Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters

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Abstract

Lysogeny (bacteria containing inducible prophages) and lytic viral infection (bacteria in a lytic stage of infection) were investigated at the community level in contrasting marine environments such as estuarine versus offshore waters, surface versus deep waters, and oxic versus anoxic waters in the Mediterranean and Baltic Seas. The frequency of lysogenic cells (FLC) in bacterioplankton communities ranged from not detectable to 84% as estimated by prophage induction due to mitomycin C, and highest values were typically found in deep waters (800–2,000 m). Transmission electron microscopy based estimates of virus-induced mortality of bacterioplankton (VMB) ranged from a few percent to 71%, and highest values were found in anoxic waters of the Baltic Sea. FLC and the frequency of infected cells (FIC) were related in form of a negative power function indicating that environments exist where one of the two viral life strategies prevails. Across all investigated environments, FLC was negatively related to bacterial abundance and production, whereas FIC showed a positive relationship with viral and bacterial parameters. FIC was higher and FLC was lower in moderately productive estuarine and offshore surface waters than in less productive mesopelagic and deep waters. Thus, lysogeny seems to be a survival strategy at low host abundance and activity, whereas high host abundance and activity seems to favor the lytic life cycle. The key process for the prevalence of lytic infection compared to prophage replication at high host abundance could be competition due to outnumbering. Between 11% and 88% (average, 35%) of the bacteria contained a functional (lytic or lysogenic) viral genome.

Viruses play a significant role in ecological and biogeochemical processes of the ocean, and their impact depends on the reproductive pathway (Fuhrman 1999; Wilhelm and Suttle 1999; Wommack and Colwell 2000). The two major viral reproductive strategies are lysogeny and lytic viral infection. In the lytic cycle, the (bacterio)phage forces the host metabolism to produce new phages, which are released during the lysis of the cell. In the lysogenic cycle, the lysogenic phage either enters the lytic cycle directly or the phage genome remains within the host in a dormant stage (prophage) and replicates along with the host until the lytic cycle is induced. In the pseudolysogenic life cycle, a fraction of the infected population is lysed (Ackermann and DuBow 1987); however, this has not been studied at the community level.

Based on studies with isolated phage–host systems and using models, it was proposed that lysogeny is a survival strategy of phages at low host densities (Steward and Levin 1984). Consequently, reproduction of lytic phages should be favored at high host abundances and when destruction rates of infectivity are low. There is a variety of methods for determining the frequency of lytically infected cells (FIC) and virus-induced mortality of bacterioplankton (VMB) (Fuhrman 1999; Wommack and Colwell 2000); however, at the community level, we cannot distinguish between lytic phages, lysogenic phages directly entering the lytic cycle after infection, and prophage induction. Viral lysis of bacteria is on average as significant as bacterivory for mortality of bacterioplankton (Fuhrman and Noble 1995), but the relative importance can vary with time and space or depend on environmental characteristics (e.g., Fuhrman 1999; Wommack and Colwell 2000). Some studies indicate that VMB might increase toward more productive systems (e.g., Weinbauer et al. 1993; Steward et al. 1996). Methods are also available to estimate the frequency of lysogenized cells (FLC) in bacterioplankton communities (Jiang and Paul 1996; Weinbauer and Suttle 1996); however, lysogeny is even less well studied than lytic infection. Estimates of FLC in bacterioplankton communities ranged from not detectable to about 80% (Jiang and Paul 1996; Weinbauer and Suttle 1996; Ortmann et al. 2002). Variability of FLC with space and time was demonstrated occasionally (Jiang and Paul 1996; Cochran and Paul 1998; Weinbauer and Suttle 1999), but the relationship between lysogeny and system productivity is even less clear than in case of lytic infection.

Viral lysis is a cause of bacterial mortality and nonrealized carrying capacity of the affected populations. Lysis of cells releases organic matter, which sustains bacterial production and respiration and increases the retention time of elements such as C, N, P, or Fe (Middelboe et al. 1996; Fuhrman 1999; Noble and Fuhrman 1999; Wilhelm and Suttle 1999; Middelboe and Lyck 2002). Viral lysis might also influence

species diversity by ''killing the winner'' (Thingstad and Lignell 1997) and keeping in check competitive dominants (Fuhrman 1999). Lysogeny confers some immunity against infection by related phages, and prophages can also change the metabolic properties of hosts and by that the fitness of lysogens (Marsh and Wellington 1994). This may result in virus-induced changes of species diversity as well. Moreover, viruses can transfer genes between host cells. The rates and ecological significance of such virus-mediated processes in the ocean remain largely unknown.

Overall, there is a lack of studies on lytic viral infection and lysogeny from contrasting marine environments, and there are almost no studies (Ortmann et al. 2002; Weinbauer et al. 2002) investigating the two viral life cycles simultaneously at the community level. Such information is necessary to assess the specific role of viruses for microbial food-web processes in different environments. Thus, we quantified FIC and FLC of bacterial communities in a wide range of contrasting marine environments, that is, estuarine versus offshore waters, surface versus deep waters, and oxic versus anoxic waters, which also comprise a trophic gradient from moderately productive estuarine waters to less productive deep waters. Our study suggests that the two viral life cycles show a tendency to exclude each other. Moreover, lytic viral infection increased and lysogeny decreased with increasing bacterial abundance and also with system productivity.

