

Spatial distribution of viruses, bacteria and chlorophyll *a* in neritic, oceanic and estuarine environments

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ABSTRACT: The spatial distribution of viruses was investigated in the coastal and oceanic waters of the Southern California Bight, USA, and the brackish waters of the Gulf of Bothnia, Sweden, using the direct harvesting technique and transmission electron microscopy. The vertical and horizontal distributions of viruses were examined in relation to bacterial abundance and chlorophyll *a*. Total virus abundances ranged from 0.3 to $52 \times 10^9 \text{ l}^{-1}$; higher concentrations of viruses were found in the upper 50 m of the water column and in coastal environments. Viruses with capsid diameters less than 60 nm dominated the virus community, were morphologically characterized as bacteriophages and were responsible for most of the observed spatial variability. Bacteria abundance alone explained 67 % of the spatial variability in virus numbers, thereby suggesting that bacteria constituted the major host organisms for viruses in these physically diverse habitats.

INTRODUCTION

Viruses often numerically dominate the microbial community in marine and freshwater environments with estimates of virus abundance generally ranging from 10^8 to 10^{11} l^{-1} (e.g. Bergh et al. 1989, Proctor & Fuhrman 1990, Hara et al. 1991, Wommack et al. 1992). The coupled dynamics of virus abundance with bacteria and phytoplankton over a spring bloom period have been demonstrated, and marked increases in virus numbers observed within an enclosed seawater sample (Børsheim et al. 1990, Bratbak et al. 1990). These observations, together with the presence of phage-infected bacteria in seawater samples (Proctor &

Fuhrman 1990) and numerous isolation of viruses with marine bacteria and algae as hosts (e.g. Cannon 1987, Moebus 1987, Cottrell & Suttle 1991, Suttle et al. 1991, Van Etten et al. 1991), strongly suggest that the majority of viruses observed in seawater by transmission electron microscopy (TEM) are indigenous to the aquatic environment, and constitute an active component of the food web.

Given the potential importance of viruses as agents of mortality for phytoplankton and bacteria, and as vectors of genetic information, there is a need for knowledge of their spatial distribution in relation to the distribution of bacteria and phytoplankton. Quantitative distribution data may demonstrate the carrying capacity for viruses in different aquatic environments, and thereby indicate the likelihood for potential virus proliferation. Few systematic studies of total virus distribution have been reported (Hara et al. 1991, Wommack et al. 1992) and only 1 with detailed depth profiles (Hara et al. 1991). Both studies were in bays

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and little is known about virus distribution in more open coastal waters. Furthermore, it is not yet clear how viral abundance relates to the biomass of bacteria and phytoplankton, although virus-to-bacteria ratios have been shown to vary by almost an order of magnitude (e.g. Wommack et al. 1992). Based on the prevailing perception that algae and bacteria are the major viral hosts, we would expect a correlation to emerge with either of these parameters. There is also a paucity of information on the *in situ* distribution and spatial variation of viral size (Bergh et al. 1989, Bratbak et al. 1990, Wommack et al. 1992); information that could be relevant to the trophic fate of viruses.

Here we present direct counts of total virus abundance by TEM as a function of depth in the water column and distance from shore in coastal and oceanic environments off southern California, USA (Southern California Bight), and the brackish water environment off northern Sweden (Gulf of Bothnia). Vertical profiles of the size distribution of viruses at the different locations are also reported. The relationships of virus abundance to bacterial abundance and chlorophyll *a* (chl *a*), and the implications for their hosts and the population dynamics of marine viruses in natural marine systems are discussed.

MATERIALS AND METHODS

Sampling. Two separate transects of stations were sampled in the Southern California Bight aboard the RV 'Robert Gordon Sproul'. A transect off Santa Monica was sampled during September 1990 and 1991 (Stns 301, 303A, 304 & 305), and a transect off La Jolla (Scripps Pier) and into the San Diego Trough (Stns T2, T5 & T8) was sampled in December 1991. Three sites

Table 1. Description of stations sampled in the Southern California Bight and Gulf of Bothnia during 1990 and 1991

Stn	Maximum depth (m)	Location	Distance from shore (km)
Southern California Bight			
305	905	33° 45.12' N, 118° 55.09' W	46
304	217	33° 54.18' N, 118° 38.26' W	18
303A	55	33° 55.71' N, 118° 31.92' W	7
301	16	33° 54.00' N, 118° 26.50' W	0.9
T8	1000	32° 52.00' N, 117° 40.00' W	37
T5	675	32° 52.00' N, 117° 28.50' W	18
T2	62	32° 52.00' N, 117° 17.30' W	2.6
Gulf of Bothnia			
US5B	229	62° 35.20' N, 19° 58.32' E	63
F9	129	62° 42.50' N, 22° 04.00' E	33
NB1	25	63° 30.50' N, 19° 48.00' E	3

were sampled in the Gulf of Bothnia off northern Sweden aboard the Swedish Coast Guard Vessel 'KBV 04' during August 1991: Bothnian Bay (Stn F9), Bothnian Sea (Stn US5B), and a coastal station off the Norrby Archipelago (Stn NB1). Station locations are shown in Table 1. Seawater samples were collected between 07:00 and 09:00 h, using 10 l PVC Niskin or Hydro-Bios bottles (both equipped with silicon springs) in California and Sweden, respectively. Samples were immediately transferred to acid-cleaned and rinsed, high-density polyethylene bottles.

Chlorophyll. Duplicate samples for chl *a* were collected on Whatman GF/F filters and stored frozen in a desiccator. Chl *a* was extracted in methanol overnight and analyzed by *in vitro* fluorometry (Holm-Hansen & Riemann 1978) using a Turner Designs Model 10 fluorometer. Reported chl *a* values have been corrected for phaeopigments determined after acidification.

Bacteria abundance. Samples for bacteria counts were fixed with borate-buffered formalin (2 % final conc.) and stored at 4 °C in the dark until counted (generally within 6 h of sampling aboard ship). Samples were enumerated by epifluorescence microscopy following 10 min staining with 4',6-diamidino-2-phenylindole (DAPI; Porter & Feig 1980), filtration on black, 0.2 µm Nuclepore filters and immediate mounting in paraffin oil on glass slides. At least 200 cells or 20 fields were counted on each filter. We did not distinguish among the various types of procaryotes (e.g. heterotrophic bacteria, coccoid cyanobacteria and prochlorophytes).

