Higher Abundance of Bacteria than of Viruses in Deep Mediterranean Sediments

Roberto Danovaro,* Elena Manini, and Antonio Dell’Anno

Institute of Marine Sciences, Marine Biology Section, Faculty of Science, University of Ancona, 60131 Ancona, Italy

Received 5 July 2001/Accepted 28 November 2001

The interactions between viral abundance and bacterial density, biomass, and production were investigated along a longitudinal transect consisting of nine deep-sea stations encompassing the entire Mediterranean basin. The numbers of viruses were very low (range, $3.6 \times 10^7$ to $1.2 \times 10^9$ viruses g$^{-1}$) and decreased eastward. The virus-to-bacterium ratio was always $< 1.0$, indicating that the deep-sea sediments of the Mediterranean Sea are the first example of a marine ecosystem not numerically dominated by viruses. The lowest virus numbers were found where the lowest bacterial metabolism and turnover rates and the largest cell size were observed, suggesting that bacterial doubling time might play an important role in benthic virus development.

Viruses are considered the numerically dominant component in all aquatic systems, and viral densities exceed bacterial densities by 1 to 2 orders of magnitude (25). Due to their abundance ($10^9$ to $10^{10}$ viruses liter$^{-1}$) and their ability to infect bacteria and phytoplankton, viruses may have profound effects on microbial loop dynamics and biogeochemical cycling of organic matter (13).

Epidemiological models predict that viral infection increases as host cell density increases (28). In this regard, marine sediments could represent the optimal environment for viral development. In fact, marine sediments are characterized by high organic matter concentrations (ca. 3 to 5 orders of magnitude higher than those in the water column) and high bacterial densities ($10^8$ to $10^9$ cells ml$^{-1}$ in sediment, compared to $10^7$ to $10^8$ cells ml$^{-1}$ in the water column); in addition, the distances between the cells of benthic bacteria are very small. All these factors exponentially enhance the probability of virus-bacterium contact in marine sediments. However, surprisingly, studies dealing with viral abundance and distribution have been restricted to the plankton domain (see references 13 and 29 for reviews), and very limited information concerning sediments is available (7, 11, 25). Therefore, factors that affect viral abundance and distribution in marine sediments are still largely unknown.

In this study we examined interactions between viral abundance and bacterial density, biomass, and production along a longitudinal transect encompassing the entire deep-sea Mediterranean basin. Our sampling strategy was designed to include a gradient of trophic conditions (in terms of pelagic primary productivity [26] and particle fluxes [5]), from the more productive western basin to the highly oligotrophic Levantine Sea of the eastern Mediterranean. The specific aims of this investigation were (i) to identify the possible interactions of viruses with benthic bacteria and (ii) to identify environmental factors that control viral distribution and the ratio of viruses to bacteria in deep-sea systems.

**Study area and sampling.** Sediment samples were collected in the Mediterranean Sea between 8 June 1999 and 1 July 1999 with the R/V Aegaeo. Sediment sampling was carried out at nine stations in the Mediterranean basin (Fig. 1). Undisturbed sediment cores were collected with a multicorer equipped with eight liners (internal diameter, 9.5 cm). The station depth ranged from 1,290 m (station S7) to 4,000 m (station S6) (Table 1). For comparative analysis, the sampling stations were grouped based on the pelagic primary productivity (26), as follows: stations S1 to S5, eastern Mediterranean; and stations S6 to S8 and S10, central and western basin (referred to below as the western basin).

To obtain bacterial and viral counts, replicate subsamples (ca. 0.5 ml) of the top 1 cm of sediment ($n = 3$) were added to 5 ml of prefiltered (pore size, 0.02 µm) seawater containing 2% formalin and were stored at 4°C until analysis (within 4 weeks of collection). Additional sediment subsamples were analyzed immediately after retrieval on board to determine bacterial production. Finally, for analysis of grain size, water content, and biochemical composition of the sedimentary organic matter, additional cores were collected and the top 1 cm of sediment was frozen at $-20°C$.

