comparable to, or higher than, abundance from other polar locations, suggesting the importance of the total viral community in ice community dynamics.

KEY WORDS: Viruses · Pack ice · Ross Sea

INTRODUCTION

Viruses influence many aspects of carbon flow in marine ecosystems (Fuhrman 1999, Suttle 2000, Wommack & Colwell 2000). For example, algal viruses have been implicated in phytoplankton bloom dynamics in lower latitude waters (reviewed by Zingone 1995). There are no similar data from polar waters where sea ice communities of eukaryotic algae and protozoans sometimes reach high densities in microhabitats that are isolated for parts of the year (Horner 1985, Garrison 1991, Palmisano & Garrison 1993). Viruses have been reported from Arctic sea ice (Maranger et al. 1994a), Antarctic marine waters (reviewed by Karl et al. 1996, Marchant et al. 2000), and Antarctic lakes (Kepner et al. 1997, 1998, Wilson et al. 2000). Although the majority of the viruses in these studies were thought to be bacteriophages, the authors noted that some could have infected algae or protozoans, especially in the lake samples.

Most ice communities reach peak abundance in spring and summer, and viruses would be most likely to affect population dynamics at those times. Autumn sea ice algal blooms, usually with smaller accumulations of biomass, have been reported from near Syowa

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Station in the Indian Ocean sector (Hoshiai 1985) and from areas in the Weddell Sea (Gradinger et al. 1993, Fritsen et al. 1994; also see Palmisano & Garrison 1993 for a review). Viral infection might be enhanced in the autumn when solar UV radiation decreases (e.g. Wilhelm et al. 1998). In addition to affecting food webs by lysing cells, viruses of both eukaryotes and prokaryotes are also potential prey for microheterotrophs (e.g. González & Suttle 1993). Protozooplankton from natural Antarctic populations have been observed with ingested virus-like particles in their food vacuoles (Gowing 1993).

On a late autumn cruise of the RV 'Nathaniel B. Palmer' we sampled Ross Sea pack ice microhabitats ranging from the ice edge to the continent. Our objectives were: (1) to determine the abundance of large (≥110 nm capsid diameter) extracellular viruses likely to infect algae and protozoans in ice surface, interior and bottom habitats and in ice of various physical structures; (2) to document infections of algae and protozoans; and (3) to examine the extent of ingestion of viruses by sea ice protozoans and by protozoans in water directly below the ice. We also report the total (all sizes) extracellular viral abundance from a few ice cores. To our knowledge this is the first study focusing on putative eukaryotic viruses.

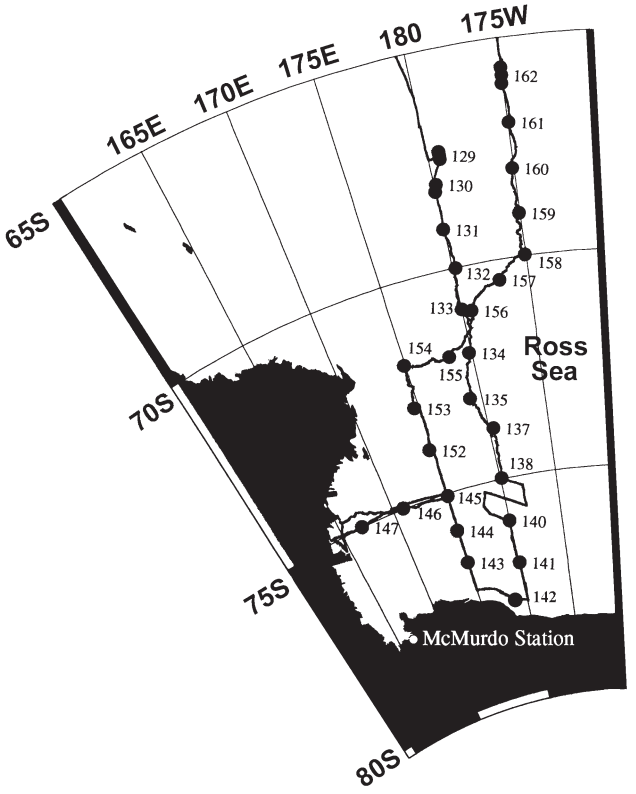

Fig. 1. Cruise track and station locations. Station number corresponds to day of the year; Stn 129 was sampled 9 May 1998 and Stn 162 was sampled 11 June 1998. Stn 142 was land-fast ice; other stations were pack ice. Two sites were sampled on Day 129, 3 on Day 130, and 3 on Day 162. This image was modified from the original cruise track provided by T. Schunck and C. Masters