Materials and methods

Study sites, sampling, and physicochemical parameters— The study site in the northwestern Mediterranean Sea was the oligotrophic station Dyfamed (a French JGOFS station), about 50 km off Nice (43.42°N, 7.85°E). Dyfamed is separated from coastal waters by the Ligurian current and the water depth is about 2,300 m. Samples along two depth profiles (2 to 2,000 m) were taken during two cruises of the R/V *Thetys II* on 22 July 1998 and on 14 June 1999 using Niskin bottles mounted on a conductivity-temperature-depth (CTD) rosette having sensors for salinity, temperature, and fluorescence. Water samples in the Baltic Sea were collected along depth profiles from oxic surface to anoxic deep waters using Niskin bottles mounted on a CTD rosette having sensors for salinity, temperature, and oxygen during a cruise of the R/V *Aranda* from 14–25 September 1998 at stations BY15 (57.19°N, 20.30°E; Gotland Deep) and Teili1 (59.26°N, 21.30°E). A water sample from the water layer about 20 cm above the sediment was collected at Teili1 along with a sediment core. Owing to its brackish waters the Baltic Sea can be considered a large estuary; however, the samples were collected from a low-productivity area in the central Baltic. A more detailed description of the study sites can be found in Brettar and Rheinheimer (1991).

Hydrogen sulfide concentrations were measured photometrically by the methylene blue method and oxygen concentrations by the Winkler method (Grasshoff et al. 1983). Samples from suboxic and anoxic waters of the central Baltic Sea were processed immediately after collection to preserve the in situ oxygen concentrations. Subsamples were taken by an overflow technique, and incubation bottles were sealed with Teflon-coated butyl rubber septa (3 mm) and aluminum crimp caps or screw caps as described in Brettar and Rheinheimer (1991).

Epifluorescence and transmission electron microscopy— Bacteria and viruses in formaldehyde (2%, final concentration) preserved samples were filtered onto 0.02-μm pore-size filters, stained with SYBR Green I (Molecular Probes, Chemical No. S-7567), and enumerated by using epifluorescence microscopy (Noble and Fuhrman 1998). The frequency of visibly infected bacterial cells (FVIC) and the burst size, that is, the number of bacteriophages set free during lysis of cells, were determined by transmission electron microscopy (TEM) (Weinbauer and Suttle 1996). Briefly, bacteria from 10 to 50 ml of seawater were collected onto electron microscope grids using ultracentrifugation and positively contrasted with uranyl acetate. Ultracentrifugation and staining conditions were the same as described in Weinbauer and Suttle (1996). Burst size was estimated for every single sample as the average number of virus particles in all visibly infected bacteria totally filled with phages. Thus, these estimates should represent maximum burst sizes (Weinbauer and Suttle 1996).

Bacterial production—Bacterial production was estimated by the thymidine (83.0 Ci mmol^{-1}, Amersham) incorporation method (Fuhrman and Azam 1980) in the Mediterranean Sea and the leucine (83.0 Ci mmol^{-1}, Amersham) incorporation method (Bell 1986) in the Baltic Sea. In oxic waters of the Baltic Sea, bacterial production estimates based on Tdr and Leu incorporation estimates were similar (data not shown). Triplicate subsamples were spiked with [³H]Tdr or [³H]Leu at a final concentration of 10 nmol L^{-1}. Duplicate control subsamples were inactivated with formaldehyde (4% final concentration) and spiked with label. For suboxic and anoxic waters, samples were filled into 50-ml serum bottles by avoiding oxygen contamination (Brettar and Rheinheimer 1991) and [³H]leucine was added by gas-tight syringes (Pressure Lok) through the Teflon-coated butyl rubber septum. We used a thymidine conversion factor of 2×10^{18} cells mol^{-1} (Ducklow and Carlson 1992) and a leucine conversion factor of 0.07×10^{18} cells mol^{-1} (Riemann et al. 1990) to relate label incorporation to cell production.

Estimation of viral production, lytic viral infection, and lysogeny of bacteria—The FVIC was related to FIC using an average conversion factor of 7.11 established for natural communities including surface waters from the study sites (Weinbauer et al. 2002). FIC was related to VMB by using a model (Binder 1999) with

$$\text{VMB}(\%) = 100[(\text{FIC} + 0.6\text{FIC}^2)/(1 - 1.2\text{FIC})]$$

assuming that infected and uninfected bacteria are grazed at the same rate and that infected and uninfected bacteria grow at the same rate. Viral production was estimated by multiplying bacterial cell production by FIC/100 and the burst size.

The approach of Jiang and Paul (1996) was used to induce prophages in bacterioplankton. Mitomycin C (Sigma Chem-
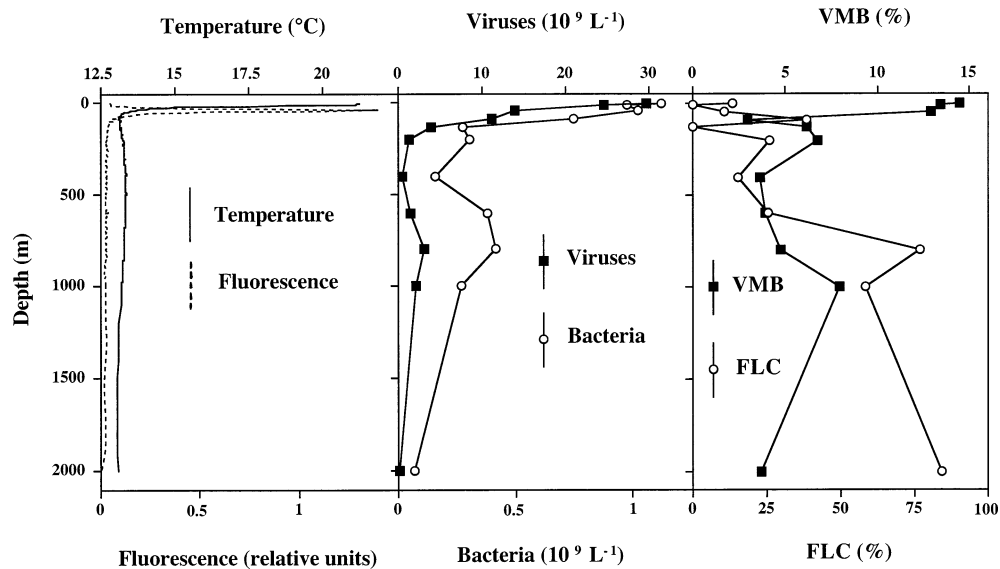
Fig. 1.   Physicochemical and microbial parameters along a depth profile in the Mediterranean Sea on 14 June 1999 at station Dyfamed. Owing to a failure to close a Niskin bottle, no samples could be obtained from 1,500 m.