**Environmental parameters.** Grain size analysis was carried out by using a dry sieve technique. Sediment water content (wc) was calculated by determining the difference between the wet and dry weights and was expressed as a percentage. Sediment porosity was determined with the following equation: (wc/1.02)/[1 - [(1 - wc)/2.64] + wc/1.02], where wc is (wet sediment weight – dry sediment weight)/wet sediment weight (6). Soluble protein and carbohydrate contents were determined as described by Dell’Anno et al. (9) and Danovaro et al. (4). For each biochemical analysis, blanks were prepared by using the same previously calcinated sediments (450°C, 2 h). All biochemical data were normalized to sediment dry weight after desiccation (60°C, 24 h).

**Viral and bacterial parameters.** Benthic bacteria and viruses were detached from sediment by using pyrophosphate (final concentration, 5 mM) and ultrasound (three 1-min treatments, 50 W power, 40 kHz)
Branson 2200 Sonifier, 60 W) to increase the extraction efficiency (8). For bacterial counting, subsamples were diluted 100- to 500-fold, stained with acridine orange (final concentration, 0.01%), and filtered on black Nuclepore 0.2-μm-pore-size filters (12). Acridine orange was used instead of SYBR Green I for bacterial analysis because it provided similar counts but did not result in the overestimation of biovolume which we observed when SYBR Green I was used. For viral counting, samples were shaken manually for 1 min and then centrifuged (800 g, 1 min) to reduce interference due to suspended particles. Aliquots of the supernatant were diluted 100- to 500-fold and filtered through 0.02-μm-pore-size Anodisc 25 membrane filters (pressure, 100 mm of Hg). The filters were then stained with 20 μl of SYBR Green I (lot no. 4967-30; diluted 20-fold in MilliQ water) for 15 min in the dark and subsequently rinsed twice with 1 ml of MilliQ water in order to eliminate background fluorescence. All filters were analyzed by epi-fluorescence microscopy using a Zeiss Axioplan microscope equipped with a 50-W lamp. Ten to 50 fields were viewed at a magnification of ×1,000, and a minimum of 400 bacterial cells or viruses were counted. Viruses were discriminated from bacteria on the basis of their dimensions (21). Bacterial biovolume was measured as described by Danovaro et al. (4) and was converted to carbon content (bacterial biomass) by using a conversion factor of 310 fg of C μm⁻³ (12). Bacterial cell size was calculated by dividing bacterial biomass by bacterial abundance. Viral counts were corrected for loss due to sampling, storage, and fixation (8). Viral and bacterial counts were normalized to sediment dry weight after desiccation (60°C, 24 h).

Bacterial production was measured by determining [³H]leucine incorporation (24) using the procedure described by van Duyl and Kop (27) for sediments. Sediment subsamples supplemented with an aqueous solution of [³H]leucine (final concentration, 0.1 nmol; specific activity, 68 Ci mmol⁻¹) were incubated for 1 h in the dark at the in situ temperature. After incubation, samples were supplemented with ethanol (80%) and were processed as described by van Duyl and Kop (27) before scintillation counting. Sediment blanks were prepared by adding ethanol immediately after [³H]leucine was added. Bacterial production was normalized to sediment dry weight after desiccation (60°C, 24 h).

![FIG. 1. Sampling area and station locations in the Mediterranean Sea.](http://aem.asm.org/)

**TABLE 1.** Station location, depth, water content, porosity, grain size, and soluble carbohydrate and soluble protein concentrations in surface sediment, (depth, 0 to 0.5 cm) at different locations in the Mediterranean Sea

