

Viral infection plays a key role in extracellular DNA dynamics in marine anoxic systems

Cinzia Corinaldesi, Antonio Dell'Anno, and Roberto Danovaro¹

Department of Marine Sciences, Faculty of Science, Polytechnic University of Marche, Via Brecce Bianche, 60131 Ancona, Italy

Abstract

To investigate the role of viruses in extracellular DNA production through cell lysis, we selected two systems where viruses are expected to be the main component controlling prokaryote dynamics. These systems include anoxic subsuperficial coastal sediments and water and sediments of two deep-hypersaline anoxic basins (i.e., DHABs) of the eastern Mediterranean. Viral production was high in both places. Viruses were responsible for 10–60% of prokaryote mortality in anoxic sediments and up to 100% in deep-anoxic waters. The daily contribution of DNA released by viral lysis to the total extracellular DNA pool was 2–11% in anoxic sediments and more than 100% in the brines of both deep-sea basins. Extracellular DNA released by viral infection was rapidly degraded by deoxyribonucleases (DNases), which were also high in permanently anoxic conditions. Overall, our data suggest that DNA released by viral lysis, because of its high lability and fast turnover, may represent an important mechanism of trophic supply for prokaryotes, particularly in systems characterized by limited availability of external trophic sources.

Extracellular DNA is a constituent of both dissolved and particulate organic matter pools (DOM and POM) in all aquatic ecosystems, including a soluble fraction represented by naked free DNA and a nonsoluble fraction made up of DNA adsorbed to detrital-organic and/or inorganic particles (Jiang and Paul 1995; Danovaro et al. 2006). Recent evidence highlighted that extracellular DNA is the largest reservoir of DNA in the world oceans (Dell'Anno and Danovaro 2005). Owing to the high lability and high nitrogen (N) and phosphorus (P) content, extracellular DNA might represent an important trophic resource for prokaryotic metabolism (Jørgensen and Jacobsen 1996; Dell'Anno and Danovaro 2005) and a source of exogenous nucleotides for the synthesis de novo of prokaryote DNA (Paul et al. 1988).

The amount of extracellular DNA in aquatic ecosystems is the result of complex interactions, involving production, release, degradation, and use processes (Paul et al. 1987; Dell'Anno et al. 2002). Potential pathways of extracellular DNA release include (1) cellular exudation and excretion from viable cells (Paul et al. 1988, 1990); (2) cellular autolysis (i.e., passive release following cell death; Proctor and Fuhrman 1990); (3) cellular lysis due to viral infection (Fuhrman 1999). Previous studies reported that viral infection can be responsible for more than 50% of the prokaryote mortality in marine environments (Steward et al. 1992; Fuhrman and Noble 1995; Hewson et al. 2001),

contributing to 2–74% of the extracellular DNA production per day (Reisser et al. 1993; Weinbauer et al. 1993).

Available studies on the role of virus mediated DNA release have been focused on the plankton domain (Suttle 2005). In the benthic compartment extracellular DNA concentrations and prokaryote abundance are three orders of magnitude higher than in the water column (Dell'Anno and Danovaro 2005), and viral production is 1–2 orders of magnitude higher, thus being potentially responsible for higher prokaryote lysis and consequent DNA release (Mei and Danovaro 2004). For these reasons the role of the viral infection on extracellular DNA dynamics in marine sediments can be far more relevant than in pelagic systems.

Here, we investigated, for the first time, the role of viral infection in extracellular DNA production and diagenesis. To do this we estimated prokaryotic DNA released by viral lysis and its contribution to the extracellular DNA pool in coastal and deep-sea sediments and in seawater and brines of two deep-hypersaline anoxic basins (DHABs). The comparison between oxygen-rich systems and others characterized by anoxic conditions, where viruses are expected to be the only agent of mortality of prokaryotes, provides new insights on the importance of viral infection in extracellular DNA dynamics and the unexpected role of viruses in sustaining prokaryotic metabolism.

Materials and methods

Study areas—The present study was carried out in two areas: (1) a coastal system located in the Southern Adriatic Sea (Manfredonia Gulf, 41°38.80'N; 16°15.80'E) and (2) two DHABs (Atalante, 21°23.37'N; 35°18.23'E, and Urania 35°13.83'N; 21°28.29'E) located in the central-eastern Mediterranean Sea.

The coastal area, located at 21 m, is characterized by high sedimentation rates (0.1 cm yr⁻¹) and relatively high organic carbon concentrations in the sediment (ca. 10 mg C g⁻¹). Oxygen penetration was confined to few

¹ Corresponding author (r.danovaro@univpm.it).

Acknowledgments

This work was financially supported by the EU within the framework of the HERMES (Hotspot Ecosystem Research on the Margins of the European Seas) program (Contract GOCE-CT-2005-511234-1) and by national funding within the framework of the NITIDA (New Indicators of Trophic State and Ecological Integrity) program. We thank E. Manini and M. Magagnini for technical support during field and laboratory work. We thank R. N. Glud and two anonymous referees for useful comments that considerably improved the quality of the manuscript.

millimeters, and anoxic conditions were constantly present in subsurface sediment layers.

The two DHABs selected for the present study (Atalante and Urania) are characterized by absence of light, elevated pressure, variable pH values and ionic compositions, and a total salinity above 30‰, close to the saturation point of NaCl. The sharp density difference between brines filling the basins and the upper normal deep-sea water acts as a barrier to the oxygen diffusion within the brines. Therefore, the brines become oxygen free and rich in hydrogen sulfide. The brines of the Atalante and Urania basins have similar dominant ion compositions, but concentrations of sulfide and methane are higher in the Urania basin. Despite the very harsh conditions, DHABs of the Mediterranean Sea are characterized by the presence of high metabolic bacterial activities, high bacterial diversity, and new phylogenetic bacterial groups (Daffonchio et al. 2006).

Sampling—Coastal sediment samples were collected by means of a spade corer. Immediately after retrieval, three independent sediment cores were vertically sliced into three layers: (1) the top centimeters, (2) 20–30 cm, and (3) 90–100 cm sediment layers. Deep-sea sediments were collected at 3,575 m by means of a multiple corer (Bowers and Connelly, inner core diameter: 6 cm) only from the Atalante basin. Three independent cores were sectioned into three horizons: (1) the top centimeters, (2) 3–5 cm, and (3) 10–15 cm sediment layers. Subsuperficial anoxic coastal sediments and all deep-sea anoxic samples were kept in strictly anaerobic conditions under N₂ atmosphere to avoid any alteration of the sample.

Deep-sea water and brine samples were collected using a rosette sampler equipped with 12-liter Niskin bottles. In the Urania basin the sampling was carried out at four depths: 3,370 m (hereafter indicated as deep water), 3,523 m (hereafter indicated as water–brine interface) and 3,553 m and 3,573 m (hereafter indicated as upper and lower brines, respectively). The same sampling strategy was utilized in the Atalante basin at 2,950 m for the deep water, at 3,450 m for the water–brine interface, at 3,483 m for the upper brines, and at 3,515 m for the lower brines.

