

## Distribution of viruses and bacteria in relation to diagenetic activity in an estuarine sediment

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### *Abstract*

The distribution of viruses and bacteria was investigated in relation to bacterial sulfate reduction and total respiration (production of dissolved inorganic carbon, [DIC]) in a coastal sediment. Viral and bacterial abundance ranged from about  $0.5 \times 10^8$  to  $8 \times 10^8$  viruses  $\text{cm}^{-3}$  and  $0.1 \times 10^8$  to  $4 \times 10^8$  bacteria  $\text{cm}^{-3}$  in the upper 16 cm of the sediment and showed large and systematic changes within scales of a few centimeters. In general, viral abundance was highest in the sediment surface (0–1 cm); however subsurface peaks at 3–5 cm depth associated with increased diagenetic activity were also observed. The virus–bacterium ratio ranged from 1.4 to 7.8 and increased significantly with depth in the upper 6 cm ( $P < 0.001$ ). Viral abundance showed significant positive correlation with both bacterial abundance and activity ( $P \ll 0.001$ ), suggesting that the distribution and abundance of viruses were closely coupled to the activity of the bacterial community and that viruses are produced by bacteria within the sediment. The significant coupling between viral abundance and sulfate reduction rate and DIC production is the first indication of viral production associated with diagenetic active bacteria in marine sediments. This coupling between viral abundance and bacterial activity and the distinct pattern of vertical distribution show that viruses are a dynamic component of the benthic community. The morphological analysis indicated that interstitial viral communities were dominated by long ( $>1 \mu\text{m}$ ) filamentous forms with a helical symmetry. Several types of these filamentous forms were observed, as well as a variety of tailed forms with icosahedral symmetry. Filamentous forms are rarely found in the water column, which suggests that they are adapted to the benthic environment and specific to the interstitial hosts.

Viruses are abundant in all aquatic environments. Studies of bacteria–virus interactions in aquatic systems have, however, almost exclusively focused on plankton communities, and investigations on benthic viruses have been performed only recently (e.g., Maranger and Bird 1996; Drake et al. 1998; Danavaro and Serresi 2000; Ricciardi-Rigault et al. 2000; Hewson et al. 2001; Danavaro et al. 2002). Consequently, even basic knowledge about the spatial distribution and the temporal dynamics of interstitial viruses is lacking. Research on the role of viruses for the mortality and structure of benthic microbial communities in the sediment is required to expand our understanding of microbial interactions in benthic environments. Benthic communities experience a different physical and chemical microenvironment than pelagic communities (e.g., retarded transport coefficients, steep concentration gradients of physical and chemical parameters, smaller spatial scales of distribution, higher organism densities), and it is possible that these differences influence the interactions between viruses and their hosts and the role of viruses in the sediment.

The majority of the previous studies of interstitial viruses have focused on surface sediments. Here it was observed that the virus abundance exceeded the number of viruses in the water column by tenfold to a thousandfold, reaching densities of  $10^7$  to  $10^9$  virus-like particles  $\text{cm}^{-3}$  (Danavaro and Serresi 2000; Hewson et al. 2001). Quantification of viral abundance in surface sediments along trophic gradients indicated an increase in viral numbers in areas with high pelagic productivity, where sediments are enriched with organic matter (Maranger and Bird 1996; Danavaro and Serresi 2000; Hewson et al. 2001; Danavaro et al. 2002). Significant correlations between viral abundance in the upper sediment and the concentration of viruses and suspended particles in the overlying water could suggest that viruses adsorbed to settling particles may contribute considerably to the viral assemblage in sediments (Maranger and Bird 1996; Hewson et al. 2001; Danavaro et al. 2002). On the other hand, an increased number of viruses in areas with high organic matter content could also be a result of enhanced bacterial activity within the sediment. This hypothesis is supported by the significant correlation between bacterial and viral abundance found in some studies (Danavaro and Serresi 2000). Recently, Danavaro et al. (2002) found a significant positive relationship between viral abundance and bacterial turnover rate in deep-water Mediterranean surface sediments. This correlation indicated that viral abundance in these sediments was influenced by the activity of the local bacterial community and, consequently, that viruses were produced within

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the surface sediment. The distribution of viruses in sediments is the result of a number of biotic and abiotic controls, and basic understanding of viral production and importance for the benthic microecology and diagenetic processes is still lacking.

The present study is to our knowledge the first to provide a description of the horizontal and vertical heterogeneity in viral abundance at a benthic location and to combine these analyses with measurements of bacterial abundance and activity. Moreover, we present a description of the morphological diversity of the viral assemblage of the investigated sediment.

## Materials and methods

**Study area and sampling**—A total of eight sediment cores (inner dimension 5.3 cm) was collected at 0.5-m water depth in Nivå Bay, Denmark, on 15 May 2001. During sampling the water temperature was 15°C and the salinity 5. Cores for abundance and activity measurements were collected pairwise on a transect with a distance of 0.5 m between the pairs (these cores were called A, B and C, D and E, F). In between, two cores were sampled for porosity and organic content measurements. Within 1 h of sampling the recovered cores were placed in aerated bottom water kept at in situ conditions.

**Sediment characteristics**—Profiles of sediment porosity and organic carbon content were measured in the two sediment cores at a vertical resolution of 1 cm. Porosity was determined from density and weight loss after drying at 105°C for 24 h. The organic carbon content was determined as weight loss after combustion (450°C, 24 h).

