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Concentration of Viruses and Dissolved DNA from Aquatic Environments by Vortex Flow Filtration

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Vortex flow filtration (VFF) was used to concentrate viruses and dissolved DNA from freshwater and seawater samples taken in Florida, the Gulf of Mexico, and the Bahamas Bank. Recoveries of T2 phage and calf thymus DNA added to artificial seawater and concentrated by VFF were 72.8 and 80%, respectively. Virus concentrations determined by transmission electron microscopy of VFF-concentrated samples ranged from 3.4 × 10^7/ml for a eutrophic Tampa Bay sample to 2.4 × 10^5 for an oligotrophic oceanic surface sample from the southeastern Gulf of Mexico. Viruslike particles were also observed in a sample taken from a depth of 1,500 m in the subtropical North Atlantic Ocean. Filtration of samples through Nuclepore or Durapore filters (pore size, 0.2 μm) prior to VFF reduced phage counts by an average of two-thirds. Measurement of dissolved-DNA content by Hoechst 33258 fluorescence in environmental samples concentrated by VFF yielded values only ca. 35% of those obtained for samples concentrated by ethanol precipitation (the standard dissolved-DNA method). However, ethanol precipitation of VFF-concentrated extracts resulted in an increase in measurable DNA, reaching 80% of the value obtained by the standard method. These results indicate that a portion of the naturally occurring dissolved DNA is in a form inaccessible to nucleases and Hoechst stain, perhaps bound to protein or other polymeric material, and is released upon ethanol precipitation. Viral DNA contents estimated from viral counts averaged only 3.7% (range, 0.9 to 12.3%) of the total dissolved DNA for samples from freshwater, estuarine, and offshore oligotrophic environments. These results suggest that viruses are not a major component of the dissolved DNA, although they may be involved in its production by lysis of bacterial and phytoplankton cells.

The occurrence of viruses and, in particular, bacteriophages in the marine environment has been known for some time (1, 2, 6, 12, 13, 18, 28, 29, 36, 38). Accurate methods for enumeration of total viruses, akin to methods for direct enumeration of bacteria (9, 14), did not exist before 1989, and estimates based on plaque assays on defined hosts implied that the occurrence of these viruses was infrequent or sporadic. Semiquantitative observations by transmission electron microscopy (TEM) (15, 26, 27, 34) suggested that viruses were abundant in the marine environment. Using ultracentrifugation to concentrate viruses from natural waters, Bergh et al. (3) reported viral abundances in natural water as high as 2.5 × 10^8/ml, or 10^3 to 10^7 times higher than had been found by the plaque assay. These observations have since been corroborated by other investigators, who used other methods to concentrate viruses (4, 5, 24, 30, 31).

For some time, we have been interested in the distribution and dynamics of dissolved (<0.2 μm) DNA in marine and freshwater environments (7, 8, 20–22). The recent observations of the abundance of viruses in aquatic environments prompted us to investigate what proportion of the dissolved DNA was attributable to nucleic acids encapsulated in virus particles. As part of this goal, we adapted vortex flow filtration (VFF) technology to the simultaneous concentration of viruses and free (soluble) DNA from the dissolved (<0.2 μm) fraction of freshwater and marine environments.

VFF is a filtration technology based upon Taylor vortices (33). Taylor vortices are established in a VFF device by rotation of a cylindrical filter inside a second cylinder. The sample is fed under pressure between these two cylindrical surfaces, forcing fluids across the filter and into the inner cylinder for collection or out to waste. The vortices constantly keep the filter surface clean, thus preventing clogging.

In this report we demonstrate the application of this technology to concentrate viruses and dissolved DNA in marine and freshwater samples. Our results suggest that the amount of DNA encapsulated in viral particles is a small fraction of the dissolved DNA in aquatic environments.

MATERIALS AND METHODS

Phage and bacterial strains. T2 phage was propagated in *Escherichia coli* B (ATCC 11303-B2) by agar overlay. The phage-containing cell lysates were eluted from overlays with sterile 0.5 M Tris-HCl (pH 8.0), sterile filtered, and stored at 4°C until use. Phage titers were determined by the plaque assay method.

