

## Virus production and life strategies in aquatic sediments

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### Abstract

Aquatic sediments host much of the bacterial biomass and biodiversity and play a key role in biogeochemical cycles. However, the potential effect of viral infection remains unknown. We present estimates of virus production in a variety of benthic habitats of the Mediterranean Sea, characterized by different contamination levels and trophic states. Viriobenthos abundance in aquatic sediments ranged  $10^8$ – $10^9$  ml<sup>-1</sup> of sediment and was ~20 times higher than virioplankton abundance. Vertical profiles in sediment cores revealed large virus numbers at 1-m depth below the sediment surface. Virus production in both marine and freshwater sediments was 1–2 orders of magnitude higher than typical values reported for virioplankton ( $0.13$ – $1.60 \times 10^8$  virus-like particles ml<sup>-1</sup> sediment h<sup>-1</sup>), which indicates that virus turnover and infection rates can be higher in aquatic sediments than in the water column. Eutrophic and contaminated sediments displayed the highest virus production rates. Virus production was significantly correlated with the abundance of active bacteria and with bacterial cell production. We estimated an average burst size (BS) of 15–18 in marine and freshwater sediments. Virus-mediated bacterial mortality was high (on average, >40% of bacterial production) and increased from surface to deeper sediment levels, down to 100-cm depth. The fraction of bacteria with lysogenic infection (range, 0.0–1.8%) increased in deeper sediment layers (3.3% at 100-cm depth). Our results suggest that high benthic virus production rates can have a significant effect on benthic bacterial dynamics and indicate that virus production should be included in biogeochemical models of aquatic sediments.

Viruses are now recognized as ubiquitous components in all aquatic environments, and they are capable of exerting control over bacterial and phytoplankton abundance and production, influencing species composition, and altering pathways of matter and energy transfer (Fuhrman 1999; Wommack and Colwell 2000).

Estimating virus production (VP) in different aquatic systems is a key issue for understanding the effect of viruses on bacterial mortality and the role of the “viral shunt” in biogeochemical cycling of organic matter (Wommack and Colwell 2000; Wilhelm et al. 2002). There is an increasing interest for the quantification of virus abundance (Maranger and Bird 1996; Bird et al. 2001) and infectivity in benthic systems (Fischer et al. 2003). However, the limited information gathered so far on benthic viruses has not allowed a clarification of the effect of viral infection in aquatic sediments (Paul et al. 1993; Steward et al. 1996; Drake et al. 1998; Danovaro and Serresi 2000; Hewson et al. 2001a), and data on factors regulating top-down bacterial production and mortality in marine sediments have been so far limited to the analysis of the nanoflagellate grazing (Wieltschnig et al. 1999).

The benthic domain should be the ideal environment for viral development; in fact, benthic bacterial and microalgal densities can be orders of magnitude higher than those in the overlying water column (Hewson et al. 2001b), thus in-

creasing the probability of phage-host contact (Wiggins and Alexander 1985; Danovaro and Serresi 2000). Therefore, it is possible that viral infection and production in the sediment might be higher than in the water column (Corinaldesi and Danovaro 2003). In this regard, Hewson et al. (2001b) investigated the impact of elevated concentrations of virus-like particles (VLPs) on microphytobenthos and reported that viruses are capable of influencing microbial dynamics in sediments. At the same time, available studies on marine sediments have reported low virus:bacterium abundance ratios (VBRs; Danovaro et al. 2001, 2002b; Hewson et al. 2001a), which could suggest a limited virus effect on benthic bacteria (Wommack and Colwell 2000; Danovaro et al. 2002b). To confirm or reject hypotheses on the relevance of viruses in benthic microbial processes, we have to (1) provide estimates of actual rates of VP in aquatic sediments and (2) identify the factors that control such rates in the benthic environment.

Viruses in the water column and sediments appear to be morphologically quite different (Danovaro and Serresi 2000; Wommack pers. comm.), and it has been suggested that viriobenthos might be mostly accounted by bacteriophages that are specific to hosts on and within the sediments (Hewson et al. 2001b). Therefore, virioplankton and viriobenthos could also be characterized by different life strategies. Fuhrman (1999) reported that the lytic cycle is the most common viral development strategy in pelagic environments, but the occurrence of a relevant fraction of lysogenic bacteria is well known in a large variety of environments (Weinbauer et al. 2003). Stewart and Levin (1984) hypothesized that lysogenic infection may be a survival strategy for virus populations threatened by poor host cell abundance. This seems to be consistent with the observation that oligotrophic environments display a high incidence of lysogenic infection (40% of bacterial isolates containing inducible prophages; Jiang

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### Acknowledgments

We thank E. Manini (CNR, Lesina, Italy), R. Noble (University of North Carolina), and two anonymous reviewers for useful discussion and suggestions on the manuscript.

This work was financially supported by the EU programs INTERPOL and MEDVEG and by the National Project MAT.

and Paul 1994, 1998; Weinbauer et al. 2003). Because sediments are characterized by a large number of actively growing (and generally nutrient replete; Luna et al. 2002) bacteria, we could expect that lysogenic strategy in the benthic environment is less important than in the water column. However, viral development depends on complex interactions between biotic and abiotic factors (including the effect of pollutants [Cochran et al. 1998; Danovaro and Corinaldesi 2003] and the presence of a variable fraction of dead and dormant bacteria [Luna et al. 2002]), so life strategies of virus-host systems can be difficult to predict, particularly in benthic systems.

In the present study, we provide the first estimates of VP and lysogeny and virus-mediated bacterial mortality in a variety of aquatic sediments. Gathering estimates of these parameters is essential to understanding whether the “benthic viral shunt” can divert organic C from the benthic food web and to quantify the effect of viruses in biogeochemical cycling of organic matter.

## Materials and methods

**Sampling**—We collected sediment samples from a variety of environments to cover different environmental conditions: (1) a highly polluted area (the Port of Ancona, Adriatic Sea), (2) a eutrophic coastal area of the Thermaikos Gulf (Aegean Sea); (3) an unpolluted oligomesotrophic coastal area (Palombina, Adriatic Sea); and (4) a freshwater environment (the Esino River, Italy). Station depth ranged from 7.7 m (Ancona Port) to 51 m (Thermaikos Gulf) for marine sediments, whereas freshwater sediments were collected at a depth of 0.3 m. Samples were collected by means of manual coring or multiple corers, depending on station depth (*see Table 1 for the location and depth of all sampling stations*), and the top 1 cm was used for the determination of all microbial parameters. At each station, five independent cores were collected, and all analyses on each sediment layer were replicated three–five times.

To investigate VP and lysogeny in deeper sediment layers, we collected a long sediment core (100-cm) in the Manfredonia Gulf (southern Adriatic Sea) by means of a spade corer. Immediately after retrieval, three sediment horizons were sliced and analyzed: (1) the top 1 cm, (2) the 10–20 cm sediment layer, and (3) the 90–100 cm sediment layer.

For bacterial and viral parameters, replicate subsamples ( $n = 3$ ; 0.5 ml) were collected using sterile syringes (Danovaro et al. 2001). Samples were fixed with prefiltered (0.02  $\mu\text{m}$ ) formaldehyde (2% final concentration) and immediately processed, to minimize storage losses.

**Environmental variables**—Temperature was measured by means of a conductivity-temperature-depth profiler (Seabird). The water content was determined by measuring the differences between wet and dry sediment weights (at 60°C until a constant weight was achieved). Sediment porosity was determined from the weight difference between wet and dry sediment samples according to the equation  $(wc/1.02)/\{[(1 - wc)/2.64] + wc/1.02\}$ , where  $wc$  is (wet – dry sediment weight)/wet weight.