O<sub>2</sub> microprofile measurements were performed within 24 h of sampling with a Clark type microelectrode equipped with an internal reference and a guard cathode (Revsbech 1989). The electrode had a tip diameter <5 μm, a stirring sensitivity <1%, and a 90% response time <2 s (Revsbech 1989; Glud et al. 2000a). The sensor was positioned with a motor driven micromanipulator, and the sensor current was recorded by a picoammeter (Unisense, PA 2000) connected to an A/D converter and a laptop (Revsbech and Jørgensen 1986). Two profiles were measured at a depth resolution of 100 μm in each of the submerged sediment cores. During measurements rotating magnets attached to the internal wall of the coreliners ensured a well-mixed overlying water phase and an approximate diffusive boundary layer (DBL) thickness of 400 μm (Rasmussen and Jørgensen 1992). The diffusive O<sub>2</sub> uptake (DOU) of the sediment was calculated from the linear concentration gradient within the DBL using Fick's first law of diffusion (Rasmussen and Jørgensen 1992). The molecular diffusion coefficient was from Broecker and Peng (1974) and corrected for temperature as described by Li and Gregory (1974).

Total exchange rates of O<sub>2</sub> and dissolved inorganic carbon (DIC) were subsequently measured in all six sediment cores. Incubations were initiated by capping the submerged cores with a lid perforated by a small hole, which allowed the insertion of a microsensors into the core water. During measurements the rotating magnets (*see above*) ensured a similar

DBL thickness as during the microprofile measurements. During incubations the microsensors tip was subsequently inserted in the respective cores to ensure that the O<sub>2</sub> concentration of the enclosures decreased linearly. Samples for DIC measurements of the enclosed water were recovered initially and at the end of the incubation. Samples were preserved in 7-ml gas-tight glass containers (exetainers) spiked with saturated HgCl<sub>2</sub> and later measured on an infrared gas analyzer. Total exchange rates were calculated from the observed concentration changes accounting for the volume of the enclosed water.

**Specific microbial activity of the sediment cores**—Following the intact core measurements, each core was cut into 1-cm slices and homogenized, and 4–5 g of sediment were transferred to a series of 12 ml, N<sub>2</sub>-flushed, gas-tight exetainers. A similar amount of sediment was put aside for enumeration of bacteria and viruses (*see below*). The individual exetainers were subsequently filled with a known amount of 0.02-μm filtered, N<sub>2</sub>-flushed bottom water from the sampling location. Glass beads were added, and the content of the exetainers was mixed thoroughly.

Subsequently, 1 ml of water was sampled in each exetainer for DIC determination, and the sampling volume was replaced by filtered bottom water. Immediately 10 μl <sup>35</sup>S-SO<sub>4</sub><sup>2-</sup> (120 kBq μl<sup>-1</sup>) was added and the exetainers were incubated for 3–4 d in darkness on a shaking table. At the end of the incubations, samples for DIC (1 ml), SO<sub>4</sub><sup>2-</sup> concentrations (1 ml), and enumeration of bacteria and viruses were retracted. Samples for DIC were stored (along with the initial probes) in small gas-tight ampoules spiked with HgCl<sub>2</sub>, while SO<sub>4</sub><sup>2-</sup> were conserved with Zn acetate (10% final solution). Samples for enumeration were conserved with glutaraldehyde (2% final solution). The remaining content of the exetainer was conserved with 2% Zn acetate for later determination of labeled reduced sulfur. DIC production rates in the different sediment slices were calculated from the concentration change during the incubation accounting for dilutions and the incubation period.

The bacterial sulfate reduction rate (SRR) in the individual sediment slices was quantified from the reduction of <sup>35</sup>S-SO<sub>4</sub><sup>2-</sup> into total reducible inorganic sulfur (TRIS = H<sub>2</sub><sup>35</sup>S, Fe<sup>35</sup>S, Fe<sup>35</sup>S<sub>2</sub>, and <sup>35</sup>S<sup>0</sup>) determined by the one-step acidic Cr-II method (Fossing and Jørgensen 1989). The Zn<sup>35</sup>S and the <sup>35</sup>SO<sub>4</sub><sup>2-</sup> radioactivity were measured in a liquid-scintillation counter. The SRRs were calculated from the fraction of reduced sulfur produced during incubation and the sulfate concentration according to Jørgensen and Fenchel (1974) using an isotopic fractionation factor of 1.06. In order to compare total DIC production rates and SRR, the latter was multiplied by two according to the stoichiometry: H<sup>+</sup> + CH<sub>2</sub>O + 0.5SO<sub>4</sub><sup>2-</sup> → CO<sub>2</sub> + 0.5H<sub>2</sub>S + H<sub>2</sub>O (Canfield et al. 1993). Sulfate concentrations were measured in diluted and filtered samples by nonsuppressed anion-exchange chromatography.

**Enumeration of bacteria and viruses**—From the initial samples (*see above*) 2–4 g sediment was weighed and transferred to 50-ml centrifuge tubes, 5 ml of 0.02-μm filtered sample water containing 3% glutaraldehyde was added, and samples were kept cold and dark until further analysis. With-

in 1 week after sampling, viruses and bacteria were extracted from the fixed sediment samples basically following the procedure by Danavaro et al. (2001). Sodium pyrophosphate was added to the sample for a final concentration of 5 mmol L<sup>-1</sup>, and after 15 min it was transferred to a sonication bath for 2 × 1 min. The sample was then centrifuged (2,000 rpm, 5 min) to precipitate the sediment particles, and the supernatant was collected in a 15-ml centrifuge tube. The sediment sample was then washed twice with 2 ml of virus-free seawater (0.02- $\mu$ m filtered). The total extracted volume from the given amount of sediment was measured. The efficiency of the pyrophosphate, sonication, and washing treatments during the extraction was analyzed at three depth intervals by quantifying the abundance of microorganisms after each individual step in the extraction procedure.

For enumeration of bacteria and viruses, 100  $\mu$ l of the extract was filtered onto 0.02- $\mu$ m filters (Anodisc) and stained with SYBR-Green I according to Noble and Fuhrman (1998). On each slide, 300–600 bacteria and viruses were counted in 10–15 fields using epifluorescence microscopy. In one sediment core (core A), duplicate filters were prepared to assess the reproducibility of the enumeration procedure. The standard deviation for two replicate measurements of bacterial and viral abundance in core A was on average 5.0% (2.2–8.8%). For a few sediment slices, virus and bacteria abundance was measured both before and after the sediment activity incubations. No significant change in the abundance occurred during the incubations ( $n = 3$ ).