Field sampling sites. Freshwater samples were collected from the Medard Reservoir, Valrico, Fla. Estuarine surface water samples were taken from Tampa Bay at Bayboro Harbor, North Shore Park, and the Pier, St. Petersburg, Fla. Samples (10 m depth) were taken in the southeastern Gulf of Mexico and the Dry Tortugas during a research cruise aboard R/V *Pelican* from 22 to 29 June 1990. Samples were taken outside the mouth of Tampa Bay (St. 1: 28°33.89'N, 82°55.03'W), in oligotrophic offshore Gulf of Mexico waters (St. 2: 26°38.96'N, 85°40.00'W; St. 3: 24°49.94'N, 85°20.00'W), on the southwestern side of 29°49.94'N Loggerhead Key in the Dry Tortugas, and in Florida Bay (24°39.00'N, 81°54.25'W). During a second research cruise aboard R/V *Cape Hatteras* (cruise no. CH-12-90), water samples were taken in the Atlantic Ocean near Miami

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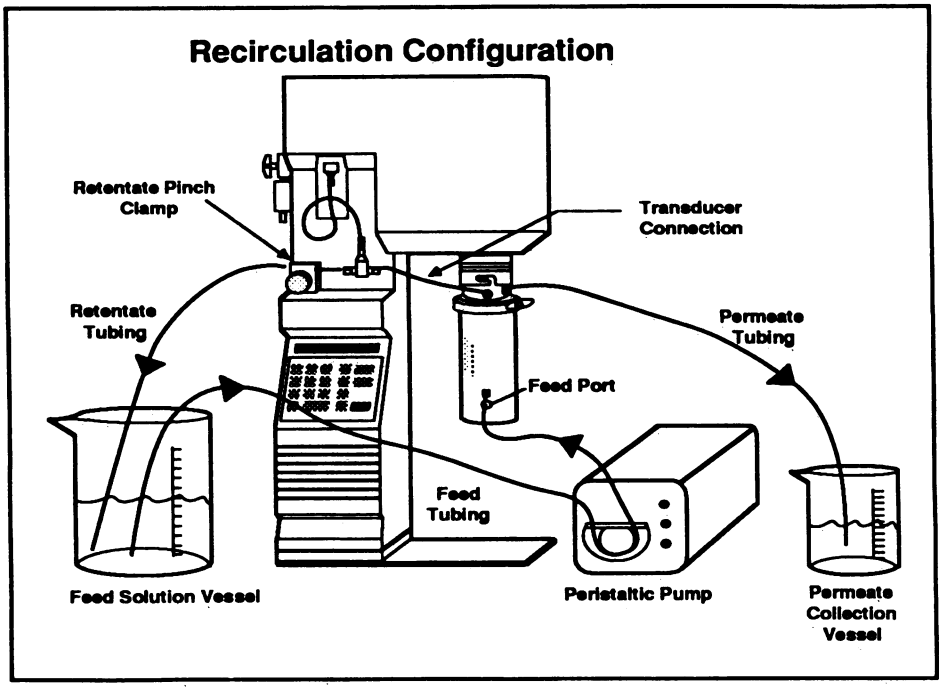

FIG. 1. Membrex Benchmark VFF device used to concentrate viruses and dissolved DNA from the marine environment. The sample is added to a feed solution vessel and pumped by a peristaltic pump through the feed port to the filter chamber. The filtrate (termed the permeate) goes to waste, and the viruses retained by the filter (termed the retentate) return to the feed solution vessel. This apparatus has been successfully used to concentrate viruses from 3 to 100 liters down to 25 ml. A larger industrial version is also available, capable of processing ca. 1,000 liters.

(25°45.10'N, 80°05.14'W); in Northwest Providence Channel, Bahamas (26°07.90'N, 78°32.50'W); in the subtropical North Atlantic Ocean at a depth of 1,500 m (25°46.90'N, 76°49.50'W); and near Mama Rhoda Rocks, Chub Cay, Bahamas (25°24.40'N, 78°55.60'W). Water samples were taken with Niskin bottles on cruises and with a dip bucket for Tampa Bay sampling.

Recovery of T2 phage and calf thymus DNA from artificial seawater. T2 phage (final concentration, ca. 10^8/ml) or calf thymus DNA (final concentration, 10 to 30 μg/liter) was added to 2 or 3 liters of autoclaved, sterile-filtered artificial seawater (19). Phage titers were determined immediately after addition to seawater prior to VFF and after concentration by VFF. The DNA content in concentrated samples was determined by the fluorometric Hoechst 33258 method (23).

Effect of prefiltration on viral recovery. Sterile artificial seawater containing T2 phage or natural water samples were filtered through Whatman GF/D and 0.2-μm-pore-size Nuclepore filters at a vacuum of <150 mmHg prior to VFF concentration. The volumes prefiltered ranged from ca. 250 ml to 1.0 liter depending on the nature of the sample (i.e., estuarine or offshore oligotrophic). A replicate sample was also concentrated by VFF without prefiltration. At the Loggerhead Key station, a third sample (>20 liters) was also passed through a 142-mm Millipore 0.22-μm-pore-size GV Durapore filter by using positive filtration pressure. The samples were immediately concentrated by VFF (see below).