Sediment chlorophyll *a* and phaeopigments were extracted

Table 1. Station location and average values of ( $\pm$  SE) sediment horizon (cm), station depth (m), surface sediment temperature ( $^{\circ}\text{C}$ ), water content (WC), porosity, chlorophyll *a* and phaeopigment (Phaeo) concentrations, chloroplast pigment equivalents (CPE), and biopolymeric carbon content (BPC).

Sta. and location	Sed. layer (cm)	Depth (m)	Temp ( $^{\circ}\text{C}$ )	WC (%)	Porosity	Chl <i>a</i> ( $\mu\text{g g}^{-1}$ )	Phaeo ( $\mu\text{g g}^{-1}$ )	CPE	BPC ( $\mu\text{g g}^{-1}$ )
Ancona Port (43°37.32'N, 13°29.97'E)	0–1	7.7	13.0	44.1 $\pm$ 4.5	0.67 $\pm$ 0.04	10.94 $\pm$ 1.30	368.4 $\pm$ 61.0	379.4	4155.6 $\pm$ 918.9
Coastal Adriatic sediments (43°38.30'N, 13°30.17'E)	0–1	8.1	14.5	23.4 $\pm$ 0.9	0.44 $\pm$ 0.01	2.03 $\pm$ 0.47	0.64 $\pm$ 0.06	2.7	308.8 $\pm$ 131.2
Gulf of Thermaikos (39°58.00'N, 22°43.20'E)	0–1	51.0	11.1	ND	ND	0.99 $\pm$ 0.12	15.55 $\pm$ 2.26	16.5	1663.9 $\pm$ 480.2
River Esino sediments (43°39.38'N, 13°31.27'E)	0–1	0.3	12.0	ND	ND	0.08 $\pm$ 0.01	2.58 $\pm$ 0.15	2.7	2701.6 $\pm$ 272.1
Gulf of Manfredonia (41°38.80'N, 16°15.80'E)	10–20	21.0	23.8	40.1 $\pm$ 0.8	0.63 $\pm$ 0.01	4.57 $\pm$ 0.45	15.01 $\pm$ 1.32	19.6	2636.3 $\pm$ 86.2
	50–100	21.0	21.4	ND	ND	0.15 $\pm$ 0.04	4.28 $\pm$ 0.36	4.4	1530.0 $\pm$ 391.0
						0.03 $\pm$ 0.00	1.22 $\pm$ 0.23	1.2	1223.4 $\pm$ 161.6

ND, not determined.

Table 2. Benthic bacterial parameters: average  $\pm$  SE of bacterial direct count (BDC), nucleoid-containing cells (NuCC), active fraction (NuCC/BDC  $\times$  100), bacterial C production (BCP), total bacterial biomass (B-biomass), bacterial C content (B. C content), and bacterial turnover (B-turnover).

Sta.	Sed. layer (cm)	BDC ( $10^8$ cell ml $^{-1}$ )	NuCC ( $10^8$ cell ml $^{-1}$ )	Active fraction (%)	BCP ( $\mu$ gC ml $^{-1}$ h $^{-1}$ )	B-biomass ( $\mu$ gC ml $^{-1}$ )	B. C content (fgC cell $^{-1}$ )	B-turnover (d $^{-1}$ )
Ancona Port	0–1	28.78 $\pm$ 11.32	17.02 $\pm$ 5.74	59.1	1.22 $\pm$ 0.18	64.8 $\pm$ 0.0	50.1	0.45
Coastal Adriatic sediments	0–1	9.32 $\pm$ 1.24	5.17 $\pm$ 0.89	55.5	0.33 $\pm$ 0.02	34.3 $\pm$ 10.4	44.7	0.23
Gulf of Thermaikos	0–1	9.44 $\pm$ 2.31	6.85 $\pm$ 1.46	72.5	0.40 $\pm$ 0.04	26.9 $\pm$ 6.5	38.1	0.36
River Esino sediments	0–1	12.82 $\pm$ 2.17	7.59 $\pm$ 0.95	59.2	0.59 $\pm$ 0.07	29.8 $\pm$ 5.1	38.4	0.47
Gulf of Manfredonia	0–1	13.80 $\pm$ 0.65	9.62 $\pm$ 0.50	69.7	0.42 $\pm$ 0.07	69.1 $\pm$ 9.0	42.7	0.14
	10–20	4.41 $\pm$ 0.42	3.35 $\pm$ 0.33	75.9	0.10 $\pm$ 0.03	17.6 $\pm$ 0.2	40.2	0.14
	50–100	5.34 $\pm$ 0.24	2.06 $\pm$ 0.16	38.6	0.10 $\pm$ 0.03	17.8 $\pm$ 3.5	38.2	0.14

in 90% acetone overnight and analyzed fluorometrically according to the method of Lorenzen and Jeffrey (1980). Chloroplastic pigment equivalents were calculated as the sum of Chl *a* and phaeopigment concentrations. The biopolymeric carbon content was determined as the sum of carbon equivalents of proteins, carbohydrates, and lipids. All biochemical components were determined spectrophotometrically according to the method of Dell'Anno et al. (2002).

*Nucleoid-containing cells and bacterial direct counts*—Counts of nucleoid-containing bacterial cells (NuCCs) were carried out as described by Luna et al. (2002). The determination of the NuCC fraction was based on a destaining/staining procedure (Zweifel and Hagström 1995). Replicate ( $n = 3$ ) sediment subsamples (1 ml) were sonicated three times (Branson Sonifier 2200; 60 W for 1 min). This procedure was checked for the possible disruption of bacterial cells using different sonication times. One minute of sonication, repeated three times (with intervals of 30 s) proved to be the most efficient treatment for bacterial extraction without damaging significant numbers of bacterial cells (69.4% of cell abundance recovered after the first extraction). A preincubation of 1 h (with Acridine Orange supplementation) was performed (Karner and Fuhrman 1997). After dilution with Milli-Q water ( $\times 250$  and  $\times 500$  for sandy and muddy sediments, respectively), samples were incubated for 2 h in the dark with Acridine Orange (0.025% final concentration) and Triton X-100 (0.1% vol/vol) and then filtered at 100 mm of Hg onto 0.2- $\mu$ m pore-size Nuclepore filters (black-stained polycarbonate). Filters were washed with 10 ml of 2-propanol for 10 min before they were mounted onto microscopic slides. NuCC counts were carried out under epifluorescence microscopy (Zeiss Axioskop 2; magnification,  $\times 1,000$ ). According to the destaining/staining procedure, filters were washed once with 5 ml of sterile seawater and then restained with Acridine Orange for total bacterial count with epifluorescence (filter beam splitter 510, long pass 520). For each slide, at least 10 microscope fields were observed, and at least 400 cells were counted per filter. Bacterial size was measured (as maximal length and width) using a micrometer on all cells counted. Bacterial biovolume was estimated by assimilating cell shape to the volumes calculated from the rotation on the main axis of the ellipsoids or geometrical surface obtained. Bacterial biovolume was then converted to carbon content under the assumption of 308 fg C  $\mu$ m $^3$  (Fry 1988). The average bacterial carbon content was calculated by as the ratio of total bacterial biomass to total bacterial abundance. Data on total bacteria and NuCC abundance were corrected for extraction efficiency.

*Bacterial carbon production*—Bacterial C production (BCP) was measured by  $^3$ H-leucine incorporation, using the procedure described for marine sediments by Danovaro et al. (2002b), slightly modified to minimize possible bias caused by sediment manipulation. Sediment subsamples (200  $\mu$ l), added with an aqueous solution of  $^3$ H-leucine (specific activity 61 Ci mmol $^{-1}$ , 0.1 nmol Leucine and 6  $\mu$ Ci final concentration; Amersham), were incubated for 1 h in the dark at in situ temperature. After incubation, samples were supplemented with ethanol (80%), washed four times

Table 3. Virus-like particles (VLPs), virus:total bacterial abundance ratios calculated on total bacterial abundance (VBR-BDC) and on nucleoid containing cells (VBR-NuCC), virus production, virus turnover, burst size, fraction of bacteria by viral infection (Bact mortality), and lysogeny.