**Virus morphology**—Samples for electron microscopy were prepared from the extracts of one sediment core at three selected depths (0–1 cm, 4–5 cm, and 14–16 cm). For each depth, two replicate samples were analyzed. Two milliliters of virus extract were filtered (0.2  $\mu$ m, Anodisc) and centrifuged onto 200 mesh formvar-coated copper grids (100,000 × g, 3 h) in an ultracentrifuge (Beckman Optima LE-80K). Grids were stained with 2% uranyl acetate for 30 s, rinsed three times in distilled water, and examined by transmission electron microscopy (Zeiss EM 900) (Hofer and Sommaruga 2001).

## Results

**Extraction of viruses and bacteria from the sediment**—The efficiency of the individual treatments for extracting viruses and bacteria was analyzed in order to optimize the extraction procedure. Addition of sodium pyrophosphate (5 mmol L<sup>-1</sup>) significantly increased dislodgement of viruses from the sediment relative to untreated samples ( $P < 0.001$ , Fig. 1A). Application of ultrasonic treatment increased the release of viruses further, and the efficiency of the combined pyrophosphate-sonication treatment was 65% to 78% of the total extractable viruses. Following the second wash, we only encountered 7% to 11% of the extractable viruses (Fig. 1A), and we conclude that the majority of the extractable viruses was released during the extraction and washing procedure.

The efficiency of bacterial extraction was not equally efficient (Fig. 1B). In general, the highest output of bacteria was observed with the combined pyrophosphate-sonication

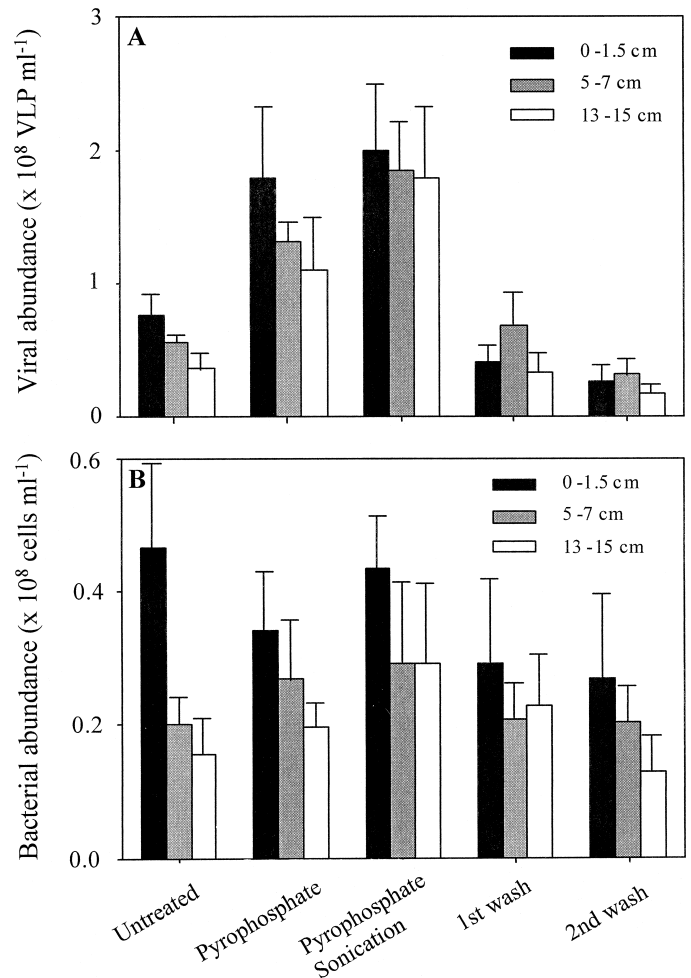


Fig. 1. Effects of extraction and washing procedures on the abundance of (A) viruses and (B) bacteria released from the sediment at different depths.

treatment, which released 42% to 45% of the total extractable bacteria. After two washes, 20% to 29% of the total extracted bacteria were still encountered, suggesting that a significant portion of the sediment bacteria was not released by the applied extraction procedure.

**Sediment characteristics and benthic exchange rates**—The relatively sandy sediment had a surficial sediment porosity of  $\sim 0.54$ , which gradually decreased to  $\sim 0.44$  at the deepest horizon (Fig. 2A). A smaller peak was observed in both profiles at a sediment depth of 8.5 cm. The surficial sediment was depleted in organic matter and reached a maximum of  $\sim 3.2\%$  dry weight at 2.5-cm depth, after which it gradually decreased with depth to  $\sim 2.5$  in the deepest sample. The values are typical for sandy estuarine sediments. The depletion in organic matter at the sediment surface was atypical but could have been caused by a recent resuspension event at the relatively exposed site. The average DIC profile reflects an increase with depth reflecting the gradual degradation of organic matter (Fig. 2B). The O<sub>2</sub> penetration depth reached  $\sim 1$  mm (Fig. 2C), and there was no significant difference between the investigated sediment cores (not shown).