For the determination of extracellular DNA concentrations, deoxyribonuclease (DNase) activity, prokaryotic abundance and carbon (C) production, and viral abundance and production, samples were collected in three replicates and immediately processed onboard.

Extracellular DNA concentrations—Water and brine samples (100 mL) were prefiltered on Anodisc Al₂O₃ filters (0.02 µm pore size) in order to retain the viral component. Samples were then added with cetyltrimethylammonium bromide (2% final concentration; Karl and Bailiff 1989) and incubated for 12 h at –20°C. After incubation, samples were centrifuged for 10 min at 7,500 × g. Each pellet containing nucleic acids was resuspended in 10 mmol L⁻¹ Tris-HCl, 1 mmol L⁻¹ ethylenediaminetetraacetic acid (EDTA) pH 8 and then purified with an equal volume of equilibrated phenol:chloroform:isoamyl alcohol (25:24:1, vol:vol:vol). Finally, extracellular

DNA was quantified fluorometrically by using Sybr Green I as described by Corinaldesi et al. (2005). The fluorescence of extracellular DNA was converted into concentrations using calibration curves obtained from standard solutions of calf-thymus DNA.

Extracellular DNA in sediment samples was extracted and quantified according to Dell'Anno et al. (2002). This method based on extracellular DNA hydrolysis by means of commercial nucleases allows the quantification of the extracellular DNA fraction without any contamination by DNA associated with living biomass. One gram of sediment (wet weight) was added with 0.1 mol L⁻¹ Tris-HCl, 0.1 mol L⁻¹ NaCl, 1 mmol L⁻¹ CaCl₂, and MgCl₂ (pH 7.5; buffer:sediment ratio of 2.5 vol:wt). Aliquots of sediment samples ($n = 3$) were added with DNase I (1.9 U mL⁻¹), nuclease P1 and S1 (4.0 and 2.3 U mL⁻¹, respectively), and esonuclease-3 (1.9 U mL⁻¹); another set of replicates was added to an equal volume of buffer, (but without enzymes) and used as control. Samples were incubated at room temperature for 2 h under gentle agitation. After incubation, all samples were centrifuged at 2,000 × g for 5 min and supernatant was used to determine the amount of DNA released from the sediments. Supernatants were dried under vacuum and analyzed fluorometrically using diaminobenzoic acid (DABA, Karl and Bailiff 1989). The fluorescence of hydrolyzed DNA was converted into concentrations using calibration curves obtained from standard solutions of calf-thymus DNA (from 0.05 to 5.0 µg DNA mL⁻¹). The amount of extracellular DNA hydrolyzed by nucleases was obtained from the difference between DNA concentrations (determined from the supernatant of enzyme-treated samples) and the supernatant of the control sample. Extracellular DNA concentrations were expressed as micrograms of DNA per milliliter of wet sediment.

DNase activity—DNase activity was determined fluorometrically by means of fluorescent DNA analog [poly (dεA), poly deoxyribo-1-N⁶ethenoadenylic acid] (Dell'Anno and Corinaldesi 2004). Poly (dεA) substrate was prepared by a chemical modification of poly (dA) (fragment length of 50 bases) by means of chloroacetaldehyde according to Cazenave et al. (1983). Deep-water, brine, and sediment samples were added with increasing concentrations of poly (dεA) (ranging from 0.02 to 10 ng mL⁻¹ for water and brines and from 10 to 100 ng mL⁻¹ for sediment samples). DNase activity in water–brine samples reached V_{\max} at poly (dεA) concentrations ranging from 0.02 to 1 ng mL⁻¹, whereas in sediment samples V_{\max} was reached at a concentration of poly (dεA) of 100 ng mL⁻¹. Autoclaved sediment, seawater, and brines were used as blanks. All samples were incubated in the dark at in situ temperature for 2, 6, 12, and 24 h. After incubation, the supernatants obtained by brine and sediment samples (centrifuged at 2,000 × g for 5 min) and seawater samples were analyzed fluorometrically at 320 nm excitation and 410 nm emission wavelengths. Fluorescence of samples was converted into amount of released mononucleotides by using calibration curves obtained from standard solutions of 1,N⁶-ethenoadenine

deoxyribose-5'-monophosphate (dεAMP, from 2.5 to 500 ng mL⁻¹). DNase activity was expressed as micrograms of DNA per milliliter per hour for sediment samples or nanograms of DNA per milliliter per hour for water and brine samples.

Extracellular DNA turnover time was calculated as the ratio between extracellular DNA concentrations and their degradation rates (i.e., DNase activity).

Prokaryote and viral abundance—Prokaryote and viral counts were carried out on the same subsamples according to Danovaro et al. (2001). Sediment samples were treated using pyrophosphate (5 mmol L⁻¹ final concentration) and ultrasound treatment (3 times for 1 min, Branson 2200, 60 W) to increase the extraction yields. Sediment, brine, and water samples were diluted 100–500 times before processing. Subsamples of water, brines, and sediment were filtered on Anodisc Al₂O₃ filters (0.02-μm pore size) stained using Sybr Green I in the dark and mounted on glass slides with a drop of 50% phosphate buffer (6.7 mmol L⁻¹, pH 7.8) and 50% glycerol containing 0.5% ascorbic acid (Noble and Fuhrman 1998).

Viral and prokaryote counts were determined by epifluorescence microscopy (Zeiss Axioplan, magnification ×1,000) by examining at least 10 fields (i.e., at least 400 prokaryote cells or viruses per replicate). Prokaryote biovolume was estimated examining at least 100 cells, and it was converted to carbon content assuming 310 fg C μm⁻³ (Fry 1988). Average prokaryote-cell size was calculated by dividing bacterial biomass by the bacterial abundance. Viral and prokaryote counts were normalized to milliliters of wet sediment, seawater, or brines.

Prokaryote DNA content (i.e., intracellular DNA) was estimated using a conversion factor of 3.2 ± 0.14 fg of DNA per cell. This conversion factor was calculated using the genome size and molar GC (guanine-cytosine) content of all prokaryotes (*n* = 197) contained in the NCBI gene bank (<http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi>).

Prokaryotic carbon production—The determination of prokaryotic C production in all samples was carried out on board, immediately after sampling by means of ³H-leucine incorporation. Additional time-course experiments were carried out for testing the linearity of ³H-leucine incorporation. Prokaryotic carbon production in water and brine samples was determined according to the procedure described by Smith and Azam (1992). Triplicate subsamples were added with ³H-leucine (final concentration 20 nmol L⁻¹) and incubated at in situ temperature for 1 h in the dark. After incubation, samples were supplemented with trichloroacetic acid (5% final concentration) and stored at 4°C. Seawater blanks were made adding trichloroacetic acid immediately after ³H-leucine addition.