Sta.	Sed. layer (cm)	VLP ( $10^8$ viruses $ml^{-1}$ )	VBR BDC	VBR NuCC	Virus production (virus $ml^{-1} h^{-1}$ )	Virus turnover ( $h^{-1}$ )	Burst size	Bact mortality (%)	Lysogeny (%)
Ancona Port	0–1	24.96±3.47	0.87	1.47	1.60±10 <sup>8</sup>	0.06	24.4	12.2	0.00
Coastal Adriatic sediments	0–1	2.09±0.28	0.22	0.40	1.30±10 <sup>7</sup>	0.06	2.5	57.3	1.80
Gulf of Thermaikos	0–1	5.63±1.30	0.60	0.82	5.90×10 <sup>7</sup>	0.10	26.2	16.1	0.00
River Esino sediments	0–1	9.83±1.86	0.77	1.30	7.70×10 <sup>7</sup>	0.08	16.6	18.4	1.48
Gulf of Manfredonia	0–1	6.65±0.75	0.48	0.69	1.50×10 <sup>7</sup>	0.02	7.2	25.3	0.66
	10–20	4.99±0.72	1.13	1.49	1.63×10 <sup>7</sup>	0.03	14.7	43.3	0.02
	50–100	6.78±1.16	1.27	3.29	2.66×10 <sup>7</sup>	0.04	20.4	42.2	3.30

in trichloroacetic acid, and immediately filtered under low vacuum (<100 mmHg) on 0.2- $\mu$ m pore-size Nuclepore filters. Filters were treated with 2 mol L<sup>-1</sup> NaOH and incubated at 100°C (for 2 h), centrifuged (for 10 min at 800 g), and added with scintillation liquid (Hionic fluor; Packard Bioscience). Sediment blanks were made stopped with ethanol immediately after the addition of <sup>3</sup>H-leucine. BCP was determined in five replicates in all experiments of VP at each sampling time (i.e., every 3 h for surface sediment and every 6 h for subsurface sediment layers).

Bacterial turnover (BT) rates were estimated as  $B - \text{turnover} = \text{BCP}/\text{bacterial biomass}$ . Bacterial cell production (i.e., the number of bacterial cells produced per hour in 1 ml of sediment) was estimated as bacterial cell production = BCP/bacterial C content. This was calculated at each sampling interval for both total bacterial counts (bacterial direct count [BDC]) and for the active fraction (NuCC), and the results were used for further calculations of the BS. Reported are average values of the 12-h (for surface sediments) and 24-h (for deeper sediment layers) incubations.

**Virus abundance**—Replicate sediment subsamples of 0.5 ml, withdrawn from each sediment sample, were transferred into sterile test tubes and detached from sediment samples using pyrophosphate (5 mmol L<sup>-1</sup> final concentration) and ultrasound treatment (three times for 1 min, Branson 2200 sonifier, 60 W) to increase the extraction yield (Danovaro et al. 2001). To eliminate uncertainties in VLP counting caused by extracellular DNA interference, we added DNase I from bovine pancreas (10 U ml<sup>-1</sup> final concentration) and incubated samples for 15 min at room temperature. Subsamples were diluted 100–250 times with sterile and 0.02- $\mu$ m pre-filtered MilliQ water. For VLP counts, aliquots of samples were stained with SYBR-Green I (Noble and Fuhrman 1998); filtered on Anodisc Al<sub>2</sub>O<sub>3</sub> filters (0.02  $\mu$ m pore size), and analyzed by epifluorescence microscopy. For each slide, at least 10 microscope fields were observed and at least 400 VLPs were counted per filter. This procedure was checked for possible disruption of VLPs. The use of 1 min of sonication for three times gave the most efficient result (average extraction efficiency, 62.5%). Data on VLP abundance were corrected for the percentage of recovery after the first extraction. The extraction efficiencies of VLP and bacteria from each sediment type were very similar and were taken into account for quantitative estimates of the two compo-

nents. Therefore, extraction efficiency cannot be responsible for any bias in estimates of VP and virus-induced bacterial mortality.

**VP**—For estimating VP, we used the dilution technique (Wilhelm et al. 2002), with a few modifications for applying the method to the sediment. This technique reduces the background of free VLPs through the addition of virus-free seawater, which allows rates of VP to be readily monitored from small changes in virus concentration (Wilhelm et al. 2002). For Ancona Port, Coastal Adriatic, Gulf of Thermaikos, and Esino River sediments, 25-ml sediment samples ( $n = 3$ ) were transferred in sterile tubes and mixed with 25 ml of VLP-free (0.02  $\mu$ m prefiltered) seawater. Subsamples (0.5 ml) for VLPs, NuCC, and BDC and a subsample (0.3 ml) for BCP were collected every 3 h for 12 h. This time interval was selected to minimize the effect of new viral infection occurring during incubation. Incubations were done in the dark at in situ temperature, using the water collected at the sediment-water interface at each site. To be sure that VLPs encountered in the VLP-free water were actually produced by viral infection and had not been simply detached from the sediment, from one of the three replicates, at each sampling time, a subaliquot of sediment was withdrawn, and VLPs were counted as described above. These control samples did not display any significant decreases in VLP abundance during incubations (performed for VP). VLP abundance in control sediments, indeed, varied within a narrow range (coefficient of variance, 7.5–11.4%), and, at certain sampling times, VLP abundance in the sediment barely increased, possibly as a result of adsorption of the newly produced VLPs on the sediment matrix.

For the analysis of VP along the vertical profile, each of the three sediment layers collected in the Gulf of Manfredonia (0–1, 10–20, and 90–100 cm depth) was incubated in six replicates, as described above. Subsamples were collected every 6 h for 24 h. VP rates were determined from first-order regression of VLP versus time for triplicate incubations. Virus turnover rates were estimated as  $V\text{-turnover} = \text{VP}/\text{VLP}$ . Virus turnover was calculated at each sampling time, and values are expressed as the average of the 12-h (for surface sediments) and 24-h (for deeper sediment layers) incubations.

**BS and virus-induced bacterial mortality**—The BS was estimated from time course experiments of VP. The BS was

calculated as the ratio of number of VLPs produced in each sampling interval per milliliter of sediment and the number of bacteria killed by infection. To provide a reliable estimate of the number of bacteria killed, we used counts of the active bacterial fraction (NuCCs), but estimates of BS based on total bacterial numbers provided similar results. The number of bacteria killed by viral infection was estimated as number of NuCCs at time 0 plus the expected increase of NuCC abundance (estimated as number of bacterial cell produced during the interval of incubation), subtracted from the number of NuCCs actually determined in the same interval. The same calculation was repeated at each sampling time (i.e., 3 h for surface sediments and 6 h for subsurface sediment layers), and values were expressed average of the whole incubation period.

The ratio of VP to number of killed bacteria, integrated to the entire time-course experiment, was used to calculate the BS in each sediment type (under the assumption that viruses did not decay significantly within each interval). Virus-induced bacterial mortality (VIBM) was calculated as follows:  $VIBM (\%) = (VP/BS) \times 100/(BT \times BDC)$ , where VP is expressed as the number of viruses produced per milliliter of sediment per hour (as a coefficient of the slope of virus production in different sediments), BS is the average of the 12-h incubation, BT is the average of the 12-h incubation (in  $h^{-1}$ ), and BDC is the average of the 12-h incubation.