ical Co. No. M-0503) was added to 10-μm Nitex prefiltered seawater samples (final concentration, 1 μg ml^{-1}), and untreated samples served as controls. Samples at oxygen concentrations <1 μg L^{-1} (>80 m at both stations in the Baltic Sea) were filled into 50-ml plastic syringes and sealed with Teflon-coated butyl rubber stoppers (Brettar and Rheinheimer 1991). This allows for sample withdrawal without creating headspace, which would influence the oxygen concentration in the sample.

Prophage induction was defined as difference of viral abundance between mitomycin C treated (V_{MC}) and control incubations (V_C) (Weinbauer and Suttle 1996). A difference between treatment and control was assumed when the range of duplicate incubations did not overlap (at least in one time point) and the difference of viral abundance in V_{MC} and V_C was calculated from mean values of duplicate incubations. FLC was estimated as

$$FLC(\%) = 100[(V_{MC} - V_C)/(BSB_{t0})]$$

with BS = burst size and B_{t0} = bacterial abundance at the start of the experiment before the addition of mitomycin C. Incubations were done in duplicates and samples for the enumeration of viruses and bacteria were removed periodically and preserved in formaldehyde (2% final concentrations). Typically, samples were collected after 6 h and 12 h and then in 12-h intervals for up to 3 d. Temperatures were kept within ±2°C by flowing seawater baths or incubation chambers. Prophage replication rates were estimated as the bacterial cell production times FLC/100.

Statistics—All data were either arcsine (% data) or log transformed for correlation and regression analyses or analysis of variance (ANOVA).

Results

Mediterranean Sea—Viral and bacterial abundances obtained during two cruises generally ranged from 0.056 × 10^6 to 29.6 × 10^6 ml^{-1} and from 0.044 × 10^6 to 1.7 × 10^6 ml^{-1}, respectively, and were higher in surface than in deep water (Fig. 1, Table 1). Viruses and bacteria did not show a continuous decrease with depth, but local abundance maxima were observed as, for example, in the mesopelagic layer. Except for 1,250 m and 1,500 m in 1998, viral abundance was always higher than that of bacteria, and the virus-to-bacterium ratio (VBR) ranged from 0.7 to 26. Prophage induction could be detected in 82% of the samples. FLC ranged from not detectable to 84% and was only determined during the 1999 cruise. FLC was less than 40% in waters down to 600 m but ranged from 58% to 84% between 800 and 2,000 m. FIC ranged from 0.7% to 12%, and VMB ranged from 0.8% to 16%. VMB dropped strongly below the deep chlorophyll maximum (DCM) layer and varied around 5% or less in deeper water.

The water column of the Mediterranean Sea was divided into three layers, surface water (<100 m), mesopelagic water (100 m to <800 m), and deep water (800–2,000 m) based on hydrological and biological data (Bethoux 1989). Values were averaged for the depth layers, and ranges were calculated from two cruises (Table 1). Microbial parameters generally showed highest values in surface and lowest values in deep waters; only FLC values were highest in deep and lowest in surface waters, whereas burst size varied only little with depth (Table 1). The difference of microbial parameters between mesopelagic and deep waters was generally low. In deep waters FLC averaged 73%, whereas in mesopelagic and surface offshore waters lysogeny averaged 21% and 12%, respectively (Table 1). VMB averaged 14%–15% in surface

Table 1. Impact of viruses on bacteria in surface, mesopelagic, and deep waters of the Mediterranean Sea. Data per cruise were averaged for depth layers, and ranges are given for two depth profiles except for data on lysogeny and prophage replication, which were obtained from a single depth profile.

	Surface	Mesopelagic	Deep
Viruses (10^9 L^{-1})	4.7–22.6	0.6–3.7	0.1–2.0
Lytic viral production (10^9 L^{-1} d^{-1})	0.082–0.091	0.0003–0.0016	0.0001–0.0016
Bacteria (10^9 L^{-1})	0.9–1.0	0.19–0.37	0.11–0.25
Bacterial cell production (10^9 L^{-1} d^{-1})	0.050–0.052	0.0024–0.0091	0.0008–0.0022
FIC (%)	9.9–11.1	2.6–4.3	2.2–5.0
VMB (%)	13.1–14.8	3.2–5.2	2.6–6.2
Viruses-to-bacteria ratio	6.1–21.7	2.5–8.1	1.1–5.6
Burst size	26–31	37	31–36
FLC (%)	12.2	21.2	73.2
Prophage replication rate (10^9 L^{-1} d^{-1})	0.0065	0.0035	0.0032

waters, 3%–5% in mesopelagic waters, and 3%–6% in deep marine waters.

