

Viriobenthos in freshwater and marine sediments: a review

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SUMMARY

1. Viruses are the most abundant biological entities on the planet, and sediments provide a highly suitable environment for them. This review presents the first comparative synthesis of information on the fresh water and marine viriobenthos and explores differences and similarities to the better known virioplankton. We present methods for studying life cycles of the viriobenthos, data on viral distribution and diversity, interactions with host microbes, and information on the role of viruses in benthic food webs and biogeochemical cycles.
2. Most approaches developed for the virioplankton are also applicable to viriobenthos, although methods for analysing benthic viruses may differ in important details.
3. Benthic viruses are very abundant in both marine and freshwater sediments, where 10^7 – 10^{10} can occur in 1 g of dry sediment. Although information on viral production (VP) and decay rates in freshwater sediments is very limited, the data suggest that VP and decay could also be high. These data highlight the potential ecological importance of benthic viruses, suggesting that they could play a key role in prokaryotic mortality and in biogeochemical cycles.
4. There is clear indirect evidence for the importance of viriobenthos in marine and freshwater ecosystems. However, large numbers of visibly infected cells have not been observed, suggesting limited effects on prokaryote population and community dynamics. The apparent paradox between high viral abundance and low impact is currently unresolved, while several aspects of viral life cycles in sediments (e.g. chronic infection) are almost completely unknown.
5. Studies on viriobenthic diversity and community structure are at a pioneering stage. First results from a few studies using pulsed-field gel electrophoresis and especially from metagenomic analyses indicate, however, that viriobenthic assemblages are both highly diverse and distinct from the virioplankton.

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6. Estimates of global viral abundance in the top 1 m of fresh water and marine sediments are 0.5 and 28.7×10^{28} viruses respectively. Similar rough estimates of production are 0.6 and 34.4×10^{28} viruses day^{-1} , suggesting an average turnover time of 20 h.

Keywords: benthos, freshwater, marine, methods, viruses

Introduction

Viruses are the most abundant biological entities in ecosystems. The estimated overall abundance in the world's oceans is on the order of 10^{30} (Suttle, 2005, 2007), a value that exceeds prokaryotic abundance (Whitman, Coleman & Wiebe, 1998) by one order of magnitude (Suttle, 2005). The total number on Earth may even be 10^{31} (Breitbart & Rohwer, 2005), and most of the viruses are prokaryote-infecting viruses also known as phages or bacteriophages. The diversity of viruses in aquatic ecosystems is also impressive: metagenomic analyses indicate that 3000–7000 genetically distinct genomes can occur in 200 L of water (Breitbart *et al.*, 2004; Angly *et al.*, 2006).

Since viruses have been recognized as the most abundant and diverse component of aquatic environments (Bergh *et al.*, 1989; Proctor & Fuhrman, 1990; Suttle, Chan & Cottrell, 1990), it has become increasingly evident that they play critical roles in shaping aquatic communities and determining ecosystem dynamics. Viruses can cause spectacular epidemics of a wide range of aquatic organisms, including large marine mammals (e.g. Harkonen *et al.*, 2006). However, it is probably that their importance in aquatic ecosystems is chiefly due to the widespread infections of single-celled organisms, such as prokaryotes and microalgae (Fuhrman, 1999). Such viral infections, which are frequently followed by death of the host cells, can have important ecological consequences. These include profound impacts on microbial population sizes and biodiversity, horizontal transfer of genetic materials and biogeochemical cycles (Suttle, 2005).

Virus-mediated mortality of prokaryotes, in both water column and sediments, is often in the range of 10–30% and can reach 100% (Heldal & Bratbak, 1991; Wommack & Colwell, 2000; Corinaldesi, Dell'Anno & Danovaro, 2007). In addition, viruses can reduce the abundance of heterotrophic nanoflagellates (González & Suttle, 1993) and contribute to the decline of phytoplankton blooms (Suttle, 1992; Fuhrman, 1999).

The integration of viruses into microbial food web models has shown, moreover, that viral lysis of microbial cells enhances the transfer of microbial biomass into the pool of dissolved organic matter (DOM) (Thingstad & Lignell, 1997; Miki *et al.*, 2008). This in turn can influence nutrient cycling, alter pathways of organic carbon use by prokaryotes (Fuhrman, 1999; Wilhelm & Suttle, 1999; Wommack & Colwell, 2000), and divert microbial biomass away from higher trophic levels (Fuhrman, 1992; Bratbak, Thingstad & Heldal, 1994). These viral-induced alterations of organic matter flows, within microbial food webs, have been termed 'viral shunt'.

At present, concepts of viral dynamics, diversity and functional importance at the population, community and ecosystem level are mainly based on data from pelagic environments (Fuhrman, 1999; Suttle, 2005). To what extent are they applicable to this compartment of aquatic ecosystems? Can insights gained from pelagic ecosystems be extrapolated to sediments and other benthic environments? Are benthic viruses similarly abundant, diverse and ecologically important? Epidemiological models predict that viral infection rates increase with increasing host cell density (Wiggins & Alexander, 1985) because infection is a direct function of the encounter rate between a pathogen and its host. Since sediments typically have high prokaryotic abundances (10^8 – 10^9 cells g^{-1} in sediments versus 10^5 – 10^6 cells mL^{-1} in the water column) and distances between cells are correspondingly short, the probability of contact between a virus and a prokaryotic cell in sediments should be especially high. This suggests that sediments may be favourable environments for viral proliferation. At the same time, the physicochemical condition of sediments (physical structure, low redox potential, pH, organic matter content, concentration of potential hosts in biofilms, etc.) might affect virus-host encounter and viral survival. Non-specific adsorption to particles, for example, has been suggested as a major mechanism of viral decay (Noble & Fuhrman, 1998). Thus, in view of the complexity of viral

interactions with their hosts and the environment, inferences on the dynamics, diversity and ecological importance of the viriobenthos require specific analysis.

Compared to the virioplankton, benthic viruses have received little attention although interest in them has recently increased. The purpose of this review is, therefore, to provide a first synthesis of information on the fresh water and marine viriobenthos gathered from studies in different habitats, including shallow marine coastal and deep-sea sediments, streams and rivers, littoral freshwater marshes and the profundal zone of lakes, at different latitudes from the tropics to the poles. This has been performed by seeking to elucidate differences and similarities with viruses in pelagic environments. Since even well-established methods are notoriously difficult to apply to benthic systems, the first section is devoted to techniques used to study the viriobenthos. Viral distribution, life cycles and diversity patterns are then presented and the relationships of benthic viruses with other microbial components, and the roles of viruses in benthic food webs and biogeochemical cycles, are discussed. A final section provides an initial attempt to estimate the quantitative importance of the viriobenthos in fresh water and marine sediments at the global scale.

Methods for studying the viriobenthos

The development and adaptation of methods for analysing environmental samples was a crucial step in elucidating the role of planktonic viruses. In principle, the suite of approaches and procedures used in pelagic ecosystems is equally applicable to benthic systems, but is often hindered by the physical and chemical matrix that characterizes sediments and other benthic environments. Nevertheless, significant progress has been made in analysing environmental samples for viruses, including those in sediments.

Sample processing and storage The standard method for preserving aquatic samples for viral counts is the addition of formaldehyde or glutaraldehyde (Wommack & Colwell, 2000). However, decreases in viral abundance in samples fixed with either preservative have been reported repeatedly (Danovaro *et al.*, 2001; Wen, Ortmann & Suttle, 2004). Significant reductions can occur after only 24 h of storage (Wen

et al., 2004). After an initial decline for up to 1 week, counts remain relatively constant for up to 3 months (Danovaro *et al.*, 2001), suggesting that sample storage is possible if viruses are counted for comparative purposes (i.e. relative abundance). In contrast, counts of absolute abundance require either application of appropriate correction factors for losses during sample storage or, preferably, immediate analysis of fresh samples. If counts cannot be made directly after sampling, significant losses of viruses can be avoided by filtering fresh samples (after viral extraction from the sediment matrix) and storing filters at -20°C . Alternatively, whole sediment cores may be frozen until analysis (Helton, Liu & Wommack, 2006).

Dislodging viruses from sediment samples is a first crucial step to maximizing their recovery from sediments (Fischer, Kirschner & Velimirov, 2005). Ultrasonication has most often been used for this purpose. Maranger & Bird (1996) used 45 s of ultrasound treatment with profundal lake sediment samples. Similarly, Fischer *et al.* (2005) used 1-min treatment with other freshwater sediment samples (water bath sonicator, Branson Sonifier 450, 70 W, Branson Ultrasonics Corporation, Danbury, CT, U.S.A.), whereas 3 min were found to be optimal for different types of marine sediments (water bath sonicator, Branson Sonifier 2200, 60 W, 47 kHz; Danovaro, Feminò & Fabiano, 1994; Danovaro *et al.*, 2001; Epstein & Rossel, 1995). As observed for benthic prokaryotes, longer sonication treatments can significantly reduce viral counts (Danovaro *et al.*, 2001).

Since viral sorption usually increases with increasing cation concentration in solution, particularly in the presence of divalent cations, the observed differences in the extractability of viruses between marine and freshwater sediments might be due to differences in cation concentrations. Moreover, as observed for prokaryotes, the optimal sonication time may strongly depend on the sonicator model, tip and settings used (Epstein & Rossel, 1995), and may therefore vary considerably among laboratories. Addition of pyrophosphate at 10 mM concentration has been observed to provide higher viral abundances than the addition of pyrophosphate at higher concentration (Maranger & Bird, 1996). However, Danovaro *et al.* (2001) found that pyrophosphate did not increase the extraction efficiency of viruses, although the coefficient of variation was about threefold lower than in untreated samples. Similar results were reported for benthic

prokaryotes (Epstein & Rossel, 1995), suggesting that the use of pyrophosphate increases counting precision. Duhamel & Jacquet (2006) reported that Tween[®] 80 (non-ionic detergent and emulsifier), in addition to pyrophosphate, increased extraction efficiency from lake sediments by *c.* 25–40%. In the protocols with pyrophosphate addition and ultrasonication proposed by Danovaro *et al.* (2001) and Fischer *et al.* (2005), the extraction efficiency of benthic viruses were about 60% and 89%, respectively, of the total viral counts.

Viral abundance Direct counts provide the most basic information to assess the abundance and distribution of viruses in ecosystems. Once viruses have been dislodged from sediments or other types of benthic samples, their total abundances can be determined by transmission electron microscopy (TEM; Bergh *et al.*, 1989; Børsheim, Bratbak & Haldal, 1990; Maranger & Bird, 1996; Paul *et al.*, 1993; Xenopoulos & Bird, 1997), epifluorescence microscopy (EFM; Suttle *et al.*, 1990; Drake *et al.*, 1998; Hara, Terauchi & Koike, 1991; Noble & Fuhrman, 1998), and flow cytometry (Duhamel & Jacquet, 2006).

