RESEARCH ARTICLE

INTERNATIONAL MICROBIOLOGY

International Microbiology (2013) 16:45-52
doi: 10.2436/20.1501.01.179 ISSN 1139-6709 **www.im.microbios.org**

Low virus to prokaryote ratios in the cold: benthic viruses and prokaryotes in a subpolar marine ecosystem (Hornsund, Svalbard)

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Received 1 February 2013 · Accepted 1 March 2013

Summary. The density and spatial distribution of benthic viruses and prokaryotes in relation to biotic and abiotic factors were investigated in sediment cores collected in Hornsund, a permanently cold fjord on the West coast of Svalbard, Norway. The cores were obtained from the mouth of the fjord to the central basin, along a longitudinal transect. The results of our analyses showed lower densities of viruses (0.2×10^8 to 5.4×10^8 virus-like particles/g) and lower virus-to-prokaryote ratios (0.2–0.6, with the exception of the uppermost layer in the central basin, where the ratio was about 1.2) at the study site than generally found in the temperate areas, despite the relatively high organic matter content in subpolar sediments. Variations in benthic viral and prokaryote abundances along gradients of particle sedimentation rates, phytopigment concentrations, and macrobenthic species composition together suggested the influence of particle sedimentation and macrobenthic bioturbation on the abundance and spatial distribution of prokaryotes and viruses in cold habitats. [**Int Microbiol** 2013; 16(1):45-52]

Keywords: viriobenthos · bacteriobenthos · marine sediment · subpolar ecosystems · stable isotopes · Svalbard archipelago (Norway)

Introduction

Marine benthic viruses are one of the most diverse and abundant components of the global ecosystem. Metagenomic analyses have shown the presence of at least 10^4 viral genotypes per kilogram of sediment [5], which is between 10- and 100-fold higher than estimated for the water column [6]. Other studies have shown that in most marine areas, viral densities are around 10^7–10^{10} g sediment^{-1} and thus 10- to 1000-fold higher in sediments than in the overlaying water column [8–10,15]. Virus-to-prokaryote ratios (VPRs) in the sediments range over four orders of magnitude, from close to 0.1 in oligotrophic and deep-sea sediments to >100 in eutrophic estuarine ecosystems [8], with viral abundance and activity correlated positively with both benthic prokaryote activity [25,40–42] and prokaryote abundance [23]. The high density and activity of prokaryotes and small distances between prokaryotic cells in the sediment is thus expected to result in a higher frequency of virus-prokaryote encounter [8] and consequently a high rate of viral pro-

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duction [40,48]. Deposition of viruses (attached to sinking particles) from the water column could also contribute to viral counts in the sediments. Biotic and abiotic factors affecting the properties of the sediment, such as sedimentation, bioturbation and irrigation processes at particular sites or regions might affect viral counts directly (by influencing delivery of viruses, viral decay, the chance for virus-host cell encounter, and the rate of viral propagation) or indirectly, influencing prokaryote growth and turnover.

Viral lysis can be responsible for as much as 50 % of mortality of prokaryotes in the sediment [37]. One effect of viral lysis is the acceleration of the transformation of particulate to dissolved organic matter. Instead of being directed to higher trophic levels through protozoan grazing on prokaryotes, organic matter released through lysis is assumed to be rapidly metabolized by the prokaryotic community, providing up to 38 % of its carbon demands [8,48].

In order to elucidate the role of viruses in controlling prokaryote mortality and biogeochemical cycling in different benthic environments, it is necessary to investigate the abundance and distribution of viruses and prokaryotes in diverse geographical regions. Only a few studies have thus far investigated the distribution of microbes along trophic gradients. For example, Hewson et al. [30], investigated the vertical and horizontal distribution of viruses and prokaryotes along a trophic gradient in subtropical estuaries (Australia). In another study [10], the top sediment layer was collected along a trophic gradient from oligotrophic Eastern to more productive Western Mediterranean. Similarly, the distribution of benthic viruses and prokaryotes was investigated along productivity gradients in the Eastern Mediterranean [13] and the Chilean upwelling zone [40]. Generally, these studies have found a decrease in the viral abundance at the surface of the sediment with decreasing organic matter supply and prokaryote activity along transects from shallow coastal sediments towards deeper open ocean environments.