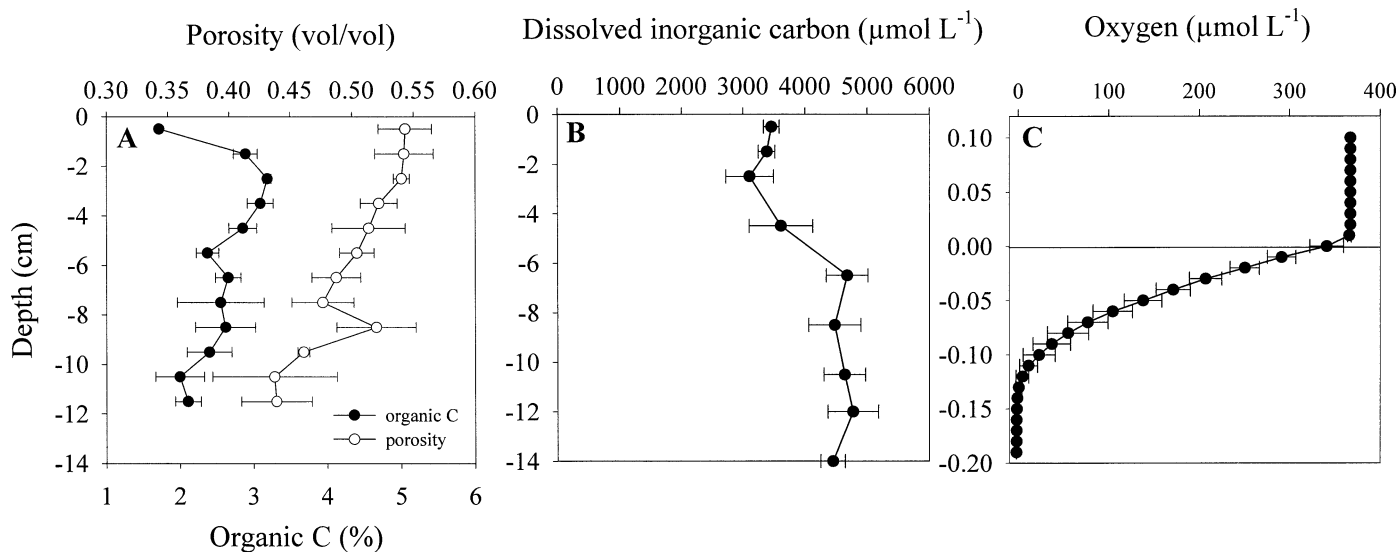


Fig. 2. Profiles of (A) sediment porosity and organic carbon content, (B) concentration of dissolved inorganic carbon, and (C) oxygen.

The same was the case for DOU calculated from the linear gradient in the DBL that reached an average value of  $41.0 \pm 4.8 \text{ mmol m}^{-2} \text{ d}^{-1}$  (Table 1).

The total  $\text{O}_2$  uptake (TOU) indicated that nondiffusive sediment–water exchange was negligible. This was confirmed by the later core sectioning, showing that large irrigating macrofauna were absent in the investigated cores. Total exchange in DIC showed more scatter than the TOU and reached an average value of  $54.9 \pm 10.3 \text{ mmol C m}^{-2} \text{ d}^{-1}$  (Table 1). The respiratory ratio (DIC exchange/TOU) for the entire benthic community was  $1.34 \pm 0.42$ , which is a typical value for coastal environments (e.g., Therkildsen and Lomstein 1993; Glud et al. 1998; Rysgaard et al. 2001 and references therein).

*Specific activity of homogenized sediment slices*—The incubated slurries generally reflected elevated degradation rates in the surface sediments. Rates gradually decreased

with sediment depth (Fig. 3, upper panel); however, a secondary peak at a sediment depth of 3–5 cm was generally observed for both DIC production and SRR. The SRR profile followed the depth profile of the DIC production (Fig. 3). In the deeper parts of the sediment, sulfate reduction explained all of the carbon oxidation in the incubated slurries (note that stoichiometry has been corrected for in Fig. 3 and Table 1). In the surface and intermediary sections of the sediment core, other electron acceptors like iron oxides probably contributed to the DIC production, even though sulfate reduction still was dominant. Core A reflected somewhat lower rates in the surficial sediment and a very distinct secondary peak as compared to the other cores (Fig. 3).

The depth-integrated DIC production varied between 105 and  $166 \text{ mmol C m}^{-2} \text{ d}^{-1}$ , with an average of  $128.8 \pm 23.2 \text{ mmol C m}^{-2} \text{ d}^{-1}$  (Table 1). With a reaction stoichiometry of 1 : 2 between sulfate and organic carbon, the depth-integrated SRR accounted for 55%–107% of the total carbon oxidation

Table 1. Bacterial respiration in samples from Nivå Bay, estimated from total core incubations and from incubations of individual depths. Units are  $\text{mmol C m}^{-2} \text{ d}^{-1}$ .

	A	B	C	D	E	F	Mean $\pm$ SD
Diffusive oxygen uptake*	40.0	39.0	43.6	45.8	36.4	—	$41.0 \pm 4.8$
Total oxygen uptake*	40.4	38.3	43.9	34.0	38.3	35.2	$38.2 \pm 3.9$
DIC exchange	57.4	50.8	65.9	65.4	49.5	42.8	$54.9 \pm 10.3$
Integrated DIC production	109.0	166.1	141.3	117.3	134.3	104.5	$128.8 \pm 23.2$
Sulfate reduction rate (SRR)†	84.7	177.3	114.5	75.9	73.6	57.2	$97.2 \pm 43.5$
Percentage of organic C mineralized via SSR†	77	107	81	65	55	55	$73 \pm 20$

\* Oxygen uptake was converted to carbon respiration using a respiratory quotient of 1.

† Sulfate reduction was multiplied by two to get the corresponding carbon respiration.