The traditional method for viral counting in environmental samples is TEM. It is the only method that provides data on both the abundance and morphology of viruses. However, counting viruses by TEM is extremely laborious, even with water samples, and it presents particular difficulties when applied to benthic samples (Bettarel *et al.*, 2006). A typical protocol involves the extraction of viruses from the sediments following the protocol set up by Danovaro *et al.* (2001). Briefly, after addition of tetrasodium pyrophosphate to a final concentration of 10 mM, viruses are detached from sediment particles by means of ultrasonic treatment (three times for 1 min). Sediment samples are then diluted 100–1000 times with virus-free water pre-filtered through 0.02 µm filters. Viruses in the supernatant are harvested by ultracentrifugation at 120 000 g for 2 h on grids (400-mesh Cu electron microscope grids with carbon-coated Formvar film). Finally, the viruses are stained with 2% uranyl acetate and dried on silica gel (Hara *et al.*, 1991; Bettarel *et al.*, 2006). Counts are carried out on at least 100 electron microscope fields from at least five grid cells at 34 000–105 000× magnification.

At present, most viral counts are routinely performed by EFM, because of much faster sample

processing and lower costs. A study comparing the efficiency of TEM and EFM protocols for counting viruses demonstrated that TEM underestimates numbers by *c.* 1 order of magnitude (Hennes & Suttle, 1995; Suttle, 2007). Moreover, EFM typically provides more accurate estimates (i.e. lower coefficients of variation among replicate counts) and greater counting efficiency when compared to the TEM method. This is probably due to the greater manipulation required for the TEM method, to the reduced area of the microscope grid effectively available for counting, and to interference by particulate and humic substances (Hennes & Suttle, 1995; Weinbauer, 2004).

The EFM method has been applied to sediment samples for more than a decade (Maranger & Bird, 1996) and has recently been optimized and standardized (Danovaro *et al.*, 2001; Fischer *et al.*, 2005). Viruses dislodged from particles are filtered onto aluminium oxide filters (Anodisc, Whatman; Maidstone, Kent, U.K.), stained with a fluorescent dye and counted under an epifluorescence microscope. For optimal counting conditions (i.e. <100 viruses per optical field), sediment dilutions ranging from 100 to 4000 times are usually appropriate. After filtration and staining, washing (twice with 1-mL Milli-Q water) is recommended to reduce fluorescence background noise. Different stains (Yo-Pro-1, DAPI, SYBR Green I, SYBR Green II, SYBR Gold; Hennes & Suttle, 1995; Weinbauer & Suttle, 1997; Noble & Fuhrman, 1998; Chen *et al.*, 2001; Buesing, 2005) have been used to count viruses in water-column and sediment samples. However, at present only SYBR Green I and II and SYBR Gold are used (Noble & Fuhrman, 1998; Chen *et al.*, 2001; Middelboe *et al.*, 2006). SYBR Gold is a sensitive fluorescence stain to detect both double- and single-stranded DNA (ssDNA) and RNA, whereas SYBR Green I yields greatest absorbance with double-stranded DNA (dsDNA) (Molecular Probes product information sheet). The latter can also be used to detect ssDNA and RNA, but the sensitivity is lower than for dsDNA (Fischer *et al.*, 2005). Conversely, SYBR Green II gives the brightest fluorescence with RNA and ssDNA (Molecular Probes product information sheet). Fluorochromes belonging to the SYBR Green family all have the advantages of: (i) being suitable for aquatic samples within a wide range of salinity; (ii) causing low background staining; (iii) high stability and (iv) fluorescing more brightly than other widespread stains, such as DAPI and Yo-Pro I

(Noble & Fuhrman, 1998; Danovaro *et al.*, 2001). SYBR Gold is becoming the most commonly used fluorochrome for counting viruses in sediments. It typically yields a bright and stable yellow–green fluorescence under EFM, although the brightness can vary due to different genome sizes among viruses (Chen *et al.*, 2001). In comparison with SYBR Green I, the fluorescence of SYBR Gold-stained viruses is typically brighter, while background noise is sufficiently reduced, thus facilitating counting (Fischer *et al.*, 2005). Stock solutions of both SYBR Green and SYBR Gold stains need to be diluted before use. Working solutions should be freshly prepared, because at low concentration these fluorochromes are unstable. Finally, for both SYBR Green and SYBR Gold staining, the use of appropriate antifade solutions is strongly recommended: a drop of 50% phosphate buffer (6.7 mM, pH 7.8) and 50% glycerol containing 0.5% ascorbic acid or 1% *p*-phenylenediamine (Noble & Fuhrman, 1998; Fischer *et al.*, 2003).

Extracellular DNA can also interfere with viral counts, but this effect can be circumvented by treating sediment samples with nucleases (Danovaro *et al.*, 2001). Danovaro *et al.* (2001) reported that viral counts in natural sediments can be improved by eliminating the interference due to extracellular DNA, by adding a mixture of 25 µL DNase I from bovine pancreas (1.9 U mL⁻¹), 10 µL nuclease P1 from *Penicillium citrinum* Link, 1809 (4 U mL⁻¹), 10 µL nuclease S1 from *Aspergillus oryzae* Micheli & Link, 1809 (2.3 U mL⁻¹) and 10 µL esonuclease 3 from *Escherichia coli* Escherich, 1885 (1.9 U mL⁻¹). However, nuclease should be used with caution, because it has also been reported to reduce viral numbers in freshwater samples by 19% (Bettarel *et al.*, 2000). In other studies, about 10% of the <0.2 µm fraction of DNA in marine water samples, which is assumed to comprise viruses (i.e. 'coated DNA'), was digested by DNase (Maruyama, Oda & Higashihara, 1993) and, in a culture containing T2 phages and marine plankton samples, 33–48% of the encapsulated viral DNA was sensitive to DNase (Jiang & Paul, 1995).

Since all these fluorochromes bind nucleic acids, some small prokaryotes (<0.2 µm) may be counted as viruses. However, as pointed out by Noble & Fuhrman (1998), even if all small prokaryotes were counted as viruses, the over-estimation of total viral abundance would be negligible. If viral and prokaryotic counts are needed from the same sample, we

suggest carrying out the counts on separate filters, because the virus-to-prokaryote ratio may vary substantially.

In the attempt to decrease sample-processing time, the use of flow cytometers has been proposed for detecting and quantifying virus-like particles and prokaryotes (Marie *et al.*, 1999; Brussaard, 2004). For sediments Duhamel & Jacquet (2006) proposed the following protocol: 0.5 mL of sediment are added to 3 mL of 0.02-µm filtered lake water, containing 0.2-µm filtered formaldehyde (2%). The viruses are separated from the sediments by using a mixture of sodium pyrophosphate (10 mM final concentration) and polyoxyethylene-sorbitan monooleate (Tween 80[®] Croda Int., Goole, East Yorkshire, U.K.; 10% final concentration) and 3 min sonication in a water bath. The best results were obtained when samples were stained with SYBR Green II.

Viral production Viral counts provide only limited information on viral dynamics in ecosystems. Accurate estimates of VP rates in different benthic environments are needed to assess viral dynamics and impacts on their hosts and, therefore, to understand the role of the benthic viral shunt in benthic food webs and biogeochemical cycles. However, the measurement of VP rates in sediments is still problematic, since there is no standardized protocol.

Four different approaches have been used to estimate VP in sediments:

1 Quantifying the increase in viral abundance over time following dilution of sediments with virus-free water (Mei & Danovaro, 2004) or sterilized sediment (Hewson & Fuhrman, 2003). The idea behind this approach is that by reducing viral and host densities, the occurrence of new infections is also reduced. At the same time, sediment dilution renders almost negligible the impact of protozoan predation on viruses, and reduces viral losses due to enzymatic degradation. The advantage of this technique is that temporal changes in viral abundance can be directly estimated without using conversion factors. The method assumes, however, that dilution and sediment manipulation does not significantly alter the activity of benthic prokaryotes (Hansen, Thampdrup & Jørgensen, 2000) and thereby viriobenthos production.

2 Quantifying increases in viral abundance over time in undiluted homogenized sediment samples incubated in Würgler bags. This approach minimizes

the stimulation of microbial activity that may occur following dilution (Glud & Middelboe, 2004; Middelboe *et al.*, 2006), but it does not take into consideration the losses of prokaryotes and viruses to their predators nor any new viral infections that may occur in undiluted sediments. The Würgler-bag method has been applied to both coastal and deep-sea sediments, and has provided values of VP about one order of magnitude lower than those obtained by the dilution technique. These two techniques are the only ones that have been used so far to estimate VP rates along with EFM counts. Both approaches require handling of the sediment (including preparation of sediment slurries in the dilution technique and sediment homogenization in Würgler-bag incubations), which can alter microbial activity (Hansen *et al.*, 2000). However, the extent to which these protocols influence viral activity is still unknown.

3 Measuring rates of viral decay (Fischer *et al.*, 2003, 2004, 2006). This approach is based on the assumption that, in steady state, viral decay is equivalent to VP. In this technique, VP in undiluted homogenized sediment is stopped by poisoning host cells with potassium cyanide (KCN) and the decrease in viral abundance is subsequently determined over time. An uncertainty associated with this approach, apart from the assumption of steady state, is the currently unknown effect of KCN on viral decay rates (VDR) and/or capsid degradation.

4 Estimating the frequency of infected prokaryotic cells (FIC) coupled with information on burst size (BS, see below) and prokaryotic production rates (Bettarel *et al.*, 2006; Filippini *et al.*, 2006). The FIC is derived from the frequency of visibly infected cells (FVIC) determined by TEM and then converted to estimates of viral-induced prokaryotic mortality (VIM) and, ultimately, VP. This approach assumes that, in steady state, infected and uninfected prokaryotes are grazed at the same rate and that the latent period equals the prokaryotic generation time (Proctor, Okubo & Fuhrman, 1993; Guixa-Boixareu *et al.*, 1996; Binder, 1999).