In this paper we present the first analysis, as far as we know, of the vertical and horizontal abundance of viruses in marine sediments collected along a trophic gradient at high latitudes. We designed our sampling strategy to capture an ecologically important gradient in productivity, sedimentation and bioturbation activity in the sub-arctic fjord Hornsund, Svalbard. We aimed to identify the biotic and abiotic factors that may influence the vertical and horizontal distribution of microbes and VPR in the subpolar benthic ecosystem.

Materials and methods

Study site and sampling. Hornsund is a 30-km long and 12- to 15-km wide fjord. It is located on the west coast of Spitsbergen (between 74º and 81º N, and 10º and 35º E), an island within the Svalbard archipelago in Northern Europe. Hornsund is the southernmost fjord of the island. The fjord is exposed to cold Arctic water transported by the Sorkapp current as well as inflows of relatively warmer Atlantic water of the West Spitsbergen current. A sea ice cover occurs between December and June, and floating sea ice from the Barents Sea from May to July [26]. About 70 % of Hornsund's catchment area is covered by glaciers. The fjord sediment consists of glacimarine mud [26]. Sedimentation is dominated by melt-water processes, which release organic and mineral particles to the water column. In the central basin, high sedimentation rates (about 1 cm/yr) increase water turbidity, reducing the euphotic zone to a depth of 5–10 m (J. Wiktor and M. Darecki, personal communication) and limiting primary production. In the mouth of the fjord, where sedimentation rates are much lower (about 0.1 cm/yr [26]), the depth of the euphotic zone is 20–55 m (J. Wiktor and M. Darecki, personal communication) and primary production is correspondingly higher than in the central basin, with estimates comparable to those for regions without ice coverage at lower latitudes (up to 120 g C m^{-2} yr^{-1}) [17,45]. Due to its pristine nature and limited anthropogenic stressors, Hornsund was selected as a European Marine Biodiversity Site (EMBS) and an All Taxa Biodiversity Inventory site (ATBI) [53].

To analyse the benthic communities in Hornsund, we used the published data collected from 89 stations located throughout the fjord between 2002 and 2007 [32]. The species composition of benthic fauna inhabiting soft sediments in Svalbard fjords has been shown to be stable on time scales of 5 years and more [32,33,46]. Arctic benthic species are predominantly sessile or undertake at most local migrations [3,52], tend to be relatively long lived (2–5 years and longer), and exhibit spatial and temporal patterns that reveal major environmental features and gradients [1,14,27,32,33,44].

We collected sediment cores at 3 locations in August 2010 (HornWest, HornMid, HornEast; Table 1, Fig. 1) along the central axis of Hornsund using a GEMAX gravity corer for soft sediments (Geological Survey of Finland, Espoo, Finland), which allows for undisturbed sampling of soft muds. The cores were cut in 1-cm-thick slices onboard and homogenized manually. Small subsamples (about 1–2 g) were packed into plastic bags, sealed, and stored at –80 °C until further analysis (about 3 months).

Virus and prokaryotes counting. The procedure for virus counting was adapted from Danovaro and Middelboe [12]. Briefly, approximately 1 g of the thawed sediment was mixed with 5 ml of ice-cold 2.5 % glutaraldehyde in 5 mM $Na_4P_2O_7$ (pH of the solution: 8.2). After a 15-min incubation on ice, the mixture was sonicated (3 cycles: 30 s of 200 J at 20 kHz, 30-s intervals with manual shaking) and then diluted to 50 ml with deionized water. Depending on the viral counts, between 20 and 35 µl of the diluted sample was filtered onto 0.02-µm Anodisc filters (Whatman). The filters were then stained with SYBR Gold (Invitrogen) for 10 min in the dark and rinsed with 0.02-µm-filtered sterile water. For each filter, a minimum of 250 prokaryotic cells and viruses (differentiated from each other by their dimensions) were counted in at least 15 fields. Replicate measurements in selected samples showed that the average abundance was determined with an error of <10 %. Similar precision was obtained before in the determination of abundance of viruses and prokaryotes using the same procedure [25,41].