VFF concentration of samples. A Benchmark rotary biofiltration unit (Membrex Inc., Garfield, N.J.) was used for VFF concentration of samples. The system was set up in the recirculation configuration (Fig. 1) with either a 200-cm^2 or a 400-cm^2 filter. The 400-cm^2 filter allowed a greater sample filtration rate (2.4 liters/h for the 30-kDa filter; 7.5 liters/h for the 100-kDa filter). Filtration was performed at 7 to 8 lb/in^2 for the 100-kDa filter and 10 to 12 lb/in^2 for the 30-kDa filter with a filter rotation speed of 2,000 rpm (200-cm^2 filter) or 1,500 rpm (400-cm^2 filter). The sample was pumped in from the feed vessel in the recirculation mode until the feed vessel was empty (Fig. 1). At this time the feed port was closed, an air pump was fitted to the retentate line, and the retentate volume was reduced under positive air pressure to 15 to 25 ml (200-cm^2 filter) or 35 to 50 ml (400-cm^2 filter). The retentate was either used directly in dissolved-DNA measurements (see below) or fixed with 2% glutaraldehyde and further concentrated for TEM by ultracentrifugation for 90 min at 70,000 × g.

Enumeration of viruses. (i) TEM. VFF-concentrated samples were diluted with deionized water (3- to 40-fold) to decrease the salt content, and 1 μl was spotted onto a Formvar-coated copper grid and allowed to dry. The area of the drop was subsequently measured with a 10× optical micrometer. The samples were stained with 2% uranyl sulfate (Polysciences, Warrington, Pa.), allowed to dry, and viewed with a Hitachi H500 TEM. Counting was performed at a magnification of ×48,000, and photography was performed at a magnification of ×70,000 to ×150,000. The magnification of the TEM was calibrated by using catalase crystals.

(ii) Epifluorescence microscopy. Virus-containing samples were stained with 10^{-5} M 4',6'-diamidino-2-phenylindole (DAPI; Aldrich Chemical Co.) for at least 20 min at room temperature in a foil-wrapped container. Then 10 μl of the stained suspension was spotted onto a clean glass slide and

TABLE 1. Comparison of filtration efficiency of the Membrex Benchmark system when using sterile artificial seawater

Sample and date (mo/day/yr)	Filter retention cutoff (kDa)	Initial titer (10^7/ml) or DNA concn (μg/liter)	Final titer (10^7/ml) or DNA concn (μg/liter)	Recovery efficiency (%)
T2 phage[a]				
3/7/90	100	10	8.1	81
7/24/90	100	11.7 ± 2.6	8.4 ± 3.6	72
7/12/90	30	20.8 ± 2.3	14.1 ± 6.6	68
7/24/90	30	11.7 ± 2.6	8.2 ± 2.4	70
Calf thymus DNA[b]				
3/13/90	100	30	3.54	12
6/7/90	50	20	3.91	20
3/19/90	30	30	23.7 ± 1	79
6/7/90	30	20	15.9	80

[a] T2 phage recovery was from 2 or 3 liters of autoclaved, sterile-filtered seawater, and the phage titer was determined by the plaque assay.

[b] Calf thymus DNA was added as 2 or 3 liters of autoclaved, sterile-filtered seawater, and the DNA content was determined fluorometrically by using Hoechst 33258 (23).

a 22-mm^2 cover glass (Corning no. 1 1/2) was placed on top of the drop. The slides were viewed with an Olympus BH-2 epifluorescence microscope under UV (360 nm) excitation by using a UV fluorite 100× objective. Viruses were found to adsorb approximately equally to both the cover glass and the slide. Thus, viruses were counted on either the cover glass or the slide, and the value obtained was multiplied by 2 to account for the surface not counted in any one field.

Measurement of dissolved DNA. Dissolved-DNA measurements were performed essentially as described by DeFlaun et al. (7). Samples (0.1 to 1.0 liter) were filtered through GF/D and 0.2-μm Nuclepore filters with the filtration apparatus kept on ice. Filtrates were ethanol precipitated, and the precipitate was solubilized as previously described (7). Two of the five replicate samples were spiked with calf thymus DNA to determine the efficiency of recovery. DNA was determined in concentrated extracts and subsamples treated with DNase (7) to detect nonspecific fluorescence.

For detection of dissolved DNA by VFF, five 0.5- or 1-liter volumes of seawater filtered through a 0.2-μm filter were each concentrated by VFF as described above. Two of these were spiked with calf thymus DNA prior to filtration to correct for losses in recovery. The feed/retentate vessel was kept on ice during VFF to minimize enzymatic hydrolysis of DNA. We used 100 μl to 0.5 ml of the retentate for fluorometric DNA determination (23). Replicate retentate samples were treated with DNase (7) prior to the Hoechst assay to assess free DNA levels and correct for nonspecific fluorescence. For certain samples, a portion of the retentate (usually 5.0 ml) was ethanol precipitated at −20°C and the precipitate was harvested by centrifugation and solubilized in 1× SSC (0.15 M NaCl, 0.015 M sodium citrate [pH 7.0]) prior to DNA determination.