<table>
<thead>
<tr>
<th>Station</th>
<th>Latitude (N)</th>
<th>Longitude (E)</th>
<th>Depth (m)</th>
<th>Water content (%)</th>
<th>Porosity</th>
<th>Silt-clay content (%)</th>
<th>Sand content (%)</th>
<th>Soluble carbohydrate (mg g⁻¹)</th>
<th>Soluble protein (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>35°46.29'</td>
<td>28°43.15'</td>
<td>3.870</td>
<td>45.8</td>
<td>0.69</td>
<td>98.3</td>
<td>1.7</td>
<td>0.18 (0.07)</td>
<td>0.24 (0.02)</td>
</tr>
<tr>
<td>S2</td>
<td>33°39.34'</td>
<td>33°18.43'</td>
<td>2.100</td>
<td>55.0</td>
<td>0.76</td>
<td>92.0</td>
<td>8.0</td>
<td>0.19 (0.12)</td>
<td>0.11 (0.00)</td>
</tr>
<tr>
<td>S3</td>
<td>33°23.18'</td>
<td>28°19.04'</td>
<td>3.055</td>
<td>37.3</td>
<td>0.61</td>
<td>84.7</td>
<td>15.3</td>
<td>0.28 (0.02)</td>
<td>0.16 (0.00)</td>
</tr>
<tr>
<td>S4</td>
<td>34°52.91'</td>
<td>22°31.97'</td>
<td>2.950</td>
<td>60.0</td>
<td>0.80</td>
<td>82.2</td>
<td>17.8</td>
<td>0.28 (0.01)</td>
<td>0.24 (0.03)</td>
</tr>
<tr>
<td>S5</td>
<td>35°42.38'</td>
<td>20°08.80'</td>
<td>3.200</td>
<td>58.9</td>
<td>0.79</td>
<td>85.6</td>
<td>14.4</td>
<td>0.20 (0.05)</td>
<td>0.24 (0.01)</td>
</tr>
<tr>
<td>S6</td>
<td>35°37.18'</td>
<td>17°23.37'</td>
<td>4.000</td>
<td>58.4</td>
<td>0.78</td>
<td>95.2</td>
<td>4.8</td>
<td>0.43 (0.09)</td>
<td>0.41 (0.01)</td>
</tr>
<tr>
<td>S7</td>
<td>36°36.66'</td>
<td>12°14.77'</td>
<td>1.290</td>
<td>51.1</td>
<td>0.73</td>
<td>88.1</td>
<td>11.9</td>
<td>0.63 (0.07)</td>
<td>0.58 (0.03)</td>
</tr>
<tr>
<td>S8</td>
<td>38°24.05'</td>
<td>06°53.72'</td>
<td>2.850</td>
<td>64.9</td>
<td>0.83</td>
<td>94.3</td>
<td>5.7</td>
<td>0.49 (0.05)</td>
<td>0.44 (0.00)</td>
</tr>
<tr>
<td>S9</td>
<td>40°33.99'</td>
<td>04°57.14'</td>
<td>2.755</td>
<td>48.0</td>
<td>0.77</td>
<td>78.8</td>
<td>21.2</td>
<td>0.28 (0.04)</td>
<td>0.41 (0.01)</td>
</tr>
</tbody>
</table>

* The values in parentheses are standard deviations.
Environmental parameters. Water content, sediment porosity, and grain size data are shown in Table 1. The water content ranged from 64.9% at station S8 to 37.3% at station S3. The porosity ranged from 0.61 at station S3 to 0.83 at station S8. The proportion of the silt-clay fraction (diameter, <0.0625 mm), which was dominant at all stations, ranged from 78.8% at station S10 to 98.3% at station S1.

Concentrations of soluble carbohydrates and soluble proteins are also shown in Table 1. The concentrations of soluble proteins and carbohydrates exhibited similar longitudinal patterns, and values were at least twofold higher at stations in the western Mediterranean basin.

Benthic bacteria and viruses. Table 2 shows data for bacterial direct counts, viral abundance, virus-to-bacterium ratios, bacterial biomass, bacterial size, and bacterial carbon production (BCP) in the deep-sea sediments of the Mediterranean Sea. Benthic bacteria did not exhibit a clear spatial pattern, and the concentrations of bacteria were similar at the western and eastern basin stations (averages, $4.34 \times 10^8$ and $4.53 \times 10^8$ cells g$^{-1}$, respectively). Similar patterns were observed for bacterial biomass. In contrast, bacterial size exhibited a clear increasing gradient eastward; the average values ranged from 0.61 at station S3 to 0.83 at station S8. The low viral abundance was very low, ranging from 0.11 (at stations S4 and S7) to 0.20 (at station S10); the only exception was station S6, where the ratio was 0.47.