Table 1. Basic characteristic of the sediment at the sampling site. The values in parentheses show the range (lowest and highest values) determined for five slices in each core

Factor	HornWest	HornMid	HornEast
Sampling date	12 Aug 2010	12 Aug 2010	11 Aug 2010
Location	76º56.54′ N, 15º18.80′ E	76º57.67′ N, 15º41.26′ E	76º59.62′ N, 15º56.54′ E
Water depth (m)	171 m	135 m	135 m
Near-bottom salinity (PSU)	34.8	34.8	34.7
Near-bottom temp. (ºC)	1.9	1.9	1.3
Water content (%)	(42.5 to 54.7)	(43.5 to 58.1)	(39.8 to 56.1)
Sand fraction (%)	(14.8 to 22.0)	(9.2 to 18.3)	(6.7 to 19.4)
Silt fraction (%)	(78.0 to 85.2)	(81.7 to 90.8)	(80.6 to 93.3)
Mean (φ)	(5.45 to 5.59)	(5.55 to 6.14)	(5.89 to 6.18)
Sorting (σ_φ)	(1.52 to 1.67)	(1.46 to 1.77)	(1.44 to 1.76)
TC (%)	(2.5 to 2.6)	(2.4 to 2.9)	(2.3 to 2.4)
TOC (%)	(1.7 to 1.9)	(1.5 to 1.6)	(1.3 to 1.4)
N (%)	(0.19 to 0.22)	(0.16 to 0.19)	(0.13 to 0.14)
P (%)	(0.13 to 0.15)	(0.12 to 0.16)	(0.08 to 0.10)
TOC/N	(10 to 11)	(10 to 12)	(11 to 13)
TOC/P	(31 to 38)	(25 to 35)	(24 to 44)
N/P	(2.9 to 3.8)	(2.2 to 3.5)	(2.2 to 3.4)
$\delta^{13}C_{TC}$ (‰)	(–17.6 to –17.1)	(–16.0 to –14.5)	(–14.6 to –14.4)
$\delta^{13}C_{TOC}$ (‰)	(–25.0 to –23.1)	(–24.8 to –24.3)	(–25.4 to –24.9)
$\delta^{15}N$ (‰)	(2.1 to 4.9)	(3.1 to 5.2)	(2.6 to 3.4)

Sediment composition. Another sediment subsample was used to determine water content, measured as weight loss after drying at 60 °C for 24 h. This treatment removes interstitial water but not water bound in hydrates. Grain size composition (range: 0.01–2000 μm) was determined using a Malvern instrument Mastersizer 2000 equipped with a Hydro MU sample dispersion unit combined with a laser diffraction system. Laser granulometry data were analyzed using GRADISTAT [4]. The concentrations of chlorophyll *a* and phaeophytin were determined fluorometrically after extraction of the pigments from freeze-dried sediment subsamples with 90 % acetone in the dark at 4 ºC for 24 h [20].