RESULTS

Efficiency of VFF. The results of efficiency studies when using the Membrex Benchmark VFF device and artificial seawater appear in Table 1. The 100- and 30-kDa filters and the 100-, 50-, and 30-kDa filters were used to concentrate T2 phage particles and calf thymus DNA, respectively. The efficiency of virus concentration (2 to 3 liters of seawater to 15 ml) ranged from 68 to 81% ($\bar{x}$ = 72.8 ± 5.7%), with no difference observed between the 30- and 100-kDa filters. Because viral enumeration in these studies was performed by plaque titer assay, the VFF concentration procedure apparently had little effect on the biologic activity (infectivity) of the virus. The efficiency of concentration of calf thymus DNA from artificial seawater was only 12 and 20%, respectively, for the 100- and 50-kDa filters. The 30-kDa filter, however, resulted in recovery of calf thymus DNA with efficiencies near 80%. In all ensuing studies, the 30-kDa filter was used for dissolved-DNA measurements. If viral enumeration only was required, the 100-kDa filter was used, which filtered at a flow rate approximately three times that of the 30-kDa filter.

Evaluation of viral enumeration techniques. The results of evaluation of three methods for viral enumeration appear in Table 2. When T2 phage was used, the plaque titer assay was used as the standard to evaluate the other methods. DAPI direct counts of phage T2 in cell-free lysates were approximately the same order of magnitude (within 0.25 log unit) as the plaque titer. The absolute value of the percent difference from the plaque titer, δ%, is given by

$$\delta\% = |\,(DC/PA \cdot 100) - 100\,|$$

where DC is the DAPI counts and PA is the plaque assay counts; it was 30.7 ± 7.6% for enumeration of T2 phage in host cell lysates and 45.8% for enumeration of T2 phage in artificial seawater. The TEM counts of T2 phage were generally closer to the plaque assay counts (δ% = 21.2 ± 3.65) than the DAPI counts were.

Enumeration of viruses by DAPI counts in natural waters resulted in values considerably lower than those obtained by TEM. This was caused in part by poor fluorescence staining of the natural viruses compared with T2. For virus enumeration in all environmental samples, counts were made by TEM.

To separate particulate DNA from dissolved DNA, all prior dissolved-DNA measurements were made by filtering samples through 0.2-μm Nuclepore filters (7). The effect of 0.2-μm filtration on viral abundance was investigated by using T2 phage in artificial seawater and natural water samples (Table 3). The effect of filtration on T2 abundance was variable, decreasing plaque titers in filtered samples by 49.1 to >99% (mean decrease, 78.9%). The effect on natural phage populations was also variable; there was either no effect or a decrease in phage counts by ca. 90%. The smallest effect was in offshore waters, and the greatest effect was in nearshore waters. A similar effect on phage abundance was noted for filtration through 142-mm Durapore filters (an 87.3% decrease in phage abundance), which have been used prior to ultrafiltration by other investigators (23, 30, 31). For all data, the average phage abundance was only 34% of the unfiltered samples.

Table 4 shows the distribution of viral abundance in unfiltered samples concentrated by VFF taken from estuarine, coastal oceanic, and oligotrophic oceanic environments. The estuarine samples exceeded 10^7 viruses per ml, the one exception being the Pier sample (from Tampa Bay), which was taken in December. The coastal oceanic samples (all measured during summer) were in excess of 10^6 viruses per ml, whereas samples from oligotrophic offshore oceanic stations (also sampled in summer) contained 2.4×10^5 to 4.5×10^5 virus particles per ml. The sample from a depth of 1,500 m in the Atlantic ocean was estimated to contain between 10^4 and 10^5 phage per ml (data not shown).

TABLE 2. Comparison of methods for enumerating phage particles

Expt or sample	No. of particles (10^6 viruses/ml ± SD) in enumeration by:		
	Plaque assay	TEM[a]	DAPI direct counts[a]
T2 phage in cell-free lysates[b]			
Expt 1	41,000 ± 11,000	ND[c]	50,400 ± 2,100 (123%)
Expt 2	29,000 ± 3,600	ND	18,000 ± 2,100 (62.1%)
Expt 3	35,000 ± 700	ND	22,000 ± 2,200 (68.9%)
T2 phage in artificial seawater[d]			
Expt 4	71.7 ± 7.0	85.9 ± 9.9 (120%)	29.2 ± 6.6 (40.7%)
Expt 5	82 ± 24.2	67.0 ± 17 (81.7%)	114.0 ± 19 (139%)
Expt 6	84.2 ± 36.0	62.9 ± 55.6 (74.7%)	117.0 ± 2.3 (139%)
Natural populations from the Gulf of Mexico[e]			
Mouth of Tampa Bay, St. 1	ND	1.8 ± 0.34	0.18 ± 0.095
Gulf of Mexico, St. 2	ND	0.28 ± 0.136	0.0154 ± 0.007
Gulf of Mexico, St. 3	ND	0.26 ± 0.09	0.017 ± 0.004

[a] Values in parentheses are percent plaque assay values.
[b] Counting and plaque assays were performed directly in phage-host system medium without concentration.
[c] ND, not determined.
[d] For experiments 4, 5, and 6, T2 phage was added to autoclaved, filter-sterilized artificial seawater and then concentrated by VFF.
[e] Samples were prefiltered through 0.2-μm filters prior to VFF; this accounts for the low viral titers.