MATERIALS AND METHODS

Sample collection. On the Ross Sea cruise NBP98-3 of the RV 'Nathaniel B. Palmer' from 9 May until 11 June 1998 we sampled sea ice at 33 stations and water and plankton from directly below the ice at some stations (Fig. 1). Sampling occurred at approximately every degree of latitude between 66 and 78° S, and 1 station was generally sampled each day. Floes were selected as representative of the ice cover in the area. On small floes requiring access via a personnel basket lowered over the side of the ship, 1 site was sampled. On floes large enough for the ship to park in, a 150 m transect was laid out to be as representative as possible of the floe's surface topographic variability. Three sites along the transect, representative of unridged ice topographic variability, were selected for ice coring for ice physics studies (e.g. Jeffries et al. 2001). If the ice physics core was visibly colored at a site, a virus core was taken there; otherwise a virus core was taken from the site closest to the ship. Ice cores of 7.5 cm diameter were taken with a motorized corer and immediately measured and split into either 10 or 20 cm sections using a saw. Where natural breaks or bands of color occurred in a core, section lengths were adjusted to avoid splitting those features. Each section was usually split longitudinally to provide aliquots for 2 different melting regimes (described below). Core sections were placed in plastic bags in an insulated box to minimize temperature changes during transport to the ship. Replicate cores for ice structure analysis, temperature and bulk salinity measurements (methods of Jeffries & Adolphs 1997), and ice community analysis (chl *a* and microscopy: Garrison et al. 1999) were taken within approximately 50 cm of the virus cores. We assumed that features of the replicate cores at each station were similar; we did not test this. Water samples (2 l) were collected from the core hole using a diaphragm pump with tubing extending about 50 cm below the bottom of the hole. Plankton tows were taken through the core hole to a depth of 5 m with a 10 µm mesh net with a 6 cm mouth. Brine samples were collected from brine holes in the vicinity of the core samples. Holes were drilled to within about 10 cm of the bottom of the ice with an ice auger, covered with pieces of plywood, and allowed to accumulate brine for a few hours. Brine salinity was measured in the field with a

refractometer; samples with abnormally low salinities (indicating that seawater had flooded the holes) or from holes that appeared flooded were discarded.

Sample processing and examination. Samples were processed for extracellular virus counts and for bulk community analysis of infected cells and ingested viruses in cell sections. A 'fast melt regime' was used to prevent grazing of heterotrophs on viruses, and a 'slow melt regime' was used to minimize loss of delicate cells from osmotic shock (Garrison & Buck 1986). Onboard ship, the 'fast melt regime' core sections (for virus counts and bulk community preparations) were placed in a mixture of 82 or 168 ml of 0.02 µm filtered seawater with 18 or 32 ml 5% borate-buffered 20% paraformaldehyde, respectively (giving a final fixative concentration of ~1%), and allowed to melt overnight at room temperature. When melting was complete, the total volume was measured to determine the dilution, and aliquots for virus counts were removed to sterile centrifuge tubes and refrigerated. 'Slow melt regime' core sections (for bulk community preparations) were placed in 1 l of 0.2 µm filtered seawater (Garrison & Buck 1986) and allowed to melt in the dark at approximately 2°C. At the few stations for which core sections were not split longitudinally for the different melting regimes, sections were melted under the slow regime using 2 l of 0.2 µm filtered seawater. After complete melting (usually 2 d) these samples were measured to determine the dilution and preserved with borate-buffered paraformaldehyde (1% final concentration).

Brine, plankton and water samples were preserved immediately upon return to the ship; 0.02 µm filtered fixative was used for aliquots for virus counts and 0.2 µm filtered fixative was used for aliquots for bulk community preparations and for plankton tows.

Samples for counts of extracellular larger viruses using TEM were shipped to the home laboratory under refrigeration via air. Viruses in aliquots of 10 or 20 ml (2 sequential 10 ml aliquots) were centrifuged (2 h at 288 000 × *g*, 25°C) in a Beckman L7-65 ultracentrifuge with a SW-41Ti rotor and stained with 0.5% uranyl acetate (the general protocol of Steward et al. 1996). We used 400 mesh carbon/formvar-coated copper grids (Electron Microscopy Sciences) that had either been coated with poly-L-lysine (Mazia et al. 1975) or had been made hydrophilic by glow discharge (Cochlan et al. 1993). Two grids were placed on a polycarbonate support for centrifugation (Wells & Goldberg 1992). Ten to 20 entire grid squares were viewed at 17 000×, and viruses of icosahedral, spherical, or 'lumpy' shape with a capsid diameter ≥110 nanometers (nm) were counted and measured using a stereoscope eyepiece reticle with concentric circles. The 'lumpy' category included particles that were intermediate between icosahedral and spherical. A commercial calibration grid was photographed at all magnifications used during each TEM session and was used to determine the true magnification (on our microscopes the true magnification typically differed from the displayed magnification by ±15%). An average grid square area was determined for each grid by measuring dimensions of grid square openings with a compound microscope; abundances of the viruses were calculated using the formula of Suttle (1993). The numbers of viruses counted per sample ranged from 0 to 357 and were generally 50 or fewer.

Ultrastructural morphology does not allow all viruses of eukaryotic cells to be distinguished from all bacteriophages. We have chosen the conservative criteria of an icosahedral, lumpy or spherical shape, dense staining, and capsid diameter ≥110 nm as indicative of viruses of eukaryotes. The size criterion was chosen because: (1) the average capsid size for viruses infecting eukaryotic algae is 152 nm (SD 108 nm, n = 46), although 28% are smaller than 60 nm (van Etten et al. 1991, cited in Cochlan et al. 1993; also see Lawrence et al. 2001); (2) the 4 divisions on our reticle used as a size cutoff gave a minimum size of 110 nm when the microscope magnifications were calibrated; and (3) a study of sizes of marine bacteriophages showed that they ranged from 30 to <110 nm (Weinbauer & Peduzzi 1994).