Baltic Sea—Physicochemical parameters of the study sites in the Baltic Sea are shown in Figs. 2 and 3. The water column in the Baltic Sea shows a complex stratification pattern; however, for the purpose of this study, several water layers were defined based on the oxygen content, that is, the surface layer with oxygen concentrations higher than 6 mg L^{-1}, the suboxic layer with oxygen concentrations between 0.5 and 6 μg L^{-1}, and the "anoxic" layer with oxygen concentrations lower than 0.5 μg L^{-1} concurring with measurable hydrogen sulfide concentrations at BY15, whereas no detectable H$_2$S concentrations could be detected at Teili1. Anoxic waters were also characterized by an increase of bacterial cell volume (data not shown). For both depth profiles, viral and bacterial abundance typically decreased with depth down to the lower suboxic layer and increased again in anoxic waters. Viral and bacterial abundances ranged from 3 × 10^6 to 32.9 × 10^6 ml^{-1} and from 0.17 × 10^6 to 3.1 × 10^6 ml^{-1}, respectively. Parameters such as viral and bacterial

abundance, production, and turnover were typically lowest in the suboxic layer, where VBR and FLC were highest (Table 2). Prophage induction could be detected in 79% of the samples. FLC ranged from not detectable to 79% and averaged 4%–9% in anoxic waters, compared to 16%–44% and 7%–8% in suboxic and oxic waters, respectively. FIC and VMB were on average lower in the oxic and suboxic layer than in anoxic waters. FIC and VMB ranged from 4% to 33% and from 4% to 71%, respectively. Virus-induced mortality of bacteria averaged 13%–18% in oxic surface waters, 12%–19% in interface waters, and 27%–51% in anoxic waters.

Across system analyses—Across systems, FLC correlated best with bacterial abundance followed by viral abundance and bacterial production (Table 3), and these relationships were negative. FIC was related best to viral and bacterial parameters, and these relationships were positive. Less significant relationships ($r < 0.5$) were found between FIC and cell-specific bacterial production and VBR. The trends of FLC and FIC with other parameters did not change, when
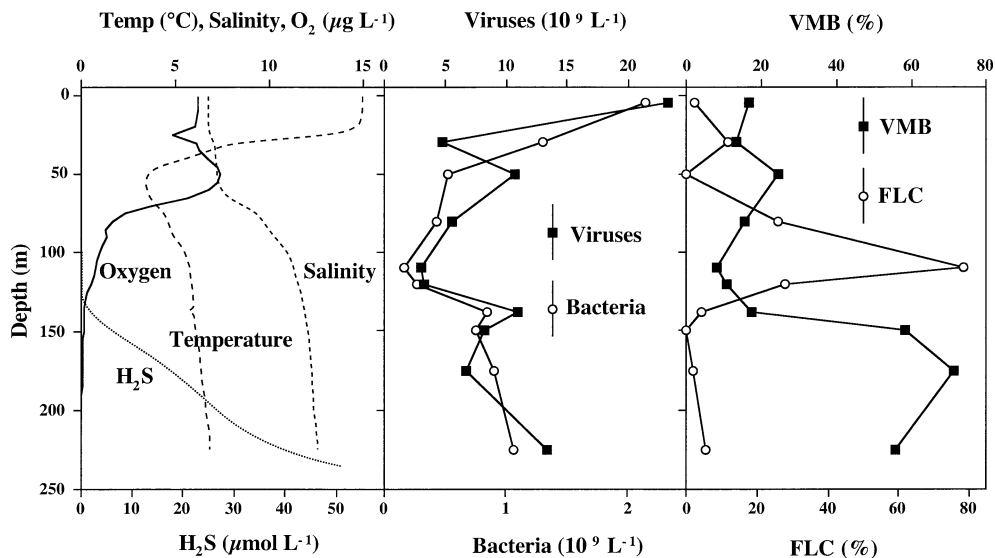

Fig. 2. Physicochemical and microbial parameters along a depth profile in the Baltic Sea in September 1998 at Sta. BY15.
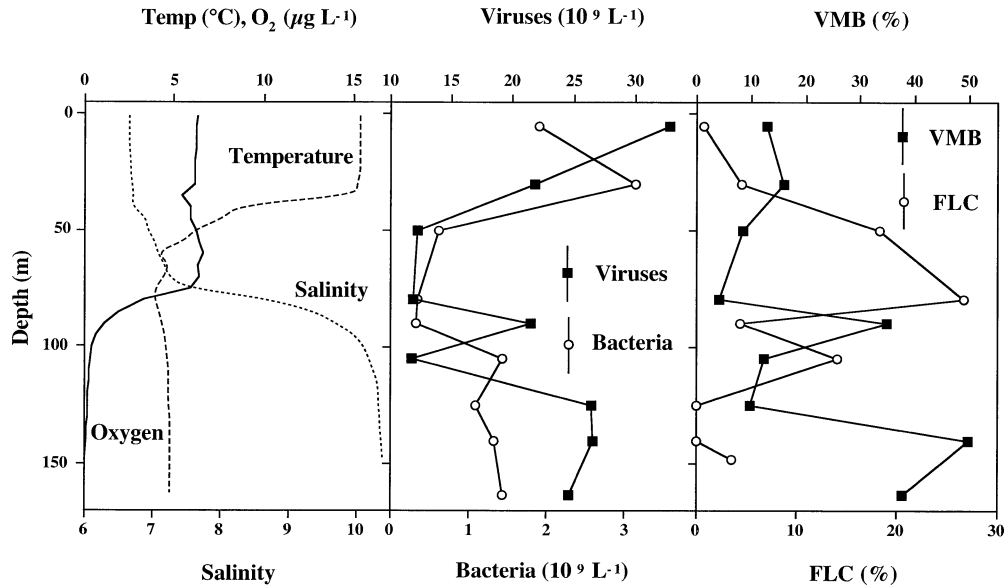
Fig. 3. Physicochemical and microbial parameters along a depth profile in the Baltic Sea in September 1998 at Teili1.

anoxic waters were excluded from the calculations (data not shown). The relationship between FIC and FLC could be best described by a negative power function in both environments (Fig. 4).