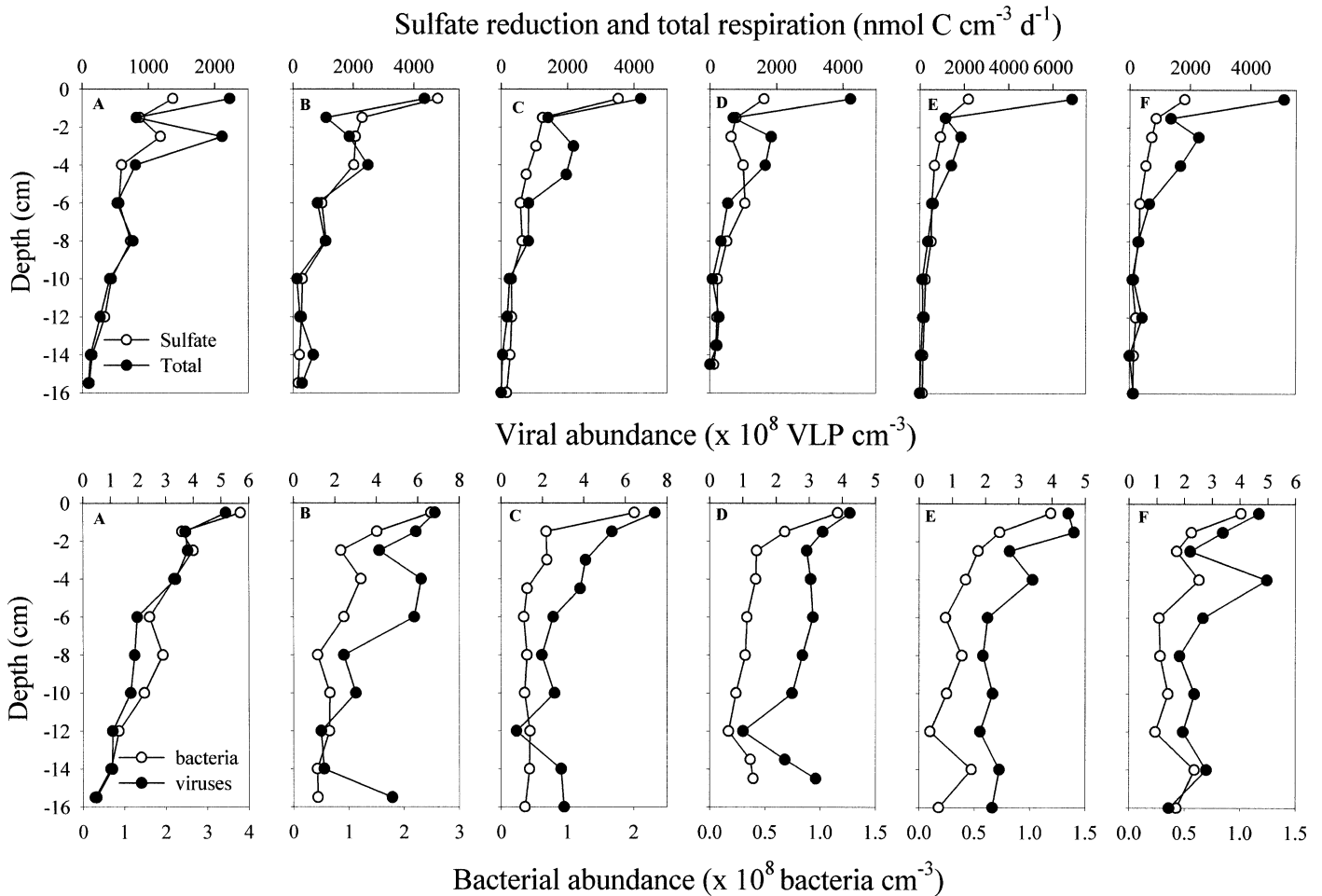


Fig. 3. Profiles of sulfate reduction rate and total respiration (upper panels) and bacterial and viral abundance (lower panels) in cores A–F.

(Table 1). Sulfate reduction was thus the most important degradation pathway in the incubated sediments.

*Distribution of viruses and bacteria*—The abundance of viruses and bacteria in the upper 16 cm of the Nivå Bay sediment ranged from  $4.9 \times 10^7$  to  $7.5 \times 10^8$  viruses  $\text{cm}^{-3}$  and  $9.9 \times 10^6$  to  $3.8 \times 10^8$  bacteria  $\text{cm}^{-3}$  (Fig. 3, lower panel). In general, there was a decrease in microbial abundance with depth, with occasional secondary peaks in the deeper sediment strata. Some samples (cores B, C, and D) showed a distinct peak in viral and bacterial abundance at the bottom of the core (Fig. 3). There was no apparent relation between the abundance profiles and the relative distance between the obtained cores.

*Coupling between viral abundance, bacterial abundance, and diagenetic activity*—As shown in Figs. 4, 5, and 6, core A differed from the other cores in the depth distribution of SRR, DIC production, virus abundance, and bacterial abundance (slope and intercept with ordinate was significantly different from the pooled data of cores B–F,  $P < 0.05$ ). Consequently, this core is treated separately in the following analysis.

Viral abundance correlated significantly with the abundance of bacteria in all the individual samples (Fig. 4A). The average virus–bacterium ratio (VBR) estimated from the slope of the regression lines was  $2.67 \pm 0.27$  and  $1.4 \pm 0.12$  for cores B–F and core A, respectively. The VBR increased significantly ( $r^2 = 0.59$ ,  $P < 0.001$ ) with depth in samples B–F from 3–5 in the surface sediment to maximum values of 5–10 at 5–6 cm depth (Fig. 4B). In sample A, on the other hand, the VBR was relatively low (1.0–1.7) and depth independent.

The diagenetic activity quantified as DIC production and SRR correlated well to the abundances of bacteria and viruses (Fig. 5A,B). Bacterial abundance explained 94% and 87% of the variance in sulfate reduction in sample A and samples B–E, respectively ( $P \ll 0.001$ , Fig. 5A), while 87% and 62% of the variance in viral abundance was explained by sulfate reduction in sample A and samples B–E, respectively ( $P \ll 0.001$ ) (Fig. 5B). Viral abundance also correlated significantly with DIC production ( $P \ll 0.001$ ); however, the power of explanation was less than for sulfate reduction ( $r^2 = 0.79$  and  $0.37$  for sample A and samples B–F, respectively).