The determination of prokaryotic C production in sediment samples was carried out according to the procedure described for marine sediments by Danovaro et al. (2002). Sediment subsamples, added with an aqueous solution of ³H-leucine (final concentration 0.2 μmol L⁻¹), were incubated at in situ temperature for 1 h in the dark. After incubation, samples were supplemented with ethanol

(80%) and processed according to van Duyl and Kop (1994) before scintillation counting. Sediment blanks were made adding ethanol immediately after ³H-leucine addition. The incorporated radioactivity in all samples was measured by a liquid scintillation counter.

The following equation was used for calculating prokaryotic C production:

$$\text{PCP} = \text{LI} \cdot 131.2 (\% \text{ Leu}) - 1 (\text{C} : \text{protein}) \text{ID}$$

where PCP is prokaryotic C production, LI is the leucine incorporation rate (mol mL⁻¹ h⁻¹), 131.2 is the molecular weight of leucine, % Leu is the fraction of leucine in protein (0.073), C : protein is the ratio of cellular carbon to protein (0.86), and ID is the isotope dilution assuming a value of 2.

Viral production and decay—Viral production in seawater and brine samples was determined using the dilution technique described by Wilhelm et al. (2002) with few modifications. Samples were diluted with an equal volume of virus-free (0.02 μm prefiltered) seawater and incubated at in situ temperature in the dark. From each replicate dilution, a 1-mL independent subsample was collected every 3 h for 24 h both for viral production and decay analyses.

Viral production in the sediment was determined as described by Mei and Danovaro (2004), after appropriate tests for assessing the accuracy of the dilution technique when applied to sediment samples. Previous studies reported that differences between viral production in nondiluted and diluted sediments varied within a narrow range (7.5–11.4%, Mei and Danovaro 2004), but it cannot be excluded that viral production was stimulated by sediment dilution. Replicate sediment samples (about 25 mL, *n* = 3) were transferred in a sterile tube and mixed with 25 mL of virus-free (0.02-μm prefiltered) seawater. Subsamples (0.5 mL) for viral and prokaryote counts were collected at 0, 3, 6, 12, and 24 h. In all samples net viral production was determined as the increment of viral abundance per milliliter of water, brine, or sediment per hour in the first 3–6 h of incubation, when the highest viral production rates were recorded.

Viral decay rates in all samples were determined following the same procedure used for the analysis of viral production without the addition of cellular metabolic inhibitors (e.g., KCN) in order to minimize biases due to sample manipulation. Viral decay was estimated as the maximum decrement of viral abundance observed during time-course experiments and expressed as viruses per milliliter of water, brine, or sediment per hour. In all samples gross viral production was estimated as the sum between net viral production and decay rates. Finally, viral turnover rates were calculated by dividing gross viral production rates by viral abundance.

Burst size and viral-induced prokaryote mortality—Prokaryote burst size was estimated from time-course experiments of viral production. The burst size was calculated as the ratio of the number of viruses produced in each sampling interval per milliliter of sample (water, brine, or

sediment) and the number of prokaryotes killed by viral infection. The number of prokaryotes killed by viral infection was estimated as follows: the number of prokaryotes at time 0 was summed with the number of prokaryotes theoretically produced on the basis of the C production (calculated as C production divided by prokaryotic cell biomass in the interval of incubation) and subtracted from the number of prokaryotes actually counted after the incubation interval. Viral-induced prokaryote mortality (VIPM) was calculated according to Mei and Danovaro (2004) as the ratio between the number of killed prokaryotes by viruses and the number of prokaryotic cells produced.

Extracellular DNA released by viral lysis—The amount of extracellular DNA released by viral lysis was estimated in two ways: (1) we assumed that prokaryote DNA was entirely released after viral lysis; (2) we assumed that a fraction of prokaryotic DNA was incorporated by the new virions (Wikner et al. 1993) and we subtracted this fraction assuming a mean bacteriophage genome size of 0.039 fg of DNA per virus (value based on the genome size and molar GC content of marine phages sequenced to date; Paul and Sullivan 2005).

Results

Extracellular DNA concentrations and DNase activity—Extracellular DNA concentrations in coastal sediments ranged from $0.5 \pm 0.1 \mu\text{g mL}^{-1}$ to $3.3 \pm 0.9 \mu\text{g mL}^{-1}$ in the 20–30 cm and 0–1 cm sediment layer, respectively. Extracellular DNA concentrations in the sediment of the Atalante basin were on average about 10 times higher than in coastal sediments, ranging from 6.7 ± 0.7 to $22.6 \pm 2.7 \mu\text{g mL}^{-1}$ in the 3–5 cm layer and in the deepest sediment horizon, respectively (Fig. 1A). Extracellular DNA concentrations in seawater decreased moving from the deep waters (4.2 ng mL^{-1}) to the water–brine interface to remain nearly constant within the brines of the Urania basin (on average $1.2 \pm 0.3 \text{ ng mL}^{-1}$; Fig. 1B). In the Atalante basin, a constant pattern was observed along the entire water column (on average $0.8 \pm 0.2 \text{ ng mL}^{-1}$; Fig. 1B).

DNase activity in surface coastal sediments was about 10 times higher than in the Atalante basin (2.9 ± 1.0 and $0.24 \pm 0.03 \mu\text{g mL}^{-1} \text{ d}^{-1}$, respectively; Fig. 2A). In coastal sediments DNase activity decreased with increasing depth, whereas in the Atalante basin it remained quite constant in all sediment horizons. DNase activity in the water column increased from the deep waters to the lower brines in both DHABs (from 1.0 ± 0.5 to $117.5 \pm 46.9 \text{ ng mL}^{-1} \text{ d}^{-1}$ in the Urania basin and from 3.6 ± 0.75 to $64.6 \pm 0.71 \text{ ng mL}^{-1} \text{ d}^{-1}$ in the Atalante basin; Fig. 2B).

Turnover times of extracellular DNA in coastal sediments were short and did not change along the entire sediment core (1.1–1.5 d; Fig. 3A). Conversely, turnover times of extracellular DNA in sediments of the Atalante basin were much longer (51–117 d) and increased with depth in the sediment (Fig. 3B). Turnover times of

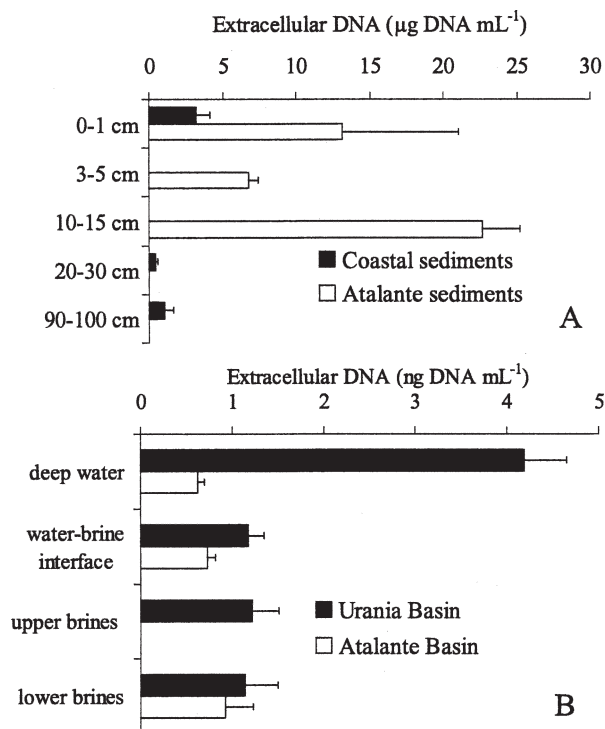


Fig. 1. (A) Extracellular DNA concentrations in different layers of coastal and deep-anoxic sediments and (B) in the deep-water column, water–brine interface, and brines of Urania and Atalante basins. Standard deviations are reported ($n = 3$).