Data on FLC and FIC in oxic waters were averaged for the estuarine system of the Baltic Sea and offshore surface and the mesopelagic and deep-water layers in the Mediterranean Sea (Fig. 5). Based on general information on these types of environments, as well as estimates on microbial parameters obtained in the present study (Tables 1 and 2), these environments represent a trophic gradient from moderately productive estuarine waters to less productive deep waters. FLC increased toward less productive environments from an average of 7% in estuarine waters to an average of 12%, 26%, and 73% in offshore surface, mesopelagic, and deep waters, respectively. FIC decreased toward less productive systems with an average FIC of 13%, 10%, 3%, and 3% in estuarine, offshore surface, mesopelagic, and deep waters, respectively. ANOVA showed that these trends were significant (FLC, $F_{13} = 7.753$; $P = 0.0062$; FIC, $F_{29} =$

14.803, $P < 0.0001$). Post hoc tests (significance level $P < 0.05$) showed that FLC differed significantly between estuarine waters and mesopelagic and deep waters and between offshore and deep waters. FIC differed significantly between all water layers except between estuarine and offshore surface waters and between mesopelagic and deep waters. The sum of FLC (only data were used, where prophage induction could be detected) and FIC infection ranged from 11% to 88% of the total bacterial community with an average of 35%.

Discussion

Viral abundance and production—Our data confirm findings from other environments (Hara et al. 1996; Noble and Fuhrman 1998) that viral abundance is surprisingly high in mesopelagic and deep marine waters with average VBR values ranging from 1 to 8 in the Mediterranean Sea. Transport of viruses in deep waters by sinking particles (Hara et al. 1996) cannot explain this, since sinking particles are rapidly

Table 2. Impact of viruses on bacteria in surface, suboxic, and anoxic waters of the Baltic Sea. Data per cruise were averaged for depth layers and ranges are given for two depth profiles. Samples with no detectable prophage induction were not included in calculating averages for parameters related to lysogeny.

	Surface	Suboxic	Anoxic
Viruses (10^9 L^{-1})	12.9–22.3	3.9–16.7	9.8–22.3
Lytic viral production (10^9 L^{-1} d^{-1})	0.53–1.84	0.026–0.175	0.57–0.99
Bacteria (10^9 L^{-1})	1.3–1.9	0.29–0.34	0.89–1.32
Bacterial cell production (10^9 L^{-1} d^{-1})	0.18–0.61	0.016–0.170	0.048–0.068
FIC (%)	10.0–13.2	9.3–12.6	16.8–25.2
VMB (%)	12.6–17.9	11.6–19.4	27.4–50.6
Viruses-to-bacteria ratio	11–15	14–50	11–17
Burst size	27–32	28	93–94
FLC (%)	6.8–7.8	15.6–44.1	3.7–8.8
Prophage replication rate (10^9 L^{-1} d^{-1})	6.8–7.8	15.6–44.1	3.7–8.8

Table 3. Correlation of FLC and FIC with other parameters. Only samples with detectable prophage induction values were used for calculations. FLC, $n = 24$; FIC, $n = 43$. NS, not significant.

Parameter	FLC		FIC	
	r	P	r	P
Viral abundance	−0.657	<0.0005	0.803	<0.0001
Viruses-to-bacteria ratio	−0.218	NS	0.680	<0.0001
Bacterial abundance	−0.723	<0.0001	0.752	<0.0001
Bacterial production	−0.557	<0.005	0.644	<0.0001

decomposed within the first 500 m at the study site Dyfamed (Turley and Stutt 2000). Decay of viral infectivity increases with increasing temperature (Cottrell and Suttle 1995), and thus the relatively low temperature of 13°C in mesopelagic and deep waters of the Mediterranean Sea and the 4°C or less in deep waters of the Indo-Pacific Ocean could have resulted in an increased survival rate of viruses and thus the high VBR values.

Another reason for sustaining high viral numbers in deep waters might be induction of prophages and pseudolysogenic viral proliferation. Jiang and Paul (1998) have formulated a numerical model to estimate the contribution of lysogenic phage production to total phage production (V_L) using the equation

$$V_L\% = 100(S_r GLB/V_n)$$

with S_r = spontaneous phage release rates, G = bacterial generation time, L = fraction of lysogenic bacteria, B = bacterial abundance, and V_n = new or total viral production. With high spontaneous phage release rates of 10^{-2} phages

per cell per generation (Ackermann and DuBow 1987) and data from this study (Tables 1 and 2), viral production due to spontaneous prophage induction was <0.1% of total viral production (averaged for depth layers). Thus, spontaneous prophage induction seems to represent a negligible contribution to viral production even in deep waters, where FLC was high and viral production was low. In 1,000–2,000 m at the Dyfamed station, bacterial production is on average higher in compressed than in uncompressed samples (Tamburini et al. 2002). This has likely resulted in an underestimation of viral production, which is based on bacterial production measurements at surface pressure. Despite that, it is unlikely that the estimates of the fraction of spontaneous prophage induction on the total viral production were impaired, since both estimates are based on the same bacterial production measurements.