Samples for bulk community preparations (~500 ml to ~2 l, depending on the ice characteristics and melting regime) were reduced to a volume of about 15 ml at sea by low vacuum filtration over 47 mm diameter, 2.0 µm pore size polycarbonate filters. Generally, the slow melt samples were embedded at sea and the fast melt, water and brine samples were embedded at the home laboratory after being shipped under refrigeration by air. Organisms were pelleted by centrifugation (16 500 × *g*) in molten agar (2% in 80% seawater) that cooled during centrifugation. The pellet was cut into ~1 mm^3 blocks that were returned to the paraformaldehyde fixative, rinsed in 0.1 M sodium cacodylate in 80% seawater, post-fixed in 1% osmium tetroxide in buffer, rinsed in cacodylate buffer, gradually brought to deionized water, dehydrated in a graded acetone series, infiltrated and then embedded in Spurr's resin (Spurr 1969). Sections of 1 µm thickness were cut from several blocks for each sample, stained with 1% toluidine blue in 1% sodium borate, and examined at 400× with a light microscope. Blocks with a diversity of cells and those with many similar cells were then thin sectioned. Thin sections were cut with a diamond knife, collected on formvar-coated grids, stained with uranyl acetate and lead citrate (Reynolds 1963), carbon-coated, and examined at 80 kV with a JEOL 100B or a

JEOL 1200EX transmission electron microscope. Sections were viewed at 12 000 to 17 000× and examined for cells with visible viral infections and for heterotrophs with viruses in their food vacuoles. All cells observed in 1 section were identified to a broad taxonomic group and counted. Additional sections were examined but not counted (to avoid counting the same cells more than once).

Phaeodarian radiolarians (*Challengeron bicorne*, *Protocystis tridens* and *P. harstoni*) from plankton tows were used as particle samplers of the water directly below the ice. These protozoans are trophic generalists (e.g. Gowing 1989, 1993) and have the unique habit of retaining their waste vacuoles in a copious mass called a phaeodium (Cachon & Cachon 1985). One to 3 individual phaeodarians from Stns 137, 138, 143, 152 to 155, and 158 to 161 were enrobed in agar and embedded for TEM as described above. Sections were viewed at 30 000×, and food and waste vacuoles were examined for viruses.

Samples for total (all sizes) extracellular virus counts on filters using light microscopy were prepared at sea using the protocol for SYBR Green I (Noble & Fuhrman 1998) or Yo-Pro-1 (Xenopoulos & Bird 1997). Replicate filters with 0.2 to 2 ml were prepared for each sample, and solution blanks were prepared each time batches of samples were processed. Filters were mounted in ProLong™ Antifade medium (Molecular Probes) on slides, frozen at –80°C, shipped surrounded by dry ice to the home laboratory via air, and again stored at –80°C. Thawed slides were viewed at 1000× with an Olympus BX-60 epifluorescence microscope equipped with an Acridine Orange filter set. A minimum of 200 virus-like particles in 20 fields of view was counted for each sample.

Terminology and data presentation. We are using the term virus for convenience. Since the particles have not been shown to cause infection, they are more correctly referred to as virus-like particles. Abundance of total viruses and viruses ≥110 nm from core sections are reported in ml^{-1} brine because we assume that these particles are suspended in brine inclusions, rather than entrapped in the ice matrix. Brine volume in the ice was calculated from the temperature and bulk salinity of the adjacent core using the formula of Cox & Weeks (1983). For total viruses, abundances ml^{-1} melted sample are also given to allow a comparison with the Arctic ice study of Maranger et al. (1994a). Brine volumes in the samples ranged from 4 to 9% of surface core sections, from 3 to 17% of interior core sections, and from 6 to 21% of bottom core sections. Thus abundances ml^{-1} brine were always higher than abundances ml^{-1} melted sample, and there is no single conversion factor. Similarly, there is no single detection limit because brine volumes varied among samples.

RESULTS

The abundance of extracellular viruses ≥110 nm in capsid diameter in core sections ranged from below detection limits to 5.6×10^5 ml^{-1} brine in surface habitats, below detection limits to 2.5×10^6 ml^{-1} brine in interior habitats, and below detection limits to 1.2×10^6 ml^{-1} brine in bottom habitats (Table 1, Fig. 2). Where large viruses were detected, abundances ranged over 3 orders of magnitude and showed no pattern related to latitude (Fig. 2). Abundances of viruses did not differ significantly as a function of ice habitat (e.g. surface, interior, or bottom habitats) or ice structural composition (e.g. frazil, congelation, or mixed structure) (Kruskall-Wallis test, $p > 0.05$; tests among habitats and structural types calculated separately for all core samples). Abundances of large viruses in core sections were positively correlated with chl *a* (Spearmann rank correlation coefficient $R_S = 0.53$, $p < 0.001$, $n = 87$ sample pairs) and with phaeophytin ($R_S = 0.44$, $p < 0.001$, $n = 87$ sample pairs), which would be expected if algae are among the hosts. Abundances in brine samples

Table 1. Summary of abundances of large extracellular viruses in different ice habitats. Abundances are ml^{-1} brine for core samples and ml^{-1} for brine hole samples. b.d.l. = below detection limits (none counted in 20 TEM grid squares; see methods section for explanation). –: not applicable. Confidence limits were calculated as described by Lund et al. (1958). Ice cores ranged in thickness from 29 to 126 cm. Brine hole depths ranged from 19 to 80 cm

Sample type	Habitat	Total no. of samples	No. of b.d.l. samples	Minimum abundance	95% confidence limits for minimum	Maximum abundance	95% confidence limits for maximum
Cores							
	Surface	26	12	b.d.l.	–	5.6×10^5	4.4×10^5, 7.3×10^5
	Interior	50	11	b.d.l.	–	2.5×10^6	2.0×10^6, 3.2×10^6
	Bottom	27	11	b.d.l.	–	1.2×10^6	1.0×10^6, 1.4×10^6
Brine holes		19	0	1.4×10^3	4.6×10^2, 4.6×10^3	3.2×10^5	2.9×10^5, 3.5×10^5

from brine holes (a mixture of viruses from surface, interior and bottom habitats) ranged from 1.4×10^3 ml^{-1} to 3.2×10^5 ml^{-1} (Table 1).