Figure 2 shows some of the phages observed in samples from various marine and freshwater environments. Although no systematic attempt was made to size phages in all samples counted, sizing of phages in photomicrographs indicated a head size range of 46.4 to 141 nm (mean, 85.8 ± 21.1 nm; $n = 30$). This result suggests that the use of T2 as a standard may be justified, because T2 has a head size of 80 to 110 nm (10), slightly larger than the mean phage head size observed for field samples. However, we cannot discount the possibility that the photographed phages were a biased subsample of the natural phage population, because large, tailed phages were more likely to be photographed than small, tailless forms. Also in Fig. 2 are viruslike particles from a 1,500-m-deep sample in the Atlantic. No tailed phages were observed in this sample, and the particles were smaller than those photographed from other samples (i.e., all 46.4 nm).

In samples that had been prefiltered through 0.2-μm filters, we observed very few bacteria (too few to enumerate), indicating that "filterable" bacteria (16, 17, 32, 35) could not account for the DNA in dissolved-DNA measurements.

In some of the samples taken for viral direct counts, bacterial abundance was also measured by direct counts. The relationship between log bacterial abundance and log viral abundance is shown in Fig. 3. The relationship between these two parameters resulted in a correlation coefficient of 0.86, indicating a significant correlation between bacterial and viral concentrations ($0.002 < P < 0.005$ [37]) for the subtropical environments sampled.

A comparison between dissolved-DNA determinations made by ethanol precipitation of 0.2-μm filtrates of seawater and VFF concentration appears in Table 5, along with estimated viral DNA contents based on viral direct counts. Dissolved-DNA measurements obtained by ethanol precipitation of samples were higher than those for samples concentrated by VFF (VFF values are 34.8% ± 9.5% of the ethanol precipitation value). Because losses occurring in the concentration process were corrected for by using calf

TABLE 3. Effect of filtration through a 0.2-μm filter on recovery of viruses prior to concentration by VFF

Expt or sample	Recovery of viruses (10^6 viruses/ml) from[a]:		% of unfiltered value
	Unfiltered samples	Filtered samples	
T2 phage in artificial seawater			
Expt 1	963 ± 209	117 ± 26	12.1
Expt 2	81 ± 16	0.23 ± 0.1	0.28
Expt 3	141 ± 23	71.7 ± 7.1	50.9
Natural phage populations			
Mouth of Tampa Bay	17.4 ± 2.5	1.78 ± 0.34	10.2
Gulf of Mexico St. 2	0.24 ± 0.06	0.28 ± 0.14	117
Gulf of Mexico St. 3	0.44 ± 0.5	0.26 ± 0.09	59.1
Dry Tortugas	1.5 ± 0.1	0.14 ± 0.03	9.3
		0.19 ± 0.12[b]	12.7

[a] All virus abundances determined by TEM.
[b] Value determined for prefiltration through a 142-mm Durapore filter.

TABLE 4. Viral abundance in Tampa Bay, the eastern Gulf of Mexico, and the Bahamas Bank as determined by TEM direct counts on VFF-concentrated water samples

Sample	Initial vol of sample (liters)	Vol after VFF (ml)	Viral abundance (10^6 viruses/ml) in initial vol
Bayboro Harbor, Tampa Bay	3	25	34 ± 14
Mouth of Tampa Bay, St. 1	3	21.3	17.4 ± 2.5
Hookers Point, Tampa Bay	20	57.0	13.3 ± 5.5
St. Pete Pier, Tampa Bay	5	46.0	6.04 ± 1.49
Florida Bay	20	18.9	2.0 ± 0.1
Loggerhead Key, Dry Tortugas	15	8.4	1.5 ± 1.0
Joulter's Cay, Bahamas	10	50.0	2.3 ± 1.1
Gulf of Mexico, St. 2	20	17.4	0.24 ± 0.06
Gulf of Mexico, St. 3	30	16.0	0.44 ± 0.15
NW Providence Channel, Bahamas	25	36.4	0.42 ± 0.06