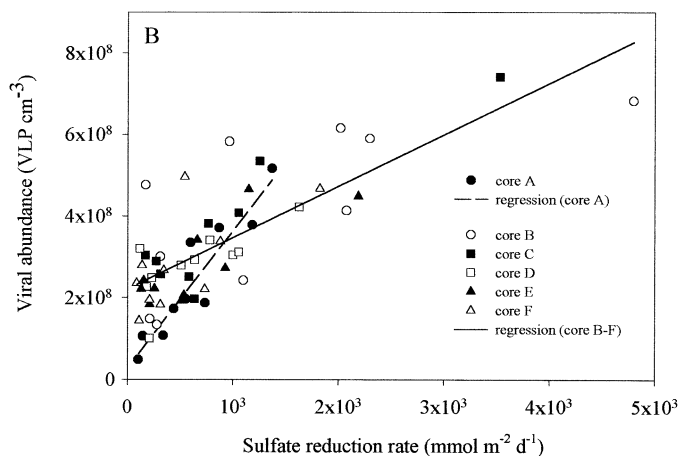
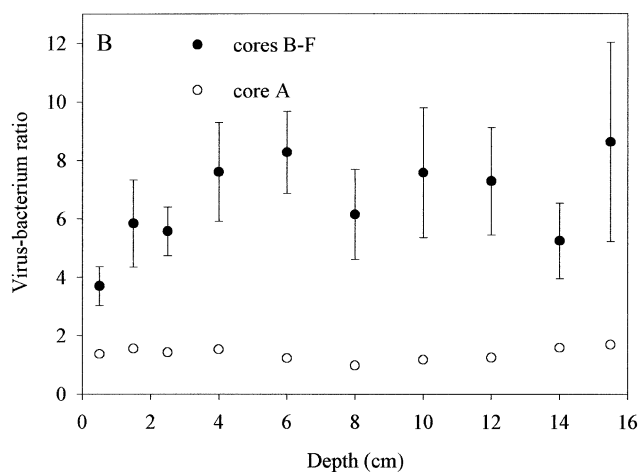
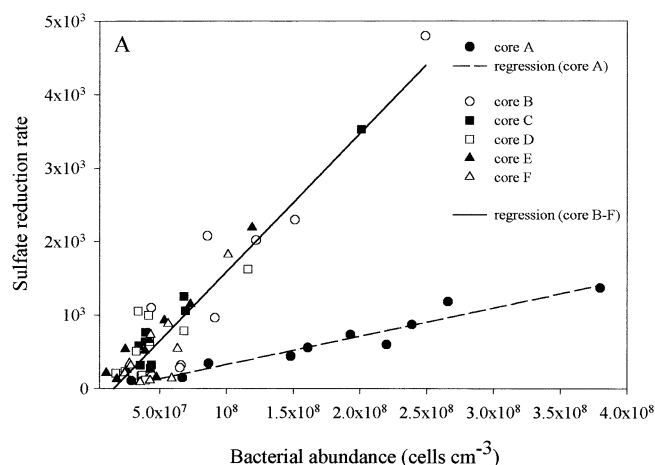
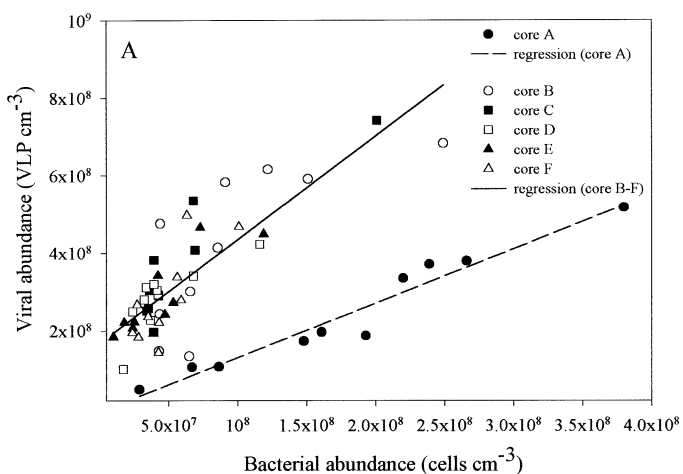


Fig. 4. (A) Viral abundance as a function of bacterial abundance. The regression for the compiled data (excluding core A) was fitted linearly as  $VA = 2.67 (\pm 0.27) \times BA + 1.7 \times 10^8 (\pm 8.5 \times 10^7)$  ( $r^2 = 0.68$ ,  $P \ll 0.001$ ), where VA and BA represent virus and bacterial abundance, respectively. For the A core the equivalent equation was  $VA = 1.40 (\pm 0.12) \times BA - 8.5 \times 10^6 (\pm 3.7 \times 10^7)$  ( $r^2 = 0.95$ ,  $P \ll 0.001$ ). (B) Changes in average virus–bacterium ratio (VBR) with depth for core A and cores B–F.

Fig. 5. (A) Sulfate reduction rate as a function of bacterial abundance and (B) viral abundance as a function of sulfate reduction rate. Regression lines show significant positive correlations for both core A and cores B–F.

**Viral morphology**—Transmission electron microscopy of samples from different sediment depths showed a large morphological diversity in the viral assemblage (Fig. 6). Several types of long ( $>1 \mu\text{m}$ ), helical filamentous forms were observed, as well as several types of tailed viruses. The differentiation of viruses from other microbial material or cell debris was made solely from observation of morphology in the electron microscope. Since we have not isolated any of the viruses, we cannot exclude that these filamentous forms may be something other than viruses. However, their structure and size are similar to that of known filamentous viruses, suggesting that these are indeed viruses (Fig. 6).