All of the above approaches make important assumptions and suffer from different biases. Furthermore, since none of the methods has been widely used to date and methodological comparisons are lacking for benthic systems, it is unclear to what extent discrepancies among results obtained with different approaches reflect real differences in viral dynamics among sys-

tems or methodological biases. The largest discrepancies appear to exist when direct measurements of VP are compared with approaches based on mathematical models (e.g. FIC approach). Estimates of VP obtained by TEM have provided the lowest values so far (Bettarel *et al.*, 2006; Filippini *et al.*, 2006). Compared with the sediment-dilution approach, low estimates of VP have also been obtained with the Würgler-bag method (Glud & Middelboe, 2004; Middelboe *et al.*, 2006). Since preparation of sediment slurries can stimulate microbial activity and, possibly, VP (Hansen *et al.*, 2000), higher production rates obtained with the dilution method may reflect an experimentally induced increase in prokaryotic activity. Alternatively, estimates of VP using the Würgler-bag method might be lower due to the fact that protozoan grazing and enzymatic degradation are not eliminated. Direct comparisons of these two approaches, and quantification of the net effect of enzymes and grazing in time-course experiments, are needed to provide a definitive conclusion. Finally, approaches based on the assumption of steady state may not be valid if short-term variation in viral and/or host dynamics is important.

Even when the same approach is used, important variations often exist within each protocol. For example, both Hewson & Fuhrman (2003) and Mei & Danovaro (2004) used the dilution approach, but diluted their sediment samples with either sterilized sediment or virus-free sea water. In addition, virus extraction techniques and other details of experimental procedures varied. Apparently, minor differences among protocols could result in significant differences in VP rates, thus influencing conclusions about the impact of viruses on benthic microbial processes. Clearly, there is a need for rigorous evaluation of available methods, intercalibration of procedures and conversion factors, and the development of widely accepted standard protocols to estimate VP in sediments.

Viral decay The term 'viral decay' is ambiguous, since it may refer either to the loss of infectivity (i.e. through damage of nucleic acids or viral receptors on the capsid) or the complete degradation of viral particles. Any virus undergoing degradation might have reduced or even lost its infectivity but remain visible under the microscope. Viral decay determined microscopically by direct counting thus often substantially underestimates the actual loss of infectivity (Wells &

Deming, 2006). Two different methods have been used to measure benthic VDR:

1 Quantifying the decline in viral abundance after stopping prokaryotic metabolism, and consequently the production of new viruses, by addition of KCN in undiluted homogenized sediment. This approach provides estimates of gross decay rates (Fischer *et al.*, 2003, 2004, 2006). A drawback is that the time course obtained during such decay experiments allows several different interpretations. Another problem is choice of the appropriate mathematical model to analyse the data. Choice of representative sampling frequencies and the total duration of incubations (e.g. 24 h) can also be problematic. Moreover, the addition of KCN inactivates both protozoan and prokaryotic metabolism (Heldal & Bratbak, 1991; Mathias, Kirschner & Velimirov, 1995), so that potential viral loss factors, such as degradation by enzymes and ingestion by protozoa, may be reduced or totally eliminated.

2 Estimating changes in virus abundance over time in undiluted homogenized anoxic sediment incubated in gas-tight plastic Würgler bags, which are flushed with nitrogen gas before incubation (Glud & Middelboe, 2004). In this approach, viral decay is measured along with microbial activity, and provides a direct relationship between prokaryotic and viral activity. A disadvantage is that Würgler-bag incubations may stimulate microbial activity (and may thereby alter virus production and decay) relative to *in situ* conditions (Middelboe & Glud, 2006; Middelboe *et al.*, 2006). Moreover, this approach can result in a net loss of viruses which is not solely due to disintegration but also includes the disappearance of viruses adsorbed to prokaryotic hosts and other particles (Glud & Middelboe, 2004).

Fraction of lysogenic prokaryotes As for pelagic samples, the approach commonly used for estimating the percentage of lysogenic cells (i.e. the percentage of cells in the prokaryotic community containing an inducible viral genome) in the entire benthic prokaryotic community is based on the induction of lysogens with UV-C light or by adding Mitomycin C (Paul & Jiang, 2001). The number of lysogenic prokaryotes (LP) is generally estimated by dividing the number of viruses produced, due to prophage induction, by the BS (i.e. the number of viruses released from a single host cell during lysis; Wommack & Colwell, 2000; Mei

& Danovaro, 2004; Glud & Middelboe, 2004). The most commonly used inducing agent is Mitomycin C, which is generally used at a final concentration of $1 \mu\text{g mL}^{-1}$ of sediment slurry or homogenate depending on the approach used for estimating VP (Glud & Middelboe, 2004; Mei & Danovaro, 2004). The percentage of LP (%LP) is then calculated as follows:

$$\%LP = \frac{VP_{\text{Mitomycin C}} - VP_{\text{control}}}{BS \times PDC_{\text{to}}} \times 100 \quad (1)$$

where $VP_{\text{Mitomycin C}}$ is the VP estimated after addition of Mitomycin C, and PDC_{to} is the prokaryote abundance at the start of the experiment (i.e. before the addition of Mitomycin C). Bettarel *et al.* (2006) used a similar approach but calculated the percentage of lysogens by viral abundance instead of production. The method for estimating the LP fraction assumes that all lysogens enter the lytic cycle after addition of Mitomycin C.

Burst size Accurate estimates of BS are critical to quantifying virus-mediated mortality of hosts. The most reliable estimates of BS measured in sediment samples have been obtained by direct TEM observations of visibly infected cells (Weinbauer, 2004). This approach has the advantage of producing images of phage formation within the prokaryotic host cells (Fig. 1), although it is sometimes difficult to recognize a phage structure and, when BS is high, to obtain accurate counts. Furthermore, this approach does not detect viruses with a chronic life cycle and lack of a visible infection stage (Middelboe & Glud, 2003). The approach is also laborious (Proctor, 1997) and therefore difficult to implement in extensive routine measurements.

The BS of virus-infected prokaryotes in sediments has also been estimated in time-course experiments with diluted sediment samples, by calculating the ratio of VP to the number of killed hosts prokaryotes over short-time intervals (Mei & Danovaro, 2004; Corinaldesi *et al.*, 2007). This indirect approach is simple and relatively fast and thus lends itself to routine use for estimating BS. The decrease in prokaryotic cell abundance is determined as the difference between the observed and expected abundance in a sample, where the expected abundance is the number of cells at time 0 (i.e. when sediments are diluted and the incubation is started) plus the increase

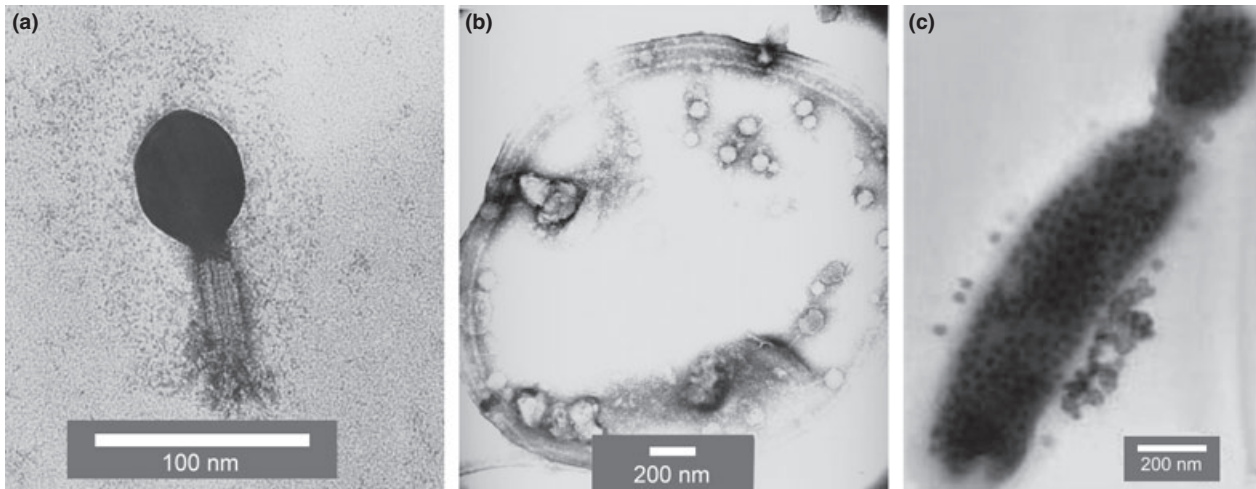


Fig. 1 Transmission electron micrographs (a) of a benthic virus isolated from coastal sediments, and examples of (b) eukaryotic and (c) prokaryotic cells displaying viral infection.

in cell abundance estimated independently by measuring prokaryotic production. The difference between observed and expected values is assumed to represent the number of cells killed by viral lysis. An advantage of this approach, in addition to its simplicity, is that the addition of inhibitors or poisons is avoided, thus excluding one important cause of potential artefacts. It is assumed that, in the short-term, prokaryotic death is due only to viral lysis and that conversion factors to estimate prokaryotic biomass production are correct.

Virus-induced host mortality The impact of phages on benthic prokaryotes can be calculated from VP (as viruses g^{-1} sediment h^{-1}) and BS, which can both be determined with any of the methods described above (Fischer *et al.*, 2003; Hewson & Fuhrman, 2003; Mei & Danovaro, 2004; Bettarel *et al.*, 2006; Filippini *et al.*, 2006; Middelboe *et al.*, 2006; Corinaldesi *et al.*, 2007). Thus:

$$PN_L = \frac{VP}{BS} \quad (2)$$

where PN_L is the number of prokaryotes lysed (cells g^{-1} sediment h^{-1}). VIM of host cells is then calculated as the percentage of cells (or biomass) removed by viral lysis in relation to the number of cells (or biomass in g) produced, i.e.:

$$VIM = \frac{PN_L}{PP} \times 100 = \frac{VP}{BS \times PP} \times 100 \quad (3)$$

where PP is the prokaryotic production (cells g^{-1} sediment h^{-1} or g biomass g^{-1} sediment h^{-1} respectively). When PP is measured by the thymidine method, which gives PP in cells g^{-1} sediment h^{-1} , eqn 3 will yield VIM as a percentage of prokaryotic cell production. Determining PP by the leucine method gives PP in biomass g^{-1} sediment h^{-1} , and eqn 3 will thus provide VIM as percentage of prokaryotic biomass production. If VIM needs to be expressed as percentage of prokaryotic cell production, then prokaryotic cell biomass must be estimated independently to convert prokaryotic biomass production to cell production. When prokaryotic turnover ($PT =$ ratio of cell production per hour to cell numbers present \approx growth rate) is determined, then total prokaryotic numbers are required. Eqn 3 then transforms into:

$$VIM = \frac{VP}{BS \times PT \times PN_T} \times 100 \quad (4)$$

where PN_T is the total number of prokaryotes (cells g^{-1} sediment).

Fischer *et al.* (2004, 2006) determined the viral turnover ($VT =$ ratio of virus particle production per hour to viral numbers present) by using an 'exponential decay' function to describe the time course of KCN decay experiments, and assuming that viral abundance is in steady state, i.e. viral decay equals VP. Eqn 4 then transforms to:

$$\text{VIM} = \frac{\text{VT} \times \text{VN}_T}{\text{BS} \times \text{PT} \times \text{PN}_T} \times 100 \quad (5)$$

where VN_T is the total number of viruses (particles g^{-1} sediment).