*Estimates of the bacterial lysogenic fraction*—To estimate the lysogenic fraction among natural populations of marine bacteria, we used one of the most effective and widely used inducing agents, mitomycin C (Weinbauer and Suttle 1996; McDaniel et al. 2002; Ortmann et al. 2002; Williamson et al. 2002). Each sediment sample (25 ml of sediment added with 25 ml of 0.02- $\mu m$  prefiltered seawater) was incubated with mitomycin C (1  $\mu g ml^{-1}$  final concentration in 0.02- $\mu m$  prefiltered seawater). No differences in VLP were observed when the mitomycin C concentration was increased from 0.1 to 10  $\mu g ml^{-1}$ . Three replicate microcosms were used for each sediment type, and analyses were carried out in three replicates per sampling time. The duration of the experiment was 12 h. A control of VP in sediments without the addition of mitomycin C was done. Lysogeny was calculated as the ratio of the number of VLPs released per milliliter of sediment per hour after the addition of mitomycin C ( $VP_{mitomycin C}$ ) and BS. The percentage of lysogenic bacteria (%LB) was then calculated as follows:  $LB (\%) = (VP_{mitomycin C}/BS)/BDC \times 100$ , where VP was estimated during the first 6 h of incubation and BS was measured for each set of sediment samples.

## Results

*Environmental conditions*—Environmental variables are reported in Table 1. Temperatures at the sediment-water interface ranged from 11.1°C in Thermaikos Gulf to 20.5°C in Manfredonia Gulf. Water content ranged from 23.4% (in coastal Adriatic sandy sediments) to 44.1% (in muddy sediments of the Ancona Port). A similar pattern was observed for porosity.

In the present study, we compared different environments characterized by evident differences in organic content (trophic conditions) and contamination levels. We also compared marine with freshwater sediments and performed a vertical profile for gathering information on integrated values of a sediment core. In particular, the Port of Ancona was characterized by high concentrations of hydrocarbons (particularly PAH (polycyclic aromatic hydrocarbons), 333.7  $\mu g g^{-1}$ , and naphthalene, 75.1  $\mu g g^{-1}$ ; data not shown). The Gulf of Thermaikos (Greece) and the Gulf of Manfredonia (Italy) are eutrophic and are subjected to heavy dumping and/or organic waste input, whereas coastal Adriatic Sea sediments can be considered to be pristine and oligomesotrophic. This was also confirmed by Chl *a* and total pigment concentrations in surface sediments: Chl *a* concentrations ranged from 0.08 (Esino River sediment) to 10.9 (Port of Ancona)  $\mu g g^{-1}$  and decreased by two orders of magnitude with depth in the sediment core of the Manfredonia Gulf. The total phytopigment content in the most eutrophic areas (Ancona Port, Thermaikos and, to a lesser extent, Manfredonia) was 1–2 orders of magnitude higher than in river sediments or in the oligomesotrophic sediments of the Adriatic Sea.

The biopolymeric carbon content ranged from 306.8  $\pm$  131.2 (coastal Adriatic Sea sediments) to 4,155.6  $\pm$  918.9 (Ancona Port)  $\mu g C g^{-1}$ , displaying a pattern similar to phytopigments, with the exception of the accumulation of organic matter in river sediments, which was reflected by high biopolymeric C concentrations. A clear decrease was observed along the vertical profile of the long sediment core collected in the Gulf of Manfredonia (from 2,636.3  $\pm$  86.2 to 1,223.4  $\pm$  161.6  $\mu g C g^{-1}$ ; top 1 cm and 90–100 cm depth, respectively).

*Bacterial parameters*—BDCs ranged 9.32–28.78  $\times 10^8$  cells  $ml^{-1}$  (in coastal Adriatic Sea and Ancona Port sediments, respectively), and relatively high BDCs were also reported for freshwater sediments (on average, 12.82  $\times 10^8$  cells  $ml^{-1}$ ). NuCC abundance in surface sediments ranged 5.17–17.02  $\times 10^8$  cells  $ml^{-1}$  (in coastal Adriatic Sea and Ancona Port sediments), which accounted for 55.5–72.5% of total bacterial counts (Table 2).

In the Gulf of Manfredonia, total bacterial abundance displayed a clear decrease from surface to deeper sediment layers (Table 2), and this decrease was more evident for the active bacterial fraction (which decreased from 69.7% of total bacterial number in the top 1 cm of the sediment to 38.6% in the deepest sediment layer; Table 2).

BCP displayed the lowest value in coastal Adriatic sediment (0.33  $\mu g C ml^{-1} h^{-1}$ ), increased in the Gulf of Thermaikos and in the Gulf of Manfredonia (0.40–0.42  $\mu g C ml^{-1} h^{-1}$ ), and it reached highest values in surface sediments from Ancona Port (1.22  $\mu g C ml^{-1} h^{-1}$ ). Along the vertical profile, a clear decrease was confirmed with a reduction of BCP by  $\sim 4$  times from surface to deeper sediment layers. Bacterial biomass followed patterns similar to those observed for bacterial density, and bacterial size varied within a narrow range (38.1–50.1 fg C cell $^{-1}$ ; Table 2). Bacterial turnover rates, calculated as the ratio of bacterial C production to bacterial biomass, ranged from 0.14 (in surface sediments from Manfredonia) to 0.45–0.47 (in surface sedi-

Table 4. Comparison of virus production in seawater, freshwater, and marine and freshwater sediments from different locations. Also reported are virus-like particle abundance (VLP), virus turnover (V-turnover), bacterial abundance (BDC), bacterial cell production (Bact. cell prod.), bacterial turnover (B-turnover), virus : bacterium abundance ratio (VBR), bacterial mortality, and burst size. For these parameters, the data set was restricted to works that also reported values of viral production.