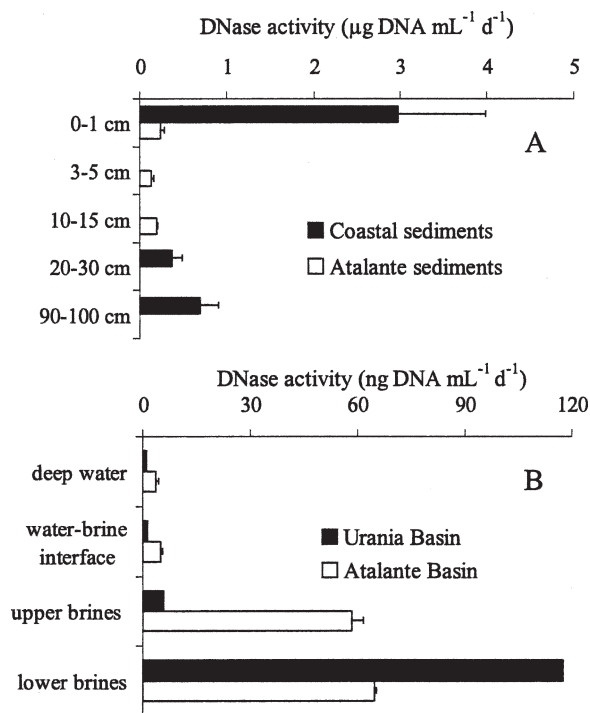


Fig. 2. (A) DNase activities in different layers of coastal and deep-anoxic sediments and (B) in the deep-water column, water–brine interface, and brines of Urania and Atalante basins. Standard deviations are reported ($n = 3$).

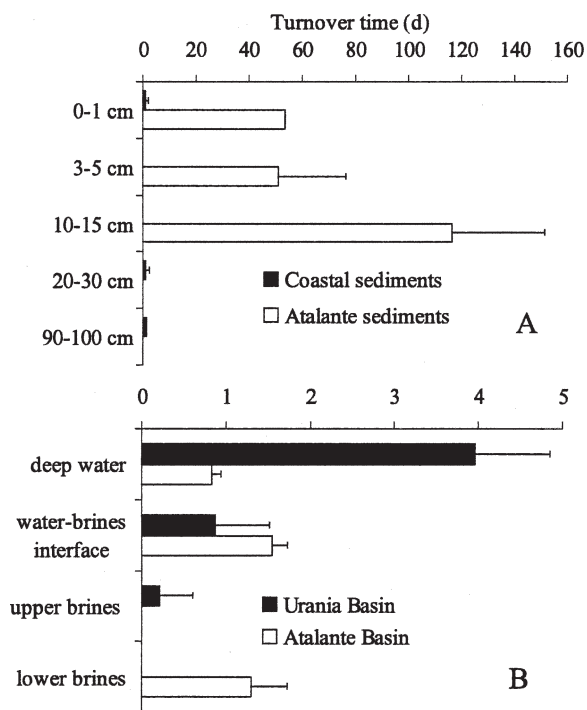


Fig. 3. (A) Turnover times of extracellular DNA in different layers of coastal and deep-anoxic sediments and (B) in the deep-water column, water-brine interface, and brines of Urania and Atalante basins. Standard deviations are reported ($n = 3$).

extracellular DNA in seawater sharply decreased, moving from the deep waters (ca. 4 d) to the lower brine of the Urania basin (0.01 d) and, in the Atalante basin, were about twice as much at the water-brine interface as in deep waters (1.6 vs. 0.8 days).

Prokaryote abundance and C production—Prokaryote abundance in coastal sediments was highest in the top 1 cm ($[13.8 \pm 0.7] \times 10^8$ cells mL^{-1}) and lowest in the 20–30 cm sediment layer ($[4.4 \pm 0.4] \times 10^8$ cells mL^{-1}). In deep-anoxic sediments, prokaryote abundance ranged from $(1.0 \pm 0.2$ to $1.7 \pm 0.3) \times 10^8$ cells mL^{-1} , with the highest values in surface sediments. Prokaryote abundance in the water column displayed the highest values in the upper brines of both Urania and Atalante basins (ca. 4×10^5 cells mL^{-1} in both systems; Table 1).

Benthic prokaryotic C production in sediment samples was about 3 orders of magnitude higher than in pelagic samples (Table 1). In coastal and in Atalante sediments prokaryotic C production decreased from the surface (0.42 ± 0.07 and 0.37 ± 0.03 $\mu\text{g C mL}^{-1} \text{h}^{-1}$, respectively) to the deepest sediment layers (0.10 $\mu\text{g C mL}^{-1} \text{h}^{-1}$ in both systems). In the Urania basin prokaryotic C production displayed a subsuperficial maximum at the water-brine interface (48 ± 4 $\mu\text{g C mL}^{-1} \text{h}^{-1}$) and minimum values in both deep water and in lower brines (about 12 $\mu\text{g C mL}^{-1} \text{h}^{-1}$ in both systems). In the Atalante waters and brines prokaryotic C production ranged from 43 ± 5 to 81 ± 18 $\mu\text{g C mL}^{-1} \text{h}^{-1}$ (in the deep waters and upper brines, respectively).

In coastal sediments, prokaryote-cell size varied from 38 to 43 fg C cell^{-1} , whereas in the Atalante basin, benthic prokaryote size ranged more widely (17–52 fg C cell^{-1} ; Table 1). In seawater, prokaryote size varied within a much narrower range (from 21 to 24 fg C cell^{-1} and from 22 to 27 fg C cell^{-1} in the Urania and Atalante basins, respectively).

Values of turnover rates of the prokaryote biomass, calculated as the ratio between prokaryotic C production and prokaryote biomass, were much higher in the deep-

Table 1. Sampling areas, prokaryote abundance (PDC), C production (PCP), cell size, turnover of prokaryote biomass, virus-induced prokaryote mortality (VIPM), and burst size (BS) in the water columns of Urania and Atalante basins and in deep-anoxic and coastal sediments. Standard deviations (\pm SD) are reported.