Data obtained in the present study indicate that deep-sea sediments of the Mediterranean basin are the first example of a marine ecosystem that is not numerically dominated by viruses. The ratio of viruses to bacteria has been used to investigate relationships between virophagotrophic and bacterioplankton populations. Previous studies carried out in marine environments revealed that even in highly oligotrophic systems, the viral number exceeded the bacterial number by a factor of 3 to 10, and much higher values for the virus-to-bacterium ratio (up to $>100$) have been reported for nutrient-rich and highly productive environments (29). This simple observation suggests that greater numbers of viruses are released by bacterioplankton host populations under environmental conditions that enhance bacterial growth and productivity (29).

The ratios of viral abundance to bacterial abundance in deep Mediterranean sediments are the lowest values reported so far (Table 3) (7, 11). Similar low values have been reported only for lake sediments (18, 19) and for pelagic deep-sea environments (14, 21).

Environmental parameters. Water content, sediment porosity, and grain size data are shown in Table 1. The water content ranged from 64.9% at station S8 to 37.3% at station S3. The porosity ranged from 0.61 at station S3 to 0.83 at station S8. The proportion of the silt-clay fraction (diameter, $<0.0625$ mm), which was dominant at all stations, ranged from 78.8% at station S10 to 98.3% at station S1.

Concentrations of soluble carbohydrates and soluble proteins are also shown in Table 1. The concentrations of soluble proteins and carbohydrates exhibited similar longitudinal patterns, and values were at least twofold higher at stations in the western Mediterranean basin.

Benthic bacteria and viruses. Table 2 shows data for bacterial direct counts, viral abundance, virus-to-bacterium ratios, bacterial biomass, bacterial size, and bacterial carbon production (BCP) in the deep-sea sediments of the Mediterranean Sea. Benthic bacteria did not exhibit a clear spatial pattern, and the concentrations of bacteria were similar at the western and eastern basin stations (averages, $4.34 \times 10^8$ and $4.53 \times 10^8$ cells g$^{-1}$, respectively). Similar patterns were observed for bacterial biomass. In contrast, bacterial size exhibited a clear increasing gradient eastward; the average values ranged from 0.61 at station S3 to 0.83 at station S8. The low viral abundance was very low, ranging from 0.11 (at stations S4 and S7) to 0.20 (at station S10); the only exception was station S6, where the ratio was 0.47.

Data obtained in the present study indicate that deep-sea sediments of the Mediterranean basin are the first example of a marine ecosystem that is not numerically dominated by viruses. The ratio of viruses to bacteria has been used to investigate relationships between virophagotrophic and bacterioplankton populations. Previous studies carried out in marine environments revealed that even in highly oligotrophic systems, the viral number exceeded the bacterial number by a factor of 3 to 10, and much higher values for the virus-to-bacterium ratio (up to $>100$) have been reported for nutrient-rich and highly productive environments (29). This simple observation suggests that greater numbers of viruses are released by bacterioplankton host populations under environmental conditions that enhance bacterial growth and productivity (29).

The ratios of viral abundance to bacterial abundance in deep Mediterranean sediments are the lowest values reported so far (Table 3) (7, 11). Similar low values have been reported only for lake sediments (18, 19) and for pelagic deep-sea environments (14, 21).

In this study, the benthic bacterial densities were in the range of densities generally reported for deep-sea sediments (10), including the deep Mediterranean Sea sediments (3, 6, 7). Consequently, the low ratio of viruses to bacteria was due exclusively to low viral abundance. Viral abundance depends upon the number of potential host cells (28), and a direct relationship between bacterial density and viral count has been reported in most studies of pelagic environments (29). However, the levels of bacteria present in the sediments of the deep Mediterranean Sea ($>10^8$ cells g$^{-1}$) were far greater than the minimum threshold level required for viral infection (28), and bacterial abundance and viral abundance were not significantly correlated.