Virus-induced mortality of benthic prokaryotes can be also estimated using empirical relationships between the FIC and VIM and conversion factors determined for water samples (Binder, 1999; Bettarel *et al.*, 2006; Filippini *et al.*, 2006). A fundamental limitation of this approach to estimating VIM is that VP is assumed to result largely from lytic infection of prokaryotes. However, micro-algae could account for a significant part of the benthic community living in well-lit systems. Therefore, VP may also originate from lysis of microalgae (Proctor & Fuhrman, 1990; Hewson *et al.*, 2001b) and other eukaryotic algae (Suttle *et al.*, 1990) or both. Detailed analyses have not yet been carried out. Consequently, it is important to interpret results with care, to use complementary methods for the same purpose where possible, and to identify the most accurate approaches for measuring VIM and other key parameters of viral dynamics in sediments.

Viral diversity The analysis of viral diversity and community structure is difficult in both benthic aquatic ecosystems and other natural environments. Morphotypes can be distinguished by TEM (e.g. Middelboe, Glud & Finster, 2003), but this approach offers very limited resolution. Molecular biological approaches have the potential to overcome this problem. Unlike the genomes of prokaryotes and eukaryotes, however, viral genomes do not share single genes across all taxa, such as 16S or 18S rRNA, precluding the possibility of monitoring viral diversity using approaches analogous to ribosomal DNA profiling. A first step towards assessing viral diversity is therefore to identify conservative regions within virus-specific genes as targets for PCR primers. These primers can then be used to amplify the target sequences from natural samples, as it has been performed with rDNA profiling (Chen & Suttle, 1995; Fuller *et al.*, 1998; Filee *et al.*, 2005). Once suitable primers have been identified, the genetic diversity and changes in viral community structure can be assessed by denaturing gradient gel electrophoresis (Muyzer, de Waal &

Uitterlinden, 1993) or similar molecular fingerprinting techniques.

Chen & Suttle (1995) developed primers specific for algal viruses. Specifically, they amplified DNA polymerase gene fragments (pol) from viruses infecting three genera of microalgae. A conserved region in the genomes of three genetically distinct cyanophages has also been identified and, based on this information, a cyanophage-specific primer targeting a gene encoding a capsid assembly protein (gp20) has been developed (Fuller *et al.*, 1998; Dorigo, Jacquet & Humbert, 2004). Similarly, Filee *et al.* (2005) designed a set of degenerate PCR primers for phage T4g23 encoding the major capsid protein in all T4-type phages, which is an important family of the tailed phages. These recent advances suggest that it should be possible to develop a suite of primers which, when used in combination, are capable of probing the structure of phage communities in natural environments, including in sediments.

A method that has become popular for analysing viroplankton communities is pulsed-field gel electrophoresis (PFGE) (Wommack *et al.*, 1999). This technique allows separation of large nucleic acid fragments on agarose gels and thus to generate fingerprinting profiles of viral communities based on differences in genome size. Resolution of this method is much lower in comparison with that obtained using molecular approaches, even if analyses of water samples suggest that major viral genotypes can be distinguished and differences in community structure resolved (Wommack *et al.*, 1999; Steward, Montiel & Azam, 2000). About 10^6 viruses of the same genome size are needed to obtain a visible band on a gel, and therefore the method only detects dominant strains. Since it is possible that several viruses have genome sizes that vary only by a few kb, a smear could be generated on the gel, making the detection of single bands difficult or impossible. Finally, this technique detects only dsDNA, as RNA and ssDNA cannot be adequately represented (Wommack *et al.*, 1999; Steward *et al.*, 2000). Thus, as for all fingerprint approaches (Danovaro *et al.*, 2007), PFGE reveals only a minimum estimate of the dominant genotypes present within a sample, typically less than 50 distinct bands. Given even greater diversity and lower proportions of dominant viral genomes in sediments than in water, application of PFGE to benthic samples

presents a major challenge. Following various modifications of protocols used for virioplankton communities, however, PFGE analyses have also been successful in both fresh water and marine sediments (Filippini & Middelboe, 2007).

Another approach is metagenomic analysis of whole viral communities in environmental samples (Breitbart *et al.*, 2004). This approach circumvents the problem of the lack of general target sequences in viruses and, in contrast to PFGE analysis, can capture the entire diversity of viral communities. Accordingly, metagenomic analyses have yielded 10^4 – 10^6 viral genotypes (e.g. Steward *et al.*, 2000; Riemann & Middelboe, 2002; Larsen *et al.*, 2004).

The usefulness of RT-PCR assays for routine monitoring of enteric viruses in waste water, sediments and shellfish has also been recognized (Schwab, De Leon & Sobsey, 1993). Green & Lewis (1999) detected enteroviruses, rotaviruses and hepatitis A viruses in different types of sediment samples and at various sampling times. However, this approach has not yet been standardized to investigate viral diversity in marine or freshwater sediments.

The viriobenthos in aquatic ecosystems

Abundance and distribution Viral abundance has been estimated in a variety of marine and freshwater sediments (Fig. 2) where they range from 10^7 to 10^{10} g^{-1} of dry sediment (Table 1). On a volumetric basis, abundances in surface and subsurface sediments exceed those in the water column (10^5 and 10^8 particles mL^{-1}) by 10–1000 times (Paul *et al.*, 1993; Maranger & Bird, 1996; Steward, Smith & Azam, 1996; Danovaro & Serresi, 2000; Danovaro, Manini & Dell'Anno, 2002; Corinaldesi & Danovaro, 2003; Fischer *et al.*, 2003). Similarly, abundances in sediment pore water tend to be higher than in the overlying water layers.

On average, the lowest viral counts in sediments have been recorded in highly nutrient-poor deep-sea sediments (17.1×10^8 viruses g^{-1} dry sediment), although very high viral abundances (up to 162.2×10^8 viruses g^{-1} dry sediment) have been observed in other deep-sea sediments (e.g. Porcupine abyssal plain; Danovaro *et al.*, 2002; Table 1). Furthermore, investigations of deep-sea trenches (e.g. from the abyssal Ierapetra Trench to Sporades basin)



Fig. 2 Location of the areas from where data on viriobenthos are available.

Table 1 Viral and prokaryotic abundance, virus to prokaryote ratio (VPR), viral production (VP), burst size (BS) and virus-induced prokaryotic mortality (VIM) in fresh water, coastal marine and deep-sea sediments

Location	Depth (m)	Viral abundance (10^8 viruses g^{-1})	Bacterial abundance (10^8 cell g^{-1})	VPR	VP (10^7 viruses $g^{-1} h^{-1}$)	Viral decay (10^7 viruses $g^{-1} h^{-1}$)	BS (virus cell $^{-1}$)	VIM (%)	Methods	Reference
Lake Gilbert, Quebec	2–14	7.2–203.3	7.8–17.8	0.8–25.7	n.a.	n.a.	n.a.	n.a.	TEM analyses	Maranger & Bird (1996)
Noosa River, Australia	n.a.	5.6–14.4	1.1	2.0–11.0	n.a.	n.a.	n.a.	n.a.	EFM analyses	Hewson <i>et al.</i> (2001a)
Brisbane River, Australia	n.a.	51.1	0.3–1.4	35.0–65.0	n.a.	n.a.	n.a.	n.a.	EFM analyses	Hewson <i>et al.</i> (2001b)
Lake Koottrabah, Australia	n.a.	2.1–13.7	1.1	1.9–12.3	n.a.	n.a.	n.a.	n.a.	EFM analyses	Hewson <i>et al.</i> (2001a)
Talladega wetland, Alabama	n.a.	>0.01–0.6	0.4–2.2	>0.1–0.7	n.a.	n.a.	n.a.	n.a.	EFM analyses	Farnell-Jackson & Ward (2003)
Kühwörter Wasser, Austria	1	80	21.1–78.9	0.9–3.2	n.a.	0–24.7	85	2.2 [†]	EFM, viral decay and cell size estimate	Fischer <i>et al.</i> (2003)
Kühwörter Wasser, Austria	1	16.1–106.4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	EFM analyses	Fischer <i>et al.</i> (2005)
Nivå Bay, Denmark	0.5	0.6–8.3	0.1–4.2	1.4–7.8	n.a.	n.a.	n.a.	n.a.	EFM and TEM analyses	Middelboe <i>et al.</i> (2003)
River Esino, Italy	0.3	10.9	14.2	0.8	8.6	n.a.	17	18.4	EFM, dilution technique	Mei & Danovaro (2004)
Senegal inland aquatic systems	n.a.	1.8–6.2 (1.0–1.7)*	0.7–4.6 (0.0–0.2)*	0.6–9.1	n.a.	n.a.	n.a.	n.a.	EFM and TEM analyses	Bettarel <i>et al.</i> (2006)
Aerobic sediment of Lake Hallwil, Switzerland	n.a.	21.1–60.0	15.6–42.2	0.9–1.8	n.a.	n.a.	38	n.a.	TEM analyses	Filippini <i>et al.</i> (2006)
Average Florida Bay	1–35	34.2	11.5	9.6	8.6	12.3	47	10.1	TEM analyses	Paul <i>et al.</i> (1993)
Chesapeake Bay	1–17	1.4–5.9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	TEM analyses	Drake <i>et al.</i> (1998)
Falconara beach, Adriatic Sea	Subtidal	3.4–8.1	0.1	n.a.	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro <i>et al.</i> (2001)
Moreton Bay, Australia	n.a.	6.7–14.4	0.4–0.7	15.0–22.0	n.a.	n.a.	n.a.	n.a.	EFM analyses	Hewson <i>et al.</i> (2001a)
Sevastopol Bay, Black Sea	n.a.	>0.1*	>0.1–0.5	>0.1–20.0	n.a.	n.a.	n.a.	n.a.	EFM analyses	Stepanova (2001)
Los Angeles Harbor	20	2.2	0.2	10.0	7.0–13.6	n.a.	100 [§]	34 [†]	EFM, dilution technique	Hewson & Fuhrman (2003)

Table 1 (Continued)