## Discussion

**Abundance and distribution of viruses and bacteria**—The measured abundances of viruses in the surface sediment of

Nivå Bay (Fig. 3) are approximately 10 times higher than pelagic viral densities measured in the same area (Riemann and Middelboe 2002) and are consistent with viral abundances measured in the surface sediments of other coastal locations, such as the Moreton Bay, Australia (Hewson et al. 2001), and Chesapeake Bay, USA (Drake et al. 1998). The virus to bacterium ratios (VBR) in Nivå Bay surface sediment were, on the other hand, considerably lower (1.4–7.8, Fig. 4B) than those obtained in the Moreton Bay (21–56) and Chesapeake Bay (29–85) (Drake et al. 1998; Hewson et al. 2001) but in the range of ratios found in deep-sea Mediterranean sediments (2–5; Danavaro and Serresi 2000). It has been argued that elevated benthic VBR is the result of higher bacterial activity associated with increased substrate availability in sediments (Hewson et al. 2001) or significant import of viruses attached to sedimenting particles (Danavaro and Serresi 2000).

In Nivå Bay, the VBR was low in the surface sediment, increased significantly ( $P < 0.001$ , Fig. 4B) with depth in the upper 6 cm of the sediment of cores B–F, and remained relatively high in the deeper parts. The increase was asso-

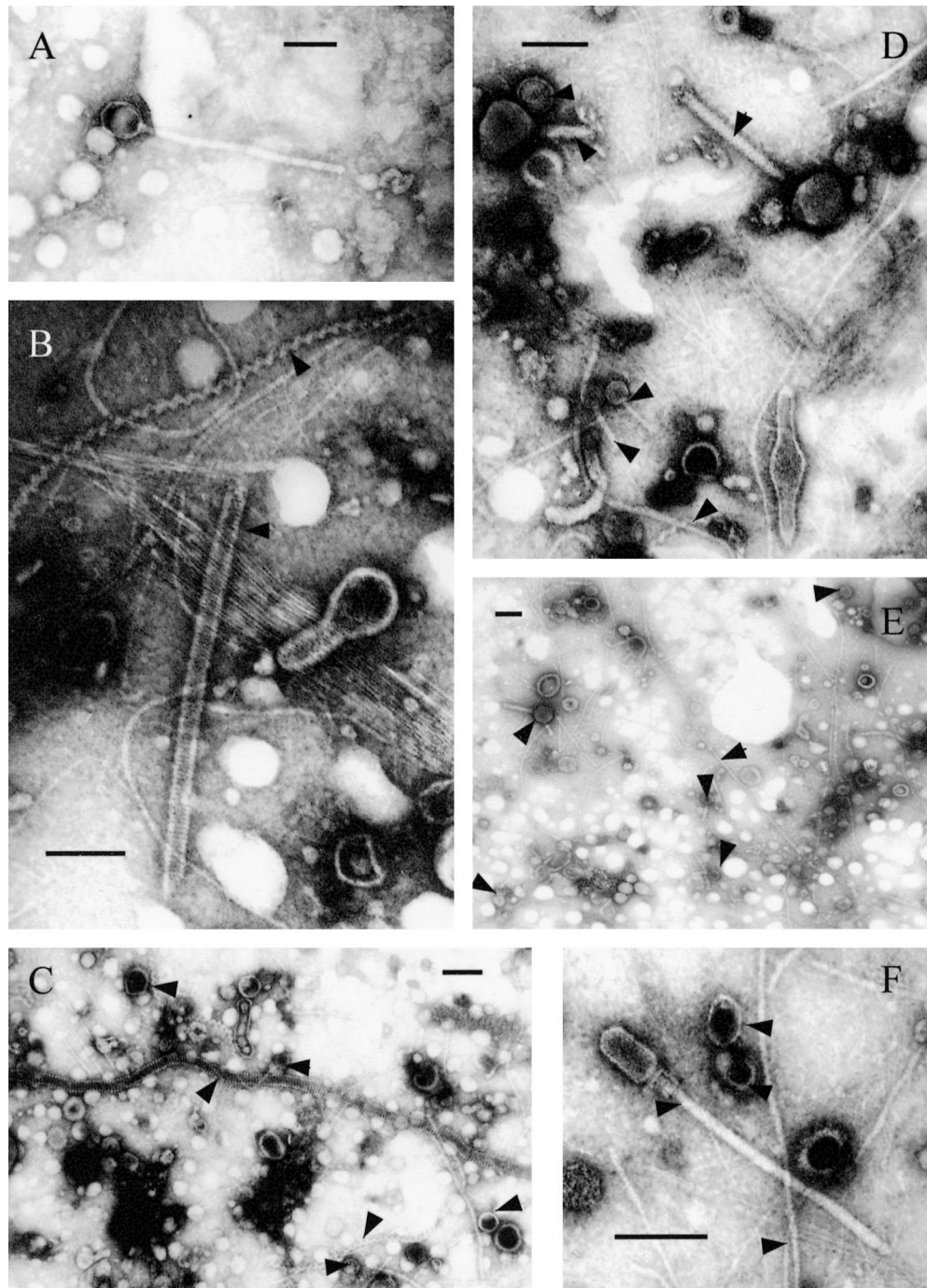


Fig. 6. Examples of the morphological diversity of viruses from the Nivå Bay sediment. Arrows indicate examples of virus-like particles. Scale bar = 100 nm. (A) 0–1 cm depth, (B) 14–16 cm depth, (C–F) 4–5 cm depth.

ciated with a decrease in the diagenetic activity (Fig. 3). This observation indicates that elevated VBR is not necessarily associated with import of viruses from the overlying water or with enhanced benthic activity in the surface sediments. Variable decay rates of viruses and the large variations in bacterial community composition with sediment depth may be additional factors contributing to variability in VBR be-

tween different depths and locations. It can not be excluded that differences in microbial extraction efficiency affect the present and previously published benthic VBR. In this study, most of the extractable viruses were released from the sediment during the initial extraction procedure, while a significant fraction of the extractable bacteria was released during the subsequent washes (Fig. 1). This indicates that viruses

are extracted more efficiently than bacteria by the applied procedure, suggesting that the VBR may have been slightly overestimated in this study.