Virus abundance. Samples (50 ml) were preserved with electron microscopy grade glutaraldehyde (2.5 % final conc.) and stored in sterile polypropylene centrifuge tubes in the dark at 4 °C. Harvesting of viruses was performed by ultracentrifugation directly onto TEM specimen grids in a procedure adapted from that of Nomizu & Mizuike (1986). The grids (200 mesh Cu, coated with carbon-stabilized Formvar film; Pelco® from Ted Pella Inc.) were rendered hydrophilic by high-voltage glow discharge (50 kV for 60 s) under vacuum; negatively charging the grid film prevents particle aggregation during drying (Hayat & Miller 1990). For each sample, 2 treated grids were placed into specially-constructed holders (details in Wells & Goldberg 1992) at the bottom of 13 ml polyallomer centrifuge tubes (Beckman). Subsamples (10 ml) were centrifuged (Beckman models L7, L8-M, L5-75B) using a swinging bucket rotor (SW41) at 41 000 rpm (288 000 × *g*) for 4 h at 25 °C. After centrifugation, the supernatant was carefully withdrawn and the grids stained with uranyl acetate (0.5 % w/v) followed by multiple rinses with 0.1 µm filtered glass-distilled water or 0.2 µm filtered Milli-Q® water. It was calculated, using Stokes Law for the velocity of sedimentation,

that 55S particles (determined at 20 °C in distilled water) of 50 nm in diameter would sediment with 100 % efficiency under these conditions. To test particle recovery efficiency, we centrifuged a monodispersed (ultrasonicated) suspension of latex microspheres (87 nm diameter; Ernest F Fullman Inc.) and compared these counts with those determined by counting (scanning electron microscopy) complete drops ($n = 6$) air-dried directly onto grids. Recovery efficiency was $93 \pm 0.2\%$ for the latex spheres under these conditions. As an additional test of particle sedimentation efficiency, we found that a marine phage isolate (50 nm capsid size, non-tailed) was completely removed from the supernatant after 2 h of centrifugation, as determined by plaque assays of supernatant sequents (1 ml) using the soft-agar overlay technique (Sambrook et al. 1989). Four-hour centrifugations were adopted to ensure adequate time for the sedimentation of the smallest viruses. Although shorter times may be adequate to sediment most viruses, centrifugation time and force have not been systematically tested on aquatic virus assemblages.

Air-dried grids were examined in a Hitachi H-500 TEM operated at 100 kV and at a magnification of 40 000 to 82 000 \times . In field samples, viruses were recognized on the basis of morphological characteristics – a regular polyhedral head with or without a tail. As in most previous reports of natural aquatic samples, we did not enumerate filamentous viruses, and were generally not able to identify the morphological details on particles much less than 30 nm in size. Hence the abundance estimates in the <30 nm class are likely underestimates as we only counted those small particles which we could distinguish clearly as viruses and excluded other similarly sized colloidal material. A minimum of 100 view fields were counted from at least

5 different grid openings. Counts were made directly from the electron microscope screen at 40 000 to 82 000 \times magnification, whereas greater magnifications (100 000 to 400 000 \times) were used for photography and measurement of virus dimensions. Viral abundance estimates were calculated taking into account the taper correction for non-parallel particle trajectories during centrifugation (Mathews & Buthala 1970).

Statistics. Correlation analyses were conducted using the data from individual stations (complete depth profiles) as well as combining the data (all stations and depths) for a particular cruise. The correlation coefficient (r) of normally distributed data was calculated by the usual Pearson product moment approach. The Spearman nonparametric rank difference correlation coefficient (r_s) was estimated for non-normally distributed data (Snedecor & Cochran 1980).