Virus replication depends on the growth and turnover rates of bacterial cells (29), so information on functional properties of benthic bacterial assemblages (i.e., bacterial size and biomass, as well as carbon production and turnover) is important (22). This is particularly true in deep-sea benthic environments, where bacterial dynamics are affected by a number of biotic variables (such as the availability of organic sources and grazing rates) and abiotic factors (temperature, redox potential, pressure, grain size, sediment compactness, turbulence), which can directly or indirectly affect virus dynamics.

In this study bacterial abundance and biomass did not exhibit a clear spatial pattern, and on average, almost identical values were obtained for the two basins (the differences were not significant, as determined by analysis of variance [ANOVA]). By contrast, bacterial secondary production was higher (ca. double [$P < 0.05$, as determined by ANOVA]) in
the western basin. Data for deep-sea benthic BCP obtained previously indicated that the BCP values in Mediterranean sediments are comparable to those reported for other deep-sea oligotrophic environments at similar depths (1, 20), but higher BCP values have been obtained for more productive deep-sea systems (2, 17, 27).

Differences in BCP values observed in this study reflect the higher organic matter quality and availability of the western Mediterranean sediments. In this regard, significant relationships between BCP and labile organic components (soluble protein concentration [R = 0.69, P < 0.05] and soluble carbohydrate concentration [R = 0.74, P < 0.01]) were found. Therefore, it is possible to conclude that input and availability of the organic sources in the deep-sea sediments of the Mediterranean largely controlled variations in BCP.

Differences between the two basins were also evident in terms of the significantly higher bacterial turnover and BCP per cell in the western basin (Table 2) (P < 0.01, as determined by ANOVA). Conversely, bacterial cell size increased eastward, indicating that there were small active cells in the western Mediterranean stations and larger slowly growing cells in the eastern basin. The lowest viral numbers were observed at stations S1 to S3, where the largest cell sizes and the lowest bacterial growth and turnover rates were observed. We also observed a significant relationship between bacterial turnover (BT) expressed as the ratio of BCP to bacterial biomass) and log-transformed viral abundance (TVN), as follows: TVN = 0.186 In(BT) + 8.397 (r = 0.749). This finding allowed us to hypothesize that bacterial doubling time may play an important role in limiting virus development in deep-sea sediments and may influence the life strategies of benthic viruses.

Fuhrman (13) reported that the lytic cycle is the most common development strategy in pelagic environments, but in some studies of oligotrophic environments characterized by low densities of slowly growing bacterioplankton, a greater incidence of lysogeny was observed (15, 16). The occurrence of a large fraction of dormant or slowly growing bacteria, generally observed in deep-sea sediments (10) and also reported in the present study, could be an important factor favoring the increase in lysogeny of benthic viruses. This factor could account, at least in part, for the lower viral numbers encountered in oligotrophic deep-sea sediments.

Previous studies have shown that viruses might be adsorbed to sinking particles and transported to the sea floor (23) so that sediments receiving higher particle fluxes might receive larger inputs of viruses from the water column. In this study the levels of soluble proteins and carbohydrates, assumed to be tracers of the labile organic matter input from the photic zone to the deep-sea floor (4), were significantly higher at the western basin stations, where higher viral abundance was also observed. We are not in a position to conclude that there is a pelagic-benthic coupling between virus distribution and particle fluxes, but the data can be the basis for future investigations of this possibility.

Results obtained in this study suggest that the number of viruses in deep-sea sediments might be dependent upon complex interactions with both abiotic factors (such as pressure, physical disturbance, and redox conditions) and biotic factors, including bacterial metabolic state and virus supply from the water column. Further studies are needed to clarify the causes of the low viral density, to estimate the actual impact of viruses on benthic microbial functioning, and to assess possible implications for biogeochemical cycles.

This work was carried out within the framework of TransMediterranean Cruise of the MATER program, which was financially supported by the European Union.

D. Marrale (University of Genoa), V. Lykoussis (NCRM, Athens, Greece), and the crew of the R/V Aegeo are gratefully acknowledged for support provided during the cruise. We thank M. L. Mei for contributing to laboratory analyses.

REFERENCES