A total of 2758 viruses ≥110 nm in capsid diameter of icosahedral (Fig. 3a–f), lumpy, and spherical morphologies were observed in the whole mount centrifuge preparations. Twenty of the icosahedral viruses had tails (e.g. Fig. 3c–f). Capsid diameters ranged up to 424 nm; the 2 most abundant size classes were 144 to 160 nm and 178 to 194 nm (Fig. 4). Large viruses also occurred in association with detrital material in the bulk community preparations examined in thin sections. These included intact viruses (e.g. Fig. 5d–f) and empty capsids (e.g. Fig. 5g).

We examined ultrathin sections of bulk community preparations of 19 brine samples, 102 core sections and 2 water samples that contained a total of more than 1054 dinoflagellates (including autotrophic and heterotrophic forms), 804 other heterotrophs, 9888 diatoms and 604 other algal cells (Table 2). Heterotrophic cells

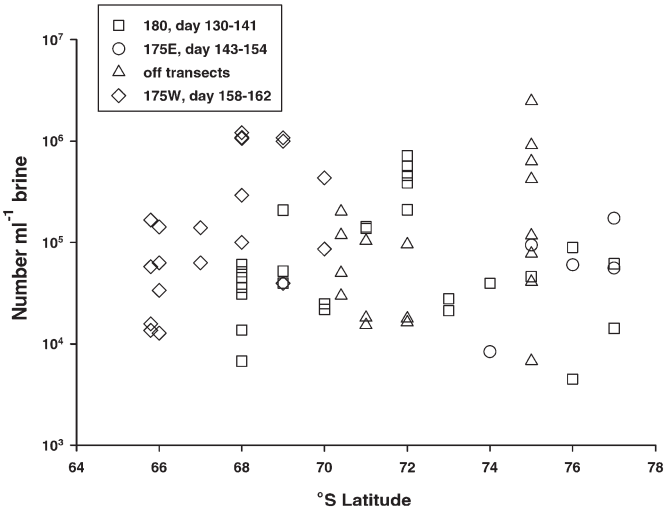

Fig. 2. Abundance of viruses (≥110 nm capsid diameter) ml^{-1} brine in all core sections (surface, interior and bottom habitats) where large viruses were detected