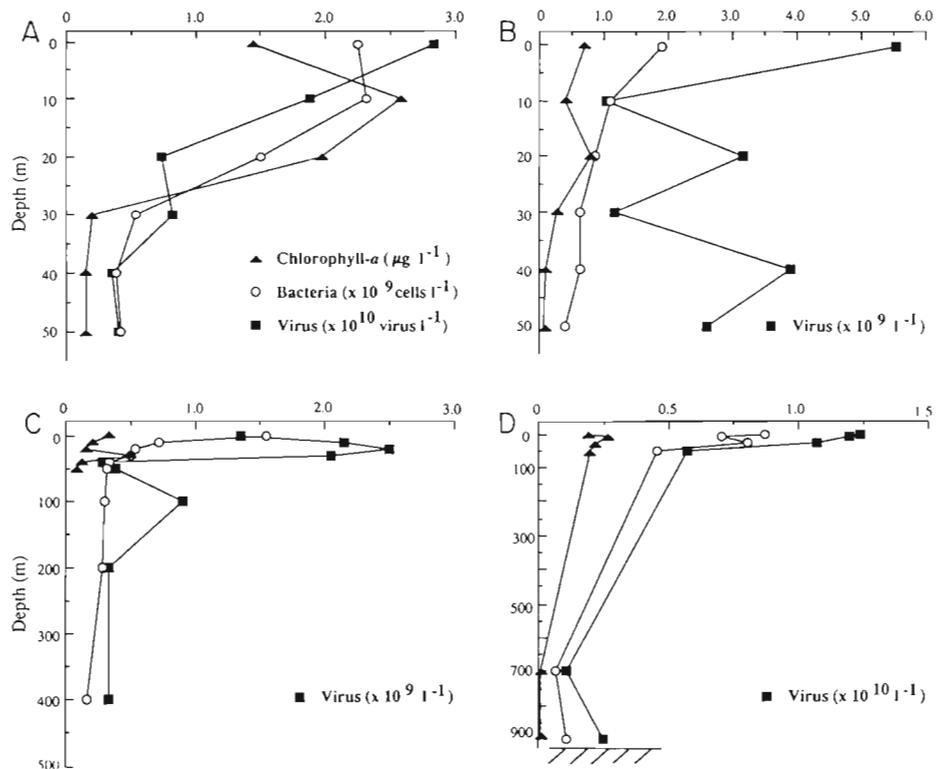


Fig 1. Depth profiles of total virus numbers, bacterial numbers and chlorophyll *a* from the Southern California Bight, Sep 1990 and 1991 (A) Coastal station 303A (1990): virus abundance covaried with bacterial numbers both according to a Pearson product moment correlation ($p < 0.05$, $r = 0.88$) and a non-parametric Spearman's rank test ($p < 0.05$, $r_s = 0.89$); chl *a* was not correlated ($p > 0.05$) with virus numbers by either method. (B) Mid-station 304 (1990): neither bacterial numbers nor chl *a* were correlated with virus numbers according to a Pearson correlation ($p > 0.05$) or a Spearman's rank test ($p > 0.05$). (C) Off-shore station 305 (1990): virus abundance covaried with bacterial numbers (non-normal distribution) according to a Spearman's rank test ($p < 0.05$, $r_s = 0.70$); chl *a* was not correlated ($p > 0.05$) with virus numbers by either parametric or non-parametric tests. (D) Off-shore station 305 (1991): virus abundance was correlated with bacterial abundance both according to a Pearson correlation ($p < 0.001$, $r = 0.98$) and a Spearman's rank test ($p = 0.02$, $r_s = 0.94$); chl *a* was correlated with virus numbers only according to a Pearson correlation ($p < 0.05$, $r = 0.88$). Shading in (D) represents sea floor

Normality of distribution was determined using the Minitab® (Ryan et al. 1980) equivalent to the Shapiro-Wilk test for normality at the 5 % level of significance.

RESULTS

Southern California Bight

The abundance of viruses generally decreased with depth at the stations in the Southern California Bight (Fig. 1). In most cases, a marked decrease in viruses

occurred at about 50 m depth, the lower part of the euphotic zone. In 1991, at the most seaward sampling (Stn 305), total virus numbers ranged from 12.4×10^9 l⁻¹ to 1.1×10^9 l⁻¹, while the previous year, corresponding numbers ranged from 10.7×10^9 l⁻¹ to 0.3×10^9 l⁻¹. Although the vertical distribution patterns of viruses were similar during the same month in 2 consecutive years, absolute numbers were > 5-fold higher in 1991. However, while the 1991 sampling of Stn 305 showed strong correlations between virus numbers and corresponding bacterial and algal parameters ($p < 0.001$ and $p < 0.05$, respectively), only virus and bacteria abun-

Table 2. Concentration and size distribution of viruses collected in the Southern California Bight. Discrete samples were collected at the depths indicated from off-shore stations (305, 304, T5 and T8), and coastal stations (301, 303A, T2 and Scripps Pier). Values in parentheses are \pm 95 % confidence limits. -: viruses were not enumerated according to this size range; ND: not detectable (detection limit: 10^4 particles ml⁻¹)

Stn	Date	Depth (m)	Virus abundance ($\times 10^6$ ml ⁻¹)					Total		
			< 30 nm	30-60 nm	60-80 nm	80-100 nm	> 100 nm			
305 ^a	1 Sep 1990	0	-	1.0 (0.3)	0.2 (0.2)	0.1 (0.1)	-	1.3 (0.3)		
		10	-	2.2 (0.5)	ND	ND	-	2.2 (0.5)		
		20	-	2.5 (0.6)	ND	ND	-	2.5 (0.6)		
		30	-	2.0 (0.5)	ND	ND	-	2.0 (0.5)		
		40	-	0.3 (0.2)	ND	ND	-	0.3 (0.2)		
		50	-	0.4 (0.2)	ND	ND	-	0.4 (0.2)		
		100	-	0.6 (0.3)	0.2 (0.1)	0.1 (0.1)	-	0.9 (0.4)		
		200	-	0.3 (0.2)	0.03 (0.06)	ND	-	0.3 (0.2)		
305	3 Sep 1990	0	2.6 (0.9)	1.6 (0.7)	6.4 (1.2)	0.1 (0.2)	0.1 (0.2)	10.7 (1.7)		
		100	0.1 (0.2)	0.5 (0.4)	1.4 (0.6)	0.2 (0.2)	0.1 (0.2)	2.4 (0.8)		
304	4 Sep 1990	0	0.9 (0.5)	2.5 (1.0)	1.8 (0.8)	0.3 (0.3)	ND	5.5 (1.4)		
		10	ND	0.9 (0.3)	0.1 (0.1)	ND	ND	1.0 (0.3)		
		20	1.1 (0.6)	1.2 (0.6)	0.7 (0.4)	ND	ND	3.2 (0.9)		
		30	0.7 (0.5)	0.5 (0.4)	ND	ND	ND	1.2 (0.6)		
		40	1.8 (0.7)	0.8 (0.5)	0.8 (0.5)	0.5 (0.4)	ND	3.9 (1.1)		
303A	2 Sep 1990	0	7.4 (1.7)	6.0 (1.3)	13.4 (2.3)	1.1 (0.7)	0.3 (0.3)	28.3 (3.2)		
		10	4.1 (1.2)	4.0 (1.1)	9.8 (2.1)	0.5 (0.4)	0.4 (0.4)	18.9 (2.7)		
		20	ND	3.9 (0.8)	2.8 (1.4)	0.6 (0.4)	ND	7.3 (1.9)		
		30	1.9 (0.8)	3.2 (1.0)	2.9 (0.9)	0.1 (0.2)	0.1 (0.1)	8.2 (1.6)		
		40	0.6 (0.5)	1.6 (0.6)	1.2 (0.6)	0.1 (0.1)	ND	3.4 (1.0)		
301	23 Sep 1991	10	1.4 (0.7)	6.0 (1.7)	3.3 (1.1)	0.4 (0.4)	0.1 (0.2)	11.1 (2.2)		
		305	24 Sep 1991	5	1.6 (0.9)	8.4 (1.6)	2.1 (0.8)	0.1 (0.2)	0.1 (0.2)	12.4 (2.0)
				10	1.4 (2.3)	7.2 (3.8)	2.5 (2.0)	0.4 (0.8)	ND	11.5 (4.9)
				30	1.0 (0.7)	7.3 (2.1)	2.4 (1.3)	ND	ND	10.7 (2.5)
				50	1.1 (0.6)	3.7 (1.1)	0.7 (0.5)	0.1 (0.2)	0.1 (0.2)	5.7 (1.4)
305	24 Sep 1991	700	0.4 (0.8)	ND	0.7 (1.0)	ND	ND	1.1 (1.3)		
		900	ND	1.1 (1.2)	1.4 (1.4)	ND	ND	2.5 (1.8)		
T2	9 Dec 1991	2	-	-	-	-	-	11.8 (3.4)		
T5	9 Dec 1991	2	3.0 (0.9)	19.2 (2.2)	2.6 (1.0)	0.1 (0.1)	ND	24.8 (2.5)		
T8	9 Dec 1991	2	-	-	-	-	-	15.4 (4.0)		
Scripps Pier	5 Dec 1991	0	2.0 (0.9)	22.6 (3.0)	3.2 (0.9)	0.2 (0.2)	0.1 (0.1)	28.2 (3.3)		