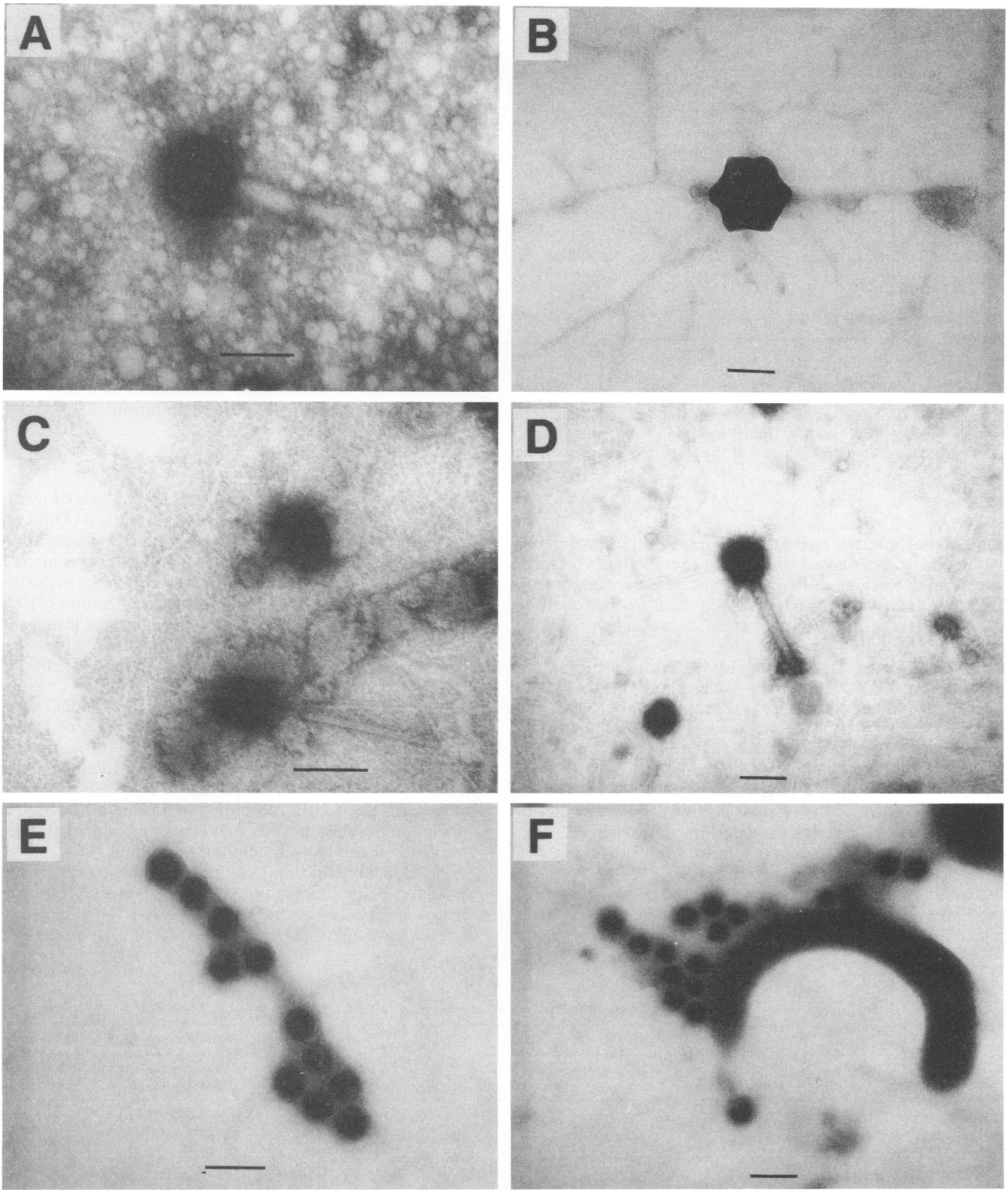

FIG. 2. Electron photomicrographs of bacteriophage and viruslike particles concentrated by VFF from the Gulf of Mexico and surrounding waters. (A) Tailed phage from the Medard Reservoir, Valrico, Fla. (B) Large viruslike particle from a sample from the Dry Tortugas near Loggerhead Key. (C) Tailed phage from Bayboro Harbor, Tampa Bay. (D) Tailed phage from outside the mouth of Tampa Bay. (E) Viruslike particles taken from a 1,500-m depth in the Atlantic ocean east of the Bahamas. (F) Viruslike particles (similar to those in panel E) on a bacterial cell surface from surface waters in the Dry Tortugas.