Location, typology (sampling depth)	PDC (10^7 cell mL^{-1})	PCP ($\mu\text{g C mL}^{-1} \text{h}^{-1}$)	Cell size (fg C cell^{-1})	Turnover (h^{-1})	VIPM (%)	BS
Seawater						
Urania basin						
Deep water (3,370 m)	0.03 ± 0.006	11.6 ± 2	22	0.002	98.6	16
Water-brine interface (3,523 m)	0.04 ± 0.001	47.5 ± 4	24	0.005	94.9	41
Upper brines (3,553 m)	0.04 ± 0.01	16.0 ± 2.8	22	0.002	96.0	59
Lower brines (3,573 m)	0.03 ± 0.01	12.2 ± 3.4	21	0.002	97.8	49
Atalante basin						
Deep water (2,950 m)	0.02 ± 0.01	42.6 ± 4.8	26	0.011	95.2	24
Water-brine interface (3,450 m)	0.01 ± 0.004	63.8 ± 7.9	22	0.027	89.0	53
Upper brines (3,483 m)	0.04 ± 0.01	81.3 ± 18.0	25	0.008	94.8	32
Lower brines (3,515 m)	0.02 ± 0.003	60.6 ± 15.2	27	0.012	92.3	37
Sediments						
Deep-anoxic (3,575 m)						
0–1 cm	16.8 ± 3.3	$36.6 \pm 2.9 \times 10^4$	52	0.042	58.7	6
3–5 cm	13.4 ± 1.4	$14.2 \pm 2.3 \times 10^4$	17	0.062	19.6	27
10–15 cm	10 ± 1.8	$10.1 \pm 3.5 \times 10^4$	17	0.059	8.6	69
Coastal sediments (21 m)						
0–1 cm	138.0 ± 6.5	$42.0 \pm 7.0 \times 10^4$	43	0.007	17.5	7
20–30 cm	44.1 ± 4.2	$10.0 \pm 3 \times 10^4$	40	0.006	30.8	15
90–100 cm	53.4 ± 2.4	$10.0 \pm 3 \times 10^4$	38	0.005	33.2	20

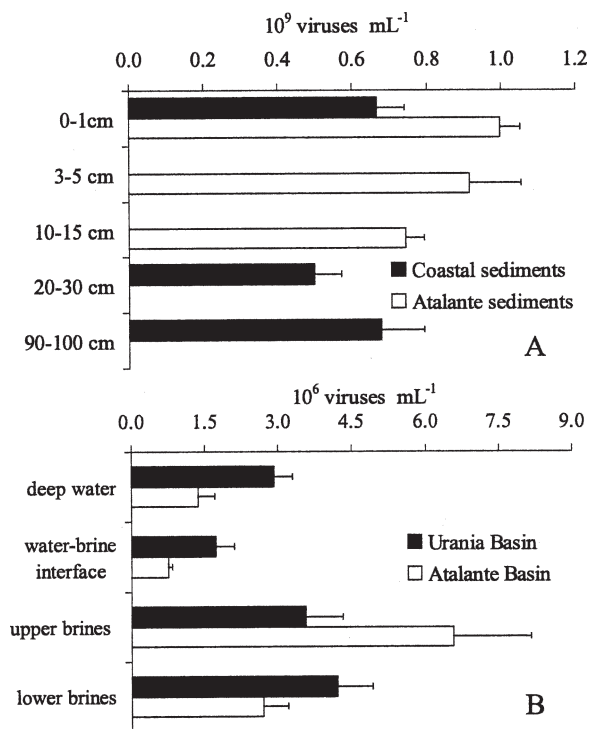


Fig. 4. (A) Virus abundance in different layers of coastal and deep-anoxic sediments and (B) in the deep-water column, water-brine interface, and brines of Urania and Atalante basins. Standard deviations are reported ($n = 3$).

anoxic benthic system than in coastal surface sediments and nearly constant along the sediment core (range: $0.04\text{--}0.06 \text{ h}^{-1}$ in deep-anoxic sediments vs. $0.005\text{--}0.007 \text{ h}^{-1}$ in coastal sediments; Table 1). Prokaryote biomass turnover ranged from 0.002 to 0.005 h^{-1} and from 0.008 to 0.027 h^{-1} in the seawater and brines of the Urania and Atalante basins, respectively.

Virus abundance, production, and prokaryote mortality—Viral abundance in coastal sediments and in the Atalante basin was almost constant along the sediment core (range: $[5.0 \pm 0.7$ to $6.8 \pm 1.2] \times 10^8$ virus mL^{-1} in coastal sediments and $[7.4 \pm 0.5$ to $9.9 \pm 0.5] \times 10^8$ virus mL^{-1} in the sediments of the Atalante basin; Fig. 4A). In the Urania and Atalante basins, virioplankton abundance displayed highest values in the brines ($[4.2 \pm 0.7$ and $6.6 \pm 1.0] \times 10^6$ virus mL^{-1} , respectively) and lowest values at the water-brine interface ($[1.7 \pm 0.4] \times 10^6$ virus mL^{-1} and $[7.6 \pm 0.7] \times 10^5$ virus mL^{-1} , respectively; Fig. 4B).

Gross viral production was high in both coastal and deep-anoxic sediments, with values about three times higher in the latter. The analysis of the vertical profiles revealed a pattern of increasing sediment depth in the coastal site (from $[1.5$ to $2.7] \times 10^7$ virus $\text{mL}^{-1} \text{ h}^{-1}$ Fig. 5A), and an opposite pattern along the vertical profile of Atalante sediments (from 6.3 ± 2.8 to $[3.8 \pm 2.4] \times 10^7$ virus $\text{mL}^{-1} \text{ h}^{-1}$ in the top 1 cm and in the deepest sediment layer, respectively; Fig. 5B). Virioplankton production in the Urania (Fig. 5C) and Atalante basins (Fig. 5D) were much higher (about three times) in the brines than in the deep

waters ($[1.3 \pm 0.3$ vs. $0.6 \pm 0.1] \times 10^6$ virus $\text{mL}^{-1} \text{ h}^{-1}$ in the Urania and $[1.9 \pm 0.06$ vs. $0.3 \pm 0.3] \times 10^6$ virus $\text{mL}^{-1} \text{ h}^{-1}$ in the Atalante basin). Along the vertical profile of deep-sea sediments, viral decay was nearly constant (range: $[1.42 \pm 0.5$ to $2.0 \pm 1.3] \times 10^7$ virus $\text{mL}^{-1} \text{ h}^{-1}$) and more than one order of magnitude higher than in the water column, where viral decay varied within a narrow range (from $[0.03 \pm 0.003$ to $0.04 \pm 0.005] \times 10^7$ virus $\text{mL}^{-1} \text{ h}^{-1}$ and from $[0.02 \pm 0.0002$ to $0.06 \pm 0.003] \times 10^7$ virus $\text{mL}^{-1} \text{ h}^{-1}$ in the brines and at the water-brine interface of the Urania and Atalante basins, respectively).

The burst size ranged, on average, from 6–69 in sediment samples to 16–59 in deep-water samples (Table 1). Virus-induced prokaryote mortality ranged from 18% to 33% in coastal sediments and from 9 to 59% in deep-anoxic sediments. Virus-induced prokaryote mortality in the Urania and Atalante waters and brines was much higher than in benthic systems, ranging from 89% to 99% (Table 1).