Environment and location	Method for virus production	Virus production (virus ml <sup>-1</sup> h <sup>-1</sup> )	VDC (ml <sup>-1</sup> )
<b>Seawater</b>			
Coastal Sta., Southern California Bight	<sup>32</sup> P <sub>i</sub> Incorporation into viral DNA	3.67×10 <sup>5</sup>	2.56×10 <sup>8</sup>
Nearshore Sta., Southern California Bight	<sup>32</sup> P <sub>i</sub> Incorporation into viral DNA	7.50×10 <sup>5</sup>	1.27×10 <sup>7</sup>
Offshore Sta., Southern California Bight	<sup>32</sup> P <sub>i</sub> Incorporation into viral DNA	2.07×10 <sup>4</sup>	9.75×10 <sup>6</sup>
Santa Monica, California	<sup>32</sup> P <sub>i</sub> Incorporation into viral DNA	1.70×10 <sup>8</sup>	2.25×10 <sup>7</sup>
Santa Monica, California	<sup>32</sup> P <sub>i</sub> Incorporation into viral DNA	1.04×10 <sup>6</sup>	1.50×10 <sup>7</sup>
Santa Catalina Island	Fluorescently labeled viruses	3.85×10 <sup>5</sup>	1.25×10 <sup>7</sup>
San Pedro Channel	Fluorescently labeled viruses	4.22×10 <sup>5</sup>	1.05×10 <sup>7</sup>
Offshore Station	Fluorescently labeled viruses	1.54×10 <sup>5</sup>	8.10×10 <sup>6</sup>
Playa del Rey Jetty	Fluorescently labeled viruses	1.17×10 <sup>6</sup>	2.99×10 <sup>7</sup>
Moreton Bay, Australia	Dilution technique	8.50×10 <sup>5</sup>	9.50×10 <sup>6</sup>
Coral Sea, Australia	Dilution technique	<0.001×10 <sup>6</sup>	1.00×10 <sup>7</sup>
Discovery Passage, Canada	Dilution technique	3.95×10 <sup>6</sup>	7.93×10 <sup>7</sup>
Strait of Georgia, Canada	Dilution technique	9.70×10 <sup>6</sup>	8.93×10 <sup>7</sup>
Chukchi Sea/Bering Sea, Alaska	<sup>3</sup> H-TdR into bacteriophage DNA	9.81×10 <sup>4</sup>	1.93×10 <sup>7</sup>
Masan Bay, Korea	Bacterial production × burst size	5.46×10 <sup>5</sup>	1.51×10 <sup>8</sup>
East Sea, Korea	Bacterial production × burst size	2.94×10 <sup>4</sup>	4.90×10 <sup>6</sup>
Northwestern Mediterranean Sea	(Bacterial production × FIC/100) × burst size	3.60×10 <sup>3</sup>	1.37×10 <sup>7</sup>
Baltic Sea	(Bacterial production × FIC/100) × burst size	4.94×10 <sup>4</sup>	1.18×10 <sup>7</sup>
Average		1.44×10 <sup>6</sup>	4.25×10 <sup>7</sup>
<b>Marine sediments</b>			
Ancona Port	Dilution technique	1.60×10 <sup>8</sup>	2.50×10 <sup>9</sup>
Coastal Adriatic sediments	Dilution technique	1.30×10 <sup>7</sup>	2.09×10 <sup>8</sup>
Gulf of Thermaikos	Dilution technique	5.90×10 <sup>7</sup>	5.63×10 <sup>8</sup>
Gulf of Manfredonia (0–1 cm)	Dilution technique	1.50×10 <sup>7</sup>	6.65×10 <sup>8</sup>
Southern California	Dilution technique	2.83×10 <sup>8</sup>	ND
Niva Bay, Denmark (0–1.5 cm)		ND	1.95×10 <sup>8</sup>
Average		1.06×10 <sup>8</sup>	8.26×10 <sup>8</sup>
<b>Deep sediment layers</b>			
Gulf of Manfredonia (10–20 cm)	Dilution technique	1.63×10 <sup>7</sup>	4.99×10 <sup>8</sup>
Gulf of Manfredonia (90–100 cm)	Dilution technique	2.66×10 <sup>7</sup>	6.78×10 <sup>8</sup>
Niva Bay, Denmark (5–7 cm)		ND	1.8×10 <sup>8</sup>
Niva Bay, Denmark (13–15 cm)		ND	1.75×10 <sup>8</sup>
Average		2.15×10 <sup>7</sup>	3.83×10 <sup>8</sup>
Marine sediments : seawater		73.5	19.4
<b>Freshwater</b>			
Lake Constance, Germany	Mortality rate × burst size	5.42×10 <sup>4</sup>	1.50×10 <sup>7</sup>
Lake Plußsee, Northern Germany	Lysed bacterial production × burst size	4.50×10 <sup>5</sup>	4.30×10 <sup>7</sup>
Brisbane River, Australia	Dilution technique	2.07×10 <sup>4</sup>	9.00×10 <sup>7</sup>
Average		9.35×10 <sup>5</sup>	4.94×10 <sup>7</sup>
<b>Freshwater sediments</b>			
River Esino sediments	Dilution technique	7.70×10 <sup>7</sup>	9.83×10 <sup>8</sup>
Oxbow lake		ND	5.75×10 <sup>9</sup>
Average		7.70×10 <sup>7</sup>	3.37×10 <sup>9</sup>
Freshwater sediments : freshwater		82.4	68.3

ments from Ancona Port and freshwater sediments, respectively; Table 2) day<sup>-1</sup>.

*Virus abundance and VP*—Numbers of VLPs ranged 2.09–24.96 × 10<sup>8</sup> VLP ml<sup>-1</sup> (in coastal Adriatic Sea and Ancona Port sediments, respectively), and, in the Gulf of Manfredonia, barely decreased with increasing depth in the

sediment core from the surface down to 10–20 cm depth (Table 3). The VBR in surface sediments ranged 0.22–0.87. When only the active bacterial fraction was considered, the VBR (based on NuCC counts) ranged 0.40–1.47 (in surface sediments) and increased 4–5 times with increasing depth in the sediment (Table 3).

VP in surface sediments from Ancona Port (on average,

Table 4. Extended.

V-turnover (h <sup>-1</sup> )	BDC (ml <sup>-1</sup> )	Bact. cell prod (cell ml <sup>-1</sup> h <sup>-1</sup> )	B-turnover (h <sup>-1</sup> )	VBR	Bact mortality (%)	Burst size	Reference
0.014	6.23×10 <sup>6</sup>	6.51×10 <sup>4</sup>	0.001	41.1	18.5	10–300	Steward et al. 1992
0.059	1.29×10 <sup>6</sup>	6.25×10 <sup>4</sup>	0.010	9.8	20.7	10–300	Steward et al. 1992
0.002	4.74×10 <sup>5</sup>	1.41×10 <sup>4</sup>	0.003	20.6	15.8	10–300	Steward et al. 1992
0.075	6.50×10 <sup>8</sup>	3.38×10 <sup>5</sup>	0.052	3.1	1.3	20	Fuhrman and Noble 1995
0.069	1.88×10 <sup>6</sup>	1.25×10 <sup>5</sup>	0.066	7.5	2.8	20	Fuhrman and Noble 1995
0.031	9.65×10 <sup>5</sup>	2.08×10 <sup>4</sup>	0.061	12.9	99.5	20	Noble and Fuhrman 2000
0.040	8.18×10 <sup>5</sup>	2.01×10 <sup>4</sup>	0.070	12.9	100.0	20	Noble and Fuhrman 2000
0.019	8.40×10 <sup>5</sup>	1.17×10 <sup>4</sup>	0.165	9.6	66.0	20	Noble and Fuhrman 2000
0.039	3.10×10 <sup>6</sup>	9.83×10 <sup>3</sup>	0.088	9.6	59.0	20	Noble and Fuhrman 2000
0.089	1.63×10 <sup>6</sup>	ND	ND	5.9	ND	ND	Hewson et al. 2001a
ND	9.00×10 <sup>5</sup>	ND	ND	11.1	ND	ND	Hewson et al. 2001a
0.050	1.29×10 <sup>6</sup>	ND	ND	61.3	ND	50	Wilhelm et al. 2002
0.109	2.08×10 <sup>6</sup>	ND	ND	42.9	ND	50	Wilhelm et al. 2002
0.005	1.16×10 <sup>6</sup>	2.22×10 <sup>6</sup>	0.209	16.7	27.7	139	Steward et al. 1996
0.004	9.00×10 <sup>6</sup>	2.08×10 <sup>5</sup>	0.165	19.9	19.2	31	Choi et al. 2003
0.006	1.35×10 <sup>6</sup>	1.27×10 <sup>4</sup>	0.631	3.9	14.5	14	Hwang and Cho 2002b
0.0003	9.50×10 <sup>5</sup>	2.13×10 <sup>3</sup>	0.002	13.9	14.0	29	Weinbauer et al. 2003
0.004	1.60×10 <sup>6</sup>	1.65×10 <sup>4</sup>	0.010	13.0	15.3	30	Weinbauer et al. 2003
0.036	2.34×10 <sup>6</sup>	2.23×10 <sup>5</sup>	0.109	17.5	33.9	35	
0.06	2.88×10 <sup>9</sup>	2.43×10 <sup>7</sup>	0.019	0.9	12.2	24	Present study
0.06	9.32×10 <sup>8</sup>	7.36×10 <sup>6</sup>	0.010	0.2	57.3	3	Present study
0.10	9.44×10 <sup>8</sup>	1.05×10 <sup>7</sup>	0.015	0.6	16.1	26	Present study
0.02	1.38×10 <sup>9</sup>	9.72×10 <sup>6</sup>	0.006	0.5	25.3	7	Present study
0.33	ND	ND	ND	ND	9.0	ND	Hewson and Fuhrman 2003
ND	4.50×10 <sup>7</sup>	ND	ND	4.3	ND	ND	Middelboe et al. 2003
0.12	1.24×10 <sup>9</sup>	1.30×10 <sup>7</sup>	0.012	1.3	24.0	15.1	
0.033	4.41×10 <sup>8</sup>	2.55×10 <sup>8</sup>	0.006	1.1	43.3	15	Present study
0.039	5.34×10 <sup>8</sup>	2.70×10 <sup>8</sup>	0.006	1.3	42.2	20	Present study
ND	2.50×10 <sup>7</sup>	ND	ND	7.2	ND	ND	Middelboe et al. 2003
ND	2.50×10 <sup>7</sup>	ND	ND	7.0	ND	ND	Middelboe et al. 2003
0.036	2.56×10 <sup>8</sup>	2.62×10 <sup>6</sup>	0.006	4.2	42.7	18	
0.08	529.9	58.0	0.11	0.07	0.71	0.42	
0.004	2.25×10 <sup>8</sup>	ND	ND	6.7	0.2	71	Hennes and Simon 1995
0.010	7.25×10 <sup>6</sup>	3.75×10 <sup>4</sup>	0.005	5.9	39.6	47	Weinbauer and Höfle 1998
0.026	4.50×10 <sup>6</sup>	ND	ND	ND	ND	ND	Hewson et al. 2001
0.013	4.67×10 <sup>6</sup>	3.75×10 <sup>4</sup>	0.005	6.31	19.9	59	
0.078	1.28×10 <sup>9</sup>	1.53×10 <sup>7</sup>	0.020	0.8	18.4	17	Present study
ND	ND	ND	ND	2.1	12.5	ND	Fisher et al. 2003
0.078	1.28×10 <sup>9</sup>	1.53×10 <sup>7</sup>	0.020	1.4	15.5	17	
5.9	274.6	406.8	3.8	0.2	0.8	0.3	