The distribution of microbial abundance and activity in the upper 16 cm of the Nivå Bay sediment showed large and systematic changes within scales of a few centimeters, which was the spatial resolution of the vertical sampling (Fig. 3). In general, microbial abundance and activity decreased with depth, and viral abundance showed significant positive correlation with both bacterial abundance and activity ( $P \ll 0.001$ , Figs. 4A and 5B). These correlations strongly suggest that the distribution and abundance of viruses were closely coupled to the activity of interstitial bacteria and that the viruses are produced by bacteria within the sediment. The significant coupling between viral abundance and sulfate reduction rate is the first indication of viral production associated with sulfate reducing bacteria in marine sediments. The coupling between the abundance of viruses and bacterial activity and the distinct pattern of vertical distribution show that viruses are a dynamic component of the sediment community. However, the turnover rate of the viral assemblage in the sediment is still unresolved.

In three samples, a distinct increase in viral and bacterial abundance was observed in the deep layers with very low or no sulfate reduction (14–16 cm depth) (Fig. 3). It can be speculated that this increase is associated to methanogenic activity or anaerobic methane oxidation, which is expected to take place below the sulfate reduction zone.

The depth-integrated DIC production was approximately twice as large as the rates measured by the whole core incubation technique. It is a common observation that homogenized samples incubated in bags or in slurries lead to higher mineralization rates as compared to whole core incubations (e.g., Kostka et al. 1999; Glud et al. 2000b; Hansen et al. 2000). The reasons are ascribed to elevated solute and particle transport rates, disruption of spatial heterogeneity within the given sample, and decay of encapsulated fauna in the homogenized samples (Dannenberg et al. 1997; Hansen et al. 2000). It is, however, generally accepted that the activity distribution remains unaltered (e.g., Canfield et al. 1993; Thamdrup and Canfield 1996; Kostka et al. 1999).

*Spatial scales of microbial variability*—The profiles of viral and bacterial abundance covered a sediment surface of 22 cm<sup>2</sup>. On that scale there were no systematic trends in the horizontal pattern; that is, the distribution of bacteria and viruses did not show a larger degree of similarity between adjacent samples than between increasingly distant samples (Fig. 3). Similarly, the variation between measured oxygen profiles was as high within individual samples as between samples (not shown). These results indicate that the horizontal heterogeneity in microbial abundance and activity in Nivå Bay varied on spatial scales smaller than the 22 cm<sup>2</sup> applied in the present study. Indeed, high-resolution two-dimensional images of aerobic activity in the investigated area show that horizontal variations take place on much smaller scales (Fenchel and Glud 2000; Glud et al. 2001).

The microbial abundances and activities at a given depth varied only by a factor of 2–3 between the six samples. Core A was the only exception, with significantly higher bacterial

abundance than the other samples but also with the lowest measured bacterial activity. Here, viral abundance was still significantly correlated with bacterial abundance and activity ( $P < 0.001$ ), and the low abundance of viruses may, therefore, have been the result of a low specific bacterial activity. This supports the suggestion that viral abundance in the sediment is affected by the abundance as well as the activity of the bacterial populations.

*Morphological diversity of viruses*—An interesting observation from the morphological analysis of interstitial viruses was the presence of large numbers of filamentous forms with a helical rather than icosahedral symmetry (Fig. 6). The only previous observations of filamentous viruses in aquatic systems were reported from an alpine lake (Pina et al. 1998; Hofer and Sommaruga 2001) and a small productive pond (Finlay and Maberly 2000). Filamentous viruses that infect bacteria are mostly described from studies of *Escherichia coli* viruses (phages M13, f1, and fd). These viruses belong to the family Inoviridae and are single-stranded DNA viruses. The life cycle of the filamentous bacterial viruses is not lytic but rather of a chronic nature. The virus DNA are maintained in the infected host cell, and new viruses are continuously released from the cells without killing the host (Madigan et al. 1997). Under conditions where particle adsorption and the impeded transport in the interstitium will counteract efficient spreading of virus particles from lytic infections (Maranger and Bird 1996), such transportation and spreading of the viruses along with their host cells may be an advantageous strategy. This may be the underlying reason for the dominance of filamentous viruses in the investigated sediment.

Apart from the filamentous viruses, we found a large number of different morphotypes of the icosahedric forms, which usually dominate viral communities of the water column (Pina et al. 1998; Wichels et al. 1998; Alonso et al. 2001). Prokaryotic diversity is known to be much higher in sediments than in pelagic systems (Torsvik et al. 2002). However, whether that is reflected by a higher viral diversity in sediments still remains to be clarified.

It should be noted that the method to measure total viral abundance in the sediment was based on staining of double-stranded DNA by the fluorochrome SYBR Green I (Molecular Probes). We may, therefore, have underestimated the total abundance of viruses if the single-stranded DNA of the filamentous viruses was less efficiently stained by SYBR Green I.

*Future directions*—It is now evident that viruses are an integral component of the benthic microbial community; however, the role of viruses in regulating and structuring the bacterial community still remains to be investigated. There exist to our knowledge no estimates of the production and decay of viruses in sediments; hence it is still an open question whether viral communities are stable, mainly consisting of persistent forms produced at a low rate, or whether they are dynamic communities with a rapid turnover. Observations of cyanophages (Suttle 2000) and viruses that infect the algae *Heterosigma akashiwo* (Lawrence et al. 2002) at 30–40 cm depth in marine sediments strongly suggest that

these algal viruses may persist and remain infectious in sediments for several decades even in the absence of proper host cells. Benthic viruses may, therefore, be stable inhabitants of sediments, waiting for the right host cell or for elevated bacterial activity in order to produce new viruses. On the other hand, the close coupling between viral abundance and bacterial activity and the observation of extensive morphological virus diversity suggest that the large and diverse viral assemblage is maintained within the sediment. It is crucial to determine the temporal dynamics of the viral assemblage and to investigate the strategies of reproduction and spreading of viruses in the sediment in order to gain further insights into the importance of interstitial viruses.

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