Discussion

Previous studies pointed out that viral abundance can be extremely high in permanently anoxic systems (Oren et al. 1997; Taylor et al. 2003; Danovaro et al. 2005) and hypothesized that in these systems, viruses can play a key role in prokaryote mortality. Virioplankton abundances in the anoxic samples collected in this study were within the range of values reported for other pelagic and anoxic systems ($10^4\text{--}10^7 \text{ mL}^{-1}$, Wommack and Colwell 2000; Taylor et al. 2003; Weinbauer et al. 2003). Moreover, viriobenthos abundances reported here for deep-anoxic and coastal subsurface sediments were two orders of magnitude higher than virioplankton abundances observed in deep waters and brines overlying the anoxic sediments and comparable to the highest values reported so far in literature for oxygen-rich benthic systems (Hewson et al. 2001; Glud and Middelboe 2004; Mei and Danovaro 2004).

The high viral abundance in subsurface coastal sediments (not affected by bioturbation) in the anoxic brines and in deep-anoxic sediments (where the strong halocline acted as a barrier for particle fluxes) might have two possible explanations: (1) high rates of in situ viral production and/or (2) low rates of viral decay (Middelboe et al. 2003; Fischer et al. 2004). Information on virioplankton production in deep-anoxic waters is almost completely lacking. In the present study rates of virioplankton production were one to six orders of magnitude higher than those reported for deep-anoxic waters (Weinbauer et al. 2003) and comparable with those reported in coastal waters worldwide (Wommack and Colwell 2000). Also, viral production values in subsurface coastal and deep-anoxic sediments were higher than those reported so far for anoxic and deep sediments (Glud and Middelboe 2004; Middelboe and Glud 2006; Middelboe et al. 2006) and comparable with the highest values reported for oxic benthic systems (see Mei and Danovaro 2004 and data therein). These results are consistent with the high prokaryotic C production, large cell size, and fast prokaryote turnover, which suggest that prokaryote assemblages in

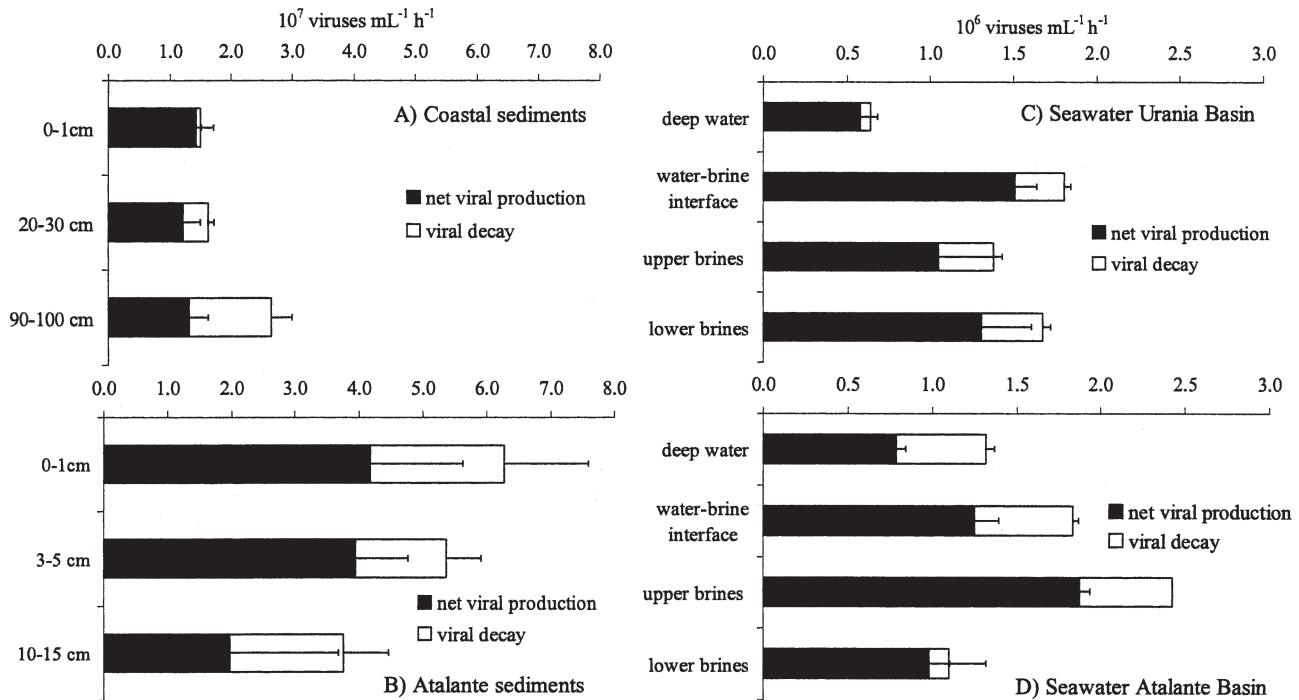


Fig. 5. (A) Gross viral production (as the sum of net viral production and viral decay) in coastal sediments, (B) deep-sea sediments, and (C) in the deep-water column, water–brine interface, and brines of Urania and (D) Atalante basins. Standard deviations are reported ($n = 3$).

anoxic conditions were metabolically very active, thus potentially sustaining a high viral production. Previously, Weinbauer et al. (2003) also reported high viral productions in shallow anoxic waters of the Baltic Sea and hypothesized that such high values were possibly coupled with relatively low viral decay rates. In order to explore the importance of viral decay rates on viral dynamics, we carried out direct measurements and we found that viral decay rates were relatively low in surface (oxygen-rich) coastal sediments (about 5% of gross viral production) and increased in subsurface anoxic layers (about 40%). Moreover, viral decay rates were about double in anoxic sediments (both in subsurface coastal layers and deep-sea samples) than in the water column (including brines and water–brine interface). Therefore, the high viral abundances observed in these systems may be due to the presence of very high gross viral production rates, able to compensate the viral decay rates.

Previous studies reported a high viral-induced prokaryote mortality (up to 71%) in different pelagic anoxic systems (such as anoxic portions of an eutrophic lake, solar saltern, and Baltic Sea; Guixa-Boixereu et al. 1996; Weinbauer and Höfle 1998; Weinbauer et al. 2003). In anoxic sediments, Glud and Middelboe (2004) and Middelboe et al. (2006) estimated a virus-induced bacterial mortality of 0.4–2% h^{-1} . However, available literature does not allow clarifying the importance of viruses in controlling prokaryotic assemblages under anoxic conditions in marine ecosystems. Our estimates of prokaryote mortality suggest that viruses are responsible for about 20% of prokaryote mortality in surface coastal sediments and for up to 33% in subsurface anoxic sediments. High values of virus-induced

prokaryote mortality were also observed in surface sediments of the Atalante basin (59%) and even much higher in pelagic compartments of the two deep-anoxic systems. All estimates reported here must be considered with caution, since they are dependent upon the method used for estimating viral production and on the estimated burst size (Suttle 2005). The dilution technique used here has been reported as one of the most reliable methods for estimating viral production (Helton et al. 2005), and the burst sizes estimated here (6–69) are within the range of those used so far in the literature (Wommack and Colwell 2000; Mei and Danovaro 2004), thus enabling a comparison with previous studies. Although standardized protocols for estimating virus production and prokaryote mortality have yet to be developed to facilitate comparisons among studies, our estimates suggest that viral-induced prokaryote mortality represents an important top-down process, controlling prokaryote assemblages, particularly in anoxic systems.