1.60 × 10<sup>8</sup> VLP ml<sup>-1</sup> h<sup>-1</sup>) was ~10-fold higher than in coastal Adriatic Sea sediment and sediment from Manfredonia Gulf and was 2–3 times higher than in other marine and freshwater sediments. In the Gulf of Manfredonia, VP slightly increased with increasing depth in the sediment (range, 1.50–2.66 × 10<sup>7</sup> VLP ml<sup>-1</sup> h<sup>-1</sup>; Table 3). Virus turnover rates ranged 0.02–0.10 h<sup>-1</sup> in surface sediments and

0.03–0.04 h<sup>-1</sup> in deeper sediment layers (Table 3). BS ranged from 2.5 in the coastal Adriatic Sea sediments to 26.2 in the Gulf of Thermaikos (Table 3).

*Estimates of VIBM and of lysogenic fraction in aquatic sediments*—VIBM ranged 12.2–57.3% but increased with depth in the sediment core from 25.3% (in the top 1 cm) to

>40% in deeper sediment layers from the Gulf of Manfredonia. In freshwater sediments, VIBM was 18.4%.

The fraction of lysogenic bacteria, induced to the lytic cycle after treatment with mitomycin C, ranged from 0.0% (in Ancona Port and the Gulf of Thermaikos) to 1.8% in coastal Adriatic Sea sediments. The lysogenic fraction increased in deeper sediment layers from the Gulf of Manfredonia (from 0.66% in the top 1 cm to 3.3% in the 90–100 cm sediment layer; Table 3).

## Discussion

*Viriobenthos abundance and distribution in aquatic sediments*—Although there have been few investigations on benthic viruses, there is increasing evidence that surface sediments contain very large numbers of virus (Table 4). Aquatic sediments could, therefore, represent reservoirs of viruses and be able to segregate or exchange virus assemblages with the overlying waters (Lawrence et al. 2002). Our results indicate that viriobenthos abundance, ranging  $10^8$ – $10^9$  ml<sup>-1</sup>, is, on average, 20 times higher than that observed in the planktonic counterparts of both marine and freshwater systems (Table 4). At the same time, benthic bacterial densities are, on average, >2 orders of magnitude higher than bacterioplankton density. The consequence is that VBR in aquatic sediments is, on average, ~10-fold lower than that in the water column (Table 4). If, as has been repeatedly reported by several authors (Wommack and Colwell 2000; Corinaldesi et al. 2003), host-cell density is a key factor favoring viral infections, why is virus abundance in sediment not much higher? The most likely explanation is that the benthic environment could be unfavorable for viral survival after cell lysis, because viruses in the sediment could remain embedded in a complex organic matrix rich with exoenzymes (Danovaro et al. 2002a,b) and rapidly decay.

Other factors can also influence virus distribution in aquatic sediments. Among these, the density, viability, and productivity of host populations (in this case, mostly benthic bacteria) or environmental conditions could be important. For instance, we observed that the abundance of VLPs was higher in productive and organically rich environments than in oligotrophic sediments (Table 1). A relationship between virus abundance and trophic conditions has been hypothesized (Hewson et al. 2001a), but because viral infection and the lytic cycle are known to affect primarily viable cells, the fraction of metabolically active bacteria is also expected to play a primary role (Wommack and Colwell 2000). In our case, a significant relationship was observed between the number of VLPs and the total or active bacterial fraction, which in turn displayed a highly significant relationship with both organic and phytopigment content (Fig. 1). The fraction of metabolically active bacteria can be highly variable, especially in marine sediments (Luna et al. 2002), but this was not the case of our study, because, in all investigated sediments, the active bacterial fraction was >50%.

The analysis of VLPs in deeper sediment layers from the Gulf of Manfredonia indicated that large numbers are still present at 1-m depth below the sediment surface. The results of redox potential profiles indicated that only the top 1 cm