Location	Depth (m)	Viral abundance (10^8 viruses g^{-1})	Bacterial abundance (10^8 cell g^{-1})	VPR	VP (10^7 viruses $g^{-1} h^{-1}$)	Viral decay (10^7 viruses $g^{-1} h^{-1}$)	BS (virus cell $^{-1}$)	VIM (%)	Methods	Reference
Santa Catalina Island, Southern California	Subtidal	27.2	0.3	11.0	4.9–12.3	n.a.	100^S	10.7 [†]	EFM, dilution technique	Hewson & Fuhrman (2003)
Central Øresund, Denmark	35	4.2	0.2	17.0	0.2–0.7	0.1–0.4	14	21**	Mitomycine addition	Glud & Middelboe (2004)
Ancona Port, Italy	8	25	32.0	0.9	19.8	n.a.	24	12.2	EFM, dilution technique	Mei & Danovaro (2004)
Coastal Adriatic sediments	8	2.1	10.3	0.2	1.4	n.a.	3	57.3	EFM, dilution technique	Mei & Danovaro (2004)
Gulf of Thermaikos, Greece	51	6.2	10.4	0.6	6.6	n.a.	26	16.1	EFM, dilution technique	Mei & Danovaro (2004)
Gulf of Manfredonia, Italy (0–1 cm)	21	7.4	15.3	0.5	1.7	0.1	7	25.3	EFM, dilution technique	Mei & Danovaro (2004)
Gulf of Manfredonia, Italy (10–20 cm)	21	5.6	4.9	1.1	1.8	0.7	15	43.3	EFM, dilution technique	Mei & Danovaro (2004)
Gulf of Manfredonia, Italy (90–100 cm)	21	7.6	5.9	1.3	3.0	1.1	20	42.2	EFM, dilution technique	Mei & Danovaro (2004)
Chesapeake Bay	n.a.	33.3–3111.1	0.4–16.7	70.0–243.0	n.a.	n.a.	n.a.	n.a.	EFM analyses	Helton <i>et al.</i> (2006)
Chile coast	14	3.3	0.6	5.5	0.4	n.a.	14 [†]	93	EFM, Würigler-bag incubations	Middelboe & Glud (2006)
Chile coast	28	2.2	0.2	11.0	0.6	n.a.	14 [†]	58	EFM, Würigler-bag incubations	Middelboe & Glud (2006)
Chile coast	35	3.1	0.2	15.5	0.4	n.a.	14 [†]	44	EFM, Würigler-bag incubations	Middelboe & Glud (2006)
Chile coast	73	2.9	0.6	4.8	0.6	n.a.	14 [†]	>100%	EFM, Würigler-bag incubations	Middelboe & Glud (2006)
Chile coast	93	2.3	0.2	11.5	0.3	n.a.	14 [†]	82	EFM, Würigler-bag incubations	Middelboe & Glud (2006)
Average		89.9	5.0	18.4	4.0	0.5	15.6	48.4		

Table 1 (Continued)

Location	Depth (m)	Viral abundance (10^8 viruses g^{-1})	Bacterial abundance (10^8 cell g^{-1})	VPR	VP (10^7 viruses $g^{-1} h^{-1}$)	Viral decay (10^7 viruses $g^{-1} h^{-1}$)	BS (virus cell $^{-1}$)	VIM (%)	Methods	Reference
Sporades Basin (Aegean Sea)	1232	23.8	11.0	2.2	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro & Serresi (2000)
Cretan Sea	1840	20.2	4	5.1	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro & Serresi (2000)
Ierapetra Trench (Aegean Sea)	4235	12.1	4.9	2.5	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro & Serresi (2000)
Porcupine Abyssal Plain	4800	12.6–162.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro <i>et al.</i> (2001)
Deep Mediterranean sediments	1290–4000	0.4–1.2	1.8–6.5	0.1–0.5	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro <i>et al.</i> (2002)
San Pedro Channel, California	900	27.2	0.02	98.0	n.a.	n.a.	100 [§]	n.a.	EFM analyses	Hewson & Fuhrman (2003)
Sagami Bay, Japan	1450	2.9–25.6	1.3–2.9	8.0–35.0	0.2	n.a.	14 ^{††}	24–48	EFM, Würger-bag incubations	Middelboe <i>et al.</i> (2006)
Topographic Hights	3225	0.6	0.4	1.5	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro <i>et al.</i> (2005)
Calabrian Rise	2255	0.5	0.2	2.5	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro <i>et al.</i> (2005)
L'Atalante basin, Ionian Sea	3363	0.5	0.5	1.0	n.a.	n.a.	n.a.	n.a.	EFM analyses	Danovaro <i>et al.</i> (2005)
L'Atalante basin, Ionian Sea	3575	11.0	1.9	5.8	7.0 [†]	2.2	6	58.7	EFM, dilution technique	Corinaldesi <i>et al.</i> (2007)
L'Atalante basin, Ionian Sea	3575	10.0	1.5	6.7	5.8 [†]	1.6	27	19.60	EFM, dilution technique	Corinaldesi <i>et al.</i> (2007)
L'Atalante basin, Ionian Sea	3575	8.2	1.1	7.5	4.2*	2.0	69	8.6	EFM, dilution technique	Corinaldesi <i>et al.</i> (2007)
Average		17.1	2.7	12.9	4.3	1.9	20	30.7		

*In parentheses TEM estimates.

†Gross viral production.

‡Estimated by assuming a BS of 25 virus cell $^{-1}$.

§TEM estimates from overlying waters.

*Calculated by assuming a prokaryotic growth rate of 3.6 day $^{-1}$.**Estimated at *in situ* temperature.

††Estimated in the coastal sediments of Central Øresund.

revealed high viral abundances, with values ranging from 12.1 to 23.8×10^8 viruses g^{-1} dry sediment (Danovaro & Serresi, 2000).

On average, the highest viral abundances have been encountered in coastal sediments (about five times higher than in deep-sea sediments), where the organic load in the water column is generally more important. For instance, at water depths between 95 and 340 m in coastal regions of the North Aegean viral abundances ranged from 2.3 to 4.1×10^{10} g^{-1} dry sediment (R. Danovaro, unpubl. data). Viral abundances in Chesapeake Bay were up to 3.1×10^{11} viruses g^{-1} of dry sediment. At other stations across the mouth of this bay, viral numbers ranged from 3.4 to 8.1×10^8 g^{-1} of sediment pore water (Drake *et al.*, 1998), comparable to values obtained from two estuarine stations in the Coral Sea (Australia; $6.7\text{--}14.4 \times 10^8$ viruses g^{-1} of sediment; Hewson *et al.*, 2001a) and from the brackish waters of Key Largo (Florida; $1.4\text{--}5.9 \times 10^8$ viruses g^{-1} of sediment; Paul *et al.*, 1993). In all these studies mean viral abundance in the sediment was almost two orders of magnitude higher than that in the overlying water column.

High abundances have also been reported for freshwater sediments (on average, 34.2×10^8 viruses g^{-1} of dry sediment). For example, in freshwater portions of the Brisbane River (Queensland, Australia), mean viral densities was 51.1×10^8 g^{-1} (Hewson *et al.*, 2001a). Similar mean values (61.2×10^8 g^{-1} of sediment) were reported for sediments of K uw orfer Wasser (Austria; Fischer *et al.*, 2005). Very low values of virus abundance ($0.01\text{--}0.6 \times 10^8$ g^{-1} of sediment) were reported for Talladega wetland (Alabama; Farnell-Jackson & Ward, 2003).

The wide range of viral abundance observed in different benthic environments might arise, in part, from differences in the methods used to extract and count viruses (see above). However, methodological differences are unlikely to be the only or even the primary reason for variations, as considerable spatial differences have been found among sites in single investigations using the same methodologies (e.g. Maranger & Bird, 1996).

One study from the deep sediments of Sagami Bay (Japan) showed that viral abundance varies substantially over short distances (Middelboe *et al.*, 2006). The fact that prokaryotic activity may vary along spatial gradients is well known, but to date only a few studies have investigated such variability of viral abundance

and dynamics (Hewson *et al.*, 2001a; Middelboe *et al.*, 2006). Moreover, available information is conflicting. In the sediments of Sagami Bay, viral distribution displayed large spatial heterogeneity because two samples taken 3 cm apart, within a single core, were no more similar than two samples taken 150 m apart. On the other hand, along a decreasing eutrophication gradient from the Brisbane River to Moreton Bay in eastern Australia, a significant decrease in benthic viral abundance was found (Hewson *et al.*, 2001a). This suggests that genuine large differences in viral abundance exist among different benthic environments. The highest viral abundance appears to be typical of fresh water and low-salinity coastal waters, whereas abundances may be up to three orders of magnitude lower in some deep-sea sediments that are largely disconnected from continental material inputs.

At a given site, viral abundance varies with increasing sediment depth. A continuous decrease with sediment depth has been reported for estuarine sediments (Hewson *et al.*, 2001a), whereas in other studies subsurface maxima (at 1–4 cm depth) and subsequent declines towards deeper sediment layers were observed (Danovaro & Serresi, 2000). In a study carried out on an entire sediment core (>100 m in length) in Holocene/Pleistocene sediments of Saanich Inlet (Canada), viral abundance decreased about $10^9\text{--}10^8$ g^{-1} dry sediment between the surface sediment and the deepest layer sampled (100 m; Bird *et al.*, 2001) suggesting that viruses persist even in the deepest sediment layers.

It has been proposed that viral abundance and the ability to infect prokaryotes increase with the productivity of waterbodies, with the highest percentage of infected cells and highest VP in highly eutrophic ecosystems (Weinbauer, Fuks & Peduzzi, 1993). This conclusion is generally supported by data reported in Table 1. However, Hewson *et al.* (2001a) investigated the spatial distribution of benthic viruses along two trophic gradients in eastern Australia: 32 stations were sampled throughout the eutrophic Brisbane River/Moreton Bay estuary and 11 stations in the oligotrophic Noosa River estuary. In both surveys, viral abundance in sediments decreased significantly from the eutrophic freshwater sites to the oligotrophic marine waters (Hewson *et al.*, 2001a). Similarly, in a large-scale study carried out along the entire deep-Mediterranean basin, Danovaro *et al.* (2002) covered a decreasing gradient of trophic state defined in terms

of pelagic primary production and vertical particle flux. This gradient extended from the relatively productive western basin to the highly oligotrophic Levantine Sea of the eastern Mediterranean. Viral abundance decreased from western to eastern stations (average of 0.82 and 0.58×10^8 viruses g^{-1} dry sediment respectively), indicating a possible causal relationship between benthic viral abundance and trophic state. Although benthic prokaryotic abundance did not show a similar spatial pattern (average of 4.3 and 4.5×10^8 cells g^{-1} dry sediment in the western and eastern basin, respectively), prokaryotic cell size increased eastwards (38 – 53 fg C cell $^{-1}$). Moreover, prokaryotic production and growth rate doubled from the eastern to the western stations. Lowest viral numbers thus corresponded with the lowest prokaryotic productivity and largest cell size, suggesting that prokaryotic metabolic status might play an important role in benthic viral dynamics. This is supported by the significant positive correlation between VP and prokaryotic respiration and between viral and prokaryote production found in different studies (Glud & Middelboe, 2004; Mei & Danovaro, 2004; Middelboe *et al.*, 2006).