^a 30-60 nm includes < 30 nm viruses, 80-100 nm includes > 100 nm viruses

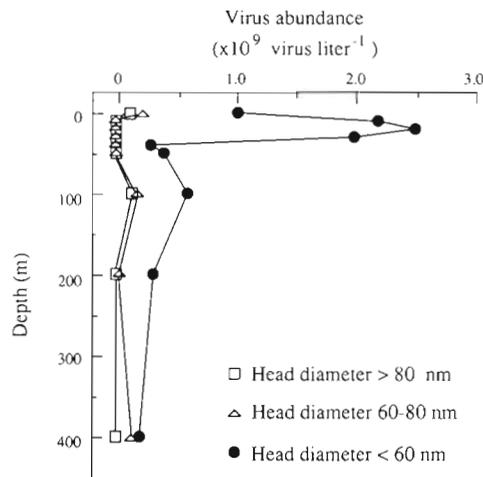


Fig. 2. Virus abundance in the various capsid size classes from Stn 305, in the Southern California Bight, 1 September 1990 (cf. Fig. 1C)

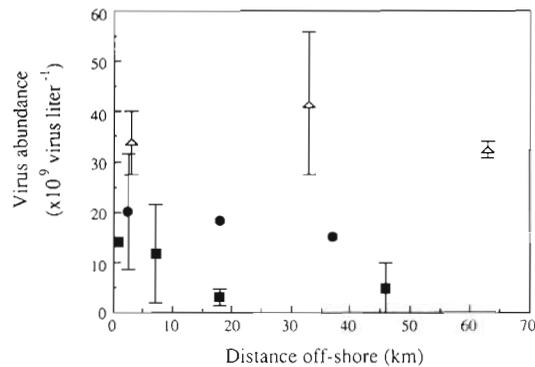


Fig. 3. Virus numbers in the upper 50 m of the water column in transects from coast (< 10 km from shore) to off-shore (> 45 km from shore). Samples are from (■) the Southern California Bight; (●) the San Diego Trough; and (△) the Gulf of Bothnia. Error bars are 95 % confidence intervals, when applicable. A statistically significant negative trend was demonstrated for the combined Pacific Ocean stations by least-squares linear regression ($p = 0.03$)

dances covaried at the 1990 sampling ($p < 0.05$). At the coastal station (Stn 303A) there was a 7-fold decrease in virus abundance with increasing depth, and virus abundance covaried with bacteria ($p < 0.05$) but not chl *a*. No significant correlations ($p > 0.05$) were found between viruses and bacteria or chl *a* at the mid-station (Stn 304). Combining the data for the upper 50 m of the 3 stations sampled in 1990, we observed that virus abundance was significantly correlated with bacteria abundance ($p < 0.05$), but not phytoplankton biomass (chl *a*, $p > 0.05$).

In general, viruses with capsid diameters less than 60 nm numerically dominated the viral assemblages (56 % of total, 1-way ANOVA, $p < 0.01$) and at the oceanic station (Stn 305) their abundance was particularly variable with depth (Table 2, Fig. 2). On 2 occa-

sions (Stn 305, 3 September 1990 and the coastal station, Stn 303A, 2 September 1990), viruses with 60–80 nm capsid size were of greater or equal importance to the < 60 nm size fraction, and made up a significant fraction of the virus communities.

A systematic decrease in average virus abundance in the upper 50 m of the water column was observed in transects off the southern California coast ($p = 0.03$, Fig. 3). Virus abundances in the surface waters off-shore (> 45 km from shore) were half those of the coastal waters (< 10 km from shore) and these viral assemblages were dominated by viruses in the < 60 nm size fraction. However, the greatest relative change in virus abundance with distance from shore was found in the > 60 nm capsid-sized viruses which averaged 5-fold lower abundance at the intermediate station (Stn 304) than at the coastal station (Stn 303A), and were not often detectable at the offshore station (Stn 305) (1-way ANOVA, $p = 0.02$; Table 2). The average decrease in total virus numbers throughout the water column was accompanied by a similar decrease in both bacteria numbers and chl *a* ($p < 0.03$, least-squares linear regression).

Gulf of Bothnia

Virus abundance was an order of magnitude higher in the estuarine waters of the Gulf of Bothnia than in the Southern California Bight (Tables 2 & 3), and there was no systematic decrease in virus numbers with distance from shore. However, in accordance with this, bacterial abundance was not significantly different at the coastal and offshore stations ($p = 0.61$, Student's *t*-test), although chl *a* was slightly higher off-shore. Total virus numbers only varied slightly with depth at the coastal station (Stn NB1) in the Gulf of Bothnia, as did the bacterial numbers and chl *a* (Fig. 4). However, within each size class a clear variation with depth could be seen for viruses with less than 80 nm diameter capsids (Fig. 5). The abundance of viruses at the offshore station F9 (Bothnian Bay) showed highest values in the euphotic zone with 5.2×10^{10} virus l^{-1} and 3-fold lower abundances at 120 m depth (Table 3). At the offshore Bothnian Sea station (Stn US5B) virus abundance was only 1.4 times lower at 210 m depth than in the surface water, although the virus abundance at this depth was remarkably high (2.3×10^{10} virus l^{-1}). Although the bacterial abundances were generally lower at the deeper depths, no significant correlation between bacteria and virus numbers could be demonstrated in the individual depth profiles. Treating all the data from the Gulf of Bothnia together we observed that total virus abundance was significantly correlated with bacteria abundance ($p < 0.02$), but not chl *a*.