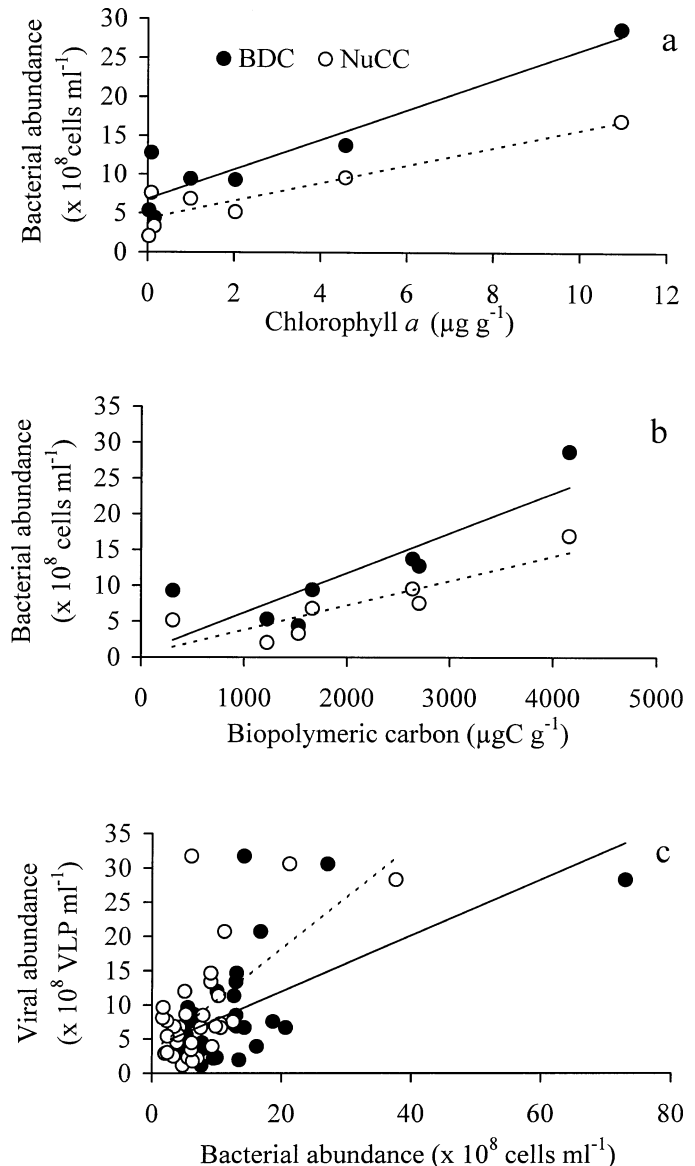


Fig. 1. Relationships between benthic bacterial abundance (BDC and NuCCs) and (a) Chl *a* content (for BDC,  $y = 1.92 \times 10^8 x + 6.84 \times 10^8$ ,  $R^2 = 0.874$ ,  $P < 0.01$ ; for NuCC,  $y = 1.15 \times 10^8 + 4.28 \times 10^8$ ,  $R^2 = 0.859$ ,  $P < 0.01$ ). (b) Biopolymeric carbon concentrations of the sediment (for BDC,  $y = 5.56 \times 10^5 x + 6.74 \times 10^7$ ,  $R^2 = 0.723$ ,  $P < 0.01$ ; for NuCC,  $y = 3.46 \times 10^5 x + 3.57 \times 10^7$ ,  $R^2 = 0.756$ ,  $P < 0.01$ ). (c) VLP abundance (for BDC,  $y = 0.41x + 3.80 \times 10^8$ ,  $R^2 = 0.385$ ,  $n = 34$ ,  $P < 0.01$ ; for NuCC,  $y = 0.75x + 3.09 \times 10^8$ ,  $R^2 = 0.340$ ,  $n = 34$ ,  $P < 0.01$ ).

of the sediment surface was oxygenated, and, below 10 cm, suboxic/anoxic conditions were constantly present ( $E_h < -200$  mV; data not shown). This indicates that viruses can also be present and potentially infective in anaerobic/reducing layers of the sedimentary matrix.

*VP and VIBM in aquatic sediments*—Estimates of VP rates in different benthic environments are needed to understand whether a benthic viral shunt can be responsible for a significant reduction of benthic bacterial production and to

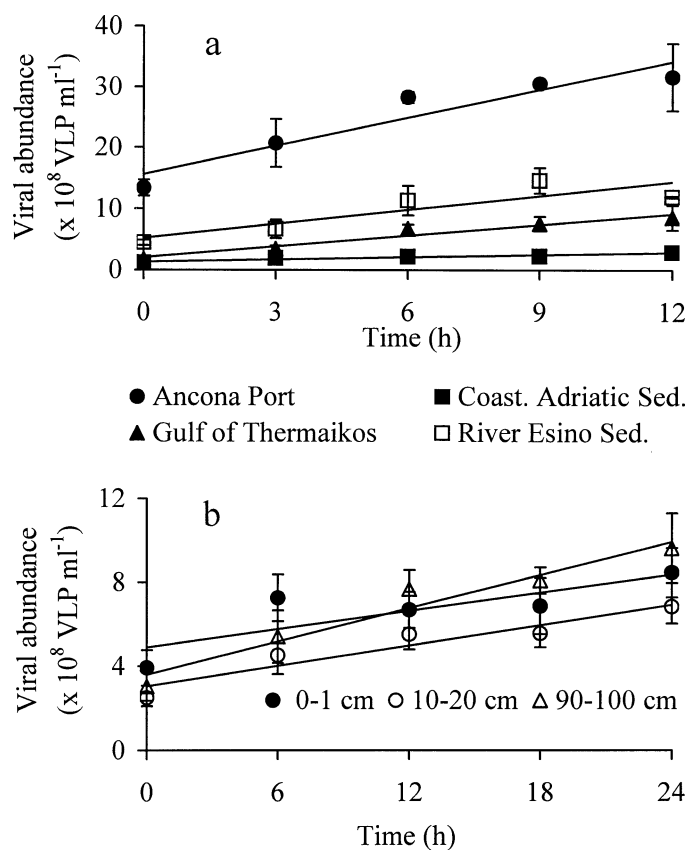


Fig. 2. VP rates, determined from the slope of the first-order regression of VLP vs. time for triplicate incubations in surface sediments (0–1 cm) from different systems. (a) Ancona Port,  $Y = 1.55 \times 10^8x + 15.6 \times 10^8$  ( $R^2 = 0.900$ ); Esino River sediment,  $Y = 7.70 \times 10^7x + 5.21 \times 10^8$  ( $R^2 = 0.770$ ); Gulf of Thermaikos,  $Y = 5.95 \times 10^7x + 2.06 \times 10^8$  ( $R^2 = 0.945$ ); Coastal Adriatic Sea sediments,  $Y = 1.25 \times 10^7x + 1.34 \times 10^8$  ( $R^2 = 0.918$ ). (b) Comparison of virus production in surface and deep layers of the Gulf of Manfredonia sediment core: layer 0–1 cm,  $Y = 1.46 \times 10^7x + 4.89 \times 10^8$  ( $R^2 = 0.681$ ); layer 10–20 cm,  $Y = 1.63 \times 10^7x + 3.04 \times 10^8$  ( $R^2 = 0.906$ ); and layer 90–100 cm,  $Y = 2.66 \times 10^7x + 3.59 \times 10^8$  ( $R^2 = 0.950$ ). SDs are reported.

quantify the effect of viruses in the biogeochemical cycling of organic matter. However, although several methods have been developed for determining VP in aquatic samples (Heldal and Bratbak 1991; Steward et al. 1992, 1996; Fuhrman and Noble 1995; Guixa-Boixareu et al. 1999; Noble and Fuhrman 2000; Hwang and Cho 2002a), no attempts have been made yet to quantify VP and VIBM in marine sediments. This can also be due to the difficulty in obtaining estimates of VP, which, being performed in sediment slurries and under aerobic conditions (even when the amount of oxygen available in subsurface sediment layers can be reduced), could be altered by manipulation.

We found that VP in all of the aquatic sediments that we investigated was extremely high, with average values that were 40–80 times higher than those observed in the water column (Table 4). Because the VLP abundance in the same sediments was only ~20 times higher, our values indicate that virus turnover in sediment can be higher than that in