Mathematical and conceptual models indicate that viruses, through cell lysis, can accelerate the transformation of nutrients from constituents of the living biomass to the dissolved form (detritus), thus contributing to their cycling in the world oceans (Suttle 2005). Here, we provide new estimates of the contribution of the “viral shunt” to extracellular DNA dynamics. To do this, first we estimated the DNA release by viral lysis, and then we calculated its contribution to the total extracellular DNA pool. Even using the most conservative approach (see *Materials and methods*), we calculated that large amounts of DNA were released from killed prokaryotes by viral lysis (0.4–2.7 $\mu\text{g DNA L}^{-1} \text{d}^{-1}$ in the water column and brines, with values up to three orders of magnitude higher in the sediments:

0.6–6.4 mg DNA m⁻² d⁻¹). Therefore, viral lysis may be a process quantitatively comparable with the input of extracellular DNA associated with vertical fluxes (Dell'Anno and Danovaro 2005).

Previous studies suggested that a relevant fraction of extracellular DNA pool in surface marine waters may originate from viral lysis of prokaryotic cells (9–28% d⁻¹, Weinbauer et al. 1993). Our results suggest that the contribution of DNA released by viral lysis to the total extracellular DNA pool was 2–4 times higher in subsurface than in surface coastal sediments and also >100% in anoxic waters of both DHABs (up to 282% d⁻¹). Here viral-induced prokaryote mortality may be, therefore, the main mechanism supplying extracellular DNA.

Previous studies highlighted that if from one side, viruses can cause the death of their hosts, from the other side, releasing organic material by cell lysis can stimulate the growth of noninfected prokaryotes (Fuhrman 1999; Middelboe and Jørgensen 2006). We measured DNase activity, which represents a key pathway for extracellular DNA use (Paul et al. 1988; Dell'Anno and Corinaldesi 2004). DNase activities were extremely high, especially in anoxic brines of the DHABs, displaying rates comparable to values previously reported for highly productive coastal systems worldwide (Paul et al. 1987; Dell'Anno and Corinaldesi 2004). These results suggest that anoxic and hypersaline conditions did not influence the degradation rates of extracellular DNA. High DNase activities were also observed in surface coastal sediments, with values up to 10 times higher than in surface deep-anoxic sediments. Although DNase activities decreased with increasing depth in the sediment, high DNA degradation rates were still present at 1 m below the sediment surface in coastal samples and similar to those reported in the sediment of the Atalante basin. Degradation activities allow estimating the rates at which extracellular DNA is potentially recycled. In coastal sediments and in the brines of the Atalante and Urania basins, the entire standing stock of extracellular DNA was potentially degraded by DNases within about 1 d.

In the deep-anoxic sediments of the Atalante basin, only a fraction (about 30%) of extracellular DNA was degraded by DNases, and the estimates of extracellular turnover time in the Atalante sediments ranged from about 50 to 120 d. Therefore, it is possible that a large fraction of extracellular DNA remained unavailable to prokaryote use and thus was preserved in deeper sediment layers.

Recent studies on deep-sea sediments worldwide reported that the extracellular DNA pool may sustain an important fraction of prokaryotic metabolism (Dell'Anno and Danovaro 2005). In this study, in order to provide additional information on the trophic role of extracellular DNA released by viral infection we calculated daily prokaryotic N and P requirement. To do this we determined prokaryotic C production and, assuming a prokaryotic C:N:P ratio = 40:10:1 (Whitman et al. 1998), we estimated prokaryotic N and P production. Then we estimated the daily potential supply of N and P derived from the extracellular DNA released by viral lysis (using the lowest rates). This was calculated assuming an average N and P content of 15% and 10% (wt:wt) of the

extracellular DNA, respectively. We estimated that extracellular DNA released by viral lysis has the potential to fulfill 65–100% of N and P requirements in the water column and 2–15% of N and P requirements in the sediments.

Overall, our results suggest that viral-induced prokaryote mortality is a key mechanism controlling the recycling of the extracellular DNA pool and that the release of DNA by viral lysis may represent an important mechanism of trophic supply for prokaryotes, especially in systems characterized by reduced availability of external trophic sources.

References

- CAZENAVE, C. J., J. TOULMÈ, AND C. HELENE. 1983. Binding of recA protein to single-stranded nucleic acids: Spectroscopic studies using fluorescent polynucleotides. *EMBO J.* **2**: 2247–2251.
- CORINALDESI, C., R. DANOVARO, AND A. DELL'ANNO. 2005. Simultaneous recovery of extracellular and intracellular DNA suitable for molecular studies from marine sediments. *Appl. Environ. Microbiol.* **71**: 46–50.
- DAFFONCHIO, D., AND OTHERS. 2006. Stratified prokaryote network in the oxic–anoxic transition of a deep-sea halocline. *Nature* **440**: 203–207.
- DANOVARO, R., C. CORINALDESI, G. LUNA, AND A. DELL'ANNO. 2006. Molecular tools for the analysis of DNA in marine environments, p. 105–126. *In* J. Volkmann [ed.], *Handbook of environmental chemistry*. Springer.
- , A. DELL'ANNO, A. TRUCCO, M. SERRESI, AND S. VANUCCI. 2001. Determination of virus abundance in marine sediments. *Appl. Environ. Microbiol.* **67**: 1384–1387.
- , M. FABIANO, AND C. CORSELLI. 2005. Viruses, prokaryotes and DNA in the sediments of a deep-hypersaline anoxic basin (DHAB) of the Mediterranean Sea. *Environ. Microbiol.* **7**: 586–592.
- , M. MANINI, AND A. DELL'ANNO. 2002. Higher abundance of bacteria than of viruses in deep Mediterranean sediments. *Appl. Environ. Microbiol.* **68**: 1468–1472.
- DELL'ANNO, A., S. BOMPADRE, AND R. DANOVARO. 2002. Quantification, base composition and fate of extracellular DNA in marine sediments. *Limnol. Oceanogr.* **47**: 899–905.
- , AND C. CORINALDESI. 2004. Degradation and turnover of extracellular DNA in marine sediments: Ecological and methodological considerations. *Appl. Environ. Microbiol.* **70**: 4384–4386.
- , AND R. DANOVARO. 2005. Extracellular DNA plays a key role in deep-sea ecosystem functioning. *Science* **309**: 2179.
- FISCHER, U. R., W. WEISZ, C. WIELTSCHNIG, A. K. T. KIRSCHNER, AND B. VELIMIROV. 2004. Benthic and pelagic viral decay experiments: A model-based analysis and its applicability. *Appl. Environ. Microbiol.* **70**: 6706–6713.
- FRY, J. C. 1988. Determination of bacterial biomass, p. 27–72. *In* B. Austin [ed.], *Methods in aquatic bacteriology*. Wiley.
- FUHRMAN, J. A. 1999. Marine virus and their biogeochemical and ecological effects. *Nature* **399**: 541–548.
- , AND R. T. NOBLE. 1995. Viruses and protists cause similar bacterial mortality in coastal seawater. *Limnol. Oceanogr.* **40**: 1236–1242.
- GLUD, R. N., AND M. MIDDELBOE. 2004. Virus and bacteria dynamics of a coastal sediment: Implications for benthic carbon cycling. *Limnol. Oceanogr.* **49**: 2073–2081.