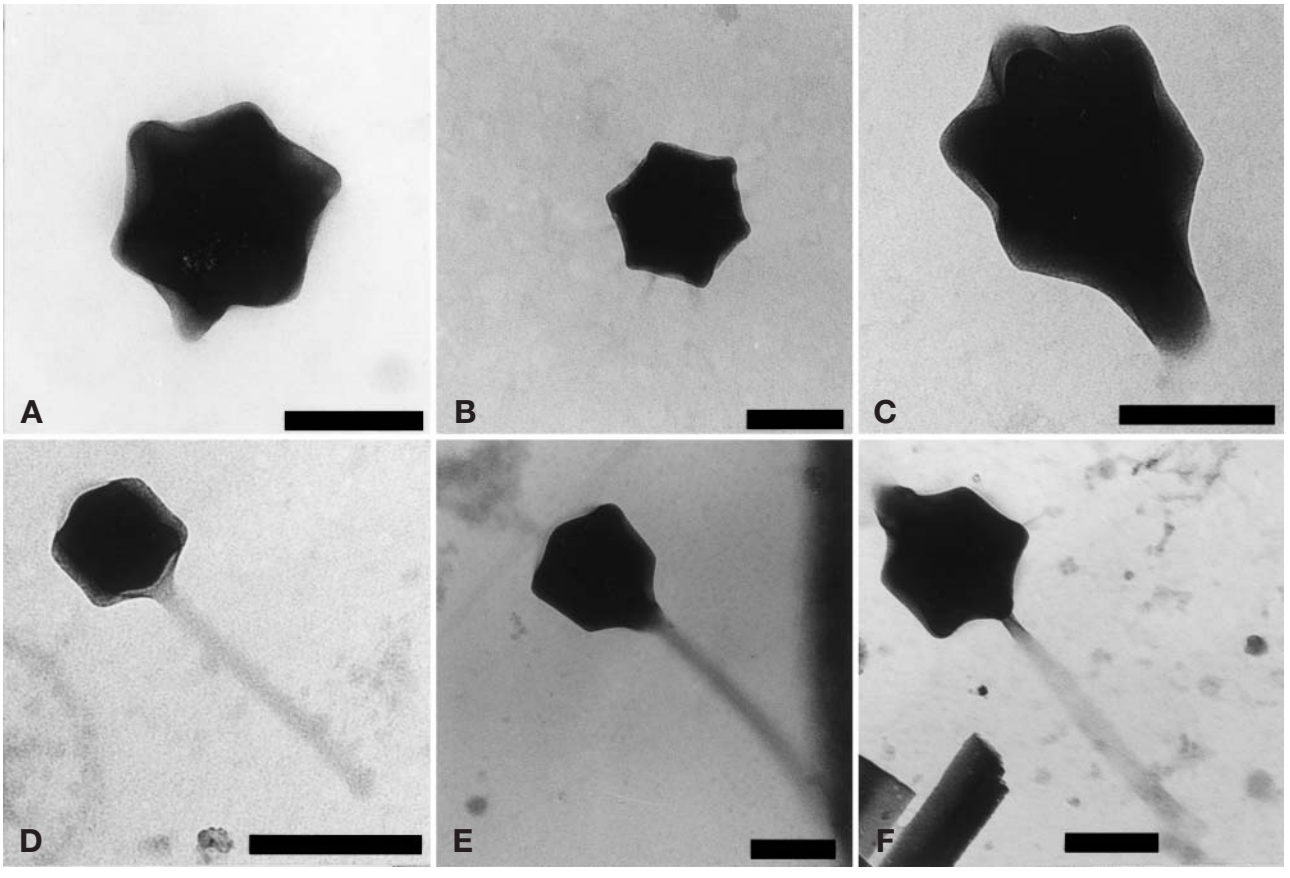

Fig. 3. Whole mounts of larger viruses of icosahedral capsid morphology. (A) Stn 159, bottom 20 cm in an 84 cm core. (B) Stn 133, interior habitat in a 63.5 cm core. (C) Stn 155, brine from brine hole. (D) Stn 156, brine from brine hole. (E) Stn 159, bottom core section. (F) Stn 160, bottom 10 cm from a 61.5 cm core. All scale bars = 200 nm