Table 3. Concentration and size distribution of viruses collected in the Gulf of Bothnia, Sweden. Discrete samples were collected at the depths indicated from off-shore stations of the northern basin (Bothnian Bay, Stn F9), southern basin (Bothnian Sea, Stn US5B) and coastal waters of the Norrby Archipelago (Stn NB1). Values in parentheses are 95 % confidence limits. ND: not detectable (detection limit: 10^4 particles ml^{-1})

Stn	Date	Depth (m)	Virus abundance ($\times 10^6 \text{ ml}^{-1}$)					Total
			<30 nm	30–60 nm	60–80 nm	80–100 nm	>100 nm	
F9	6 Aug 1991	0	3.5 (1.1)	23.4 (2.5)	3.0 (0.9)	0.4 (0.3)	0.9 (0.5)	31.2 (2.8)
		6	9.5 (2.7)	35.4 (0.9)	4.5 (1.3)	1.2 (0.9)	1.6 (0.7)	52.2 (9.8)
		120	9.1 (11.6)	6.5 (1.5)	1.7 (0.7)	ND	0.1 (0.2)	17.5 (1.9)
US5B	7 Aug 1991	0	4.7 (1.3)	25.4 (0.3)	2.8 (0.8)	0.2 (0.2)	0.2 (0.2)	33.3 (3.0)
		6	4.2 (1.2)	24.2 (0.3)	2.4 (0.9)	0.1 (0.2)	0.3 (0.3)	31.2 (2.9)
		210	4.1 (2.3)	13.8 (1.9)	0.9 (0.5)	4.3 (8.1)	0.3 (0.3)	23.4 (2.2)
NB1	8 Aug 1991	0	4.7 (1.2)	35.8 (2.7)	8.7 (9.4)	0.6 (0.5)	0.6 (0.4)	50.4 (3.2)
		6	0.7 (0.5)	29.2 (3.1)	5.3 (2.0)	0.2 (0.2)	0.4 (0.3)	35.8 (3.4)
		23	9.0 (8.1)	24.4 (2.8)	2.9 (0.8)	0.1 (0.1)	0.1 (0.2)	36.5 (2.7)
NB1	22 Aug 1991	0	6.2 (1.5)	19.4 (2.3)	7.1 (1.6)	0.4 (0.3)	ND	33.1 (8.6)
		4	6.0 (1.4)	23.0 (3.0)	6.8 (1.4)	0.3 (0.3)	ND	36.1 (3.2)
		8	11.2 (2.2)	14.5 (2.4)	7.3 (1.3)	0.2 (0.2)	0.4 (0.3)	33.6 (3.6)
		20	7.6 (1.8)	15.2 (2.5)	3.1 (0.9)	0.2 (0.2)	ND	26.1 (3.5)
Average			6.2	22.3	4.4	0.6	0.4	33.9
± SD			3.0	8.5	2.5	1.1	0.4	9.5
% of total			18	66	13	1.8	1.1	–

Viruses with capsid diameters of 30–60 nm accounted for 66 % of the total, and numerically dominated virus assemblages in the Gulf of Bothnia (1-way ANOVA, $p < 0.001$, Table 3). As a consequence, the major part of the difference in virus abundance estimates between the Gulf of Bothnia and the Southern California Bight was due to this size fraction. Viruses larger than 80 nm made up only a small fraction of the virus assemblages in both the Gulf of Bothnia and

Southern California Bight waters. In the Gulf of Bothnia, viruses in the >100 nm size fraction were most abundant at Stn F9 while those 80–100 nm in size were most abundant at Stn US5B (1-way ANOVA, $p < 0.01$). Virus abundance in the fractions <80 nm did not differ significantly between stations.

Combined data

Treating the data from the Gulf of Bothnia and the Southern California Bight together, we found that

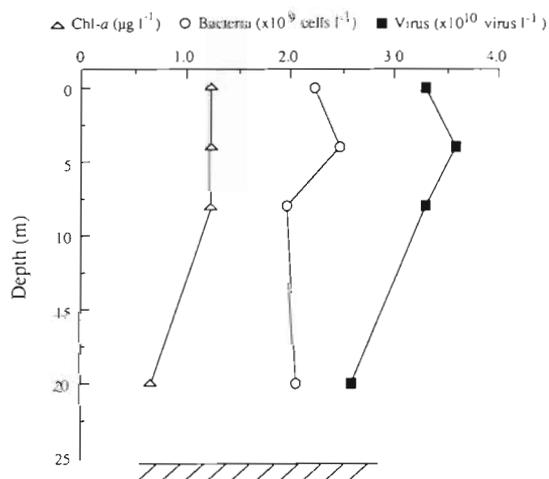


Fig. 4. Depth profile of total virus numbers, bacterial numbers and chl *a* from the Norrby Archipelago coastal station (Stn NB1), 22 August 1991 in the Gulf of Bothnia. Virus abundance did not covary with bacteria abundance or chl *a* ($p > 0.05$). Shading represents sea floor

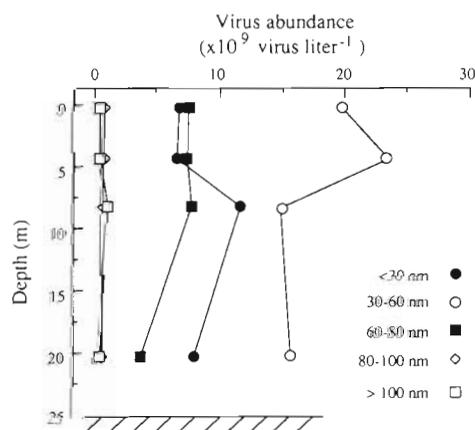


Fig. 5. Virus number in capsid size classes from Norrby Archipelago coastal station (Stn NB1) August 1991 in the Gulf of Bothnia (cf. Fig. 4). Shading represents sea floor

virus numbers were positively correlated with bacterial numbers (coefficient = 10.9, $p < 0.001$, $r^2 = 0.67$), whereas chl *a* did not show a coefficient different from zero (coefficient = 1.2, $p = 0.73$), as determined by a multiple regression between the 3 variables. The *x*-intercept was less than 2.5×10^8 bacteria l^{-1} , and not statistically significantly different from zero, based on 95 % confidence limits. As expected, bacterial numbers and chl *a* were intercorrelated in this data set ($p < 0.001$, $r^2 = 0.63$).