Positive correlations between viruses and trophic state have not been found in all investigations. In a study carried out in the southern and northern Aegean Sea, viral abundance decreased along a trophic gradient, whereas benthic prokaryotic abundance increased threefold (Danovaro *et al.*, 2001). However, correlation analyses do not allow inferences on cause–effect relationships between viral

abundance and environmental variables, as it might be that trophic state is more important in controlling the distribution of hosts than the viruses themselves.

Viral life cycles The lack of metabolic activity and independent replication sets viruses apart from other self-replicating systems. Viruses thus do not represent living entities according to the standard definition of life. A schematic view of the different viral model of life (i.e. chronic, lytic, lysogenic and pseudolysogenic) is illustrated in Fig. 3. Among these life cycles, lytic and lysogenic infections are most often considered. Both involve host-cells lysis. However, in the lytic cycle, viruses lyse their hosts immediately after infection, whereas in the lysogenic cycle the viral genome is typically integrated into the host genome as a prophage or provirus, which is subsequently replicated along with the host genome until host lysis is induced by an agent, such as UV radiation, a chemical or other factors. Conversely, pseudolysogenic and chronic infections have been poorly defined and investigated. Some authors appear to equate the two life cycles (Paul & Kellogg, 2000), whereas others (Fuhrman & Suttle, 1993; Weinbauer, 2004) consider them separate types of interactions (Fig. 3). In pseudolysogeny, the viral genome remains in the host cell for an extended period but is not integrated and replicated in the infected cell. Therefore, the pseudolysogenic state of a prokaryotic cell is sometimes equated with the ‘carrier state’. In this case the prophage is not inducible, as it cannot be stimulated with chemical- or physical-inducing agents. In chronic

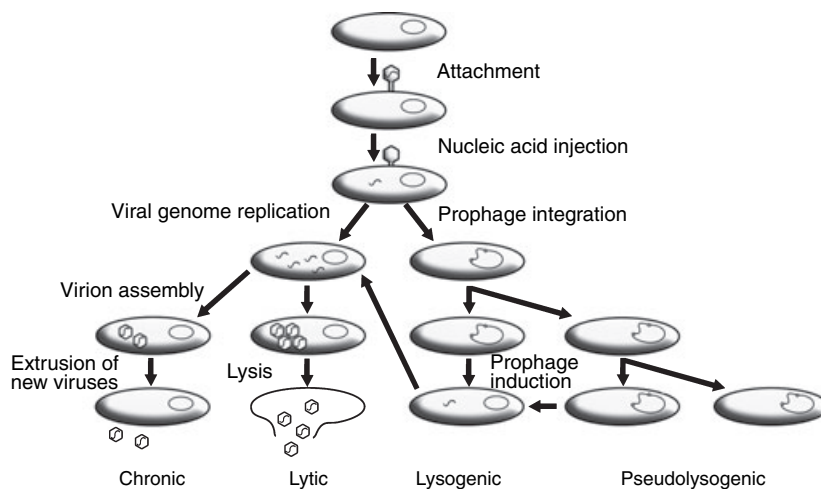


Fig. 3 Conceptual scheme of the different life cycles of virobenthos: chronic, lytic, lysogenic and pseudolysogenic.

infections, host cells are not lysed during the viral life cycle, but living host cells release filamentous viruses by budding or extrusion.

Growth of a virus population requires a rate of successful virus-host encounter that exceeds the rate of viral destruction and inactivation. This is most critical for lytic viruses, which should be favoured when host abundance is high. Conversely, the production of temperate viruses is less dependent on host cell density. In fact, this only requires a relatively small number of lysogenic cells and the occasional action of lysis-inducing agents to release free viruses. A key factor favouring lysogeny over the lytic cycle may therefore be the much greater probability for temperate viruses to survive at low host-cell abundances (Levin & Lenski, 1983). Consequently, lysogeny would be expected to be successful for viral propagation when conditions for growth and replication of hosts are unfavourable (Fuhrman, 1999; Weinbauer, 2004). Therefore, lysogeny might be less important than the lytic cycle in sediments, as they generally provide abundant resources for the growth of heterotrophic prokaryotes.

In contrast to this expectation, visibly infected cells were notably scarce in two studies investigating viral infection of prokaryotes in freshwater sediments by TEM (Bettarel *et al.*, 2006; Filippini *et al.*, 2006). Only one out of 4269 cells extracted from the surface sediments of a freshwater marsh was visibly infected (Filippini *et al.* (2006), and none out of 5840 cells (Bettarel *et al.*, 2006) in sediment samples collected from shallow African lakes. Similar observations were made for biofilms on submerged plant surfaces, where none out of 4970 inspected cells was infected by phages, and only three out of 5145 cells associated with decaying plant litter contained visible phages (Filippini *et al.*, 2006). At the same time, data from both marine and freshwater sediments indicate that the lytic life cycle is the main route of VP, while temperate phages are less important (Glud & Middelboe, 2004; Mei & Danovaro, 2004). The few available studies based on the lysogenic fraction estimated by using Mitomycin C, reported only a small fraction of lysogen prokaryotes ranging from undetectable to 14% (Glud & Middelboe, 2004; Mei & Danovaro, 2004). Mei & Danovaro (2004) found that, at most, 3.3% of the community hosted prophages and that lysogeny at various sites in the Mediterranean Sea ranged from 0% to 1.8%. The

lysogenic fraction increased with increasing depth in the sediment. In fact, in the oxygenated top 1 cm, the lysogenic fraction was five times lower than in the anoxic deepest sediment layer (50–100 cm depth).

Several hypotheses have been proposed to explain why the lysogenic life cycle appears to contribute little to benthic virus production. The most promising explanations are: (i) the accumulation in sediments of some unknown contaminants that could induce the lytic cycle in LP; (ii) the agents used to induce lysis in planktonic prokaryotes (i.e. Mitomycin C) are ineffective for benthic prokaryotes; (iii) the high host-specificity of phages, coupled with the high diversity of both prokaryotes and phages in sediments, decreases the likelihood for viruses, including temperate phages, to find a suitable hosts.

Review of the data leads to the following line of arguments: while viral abundance and production suggest a dominance of the lytic cycle in marine sediments (supported by the results of Mitomycin C experiments), the number of visibly infected cells is negligible in all samples examined so far. According to Filippini *et al.* (2006), VP and impact in the freshwater sediments examined is much lower than expected. However, the failure to detect infected cells does not imperatively imply that infection does not take place. The protocol used for the extraction of prokaryotes from sediment matrix involved vortex and sonication steps, which could alter the percentage of lysogens and cause the premature burst of infected cells. Moreover, the lack of visibly-infected cells, and the occurrence of VP in sediments, might be an indication of the importance of alternative viral life cycles, such as chronic infections or pseudolysogeny (see below). Finally, since all available data on benthic sediments indicate a high viral abundance, it is also worth mentioning the possibility of multiple infections, such as polylysogeny (a lysogen containing two or more different viral prophages; Hurst, 2000). Data on polylysogeny in benthic systems are still lacking, and therefore this mode of life is assumed to be unimportant.

Pseudolysogeny and chronic infection are poorly investigated and this is especially true for sediments. Nevertheless, chronic infection has been observed not only in cultures but also in alpine lakes (Hofer & Sommaruga, 2001) and it might be an important life strategy in benthic environments (Filippini *et al.*,

2006). The significance of pseudolysogeny in sediments is not clear either. In the marine bacterium H24, lysogeny was favoured at low nutrient concentrations, whereas high nutrient concentrations, analogous to situations in many sediments, triggered a phage mutation that led to pseudolysogeny (Moebus, 1997). Conversely, *Pseudomonas aeruginosa* phage UT1, gave rise to pseudolysogeny under extreme starvation conditions (Ripp & Miller, 1997), which suggested that the lack of available energy kept the phage genome in a state where neither lysogeny nor virulence could occur.

Given the scarce information available at present, it is premature to draw general conclusions about viral life cycles in benthic habitats. All types of life cycle may contribute to the observed VP and abundances. Determining their relative frequency and contribution to the dynamics of viruses in sediments will require a major effort, including the development of new methodologies.

Viral diversity The diversity of the viriobenthos has received only cursory consideration in reviews on viruses in aquatic ecosystems (Fuhrman, 1999; Wilhelm & Suttle, 1999; Wommack & Colwell, 2000; Sime-Ngando *et al.*, 2003; Weinbauer, 2004; Weinbauer & Rassoulzadegan, 2004; Breitbart & Rohwer, 2005; Hambly & Suttle, 2005; Suttle, 2005; Jackson & Jackson, 2008). Recent studies, however, have begun to fill this gap (e.g. Breitbart *et al.*, 2004; Filippini & Middelboe, 2007) and the information available to date is summarized here. Enteroviruses and other viruses of terrestrial origin are not considered as they have been described elsewhere (LaBelle & Gerba, 1979; Lewis, 1985; Rao & Melnick, 1986; Bosch, Girones & Jofre, 1988; Green & Lewis, 1999).

The first study to identify and isolate a phage from sediment was conducted by Wiebe & Liston (1968), who used a standard plaque assay with surface sediment samples taken at 825 m depth in the North Pacific Ocean. The isolated bacteriophage was found to infect an *Aeromonas* strain. Use of the same technique revealed that benthic and pelagic communities of coliphages, close to a coral reef, were diverse (Paul *et al.*, 1993), providing the first evidence that viral community structure in sediments may differ from that of the viroplankton. A corollary of this finding is that benthic viruses are autochthonous and do not originate from the overlying waters. However,

the lack of quantitative information on viral input from the upper water layers (as input of viruses attached to settling) does not allow us to make inferences about the extent to which benthic viral communities are autochthonous.

Some viruses, such as those infecting the bloom-forming alga *Heterosigma akashiwo* (Raphidophyceae; Nagasaki, Tarutani & Yamaguchi, 1999a,b), have been isolated from sediment samples at a variety of locations. Proliferation of these viruses is linked to the germination of benthic resting cysts of flagellated phytoplankton (Lawrence, Chan & Suttle, 2002). This suggests that some viruses detected in sediment are not active, but persist in infected host cells that reached the bottom prior to lysis and constitute a reservoir during host dormancy (Lawrence & Suttle, 2004).