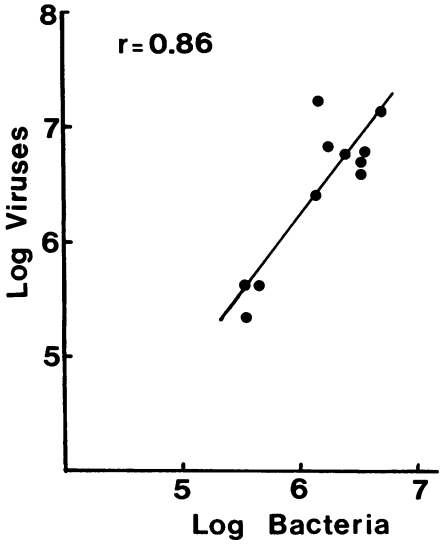

FIG. 3. Relationship between virus abundance and bacterial abundance for samples taken in Tampa Bay and surrounding waters. The correlation coefficient appears in the upper left corner of the figure.

thymus DNA internal standards, observed differences were not thought to be related directly to the efficiency of concentration. DNase treatment of replicate samples concentrated by the two methods showed that neither method preferentially concentrated spurious fluorescing materials. Ethanol precipitation of the samples concentrated by VFF led to an increase in the amount of measurable DNA, averaging ca. 80% of the DNA measured in the direct ethanol-precipitated (standard dissolved-DNA) method. Ethanol precipitation of naturally occurring dissolved DNA (in contrast to highly purified calf thymus DNA) apparently released DNA from a "bound" form or from a form inaccessible to nucleases and Hoechst stain. The DNA may be bound to histones or histonelike proteins, polyphenolic or humiclike proteins, or encapsulated in viral particles. This type of binding was not noted prior to the use of VFF filtration to concentrate dissolved DNA.

Ethanol precipitation of bacterial lysates containing T2 phage also resulted in an increased Hoechst DNA fluorescence reading (data not shown). This suggests that encapsulation of nucleic acids in viral capsids may impede access to DNase and Hoechst stain. To determine whether this increase in Hoechst fluorescence observed in natural dissolved-DNA samples was the result of phage particles, we estimated the viral contribution to dissolved DNA on the basis of phage counts and an average phage DNA content (10) (Table 5). The phage DNA content was expressed as a percentage of the dissolved-DNA value determined by the three methods described above (i.e., direct ethanol precipitation, VFF concentration, and VFF concentration followed by ethanol precipitation). Estimated phage DNA contents (i.e., those in 0.2-μm filtrates) ranged from 0.036 to 0.44 μg/liter, or 0.9 to 12.3% ($\bar{x}$ = 3.7 ± 3.8%) of the dissolved DNA. The largest proportion of dissolved DNA attributable to viruses was found in estuarine samples, with lower percentages in offshore oligotrophic environments. These results suggest that the amount of dissolved DNA accounted for by virus particles is seldom more than 10%, and is usually less than 5%. These data also indicate that approximately half of the dissolved DNA found in aquatic environments is in a form that is inaccessible to nuclease digestion but that can be liberated upon ethanol precipitation.

DISCUSSION

VFF has been shown to be an efficient and rapid means of concentrating phage particles and dissolved DNA from seawater. The efficiency of harvesting T2 phage and calf thymus DNA was between 70 and 80%, in agreement with claims of the VFF manufacturer for biologic materials in general. Although any of the pore-sized ultrafilters (100, 50, and 30 kDa) could be used to efficiently harvest viruses, only the 30-kDa filter collected DNA efficiently. Although the efficiency of the ethanol precipitation method for dissolved-DNA concentration has been reported to be over 90% (7), these efficiencies were generally obtained for small samples (100 ml) spiked with relatively large amounts of DNA (10 μg). Ethanol precipitation of large samples of seawater (i.e., 0.5 to 1.0 liter) in this study resulted in an average recovery of 79.3% ± 10.96%. An advantage of the VFF procedure is its general speed for obtaining a dissolved-DNA value, which could be as short as 4 to 6 h, compared with the 5 to 7 days for the ethanol precipitation method.

The decrease in viral abundance caused by filtration through 0.2-μm filters is not surprising because collection of viruses on 0.2-μm filters has been used for some time by aquatic virologists (11). Viruses are known to adsorb to

TABLE 5. Dissolved-DNA values and estimates of viral DNA content in natural waters

Sample	Dissolved DNA μg/liter) determined by:			Viral direct counts (10^6/ml)[a]	Estimated viral DNA content[b] (μg/liter)	Viral DNA as % of dissolved DNA for:		
	Ethanol precipitation[c]	VFF[d]	VFF and ethanol precipitation[e]			Ethanol precipitation	VFF	VFF and ethanol precipitation
Medard Reservoir	12.1 ± 1.2	4.2 ± 0.4	ND[f]	4.9 ± 2.5	0.44	3.6	10.5	ND
Bayboro Harbor	3.1 ± 0.2	ND	ND	4.2 ± 0.7	0.38	12.3	ND	ND
Mouth of Tampa Bay (St. 1)	11.0 ± 0.53	5.0 ± 1.5	11.0 ± 1.0	1.8 ± 0.34	0.16	1.5	3.2	1.5
Sea Buoy, Miami	11.5 ± 2.7	2.6 ± 1.3	7.5 ± 1.1	0.74 ± 0.15	0.067	0.58	2.6	0.90
Chub Cay, Bahamas	2.5 ± 0.93	0.9 ± 0.67	1.8 ± 0.9	0.4[g]	0.036	1.44	4.0	2.0