Viral abundances in anoxic marine waters have been reported during anoxia events in the Chesapeake Bay (Wommack et al. 1992) and in deep waters of the Cariaco Trench (Taylor et al. 2001). In the Chesapeake Bay, viral abundance was not related to oxygen content, whereas in the Cariaco Trench viral abundance was high close to the interface and in deep anoxic water. Higher viral abundance in anoxic than in oxic water layers has been shown for a eutrophic lake
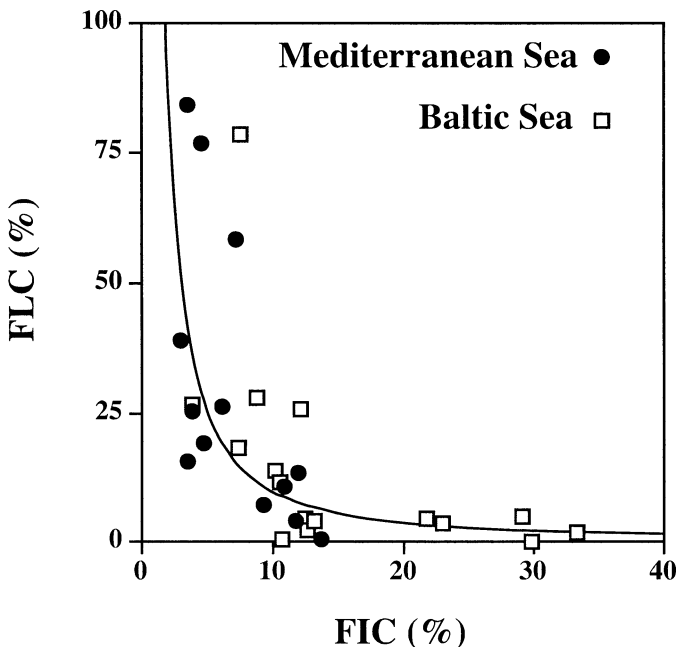

Fig. 4. Relationship between lytic-stage viral infection (frequency of infected cells) and lysogeny (frequency of lysogenic cells) of bacteria plankton. All data: $y = 252.4x^{-1.419}$, $r = 0.684$, $P < 0.0001$. Mediterranean Sea: $y = 429.9x^{-1.765}$, $r = 0.715$, $P < 0.005$. Baltic Sea: $y = 230.6x^{-1.348}$, $r = 0.610$, $P < 0.005$.
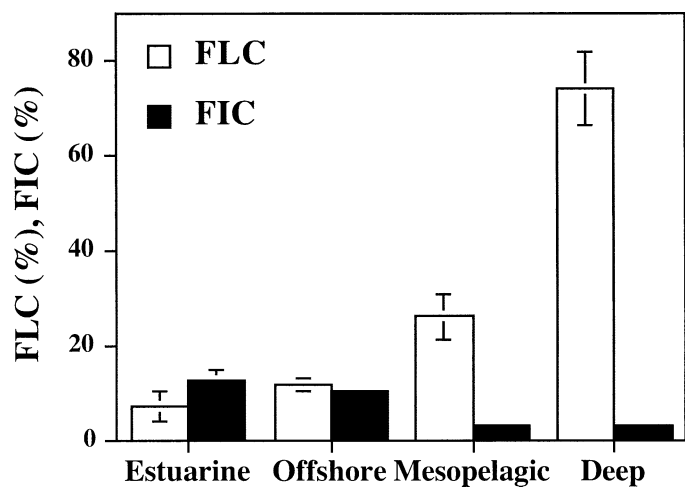

Fig. 5. Percent lysogeny (frequency of lysogenic cells) and viral infection of bacterioplankton (frequency of infected cells) in different oxic environments. This also represents a trophic gradient from moderately productive estuarine to less productive deep waters. Data from oxic waters of the Baltic Sea were used for estuarine systems. Data are averages for single environments, and error bars are standard errors.

(Weinbauer and Höfle 1998). The low temperature in the anoxic waters of the Baltic ($<8°C$; Figs. 2 and 3) and the lake (Weinbauer and Höfle 1998) could have slowed down viral decay rates and thus sustained high VBR and viral production.

Lysogeny—Mitomycin C does not induce all lysogenic isolates (Ackermann and DuBow 1987), and it has been shown for bacterial communities that environmental pollutants can be more efficient inducing agents than mitomycin C (Cochran et al. 1998). Thus, it is possible that mitomycin C resulted in an underestimation of FLC in some environments, such as surface waters. However, the finding that the majority of deep-water bacteria was lysogenized indicates that mitomycin C is a potent inducing agent in natural bacterial communities.

Jiang and Paul (1994, 1998) have shown that the percentage of lysogenized isolates was higher in offshore than coastal stations. Also, FLC of bacterioplankton as determined by prophage induction and burst size estimates was higher in offshore than in coastal habitats (Weinbauer and Suttle 1996, 1999) but highest in estuarine environments (Jiang and Paul 1996). However, a high FLC value of 80% was reported from coastal (although pristine) waters in the Strait of Georgia (Ortmann et al. 2002). This suggests that there might be no simple relationship between FLC and system productivity. In most studies FLC varied strongly between samples but was on average below about 15% in estuarine, coastal, and offshore surface waters (Tables 1 and 2) (Jiang and Paul 1996; Weinbauer and Suttle 1996, 1999; Cochran et al. 1998; Cochran and Paul 1998; Weinbauer et al. 2002), indicating that differences of average FLC values between such environments can be moderate. In contrast, the average FLC was six times higher in deep than in surface waters of the Mediterranean Sea (Fig. 5), indicating that the level of lysogeny is significantly different between deep and surface waters. Our finding that FLC was inversely related to bacterial abundance and production (Table 3) supports the hypothesis that lysogeny is a survival strategy, when host abundance is low (Steward and Levin 1984) at the community level.