Use of TEM has revealed different morphologies and sizes of viruses in water and sediment samples (Danovaro & Serresi, 2000; Middelboe *et al.*, 2003) and a higher morphological diversity in the latter habitat. Moreover, RNA and ssDNA phages, which are rarely observed in the water column, were commonly found in sediments (Middelboe *et al.*, 2003). Bettarel *et al.* (2006) reported that viruses in sediments of West African lakes displayed a great variety of sizes, with viruses <60 nm dominating (59%) and viruses >95 nm being relatively rare (3%). The proportions of viruses <60 nm and between 60 and 95 nm were similar in the pelagic and benthic environments, but the abundance of larger viruses was clearly greater in the benthos. However, differences in the structure of viral communities in the water column and sediment may not always be evident (Demuth, Neve & Witzel, 1993), although this lack of differentiation may have been due to the low resolution of morphotype analyses.

Given the small number of viral morphotypes, only a small fraction of total viral diversity is probably to be captured by TEM. Metagenomic approaches overcome this limitation and have provided evidence of an enormous genetic diversity in benthic viral communities; as many as 10^4 – 10^6 viral types have been found in 1 kg of near-shore marine sediment (Breitbart *et al.*, 2004). These empirical estimates are similar to results obtained in Monte Carlo simulations, which suggest that sediments containing 10^{12} viruses could host at least 10^4 viral genotypes (Breitbart *et al.*, 2004). Comparisons of viral sequences from sediment and water

column samples suggest a common phylogenetic origin of marine phages, whereas benthic and pelagic viral communities display completely different compositions. These findings provide further evidence that the majority of sediment viruses do not originate from the water column (Paul *et al.*, 2002; Fischer *et al.*, 2004).

As many as 75% of the 1156 sequences analysed by Breitbart *et al.* (2004) were unknown and most of the others belonged to dsDNA phages. Sequences of phages were the most common (44%) hits in GenBank, whereas sequences of viruses infecting eukaryotes were rare (3%). Viruses belonging to the family of Siphoviridae, which are mainly temperate phages, were more common (45%) than those belonging to myo- and podoviruses families. Even the most abundant genotypes in sediments appear to be extremely rare, comprising only 0.01–0.1% of the total viral community, compared to 2–3% in water samples (Culley, Lang & Suttle, 2006). Nevertheless, rare viruses can become abundant when their hosts are dominant. This may occur, for example, in response to changes in environmental conditions. The high richness and evenness of viruses in sediments results in extremely high values of the Shannon diversity index, the highest values reported in the literature ever (Breitbart *et al.*, 2004). This implies that viruses might constitute the largest reservoir of genetic diversity on the planet. This viral diversity remains largely uncharacterized, with most viruses belonging to novel groups that do not have culturable representatives. A very large effort would be needed to assemble complete genomes of these unculturable phages.

Both culturing and molecular studies indicate that viral populations can move over large distances and occur in vastly different environmental conditions (Breitbart & Rohwer, 2005). This notion is supported by the finding that the same viral genotypes can be found in marine, fresh water and soil environments. For example, a phage-encoded DNA polymerase sequence named HECTOR has been detected in marine water, soil, rumen fluid, in association with corals and in water of solar salterns (Breitbart & Rohwer, 2005). This viral sequence moved through different environments during the last 1000–2000 years. Sano *et al.* (2004) incubated the same marine microbial communities with viruses from soil, sediment and fresh water and observed viral proliferation after about 2 days. This result demonstrates

that viruses have the potential to find and infect suitable hosts in environments vastly different from those of their origin.

Using PFGE, Filippini & Middelboe (2007) compared viral community diversity in the sediment and water column of marine, brackish and freshwater systems. Four genome size classes (12–19, 30–48, 50–70 and 90–200 kb) dominated the communities in all these systems. Most of the genomes ranged in size from 30 to 50 kb. The band patterns of PFGE suggested that some virus phylotypes may occur in contrasting aquatic environments, including pelagic and benthic systems, while others may be restricted to specific environments or conditions. In line with the conclusion above, clear differences in viral communities were observed between sediment and the overlying water, as well as between fresh water and marine or brackish sediments. Moreover, viral community structure changed with sediment depth. More bands <145 kb were observed in lake than sea water, and marine sediments contained several genomic sizes completely absent in lake sediments (Filippini & Middelboe, 2007). Since large genomic sizes probably belong to algal viruses (Schroeder *et al.*, 2002), these observed differences could be related to differences in algal community composition.

A virtually unexplored component of viral diversity are the viruses infecting Archaea. Cultured archaeal viruses to date, which have solely dsDNA genomes, exhibit a wide range of peculiar morphotypes, including fusiform, droplet and bottle shapes and linear and spherical virions (Prangishvili, Garrett & Koonin, 2006). Moreover, genome-sequence analyses have demonstrated that most of these archaeal viruses are very different from those of other known bacterial viruses, suggesting that they might have different, and possibly multiple, evolutionary origins (Prangishvili, Forterre & Garrett, 2006).

Viral production and decay Viral production rates in freshwater sediments estimated with the decay method range from undetectable to 2.5×10^8 viruses $g^{-1} h^{-1}$ (Fischer *et al.*, 2003, 2004, 2006). The dilution approach, which has been applied more widely to fresh water, coastal and deep-sea sediments (Hewson & Fuhrman, 2003; Mei & Danovaro, 2004; Corinaldesi *et al.*, 2007), has generally given higher values, ranging from 1.4 to 19.8×10^8 viruses $g^{-1} h^{-1}$ (Table 1). The Würgler-bag method has also been applied to

both coastal and deep-sea sediments. In both cases VP estimates were about one order of magnitude lower than those obtained by other approaches, with values varying between 0.2 and 0.6×10^8 viruses $g^{-1} h^{-1}$, respectively, in deep-sea and coastal sediments (Middelboe & Glud, 2006; Middelboe *et al.*, 2006). BS determined for marine viriobenthos in time-course experiments with diluted sediment samples range from three to 69 with a modal value of 20 viruses $cell^{-1}$. These are within the range of 10–100 viruses $cell^{-1}$ commonly observed in water samples (Wommack & Colwell, 2000; Weinbauer, Brettar & Höfle, 2003).

Viral decay in sediments has been measured with the KCN method in surface sediments of two oxbow lakes (Fischer *et al.*, 2003, 2004, 2006). In an *in situ* study over 13 months, VDR ranged from undetectable to $0.036 h^{-1}$, corresponding to 0 – 24.7×10^7 viruses $g^{-1} h^{-1}$, and in microcosms VDR reached values up to $0.078 h^{-1}$ (Fischer *et al.*, 2003). The average VDR in these freshwater sediments was two orders of magnitude higher than in the water column (Mathias *et al.*, 1995). In anoxic enclosures of coastal sediments VDR were lower than in freshwater surface sediments, with values ranging from 0.1 to 0.4×10^7 viruses $g^{-1} h^{-1}$, as determined by the Würigler-bag method (Glud & Middelboe, 2004).

Factors known to influence viral persistence (or decay) in aquatic systems include: solar radiation, temperature, pH, organic matter, salts, heavy metals, protozoan grazing and enzymes (e.g. Gerba & Schaiberger, 1975; Bitton, 1980; LaBelle & Gerba, 1980, 1982; Suttle & Chen, 1992; Noble & Fuhrman, 1997; Rossi & Aragno, 1999; Bongiorno *et al.*, 2005). Most studies have focussed on the loss of infectivity of various culturable viruses in sediments rather than decay rates of the entire viriobenthos. As the impact of environmental factors varies among types of virus, extrapolation from studies on single virus types to whole communities is problematic. However, it appears that the presence of sediment particles (especially clay minerals) can retard the loss of viral infectivity (e.g. Bitton, 1980; LaBelle & Gerba, 1980; Toranzo, Barja & Hetrick, 1982; Gerba, 1984; Sakoda *et al.*, 1997). The mechanism of this protection is largely unknown but it could be related to: (i) adsorption of the substances causing viral decay (e.g. proteolytic enzymes) and thus prevention of interaction with the virus; (ii) adsorption of the virus

itself, leading to stabilization of the viral structure by electrostatic forces, and/or trapping of the virus in a surface opening and thus reduced exposure to substances causing decay (e.g. Mitchell & Jannasch, 1969; Gerba & Schaiberger, 1975; Stagg, Wallis & Ward, 1977; Bitton, 1980; LaBelle & Gerba, 1982; Gerba, 1984).

Viral decay in sediments has a number of ecologically relevant consequences including: (i) the decrease of viral-mediated mortality of benthic prokaryotes and other organisms that are infected; (ii) the increase of the supply of extracellular DNA and RNA as well as peptides and amino acids, with consequences particularly for N and P cycling (see below); (iii) it shifts the processes of genetic recombination within the microbial compartment from generalized and specific transduction towards transformation. The factors controlling viral decay may provide selective pressures that influence the composition of viral communities. Such changes in viral community structure may also have consequences for microbial diversity and this, in turn, may affect the flow of energy and nutrients in aquatic ecosystems (Wommack & Colwell, 2000). Thus, viral decay plays an essential role in the dynamics of microbial food webs and the flow of genetic information within microbial communities.

Virus-induced host mortality The role of viruses in benthic processes depends largely on the virus-mediated prokaryote mortality but available results are conflicting and hardly comparable, as these have been obtained using different approaches (see paragraph 'Virus-induced host mortality' in Methods section). Therefore, to allow meaningful comparisons, data reported in Table 1 have been transformed, when needed, to percentages of prokaryote loss relative to total prokaryotic abundance.

Information on VIM in freshwater sediments is scant, but the available data suggest that prokaryote mortality mediated by viruses is three- and fivefold lower on average than in marine deep and coastal sediments respectively. Fischer *et al.* (2003) found the lowest values of VIM ranging from 0% to 5.8% (mean, 2.2%). Similarly, Bettarel *et al.* (2006) and Filippini *et al.* (2006) reported near-zero values in tropical lakes and freshwater marshes. The highest VIMs (up to >100%) were found in coastal sediments off Chile (Middelboe & Glud, 2006) and in coastal Adriatic

sediments (57.3%; Mei & Danovaro, 2004). Only two studies reported data on VIM in deep-sea sediments (Middelboe *et al.*, 2006; Corinaldesi *et al.*, 2007). Both of these studies reported that viruses play a crucial role in the mortality of deep benthic prokaryotes, being responsible for up to 60% of their loss.

Mei & Danovaro (2004) found a higher viral impact on prokaryotes in subsurface (42% of total prokaryotic abundance killed by viral infection) than in surface sediments (25%). These findings may be related to the probable absence, or extremely low density, of grazers that compete with viruses for prokaryotic cells (Fenchel, Kristensen & Rasmussen, 1990; Corinaldesi *et al.*, 2007).