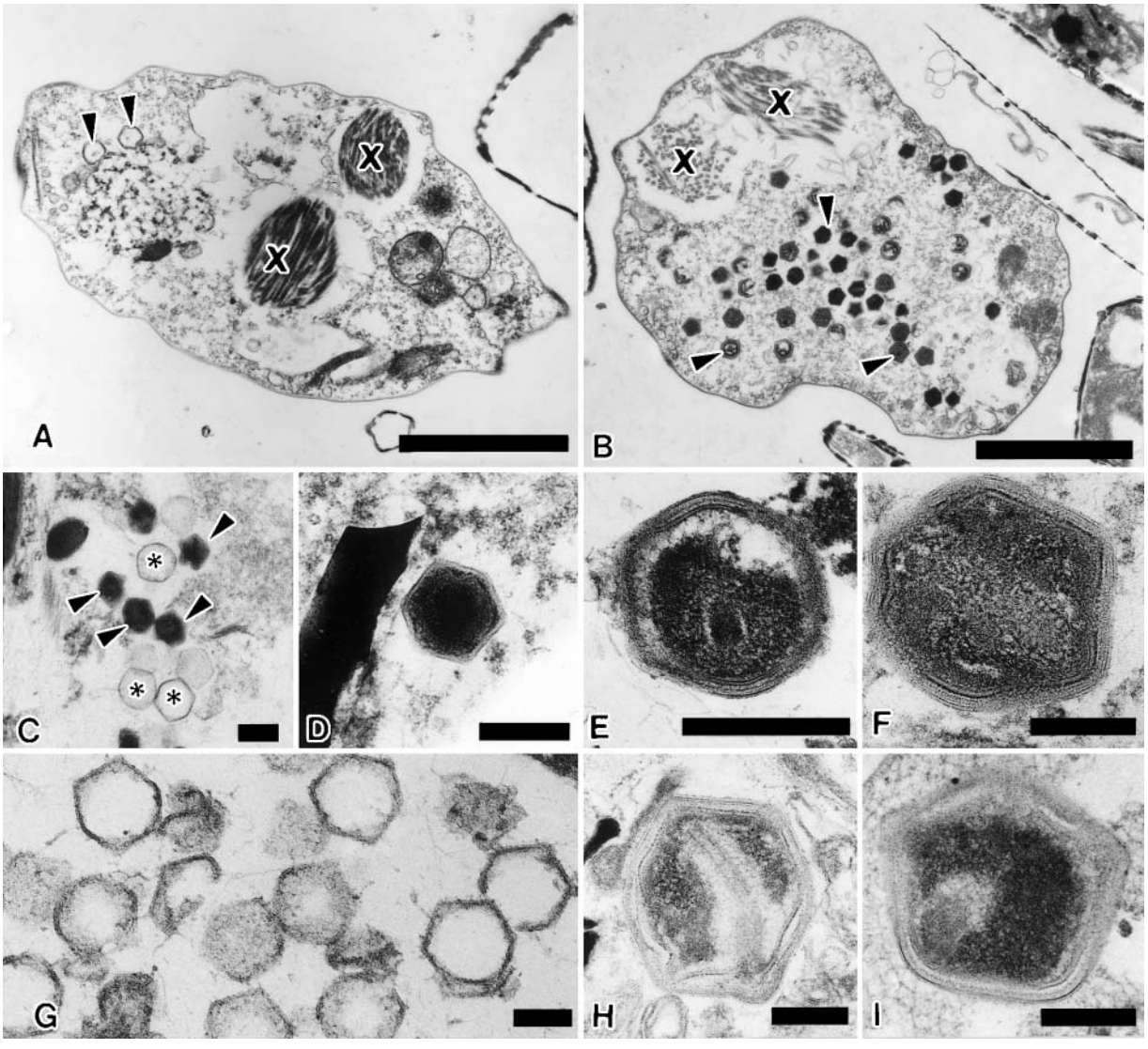

Fig. 5. Thin sections of viruses ≥110 nm capsid diameter. Arrows: viruses. X: food vacuoles containing trichocysts. (A) Section through an infected microheterotroph from the bottom core section at Stn 160. Scale bar = 2 µm. (B) Section through an infected microheterotroph from the bottom core section at Stn 159. Scale bar = 2 µm (C) Viruses and empty capsids (asterisks) from a fecal pellet from an interior habitat at Stn 134. Scale bar = 200 nm. (D) Virus in detrital material from the bottom core section at Stn 129-3. Scale bar = 200 nm. (E) Virus in detrital material from a brine hole sample at Stn 159. Scale bar = 200 nm. (F) Virus in detrital material from the bottom core section at Stn 140. Scale bar = 200 nm. (G) Empty capsids in detrital material from a brine hole sample at Stn 159. Scale bar = 200 nm. (H) Virus from a food vacuole of a phaeodarian radiolarian directly below the ice at Stn 158. Scale bar = 200 nm. (I) Virus from a food vacuole of a phaeodarian radiolarian from directly below the ice at Stn 138. Scale bar = 200 nm

included ciliates, heliozoans, *Cryothecomonas* spp., *Telonema* spp., choanoflagellates, aplastidic cryptophytes and other flagellates. Autotrophic cells included non-motile *Phaeocystis*-like cells, Parmales cells, chrysophyte cysts, motile and non-motile *Pyramimonas* cells, and other flagellated and non-flagellated cells. We observed only 5 infected cells. These were roughly similar heterotrophs with no specific diagnostic features (e.g. Fig. 5a,b). Two occurred in the bottom 20 cm of the core from Stn 159, and 3 occurred in the bottom 20 cm of the core from Stn 160. Their viruses were icosahedral, 181 to 397 nm in capsid diameter and were in the cytoplasm. No sections contained a nucleus, but the entire organisms were not serially sectioned.

We observed intact large viruses in fecal pellets from ice cores at 3 stations (e.g. Fig. 5c); capsid diameters ranged from 128 to 513 nm. In fecal pellets at 8 stations we observed empty capsids ranging from 186 to 560 nm in diameter. Smaller viruses (capsid diameters of 50 to 88 nm) were observed in fecal pellets from ice

Table 2. Summary of cells counted in TEM sections. For each station cell counts from core sections and brine hole samples have been totaled. Dinoflagellates include autotrophic and heterotrophic forms. P: present in low abundance and not counted. Diatom counts are shown as '>' when counts were stopped before all cells in a TEM section had been counted. Some other cell counts are shown as '>' when additional cells were present but not counted

Stn	Diatoms	Dino-flagellates	Other algal cells	Other heterotrophs
129	>214	10	21	10
130-1	>877	9	6	10
130-2	>579	10	1	8
131	>316	42	31	17
132	>487	23	33	30
133	>192	31	14	23
134	>790	27	17	27
135	>37	19	3	8
137	16	32	4	7
138	>15	11	3	5
140	>19	4	2	8
142	27	31	11	23
144	P	5	4	1
145	P	10	0	0
146	>406	11	3	19
152	P	8	1	4
153	P	23	1	2
154	>19	64	12	21
155	>19	23	1	9
156	>534	>23	>6	>28
157	>600	>9	45	>25
158	>267	9	>74	80
159	>1503	266	>158	194
160	>1562	223	133	185
161	>165	30	6	11
162-1	>668	43	10	14
162-2	>484	43	2	30
162-3	>92	15	2	5

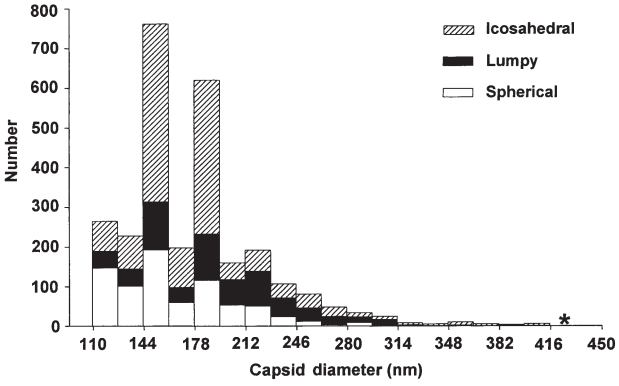

Fig. 4. Size-frequency histogram of viral capsid diameters. Diameters were the maximum diameter. Values are plotted at the mid-point of the size class. Size classes were 110–126, 127–143, 144–160 nm, etc. The largest specimen (424 nm capsid diameter) was a single icosahedral one denoted by the asterisk because a frequency of 1 does not show on the plot. Total n = 2758

cores at 6 stations. We observed intact larger viruses (capsid diameter range 152 to 607 nm) in 1 phaeodarian radiolarian each from plankton tows at 3 stations (e.g. Fig. 5h,i). Empty capsids (diameter range 177 to 593 nm) were observed in 1 phaeodarian each from 4 stations.

Total (all sizes) extracellular viruses in core sections ranged from 1.0×10^8 to 1.5×10^9 ml^{-1} brine (Table 3). The data set is small because problems with filter preparation rendered many filters uncountable; we show only data for samples for which at least 2 replicate filters were counted. At 2 stations entire cores were examined, and abundance did not differ significantly with position in the core. For the 8 core sections for which larger viruses were detectable on TEM grids and for which total counts were made on filters, the larger viruses ranged from 0.002 to 1% of the total viruses.