Lytic infection and virus-induced mortality of bacteria— With none of the methods for estimating virus-induced mortality of bacterioplankton is it possible to distinguish viral infection of bacteria due to lytic phages from induced prophages or lysogenic phages immediately entering the lytic pathway after infection. Despite this problem, it is possible to estimate virus-induced mortality of bacterioplankton. In surface waters of the Mediterranean Sea VMB was moderate (13%–15%), and in mesopelagic and deep waters it was low (3%–6%). In the Mediterranean Sea, it was estimated that viruses remove $<20\%$ of the bacterial production in surface waters (Guixa-Boixareu et al. 1999), which is similar to our data. Data on viral infection of bacterioplankton in mesopelagic and deep marine waters have not been reported before. Our estimates suggest that viral infection is not a significant factor for bacterial mortality in this environment.

The VMB values from anoxic waters in the Baltic Sea (up to 71%; Fig. 2) are among the highest reported for marine systems using the TEM approach (Weinbauer et al. 2002). High viral mortality of prokaryotes in anoxic waters is in accordance with low grazing rates on bacteria by protists in this environment (Fenchel et al. 1990). High viral mortality of bacteria at low grazing rates was also found in the anoxic water layer of a eutrophic lake (Weinbauer and Höfle 1998) and in solar salterns at the highest salinity values (Guixa-Boixareu et al. 1996), suggesting that viruses are the major cause for bacterial mortality in these environments. In addition to low grazing rates, the low bacterial diversity found in anoxic waters of the Baltic Sea (Höfle and Brettar 1995) may have sustained high viral infection frequencies.

Parameters such as bacterial abundance and production showed a significant positive relationship with FIC (Table 3). Also, FIC increased with system productivity (Fig. 5). An increase of FIC with increasing bacterial abundance or production was also found occasionally in other environments (e.g., Weinbauer et al. 1993; Steward et al. 1996). In our study, this relationship was found across a large range of marine environments such as estuarine, offshore, deep, and anoxic waters with bacterial abundances ranging over two orders of magnitude. This empirical relationship suggests that lytic-stage infection dominates at high total contact rates between bacteria and viruses and at high bacterial productivity.

Relationship between lysogeny and lytic viral infection— The finding that the relationship between FLC and FIC was described best by a power function (Fig. 5) indicates that environments might exist where one of the two predominates, such as lytic viral infection in anoxic waters and lysogeny in deep marine waters. This would imply that there are environmental characteristics favoring one of the two viral life cycles as, for example, the number and activity of host cells, the resource for phages. While it makes evolutionary sense that lysogeny is an adaptation to low host abundance, an explanation is required for the finding of low FLC values at high bacterial abundance, since there is no a priori reason why lysogeny should be disadvantageous in such conditions. The number of genomes of a prophage population depends on the replication rate of the host resulting in one additional viral genome per cell division (Marsh and Wellington 1994), whereas lysis results in multiple genome copies determined by the burst size. At the community level, prophage replication rate was only two times higher in surface waters than in mesopelagic and deep waters, whereas viral production was about two orders of magnitude higher (Table 1). This could indicate that lytic phages (and lytic-stage infection) predominate at high contact rates by outcompeting of prophages due to faster reproduction. However, free viruses are likely more rapidly destroyed than prophages, and free phages have to find a host. Thus, more information on viral replication and destruction rates is required to assess the competitive ability of lytic and lysogenic phages at the community level.

The data obtained in this study indicate that a high percentage of bacteria (average, 35%) contained a functional viral genome. Although the specific role of viruses may differ between environments depending on the predominance of lysogeny or lytic-stage infection, the high total infection

frequencies suggest a significant role of viruses in marine microbial food webs. In addition to influencing food-web processes, nutrient cycling, and metabolic properties of bacteria, phages can transfer genetic material between host cells. Owing to the high total infection rates, it is conceivable that this influences short-term adaptation and population dynamics of bacterioplankton (Fuhrman 1999). It remains a challenge for future research to elucidate the ecological role of such processes and its relationship to viral reproductive strategies.

References

ACKERMANN, H.-W., AND M. S. DuBow. 1987. Viruses of prokaryotes, v. I., General properties of bacteriophages. CRC.

BELL, R. T. 1986. Further verification of the isotope dilution approach for estimating the degree of participation of [^{3}H] thymidine in DNA synthesis in studies of aquatic bacterial production. Appl. Environ. Microbiol. **52:** 1212–1214.

BETHOUX, J. 1989. Oxygen consumption: New production, vertical advection and environmental evolution in the Mediterranean Sea. Deep-Sea Res. **36:** 769–781.

BINDER, B. 1999. Reconsidering the relationship between virally induced bacterial mortality and frequency of infected cells. Aquat. Microb. Ecol. **18:** 207–215.

BRETTAR, I., AND G. RHEINHEIMER. 1991. Denitrification in the Central Baltic: Evidence for H$_2$S-oxidation as motor of denitrification at the oxic-anoxic interface. Mar. Ecol. Prog. Ser. **77:** 157–169.

COCHRAN, P. K., C. A. KELLOGG, AND J. H. PAUL. 1998. Prophage induction of indigenous marine lysogenic bacteria by environmental pollutants. Mar. Ecol. Prog. Ser. **164:** 125–133.

———, AND J. H. PAUL. 1998. Seasonal abundance of lysogenic bacteria in a subtropical estuary. Appl. Environ. Microbiol. **64:** 2308–2312.

COTTRELL, M. T., AND C. A. SUTTLE. 1995. Dynamics of a lytic virus infecting the photosynthetic marine picoflagellate *Micromonas pusilla.* Limnol. Oceanogr. **40:** 730–739.

DUCKLOW, H. W., AND C. A. CARLSON. 1992. Oceanic bacterial production. Adv. Microb. Ecol. **12:** 113–181.