Total carbon (determined as $\delta^{13}C_{TC}$; TC), total organic carbon ($\delta^{13}C_{TOC}$; TOC), and total nitrogen (with $\delta^{15}N$; N) were measured in subsamples using an elemental analyzer (Flash EA 1112 series coupled with the isotopic ratio mass spectrometer IRMS Delta V Advantage, Thermo Electron, Bremen, Germany). The freeze-dried sediment was weighted in two silver capsules (with 1-μg accuracy). One capsule was used to determine TC, $\delta^{13}C_{TC}$, N, and $\delta^{15}N$. The other capsule was used to measure TOC and $\delta^{13}C_{TOC}$ by first soaking the sample portion in 2 M HCl in order to remove inorganic carbon species, followed by drying at 60 °C for 24 h. Quality control standards (EA instruments calibration and recovery tests), purchased from Thermo Electron, were: acetanilide (C = 71.09 %, H = 6.71 %, N = 10.36 %, O = 11.84 %), atropine (C = 70.56 %, H = 8.01 %, N = 4.84 %, O = 16.59 %), and cyclohexanone 2–4 dinitrophenyl hydrazone (C = 51.79 %, H = 5.07 %, N = 20.14 %, O = 23.00 %). Additionally, certified reference materials (HEKAtech GmbH, Wegberg, Germany), consisting of environmental samples (including marine sediments) with an established concentration of analyzed chemical elements, were used to assess the accuracy of the analytical method. The average recovery for the standard and certified reference materials was 99.2 % for TC, 99.3 % for TOC, and 98.8 % for N; standard deviations were less than 0.5 %, 0.5 %, and 0.7 % for TC, TOC, and N, respectively. The results obtained for $\delta^{13}C_{TC}$, $\delta^{13}C_{TOC}$, and $\delta^{15}N$ are given in the conventional delta notation versus Pee Dee Belemnite for $\delta^{13}C_{TC}$ and $\delta^{13}C_{TOC}$ and versus air for $\delta^{15}N$. Pure CO_2 and N_2 calibrated against an IAEA (International Atomic Energy Agency) standard (CO-8 and USGS40 for $\delta^{13}C$ and N-1 and USGS40 for $\delta^{15}N$) were used to calculate stable isotope ratios. Standard deviations for replicate samples were less than 0.14 ‰, 0.12 ‰, and 0.17 ‰ for $\delta^{13}C_{TC}$, $\delta^{13}C_{TOC}$, and δ^{15}, respectively. To measure phosphorus