Morphology

In both environments, the 30–60 nm size fraction was numerically dominated by phage-like particles with long non-contractile tails (Bradley group B; Bradley 1967), but viruses with contractile tails (Bradley group A) were also present (Fig 6). The hexagonal heads were generally isometric (B1) or moderately elongated (B2). The average headsize in the largest size group was ca 100 to 120 nm, although viruses as large as 200 nm in diameter with tails ca 1800 nm in length were observed occasionally at depth in the Southern California Bight (Fig. 6C).

DISCUSSION

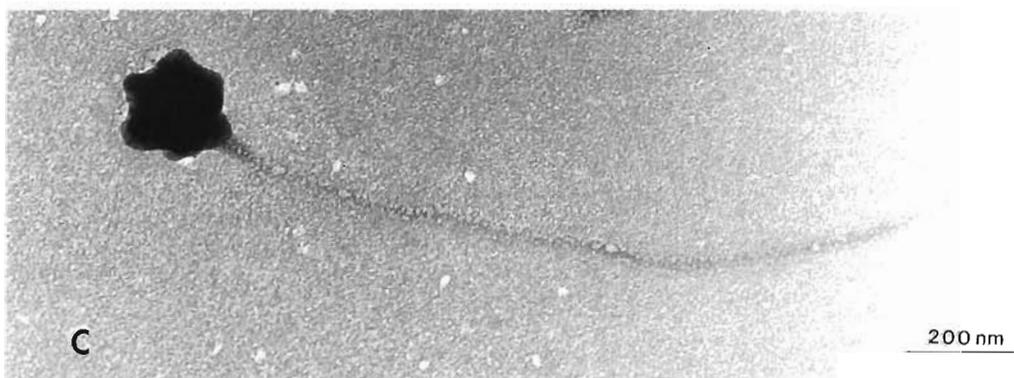
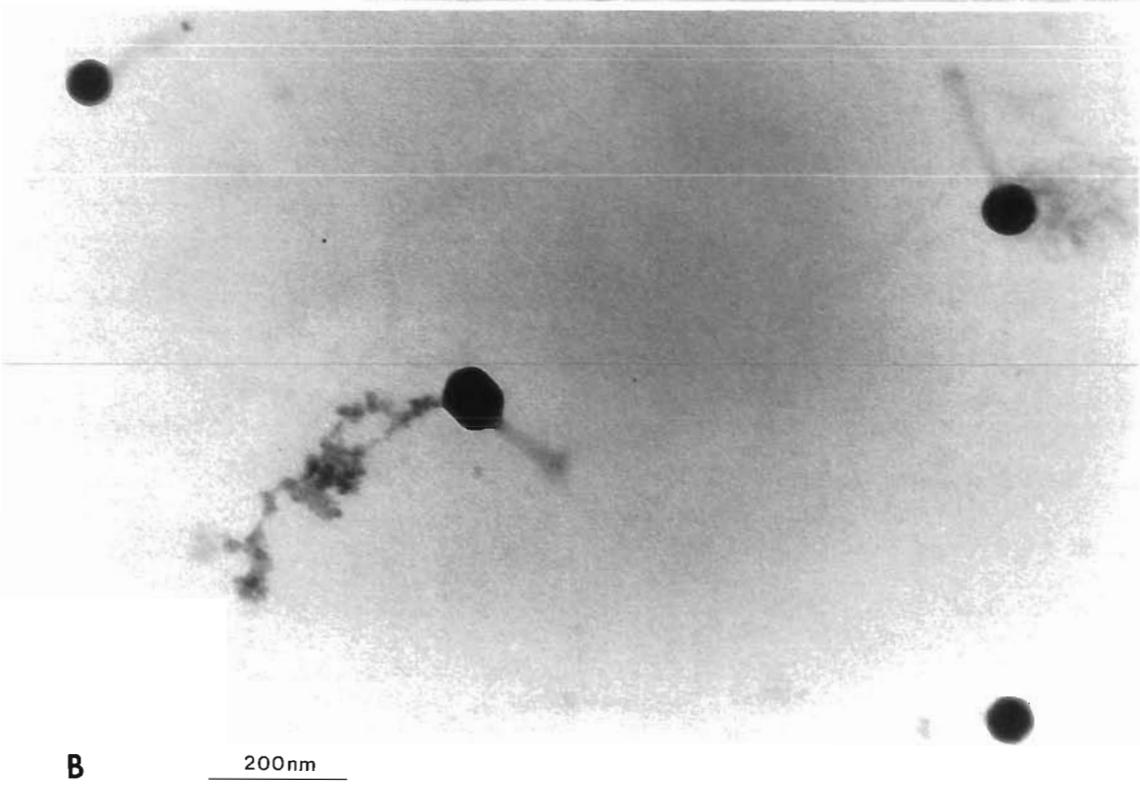
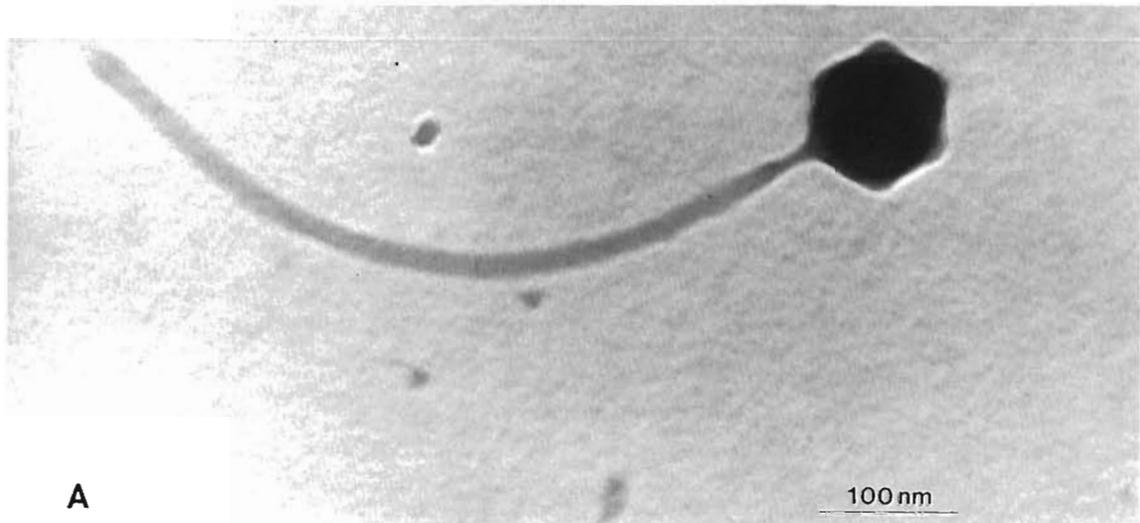
The spatial distribution of viruses in the marine environment was similar to the distribution of bacteria and phytoplankton (chl *a*), with an especially strong correlation to bacterial numbers. In the Southern California Bight, virus abundance was generally found to be higher in the surface and coastal waters. In addition, the viruses were clearly more abundant in the brackish waters of the Gulf of Bothnia (salinities 3.5 to 7 ppt) than in the oceanic environment of the Southern California Bight (salinities > 33 ppt). Our results agree with Hara et al. (1991) who reported higher abundance of viruses and bacteria in the surface waters of a 200 m depth profile in Sagami Bay, Japan. Our estimates of virus abundance are also in good agreement with recent reports of virus abundance in other marine environments (e.g. Bergh et al. 1989, Børsheim et al. 1990, Bratbak et al. 1990, Proctor & Fuhrman 1990, Hara et al. 1991, Wommack et al. 1992). Based on the observed distribution of viruses, we infer that the carrying capacity of marine viruses was higher in the euphotic zone and in coastal waters of oceanic environments, as previously established for bacteria and phytoplankton (e.g. Fuhrman et al. 1980). Thus, the viroplankton community appears to be favored by higher energy input and increased biological productivity in the pelagic ecosystem and likely constitutes an