Overall, it appears that mean VP rates are highest in freshwater sediments where the viral contribution to prokaryotic losses are lowest, while the opposite is true for coastal and deep-sea sediments (Table 1). Another discrepancy between marine and freshwater virobenthos was observed when experiments on the effect of temperature were carried out (Fischer *et al.*, 2003; Glud & Middelboe, 2004; Middelboe *et al.*, 2006). In freshwater sediments, the highest mortality of prokaryotes occurred at temperatures below 10 °C and the lowest mortality above 12 °C (Fischer *et al.*, 2003). Conversely, a positive relationship between temperature and VIM was found in surface marine sediments, probably as a result of stimulated prokaryotic production (Glud & Middelboe, 2004), whereas the opposite was observed in deep-sea sediments (Middelboe *et al.*, 2006). Experiments involving DOC addition to marine sediment samples indicated that the associated stimulation of prokaryotic production decreased viral impact on their hosts (Middelboe *et al.*, 2006).

To evaluate the reliability of these data on VIM and the extent to which environmental variability affects VIM, a larger data set is needed as well as systematic comparisons of the methods used for estimating virus production, BS and VIM.

The mortality of benthic microalgae is generally attributed to grazing, burial, resource exhaustion or apoptosis (Fenchel & Staarup, 1971). Only in the last few years has viral infection been recognized as an additional important source of microalgal mortality in sediments. Viruses could directly reduce primary production by infecting microalgae and cyanobacteria (Milligan & Casper, 1994). Information on lysis rates of benthic cyanobacteria and micro-algae is restricted

to the studies of Suttle *et al.* (1990) and Hewson *et al.* (2001b). Suttle *et al.* (1990) reported a decrease in the biomass of a benthic pennate diatom culture after addition of a high-molecular weight compound. Hewson *et al.* (2001b) tested experimentally the effect of viral infection on microphytobenthos abundance in sediments by enriching the natural community with viral concentrates. A 90% decrease was reported in the biomass of benthic pennate diatoms and a 20–60% decrease for benthic microalgae (Euglenophytes). Chlorophyll fluorescence and photochemical efficiency were also reduced. These data indicate a tremendous potential for viruses to control the abundance of benthic microalgae. However, the very limited number of studies carried out to date is insufficient to assess whether the observed values are generally representative for virobenthos infecting microalgae.

The role of viruses in benthic food webs and biogeochemical cycles Sediments are the main reservoir of organic carbon and nutrients in the oceans. However, knowledge of the role of viruses in biogeochemical cycles is still scant. Recent evidence that viral lysis in sediments can cause a large proportion of prokaryotic mortality (>50% in coastal sediments; Mei & Danovaro, 2004) poses the key question of the relevance and implications of viruses for benthic food webs and biogeochemical cycles.

Theoretical modelling suggests that if the main control of prokaryotic abundance is via protozoan grazing, most of the carbon will be channelled to higher trophic levels in the food web (Wommack & Colwell, 2000). Conversely, if viral infection accounts for most prokaryotic losses, the flow of carbon and nutrients can be diverted away from larger organisms (Bratbak *et al.*, 1990; Proctor & Fuhrman, 1990; Fuhrman, 1992, 1999), thus accelerating the transformation of nutrients from particulate (i.e. living organisms) to dissolved states. Only a single study has compared the impact of viruses and protozoan grazers on prokaryotes in freshwater sediments and found that viral lysis prevailed over protozoan grazing by a factor of 2.5–20 (Fischer *et al.*, 2006). If this finding can be generalized, it would suggest that the impact of viruses in sediments may be more important than that of virioplankton.

Viral infection has the potential to stimulate prokaryotic production and respiration, and to increase

nutrient regeneration through the liberation of products from cell lysis (i.e. soluble cytoplasmic components and structural materials, DOM, extracellular DNA and nutrients). This in turn might have important ecological and biogeochemical consequences. When viruses are included in food web models it is generally assumed that lysates are rapidly metabolized within the microbial community (Middelboe, Jørgensen & Kroer, 1996; Gobler *et al.*, 1997; Fuhrman, 1999; Wommack & Colwell, 2000).

Hewson *et al.* (2001b) added viral concentrates to marine benthic microcosms and observed a net decrease in prokaryote abundance and an increase in aggregates, probably resulting from the growth of uninfected prokaryotic cells due to products released by viral lysis. This result suggests that viral lysis stimulated DOM recycling in the sediments. Virus-induced C production was estimated to range from 7.5 to 38 nmol cm⁻³ h⁻¹, but these rates were equivalent to only 6–11% of the average carbon sedimentation rate in the study area (Hewson *et al.*, 2001a). For estuarine sediments, Glud & Middelboe (2004) estimated a DOC release rate of 1.0–1.9 nmol cm⁻³ h⁻¹, which could sustain 4.1–7.9% of the total prokaryotic carbon demand. Similar rates of virus-mediated DOC release were observed in sediments off the Chilean coast (0.3–3.5 nmol C cm⁻³ h⁻¹), sustaining 1–8% of the prokaryotic respiration (Middelboe & Glud, 2006). Further, in deep-sea sediments of a very productive ocean area, where viral infection accounted for the loss of a large fraction (24–48%) of total cell production, DOC release ranged from 0.5 to 2.1 nmol C cm⁻³ h⁻¹ (Middelboe *et al.*, 2006). On the basis of these results Middelboe *et al.* (2006) and Glud & Middelboe (2004) concluded that virus-mediated recycling of organic carbon played a minor role in the marine sediments they studied.

The only comparable data on virus-mediated release of C in freshwater sediments are higher than those obtained in marine sediments, ranging from 1.7 to 31 nmol C cm⁻³ h⁻¹ with an average of 12.5 nmol C cm⁻³ h⁻¹ (Fischer *et al.*, 2006). If this carbon released through virus-induced cell lysis was converted to new prokaryotic biomass with an efficiency of 31% (Kristiansen *et al.*, 1992), 4% of the prokaryotic production could have been sustained by viral lysates (range 0.1–11%). This was equivalent to a contribution of 8.9% (range 0.2–25%) to prokaryotic respiration. Fischer *et al.* (2006) also concluded, for the

freshwater sediments they studied, that virus-mediated lysis of prokaryotes did not contribute significantly to the DOM pool or to prokaryote production. Overall, these estimates suggest that virus-mediated recycling of organic carbon is insufficient (average generally <10%) to satisfy a substantial fraction of the carbon demand of heterotrophic prokaryotes in marine and freshwater sediments. However, considering the highest values in the study by Fischer *et al.* (2006), there is potential that virus-mediated DOC release contribute considerably to C cycling in sediments, since the total C supply for biomass production and respiration was up to 35%.

Even though virus-mediated lysis of prokaryotes generally does not satisfy a large fraction of the microbial C demand in sediments, it could be an important pathway of nutrient regeneration, particularly in systems characterized by limited external nutrient loading (Blackburn, Zweifel & Hagström, 1996; Zweifel, Blackburn & Hagström, 1996). Among the cell products released by viral lysis, extracellular DNA (due to its high lability and high content of organic nitrogen and phosphorus), might represent a particularly important source of nutrients for prokaryotic metabolism (Turk *et al.*, 1992; Jørgensen & Jacobsen, 1996; Danovaro *et al.*, 1999; Corinaldesi *et al.*, 2007) or a direct source of exogenous nucleotides for *de novo* synthesis of DNA (Paul, Deflaun & Jeffrey, 1988; Paul *et al.*, 1989). Recent studies carried out in deep-sea anoxic sediments reported that extracellular DNA released by viral lysis had the potential to fulfil 2–15% of the nitrogen and phosphorus requirements of prokaryotes, suggesting that viral lysis may represent an important nutrient source, especially in systems characterized by reduced external supply (Dell'Anno & Danovaro, 2005; Corinaldesi *et al.*, 2007).

A global estimate of viriobenthos

At the present state of knowledge, the data available on viral abundances and production in fresh water and marine sediments are still scant (Table 1). Nevertheless, we have attempted a first estimate of the global abundance and production of benthic viruses. To this end, we only considered the top layer of 1-m thickness of marine and lake sediments. Rivers were not included in the estimate since their total surface area is not well known and accounts for only

between 0.001% and 0.1% of the global land surface (Wetzel, 2001). Only two investigations so far have reported vertical profiles of viral abundance and production in surface sediments (Mei & Danovaro, 2004; Middelboe & Glud, 2006), and both found a decreasing trend with sediment depth. In spite of the scant data, we assumed that these vertical patterns are characteristics of sediments, and we also assumed a homogenous horizontal distribution of viruses within the sediment layer considered. All viral abundances originally expressed as number of viruses mL⁻¹ of wet sediment were transformed to number of viruses g⁻¹ of dry sediment (Table 1). To do this, we used a conservative factor of 0.9 to convert millilitres to grams, based on the assumption that the sediment water content is typically 50% and the sediment density is 1.8 g cm⁻³ (R. Danovaro, unpubl. data). Furthermore, we considered a total water surface of 362 × 10⁶ km² for the oceans (Dietrich *et al.*, 1975; Seibold & Berger, 1982) and 2.5 × 10⁶ km² for lakes and reservoirs (Wetzel, 2001) as representative of the global sediment surface. We refrained from applying a conversion factor for the global sediment surface, which due to its topographic complexity, is probably considerably larger than the global water surface area.

Using the simplifications described above, we calculated the total viral abundance in marine surface sediments at 28.7 × 10²⁸ viruses. The corresponding value for lake sediments is 0.5 × 10²⁸ viruses, resulting in a global abundance of 29.2 × 10²⁸ benthic viruses in the sediment layer up to 1 m depth. Remarkably, although the estimated total surface area of the oceans is 145 times greater than that of all lakes, viral abundance normalized per unit area of marine sediment is 2.5-fold smaller than viral abundance calculated for lakes.

Using the same approach, we also estimated VP in the top 1-m layer of the world's marine and freshwater sediments. We obtained 34.4 × 10²⁸ viruses day⁻¹ for marine and 0.6 × 10²⁸ viruses day⁻¹ for freshwater sediments. Thus, a total VP for the global viriobenthos would amount to 35 × 10²⁸ viruses day⁻¹, implying that the viriobenthos in both marine and freshwater ecosystems roughly turns over once every 20 h.

We are aware of the fact that marine sediments are much thicker than 1 m, generally reaching vertical extensions between 100 and 1000 m, and most sediment layers of large lakes have a thickness ranging

between 1 and 100 m. Given that relatively high viral abundance have been reported to a depth of 100 m in the sediment cores (Bird *et al.*, 2001), our estimates are probably to be extremely conservative.

Although our knowledge on viriobenthos is still far from complete, data provided here allow us to hypothesize that viruses constitute a key component of sediments, are potentially important agents of host mortality and major players in global biogeochemical processes. Considerably more information is needed to quantify their contribution in different ecological processes on a global scale and to provide accurate stratigraphic or region-specific estimates of viral abundance and production.

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