DISCUSSION

To provide an overview of the late autumn environment, a synopsis of conditions in the study area follows; detailed analyses of ice structure are reported in Jeffries et al. (2001), and abundance and biomass of organisms of ice communities will be reported elsewhere (Garrison et al. unpubl., Gibson et al. unpubl.). The total ice concentration exceeded 90% throughout much of the pack ice except for the marginal ice zone immediately adjacent to the open ocean where the

Table 3. Total (all sizes) viral abundances in ice cores. Ranges are the means of counts of 2 filters for all samples except for 1 bottom sample for which 3 filters were counted. Abundance per ml melted sample is included for comparison with the previous literature

Habitat	No. samples	Stns	Range $\times 10^7$ (ml^{-1} brine)	Range $\times 10^7$ (ml^{-1} melted sample) $\pm$ SE $\times 10^7$
Surface	4	135, 137, 140, 153	37–150	2.1 ± 0.01 to 10 ± 0.16
Interior	10	130-1, 132, 134, 135, 137, 147	10–120	1.0 ± 0.04 to 7.8 ± 0.77
Bottom	3	135, 137, 147	11–30	1.5 ± 0.41 to 3.4 ± 0.001

concentration was 70 to 80% at the beginning and end of the cruise (Morris et al. unpubl.). The pack ice was primarily composed of first-year ice from 0.3 to 0.7 m thick (Morris et al. unpubl.). Snow cover ranged from 0 to 36 cm. Frazil ice predominated north of 70° S, and congelation ice predominated south of 70° S (Jeffries et al. 2001). Chl *a* concentrations were generally higher north of 70° S, reaching a maximum of >20 mg m^{-2} (Garrison et al. 1999). Air temperatures ranged from 0.5 to –33.0°C and generally decreased with increasing latitude. Solar radiation decreased with latitude and with date, ranging from about 6 h at 68° S on May 1 to 0 h of light at 76° S on June 1 (Garrison et al. 1999).

Abundance (from below detection limits up to 2.49×10^6 ml^{-1} brine, equivalent to 8.2×10^4 ml^{-1} melted sample) of the large viruses in autumn sea ice is generally comparable to the few data for this size class from other polar locations sampled in different seasons. Large viruses (100 to 120 nm capsid diameter) in Arctic sea ice spring bloom samples peaked at about 7.5×10^6 ml^{-1} melted sample, equivalent to 10% of the total viruses on that date, and were otherwise <1% of the total viruses (Maranger et al. 1994a). Kepner et al. (1998) reported a November abundance of 6.7×10^3 ml^{-1} at 8 m in 1 Antarctic lake for viruses with capsids >100 nm in diameter; this size class was roughly 1% of the total viruses in 2 Antarctic lakes. Wilson et al. (2000) remarked that viruses 100 to 180 nm were common in their Signy Island February lake samples and that a few tailed viruses with 330×370 nm capsids were observed in 1 lake.

Various types of communities occur at different vertical positions within sea ice (reviewed by Palmisano & Garrison 1993), and physical processes occurring during sea ice formation affect sea ice communities (reviewed by Garrison 1991, Ackley & Sullivan 1994). These processes could also directly or indirectly affect viral abundance. Frazil ice forms under turbulent conditions, and algal cells can be harvested by the rising ice crystals or incorporated by propagating wave fields (Garrison et al. 1983, Gradinger & Ikävalko 1998 and references therein). Bacterial enrichment in newly forming ice can occur (see Gradinger & Ikävalko 1998); there are no data for viruses. Congelation ice grows under quiescent conditions or after an ice sheet forms, so increases in cell or viral abundance would presumably be due to growth or release, respectively, *in situ*. We found no difference in viral abundance in ice core sections consisting of frazil, congelation, or mixed frazil and congelation ice. Additionally, in our data set viral abundance did not differ among surface, internal, and bottom habitats. These results and the variability among samples (Fig. 2) illustrate the heterogeneity of habitats within sea ice. This differs from, for example, the water column where organisms and viruses typically show an abundance pattern related to depth.

Because viruses require host organisms for production and because extracellular viruses are lost over time by a variety of processes (see Wommack & Colwell 2000), the data indicate that viral infections occurred from 66 to 78° S in late autumn. Infected cells were therefore present going into winter. Although we are not absolutely certain that these viruses infect algae and protozoans rather than bacteria, their large size and morphologies are consistent with characteristics of algal and protozoan viruses (see reviews by van Etten et al. 1991, Reisser 1993, Proctor 1997), and they are larger than marine viruses known to infect bacteria (e.g. Weinbauer & Peduzzi 1994). As noted in the methods section, we did not count viruses <110 nm that infect algae and protozoans; therefore we did not sample total eukaryotic viruses. Abundances of the large viruses were positively correlated with chl *a*, a bulk measure of algal biomass, and with phaeophytin, an indicator of degradation of chlorophyll by grazing or senescence. Because viruses must attach to host cells, viral abundance should be highest where host cell abundance is highest, and the highest abundance ($>9 \times 10^5$ ml^{-1} brine) of larger viruses did occur in core sections that were visibly green at Stns 146, 159 and 160. Pigment measurements and cell counts were made at Stns 159 and 160. Chl *a* at the depths of highest viral abundances ranged from 16 to 73 µg l^{-1}, and phaeophytin ranged from 0.5 to 27 µg l^{-1}. The most abundant ($>10^5$ cells ml^{-1} melted sample) autotrophs included non-motile *Phaeocystis* spp. cells, chrysophyte cysts, *Mantoniella* spp., *Gymnodinium* spp., and several species of diatoms. Several different species of diatoms were present in abundance $>10^4$ ml^{-1} melted sample. The most abundant ($>10^5$ ml^{-1} melted sample) heterotrophs included choanoflagellates, unidentified flagellates, euglenoids, *Cryothecomonas* spp. and *Telonema antarctica*. Therefore there was a diversity of eukaryotes present in high abundance that were possible sources of the large viruses.