active part of the marine food web as previously proposed (Bratbak et al. 1990, Proctor & Fuhrman 1990).

An important observation was a numerical dominance of the virus assemblages by viruses with capsids < 60 nm in diameter. Viruses in this size class were responsible for the major part of the spatial variability, both vertically within the water column and horizontally with distance from shore, in both environments (Tables 2 & 3). Viruses larger than 80 nm generally showed a different depth distribution than the smaller viruses, and their contribution to the virus community varied unsystematically with sampling site in the Gulf of Bothnia.

Our observations agree with those of Bergh et al. (1989) and Wommack et al. (1992), who reported that viruses of 30–60 nm capsid size dominated their water samples collected from the North Atlantic Ocean and the Norwegian coastline, and from the Chesapeake Bay, respectively. In addition, Bratbak et al. (1990) found that small viruses (< 60 nm) were the most abundant size class during a spring bloom in coastal Norwegian waters. These observations suggest that marine viruses in general are dominated by the < 60 nm size fraction, which also constitutes the most dynamic component of the virus community. The numerical dominance of the viroplankton community by small viruses suggests that larger viruses are produced at relatively slower rates and/or degraded at higher rates. Since flagellates are capable of ingesting material in the colloidal size range (Sherr 1988, González & Suttle 1993), the lower abundance of viruses greater than 80 nm may reflect a greater efficiency of grazing on larger viruses by heterotrophic flagellates. A previous study has demonstrated similar size-selective grazing pressure on the bacteria community by flagellates (Andersson et al. 1986). The potential significance of relative grazing pressure on viruses of different sizes has yet to be determined, but would add yet another level of complexity to the trophic dynamics of the microbial loop.

The capsid-size distributions of the natural marine virus assemblages reported in our study are more similar to that of bacteriophages than that of viruses infecting eucaryotic algae. The average capsid size for viruses of eucaryotic algae is reported to be 152 nm (SD = 108 nm, $n = 46$), although 28 % of these are less than 60 nm (Van Etten et al. 1991). Generally bacteriophages are smaller with an average capsid size of 70.0 nm (\pm SD 18.1 nm) based on 183 bacteriophages (Ackermann & DuBow 1987b). In the Pacific Ocean samples, capsid diameters of < 60 nm predominated, although viruses 60–80 nm in size made up a significant proportion of the total (Table 2). In the Gulf of Bothnia, viruses in the < 60 nm size class were clearly the most abundant (84 % of the total on average).



Although these smaller capsid sizes may be expected in a bacteriophage-dominated assemblage, there is, clearly, considerable overlap in the capsid diameters of eukaryotic and prokaryotic viruses. While suggestive, capsid size alone is not a reliable criterion for distinguishing between bacteriophages and algal viruses.

The viruses in the numerically dominant size fraction (30–60 nm) appeared phage-like with tails morphologically similar to both contractile and non-contractile types (Fig. 6). Thus, most of the marine viruses could be classified as bacteriophages of the Bradley B and A groups (or *Siphoviridae* and *Myoviridae*, respectively; Ackermann & DuBow 1987a). The viruses in the largest and smallest size groupings in our study were generally tail-less, had an isometric hexagonal outline, and probably could be assigned to Bradley groupings D and E, respectively, of the bacteriophages. On the basis of these morphological descriptions, we cannot ascertain the host (whether eucaryotic or procaryotic) specificity of the viruses, but it appears likely that at least members of the mid-sized groups were predominantly bacteriophages. Our observation that most of the viruses found in the 2 marine environments showed morphological characteristics common to bacteriophages is in agreement with previous reports (Torella & Morita 1979, Bergh et al. 1989, Bratbak et al. 1990, Proctor & Fuhrman 1990). In contrast, Wommack et al. (1992) found tail-less viruses to be numerically dominant in the 30–60 nm as well as the <30 nm size ranges of viruses in the Chesapeake Bay. Tailed viruses were dominant only in the >60 nm range in their study.