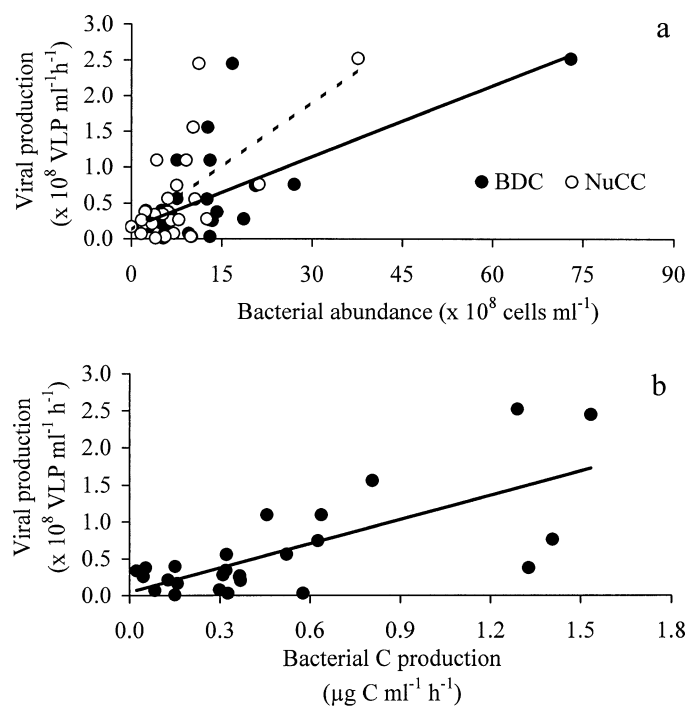


Fig. 3. Relationship between VP and (a) bacterial abundance (for BDC,  $y = 0.06x + 1.17 \times 10^7$ ,  $R^2 = 0.447$ ,  $n = 34$ ,  $P < 0.01$ ; for NuCC,  $y = 0.03x + 1.51 \times 10^7$ ,  $R^2 = 0.442$ ,  $n = 34$ ,  $P < 0.01$ ) and (b) BCP  $y = 1.10 \times 10^6x + 4.49 \times 10^6$ ,  $R^2 = 0.516$ ,  $n = 34$ ,  $P < 0.01$ ) in all benthic systems investigated.

the water column and that viriobenthos virulence can be even higher than in the water column. Similarly high values of VP have been recently observed in coastal sediments of the Southern California region (Hewson and Fuhrman in press). Hewson et al. (2001a) compared VP in different water bodies characterized by trophic gradients and observed the highest VP rates in eutrophic waters (Brisbane River) and the lowest values in oligotrophic coastal waters (Moreton Bay). They also reported that the addition of inorganic nutrients significantly stimulated VP. In the present study, we found a similar pattern. The sediments of the Port of Ancona and the Gulf of Thermaikos displayed the highest VP rates, whereas the more oligotrophic coastal Adriatic Sea sediments had values that were 5–10 times lower (Fig. 2). The significant relationships between VP and bacterial abundance and C production (Fig. 3), together with a bacterial cell production (number of bacteria produced per milliliter of sediment per hour) rate that was 50–400 times higher in the sediment than in the water column, indicate that virus-mediated processes can increase substantially in the sediment (Table 4).

The high values of benthic VP also indicate that sediments present favorable conditions for viral infection. Therefore, the discrepancy between high VP and relatively low VBR in all of the sediments that we investigated can be explained by high rates of viriobenthos decay and turnover rates, and further studies are needed in this area.

High VP in aquatic sediments is expected to cause a high rate of bacterial mortality, but the fraction of bacteria lysed by viral infection also depends on the BS. In the present

study, we made an attempt to estimate the BS in different sediment types, using intensive time-course experiments in which we monitored contextually the number of dead bacteria and the number of VLPs released into the medium. We found an average BS of 15–18 (in marine and freshwater sediments, respectively; range, 3–26). These values fall within the range of those generally used in water-column studies (Fuhrman and Noble 1995; Noble and Fuhrman 2000; Wommack and Colwell 2000), thus making our results comparable with estimates of bacterial mortality reported so far in literature (Table 4). We also observed that BSs increased with increasing VP and virus turnover but were not related to bacterial C content (or biovolume). Moreover, the highest BSs were observed in eutrophic and/or contaminated environments (Gulf of Thermaikos and Ancona Port), whereas the lowest values were encountered in oligomesotrophic systems (Adriatic Sea coastal sediments and the Gulf of Manfredonia). This suggests a coupling between BS and bacterial production/activity, given that these parameters were higher in the contaminated/eutrophic areas.

The results reported in the present study indicate that VIBM in marine and freshwater surface sediments is high (i.e., on average, ~30% and 20%, respectively; Table 4). Bacterial mortality increased significantly in deeper sediment layers (consistently >40%; *t*-test,  $P < 0.005$ ), so, by integrating our results in the top 20 cm (where most of benthic fauna are confined) or along the whole sediment core (100 cm), it is possible to estimate that viruses are responsible for >40% of bacterial mortality.

Virioplankton has been shown to be responsible for an important fraction of bacterioplankton mortality (up to >50%) in several aquatic environments (Steward et al. 1992, 1996; Fuhrman and Noble 1995; Hewson et al. 2001a; Hwang and Cho, 2002b; Wilhelm et al. 2002; Choi et al. 2003). Data summarized in Table 4 indicate that the average bacterial mortality rate in marine and freshwater sediments is comparable with mortality rates reported here for the overlying water bodies (~37%). Recent estimates of VIBM in shallow lake sediments (0–25%; average, 6%; Fischer et al. 2003) were lower than the average values that we observed for freshwater sediments (average, 18.4%); further studies are needed to better understand the factors that affect VP and viral infection in freshwater sediments. Nonetheless, our results suggest that high benthic VP rates can influence benthic bacterial dynamics, particularly in marine systems, and provide new elements for including benthic viruses in models of the flow of energy and organic material in aquatic sediments.

*Life strategies of viruses in aquatic sediments*—The factors that regulate the occurrence of lysogeny are poorly understood. It is known that lytic infection is the most common life strategy among virioplankton (Fuhrman 1999), but recent investigations have reported that lysogens comprise a significant portion of the heterotrophic microbial population, especially in deep waters (Weinbauer et al. 2003), and that several marine environments contain inducible prophages (Jiang and Paul 1996, 1998; Ortmann et al. 2002). Conversely, other studies from both marine and freshwater environments have reported that lysogeny is not an important virus-

host interaction, because only a minor fraction of bacteria contains inducible prophages (Weinbauer and Suttle 1996; Tapper and Hicks 1998). Generally, it is assumed that certain environmental factors can directly influence viral replication and/or prophage induction, whereas others can only indirectly influence the lysogenic decision (Williamson et al. 2002), and that lysogenic infection may be more common in oligotrophic than in eutrophic waters (Wommack and Colwell 2000; Hewson et al. 2001a).

Our results indicate that, in the Port of Ancona and the Gulf of Thermaikos, only lytic infection occurred, and that, in all other aquatic sediments, lysogens accounted for <2% of total bacterial abundance. Lysogeny was barely detectable only in oligotrophic systems, as it was in coastal Adriatic Sea sediment (1.8%), in freshwater sediments (~1.5%), and in the Gulf of Manfredonia, which also displayed an increase of the lysogenic fraction in deeper sediment layers (~0.66–3.30%; Table 3).

Several hypotheses can be proposed for explaining the lack of a relevant lysogenic strategy in aquatic sediments: (1) high bacterial densities (with large cell size) and fast bacterial growth rates, which would increase the contact rate with host cells; (2) different virus species composition in water column and sediments; (3) an accumulation of contaminants in aquatic sediments and its consequent induction of the lytic cycle in lysogenic bacteria; and (4) that the factors responsible for environmental stress, and the therefore induction of sediment bacteria, are not well understood and induction agents have not been successfully identified. In fact, it has been demonstrated that several contaminants (heavy metals, hydrocarbons, pesticides, and micropollutants) induce lysogenic cells to the lytic cycle in the marine environment (Jiang and Paul 1996; Cochran et al. 1998; Danovaro and Corinaldesi 2003). In our study, no lysogens were found in eutrophic/contaminated sites. Because both the Port of Ancona and the Gulf of Thermaikos contained high levels of PAH and heavy metals, it is possible that these contaminants have induced lysogenic infections to lytic development. However, at present, we have no information on the effect of mitomycin C on benthic bacteria, and the low lysogenic fraction could have been due to the inefficacy of this treatment in the sediment. Further studies are therefore needed to elucidate the potential effect of contaminants on viriobenthos life strategies.

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*Received: 17 May 2003*

*Accepted: 23 October 2003*

*Amended: 7 November 2003*