[a] Viral direct counts made on filtered (0.2-μm filter), VFF-concentrated samples.
[b] Viral DNA content estimated by multiplying the viral abundance found by 9×10^{-17} g of DNA/phage (10).
[c] Dissolved DNA determined by ethanol precipitation of filtrates (0.2-μm filter) of seawater (7).
[d] Dissolved DNA determined by direct Hoechst fluorescence reading of VFF-concentrated samples.
[e] Dissolved DNA determined on VFF-concentrated samples that had been ethanol precipitated.
[f] ND, not determined.
[g] Viral counts taken from a nearby station.

particulate matter, and they would therefore be collected on 0.2-μm filters. It seems that a greater percentage of viruses passed the filters in samples from oligotrophic waters, perhaps because of the smaller amount of particulate matter available for viral adsorption. These results are important because most of the viruses (an average of 60%, and often 90%) are retained on the 0.2-μm filters in the process of filtering water for dissolved-DNA measurements and would not be measured as dissolved DNA. A second result of these findings is that if samples are prefiltered through 0.2-μm filters to remove bacteria and other microorganisms for viral enumeration, the total viral population may be underestimated.

A significant linear relationship was found between phage and bacterial abundance. It may be argued that the log-log relationship reflected only differences between offshore and nearshore environments and that any biologic parameter will decrease as a function of distance from the shore, and hence will correlate. However, the occurrence of phage in any environment is contingent upon the presence of bacterial hosts. Our data suggest that viral abundance exceeds bacterial abundance by approximately 0.5 log unit when both are high, as in estuarine environments. In oligotrophic environments, the bacterial abundance is fairly close to the viral abundance (i.e., both at 2×10^5 to 4×10^5/ml). Borsheim et al. (4) found the viral abundance to exceed the bacterial abundance by approximately 1 order of magnitude for samples taken in a Norwegian fjord. Although our viral abundances for coastal samples are similar to theirs (i.e., 10^6 to 10^7/ml), their bacterial abundances were only 1×10^5 to 4×10^5/ml, which are considerably lower than those found in this study. It is not known why bacterial abundances were so low in the coastal fjord environments sampled.

Our work also demonstrates the presence of viruslike particles in mesopelagic environments (i.e., 1,500 m deep). We estimate the abundance of such particles at 10^4 to 10^5/ml, with uncertainties present in the recovery of the large-scale VFF device (Pacesetter; Membrex Inc.) used to concentrate the samples (data not shown). Proctor and Fuhrman (25) demonstrated the presence of phages in samples from sediment traps moored at a depth of 400 m. These investigators suggested that 2 to 37% of the bacteria in traps had been killed by phage infection.

Dissolved DNA has been operationally defined as DNA which passes a 0.2-μm Nuclepore filter (7). This could therefore include free or soluble DNA, DNA in virus particles, DNA in ultramicrobacteria (35), and DNA in a "colloidal" or humic-bound form. TEM observation of filtrates from 0.2-μm filters indicated that this fraction contained far too few ultramicrobacteria to enumerate (22a). We therefore deem these an insignificant portion of the dissolved DNA. Concentration of dissolved DNA by VFF has demonstrated a difference in naturally occurring dissolved DNA and purified (i.e., calf thymus) DNA in terms of staining with Hoechst 33258 and susceptibility to nuclease digestion. For example, ethanol precipitation of calf thymus DNA in either filtered artificial seawater or filtered ambient seawater resulted in fluorescence values equal to the amount of DNA added, as determined by standard curves. When natural dissolved-DNA samples were concentrated by VFF, the amount of DNA detected was less than half of that determined in samples concentrated by ethanol precipitation. Ethanol precipitation of VFF-concentrated samples resulted in an increase in Hoechst fluorescence and hence in DNA concentration, approaching the values determined in samples originally concentrated by ethanol precipitation. Since all prior dissolved-DNA measurements have been made by ethanol precipitation, we had not previously detected this form of sequestered DNA. A tempting hypothesis is that the bound or sequestered DNA existed in viral capsids. However, viral DNA estimates on these same samples indicated that viral DNA could be no more than 10% of the dissolved DNA and, in most cases, less than 5%. A possible explanation for these observations is that a portion of the dissolved DNA is in a combined form (i.e., bound with histones or histonelike proteins or some other polymer) that hinders accessibility to strains and DNase. A second possible explanation is that the microscopic enumeration of viruses is too low by 1 to 2 orders of magnitude. Because of the agreement with phage standards, we consider this alternative unlikely. A third possibility is that aquatic phage populations contain 1 to 2 orders of magnitude more DNA than those previously described for phage in culture. Of these hypotheses, we think that the bound-DNA hypothesis is the most likely.

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