Based on a multiple linear regression, variation in bacteria numbers was found to explain 67 % of the variability in viral abundance and could be used as the sole predictor to explain change in virus numbers. The variation in chl *a*, indicating phytoplankton biomass, did not show a regression coefficient different from zero, according to the multiple regression. In a simple linear regression between the parameters, bacterial numbers demonstrated a better degree of explanation ($r^2 = 0.69$) than chl *a* ($r^2 = 0.45$) and virus abundance was on average 11.6 (95 % CI ± 4.4) times higher than bacterial abundance (Fig 7). A similar correlation between virus and bacteria abundance was also reported by (Paul et al. 1991) based on 11 samples from the Gulf of Mexico. A positive correlation between viruses and the major host organisms may be expected,

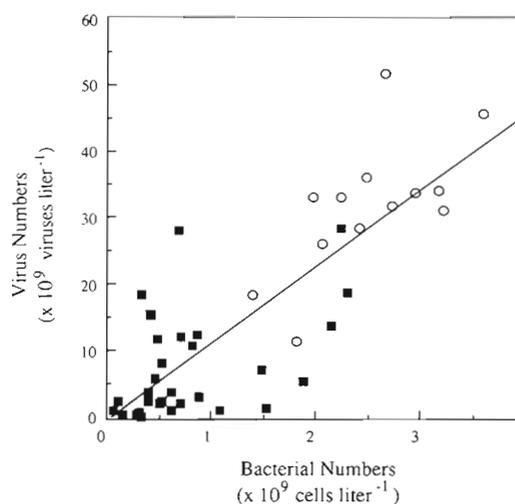


Fig. 7. Least-squares linear regression of the abundance of viruses and bacteria. Squares represent samples from the Southern California Bight and circles are samples from the Gulf of Bothnia. The slope coefficient is 11.6 (± 4.4 95 % CI), $p < 0.001$, $r^2 = 0.69$. The x-intercept is not statistically different from zero ($< 2.5 \times 10^8$ bacteria l^{-1} , $p > 0.05$)

since higher host density increases the adsorption rate of viruses, an important step in virus proliferation. It could be argued that an inverse correlation between virus and host abundance may occur as a result of predator-prey cycling. While this could be true for a given phage-host system on a temporal scale, it does not necessarily follow for a complex community with numerous different hosts and viruses examined on a spatial scale. Rather, in such a case, we argue that virus abundance will be driven by virus production, which will increase with increasing host density (Steward et al. 1992).

The generally poorer correlations between virus abundance and chl *a* concentration does not discount the possibility that phytoplankton are significant host organisms of the viruses in our study areas. Since chl *a* is only a proxy for phytoplankton biomass, it may not accurately reflect phytoplankton abundance; variations in chlorophyll content per cell may occur as a result of changes in depth and/or species composition.

We could not demonstrate a statistically significant threshold (x-intercept $< 2.5 \times 10^8$ bacteria l^{-1} , $p > 0.05$) of bacterial density for virus abundance, as has been proposed by other studies (Wiggins & Alexander 1985). We can only speculate that a threshold, if present, was

Fig. 6. Viruses observed by TEM in natural water samples from the Southern California Bight and the Gulf of Bothnia. (A) Single bacteriophage from 100 m depth in the northern Gulf of Bothnia (Stn F9) with capsid diameter of ca 100 nm and a long (0.58 μm) contractile tail. (B) Four tailed-viruses from the surface waters of Santa Monica Basin (Stn 303A), sample taken relatively close (3 to 6 km) from Hyperion waste treatment outfalls for the City of Los Angeles, California. Capsid diameters are 60 to 80 nm and tail lengths 130 to 180 nm. (C) Large virus with a capsid diameter of ca 200 nm and a long tail of ca 1.75 μm collected from 100 m depth in Southern California Bight (Stn 305) in September 1990

below our detection limit (10^7 l^{-1}), or that lysogenic bacteria are common, giving rise to production of phages even at low bacterial concentrations. Alternatively, at lower bacterial abundances representative of the oceanic mesopelagic depths in our data set, high bacterial densities on decomposing particles, not recorded by conventional microscopy, may comprise microhabitats of high phage production (Proctor & Fuhrman 1991). In a deep profile of our most seaward Southern California Bight station (Stn 305, Fig 1D) both the abundance of viruses and bacteria declined with depth, but increased in close proximity to the ocean bottom. Relatively high virus abundances have also been reported at 2000 m in the North Atlantic Ocean by Paul et al. (1991) which raises an important question as to the maintenance of these deep virus assemblages. Preliminary results of Steward et al. (1992) have shown a positive correlation between bacteria abundance and virus production rates. The enriched nutrient supply available for bacterial utilization in the benthic boundary layer can support larger bacterial populations (e.g. Smith et al. 1986) and thereby support *in situ* phage production rates greater than those of the overlying mesopelagic waters. Alternatively, since viruses are known to be associated with sinking particulate organic matter (POM) (Proctor & Fuhrman 1991), the maintenance of a large virus community at depth may reflect the solubilization of POM at depth with the concomitant release of free viruses by desorption or by lysis of particle-associated bacteria.

The correlations observed in this study between total virus numbers and bacterial numbers, and the size distribution and morphology of viruses are consistent with the conclusion that bacteria are the major host organisms of marine viruses. The suggestion that marine viruses are mainly bacteriophages has been assumed in previous recent studies based primarily on morphological criteria (Bergh et al. 1989). However, the use of morphological characteristics as the sole criterion to distinguish between bacteriophages and other viruses is questionable, since viruses which infect both heterotrophic bacteria and cyanobacteria, as well as eucaryotic algae, show isometric hexagonal capsids, and tailed virus may be present in all categories (Ackermann & DuBow 1987a, Cannon 1987, Van Etten et al. 1991, Bratbak et al. 1992). Additionally, we can not rule out the possibility that tails or tail-like structures were lost during either centrifugation or TEM preparation. Therefore direct quantitative evidence favoring either bacteria or algae as the major host organisms is presently not available. Attempts to quantify bacteriophages by the soft agar overlay technique have shown that there are phages against marine bacteria, but the concentration of plaque forming units

(PFU) obtained is usually less than 1 % of recent total virus counts (Moebus 1987). A similar situation exists for algal viruses and cyanophages in the few reports of PFU's in field samples against a specific host (Cannon 1987, Cottrell & Suttle 1991). The prevailing view that most viruses are bacteriophages therefore relies on indirect evidence such as morphology, capsid size and correlation with, and the abundance of, potential host organisms. It is noteworthy, however, that none of the indirect evidence in our study favored eucaryotic algae as the major host organisms of marine viruses.

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