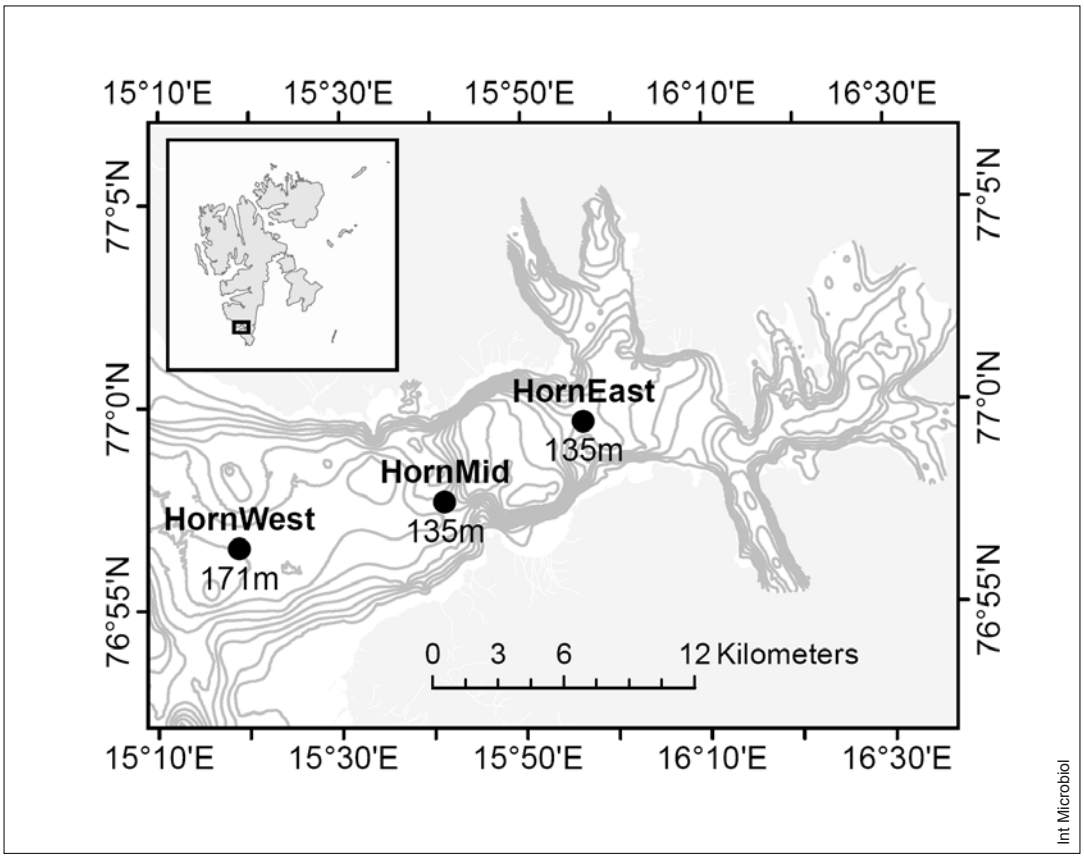

Fig. 1. Sampling area and station locations at Hornsund fjord (Svalbard, Norway).

(P) concentrations, dry sediment samples (100 mg) in glass tubes were first mineralized in a temperature gradient from 100 to 300 °C with 1.6 ml of 10 N acid mixture ($HClO_4$ and H_2SO_4, 1:3.85 molar ratio). The mineralized samples were diluted with Milli Q water and filtered through MN GF5 glass-fiber filters (Macherey Nagel, Düren, Germany). The filtrates were transferred into 100-ml flasks and neutralized with NaOH in the presence of phenolphthalein. The flasks were then filled up to 100 ml with Milli Q water, 2 ml of 0.02 M $(NH_4)_6Mo_7O_{24}$ (in water), and 0.5 ml of 0.11 M $SnCl_2$ (in glycerine). After 10–12 min, P concentrations were measured colorimetrically. The estimated relative standard deviation of the measurement was <2.1 %.

Statistical analysis. The R package [http://www.r-project.org/] was used for the statistical analysis of the data, including a linear model for regression analysis and a one-tailed *t*-test to confirm the null hypothesis that measurements in the uppermost layer were based on a normal distribution estimated using the remaining measurements for the same core. The level of significance throughout the analysis was 0.05.

Results and Discussion

The sediments collected at the three locations had similar general properties, consisting mostly of silt with an admixture of sand (Table 1). The sediment was poorly sorted, with sorting values (σ_φ) characteristic of sediments originating from glacier material (Table 1). The arithmetic mean in the φ scale for all slices was >2 φ, which indicates that the sediments were transported as suspension. The TC, TOC, N, and P contents were relatively high, as observed before [24] and there was little variation between slices or sites. In general, the concentrations were higher in the outer fjord than towards the east. This likely reflects the combined higher production and greater sedimentation of organic matter in the west and supports previous observations of higher inorganic matter sedimentation in the central basin, resulting in a "dilution effect" of organic matter by mineral particles [26].

Based on the carbon/nitrogen molar ratios (around 10 in all samples, Table 1), the organic matter is mostly of marine origin and relatively freshly deposited. The slight increase in the TOC/N ratios towards the east (up to 13 at HornEast) suggests, however, the increased contribution of terrestrial organic matter in the bulk of sedimentary organic material along the fjord's latitudinal axis [38,39]. This is supported by the $\delta^{13}C_{TOC}$ values, which decreased towards