FENCHEL, T., L. D. KRISTENSEN, AND L. RASMUSSEN. 1990. Water column anoxia: Vertical zonation of planktonic protozoa. Mar. Ecol. Prog. Ser. **62:** 1–10.

FUHRMAN, J. A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature **399:** 541–548.

———, AND F. AZAM. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. **39:** 1085–1095.

———, AND R. T. NOBLE. 1995. Viruses and protists cause similar bacterial mortality in coastal seawater. Limnol. Oceanogr. **40:** 1236–1242.

GRASSHOFF, K., M. EHRHARDT, AND K. KREMLING. 1983. Methods of seawater analysis. Verlag Chemie.

GUIXA-BOIXAREU, N., J. I. CALDERÓN-PAZ, M. HELDAL, G. BRATBAK, AND C. PEDRÓS-ALIÓ. 1996. Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. Aquat. Microb. Ecol. **11:** 215–227.

———, D. VAQUÉ, J. GASOL, AND C. PEDRÓS-ALIÓ. 1999. Distribution of viruses and their potential effect on bacterioplankton in an oligotrophic marine system. Aquat. Microb. Ecol. **19:** 205–213.

HARA, S., I. KOIKE, K. TERAUCHI, H. KAMIYA, AND E. TANOUE. 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. **145:** 269–277.

HÖFLE, M. G., AND I. BRETTAR. 1995. Taxonomic diversity and metabolic activity in the water column of the central Baltic Sea. Limnol. Oceanogr. **40:** 868–874.

JIANG, S. C., AND J. H. PAUL. 1994. Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. Mar. Ecol. Prog. Ser. **104:** 163–172.

———, AND ———. 1996. Occurrence of lysogenic bacteria in marine microbial communities as determined by prophage induction. Mar. Ecol. Prog. Ser. **142:** 27–38.

———, AND ———. 1998. Significance of lysogeny in the marine environments: Studies with isolates and a model of lysogenic phage production. Microb. Ecol. **35:** 235–243.

MARSH, P., AND E. WELLINGTON. 1994. Phage-host interactions in soil. FEMS Microbiol. Ecol. **15:** 99–108.

MIDDELBOE, M., N. O. G. JØRGENSEN, AND N. KROER. 1996. Effects of viruses on nutrient turnover and growth efficiency of non-infected marine bacterioplankton. Appl. Environ. Microbiol. **62:** 1991–1997.

———, AND P. LYCK. 2002. Regeneration of dissolved organic matter by viral lysis in marine microbial communities. Aquat. Microb. Ecol. **27:** 187–194.

NOBLE, R. T., AND J. A. FUHRMAN. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. **14:** 113–118.

———, AND ———. 1999. Breakdown and microbial uptake of marine viruses and other lysis products. Aquat. Microb. Ecol. **20:** 1–11.

ORTMANN, A., J. LAWRENCE, AND C. SUTTLE. 2002. Lysogeny and lytic viral production during a bloom of the cyanobacterium *Synechococcus* spp. Microb. Ecol. **43:** 225–231.

RIEMANN, B., R. T. BELL, AND N. O. G. JØRGENSEN. 1990. Incorporation of thymidine, adenine and leucine into natural bacterial assemblages. Mar. Ecol. Prog. Ser. **65:** 87–94.

STEWARD, F. M., AND B. R. LEVIN. 1984. The population biology of bacterial viruses: Why be temperate? Theor. Popul. Biol. **26:** 93–117.

———, D. C. SMITH, AND F. AZAM. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. Mar. Ecol. Prog. Ser. **131:** 287–300.

TAMBURINI, C., J. GARCIN, M. RAGOT, AND A. BIANCHI. 2002. Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2,000 m water column in the NW Mediterranean Sea. Deep-Sea Res. II **49:** 2109–2123.

TAYLOR, G., AND OTHERS. 2001. Chemoautotrophy in the redox transition zone of the Cariaco basin: A significant midwater source of organic carbon production. Limnol. Oceanogr. **46:** 148–163.

THINGSTAD, T. F., AND R. LIGNELL. 1997. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquat. Microb. Ecol. **13:** 19–27.

TURLEY, C., AND E. STUTT. 2000. Depth-related cell-specific bacterial leucine incorporation rates on particles and its biogeochemical significance in the Northwest Mediterranean. Limnol. Oceanogr. **45:** 419–425.

WEINBAUER, M. G., D. FUKS, AND P. PEDUZZI. 1993. Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. Appl. Environ. Microbiol. **59:** 4074–4082.

———, AND M. G. HÖFLE. 1998. Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. Appl. Environ. Microbiol. **64:** 431–438.

———, AND C. A. SUTTLE. 1996. Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. Appl. Environ. Microbiol. **62:** 4374–4380.

———, AND ———. 1999. Lysogeny and prophage induction in

coastal and offshore bacterial communities. Aquat. Microb. Ecol. **18:** 217–225.

———, C. WINTER, AND M. HÖFLE. 2002. Reconsidering transmission electron microscopy based estimates of viral infection of bacterioplankton using conversion factors derived from natural communities. Aquat. Microb. Ecol. **27:** 103–110.

WILHELM, S. W., AND C. A. SUTTLE. 1999. Viruses and nutrient cycles in the sea. Bioscience **49:** 781–788.

WOMMACK, K. E., AND R. R. COLWELL. 2000. Virioplankton: Vi-

ruses in aquatic ecosystems. Microbiol. Mol. Biol. Rev. **64:** 69–114.

———, R. T. HILL, M. KESSEL, E. RUSSEK-COHEN, AND R. R. COLWELL. 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. **58:** 2965–2970.