Viruses are only visible in a host during a short part of the lytic cycle (see discussion in Binder 1999), and in natural samples only a small percentage of cells may be undergoing lysis. During plankton blooms, when viral infections are known to occur, the percentage of visibly infected cells varies from <1 to 50 and is commonly <10 (e.g. Bratbak et al. 1993, Nagasaki et al. 1994, Brussaard et al. 1996). We looked at sections through more than 9000 diatom cells, and given the high abundance (from cell counts), we probably examined an adequate number of several species and saw no viruses in any of them. Interestingly, there is only one report (Proctor & Fuhrman 1991) of viruses infect-

ing diatoms; the putative viruses causing lysis of the pennate diatom (*Navicula* sp.) culture of Suttle et al. (1990, 1991) were subsequently determined to be 0.2 µm filterable, species-specific pathogenic bacteria (Chan et al. 1997, Suttle 2000). Diatoms and dinoflagellates may be less susceptible to viral infections than other algae because of their cell covering and exudate production (Zingone 1995). Diatoms would seem to have been an unlikely source of the larger viruses in our samples.

The lack of infected heterotrophs and non-diatom algal cells may have been because we simply did not look at enough cells of the numerically dominant species or because infected cells were not present. In most samples we probably only looked at a few specimens of any species. For Stns 159 and 160 (Table 3) where we looked at a large number of heterotrophs, there was also a high diversity of heterotrophs. It is also possible that our preparation methods caused infected cells to lyse (and thus not be observed) because processing ice cores did involve dilution along with osmolarity changes. However, we used the same methods during a summer study and observed numerous infected cells of 3 (albeit different) flagellate taxa (Gowing 1999). The sizes of the viruses in the late autumn infected heterotrophs were generally larger than most of the free viruses (Fig. 4). This suggests that there were other infected taxa in the ice.

Viruses were being consumed in late autumn, as evident by their presence in fecal pellets from ice samples and within food vacuoles of phaeodarian radiolarians from water directly below the ice at several stations. Based on size and contents, most of the small fecal pellets observed with viruses were probably produced by protozoans, although a few contained pulverized silica, suggesting a metazoan producer. Some of the viruses may have been digested, as only their outer boundaries remained, but others looked ultrastructurally intact. In most cases, the intact larger viruses appeared to have been ingested as individuals or as part of a particle rather than as infected cells because only 1 or a few viruses were observed in a section. One pellet contained a cluster of >24 uniform (ca. 186 nm diameter) empty capsids. That is consistent with digestion of an infected cell; however, no remnants of the cell were recognizable. Three pellets contained clusters of small (50 to 80 nm) viruses, consistent with digestion of infected bacteria. We observed sections through more than 804 microheterotrophs of a variety of taxa from the ice cores and brine hole samples but saw no viruses in food vacuoles. Viruses thus do not appear to be an important food source for ice community microheterotrophs at the time of our study. Whether this is due to avoidance of viruses or to their low abundance is not known.

Viruses in sea ice have only been investigated in one Arctic study (Maranger et al. 1994a). Abundance of viruses (<50 nm to 120 nm capsid diameter) in the bottom of the ice ranged from 9.0×10^6 to 1.5×10^8 ml^{-1} melted sample during a month of the spring algal bloom (Maranger et al. 1994a). Total (all sizes) viruses in the late autumn Ross Sea core sections were within this range (Table 3). Maximum viral abundance in Southern Ocean water column samples generally ranged from 10^5 to 10^7 ml^{-1} (Smith et al. 1992, Bird et al. 1993, Maranger et al. 1994b, Marchant et al. 2000). Maxima in Antarctic lake samples ranged from ca. 10^6 to 10^8 ml^{-1} (Kepner et al. 1997, 1998, Wilson et al. 2000). In contrast, abundance in the 17 core sections from the Ross Sea (Table 3) was generally higher, ranging from 10^8 to 10^9 ml^{-1} brine. Thus Antarctic sea ice is a habitat of high viral abundance, and the total viral community is likely to play a role in ice community dynamics.

In summary, large viruses are present in a variety of Antarctic sea ice microhabitats in late autumn and from the ice edge to the continent. Size data indicate that several eukaryotic taxa are probably infected, although we saw direct evidence of infection for only an unidentified heterotroph. Diatoms were unlikely to have been the source of the large viruses. Abundances of large viruses were positively correlated with chl *a*, but did not show an obvious relationship with microhabitat or ice structure. Abundances of large viruses reached a maximum of 2.5×10^6 ml^{-1} brine, whereas total (all sizes) viruses from a subset of samples ranged from 10^8 to 10^9 ml^{-1} brine. These abundances suggest roles for viruses in sea ice communities comparable to those in other ecosystems, but not as a food source for ice heterotrophs.

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