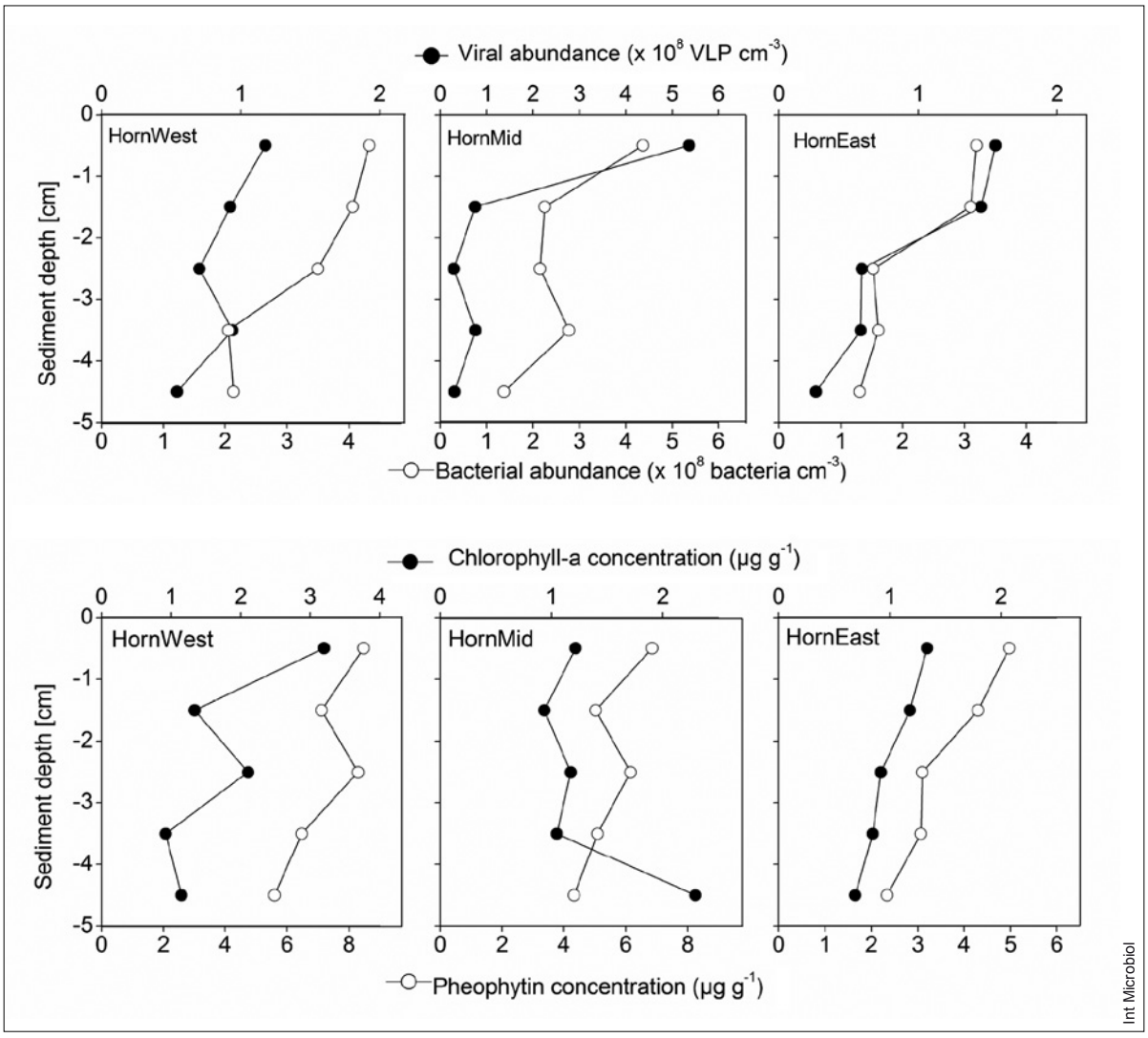

Fig. 2. Vertical distribution of viral and prokaryote abundance and concentrations of chlorophyll *a* and phaeophytin in Hornsund cores (Svalbard, Norway).

the east, from –23.1 ‰ at HornWest to –25.4 ‰ at HornEast (Table 1) [16,18,19,47,49–51]. A latitudinal trend was observed also for inorganic carbon concentrations (the difference between TC and TOC; Table 1): the highest values (1.1 %) occurred at HornEast and the lowest (0.7 %) at HornWest. The $\delta^{13}C_{TC}$ values also increased towards the east, consistent with an increasing input of terrestrial carbonates (e.g., from limestone formations [28]). By contrast, there were no readily apparent latitudinal trends for $\delta^{15}N$ and TOC/P, which suggests similarities in the food web structure and in processes affecting P preservation in sediments, respectively [31,54].