- GUIXA-BOIXEREU, N., J. I. CALDERON-PAZ, M. HELDAL, G. BRATBAK, AND C. PEDROS-ALIO. 1996. Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. *Aquat. Microb. Ecol.* **11**: 215–227.
- HELTON, R. R., M. T. COTTRELL, D. L. KIRCHMAN, AND K. E. WOMMACK. 2005. Evaluation of incubation-based methods for estimating virioplankton production in estuaries. *Aquat. Microb. Ecol.* **41**: 209–219.
- HEWSON, I., J. M. O'NEIL, J. A. FUHRMAN, AND W. C. DENNISON. 2001. Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries. *Limnol. Oceanogr.* **46**: 1734–1746.
- JIANG, S. C., AND J. H. PAUL. 1995. Viral contribution to dissolved DNA in the marine environment as determined by differential centrifugation and kingdom probing. *Appl. Environ. Microbiol.* **61**: 317–325.
- JØRGENSEN, N. O. G., AND C. S. JACOBSEN. 1996. Bacterial uptake and utilization of dissolved DNA. *Aquat. Microb. Ecol.* **11**: 263–270.
- KARL, D. M., AND M. D. BAILIFF. 1989. The measurement and distribution of dissolved nucleic acids in aquatic environments. *Limnol. Oceanogr.* **34**: 543–558.
- MEI, M. L., AND R. DANOVARO. 2004. Viral production and life strategies in aquatic sediments. *Limnol. Oceanogr.* **49**: 459–470.
- MIDDELBOE, M., AND R. N. GLUD. 2006. Viral activity along a trophic gradient in continental margin sediments off central Chile. *Mar. Biol. Res.* **2**: 41–51.
- , ———, AND K. FINSTER. 2003. Distribution of viruses and bacteria in relation to diagenetic activity in a estuarine sediment. *Limnol. Oceanogr.* **48**: 1447–1456.
- , ———, F. WENZHOFFER, K. OGURI, AND H. KITAZATO. 2006. Spatial distribution and activity of viruses in the deep-sea sediments of Sagami Bay, Japan. *Deep-Sea Res. I* **53**: 1–13.
- , AND N. O. G. JØRGENSEN. 2006. Viral lysis of bacteria: An important source of dissolved amino acids and cell wall compounds. *J. Mar. Biol. Assoc. UK* **86**: 605–612.
- NOBLE, R. T., AND J. A. FUHRMAN. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquat. Microb. Ecol.* **14**: 113–118.
- OREN, A., G. BRATBAK, AND M. HELDAL. 1997. Occurrence of virus-like particles in the Dead Sea. *Extremophiles* **1**: 143–149.
- PAUL, J. H., M. F. DEFLAUN, AND W. H. JEFFREY. 1988. Mechanisms of DNA utilization by estuarine microbial populations. *Appl. Environ. Microbiol.* **54**: 1682–1688.
- , W. H. JEFFREY, AND J. P. CANNON. 1990. Production of dissolved DNA, RNA, and protein by microbial populations in a Florida reservoir. *Appl. Environ. Microbiol.* **56**: 2957–2962.
- , ———, AND M. F. DEFLAUN. 1987. Dynamics of extracellular DNA in the marine environment. *Appl. Environ. Microbiol.* **53**: 170–179.
- , AND M. B. SULLIVAN. 2005. Marine phage genomics: What have we learned? *Curr. Opin. Biotechnol.* **16**: 299–307.
- PROCTOR, L. M., AND J. A. FUHRMAN. 1990. Viral mortality of marine bacteria and cyanobacteria. *Nature* **343**: 60–62.
- REISSER, W., S. GREIN, AND C. KRAMBECK. 1993. Extracellular DNA in aquatic ecosystems may in part be due to phycovirus activity. *Hydrobiologia* **252**: 199–201.
- SMITH, D. C., AND F. AZAM. 1992. A simple, economical method for measuring bacterial protein synthesis rates in sea using ³H-leucine. *Mar. Microb. Food Webs* **6**: 107–114.
- STEWART, G. F., J. WIKNER, D. C. SMITH, W. P. COCHLAN, AND F. AZAM. 1992. Estimation of virus production in the sea: 1. Method development. *Mar. Microb. Food Webs* **6**: 57–78.
- SUTTLE, C. A. 2005. Viruses in the sea. *Nature* **437**: 356–361.
- TAYLOR, G., C. HEIN, AND M. IABICHELLA. 2003. Temporal variation in viral distribution in the anoxic Cariaco Basin. *Aquat. Microb. Ecol.* **30**: 103–116.
- VAN DUYL, F. C., AND A. J. KOP. 1994. Bacterial variation in North Sea sediments: Clues to seasonal and spatial variations. *Mar. Biol.* **120**: 323–337.
- WEINBAUER, M. G., I. BRETTAR, AND M. G. HÖFLE. 2003. Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. *Limnol. Oceanogr.* **48**: 1457–1465.
- , D. FUKS, AND P. PEDUZZI. 1993. Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. *Appl. Environ. Microbiol.* **59**: 4074–4082.
- , AND M. G. HÖFLE. 1998. Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. *Appl. Environ. Microbiol.* **64**: 431–438.
- WHITMAN, W. B., D. C. COLEMAN, AND W. J. WIEBE. 1998. Prokaryotes: The unseen majority. *Proc. Natl. Acad. Sci. USA* **95**: 6578–6583.
- WIKNER, J., J. J. VALLINI, G. F. STEWART, D. C. SMITH, AND F. AZAM. 1993. Nucleic acids from the host bacterium as a major source of nucleotides for three marine bacteriophages. *FEMS Microbiol. Ecol.* **12**: 237–248.
- WILHELM, S. W., S. M. BRIGDEN, AND C. A. SUTTLE. 2002. A dilution technique for the direct measurement of viral production: A comparison in stratified and tidally mixed coastal waters. *Microb. Ecol.* **43**: 168–173.
- WOMMACK, E., AND R. R. COLWELL. 2000. Virioplankton: Viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.* **64**: 69–114.

Received: 26 June 2006

Accepted: 13 October 2006

Amended: 22 November 2006