While TC, TOC, N, and P showed little vertical variation, chlorophyll *a* and pheophytin concentrations varied both vertically and horizontally. The concentration of chlorophyll *a* was higher in the uppermost layer at HornWest than at other stations, reflecting the fact that HornWest lies in the region with the highest primary production (J. Wiktor and M. Darecki, personal communication).We hypothesize that organic matter delivered to the sediment in the central basin originates in part from the outer fjord and that the phytoplankton undergoes a more pronounced degradation during delivery. At HornWest and HornEast the chlorophyll *a* concentrations were significantly higher in the uppermost layer than in the lower sediment layers (one-sided t-test, $P = 0.0029$, and $P = 0.0013$, respectively, with the null hypothesis that measurements of all the layers have the same distribution). At all stations, pheopigment

concentrations were higher in the uppermost layer than in deeper sediment layers (HornWest: $P = 0.033$, HornMid: $P = 0.010$, HornEast: $P = 0.011$). The HornEast data could be fit to a linear model relating pigment concentrations with sediment depth (chlorophyll *a*: $P = 0.0018$, phaeophytin: $P = 0.0063$). These observations suggest that following its depostion, organic matter undergoes further degradation. High concentration of chlorophyll *a* at 4.5 cm sediment depth in the core collected at HornMid may correspond to the burial of the organic matter produced during the extremely warm 2005–2006 period in the European Arctic [7], quickly overlaid with the sediment that is deposited at about 1 cm/yr in this region [26].

In general, the prokaryote and viral counts determined in Hornsund (Fig. 2) were low (around 10^8 cells/viruses g^{-1}) but still within the range reported for marine sediments [8]. Note that the VPRs in this study were very low (0.2–0.6, with the exception of the uppermost layer at HornMid, Fig. 2). The results of our linear regression analysis of the relationship between prokaryote and viral density indicated that the data from all cores could be described using a linear model ($P = 0.015$). For all stations, prokaryote density was significantly higher in the uppermost layer than deeper in the sediment (one-sided t-test, HornWest: $P = 0.034$, HornMid: $P = 0.0022$, HornEast: $P = 0.025$), as was the case for viruses (HornWest: $P = 0.012$, HornMid: $P = 0.000022$, HornEast: $P = 0.023$). In the uppermost layer at HornMid, the VPR significantly differed from the ratio determined in the other layers from this core ($P = 0.000082$) and indeed from the ratios of all the other samples ($P = 7.405 \times 10^{-14}$).

Prokaryotes and viruses in surface sediments originate either from production within the sediments or from sinking particles that act as vectors for the vertical transport of pelagic microorganisms. Previous studies suggested that the inherent production of viruses is the dominant source of benthic viruses in coastal sediments [29,40] and that higher benthic viral production corresponds to higher benthic productivity and thus to the trophic state of the sediment [40]. In Hornsund, however, viral and prokaryote abundances were uncoupled from the sediment trophic state. In other words, the higher viral and prokaryote abundances in the surface sediments at HornMid than in the more productive HornWest did not correspond with the relatively low sediment chlorophyll *a* concentrations and the low chlorophyll/pheophytin ratios at HornMid. We speculate that the high abundance of prokaryotes and viruses in the central basin reflects the 10-fold higher sedimentation of particles which may scavenge viruses and prokaryotes from the water column and contribute significantly to their abundance in the surface sediment at this station. This hypothesis is supported by the rapid decrease in viral and prokaryote abundances with sediment depth at HornMid. This decrease suggests that the imported prokaryote populations are not maintained in the sediment due to limited substrate availability and prokaryote growth, and rapidly decay.

The relatively low VPR in the subpolar sediments might stem from very low viral production or from physicochemical conditions in these environments that enhance viral decay. Viral production depends on the growth and turnover rates of prokaryote cells [55], which are affected by abiotic and biotic factors. Conditions in which prokaryotes grow slowly, e.g., low temperature or nutrient limitation, may limit viral production and lysogeny, and favour pseudolysogeny and chronic infection [8,11].

Long-term data on the composition of the benthic communities in Hornsund [32] indicate a pronounced difference between stations of the outer fjord (close to HornWest) and central basin (HornMid and HornEast), with higher species diversity and a higher degree of bioturbation in outer fjord sediments. By contrast, the total abundance of infauna in the sediment is similar for the outer fjord and the central basin (about 600 individuals 0.1 m^{-2}, the data here and further are from the 2007 sampling campaign), but the abundance of subsurface deposit feeders (burrowers) in the outer fjord sediments is about two times higher than in those of the central basin (over 300 ind. 0.1 m^{-2} vs. about 150 ind. 0.1 m^{-2}, respectively). Outer fjord sediments are dominated by *Leitoscoloplos mammosus* and *Cossura longocirrata* (15 % and 8.5 % of total infaunal abundance, respectively), which are non-selective deposit-feeders that burrow freely through the sediment [21].

A decrease in viral abundances with sediment depth has been reported for a number of temperate and subtropical sediments [10,13,30,41,42]. This decline generally reflects the high prokaryote activity in the benthic surface layers in response to the input of organic matter from the water column. The higher degree of bioturbation in outer fjord sediments might be responsible for the more even depth distribution of viruses in our HornWest core (Fig. 2) than in the cores we collected in the central basin, in which viral and prokaryote densities clearly decreased with sediment depth. Although further analysis of sediments in other fjords is necessary, we propose that differences in the distribution of viruses and prokaryotes in the Hornsund sedi-

ments reflect functional differences in the benthic invertebrate community along the west-east axis, i.e., a decrease in the abundance of subsurface burrowers and a corresponding increase in the abundance of mobile surface deposit feeders (from about 100 ind. 0.1 m^{-2} in the outer fjord to over 300 at stations close to HornEast). According to our hypothesis, the higher activity of burrowers in the outer fjord (HornWest) results in the delivery of viral particles and prokaryotes to lower sediment layers. Less bioturbation at HornMid is supported by the presence of a chlorophyll-rich layer at 4.5 cm, perhaps corresponding to the warm 2005–2006 period. Finally, at HornEast, still more degraded organic matter and presumably even lower bioturbation rates account for the almost continuous decrease in prokaryote and viral counts and in pigment concentrations with sediment depth.

Higher abundances of prokaryotes than of viruses (VPR <1.0) were previously recorded in oligotrophic marine sediments in the temperate zone [11,37] and in inland sediments [2,22,35–37]. In nutrient-poor deep-sea sediments, low rates of prokaryotic metabolism were suggested as an explanation for the low virus-bacteria ratios [11]. We found virus-bacteria ratios similar to those of deep sea sediments, despite the higher organic carbon contents. The low VPRs in polar environments might be indicative of prokaryotic activity that is too low (e.g., with temperature as a limiting factor for prokaryotic metabolism) to maintain a high level of virus production [34,43]. Nonetheless, viruses may still be important in controlling prokaryote populations in subpolar sediments. In fact, there is evidence of high virus-induced prokaryote mortality (>40 %) even in oligothropic sediments, where the VPR is below 1.0 [37]. Further research will contribute to our ability to quantify the role of virus-induced prokaryote mortality in high-latitude sediments.

Acknowledgements. This study was funded by the Polish Ministry of Science and Higher Education (AOD/W6/2008 and AODP/09/2010/0 to BW) and a grant from the Danish Council for Independent Research to MM. We are grateful to Mirosław Darecki, Ilona Goszczko, Witold Szczuciński, Joanna Przytarska, Jan Marcin Węsławski, Józef Wiktor, and the crew of the r/v *Oceania* for their help during sampling, laboratory procedures, and/or discussion of the results.

Competing interest: None declared

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